IPNC 2022

International Plant Nutrition Colloquium

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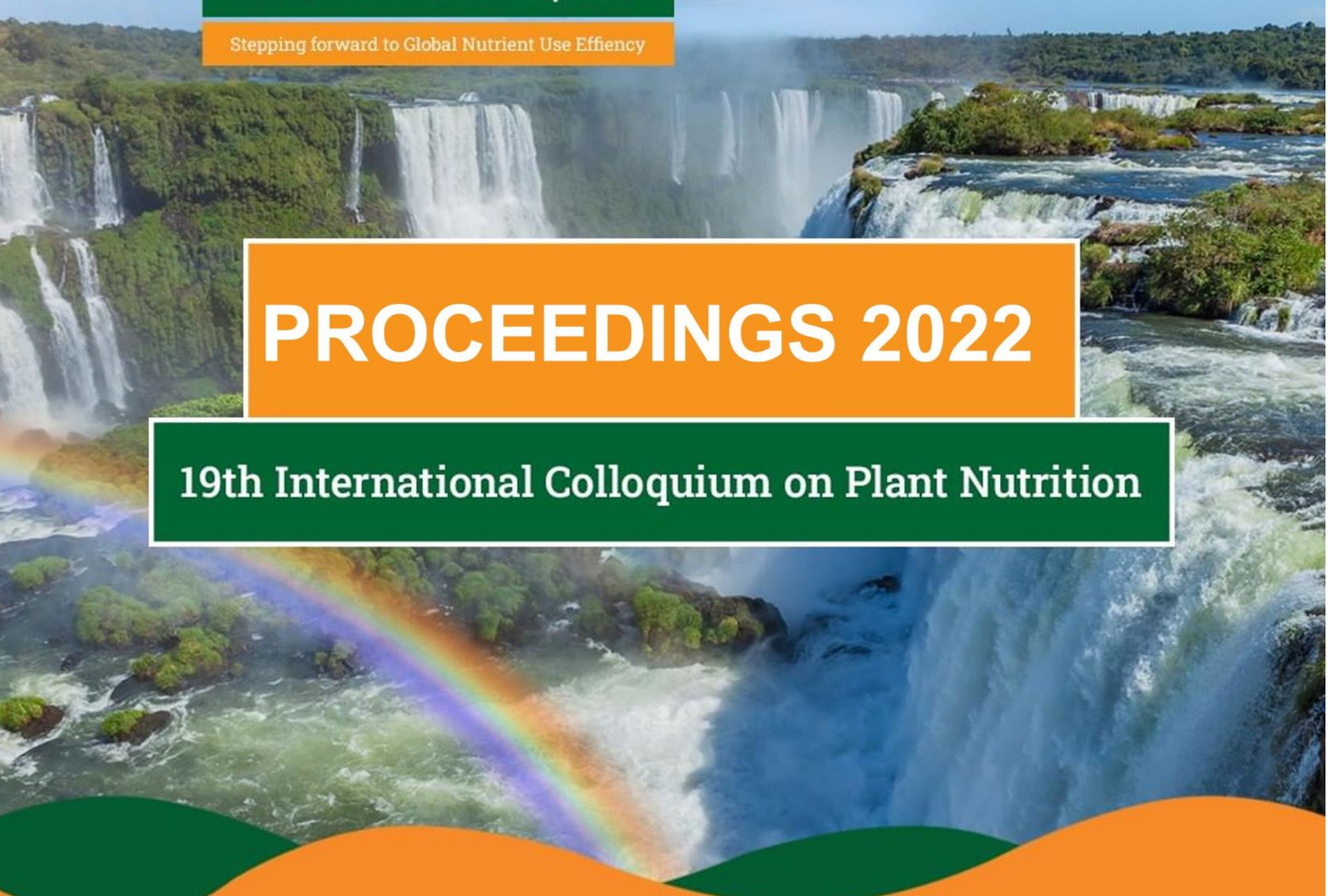
Stepping forward to Global Nutrient Use Effiency



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PRESENTATION

Since its beginning in the early 1950s, the IPNC has grown to become the most important international meeting on fundamental and applied plant nutrition. Building on the success of previous IPNC meetings, more than 800 participants from all over the world was expected in Iguazu Falls. The main theme of the 19th International Plant Nutrition Colloquium is: "Stepping forward to Global Nutrient Use Efficiency". This theme has been chosen to highlight that plant nutrients, fundamental for successful intensification of global crop production can pose an environmental risk if not well used. This intensification is required to meet the demands of the future bio-based society for nutritious food, feed, raw materials, and energy.

The colloquium has provided an excellent forum for the exchange and transfer of knowledge, information and ideas as well as for the creation of new collaborations and fostering synergies in the fields of plant mineral nutrition, plant molecular biology, plant genetics, agronomy, horticulture, ecology, environmental sciences and fertilizer use and production. We invite you to present the recent results of your research related to the different aspects of plant mineral nutrition.

Professor Ciro A. Rosolem
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Biostimulants and plant nutrition

THE EFFECT OF SILICON ON THE ORGANICALLY GROWN LEAF CELERY TRANSPLANTS GROWTH AND QUALITY

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Keywords: Silicon; tomato transplant; growth and quality

Introduction

The role of Si in plant growth and development was overlooked until the beginning of the 20th century (Shakoor & Bhat, 2014). However large amounts of field studies have shown that supplying crops with adequate plant-available Si can suppress plant disease, reduce insect attack, improve environmental stress tolerance, and increase crop productivity (Heckman, 2013). Silicon plays important roles in mitigating the biotic (insects, pests, pathogens) and abiotic (metal, salinity, drought, chilling, freezing) stresses (Guntzer et al., 2012; Thilagam, 2014). The application of stabilized silicic acid is called the 'silicic acid agro technology' (SAAT) (Bent, 2014). The purpose of the investigations was to look the effect of Silicon on the leaf celery transplant growth and nutrient content.

Materials and Methods

There were two treatments: 1. stabilized silicic acid treatment; 2. control. Seedlings and transplants were grown in Novarbo B2 Organic Biolan substrate for organic cultivation. Silicic acid treatment was carried through as followed:

Item	Si treatment	Control
1st true leaf stage spraying	Silicic acid (2 ml L^{-1}) solution	water
2 weeks later	Silicic acid (2 ml L^{-1}) solution	water
2 weeks later	Silicic acid (2 ml L^{-1}) solution	water

Each treatment consisted of 16 plants, within that one plot consisted of 4 plants in four replications. The experiment was repeated at the same time. The greenhouse lighting at a plant level was approximately 12000 lux from high pressure sodium lamps. The plants were additionally lighted in the period of 18 hours (04.00 - 22.00). All plants were grown with a minimum day and night temperature of 20 °C and 18 °C, respectively. On 12.05.2014 the height and stem diameter were recorded. The contents of Nitrates, Nitrogen, Phosphorus, Potassium, Calcium and Magnesium were determined. Analyses of variance were carried out on the data obtained using programme Excel. Used signs: *** p<0,001; ** p= 0,001 - 0,01; * p= 0,01 - 0,05; NS not significant, p>0,05.

Results

Silicic acid improved the quality of leaf celery transplants: leaf celery transplants were taller and stem diameter was greater in silicic acid treatment compared to control (Figure 1). Nutrient content is in table 1. The content of nitrates in raw leaf celery transplants was not statistically different between treatments. The content of Nitrogen, Calcium and Magnesium in leaf celery transplants dry matter were higher in silicic acid treatment compared to control treatment. The content of Phosphorus and Potassium in leaf celery transplants dry matter were not significantly different between treatments.

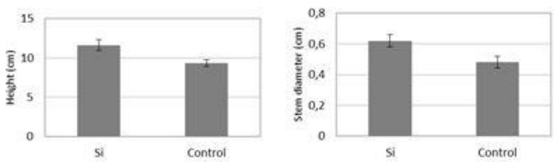

Fig. 1. The height (cm; *) and stem diameter (cm; ***) of leaf celery transplants treated with silicic acid.**

Table 1. The content of Nitrates (mg/kg) in raw leaf celery transplants and the contents of Nitrogen (%), Phosphorus (%), potassium (%), Calcium (%) and Magnesium (%) in leaf celery transplants dry matter.

	Nitrates (mg/kg)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Si	6.74	1.85	0.66	5.72	1.62	0.33
Control	6.33	1.68	0.67	5.72	1.43	0.27
p	NS	*	NS	NS	**	**

Discussion

The height of leaf celery transplants was significantly higher in Si treatment compared to control treatment. Similarly, the research data with cucumber, tomato, iceberg lettuce and leaf lettuce (Olle, 2016) confirmed that the height of transplants was higher in Si treatment than in control treatment. Treatment with silicon has been reported to increase nutrient uptake generally (Bent, 2014). In present investigation also Si treatment increased the content of Nitrogen, Calcium and Magnesium in leaf celery.

Conclusion

Silicic acid improved the quality of leaf celery transplants: leaf celery transplants were taller and stem diameter was greater. The content of nitrates in raw leaf celery transplants and the contents of Phosphorus and Potassium were not statistically different between treatments. The content of Nitrogen, Calcium and Magnesium in leaf celery transplants dry matter were higher in silicic acid treatment.

Acknowledgements

The present research was carried through with financial support from Estonian Agricultural Registers and Information Board and with the help of the Jaagumäe Agro OÜ and Estonian Crop Research Institute. ReXil Agro BV have supported by free of charge silicic acid, provided the training for using SAAT technology and issued a travel grant to attend IPNC 2022.

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PHYSIO-AGRONOMIC CHARACTERIZATION OF UPLAND RICE INOCULATED WITH MIX OF MULTIFUNCTIONAL MICROORGANISMS

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Keywords: nutrient uptake; beneficial microorganisms; sustainable agriculture

Rice producer will need sustainable inputs to reach the world and economics demands. In this sense, the use of beneficial microorganisms that can contribute to crop growth and health consist in a good strategy for a sustainable agriculture. Plant Growth Promoting Rhizobacteria (PGPR) and *Trichoderma* spp. fungus are beneficial microorganisms that interact with root plants and can promote plant development. The effects of these beneficial microorganisms can be direct, such as hormones and siderophores production, phosphate solubilization and nutrient mobilization; or indirectly, by suppressing various pathogens in the forms of biocontrol agents, and by providing greater efficiency in the gas exchange process. In addition, these microorganisms could provide protection against biotic stress, such as the induction of resistance and direct antagonism. The objective of this study was to determine the effect of bioagents applied alone or in mix on the performance of upland rice. The experiment was conducted in a greenhouse in a completely randomized design with four replications. The 26 treatments consisted of the rhizobacteria *Bacillus* sp. (BRM 32109, BRM 32110 and 1301), *Azospirillum* sp. (1381), *Azospirillum brasilense* (Ab-V5), *Pseudomonas* sp. (BRM 32111), *Pseudomonas fluorescens* (BRM 32112), *Burkholderia pyrrocinia* (BRM 32113), *Serratia* sp. (BRM 32114), and a fungal genus formed by a pool of *Trichoderma asperellum* (T-06, T-09, T-12, and T-52), alone or in mix, plus a control treatment without microorganism application. The largest accumulations of N, P and K in the shoots were observed in rice plants treated with the mixed application of the microorganisms BRM 32114 + BRM 32110 (Figure 3). However, this treatment provided a significant difference only for N and P accumulations in rice plants, and for nutrient K it did not differ significantly from the control treatment. In terms of nutrient accumulation in the root system, treatment 1301 + BRM 32110 stands out, which provided a greater increase in nutrients N and P (12 and 98%, respectively) compared to the control treatment, however, there was a significant difference in relation to control treatment only for nutrient P. The highest accumulation of K in the root system was obtained with plants treated with 1381 + Pool of T. asperellum, which differed significantly from the control treatment. Based on our results, it appears that, in general, treatments containing mix of microorganisms provided greater gains in nutritional accumulation in rice plants, which allows us to infer that the use of mixture of microorganisms is promising and provides better results than application of only one microorganism. The most effective treatments were the mixes of microorganisms 1301 + Ab-V5 and BRM 32114 + pool of *Trichoderma asperellum,* as they provided an average increase of 123 and 88% in the number of panicles and 206 and 167% in the grain yield of upland rice plants, respectively. Mixes of 1301 + Ab-V5, BRM 32114 + *Trichoderma asperellum* pool, BRM 32110 + BRM 32114, BRM 32110 + Ab-V5, 1301 + BRM 32110 and 1381 + *Trichoderma asperellum* pool also provided better morphophysiological performance in rice plants (photosynthetic rate, carboxylation efficiency, number of tillers, shoot dry biomass and nutrient content in shoot and root). Therefore, the use of multifunctional microorganisms in the management of upland rice was efficient in its ability to improve plant performance.

Financial Support

To Embrapa Rice and Beans for the support and concession of the study area. To the Ministry of Agriculture and Renovagro Company for supporting the attending in this event.

THE INFLUENCE OF HUMIC SUBSTANCES AND AMINO-ACIDS ON GROWTH AND NITROGEN NUTRITION OF ARABIDOPSIS PLANTS

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Keywords: N fertilization; Arabidopsis; Biostimulants

Like all living organisms, plants need to finely tune their growth and development in accordance with available nutrients. Moreover, nutrients such as nitrogen (N) are not only the substrates but also the signals for growth. N is an essential macro-element for plants and, for most of them, is taken up as inorganic N source from the soil (nitrate or ammonium). Therefore, its availability can largely vary and has a strong effect on crop yield but also on resistance to various stresses. However, the use of high quantities of inorganic N fertilizers has also a strong impact on the environment. There is thus an urgent need for strategies allowing a better N use efficiency in crops. For this goal, the use of molecules, such as humic substances or amino-acids, stimulating and optimizing N use is a promising strategy. However, the links between humic substances, growth and N metabolism are not well documented in plants.

We have studied the influence of humic substances and amino-acids on growth, N uptake and metabolism of the model plant Arabidopsis thaliana and of wheat plants either in controlled in vitro conditions or in hydroponic cultures. Addition of both humic substances and amino-acids results in effects on root growth and development in a dose-dependent manner. We have also explored the effects of these molecules in response to variations in N nutrition.

Financial Support

This project was partly supported by Fertinagro France.

EFFECTS OF ASCOPHYLLUM NODOSUM AND SOIL AMMENDMENTS ON THE DEVELOPMENT OF MAIZE SEEDLINGS CULTIVATED UNDER ACIDIC OXISOL

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Keywords: biostimulants; gypsum; lime

INTRODUCTION

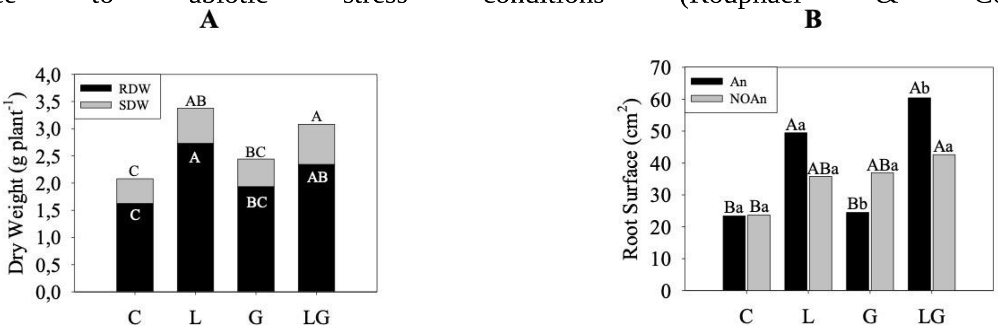
Maize (Zea mays L.) plays a fundamental role in Brazilian agriculture, and it's mostly cultivated in Brazilian Savannah areas, under low nutrient availability, high Al content and acidic pH. To mantain high yield and grain quality, a routine of soil fertilization and amendment for mitigation of the aluminum toxicity and low pH is crucial (Fageria et al., 2014). With the rise in environmental awareness over the risks of soil contamination due to the excess of mineral fertilizers, new sustainable nutrient sources, such as algae extracts, have been increasing in the market. The use of seaweed in agriculture as a source of organic matter or soil amendment is quite old, but its biostimulant effect has only been studied in the past few decades (Mukherjee & Patel, 2020). They also increase nutrient absorption, as they affect both soil processes, such as pore structure and micronutrient solubility, and plant physiology, through changes in root morphology (Rouphael & Colla, 2020). Therefore, the aim of this work was to evaluate the effects of A. nodosum extract combined with gypsum and lime soil amendment treatments on the initial development of the root system in an acid Ferralsol.

METHODS

The plants were sown in glass rhizotrons of 60cm of height, 40cm width and 3cm depth. They were filled a sandy loam Ferralsol (IUSS Working Group WRB, 2015), with 51% of Al saturation (m) at 1,2 g cm^{-3} density. The experimental design was randomized 4x2 factorial, with the following treatments: lime (L), gypsum (G), lime + gypsum (LG) and control (C), cultivated with and without seed treatment and foliar application of the *Ascophyllum nodosum* "Shropshire seaweed" (Sea-chem Limited), in six replicates. For the soil amendment treatments, the "lime" used was calcium oxide (CaO), calculated to increase soil base saturation (V%) to 70 and the "gypsum" used was calcium sulfate ($CaSO_4.2H_2O$), calculated according expression: Gypsum (Mg ha^{-1}) = 50 X Clay (%). The experimental evaluations were carried out fifteen days after plant emergence. The glass surface of the rhizotrons was photographed. The images were processed in the QUANT 1.0.1 program (Vale et al., 2001) to estimate the root surface in 2400 cm^2 of rhizotron area. The root depth was determined, and subsequently, the rhizotrons were disassembled. Plant were hasrvest and separated into shoot and root, then washed and dried until constant weight, weighed for shoot (SDW) and root (RDW) dry weight determination. Data were analyzed using ANOVA, with a p-value of $\leq$ 0,05 using the software SISVAR (Ferreira, 2014). When significant effects of treatments were found, multiple means comparison was carried out using Fisher's LSD analysis with a 95% confidence interval.

RESULTS AND DISCUSSION

The variables shoot (SDW) and root dry weight (RDW) were significant (Table 6). The root depth did not change with the treatments, and their means were 35,85 cm. The plants that had higher shoot and root dry weight were the ones under L and LG treatments, while those with the lowest dry weight were those of the control treatment (Figure 1A). The plants that had higher shoot and root dry weight were the ones under L and LG treatments, while those with the lowest dry weight were those of the control treatment (Figure 1A). The root surface was under the interaction between soil treatments with and without the application of A. nodosum. The soil amendment treatments that provided the largest root surface with A. nodosum were L and LG, and in the absence of the algae extract, LG, L and G were the ones with the largest root surface (Figure 1B). The positive correlation between L and LG and algae application demonstrates that, in these treatments, with the application of A. nodosum the plant developed its root system better. Root having a larger area and increasing its contact surface with the soil, which is essential for greater absorption of water and nutrients, and greater tolerance to abiotic stress conditions (Rouphael & Colla, 2020).



Capital letters differ the soil amendment treatments from each other, regardless of whether with or without the application of *A. nodosum*, and lowercase letters differ the treatments with *A. nodosum*, regardless of corrective treatments, by the t LSD test (5% probability).

Fig. 1. Initial maize growth: (A) shoot (SDW) and root dry weight (RDW) (g plant-1) and (B) Root surface (cm²) varying according to soil amendment treatments and A. nodosum application. Rio Verde, 2019.

CONCLUSIONS

The seed treatment with *A. nodosum* extract and its application on maize seedlings led to an increase in the root surface in treatments with lime.

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Biotic stress and plant nutrition

RESPONSES OF PURPLE RICE TO LOW LIGHT INTENSITY ON YIELD AND GRAIN BIOACTIVE COMPOUNDS

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Keywords: purple rice; bioactive compound; shading

INTRODUCTION

Purple rice has garnered increasing attention as a health food, especially among health-conscious consumers, as reflected in the rising demand and production by farmers. Although purple rice can be cultivated throughout the year by planting non-photosensitive varieties, the available information concerning optimum light conditions for productivity and grain nutritional quality is limited. Light is an extremely essential factor in potential productivity; low light conditions during cloudy weather can suppress physiological processes such as photosynthesis and the activities of enzymes involved in starch synthesis and the translocation of carbohydrates (Liu et al., 2020). Purple rice is a source of bioactive compunds, having antioxidant and anti-inflammatory properties. Previous study has reported that the anthocyanin concentration and antioxidant capacity in purple rice grains were affected by growing elevation in the highlands and lowlands (Yamuangmorn et al., 2021), suggesting that the variation in climate factors such as light intensity may be the key for stimulating compound synthesis. However, limited information is available concerning how light intensity would affect the bioactive compounds in purple rice. This study was initiated to evaluate the responses of purple rice to light intensity in terms of productivity and synthesis of bioactive compounds.

METHODS

The K4 rice variety *(Oryza sativa* L.), an advanced line of non-photosensitive rice with purple color in the shoot and grain derived from the Agronomy Division at Chiang Mai University was used in this experiment. A randomized complete block design with four replications was arranged. The two light intensities applying shading were (i) treatment without shading for the entire seed development period as a control light condition, designated as unshaded (13.1-61.0k lux) and (ii) depressed light intensity by applying shading on the first day after flowering and continuing for 14 days, designated as shaded at early seed development (5.5-23.0k lux). The samples were harvested at maturity stage for yield measurements. Grain phenolic compounds were determined by HPLC by using the modified method of Ryu et al. (1998) for anthocyanin, and the modified method of Mighri et al. (2019) for phenolic acid and flavonoid analysis.

RESULTS AND DISCUSSION

Grain yield and yield components were significantly affected by shading treatment (Table 1). Grain yield was reduced by 17% compared to the unshaded. This response was similar to the 1,000-grain weight and the filled grain in the shaded application; the values were decreased by 4.3% and 5.9% compared to the control with no shading. The results demonstrated that shading resulted in significant yield loss due to the reduction of grain filling and individual grain weight compared with the unshaded treatment. A previous study mentioned that shading of rice plants at the grain filling stage affected the major physiological processes of electron transport and photosynthesis and consequently reduced productivity (Wang et al., 2015). Similarly, shading at heading stage was found to decrease grain filling in the spikelets at the bottom and middle positions of the panicle (Li et al., 2020).

Table 1. Yield and phenolic compounds in purple rice grains grown with unshaded and shaded applications.

Yield	Unshaded	Shaded
Grain yield (g plant^{-1})	7.07 ± 0.32 a	5.88 ± 0.28 b
1000-grain weight (g)	21.26 ± 0.29 a	20.33 ± 0.15 b

Filled grain (%)	91.9 ± 2.07 a	86.5 ± 3.07 b
Phenolic compound		
Total anthocyanin (mg kg^{-1})	506.99 ± 27.68 b	1,164.9 ± 70.61 a
Total phenol (g GAE kg^{-1})	3,204.6 ± 117.1 b	4,472.7 ± 194.7 a
DPPH (g trolox kg^{-1})	28.63 ± 2.30 b	47.6 ± 2.36 a
Anthocyanin (mg kg^{-1})		
Cyanidin-3-glucoside	165.25 ± 19.75 b	344.87 ± 2.12 a
Peonidin-3-glucoside	60.59 ± 8.60 b	118.17 ± 1.59 a
Phenolic acid (mg kg^{-1})		
Chlorogenic acid	822.71 ± 15.93	872.76 ± 31.79
p-Coumaric acid	264.63 ± 0.49	272.02 ± 7.40
Ferulic acid	111.85 ± 0.40	105.78 ± 8.93
Epigallocatechin-3-gallate	218.26 ± 8.24	251.03 ± 27.73
Hydroxybenzoic acid	206.94 ± 3.68 a	143.40 ± 0.92 b
Flavonoid (mg kg^{-1})		
Quercetin	1,121.1 ± 9.25	1,165.5 ± 14.43
Rutin	465.63 ± 18.46	370.49 ± 2.82
Naringin	371.01 ± 5.53	324.24 ± 10.61

Values within each row followed by different letters are significantly different at $P < 0.05$. The values are expressed as mean ± Sd.

The concentration of specific phenolic compounds in the grains grown with unshaded and shaded applications is shown in Table 1. Cyanidin-3-glucoside and peonidin-3-glucoside concentrations were significantly increased by 109% and 95% in grains treated by shading in comparison to the unshaded treatment. On the other hand, the response of phenolic acids and flovoniods to shading treatment was trended to be decreased. For instance, the reduction of 31% of chlorogenic acid was found in the shaded compared to the unshaded control. Rutin in the grains shaded was reduced by 21% compared to the unshaded control. However, other phenolic compounds were not affected by shading treatment. Shading during reproductive growth increased grain anthocyanins, in contrast to the response in phenolic acids and flavonoids. Anthocyanins are the major phenolic compounds involved in the antioxidant response, and their attenuation in the stressed plants is mainly caused by high light intensity (Zheng et al., 2019). The present study noted that anthocyanins are sensitive to light intensity; this can be used as a key parameter to identify low light tolerance.

CONCLUSION

The shading at seed development decreased grain yield; shading resulted in the reduction of individual grain weight and filled grain. Remarkably, the increased anthocyanin in the grains were strongly enhanced by shading, suggesting that the bioactive compounds are highly sensitive to light. These results provide valuable information for purple rice cultivation, especially during cloudy and rainy weather, and the findings can be used as a guide to stabilize and balance grain yield and grain bioactive compounds for the benefit of farmers and consumers.

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DEVELOPMENT PARAMETERS OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) ARE AFFECTED BY PHOSPHORUS DEFICIENCY IN SOYBEAN PLANTS

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Keywords: phosphorus; soybean; *Spodoptera frugiperda*

INTRODUCTION

Phosphorus (P) is a crucial macronutrient for plant metabolism because it is a structural component of many biomolecules, such as nucleic acids, carbohydrates, phosphorylated proteins, and phospholipids (Chiou and Lin, 2011; Veneklaas *et al*., 2012). Plants are extremely sensible to phosphate (Pi) deficiency, constituting a limiting factor for crop growth and yield, especially in highly weathered soils (Kochian *et al*., 2015). Plants under Pi deficiency in natural and agricultural ecosystems show biochemical, morphological, and physiological changes associated with plant-insect interaction. For example, *Arabidopsis thaliana* and *Solanum lycopersicum* reduced the fitness of lepidopterans when larvae fed on these plants under P starvation (Khan *et al*., 2016). The effect of this interaction has not yet been explored in soybean (*Glycine max*) which is an important crop species in Brazil. In future scenario, expected that Pi fertilizers get scarce due to the high world demand and that insect herbivory might be enhanced by environmental change. This work aimed to evaluate the biological parameters of fall armyworm larvae (*Spodoptera frugiperda*) feeding on soybean plants under P deficiency.

METHODS

Spodoptera frugiperda immatures (n = 25; 3rd instar) obtained from the Embrapa Soybean Entomology Laboratory (Hoffmann-Campo *et al*., 1985) were transferred and kept in confinement for 15 days in individualized soybean plants (cv. NA 5909 RG; n = 25) at V4 stage (Fehr and Caviness, 1977) wrapped in transparent polypropylene bags (28.0 x 42.0 cm) with one side made of tulle fabric for transpiration. These plants have grown polypropylene tubes with sand substrate irrigated with the nutrient solution proposed by Broughton and Dilworth (1970), prepared in deionized water (pH 6.6-6.8). The concentration of phosphorus (P) in the nutrient solution was adjusted to 0.01 mmol L^{-1} (phosphorus deficiency; -P) and 0.5 mmol L^{-1} (phosphorus sufficiency; +P) of KH_2PO_4, aiming to find phenotypic differences under contrasting conditions (Tawaraya *et al*., 2014; Khan *et al*., 2016). The K^+ content in (-P) treatment was balanced by a 0.49 mmol L^{-1} KCl solution. After 15 days, *S. frugiperda* larvae were sampled and fresh mass (mg) and population survival (%) parameters were measured. The fresh mass data were analyzed by Student's t-test (fresh mass) and larvae survival by Pearson's chi-square test using R software (R Stats Package).

RESULTS AND DISCUSSION

S. frugiperda larvae growing on phosphorus-deficient soybean plants had a reduction of 267% in fresh weight (Table 1) when compared to insects that fed on plants under P sufficiency condition (t = 17.317; df = 34.7; p-value < 0.001). Larvae survival in the phosphorus-limited soybean plants was significantly lower (76%) when compared to the *S. frugiperda* population in the non-deficient plants (96%) by the Pearson's chi-squared test ($\chi^2 = 4.1528$; df = 1; p-value = 0.020). Pi is one of the most common nutrient deficiencies limiting plant growth, while insect herbivory accounts for major losses in plant yield and impacts ecological and evolutionary changes in plant populations. *Arabidopsis* and tomato plants under P deficiency also triggered increased resistance to *Spodoptera littoralis* (Khan *et al*., 2016). These results reveal that the link between Pi deficiency and enhanced herbivory resistance is conserved in a diversity of species. The results also indicate that soybean plants possibly use biochemical mechanisms of defense to deal with herbivory.

Table 1. Development parameters of *Spodoptera frugiperda* larvae (6th instar) feeding on soybean plants under contrasting phosphorus nutritional conditions. Means and standard deviations for 25 samples are provided. *** p <0.001.

Treatment	Fresh Weight	Population Survival
	(mg)	(%)
-P	84.62 ± 27.07	76
+P	310.42 ± 56.16***	96

CONCLUSIONS

The results show a reduction in biological parameters of the generalist caterpillar *Spodoptera frugiperda* growing on soybean plants under phosphorus deficiency, suggesting that defense mechanisms against herbivory are reprogrammed in soybeans under phosphorus stress. These results are important for understanding how soybean modulates its defense mechanism in responses to P starvation when challenged by insects, therefore aiding in the management of this crop under stressful conditions.

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APPLICATION OF FERROUS IONS TO CITRUS TREES INFECTED WITH CITRUS GREENING DISEASE (HUANGLONGBING) ENABLES RECOVERY FROM FE DEFICIENCY

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Keywords: Fe; Huanglongbing; Citrus

INTRODUCTION

Huanglongbing (HLB) , also known as Citrus Greening Disease, is a serious world-wide bacterial disease of citrus [1,2]. There is currently no practical method of preventing the disease, and infected trees must be destroyed to halt further spread of the pathogen.

Iron (Fe) is an essential nutrient for plant growth. Leaves of HLB-infected trees contain less Fe than those of healthy trees (Masaoka et al., 2011), suggesting that HLB infection causes Fe deficiency. The symptoms of HLB are similar to Fe-deficiency-induced chlorosis, and HLB-infected citrus trees are more commonly found in alkaline than in acidic soils in the same region (Inoue et al., 2020a). Plants absorb Fe as ferrous ion across cell memblane via Iron-regulated transporters (IRTs).

In this study, we investigated the efficacy of applying Fe^{2+} solutions to reverse the symptoms of HLB infection. Experiments were performed with potted trees in a closed greenhouse, as well as with trees grown in the field.

METHODS

1.Pot experiment 1 (closed greenhouse)

Ten infected and ten healthy citrus trees were used in each of five replicate experiments. A solution (Fe^{2+} Solution 1) of organic acid containing 15 mg Fe /L (Fe^{2+} was 30% of the total Fe) was applied to the soil and leaves of the infected trees once every five days.

2. Pot experiment 2

Eighteen infected trees were studied in each of three replicate experiments. A solution of organic acid (Fe^{2+} Solution 2) containing 15 mg Fe /L (Fe^{2+} was 70% of the total Fe) was applied to the leaves once every five days.

3. Field experiments (open space)

A total of 36 three-year-old citrus trees were studied in each of twelve replicate experiments. Most of the trees in the test area exhibited symptoms of HLB infection. Fe^{2+} Solution 2, containing either 30 or 60 mg Fe /L, was applied to the foliage at regular intervals (weekly or monthly at 30 mg Fe /L, or biweekly or bimonthly at 60 mg Fe/L) for 2.5 years.

RESULTS AND DISUCUSSION

Pot experiment 1

*?*Application of Fe^{2+} solution 1 to HLB-infected trees for 160 days enabled recovery of growth comparable to the control trees (Fig. 1A), and the leaves became greener (Inoue et al., 2020b).

Pot experiment 2

Application of Fe^{2+} solution 2 to HLB-infected trees for 40 days caused a gradual increase in the Soil Plant Analysis Development (SPAD) values of the leaves, although other Fe-chelate compounds gradually decreased throughout the treatment (Inoue et al., 2020b).

Field experiment

Application of Fe^{2+} solution 2 to field-grown citrus trees over a period of 2.5 years increased fruit production by approximately 70% in the case of 60 mg Fe /L, and 20% in the case of 30 mg Fe /L. These data suggest that continuous Fe^{2+} application to HLB-infected citrus trees is an effective treatment for recovery from Fe deficiency caused by HLB infection.

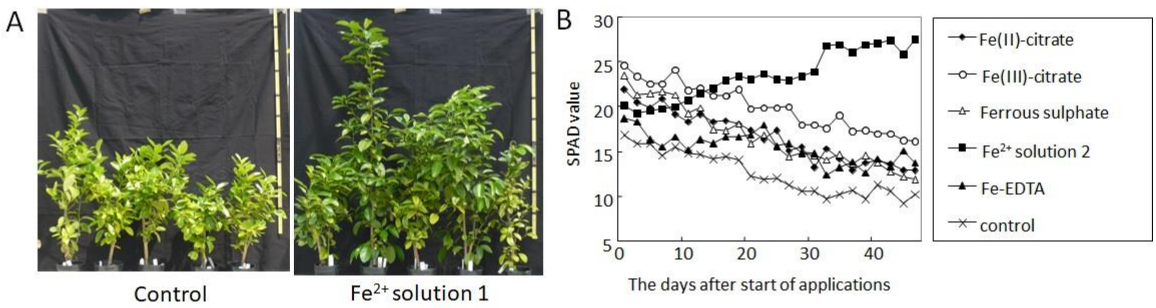

Fig. 1. The effect of Fe-solution applications on the growth of HLB-infected trees grown in pots.

A. Tree growth after application of Fe^{2+} solution 1 (Fe^{2+} is 30% of the total Fe) every five days for 160 days.

B. The chlorophyll index (as SPAD value) during the application of Fe^{2+} solution 2 (Fe^{2+} is 70% of the total Fe) every five days for 44 days.

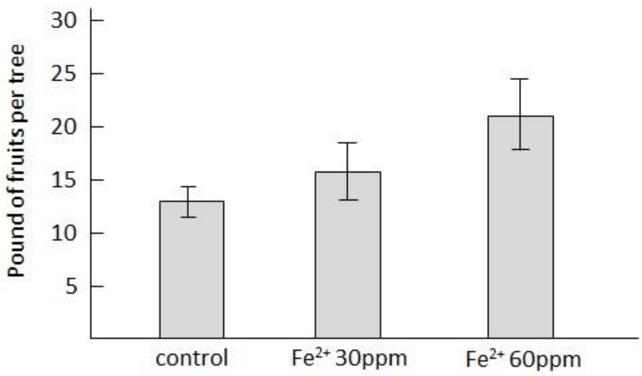

Fig. 2. The fruit-yield of field-grown citrus trees after 2.5 years of regular Fe-solution application.

CONCLUSIONS

Growth was restored to HLB-infected citrus plants following regular application of Fe solutions, presumably due to improved Fe nutrition. Thus, foliar application of divalent Fe which is preferable form to absorb Fe across cell memblane may be an effective treatment for HLB-infected citrus trees.

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Boron Workshop

ENHANCE SIZE AND QUALITY OF PEANUTS THROUGH BORON FERTILIZERS

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Keywords: B fertilizers ; seed size; pod filling

INTRODUCTION

Peanut (*Arachis hypogaea* L.), a high-energy and protein at a low cost consumed by a large populations in the world, is an important food legume crop of tropical and subtropical world, mainly grown as rain-fed crop in Asian and African countries on low fertility conditions where deficiencies of boron (B) and calcium (Ca) are the important factors causing low pod filling (Singh et al 2009, 2017). The Indian peanuts are world famous for its crunchiness and flavors, which calls for the production of well filled seed in the recent cultivars having its export market.

Monitoring of B deficiencies in peanut cultivars in various parts of India and their remedies through soil and foliar applications of boron using various sources are the regular features of this Institute for the past 30 years. The experiments conducted till 2004 are summarized earlier (Singh et al 2007). Here we share the outcome of several field experiments on boron improving the quality of peanut.

METHODS

A number of field and pot experiments were conducted at this institute as well as at various locations under varied agroecological situations, right from north to south, and acid soils of northeastern to alkaline soils of western part of India the results of which are summarised here.

RESULTS and discussion

In peanut, though the B deficiency caused retarded growth of apical portion, malformation of the leaf vein, chlorosis, necrosis of basal margins in emerging leaves and finally death of the stem apex under sand culture, these symptoms were not observed in field grown crop on B deficient soils. The sufficiency level of hot water soluble B in soil for peanut was worked out to be 0.5 ppm and depending on soil and genotype the critical limits of B vary from 0.2-0.4 ppm. In soils having less than 0.4 ppm hot water soluble B, shriveled seeds with low pod filling and hollow in the center of the seed were commonly observed symptoms of B deficiency in field which depending upon the severity of the malady, soil types and cultivars caused 10-54 % yield losses. Application of 0.5-2.0 kg ha^{-1} B, depending on the solubility of B sources and size of peanut seed, alleviate the B-deficiency disorder in the field. However, the B doses of more than 5 kg ha^{-1}, showed its toxicity in leaves, which need careful application.

In light texture soils of neutral to alkaline in reaction, though field application of 1.0 kg B ha^{-1} is ideal for small to medium seed-size peanut cultivars, the large seed size peanuts require 2 kg B ha^{-1}. Boron facilitates translocation of sugar and fat synthesis and plays an important role in pollen germination, maturation and seed production. Application of B caused early flowering by 1-6 days, increased number of pods by converting more peg to pods, increased shelling out turn and 100-seed mass which finally had their cumulative effects on pod and seed yields. These parameters, however, slightly varied with the B sources, their modes of applications (soil, seed dressing, foliar) and locations. Also, genetic variations for the response of B in the field were observed among peanut cultivars.

The effectiveness and feasibility of twelve commercial or agriculture grade B fertilizer sources were evaluated for large seed size peanuts where most of them showed positive response with slight variations. Soil application of 2.0 kg B ha^{-1} as commercial grade Agricol, Solubor and Borosol and many other B sources containing borax showed a similar response, but were superior over their foliar applications. The responses of foliar applications of 0.05-0.1% aqueous solution of 1.0 kg B ha^{-1} as Chemiebor, Solubor and Borosol were at par and more effective in humid areas only. However, foliar applications showed scorching of peanut leaves during dry weather in arid and semi-arid region.

The effectiveness of various methods of application of various B sources containing borax and boric acid when compared in field by applying them as seed dressing, in the soil as basal or top dressing, as foliar sprays and also along with drip irrigation, the maximum response was observed when at 1 kg B ha^{-1} was applied along with drip irrigation, followed by soil application. Further split application of 50 % in soil as basal and 50% at 30 days after emergence was superior over basal application alone or foliar application. The boron sources containing boric acid caused scorching of leaves when applied on foliage of 0.05-0.1% aqueous solution, and as seed dressing it caused damage to seed reducing field germination. Hence these practices should be avoided in peanut.

Boron helps in nitrogen absorption and acts as a regulator of K/Ca ratio in the plant. The sufficiency level of B in the leaves during flowering and fruiting was 25-60 ppm and the critical level 20 ppm and peanut seeds containing less than 17 ppm B showed the incidence of hollow-heart. The seed B content was 12-15 ppm in the peanut grown without B application at most of the places, which increased to 15-19 ppm with application of B fertilizers.

With increase in population the demand of peanut, both as oilseeds and foods, is increasing which demand increase its production, essentially through the recently released peanut, where blanket application of boron fertilizer is must.

CONCLUSIONS

To obtain well filled quality peanuts, it is mandatory to apply 1.0 kg B ha^{-1} every year in the soil 50 % as basal and 50% at 25-30 days after emergence. While for growing large seed peanuts, this dose can be increased upto 2.0 kg B ha^{-1} onl

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BORON FERTILIZATION IMPROVES PEANUTS NUTRITION AND YIELD IN A SANDY SOIL

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Keywords: *Arachis hypogea* L.; Sufficiency range ; Micronutrient

INTRODUCTION

Boron is the most limiting micronutrient for peanut production, especially in intemperized and sandy soils with low organic matter content (Mantovani et al., 2013). Boron deficiency in peanuts causes abscission of flowers, poor pod formation and grain filling, resulting in low yield (Singh et al., 2017). Thus, in most cases it is necessary to apply boron via fertilizer to peanuts. However, it is still not known what is the best way (via soil or foliar fertilization), rates and sources of boron for peanuts. Additionally, the boron leaf sufficiency range for peanuts in Brazil was defined for the old upright cultivars from Valencia and Spanish group, which had lower yield, and for modern runner-type and Virgínia group cultivars, the sufficiency range could be higher, requiring further studies. Thus, the objective of the study was to evaluate the yield and concentration of boron in peanut leaves as a function of the boron fertilization management.

METHODS

The field experiment was carried out in the field in Regente Feijó, São Paulo, Brazil (2020/2021 crop season), in a sandy soil (Oxisol) with low boron content 0.07 mg dm^{-3} (0-0.2 m). The cultivar Granoleico (runner-type) was used. The experimental design was a randomized complete block with split plot scheme with four replications. Plots were 3.6 m wide and 6.0 m long. Treatments included the application of boron via soil: control (unfertilized), boric acid at 1.5 kg ha^{-1}, Ulexite (1.5 and 3.0 kg ha^{-1}), and sodium tetraborate (Granubor) (1.5 and 3.0 kg ha^{-1}) combined with foliar fertilization (0, 400, 800 and 1200 g ha^{-1} as boric acid). The application via soil was carried out on the day of sowing, manually. Foliar application was splitted in four applications (21, 28, 35 and 42 days after emergence) with a pressurized CO_2 sprayer at a volume of 200 L ha^{-1}. At 48 days after emergence (R3- beginning of pod formation) 15 leaves were collected from each plot, washed in running water, and then the boron content in the leaves was determined. At pod maturity (70% of mature pods (R8 and R9) two linear meters were harvested from each plot to evaluate pod yield. Data were submitted to ANOVA and means were compared by the LSD test ($p < 0.05$).

RESULTS and discussion

Soil boron fertilization increased peanut pod yield, regardless of boron source or rate (average increase of 18% or 1038 kg ha^{-1}). Boron foliar fertilization increased yield only under the absence of boron via soil and yield increase was 10% (618 kg ha^{-1}). Additionally, associating soil and foliar boron fertilization, there was a yield decrease when applying foliar rates above 400 g ha^{-1} (Fig. 1a), which may caused leaf boron fitotoxicity in these treatments (> 60 mg kg^{-1}) (Fig. 1b). Singh et al. (2017) also reported a greater yield increase through soil boron fertilization at 2 kg ha^{-1} and high foliar rates can cause toxicity.

In the past, it was determined in Brazil that, for peanut cultivars of the Valencia and Spanish group, the range of boron sufficiency in leaves was between 20 and 50 mg kg^{-1} (Nakagawa and Rosolem 2011), with higher levels causing toxicity. However, in treatments with higher pods yield, the boron content in the leaves was between 40 and 60 mg kg^{-1} and the control treatment (low pods yield) had a boron content of 35 mg kg^{-1} (Fig. 1b). This suggests that for modern peanut cultivars of the Virgínia group with high yield potential, the sufficiency range should be between 40 and 60 mg kg^{-1}.

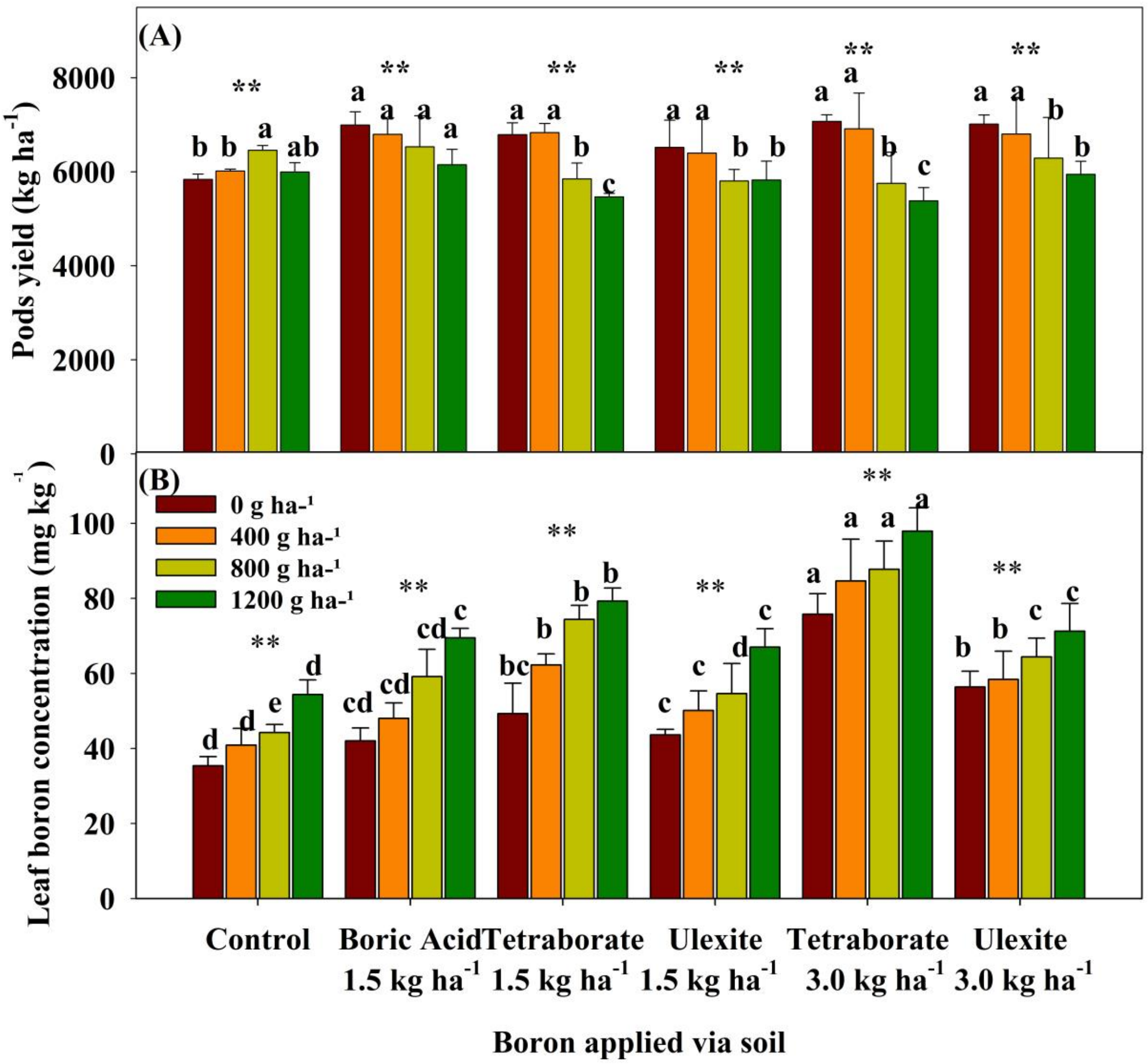

Fig. 1. Pods yield and boron concentration in peanut leaves as a function of boron fertilization via soil and foliar feeding. Vertical bars represent the mean standard error. Lowercase letter compare soil treatments in each foliar rate.

CONCLUSIONS

Soil boron fertilization at 1.5 kg ha^{-1} at sowing is the best option to improve peanut boron nutrition and pods yield in soils with low boron content, with little difference among boron sources. Foliar fertilization is an option only when boron is not applied via soil, at the optimal rate of 800 g ha^{-1}, but with a lower yield increase potential. More studies are needed to define a new boron leaf sufficiency range for peanuts.

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IMPROVING PEANUT SEED GERMINATION PARAMETERS BY BORON FERTILIZATION

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Keywords: boron sources; root length; shhot length

INTRODUCTION

Peanuts are grown predominantly in sandy soils, which are poor in organic matter and nutrients, including boron. Boron supply improves the formation of vascular bundles, improving the transport of photosynthetic products and increasing seed quality (Li et al., 2017; Pandey and Gupta, 2013). In addition, boron increases the germination of peanut seeds, accelerates the germination process and makes seedlings more vigorous (Quamruzzaman et al., 2016; Rerkasem et al., 1990). We hypothezed that the application of boron, mainly via soil, improves the physiological quality of peanut seeds. The objective of this work was to evaluate the effect of boron fertilization via soil combined or not with leaf application on the physiological attributes of seeds.

METHODS

The field experiment was conducted in Regente Feijo, São Paulo, Brazil (2020/2021 season), in a sandy soil (Oxisol) with low boron content - 0.07 mg dm-3 (0-0.2 m). The cultivar Granoleico (runner type) was used. Treatments included the application of boron via soil (unfertilized control, Boric Acid at 1.5 kg ha-1, Ulexite (1.5 and 3.0 kg ha-1), and boron tetraborate (1.5 and 3.0 kg ha-1)) combined with foliar fertilization (0, 400, 800 and 1200 g ha-1 as boric acid). The germination test was installed on paper rolls with 25 seeds per repetition. The substrate, consisting of 3 sheets of paper, two as a base and one for seed cover, was damined with distilled water in the proportion of 2.5 times the weight of dry paper. The rollers were kept in a constant Mangelsdorf type germinator at 25ºC. The evaluations were daily considering the seed with root protrusion above 0.5 cm. Germination stabilized 6 days after sowing and daily evaluations were inserted in germinator software to obtain the maximum germination values, area under the germination curve, time for 10% germination, mean germination time. At 6 days after sowing, normal seedlings with root greater than 3 cm were counted and separated. The roots were measured to obtain the average length and dry mass after drying the seedlings in an oven at 65ºC for 48h.

RESULTS AND DISCUSSION

Seed germination was improved by fertilization of boron on ground with boric acid (1. 5 kg ha-1) (78%) or boron tetraborate (3. 0 kg ha-1) (80.5%) in the absence of leaf fertilization. Leaf fertilization at 1200 g ha-1 resulted in poor seed germination when combined with ulexite. The application of boron from ulexite soil (3. 0 kg ha-1) decreased the time to reach 10% of seed germination (T10) (33. 3 h) and in the absence of fertilization of soil B, the application of 400 and 1200 g ha-1 via leaf feeding resulted in a reduction of 14 and 11% in T10 compared to the average of soil B treatments, respectively. Boric acid applied to the soil (1. 5 kg ha-1) reduced the mean germination time in relation to ulexite or boron tetraborate in 11%, but there was no difference in relation to the control. Boric acid (1. 5 kg ha-1) and boron tetraboboly (3.0 kg ha-1) in the absence of leaf feeding resulted in normal seedlings with primary root length greater than 3.0 cm above 50%. The dry weight of the seedling was improved by ulexite in 1. Five and three. 0 kg ha-1, both in the absence of leaf fertilization, but spraying 400 or 800 g ha-1 in the absence of soil B also resulted in a higher dry weight of the seedling. The length of the seedling was improved by fertilization of soil B and boron tetrabobode (3.0 kg ha-1) in the absence of leaf fertilization. In addition, in the absence of soil fertilization, leaf spray of 400 g ha-1 improved seedling length. Boron application improved seed quality parameters, especially when applied to soil. This is because boron is a nutrient of low remobilization in phloem, which ends up being more efficient when applied to the soil, because it moves upwards, from roots to leaves (Balota, 2014). Moreover, according to that same author, excessive application may cause toxicity to peanuts, which may have caused poor seed germination when applied the fertilizer to the cominated soil foliar application at a high dose (1200 g ha-1). However, fertilization with boron in correct rates can accelerate the germination process (Rerkasem et al., 1990), as shown in our results.

WB: Without boron; TB 1.5: 1.5 kg ha-1 boron tetraborate; TB 3.0: 3.0 kg ha-1 boron tetraborate; ULE 1.5: 1.5 kg ha-1 ulexite; ULE 3.0: 3.0 kg ha-1 ulexite; BA: 1.5 kg ha-1 boric acid.

	Germination (%)				**Germination time (h)**			
Rate	**0**	**400**	**800**	**1200**	**0**	**400**	**800**	**1200**
	g ha^{-1}	**g ha^{-1}**	**g ha^{-1}**	**g ha^{-1}**	**g ha^{-1}**	**g ha^{-1}**	**g ha^{-1}**	**g ha^{-1}**
WB	74.5 ABa	74.0 Aa	68.0 ABa	69.0 Aa	37.2 BCa	37.5 Aa	39.0 Aa	34.5 Ba
TB 1.5	69.0 Ba	59.7 Ba	65.0 Ba	66.0 Aa	43.1 Aa	41.8 Aa	38.2 Aa	38.8 ABa
TB 3.0	80.5 Aa	66.5 ABb	76.5 Aab	73.0 Aab	41.1 ABab	45.6 Aa	39.2 Ab	38.7 ABb
ULE 1.5	71.0 ABa	72.0 Aa	74.5 ABa	49.0 Cb	43.0 Aab	44.0 Aa	38.0 Ab	38.9 ABab
ULE 3.0	77.0 ABa	73.5 Aab	75.0 ABa	63.5 ABb	33.3 Cb	43.5 Aa	39.2 Aa	39.8 Aa
BA 1.5	78.0 ABa	64.0 Ab	54.0 Cb	54.0 BCb	35.3 Cb	41.9 ABa	38.5 Aab	37.1 ABab
CV %	**11.15**				**9.37**			
Rate	**Seedling length (cm)**				**Seedling dry weigth (g)**			
WB	5.0 Cb	5.8 ABa	5.3 Bab	4.6 Bb	45.8 Bb	56.3 Aa	55.2 Aa	52.5 Aab
TB 1.5	5.4 BCa	5.2 Ba	5.0 Ba	5.0 Ba	46.1 Ba	37.8 Da	38.2 Dab	39.7 Cab
TB 3.0	6.1 ABa	5.4 ABab	5.7 Ba	4.9 Bb	50.3 ABa	43.2 CDa	43.5 BCDa	42.6 ABa
ULE 1.5	6.3 Aa	5.4 ABb	5.3 Bb	4.9 Bb	56.9 Aa	43.9 CDb	42.6 CDb	26.0 Dc
ULE 3.0	5.9 ABa	5.9 Aa	6.5 Aa	6.1 Aa	57.1 A	52.6 AB	51.5 AB	44.6 BC
BA 1.5	5.5 BCa	5.3 ABa	5.4 Ba	4.9 Ba	50.2 ABa	47.4 BCa	48.5 ABCa	47.9 ABa
CV %	9.31				12.11			

Table 1. Germination, germination time, root length and seeding dry weigth of peanut on the effect of boron fertilization on soil combined or not with foliar application.

CONCLUSIONS

In conclusion, soil boron fertilization (3.0 kg ha-1 - ulexite or boron tetraborate) or leaf feeding at 400 g ha-1 (boric acid) improved peanut germination parameters.

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BETTER BORON NUTRITION REDUCES THE NEED FOR NITROGEN AND POTASSIUM IN COTTON

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Keywords: nutrition efficiency; fiber yield; physiology

INTRODUCTION

The balanced nutritional management of cotton (*Gossypium Hirsutum* L.) is fundamental to explore the yield potential, reduce costs, nutrient losses and ensure the quality of the fiber produced. Thus, the rates applied should explore the nutrient's antagonistic and synergistic relationships. Currently, the use of nitrogen (N) and potassium (K) has been, in most crops in Brazilian Cerrado, above the recommended, which in addition of increasing production costs, may be compromising the efficiency of use of these nutrients as well as boron uptake. The aim of this study was to evaluate the cotton nutrition and yield as affected by nitrogen and potassium rates under two boron levels.

METHODS

The study was conducted at the Experimental Farm (UNOESTE) in a sandy soil, and the cultivar used was FM 985GLTP. The experimental design used was a randomized blocks in a 4x3 factorial scheme with four replications, and the treatments were the result of the combination of N (0, 70, 140 and 210 kg ha^{-1} N) and K (60, 120 and 180 kg ha^{-1} K_2O). Two experiments were conducted, one with the rate of 1.5 kg ha^{-1} of boron (at sowing) and the other with the dose of 3 kg ha^{-1} of boron (50% at sowing and 50% at 25 DAE). The application of N (urea) and K (potassium chloride) were performed in two times; and the boron source used was ulexite (10% B).

Leaf sampling for leaf diagnosis of nutrients was performed as described by Malavolta et al., (1997) at full bloom time, which occurred at 76 DAE. Upon reaching physiological maturity, cotton was harvested, weighed and ginned, to determine fiber yield.

RESULTS AND DISCUSSION

Fiber yield with 1.5 kg of B ha^{-1} (Fig. 1a) was higher with the maximum rate of N (210 kg ha^{-1}) and K (180 kg ha^{-1}). On the other hand, the highest yield with 3.0 kg of B ha^{-1} (Fig. 1b) was achieved with a lower rate of N (140 kg ha^{-1}) and K (60 kg ha^{-1}). This shows that the better nutrition of cotton with boron improved the efficiency of nitrogen and potassium fertilization, as a rate of 33.3% lower of N and 66.6% lower of K was required to obtain similar yields. This can be explained due to the effect of boron on N metabolism, because under boron deficiency conditions, the efficiency of nitrate reductase enzyme activity is lower, which reduces the efficiency of N assimilation by plants (Shen et al., 1993).

As expected, the increase in potassium and nitrogen rates increased the concentration of these nutrients in the leaves (Table 1). Under lower N supply, even with application of the maximum potassium rate, there was no increase in potassium concentration in the leaves, regardless of boron rate. This is because there is a synergism between the uptake of these nutrients, under conditions of low supply of N, potassium absorption is limited (Zhang et al., 2010), that is, it is not recommended to increase the potassium rate when there is a nitrogen deficiency.

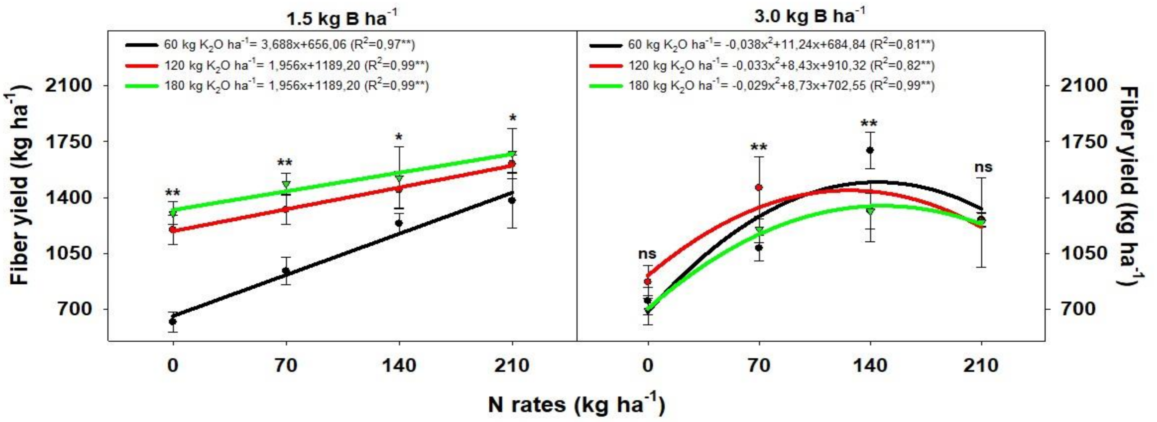

Fig. 1. Fiber yield as a function of boron, potassium, and nitrogen (N) rates. **= Significant at 1%. *= Significant at 5%. NS= Not significant by Tukey's test ($p<0.05$). Vertical bars represent standard error of means.

Table 1. Leaf content of total nitrogen, potassium and boron as a function of boron rate (B), potassium rates, and nitrogen rates (N). Means followed by the same letter did not differ by Tukey's test ($p<0.05$). Lowercase letters compare doses of N and uppercase letters compare K rate within each dose of B.

K_2O (kg ha^{-1})	1.5 kg B ha^{-1}				3.0 kg B ha^{-1}			
	0	70	140	210	0	70	140	210
	N rate (kg ha^{-1})							
	Total nitrogen (g kg-1)							
60	37,5 Ac	44,3 Bb	47.5 Ba	47,6 Aa	48,3 Aab	48.6 ABb	52 Aa	50,1 Aab
120	35,3 Ac	44,0 Bb	50,5 Aa	48,1 Aa	46.3 Ba	47.5 Ba	48.1 Ba	48,4 Aa
180	35,8 Ac	47,2 Aab	45,5 Bb	49,1 Aa	47.5 ABb	50,6 Aa	50,2 ABa	49,6 Aab
CV%	3.63				2.56			
	Potassium (g kg^{-1})							
60	16,1 Aa	15,7 Aa	14,7 Aa	14.1 Ba	13,1 Aab	10,0 Bb	12.8 Aab	13,6 Aa
120	13,9 Aa	12.5 Ba	14,5 Aa	14,8 ABa	11.5 Aab	13,6 Aa	9,4 Bab	10,5 Bb
180	14,4 Aab	13.5 ABb	15,2 Aab	16,8 Aa	14,0 Aab	16,5 Aa	13.1 Ab	14.5 Aab
CV%	10.48				13.54			
	Boron (mg kg^{-1})							
60	41,7 Aa	45,0 Aa	46,1 Aa	44,3 Aa	48,2 Aa	45,0 Bb	44,5 Bb	46,1 Aab
120	44,3 Aa	44.6 Aa	45.7 Aa	46,7 Aa	46,5 Aa	48,3 Aa	47,8 Aa	47,7 Aa
180	45,0 Aa	44,6 Aa	43,0 Aa	45,3 Aa	48,0 Aa	45,1 Bb	46,7 ABab	45,5 Aab
CV%	5.38				2.48			

CONCLUSIONS

An important strategy to reduce the need for nitrogen and potassium fertilization is better nutrition with boron in cotton.

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BRASSICA NAPUS BNABOR1;3C IS CRITICAL FOR THE STEM DEVELOPMENT AND THE FORMATION OF REPRODUCTIVE ORGANS UNDER BORON DEFICIENCY

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Keywords: *Brassica napus*; Boron; *BnaBOR1;3c*

INTRODUCTION

Boron(B) is an essential nutrient for plant growth and development. B deficiency usually inhibits root growth, leaves expansion and the formation of flower organs. In Arabidopsis, the nodulin 26-like intrinsic protein (NIP), NIP5;1 and NIP6;1, and the BOR1 are required for B absorption and distribution in plant seedling and floral organs under B deficiency (Yoshinari and Takano, 2017).

Brassica napus (*B. napus*) has high B demand and is sensitive to B deficiency due to the great biomass. B deficiency causes typical growth defects and distinct growth defects such as multi-stems and stem cracking. Although the *B. napus* homologs of Arabidopsis NIP5;1, NIP6;1 and BOR1 were identified and their importance for B absorption and distribution was revealed (Zhang et al., 2017; Wang et al., 2021; He et al., 2021; Song et al., 2021), the mechanism of fine-tuning B distribution in stem and floral organs remains not fully known. Here, we report an important role of *BnaBOR1;3c* for B distribution in stem and floral organs in *B. napus*.

METHODS

Rapeseed materials and growth conditions

The *Brassica napus* cultivar (QY10) and its *BnaBOR1;3c* mutants were kept in this research. For the hydroponic experiment, the plants were cultured in a standard procedure with Hoagland's solution (Hoagland & Arnon, 1950) supplemented with100 or 0.25μM B for 14 d. For the pot experiment, low B containing grey purple sandy soil was used. Total B was 0.27 mg B/kg soil (low B) or 1.5 mg B/kg soil (normal B) by adding boric acid.

RESULTS and DISCUSSION

To elucidate the physiological effects of *BnaBOR1;3c* on plant growth and B transport, 14 d seedlings cultured in a hydroponic system supplemented with 100 or 0.25 μM B were used for phenotypical comparison (Fig. 1 A). We did not observe distinguishable differences among QY10 and mutants under 100 μM B, while growth defect was clear in the mutants under 0.25 μM B conditions (Fig. 1 A). This result indicates that function disruption of *BnaBOR1;3c* reduced low B tolerance of *B. napus*.

Based on a qRT-PCR analysis, *BnaBOR1;3c* was strongly detected in the stem, floral organs, especially in stigma, anther and pollen grain, suggesting that *BnaBOR1;3c* might play a critical role in the stem development and the formation of floral organs. A pot experiment was performed by culturing plants in soil (0.37 mg B /kg soil or 1.5 mg B/ soil) to investigate the growth at the bolting stage and reproductive stage. In the bolting stage, the mutants height was significantly lower than that of QY10, and showed typical boron deficiency symptoms, such as reduced branches, inhibited inflorescence growth, wrinkled leaves and stem cracking under B deficiency (Fig. 1b). At the reproductive stage, a large number of abnormal flowers with dried-up and dropped floral buds were found in the mutant compared with QY10 (Fig. 6c). our findings highlight the important role of BnaBOR1;3c in the stem development and the formation of floral organs.

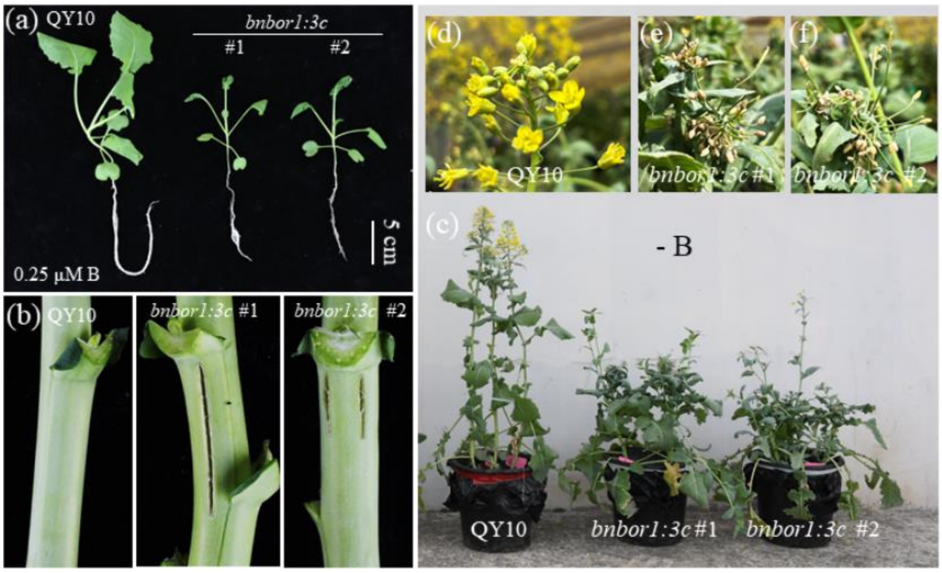

Fig. 1. *Bnabor1;3c* mutants showed growth defects under B deficiency.

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INTERACTION BETWEEN CULTIVARS, BORON RATES AND LONG-TERM GYPSUM IN THE BARLEY CROP GRAIN YIELD (*HORDEUM VULGARE*)

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Keywords: Boric Acid; Phosphogypsum; No-till

INTRODUCTION

The cultivation of barley (*Hordeum vulgare*) has increased in Brazil, especially in subtropical environments in the South Region. Fertilization with B in barley has been associated with improvement in grain quality and productivity, although there is still no established technical criterion for the use of this nutrient. The refinement in the recommendation and use of doses of B will help barley producers to use more rationally this nutrient.

The objective of this research was to evaluate the grain yield of two barley cultivars, under four doses of B and the residual effect of five doses of gypsum during the 2021 season.

METHODS

The study was carried out in Guarapuava-Paraná, in a Typic Hapludox area. The gypsum doses were 0, 3, 6, 9, and 12 Mg ha^{-1}, applied to the soil surface in November 2009, for a long-term experiment on the effects of this soil conditioner in a grain production system under no-till, which included soybean, corn, common bean, and winter cereal crops.

The experimental design was randomized blocks, with four replications. During the 2021 barley season, a sub-divided plot scheme was used for the treatments. The doses of gypsum constituted the main plots, the doses of boron were applied in the sub-plots, and the barley cultivars were planted in the sub-sub-plots.

Barley cultivars Danielle and Imperatriz were sown on June 25, 2021, with 40 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 60 kg ha^{-1} K_2O, and 0,17 m between rows. On the average in the experimental area, the soil test for B (barium chloride extractant) showed levels close to 0,30 mg dm^{-3}, and the applied B doses were: 0, 1, 2, and 4 kg ha^{-1}. The equivalent boric acid (17% B) doses in relation to each experimental unit area were diluted in water and applied to the soil surface using a watering can.

To evaluate crop yield, after physiological maturity the grains were harvested in an area of 3.2 m^2, and the weight values were corrected to 13% of humidity. Data were submitted to analysis of variance, and when there was a significant difference between the treatments ($p < 0,05$), means were compared by Tukey's test.

RESULTS and discussion

The grain yield responded to the interactions between: boron rates and cultivars, gypsum rates and boron rates, as well as gypsum rates and cultivars (Table 1). Within the Danielle cultivar, the rates of 1, 2, and 4 $kg_{[B]}$ ha^{-1} did not differ from each other and resulted in higher yields compared to the control with 0 $kg_{[B]}$ ha^{-1}. Within the cultivar Imperatriz, the yield was higher at the rate of 2 $kg_{[B]}$ ha^{-1} compared to the rate of 4 $kg_{[B]}$ $ha^{-1,}$ and the other rates did not differ from each other.

Within all boron and within all gypsum treatments, the Imperatriz cultivar showed a higher grain yield than the Danielle cultivar (Table 1).

The yield of the cultivar Imperatriz did not differ between the gypsum rates, while in the cultivar Danielle the yield was lower at the rate of 6 $Mg_{[gypsum]}$ ha^{-1}, which shows a difference in the root system of these cultivars, and/or a difference in response to the long term effects of gypsum in the soil chemical attributes. Between the gypsum application in 2009 and the barley season of 2021, Poaceae grains crops like corn, wheat, and previous barley, as well as black oat cover crop presented higher yields (grains, aboveground biomass) and nutrient exportation under gypsum application, which in the case of magnesium (Mg^{2+}) turned to be a limiting factor for higher yields, once gypsum also caused Mg^{2+} leaching, depending on the rate and timelapse after application.

In the absence of B application, barley grain yield was higher in the absence of gypsum in relation to the applied rates of 3, 6, 9, and 12 $Mg_{[gypsum]}$ ha^{-1}. At doses of 1, 2, and 4 $kg_{[B]}$ ha^{-1}, there was no difference in grain yield at all rates of gypsum.

Table 1. Grain yield of barley cultivars submitted to boron rates applied at the surface of a Typic Hapludox, under the residual effect of gypsum rates in Guarapuava, Paraná state, Brazil.

Cultivar	Boron (kg ha^{-1})				
	0	1	2	4	Avg.[1]
	Grain yield (Mg ha-1)				
Daniele	2.32 bB[2]	4.23 bA	4.27 bA	4.47 bA	3.82 b
Imperatriz	4.93 aAB	4.95 aAB	5.22 aA	4.82 aB	4.98 a
Avg.	3.62 B	4.59 A	4.75 A	4.64 A	

p-value	Boron (B)	**<0.001**
	Cultivar (C)	**<0.001**
	B x C	**<0.001**

Gypsum (Mg ha^{-1})	Boron (kg ha^{-1})				
	0	1	2	4	Avg.
	Grain yield (Mg ha^{-1})				
0	4.44 aA	4.57 aA	4.48 aA	4.23 aA	4.43 a
3	2.99 bB	4.72 aA	4.59 aA	4.85 aA	4.29 a
6	3.48 bB	4.63 aA	4.84 aA	4.45 aA	4.35 a
9	3.64 bB	4.65 aA	4.89 aA	4.89 aA	4.52 a
12	3.57 bB	4.40 aA	4.94 aA	4.79 aA	4.43 a
Avg.	3.62 B	4.59 A	4.75 A	4.64 A	

Gypsum (Mg ha^{-1})	Cultivar		
	Daniele	Imperatriz	Avg.
	Grain yield (Mg ha^{-1})		
0	4.12 aB	4.74 aA	4.43 a
3	3.71 abB	4.87 aA	4.29 a
6	3.65 bB	5.06 aA	4.35 a
9	3.94 abB	5.09 aA	4.52 a
12	3.70 abB	5.15 aA	4.43 a
Avg.	3.82 B	4.98 A	

p-value	Gypsum (G)	n.s.	Gypsum (G)	n.s.
	Boron (B)	**<0.001**	Cultivar (C)	**<0.001**
	G x B	**<0.001**	G x C	**0.006**

[1]Average; [2]Uppercase letters in the row and lowercase letters in the column rank means by Tukey's test at 5%, n.s. = non-significant. Significant *p*-value data are in bold.

CONCLUSIONS

The barley cultivars in this study presented genotypic differences in terms of yield level and response to B application. The cultivar Imperatriz showed a higher yield and the dose of 2 $kg_{[B]}$ ha^{-1} was suitable, while cultivar Daniele response up to 1 $kg_{[B]}$ ha^{-1}. In the presence of the residual effect of gypsum rates and without boron application, barley grain yield was lower, which highlights the need to evaluate the availability of B in soils subjected to gypsum application.

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BORON (^{10}B) FOLIAR SOURCES: UPTAKE AND MOBILITY IN SOYBEAN

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Keywords: Micronutrient; translocation; foliar fertilization

INTRODUCTION

Soybean is one of the most important crops in Brazil due to its importance in the livestock feed and food industry. The mobility of boron (B) in the phloem varies among species. In plants that synthesize enough amounts of polyols, the B forms complexes that facilitate the micronutrient redistribution in the phloem, which does not occur in soybeans. The aim of this work was to evaluate if a B foliar source complexed with polyol favors the micronutrient mobilization in the soybean plants, in addition to studying whether the nutritional status of the plant influences the absorption and redistribution processes of B.

METHODS

The trial was carried out in 7 L pots with 2 soyabean plants, in a 2x4 factorial design, with 2 levels of B supply in the substrate (adequate and high), and 4 foliar sources of B (boric acid, B-MEA, B-Polyol and Control) enriched in ^{10}B, with 5 replicates each treatment. When the plants reached the R2 stage of development, the application of foliar solutions was performed. All the branches that grew after the foliar sprayed were identified, when the soybean reached the R6 stage the plants were harvested in a destructive way and separated into root; parts (leaves and branches) that received the spraying with B; and new parts (leaves, branches, and pods) that grew after foliar application. Biometric parameters, total dry matter, total B concentration, B absorption and remobilization, chlorophyll content and total proteins were evaluated.

RESULTS AND DISCUSSION

No differences were observed in the biometric parameters, total dry matter, chlorophyll content and total proteins. The new parts (leaves, branches, and pods) that grew after B foliar fertilization represented two third (66%) of the total biomass accumulated in the plants. Higher B concentrations were verified in those parts of the plants that received foliar sprays, and in the new branches of the plants when cultivated in a substrate with high supply of B. The high supply of B in the soil reduced the efficiency of foliar uptake for all fertilizer sources. Between 13 and 27% of the B sprayed on soybeans was absorbed by the plants, the highest values were obtained when the source used was B-Polyol and boric acid than when it was B-MEA (Fig. 1A). The B redistribution did not vary between treatments, remaining close to 60% (Fig. 1B), however, higher levels of B derived from fertilizer in the new parts of the plants were observed in treatments that did not receive fertilization with B in the substrate (Fig. 1C), due to lower absorption of B by leaves in the treatments that received high B in the substrate. Approximately 1% of the B absorbed by the aerial part was found in the roots. The amount of B mobilized provided an increase in the B concentration of 4-5 mg kg^{-1} in the new part of the plant (Fig. 1D).

A	**B**
C	**D**

Figure 1. A) Percentage of B absorbed by the plant of the total sprayed; B) Percentage of B sprayed on the plant that was mobilized to other parts of the plant (roots + new parts); C) Percentage of Boron derived from fertilized (Bdff) in the new parts of the plant; and D) Concentration of Bdff in the new parts of the plant.

Bars marked with different letters are significantly different according to Tukey test ($p < 0.05$). Asterisk (*) indicates significant difference between levels of B supply in the substrate (adequate and high).

CONCLUSIONS

Although soybean plants do not synthesize polyols, B redistribution can be considered high, which can be explained by the drain strength of new plant parts. Foliar application of complexed B sources did not favor nutrient mobility in soybeans when compared to boric acid. Despite the intermediate mobility of B in soybean, the amount mobilized is not enough to deliver the B demand for the plant parts grown after foliar spraying.

EFFECTS OF BORON ON GLOBAL TRANSLATIONAL IN ARABIDOPSIS

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Keywords: Ribosome sequencing; mRNA sequencing; translation termination

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INTRODUCTION

Boron transporters of Arabidopsis thaliana have been well studied for their regulation of expression in response to boron conditions. BOR1 the efflux transporter of boron (Takano et al 2002) is regulated at the levels of protein degradation under high boron conditions (Takano et al 2005, 2010?Kasai et al 2011) and it has been suggested that BOR1 acts as a sensor of boron (Yoshinari et al 2020). NIP5;1, diffusion facillitator of boron (Takano et al 2007) is regulated through mRNA degradation in response to high boron (Tanaka et al 2011). This degradation of NIP5;1 mRNA has been demonstrated to be mediated by translational regulation; ribosome stalling at the AUG-Stop sequence in the 5' UTR of NIP5;1 mRNA (Tanaka et al 2016). This regulation occurs in in vitro translation systems (Tanaka et al 2016), suggesting that translational machienary works as a sensor of boron concentration in the cytoplasm. It is also found that BOR1 is also regulated at translation. In this case, a boron-dependent regulation involves multiple uORFs, including an AUG-stop (Aibara et al., 2018) in the 5' UTR of BOR1. It is likely that translational activities are affected by boron concentrations in the cytoplasm, suggesting that changes in boron concentration not only affect traslation of boron transporter genes but also have a wide range of impacts on translation at genomic levels.

In this study, we utilized ribosomal sequencing to reveal impacts of boron concentration on translation at genomic levels and found that boron affect translation of a number of genes and also the process of translational termination.

METHODS

Arabidopsis thaliana Col-0 were used for the study. Plants were grown under different cocentrations of boron and subjected to ribosomal sequencing analysis. In comparison, mRNA sequencing were also conducted and effects of boron conditions on transcript accumulation and translational efficiencies were compared for each gene detected. Outline of the analysis is described in Fig. 1. Details of the procedure is described by Sotta et al (2021).

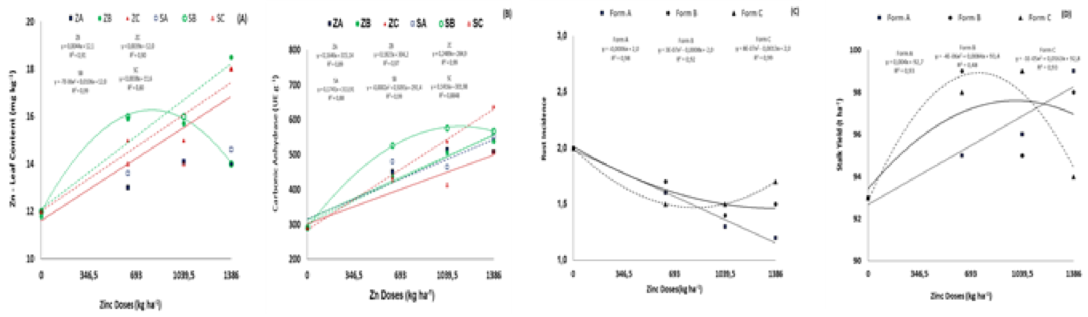

Fig. 1. Outline of the Ribo-seq and RNA-seq analysis in this study. Arabidopsis grown under different boron conditions were subjected to RNA seq and Ribo-seq. Ribo-seq is a procedure to detect mRNA sequence covered by ribosome (Repoduced from Sotta et al 20201)

RESULTS AND DISCUSSION

In this study, hundreds of translationally regulated genes have been found. Under the high boron conditions many genes with enriched uORFs were translationally regulated while genes with downregulated translation often contained AUG-stops in their uORF. These findings suggest that boron conditions have a wide range of translational effects and AUG-stops play an important role in a number of genes. More interestingly, it was revealed that boron increased the ribosome occupancy of stop codons. We have previously demonstrated the importance of AUG-Stop sequence in boron-dependent translational regulation and the present study revealed boron also affect ribosome behavior at the stop codons in general. The details are described in Sotta et al (2021) and in this presentation we would like to introduce additional findings on the effects of boron on translation.

CONCLUSIONS

Boron has global effects on translational regulation and it affects translational termination.

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FOLIAR APPLICATION OF BORON IS A STRATEGY TO ATTENUATE DROUGHT STRESS IN SOYBEAN DUE TO PHYSIOLOGICAL RESPONSES

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Keywords: Abiotic stress tolerance; Micronutrient; Foliar fertilization

INTRODUCTION
Drought stress is the major abiotic stress condition that limits growth, development and yield of crops around the world. In this scenario, balanced supply of nutrients have been promising for increasing the tolerance of plants to different abiotic stresses due to their physiological roles in plants.

The adequate or additional supply of nutrients to the crops has been promising to increase tolerance of plants to abiotic stress. In this context, boron (B) can act in physiological and/or biochemical processes, and some can participate in the alleviation of the effects caused by drought stress in different crops (Tavanti et al., 2021). However, it is still not well elucidated how B in fact contribute to relieving water stress. In the present work, we aimed to evaluate the viability of foliar supplementation of boron (B) in either relieving or reducing the physiological damages caused by water stress in soybean (*Glycine max* L.).

METHODS
A greenhouse trial was carried out with soybean in a 2x3 factorial scheme, with two water conditions (well-watered and drought stress) and three rates of B applied foliar (0; 150, and 300 mg B L^{-1}). Thirty days after emergence, when the plants presented the second fully developed trifoliate leaf, the rates of B were applied with the foliar fertilizer formulated with monoethanolamine borate (YaraVita BORTRAC, Yara International). The water stress was initiated ten days after the foliar application of B. To induce drought stress, the volume of water applied to the pots was first reduced to 60% of the volume placed in the irrigated plants for 10 days. Subsequently, it was further reduced to 50% for another 10 days. The period of "maximum stress" was considered at the 20^{th} day after the onset of water restriction. After this period, plants were rehydrated. Plants were evaluated according to the water conditions: at the maximum stress, 5 days after rehydration and just before harvesting.

RESULTS AND DISCUSSION
During the maximum stress period, plants supplied with B and under drought stress did not showed any differences on the shoots and roots dry mass (DM; Table 1). However, well-watered plants presented higher shoots and roots DM when supplied with foliar application of B (Table 1). During this period, no B supply and drought stress had the same effect on the DM of soybean.

Drought stress increased the levels of lipid peroxidation (MDA) in the soybean leaves. However, plants supplemented with B via foliar spray showed lower MDA levels, either under well-watered or drought stress condition (Table 1). Conversely, B supply increased the activity of ascorbate peroxidase (APX) in well-watered plants, as well as the catalase (CAT) activity in well-watered plants and under drought stress (Table 1).

After rehydration the soybean plants did not were able to recover the growth from the drought stress and presented lower shoots and roots DM when compared to well-watered plants (Table 2). However, well-watered plants suplied with 300 mg of B L^{-1} exhibited greater roots DM compared to untreated plants and the ones supplied with 150 mg of B L^{-1}.

Table 1. Shoots and roots dry mass (DM), lipid peroxidation (MDA) and the activities of ascorbate peroxidase (APX) and catalase (CAT) in the leaves of soybean after the application of rates of B and under the "maximum stress" for drought.

Rates of B	Shoots DM (g)	Roots DM (g)	MDA (nmol g^{-1} FW)	APX (nmol g FW^{-1} min^{-1})	CAT (nmol g FW^{-1} min^{-1})
---- Well-watered ----					
0 mg L^{-1}	3.2 Ba	1.78 Ca	6.0 Ab	7.8 ABa	2.33 Ba
150 mg L^{-1}	10.3 Aa	3.12 Ba	4.2 Ab	5.8 Ba	2.59 ABa
300 mg L^{-1}	9.8 Aa	5.26 Aa	2.8 Bb	9.6 Aa	3.22 Ab
---- Drought stress ----					
0 mg L^{-1}	2.4 Aa	0.88 Ab	9.0 Aa	8.2 Aa	2.43 Ba
150 mg L^{-1}	3.5 Ab	1.52 Ab	7.2 Ba	8.1 Aa	2.44 Ba
300 mg L^{-1}	2.9 Ab	1.27 Ab	7.0 Ba	6.8 Ab	4.47 Aa

Uppercase letters compare foliar B application treatments and lowercase letters compare water conditions within each treatment, according to Fischer's LSD test (*p*<0.05).

In general, soybean plants supplied with B exhibited higher activities of APX and CAT after the rehydration. Consequently, the levels of MDA in the soybean leaves were lower when supplied with the highest rate of B and under well watered condition (Table 2).

At the harvesting time, the parameters directly related to yield were positively influenced by the B supplementation: dry mass of pods, harvest index and the number of grains and pods per plant, especially in plants under drought stress (data not shown).

Table 2. Shoots and roots dry mass (DM), lipid peroxidation (MDA) and the activities of ascorbate peroxidase (APX) and catalase (CAT) in the leaves of soybean after the application of rates of B and "after rehydration".

Rates of B	Shoots DM (g)	Roots DM (g)	MDA (nmol g^{-1} FW)	APX (nmol g FW^{-1} min^{-1})	CAT (nmol g FW^{-1} min^{-1})
---- Well-watered ----					
0 mg L^{-1}	7.9 Aa	4.9 Ba	5.2 Aa	1.3 Ba	0.10 Ba
150 mg L^{-1}	8.8 Aa	5.2 Ba	5.6 Aa	3.5 Aa	0.14 ABa
300 mg L^{-1}	11.1 Aa	7.7 Aa	4.5 Ab	4.2 Aa	0.15 Aa
---- Drought stress ----					
0 mg L^{-1}	3.5 Ab	2.0 Ab	6.4 Ba	2.2 Ba	0.07 Ba
150 mg L^{-1}	4.9 Ab	3.0 Ab	5.2 Ba	2.4 ABa	0.13 Aa
300 mg L^{-1}	5.3 Ab	3.0 Ab	8.8 Aa	4.1 Aa	0.15 Aa

Uppercase letters compare foliar B application treatments and lowercase letters compare water conditions within each treatment, according to Fischer's LSD test (*p*<0.05).

CONCLUSIONS

The foliar application of B has shown as an alternative management to mitigate the damages caused by drought stress in soybean.

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ROOT AND FLOWER RESPONSES OF BORON-EFFICIENT AND -INEFFICIENT *BRASSICA NAPUS* ACCESSIONS TO BORON DEPRIVATION

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Keywords: boron; deprivation; vasculature

INTRODUCTION AND RESULTS

Boron (B) is an essential element for vascular plant nutrition. Soils in diverse regions contain insufficient B for adequate plant growth and development [Shorrocks, 1997]. In addition, during periods of drought, mass flow of B from the soil solution to roots and its translocation to shoots is extremely limited. Deficiency symptoms can include inhibited root and shoot elongation, progressing to reduced fertility and flower abortion, which can limit crop yield considerably. Acute periods of B deficiency can have long-lasting effects on vascular function, further negatively affecting the uptake of B, other nutrients and water [Wimmer and Eichert, 2013]. *Brassica napus* is a significant global source of edible oil and animal feed, and is highly sensitive to B deprivation. Breeding for increased B-efficient cultivars represents a sustainable solution to improve yield stability in a changing climate. However, identifying mechanisms controlling B efficiency has historically proven difficult, largely because of experimental difficulties. In response, we developed systems to study genotypic effects on plant development in repeatable B-deficient conditions, using a "B-free" peat-limestone-based "zero-soil" [Eggert and von Wirén, 2017; Pommerrenig et al., 2018]. Precise tailoring of this substrate has enabled us to study B-deficiency induced specifically during flowering in *B. napus*, which represents a crucial developmental stage reflecting final yield. Through RNA-seq of multiple inflorescence tissues at different time points, we identified mechanisms controlling the response to B deficiency during flowering. In a parallel study, we were able to describe an abnormal vasculature differentiation as an irreversible symptom of B deficiency in *B. napus*. For a morphological and structural characterization plants were used grown under control and B-limiting hydroponic conditions. Over the timespan of 3-14 days after germination tissue samples were collected and investigated by light and electron microscopy. Significant structural modifications especially occurred in the xylem and phloem affecting cell structure and tissue organisation. The morphological alterations were much more prominent in the B-inefficient accession and differed clearly from those reported for other nutrient deficiencies. First changes in the vascular and meristematic tissue due to B-efficiency were detected after three days in root tips, and after 7-9 days in hypocotyl and primary leaf.

Continued analysis of these complementary studies offers significant opportunity for elucidating mechanisms controlling the response to B-deficiency in *B. napus*, which can inform breeding strategies for increasing B-efficiency in the face of a changing climate.

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Breeding for a better plant nutrition

MICRONUTRIENT BIOFORTIFICATION OF CASSAVA: ACHIEVEMENTS AND CHALLENGES

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Keywords: Cassava; Biofortification; Iron

Micronutrient Biofortification of Cassava: Achievements and Challenges

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INTRODUCTION

Cassava (*Manihot esculenta Crantz*), which was first domesticated in west-central Brazil, provides dietary calories for more than half a billion people worldwide. Although an excellent source of energy in the form of starch, cassava storage roots provide less than 10% and 15% of the estimated average requirements (EAR) for iron and zinc respectively (Hefferon, 2015; Zimmermann and Hurrell, 2007). For populations that rely on starchy staple crops such as cassava, the 'hidden hunger' posed by micronutrient deficiencies represents a significant threat to human health (Gegios *et al*., 2010). Thus, efforts to enhance iron and zinc concentrations of cassava storage roots through traditional breeding have been considered, but they are constrained by insufficient genetic variation for these traits within the existing germplasm. A transgenic approach, however, using the overexpression of iron-regulated genes under the control of the patatin promoter resulted in higher iron concentration in storage roots (Narayanan et al., 2019). These transgenic lines could potentially provide a significant beneficial impact on the iron and zinc status of cassava-consuming populations. Transferring this technology to farmer-preferred cassava cultivars generated interesting results. Different farmer preferred cassava cultivars have been found to respond differently to co-expression of *AtIRT1* (iron transporter) and *AtFER* (ferritin). Overexpressing *AtIRT1* alone is toxic in some cases, preventing the recovery of transgenic plants. Overexpressing *AtFER* alone in cv. TMS 98/0505 causes upregulation of endogenous *IRT1* and *FRO*, but this does not occur in cv. TME 419 and TMS 91/02324. Efforts are ongoing to better understand these differential responses and to produce mineral biofortified cassava for consumers.

RESULTS and discussion

Fourteen constructs comprising seven different transgene combinations were introduced into cassava. Strategies reported to be efficacious in cereals, including upregulation of genes involved in long-distance transport (*OsNAS2* and *OsYSL)*, were not successful in cassava. Co-expression of four mineral-related genes (*AtIRT1+ OsNAS1+ AtFER+AtVIT1*) was toxic in callus tissues and prevented the recovery of transgenic plants. Alternative strategies were therefore designed to enhance mineral source-sink dynamics in cassava storage root tissues.

Figure 1 Storage root mineral concentrations of *VIT1* and *IRT1+FER* transgenic cassava plants at harvest 12 months after planting (Narayanan et al., 2019).

Under field conditions, 10 out of 15 VIT1 (vacuolar transporter) lines showed storage roots with a significant 2-5 fold increase in iron concentration (Fig. 1a). *IRT1+FER* (major iron transporter and storage protein) transgenic plants showed 6-12 times higher iron and 3-9 times higher zinc concentrations compared to non-transgenic controls (145 and 40 µg/g dry weight, respectively) (Fig. 1c and d). Retention and bioaccessibility of the transgenically accumulated iron and zinc in the West African cassava foodstuffs gari and fufu indicated that *IRT1+FER1* plants could provide 40-50% EAR iron and 60-70% EAR zinc for 1- to 3-year-old children and non-lactating, non-pregnant women (Narayanan et al., 2019). Farmer preferred cassava cultivars showed differential iron regulation. TMS 98/0505 showed higher iron deficiency responses compared with TME 419 and TMS 91/02324 when overexpressing ferritin by showing an abnormal phenotype under greenhouse (Fig. 2a) and also an increase in endogenous FRO and IRT expression in fibrous roots (Fig. 2b).

Figure 2 (a). Phenotypes of 10-12 week old cassava plants in greenhouse when expressing *FER* and *IRT1+FER* in different farmer preferred cultivars. (b). Endogenous *MeFRO* and *MeIRT* expression in fibrous roots

CONCLUSIONS

Delivering the benefits of agricultural biotechnology to smallholder farmers requires that resources be directed towards staple food crops. This study shows evidence that mineral biofortification is well demonstrated in farmer preferred cultivars with iron and zinc levels elevated in storage roots. Studies are in progress to better understand differential responses of iron regulation in different cassava cultivars and to produce mineral biofortified cassava for consumers around the world.

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Diagnosis of nutrient status and analytical methods

INCLUSION OF POTENTIALLY TOXIC ELEMENTS IN DRIS FUNCTIONS FOR ASSESSING THE NUTRITIONAL STATUS OF *EUCALYPTUS*

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Keywords: Forest nutrition; DRIS; Sewage sudge management

INTRODUCTION

The use of sewage sludge (SS) in forest plantations increases soil chemical, physical, and biological fertility, which can increase the productivity of *Eucalyptus* wood (Abreu-Junior et al., 2020). However, the presence of potentially toxic elements (PTEs) in sewage sludge, such as As, Ba, Cd, Cr, Hg, Pb, and Se, can make it unfeasible or restrict its use in forestry. Given the possibility that PTEs can cause imbalances in the physiological functions of plants, there is a need for a nutritional assessment method that considers the interference of these elements. Thus, the use of tools to assess the nutritional status of plants, such as the Diagnosis and Recommendation of Integrated System (DRIS), can be important in assessing the influence of the PTEs on plant nutrition and development. DRIS allows the calculation of indices for each nutrient, using its binary relationships with the others (bivariate method), and comparing them with a reference population, aiming to classify the nutrients in the order of limiting plant growth (Costa, 1999). Therefore, we aim to model the DRIS functions with the inclusion of PTEs in its norms for assessing the nutritional status of *Eucalyptus* treated with sewage sludge.

METHODS

In February 2015, an experiment was carried out in a commercial plantation of *Eucalyptus urograndis* in the municipality of Boa Esperança do Sul/SP. The experiment presented a randomized block design with four replications, with ten treatments: control (C); mineral fertilization (MF1 and MF2); 14.5 Mg ha^{-1} of SS + 22 kg ha^{-1} of P, with (S1P1B) or without (S1P1) 6.5 kg ha^{-1} of B; 29 Mg ha^{-1} of SS + 17.5 kg ha^{-1} of P, with (S2P2B) or without (S2P2) 6.5 kg ha^{-1} of B; 29 Mg ha^{-1} of SS + 6.5 kg ha^{-1} of B (S2B); and 43.5 Mg ha^{-1} of SS, with (S3B) or without (S3) 6.5 kg ha^{-1} de B. The sampling of leaf used for nutritional diagnosis and data collection to estimate *Eucalyptus* production were carried out at 54 months after planting (48 months after SS application). As it is a residue with an unbalanced concentration of nutrients in relation to the needs of the crop, we chose to adopt the adapted method according to Wadt et al. (2007), in which it provides the division of nutrients into groups (N, P, and K; Ca, Mg, and S; B, Cu, and Zn; and Fe and Mn). Due to the inclusion of PTEs, another group is proposed (Al, As, Ba, Cd, Cr, Ni, Pb, and Se). In order to overestimate and/or underestimate nutritional deficiencies and excesses, according to the nutrient group, it was used the DRIS formulas proposed by Wadt et al. (2007). With the PTEs group, the proposed calculation aims to underestimate the "deficiency" and overestimate the excess of PTEs. With the indices of each element generated from the DRIS functions, the mean nutritional balance index (NBIm) was calculated. Additionally, to interpret the values of the generated indices, the criterion of potential response to simplified fertilization was adopted, which classifies the nutritional status of the plant for each nutrient as insufficient, balanced, and in excess (Pinto et al., 2010). Afterwards, the classifications with and without the addition of PTEs were compared by the chi-square test ($p < 0.05$) with the aid of SigmaPlot software version 14.0 (Systat Software, 2020). This analysis aims to verify the agreement of classification of nutritional status when there was or was not the addition of PTEs in DRIS functions.

RESULTS AND DISCUSSION

The reference population (RP) had an average productivity of 57 m^3 ha^{-1} $year^{-1}$, 10% higher ($p<0.05$, t-test) than the low productivity subpopulation (LPP). In which, 86% of the plots that made up the RP corresponded to treatments with SS application and 50% of the plots corresponded to the application of SS to provide 100% of the N recommendation (S2P2, S2P2B, and S2B). By placing the nutrient indexes in ascending order, it is possible to verify the most limiting nutrient by deficiency and excess (Pinto et al., 2010). For LPP, B was the most limiting due to deficiency, with or without the inclusion of PTEs, approximately, 27.8% of the plots

(Table 1). However, these plots correspond to treatments that did not receive B application or with a low SS dose, suggesting the need to apply B with SS. Furthermore, 29% of LPP was limited by Pb excess.

Table 1. Order of nutrients, in deficiency and excess, limiting the productivity and respective percentages of occurrence in *Eucalyptus* plots in the low productivity subpopulation

Status	PTEs	Limitation order										
Deficiency	Without	B >	K >	N =	P =	Zn >	Cu >	Fe =	Mn >	Ca =	Mg =	S
		26.7	17.8	11.1	11.1	11.1	8.9	6.7	6.7	0.0	0.0	0.0
	With	B >	K >	Cu =	Fe =	Zn >	P >	N >	Ca =	Mn >	Mg =	S
		28.9	13.3	11.1	11.1	11.1	8.9	6.7	4.4	4.4	0.0	0.0
Excess	Without	Ca >	Fe >	N >	Zn >	Mg =	Cu >	P =	K =	Mn >	B >	S
		20.0	15.6	13.3	11.1	8.9	8.9	6.7	6.7	6.7	2.2	0.0
	With	N >	Fe =	Zn >	Ca >	P =	Mn >	B >	K =	Mg =	S >	Cu
		22.2	13.3	13.3	11.1	8.9	8.9	6.7	4.4	4.4	4.4	2.2
		Pb >	Cd >	Al >	As =	Cr =	Ni >	Ba =	Se			
		28.6	19.0	14.3	9.5	9.5	9.5	4.8	4.8			

Considering that the situation of "deficiency" and nutritional balance for PTEs is desired because they present values ??lower or similar to the reference population, the situation of excess of these elements is not desirable. The frequency of plots in situation of excess varied from 8 to 50% and that Ba caused an increase in the portions in a situation of imbalance (excess). The agreement of nutritional diagnosis with or without the PTEs inclusion in DRIS functions ranged from 79.8 to 97.5% ($p<0.05$, chi-square test). Thus, the PTEs inclusion in DRIS functions can provide important information as to which element is causing more damage to plants.

CONCLUSIONS

Modeling DRIS functions with the inclusion of PTEs was able to underestimate the "deficiency" and overestimate the excess of PTEs, contributing to the assessment of the *Eucalyptus* nutritional status, generating indices capable of ordering the limiting elements in the *Eucalyptus* productivity.

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BIOCHEMICAL ASPECTS OF COPPER DEFICIENCY INDUCED BY HIGH NITROGEN FERTILIZATION IN CITRUS

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Keywords: micronutrient; enzymatic activity; anatomy

INTRODUCTION

Copper (Cu) is an essential element for several enzyme systems, photosynthesis electron transport and lignin synthesis affecting plant metabolism and growth. Cu deficiency has been observed either on citrus nursery trees production or newly filed planted orchards, likely induced by interaction with other essential mineral elements. However, visual symptoms of Cu deficiency are not easily recognized in plants since those are not limited to leaves as chlorosis or necrosis typically described by other mineral nutrients. In this context, we characterized Cu deficiency in orange trees, as induced by high nitrogen fertilization by evaluating biochemical parameters, gas exchange and plant leaf anatomy.

METHODS

Young sweet orange trees cv. Valencia grafted onto Rangpur lime were grown with under two levels of Cu supply via fertigation (control without Cu or with 2.4 g per plant of Cu) and two levels of nitrogen (N) (medium: 8.6 or high: 25.9 g per plant of N) in addition to foliar spraying, for 210 days.

RESULTS AND DISCUSSION

Plants with Cu exhibited higher rate of electron transport (ETR), CO_2 assimilation (PN), transpiration (E) and stomatal conductance (gs) compared to the control without Cu. Plants with high N exhibited higher ETR than those with medium N. Plants without high Cu and N showed the lowest nitrate reductase (RNase) activity, while plants with high Cu and N showed the greatest increase in the activity of this enzyme, in addition to the highest levels of total free amino acids. Furthermore, plants lacking Cu and under high N growing regime exhibited the lowest superoxide dismutase (SOD) activity, mainly of the Cu/Zn-SOD II isoform. Also, it was observed that anatomical aspects of loose parenchyma structures of leaves and branches were associated with visual symptoms of Cu deficiency.

CONCLUSIONS

The plant responses to the differential supply of Cu and N characterized in the present work demonstrate, in part, how Cu deficiency is induced in citrus plants by high doses of N, thus highlighting the importance of establishing better nutrient management practices in citrus production what will contribute with the maintenance of crop production efficiency.

RELATION BETWEEN PLANT PHYSIOLOGY, SAP NUTRITIONAL ASSESMENT AND MD-2 HYBRID PINEAPPLE YIELD AND QUALITY RELATED VARIABLES: NEW TOOLS TO MEASURE AND ENHANCE THE UNDERSTANDING AND INTEGRATION OF NUTRITIONAL STATUS AND ITS RELATION WITH PHYSIOLOGICAL DYNAMICS

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Keywords: Plant Nutrition ; Crop Physiology ; Pineapple

Relation between plant physiology, sap nutritional assesment and MD-2 Hybrid Pineapple yield and quality related variables: New Tools to measure and enhance the understanding and integration of nutritional status and its relation with physiological dynamics

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INTRODUCTION

The understanding of pineapple physiology based on variables like stomatal conductance, transpiration and CO2 net assimilation rate and its relation with the nutritional dynamics of the crop is very important to determine the factors that have more impact on yield. Furthermore, including sap nutrition assesments as a real time tool allows a better resolution of the impact of fertilization programs because variables like Ca+2, K+, NO3-, pH and electrical conductivity (EC) in the sap have a direct relation with biometric variables like D leave, stem and plant weight and with concentration of metabolites like starch and metabolized nitrogen. The holistic integration and analysis of all this factors evidenced a direct relation between sap composition, foliar nutrition and biometric and quality variables in pineapple crop that end up contributing to overall crop yield.

METHODS

In the last couple of years, a continous survey of physiological and nutritional variables have been done in many pineapple farms of Costa Rica during th entire crop phenology. Stomatal conductance, transpiration and CO2 net assimilation rate have been the focus in terms of plant physiology related factors. On the other hand, soil and tissue chemical analysis and sap composition assesment have the main nutritional tools to provide the integration of both areas and yield.

Physiological variables

Stomatal conductance (mmol/m2/s), transpiration (mmol//m2/s) and CO2 net assimilation rate (µmol/m2/s) were measured during the whole crop cycle using a Ci-340 (CID-Bio Science) equipment.

Nutrional variables

Soil and foliar tissue chemichal analysis were done at different certified labs in Costa Rica. For soil analysis Olsen modified and KCl 1N solutions and for tissue analysis a complete diggestion process using the ICP-MS technique was done. Sap nutritional composition assesment was done using a Horiba Laquatwin pocket meters (Ca-11, K-11, NO3-11). Sap pH and EC were measured isung the HANNA HI9811-5 equipment.

RESULTS AND DISCUSSION

The evaluation of physiological variables of MD-2 Hybrid Pineapple, showed how stomatal conductance and transpiration are closely related to CO2 net assimilation rate creating a sink source dynamic that increases after flower induction (Fig 1). That dynamic is critical for nutrient uptake and movement in the plant and that phenomenom was evident in the sap composition assesments (Fig 2). By integrating both type of factors we can observe that their dynamics are closely related with yield and quality variables of the crop (Fig 3).

Fig. 1. Preforcing and postforcing relation between CO_2 net assimilation rate and Stomatal conductance in MD-2 Hybrid Pineapple.

Fig. 2. Preforcing and postforcing sap levels of Ca^{+2}, K^+, NO_3^- in MD-2 Hybrid Pineapple.

Fig. 3.Relation between sap nitrate levels and °brix in MD-2 Hybrid Pineapple fruit.

CONCLUSIONS

The integration and understanding of the dynamics of physiology and nutrition in MD-2 Hybrid Pineapple crop is key to optimize fertilization management allowing improvements in yield and quality related variables.

READILY SOLUBLE SILICON POOLS, CARBON FRACTIONS AND PHYTOLITH DISTRIBUTION IN RICE AND SUGARCANE SOILS OF TROPICAL REGION OF INDIA

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Keywords: Plant available silicon; Phytolith; Rice and sugarcane soils

Readily soluble silicon pools, carbon fractions and phytolith distribution in rice and sugarcane soils of tropical region of India

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INTRODUCTION
The knowledge about silicon (Si) availability in Indian soils is limited and scattered. Since no extractant has been found to work equally well on all soils, there is no precise and consistent method which can easily be used for predicting the necessity for Si fertilization in tropical soils. Additionally, besides primary crystalline and secondary silicates, Si also occurs as readily soluble Si pools which includes dissolved Si (DSi), adsorbed Si (AdSi) and amorphous Si (ASi). However, the influence of rice and sugarcane on the readily soluble Si pool in soil has not been investigated so far under tropical condition of India. Hence, there is a necessity for evaluation of simple and rapid extractants for determination of readily soluble pools of Si in Indian soils. Further, there is no database on systematic quantification and description of phytolith in intensively cultivated rice and sugarcane soils in India. Since little is known about the interrelationship between Si and carbon (C) in intensively cultivated rice and sugarcane crops, there is a need to explore the prospects of any possible relationships between the Si and C fractions. In view of this, the present study was planned to quantify the readily soluble silicon pools, carbon fractions and distribution of phytoliths in rice and sugarcane soils.

METHODS

To assess the readily soluble silicon pools such as DSi, AdSi and ASi; two soil profiles each from rice and sugarcane, where rice and sugarcane were being intensively cultivated for more than a decade, representing four different agro-climatic zones viz., central dry zone, southern dry zone, southern transition zone and coastal zone were collected. The following standard methods were used for extraction and estimation of readily soluble silicon pools:

Table 1. Standard methods and extractant used for estimation of readily soluble silicon pools

Sl. No	**Type of readily soluble silicon**	**Extractant used**	**Reference**
1	Dissolved silicon	0.01 M $CaCl_2$	Haysom and Chapman (1975)

2

Adsorbed silicon

0.5 M CH_3COOH

Narayanaswamy and Prakash (2009)

3

Amorphous silicon

1% Na_2CO_3

Modified DeMaster (1981); Majumdar and Prakash (2020)

Collected soil samples were processed and physical separations of phytoliths were performed on surface and sub-surface soil sample using a gravimetric separation method by using sodium polytungstate as a heavy liquid having density of 2.3 g cm^{-3}. Later on, total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) was analyzed by adopting standard procedure of Yeomans and Bremner (1991).

RESULTS and discussion

In this study, 0.5 M acetic acid, due to low pH of the extractant, extracted higher amount of AdSi compared to DSi extracted by 0.01 M calcium chloride. Adsorption is a very complex process that includes physisorption, chemisorption, organo-mineral complexation, and complexation to bacteria. The higher values of AdSi content compared to DSi content in the present study indicates that calcium chloride extractant gives favored access to the physiosorbed Si, while acetic acid extractant gives access to physiosorbed and chemisorbed Si. The actual procedure of ASi determination (DeMaster, 1981) mainly called for sub-sampling at 2, 3 and 5 hours by using 1% Na_2CO_3 as an extractant. However, initial batch experiment study conducted in this research indicated that 5 hours extraction period as described by DeMaster (1981) is not sufficient enough for ASi determination for the tropical soils. Hence, it was suggested that 1% Na_2CO_3 method can be adopted for tropical soils with a modification of digestion up to 6 hours instead of 5 hours (Fig. 1).

Fig 1. Schematic diagram explaining the methodology used to account for the simultaneous dissolution of mineral silicates during amorphous silicon (ASi) extractions in tropical soils by 1 per cent Na_2CO_3 at 85 °C as a function of time (Majumdar and Prakash, 2020).

The dominant phytolith morphotypes observed in rice and sugarcane soils were bulliform, bilobate, elongate sinuous, elongate echinate, trapezoid and rod shaped (Fig. 2).

Fig 2. Different phytolith morphotypes observed in rice and sugarcane soils (A) Short cell bilobate (a), Trapezoidal (b); (B) Trapezoid; (C) Bulliform; (D) Bulliform (a), Rod shaped (b); (E) Trapezoid (a), Lanceolate (b), Elongate smooth (c), Bulliform(d); (F) Elongate smooth; (G) Rod shaped; (H) Trapezoid (I) Rod shaped (a), Trapezoid (b).

The results from this study exposed that irrespective of crop, a positive correlation existed between DSi and TOC content, whereas TOC correlated negatively with AdSi content. It suggested that TOC and IC may act as the main and/or potential source of DSi and AdSi content, respectively in rice and sugarcane soils (Majumdar and Prakash, 2021).

CONCLUSIONS

The findings of this study would assist to assess the status of plant available Si in soils in relation geology and climatic conditions. The electron micrographs of phytoliths may serve as direct evidence to ascribe due to the changes in crops and/or soils. It is necessary to further investigate to authenticate the functional relationship of carbon fractions with readily soluble Si pools.

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CHLOROPHYLL *A* FLUORESCENCE ANALYSIS TO MONITOR P RESTORATION IN DEFICIENT PLANTS WITH FOLIAR FERTILIZATION

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Keywords: Phosphorus deficiency; Foliar fertilization; Diagnosis

INTRODUCTION

Phosphorus (P) deficiency is a global challenge. Standard soil fertilization practices are often problematic as a large part of the applied P becomes plant unavailable in the soil. P is furthermore a limited natural ressouce in risk of depletion, which calls for efficient supplemental fertilization strategies. Foliar fertilization can be a valuable supplementary technique to ensure fast and efficient uptake of P as nutrients are applied directly to the foliage, thereby bypassing adverse soil processes.

P deficiency is often latent, and efficient methods are thus needed to detect P deficiency and restoration after e.g. foliar fertilization. Analysing whether applied foliar fertilizers have become bioavailable and restored P functionality in a plant can be challenging, as most analytical methods does not distinguish between bioactive and bio-unavailable P in the foliage. On this background, a nutrient specific assay for P deficiency was developed based on chlorophyll *a* fluorescence. In high intensity light, the thylakoid lumen in plants acidifies, which triggers induction of non-photochemical quenching (NPQ). In P-deficient plants the lumen acidification is induced more rapidly due to their existing deficiency (Carstensen et al 2018). This leads to a faster induction of NPQ (Arsic et al 2020), as well as specific changes in the pattern of the fluorescence transient (Carstensen et al 2018), both of which can be used to diagnose plant P status. In this study, the assay has been used to image NPQ across leaves to trace restoration and translocation of foliar applied P in barley.

METHODS

Barley plants were grown in a hydroponic system, under P deficiency (P-), or as control (P+). Foliar fertilizer treatments with 0.2 M KH_2PO_4 + 0.1% tween20 was applied by spraying entire plants until the drip point. NPQ and P concentration was measured 1, 4 and 7 days following the application. To image NPQ across the leaf surface, entire leaves were dark-adapted and placed in a Pulse-Amplified Modulation (PAM) system measuring chlorophyll a fluorescence. Saturating light pulses were given to measure the maximum fluorescence while darkadapted (Fm) and during actinic light (Fm'). NPQ was induced by high light intensity (926 PAR), and calculated as (Fm-Fm')/Fm'. P concentration in leaves was measured with ICP-MS after drying and digestion of leaves. Methods further described in Arsic et al 2020.

RESULTS AND DISCUSSION

The assay can quickly and reliably diagnose P deficiency. Differences between P-deficient and P-sufficient plants was significant after 15-60 seconds in sustained high intensity light, and could be used for diagnosis of P deficiency (Figure 1). The highest ratio between NPQ in P+ and P- leaves occurred after 15 seconds, so this time point was chosen (Figure 1C). NPQ development in P-deficient plants is a nutrient specific response, as other nutrient deficiencies do not increase the rate of NPQ induction without simultaneously affecting other chlorophyll fluorescence parameters.

To trace the bioavailability of foliar applied P, NPQ development was followed after treating P- barley plants with a foliar spray of KH_2PO_4. After 1 day, P status in the youngest fully evolved leaves (YFEL) was almost restored to P+ level. This was analysed with both NPQ (Fig 2A), and P concentration (Fig 2B). However, this restoration was transient, as the sprayed leaves returned to P deficiency after 7 days (Figure 2). After 7 days, the emerging leaves were not deficient in the foliar treated P- plants, indicating a translocation from the treated source tissue to sink tissues (data not shown).

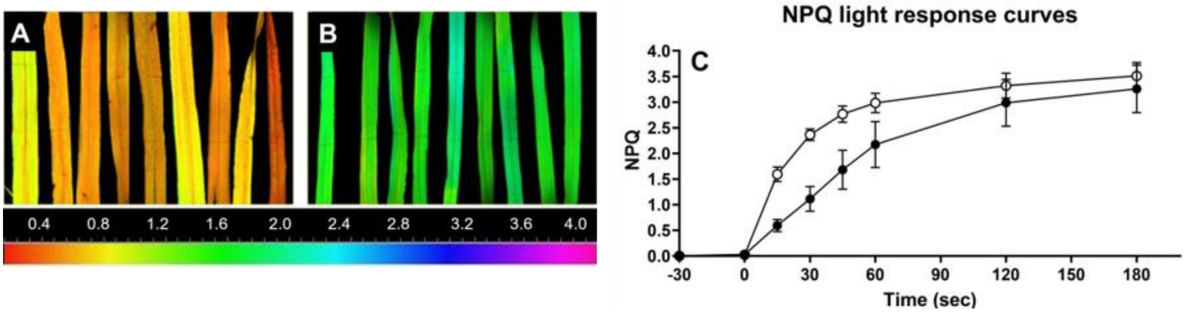

Figure 1: NPQ assay. False-color imaging of NPQ after 15 seconds in P-sufficient (A) and P-deficient (B) barley YFEL. The color correspond to the measured NPQ, as seen in the color scale. NPQ light response curve in C) shows NPQ development in P-sufficent (white circles) or P-deficient (black circles) following the light-induction at 0 sec. N=9, error bars represent sd. (Arsic et al 2020)

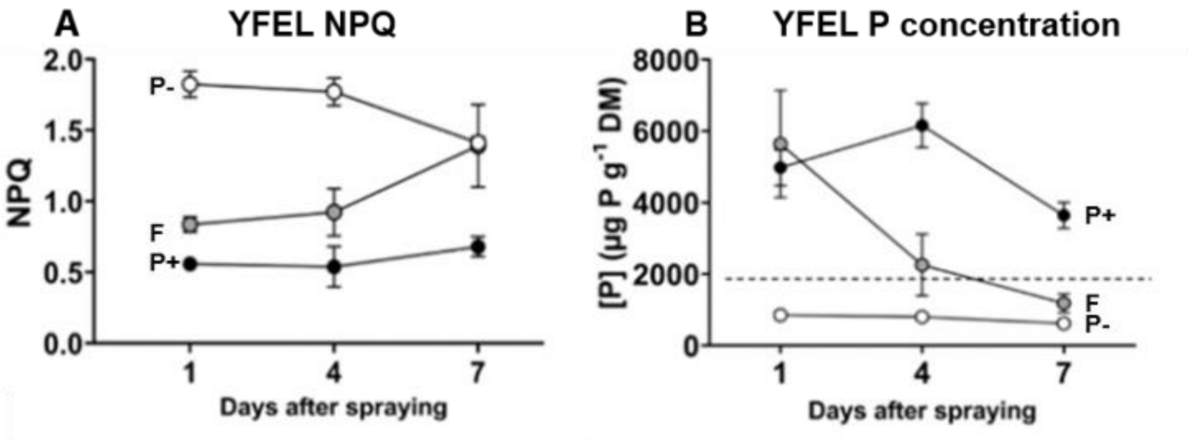

Figure 2: NPQ (A) and P concentration (B) in YFEL of P-deficient (P-, white circles), P-sufficient (P+, black circles) and foliar P-treated (F, grey circles) barley 1, 4 and 7 days after treatment. Dotted line in (B) represent level of deficiency. N=24, error bars represent sd. (Arsic et al 2020)

The NPQ assay are able to identify P deficiency in potato, and likely a wide range of other crops. Work is currently ongoing in potato to elucidate differences relative to barley, which will be presented at the IPNC. Potato and barley differ markedly in leaf morphology, with potato having multiple types of trichomes, and a higher stomata and trichome density. Potatoes are furthermore a highly P demanding crop, and one of the most globally important food crops.

CONCLUSIONS

Analysis of chlorophyll *a* fluorescence with the NPQ assay is a fast, low cost and nutrient specific method to diagnose P deficiency and trace restoration of P status following foliar fertilizers. The NPQ assay can be used to evaluate the efficiency of different foliar P fertilizers, the speed at which the nutrients become biofunctional, and the translocation patterns of the applied foliar P. It was shown that following foliar fertilization with KH_2PO_4, P status in barley was restored after 1 day, and after 7 days the applied nutrients were translocated from the treated tissues to untreated sink tissues.

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PHOSPHORUS QUANTIFICATION IN SUGAR CANE LEAVES IN VIVO BY PORTABLE X RAY FLUORESCENCE SPECTROSCOPY

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Keywords: Plant P nutrition; *Saccharum officinarum*; sustainable chemistry

INTRODUCTION

One of the most efficient ways to evaluate the nutritional status of the plants and indicate zones of nutrient deficiency or surplus is by leaf analysis, once the plant is the best indicator of nutrient availability. However, it demands for sampling in short periods, in a large number of plants, and in a specific stage of plant development, which may not be feasible. For phosphorus (P) determination in plant tissue, X-ray fluorescence should offer adequate limits of quantification, maximum accuracy, reproducibility, portability, and minimal sample preparation, measuring the flux and energy photon, which is characteristic of each element, and the element concentration in the sample [(1, 2)]. Previous studies have reported good results for P quantification in the leaves ground powder, pelletized or detached from plants [(3, 4)]. In the presented study, we intend to adapt the use of pXRF for P quantification on leaves "in vivo" rather than on detached ones, which broadens the range of applications of the technique. This study aimed to evaluate the potential of pXRF to quantify P in sugar cane leaves in vivo.

METHODS

Sugar cane seedlings (RB96-6928), were transplanted into PVC pots filled with 3 kg of *a Latossolo Vermelho-Amarelo distro?fico*, medium texture, with low available P content (5 mg kg^{-1} P resin). Increasing doses of P_2O_5 (0, 30, 60, 90, 120, 150, 200, 400, and 500 mg kg?[1]) were applied as triple superphosphate and plants were grown for 60 days in a greenhouse. In each plant, the leaves (not detached from the plants) were placed on a 5 µm polypropylene film and three points were measured in the abaxial surface of the limb of the sugar cane Top VisibleDewlap, with a data acquisition time of 60 s per scan, Rh X-ray tube at 40 kV and 30 µA, and under vacuum, using a pXRF spectrometer (Tracer III-SD model, Bruker) equipped with a 4 W Rh X-ray tube and a 10 mm^2 X-Flash Peltier cooled silicon drift detector. The pXRF intensity was obtained at the P Kα peak (2.01 keV) and divided by the lifetime, yielding the intensity in counts per second. Three strategies to convert the P Kα XRF signal into P concentration in the leaves were employed: (i) external calibration curve using cellulose pellets with chemical composition similar to the samples, (ii) external calibration curve in which the pXRF counting rate was normalized by the ratio between the density of cellulose pellets and the density of sugar cane leaves (DC:DL) and (iii) external calibration curve by cross calibration using inductively coupled plasma optical emission spectrometry (ICP OES). The experimental design was completely randomized with nine treatments and three repetitions. The pXRF instrumental settings were analyzed on the basis of the SNR and the CV (%). The comparison between the pXRF and ICP OES methods was performed using simple linear regressions and Pearson's correlation test. Statistical analysis was performed using the Statistical Analysis System version 9.3 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

The intensity of the P Kα peak increased with the dose of P_2O_5 applied to the soil, showing the dependence of the pXRF signal on the elemental quantity per unit of volume, which demonstrates the capacity of the technique to identify differences in the content of P in sugar cane leaves in vivo. If one considers the external calibration curve in which the pXRF counting rate was normalized by the ratio between the densities of standard cellulose pellets and the density of sugar cane leaves (y= 0.084x + 8.149) to convert the P Kα signal from pXRF into the P concentration in the leaves, we found a good linear relationship between the P concentration obtained by the ICP OES destructive reference method and pXRF (Fig. 1A). Nevertheless, a slight underestimation of the P content by pXRF remains (Fig. 1B), which can be explained by the high silicon (Si) content in the leaves, that probably attenuated the P Kα X-rays produced. An alternative to overcoming the matrix differences issues is the cross-calibration, in which the P signal obtained in the leaves by pXRF is correlated to the P concentration obtained by a reference method of analysis (ICP OES). In the framework of this study, this strategy proved to be as good as the use of the external calibration curve in which the pXRF counting rate was normalized by the DC:DL ratio (y= 0.0204 + 8.31, R^2= 0.865 and y= 0.084x + 8.149, R^2= 0.994, respectivily).

Fig. 1.

Comparison of the P content in sugarcane leaves obtained by pXRF ICP OES using external calibration curve considering the normalization by the DC and DL (A). Total P content in sugarcane leaves determined by pXRF and ICP OES in relation to the P concentration applied to the soil. black **: linear regression obtained to ICP OES. orange: linear regression obtained to pXRF (B). Vertical error bars correspond to standard deviation. R^2= Coefficient of determination; r= Pearson´s correlation coefficient.**

CONCLUSIONS

Despite the difficulties caused by the matrix effect, the quantification of P in sugar cane leaves in vivo and in situ by pXRF was possible and accurate by using cross-calibration and if the density of the standards pellets is considered to normalize the fluorescence intensities of P. The use of this technique provided a cheaper and faster analysis, with little sample preparation, high spatial resolution, and consistent with the precepts of sustainable chemistry.

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COMBINED STRESS DIAGNOSTIC TRANSCRIPTOMICS IN POTATO

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Keywords: Combined Stress; Water; Phosphorus

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a major global crop that has an important role to play in food security, reducing poverty and improving human nutrition. But, its production currently requires large amounts of nitrogen (N), potassium (K) and phosphorus (P) fertilizer and irrigation water. In the interests of sustainable crop production, it is important to reduce these resource inputs and make the system as efficient as possible in the face of climate change, where interactions between the stresses will become more acute. This can be achieved by improving management practices and developing genotypes with better root systems and greater physiological water and nutrient use efficiencies. Here we describe research, performed in the EU Horizon 2020 project SolACE, which aims to generate a transcriptional diagnotic tool to assess the status of potato plants under combined stress.

METHODS

To determine a diagnostic transcriptome for combined stress, RNAseq analysis was performed on tissue samples of plants grown in a range of conditions including hydroponics, mesocosms in controlled conditions and in the field. In mesocosm experiments, plants were grown from tubers and irrigated to the soil water holding capacity until established, then the irrigation of one set of plants was reduced by half whilst the other plants continued to be irrigated to soil water holding capacity. Shoots and diagnostic leaves from three individual plants from both sets of plants were harvested on the day before they received different irrigation treatments and a further eight times over a period of 28 days. After 28 days of treatment, the irrigation was restored to both sets of plants to achieve soil water holding capacity and shoots and diagnostic leaves from three individual plants from each set were sampled again three times over the next seven days. Dry weights of the harvested shoots were measured and their mineral composition was analysed using ICP-MS to determine the effects of drought on the shoot ionome. In the field, the ionodes of diagnostic leaves, haulms and tubers of plants from a full-factorial experiment to test the effects of combinations of P, N and water stress on two potato cultivars, one with a large root system (cv Desiree) and one with a small root system (cv Pentland Dell), was performed. Phosphorus stress was imposed by the omission of phosphate-fertiliser, nitrogen stress by reducing the N-fertiliser applied, and water stress by reducing irrigation. Soil moisture status was monitored throughout the experiment. Canopy development was monitored, and the fresh and dry weights of haulms and tubers were measured at harvest to determine the effects of combined stresses on plant growth and crop yield. Diagnostic leaves, haulms and tubers were analysed for mineral composition by ICP-MS and EA for nitrogen, allowing comparisons of nutrient use efficiency. From all experiments, diagnostic leaves were sampled, and RNA extracted for transcriptomic analyses to design and test the efficacy of diagnostic transcriptomes for N, P, drought and combined stress.

RESULTS and discussion

Generally, potato plants with adequate water were larger than droughted plants, but only by a few percent. There were changes in the plant ionome related to the drought treatment, with water having little effect on N concentration in leaves, but a discernible effect on P and K concentrations. In general, shoot concentrations of N, P and K decreased with age, but P and K concentrations were larger in watered than in droughted plants. There was a large increase in shoot P and K concentrations when plants were rewatered after being allowed to dry (Figure 1). In the field reduced irrigation limited the growth and tuber yield of both the large-rooted genotype (Desiree) and the small-rooted genotype (Pentland Dell) whether alone or in combination with N or P stresses, suggesting drought was always the limiting stress. Reducing N and P supply had mixed impacts on haulm biomass production, but generally led to a smaller reduction than did a lack of water, while reducing N and P had little effect on tuber yield. The effect of reduced irrigation on tuber yield was generally less in Desiree than in Pentland Dell, suggesting the importance of the size of the root system in mitigating drought stress. Reducing the application of P or N fertilisers limited the growth of both genotypes, but only reduced tuber yield of Desiree when droughted and did not affect tuber yield of Desiree when irrigated or Pentland Dell when irrigated or droughted, suggesting that the harvest index (tuber weight / plant weight) of plants was altered by combined stresses to mitigate their effects on tuber yield. N concentrations in diagnostic leaves, haulms and tubers were affected in the same way by the treatments, being greater in droughted plants. When N supply was reduced, N concentration of tissues was also reduced, with the effect being greatest in irrigated plants and in Desiree. There was no effect on N concentration when P supply was reduced. The results for tissue P concentration contrasted with those for tissue N concentration. The P concentration of diagnostic leaves was increased by drought, and this was more apparent in Pentland Dell than in Desiree, while haulm P concentration was reduced by drought. The P concentrations in diagnostic leaves and haulms were reduced more by lack of P fertiliser under drought. Based on these observations, one might expect the transcriptome of droughted plants to be confounded by P related genes, but not by N related responses, the transcriptomic data will be presented.

CONCLUSIONS

Effects of combined stresses on tuber yield followed an approximation of Leibig's law, with drought having the biggest effect of all the stresses. Elemental composition of the two varieties with different root sizes differed, but they responded similarly to the combined stress treatments. Drought generally increased N, P and K concentrations in diagnostic leaves and haulms with the exception of haulm P concentration which was decreased by drought. In tubers there was little effect of water supply on nutrient concentrations, except when P was reduced by drought. Remobilisation of nutrients within the plant appears to buffer the nutrient content of both diagnostic leaves and tubers. These responses are expected to be reflected in the diagnostic transcriptome and may impact the utility of such diagnostic tools in conditions of combined stress.

Financial Support

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SINGLE CELL AND SINGLE LIPOSOME ANALYSIS FOR OPTIMIZATION OF FOLIAR NANO FERTILIZATION

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Keywords: Nano; Liposome; Fertilisation

INTRODUCTION

Poor mobility poses a challenge for some of the essential plant nutrients, both in the soil and inside the plant. Foliar fertilization has a range of advantages that, if succesful, may circumvent and improve not just the poor mobility, but also the timing of the added fertilizers. In order for this strategy to be successful there must be an efficient transfer of nutrients across the cuticle of the leaf and further on to the target sites in the plant. Due to the lipophillic nature of the plant cuticle, the water-soluble nutrients applied to leaf surfaces need help to attach to and cross this barrier. Detergents and surfactants have since long been added to foliar formulations to overcome the cuticle barrier and this has in many cases improved the uptake of nutrients into the leaf. Nevertheless, foliar fertilization still suffers from huge variability in terms of its efficiency, and the mechanisms for foliar uptake remain enigmatic.

Liposomes have been used as vehicles for drug delivery for many years, e.g. in the fight against the Corona Virus, acting as a stealth carrier of the active substance (mRNA). In this project, we aim to utilize liposomes, derived from soybean lechitins, as vehicles of plant nutrients. We hypothesize that the amphiphilic liposomes will be highly compatible with the cuticle and, like a trojan horse, will enable robust nutrient delivery deep inside the leaf tissue. In our project, we are producing 70-100 nm liposomes in different formulations, with various nutrient cargos. In the initial stages we have worked with Mn, since we have a number of powerful bioassays (Schmidt *et al,* 2016) that can document if the nutrient is actually able to restore functionality and reverse the deficiency.

In order to understand how liposomes deliver its cargo, we need to know exactly how much cargo each liposome carry, and how the liposomes interact with the target cells, i.e. with the cell wall and plasmamembrane. For these reasons, we have developed a single particle-inductivly couple plasma-mass spectometry (SP-ICP-MS) method that enables Mn (cargo) and P (membrane) detection and quantification in a single mesophyll cell (protoplast) or liposome. With this method at hand, we can both quantify the uptake and document the Mn concentrations needed to restore deficiency.

METHODS

The liposome were produced using a high-pressure-extrusion method at 65-70ºC (Trucillo, P, 2020). Briefly, the formulation consisted of an aqueous mixture of phospholipids (HSPC; L-α-phosphatidylcholine, Merck, DK) from soy beans and cholesterol (Merck, DK) from sheep's wool. Manganese (Mn) was added as a cargo mineral to the formulation. Homogenity of the self-assembled liposomes was ensured by emulsification and extrusion (EmulsiFlex-C5, Avestin, Åge Christensen, DK). Excess cargo was removed using tangential flow filtration (TFF) (Repligen, NL). Average size and the zeta potential was determined by DLS (Malvern).

Single liposome and single cell analysis were performed on an ICP-MS (7900 Agilent Technologies) running with a circle time of 100 µs (mass/s). Samples were diluted so that one particle at the time could be analysed.

Leaves from three weeks old barley plants where chopped and infiltrated with incubation buffer for 3 hrs at RT. Protoplast could hereafter be extracted and purified using a repeated spin procedure at 200g at 4ºC. The final concentration of protoplasts were found through counting with a *SIGMA* Bright-Line™ Hemacytometer placed in an optical microscope.

RESULTS and DISCUSSION

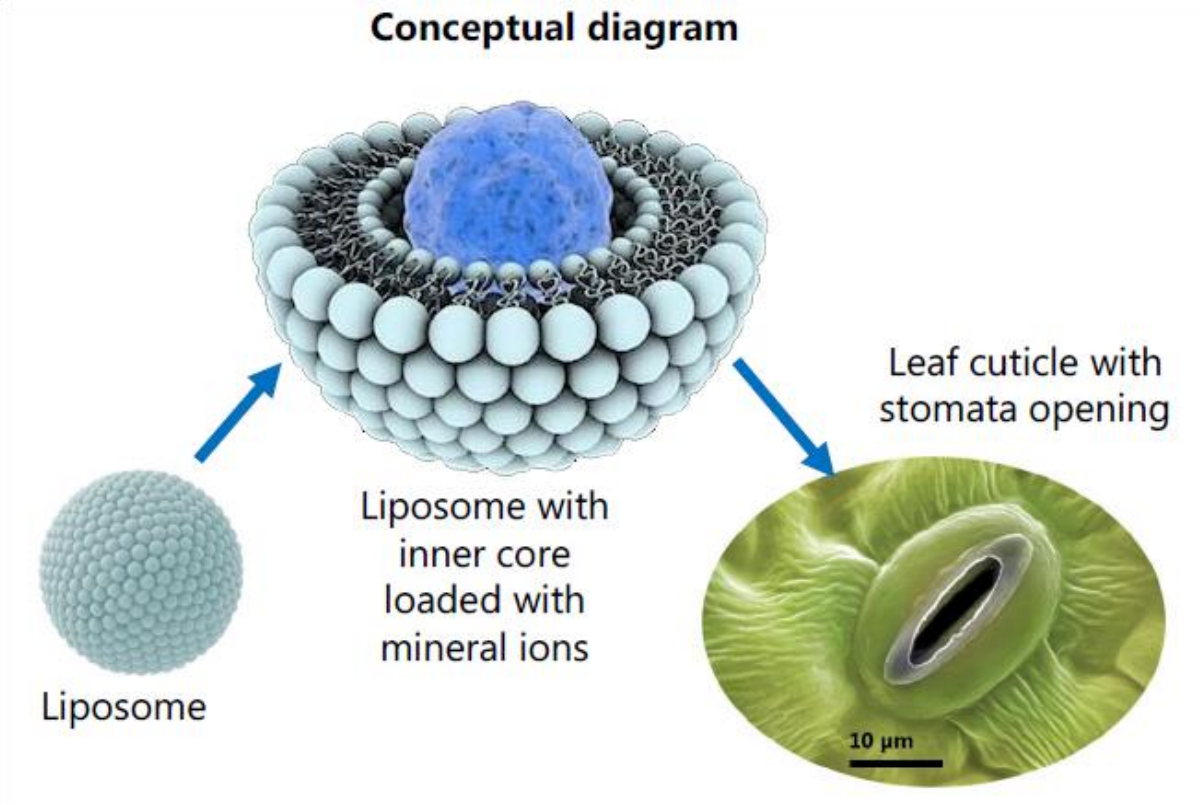

Fig. 1. Aconceptual diagram of the proposed gateway for liposomes transport into the leaf through an open stomata.

In order to document the exact amount of Mn needed to restore functionality at the cellular level, protoplasts were isolated from Mn deficient and sufficient barley plants and then treated with Mn-salts. Using the SP-ICP-MS method, the uptake of Mn could be followed and it was found that the deficient protoplasts took up more Mn than the control samples.

Initially the cell concentration of each batch of protoplasts was diluted to an equal concentration based on cell count. However, further standardization could be accomplished based on the phosphorous (P) signal of single protoplasts. This strengthened the comparative analysis of data between batches and days. Combining restoration data with the uptake of Mn, the method now enables calculations of how much Mn a protoplast requires in order to restore its functionallity.

Liposomes with a theoretical cargo of 30 mM of Mn-salts were washed for removal of external Mn-salts, then quantified using the SP-ICP-MS method, showing that only negligible amounts of Mn leaked out of the liposomes.

Currently, we are loading liposomes with a Mn cargo to deficient protoplasts with the aim to unravel uptake and restoration of the Mn deficiency at the mechanistic level

Though we so far have concentrated our effort on Mn, liposomes can be filled with a range of different essential plant nutrients, making liposome-based foliar fertilisation both more flexible and efficient than current strategies.

CONCLUSIONS

A single-cell ICP-MS method has been developed, enabling detection and quantification of the Mn-content of both single protoplasts (25 um) and single liposomes (70-100 nm). This system enables detailed studies of plasmamembrane-liposome interactions and the delivery of Mn into Mn-deficient plant cells. The SP-ICP-MS method is crucial in our further studies on liposomes as efficent vehicles for smart plant nutrient delivery via the foliage.

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Digital plant nutrition

BORON AND MAGNESIUM CONTENT PREDICTION IN GRAPEVINE THROUGH HYPERSPECTRAL PROXIMAL SENSING

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Keywords: Neural network; Micronutrients; Spectroradiometry

INTRODUCTION

Optimizing yield quality while reducing fertilizer input is a key goal for precision agriculture. With the development and adaptation of higher resolution methods to assess plant nutrient status, there will be more opportunities for area-specific fertilization within a single field. One such method is spectroradiometry, which measures light energy at different wavelenghts within the electromagnetic spectrum. For plant leaves, the energy of reflected light is a function of their biochemical composition, internal structure and surface properties, which are in turn determined by soil characteristics, temperature, humidity, time of measurement, presence of diseases, nutrient status or any other factor affecting plant metabolism (Gitelson et al., 2003; Merzlyak et al., 2003). Because of their high resolution, spectroradiometers can be used to predict many different parameters like chlorophyll concentration, leaf water content and others (Thorp et al., 2018). In the present work, we used the leaf's reflectance spectrum as proxy for the prediction of Magnesium and Boron content.

METHODS

Field Measurements

In 4 different vineyards in Belgium, 382 leaf samples were collected during the summer of 2020. To measure the reflectance spectrum and assess the micronutient content, at least 32 plants were randomly chosen throughout each vineyard. Five individual leaves per plant were spectroradiometrically measured. These leaves were then detached, pooled and stored in ziploc bags to gather enough matter for nutrient analyses. Leaves were subsequently washed with 0.1% HCl and deionized water to remove polluants or residues of foliar fertilizers. Then, leaves were dried for several days at 60°C. Afterwards, mineral nutrient contents, including Magnesium and Boron, were measured through ICP-OES.

Data Analysis

As the nutrient analyses were done on pooled leaves, each five different hyperspectral measurements were linked to one representative mineral nutrient content of a plant. Each set of 5 spectral measurements was assigned randomly to train (56%), validate (14%), or test (30%) a neural network model. A custom 1D LeNet-style neural network was trained to predict mineral nutrient content from the first derivative of the reflection spectrum (LeCun et al., 1995). During training, each spectral measurement was treated as a single sample. In testing, the prediction of each of the 5 measurements was averaged to reduce the risk of outliers significantly affecting the result. The model predicts several nutrients simultaneously.

RESULTS AND DISCUSSION

A custom LeNet-style neural network predicts the boron and magnesium contents in a leaf with RMSE values of 5.06 mg/kg and 437.00 mg/kg and R^2 values of 0.62 and 0.82 respectively (Figure 1). This is an intermediary result and will likely be improved as more samples are available, new strategies are tested, models are trained further and understanding of the dataset grows. Nevertheless, this result already shows that the features measured by a spectroradiometer suffice to predict the B and Mg content in grapevine leaves with a reasonable accuracy. This suggests that the necessary information to accurately estimate nutrient levels in plant leaves is present in the dataset.

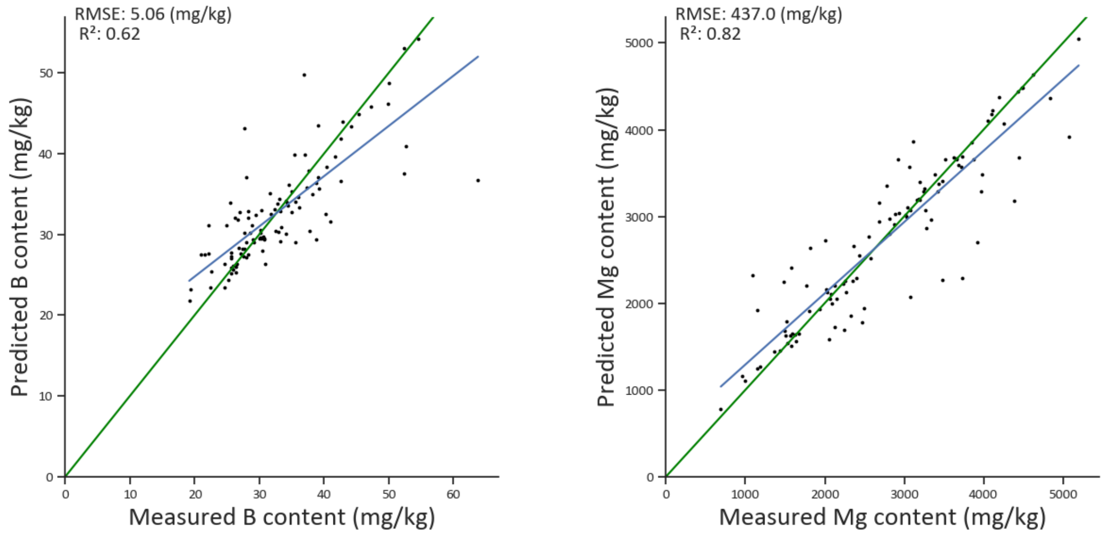

Figure 1: Prediction results for B and Mg. Each point is the average of 5 different spectral measurement of different leaves from the same plant. Green line represents a 1:1 ratio,. Blue line represents a linear fit between measured and predicted values.

CONCLUSIONS

Spectroradiometry is a promising method to provide accurate diagnosing of nutritional problems at the plant level. The high spectral resolution makes it possible to identify and quantify a wide variety of different stressors. The next challenge is to further improve the model and to figure out what wavelenghts are important for estimating the nutrient content levels.

Financial Support

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USE OF A DIGITAL TOOL FOR NUTRIENT RECOMMENDATION IN AGRICULTURE

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Keywords: Digital tools; Soil analysis; Potassium

INTRODUCTION

The evaluation of soil chemical analysis for nutrient recommendations in agriculture results in great agronomic benefits (Costa, 2017). However, soil analysis interpretation is time consuming and could be a tough task, especially when different crops and regions are considered. In addition to soil analyses, nutrient recommendation must also consider several other factors in order to calculate the nutrient demand of crops. The advancement of digital tools in agriculture can facilitate this complex task in order to achieve precise nutrient recommendation (Matias et al., 2021), which in turn may result in nutrient savings. The objective of this study was to test the hypothesis that the use of a digital tool that takes into account soil chemical analysis to generate nutrient recommendations may result in nutrient savings, specifically potassium (K), for different crops.

METHODS

Data captured from actual nutrient recommendations for crops grown in Brazil were evaluated. The recommendations evaluated were generated through a decision-making tool based on digital intelligence designed to support agronomists. Specific algorithms take into account different factors to provide a nutrient recommendation for crops, such as expected yield, soil analysis, region, and nutrient uptake and removal, following local literature. A sample of 350 recommendations based on soil analysis from 0-0.20 m depth was taken from the tool's database, from April 2021 to April 2022. Out of the 350 recommendations, 23, 9, 8 and 60 % were for coffee, citrus, sugarcane and soybean fields, respectively, from different States of Brazil (AL, GO, MA, MT, MS, MG, PI, RS, SP and TO).

Soil analysis interpretation of all recommendations were taken to identify the percentage of soil samples below and above the critical level of some plant nutrients (P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn). The critical level was considered as the nutrient level in the soil to achieve 80-90 % of relative yield. In the case of K, the distribution of soil nutrient levels was analyzed per crop, considering levels from very low to very high, where the critical level corresponds to the upper limit of the medium class.

For the recommendations that take soil analysis into account, the average recommended K rate per crop and soil K level was calculated. The weighted average K rate per crop was then calculated according to the percentage of soil samples from very low to very high K level. The average recommended K rate per crop from recommendations without soil analysis was calculated, considering the same expected yield observed in the recommendations with soil analysis. Finally, differences between recommended K rates with and without soil analysis were calculated.

RESULTS AND DISCUSSION

Most of the soil samples presented nutrient contents above the critical level, except for boron (B) (Fig. 1a). This reveals a potential to optimize the recommendations and to save nutrients. In a closer look at the K availability, it is possible to observe that citrus, coffee and soybean have a higher percentage of soils with K above the critical level in relation to sugarcane (Fig. 1b).

The average K rates are higher in cases where soil K level is below the critical level than in cases above the critical level (Table 1). As citrus, coffee and soybean, have a great part of the samples above the critical level, the weighted average K rate is lower than the average K rate observed in recommendations without soil analysis, resulting in a saving of K. However, the savings for soybean are quite small and for sugarcane are negative due to the higher rates for the corrective recommendation to increase soil K in fields below the critical level than the rates usually observed in recommendations without soil analysis. It is important to mention that the expected yield may not be achieved without raising K in poor K soils. Therefore, savings in K rates from fields with high and very high K levels should be allocated to fields with soil K below the critical level.

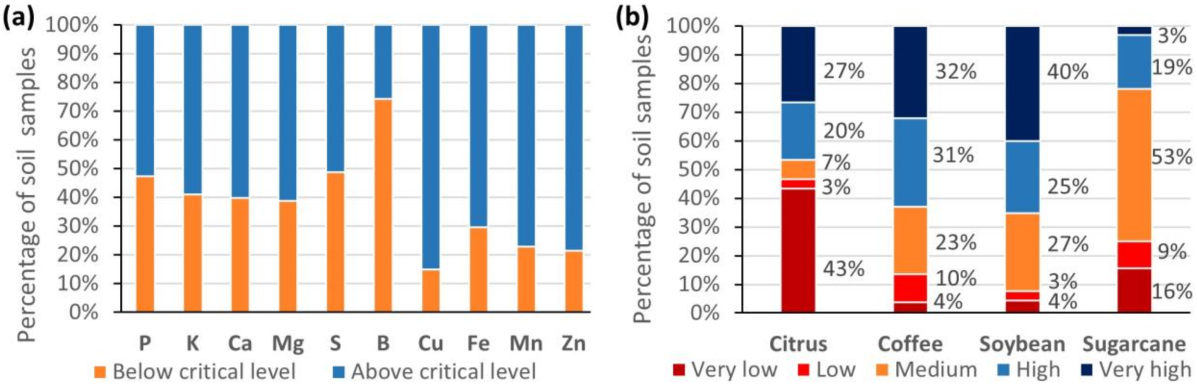

Fig. 1. Percentage of soil samples below and above the critical level according to different nutrients, regardless of crop (a). Percentage of soil samples with very low to very high soil K interpretation according to different crops (b).

Table 1. Average K rates for different crops with and without soil analysis. Weighted average K rate considering the percentage of soil samples with very low to very high soil K level. Savings in K rates from recommendations based on soil analysis interpretation compared to K rates from recommendations without soil analysis.

Soil K interpretation	Citrus	Coffee	Soybean	Sugarcane
	Average K rate according to soil K interpretation (kg ha^{-1})			
Very low	240	366	208	140
Low	240	347	190	140
Medium	193	206	109	120
High	153	197	110	100
Very high	109	182	82	100
	Weighted average K rate according to soil samples (kg ha^{-1})			
	185	215	105	121
	Average K rate without soil K interpretation (kg ha^{-1})			
	203	349	106	116
	Saving (kg ha^{-1})			
	18	133	1	-5

CONCLUSIONS

The use of a digital tool that takes into account soil analysis to support nutrient recommendation not only results in K savings for fields with high and very high K levels, but also highlights the need to correct soil K level in poor K soils, resulting in a better nutrient allocation.

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POTENTIAL USE OF THE LEAF-CLIP SENSOR DUALEX IN ESTIMATING NITROGEN STATUS IN CORN

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Keywords: SPAD; Fertilizer; Digital tool

INTRODUCTION
Nitrogen (N) is the most demanded nutrient and one of the main factors related to the productivity of corn (Zea mays, L.), so that its efficient management becomes more and more necessary, especially to meet specific adjustments and in real time in order to seek economic and environmental benefits (Thenkabail, 2019). In this context, digital solutions able of monitoring N status are powerful tools, such as embedded platforms with different sensors. Among the portable sensors, the SPAD-502 chlorophyll meter (Konica Minolta Inc., Japan) is very likely the most known for N management in corn, since it was calibrated for the crop (Markwell et al., 1995) and is been on the market for a long time.

Other diverse sensors are on the market and may also have interesting potential, such as the Dualex (Force-A, Orsay, France), a leaf-clip sensor that combines fluorescence and light transmittance to determine indices associated with the N status in plants, but still with few results for the corn crop - despite having presented promising results, particularly when associated with SPAD (Yu et al., 2010). Thus, the objective of this work was to evaluate the potential of Dualex in estimating the N content in corn leaves (V6 stage) cultivated in off-season, in the Cerrado region of Brazil.

METHODS
The study was carried out under field conditions, in five grain producing areas in southwest Goiás, Brazil, in the 2019 and 2020 seasons. In each of the areas, a N management trial was carried out, with five treatments and eight replicates. The treatments consisted of doses of N (0, 75, 150, 225 and 300 kg ha-1) applied in topdressing, at the V3 stage and in the form of urea protected with urease inhibitor. The same corn hybrid (DKB 390) was used, mechanically seeded with an expected population of 60,000 plants ha^{-1}.

The readings with the SPAD-502 and Dualex sensors were obtained when the plants were at the V6 stage. Measurements were performed between 8:30 am and 11:00 am, in three plants per plot, on both sides of the middle third of the leaf that defines the stage. After the readings with sensors, the leaves were collected and washed in distilled water to be analyzed for N content via micro Kjeldahl distillation and determination by titration (Silva et al., 2009).

Data were submitted to simple linear regression analysis to assess whether N doses were able to change N status in the corn plants, followed by Pearson's correlation analysis (r) between the different indices (SPAD, Chl , Anth, Flav and NBI Dualex) with the levels of N determined in laboratory. Statistical analyzes were performed using the statistical program RStudio R v.4.0.3 (R Core Team, 2013), and the values ??of Pearson's correlation determination coefficients (r) were classified according to Hinkle et al. (2003).

RESULTS AND DISCUSSION
The response of corn plants in V6 to N doses applied in V3 was significant and linearly positive in the five study areas, so that the increase in N dose also resulted in an increase in foliar N content, with coefficients of determination varying between of 0.45 and 0.83. This variation in N status in V6 leaves was a conditioning factor for the results obtained with the portable sensors.

In over all, the Flav and Anth indices - given by the Dualex and which estimate flavonoid and anthocyanin levels in plants - were negatively correlated with the leaf N content. This negative correlation is already reported in the literature (Gabriel et al., 2017), since flavonoids are carbon-based secondary metabolites whose content increases with lower N availability and which is generally inversely related to chlorophyll content (

Padilla et al., 2014). In addition to being negatively correlated, the Anth and Flav indices showed a low to moderate correlation (average of the five trials) with the N content (*r* Flav = 0.39 and *r* Anth = 0.52).

On the other hand, the SPAD, Chl and NBI indices showed a positive correlation with the N content. In terms of average results, the best correlations - even moderate - with the N content were found when using the SPAD and Chl indices. The NBI composite index showed a mean correlation (*r*) of 0.55. The SPAD and Chl indices, on the other hand, presented averages of 0.60 and 0.65, with correlations (*r*) in three of the five areas above 0.70, considered as high (Hinkle et al., 2003). The differences observed between the study areas affected the sensors in a similar way, so it may be related to the amplitude of the N status variation obtained, which does not depend solely on fertilization and can be mainly affected by soil conditions, and local climate. Also, the combined ratios with SPAD and Dualex readings could be another option to improve the efficiency, once has presented very sensitive results in estimating the N status in corn, particularly at earlier stages (Tremblay et al, 2007).

CONCLUSIONS

Among the indices obtained by the portable Dualex sensor, Chl is the one that best correlates with the N content in corn leaves, presenting a very similar result to that obtained by SPAD-502 chlorophyll meter.

ACKNOWLEDGEMENTS

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Mineral nutrition of wild plants

NITROGEN CONCENTRATION OF SOYBEAN INFLUENCED BY FOLIAR FERTILIZATION

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Keywords: Fertilization ; macro and micronutrients; Soybean

INTRODUCTION

Nitrogen (N) is the main nutrient for plant growth and good development (Maschner, 2012), and the soybean is one of the principal legumes and sources of oil and protein in the world (Kamali et al., 2017). The objective of this study was to evaluate if the N, cobalt (Co), molybdenum (Mo), and nickel (Ni) application improve N concentration in soybean foliar and grains.

METHODS

The study was carried out under field conditions on no-till management at Embrapa Soybean, Londrina, Paraná State, Brazil. The experimental arrangement was completely randomized blocks, with six treatments and eight replicates. The treatments consisted of; 1 - Control; 2 - Co + Mo (5 + 25 g ha^{-1}) applied at vegetative growth stage (2.5 + 12.5 g at V3 growth stage and 2.5 + 12.5 g at V5 growth stage); 3 - treatment 2 + Ni 60 g ha^{-1} applied at reproductive stage (30 g Ni at R1 and 30 g Ni at R5 stage); 4 - Treatment 2 + Ni 120 g ha^{-1} applied at reproductive stage (60 g Ni at R1 and 60 g Ni at R5 stage); 5 - Treatment 2 + N 10 kg ha^{-1} applied at reproductive stage (5 kg N at R1 and 5 kg N at R5 stage) and 6 - Treatment 2 + 4 + 5. Two growing season (2019-2020 and 2020-2021) were evaluated.

The Co+Mo source used the CoMoBIOLÒ fertilizer, Ni as $NiCl_2$ (45.2% Ni), and N as urea (44% N). The foliar application was carried out using a CO_2 sprayer with a continuous flow of 160 L ha^{-1}. All treatments including control were inoculated with *Bradyrhizobium elkanii + B. japonicum* to deliver 1.2 x 10^6 cells per seed, and in the sowing was applied 300 kg ha^{-1} N-P_2O_5-K_2O (0-20-20, NPK) in broadcast in two soybean cultivars (transgenic, BRS 399 RR and conventional, BRS 284). At reproductive stage, foliar and physiological maturation R8, and grains (R8.1) were collected for N analysis. N was quantified by spectrophotometry (Searle, 1984).

RESULTS AND DISCUSSION

The Co+Mo+Ni, Co+Mo+N, and Co+Mo+Ni+N foliar application significantly increased 25.1, 17.0, 15.1, 10.6 and 7.1% the foliar N concentration (2019-2020, BRS 399 RR, Figure 1A). Also, the Co+Mo, Co+Mo+Ni, Co+Mo+N, and Co+Mo+Ni+N foliar application increased 20.3, 15.8, 25.3, and 25.9% the N concentration in leaf (2020-2021, BRS 284). However, on 2019-2020 season, only the Co+Mo+N application increased 9.8% the N concentration (Figure 1B). The Co+Mo+Ni, Co+Mo+N, and Co+Mo+Ni+N application significantly increased the N concentration in grains (2020-2021, BRS 284, Figure 1D) and the Co+Mo+Ni+N application significantly reduced the N concentration in grain (BRS 399RR, Figure 1C).

The adequate Co, Mo, Ni, and N supplementation improved N and soybean health because of the functions of Co, Mo, and Ni in the N_2 fixation; those elements increase the biological N fixation by being part of the coenzyme cobalamin, leghemoglobin, nitrogenase and hydrogenase enzyme in the root nodules; also, Mo is required for nitrate reduction, for its functions in nitrate reductase activity (Marschner, 2012). While, Ni is essential for the transformation of urea by ammonium, as a component in urease (Freitas et al., 2019). Besides, N is the most required nutrient for soybean and other plant growth. For instance, the good N supplementation at the reproductive growth stage of soybean retarding leaf senescence, for enhanced soluble N, sugar and carbohydrates, consequently stimulated growing roots by continuous supplying photoassimilates to prevent senescence (Rodrigues et al., 2021). And it can increase in foliar and grains N concentration, as observed in this study.

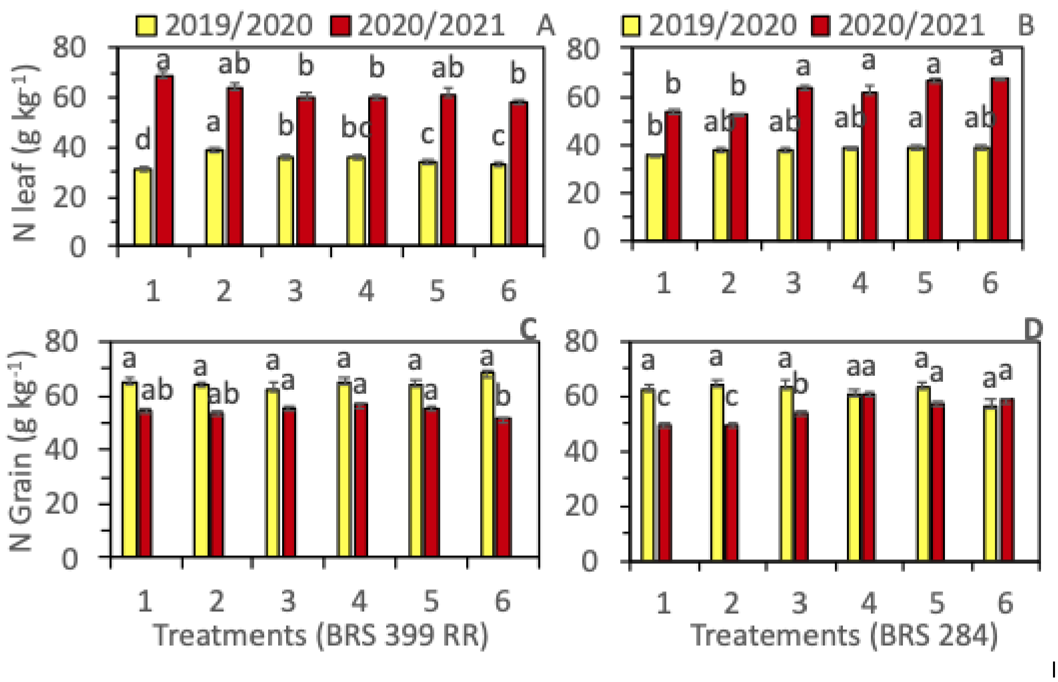

Fig. 1. Foliar and grains N concentration in soybean influenced by Co, Mo, Ni, and N foliar application. Means followed by the same letter, year and soybean genotype do not differ significantly by Tukey teste (5%). 1 = control; 2 = Co+Mo; 3 = Co+Mo+Ni (60 g); 4 = Co+Mo+Ni (120g); 5 = Co+Mo+N, and 6 = Co+Mo+Ni (120g)+N. Vertical bars represent the standard deviation.

CONCLUSIONS

The Co, Mo, Ni and N foliar application have positive effect on N concentration, acquisition, and metabolism of soybean inoculated with *Bradyrhizobium*. In the edaphoclimatic conditions studied, the benefits of this practice on the nutritional status of N improve grain yield.

ACKNOWLEDGEMENTS

To CAPES and CNPq for the finantial support.

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THE EFFECTS OF PREVIOUS FALL-WINTER CROPS ON SOYBEAN NUTRITION AND SEED YIELD

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Keywords: *Glycine max* L; Sucession of crops; Nutrition

INTRODUCTION

The cropping systems used in Brazil have soybean as one of the main crops grown in soils managed under the no-till system, with sowing being carried out in the spring/summer seasons in succession to maize, sorghum, millet and sunflower that are cultivated during the fall/winter seasons. In addition to these, other crops such as safflower and crambe have characteristics that allow their cultivation in the fall/winter seasons and the cultivation of soybeans in succession in the spring/summer seasons. Although studies have evaluated soybean seed yield in different cropping systems, others studies need to be carried out, especially with the cultivation of new crops such as safflower and crambe preceding soybean. Therefore, the objective of this work was to evaluate the nutrition and yield of soybeans cultivated during three agricultural years in different cropping systems.

METHODS

The experiment was carried out during the agricultural years of 2014/2015, 2015/2016 and 2016/2017, under field conditions at the Lageado Experimental Farm of the College of Agricultural Sciences, São Paulo State University, located in the municipality of Botucatu, state of São Paulo, Brazil. The experimental design used was complete randomized blocks, with four replications. The treatments consisted of sowing soybean in succession to safflower, crambe, sunflower, maize, sorghum, fallow and fallow + millet, totaling seven treatments. Each experimental unit consisted of ten rows of 11 m long soybeans, with a spacing of 0.45 m between the rows.

At the time of full flowering of soybean (R2) in the three agricultural years, samplings were carried out for foliar diagnosis, collecting the third leaf with petiole from the apex of the main stem of 15 plants per plot. After being washed, these samples were subjected to drying in an oven with forced air circulation at 65 ºC for 72 hours, ground in a Willey mill and subjected to analysis of the total contents of N, P, K, Ca, Mg and S (Malavolta et al., 1997). At the R8 stage of soybean (full ripening) were collected, in the three agricultural seasons, the soybean plants present in 4 m of rows were sampled in each experimental unit to determine seed yield, with humidity corrected to 130 g kg^{-1}. The data obtained were submitted to analysis of variance and the means of the treatments were compared by the Tukey test at 5% of probability, using the statistical program SISVAR 5.4.

RESULTS and discussion

The analysis of variance for the foliar diagnosis results revealed a significant interaction of the variables tested (growing season x previous crop) only for the leaf K concentration. Leaf P, Ca, Mg and S concentrations were influenced separately by the growing season, and the previous crop affected only the S concentration. Leaf P and S concentrations were higher in the 2014/2015 and 2016/2017 growing seasons, while the highest concentrations of Ca and Mg occurred in the 2015/2016 growing season (Table 1). Regarding the isolated effect of the previous crop, it was found that the cultivation of safflower resulted in lower leaf S concentration compared to the cultivation of maize and sorghum; however, there was no significant difference for the other species. Higher leaf N concentration in soybean were obtained when crambe was cultivated in previous fall-winter. In summary, all the nutrients analyzed presented concentrations within the range considered adequate for the soybean crop.

Table 1. Nutrient concentrations (N, P, K, Ca, Mg, and S) in leaves of spring-summer soybean crop following different fall-winter crops in three growing season.

Treatment	N	P	K	Ca	Mg	S
			$g\ kg^{-1}$			
Growing season						
2014-2015	48.6a	3.0a	23.8	12.8b	3.5ab	2.5ab
2015-2016	48.7a	2.8b	26.1	14.5a	3.7a	2.3b
2016-2017	49.2a	3.1a	21.4	11.3c	3.4b	2.6a
Previous crop						
Fallow	48.6ab	2.8a	22.8	12.6a	3.4a	2.4abc
Crambe	50.5a	2.9a	23.4	12.9a	3.7a	2.4abc
Maize	46.6b	3.1a	23.9	12.6a	3.7a	2.8a
Safflower	49.6ab	3.0a	24.5	12.3a	3.4a	2.1c
Sorghum	48.2ab	3.0a	24.6	13.5a	3.7a	2.6ab
Sunflower	49.2ab	2.9a	23.3	13.2a	3.4a	2.4abc

Mean values followed by the same letter in the column are not significantly different (P<0.05) by Tukey's test.

For K concentration in soybean leaves, there was a difference among previous crops only in the 2016/2017 growing season, with higher K concentrations with sorghum cultivation (Figure 1A). Comparing the growing seasons, it was found higher K concentrations in the 2015/2016 than in the other growing seasons, when fallow, crambe, maize, safflower, and sunflower were cultivated as previous crops. In 2015/2016, the soybean seed yield showed higher values when cultivation was carried out following maize and safflower (Figure 1B). In 2016/2017, the higest seed yields were found with sunflower, sorghum, and safflower as previous crops. On average, sunflower, safflower, and maize promoted the highest seed yield of following soybean crop.

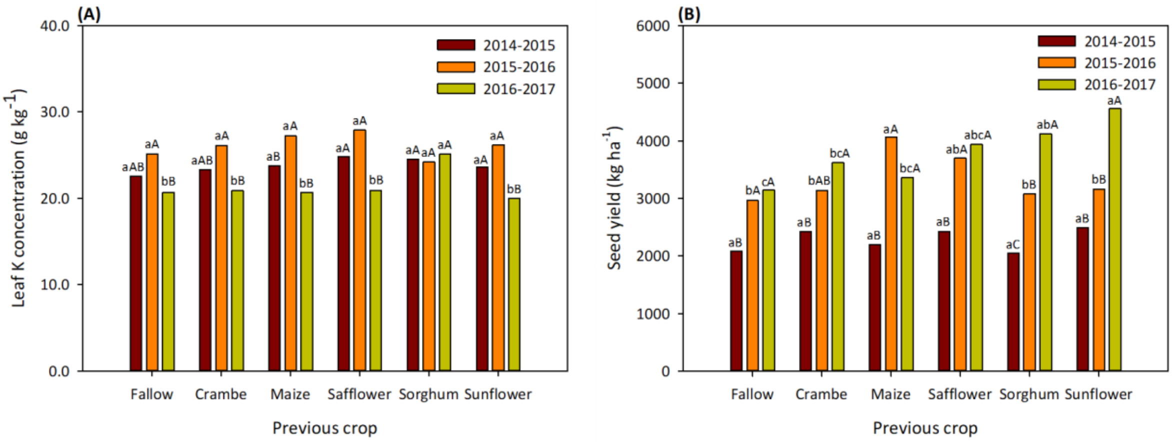

Figure 1. Growing season × previous crop interaction for leaf K concentration (A) and seed yield (B) of spring-summer soybean crop. Lowercase letters compare previous crops and uppercase letters compare growing seasons, whose values followed by the same letter are not significantly different at *P* < 0.05 according to Tukey's test.

CONCLUSIONS

Soybean grown in succession to safflower and maize showed the highest concentration of N and S nutrients, respectively. Soybean yiedl was higher in the cropping system with sunflower, safflower, and maize as previous crops.

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DIAGNOSIS OF SOIL FERTILITY BY THE DRIS METHOD IN SUGARCANE IN THE STATE OF GOIÁS, BRAZIL

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Keywords: nutritional balance; *Saccharum officinarum*; nutritional diagnosis

INTRODUCTION

Sugarcane is a perennial crop and has multi-annual cultivation with cutting of the stem and maintenance of the ratoons, making its fertilization management and cultural treatments similar to what occurs in annual crops (Silva et al., 2020). In the Brazilian Midwest region, the main way of monitoring soil fertility and recommending fertilization for the cultivation of sugarcane or ratoons is through recommendation bulletins, mainly the one proposed by Sousa and Lobato (2004).

An alternative for interpreting the results of soil analysis is through the DRIS indexes, which uses a bivariate methodology, which, through a set of norms, evaluates the double interaction (direct and indirect) between two nutrients, such as the DRIS method (Diagnosis Recommendation Integrated System) (Beaufils, 1971). This study aimed to evaluate and compare the efficiency of the DRIS methods and the traditional methods of interpreting the nutrient content in the soil of sugarcane cultivated in commercial plantation areas in the state of Goiás.

METHODS

Study location

The research was carried out in the 2018/2019 harvest in commercial sugarcane plantations, located at the Mills: Jalles Machado (UTM: Zona 22, N 8,316,968.68, E 716,170.15), headquartered in the municipality of Goianésia and in Vale do Verdão (UTM: Zona 22, N 8,013,152.13, E 572,297.64), located in the municipality of Maurilândia, both in the state of Goiás, Brazil.

Database creation

400 soil samples composed of 5 simple samples were collected and the contents of P, K, Ca, Mg, Cu, Fe, Mn, Zn, organic matter (OM) were analyzed and the cation exchange capacity (CEC) was calculated. At the same points, 400 yield samples were collected and the stems were weighed after separating the leaves and tops. The samples were divided into two subpopulations: low productivity (<80 t ha^{-1}) and high productivity (≥80 t ha^{-1}), this being the reference population.

DRIS calculation

Based on the reference population, DRIS indices and norms were calculated following the methodology proposed by Beaufils (1971). Descriptive statistics of the samples (mean, variance and coefficient of variation) were also performed. Based on the DRIS indexes, the Nutritional Balance Index of the areas was calculated.

RESULTS AND DISCUSSION

When classifying nutrients in the soil based on traditional methods of interpretation, according to the order of limitation by excess, in the high productivity subpopulation we have: K>Mn>Fe>Cu>Mg, and for the low productivity subpopulation, we have: Mn>K>Fe>Cu>Mg. Regarding disability limitation, the classification was the same in both subpopulations, as follows: MO>Zn (Table 1).

Table 1. Classification of soil P, K, Ca, Mg, Cu, Fe, Mn, Zn, OM and CEC in sugarcane cultivated in the municipalities of Barro Alto, Castelândia, Porteirão and Turvelândia, both in state of Goiás, Brazil, in the harvest 2018/19.

Soil content	Suitable concentration range and critical level [1]	Reference subpopulation [2]	Low yield subpopulation [3]
P (mg dm^{3})	8,1 – 18,0	10,65	9,75
K (mg dm^{3})	51,0 – 80,0	125,78	109,66
Ca (cmol$_c$ dm^{3})	1,5 a 7,0	2,70	2,88
Mg (cmol$_c$ dm^{3})	0,50 – 2,00	1,10	1,11
Cu (mg dm^{3})	0,50 - 0,80	1,32	1,44
Fe (mg dm^{3})	5,00 - 12,00*	24,68	20,44
Mn (mg dm^{3})	2,00 - 5,00	42,95	43,68
Zn (mg dm^{3})	1,10 - 1,60	1,04	0,96
OM (%)	2,50 – 3,00**	1,84	1,81
CEC (cmol$_c$ dm^{3})	4,8 – 9,0	6,41	6,60

[1] Suitable concentration range described by Souza e Lobato (2004), * Raij et al. (2011), ** Ronquim (2010); [2] Subpopulation with yield equal to or greater than 80 t ha^{-1}; [3] Subpopulation with yield below 80 t ha^{-1}. Caption: Excess (color blue), Adequate (color green) and, Deficiency (color red).

The DRIS indices calculated based on the reference population demonstrate that the nutrients magnesium and iron are in balance in the soil (DRIS = 0). The scale of limitation by excess was classified in descending scale by CEC>Ca=Cu=OM, and in deficiency by Mn>P=K=Zn (Fig. 1).

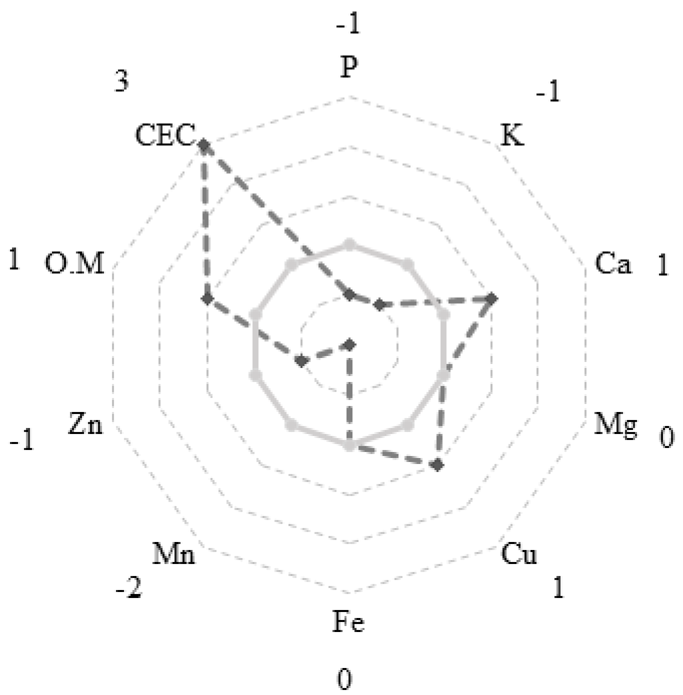

Fig. 1. Average DRIS indices calculated as a function of soil nutrient content in sugarcane cultivated in the municipalities of Barro Alto, Castelândia, Porteirão and Turvelândia, both in stato of Goiás, Brazil, in the 2018/2019 harvest.

CONCLUSIONS

The interpretation of the soil analysis by the DRIS method presents a greater amount of nutrients with indices close to balance in relation to the interpretation by the traditional methods. Thus, being less restrictive, allowing a wider range of nutrient content in the soil that results in a higher final yield of industrialized stalks.

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THE EFFECTS OF BIODIVERSITY AND WATER AVAILABILITY UPON ACID PHOSPHATASE ACTIVITY AND MYCORRHIZAL COLONIZATION IN CERRADO HERBACEOUS SPECIES

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Keywords: Cerrado; biodiversity; phosphatase

INTRODUCTION

Soils of the Brazilian Cerrado are naturally acidic and nutrient-poor (Goedert et al., 1983). The activity of root acid phosphatase together with the association of mycorrhizal fungi are mechanisms used by the plants of this biome to extract nutrients from these soils. In biodiverse environments, these mechanisms are more efficient than in monocultures (Hacker et al., 2015) and may even be a contributing factor for the success of plant invasions (Lannes et al., 2020). In addition, local biodiversity can affect the amount of moisture in the soil, which can increase or decrease activities underground (Walker et al., 2003). This study investigated water availability in pots with different numbers of species as a determining factor for acid phosphatase activity and mycorrhizal colonization of herbaceous plants living in the Cerrado.

METHODS

The experiment was conducted in a greenhouse, with herbaceous species found in the Cerrado: the naturalized grass *Melinis repens*, the common invasive exotic grass *Urochloa decumbens*, and the native legume *Calopogonium mucunoides*. Pots of 1.5 liters containing Cerrado soil (red dystrophic Latosol) were set up with three individuals in each, with monocultures, and combinations of two and three species in five replicates each. Pots were weighed and watered daily with the same amount of water. After 90 days, total biomass, root acid phosphatase activity (Olde Venterink, 2011) and mycorrhizal colonization (Vierheilig et al., 1998) for each plant were recorded.

RESULTS AND DISCUSSION

Water mass decreased as biodiversity increased in the pots, demonstrating the effect of niche complementarity to water use (Figure 1), as observed by Ceulemans et al. (2017) for nutrient absorption. For all species analysed together, there was a negative effect of water availability on root acid phosphatase activity, suggesting that the occurrence of droughts can interfere with the phosphorus cycle (Walker et al., 2003). Classically, we have seen in the literature that mycorrhizal association can favor plant diversity (Tedersoo, Bahram and Zobel, 2020), but here we observed that the increase in diversity also promotes an increase in mycorrhizal colonization in the three species studied (Figure 1). The highly invasive grass *Urochloa decumbens* profited from a positive effect of biodiversity on mycorrhizal colonization and of the effect of acid phosphatase activity on biomass production, in accordance to Lannes et al. (2020). The exotic grass *Melinis repens* alternated the increase on phosphatase and mycorrhizal colonization as biodiversity increased, which suggests phenotypic plasticity for this species in relation to nutrient acquisition and raises a red flag in relation to its invasive potential in the highly biodiverse Cerrado biome.

CONCLUSIONS

Biodiversity affects water availability and these influence processes related to nutrient acquisition in various ways for native and exotic species in the Cerrado.

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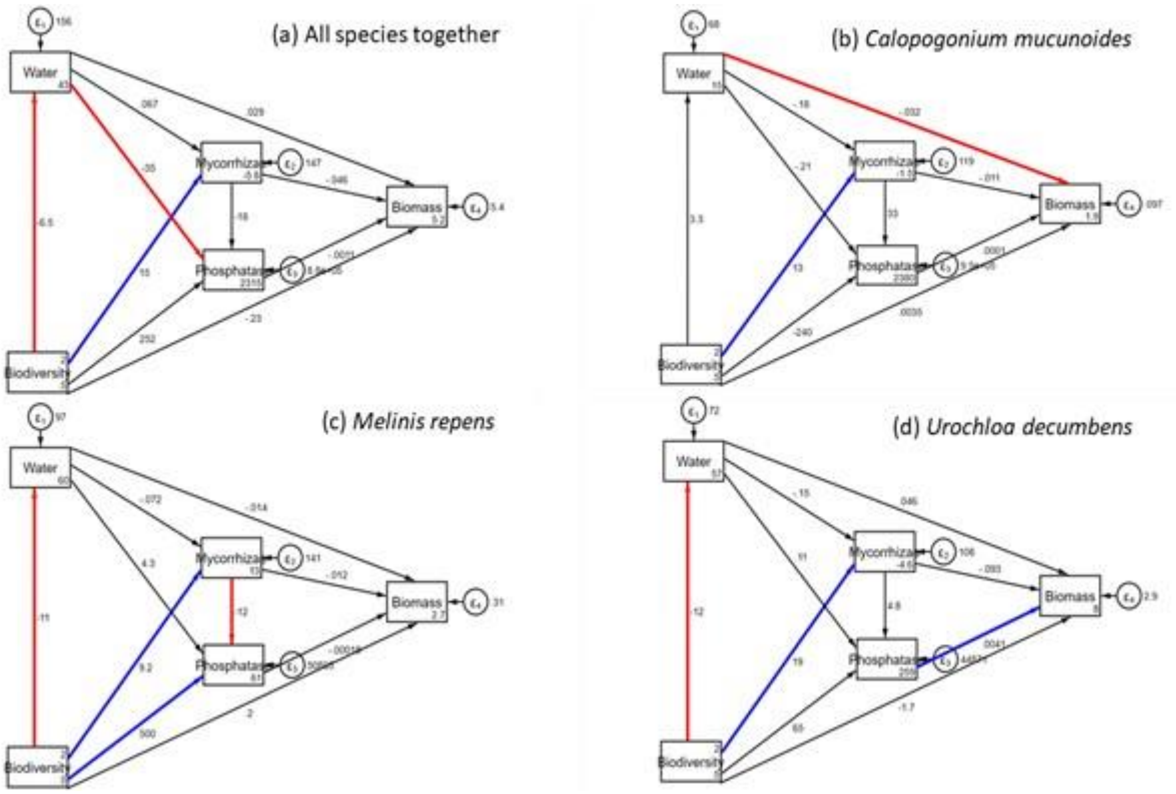

Fig 1. Structural equation modeling (SEM) showing the effects of water content and biodiversity on root acid phosphatase activity, mycorrhizal colonization and biomass of Calopogonium mucunoides, Melinis repens, Urochloa decumbens. Numbers next to the arrows are the standardized values (scaled by the standard deviations). Blue and red arrows respectively indicate positive and negative significant relationships. Goodness of fit of all models: p $\chi 2 > 0.05$.

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New fertilizers and fertilizer recommendation

THE USE OF BIO-NANOTECHNOLOGY TO MANIPULATE FOLIAR UPTAKE AND TRANSLOCATION OF MANGANESE IN CROPS.

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Keywords: Manganese; Nano-fertilization; Translocation

INTRODUCTION

Conventional Mn fertilization strategies are remarkably inefficient due to the inherent chemical properties of Mn. Especially in well-drained sandy soils rich in organic matter, soluble Mn^{2+} is rapidly converted into plant unavailable Mn oxides. To prevent or alleviate Mn deficiency, Mn is often supplied *via* foliar spray application as it is readily assimilated by crop leaves. However, poor mobility of Mn in the phloem prevents any significant remobilization from older to developing organs. Hence, multiple foliar applications are required during a growing season to supply adequate Mn to crops, representing a costly and time-consuming approach for farmers.

Nanotechnology offers valuable tools for developing new Mn delivery strategies. Several studies have reported translocation of foliarly applied nanoparticles (NPs) thorugh the phloem, highlighting a clear potential for translocation of nutrients that are phloem immobile. NPs can accommodate Mn ions and promote their transport within plants, bypassing the limitations of Mn mobility and enhancing its use efficiency. To further advance science in this direction, a better fundamental understanding of the fundamental interactions between Mn-based NPs and plants need to be established. In this work we highlight differences in Mn functionality recovery when Mn(II) oxide (MnO) NPs and convential $MnSO_4$ salts are supplied to deficient barley (*Hordeum vulgare*) plants and protoplasts.

METHODS

Synthesis and characterization of polyacrylic acid-coated MnO NPs

Polyacrylic acid-coated MnO (PAA-MnO) NPs were synthesized following a one-pot polyol method[1] and their physicochemical properties (*i.e.* size, shape, charge, surface functionality, Mn content and Mn oxidation state) were thoroughly characterized using different techniques (dynamic light scattering (DLS), cryo-TEM, ICP-MS, FTIR, EPR and XPS). PAA-MnO NPs were also labelled with DiI dye (DiI-PAA-MnO) to allow confocal microscopy bioimaging with cellular resolution.

Mn-deficient plant growth and protoplast isolation

Mn-deficient barley plants were grown in a greenhouse under low Mn concentration for 21 days. The quantum yield efficiency of PSII (F_v/F_m), determined through chlorophyll a fluorescence analysis, is representative of Mn status and was used to diagnose latent Mn deficiency in plants. 21 days-old Mn-deficient leaves were used for protoplast isolation adapting a protocol by Shan *et al.* (2014)[2].

Application of PAA-MnO NPs

Prior to application, the surface tension of MnO NPs and $MnSO_4$ formulations containing progressive concentrations of Silwet surfactant was syistematically investigated with an optical tensiometer. To assess the impact of surfactant concentration on NP uptake efficiency, distinct DiI-PAA-MnO NP solutions containing respectively high and low Silwet concentrations were indipendently applied onto plant leaves and imaged using CLSM. Mn nutritional status of barley leaves was monitored over time after exposure to $MnSO_4$ and PAA-MnO NPs solutions containing high Silwet concentration using PAM fluorometry. In parallel, protoplast ability to internalize and metabolize MnO NPs was verified using CLSM and chlorophyll *a* fluorescence analysis, respectively.

RESULTS AND DISCUSSION

After 21 days, moderately Mn-deficient plants ($F_v/F_m = 0.56 \pm 0.05$) were exposed to NPs. Following 6 hours of leaf exposure to 20 nm DiI-PAA-MnO NPs, CLSM analysis revealed greater penetration through the cuticle for the NP solution containing high Silwet concentration. Furthermore, imaging analysis showed that NP foliar uptake occurred through stomata, followed by short-distance radial spread of NPs from the stomatal cavity area.

PAM analysis performed after exposure to foliar $MnSO_4$ showed restoration of Mn-status to control levels. Despite being slower, leaf treatment with PAA-MnO NPs led to partial restoration of Mn functionality in the tissue surrounding the droplet deposition areas, highlighting NP ability to provide bioavailable Mn upon dissolution.

In Mn-deficient protoplasts, NP internalization was reported already 10 minutes after incubation with DiI-PAA-MnO. Furthermore, the presence of round vesicles surrounding the fluorescent NPs suggests the involvement of endocytosis in the uptake process.

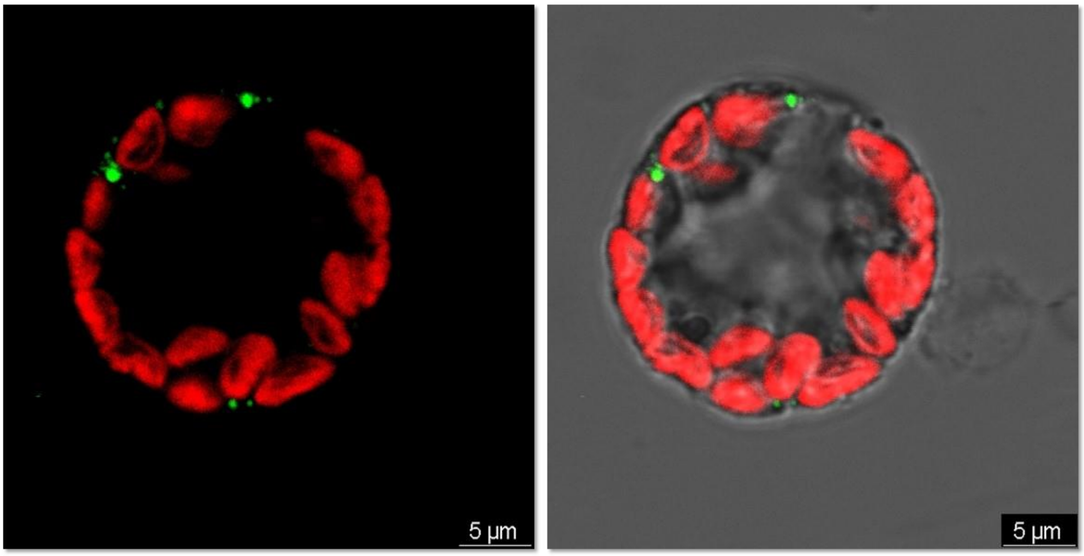

Fig. 1. Confocal imaging of DiI-PAA-MnO uptake by barley meshopyll protoplast. Fluorescent signal from DiI is visible in green, while the autofluorescing chloroplasts are shown in red.

Once established the ability of protoplasts to internalize MnO NPs, these were exposed to either $MnSO_4$ or MnO NPs. Positive results were obtained in both cases, showing that protoplasts can utilize MnO NPs as a source of Mn to restore Mn metabolic functionality.

CONCLUSIONS

Herein, we show that bioavailable MnO NPs can be utilized at different biological levels to restore Mn functionality in photosynthesis. Importantly, CLSM and PAM imaging analyses indicate that MnO NPs posses considerable mobility within the mesophyll tissue. However, whether MnO NPs can be loaded in the phloem and translocated to other plant organs has yet to be determined.

In summary, this work supports the potential of MnO NP to serve as future carrier for the efficient delivery of Mn in plants.

ACKNOWLEDGEMENTS

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USE OF POLYHALITE IN SUGARCANE

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Keywords: *Sacharum* spp.; Fertilization in sugarcane; Alternative sources potassium

INTRODUCTION

Despite the proven beneficial effect of potassium on sugarcane, we found in the literature, especially on ratoons, a lack of responses to K fertilization (Zambello Júnior et al. 1981), which may be associated with an imbalance of K, with Ca, Mg and S (Rosseto et al.,2008, Lifang et al. 2001; Tan et al. 2005). This can increase with the use of KCl in the fertilization, which does not provide Ca, Mg and S and provides Cl, which is harmful to the industrial quality of the stalks (Moraes e Almeida 1938). An alternative widely used by producers is the application to KCl and gypsum on the ratoons, but this practice does not provide Mg and requires one more agricultural operation to gypsum application, which increases carbon footprint and soil compaction, reducing longevity of the cane fields. Thus, the use of Polyhalite becomes an alternative in the management of potassium fertilization to supply Ca, Mg and S. Polyhalite is a new fertilizer that contains 19% S, 14% K2O, 17% CaO and 6% of MgO available to plants (Mello et al. 2018). Recent studies comparing the use of polyhalite with KCl and gypsum in sugarcane ratoons in Brazil concluded that there is no difference in the efficiency of polyhalite compared to other sources to provide K, Ca and S (Herrera 2019, Vale and Sério 2019). However, the authors emphasize that the total replacement of KCl by polyhalite may not be economically viable for sugarcane. Despite this, the authors emphasize that the total replacement of KCl by polyhalite may not be economically viable for sugarcane. Therefore, studying the use of KCl + Polyhalite blends is essential to determine if this technology will be viable for sugarcane producers in Brazil.

The aim of this study was to evaluate the effect of applying blends of potassium, KCl and polyalite sources, replacing fertilization with KCl and KCl + gypsum, on the nutrition, production and quality of sugarcane.

METHODS

The study was carried out on the first and second ratoons (2019/2020 and 2020/2021) in the State of São Paulo in Assis (Oxisol sandy). The variety cultivated was RB867515. The statistical design was in randomized blocks with seven treatments and six replications, as follows: T1= control (without application of K); T2=100%KCl + gypsum; T3=100% KCl; T4= 25%Polylite + 75%KCl; T5= 50%Polyalite + 50%KCl; T6=75%Polylite + 25%KCl; T7=100%Polyalite. Equal doses of NPK were applied in all treatments, as recommended by Bulletin 100, in order to reach a productivity higher than 100 t ha^{-1}. In T2, 1250 kg ha^{-1} of gypsum was applied, which represents the same amount of S provided by T7. Were evauated the effects of treatments on sugarcane nutrition (teor foliar), stalk yield and industrial quality (total recoverable sugar and ton of sugar per hectare). The results were statistically analyzed using analysis of variance (ANOVA) ande the means were compared by the t-student test ($P<0.10$).

RESULTS AND DISCUSSION

The substitution of KCl for polyalite increased the availability of S in the soil proportionally to the amount of polyalite applied after two years. The application of the highest percentages of polyalite provided sulfur in an equal way to the application of gypsum (Table 1). In addition, the use of polyalite decreased the leaching of Mg to deeper layers, mainly in relation to the KCl+gypsum treatment. In this way, the substitution of the use of KCl+gypsum for polyalite may be interesting in sugarcane ratoons. Regarding Ca and K, there were no statistical differences between treatments, which indicates that polyalite was as efficient as KCl and gypsum to supply these macronutrients to the sugarcane. Huang et al (2020) studied nutrient leaching in two soils with a contrasting texture, clayey and sandy, and concluded that polyalite presents lower leaching of K, Ca, Mg and S, due to its slower mineralization. The substitution of KCl for polyalite reduced the chlorine content in the sugarcane leaves in the two studied ratoons (Figure 1). Some authors report that excess Cl negatively influences the accumulation of sugar in sugarcane stalks (Ramos, 2022). Despite reducing the absorption of chlorine in the leaves, there was no effect of treatments on the TRS accumulated in two ratoons. However, the substitution of KCl for polyalite increased SY and TSH (Figure 1). There are no differences between the treatments with the highest proportions of polyhalite and the KCl+Gypsum treatment. The treatment that provided the greatest gain in stalk production in relation to the control and the 100% KCl treatment was the 50%Poly 50%KCl, which increased SY by 13% and 7.5%, respectively. TSH increased by 11.6% in relation to the control with the application of the treatments 100% KCl+gypsum and 50%Poly 50%KCl. In relation to the 100% KCl treatment, this gain was 6.6%. Pavinatto et all (2020) also compared the replacement of KCl and gypsum with polyalite in the fertilization of sugarcane and observed differences in productivity between polyhalite and the other sources.

Tabela 1. Chemical parameters in soil samples collected at depths 0-0.2m and 0.2-0.4m.

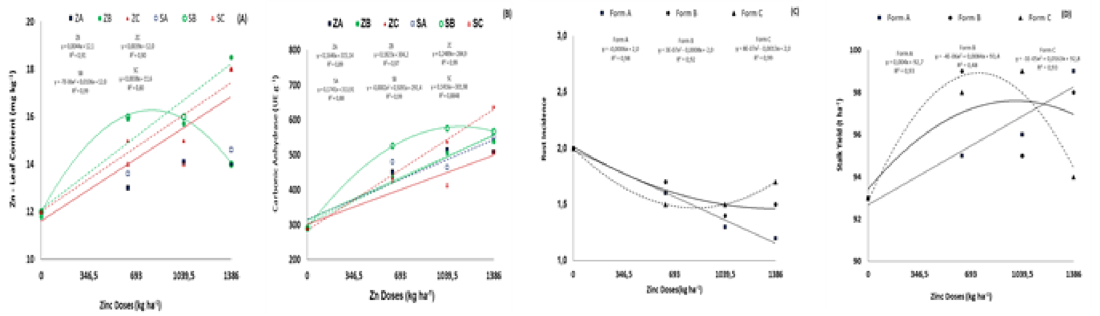
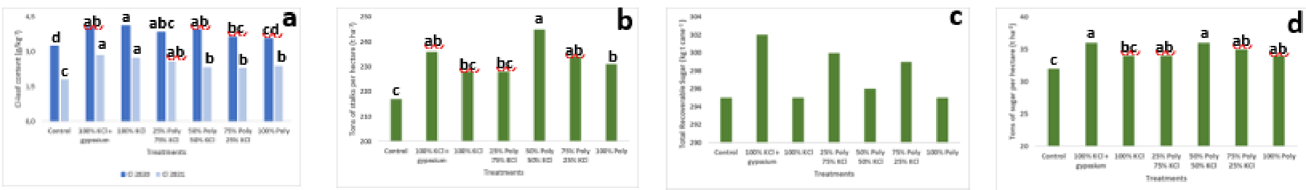


Figura 1. Cl-leaves content (A); Tons of stalks per hectare (B);Total Recoverable Sugar (C); Tons of sugar per hectare(D)



CONCLUSIONS

Polyalite has the potential to partially replace the use of KCl in sugarcane and can even meet the Ca and S needs of ratoons in a similar way to gypsum. Mg leaching can be reduced with the use of polyalite instead of gypsum. Despite the potential, it is necessary to conduct experiments throughout the cycle to verify if the use of polyalite in substitution of KCl and gypsum is economically viable for sugarcane.

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ZN FOLIAR SPRAY INCREASES THE HEALTH AND YIELD OF SUGARCANE RATOON

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Keywords: micronutrients; fertilizer; bioenergy

INTRODUCTION

Sugancane Zn deficiency affects tillering and plant growth that are two fundamental factors to the productivity of sugarcane. In metabolism plants, Zn is essential for the synthesis of tryptophan, which is the precursor indoleacetic acid (AIA), responsible for the production of enzymes that will promote cell stretching and growth. This micronutrient is also involved in the activation of diverse enzymes that act on photosynthesis and sugar formation (Orlando Filho et al., 2001). Among these enzymes, carbonic anhydrase is directly involved in the photosynthesis of sugarcane with a reduction in carbonic anhydrase activity and photosynthesis rate when the plant is deficient in Zn (Alloway, 2008). In addition, sugarcane Zn deficiency can increase the incidence of plant diseases with red spots on the leaves due to the attack of fungi (Orlando Filho et al., 2001). The Zn is importance on crop production is well known, but studies with Zn on sugarcane are still scarce in tropical soils, especially to foliar spray. The aim of this study was to evaluate the best source, rate and form foliar application of Zn for sugarcane fisrt ratoon.

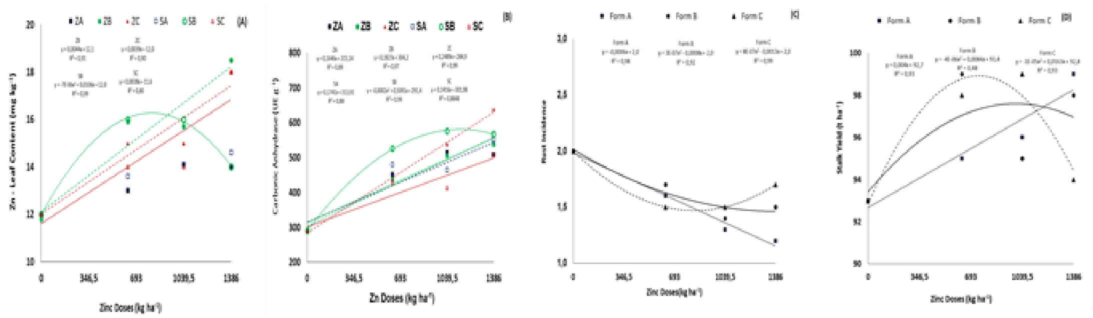
METHODS

A study was carried out during ratoon cane in three sites, Assis, Ourinhos, and Serrana, São Paulo State, Brazil. The experiment was installed between October (2019) and January (2020) in the first ratoon of sugarcane with the varieties RB 867515 (Site 1), CTC 9003 (Site 2), and RB 855156 (Site 3). In all Sites, the experiments were set up in a randomized block experimental design, arranged in a factorial 4 x 2 x 3, corresponding to (i) four Zn doses (0; 639; 1,039; or 1,386 g ha^{-1}), (ii) two Zn sources (Zn sulphate or Micronized Zn oxide (Zintrac)), and 3 forms to foliar spray (Form A- application on plant with height of 0.5 (100%) and Form B- 1.0 (100%), or Form C- 0.5 (50%) + 1.0 m (50%)). Each treatment had four replications, totaling 96 experimental plots, where each plot was composed of 6 rows with 15 m long and distance between rows. The effect of treatments on the stalk yields of sugarcane (SY) and industrial quality (total recoverable sugar - TRS), Leaf Zn content, Carbonic anhydrase activity (CA), severity levels of orange rust, and sugarcane-borer were monitored after the foliar Zn application were evaluated. For a better conclusion of the results, the data were statistically analyzed together, through analysis of variance (F test). To compare the means of the factors, the Tukey test and the doses analyzed by regression were used, both at 10% probability.

RESULTS and discussion

The foliar spray of Zn influenced the yield and health of sugarcane ratoon, regardless of the source used. Studies with foliar applications of Zn oxide are still scarce, especially for sugarcane, and there are still doubts about the effectiveness of the application of this source via foliar in crops. The foliar application of Zn in the ratoons of sugarcane increased the foliar contents of this micronutrient. The largest increments occurred in plants sprayed with Zintrac. The application of this source increased the Zn content linearly, both in the single application application on form B and C.The foliar content of Zn in these treatments increased 20% in relation to the control (Figure 1A) The application of Zn in the leaves increased the activity of the enzyme carbonic anhydrase. Although there is a significant effect between sources, forms and doses, it is observed that the difference in enzymatic activity in each applied dose differs very little between sources and forms. However, the increasing effect on anhydrase activity with foliar application of zinc as a function of doses is evident, with increases above 100% (Figure 1B). Regarding SY, the dose x form interaction, it can be seen in figure 1 C that all application forms showed gains in relation to the control treatment (without foliar application of Zn), however the response was different between them. The highest productivity gain was obtained with the application divided into two seasons (Form C). The maximum yield dose calculated was 815 g ha^{-1}, which resulted in a 6% increase in SY. The Zn foliar spray in forms A and B increased sugarcane productivity by 5%, however, to obtain this gain, it was necessary to apply a dose of Zn greater than 1000 g/ha. The foliar application of Zn reduced the incidence of rust in the first ratoon. The greatest impact was observed in the only treated plots at 0.5 n height (Form A). Figure 10 shows that form A, ie, foliar application of Zn at the beginning of ratoon sprouting, decreased the incidence of rust in a linear. The application of the maximum dose of Zn studied (1386 g ha^{-1}), the incidence of rust in this form of application decreases from 2.0 (whitout Zn spray) to 1.2. The Zn spray in the form B and C also reduced the incidence of rust, however the percentage decrease of the rust grade occurred with smaller treatments. While in the form A the severity of the rust dropped 40% with the application of the maximum dose of Zn, these treatments, form A and C, the reduction was around 25%. Studies comparing the foliar application of Zn in crops such as pepper, soybean and beans, found that the foliar application of ZnO was as efficient as the application of Zn sulfate (Mahdieh et al., (2018); García-López et al, (2020). These results corroborate the results obtained in this study. Although Zintrac is not classified as nanoparticulate, the application of this source was as efficient as the sulfate in sugarcane.

Fig. 1. Effect of foliar application of Zn on foliar content (A), carbonic anhydrase activity (B), orange rust incidency (C) and stalk yield (D) of the sugarcane first ratoon.



CONCLUSIONS

The foliar application of micronized Zn oxide was as efficient as Zn sulfate. Zn foliar spray in first ratoon increased the activity of carbonic anhydrase and foliar content Zn in sugarcane. Zn foliar spray increased the yield of the first ratoon, but the split application in two doses of 815 g ha^{-1}, was a way that allowed the greatest gain in stalk production. The best nutrition of the sugarcane with Zinc reduces the severity of the orange rust. The application at the beginning of ratoon development (Forma A-stems at 0.5 m high) was more efficient than the other forms.

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IRRIGATED RICE YIELD INFLUENCED BY SULFUR SOURCES IN FERTILIZATION

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Keywords: Rice; Sulfur; Polyhalite

INTRODUCTION

The yield of irrigated rice grains in Brazil has increased considerably in the last decade, reaching a level around 9 t/ha in 2019/2020 harvest. This progress is a result of the red rice control with the "Clearfield" technology and the implementation of a set of management practices including the use of sulfur (S) in balanced fertilization (MENEZES et al. 2012).

Around 90% of the sulfur in plants is found on amino acids that are essential for plant development. The S demanded for one ton of irrigated rice is around 2.1 kg, of which 1.1 kg is exported in grain and 1 kg is in straw. The aim of this experiment was to evaluate the use of different S sources in the yield and quality of irrigated rice.

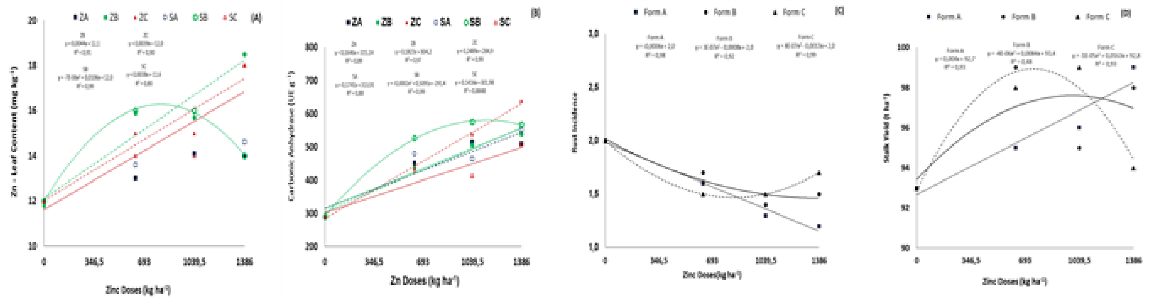
METHODS

The experiment was conduced at Chasqueiro Technological Center, Arroio Grande, Rio Grande do Sul state in Brazil, 2020/2021 growing season. The soil was an Planossolo Háplico Eutrófico Solódico according STRECK et al. (2008) (Alfisol in american soil taxonomy) and contained 280 g kg^{-1} of clay; 9.0 g kg^{-1} of organic matter; 8.5 mg dm^{-3} of P, 69 mg dm^{-3} of K; 11.40 mg dm^{3} of S; 3.7 $cmol_c$ dm^{-3} of Ca and 2.5 $cmol_c$ of Mg. CEC was 7 $cmol_c$ dm^{-3} and pH 5.3. Experimental design was in randomized blocks and contained 7 treatments and 4 replications and consisted in blends among urea, MAP, KCl and other sources in order to supply 185 kg ha^{-1} of N, 108 kg ha^{-1} P_2O_5, and 102 kg ha^{-1} of K_2O, except Treatment 1 without any fertilization. The S sources to supply 25 kg ha^{-1} of S are described in Table 1, except treatments 1 and 2 without S application.

The crop was implanted on conventional cultivation system and management was carried out based on research indications for irrigated rice cultivation (SOSBAI, 2018). The variety IRGA 424 RI was sown in the amount of 60 kg ha^{-1} of seeds, with a 17 cm spacing between rows. Irrigation was carried out with permanent water depth in all plots simultaneously.

It was evaluated the effect of S sources on the height of rice plants, grain yield, number of grains per panicle, spikelet sterility, and percentage of broken grains. Data were submitted to analysis of variance using F test, and the means compared by the t test at the level 5% of probability.

Table 1. Sulfur sources and rates of nutrients applied in the experimental plots.



RESULTS AND DISCUSSION

Table 2 shows the results found for each variable. The development of plants, evaluated according to their height, was significantly affected by the application of fertilizers, and the use of Polyhalite as a source of S favored the formation of taller plants. The yield of irrigated rice grains have significant differences between the S sources, and Polyhalite was the only source with a positive response in relation to the treatment without S.

The application of Polyhalite also favored the development of a greater number of grains per panicle. No differences were observed related to the application of fertilizers or for the sources of S in relation to the spikelet sterility. However, the use of fertilizers significantly reduced the amount of broken grains, and the sources of S showed good results, highlighting the use of ammonium sulfate.

Table 2. Height of rice plants (cm), grain yield (Mg ha^{-1}), number of grains per panicle, spikelet sterility (%), an amount of broken grains (%), as a function of treatments with different sulfur sources

TREATMENTS	Height of plants, cm	Grain yield, Mg ha^{-1}	grains per panicle	Spikelet sterility, %	Broken grains, %
T1. NO FERTILIZATION	99.3 b	14.14 c	113.6 b	17.6 a	10.2 a
T2. NO SULFUR	103.8 a	16.37 b	122.2 ab	14.7 a	7.8 ab
T3. ELEMENTAL S (90%S)	102.5 a	17.49 ab	128.1 ab	15.1 a	9.3 ab
T4. SINGLE SUPERPHOSPHATE (10%S)	103.4 a	17.51 ab	128.1 ab	15.7 a	8.0 ab
T5. AMMONIUM SULPHATE (21%S)	103.5 a	17.42 ab	127.9 ab	16.2 a	6.6 b
T6. BLEND 08-40-00 + 9.3%S	102.0 ab	17.05 ab	122.2 ab	17.0 a	7.1 ab
T7. POLYHALITE (19.2%S)	102.5 a	18.13 a	128.9 a	15.4 a	7.7 ab
CV%	1.88	4.83	8.05	15.02	26.55

Means followed by the same letter do not differ from each other by Test t at 5% probability

CONCLUSIONS

The dataset analyzed allows us to state that rice cultivation responded to sulfur, especially when high yields are expected or when soils are deficient. The greatest potential was obtained with the Polyhalite source.

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NANO-HYDROXYAPATITE AS A PHOSPHORUS FERTILIZER

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Keywords: Nano-fertilization; Phosphorus; Nanoparticle

INTRODUCTION

Conventional fertilization practices are remarkably inefficient with respect to phosphorus (P) use. When common P-based inorganic fertilizers are applied to agricultural soils, the largest portion of P released is quickly made plant-unavailable due to chemical fixation or microbial immobilization. Foliar application of P bypasses some of the limitations associated with soil application, but comes with other problems such as leaf scorching and poor uptake efficiency [1].

The introduction of nanotechnology in plant science paves the way for the creation of smart nanofertilizers capable of overcoming these challenges, as nanoparticles (NPs) possess flexible surface properties that can be tailored to deliver essential nutrients in multiple chemical forms compared to commercial mineral salts.

Our work investigates the potential of hydroxyapatite nanoparticles (NPs) as a P fertilizer. In two separate studies, we applied nano-hydroxyapatite (nHAP) to either the roots [2] or the foliage of P-deficient barley plants (*Hordeum vulgare* L.). Then, using a combination of techniques to assay P functionality in metabolism and for bioimaging of NPs with cellular resolution, we monitored the fertilizing effects of nHAP as well as the mechanistic interactions occurring between NPs and plant tissue.

METHODS

P-sufficient (+P) and P-deficient (--P) barley plants were grown either in hydroponics or in vermiculite. Different types of surface-modified nHAP particles were applied to either the roots or the foliage of 2-3 weeks-old --P plants.

To monitor the nutritional effects of nHAP and the evolution of P content in treated plants, we used chlorophyll *a* fluorescence, PAM fluorometry and ICP-MS analyses. To follow the entrance, transport and dissolution of NPs inside treated plants, we used confocal laser scanning microscopy (CLSM), transmission electron microscopy-energy-dispersive X-ray spectroscopy (TEM-EDXS) and laser ablation-ICP-MS (LA-ICP-MS) imaging techniques. To characterize the different types of NPs prepared, we used TEM-EDXS, ICP-MS and dynamic light scattering (DLS) analyses.

RESULTS AND DISCUSSION

(a) Root application of nHAP

The nutritional status of --P plants was restored in less than 48 hours after exposure to nHAP; here, the P content of youngest fully evolved leaves increased by >3 folds compared to non-treated plants, reaching the same tissue concentrations found in +P treatments. The same result was obtained with the use of nanosized-phosphate rock. No effects were instead observed after the use of hydroxyapatite or phosphate rock micro-particles.

Bioimaging results indicate that NPs (rod-shaped, approx. 40x13 nm) initially aggregate onto the root surface, and subsequently penetrate into deeper cell layers through the apoplast of mature epidermal and cortical cells. The acidic environment found in these compartments causes the NPs to dissolve and release their P cargo in form of orthophosphate ions, which are promptly translocated and metabolized by other sink organs.

The sticky pectin-rich mucilage matrix surrounding the root cap appears to strongly inhibit, if not prevent, the uptake of nHAP, which dissolves without penetrating into deeper cell layers of the root tip (Fig. 1A).

(b) Foliar application of nHAP

Bioimaging results indicate that stomata are the main entrance gateways utilized by the NPs to penetrate the leaf surface. After accessing the sub-stomatal cavity, the NPs freely diffuse into the mesophyll apoplast (Fig.

1B). Interestingly, the choice of the surfactant exerts a strong influence on the entrance pathway and the transport of nHAP inside the plant; nHAP dispersed in formulations with lower surface tension appear to penetrate a greater number of stomata, likely "activated" by the formation of a water film that connects the exterior with the interior of the leaf.

We tested the fertilizing effect of both conventional P salts and nHAP solutions, either applied as micro-droplets on the adaxial page of barley leaves, or infiltrated using a needleless syringe. Overall, results indicate that infiltration was the only application method that allows for restoration of P functionality, whereas leaf spraying only leads to minor P recovery, suggesting only partial leaf uptake of the nanofertilizer.

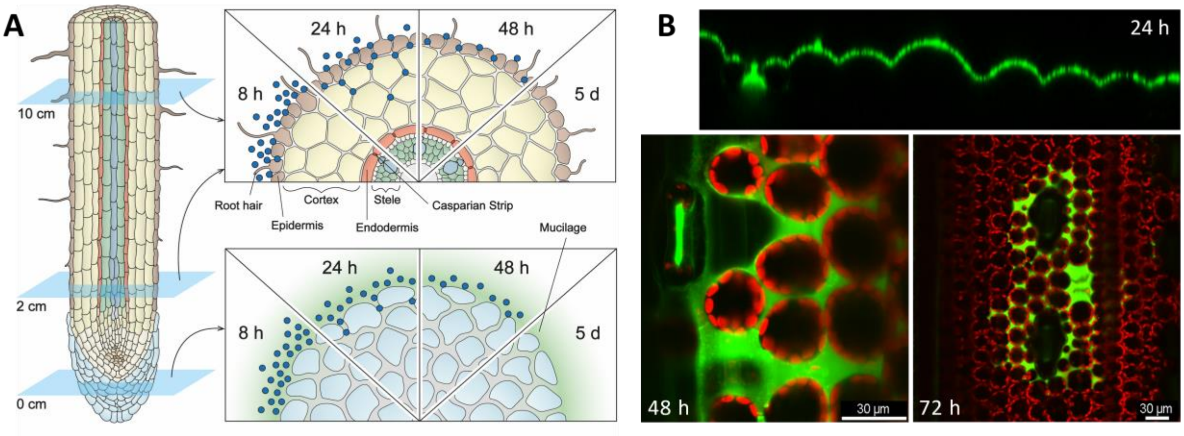

Fig.1. Overview of uptake and dissolution mechanisms for nHAP in barley roots (A) [2]. Confocal images of nHAP (green signal) penetrating stomata of barley leaves and diffusing into the mesophyll apoplast. The red signal is autofluorescence from chloroplasts inside mesophyll cells.

CONCLUSIONS

This is the first work, to our knowledge, demonstrating that plants diagnosed with a nutrient deficiency are capable of restoring their physiological status after treatment with nanofertilizers. Being a bioavailable source of P for agricultural crops, nHAP shows great potential for both root and foliar application in modern agriculture. Work is in progress to address the following challenges for the implementation of nHAP in real-life agriculture:

(a) *Root application*: development of NP structures capable of mass-flow transport in the soil solution across different soil types;

(b) *Foliar application*: development of NP structures with high capacity of spontaneously diffusing across the leaf cuticle; optimized NP surface tailoring to promote cellular internalization and subcellular delivery, followed by programmed nutrient release.

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SOURCES AND TIMING OF NITROGEN FERTILIZATION ON COTTON GROW IN SANDY SOILS

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Keywords: Nitrogen; Sources; Controlled release urea

INTRODUÇÃO

Nitrogen (N) is one of the macronutrients most required by cotton. For each ton of seed cotton produced, from 57 to 58 kg ha^{-1} of N is took up (Vieira et al., 2018). N fertilization management (sources, rates and timing) is essential to increase yield and assure fiber quality (Bono et al., 2006). Urea is the most widely used source of N in agriculture because of its high concentration (45%N) and lower cost value per unit of N. Controlled release urea (CRU) has shown great potential to increase the efficiency of nitrogen use (NUE) and reduce costs (Tian et al., 2017). Despite that, research results of CRU on cotton yield and quality sandy soils are scarce . The objective of this work was to evaluate the effect of nitrogen management (sources and timing) on yield and fiber quality in a sandy soil.

METHODS

The experiment was carried out in the field in Presidente Bernardes-SP in the 2020/2021 season. The experimental design was in a randomized blocks with five replications. Treatments were: 1-CRU 100% incorporated at sowing (CRU 100I); 2- CRU 100% broadcasted at sowing (CRU 100B); 3-CRU 70% incorporated at sowing and 30% at 50 days after emergence (DAE) (CRU 70/30); 4-CRU 70% incorporated at sowing and 30% with urea at 50 DAE (CRU/CU 70/30); 5- Urea at 20, 40 and 60 DAE (CU) and 6- Ammonium nitrate at 20, 40 and 60 DAE (AN). N rate was 100 kg ha^{-1} for all treatments. Yield, yield components (boll weight, boll number and gin turnout) and fiber quality (micronaire, strength, length and short fiber index) were evaluated. Treatment means were compared by LSD test ($P<0.05$).

RESULTS AND DISCUSSION

Conventional urea increased the number of bolls (109) in relation to treatments fertilized with CRU splited at sowing and topdressing (70/30) and CRU/CU (70/30) splited at sowing and topdressing (Fig. 1). However, there was no difference when CRU was fully applied at sowing and when ammonium nitrate was applied (Fig. 1). There was no difference between sources and timing of N application on boll weight, gin turnout, fiber yield and fiber quality parameters as well as N content in cotton leaf (Table 1). Our results show that cotton with the application of CRU totally in sowing has the same yield and fiber quality as urea splited three times. These results are encouraging, because a single application at the time of sowing can minimize the production costs of the crop (Tian et al., 2018). The positive result of CRU is due to the slow release of N over a period of time, which may coincide with the nutritional demand of the crop (Grant et al., 2012).

Trataments	N leaf	Micronaire	Resistance	Length	SFI
	g kg-1	e pol-1	gf TEX-1	mm	%
CRU 100I	40,22 a	3,79 a	35,16 a	31,40 a	7,39 a
CRU 100B	39,90 a	3,96 a	35,74 a	31,59 a	7,19 a
CRU 70/30	40,45 a	3,76 a	35,08 a	31h30	7,45 a
CRU/CU 70/30	40,20 a	4,06 a	35,51 a	31.21 a	7,69 a

COM	39,76 a	3,69 a	35,80 a	30,97 a	7,83 a
N / D	39,58 a	3,84 a	36,52 a	31,39 a	7.11a
CV%	3.98	9.00	3.15	1.34	8.73

Table 1. N content in the a leaf and fiber quality (micronaire, resistance, length and short fiber index - SFI) of cotton in different sources of N and timing of application.

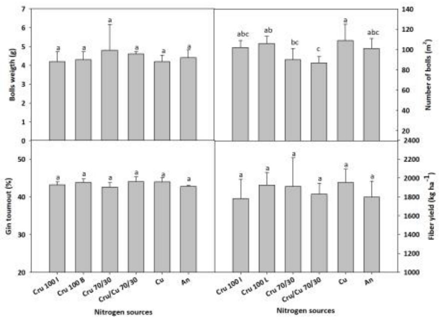

Fig 1. Yield content in components of cotton in different sources of N and timing of application.

CONCLUSIONS

CRU fully at sowing assured fiber yield and quality of cotton grown in a sandy soil.

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YIELD AND COTTON FIBER QUALITY AS AFFECTED BY CALCIUM SOURCES IN A SANDY SOIL

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Keywords: Calcium; calcium nitrate; fiber

INTRODUCTION

Liming is used to reduce soil acidity and it is the main source of calcium (Ca) to plants, however, its solubility in water is low. Other sources of Ca include gypsum, which is relatively soluble (Caires et al., 1999), and also calcium nitrate (CN) which presents higher solubility (Easterwood, 2002). Cotton plants are very sensitive to soil acidity and require great amounts of Ca, so it is usually grown in corrected pH soils. However, we do not know about the best source of Ca to be used in situations where soil acidity is corrected and the Ca level is low considering that its desirable participation in soil cation exchange capacity (CEC) should range from 50 to 65% (Pauletti, 2020). Therefore, the objective of this study was to evaluate the effects of different Ca sources and rates on cotton yield, fiber quality and plant nutrition in a sandy soil, under no-till system.

METHODS

The experiment was carried out in the field at the Experimental Farm of the University of Western São Paulo, in Presidente Bernardes-SP, in sandy soil in the 2020/2021 season. Soil Ca content and percent saturation of Ca in the CEC was 8.3 $mmol_c$ dm^{-3}[TC1] [GA2] and 28%, respectively. The experimental design was in a randomized block with five replications. The treatments included: 1- Control (No calcium); 2- Limestone at 30 kg Ca ha^{-1} (L30); 3- Calcium nitrate at 30 kg Ca ha^{-1} (CN30); 4- Gypsum at 30 kg Ca ha^{-1} (G30); 5- Limestone at 60 kg Ca ha^{-1} (L60); 6- Calcium nitrate at 60 kg Ca ha^{-1} (CN60); 7-Gypsum at 60 kg Ca ha^{-1} (G60); 8- Gypsum at 180 kg Ca ha^{-1} (G180); 9- Gypsum at 180 + Calcium nitrate at 30 kg Ca ha^{-1} (G180+CN30); 10-Gypsum 180 + Calcium nitrate at 60 kg Ca ha^{-1} (G180+CN60). The applications occurred 50 days after the emergence of the cotton, except for doses with 180 kg via gypsum, which preceded sowing. Yield, and yield components (boll weight, boll number) and fiber quality parameters (micronaire, length, strength and maturity - HVI method) were evaluated. Treatments means were compared using LSD test ($P<0.05$)

RESULTS AND DISCUSSION

The highest yields were achieved with CN60 and G180+CN60 treatments, 23 and 22% higher than the control (Table 1), which was a result of the increased boll number in these treatments. Ca contents in the leaves (Table 1) were 47%, 43% and 35% higher in G30, G180+CN60 and CN60, respectively, compared to the unfertilized Ca treatment. These results show that the application of Ca to the soil increased Ca contents in leaves (except for treatment L30) and should be considered for nutrient management. The sufficiency range of Ca contents on cotton leaves is from 25 to 35 g kg^{-1} (Borin et al., 2014). Fiber length decreased by 2% in the treatment of unfertilized Ca (Table 2). Micronaire was reduced by 21% in CN60 and G180+CN60 and resistance increased 7% in control treatment (Table 2). The results may be related to the number of bolls m^{-2} (G180+CN60) and productivity (CN60 and Control). According to Echer et al (2020), the number of reproductive structures and yield can affect fiber quality. Despite the reduction in resistance in CN60 and G180+CN60, the values were within the appropriate standard (> 27 gf TEX^{-1}), however, the micronaire was below (3.5 to 4.9 μg pol^{-1}). Fiber length and maturity were also within the ideal standards (> 27.4 mm and > 81%, respectively).

Trataments	Ca Leaf	Bolls weigth	Number of bolls	Fiber yield
	g kg -1	g	m2	kg ha-1

Control	24.23 c	4.3 a	115.31 abc	1858 c
L30	28.76 bc	4.4 a	111.63 abc	1889 bc
CN30	31.20 ab	4.3 a	118.85 ab	2242 ab
G30	35.66 a	4.4 a	114.76 abc	2055 abc
L60	31.10 ab	4.4 a	117.70 ab	2036 abc
CN60	32.74 ab	4.6 a	122.10 ab	2299 a
G60	29.06 b	4.7 a	102.30 c	2055 abc
G180	31.20 ab	4.4 a	116.96 abc	2098 abc
G180+CN30	31.46 ab	4.6 a	111.10 bc	2055 abc
G180+CN60	34.80 a	4.3 a	126.31 a	2283 a
CV%	8.69	11.93	7.48	10.65

Table1. Calcium contents in the leaves, avarage weigth of bolls, bolls m2 and fiber yield in differents rates and sources of calcium.

Tratamens	Micronaire	Length	Resistance	Maturity
	ug pol-1	mm	gf TEX-1	---
Control	3.7 ab	30.82 d	32.90 a	85 a
L30	3.7 ab	31.34 ab	31.01 bcd	85 a
CN30	3.7 ab	31.27 bc	32.18 abc	85 a
G30	3.6 ab	31.30 b	31.54 cd	85 a
L60	3.5 ab	31.30 b	31.35 cd	86 a
CN60	3.3 b	31.26 bc	31.77 bcd	86 a
G60	3.6 ab	31.53 ab	31.55 cd	85 a
G180	4.0 a	31.21 bc	32.50 ab	85 a
G180+CN30	4.0 a	31.68 a	30.47 e	85 a
G180+CN60	3.3 b	30.94 cd	31.51 cd	85 a
CV	8.80	0.66	1.50	84 a

Table 2. Cotton fiber micronaire, length, resistance and maturity at different rates and calcium sources.

CONCLUSION

Calcium fertilization increases Ca content on cotton leaf. Higher yields were obtained with the application of calcium nitrate at a rate of 60 kg ha^{-1} and with gypsum + calcium nitrate at a rate of 180 + 60 kg ha^{-1} of calcium. However, higher yields can negatively affect fiber quality.

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AGRONOMIC EFFICIENCY OF FLUID FERTILIZER OBTAINED FROM HUMIC SUBSTANCES EXTRACTED FROM POULTRY BEDDING

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Keywords: avena sativa; biofertilizers; humic acids

The world population grows annually, promoting an increase in the demand for food, in this way, fertilizers are essential to guarantee production. However, Brazil's external dependence on fertilizers leads to the need to increase its efficiency in the use of applied nutrients. One of the alternatives pointed out to optimize resources from chemical alterations may be the use of humic substances extracted from chicken litter, which is abundant in some regions of the country. They are commonly extracted from peat, and there are few studies of chicken litter extraction, therefore, the objective of the study was to quantify the extractable humic substances from chicken litter, defining extractor and concentration with higher yield and, to evaluate the agronomic efficiency of a fluid fertilizer enriched with substances. Two experiments were performed. The first, extraction, in a randomized design, 3x3+1 factorial with three extractors (Sodium hydroxide (NaOH); Potassium hydroxide (KOH); Ammonium hydroxide (NH4OH) and three concentrations: (0.1; 0.25 and 0,5 mol L^{-1}) + control. The total organic carbon of fulvic and humic acids was quantified. The second experiment was carried out in a greenhouse, in a randomized block design in a 3x5 scheme, with three fertilizers (without fertilization, poultry litter, mineral fertilizer) and five doses of fluid fertilizer (0; 50; 100; 150 and 200 mg of C-SH Kg^{-1} of soil), with five repetitions. White oat was cultivated in Nitosol Vermelho and crop yield, nutrient export and soil chemical attributes were evaluated. The highlight in the extraction was for KOH, which showed an increasing linear behavior for fulvic and humic acids, as well as NaOH for humic acids. Unlike NH_4OH, whose response was decreasing. In total, 0.5 mol L^{-1} KOH showed the highest SH yield. The higher solubility of mineral fertilizer on litter allows better oat development and nutrient absorption, with the exception of P. Dry biomass production did not vary when sources were combined with humic substances, however, the fluid fertilizer alone showed a linear increase. on productivity and uptake of N, P and K. Poultry litter + FF increases the efficiency of phosphorus use. FF with SH allows increasing the efficiency of P and K availability and higher levels of P, CEC and V% in the soil when associated with litter, and of K when associated with the mineral, which characterizes better oat nutrition. The best extractor for SH chicken litter was KOH 0.5 mol L^{-1}, still allowing 1.65% of K in the fertilizer composition and the synergy between FF with SH and mineral or organic fertilizers promotes benefits of nutrient absorption in the plant and increases soil fertility, with greater emphasis on P and K, and promotes benefits in CEC and V% in conjunction with organic fertilization.

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To Ferticel Fertilizer Company

THE RESPONSE OF CORN YIELD TO POLY4 FERTILISER PROGRAMMES COMPARED TO CONVENTIONAL PROGRAMMES IN MEXICO.

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Keywords: POLY4; Maize; multinutrient

INTRODUCTION

Corn is the most important crop in Mexico, with applications ranging from human consumption to animal feed. Depending on the technology input, average yields range from 2 to 14 t ha^{-1} depending on the area and state.

Standard practice is to supply S as ammonium sulphate and the majority of farmers do not apply sufficient rates of K.

POLY4 supplies potassium associated with sulphur in the form of sulphate as well as Mg, Ca and micronutrients. POLY4 gives a sustained nutrient delivery throughout the growing season, matching the crops demands.

The aim of this study was to evaluate corn production fertilized with POLY4 and conventional NPK fertilizers.

METHODS

In Mexico, many farmers grow corn in a low NPK input system, while larger scale farmers have higher input systems.

The use of POLY4 to improve these two types of systems was tested. In total, 16 corn trials were conducted in Jalisco, Sinaloa, and Guanajuato in central and western Mexico between 2018 and 2020.

Table 1. Average fertiliser and nutrient application rates at low input trial sites (kg ha^{-1})

Treatment	Product rate	POLY4 rate	N	P_2O_5	K_2O	S	MgO	CaO
Standard programme	294	0	80	25	0	21	0	9
POLY4 programme	463	214	80	25	30	48	13	46

Table 2. Average fertiliser and nutrient application rates at high input trials sites (kg ha^{-1})

Treatment	Product rate	POLY4 rate	N	P2O5	K2O	S	MgO	CaO
Standard (MOP + AS)	465	0	80	80	60	36	0	0
POLY4 (143 kg/ha) + MOP + AS	511	143	80	80	60	36	9	24
POLY4 (286 kg/ha) + MOP	600	286	80	80	60	55	17	49
POLY4 (429 kg/ha)	709	429	80	80	60	82	26	73

RESULTS AND DISCUSSION

Low input systems

The median yield of the standard practice was 11.8 t ha^{-1} When 214 kg POLY4 ha^{-1} was included in a low input fertiliser programme, yield was increased in 15/16 trials. The median yield improvement was 656 kg ha^{-1} or 5.6%.

High input systems

The median yield of the standard practice (MOP+AS) was 12.6 t ha^{-1} With 143 or 286 kg POLY4 ha^{-1} yield was increased in 11/16 trials. With 429 kg POLY4 ha^{-1} yield was increased in 14/16 trials.

(a) With 143 kg POLY4 ha^{-1} the median yield improvement was 372 kg ha^{-1} or 2.9%

(b) With 286 kg POLY4 ha^{-1} the median yield improvement was 298 kg ha^{-1} or 2.4%

(c) With 429 kg POLY4 ha^{-1} the median yield improvement was 572 kg ha^{-1} or 4.5%

(d) Across the three POLY4 rates the median yield response was 364 kg ha^{-1} or 2.8%

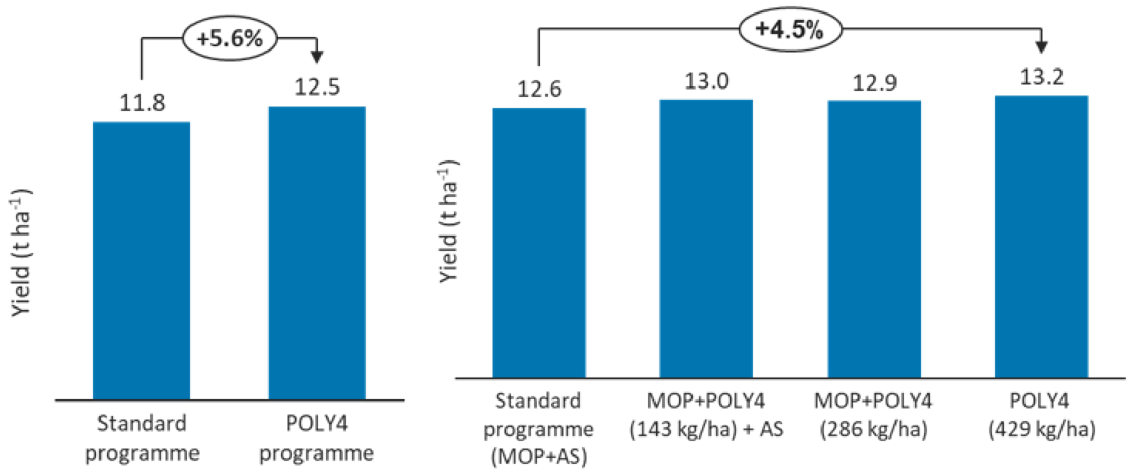

Fig. 1. Median yield improvement of corn across 16 low input trials (left) and high input trials (right)

CONCLUSIONS

Yield was consistently improved with POLY4 both in low input systems that received little or no P and no K, and higher input systems that received the recommended rates of P and K. This yield improvement was seen in three states in Mexico (Sinaloa, Jalisco and Guanajuato) across 13 different corn varieties.

As expected overall yields were lower in the low input systems, but POLY4 gave a greater yield improvement (656 kg ha^{-1} from 214 kg POLY4 ha^{-1}) compared to high input systems where similar POLY4 applications rates of 143 and 286 kg POLY4 ha^{-1} gave 372 and 298 kg ha^{-1} median yield improvements. By increasing the POLY4 input to 429 kg ha^{-1} yield improvement of high input systems was further increased to 572 kg ha^{-1}.

RESPONSE OF A NEW BIOLOGICAL ADDITIVE TECHNOLOGY FOR FERTILIZERS ON SOYBEAN YIELD ACROSS DIFFERENT REGIONS IN BRAZIL

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Keywords: phosphate; phosphorus management; soil health

INTRODUCTION

Soybean yields have increased over the last decades in Brazil, as consequence of many technological advances such as the adoption of transgenic soybean, improvements on planting and harvesting machinery, implementation of the agro-climatic zoning for the crop, massive adoption of no-till system, but mainly due to several technologies adopted for Cerrado production systems, including improvements on soil fertility and nutrients management (Cattelan and Dall'agnol, 2018).

Improved agricultural systems often present sufficient levels of nutrients in the soil, with low restrictions in terms of physical properties. However, agricultural intensification with low diversity and restricted crop rotation systems may result in losses on biological diversity to the soil, reducing its biological quality (Doran and Zeiss, 2000). In this context, several practices and technologies have been suggested as strategy to improving soil microorganisms diversity for both minimizing pressure of pathogenic microorganisms in soils and providing additional biostimulant effects and, ultimately, contributing with soil health (Doran and Zeiss, 2000).

The present research was developed aiming to evaluating the combined adoption of improved fertilizers and a new technology with potential related to promoting activity of several benefic soil microorganisms and biostimulant effects. The phosphorus (P) uptake and soybean yield across several Brazilian agricultural regions were evaluated in the present study.

METHODS

To evaluating the soybean response in different regions of Brazil, five field experiments were carried out in the 2020-2021 growing season. The experiments were stablished in Botucatu-SP; Uberlândia-MG; Londrina-PR; Rondonópolis-MT and Jataí-GO.

The experimental design adopted was the randomized block design, with four replicates for each treatment. Individual plots size varied from $24m^2$ up to $40m^2$, with 8 - 10 rows, with row space of 0.5m. Treatments consisted of P fertilizers with different compositions and production technologies: T1- Control; T2- MAP (monoammonium phosphate) [(11-52-0-0), (N-P_2O_5-K_2O-S)]; T3-MicroEssentials® (monoammonium phosphate with sulfur in elemental and sulfate forms [(10-46-0-9), (N-P_2O_5-K_2O-S)]; T4-MicroEssentials® + plus biological additive.

The P fertilizers were applied at the rate of 80 kg ha^{-1} of P_2O_5. For all the treatments, broadcast MOP (muriate of potash) was applied as source of potassium for soybean, at the rate of 90 kg ha^{-1} of K_2O. The plant P uptake at R2 stage and soybean grain yield were evaluated. The agronomic efficiency index was calculated for P sources according Fageria and Baligar (2005).

The effect of the fertilizers and the biological additive on the P uptake, grain yield, relative yield over the control treatment and agronomic efficiency were verified by ANOVA analysis. When significant ($p<0.10$), for each analyzed variable, the treatments mean values were compared by the T test (LSD, $p<0.10$). For relative yield and agronomic efficiency, the 5 sites and the 3 tested P sources were considered for the statistical analysis, in a 3x5 factorial design.

RESULTS AND DISCUSSION

There was significative effect of P fertilizer addition for P uptake at R2 in all the studied sites (Table 1). Overall, the use of MicroEssentials® (T3) resulted in higher P uptake, but with no significative difference being observed for the biological additive. Soybean yield was affected by the treatments in almost all the studied sites, except in Jataí, GO. The use of MicroEssentials® + biological additive (T4) showed positive increment on soybean yields, with increments from +63 kg ha^{-1} up to +349 kg ha^{-1}, when compared to the treatment without the biological additive application (T3).

The agronomic efficiency showed differences on the P use efficiency among the tested fertilizers. Whereas the MAP P source (T2) resulted in average of 3.2 kg of soybean grains per kg of applied P_2O_5 kg, greater indexes values were observed for MicroEssentials® (T3) and MicroEssentials® + biological additive (T4), average values of 6.3 and 9.3 kg kg^{-1} respectively.

Table 1. Soybean P uptake (R2 stage), grain yield and agronomic efficiency index as response of P sources and biological additive in different regions of Brazil. 2020/2021 crop season.

Treatment	Botucatu, SP	Uberlândia, MG	Rondonópolis, MT	Jataí, GO	Londrina, PR	Average
	-- P Uptake[1] (kg ha^{-1}) --					
T1	6.9 b	6.3 b	16.9 a	4.3 b	8.9 c	-
T2	8.3 a	9.1 a	20.9 a	5.1 a	12.9 b	-
T3	8.8 a	8.5 a	19.7 a	5.1 a	16.7 a	-
T4	8.4 a	8.5 a	16.0 a	5.6 a	16.5 a	-
	-------------------------------------- Soybean Grain yield (kg ha^{-1}) --------------------------------------					
T1	4285 c	3198 c	3537 c	4414^{ns}	3457 d	-
T2	4418 bc	3658 b	3795 bc	4661	3627 c	-
T3	4717 ab	4100 a	4088 ab	4787	3724 b	-
T4	4780 a	4376 a	4367 a	5034	4073 a	-
	--------------------------------- Soybean yield (Over Control, kg ha^{-1}) ---------------------------------					
T1	-	-	-	-	-	-
T2	133	460	258	247	170	253 c
T3	432	902	551	373	267	505 b
T4	495	1178	830	620	616	748 a
Average	353 B	847 A	546 B	413 B	351 B	
	-------------------------------- Agronomic Efficiency Index (kg kg^{-1}) --------------------------------					
T1	-	-	-	-	-	-

T2	1.7	5.8	3.2	3.1	2.1	3.2 c
T3	5.4	11.3	6.9	4.7	3.3	6.3 b
T4	6.2	14.7	10.4	7.8	7.7	9.4 a
Average	4.4 B	10.6 A	6.8 B	5.2 B	4.4 B	

[1]1R2 soybean growth stage. [ns] not significant. T1: Control (no P); T2: MAP [(11-52-0-0), (N-P_2O_5-K_2O-S)]; T3: MicroEssentials® [(10-46-0-9), (N-P_2O_5-K_2O-S)]; T4: MicroEssentials® + biological additive.

Means followed by same letters, uppercase in the row and lowercase in the column, do not differ statistically by the T test (LSD, $p<0.10$).

CONCLUSIONS

Soybean yield was improved as response of P fertilization, with significative increments as consequence of NPS source (MicroEssentials®).

The tested combination of MicroEssentials® + biological additive showed significative results on soybean yield and agronomic efficiency, being a promisor technology for utilization in fertilizers.

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"WHAT IS A PLANT NUTRIENT?"

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Keywords: plant nutrient; benificial elements; plant nutrition

INTRODUCTION

A recent paper by Brown et al (2021)[1] proposed the adoption of a new definition and utilization of the term 'Plant Nutrient' that has important implications for the science of plant nutrition, the optimization and remediation of agricultural and natural ecosystems, fertilizer development and the development of regulations governing the sale and use of fertilizers. The new definition is built on the principle of 'one-health' that suggests that the optimal function of any ecosystem requires optimization of all parts of that ecosystem. Specifically "*a mineral plant nutrient is an element which is essential or beneficial for plant growth and development or for the quality attributes of the plant or harvested product, of a given plant species, grown in its natural or cultivated environment."* This new definition represents a paradigm shift, by defining plant nutrients in the context of their role in optimizing plant growth and development or the quality attributes of a plant (or its harvested product) and not solely in the context of a demonstrated 'essential' role in the plant life cycle.

Changing the definition of a plant nutrient has scientific and commercial relevance. The broadening of the definition of a plant nutrient will encourage a more holistic view of the full suite of elements that might interact with the plant in its natural or cultivated environment. From a commercial perspective many jurisdictions restrict the term 'fertilizer' to only the established 'plant essential elements', while elements that may benefit some species under some conditions or as a consequence of indirect effect on the whole ecosystems, or that 'merely' benefit the health of the organisms that consume the plant, are not considered 'plant-nutrients' and hence cannot be labeled as fertilizers. The historic adherence to the narrow 'essentiality' based definition of a plant nutrient has undoubtedly also constrained academic inquiry and reduced commercial investment in the full suite of potentially relevant elements.

The past 100 years has seen a significant number of studies demonstrating positive responses to a range of elements not known to be essential for plants. These include elements widely recognized as 'beneficial' such as sodium (Na), silicon (Si), cobalt (Co), selenium (Se) and aluminum (Al) [2,3,4,5]. Some 'beneficial' elements have also been shown to be essential but only for specific plants, or under specific growth conditions, such as Al^{3+} in *Camelia sinensis* [6] or Na^{+} for C4 or CAM plants [2]. A 'plant nutrient' may also be an element valuable solely for its impact on the quality of the consumed product: iodine (I) is essential for human health as constituent of thyroid hormones [7] and its deficiency contributed to the establishment of mandatory salt-iodization program in several countries [8]. Selenium is essential for the thyroid hormone metabolism [9], and a program of simultaneous biofortification of I and Se could help prevent hypothyroidism [7]**.** While strong evidence for a practical agronomic benefit is lacking there are a number of elements, present at trace levels in plants, that have been shown to have a direct and specific function in plant metabolic pathways but that have not been shown to satisfy the 'essentiality' criteria, including elements such as titanium (Ti), iodine (I), and vanadium (V) [10,11,12,13]. There is also a large body of literature suggesting beneficial effects of the rare earth elements (REEs): cerium (Ce), lanthanum (La) and neodymium (Nd) [14,15,16,17].

The growing recognition of the role of the microbiome in ecosystem function also suggests that any element essential for a critical microbial mediated process will also be important for ecosystem function and agricultural productivity. Vanadium, for example, is known to be a critical cofactor for N fixation in a number of free living N fixing bacteria (Azotobacter vinelandii) [18] hence in any plant system dependent upon this source of fixed N, the optimization of V status would in turn optimize plant production. Wackett et al. (2004) [19] extends this concept in his novel 'biological' table of elements that represents all elements known to be biologically active in organisms.

If the goal of plant nutrition is to optimize plant productivity and quality, then any element that contributes to this goal, could legitimately be considered a plant nutrient. In this paper we will review evidence for the biologically relevant role of elements not currently defined as essential or beneficial [20] in plant productivity, ecosystem services or the quality of the harvested plant part.

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POLYHALITE AS A SOURCE OF POTASSIUM AND MULTINUTRIENTS FOR COMMON BEAN AND SOIL AMMENDMENT

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Keywords: Brazilian cerrado; secondary nutrients; potassium management

INTRODUCTION

More than 50% of grain production in Brazil, including common bean, come from cultivation in the Cerrado biome region. Most soils of this region do not have sufficient reserves of nutrients, including potassium (K) to sustain agricultural production without fertilization. The amount of potassium (K) exported per ton of grains of soybean (*Glycine max* L.), maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris L.*) are an average of 22 kg, 5 kg and 18 kg, respectively. Thus, the maintenance fertilization with potassium plays an important role for the sustainability of production. I the Brazilian fertilizer market most importance is given to nitrogen, phosphorus and potassium (NPK), which are the basis of the commercial formulations. However, in the Cerrado soils besides the NPK fertilizers it is essential that sufficient amounts of sulfur (S), calcium (Ca), magnesium (Mg) and micronutrients are provided through the application of limestone and fertilizers. A deficiency in any of these secondary macronutrients or micronutrients reduces the agronomic efficiency of NPK fertilizers due to limitation of crop production.

The objective of this study was to evaluate the performance of a granulated polyhalite-based fertilizer as a source of potassium and secondary macronutrients for common bean and its additional benefit as a soil ammendment.

METHODS

The study was conducted during the 2016/17, 2017/18 and 2018/19 growing seasons at the Embrapa Rice and Beans experimental site (16°29'21"S 49°18'00"W), minicipality of Santo Antônio de Goiás, Brazil, under no-tillage system, in a corn-common bean crop rotation. The soil was classified as acric Red Latosol, with a clayey texture. Soil K contents measured at the beginning of experimentation at the 0-10 cm, 10-20 cm and 20-40 cm soil layers were 110 mg kg^{-1}, 73 mg kg^{-1} and 44 mg kg^{-1}, respectively. In this work we present the results of common bean grain yield in 2018/2019 growing season as well as the chemical changes in the soil profile up to 1.0 m deep resulting from the accumulated application of fertilizers at a dose of 90 kg ha^{-1} of K_2O (180 kg K_2O ha^{-1} applied annually).

The experimental design was a randomized complete block layout arranged in a 2x2x4+2+1 factorial scheme with four replications. The fertilizer treatments for both maize (summer season) and common bean under center pivot (winter season) included K_2O applied either at planting in furrow or as a top dressing broadcast with four rates - 30, 60, 90 and 180 kg K_2O ha^{-1}. These treatments were compared to a control (without K, S, Ca and Mg) and to a KCl+gypsum+kieserite treatment applied at planting and another applied at top dressing for both maize and common bean at the 90 kg K_2O ha^{-1} application rate with the kieserite rate set to equal the Mg applied in the same rate for polyhalite and the gypsum rate to match the S rate in that same polyhalite treatment adjusted for the S in Kieserite. Nitrogen, phosphorus, and micronutrients were also applied according to the regional recommendation. Crop management was performed according to the recommendations to keep the area free of weeds, diseases and insects. At the end of 3rd year of trial, after harvest of common bean, soil was sampled at 0-10, 10-20, 20-40, 40-60 and 80-100 cm depth in all repetitions of the treatments control and with the application of K sources at the rate of 90 kg K_2O ha^{-1}. Soil sampling was performed in trenches (1.2 m wide, 1.5 m long, and 1.0 m deep) opened perpendicularly to the common bean rows. The soil was air dried, passed through a 2-mm sieve and kept for chemical analysis, according to methods described at Embrapa (1999).

RESULTS AND DISCUSSION

The comon bean grain yield was influenced by the interaction between K sources and K rates (Fig. 1). Regardless of the timing of K fertilizer application, grain yield did not increase with K rates above 30 kg ha^{-1} of K_2O as KCl. However, there was a quadratic response of grain yield to K rates with the application of polyhalite, indicating that this increase was due to other beneficial effects of polyhalite for common bean in addition to K-supplying. The differences in favor of polyhalite, in comparison with KCl, were equivalent to 12%, 14%, and 15% at the rates of 60 kg ha^{-1}, 90 kg ha^{-1}, and 180 kg ha^{-1}, respectively, considering the average of the two K application timing. After three years and six crops grown cycles, the cumulative application of polyhalite at top dressing was the best treatment for increasing exchangeable Ca and Mg contents and base saturation in soil profile, promoting a better balance among basic cations, compared with KCl or KCl+ Gypsum + Kieserite (Fig. 2). The polyhalite also increased the SO_4^{2-}-S content in all soil layers at 0-100 cm profile (Fig. 2), which was retained mainly in the 20-80 cm deep layer, with a greater proportion in the 40-60 cm layer, probably due to the presence of larger proportion of positive net charge, as occur in highly-weathered soils of Cerrado.

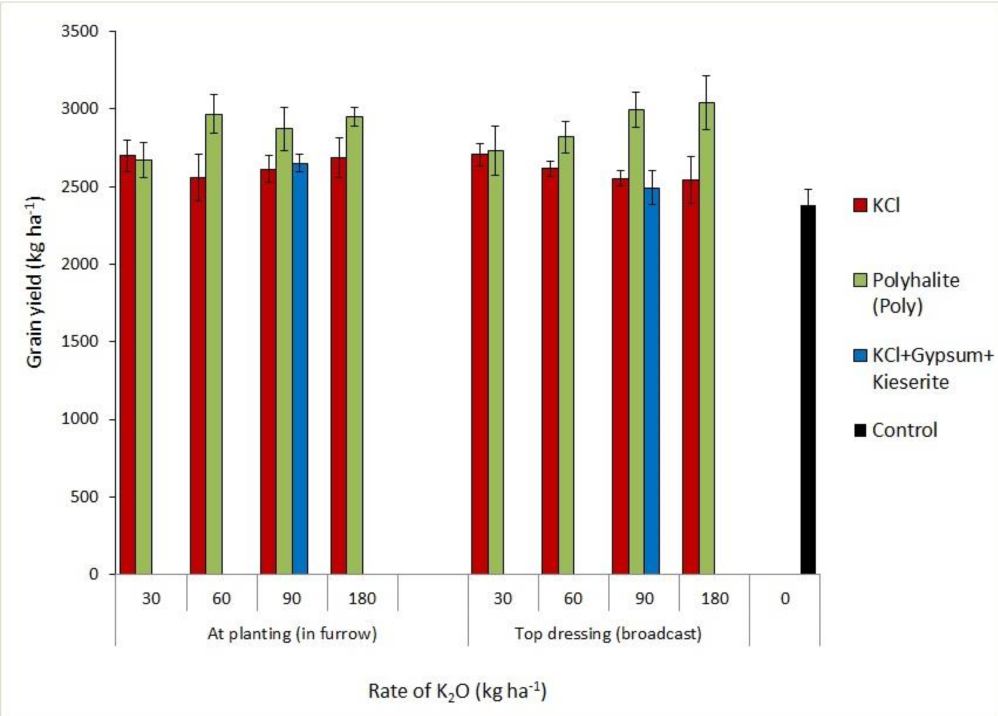

Fig. 1. Grain yield of common bean as affected by potassium rates and sources in 2018/19 growin season.

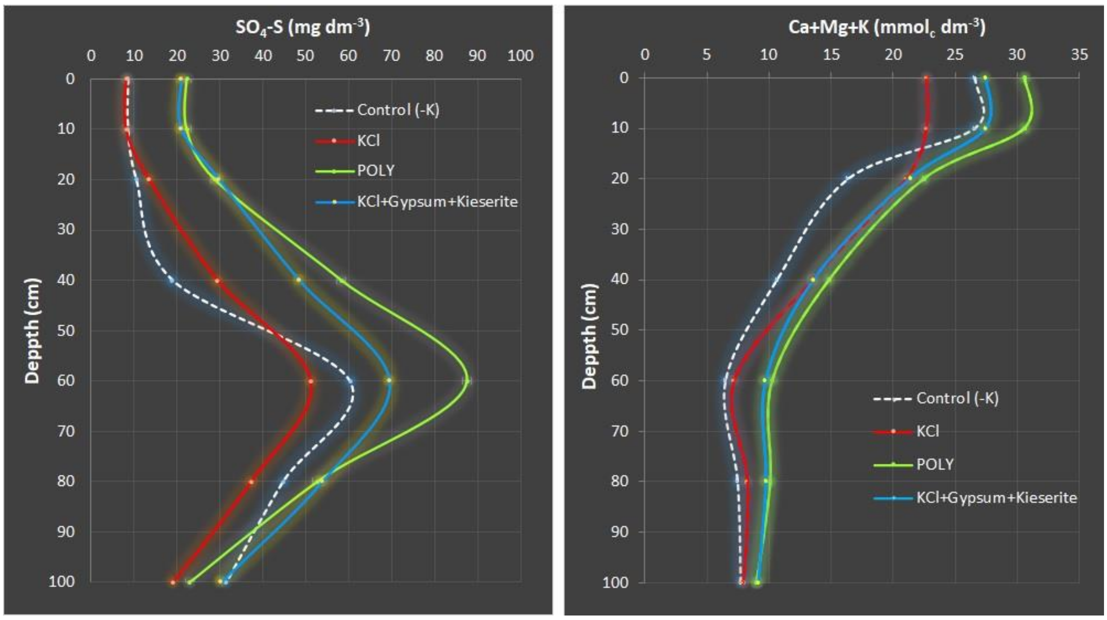

Fig. 2. Distribution of SO_4-S and basic cations in a clayey Typic Acrustox soil profile after three years of annual application of 180 kg ha^{-1} of K_2O as KCl, polyhalite (POLY), and KCl+gypsum+kieserite.

CONCLUSION

The results indicate that farmers of the Brazilian Cerrado could consider polyhalite as a good technical option to meet K and secondary macronutrient requirements for common bean and amelioration of subsoil for root growth.

NITROGEN AND PHOSPHORUS FERTILIZATION OF MAIZE AS A SECOND CROP IN THE CERRADO: A CASE STUDY FOR A BETTER MANAGEMENT STRATEGY

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Keywords: Soybean-maize rotation; Soil available phosphorus content; Nitrogen management

INTRODUCTION

Maize produced as a second crop (also called *safrinha*), usually after soybean harvest, represents about 70% of the production of this cereal in Brazil and 95% in the Midwest region. The risk factors associated with drought conditions, especially when maize (*safrinha*) is planted outside the optimum window, discourage farmers to make higher investments in the crop, such as applying phosphorus and, mainly, nitrogen fertilizers However, with the release of early maturing soybean cultivars, *safrinha* maize can be planted earlier. In this way, climate risks are minimized, yield potential is increased, and farmers find better opportunities to increase their investments in *safrinha* maize.

Nitrogen (N) is the nutrient mostly required by maize, which extracts about 28 kg of N per ton of grains. Thus, depending on the availability of phosphorus (P) in the soil, the investments in N fertilization may be more advantageous, when compared to the application of P fertilizers. Research results considering these aspects will allow producers greater flexibility in decision making.

This research aimed to discriminate the importance and impact of N and P fertilization on the yields of *safrinha* maize following soybean harvest in a Cerrado soil with different levels of available P.

METHODS

The study was conducted during the 2019/20 and 2020/21 seasons at the Embrapa Rice and Beans experimental site, Santo Antônio de Goiás, Brazil, under no-tillage system. The soil was classified as acric Red Latosol, with a clayey texture.

The experimental area has been cultivated since the 2010/11 season with maize as 1st season crop followed by irrigated common bean in the winter, as a third season crop. These two crops received three levels of P fertilization (0, 60 % and 120% of the recommended rate) over eight years, so that after soybean harvest, in February 2020, there were three levels of available P in the soil (measured by the Mehlich extractor) in the 0-20 cm soil layer: i) 4 mg dm^{-3}, ii) 9 mg dm^{-3} and iii) 16 mg dm^{-3}. The soil organic matter content was 26 mg kg^{-1} and the other nutrients showed values above the critical level. The soybean cultivars cultivated during the 2019/20 and 2020/21 seasons were BRS 6970 IPRO and NS 6906, respectively, and their seeds were inoculated with *Bradyrhizobium japonicum*.

For both seasons, the experiment was conducted with maize *safrinha*, sowed shortly after soybean harvest, in the middle of February. A randomized block design was used, with four replications and a 3x2x2 factorial scheme. The 12 treatments consisted of three levels of P availability in the soil, two levels of fertilization with P (0 and 50 kg ha^{-1} of P_2O_5) and two levels of fertilization with N (55 and 110 kg ha^{-1} of N). The rates of P and N used in the trial were defined based on the total amount of P and N that is exported by maize grains and considering a yield of 8,000 kg ha^{-1}. The hybrid AG 7098 PRO 2 was used for both seasons, sowed with three viable seeds per meter of line, and spaced 0.50 m between rows. P was applied to the sowing line, whereas the N was split - 20 kg ha^{-1} applied in the sowing line and the rest was broadcasted on the soil surface shortly after sowing. The N and P sources were calcium ammonium nitrate (CAN) and triple superphosphate, respectively.

RESULTS AND DISCUSSION

The average soybean grain yields obtained in the treatments within the different soil P levels (4 mg dm^{-3}, 9 mg dm^{-3} and 16 mg dm^{-3}) were 2158, 3688 and 3809 kg ha^{-1}, in the 2019/20 season, and 3015, 4339 and 4457 kg ha^{-1} in the 2020/21 season, respectively. As expected, the results show that phosphate fertilization in soil with low phosphorus content is a determining factor to increase grain yields. On the other hand, soybean yields were similar when comparing the levels 9 mg dm^{-3} and 16 mg dm^{-3}, indicating that it is possible to adjust the dose of phosphorus applied in the maintenance fertilizations to quantities closer to those exported in the grains.

Maize grain yields were strongly affected by the different levels of P in the soil and by the N rates (**Fig. 1**). The higher yields were achieved with the highest rate of N applied (110 kg ha^{-1} of N), regardless of soil P content or P fertilization. In soil with low P content (4 mg dm^{-3}), fertilization with 50 kg ha^{-1} of P_2O_5 showed the greater yield when combined with the highest rate of N. Under this circumstance, the management strategy should be to increase the P content of the soil, with P fertilization above the exported quantities, before making greater investments with N fertilization.

In the soil with a content of 9 mg dm^{-3}, P fertilization increased yields only when combined with the highest rate of N. In the soil with higher P content (16 mg dm^{-3}), the yield increase was only observed when N rate was increased, and there was no response to P fertilization. Fig. 1. Maize *safrinha* grain yields for cropping seasons 2019/20 and 2020/21 in response to fertilization with P and N in a Cerrado soil with different levels of availability of P. Vertical bars represent the standard error of the means accumulated in the two growing seasons.

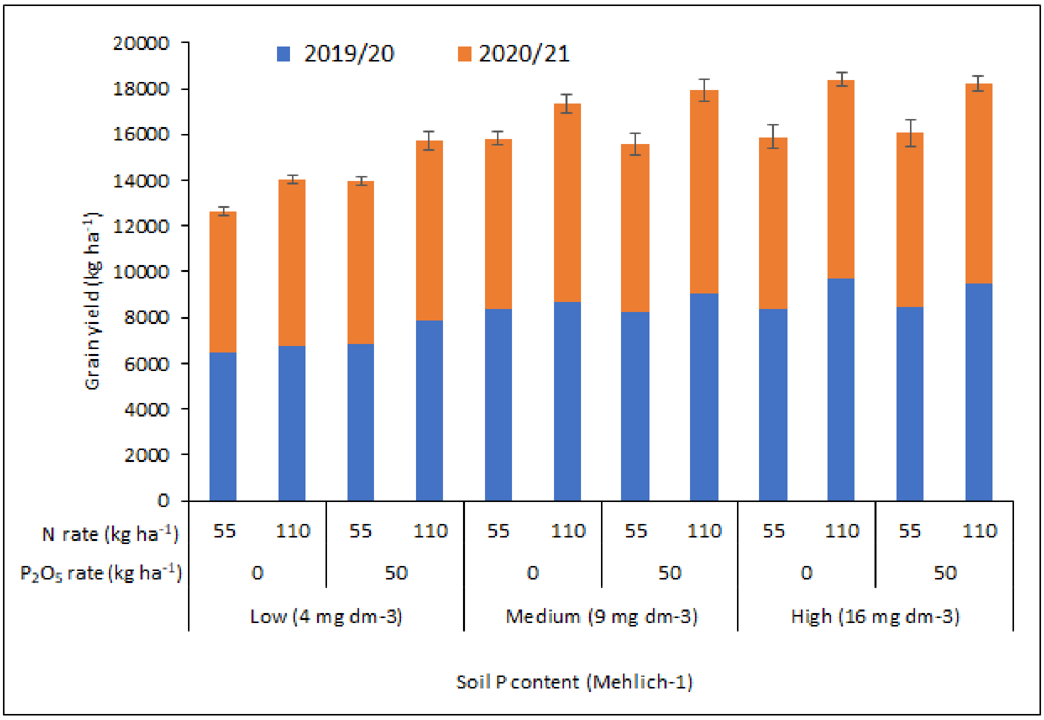

Fig. 1. Maize *safrinha* grain yields for cropping seasons 2019/20 and 2020/21 in response to fertilization with P and N in a Cerrado soil with different levels of availability of P. Vertical bars represent the standard error of the means accumulated in the two growing seasons.

CONCLUSION

The increase in the N rates aiming to replace 100% of the amount exported in 8,000 kg ha^{-1} of grains provided an increase in yield for maize s*afrinha* in all situations of P availability in the soil (low, medium and high), indicating that it is a good strategy, especially in regions with potential to produce above 7,000 kg ha^{-1} of grains and with high P content in the soil, to reduce or even suppress P fertilization.

ONION YIELD AS A FUNCTION OF DOSES AND SOURCES OF NITROGEN IN TOPDRESSING

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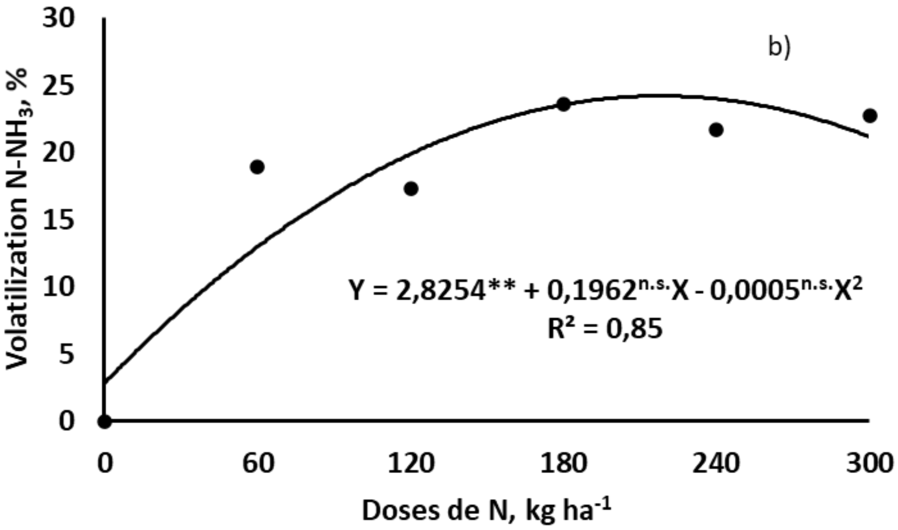
Keywords: Fertilizer; Nitrogen; Topdressing

INTRODUCTION

The onion crop represents social and economic importance role in the Brazilian scenario. Being cultivated by smallholders or in large plantations, the culture demands a lot of labor, presenting significant importance in the generation of jobs directly and indirectly. According to the results of the Brazilian Institute of Geography and Statistics (IBGE, 2020), Brazil, in 2020, reached the production of 1.495.618 tons of onions in an area of ??47.507 hectares, with an average yield of 31.495t ha^{-1}. The expansion of its cultivation in different regions of the country generates the need for research on the culture, especially its management (Rodrigues, 2014). According to Pôrto et al (2007), nitrogen is the second most important nutrient for onions after potassium. Keeping in view the importance of production as well as the response of the crop to nitrogen, the present study aimed to evaluate the productivity, contents and accumulation of nutrients in onions as a function of nitrogen sources and doses.

METHODS

To achieve the proposed objectives, the experimental fields were carried out at the Experimental Unit of IPACER - Instituto de Pesquisa Agrícola do Cerrado, located in the municipality of Rio Paranaíba, state of Minas Gerais. The crop of the installed installation, Aquarius variety, was planted on 01/26/2021 and harvested on 05/26/2021. The treatments were combined as follows: nitrogen doses combined with three sources (Ureia + NBPT, Ammonium Nitrate and Calcium Nitrate). In pre-sowing, all treatments received 80 kg/ha of fertilizer via fertilizer 04.36.06. The remaining doses of treatment plots of each were applied according to (Table 1).



The planting fertilizer used was 04-36-06 (except for the Control treatment), which contains 0.05% of B and 0.25% of Zn at a rate of 2,000 kg ha. 200 kg/ha of 00-00-58 fertilizer + 0.5% B was applied to

leaf 5 and bulbing, except for the two treatments (Control + Control N) totaling 120 kg/ha of K2O in Control N and 360 kg/ in the other treatments.

Sampling Observations

A randomized block with five replications. The plots were formed by an eight meter long bed in which three double rows of plants were arranged. The parameters evaluated were nutrient content and accumulation of onion in full vegetative growth and bulb filling and productivity and commercial classification of bulbs according to equatorial diameter. The data were submitted to analysis of variance and the means of the sources compared by the Scott-Knott test at 10%. Regression analysis was performed for the nitrogen dose factor.

RESULTS AND DISCUSSION

The nitrogen source factor was significant indicating that Calcium Nitrate resulted in higher maximum productivity. The amide source (Urea + NBPT) treated with urease inhibitor can control the nitrogen losses of this source by volatilization and result in performance similar to that obtained with nitric sources (not prone to volatilization loss), such as Ammonium Nitrate (Figure 2).

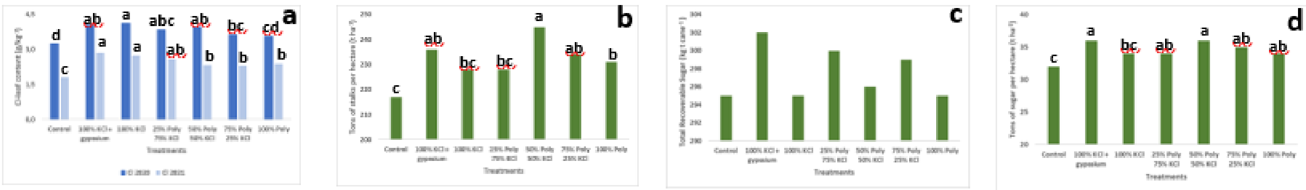


Fig. 2. Onion yield as response of sources and doses of nitrogen, 2021 crop season.

Dry matter accumulation was optimized with 185 or 167 kg/ha of nitrogen with application of nitrogen in coverage by Urea + NBPT or Calcium Nitrate, respectively. Most of the bulbs were classified in the noble classes (class 3, 3+ and 4), with no effect of the N source applied in onion cover.

Regarding the omission of topdressing, it reduced the production of larger diameter bulbs, which are more valued and have a greater impact on productivity gains.

CONCLUSIONS

It can be concluded that the higher increase in the variables analyzed was due to the sowing fertilizer. The nitrogen sources Urea + NBPT, Ammonium Nitrate and Calcium Nitrate resulted in bulb proportions in different commercial classes and similar onion yields. In relation to the maximum onion yields, they were reached with 207, 216 and 204 kg/ha of nitrogen when the sources of nitrogen in coverage were Urea + NBPT, Ammonium Nitrate and Calcium Nitrate respectively. This productivity was 6.89% higher when the calcium nitrate source was used as a top dressing.

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DEVELOPMENT OF BIODEGRADABLE PHYTOSIDEROPHORE ANALOG AS FE AND ZN FERTILIZER BOTH FOR POACEAE AND NON-POACEAE PLANTS

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Keywords: iron; zinc; phytosiderophore

INTRODUCTION

Iron (Fe) and zinc (Zn) deficiency is an important problem in agriculture, particularly in calcareous and alkaline soils. Synthetic chelating agents can be used to address this problem; however, these are often not biodegradable and are sometimes unsuitable for promoting Fe acquisition in Poaceae species because they absorb Fe-phytosiderophore complex through yellow stripe 1 (YSL) and YS1-like transporters. By contrast, mugineic acid family phytosiderophores (MAs) are highly biodegradable, although their ingredients are too expensive for large-scale synthesis. Therefore, in this study, we developed a synthetic chelating agent as an analog to proline deoxymugineic acid (PDMA) (Suzuki et al., 2021) in which deoxymugineic acid (DMA) is synthesized from L-proline instead of 2-azetidine carboxylic acid as described previously (Namba et al., 2007). Next, we examined the biodegradability, transport activity, and efficacy of PDMA for plant growth.

METHODS

The biodegradability rates of citrate, EDTA, and PDMA were evaluated in accordance with the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals (301A). Rice and pumpkin seedlings were transplanted to calcareous soil. PDMA and chelating agents for Fe or Zn were applied to the soil, and then Soil Plant Analysis Development (SPAD) values and metal concentrations were measured. YS1 and YSL transporter activity were measured using an oocyte assay. The reducibility of Fe-PDMA and other chelating agents was evaluated using Fe-deficient cucumber roots that were grown hydroponically (Ueno et al., 2021).

RESULTS and discussion

Biodegradivility

Compared with citrate, PDMA was biodegraded by microorganisms much less rapidly, which is likely to be due to differences in microbial community composition. By contrast, no biodegradation of EDTA was observed.

Efficacy of PDMA for Poaceae plants in calcareous soil

Rice plants suppled with Fe-PDMA recovered from Fe deficiency to a greater extent than those supplied with chelating agents and Fe complex. The application of metal-free PDMA to calcareous soil also led to recovery from Fe deficiency. The application of Zn-PDMA increased Zn concentrations, indicating that it also acts as a Zn fertilizer. Fe-PDMA was taken up through the OsYSL15, ZmYS1, and HvYS1 transporters. These findings suggest that PDMA has the same functions as natural MAs.

Efficacy of PDMA for non-Poaceae plants in calcareous soil

Pumpkin plants supplied with Fe-PDMA recovered from Fe deficiency to a greater extent than those supplied with Fe-EDDHA, according to increasing Fe and Zn concentrations in true leaves. A reducibility assay in cucumber roots showed that the PDMA-Fe reduction level was greater than that of EDTA-Fe and citrate-Fe under alkaline pH.

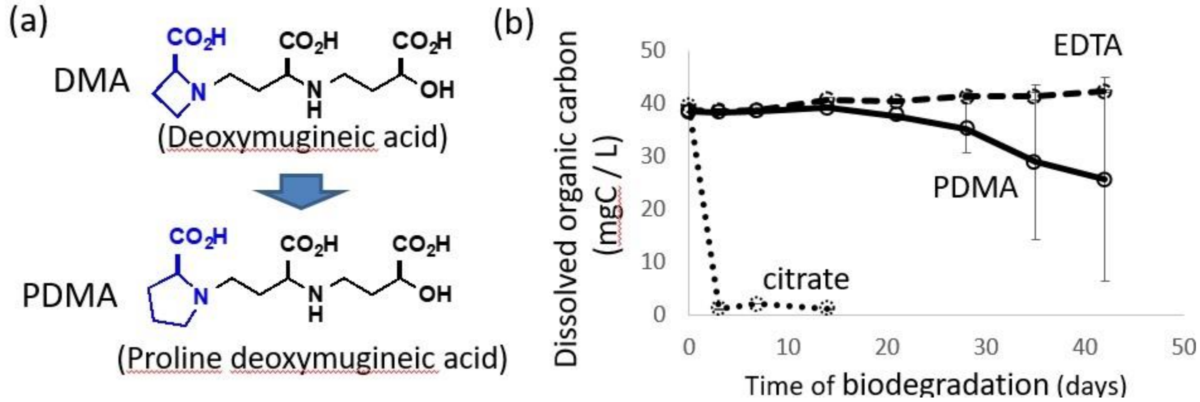

Fig. 1. Structure of PDMA and its biodegradability

(a) Proline deoxymugineic acid (PDMA) is analog of deoxymuginienc acid (DMA) and is syntheseized from L-proline. (b) Biodegradibilities of PDMA, citrate and EDTA in accordance with OECD301A.

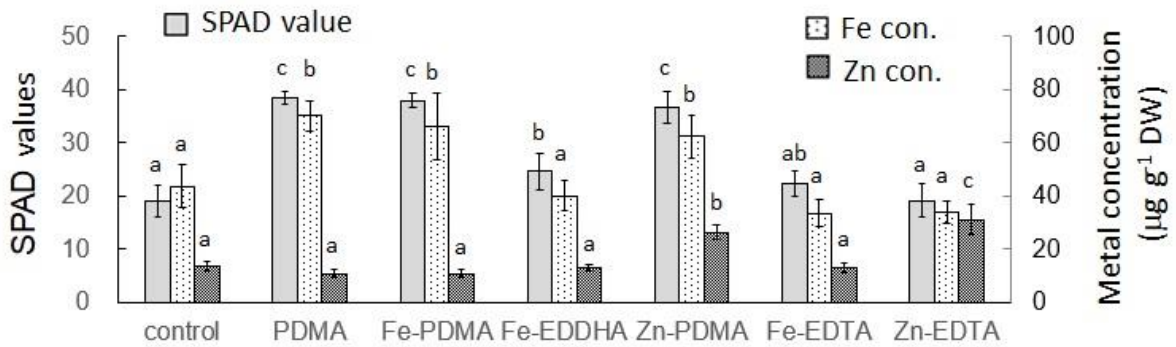

Fig. 2. SPAD values and metal concentrations in rice leaves with applications of chelate agents in calcareous soil

SPAD values of newest leaves were measured 7 days after a single application of chelate agents and their metal complexes. The leaeves were collected in same time, and metal concentrations were measured.

CONCLUSIONS

PDMA is biodegradable, is adapted to both strategy I and II Fe uptake systems, and chelates both Fe and Zn; therefore, it appears to be a promising micronutrient fertilizer.

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ORGANIC AMENDMENTS FOSTER A CARBON FARMING AND ENHANCE BIOLOGICAL PROPERTIES OF ANDISOLS

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Keywords: Organic amendments; Soil phosphatase activity; Carbon farming

Soil degradation have led to losses of 1.16 tons of soil organic carbon (SOC), 52.3 kg of nitrogen (N) and 0.28 kg of phosphorus (P) per hectare. In accordance, to increase productivity in degraded soils, fertilizer sources must be capable of improving both the biomass production and soil quality, as well as, to recover the potential productivity on degraded soils, which will contribute to ensure food security. To evaluate the long-term effect of organic and inorganic fertilizer source application on the physicochemical and biological soil quality indicators, five sites of pasture productions were sampled. Plant biomass, P concentration in shoots and roots, and lipid peroxidation in shoots were analyzed. In addition, soil organic matter, SOC, particle sizes, and phosphatase activity were measured. Organic fertilizers sources highgly increased phosphatase activity, SOC and SOM, as well as soil particle size. Moreover, soils under organic management were lower in lipid peroxidation as compared to inorganic fertilization, and similar biomass production was found. Thus, we can conclude organic amendments might be efficient to enhance both, crop production and favourable conditions for plant growth.

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UPGRADING CONVENTIONAL MAP AND MOP SOYBEAN FERTILIZATION WITH HIGH-TECH FERTILIZERS CONTAINING SULFUR AND BORON

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Keywords: Technology; Nutrition; Extended

INTRODUCTION

A large portion of soybean producers conventionally uses MAP (Ammonium Monophosphate) and MOP (Muriate of Potash) as a standard for fertilization of this crop. However, there are new developed technologies in fertilizers to improve crop nutrition and nutrients use efficiency, mainly in tropical agriculture. As example of improved technologies in fertilizers are the NPS sources, providing phosphorus, nitrogen and sulfur (S) in their composition. Microessentials©, a The Mosaic Company patented tecnology, presents S in two forms: sulfate (fast release) and elemental (gradual release). Aspire© is an another patented technology from Mosaic that includes two forms of boron (B), with fast and gradual release, in MOP granules. Both technologies guarantee extended nutrition and enhanced efficiency for nutrient delivery to plants.

In general, tropical soils have low S and B levels and present response to these nutrients inputs through fertilization. This occurs because such nutrients have high mobility in the soil, being susceptible to loss by leaching (Dantas, 1991 and Ghiberto, 2009). On the other hand, they are essential nutrients for soybean development and productivity. Whereas S participates in the activation of plant defense mechanisms, as well as protein synthesis (Broch, 2011), B is essential for plant growth, composing cell walls and is also important in the reproductive phase as it is essential for flower fertility and grain filling (da Silva, 2017).

The present research was developed to to evaluating the soybean yield and the crop production components related to the use of high technology fertilizers (which provide S and B in extended availability) compared to conventional fertilizers.

METHODS

Characterization of the experimental area

The experiment was carried out in Balsas-MA (latitude 8°25'29"S and longitude 46°38'10"W), average altitude of 448 m, in 2020/2021. The predominant climate in the region is Aw (Köppen).The soil is classified as Xanthic Ferralsol, with a history of 18 years of cultivation.

A survey of soil fertility and granulometry was carried out before the experiment was set up, using the methodology proposed by van Raij et al. (2001) and Embrapa (1997), at a depth of 0 to 0.20 m (Table 1).

Table 1. Chemical and textural characterization of the soil.

pH	MO	P	S	Al^{3+}	$H+Al^{3+}$	K	Ca	Mg	CEC	V	Fe	Mn	Zn	B	Sand	Clay
$CaCl_2$	g.dm^{-3}	mg.dm^{-3}			mmol$_c$.dm^{-3}					%		mg.dm^{-3}			%	
4,9	31,2	21	13	0	47	1,9	23	9,1	82	42	142	9,0	2,1	0,4	45	41

Assessments, statistical analysis and treatments

At harvest, ten plants were collected per experimental unit and the total number of pods per plant (NPP), of grains per plant (NGP), the average number of grains per pod (NGPOD) and the weight of 100 grains (W100) (13% wet base). Grain yield (GY) was determined by harvesting all plants in the useful area of the plot. The results were submitted to ANAVA using the F test and when significant ($p \leq 0.10$), means were compared using the LSD test ($p \leq 0.10$). The treatments and nutrient supply are described in table 2.

Table 2. Treatments (fertilizers and doses) and nutrient supply per treatment.

	Fertilization		Nutrient supply (kg.ha^{-1})				
Treat	**Sowing (V0)**	**Top dress (V4)**	**N**	**P_2O_5**	**K_2O**	**S**	**B**
T1	Control (no fertilizer)	Control (no fertilizer)	0	0	0	0	0
T2	Control (no fertilizer)	MOP (155 kg ha^{-1})	0	0	93,0	0	0
T3	MAP (180 kg ha^{-1})	MOP (155 kg ha^{-1})	19,8	93,6	93,0	0	0
T4	Microessentials© (200 kg ha^{-1})*	MOP (155 kg ha^{-1})	20	92,0	93,0	18	0
T5	Microessentials© (200 kg ha^{-1})	Aspire© (160 kg ha^{-1})**	20	92,0	92,8	18	0,8

*MicroEssentials© (monoammonium phosphate with sulfur in elemental and sulfate forms [(10-46-00-09), (N-P_2O_5-K_2O-S)]

*Aspire© (Muriate of Potash with Boron in Sodium Borate and Calcium Borate forms [(00-00-58-0.5), (N-P_2O_5-K_2O-B)]

RESULTS AND DISCUSSION

The treatments evaluated significantly influenced the NPP, NGP, NGPOD, W100 and GY (Table 3). The treatments without fertilization (T1) or with reduced fertilization (T2) provided the lowest values for the evaluated attributes, demonstrating the responsiveness of the environment to fertilization. NPP and NGP were higher in T5, however, for NGPOD, there was no statistically significant difference between treatments that received fertilizers. T5 can be highlighted as the most efficient treatment in promoting an increase in production (NPP, NGPOD and NGP) and soybean grain weight (W100), consequently providing higher GY (3918 kg ha^{-1}) compared to other technologies evaluated.

Table 3. Average values of, number of pods per plant (NPP), number of grains per pod (NGPOD), number of grains per plant (NGP), weight of 100 grains (M100) and grain yield (GY) of soybean as a function of the application of different fertilizers. Balsas/MA, 2020/2021 harvest.

	Fertilization		Assessments				
Treat.	**Sowing (V0)**	**Top dress (V4)**	**GY (kg.ha^{-1})**	**NPP (#)**	**NGP (#)**	**NGPOD (#)**	**W100 (g)**
T1	Control	Control	2814 c*	55,4 c	115 c	2,08 b	14,5 c
T2	Control	MOP	3377 b	61,8 ab	137 ab	2,23 a	15,1 b
T3	MAP	MOP	3341 b	56,6 bc	126 bc	2,23 a	15,8 a
T4	Microessentials©	MOP	3522 ab	59,6 abc	134 b	2,25 a	15,8 a
T5	Microessentials©	Aspire©	3918 a	65,0 a	147 a	2,27 a	15,9 a

Pr > Fc	-	-	0,012	0.072	0.006	0.0004	0.0021
CV(%)	-	-	10,3	7,76	7,48	1,96	2,69

*Means followed by the same letter are not significant different according to the LSD test ($p \leq 0.10$).

CONCLUSIONS

1. The addition of MicroEssentials© + Aspire© in soybean crop management, provided a relative gain in productivity of 1104 kg ha^{-1}, with a 39% increase in production when compared to T1 that did not receive any type of fertilization. 2. Even when compared to the conventional treatment most used by producers (T3 - MAP seeding (180 kg ha^{-1}) + KCl top dress (155 kg ha^{-1}), T5 was superior.

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NITROGEN MOBILITY INTO THE SOIL IN INTERACTION WITH DIFFERENT PHOSPHORUS CONCENTRATION IN ORGANOMINERAL FERTILIZER

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Keywords: Poultry Litter; relation; displacement

The mobility of inorganic nitrogen (N) in response to application of organomineral fertilizers in interaction of phosphorus (P) is changed around the granules in variable charge soils. The aim of this research was to evaluate the mobility of inorganic N in the form of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in a Typic Hapludox (Oxisol) in response to phosphorus (P) different concentrations in organomineral fertilizers. Incubation experiment in petri dishes was carried out in a completely randomized design, with six replications. Treatments were consisted of eight P doses (0; 0,7; 1,3; 1,8; 2,5; 3,5; 5 and 10 mg/dishes) present in organomineral fertilizers, besides the control (unfertilized) and urea (without P), with the N dose interaction in all fertilizers being fixed in 5 mg/dishes of total N. For the N analysis were sampled in petri dishes, the soil in concentric cylinders in four different distances from the central deposition point of fertilizers (0?7,75; 7,76?13,5; 13,51?25,5; and 25,51?43 mm) in five times of incubation (7, 14, 21, 28 and 35 days after fertilizer application). The P content in organomineral fertilizer and the doses applied interferes in the NH_4^+-N and NO_3^--N availability and displacement, and in the process of immobilization and nitrification, with different behavior to urea in the soil region around the fertilizers granules. Up to 21 days after fertilizers application there are predominance of N in the NH_4^+-N form with displacement up to section 2 (7,76?13,5 mm), while, the NO_3^--N form had a higher proportion from 28 days and shown mobility up to section 4 (25,51?43 mm). The higher availability of mineral N and displacement of mineral N of NH_4^+-N and NO_3^--N in soil occurs in the treatments with urea and organomineral in the P doses interaction of 0,7 mg/dishes, whereas, the lower contents are demonstrated in intermediate formulations in interactions doses of 2,5, 3,5 e 5 mg/dishes of P.

ORGANIC FERTILIZERS IN HYDROPONIC PLANT PRODUCTION

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Keywords: Hydroponics; Nutrients; Organic fertilizers

INTRODUCTION

Hydroponic growth systems are often used for indoor production of vegetables and herbs in areas with little arable land or water scarcity. Hydroponically grown crops are most often fertilized with inorganic fertilizers, but the use of various organic fertilizers have been studied to achieve a more circular and sustainable indoor plant production. However, the use of organic fertilizers in hydroponics is challenged by: *i)* a low availability of essential plant nutrients, *ii)* suboptimal ratios of essential plant nutrients, *iii)* a high content of non-essential elements and toxic compounds, *iv)* formation of biofilms on roots and, *v)* fluctuating pH values in the nutrient solution (Williams & Nelson 2016). Most nutrients in organic fertilizers are organically bound, and are thus unavailable for plant uptake. Optimal plant growth therefore depends on mineralization to increase nutrient availability. The addition of microorganisms via an inoculum, for example in the form of a soil extract, has previously shown to effectively increase nutrient availability in hydroponics (Shinohara *et al.* 2011).

METHODS

Two experiments were conducted to study the use of organic fertilizers in a hydroponic system. In the first experiment (Exp. 1), Pak Choi (*Brassica rapa* subsp. *chinensis)* was cultivated in hydroponics (aerated deep-water culture, 3 replicates per treatment) and fertilized with inorganic fertilizer or different levels of commercially available plant-based liquid organic fertilizer. Treatments were: inorganic fertilizer including all essential nutrients (NO_3^--N=40 mg/L) (IF), organic fertilizer with a total nitrogen (N) level corresponding to IF (OF1) and double amount of organic fertilizer (OF2). All plants were fertilized with inorganic fertilizer for the first 2 weeks after transplantation to the hydroponic system following induction of fertilizer treatments. Plants were harvested 38 days after transplanting and essential plant nutrients in dried leaves were measured using inductively coupled plasma optical emission spectroscopy and dumas combustion. In the second experiment (Exp. 2), the organic fertilizer was inoculated with an agricultural soil extract and left to incubate for 1 week. Plants were then fertilized with inorganic fertilizer (IF), organic fertilizer (OF2) or the incubated organic fertilizer (INOC) and were harvested 30 days after transplantation.

RESULTS AND DISCUSSION

Pak Choi with organic fertilizer corresponding to the N level in the inorganic fertilizer had stunted growth and clear visual symptoms of severe nutrient deficiencies (Fig. 1A). Plants that received twice as much organic fertilizer grew better, but shoot biomass was still significantly reduced compared to the IF treatment in both experiments. Tissue analysis revealed that plants were severely deficient in calcium (Ca) and magnesium (Mg), but had N and phosphorus (P) contents within normal levels (Table 1) (de Bang *et al.* 2021). In both OF treatments the roots were brown and had a biofilm but most severely in OF2. This may have negatively affected nutrient uptake. In the second experiment the INOC plants produced the same fresh weight as the IF plants, although they did show minor visual signs of deficiencies with beginning chlorosis on the lower leaves (Fig. 1B). Based on these findings, it appears that even a short incubation period, with a soil-derived inoculum, can effectively improve growth of Pak Choi in hydroponics with organic fertilizer. Results from further studies including incubation experiments and hydroponic growth of other plant species will be presented at the conference.

Fig 1. Pak Choi shoot biomass from experiment 1 (A) and experiment 2 (B). Treatments are inorganic fertilizer (IF), organic fertilizer (OF1-2) and organic fertilizer + inoculum (INOC).

Table 1. Fresh weight (FW) of shoots per plant for experiment 1 (Exp. 1) and experiment 2 (Exp. 2) and nutrient contents in dry matter from experiment 1. Values are averages ± SD (n=3). Statistically significant differences are shown by different letters (Tukey test, significance level=0.05).

Treatment	Shoot FW (Exp. 1)	Shoot FW (Exp. 2)	Nitrogen (Exp. 1)	Phosphorus (Exp. 1)	Calcium (Exp.1)	Magnesium (Exp. 1)
	(g/plant)	(g/plant)	%	µg/g	µg/g	µg/g
IF	82.2±3.4 [a]	62.9±5.3 [a]	2.8±0.6[a]	2845±625[b]	11273±2152[a]	3856±722[a]
OF1	24.6±1.1 [c]	-	2.6±0.6[a]	2974±325[b]	1996±661[b]	450±77[b]
OF2	38.5±4.7 [b]	27.3±1.4 [b]	3.5±0.7[a]	4418±648[a]	1574±310[b]	1069±125[b]
INOC	-	63.6±11.3 [a]	-	-	-	-

CONCLUSION

Plants can be grown hydroponically with liquid organic fertilizer, but to achieve high growth rates it is necessary to incubate the organic fertilizer with an inoculum (e.g., agricultural soil) to initiate mineralization and increase the availability of organically bound nutrients. It was shown that nitrogen is not always the main limiting nutrient when using organic fertilizer. Nitrogen contents of plants were within the normal range while plants fertilized with organic fertilizer were Mg and Ca deficient.

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MARANDU GRASS PRODUCTIVITY FERTILIZED WITH UREA TREATED WITH DUROMIDE TECHNOLOGY

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Keywords: Fertilizer; Nitrogen; Pasture

INTRODUCTION

The forages used for pastures are predominantly grasses in Brazilian agricultural production, and highly responsive to nitrogen fertilization. However, pastures still have a reduced share of fertilizer consumption in the Brazilian market, representing about 1.5% of the total (Francisco et al. 2017). On the other hand, due to the large territorial extension of the country occupied by forage grasses, the demand for fertilizers in the sector is growing and requires good agronomic practices and technology to improve the use of nitrogen by forages.

Urea is the most used nitrogen source to improve pasture productivity, mainly due to its low cost. However, it has a high susceptibility to volatilization loss that can reduce the production potential of crops (Faria et al., 2020).

NBPT [N-(n-butyl) thiophosphoric triamide] is a urease inhibitor commonly used as an additive in urea, which in acid soil pH conditions and temperatures above 30 °C has reduced efficiency. Duromide is a new urease inhibitor active principle, which consists of a newly developed molecule with a chemical function similar to that found in the NBPT molecule, which gives it the property of binding and inactivating the urease active site. However, it has a different chemical structure compared to NBPT, with radicals that make the molecule more stable, providing a greater protection in the most varied types of soil and climatic conditions, making it more efficient in inhibiting urease and, consequently, in the reduction of nitrogen loss by volatilization.

Considering the need to make nitrogen fertilization in pastures more efficient and increase forage production, a study was carried out to evaluate sources and rates of nitrogen fertilizers in pasture fertilization of Urochloa brizantha cv. Marandu grass and its effects on dry mass production.

METHODS

The experiment was carried out with *Urochloa brizantha* cv. Marandu, in the experimental field of the College of Technology and Agricultural Sciences, UNESP, in a dystrophic Ultisol. The experimental design was in randomized blocks with four replications, in a 3x2+1 factorial scheme, with three nitrogen sources (urea, ammonium nitrate and urea+Duromide), and two nitrogen rates (100 and 200 kg ha^{-1} $year^{-1}$), plus a treatment without nitrogen fertilization (control), totaling 28 plots. Nitrogen rates were divided into four applications, representing 25 and 50 kg ha^{-1} of nitrogen per application, distributed by broadcast and without incorporation into the soil, the first being 30 days after sowing and the others after each subsequent cut. Four cuts were performed, with intervals regulated when the best treatment reached 28 cm in height (95% light interception). The dry mass evaluation was as described by Silva; Queiroz (2002). Data were submitted to ANOVA and, when significant, the means were compared using the Tukey test ($p < 0,05$).

RESULTS AND DISCUSSION

There was a significant interaction between N rate and source in the accumulated production of Marandu grass. The highest dry matter productivity was verified for urea+Duromide, intermediate for ammonium nitrate and the lowest for urea, for the two N rates (Table 1). Productivity with urea+Duromide was 17% and 69% higher compared to ammonium nitrate and 52% and 78% compared to urea, respectively, for doses of 100 and 200 kg ha^{-1} of N.

The positive results in the production of dry mass in fertilization with urea+Duromide can be supported by the high volatilization of ammonia with the use of urea (Cassimiro, 2020) and possible losses of N-NO_3 by leaching in the application of ammonium nitrate. Regarding the doses, it was found that marandu grass is highly responsive in the production of dry mass with the supply of nitrogen in the management of fertilization. These results confirm the importance of nitrogen fertilization associated with fertilizer sources with incorporated technology to increase efficiency, to increase forage production (Patzlaff et al. 2020).

Table 1. Accumulated dry mass production in four cuts of Marandu grass, fertilized with three nitrogen sources at two rates. Agricultural year 2018/2019.

Sources of N	Nitrogen doses (kg ha^{-1})		
	0	100	200
	---------- kg ha^{-1} ----------		
Urea	5903B	5642Bc	8310Ab
Ammonium nitrate	5903C	7363Bb	8755Ab
Urea+Duromide	5903C	8599Ba	14798Aa

Means followed by distinct letters, lowercase in the columns and uppercase in the row, differ from each other by Tukey's test at 5% probability.

CONCLUSIONS

Urea+Duromide, resulted in higher productivity of Marandu grass dry mass in relation to the application of urea or ammonium nitrate, indicating that it is an important technology for pasture fertilization, and to increase the utilization of nitrogen by plants.

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GROWING COFFEE WITH POLY4 IN BRAZIL & COLOMBIA

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Keywords: Coffee; polyhalite; POLY4

Brazil is the biggest coffee producer globally whilst ranks Colombia third. Brazilian coffee is often cultivated in several classes of soils, mostly low natural fertility soils which are predominantly acidic, with high levels of Al and Mn and low soil organic matter, low available P, Ca, K, and micronutrients, and low base saturation. Crop nutrition is conventionally provided to coffee from a range of products, with K being supplied as KCl (MOP), K_2SO_4 (SOP), or KNO_3; Mg supplied as MgO or $MgSO_4$, and Ca as $CaSO_4$ (gypsum) or calcium ammonium nitrate (CAN). These nutrients can also be supplied from POLY4, which is a product that provides low-chloride potassium, magnesium, sulphate-sulphur and calcium. The use of POLY4 can simplify the fertilizer programme and provide plant available nutrients throughout the growing season. The response of coffee yield to POLY4 programmes was compared to standard fertilizer programmes between 2016 and 2020 at two locations in Colombia and at three locations in Minas Gerais, the largest coffee producing state of Brazil. In both countries the high-value crop responded well to the nutrients provided in POLY4. Using POLY4 increased median yield by 2.8 bags/ha (168 kg/ha) in Brazil and by 3.33 bags/ha (200 kg/ha) in Colombia when compared to local NPK practices. In Colombia, POLY4 was also compared to MOP+MgO+Gypsum where it increased yield by 3.25 bags/ha (195 kg/ha).

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POLY4 is the trademark name for the polyhalite fertiliser from Anglo American's Crop Nutrients business. Trials were conducted by independent researchers from Cenicafe in Colombia, and Instituto de Pesquisa Agrícola do Cerrado and Universidade Federal de Lavras in Brazil.

EFFECTS OF NICKEL FERTILIZATION ON SOYBEAN PHOTOSYNTHETIC APPARATUS

Luiz Gustavo Moretti [1]; Carlos Alexandre Costa Crusciol [2]; João William Bossolani [1]; José Roberto Portugal [1]; Mariley Fonseca [1]; Mariangela Hungria [3]

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Keywords: Glycine Max (L.) Merrill; Micronutrient; Plant Nutrition

INTRODUCTION

Nickel (Ni), a cofactor for urease and hydrogenase, is the most recent micronutrient to be recognized as an essential element for plants. However, there are no reports of Ni deficiency in annual species grown under field conditions, possibly because such a deficiency is hidden (or latent) and lacks obvious and distinct symptoms (Lavres et al., 2016). In Brazil, soybean [*Glycine max* (L.) Merrill] cultivation is highly dependent on biological nitrogen fixation (BNF), in which Ni plays a fundamental role (Macedo et al., 2016). The treatment of seeds with other micronutrients that are essential to BNF, such as cobalt and molybdenum, and annual inoculation with strains of *Bradyrhizobium* spp. are widespread practices in Brazil, but information on the potential role of Ni is scarce (Freitas et al., 2018). Given the possibility of hidden Ni deficiency, we hypothesize that fertilization with Ni will optimize the efficiency of BNF and plant photosynthetic parameters.

MATERIAL AND METHODS

Site description, experimental design and treatments

The field experiment was carried out in the 2021/2022 growing season with soybean at the Lageado Experimental Farm of São Paulo State University (UNESP), located in the municipality of Botucatu in the southeastern region of São Paulo State, Brazil (48°26'W, 22°51'S, elevation of 786 m above sea level), and the soil is classified as a Oxisol. According to the Koppen-Geiger climatic classification system, the region has a mesothermal climate (Cwa), that is, a humid subtropical climate with dry winters and hot summers. The average rainfall is ~1,360 mm year^{-1}, and the mean annual air temperature is 20.7°C (50-year average). The experiment was performed in a randomized complete block (RCB) design with six treatments and four replicates. The doses of Ni (0;3; 6; 9; 12 and 15 g ha^{-1}) were applied via seeds in the form of nickel chloride ($NiCl_2$).

Gas exchange

Gas exchange was evaluated via nondestructive analysis with a portable gas exchange device - infrared gas analyzer (IRGA). Samples were taken from the central leaflet of the third fully expanded leaves and intact trifoliate leaf from the plant apex of the main stem of 10 plants per plot. The parameters of the instrument were as follows: 380-400 mol mol^{-1} atmospheric CO_2, 1100 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) supplied by LED lamps, 25-27°C leaf chamber temperature, and 60-70% relative humidity. The minimum equilibration time for each set of measurements was 3 min. The measurements were performed between 10:00 and 12:00 am. The following parameters were determined: net photosynthesis rate (*A*; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gS*; mol H2O m^{-2} s^{-1}), internal CO_2 concentration in the substomatal cavity (*Ci*; μmol mol^{-1}), and transpiration (*E*; mmol H_2O m^{-2} s^{-1}).

Statistical Analyses

The normality and homoscedasticity of the data in all datasets were checked using the Anderson-Darling test, and homoscedasticity was analyzed with the variance equation test (or Levene's test). Subsequently, the means were subjected to analysis of individual variance (ANOVA) by the F test ($p \leq 0.05$) and, when significant, were analyzed using the Fisher's protected least significant difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Most studies in the literature associating Ni and photosynthesis are related to its toxic efect by limiting the electron transport chain, damaging photosystems, disrupting chloroplast structure, decreasing the synthesis of chlorophyll (Polacco et al. 2013), and inactivating the Calvin cycle enzymes (Reis et al. 2017). However, some studies with corn and oat reported increased photosynthetic rate of plants mainly due to an increase in photosynthetic pigment content. In the present study, the application of the Ni doses via seeds influenced positively the net photosynthetic rate (increased significantly ~12.3%) and internal CO_2 concentration in the substomatal chamber (decreases significantly by up to ~14.1%), when compared to treatments with fertilization equal to or greater than 9 g Ni ha^{-1}, in relation to plants with lower fertilization or no fertilization. The lower *Ci* reflects the higher C-fixation by the photosynthetic process, marked by the high *A*.

Table 1. Net photosynthetic rate (A), stomatal conductance (gS), internal CO_2 concentration in the substomatal chamber (Ci), and transpiration (*E*) of soybean leaves at the R_2 reproductive stage as a function Ni fertilization.

Doses	Net photosynthesis rate (*A*)	Stomatal Conductance (*gS*)	[CO_2] Substomatal	Transpiration (*E*)
	μmol CO_2 m^{-2} s^{-1}	μmol mol^{-1}	mmol H_2O m^{-2} s^{-1}	
0	16.3 b	198	270 a	3.5
3	16.9 b	203	268 a	3.4
6	17.1 b	205	253 a	3.5
9	18.3 a	197	232 b	3.2
12	18.5 a	208	235 b	3.2
15	18.9 a	210	229 b	3.1
C.V.	12.1	9.8	13.2	6.3
$p \leq$	0.05	^{ns}	0.01	^{ns}

Means followed by the same letter do not differ (^{ns}) by the LSD test (Fisher's least significant difference) at $p \leq 0.05$ and $p \leq 0.01$ probability.

CONCLUSIONS

There was no significant effect of treatments on the transpiration and stomatal conductance in the leaves of soybean. The best results for net photosynthetic rate and internal CO_2 concentration in the substomatal chamber for soybean crop were similarly observed from 9 g Ni ha^{-1}.

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Nutrient availability in soils, toxicity and remediation

EFFECTS OF SUMMER AND WINTER CROP ROTATIONS ON CROP YIELD OF THE SUCCEEDING WINTER CHICKPEA (*CICER ARIETINUM,* L) AND SOIL PROPERTIES

Keywords: chickpea; soil properties; crop rotation

INTRODUCTION

Rainfed crop yields are generally low in South Africa due to low and erratic rainfall coupled with poor soil fertility; these together with a rapidly growing population (United Nations, 2019) and climate change (Godfrey and Tunhama, 2020) pose a serious threat to food security. Therefore, there is a need for agricultural technologies that play an important role in conserving moisture and improving and maintaining soil quality. Cropping systems such as crop rotation may result in higher and stable crop yields, save production costs, and increase carbon inputs into the soil. The residual effects of summer crops on winter-chickpea crop and soil properties in this region is unknown. Therefore, the study assessed the residual effects of summer crops (and fallow) on the succeeding winter crop and soil properties.

METHODS

The study is based on the first (2017) and second (2018) winter seasons, focusing on chickpea, of a long-term summer and winter crop rotation field experiment established at Thohoyandou (22° 58' S and 30° 26' E, and 595 masl), South Africa in summer 2016/2017. Treatments consisted of summer crops [maize (*Zea mays*), bambara groundnut (*Vigna subterranea*), cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*)], fallow and winter crops [chickpea (*Cicer arietinum*) - mustard (*Brassica rapa*)]. Three soil samples were collected randomly from two depths (0-20 and 20-40cm) in each plot. Grain yield and yield components were determined at harvest maturity. The data were subjected to analysis of variance, and independent samples t-test was used to compare the differences between the two winter seasons, and soil depths.

RESULTS AND DISCUSSION

Summer rotations had no effects on selected soil chemical properties, and grain yield and yield components. However, an independent t-test revealed greater soil $_{NO3}^{-}$ values in winter 2018 compared to winter 2017 (Fig. 1). A similar trend was observed with P, $_{NH4}^{+}$ and SOC; where greater mean soil P, $_{NH4}^{+}$ and SOC were recorded in the second compared to the first winter season. Our results show that are consistent with previous findings that the effects of crop rotation on soil properties tend to be long-term and are unlikely to be observed during the first few years of rotation (Bassegio et al., 2015; Rosa et al., 2009).

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Conclusion

Treatments had no significant effects on selected soil properties and crop yield of the succeeding winter chickpea. However, greater soil NO_3^- , NH_4^+, P and SOC were observed in the second compared to the first winter season. Clearly, the importance of long?term experiments to assess the productivity and sustainability of crop rotational systems cannot be gainsaid.

ACKNOWLEDGEMENTS

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PHOSPHORUS SEQUENTIAL EXTRACTIONS IN SOIL AS WAY TO EVALUATE SLOW RELEASE FERTILISER: MEHLICH 3

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Keywords: monoammonium phosphate; kimcoat; Ferrasols

INTRODUCTION

The correct management of fertilization ensures an increase in the efficiency of fertilizers and is consequently a guarantee of food security and reduction of the risks of environmental contamination. In this sense, some technologies are being associated with fertilizers to increase the efficiency of nutrient use with the reduction of solubilization. In tropical soils, due to the high adsorption capacity on the surface of inorganic colloids and precipitation with Fe, Al and Ca in solution, the doses of phosphate fertilizers are up to four to five times more than the total exported by the crops.

To reduce losses it is necessary to use technologies that reduce the solubilization of fertilizer and thereby increase the use efficiency by plants throughout the cycle and reduce adsorption and precipitation in the soil. However, there is no routine laboratory way that can prove whether or not there is the slower release of phosphate fertilizer.

In this sense, sequential xxtraction of phosphorus by the Mehlich 3 way was evaluated in a dystrophic Latossolo Vermelho distroférrico with the application of monoammonium phosphate with and without protection to validate the method as a technique to evaluate whether or not there is a reduction in fertilizer solubilization.

METHODS

Soil samples were collected at a depth of 0 to 0.1 m after 15 days of sowing fertilization to perform sequential extractiond at a depth of 0 to 0.1 m after 15 days of planting fertilization to perform sequential extraction. The samples were collected in a field experiment in the 2020/2021 season with the broadcast phosphate fertilizer.

The experimental design was randomized in a factorial scheme 5 x 2 + 1 and, five doses of P (23.5, 47.0, 70.5, 94.0 and 117.5 kg ha$^{?1}$ of P) and two sources (monoammonium phosphate - 11% N and 22.3% of P and monoammonium Kimcoat phosphate - 10% Of N and 21.8% of P) and one treatment without phosphate fertilization (Control). Before the experiment, soil sampling was carried out at a depth of 0 to 0.1 m for chemical characterization: pH ($CaCl_2$): 5.9; P - Mehlich 3: 21.8 mg dm^{-3}; P - Mehlich 1: 5.81 mg dm^{-3}; K - Mehlich 1: 202.8 mg dm^{-3}; Ca: 5.01 cmol$_c$ dm^{-3}; Mg: 2.92 cmol$_c$ dm^{-3}; Al: 0.02 cmol$_c$ dm^{-3}; H+Al: 3.27 cmol$_c$ dm^{-3}; MO: 51.9 dag dm^{-3}; Arliga: 556.4 dag kg^{-1}; Remanescent P - 37.39 mg dm^{-3}; maximum phosphorus adsorption capacity (MPAC), by Langmuir isotherm: 0.64 mg g^{-1}.

Phosphorus (P) extraction was performed using the Mehlich 3 (Mehlich 2008) extractor in 1:10 soil:extractor solution ratio. The P was determined by colorimetric way (Murphy and Riley 1962).

Decreasing exponential models have been adjusted from P-Mehlich 3 contents as a function of extractions in Sigmaplot v.11.0 and estimated the inflection points (IP) of each model.

RESULTS AND DISCUSSION

The adjustment of exponential equation models with sequential extractions of P is already used to understand part of the processes of transformationof P in the soil. The inflection points (IP) are associated with higher or lower P adsorption capacity in the soil, when the lower the IP greater the P adsorption capacity in the soil, and the lower the availability of the nutrient for the plants (NOVAIS and SMYTH 1999). As in the present study there was no variation in MPAC (0.62 mg g-1), the alterations that occurred were nthe available P contents with the doses and the difference between the sources.

At 15 days after fertilization, the highest levels of available P were obtained with the application of MAP-Kimcoat, which can be explained by the reduction in soil losses. The IP of exponential models adjusted to sequential P extractions is equivalent to the restriction to soil P desorption (NOVAIS and SMYTH 1999). The lower the IP, the greater the P release restriction in the soil solution. In the experiment, the higher the P dose, the higher the IP, i.e., the higher the P dose, the lower the restriction of P availability in the soil (Fig. 1). When comparing the sources at the same dose, the highest IP values were obtained when MAP-Kimcoat was used (Fig.1). Thus, the higher the dose and when the MAP-Kimcoat is used, the lower the restriction in the availability of P in the evaluated soil.

The use of IP of exponential equations adjusted for the p contents available in the soil using the Mehlich 3 extractor as a function of sequential extractions proved to be a good indicator to evaluate the restriction of P availability in the soil for the plants, being a good indicator for the evaluation of technoilogies to increase the efficiency of phosphate fertilizers.

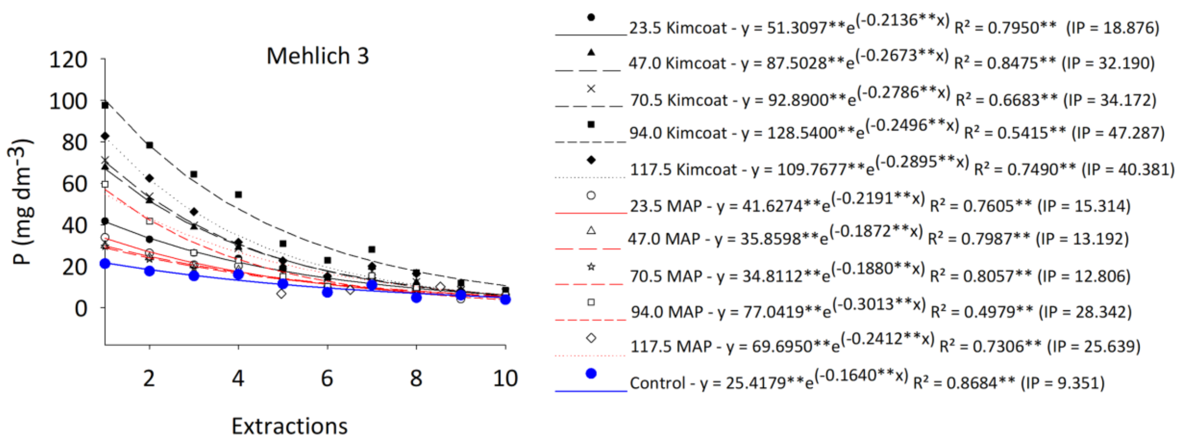

Fig. 1. Exponential models of soil P (Mehlich 3) available as a function of sequential extractions and inflection points (IP) of each equation in a Latossolo Vermelho Distroférrico (Ferrasol) with different P doses (23.5; 47.0; 70.5; 94.0 and 117.5 kg ha-1) and soruces (monoammonium phosphate - MAP and protected MAP - Kimcoat).

CONCLUSIONS

The highest values of inflection points, or lower restriction of P availability, were obtained with the higher phosphorus doses and with the use of MAP-Kimcoat.

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DYNAMICS AND AVAILABILITY OF CALCIUM, MAGNESIUM AND POTASSIUM APPLIED BY DIFFERENT SOURCES IN A CLAY SOIL

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Keywords: POLY4; Polyhalite; Nutrient Availability

INTRODUCTION

The POLY4 product is mainly produced with Polyaline ore. In the United States, in the early 1930s, there was strong interest in Polyaline (POLY), due to the fact that it is the most abundant mineral present in salt deposits. Since then, POLY has not been fully commercially available as fertilizer and, consequently, little information has been published on poly's performance as fertilizer for agricultural production. However, the potassium fertilizer industry is changing again, and the POLY product is emerging as a potential source of fertilizer (POLY4 includes four of the six main macronutrients: potassium, sulfur, magnesium and calcium).

In that sense, the availability of these nutrients will be verified in two tropical soil profile (segmented soil column) (sandy and clay texture), a benchmark soil of the region of the São Paulo state, over a certain period of time (8 months) under the rainfall regime of the region (a simulated rainfall application); and compare POLY4 fertilizer with KCl + Gypsum, KCl and Calcium Nitrate ($Ca(NO_3)_2$), seeking to understand the dynamics and transport of potassium (K), magnesium (Mg) and calcium (Ca) ions.

METHODS

Experimental Set up

The research proposal was carried out with Sirius Minerals at the Luiz de Queiroz College of Agriculture - ESALQ / USP, Department of Biosystems Engineering, located in Piracicaba, São Paulo, Brazil. The mais stage was monitoring the "availability" of nutrients under sectioned soil columns condition of unsaturated soil (sandy and clay texture) (simulating a soil profile).

Nutrient Distribution Over Time

Altogether, 24 sectioned soil columns of 5 cm in diameter and 70 cm in height, sectioned in 10 rings with 7 cm high shall be used (Figure 1). Comparisons will be made between POLY4 (3 replicates for each soil type), with (KCl + Gypsum) (3 replicates for each soil type), KCl (3 replicates for each soil type) and Ca(NO3)2 (3 replicates for each soil type).The columns will have the soils under a non-saturated condition and will be in a rest period, under laboratory conditions, for a period of 6 months, so that the process of solubilization of fertilizers in the soil occurs and for interactions with the chemical exchange complex with the soil material. After assembling the soil columns with the previously incorporated fertilizers, a water depth corresponding to the average precipitation in the rainy season of the Piracicaba region will be applied. The application of water slides will follow a distribution of rainfall regime in the region of Piracicaba, SP. Rainfall data from the Piracicaba region from 1917 to 2020 were analyzed. At the end, to simulate the rainfall regime, the application of 86 mL to each soil column every week was defined.

RESULTS AND DISCUSSION

In the segmented soil column profile from 7 to 70 cm, the average value of calcium concentration in the soil, applied by POLY4, was 18.8533 mmolc dm^{-3} (sandy soil) and 30.85 mmolc dm^{-3} (clay soil). In the case of KCl + Gypsum, the concentrations found were 19.12 mmolc dm^{-3} (sandy soil) and 34.2933 mmolc dm^{-3} (clay soil). In this case, the difference was not significant for a sandy soil (only 1.4% difference), being a little more evident, in KCl + Gypsum, in the calcium supply to the clay soil. Analyzing average values in the soil profile from 7 to 70 cm of segmented soil columns, the magnesium concentration, applied by POLY4, was 6.9467 mmolc dm^{-3} (sandy soil) and 9.91 mmolc dm^{-3} (clay soil). The KCl + Gypsum, the concentrations founded were 6.4667 mmolc dm^{-3} (sandy soil) and 9.0267 mmolc dm^{-3} (clay soil). Evidencing the efficiency of POLY4 over KCl + Gypsum, in terms of 7% more in sandy soil and 9.7% more in clay soil. By the results from the segmented soil column from 7 to 70 cm, in average terms, potassium concentration, applied by POLY4, was 1.54 mmolc dm^{-3} (sandy soil) and 1.39 mmolc dm^{-3} (clay soil). For KCl + Gypsum, the concentrations were 1.45 mmolc dm^{-3} (sandy soil) and 1,33 mmolc dm^{-3} (clay soil). Evidencing the efficiency of POLY4 over KCl + Gypsum, in terms of 6% more in sandy soil and 4% more in clay soil.

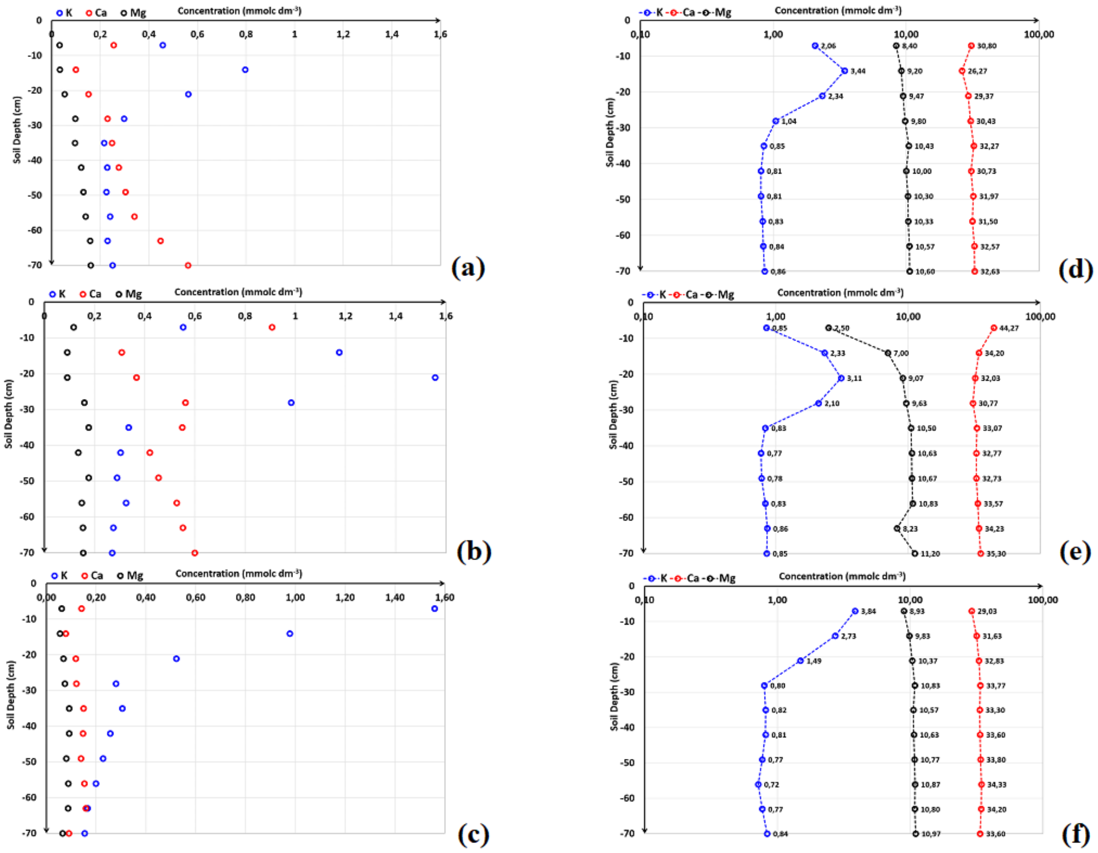

Fig. 1. Distribution of potassium, calcium and magnesium applied by different sources in a sandy soil: (a) POLY4 (soil solution), (b) KCl + Gypsum (soil solution)and (c) KCl (soil solution). (d) POLY4 (Soil Exchangeable), (e) KCl + Gypsum (Soil Exchangeable) and (f) KCl (Soil Exchangeable).

CONCLUSIONS

POLY4 when compared to KCl + Gypsum and KCl, keeps an excellent performance in nutrient availability in a clay soil profile.

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The authors would like to thank AngloAmerican for the financial support and the team of the Laboratory of Soil Physics of the Department of Biosystems Engineering, Luiz de Queiroz College of Agriculture (ESALQ/USP).

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Lino Ricardo Rios Furia [3]; Bruna Marques de Queiroz [2]; Isabela Klefenz Rabello de Oliveira [2]; Rafaela Klefenz Rabello de Oliveira [2]; Jarbas Honório de Miranda [1]

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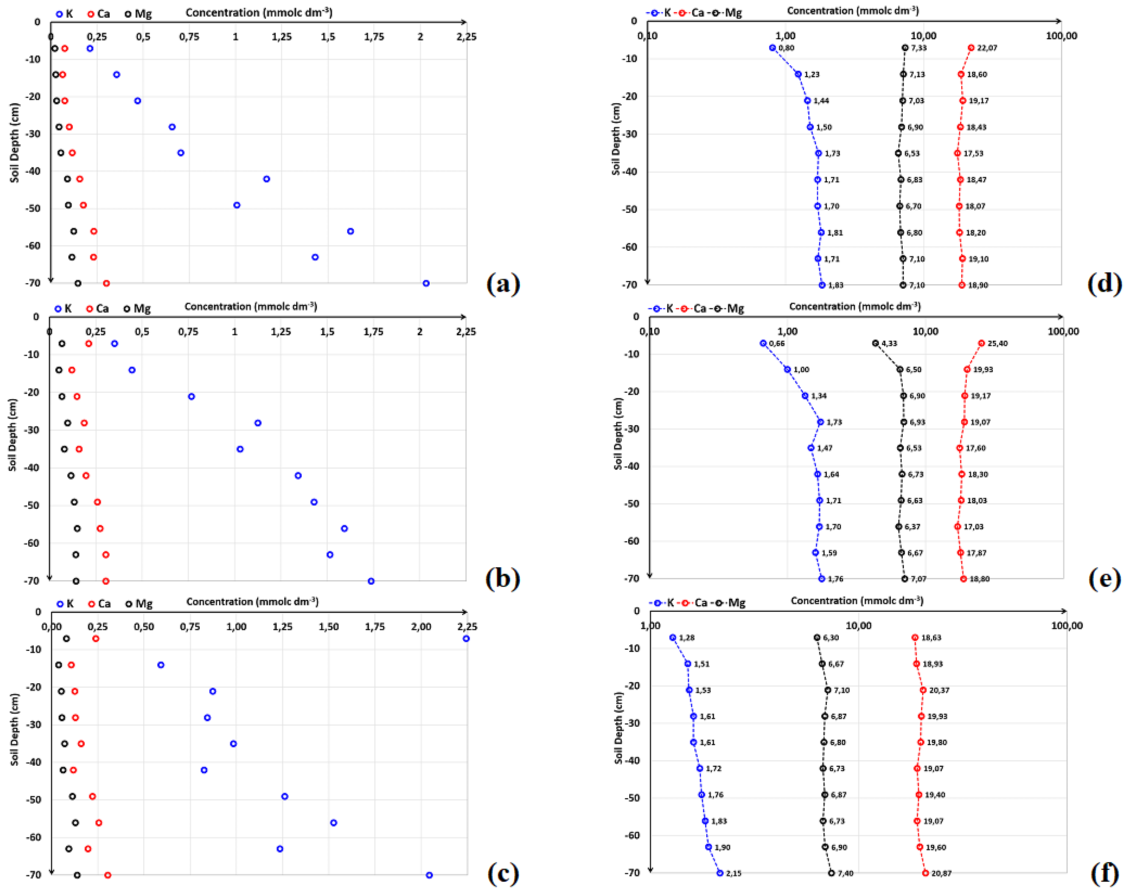

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ALTERNATIVE SPLICING IN RICE LEAVES EXPOSED TO IRON EXCESS

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Keywords: Iron; Stress; Splicing

INTRODUCTION

Iron (Fe) is a micronutrient required for plant metabolism. It acts as cofactor for several enzymes involved in vital processes, such as respiration and photosynthesis. However, when in excess, Fe can result in the production of reactive oxygen species due to the occurrence of Fenton's reactions. In this context, Fe toxicity is a common nutritional disorder found in flooded soils, affecting rice (*Oryza sativa*) plants and consequently their productivity (Aung & Masuda, 2020). Within the *Oryza* genus, *O. meridionalis* is a wild species that has potential as a source for Fe tolerance gene discovery because of its natural tolerance to Fe toxicity (Bierschenck *et al*., 2020). An introgression line generated from crosses of *O. sativa* with *O. meridionalis* showed Fe toxicity tolerance (Wairich *et al.*, 2021). In order to identify possible candidate genes for Fe tolerance, plants of parents and the introgression line were exposed to Fe excess, and transcriptomic data was generated from leaves. Here, we used the data to identify differential gene expression and alternative splicing, which could be related to Fe toxicity response among genotypes with different Fe tolerance levels.

METHODS

Plant growth, RNA extraction and transcriptomic analyses were performed as described by Wairich *et al*. (2021). Seeds of the introgression line CM23 and its parental backgrounds, *O. sativa* cv. Curinga and *O. meridionalis* Ng. accession W2112, were germinated, and seedlings were grown in meshes floating on solution containing 0.5 mM $CaCl_2$ and 10 µM $FeCl_3$ for 2 weeks at 28 °C. The solution was renewed twice a week. Eight seedlings per genotype per treatment were transplanted into 60-liter containers, each containing 40 seedlings, filled with nutrient solution (Yoshida *et al.*, 1976). The pH was adjusted to 5.5 every 2 days. After 3 weeks of growth, plants were exposed to Fe excess treatment (+Fe; 4.475 mM) or remained in control condition (CC) for 15 days.

Leaf samples for RNA extraction and transcriptomic analysis were harvested 7 days after the onset of the experiment. RNAs derived from three biological replicates were used to generate the sequencing libraries. Differential gene expression (DE), differential alternative splicing (DAS) and differential transcript usage (DTU) were determined according to Calixto *et al*. (2018).

RESULTS AND DISCUSSION

Out of the 3824 genes that were significant DE and/or DAS, 2% (85 genes) were differentially alternatively spliced in response to Fe excess (Fig. 1A). 11 DAS genes were also DE genes (differentially expressed and alternatively spliced genes) and 74 were not DE (regulated by AS only). Among these DAS-only genes, we highlight LOC_Os03g05390 (Os03g0147400; Fig. 1B) and LOC_Os12g37530 (Os12g0562100; Fig. 1C). The first one, also named Zebra3, encodes a putative citrate transporter (Kim *et al*., 2018). Citrate has a crucial role in root-to-shoot Fe translocation, being the main Fe chelator in the xylem sap (Wu *et al*., 2018). It is plausible that changes in citrate levels are relevant for tolerance to Fe toxicity. The second gene is a predicted Fe-regulated metal cation transporter, belonging to the Ferroportin (FPN) protein family. FPN proteins are involved in mobilization of Fe into the vacuole or efflux from xylem parenchyma cell, therefore regulating Fe root-to-shoot translocation (Morrissey *et al*., 2009). Wairich *et al*. (2021) observed that CM23 plants showed higher Fe contents in leaf blades and culms in comparison with its parental backgrounds. Thus, our results indicate the occurrence of chelation and/or compartmentalization as strategies to avoid Fe toxicity in the aerial parts of the introgression line plants.

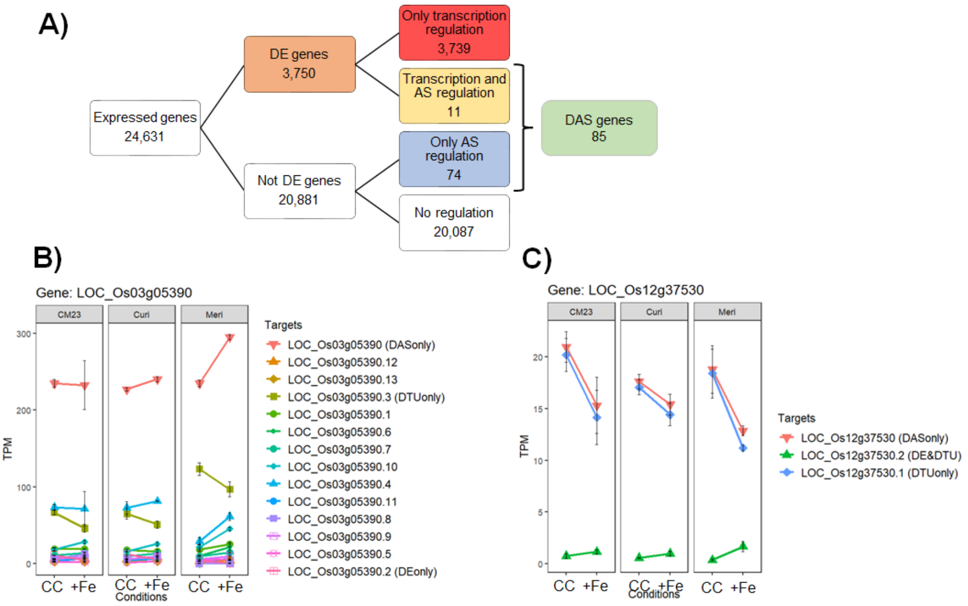

Figure 1. DE and DAS Analyses of Rice Response to Fe excess. A) Flow chart showing the distribution of the 24,631 expressed genes. B) Abundance plot of LOC_Os03g05390 C) Abundance plot of LOC_Os12g37530

CONCLUSIONS

We found AS regulation in dozens of rice genes upon Fe excess. We plan to follow up these findings with gene expression experiments to confirm Fe regulation by AS of these two candidate transporter genes, and explore their possible roles in Fe tolerance in rice.

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INCREASED SOIL MOBILITY AND PLANT AVAILABILITY OF PHOSPHORUS FERTILIZERS BASED ON FUNCTIONALIZED NANOPARTICLES

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Keywords: Nanofertilizer; Hydroxyapatite; Rhizosphere

INTRODUCTION

Phosphorus (P) fertilizers are applied in agricultural systems worldwide to achieve optimal crop growth. However, P is a soil immobile nutrient, which moves primarily via diffusion and rapidly engages in complexation reactions with soil components. As a result, many fertilizers have a low P use efficiency (PUE) and are often applied in excess to ensure sufficient plant availability. As P generally accumulates in soils receiving a high input of fertilizer, a common externality of P application is eutrophication of surface waters due to P losses from the fields. The current fertilization practices are thus both costly and unsustainable, and even more so considering that P is a non-renewable resource. Hence, improved PUE is essential to secure the nutrient supply for future crop production.

A potential solution to this P-problem could be found within the field of bio-nanotechnology. In this study, hydroxyapatite (HAP), which is a calcium phosphate mineral, was subjected to nanosizing and surface modification using citrate, in order to create a P fertilizer with improved soil mobility. Modification of the physicochemical properties of HAP, allows it to remain as intact nanoparticles (NPs) and bypass the mechanisms that would otherwise inhibit mobility of phosphate ions in the soil. Reducing soil adsorption of P and allowing it to move as NPs via mass flow could increase the plant availabity and potentially reduce P fertilizer application rates.

METHODS

Synthesis and characterization of hydroxyapatite nanoparticles

Nanohydroxyapatite (nHAP) particles were synthesized and prepared according to the multi-step method described by Szameitat *et al.*[1]. The physicochemical properties of nHAP (i.e. size, zeta potential, morphology and elemental composition) was measured using various techniques (dynamic light scattering (DLS), transmission electron microscopy (TEM) and inductively coupled plasma mass spectrometry (ICP-MS)).

Soil columns and rhizoboxes

To examine the soil mobility of different P fertilizers, a packed column study was conducted. Glass columns (15 mm x 185 mm) were packed with six different soils and received P either in the form of a water soluble phosphate salt (K-PO_4) or nHAP (citrate-stabilized or unstabilized). Water was pumped through the columns for 24 hours and the P concentration in the leachates were determined using a colorimetric molybdenum-blue assay.

The soil mobility of nHAP and a conventional P fertilizer, triple-superphosphate (TSP), was also compared in a rhizobox setup with spring barley (*Hordeum vulgare*). P-33 isotopes were incorporated into the fertilizers and their movement in the soil over time was detected with a Phosphor imaging screen.

Greenhouse pot experiment

The bioavailability of nHAP vs. K-PO_4 was examined in a pot experiment where spring barley was cultivated for six weeks in a greenhouse. A root-impermeable polyester filter was inserted in the soil to prevent direct contact between root and fertilizer, unless the fertilizer displayed mobility and migrated across the filter. Chlorophyll *a* fluorescence was used as a tool for predicting the P-status of the plants and inductively coupled plasma optical emission spectrocopy (ICP-OES) was used to measure the P content of the biomass. Other growth parameters (number of tillers, growth stage, height, dry weight) were also measured.

RESULTS AND DISCUSSION

In the soil columns, nHAP was more mobile than K-PO_4 in five of the six soils and they were equally mobile in the sixth soil. K-PO_4 was retained according to the P saturation of the soil, which depended on previous fertilizer applications, whereas nHAP was less affected by the soil's P status. The leaching profiles (fig. 1) were remarkably different, implying that nHAP did not dissolve into phosphate ions but remained largely intact as particles.

In the pot experiment, nHAP and K-PO_4 applied above the polyester filter, were equally efficient as P fertilizers, whereas nHAP was more efficient than K-PO_4 when applied below the filter. Similarly, the rhizobox experiments revealed that nHAP was more mobile than TSP.

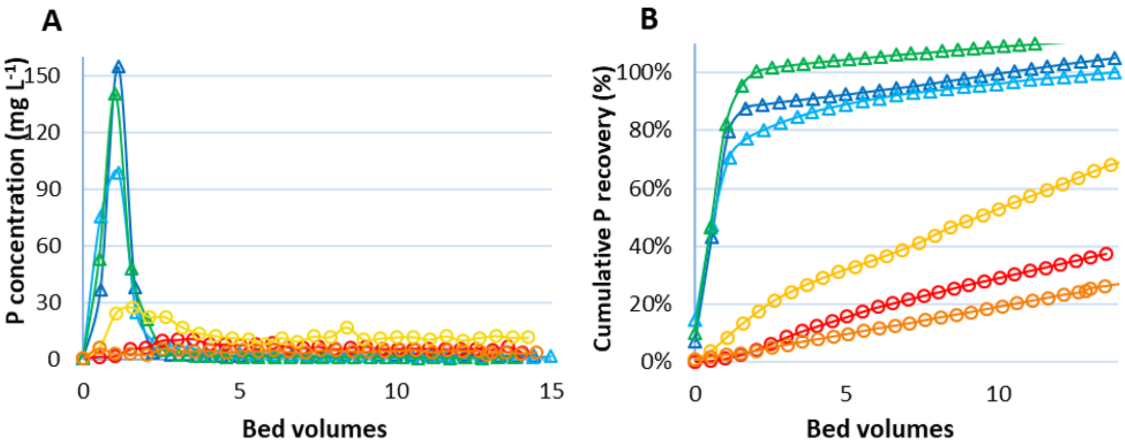

Fig. 1. A) P concentration [mg/L] and B) cumulative P recovery [%] in leachates as a function of bed volumes of water pumped through the soil columns. Results shown for three soils previously receiving different inorganic P fertilizer rates (0-40 kg P/ha), treated with either nHAP or K-PO4. Blue/green curves respresent nHAP treatments and red/yellow curves are K-PO4 treatments.

CONCLUSIONS

Nanohydroxyapatite stabilized in citrate was more soil mobile than conventional water soluble phosphates (K-PO_4 and TSP) as well as the unstabilized NPs. nHAP restored P functionality to the same extent as K-PO_4 when applied in close proximity to plant roots, but was more efficient than K-PO_4 when applied below a root-impermeable filter, indicating a superior soil mobility. These findings underline the potential for development of P nanofertilizers with low adsorption to soil and improved plant availability.

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EFFECTS OF SOIL AMENDMENT WITH LIME AND GRANULATED POLYHALITE ON NO-TILL MAIZE ROOT GROWTH, NUTRITION, AND YIELD

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Keywords: *Zea mays*; Fertilizer; Poly4

INTRODUCTION

No-till systems increase soil organic carbon (SOC) content mainly in the top layers of the soil, via increased plant residue deposition and decreased soil disturbance. The increase in SOC under no-till may reduce aluminum (Al) toxicity. Calcium (Ca) also ameliorates the toxic effects of Al on root growth (Brady et al., 1993). In addition, the addition of base cations to highly acidic soils can increase microbial growth, thus aiding with carbon sequestration in these agricultural soils (Horn et al., 2021).

Polyhalite supplies K associated with sulfur (S) in the form of sulfate, which can be an important alternative source of K, with the added benefit of providing S in a form that does not acidify the soil. Traditional sources of fertilizers used in Brazilian agriculture usually do not supply S. In addition, since polyhalite also contains Ca and magnesium (Mg) as sulfates, it is possible that its use may reduce Al toxicity to crops grown under no-till. We hypothesized that maize yield in an acid soil under a no-till system would be similar with the use of polyhalite without liming, or with surface application of lime followed by application of potassium chloride (KCl) or KCl + elemental S.

METHODS

A field experiment was carried out in Ponta Grossa, Parana State, Brazil, on an acid Oxisol under a no-till system. Topsoil (0-20 cm) had $pHCaCl_2$ 4,,4 and exchangeable Al, Ca, Mg, and K contents of 8, 15, 4, and 1.1 $mmol_c$ dm^{-3}, respectively. The climate at the site is categorized as mesothermal, humid, subtropical. The average altitude is 970 m and the annual precipitation is about 1550 mm.

A randomized complete block design was used, with four replications in a 4 × 2 factorial arrangement. Plot size was 8 m by 6 m. The treatments consisted of a control and K applications as KCl, KCl + elemental S, and granulated polyhalite on soil without and with surface application of dolomitic lime. Lime was broadcast on the soil surface at a rate of 4 t ha^{-1} in a single application during early development of black oat. Maize, hybrid AG 9025 PRO3, was sown following black oat at a seeding rate of 86000 seeds ha^{-1}, and row spacing of 0.45 m. Applications of K as KCl and polyhalite were performed on the soil surface at a rate of 116 kg K ha^{-1} just before sowing maize. The amount of S added by the elemental S was the same as that of polyhalite (190 kg S ha^{-1}). Fertilizers were applied at rates of 250 kg ha^{-1} of 11-52-00 (N-P_2O_5-K_2O) as mono-ammonium phosphate (MAP) in the sowing, and 225 kg ha^{-1} of N as urea in top dressing at the V_5 development stage.

Maize root samples were collected during the flowering period by means of a sampling tube of 3.5 cm diameter, at depths of 0-10, 10-20, 20-40, and 40-60 cm. Six sub-samples were taken in the plot to form a composite sample. The roots were separated from the soil by dispersion in water through a 0.5 mm mesh sieve. Root length was estimated using WinRhizo software. Leaf samples of maize included 30 leaves per plot that were collected during the crop flowering period. The maize leaf below and opposite to ear was taken. Leaf concentrations of N, P, K, Ca, Mg, and S were determined. Maize was harvested from a 7.2 m^2 area on each plot and grain yield was expressed at 130 g kg^{-1} moisture. Data were analyzed using ANOVA and significant differences between the means were determined using an LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

The maize root length per unit soil surface area to a depth of 60 cm was not influenced by the treatments, with an average value of 108 cm cm^{-2}. However, the distribution of maize root length in the soil profile showed important changes. On unlimed plots, there was a better distribution of root length in the soil profile with the application of polyhalite, while on surface-limed plots the root length was better distributed in the no-K control treatment. These effects could be related to a reduction in Al saturation along the soil profile.

Leaf nutrient concentrations of maize were not significantly influenced either by the surface liming or by the interaction effect of surface liming x K applications. Potassium-leaf concentration of maize was higher with the application of polyhalite (37.2 g kg^{-1}) compared to the no-K control (26.6 g kg^{-1}) and KCl application (33.7 g kg^{-1}). However, the K-leaf concentration in the treatment with polyhalite did not differ from that with KCl + elemental S (36.0 g kg^{-1}). Magnesium-leaf concentration of maize was lower with the K applications as KCl (2.8 g kg^{-1}), KCl + elemental S (2.8 g kg^{-1}), or polyhalite (2.8 g kg^{-1}) than in no-K control (3.7 g kg^{-1}). A reduction in Mg-leaf concentration of maize with K fertilization was certainly caused by higher K uptake by plants. A significant negative correlation between the K-leaf concentration and the Mg-leaf concentration ($r = -0.79$) of maize was found showing the occurrence of a substitutive effect of Mg by K. The applications of polyhalite (2.2 g kg^{-1}) and KCl + elemental S (2.3 g kg^{-1}) provided a higher S-leaf concentration of maize compared to the no-K control (1.9 g kg^{-1}) and KCl application (1.9 g kg^{-1}).

Maize yield was not significantly influenced either by the surface liming or by the interaction effect of surface liming x K applications. Maize average yields within unlimed and limed plots were 14614 and 15086 kg ha^{-1}, respectively. The non-response of surface liming on maize nutrition and yield could have been caused by the short-term reaction of the surface-applied lime. However, maize yields were significantly affected by the treatments with K applications. Although maize yield was higher with the application of KCl (14489 kg ha^{-1}) compared to the no-K control (12475 kg ha^{-1}), important increases in the maize yield were obtained with the applications of polyhalite (16265 kg ha^{-1}) or KCl + elemental S (16173 kg ha^{-1}). The increase in maize yield by applying polyhalite or KCl + elemental S was 30% compared to the no-K control treatment and 12% when compared to the KCl application. Such increases in maize yield with the applications of polyhalite or KCl + elemental S could have been due to greater uptake of K and S by plants. An advantage of using plyhalite compared to KCl + elemental S is that a similar gain in grain yield would be achieved with a single application of polyhalite, while KCl + elemental S would require two applications, increasing the cost of production.

CONCLUSIONS

The application of granulated polyhalite on unlimed plots improved the distribution of maize root length in the soil profile by alleviating Al toxicity.

The applications of granulated polyhalite or KCl + elemental S increased maize grain yield by 30% compared to the no-K control and 12% compared to the KCl application due to greater uptake of K and S by plants, regardless of the surface application of lime.

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PHOSPHORUS DINAMIC IN TROPICAL SOILS UNDER DIFERRENTS SOURCES: USE OF STRUCTURAL EQUACION MODELING

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Keywords: monoammium phosphate; superphosphates; natural phosphates

INTRODUCTION

Brazil is highly dependent on imported fertilizers, with approximately 46% of all the phosphate fertilizers used are come from the international market. High phosphate fertilisers doses have always been recommended in the Brazilian Cerrado, which over the years created a stock, "legacy", of Soil P (WITHERS ET AL. 2018; PAVINATO ET AL. 2020). It is important to understand how this legacy of P in the soil works, in order to be able to adjust the management and, or even, develop new technologies so that part of this stock can be available to the plants. To understand how the management of phosphate fertilization can change soil P dynamics studies have used the chemical fractionation proposed by Hedley (Hedley et al. 1982), with adaptations to tropical soils (GATIBONI ET AL. 2013). The data of P chemical fractionation in the soil have difficult interpretation (GAMA-RODRIGUES ET AL. 2014). Thus, to aid understanding there may be unmeasured variables, such as latent variables. With this, through the latent variants one can better understand the interrelations between the P fractions in the soil (GAMA-RODRIGUES ET AL. 2014; SALES ET AL. 2015, 2017; DE OLIVEIRA VIANA ET AL. 2018). The present study aimed to use structural equations modeling (SEM) to evaluate hypothetical models of the P cycle, determine the interactions between P *Pools* and identify which pools act as a P sink or source in tropical soils. METHODS The structural equations modeling (SEM) of the phosphorus fractions data obtained by the Hedley method, using the soil of a field under long-term experiment for four harvest seasons (2014/2015 to 2017/2018) was performed under Latossolo Vermelho Distroférrico, correlated to Oxisol in the Soil Taxonomy (SANTOS ET AL. 2018). After soybean harvest were sampled soil at 0 to 0.1 m deep to extract phosphorus chemical fractionation by Hedley (HEDLEY ET AL. 1982) method, with adaptations to tropical soils (GATIBONI ET AL. 2013). In Hedley's method were estimated the following phosphorus fractions: inorganic phosphorus in soil solution (Pi.sol); inorganic labile P (Pi.Labile); inorganic moderate labile P (Pi.Labile); P precipitaded with Ca (Pi.Ca); occluded P in soil minerals structures (Pi.occluded); organic labile phosphorus (Po.Labile); moderated to non labile organic phosphorus (Po.NLabile) and organic phosphorus in the microbiote (Po.mic) The maximum likelihood method was used to estimate the indexes of the models using the *Lavaan* package (ROSSEEL 2012). The graphic presentation of the models was used the *lavaanplot* package (LISHINSKI 2021).

For the overall adjustment of the models, were used the chi-square test (X^2), degrees of freedom (df) and probability level (P) associated with the model; the quality fit index (GFI), the comparative fit index (CFI), the root mean square error approximation (RMSEA) and Akaike information criteria (AIC) (GRACE 2006; IACOBUCCI 2010).

RESULTS AND DISCUSSION

Pi.sol positively correlated with Pi.Labile (r = 0.29), Pi.NLabile (r = 0.52), Pi.Ca (r = 0.49), Po.mic (r = 0.33) and Po.NLabile (r = 0.14). The P fractions Pi.occluded (r = -0,09) and Pio.Labile (r = -0,08) did not correlate with Pi.sol. Therefore, for the study conditions it can be affirmed that none of the fractions studied were P drain in the soil solution. Fractions of moderate to low lability such as P precipitated with Fe, Al (Pi.NLabile) and Ca (Pi.Ca) were the main sources of inorganic P for soil solution.

For the adjustment of SEM, the theoretical Model A described by Gama-Rodrigues et al. (2014) was tested. In this model, the hypothesis was to design a basic structural model with five latent variables (Available *Pool*, Inorganic *Pool*, Primary Mineral *Pool*, Occluded *Pool* and Organic *Pool*) to evaluate the relative importance of P *Pools* affecting Pi.sol (Model 1 - Figure 1 A). The Occluded and Organic *Pool*s did not explain the variation of Pi.sol and Model 1 obtained adjustment indexes outside what is indicated by the literature (GFI, CFI and TLI < 0.9 and RMSEA and SRMR > 0.05). Then, Model 2 was obtained with the respecification of Model 1 (Figure 1 B).

In Model 2 the Occluded *Pool* was removed. The Organic *Pool* was maintained only with Po.mic, because this fraction of P was the one that obtained the third highest correlation coefficient (Pearson) with Pi.sol. Model 2 obtained the adjustment indexes within what is suggested in the literature (GFI, CFI and TLI < 0.9 and RMSEA and SRMR > 0.05), with all significant coefficients. The Inorganic Pool followed by Mineral and Organic Pools acted as a source, i.e., "quantity factor" of soil P. Thus, in the soil under study, the principal stock of P, "P Legacy", lie in the inorganic fractions with moderate to low labile (Pi.NLabile) and the P precipitated with Ca (Pi.Ca).

CONCLUSIONS

In Latossolo Vermelho Distroférrico fertilized with high doses of P in four consecutive harvest seasons, the largest stock of P are the Inorganic, labile and moderate to low lability, Mineral and Organic Pools, composed only by Po.mic; and that all these fractions compose the P quantity factor in the soil.

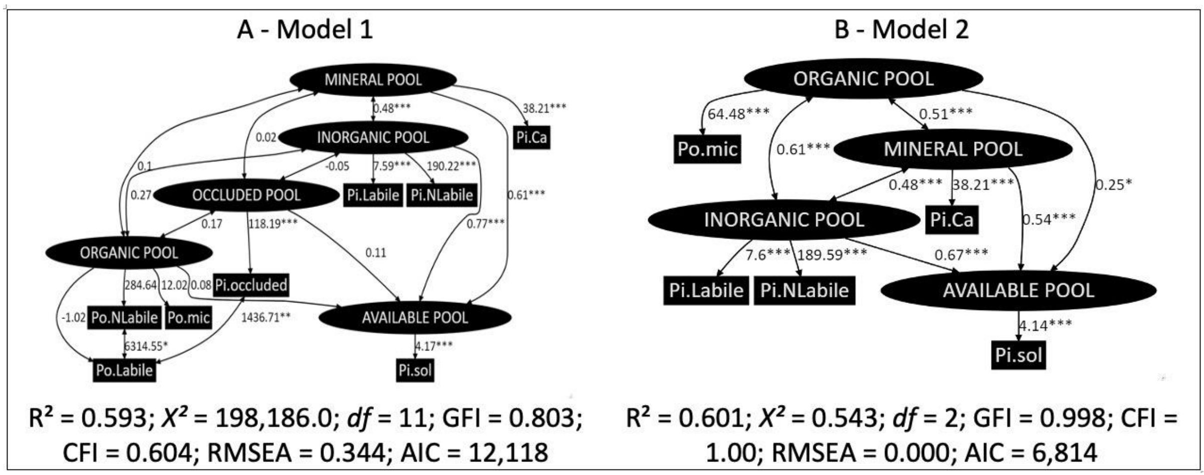

Fig. 1. SEM "1" (A) and "2" (B) for soil phosphorus dynamics obtained with data from P fractions according to Hedley method. All variables measured (in rectangles) are represented as indicators of effects associated with latent variables (in ellipses). the numbers close to the arrows were the estimated indexes of the models and their significance (***pvalue <0.001; **pvalue < 0.01 and *pvalue <0.05). Indexes without asteristics pvalu >0.05.

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CATION MOBILITY WITH POLY4 COMPARED TO OTHER NUTRIENT SOURCES

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Keywords: Calcareous; Fertilizer; Polyhalite

INTRODUCTION

Polyhalite (POLY4) is a multi-nutrient fertiliser containing potassium, calcium, magnesium and sulphur ($K_2Ca_2Mg(SO_4)_4{\cdot}2H_2O$). The mineral has a sustained dissolution rate which can deliver nutrients throughout the crop growing season. A number of leaching column studies were conducted with different soils at different institutions to evaluate POLY4 under different conditions. The aim was to assess the release rate and quantity of the nutrients contained in POLY4, compared to conventional fertilisers that also supply K, S, Ca and/or Mg.

METHODS

Column trials were conducted with sandy loam soil from Aberdeen, Scotland and from silt loam, loamy sand, and calcareous soils in Florida. Columns were 30 cm deep and had no plants growing in them, therefore leaching is considered a proxy for nutrient availability.

The trials compared different potassium containing fertilisers: POLY4 and MOP (KCl) in both Florida and Aberdeen. SOP (K_2SO_4) and SOP-M ($K_2Mg_2(SO_4)_3$) in Florida only.

The potassium application rate was balanced among treatments within each trial. In addition, a control that did not receive K was used to measure the baseline nutrient mobilisation of the soil.

Nutrient leachate was measured from each column and converted to kg ha^{-1}. The baseline control was subtracted to give the change in leachate with nutrient application. This value was then divided by the application rate to give the percentage of nutrient applied than was mobilised.

RESULTS AND DISCUSSION

Potassium mobilisation

With the Ankona silt loam and Norfolk loamy sand all fertilisers mobilised K. POLY4 mobilised more K (>100%) than was applied on both soils, while MOP had lower K mobilisation, with only 63% of K mobilised on the loamy sand.

For the Calcareous and Aberdeen soils, only POLY4 mobilised a substantial amount of the K applied. On the calcareous soil, 18% of the amount of K supplied with POLY4 was mobilised, but less than 7.5% of K supplied by MOP, SOP and SOP-M was mobilised

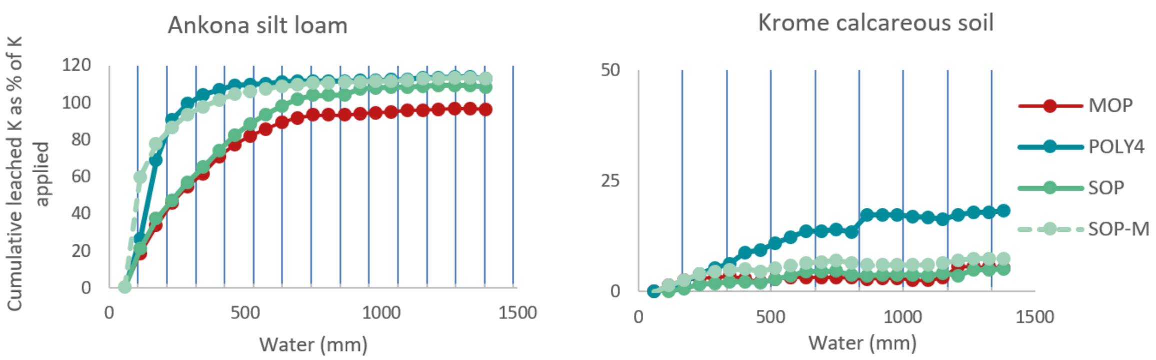

Fig. 1. K mobilisation with different fertiliser sources through a loamy sand (left) and calcaerous soil (right). Verticle lines indicate the pore volume

Distribution of nutrients mobilised

With the Ankona silt loam and Norfolk loamy sand the distribution of nutrients applied was reasonably similar to the distribution of nutrients measured in the leachate. However with the Aberdeen soil and Krome calcareous soil a large amount of Ca was mobilised. However, the POLY4 treatment had a greater quantity of potassium and magnesium mobilised despite supplying the same rate of K, and less Mg than SOP-M.

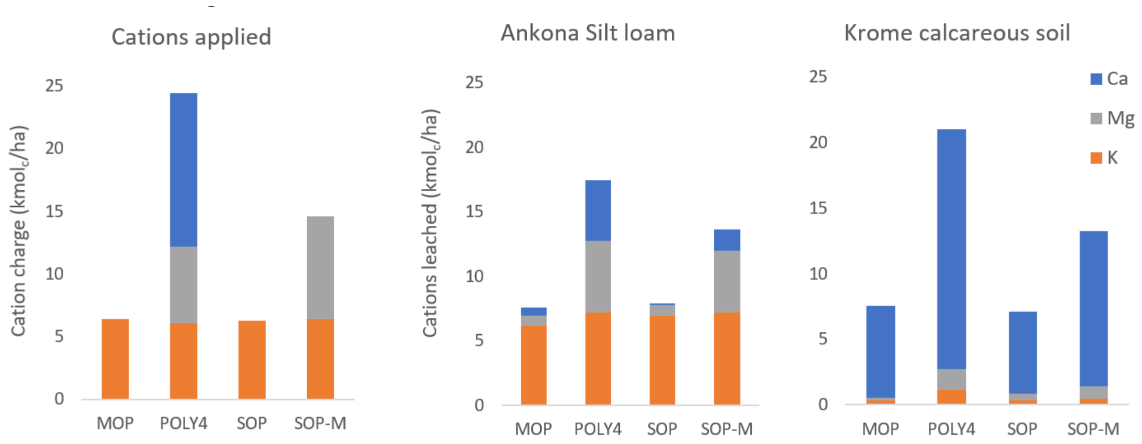

Fig. 2. Cations applied (left) versus cations leached from Ankona silt loam (centre) and from calcareous soil (right)

CONCLUSIONS

(a) POLY4 consistently mobilised a greater proportion and overall quantity of potassium than other K sources.

(b) POLY4 was the only treatment in Florida that leached more K than it put in.

(c) POLY4 mobilised K in the calcareous soil and the Aberdeen soil. In these soils other K treatments only mobilised Ca.

(d) POLY4 consistently leached the greatest Mg, even when the Mg rate was lower with POLY4 than the comparative treatment (i.e. SOP-M in Florida).

Financial Support

Trials conducted by Professor Graeme Paton and Professor Paul Hallett (University of Aberdeen) and Dr Marcel Barbier (University of Florida)

Nutrient cycling, agroecosystems and sustainability

SOIL ACIDITY ALLEVIATION AND FATE OF NITROGEN IN A MAIZE/FORAGE INTERCROPPING

Keywords: lime+gypsum ; ^{15}N; maize

INTRODUCTION

Liming alone has not been effective in alleviating of sub soil acidity in systems under no-till (Caires et al., 1998). However, gypsum application can raise cation concentration and decrease Al toxicity in the subsoil, favoring deep root growth (Rosolem et al., 1998).

Under no-till lime is applied on the soil surface, resulting in an overdose in the first cm of the soil, leading to very high pH, and increasing nitrification. Then, nitrate can be leached deeper into the soil profile. If there are root in this region, N use efficiency will be enhanced (Rosolem et al., 2017).

Growing maize intercropped with a forage as a relay crop after soybean under no-till has been used as an effective conservation system, and the introduction of legumes and/or forage grasses with deep, vigorous roots in the system can prevent nitrate leaching. However, it is not known the fate of the N used is these systems. We hypothesized that soil acidity alleviation with gypsum associated to lime would enhance N use efficiency in a system with maize intercropped with a forage grass by increasing root growth in the soil profile. The objective of this work was to determine how lime, lime+gypsum and N fertilization affects N use and distribution in a cropping system where maize was intercropped with Guinea grass (*Megathyrsus maximus)* and grown as a relay crop after soybean.

Financial Support

Department of Crop Science, School of Agricultural Sciences, São Paulo State University, 3780 Universitária Av., Botucatu, SP, 18610-034, Brazil.

RECOVERY AND STORAGE OF NUTRIENTS DUE TO THE INTRODUCTION OF *UROCHLOA* IN INTENSIFIED GRAIN PRODUCTION SYSTEMS: A STRATEGY FOR CONSERVATIVE CYCLING

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Keywords: Intercropping; Brachiaria; No-till system

INTRODUCTION

The use of fertilizers is essential to obtain high grain yields in the Brazilian savannah (Cerrado), but it represents a significant part of production costs. It also has environmental implications, as nutrient losses from the soil-plant system become potential water or atmosphere contaminants.

The introduction of forage grasses aiming at the diversification and intensification of annual crop systems has been shown to be a promising practice, with economic and sustainability gains. Plants with a robust root system, such as brachiaria (*Urochloa* spp), contribute to the greater recovering of nutrients (Crusciol and Soratto, 2010; Salton and Tomazi, 2014; Crusciol et al., 2015).

This study aimed to assess the biomass production and the N, P and K accumulation in plant shoots and roots in a cycle of off-season grain sorghum, when monocropped or intercropped with *Urochloa ruziziensis* (ruzigrass), in an oxisol of the Cerrado region.

METHODS

The evaluations were carried out from fertilization strategy trials that included the off-season sorghum cultivated in monocropping or intercropping with ruzigrass, in succession to soybean, at the Fazenda Decisão, municipality of Unaí, Minas Gerais State. The soil is classified as very clayey Yellow Red Latosol (Oxisol), with high fertility, presenting 41 and 34 g kg^{-1} of organic matter, 38 and 23 mg dm^{-3} of Mehlich P, and 183 and 157 mg dm^{-3} of exchangeable K, in the 0-10 and 10-20 cm layers, respectively.

The selected treatments were three NPK fertilization options (1 - control without NPK, 2 - replacement of nutrients removed at harvest, and 3 - standard management of the farm) in monocropped and intercropped systems, with four replications.

At harvest, the biomass of stem, leaves and grains of sorghum, as well as the shoot of ruzigrass, was determined. Roots were collected in trench profiles, sampling from soil monoliths that were 25 cm wide by 10 cm thick and at a depth of 100 cm, from the sorghum row towards the center of the interrow. Samples of plant materials were analyzed to determine the concentration of nutrients and subsequent calculation of the amounts accumulated in each compartment.

RESULTS AND DISCUSSION

The introduction of ruzigrass reduced sorghum grain yield by 15%, regardless of fertilization level, indicating the need for adjustments in the management of these species to decrease the competitive pressure for resources. However, the intercropping increased the amount of aboveground plant residues by 44%. In addition to incorporating more carbon, the presence of ruzigrass promoted a substantial increase in the system's capacity to acquire and store N, P and K (Figure 1, Table 1), a fact that ensures a greater flow of these nutrients in the cycling process and subsequent availability to soybeans in the following season.

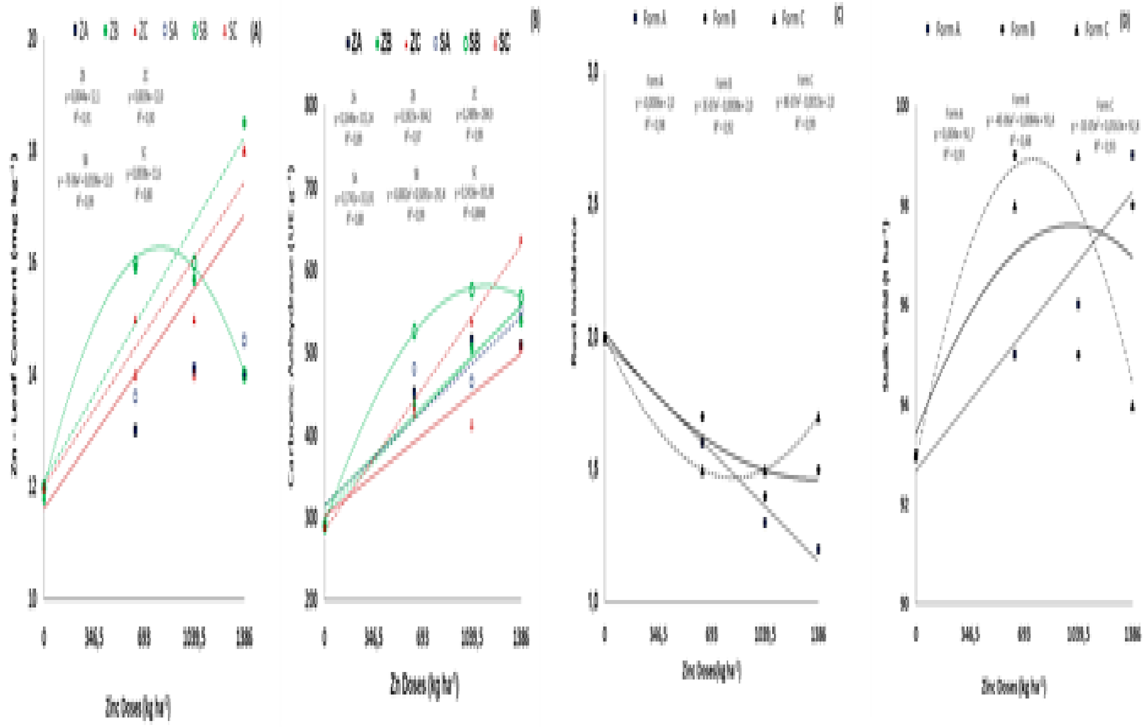

Fig. 1. Biomass production and nutrient accumulation in N, P_2O_5 and K_2O equivalents (kg ha^{-1}) in shoot and root components, in systems of grain sorghum intercropped with ruzigrass and monocropped. Average of three fertilization treatments. 2019.

Table 1. Percent difference (%) of the intercropped versus the monocropped sorghum system in terms of grain yield[(1)], shoot[(2)] and root[(3)] biomass production and nutrient storage in plant residues (shoot + root)[(4)]. Average of three fertilization treatments.

Grain[(1)]	Shoot[(2)] (+ grain)	Shoot[(2)] (- grain)	Root[(3)]	N[(4)]	P[(4)]	K[(4)]
-15	11	44	198	65	101	54

CONCLUSIONS

The intensification of grain production systems by introducing *Urochloa* modifies the nutrient fluxes in the soil-straw compartment, by increasing plant residues and nutrient accumulation that will serve as a source for subsequent crops, while helping to prevent nutrient losses.

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RESPONSE ON SOIL CHEMICAL PROPERTIES AND PLANT NUTRIENT UPTAKE BY ADDITION OF ORGANIC AMENDMENTS IN A GREENHOUSE EXPERIMENT

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Keywords: Organic residues; Phosphate rock; Plant nutrition

INTRODUCTION

Accelerated population growth has implied a growing demand for food, leading to increased consumption of energy and non-renewable resources. In this context, to meet the high energy crisis and support the environmental sustainability of non-renewable resources, the use of agricultural residue appears to be a suitable alternative (Kumar el al. 2022) . Among the agricultural residues most used as organic fertilizers are cattle and poultry manure. These residues have shown the ability to provide plants with an optimal combination of macro and micronutrients concentrations, despite high total phosphorus (P) levels (Pagliari et al. 2020). The aim of this study was to evaluate the effect of two organic amendments, Cattle (CM) and poultry manure (PM), and Phosphate Rock (PR), as a phosphorous (P) source and two-level of Nitrogen (N) applied as urea (100 and 200 mg kg soil^{-1}) on the performance and quality of plant biomass of Lolium perenne L. (cv. Base) under greenhouse conditions.

METHODS

Sampling and chemical characterization of soil CM and PM samples

The soil used for the experiment was an Andisol collected from the Mahuidache locality of southern Chile, collected from 0 - 20 cm depth, air dried and sieved <2 mm. Semifresh CM was collected from Santa Elena Farm located in the Barros Arana locality. The samples were air-dried , milled and sieved through a 2-mm sieve for subsequent analysis. PM compost was provided by Pucalan LTDA. Chemical analysis of soil was carried out according to Sadzawka et al. (2006) , while CM and PM were carried out according to Sadzawka et al. (2004).

Greenhouse experiment

The pot experiment included the following treatments: Control soil; soil + CM; soil + PM; soil+ PR; and triple superphosphate (TSP) addition was included as chemical control. One hundred and forty seeds of ryegrass (Lolium perenne L.) cv. Base were sown in each pot containing 1.0 kg of soil. After germination, the assay was thinned to 115 seedlings per pot. Harvesting was carried out after 30 days.

Plant biochemical analyses
The dried shoot and root tissues were ground to a fine powder, ashedin a muffle furnace at 500 ?C for 8 h, digested with 2 M HCl and filtered. In the extract, P, Al, Ca, Mg, K were determined according to Sadzawka et al. (2004). In fresh shoot material, lipid peroxidation was measured using the thiobarbituric acid-reacting substances (TBARS) assay according to Du and Bramlage (1992). The total SOD was determined by measuring the photochemical reduction of nitroblue tetrazolium (NBT) according to Donahue et al. (1997)

RESULTS AND DISCUSSION
In the soil, the highest P content (8.34 mg kg^{-1}) was observed in PM treatment at 200 N level and the lowest P content (3.63 mg kg^{-1}) was obtained from the CD treatment at 100 N level. Similarly, soil physical properties were positively influenced by CD, PM, and PR treatments under different N regimes. Organic residues have been reported to increase P availability in soils with high P retention (Guppy et al., 2005), probably due to the release of byproducts of residue decomposition, such as low-molecular-weight (LMW) organic acids.

Moreover, these residues increase soil fertility due to the addition of soluble base cations, which are released from the mineralization.

The highest ryegrass biomass (4.08 g) was observed in PM treatment at 200 N level and the highest ryegrass P uptake (4.27 mg $g^{?1}$) was obtained from the PR treatment at the same level of N (Fig.1 A;B).

Lipid peroxidation in shoots showed a significant decrease in response to organic amendment except for treatment with CD at 100 N dose. When plants were grown in PR and PM treatments at the same N dose, the amount of TBARS decreased 1.3 and 1.4-fold respectively, while at dose of 200 N decreased 1.1 and 1.3-fold. Moreover, regarding the enzymatic activity, differential SOD activity in response to organic amendments was observed in shoots. Control shoots at dose of 100 N, exhibited the highest SOD activity compared to the other treatments. The application of CD caused a decrease in SOD activity by 11% in comparison to the control. While PR and PM treatments, reduced SOD activity by 17 and 23% respectively.

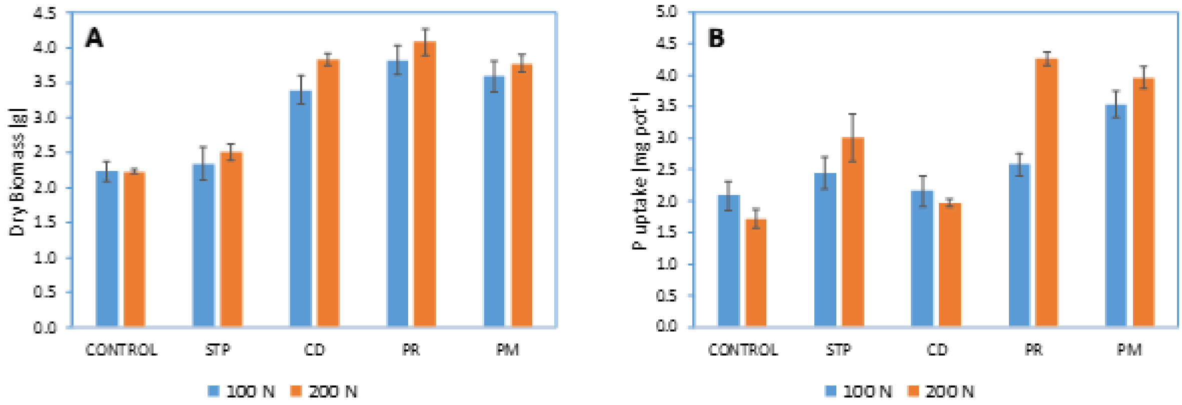

Fig. 1. Dry weight biomass (A) and shoot P uptake (B) of ryegrass plants under cattle manure (CM),phosphate rock (PR), poultry manure (PM) and supertriplephosphate (STP) treatments. Data are means of three replicates ± SD.

CONCLUSIONS

These findings revealed that the organic amendments and PR treatments under different N levels showed a beneficial effect on P uptake and oxidative stres with a concomitant increase in ryegrass yield.

ACKNOWLEDGEMENTS

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DRY MATTER ACCUMULATION IN SOYBEAN IN A MEDIUM AND HIGH POTENTIAL AREA IN POCONÉ - MATO GROSSO

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Keywords: Nutritional demand; Productive potential; Management practices

INTRODUCTION

The constant modernization of agriculture coupled with the high international and national demand, among other economic factors have enabled the increasing advance of soybean production in Brazil (CAMPOS, 2010). However, the biggest challenge is in maintaining the capacity of the soil to provide resources through the preservation of the physical, chemical, and biological properties of the soil (JÓNSSON et al., 2016). Pricipally in face of the restrictions on land use expansion in Brazil, therefore, increasing productivity presents itself as a necessary path for the expansion of the food supply. Within this context, in Brazil there are areas with greater production potential, characterized as areas of high productivity and present higher average production than the average of the state of Mato Grosso. Therefore, the difference between actual and potential productivity demonstrates that the management and edaphoclimatic conditions can lead to yields around to the crop's potential.

The knowledge of the nutritional demand throughout the vegetative stages of soybean is an important study to understand the needing of nutrients in the different phases, allowing the right moment of fertilizer application to be defined in order to improve production rates. Thus this research aimed to verify the accumulation of dry matter mass in the parts of indeterminate growth soybean plants grown in two areas of commercial tillage.

METHODS

The present study was conducted on the farm Lagoa Dourada, located in the municipality of Poconé, in the state of Mato Grosso, in two areas: one considered high (>70 sc/ha) and the second medium (<70 sc/ha) productivity. The chemical and physical characterization of the soil of the two areas was performed and the management for planting the cultivar BMX Ultra 75i77RS Ipro with a 120-day cycle was done with the same parameters. To determine dry matter, 5 whole plants per plot were collected at the following crop stages: V7, R4, R5.1, R6, R7, and R8. Each plot was defined by 8 rows of 10 meters, and for this collection a useful area of 2 central rows of 5 meters was delimited, discounting 0.5 meter of border per plot. The plant parts were separated into leaves, stems and pods, washed and placed in paper bags properly identified, then sent to the oven with forced air circulation at 65º C until constant weight.

RESULTS AND DISCUSSION

The nutritional demand of the areas can be observed in Figure 1 that shows the production of dry matter mass of the crop in six stages (V7, R4, R5.1, R6, R7 and R8). In Figure 1a, over the high potential area, it can be observed that initially the dry matter mass was accumulated in the leaves and stem until the R4 stage, where the accumulation starts in the pods and in the R5.1 stage the accumulation starts in the grains and decreases in the leaves and stem. The accumulation in the pods has a gradual increase until R8 and the increase in the grains occurs until R7, after this there is a gradual decrease until R8. Figure 1b shows the accumulation of dry matter in the medium yield potential area, such as it happened in the high potential area, the initial accumulation is in the leaves and stem, in the R4 stage the accumulation starts in the pods and in the R5.1 stage the accumulation starts in the grains and decreases in the leaves. The accumulation in the grains and pods increases until R8. It can be seen that in R5, both areas, there was a decrease of the leaves contribution in the accumulation of dry matter mass. Both areas are characterized by the period of grain filling, which photoassimilates translocate from the leaves to grain formation (SFREDO, 2008).

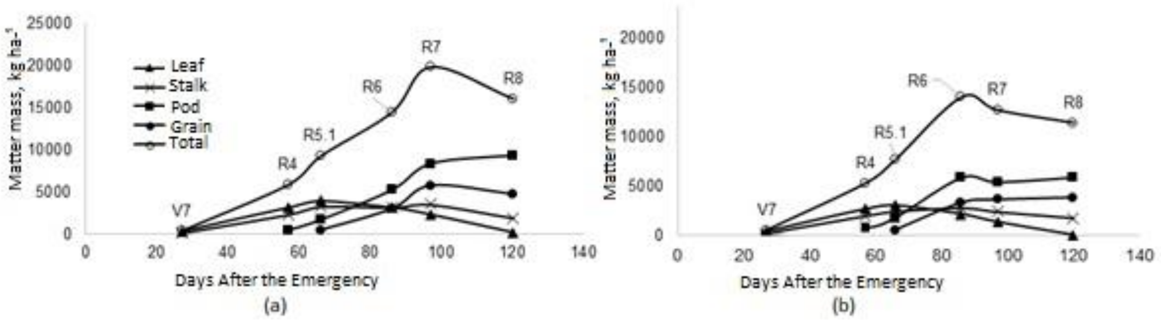

Fig. 1. Acúmulo de massa de matéria seca nas áreas de alto potencial (a) e médio potencial (b)

The accumulation of total dry matter mass in the high potential area (figure 1a) was at the R7 stage with 19,814 kg ha-1 and in the medium potential area (figure 1b) occurred at the R6 stage with a total of 14,004 kg ha-1 . In studies conducted by Oliveira et al. (2014) in soybeans of indeterminate growth type in the 2010/2011 crop the highest accumulations found by these authors were at the R7 and R6 stages with 8,000 kg ha-1 , different from the results found in this study that obtained higher values.

CONCLUSIONS

The chemical condition of the soil combined with the climatic condition in both production environments interfered directly in the nutritional contents accumulated in the plant. Therefore, through adequate management practices it is possible to reach the maximum yield potential.

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WIDESPREAD POTASSIUM DEFICIENCIES IN INTENSIVE RICE SYSTEMS IN SOUTHEAST ASIA

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Keywords: Potassium; crop nutrition; food security

Potassium (K) is perhaps the most neglected plant essential nutrient despite negative K balances are common across major cropping systems around the world. Conditions that favored K deficiencies include: (i) little K inputs, (ii) high K removal with gran and/or straw, and (iii) soils with high K-fixing capacity. Here we assessed K deficiencies in intensive rice systems in Indonesia, where K inputs are relatively small while K removal is high due to whole-plant harvesting and subsequent burning. To do so, we followed four independent approaches: (i) quantification of partial K balances using farmer-reported data (n = 1147 fields), (ii) in-situ diagnosis based on flag-leaf nutrient concentration measured in a subset of fields (n = 335 fields), (iii) existing data from a long-term field experiment (n = 40 consecutive rice crops), and (iv) data from on-farm K-omission plots (n = 172 trials). We found that partial nutrient balances were positive for N and P, but highly negative for K. Similarly, leaf nutrient concentration was within or above the sufficiency range for N and P, but well below for K in most cases. In contrast, leaf P and K concentrations were within the sufficiency range for maize, but N was deficient for some provinces. Long-term experiments showed that the yield gap due to K increases slowly over time and can help explain up to one-third of the current exploitable yield gap. On-farm field trials showed that *ca*. half of the fields exhibited yield reductions when K fertilizer was not applied. Removing the K limitation would require higher (and costly) K fertilizer inputs and/or changes in current straw management.

CROP ROTATION IS AN EFFECTIVE WAY TO UNLOCK RESIDUAL SOIL PHOSPHORUS FOR WHEAT

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Keywords: phosphorus; wheat; crop rotation

INTRODUCTION

Phosphorus (P) inputs as soluble fertilisers (mainly triple superphosphate, mono- and di-ammonium phosphates) in Australia surpassed 333,340 thousand metric tonnes of P over the last 20 years. More than 38% of this amount has been applied in Western Australia (WA) - the largest consumer of P fertilisers in Australia (ABARES, 2021). Positive P balances (inputs greater than outputs) resulted in soil P accumulation (Harries et al., 2021), and while this 'legacy' or residual P represents a major P pool, more nuanced fertiliser recommendations are needed to ensure a sustainable and targeted use of P resources. Additionally, market volatility, frequent supply chain disruptions and increasing rock phosphate commodity prices require an integrated approach to enhance residual soil P recycling, and thus buffering available P levels to ultimately improve P-use efficiency in agriculture (Haygarth and Rufino, 2021).

METHODS

We conducted two 4-year field trials in contrasting regions of the Western Australia wheatbelt (Wongan Hills, mean annual [2018-2021] rainfall 328 mm $year^{-1}$; Esperance, 441 mm $year^{-1}$) to test the effects of crop rotation strategies and P fertiliser supply on wheat (*Triticum aestivum* L) (main cash crop) grain yields. The crop rotation systems evaluated were either continuous wheat for four years (indicated as "WWWW"), rotation of narrow-leafed lupins (*Lupinus angustifolius* L) and wheat ("LWLW") or brown manuring with a faba bean (*Vicia faba* L) crop in the first year (2018) followed by three consecutive years of wheat crop ("BWWW").

Phosphorus treatments tested were nil-P and various replacement rates (half, full and two-fold) drilled with seed each year.

RESULTS AND DISCUSSION

Results demostrated that the benefits of brown manuring were evident at both sites and extended for the 3 years. Fig. 1 shows the wheat grain yield responses by crop rotation treatment at both sites during the years of the trials.

At the Esperance trial, wheat yield was 38% higher in the first year (2019) following brown manuring in comparison to wheat after wheat. The residual effects of brown manuring were still significant in the second and third wheat crops, with increases of 32 and 23%, respectively, compared to wheat monocropping. Rotating lupins and wheat was shown to increase the yield of the following wheat crop by 33%, and up to 74% on average in 2019 and 2021, respectively, compared to wheat alone.

At the Wongan Hills site, compared with wheat monoculture, brown manuring increased grain yields of following wheat from 24 to 33%, with clear effects even after 3 years. Following a lupin crop, wheat yields were 24 and 38% higher in 2019 and 2021, respectively, compared to sequential wheat crops at this site. In Esperance, compared with the nil-P fertiliser applied, P uptake by wheat was 91% higher at anthesis in 2019, the first year after brown manuring, and 47% higher after a lupin crop compared to wheat followed by wheat. Similarly, at Wongan Hills, P uptake by wheat was 53% higher following the brown manure and 26% higher following lupins.

The agronomically optimal P rates (to achieve 90% of the maximum yield) varied from less than the half replacement (in Esperance) to about replacement rates (in Wongan Hills), but the responses were highly dependent on the crop rotation system employed.

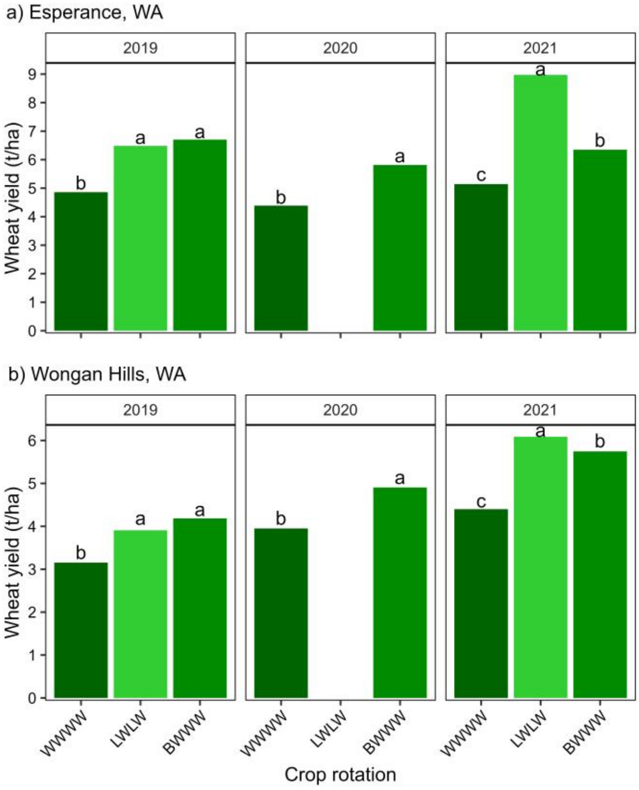

Fig. 1. Wheat grain yield responses as an effect of crop rotation treatments at Esperance (a) and Wongan Hills (b). The bars represent the mean values for each treatment. For each year, lower-case letters indicate significant differences between means (Fisher's LSD test, α = 0.05).

CONCLUSIONS

Combined, the results of these experiments highlight the potential for achieving high wheat yields by introducing legume species, such as lupins, in the crop rotation systems and brown manuring as an effective way of enhancing soil P mobilisation and recycling.

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INJECTION OF LIQUID MANURE IN MAIZE CROPPING: REDUCING NITROGEN LOSSES AND ENHANCING NUTRIENT USE EFFICIENCY

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Keywords: soil mineral nitrogen; N_2O losses; fertilizer placement

INTRODUCTION

Agriculture in northwestern Germany is characterized by intensive livestock farming based on extensive cultivation of maize. Broadcast application of the resulting animal slurries already covers the nutrient demand of maize. However, most farmers band-apply a mineral NP fertilizer close to the maize seed as a "starter fertilizer" to ensure proper early growth development. This often leads to nutrient surpluses at field level, which are at risk to be lost into non-agricultural ecosystems. Slurry injection below the maize seeds might be an option to replace mineral starter NP fertilizer without impairing early maize growth and yields.

METHODS

In a 3-year field trial series (8 sites per year), slurry injection (with/without addition of a nitrification inhibitor [NI]) was compared with slurry broadcast application and a control treatment without slurry application (all 4 treatments combined with/without mineral NP starter fertilizer). Biomass accumulation and nitrogen uptake were assessed at harvest.

An additional 2-year field trial on a sandy soil with four treatments (unfertilized control, broadcast application + NP starter fertilizer, injection and injection + nitrification inhibitor) was conducted to investigate the spatial and temporal soil mineral nitrogen dynamics. Soil samples were taken from three soil layers at 30 cm intervals down to 90 cm, and at three positions (below the maize row, 15 and 30 cm distance to the row) at several dates during the maize growing season. Furthermore, N_2O emissions were measured using gas-sampling chambers (70 x 70 cm; 51 cm high) installed centrally above the maize row (weekly gas-samplings for 1 year). In later growth stages of maize "split-chambers" (Olfs et al. 2018) were used to enable N_2O measurements.

RESULTS AND DISCUSSION

Yield And N Uptake

Averaged over all experimental sites slurry injection resulted in equal (without NI) or slightly higher (with NI) dry matter yields compared to slurry broadcast application. Addition of a NI to the slurry increased N uptake (up to + 9 %) compared to slurry broadcast application with starter fertilizer. These observations go along with other studies (e.g. Schröder et al. 2015) and show the higher N use efficiency (NUE) from injected slurry. Reasons for this NUE increase may be lower ammonia losses (Sommer and Hutchings 2001), although the broadcast applied slurry was incorporation shortly after surface application as well as decreased nitrogen immobilization when manure is injected in a band due to a reduced soil/slurry interaction (Sørensen and Amato 2002). Finally, the addition of a nitrification inhibitor leads to a mitigation of nitrate leaching and N_2O emissions (Ruser and Schulz 2015).

Spatial And Temporal Soil Mineral N Dynamics

The detailed studies on soil mineral N dynamics revealed that in a year with heavy rainfall all fertilized N of the slurry broadcast treatment was leached out from the top soil layer until 6-leaf stage of the maize crop. Displacement of N was significantly smaller after slurry injection leading to an increased NUE for the maize crop after slurry injection as revealed by Federolf et al. (2016). However, in a year with average rainfall, no displacement of fertilized N out of the top soil layer occurred independently of treatments. The addition of a NI led to significantly increased ammonium N concentrations in the injection zone throughout the early growth stages, reduced nitrate leaching further, and finally resulted in a higher N uptake (Westerschulte et al. 2017). In addition, the plants take up more N as ammonium followed by a pH decrease in the soil surrounding the roots. This leads to an improved availability of P and micronutrients (e.g. zinc) resulting in an overall better crop growth (Westerschulte et al. 2018).

N_2O Emissions

While slurry broadcast application only slightly increased N_2O emissions compared to the control treatment without any slurry application, injection of slurry increased N_2O losses significantly during the early growth stages of maize. Most probably high concentrations of easy available carbon in the slurry band has led a depletion of O_2 in this soil zone with the consequence of increased denitrification (Ruser and Schulz 2015). Addition of a NI to the injected slurry resulted in ca. 50 % lower N_2O emission.

CONCLUSIONS

Due to slurry injection, the applied nitrogen is located in a soil zone with better spatial availability for plant roots compared to broadcast application. Especially when a nitrification inhibitor is mixed into the slurry, the risk of nitrate leaching is significantly reduced. Obviously, slurry injection is a reliable option to mitigate nutrient surpluses in maize growing resulting in beneficial effects for the environment.

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QUANTIFICATION OF NH_3 AND N_2O EMISSIONS AND CARBON FOOTPRINT OF N FERTILIZERS IN SUGARCANE

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Keywords: greenhouse gas; carbon market; biofuel

INTRODUCTION

The biofuel carbon market in Brazil has been undergoing strong transformations and making commitments to reduce greenhouse gas (GHG) emissions throughout the production process. The efficient use of fertilizers, especially nitrogen (N), will significantly contribute to the success of the established goals. Therefore, it will be necessary to implement a set of good practices that increase nutrient use efficiency and restore soil quality. Fertilizers with a low carbon footprint (CFP) and efficient management that provide lower losses will be key in this process.

Urea (UR), the most-used N fertilizer in sugarcane, is subject to high losses of N through ammonia (NH_3) volatilization when surface-applied to soils, especially when applied to ratoon cycles, in which a thick mulch of plant residues remains on the soil surface. Recent studies show that the nitrous oxide (N_2O) emissions in sugarcane fields occur mainly due to the nitrification process and, in a lesser extent, in denitrification. For this reason, amidic and ammoniacal N sources may have higher N_2O emission than those containing nitrate. Emissions of GHG, especially N_2O, can represent up to 40% of the total GHG emitted in the production of ethanol from sugarcane. In this context, calcium ammonium nitrate (CAN) is a promising N fertilizer for sugarcane. Therefore, the objective of this study was to evaluate the effect of N sources and rates on yield, NH_3 volatilization, N_2O emissions, and CFP of sugarcane production in Brazil.

METHODS

A long-term field trial with sugarcane variety IAC-5000 was set up in Piracicaba, São Paulo State - Brazil, in an Alic Clayey Red Latosol. The experiment consisted of a factorial of seven treatments, with two sources (UR and CAN), three rates of N (30, 60, 90 kg ha^{-1} plant cane, and 60, 120, and 180 kg ha^{-1} in ratoons cycles), and a control treatment without N application. The N was applied annually to the planting furrow, starting in the plant cane cycle in April 2013, until the 7th ratoon in 2020-2021 cycle. The crop was managed with the most current practices adopted in Brazil, which included the harvest without burning so that the straw remains on the soil.

Evaluations included sugarcane yield and N losses as NH_3 and N_2O. In the 2nd, 3rd, and 6th ratoon cycles, the NH_3 volatilized was measured during 27 days in the field according to the procedure described by Cantarella et al. (2003). This was made in all UR plots and in one CAN plot (180 kg ha^{-1}) as well as in the control plots. In the plant cane, 2nd, 3rd, and 7th ratoon cycles, chambers were installed in the field to collect N_2O emissions from all N treatments and from the control plots as described by Soares et al. (2015). Data were submitted to analysis of variance (ANOVA) and average compared by the Tukey test ($P \leq 0.05$). The data was also used to calculate the CFP of sugarcane production as affected by N fertilization.

RESULTS AND DISCUSSION

Considering the whole cycle of evaluation, from plant cane to 7th ratton, the sugarcane stalk yield of the control treatment (no N) was 66.1 t ha^{-1}, whereas the average of the three N rates for the UR and CAN treatments was 85.0 t ha^{-1} for both sources. There was a significant linear effect for N rates, but no difference in yield was observed between UR and CAN. The N balance, where the amounts of N fertilizer applied and removed with the stalk harvest were equal, was around 60 kg N ha^{-1}. Therefore, at least for the rates 120 and 180 kg N ha^{-1}, there should be an N surplus in the system regardless N sources, and this could explain in part the small yield difference between sources.

The NH_3 losses for UR, expressed as a percentage of the applied N, varied from 10.8 to 16.0% in the 2nd ratton, 6.9 to 10.8% in the 3rd ratton, and 2.8 to 3.2% in the 6th ratton. For CAN, NH_3 losses were significantly lower at the rate of 180 kg ha^{-1}, varying from 0.4 to 0.7% in all cycles evaluated (Fig. 1a). No significant difference was observed between CAN and control plots. In the 6th ratton cycle, low NH_3 losses were observed as compared to the other cycles, and this was due to a rain event of 63 mm that occurred after fertilizer application.

The average cumulative N_2O emissions for the three N rates applied as CAN were 315, 675, 427, and 298 mg m^{-2} $year^{-1}$ in the plant cane, 2nd, 3rd, and 7th ratoon cycles, respectively. The corresponding values for the UR treatments were 528, 884, 668, and 770 mg m^{-2} $year^{-1}$. The N_2O emissions from UR were significantly higher than CAN; the fertilizer emission factor for UR was on average 0.90% and 0.47% for CAN throughout the evaluation period (Fig. 1b).

Based on the data from Fertilizer Europe (2011, Ecoivent 2.0), sugarcane stalk yield, and the real N_2O emissions from the field trial, the CFP for producing sugarcane was lower when CAN was the N source than UR. The total in kilograms of CO_2 equivalent emitted per hectare was on average 2,610 for CAN and 3,729 for UR.

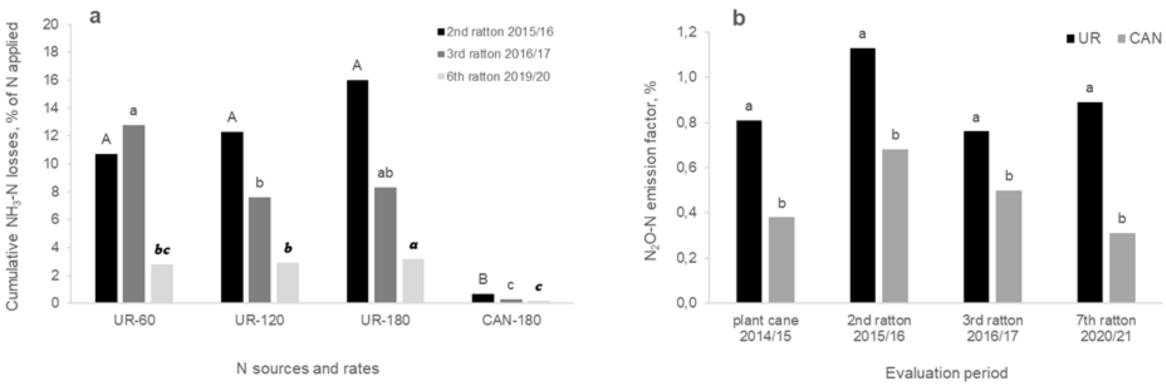

Fig. 1. (a) Cumulative ammonia volatilization losses as affected by sources and rates of N. Uppercase, lowercase, and bold letters compare the means (Tukey, $P \leq 0.05$) for the 2nd, 3rd and 6th ratoons, respectively. **(b)** Nitrous oxide emission factor for UR and CAN during the evaluation period. Letters compare the means (Tukey, $P \leq 0.05$) for N sources in each evaluated cycle.

CONCLUSIONS

From an environmental point of view, CAN should be the preferred source of N for sugarcane due to the lower NH_3 volatilization and N_2O emission and because it reduces the CFP in the sugarcane production system.

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EXPLORING LEGACY P WITH COVER CROPS: SOIL PHOSPHORUS DYNAMICS IN LONG-TERM

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Keywords: P fractions; P suppression; Sustainability

INTRODUCTION

Frequent use of phosphate fertilizers results in the accumulation of P in soils (also known as Legacy P) organic (Po) and inorganic (Pi) forms. However, most of these P forms are not available for absorption by cash crops (Lambers, 2022). In the off-season, the cultivation of cover crops can increase the availability of P for cash crops because they have different P acquisition strategies that can access the Legacy P of the soil (Hallama et al., 2019; Pavinato et al., 2020).

However, few studies evaluate the long-term effects of P suppression with cover plants in the P dynamic. Thus, the objective was to evaluate the fractions of P of the soil in a long-term experiment with cover crops and phosphate sources.

METHODS

The study was conducted from 2009 to 2021 (12 harvests) at the Federal Technological University of Paraná (UTFPR), Dois Vizinhos - PR, Brazil (25° 44? 05? S, 53° 03? 31? W).

The experimental split-plot randomized block design was established in a 3 x 6 factorial scheme with three replications. Cover crops used were common vetch (*Vicia sativa*), white lupin (*Lupinus albus*), fodder radish (*Raphanus sativus*), ryegrass (*Lolium multiflorum*), black oat (*Avena strigose*), and fallow, cultivated every season from 2009 to 2021, preceding by maize or soybean as cash crops in the summer. From 2009 to 2015, phosphorus sources (single superphosphate [SSP] - 18% soluble P_2O_5, rock phosphate [RP] - 9% soluble, and control) were applied at cash crop sowing. From 2016 and so on, the residual effect of the previous fertilization was evaluated.

After the 12th harvest, the soil samples were collected in the depth of 0-5, 5-10, 10-15, and 15-30 cm to determine the P fractions. The data were evaluated by analysis of variance (ANOVA, $p \leq 0.05$), and the means were compared by the LSD test ($p \leq 0.05$), unfolding the factors when there was significant interaction.

RESULTS AND DISCUSSION

Thes most significant statistical differences ($p \leq 0.\ 05$) occur in the depth of 0-5 and 5-10 cm deep (regardless of fractions). The labile P distributions in the soil were strongly influenced by the phosphate source, where RP always presented the highest contents (Fig. 1-B), at 0-5 cm showed a difference of 54. 9 mg kg^{-1} with SSP and 72. 8 mg kg^{-1} with Nil-P. Similar responses were observed in total inorganic P and, interestingly, total organic P showed higher treatments without phosphate fertilization for 12 years (Fig. 1-F)

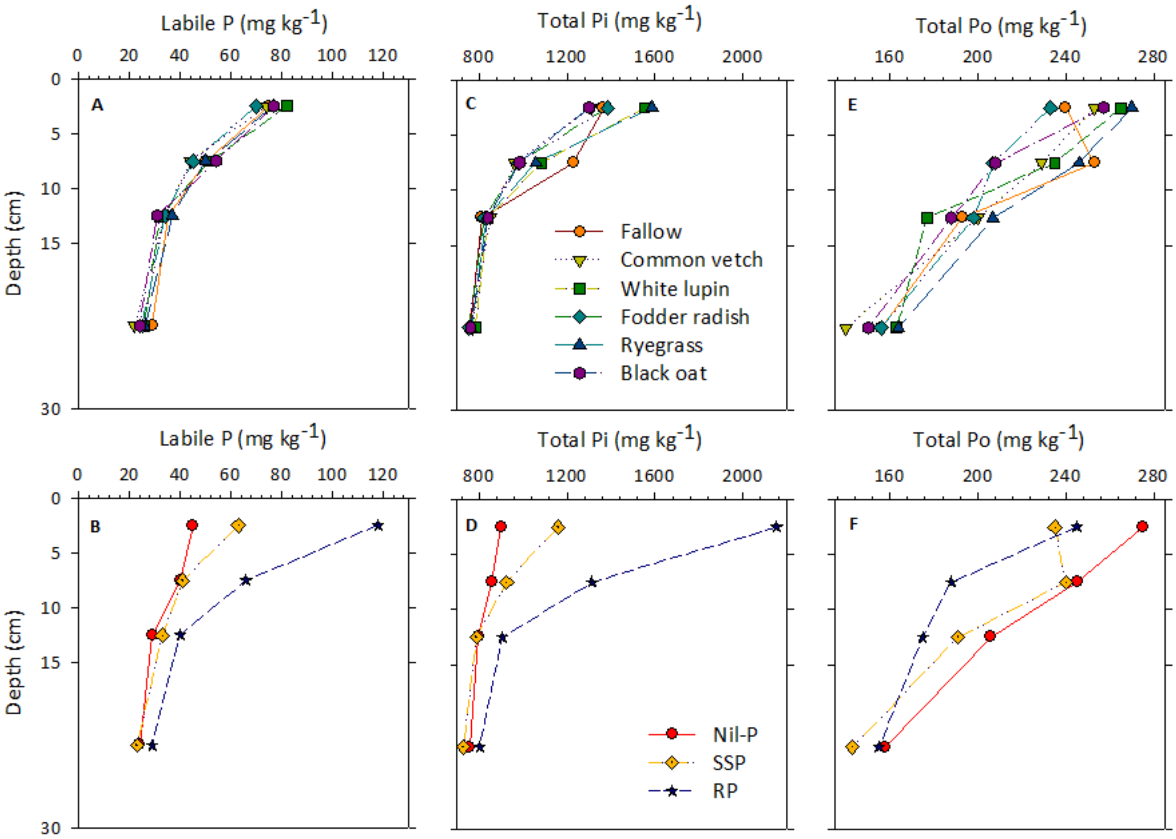

Fig. 1. Total P labile contents (A and B), total Pi and Po of the simple effects of cover crops (C and E), and phosphates sources (D e F) after 12 crop harvests (last 5 with P suppression).

The cover crops showed similar effects on labile P (Fig. 1-A); this indicates that phosphate sources imply more significant differences for this fraction of P. This same effect does not occur for the total Pi (Fig. 1-C) and, mainly, for the total Po (Fig. 1-E). In the total Po, depth 0-5 cm, only fudder radish was lower than the fallow ($p = 0.04$), and the other cover crops showed an average increase of 22 mg kg^{-1}. We jump that strong frosts interrupted the cycle of fudder radish in the seedling this off-season, justifying these results.

CONCLUSIONS

Phosphatic rock is sustainable for suppressing P, ryegrass, and white lupin alters phosphorus fractions in the soil (Pi and Po), except for total P labile.

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COVER CROPS IN SOYBEAN-WHEAT OFF-SEASON INCREASE NUTRIENT CYCLING AND GRAIN YIELDS

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Keywords: Cropping systems; Pearl millet; Sunn hemp

INTRODUCTION

In Southern Brazil a large area of wheat is preceded by soybean. The time between the wheat harvest and the soybean sowing can be as long as 60 days. However, most of producers leave the soil on fallow. The insertion of cover crops in this off-season period is a strategy for protect the soil from erosion and nutrient losses, specially the nitrogen (N).

Cover crops can help to prevent N losses by taking up high amounts of N or by having C/N ratios that balance N mineralization and immobilization resulting in better synchrony of mineralization with N uptake by the subsequent crop (Lara Cabezas et al., 2004). Grasses may prevent losses by taking N up and immobilizing it in the biomass. On the other hand, legume cover crops add N to the system, which could offset losses. Furthermore, according to the cover plant and the subsequent crop in succession, there may be a need for changes in the management of N fertilization.

The aim of this work was to evaluate the effect of cover crops grown in soybean-wheat off-season and sidedress N rates on agronomic performance of subsequent wheat and soybean.

METHODS

The experiment was carried out in Nova Aurora (PR), in an Oxisol with 175, 162, and 663 g kg^{-1} of sand, silt, and clay. The chemical properties were: pH(Cl_2Ca) 5.2, P 15.8 mg dm^{-3}, OC 16.9 g dm^{-3}, and exchangeable Ca, Mg, K, and Al values of 6.7, 2.5, 0.56, and 0.00 $cmol_c$ dm^{-3}, respectively.

The experimental design was a randomized block in a 3x4 factorial scheme, with four replications. The first factor was composed by the cover crops sunn hemp, and pearl millet, besides fallow. The second factor was the sidedress N rates, 0, 30, 60, and 90 kg N ha^{-1}, applied on wheat, using urea as a source of N.

The cover crops were sowed in January 31, 2019 (pearl millet - ADR 300®; sunn hemp - IAC KR1®). In April 4, 2019 the cover crops were collected for N, P, K, and C analysis, and subsequently were dessicated.

The wheat (TBIO Toruk®) was sowed by April 16, 2019. The sowing fertilization was 27, 8, and 15 kg ha^{-1} of N, P, and K, respectively. The sidedress N rates were applied at tillering stage. The wheat was harvested in August 28, 2019. The soybean (Basf 2606 Ipro®) was sowed in mid-September and harvested in mid-February.

Treatment effects were analyzed using ANOVA. When significant differences were found (F test, $P < 0.05$), means were compared using Tukey test for crops, and regression analyzis for N rates.

RESULTS AND DISCUSSION

The pearl millet showed a higher capacity for growth and nutrient recycling than sunn hemp (Table 1). As expected, the sunn hemp showed higher N concentration, but due to its lower dry matter, the pearl millet accumulated more N. Despite of higher C/N ratio (Table 1), the pear millet did not show N immobilization, since the wheat yield at absence of sidedress N was higher after the pearl millet (Figure 1). Also, the similarity between the slopes of millet and sunn hemp regressions reinforces this hypothesis.

Table 1. Dry matter (DM), nutrient content, and nutrient concentration of cover crops.

Cover crop	DM	K	P	N	K	P	N	C/N
	(kg ha^{-1})	Nutrient concentration (g kg^{-1})			Nutrient content (kg ha^{-1})			
PM	9994 a	25.9 a	2.4	12.1 b	259 a	24 a	121 a	38 a
SH	2783 b	14.3 b	2.7	22.6 a	40 b	8 b	63 b	21 b
CV (%)	12.6	8.7	10.5	28.5	20.8	28.9	14.8	16.5

PM: Pearl millet; SH: Sunn hemp; Means followed by different letters differ significantly by F test, at 5% probability. CV = coefficient of variation.

It seemed that the main factor that affect the wheat yield was the water availability, demonstrated by very low yields. Probably, the pearl millet straw improved soil moisture maintenance, which even helped the wheat to respond to N supplied. This effect also occurred after the sunn hemp, but to a lesser extent.

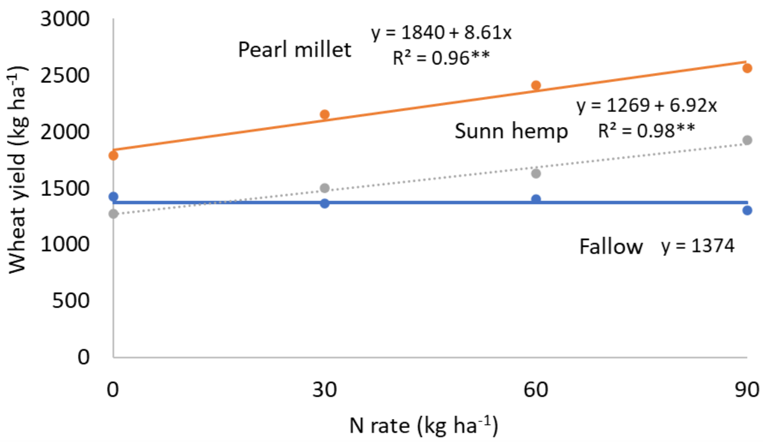

Fig. 1. Wheat yield as affected by previous crop and sidedress N rates.

The soybean was not affected by the residual N rates applied on the wheat, but the grain yield was significantly higher in rotation with pearl millet (5258 kg ha^{-1}) than the fallow (4407 kg ha^{-1}), with intermediate values in rotation with sunn hemp (4650 kg ha^{-1}). Otherwise, Calonego et al. (2017), in an average of ten seasons, observed higher soybean yield after sunn hemp than the fallow or pearl millet. However, the authors did not apply N to any of the crops in system, what favors the effect of sunn hemp.

CONCLUSIONS

The sidedress N in wheat has no effect on subsequent soybean crop. The use of cover crops in soybean-wheat off-season, specially the peal millet, is a suitable strategy to increase the nutrient cycling and grain yield of the subsequent crops.

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CONTRIBUTION OF ROOT ACID PHOSPHATASE ACTIVITY TO MAIZE (*ZEA MAYS*) GRAIN FILLING

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Keywords: Phosphorus; Sustainable; Fertilization

INTRODUCTION

One of the plant's strategies for nutrient acquisition is root acid phosphatase activity (PMEr), which increases the plant overal phosphorus (P) uptake, making it possible to reduce the use of phosphate fertilizers in maize (Wei *et al*., 2020). González-Munoz *et al*. (2015) identified 33 genes related to acid phosphatase activity in maize, showing that the expression of this trait is quantitative and confirming that there are important functional variations within maize germplasm. Therefore, PMEr can be an excellent way to optimize the management of organic P in the soil, resulting in better soil conservation and agricultural sustainability, since a considerable part of the assimilated P is produced from P mineralization through PMEr. We aim at investigating whether PMEr of maize genotypes affect the weight of grains under different soil inorganic P availabilities in central Brazil.

METHODS

The experiment was conducted in central Brazil (20°20'50.65"S 51°24'06.32"O, 344 m elevation). We used 13 commercial hybrids grown in three randomized blocks in the field and two treatments: Control - NK addition (250 kg.ha^{-1} of 8(N): 0(P_2O_5): 16(K_2O)) and treatment - NPK fertilization (250 kg.ha^{-1} of 8(N): 28(P_2O_5): 16(K_2O), equivalent to 70 kg.ha^{-1} (P_2O_5)). In the 10-leaf stage, we measured PMEr in one random plant per hybrid from each block (total of 78 plants) following Olde Venterink (2011). The effect of fertilization upon measured variables was analysed through Student t tests, the differences amongst genotypes within treatments were tested by means of ANOVA+Tukey test and linear regression analyses were employed to assess the effects of PMEr on weight of 100 grains.

RESULTS AND DISCUSSION

There were no significant effects of fertilization on total mean values of PMEr (means and standard deviations: control 860 (313), NPK-fertilized 930 (330) μmol pNPP g-root^{-1} h^{-1}) and on weight of 100 grains (control 31.7 (4.5), NPK-fertilized 33.7 (5.1) g). Out of 13, PMEr of only hybrid 11 differed ($P<0.05$) between treatments, with higher activity in the treatment with NPK fertilization in relation to the control (947 (66) and 727 (90) μmol pNPP g-root^{-1} h^{-1}). Only hybrids 1 and 9 had higher weight of 100 grains under NPK-fertilization in relation to the control (respectively 40.8 (1.1) / 35.7 (2.8) g and 32.2 (1.9) / 28.6 (1.3) g).

A significant relationship was found between PMEr and weight of 100 grains when all hybrids in the control plots were analysed (Fig. 1). This demonstrates, for the first time, the importance of the PMEr for productivity enhancement in maize under low P conditions and reinforces the need to consider this variable for P acquisition in natural, non-P fertilized agricultural systems. This might explain why the study by Wei *et al*. (2020) didn't show a significant loss of productivity after a 20% P fertilization reduction. Considering that PMEr is a quantitative trait, it is expected that it will be possible to generate genotypes with high variability, explaining the case of the hybrid 11, higher PMEr in P-fertilized soil, oppositely to the expected, since higher P availability inhibits PMEr (Olde Venterink and Güsewell, 2010). However, hybrids showed no difference for PMEr within treatments, suggesting that indirect selection has occurred in the genetic improvement process. Since it is not common for plant breeding companies to test their hybrids in unfertilized natural conditions, this trait has a relationship with productivity in low P availability. This positive effect of PMEr on this important productivity factor in conditions of low P availability in the soil shows that PMEr is an important feature to make maize production more sustainable, which can contribute to the use of less phosphate fertilizers. We believe that due to the fact that PMEr has a large number of genes involved in its expression, which allows the generation of genetic variability, and the high number of genotypes currently available in plant breeding companies, it is possible that some of these genotypes already have high PMEr that could make them suitable for more sustainable systems without problems associated to productivity loss.

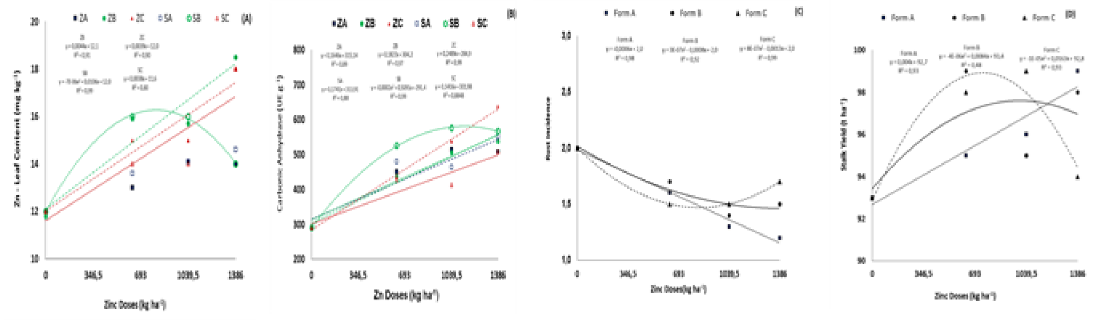

Fig. 1. Effect of root phosphatase activity (PMEr) on weight of 100 grains in P-unfertilized control plots in 13 commercial hybrids of maize (*Zea mays*) cultivated in the field in central Brazil. N= 39.

CONCLUSIONS

Root phosphatase activity positively influenced the weight of 100 grains in maize growing in low P soils, making it a promising variable to be inserted in programs of maize genetic improvement to develop genotypes adapted to more sustainable production systems in P impoverished areas.

ACKNOWLEDGEMENTS

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SOIL CARBON AND NITROGEN STOCK IN A TROPICAL INTEGRATED CROP-LIVESTOCK-FLOREST SYSTEM WITH ORGANIC AND MINERAL FERTILIZER

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Keywords: Poultry Litter; Slurry Manure; Fertilizer

Integrated production systems are promising alternatives for sustainable agriculture, allowing a positive interaction between animals and plants, which results in environmental benefits and economic viability. The objectives of this study were to investigate the contribution of organic and mineral fertilizers to carbon sequestration, and the microbiological properties of the soil in two integrated agricultural production systems. The treatments included two conservation production systems: Crop-Livestock Integration (ICL) and Crop-Livestock-Forest Integration (ICLF) and interaction with three different fertilizers (liquid swine manure, poultry litter and mineral) and control without fertilization. Soil samples were collected in trenches for analysis of C and N content and for microbiological analysis. The C and N analyzes were performed using the elemental analyzer (CHN) and the microbiological using the PLFA methodology. For the analysis content of C and N contributed by the cultures samples were collected and analyzed through the elementary analyzer. Eucalyptus dendrometric variables were collected to estimate the C content. Greater efficiency in the C input in 2018 was found in the iLP system (2590 Kg ha^{-1}) over the ICLF (2030 Kg $ha^{?1}$) in the control treatment, and no other difference was found when comparing the production systems. In relation to fertilizers, the largest contributions of C and N in 2018 were observed in poultry litter and liquid swine manure, with efficiency of 56% for C in the ICL system and 59% in ICLF, and 60% for N in ICL and 62 % in ICLF. However, in 2019 the mineral fertilizer showed a difference in ICL and poultry litter in ICLF (both with an efficiency of 27%) for both C and N. Considering the total soil profile in the comparison between production systems, the system iLPF showed an efficiency of 20.7% over iLP in relation to the C stock in the control treatment, and the N stock was more efficient over the iLP in poultry litter and mineral fertilizers. When there is no fertilization in the production systems, iLPF is superior to iLP for C input, however when there is fertilization with poultry litter and mineral, the iLP system is superior to iLPF for N input. Mineral or organic fertilizer increased the input of C only in the iLPF system, demonstrating that there is another C dynamic when eucalyptus is present in the iLPF system.

CLOSING PHOSPHORUS CYCLES IN MAIZE-BASED CROPPING SYSTEMS

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Keywords: Phosphorus; maize; nutrient cycles

INTRODUCTION

Phosphate (P) is a limited essential nutrient with a very uneven global distribution. Closing the P-cycle and reducing primary P consumption are fundamental future challenges. Maize has a comparatively high demand for P. China and Germany together cover the whole range of maize production systems and complement each other in all aspects of production and utilization (Fig. 1). The DFG-funded Sino-German international research training group *"Adaptation of Chinese and German maize-based food-feed-energy systems to limited phosphate resources" (AMAIZE-P, DFG 328017493/GRK 2366)* was initiated in 2018 as a joint venture of the China Agricultural University (Beijing) and the University of Hohenheim (Stuttgart, Germany). The research is driven by the hypothesis that *"under P limited conditions, high productivity and high P use efficiency can be achieved simultaneously by adapting P cycling and availability (sources) to the multipurpose P demands (sinks) in maize based food-feed-energy systems"* [1]. AMAIZE-P will last for a maximum of nine years ending in 2027 after three cohorts of roughly 100 doctoral researchers in total.

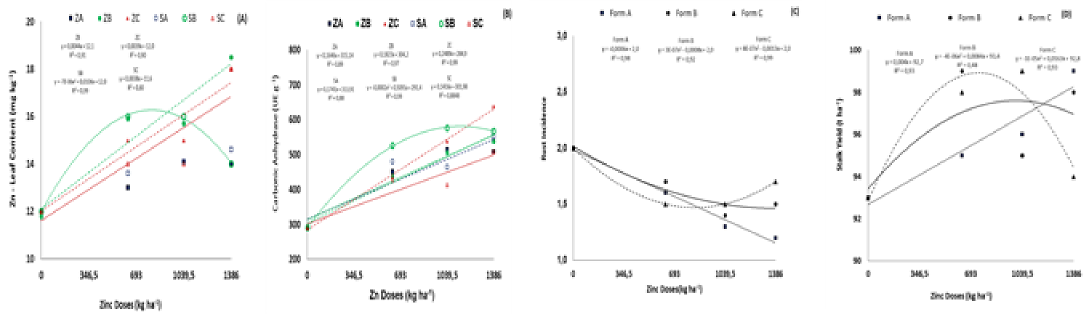

Fig. 1: Maize-based agricultural food-feed-energy systems in China and Germany together represent all aspects of maize production in contrasting and complementing situations.

RESULTS AND DISCUSSION

Here, we mainly report the first results from two research subjects (RS): *RS 2.2 Increasing soil P availability and P fertilizer efficiency*; *RS 4.2 Synthesis and field experiments*. RS 2.2 initially focuses on improving basic understanding on soil P species and fraction sizes, turnover processes and plant availability. We used four standard wet extractive analytical procedures for soil P in a broad range of 50 soils across Europe. The P concentrations in the extracts were detected by both colorimetry and ICP-OES. For the increasingly popular ICP-OES detection method, the calcium acetate lactate (CAL) extraction method proved to be superior to the Olsen method due to the overestimation of soil P by the latter, which is owed to the high amounts of extracted soil organic P using the alkaline Olsen extractant (Fig. 2). As shown, the gap between the red theoretical 1:1 line (representing equal P concentrations by both detection methods) and the actual regression line between both detection methods is narrower in CAL than in Olsen extracts. Solution ^{31}P-NMR studies are underway to elucidate organic P species in soil.

Volatilization of ammonia after application of urea on Marandu grass

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Keywords: Urea; Brachiaria brizantha; Nitrogen

INTRODUCTION

Nitrogen fertilization, after adjusting the level of the other nutrients in the soil to provide favorable conditions is considered to be one of the most important practices to obtain good yields from pastures. Depending on the source of N of the fertilizer applied, the volatilization of ammonia (N-NH_3) can represent a loss of up to 60% of the N from the fertilizer applied to pastures (CANTARELLA et al., 2001). We quantified the ammonia volatilized from the urea applied as top dressing in a pasture by using a semi-open static chamber (SOSC) after application of six urea doses.

METHODS

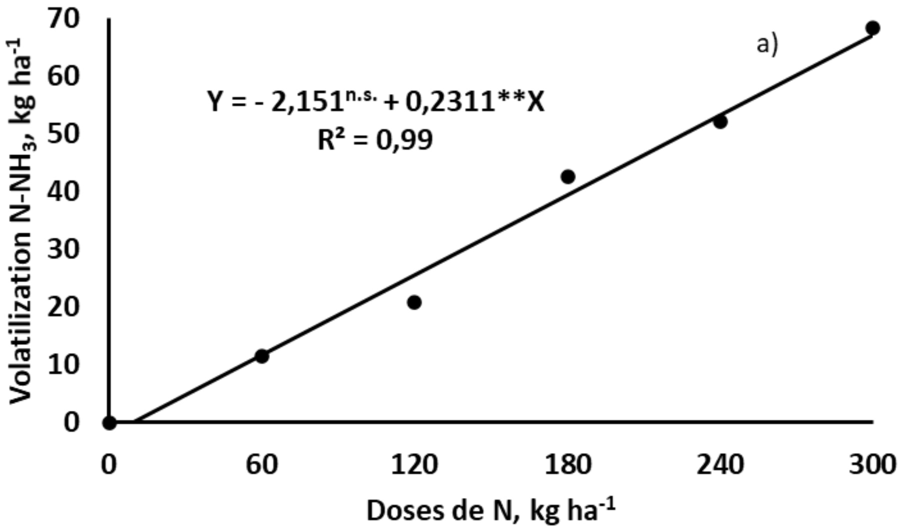
The experiment was conducted in a experimental area of Federal University of Mato Grosso (UFMT), Araguaia Campus, located in the municipality of Barra do Garças, Mato Grosso, located at coordinates 15°52'28.64"S and 52°18'36.86"W. The experimental design used was randomized blocks with six N doses (0, 60, 120, 180, 240 and 300 kg ha^{-1}) supplied by urea (46% N), with four repetitions.

In each plot, six PVC pipes with diameter of 10 cm and height of 10 cm were installed in the soil, to which a semi-open static chamber (SOSC) was attached to collect ammonia. The model used was similar to that described by Araújo et al. (2009). The solution to capture the volatilized ammonia was H_2SO_4 1 mol dm^{-3} + glycerin 2% v/v. The foam was collected on days 1, 3, 5, 9, 15 and 20 after application of the treatments. The N-NH_3 retained in the foam was quantified by distillation and titration by the Kjeldahl method. The accrued losses of N-NH_3 were calculated in kg ha^{-1} and in percentage of total N applied per hectare. The data were submitted to analysis of variance and when significant by the F-test, were used in regression analysis employing the SISVAR 4.6 statistical progam (Ferreira, 2019).

RESULTS AND DISCUSSION

The accrued losses of N-NH_3 in kg ha^{-1} of total N added were nearly 6-fold lower for the dose of 60 kg ha^{-1} than for the dose of 300 kg ha^{-1}, and for the lowest N dose applied (60 kg ha^{-1}), the maximun daily loss of ammonia was 4.81 kg ha^{-1}, while for the largest dose (300 kg ha^{-1}), the maximum dail ammonia loss was 18.8 kg ha^{-1} (Figure 1a). In percentage terms, the cumulative ammonia lost due to volatilization was 23.64% for the N dose of 180 kg ha^{-1} de N (Figure 1b).

Primavesi et al. (2001) reported ammonia losses ranging from 4.6% to 61.6% for doses of 25, 50, 100 and 200 kg ha^{-1} of N supplied by urea. Lara Cabezas, Korndörfer and Motta (1997) found cumulative loss values up to 78% with application of 100 kg ha^{-1} of N as top dressing of corn plants in a no-till system, utilizing urea as N source. Cantarella et al. (2001), in an experiment conducted in the Brazilian state of Mato Grosso do Sul in a pasture planted with *Brachiaria brizantha* with application of 100 kg ha^{-1} of N, found losses of 28% of N in the form of ammonia.



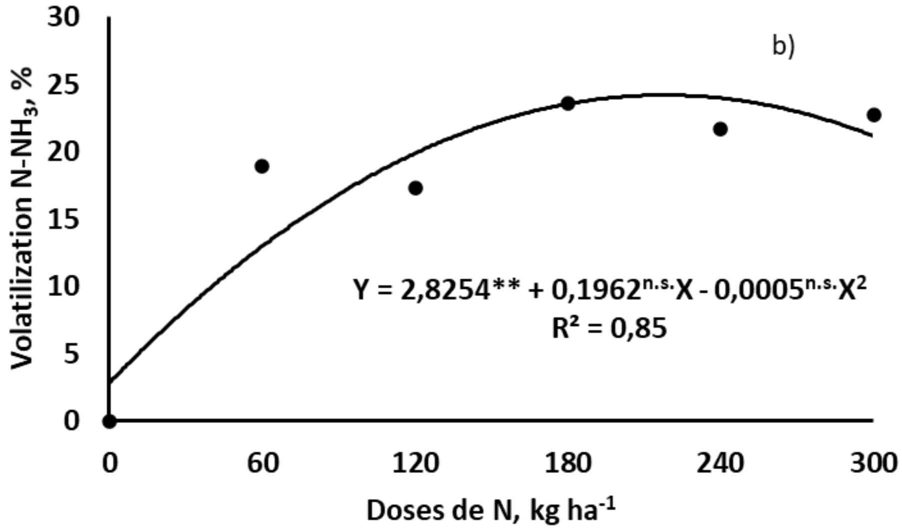


Figure 1. Cumulative losses of N-NH_3 in kg ha^{-1} (a) and percentage of total N applied (b) in each dose of urea as top dressing of Marandu grass.

CONCLUSIONS

The losses of ammonia by volatilization increased as the urea doses applied increased.

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NUTRIENT REMOVAL OF ENERGY CANE GENOTYPES IN BRAZILIAN CONDITIONS

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Keywords: Macronutrient; NPK; Nutrient Exportation

INTRODUCTION

Fossil fuels represent a finite source of energy and are directly related to global climate changes caused by emissions of greenhouse gases (Cheng, 2017). Thus, the use of bioenergy sources is strategical to meet environmental, economic, and social requirements for the global sustainable development (Santoyo-Castelazo and Azapagic, 2014).

Brazil is an example of having successfully reduced the gasoline consumption by partially replacing it by biofuel, making the country the world major producer of sugarcane-ethanol producer, reaching a 35 million m3 production in the 2019/2020 sugarcane harvesting season (MAPA, 2020). However, it is estimated that only one third of the sugarcane crop energy potential derives from sucrose broth (which has been used the production of in sugar and first-generation ethanol), since the greatest energy potential of sugarcane is associated with crop residues (Carvalho et al., 2017). Therefore, the objective of this study was to quantify the nutrient removal of 26 energy cane genotypes and compare with tradictional sugarcane varieties cultivated in Brazilian conditions.

METHODS

The experiment was carried out under field conditions in the city of Paulicéia (21º16? "S; 51º46' "W, 341 m altitude), located in the western region of the state of São Paulo. The experimental design was arranged in randomized blocks with three replicates containing 28 genotypes, of which 26 energy cane genotypes from the GranBio breeding program and two commercial genotypes of sugarcane (RB867515 and RB92579). At the end of plant-cane and first ratoon cycles, biomass evaluations were performed to estimate dry biomass, and the accumulation of macronutrients in each plant compartment (stalk, top, and dry leave). Results of nutrient extraction were submitted to variance analysis through the GLM procedure of SAS (SAS Institute Inc., Cary, NC, EUA) software, by means of F test, followed by the Scott Knott test for comparison of means with $P \leq 0{,}05$ significance.

RESULTS and discussion

In the cane plant cycle, N accumulation on plant shoot was ranged 191 to 383 kg ha^{-1} of N. P accumulation varied from 34 to 98 kg ha^{-1} and K extraction by plant-cane plant was highest for all macronutrintes varying from 295 to 607 kg ha^{-1}. In the second year of cropping, the primary macronutrientes varying from 219 to 346 kg ha^{-1} of N, from 21 to 46 kg ha^{-1} of P and export of K exceeded 1,000 kg ha^{-1}. For extraction and accumulation of secondary macronutrients in both cycles, two and three contrasting groups were created. However, the order of extraction and accumulation was different in both cycles, with Mg>Ca>S for the first cycle (cane plant), and S>Mg>Ca for the second cycle (first ratoon). Furthermore, average nutrient accumulation in aboveground biomass plant shoot was also higher in energy cane genotypes in comparison with sugarcane, reaching average increments of approximately 25% of N, 45% of P and K, 30% of S, 90% of Ca and Mg. The amounts of N, P and K accumulated by energy cane shoots were higher than those obtained in three sugarcane and energy cane crops in the United States, having reached averages in the order of 179. 41 and 279 kg ha^{-1} year^{-1} of N, P and K, respectively (Singh et al., 2015). Differences can be attributed to higher biomass production under Brazilian conditions. In the present study, total removal of macronutrients by energy cane genotypes varied from 77 to 244 kg ha^{-1} for N; 12 to 58 kg ha^{-1} for P; 278 to 755 kg ha^{-1} for K; 32 to 96 kg ha^{-1} for Mg; and 26 to 77 kg ha^{-1} for o S.

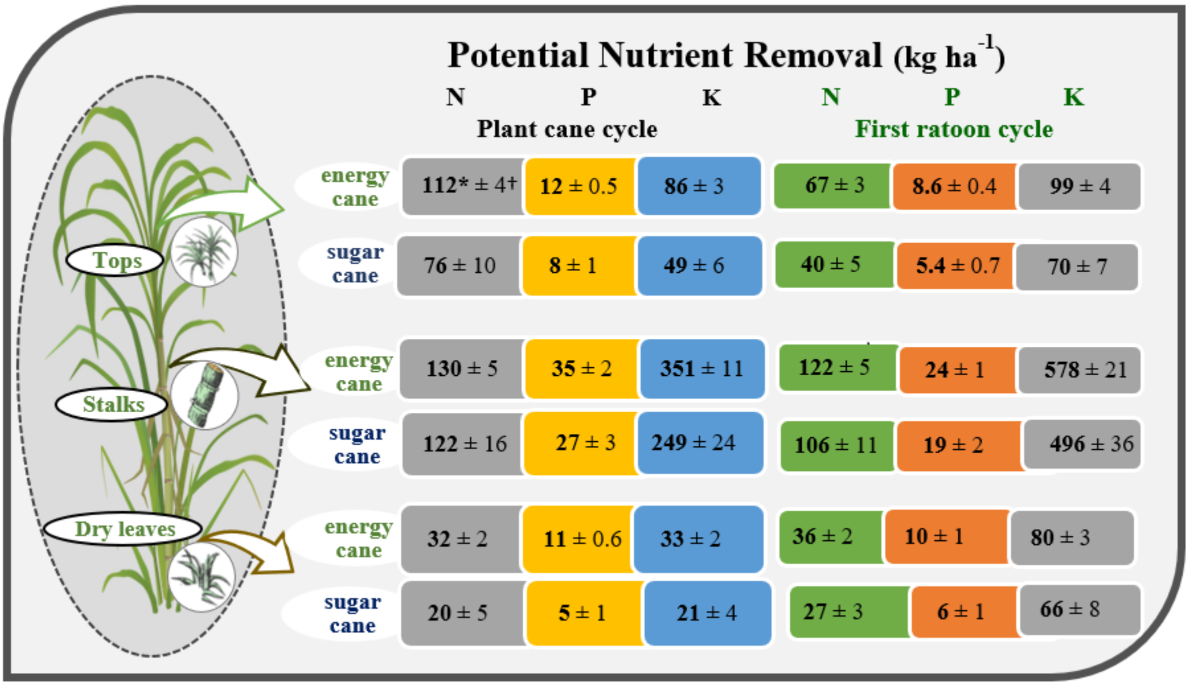

Figura 1. Overall balance of potential N, P and K nutrient removal by energy cane and sugarcane first crop 2016-2017 and second crop 2017-2018, Paulicéia, Brazil.

CONCLUSIONS

Information regarding agronomic management practices for energy cane crop, such as nutrition and fertilization, is still incipient, even considering the evaluation performed by several research studies over the last decade. Therefore, a set of agronomic recommendations containing information on nutritional supply (fertilizer input) is highly needed, which should also include comparisons with sugarcane as it presents higher content of nutrient extraction, especially if stalks and dry leaves are removed from the field for industrial utilization.

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LAND USE AND MANAGEMENT OUTCOMES ON SOIL HEALTH AND QUALITY

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Keywords: Agroforestry; conventional wheat; soil properties

Land use and management outcomes on soil health and quality

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INTRODUCTION

Soil health assessment has gained increasing attention for monitoring soil productivity for sustainable land management and restoration of degraded land (Bünemann et al., 2018). However, soil health assessment remains a challenging issue (Klimkowicz-Pawlas et al., 2019) due to diversity of local climate, environment and management regimes affecting the inherent and dynamic characteristics of soils. To capture effects of the different land use and management systems on soil health, it is of importance to apply integrated assessment approaches that characterize the physical, chemical, and biological conditions of the soil (Idowu et al., 2008). Hence, the objective of the study was to assess and compare the effects of diverse land use and management regimes on soil health

METHODS

For the study, four diverse production systems viz. agroforestry biomass belts (BB), crop alleys (CA), natural forest (NF) and conventional wheat (CW) (*Triticum aestivum* L.) fields were identified, representing diverse land use and management regimes at an experimental farm in Tasstrup under the University of Copenhagen in Denmark. Out of the four production systems, 3 production systems viz. BB, CA and NF were located within a combined food and energy production system (CFE) (Fig. 1), an agroforestry system established at the experimental farm in Taastrup. Soils were sampled at 0-30 cm depth and measurements included soil bulk density, soil pH (water), electric conductivity (EC), phosphorus (Mehlich, M3), potassium (exchangeable), soil organic carbon, active carbon, potentially mineralizable nitrogen (PMN), and soil organic matter content. The measured soil physical, chemical and biological properties were integrated into a Soil Quality Index (SQI) using the "Soil Management Assessment Framework" (SMAF) for comparison of soil quality across land use and management regimes. SMAF can integrate soil chemical, physical and biological properties and one of the most widely used soil quality evaluation tool (Karlen et al., 2006). SMAF involves three steps: (a) indicator selection, (b) interpretation of indicator, and (c) integration of the SQI (Karlen et al., 2008; Wienhold et al., 2009). Karlen et al., 2008 recommended a minimum of five soil properties with at least one each of the soil chemical, physical, and biological properties to evaluate the soil quality. Then using SMAF algorithms, the indicators of soil are converted into individual scores that take into account the intrinsic and dynamic properties of soil due to management and environmental conditions. At last, the individual scores are combined to create overall SQI (Wienhold et al., 2009).

RESULTS AND DISCUSSION

Bulk density is an indicator of the soil compactness, which affects the root growth and availability of plant nutrients and soil moisture for optimal crop growth. Bulk density was lower in NF and BB compared to CA and CW (Table 1), which indicated that there were more air spaces for the plant roots to grow with ample aeration for supply of oxygen for optimal growth in BB and NF compared to CA and CW. Of the nine soil parameters, seven soil parameters were used for calculation of SQI except active carbon and soil organic matter content. Active carbon and soil organic matter contents were found higher in BB and NF indicating higher soil fertility and productivity (Table 1). As soil organic matter is positively correlated to soil moisture content, BB and NF systems had the higher capacity to store soil moisture and make it plant-available compared to the CA and CW fields. The SQI was found highest in the BB (0.93) followed by NF (0.90), CA (0.80), and CW (0.76) fields. The study demonstrated that the BB and NF systems had higher SQI, indicating better soil health and fertility, compared to CA and CW fields, highlighting the importance of land use and management on soil quality and health for food, feed and biomass production to meet the demands of the growing population.

Table 1: Key soil indicators (Mean±SD) and SQI scores in diverse production systems

Soil parameters	Biomass belts (BB)	Crop alleys (CA)	Conventional wheat (CW)	Natural Forest (NF)	P Value
BD [g/cm^3]	1.28±0.06	1.38±0.09	1.42±0.06	1.23±0.18	0.10
SQI [BD]	0.971± 0.02	0.834± 0.14	0.771± 0.13	0.899± 0.19	0.255
Soil organic carbon [%]	2.22±0.54 [b]	1.62±0.12 [a]	1.39±0.04 [a]	2.35± 0.11 [b]	≤***
SQI [Soil Organic Carbon]	0.799±0.189 [b]	0.561± 0.079 [a]	0.421± 0.028 [a]	0.894 ± 0.023[b]	≤***
Soil organic matter [%]	3.8[b]	2.78[a]	2.39[a]	4.04[b]	≤***
PMN [mg/kg]	64.1± 5.31 [b]	51.0±2.77 [a]	45.0±1.84 [a]	61.1±4.50 [b]	≤***
Active carbon[mg/kg]	933 ± 219 [c]	591±57.8 [a]	571± 78.1 [a]	1260± 181 [b]	≤***
Overall SQI	0.926± 0.033 [b]	0.798 ± 0.0239[a]	0.764± 0.0313[a]	0.901±0.0429 b	≤0.001***

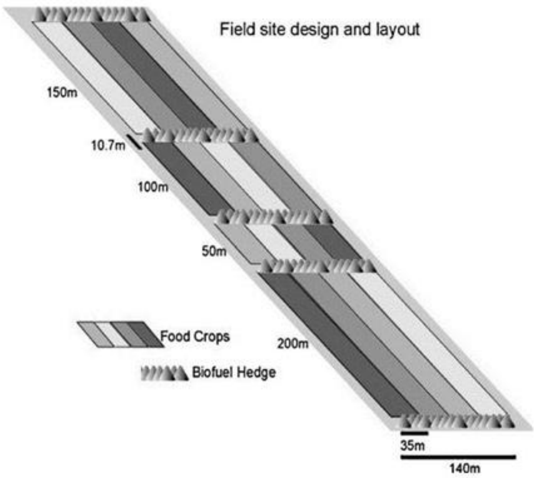

Figure 1: Field layout of the Combined Food and Energy (CFE) agroforestry system

CONCLUSIONS

Soil properties differed significantly among four different land-use and management systems. The key soil indicators such as bulk density, soil organic carbon, active carbon and soil organic matter content and PMN were found higher in the BB and NF demonstrating the role of trees for enhanced soil quality. Trees play important roles in the enhancement of soil quality since they contribute to the significant addition of organic matter by decomposing leaves, litter and roots as well as bringing up nutrients from deeper layers of soil resulting in overall improvement in the physical, chemical, and biological indicators of soil.

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MACADAMIA HUSK COMPOST ENHANCED ROOT GROWTH AND LEAF YIELD OF CHINESE CABBAGE (*BRASSICA RAPA* L. CHINENSIS) BY IMPROVING SOIL PHYSICO-CHEMICAL PROPERTIES

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Keywords: Leaf biomass; Root biomass; Root length

INTRODUCTION
Poor soil fertility caused mainly by low and declining soil organic carbon is one of the major constraints limiting crop productivity in tropical and subtropical regions of South Africa. We evaluated the effect of macadamia husk compost (MHC) on physico-chemical properties of soil, and root growth and leaf yield of Chinese cabbage on sandy loam soil in NE South Africa.

METHODS
A field experiment, consisting of four treatments, i.e., zero control, inorganic fertilizer (IF), 15 t ha$^{?1}$ macadamia husk compost (MHC1), and macadamia husk compost at 30 t ha$^{?1}$ (MHC2), was conducted at Levubu (25? 270 S and 30? 580 E, and 877 m asl), South Africa. MHC was incorporated into the soil a month before planting at a depth of 15 cm and inorganic fertilizer was applied 2 weeks after planting by ringing around Chinese cabbage (*Brassica rapa* L. Chinensis).

Selected soil chemical (pH, EC, and soil organic carbon, Total N, available P, K , Ca, Mg, Na, Zn, Mn, and Al) and physical (bulk density and water holding capacity) properties were determined prior to planting (just after ploughing) and after harvesting using standard procedures.

Six plants from two inner rows were randomly selected at harvest and used for determination of yield (number of leaves and leaf biomass). During harvesting, roots were carefully uprooted and gently washed with tap water. Root length was measured and thereafter the roots were dried in an oven for 48 h at 65 ^{O}C for determination of root dry weight.

The data were subjected to analysis of variance. Where siginifcant, means were separated using LSD. Pearson's correlation analysis was conducted to assess the relationship between (soil pysicohemical properties & root length and root biomass) and root length and root biomass & yield biomass.

RESULTS and discussion
There was a significant relationship between soil propeties and root growth (Table 1) which suggests that increase in root length and biomass with application of MHC (Maselesele et al., 2022) was due to similar improvement in soil physico-chemical properties with MHC application (Maselesele et al., 2021).

We observed a positive correlation between leaf biomass and root biomass (Table 2) which implies that the increase in leaf yield with MHC (Maselesele et al., 2022) could be attributed partly to a similar increase in root biomass with MHC application (Maselesele et al., 2022).

Table 1. Correlation coefficients between root biomass, root length and soil physicochemical properties

Variables pH EC OM SOC BD WHC N P K Ca Mg Na Al Zn Mn

Root length 0.28ns 0,16ns **0,33* 0,52** -0,62**** 0,53* **0,51* 0,58* 0,79*** 0,64** 0,58** 0,58** 0.43* 0.54** 0.39***

Root/Shoot biomass 0.29ns -0.08ns 0.03ns 0.19ns -0.26ns 0.29ns 0.15ns 0.21ns 0.27ns 0.28ns 0.22ns 0.25ns 0.22ns 0.22ns 0.26ns 0.17ns

Root biomass **0.57** 0.39* 0.54** 0.73** -0.60** 0.67** 0.77*** 0.73** 0.85*** 0.83*** 0.82*** 0.70** 0.65** 0.54** 0.39***

Values are means ± standard deviation; n= 12; within a column means bearing the same letter are not statistically different; Control = no fertilizer; IF = 100:60:60 kg NPK $^{ha-1}$; MHC1 = Macadamia husk compost at 15 t $^{ha-1}$; MHC2 = Macadamia husk compost at 30 t $^{ha-1}$.

Table 2. Correlation coefficient between root biomass, root length and yield componets

Variables Number of leaves Leaf Biomass (DW) Leaf area index

Root biomass (DW) **0.63** 0.74**** ** **0.86*****

Root length **0.72** 0.86*** 0.80*****

Values are means ± standard deviation; n= 12; within a column means bearing the same letter are not statistically different; Control = no fertilizer; IF = 100:60:60 kg NPK $^{ha-1}$; MHC1 = Macadamia husk compost at 15 t $^{ha-1}$; MHC2 = Macadamia husk compost at 30 t $^{ha-1}$.

CONCLUSIONS

Application of macadamia husk compost improved soil physico-chemical properties and consequently increased root length, root biomass and leaf yield of Chinese cabbage

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THE SOYBEAN CROP PROFITABILITY DUE APPLICATION PHOSPHORUS DOSES AND SOURCES

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Keywords: Fertilizer Price; Efficiency; Yield

INTRODUCTION

As a consequence of modernization, the use of new technologies, there has inevitably been an increase in production costs over the years and the need for increasingly effective and efficient rural management (Alvez, 1998 apud Artuzo et al, 2017). Recently, the conflict between Russia and Ukraine, mainly due to the increase in fertilizer costs, impacted, for the 2021/2022 crop, the cost of production of this commodity, demonstrating that, in addition to the technological factor, the "human factor" ended up further entering the soybean producer. According to data published by CONAB, fertilizers of 72% compared to the 2020/2021 harvest. Within a scenario, the present work aims to analyze and identify the relationship between soybean production costs and the revenue of its productive activity, focusing on the use of fertilizers in a rational and situational manner, with a focus on the phosphorus element.

METHODS

This study was conducted in the state of Paraná, in the city of Bandeirantes, which is located in the north portion of the state, employing the soybean productivity data collected from an experimental area in the State University of Northern Paraná (UENP), *campus* Luiz Meneghel. The experiment consisted of different phosphorus leves applications with an organomineral source, distributed in four repetitions per treatment, as shown: control (T1), recommended P dose (T2); recommended P doses by the "organomineral' fertilizer (T3); 50% less of the recommended dose (T4); and 50% more than the recommended dose (T5). The estimation of the recommended dose was based on soil analysis, making use of the 'Liming and Fertilization Recommendation Manual for the State of Paraná". The doses were composed of: 40 kg ha^{-1} of P and 80 kg ha^{-1} in the form of potassium chloride (KCl). The K doses were the same for all treatments.

The further data, considering only crop costing expenses: costs with the machinery operation, seeds; the agricultural defensives and labor costs were obtained through The National Supply Company (CONAB), compiled from the Londrina region data for the 2021/2022 harvest, taken as constant in all treatments. The sale price was obtained from the Center for Advanced Studies on Applied Economics (CEPEA), being an annual price average of a 60 kg bag for 2021. The productivity data were subjected to ANOVA analysis of variance, and when significant, they were compared by the Tukey test ($P<0.05$), by using the SISVAR software, with a significance level of 5%.

RESULTS AND DISCUSSION

There was not any statistical difference between the treatments' soyben yield averages, sustaining the hypothesis that the hydric stress period plus a low mulch condition and a non-existent soil fertility profile contributed to a non-response by the crop to different phosphorus doses variations and the use of an organomineral formulate. All the treatments achieved a positive net income. Nevertheless, they attained under productivity average results regarding to the state of Paraná, with 3.535 kg/ha in the 2021/22 harvest, according to CONAB. It is known that the elements to be transported and absorbed must be in the soil solution, in their ionic forms, so the transport mechanisms can act, considering that the nutritional deficiency symptoms can be intense in low water disponibility conditions and decrease or disappear throughout rainy periods (MELLO; NOVAES, 2007). Reinforcing the water importance in the soil/plant relation, especially in the absorption and transport dynamic of nutrients.

Table 1. Soybean yield, weight of a thousand seeds , and plant height related to different P application rates and ways in the soybean crop in Bandeirantes - PR.

Treatment	Yield	weight of a thousand seeds	Plant height
	Kg/ha	g	(cm)
T1	1967,41 a	138 A	60A
T2	2057,93 a	136 A	63A
T3	1766,67 a	143 A	60A
T4	2328,89 a	140 A	64A
T5	1967,93 a	142 A	65A

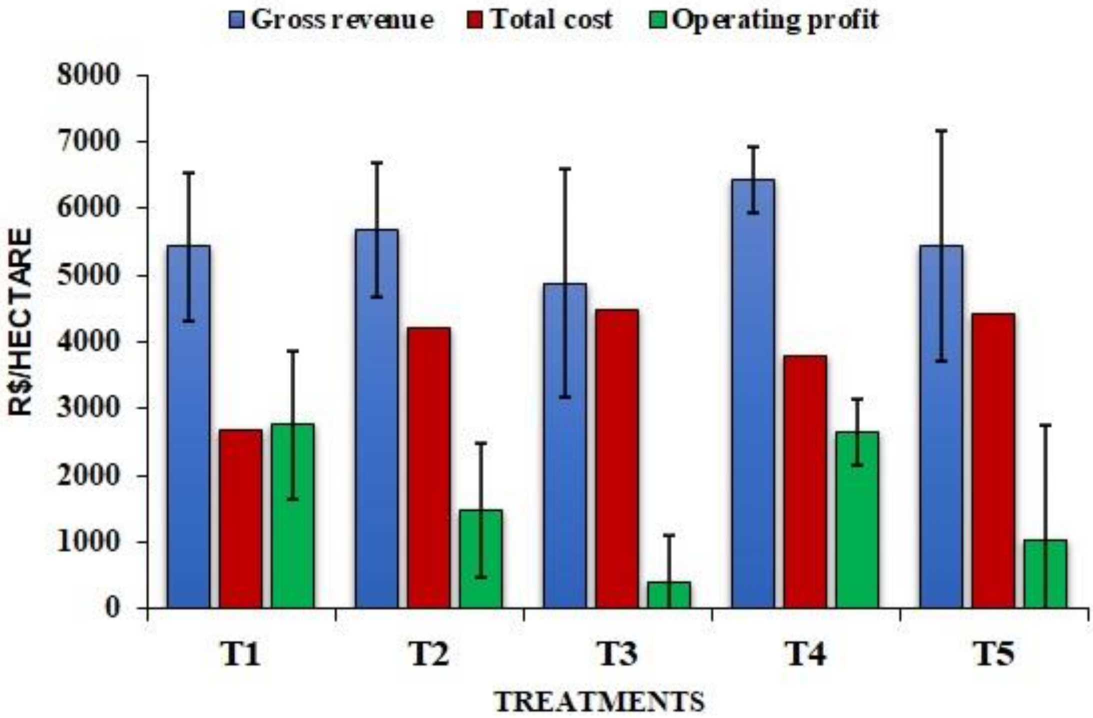

Fig. 1. Gross revenue, total cost and operating profitelated to different P application rates and ways in the soybean crop in Bandeirantes - PR.

CONCLUSIONS

Under a low crop residues volume condition and low pluviosity - considering the fertilizer price increase as a determinant factor - the results indicated that the half recommended dose or "dose zero" application demonstrated better rentabilities. However, it is not recommended in the agronomic sector and is unable to sustain for a long period, being an extremely conditional measure and deprived of more data to corroborate its real efficacy. It is important to mention that the technical basis for the decision-making is composed of the particular soil fertility guidelines, dismissing any decision besides that.

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Nutrient uptake, transport and use efficiency

EVALUATING ROOT-ARCHITECTURAL VARIABILITY AND NO_3^- REDUCTASE ACTIVITY IN WHEAT GENOTYPES REFERRING N USE EFFICIENCY

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Keywords: nitrate; N use effciency; wheat

INTRODUCTION

Overuse of Nitrogen (N) fertilizer, a crucial productivity factor in world cropping systems, is detrimental to the sustainability of crop production from an economic and environmental perspective (Shahzad *et al.,* 2019). The generalized recommendation of N fertilizer without the proper soil analysis is the prime reason of low N use efficiency (NUE) in crop plants (Wakeel *et al*., 2021). The uptake and utilization efficiency of N is closely related to root architectural traits and nitrate (NO_3^-)-ammonium (NH_4^+) assimilation pathways respectively. According to Sinha *et al*., (2015) in wheat seedlings the presence of specific NO_3^- transporters coordinated with N metabolism paves the way for root architectural variation under limited N regimes to enhance the N use efficiency. Root system architecture (RSA) is the spatial configuration and distribution of roots includes total root length, root volume and root length density which affects proper acquisition of nutrients and ultimately plant biomass. It was hypothesized that RSA and NO_3^- reductase activity concomitantly enhance NUE in wheat genotypes and present study evaluates genotypic variation for NUE based on inherent variation in RSA traits regulated by nitrate reductase activity (NRA) under low and high nitrate and ammoniacal form of N supply.

METHODS

The 20 wheat genotypes used for the experiment collected from Ayub Agricultural Research Institute and National Agriculture Research Center. The genotypes were grown in Hoagland solution. Images of 28 days old wheat seedling were collected using high resolution digital camera by spreading on flatbed covered with black sheet. All traits were analyzed by importing the images on ImageJ based smart root software. For Nitrate reductase activity (NRA), the fresh leaf samples were frozen at -80?C and activity was determined following Baki *et al.* (2000). Plant total N was determined using the method of Jackson (1962).

The N-use related traits were determined as by Merigout *et al.* (2008) and Wang *et al.* (2017).

NUE (%)=Plant dry biomass/N supplied x 100

R-studio of R software 4.1.0 (Team, 2020) was used to statistically analyze the data. Whole data set was statistically analyzed by applying analysis of variance techniques using 2-factor factorial design under CRD. LSD test was applied to check the significance. Correlation analysis was performed by using Pearson correlation coefficient method.

RESULTS AND DISCUSSION

Results revealed that genotypes Pirsabak-13, AAS-2011 and Shakar-13 have shown better N uptake and utilization owing to vigorous RSA and higher NRA. The enhanced nitrogen use efficiency (NUE) is evident that RSA and NRA have deemed potential indicators to characterize N efficient genotypes and selected characters can be used in breeding program for development of N efficiency varieties.

Increment in root length is the indication of the foraging capability of the genotypes under reduced Ni concentration in solution culture, and N deficiencies significantly affects a number of metabolic pathways like primary photosynthesis and carbohydrate partitioning between source and sink tissues (Sinha *et al.*, 2015). So, at seedling stage changes in root/shoot length, fresh and dry weight defines its genetic variability. A significant effect of treatments on the genotypes Pirsabak-13, AAS-2011 and shakar-13 have been observed in the present study (Fig. 1a). The NR is stimulated with the exposure of plants with Ni and its activity reduces with Am supply as shown by genotype Pirsabak-13 in the present study (Fig. 1b).

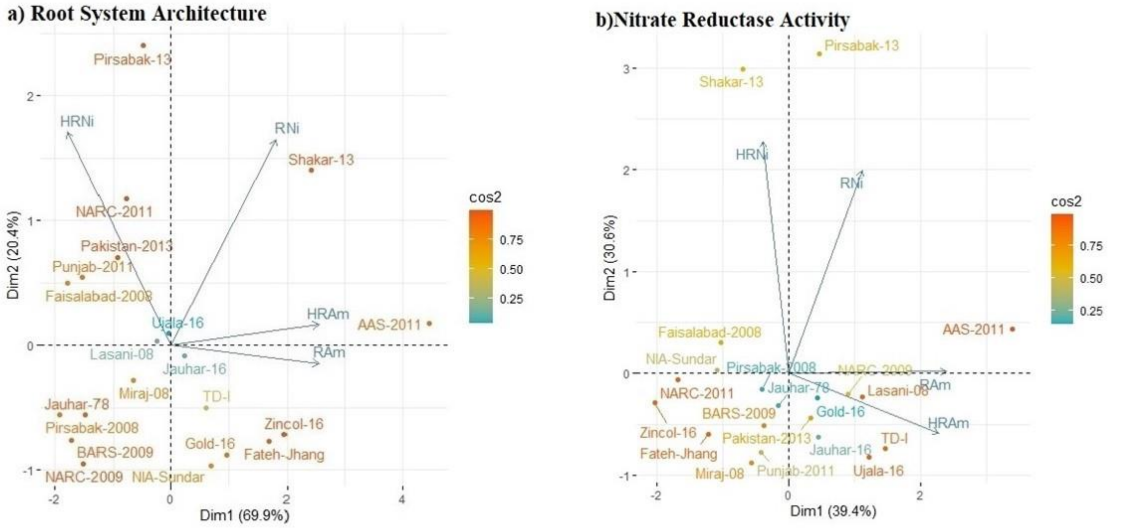

Figure 1. Biplot generated to determine the root system architectural behavior (a), for nitrate reductase activity (b). R_{Ni}: Recommended nitrate nitrogen, HR_{Ni}: Half of recommended nitrate nitrogen, R_{Am}: Recommended ammoniacal nitrogen, HR_{Am}: Half of recommended ammoniacal nitrogen.

CONCLUSIONS

Three genotypes Pirsabak-13, AAS-2011 and Shakar-13 with better N uptake and utilization due to vigorous RSA and higher NRA leading to enhanced NUE by wheat plants. These genotypes can be used in breeding program to develop N efficient cultivar with better yield characteristics. However further studies are needed to estimate their full genetic potential in terms of yield under field conditions.

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NITROGEN FERTILIZATION BOOSTS ESTIMATED MEAT PRODUCTION IN AN INTEGRATED MAIZE/FORAGE CROPPING SYSTEM

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Keywords: nitrogen; cropping system; forage grass

INTRODUCTION

Remediation of degraded pastures and high fertilizer use efficiency is essential to increase agricultural systems sustainability. Integrated systems, such as the intercropping of maize with forage grasses that could be pastured after maize harvest have been important for tropical agriculture, extending land use throughout the year. However, in maize-grass intercropped systems competition for N between species can compromise maize and/or forage production (Borghi et al. 2014), and it is not known whether the rate of N fertilizer applied to maize would be enough to meet the requirements of the system as whole. Palisade grass and Guinea grass are among the species most used due to their high dry matter yields and palatability (Machado et al., 1998), but forage production is directly associated with direct light interception, which could be impaired by competition with maize and N availability. We hypothesized that N fertilization, besides increasing maize yield, could improve forage yield and quality, eventually increasing meat production. The objectives of the present short-term experiment were to evaluate maize grain yield when intercropped with grasses and assess forage yield and bromatological quality as affected by nitrogen fertilization and grass species.

METHODS

A field experiment where maize was intercropped with Guinea grass (*Megathyrsus maximus*) or palisade grass (*Urochloa brizantha*), and fertilized with 0.0 to 270 kg ha^{-1} of N was carried out in Botucatu, Brazil (22º49'27.68" S, 48º25'46.75" W, 700 m asl). Maize and forage yields were determined, and bromatological characteristics were assessed at maize harvest (April) and when grasses were cut in mid-October/November. After maize harvest, light interception (LI) of the forage grasses was measured weekly. When each treatment reached 95 % LI, the SPAD index was determined. Although grazing by animals was not performed, meat production was estimated based on the Cornell Net Carbohydrate and Protein System (CNCPS) version 5 (Fox et al., 2004).

RESULTS AND DISCUSSION

The importance of N fertilization for forage grasses was remarkable in our experiment, since dry matter yields were increased up to 5-fold with N fertilization. Despite maize response N fertilization, there was an apparent competition between species when it was intercropped with Guinea grass. Probably, this is due to the vigorous development of Guinea grass, which ends up inhibiting maize growth in intercropping systems. which may have competed with maize for plant nutrients. Competition for water must be discarded, because no drought was observed during the maize growing season.

Due to the high maize requirement of N, between 180 and 200 kg ha-1, differences between the growth of different species of forage grasses after maize harvest can be insignificant, although Guinea grass has a vigorous development. The LI equations that fit the forage grasses in this study were very similar for both species. As N is part of the chlorophyll molecule, the increase in N fertilization directly reflected in the SPAD index reading.

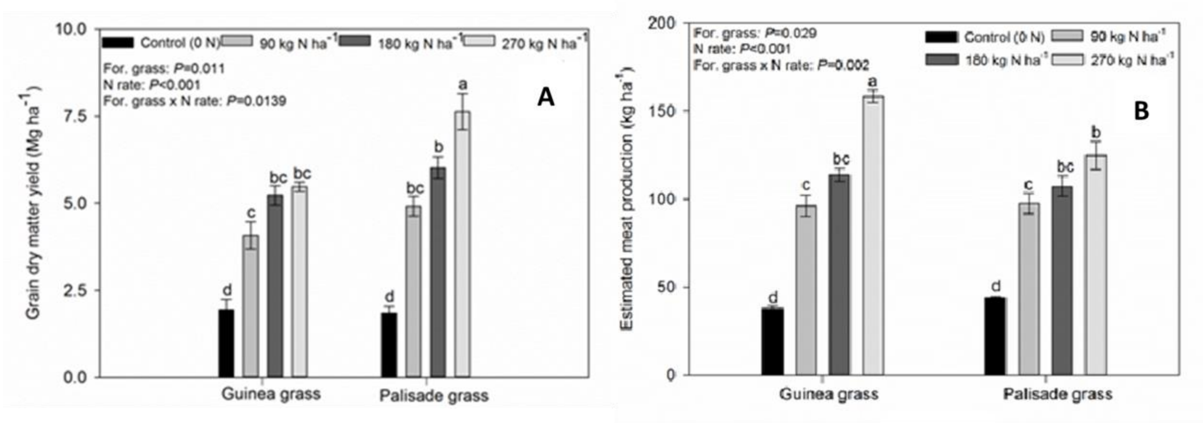

Fig 1. Maize grain dry matter yields (A), and estimated meat production (B) as affected by forage grass species and N fertilization. Different letters show significant difference between N rates (Tuckey, *p* < 0,05).

The forage dry matter yield assessed in October and November responded to N fertilization. Under low light, forage plants change their structure and nutrient concentration (Carvalho, 1997) because the plant is not metabolizing all N taken up and converting it into dry matter. From May to September the luminosity and temperature are lower in tropical Brazil.

As N fertilization generally increases the forage dry matter yield by enhancing tillering and leaf production, as well as the expansion of roots, this may explain why cellulose, NDF, and ADF were higher in Guinea grass compared with palisade grass, since the former has a higher N requirement. Indeed, Guinea grass fertilized with 270 kg N ha^{-1} showed the highest estimated meat production.

CONCLUSIONS

This study proves the potential of Integrated Crop Livestock Systems for meat production in the off-season (May to September), when grain production is also targeted. Nitrogen application is paramount in this system, since N fertilization positively affects forage growth and nutritional quality, resulting in a higher maize grain yield, higher forage production and quality, and eventually higher estimated meat production. Guinea grass resulted in the highest estimated meat production when fertilized with 270 kg N ha^{-1}, despite the high values for NDF, ADF and cellulose. REFERENCES Borghi, E., Crusciol, C.A.C., Trivelin, P.C.O., Nascente, A.S., Costa, C., Mateus, G.P., 2014. Nitrogen fertilization ($^{15}NH_4NO_3$) of palisadegrass and residual effect on subsequent no-tillage corn. Revista Brasileira de Ciência do Solo. 38, 1457-1468.

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THE F-BZIP-REGULATED ZN DEFICIENCY RESPONSE IN LAND PLANTS

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Keywords: Zn deficiency ; F-bZIP transcription factors; ZIP transporters

INTRODUCTION
All living organisms require zinc (Zn) as an essential micronutrient. This is due to its structural and catalytic role in many proteins, as a cofactor in enzymes, transcription factors and protein-interaction domains (Colvin et al., 2010). An appropriate zinc distribution and intracellular availability relies on membrane transporters and low-molecular-weight ligands, and requires a tight regulation of the zinc homeostasis network in order to avoid zinc deficiency or toxicity (Clemens, 2001).

In *Arabidopsis thaliana*, bZIP19 and bZIP23 transcription factors are the central transcriptional regulators of the zinc deficiency response (Assunção et al., 2010). They belong to the F group of basic leucine-zipper (F-bZIP) proteins, which are characterized by a cysteine(Cys)/histidine(His)-rich motif at the protein N terminus (Assunção et al., 2013). Recently, we unravelled that bZIP19 and bZIP23 transcription factors also function as sensors of intracellular Zn concentration, through a direct binding of Zn^{2+} ions to the Cys/His-rich motif, now referred to as the Zn-sensor motif (ZSM) (Lilay et al., 2021). bZIP19/23 activity regulates the plant zinc levels through Zn-dependent changes in the expression of their target genes, which include members of the Zrt/Irt-like protein (ZIP) transporter family, involved in cellular zinc uptake and the NAS enzymes that produce the Zn ligand nicotianamine (NA) (Figure 1).

We have found evidence for evolutionary conservation of the F-bZIP transcription factors and their function in the Zn deficiency response across land pants (Castro et al., 2017). Thus, fundamental knowledge of the Zn deficiency response in the model *Arabidopsis thaliana* and translational research with crop species, anchored at the F-bZIP transcription factors, can contribute to develop crops with improved Zn nutritional content (biofortified) and improved resilience to Zn-deficient soils. This is significant considering that Zinc-deficient soils are widespread globally and human Zn malnutrition affects about one-third of the world's population (White and Broadley et al., 2009).

Here, I will present an overview of the research developed at my Lab, where we investigate the F-bZIP regulatory network, its functional knowledge and its role in the Zn deficiency response in plants. Important new findings include showing that deregulation of the bZIP19/23 regulatory network, through mutations in the ZSM, leads to a significant increase in Zn concentration of shoots and seeds, the latter by 50% (Lilay et al., 2021). I then discuss the effect of such deregulation of the bZIP19/23 regulatory network on plant performance, ionome profile, and how to integrate this knowledge in evolutionary and applied research.

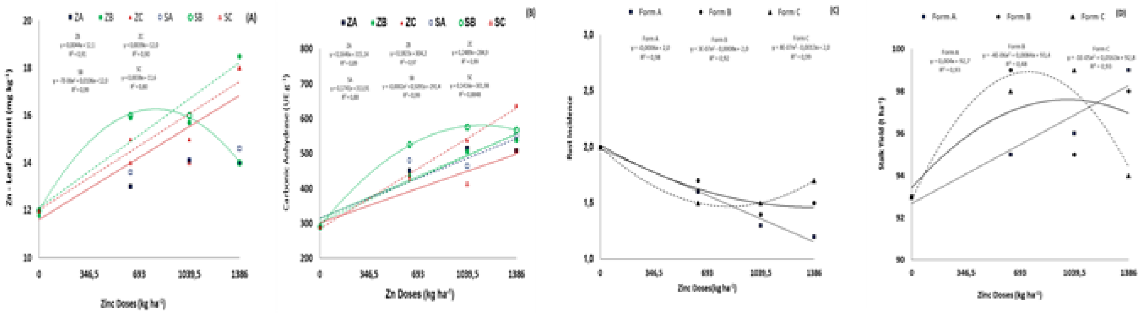

Fig. 1. Simplified scheme of cellular Zn sensing in *Arabidopsis thaliana*. bZIP19 and bZIP23 belong to the bZIP family of transcription factors, which is characterized by a conserved bZIP domain that is involved in protein binding to DNA as a dimer (Deppmann et al., 2006). bZIP19 and bZIP23 are the central regulators of the Zn-deficiency response, and are shown as F-bZIP. Under Zn-deficiency conditions, the transcription factors bind to the Zn Deficiency Response Element (ZDRE) in the promoter of their target genes, activating their transcription. These include ZIP transporters and NAS genes that contribute to Zn uptake and NA-mediated Zn distribution (Assunção et al., 2010). Under Zn-sufficient conditions, cellular free or loosely bound Zn ions bind to the characteristic Cys/His-rich ZSM of bZIP19 and bZIP23, and this binding of Zn to the protein represses the activity of the transcription factors (Lilay et al., 2021). (Image from Lilay et al., 2021)

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IMPROVING ESTIMATES OF CROP NUTRIENT REMOVAL FOR MORE LOCALLY RELEVANT NUTRIENT BALANCES.

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Keywords: nitrogen; machine learning; nutrient concentration

INTRODUCTION

Developing more sustainable food systems requires insights into current nutrient balances across regions, farms and fields, to identify hotspots of nutrient deficiency or nutrient surplus. In addition, monitoring nutrient balances through time is needed to verify expected impacts of implemented management practices or policy schemes. Calculations of crop nutrient balances require reliable estimates of nutrient concentrations of crop products and residues and the total biomass. Values of crop product yields are relatively well known compared with the nutrient concentrations of crop products and residues, or the yields of crop residues. It is now common to use global mean harvest index values to estimate crop residue yields based on crop product yield data, and to use default constant nutrient concentrations of crop products and crop residues for a wide range of conditions to estimate nutrient uptake and offtake. However, both harvest index values and nutrient concentrations in crop products and crop residues are known to vary by location and crop management. The aim of this study was to assess whether estimates of crop harvest index and nutrient concentration of crop products and residues could be improved using statistical and machine learning methods based on only a limited set of widely available predictor variables. Our methodology for improving estimates of the harvest index and nutrient concentration of crop products and crop residues was first tested on maize, given its importance globally. We focussed on nitrogen (N) as the nutrient of interest given its relative importance in global nutrient balances.

METHODS

Field experiment data for maize were collated from 1520 locations across 31 countries. Data were collated from the literature as well as from requests to various researchers and organizations. These data were used to calibrate and test the accuracy of mixed-effects and random forest models (a machine learning method) for predicting harvest index and N concentration of crop products and crop residues. Crop product yield, yield potential, fertilizer application rates, and location information were used in the models as they are widely available at the global level. Various combinations of predictor variables were tested in the mixed-effects models with region as a random factor. Akaike information criteria and Nakagawa R^2 (Nakagawa et al., 2017) values were used to compare the different mixed-effect models for suitability for each dependent variable. The models were trained on an 80% sub-selection of the total data set and tested on the remaining dataset for prediction accuracy. Prediction accuracies of the best mixed-effects models were compared with those of random forest models using regressions of predicted and actual values.

RESULTS AND DISCUSSION

Random forest models were more accurate in predicting harvest index and N concentrations of maize, compared with the mixed-effects models (Table 1). However, the random forest models included a greater number of variables. For harvest index, random forest models relied on information on crop product yield, N and P application rates and region. For N concentrations, random forest models relied on product yield, N and P application rates, region and the yield potential.

For the mixed-effects models, harvest index of maize was best predicted using crop product yield in a logarithmic mixed-effects model (Table 1; R^2 =0.46). N concentrations of both product yield and crop residues were best predicted using a combination of yield potential and N application rates (Table 1; R^2=0.40 and R^2=0.35).

Table 1. Prediction accuracy of mixed-effects and random forest models for various dependent variables for maize.

Dependent variable	Prediction accuracy (R^2) for each model	
	Mixed-effects	Random forest
Harvest index	0.46	0.64
Crop product N concentration	0.40	0.69
Crop residue N concentration	0.35	0.57

Our analysis shows that random forest models can be accurate in predicting variables relevant to calculate N offtake, but they also require more input variables than mixed-effects models. Moreover, the random forest models were more difficult to interprete than the mixed-effects models, with the latter providing specific equations with coefficients.

The decision of which method to use for predictions will need consideration of the relative benefits of prediction accuracy (of random forest) with the benefits of interpretability (of mixed-effects models) of the models.

CONCLUSIONS

The random forest and mixed-effects models showed potential for use in improving predictions of harvest index, and N concentrations of crop products and residues of maize worldwide. In a follow-up study, methods described will be applied to other crops (wheat, rice soybean) and nutrients (phosphorus and potassium). This will allow for more locally relevant estimates of crop nutrient offtake and enable more precise nutrient balances at a national, regional or global level.

Financial Support

This work was supported by the International Fertilizer Association (IFA). The authors wish to acknowledge all those who have contributed raw field experiment data for maize included in this study. Those include Fernando Garcia (Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata), Rachel Fields and Timothy Lewis (AngloAmerican), Tek Saptoka (CIMMYT) and Maryam Rahimi Jahangirlou. The authors also wish to acknowledge the Global Yield Gap Atlas team (www.yieldgap.org) for providing yield potential data, as well as those who converted data from the literature into a standardized format including, Joost Krooshof, Bert Rijk, Tobias Bader and Victoria Miles-Hildago.

IMPROVING PALM OIL PRODUCTION THROUGH CULTIVATION OF KUE-GENOTYPES AND AGRONOMIC EFFICIENCIES

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Keywords: Oil Palm; Potassium Use Efficiency; Polyhalite

Oil palm (E. *guineensis* Jacqs), despite being the highest oil yielding crop, its oil yield on per hectare basis did not show an improvement parallel to improvements made by other oil crops. Such a phenomenon was attributed to the planting of oil palms on marginal land as less good arable land is available for agricultural developments across multiple countries. Conventional approaches of using higher fertilization inputs to sustain profitable yields are often uneconomical and produce inconsistent results. In addition, its biological yield potential of attaining much higher oil yield is often limited in such marginal environments. Realizing such limitations, a gradual effort is now being concentrated into improving the oil palm productivity via adopting Nutrient Efficient Genotypes and fertilizers with high agronomic efficiencies could potentially lead to higher productivity on marginal land and eventually sustainable use of resources and production of sustainable palm oil.

Enhancing Potassium Use Efficiency (KUE) in Oil Palm Genotypes

The development of nutrient efficient oil palm as a solution to marginal growth environment is a long-term proposition and can be achieved by genetically improving the nutrient acquisition by roots and nutrient utilization capacity to achieve the higher potassium use efficiency in oil palm. Variations in potassium acquisition and the utilization efficiency have been observed within the same species and different genotypes in oil palm. Selection genotypes with higher potassium efficiency in acquisition and utilization enable oil palm breeding genetically enhanced cultivars. Despite the potential impact of improving nutrient use efficiency on oil yield, little progress has been made in the oil palm breeding programme in the development of nutrient use efficient cultivar. Oil palm conventional breeding programme in the past decades has focused primarily on yield improvement and has achieved a boost in oil yield in the Dura x Pisifera hybrid cultivar through improvement of a single gene inheritance on the shell. However, slow progress in oil palm conventional breeding is mainly contributed by the challenging limitations with respect to its high variability in the genetic pool and less effective in low heritable traits, random genetic combination within crosses, bias evaluation based on phenotypic diversity and requires a long time to develop pure lines possessing the desired traits. Hence, a holistic strategy integrating oil palm breeding and biotechnology approaches including marker-assisted breeding, molecular biology, genetic modification, and genome editing to establish nutrient use efficient oil palm provide opportunities to overcome the genetic features that limit conventional breeding.

In-depth molecular function of variant genetics contribute to the phenotypes are becoming increasingly important for understanding phenotypic diversity in plants. Functional genomics or molecular biology approaches study the function of genes and uncover the biological mechanisms underlying the complex relationship between genotype and phenotype. This approach has increased the understanding of molecular mechanisms underlying potassium uptake and distribution in plants, and plants' response to diverse potassium availability, hence, presenting new opportunities in the prospect of developing new genetically improved or engineered crops for efficient potassium use. Understanding and enhancing the mechanisms for potassium acquisition, transport and translocation, and root growth or morphology are strategies for improving potassium use efficiency in various plants. Despite the progress in deciphering the regulatory underlying key physiological processes and potassium utilization mechanisms in Arabidopsis and other annual crops, little progress has been made in oil palm. Thus far, only oil palm EgKUP8 has been identified to be involved in low-affinity transport in potassium sufficient conditions (Husri and Ong-Abdullah, 2018). A deeper understanding of potassium channels and transporters in oil palm will be necessary to leverage on the opportunities in current disruptive technologies such as genetic modification and genome editing as a potential strategy towards manipulating potassium use efficiency for significant and positive impact on oil palm yield.

In addition, potassium acquisition by plant roots relies significantly on the availability of soluble potassium in the rhizosphere. Beneficial soil microorganisms essentially the potassium-solubilizing microbes and plant growth-promoting rhizobacteria have been reported to enhance the content of soluble potassium in the soil, enabling crops to acquire more potassium (Meena et al., 2016). In maize and wheat, inoculation with plant growth-promoting rhizobacteria (PGPR) including Bacillus mucilaginous, Azotobacter chroococcum and Rhizobium spp improved potassium assimilation from waste mica and resulted in higher biomass accumulation and potassium uptake by plants (Singh et al., 2010). Along with the recent plant microbiome approach that unveils the biodiversity of root microbiome and their abundance in soil, exploration into oil palm microbiome will enable the identification of plant roots and microbe interactions to maximize potassium availability, acquisition and plant health. The current limitation lies in the comprehensive mechanistic understandings in oil palm-microbiome interaction and the efficiency of microbe in solubilizing potassium in the rhizosphere. The future aspects could consider the application of genetic engineering techniques to manipulate the beneficial microbes for improvement in their efficiency and performance in mineral potassium solubilization and to prolong their strain lifespan in the rhizosphere. In a nutshell, improvement of potassium use efficiency in oil palm using multidisciplinary approaches in various fields of discipline are urgently required.

Enhancing Potassium Use Efficiency (KUE) with Agronomic Efficiencies

From a fertilizer perspective, the use of controlled-release fertilizers has not been popular due to the high price of coated muriate of potash (KCl). Recently, a UK-mined mineral called polyhalite ($K_2Ca_2Mg(SO_4)_4.2(H_2O)$) which contains K, Mg, Ca and S (14% K_2O, 6% MgO, 17% CaO, and 45% SO_3) has opened up new opportunities in potassium use efficiency research. The prolonged-release mechanism of the fertilizer was recently shown to improve crop yields, and recent trials in oil palm have shown some positive indications of enhancing potassium use efficiency using polyhalite. A study in Colombia in a soil classified as Ultisol with a pH of 4.4, sandy loam texture, and high Al concentrations, showed that fresh fruit (FF) bunch yields were highest when 38% of K_2O was supplied in the form of polyhalite as a complementary K source combined with KCl. The agronomic efficiency of K fertilizers increased with polyhalite indicating a better supply of nutrient requirements for oil palm crops under balanced N, P and Mg inputs. The agronomic efficiency of K fertilizers increased with polyhalite indicating a better supply of nutrient requirements for oil palm crops under balanced N, P and Mg inputs (Figure 1). Palms from the high yielding blocks recorded larger frond sizes and leaf area index, which could have maximised photosynthesis and led to better yields.

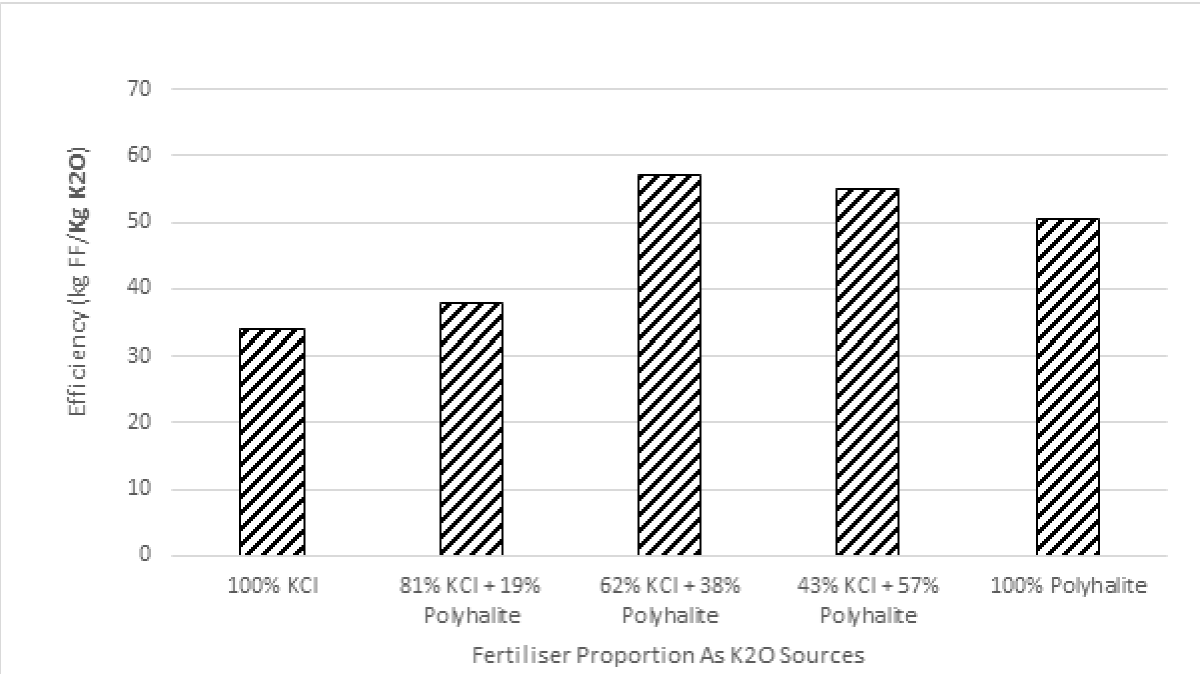

Figure 1. Agronomic efficiency of K fertilizers in a trial in Colombia to evaluate the effects of complementing KCl with polyhalite.

A separate study in Indonesia, in a soil classified as Ultisol with a pH 4.2 - 4.4, loamy texture showed that fresh fruit bunch yields of oil palm increased when half of the K_2O requirements were supplied with polyhalite, either through straight fertilizer applications (+17.6% increase) or applications of a compacted form of KCl and polyhalite (+19.9% increase). In all treatments, N, P, K, Mg and Ca input were all balanced. When compared to the practice of KCl applications through compound fertilizers in the same study, yields were 8.3 - 10.0% higher. Palms applied with polyhalite had better vegetative growth and were generally more productive. Hence, polyhalite was concluded to be a suitable complementary fertilizer to KCl. In both trials, soils were poor in fertility and contained low amounts of exchangeable K and Mg. The gradual supply of K and secondary macronutrients could have contributed to better yields even though K and Mg input were balanced in the treatments of the studies. In terms of Ca and S, their roles remain poorly studied in oil palm. A trial in an oil palm nursery in Malaysia showed increasing input of polyhalite improved the biomass of palms (above ground, bole and root) by 18.3 - 28.1%. Although the agronomic efficiency of K fertilizer was not evaluated, it is interesting to note that applications of polyhalite led to improved uptake of nitrogen, phosphorus and boron despite similar levels of input.

UNCOVERING THE IONOME BY MAGIC: IDENTIFICATION OF GENES CONTROLLING NATURAL VARIATION OF ELEMENTAL CONCENTRATION IN ARABIDOPSIS THALIANA

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Keywords: Zinc; Ionome; Transporter

INTRODUCTION

Plants must be efficient in searching for nutrients in the soil. To understand the mechanisms involved in such control, the ionome is a useful concept for plant nutrition: it is defined as the inorganic composition of an organism, its organs, tissues or single cells (Huang and Salt 2016). Ionomics is the integrated study of an organism's nutrients and trace elements, and consist in a series of multi-element quantification and visualization methods that allows us to study the ionome. In this work, we used Multiparent Advanced Genetic InterCrossed (MAGIC) lines combined with simultaneous quantification of 22 elements inin leaves to identify genes associated with natural variation in *Arabidopsis thaliana* (Kover et al 2009). We found several *loci* that are linked to high/low concentration of nutrients and trace elements in *A. thaliana* leaves. Among them, we identified and characterized one gene associated with increased zinc (Zn) concentrations in leaves and seeds, AtMTP3, a protein expressed in roots and localized to the tonoplast (Arrivault et al 2006). One parental accession used in the construction of the MAGIC lines, Kn-0, is the donor of a weak allele of AtMTP3, leading to decreased Zn sequestration in roots and higher root to shoot translocation. The allele from Kn-0 is unique among +1000 *A. thaliana* genomes sequenced. Our work demonstrates the power of combinining high-throughput ionomics with genomics to uncover the genetic basis of plant nutrition.

METHODS

Soil plant growth for ICP-MS analyses was performed as described (Campos et al 2021). Plant growth in plates was performed as described (Ricachenevsky et al 2018) Quantitative trait loci were analyzed as described (Kover et al 2009). For yeast complementation, *zrc1cot1* mutants were transformed with pDR196-AtMTP3 clones derived from either Col-0 or Kn-0 genomes (Ricachenevsky et al 2018). Complementation of Kn-0 with Col-0 genomic sequence was performed with the full length AtMTP3 locus in pCAMBIA1300, and *Agrobacterium tumefaciens* transformation by floral dipping.

RESULTS AND DISCUSSION

QTL analyses identified several regions associated with variation in elemental concentration. Genes already described before, such as AtMOT1 for molybdenum, AtHKT1 for sodium, AtHMA3 for cadmium and AtFPN2 for cobalt, and candidate genes not yet characterized. We found that AtMTP3 is within a region associated with high Zn concentration. Among all the 19 parental accessions used to generate the MAGIC lines, Kn-0 contributes with the high Zn phenotype: Kn-0 has high Zn concentration in three independent experiments compared to Col-0 (Fig 1A). The phenotype is caused by a recessive allele, since Col-0 X Kn-0 F1 plants have leaf Zn concentrations similar to Col-0 (Fig 1B). Kn-0 seeds have high Zn compared to Col-0 (Fig 1C). We complemented Kn-0 plants with Col-0 AtMTP3 genomic locus. Kn-0 plants expressing Col-0 AtMPT3 have Zn concentrations similar to Col-0 and lower that wild type Kn-0 (Fig 1D), showing that AtMTP3 is causative the Zn variation in these accessions.

We identify six nucleotide changes in Kn-0 AtMTP3 region compared to Col-0. Using other accessions that share different combinations of mutation with Col-0 and Kn-0, we narrowed down the causative mutation to a

T to A change that results in a Isoleucine to Lysine change in the protein sequence. We showed the AtMTP3 allele from Kn-0 is a weak/hypofunctional allele, as it cannot detoxify Zn at high concentration compared to Col-0 AtMTP3 when expressed in yeast mutants (Fig 1E). We found that the causative mutation found in Kn-0 is a rare allele, not shared with any other *A. thaliana* accession among all genomes sequenced. Our data adds several new candidate genes for natural variation in the ionome, and describes a rare allele that can be explored to increase Zn in leaves and seeds in plants.

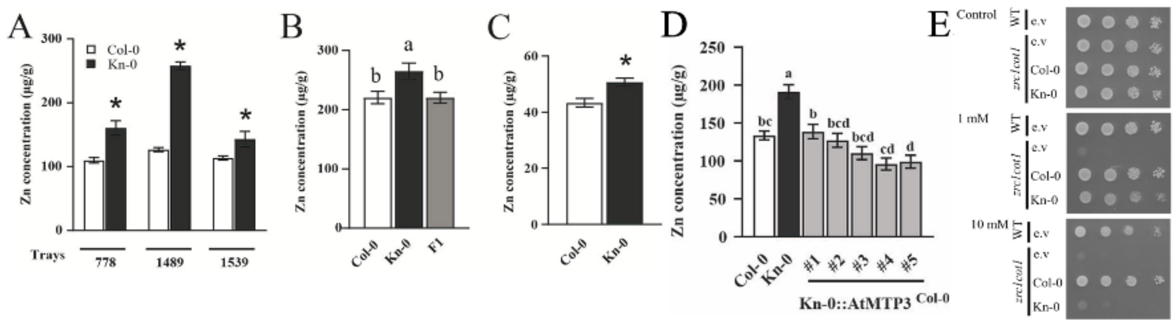

Figure 1. AtMTP3 is the causative allele of high Zn concentration in Kn-0. (A) Experiments show that Kn-0 has higher leaf Zn concentration than Col-0. (B) Col-0, Kn-0 and F1 crosses Zn concentration. (C) Zn concentration in seeds. (D) Complementation of Kn-0 with AtMTP3 genomic sequence results in Zn concentration similar to Col-0. (E) Yeast mutant complementation shows that Col-0 and Kn-0 AtMTP3 are functionl, but Kn-0 AtMTP3 is hypofunctional. Statistical analyses used Students's t test or ANOVA followed by Tukey HSD ($p \leq 0.05$).

CONCLUSIONS

MAGIC lines as excellent tools to discover new genes linked to complex phenotypes such as nutrient and trace-element concentration variation in natural populations. AtMTP3 is important to determine Zn concentration in leaves and seeds of *A. thaliana*.

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SUFFICIENCY RANGE AND RESPONSE OF MAIZE HYBRIDS SUBJECTED TO POTASSIUM DOSES IN NUTRIENT SOLUTION

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Keywords: efficiency; *Zea mays* L.; critical level

Maize (*Zea mays* L.) is a crop that requires high soil fertility for maximum yield (Paponov and Engels 2003) and consequently has 1 of the highest fertilizer requirements (Bonfim-Silva and Monteiro 2010). The metabolism of potassium (K) in the cytoplasm (Xu et al. 2006), which is an important nutrient for crop yield. The critical level is the nutrient concentration in a particular phenological stage that is associated with a 5% or 10% yield reduction (i.e., it is a transitional concentration between nutritional deficiency and the sufficiency levels of the nutrient) (Ulrich and Hills 1973). Knowing exactly where and how grain production is hindered by nutrient limitations contributes to better management of nutrient deficiency in crops. The objective of this study was to determine the sufficiency ranges of K in maize hybrids (*Zea mays* L.) with high productive potential in 2 phenological stages grown with different K doses in nutrient solution. The crop used was maize (*Zea mays* L.) grown hydroponically in a Hoagland and Arnon (1950) nutrient solution. Three maize hybrids [DKB390 ProR2®(H1), Pioneer 30F35®(H2) and Syngenta Status®(H3)] were treated with 4 doses of nutrients for each element studied (D1 = 5%, D2 = 20%, D3 = 100% and D4 = 200% of the full dose by Bataglia et al.,1983) with 4 replications (Tukey 5%). The plants were evaluated at stages V4 and R1. The shoot+root and the leaf of interest (FI) corresponding to leaf 4 (in V4) and the leaf opposite and below the cob (in R1) were separated. Relative dry mass (DMrel) expressed as a percent (%) was calculated based on the relationship between the total dry mass (DMtot) of each treatment. The sufficiency ranges for K were obtained by calculating the concentration of the element in the nutrient solution or in the plant needed to promote 90% of the maximum shoot dry mass. Omitting K from the nutrient solution resulted in a foliar K concentration lower than that of the plants that received this nutrient in solution. In the hybrids grown with 5% and 20% K in solution in R1, the foliar concentration of this nutrient was below the foliar levels suitable for maize in this stage of development (Marschner, 2012). The foliar concentration of K for the hybrids grown without nutrient omission was within the range suitable for maize. The differences between the sufficiency ranges of the hybrids indicated differences in nutritional requirements among them. The K concentrations in solution that led to a maximum reduction in yield of 10% were 3.4 (H2) and 3.2 (H3) mMol L^{-1} in stage V4. The sufficiency range of K for the hybrid maize ranged from 3.4 to 11.2 mMol L^{-1} in H2 and 3.2 to 10.0 mMol L^{-1} in H3 in stage V4, above which the probability of a response to K fertilization was small with no need for the addition of K. H3 had a narrower K sufficiency range than H2, with amplitudes of 7.8 and 6.8 mMol L^{-1}, respectively. In R1, the sufficiency range varied from 5.4 to 11.6 and 4.9 to 12.0 mMol L^{-1} K in H1 and H2, respectively. H3 reached 90% relative dry mass with only 6.0 mMol L^{-1} K in the solution. Above the maximum values established in this sufficiency range, the probability of a response to the increase in K in the solution is very low. The sufficient ranges of K are variable among hybrids and between phenological stages of maize, indicating that the nutritional requirements for these elements also varies among hybrids and between developmental stages. Pioneer 30F35® (H2) is the most demanding hybrid in terms of K in stage V4. However, in stage R1, Syngenta Status® (H3) are the most demanding in K. Thus, attention must be paid to fertilization for the different hybrids studied and to the phenological stage.

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^{10}B USED AS A TRACER TO STUDY ABSORPTION AND MOVEMENTS IN CHERRY TREES: PRELIMINARY RESULTS

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Keywords: ^{10}B tracer ; Boron movement; cherry bud

Boron has been correlated with fruit set and productivity, and as a consequence, commercial orchards carry out foliar applications at different seasonal plant stages. A cherry tree experiment located in the central valley of Chile was carried out to identify the absorption and movement of foliar B applications, applied before bud break, to other organs.

Boron application consisted in treating Cherry buds with B solution enriched boric acid with the stable isotope 10B. An aliquot (5 µL) of 10B was applied with a micropipette to the surface of the buds in August (southern hemisphere) in five trees as replicates. Ninety days after the 10B application, leaves of the spur, wood of the spur base, wood between spurs and fruits were collected, dried at 65°C, digested in a microwave (CEM-Mars 5), and extracts analyzed for isotopic determinations using an ICP-MS (7500 Series, Agilent).

Results indicated that 10B applied on the surface of buds was absorbed and retained primarily in fruit tissues and to a less extent on leaves and wood between spurs. An appreciable amount of 10B was also detected in the wood of the base of the spurs.

At the same 10B application time, additional bud samples were collected and observed using electron microscopy (SEM) to determine tissue morphology showing the presence of scales, trichomes, and spaces on the buds where absorption could occur.

Further studies are needed to understand boron movements within Cherry tree buds better.

SYSTEMS BIOLOGY OF SOYBEAN RESPONSE TO PHOSPHORUS STRESS AT THE QTL LEVEL AND THEIR INFLUENCE ON QUALITY-RELATED TRAITS

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Keywords: phosphorus; QTL; soy genetics

Soybean (*Glycine max*) seed size and composition are important in food crop plants and can be affected by nutrient availability in the soil. Phosphorus (P) is an essential macronutrient, and its deficiency significantly limits soybean yield and quality. Soybean recombinant inbred lines (RILs) from the cross of Fiskeby X Mandarin were grown under contrasting P availability environments to investigate the association of seed and plant traits in low and high P environments. Traits including seed weight, seed number, and pod plus seed weight were significantly affected by soil P levels and showed transgressive segregation among the RILs. The RIL phenotypes were used to identify and map quantitative trait loci (QTLs) controlling seed size and composition traits. Broad-sense heritability estimates were 0.78 (individual seed weight), 0.90 (protein), 0.34 (oil), 0.98 (seed number). Five QTLs were significant in low P environments, and one QTL was significant in optimal P environment. QTLs were detected for seed weight (SeedwtQTL4.1; SeedwQTL4.3; and SeedwQTL6.1), pod plus seed weight (PodplswtQTL4.2), volume (VolQTL6.2), and and protein (ProtQTL20.1). In summary, detection of specific QTLs under contrasting P levels together with transgression of seed and plant characteristics will help our understanding and improvement of soybean performance under limited P conditions. The QTLs identified can be used for molecular marker assisted breeding with their differential expression contributing to seed traits and low P tolerance in soybean. The current status of this project will be presented including the further research results.

EFFECT OF CRISPR/CAS9-MEDIATED KNOCKOUT OF OSBZIP48 TRANSCRIPTION FACTOR IN RICE ZN DEFICIENCY RESPONSE AND ZN ACCUMULATION

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Keywords: Zinc; Rice; Biofortification

SUMMARY

Zinc (Zn) is an essential micronutrient for all living organisms, and its deficiency in agricultural soils adversely affects the yield and nutritional quality of crops globally. While Zn uptake, transport, and storage is controlled by several factors, the response to zinc deficiency is regulated by F-bZIP transcription factors. The Arabidopsis group F bZIP (F-bZIP) transcription factors, bZIP19 and bZIP23, and their homologs from rice, wheat and barley have been identified as the key regulators of the zinc deficiency response, supporting the evolutionary conservation of this regulatory network across land plants[1-6]. Recently, we showed that the Arabidopsis F-bZIPs, bZIP19 and bZIP23, act as Zn sensors by directly binding Zn^{2+} ions to a Cys/His-rich Zn sensor motif (ZSM). Deletions or modification of the ZSM disrupts Zn binding, leading to a constitutive transcriptional activation of the Zn deficiency response. This, in turn, results in a significant increase in plant and seed Zn content[2]. Since F-bZIPs, and their ZSM, are highly conserved across land plants[6], we sought to translate this fundamental knowledge from the model plant Arabidopsis to the important crop rice. Previously, we identified the rice F-bZIP OsbZIP48 as the functional homolog of the Arabidopsis bZIP19 and bZIP23 transcription factors[3]. Here, we will show results of genetically modified rice lines where CRISPR/Cas9-mediated knockout was employed targeting rice OsbZIP48. The response to Zn deficient supply in hydroponic nutrient solution and the effect on Zn uptake and accumulation in these lines was analyzed. Our results show the critical role of rice F-bZIPs in the Zn deficiency response and provide new promising avenue for the development of plant-based biofortification strategies to tackle the global Zn-deficiency health problem.

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FOLIAR-APPLIED PHOSPHORUS AND MANGANESE IN DEFICIENT BARLEY: ABSORPTION PATHWAYS AND PHOTOSYNTHETIC RESPONSES

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Keywords: Foliar fertilization; Phosphorus; Manganese

INTRODUCTION

Standard soil fertilization strategies are in many cases inefficient due to nutrient immobilization in the soil or losses of nutrients by leaching and volatilization. Foliar fertilization offers an attractive supplementary strategy as it bypasses adverse soil processes and provides a tool for rapid restoration of the physiological processes affected by nutrient deficiencies. However, implementation of foliar fertilization is restricted by poor and unpredictable penetration through leaf surface barriers. The aim of the present study was to investigate how latent Mn and P nutrient deficiencies specifically alter barley leaf surface properties and the consequences this has for the absorption pathways and efficiency of foliar-applied Mn and P, respectively.

METHODS

Foliar solutions were applied to the adaxial surface of the youngest fully expanded leaf (YFEL) of hydroponically grown barley plants at tillering and flag leaf emergence (21 and 45 days after sowing, respectively). The middle section of an YFEL still attached to the plant was slid into a Petri dish lined with a moist filter paper to maintain high humidity. The part of the leaf inside the Petri dish was labelled and 30 x 5 µL droplets were applied to the leaf surface. The Petri dish lid was replaced and relative humidity rapidly increased (>95%). Droplets were left on the leaf for 6 h and remained liquid throughout the exposure period. Upon reopening the dish, drops were carefully blotted using a paper towel. Three types of foliar solutions were prepared: 0.2 mol L^{-1} KH_2PO_4 with 1 mmol L^{-1} Na_3VO_4 (vanadium as a foliar-P tracer; Arsic et al., 2020), 20 mmol L^{-1} $MnSO_4$, and a control (deionized water). All solutions included 0.05% (v/v) Tween-20 and were adjusted to pH 6 using either 1M NaOH or HCl.

Leaves were excised at the base for the following Imaging-PAM measurements. Nutrient specific chlorophyll a fluorescence assays were used to visualize leaf nutrient status, while laser ablation-inductively coupled plasma-mass spectrometry was used to visualize foliar absorption pathways for P and Mn ions (Frydenvang et al., 2015; Schmidt et al., 2016; Arsic et al., 2020). Leaf surface morphology was imaged using a FEI Quanta 200 Scanning Electron Microscope. The YFEL adaxial surface biopolymer composition was analysed using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy.

RESULTS and discussion

Rapid Mn absorption was facilitated by a relatively thin cuticle with a low abundance of waxes and a higher stomatal density in Mn deficient plants. Following absorption, Mn accumulated in epidermal cells and in the photosynthetically active mesophyll (Fig. 1, right), enabling a fast (6h) restoration of Mn dependent photosynthetic processes (Fig. 1, left). In contrast, P deficient plants had thicker cuticles and epidermal cell walls which reduced the penetration of P across the leaf surface barriers. Foliar-applied P accumulated in trichomes and fiber cells above leaf veins without reaching the mesophyll. Consequently, foliar P applications were not able to restore functionality within the same time frame as Mn. The 6 h application period was likely too short to allow for sufficient P penetration to restore physiological function, as we in a previous study under similarly high humidity showed restoration of P functionality in P-deficient barley after 24 h (Arsic et al., 2020). Higher nutrient demand, lower foliar-applied concentration gradients, ion radius, and ionic charge could also limit foliar-applied P absorption and increase the time required for restoration of functionality relative to Mn.

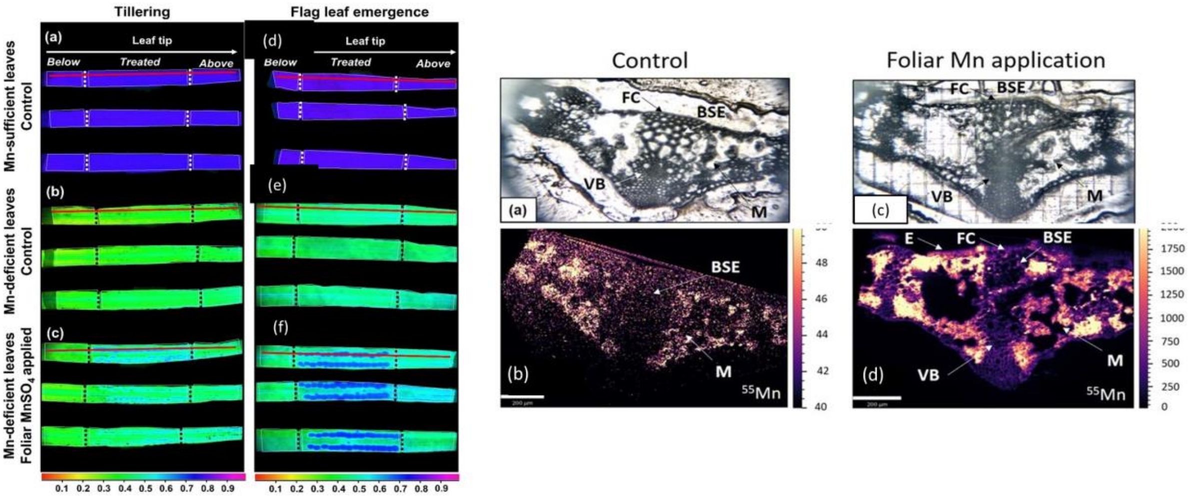

Fig. 1. Left: Fv/Fm Imaging-PAM assay of physiological plant Mn status in YFEL at tillering and flag leaf emergence of Mn-sufficient control leaves (a, d), Mn-deficient control leaves (b, e) and 6 hours after foliar Mn applications to Mn-deficient plants (c, f). Coloured scale bar shows Fv/Fm values from 0-1. Note green colour shows Mn deficiency and blue colour Mn sufficiency.
Right: Representative LA-ICP-MS scans of Mn-deficient leaf midrib cross-sections at flag leaf emergence, where (a, c) are the bright field microscopy images, and (b, d) show ^{55}Mn element distributions. White scale bars indicate 200 µm for microscopy images. Element scale bars indicate counts (signal intensity)

CONCLUSIONS
A relatively thin cuticle with a low abundance of waxes in manganese deficient leaves promoted rapid restoration of photosynthetic processes by foliar-applied Mn, while P predominantly accumulated in trichomes and fiber cells above leaf veins in P deficient leaves with thicker cuticle and epidermal cell walls. Foliar P applications were therefore not able to restore functionality within the same time frame as Mn.

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COMBINING LIME WITH PHOSPHOGYPSUM TO IMPROVE ^{15}N-FERTILIZER RECOVERY IN A CROP ROTATION SYSTEM

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Keywords: Soil amendments; $(^{15}NH_4)_2SO_4$; Nitrogen losses

INTRODUCTION

Liming is a usual management practice to correct soil acidity worldwide (Holland et al., 2018). However, as lime is poorly soluble with limited mobility in the soil, the action of surface liming is usually restricted to the uppermost soil layers (Crusciol et al., 2019). On the other hand, phosphogypsum ($CaSO_4.2H_2O$), a by-product of the phosphoric acid industry, although not capable of correcting soil pH, is more soluble than lime and provides calcium (Ca) and sulfur (S) to the system, in addition to precipitating aluminum (Al^{3+}) free (Zoca and Penn, 2017). Aluminum is the major element that causes detrimental effects on root deepening, impairing water and nutrient (soil and fertilizer) uptake (Zoca and Penn, 2017). These soil amendments can also improve the recovery of and efficiency of N-fertilizers by crops (Crusciol et al., 2019). Although increasing soil pH increases nitrification rates and, consequently, risks of loss of this nutrient (denitrification and/or leaching), this will only occur if plant demand is low. Therefore, combining lime with phosphogypsum can be a viable alternative to stimulate nitrification of ammoniacal fertilizers and, at the same time, improve N-fertilizer uptake by crops. Here, we aimed to verify the recovery of ^{15}N-fertilizer by maize intercropped with ruzigrass and soybean established in succession, in long-term experiment managed with lime and/or phosphogypsum applied to soil surface.

METHODS

A long-term (18 years) field experiment based on surface applications of lime (L) and phosphogypsum (PG), has been carried out in Botucatu, São Paulo State, Brazil, since 2002. The climate in this region is classified as mesothermic type (Cwa), presenting dry winters and hot summers. The soil is classified as Typic Haplorthox (particle size composition: 347 g kg^{-1} clay, 108 g kg^{-1} silt, and 545 g kg^{-1} sand). Four treatments with four replicates were tested in a randomized complete block design. Treatments were consisted by: (*i*) control (no soil amendments applied); (*ii*) exclusive application of PG; (*iii*) exclusive application of L; and (*iv*) combined application of L and PG (LPG). Over the 18-year experimental period, the field area received four reapplications of treatments (2002, 2004, 2010, and 2016). In 2016, the doses of L and PG were 13 and 10 Mg ha^{-1}, respectively. This study characterizes the residual effects of L and PG application in the third year after the last soil amendment reapplication. Maize sowing occurred in March 2019. The grain crop was intercropped (same row) with ruzigrass (*Urochloa ruziziensis*). After the maize harvest, the ruzigrass remained vegetating until October, where it was desiccated and managed for soybean sowing. Unconfined microplots (1.8 × 1.2 m) were set up in each treatment during the maize + ruzigrass season. ^{15}N-labeled ammonium sulfate fertlizer (6.31 atom % ^{15}N excess) was applied in topdressing (100 kg ha^{-1} N) at V_5 maize stage. Maize stover returned to the microplot after lab processing to ensure the cycling of the ^{15}N present in the plant residues. After maize harvesting, the microplot remained identified to assess the recovery of residual ^{15}N-labeled fertilizer by soybean. Determination of ^{15}N abundance occurred in plant tissues, and in soil (down to 100 cm depth). Details of the samplings procedure and ^{15}N abundance calculations in soil and plant samples can be found in Rocha et al. (2019). All dataset were tested for normality (Anderson-Darling test) and homoscedasticity (Levene's test). When significant, means were separated using the modified t-test [Fisher's protected least significant difference (LSD), at $p \leq 0.05$].

RESULTS AND DISCUSSION

The cascading effects of soil amendments in soil fertility, and crop growth are usually reported (Crusciol et al., 2019), and these changes can alter ^{15}N fate. LPG-amended soil not only increased ^{15}N recovery by maize grains (232%, 150%, and 16% higher than control, PG, and L, respectively), but also increased ^{15}N accumulation in ruzigrass biomass (55%, 37%, and 13% higher than control, PG, and L, respectively) (Fig. 1A). While the ^{15}N in the ruzigrass biomass and maize stover act as N pools that potentially return to system, N in grains is exported after harvest. Interestingly, the ^{15}N recovery by maize and ruzigrass shows an inverse trend to that obtained in soybean cultivated in rotation (Fig. 1B). Overall, ~70% of the total N accumulated by soybean comes from biological N fixation (Ciampitti and Salvagiotti, 2018), but the ^{15}N recovery was higher in the control and PG treatments. This effect is underpinned by the following rationale: (*i*) the smaller amount of ^{15}N-fertilizer remaining in the soil from L and LPG treatments, and/or more likely (*ii*) liming increased the efficiency of BNF (Alves et al., 2021), reducing dependence on soil fertilizer-derived ^{15}N. At the end of the experiment, the total amount of unrecovered ^{15}N (sum of the first and second growing seasons) had this series: control (55%) > PG (45%) > L (29%) > LPG (22%) (Fig. 1C).

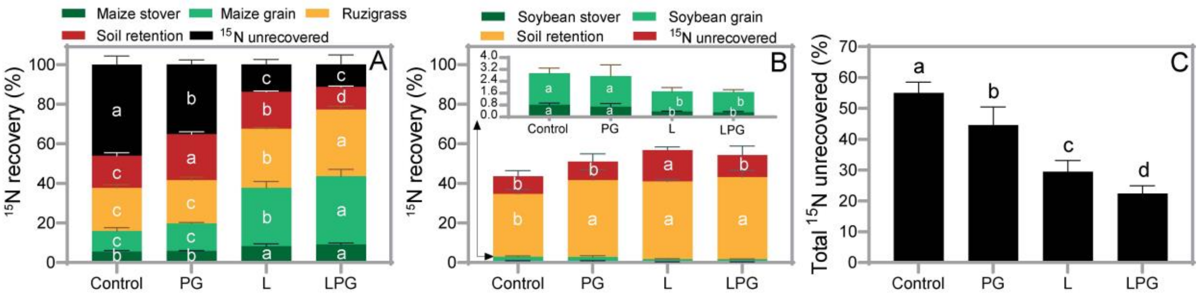

Fig. 1. ^{15}N-fertilizer recovery from maize+ruzigrass (A) and soybean (B), and total ^{15}N unrecovered (C) in the soil-plant system in response to soil amendments. Different lowercase letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$.

CONCLUSIONS

Limed soils, especially when combined with phosphogypsum, increases the ^{15}N-fertilizer recovery by maize and ruzigrass and reduces their losses to the environment. Our results suggest that increasing the recovery of N-fertilizers by the crops by combining lime with phosphogypsum can be a promising alternative to increase crop yield, and make agriculture environmentally safe by recovering the most part of N-fertilizers applied.

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ATBOR1 IN TAPETUM CELLS TRANSPORTS BORON TO SUPPORT POLLEN DEVELOPMENT

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Keywords: Boron; Transport; Pollen

INTRODUCTION

Boron (B) is required for cell wall structure by cross-linking the rhamnogalacturonan II component of the pectic pollysaccharides in higher plants. It has been well known that boron deficiency affects fertility of various crop plants due to relatively immobile nature of B.

In roots of *Arabidopsis thaliana* under B limitation, a boric acid channel NIP5;1 and a borate exporter BOR1 are responsible for B uptake and translocation toward stele (Yoshinari and Takano, 2017). NIP5;1 and BOR1 are localized in the plasma membrane of epidermal and endodermal cells in polar manners toward the soil- and stele-side, respectively. Expression and accumulation of NIP5;1 and BOR1 are tightly regulated by B conditions to optimize B transport. Upon sufficient-B supply, BOR1 undergoes polyubiquitination and is transported from the plasma membrane to the vacuole for degradation, to avoid overaccumulation of B. We recently proposed that BOR1 is a transporter-receptor, "transceptor", directly senses the B concentration and promotes its own polyubiquitination and vacuolar sorting for quick and precise maintenance of B homeostasis (Yoshinari et al., 2021).

The *bor1-1* mutant, a loss of function mutant of BOR1, was originally identified by its sterility under normal B supply (30 µM, Noguchi et al., 1997). Even when the *bor1-1* mutants grow normally in the vegitative and early following stages, the mutants did not develop siliques. The defect was recovered by supply of higher concentration of B (150 µM). These results suggested the functions of BOR1 in preferential distibution of B toward reproductive tissues.

Recently, a boric acid channel NIP7;1 was shown to function in B transport in tapetum cells in developing anther (Routray et al., 2018). Under B limitation, the *nip7;1* mutants showed reduced fertility, including shorter siliques and an increase in aborted seeds, compared with the wild type. Here, we investigated the localization and function of the borate exporter BOR1 expressed in the tapetal cells. Our results suggest that BOR1 transports B from tapetum cells to locule to support development of pollen microspore cell wall.

METHODS

B content in each pollen grain was measured by a laser ablation (NWR213) hyphenated to an inductively coupled plasma-mass spectrometer (Agilent 8800 ICP-MS/MS) in Chiba University.

RESULTS AND DISCUSSION

To investigate localization of BOR1 in reproductive tissues, we analyzed the localization of BOR1-GFP in the transgenic *bor1-1*mutant plants harboring a *promoterBOR1:BOR1-GFP* construct. Expression of BOR1-GFP complemented the low-B sensitive phenotype of the *bor1-1* mutants. BOR1-GFP was detected in developing anther specifically in tapetum cells under low B supply (0.3-30 µM B). In the early developmental stages of anther, BOR1-GFP was localized in the plasma membrane in a polar manner toward the locule side (inner side). However, in the later developmental stages, GFP derived from BOR1-GFP was observed in endosomes and vacuole, indicating the degradation via endocytosis. We then analyzed localization of BOR1(K590R)-GFP, which is not subjected to high B-dependent ubiquitination (Yoshinari et al., 2021). The BOR1(K590R)-GFP also showed accumulation in the plasma membrane in early stages and degradation in the vacuole in the later stages. These results suggest the contribution of BOR1 in early developmental stages of anther and the control of BOR1 turnover dependent on the devlopmental cues.

To invesitage the function of BOR1 in pollen development, we observed the structure of pollen grains of Col-0 wild-type and the *bor1-1* mutant plants under a scanning electron microscope. Under 30 µM B supply, the *bor1-1* mutants showed normal growth in the vegitative stage and developed flowers, however, a large portion of pollen grains showed shurunk structure. We then measured B content of each pollen grain by laser ablation ICP-MS and found that B content of pollen grains of the *bor1-1*mutants was drastivally reduced compared to those of wild-type.

CONCLUSIONS

Our results suggest that BOR1 in tapetum cells in developing anther is responsible for B export toward locule to provide B for pollen cell wall development under low B supply. Further genetic studies are ongoing to confirm the contribution of BOR1 expressed in tapetum cells.

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NOVEL TECHNIQUE FOR BIOFORTIFICATION OF CEREAL GRAINS WITH ZINC THROUGH ENGINEERED ZNO NPS

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Keywords: Grain Zn-Concentration; Nano fertilizer; ZnO nanoparticles

Abstract

Zinc deficiency in agricultural soils is a well-recognized global, micronutrient problem resulting in sub-optimal production of cereals and leading to poor human health. The soil Zn deficiency not only limits the productivity of crops but also lowers grain nutritional quality (Chen et al., 2017). Nanomaterials can be used in producing more soluble and diffusible sources of Zn fertilizers. The varying dosages (0, 100, 200 and 500 mg/L), sizes (30-100 nm), and the spherical shape of ZnO NPs were synthesized, characterized, and evaluated in comparison to ZnSO4.7H2O levels in rice and wheat crops (Madhusudanan et al., 2019, Singh et al., 2021). Foliar fertilization of ZnO NPs (100ppm) increased rice grain zinc content by 75.2 per cent and in wheat, it increased by 84.1 percent over control. Application of ZnO NPs at relatively lower doses showed positive effects on both crops and increased grain zinc concentration significantly. There is a possibility of reducing the dose of Zn fertilizer by using ZnO NPs instead of bulk ZnSO4. Further, delivery of Zn through ZnO nanoparticles in right dose, right time and of right size could be beneficial and effective in enhancing growth and yield attributes of rice and wheat crops. This study proves the application of engineered ZnO nanoparticles as efficient means for biofortification of cereal grains. It is concluded that foliar application of nanostructured fertilizer like ZnO NPs is a useful and cost-effective solution to address the Zn deficiency problem in malnourished crops vis-à-vis human population.

Keywords: Grain Zn-Concentration; Nano fertilizer; ZnO nanoparticles; Rice; Wheat.

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New approach to determining coffee nutrient demand

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Keywords: Biomass; Nutrient concentration; *Coffea arabica* L.

INTRODUCTION

Brazil imports more than 80% of the fertilizers for agricultural production (Brasil, 2021), which causes insecurity to the sector due to heavy dependence from other countries, since fertilizers are strategic products for food security. Awareness and interest in improve nutrient use efficiency has never been greater and has been a prominent issue in national politics. One way to enhance this efficiency is to apply nutrients at the right rate, which requires knowing the plant nutrient demand. Nevertheless, the adequate nutrient demand is still unknown in coffee crops.

Fruits and active vegetative growth are the main sinks in mature coffee trees; thus, the annual nutrient demand in coffee crops can be predicted by multiplying the total plant biomass produced in a growth cycle by the nutrient concentration. However, fruits and active vegetative have different biomasses and nutrient concentration. In addition, fruits are the major physiological sink, regulating carbohydrates allocation (Bote and Jan, 2016). The relationship between fruit load and active vegetative growth provides a simple and secure method to estimate total biomass produced, as well as to predict the nutrient demand. Hence, this study aimed to develop a model to predict active vegetative growth regarding fruit load for a better description of the competition between these sinks in coffee trees, as well as determine the nitrogen (N), phosphorus (P), and potassium (K) concentration to quantify plant nutrient demand.

METHODS

Plant material, growth conditions, and experimental design

The experiment was conducted on arabica coffee (*Coffea arabica* L.) in the municipality of Jacuí, Minas Gerais State, Brazil (Cwb - humid subtropical with dry winter and temperate summer), in the third production cycle with a tree arrangement of 3.5 x 0.7 m spacing. The experimental design was completely randomized with six levels of fruit load (no fruit, 20, 40, 60, 80, and 100%) and five replicates. Each tree consisted of an independent experimental unit. For the fruit load manipulation, trees were selected based on uniformity and vigor. In early November, after flowering, when the fruits were in pinhead development stage, the fruit loads were manually imposed in all reproductive branches of the respective tree. The insertion of the last pair of fully expanded leaves of each branch was labeled for further evaluation of active vegetative biomass in the growth cycle.

Crop yield, measurements, and data analysis

The harvesting occurred in early June when all branches (vegetation biomass) were cut from the wire-label and fruits were collected. Vegetative biomass and fruit samples were oven-dried at 65°C to constant weight. After, the samples were weighed, fruits were de-husked, and the bean mass was corrected to 12% of water concentration (moisture of commercialized bean) to obtain coffee beans, hereafter called green beans. Subsequently, the materials were ground for the quantification of N according to the analytical semi-micro Kjeldahl method and P and K by X-ray diffraction. The datasets were subjected to the residual normality and variance homogeneity. The assumptions were met and the Analysis of Variance with F-test was performed considering <0.05 of probability.

RESULTS AND DISCUSSION

Fruit load significantly affected active vegetative biomass and biomass of green beans. However, no significant effect was observed in the N, P, and K concentrations evaluated in vegetation and fruiting organs (Table 1). As expected, reduction of fruit load significantly increased vegetative biomass and decreased coffee production linearly under field-growing conditions (Figure 1).

Table 1. Summary of the variance analysis of fruit load effects on variables. Variables: biomass of green beans, vegetative biomass, nitrogen (N), phosphorus (P), and potassium (K). Acronyms: DM = dry matter, and ns = not significant.

	Average	*P*		Average	*P*	Average	*P*
				---------- Vegetation ----------		---------- Fruiting ----------	
	g per tree			g kg^{-1} $_{DM}$		g kg^{-1} $_{DM}$	
Biomass of green beans	434.49	0.0016**	N	15.48	0.8418^{ns}	13.58	0.1611^{ns}
Vegetative biomass	678.72	0.0028**	P	1.53	0.2174^{ns}	1.39	0.0525^{ns}
			K	18.00	0.0501^{ns}	18.87	0.2844^{ns}

** significant below 1% of probability of error by the F test.

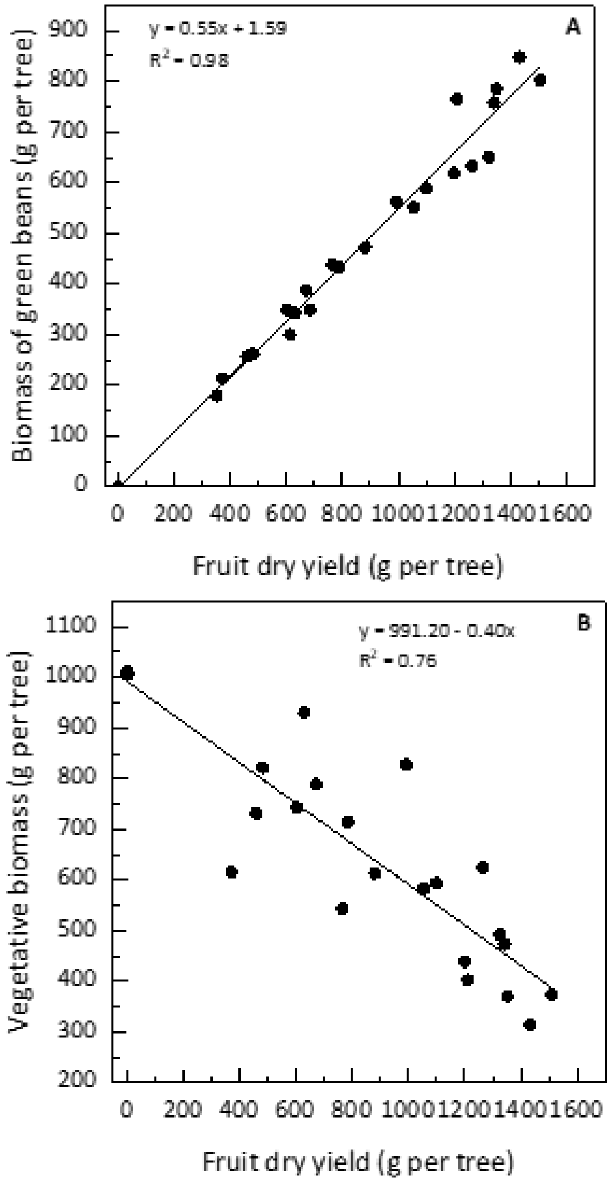

Fig. 1. Biomass of green beans (A) and vegetative biomass (B) per tree regarding fruit dry yield. Data consist of observations of each coffee plant under coffee-growing conditions and manipulation of fruit load.

CONCLUSIONS

Total Nutrient Demand (TND) for arabica coffee can be predicted for any fruit load by the product between the Fruit Biomass (FB) and its Nutrients Concentration (NC) added the product between the active Vegetative

Biomass (VB) and its Nutrients Concentration (NC): **TND = (FB x NC) + (VB x NC)**. Fruit and vegetative biomasses can be estimated according to fruit load predicted for the growth cycle by the regressions and multiplied by nutrient concentrations found in this research. For instance, N, P and K demand to produce 3 Mg ha^{-1} green beans is 103 kg N, 10 kg P and 136 kg K, by multiplying the nutrient demand per tree for active vegetative growth and fruit, which corresponds to 25.2 g N, 2.5 g P and 33.4 g K, by the number of trees per hectare (4082 trees ha^{-1}).

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ABILITY TO EXPLORE SOIL LEGACY PHOSPHORUS BY DIFFERENT CROP SPECIES

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Keywords: phosphate; Soil fertilization; Plant nutrition

INTRODUCTION

Phosphate fertilizers have been overused in agriculture for decades; and, whenever the P use efficiency is less than 100%, remaining P is accumulated in our soils. The accumulated P is called legacy P, determined by the difference between P inputs and outputs of the system (Pavinato et al., 2020). In this sense, the exploitation of legacy P can be a promising strategy to improve the sustainability and profits of agricultural system, in addition to reducing the demand for P fertilizers (Withers et al., 2018). However, the main barrier for this is to detect whether the mobilization is sufficient to supply all or part of the plant demanded P and in what period plants can use it.

Thus, considering that plants have different mechanisms to absorb and solubilize P in the soil, the aim of this study was to evaluate the ability of plant species in accessing legacy P and how much of the legacy P can potentially be exploited by agricultural crops.

METHODS

The experiment was carried out in a greenhouse using a Latossolo Vermelho Distrófico (Oxisol), clayey texture (64% of clay), from Planaltina, Distrito Federal. Previously, it was cultivated under no-tillage with annual inputs of 80 kg ha^{-1} of P_2O_5 (triple superphosphate - TSP) for a period of 17 years, followed by 8 years of P suppression. The soil was sampled at 0-10 cm layer, air-dried, ground, sieved (< 2 mm) and placed in 2 kg pots. The P content (Mehlich-1) after this 25 years of cultivation was 1.9 mg kg^{-1}.

The greenhouse experiment consisted of a sequence of 10 crop cycles (30 to 40 days each), arranged in a completely randomized design, in a split-plot scheme: the plot was composed by three plant species: braquiária (*Urochloa ruziziensis*), maize (*Zea mays* L.) and soybean (*Glycine max* L.); and the subplot by doses of P fertilizer (TSP): 0, 25 and 50 mg kg^{-1}, corresponding to approximately 0, 50 and 100% of P uptake by plants, respectively. Other macro and micronutrients were applied similarly without restriction. Six plants of brachiaria, two plants of maize or soybean composed each experimental unit in each cycle.

After 30-40 days, plants shoot were harvested to determine the biomass, estimated after drying in an oven at 65°C. After, tissue was ground and the leaf P content was obtained by nitroperchloric digestion (Embrapa, 2009). Exported P in each cycle was obtained by leaf P content and biomass data. The data were submitted to analysis of variance, and when significant, the means were compared using the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

Biomass production and P export were strongly influenced by P doses (Fig. 1). In general, brachiaria and maize were more effective in producing biomass using only the soil legacy P in the first two crop cycles, probably due to the root architecture and abundance. In the following cycles, there was no difference between species at zero P. The highest yield was obtained with the highest P dose, and, in general, in brachiaria plants (Fig. 1a,b,c).

Regarding P export, there was a difference only in first cycles at dose 0; however, considering the average data (data not shown), maize presented greater P export than other species. At the dose 25 and 50 mg kg^{-1},brachiaria was more efficient in exporting P (Fig. 1d,e,f). In addition, it stands out that in two first cycles, soybean had high P exports, especially at higher doses, but this behavior was observed in the following cycles.

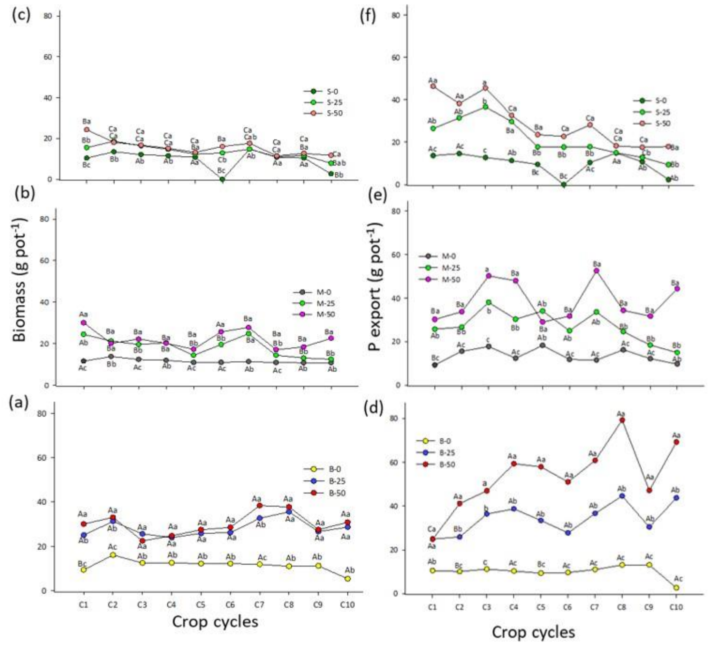

Fig. 1. Dry mass and exported P in each crop cycle of brachiaria (B) (a, d), maize (M) (b, e) and soybean (S) (c, f) for distinct P doses (0, 25 and 50 mg kg^{-1}). * Means followed by different capital letters differ by the tukey test at 5% for species within each dose; means followed by different lowercase letters differ by the tukey test at 5% for doses within each species.

CONCLUSIONS

Brachiaria and maize were more efficient in accessing legacy P for producing biomass, and maize was more efficient in exporting P, but only in the first cycles. When P was added, brachiaria performed better in biomass production.

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EVALUATION OF N USE EFFICIENCY AND RECOVERY ASSOCIATED WITH MO SOURCES

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Keywords: Urea; Ammonium molybdate; Sugarcane

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RELATIVE EXPRESSION OF *DUR3* GENE AND ROOT GROWTH IN *MEGATHYRSUS MAXIMUS* UNDER DIFFERENT NITROGEN SOURCES

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Keywords: Urea transporter; Grass; Nitrogen use efficiency

Nitrogen (N) is an essential macronutrient for plant growth and its deficiency is a limiting factor for forage grass productivity. Plants absorb N preferentially in the form of nitrate and ammonium, but urea can be directly acquired by the roots through a high-affinity transporter called *DUR3*. Increasing urea uptake by plants can minimize N losses by volatilization and improve nitrogen use efficiency. In the present study, we evaluated the influence of nitrogen sources on the levels of gene expression of the urea transporter *DUR3* and on root growth of *Megathyrsus maximus*. Seeds of *Megathyrsus maximus* grass (Syn. *Panicum maximum*), cultivar Mombaça and Aruana, were germinated in autoclaved substrate. After initial growth, uniform 15 day-old seedlings were transferred to plastic pots containing of nutrient solution. For the analysis of the relative expression of the *DUR3* gene, samples of roots were collected after 3 and 24 h of re-supplement with 2 mM of N in the forms of nitrate Ca $(NO_3)_2$, ammonium $(NH_4)_2SO_4$ and urea $CO(NH_2)_2$. For the root growth analysis, the plants remained in greenhouse for additional 30 days. The roots were scanned and analysed using WinRHIZO software. After 3 h of ammonium re-supplement, there was a significant increase in *DUR3* transcripts in roots compared to the nitrate source. Urea re-supplement also increased *DUR3* transcription in roots, with a higher expression level after 3 h in Aruana and after 24 h in Mombaça cultivars. Root morphological characteristics in both grass cultivars were significantly affected by N sources. The Mombaça cultivated under nitrate source presented the highest values average for all variables analyzed. Plants grown on ammonium and urea had lower root growth compared to plants under nitrate. There was no difference between root plant growth under ammonium or urea. The relative expression of the *DUR3* gene is positively regulated in plants that have undergone the N deficit. The nitrate was the best source for root growth.

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PHOSPHORUS ABSORPTION AND USE IN EUCALYPTS UNDER SPATIAL HETEROGENEOUS PHOSPHATE AVAILABILITY IN SPLIT-ROOT SYSTEM

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Keywords: Eucalyptus; Phosphorus; Nutrition

INTRODUCTION

Due to its high adsorption capacity to soil particles, phosphorus (P) is one of the least available macronutrients and, even in soils that receive phosphate fertilization, only around 20% of the P supplied is used by plants (Neumann 2016). Plant growth is related to the amount of nutrients it can acquire and can be limited by low root development and nutrient absorption, and low efficiency in their use. Nutrients are not evenly distributed in the soil around the roots, causing plants to trigger the functioning of several gene signaling pathways to coordinate adaptive responses to the most diverse types of stress, including nutritional limitation. Split-root has been used to analyze transcriptional, biochemical, and physiological changes in roots in response to nutritional challenges (Torres et al. 2021). This study aimed to verify the signaling between roots and shoots in different situations of P supply.

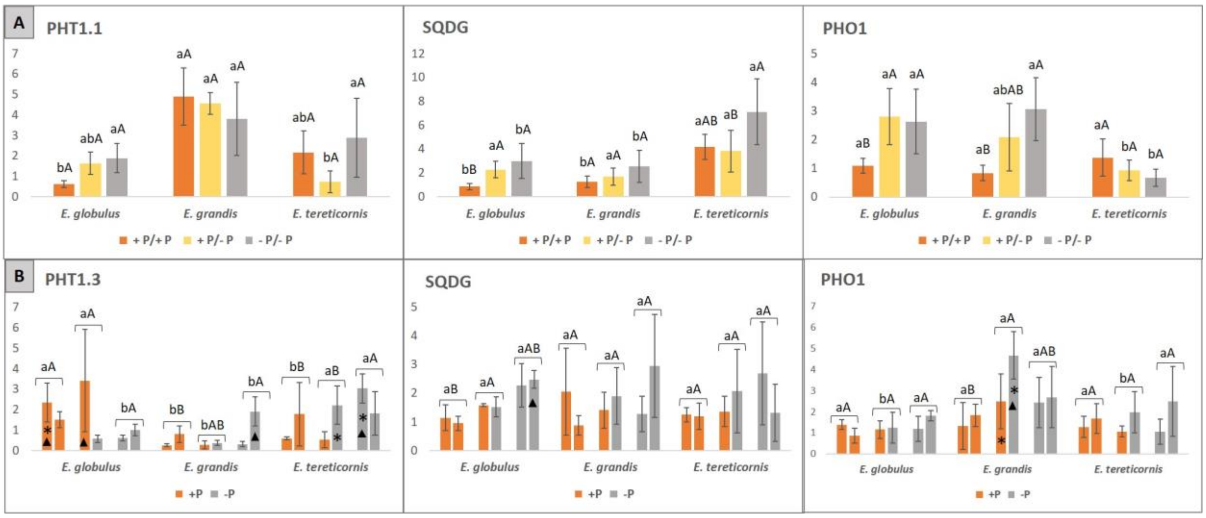
METHODS

Seeds *of Eucalyptus grandis*, *E. globulus*, and *E. tereticornis* were germinated in vermiculite and after 2 months in a growth chamber, they were transferred to a greenhouse, when each seedling was structured into a split-root system consisting of two plastic cups of 290 mL in which half of the root system of a plant were placed. The P treatments were: +P/+P (P-repleted homogeneous environment), +P/-P (heterogeneous P environment), -P/-P (P-deprived homogeneous environment), with absent level (0 μM) and sufficient level (440 μM) of P (Bahar et al. 2018). The experiment was collected after six weeks in this condition. The expression of genes related to P uptake, transport and, use (*PHT1, PHO1 and, SQDG)* was evaluated by RT-qPCR.

RESULTS AND DISCUSSION

Members of the *PHT1* phosphate transporters are involved either in the direct uptake of phosphate from the soil or in the transport of P within the plant (Wang et al., 2017). For eucalypts leaves, *PHT1.1* transcript levels were similar in plants that contained the -P and +P treatments. Plants under the -P/-P produced similar biomass than plants that receive +P in both or one of the root compartments, indicating efficient use of internal P (data not show). *E. tereticornis* showed lower leaf biomass in +P/+P treatment and higher root biomass in -P/-P and +P/-P (data not show), which indicates preferential carbon allocation to the roots when P is limiting even in a heterogeneous environment.

For roots, the expression of the *PHT1.3* gene, especially in *E. globulus*, suggests possible signaling between the splitted roots in the heterogeneous system +P/-P. Once in the -P side the expression of this *PHT1* transporter was lower than on the homogeneous -P/-P treatment, while in the +P side the expression was induced and higher than in the -P side (Fig. 1). Under P-deprivation plants may promote changes in the composition of membrane lipids inducing sulpholipids synthesis as a form to improve P use efficiency (Sun et al., 2020). In leaves and roots, the expression of *SQDG* gene, involved in sulpholipids synthesis, was higher when plants were exposed to P-deprivation, in the -P/-P treatments. The phosphate transporter1 (*PHO1*), involved in the loading of P into the xylem, is important in the long-distance transport of P. In the leaves of *E. globulus* and *E. grandis* this gene was more expressed in plants under at least one side in the -P treatment. In *E. tereticornis*, there was no difference in *PHO1* expression between treatments in leaves and roots, and as previously mentioned this species showed the highest root biomass, suggesting that larger roots for better P absorption may have contributed to no changes in *PHO1* gene expression.



ABSTRACT LUANA

Fig. 1. Relative expression of genes upregulated in eucalypts leaves (A) and roots (B). The relative quantification of each transcript in each treatment was normalized against the constitutive gene *Ef1*. Values represent the means of three replicates and bars represent ± standard deviation. Different lower-case letters represent significant differences between species in each treatment. Different capital letters represent significant differences between treatments within each species. Asterisk represents the most significant expression among the species in each treatment with absence or presence of P. Triangle represents a significant difference between treatments with absence or presence of P within the species (Tukey Test, $p < 0.05$).

CONCLUSIONS

Through this split-root system in eucalyptus, we observed that root-to-root signaling in P heterogeneous environments depends on the species, and it is assumed that complex interactions of the analyzed genes with other factors may have determined these responses.

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USE OF NITROGEN BY SOYBEAN IN ROTATION WITH MAIZE INTERCROPPED WITH GRASSES

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Keywords: Nitrogen absorption; ^{15}N; N losses in the soil

INTRODUCTION

Maize (*Zea* mays L.) intercropped with grasses grown as a relay crop after soybean (*Glycine max* L, Merril) has been a sound environmental and economic practice in tropical areas. In this system, nitrogen (N) dynamics is very complex and it is not fully understood, because it is affected by soybean and can be affected differently by the grasses intercropped. Understanding the fate of N in the cropping system is critical to define fertilizer rates and improve N use efficiency. It was hypothesized that a grass intercropped with maize grown in rotation with soybean could improve grain yield and N recovery from the fertilizer in the system by decreasing loss. The objective was to evaluate the effect of Guinea grass (*Megathyrsus Maximus)* and ruzigrass (*Urochloa ruziziensis*) on N recovery from the fertilizer by soybean and maize grown as a relay crop in a two-year field experiment in southeastern Brazil.

METHODS

A dryland field experiment was conducted in Botucatu, SP, Brazil, where maize was grown as a relay crop after soybean for two years. The soil is a loamy Rhodic Hapludox. Maize was intercropped with the forages Guinea grass (*Megathyrsus maximum,* cv Tanzânia) and ruzigrass (*Urochloa ruziziensis* cv. comum), plus a control without grass, and fertilized with up to 180 kg ha^{-1} of N. Labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$] enriched at 6.8% and 7.4% excess ^{15}N atoms in the 1st and 2nd seasons, respectively, was applied to maize in microplots, only to the treatment with 120 kg ha^{-1} of N. Regular ammonium sulphate was applied at sowing (20 kg ha^{-1}), next to the plant rows, and topdressed at V5 (100 kg ha^{-1}) at the V5 stage of corn. The enriched fertizer was used only at topdressing. No N was applied to soybean. Total N concentration and ^{15}N abundance were determined in maize, grasses and soybean, and also in the soil up to the depth of 80 cm.

RESULTS AND DISCUSSION

In the first season maize yield responded up to 60 kg ha^{-1} of N, with no response in the second season, when there was less water available and yields were lower. Ruzigrass impared maize grain yields. There have been reports of ruzigrass impairing maize yields, mainly in the absence of N fertilization, what was attributed to the effect of the grass on soil N dynamics (Marques et al., 2019). The response was limited to 60 kg ha^{-1} because there was water shortage, mainly in the second crop. Soybean yield was increased by N rates applied to the preceeding maize. Despite several reports of no reponse of soybean to N (Hungria et al., 2005), it has been shown that the soil native or residual N could favor soybean yields (Salvagiotti et al., 2008), what could explain our results. The average soybean yield were 4.08 and 4.16 t ha^{-1} in the first and second seasons, respectively. Soybean grain yields were increased with up to 60 kg ha^{-1} of N applied to maize + Guinea grass in both years, but there was no response in the control and after ruzigrass in the first year. In the second year soybean responded up to 120 kg ha^{-1} of N in the control and up to 180 kg ha^{-1} after ruzigrasss.

When grown after maize intercropped with grasses, N recovery by soybean was increased by 27 % and 380 % in over the control without grasses the first and sencond seasons, respectively (fig 1). This greater recovery was likely due to a higher availability of N in the system, since the plant residues from maize and the forages grown in the second maize crop accumulated 6% more ^{15}N, which was later released slowly to the soil

(Rosolem et al., 2017). Furthermore, maize and grass yields were lower in the second year, leaving more N in the system to be used by soybean. The greater accumulation of ^{15}N by the grasses and in the soil explain its recovery by soybean. Therefore the plant residues left on the soil surface acted as a slow release fertilizer, thus representing an opportune strategy to increase the efficiency of nutrient use in the agroecosystems.

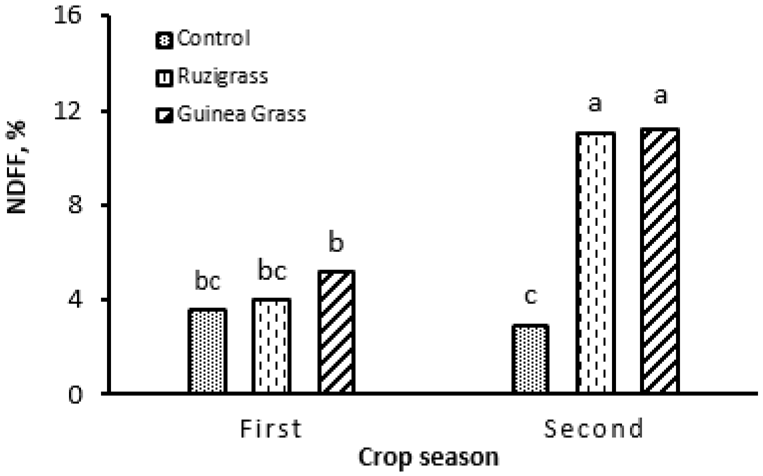

Figure 1 - Percentage of ^{15}N-residual (Nitrogen derived from the fertilizer applied to maize - NDFF) recovery in the soybean shoots. Different letters show significant differences (LSD, $p < 0.05$)

CONCLUSIONS

The use of grasses intercropped with maize is an interesting practice to preserve N in the cropping system. The amount of N applied to maize that will be available to the subsequent soybean is inversaly proportional to maize productivity, but it is higher in the presence of forage grasses, mainly Guinea grass. However, when maize yields are low and the N surplus in the system is higher, the grasses are able to avoid loss, and the nutrient will be used by soybean. Ruzigrass has a detrimental effect on N use by maize and by the following soybean.

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ENDODERMAL SUBERIN DEPOSITION RESTRICTS POTASSIUM LEAKEAGE FROM ROOTS

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Keywords: Suberin; Potassium; Endodermis

INTRODUCTION

The endodermis serves as a checkpoint for ions, including K, and water escaping or entering the root. It has been hypothesized that suberin acts as a physical barrier preventing K leakage from the stele during root to shoot translocation. However, contrasting observations has been reported regarding shoot ionomes of root suberin mutants (Barberon *et al.*, 2016; Cohen *et al.*, 2020; Calvo-Polanco *et al.*, 2021; Shukla *et al.*, 2021). Accordingly, the role of suberin with respect to uptake and translocation of mineral ions remains enigmatic. In order to resolve the contrasting data obtained by mutant studies there is a need to directly investigate the K transport dynamics across the suberin layer, instead of solely relying on shoot ionomic data. In the current study, we present a method for the direct measurement of K transport across the endodermis, using a novel approach which includes Laser Ablation ICP-MS (LA-ICP-MS) and the use of a proxy element for K; Cs. Our data show that Cs is an excellent proxy for K in this system, which enabled us to clarify the mechanistic role of suberin with respect to K transport across the endodermis.

METHODS

Using LA-ICP-MS, we developed an experimental setup allowing us to image and mimic the biodistribution of K by use of a proxy, Cs. The use of Cs was necessary since the background K concentration is very high in plant tissue, which make it difficult to distinguish between added and native K. With similar chemical properties, similar biodistribution *in planta* and a negligable toxic effect to the plants, we show that Cs is an excellent proxy for K studies. By imaging the distribution of Cs (representing K) in various root regions and by analysing changes in Cs gradients across the endodermis, the role of suberin could be unravelled in root sections with contrasting suberization. Accordingly, we employed this technique to study two root types of barley with contrasting suberin deposition and three known mutants of *Arabidopsis thaliana* with different suberin content; Col-0, CASP1::CDEF1 and esb1.

RESULTS AND DISCUSSION

Current interpretations of shoot ionomic data suggest that the impact of suberin on net K uptake is related to the ability of suberin to prevent leakage from the vasculature during root-to-shoot translocation (Barberon *et al.*, 2016). In this study we show that the mature parts of both nodal and seminal roots, where most suberin is deposited, indeed were able to secure K within the stele during translocation (Fig. 1, A & B) On the contrary, closer to the tip, where both root types deposited less suberin (Fig. 1, C & D), both seminal and nodal roots lost K from the stele to the cortex during translocation. The same conclusion was found in suberin mutants of Arabidopsis differing in suberin deposition, which supports the results from other studies (Barberon *et al.*, 2016). We also observed that nodal roots of barley represent a special case, where the endodermis remained incompletely suberized also in the mature parts (i.e. the phloem-facing zone), yet successfully prevented K leakage. The exact reasons for this will be discussed and is currently further pursued in our laboratory.

CONCLUSIONS

Collectively, direct measurements of K transport across the endodermis was made possible by combining LA-ICP-MS measurements with the employment of Cs treatments. Our results provide evidence to support that suberin, in the mature parts of barley and Arabidopsis roots, facilitate net uptake of K by preventing K leakeage from the stele to cortex during translocation from roots to shoots.

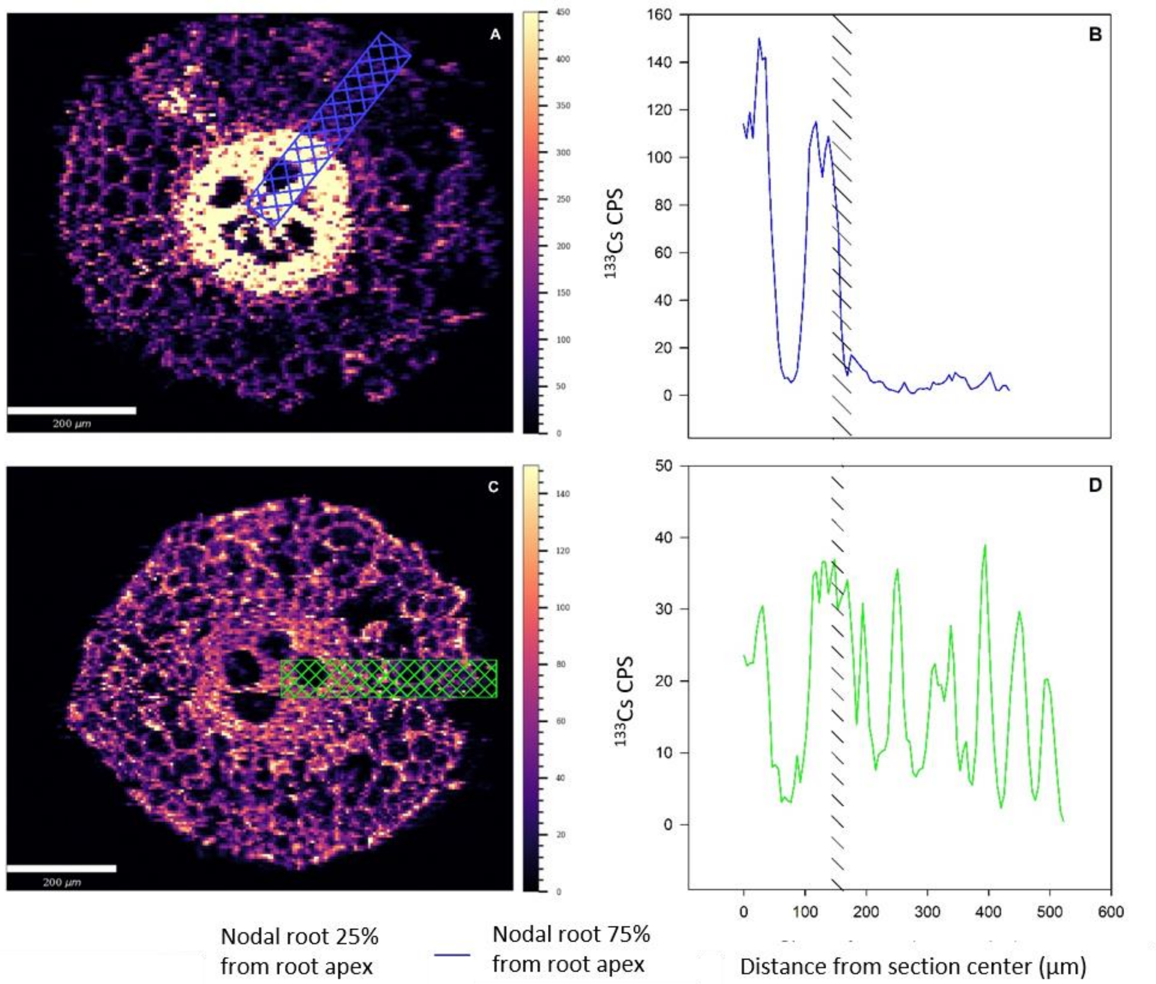

Figure 1. Tissue distribution of the Cs (representing K) in the phloem facing and patchy-suberized zones of barley nodal roots. A and C, Images of Cs distribution in root cross sections of root sampled in the phloem facing zone (A) and patchy-suberized zone (C) sampled 75% and 25% distance from the root apex respictibly. Root sections were sampled following 10 min. incubation with 10 mM 133Cs. The sections were sampled one cm from the incubated area towards the root base. B and D, Line scans of Cs intensities across root cross sections in A and C. Colored crosshatched boxes represent line scans at the broadest diameter of root cross sections and reported values are average intensities across 40 µm. Crosslines represent when the extracted line scan is coinciding with the endodermis.

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FUNCTIONAL CHARACTERIZATION OF AGRONOMICALLY IMPORTANT OSSULTR3 ISOFORMS

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Keywords: SULTR; Membrane Transport; Plant Nutrition

INTRODUCTION

The plant Sulfate Transporter (SULTR) gene family was characterized for sulfate uptake from the soil and its re-distribution within plant tissues. The proteins encoded by the SULTR gene family form 4 sub-groups based on amino acid sequence identity that largely correlates with localization and function. Despite these seemingly clear distinctions, SULTR3 protein isoforms have evaded a clear functional description. *Oryza sativa* contain six SULTR3 isoforms, and several have been shown to fill non-canonical, yet agronomically relevant roles, including phosphate transport (OsSULTR3;4), and underlying rice susceptibility to bacterial leaf streak (OsSULTR3;6; Cernadas et al., 2014; Yamaji et al., 2017). Several OsSULTR3 isoforms lack in-depth functional analysis and therefore, their their full contributions to mineral nutrition is unknown. This talk will highlight recent work on the functional characterization of OsSULTR3;6 and future steps to better understand OsSULTR3 functional diversity.

METHODS

Transport function of OsSULTR3;6 was assessed through two-electrode voltage clamp (TEVC) in *Xenopus laevis* oocytes. Briefly, de-folliculated oocytes at stage V and VI were micro-injected with 50 nL of water or RNA encoding OsSULTR3;6 (25 ng RNA). Cells were incubated in a modified Ringer's solution for 3-4 days prior to TEVC or confocal microscopy.

Transient expression in tobacco (*Nicotiana benthamiana*) was achieved using the *Agrobacterium tumefaciens* strain GV3101 transformed with YFP::OsSULTR3;6. Activated *A. tumefaciens* carrying YFP::OsSULTR3;6 was syringe-infiltrated into the abaxial leaf surface of young, fully expanded leaves. Tobacco plants were incubated for 3-5 days prior to confocal imaging.

RESULTS AND DISCUSSION

Expression of YFP-tagged OsSULTR3;6 (YFP::OsSULTR3;6) in *N. benthamiana* and *X. laevis* oocytes revealed plasma membrane localization in both systems (Figure 1A,B). For functional analysis, *X. laevis* oocytes were impaled with microelectrodes to measure their resting membrane potential (RMP). Less-negative RMPs were recorded in oocytes microinjected with OsSULTR3;6 RNA compared to water-injected controls (Figure 1C), suggesting that OsSULTR3;6 facilitates the net movement of charge across the membrane. To validate OsSULTR3;6 as an electrogenic membrane transporter, OsSULTR3;6-expressing oocytes and water-injected controls were assessed using the two-electrode voltage clamp (TEVC) technique. Compared to water-injected control oocytes, OsSULTR3;6-expressing oocytes displayed large, inward currents at negative clamp potentials that was enhanced at lower extracellular pH (Figure 1 D,E). These data demonstrate that OsSULTR3;6, a previously uncharacterized SULTR3 member, functions as an electrogenic, pH-enhanced membrane transporter.

FIGURES

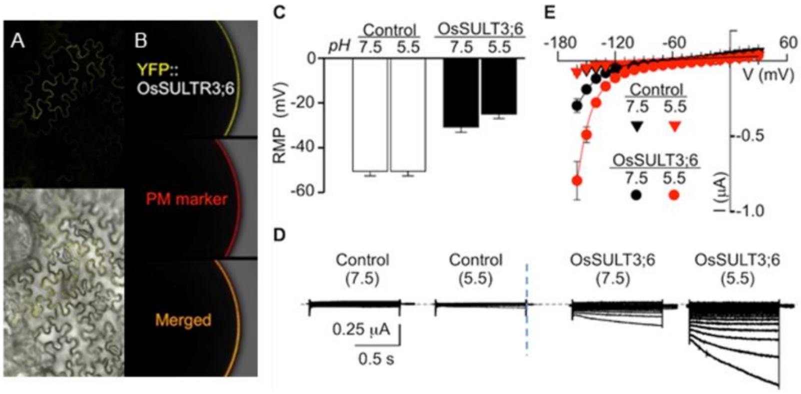

Fig. 1. Cellular localization (confocal microscopy) and electrophysiological (two-electrode voltage clamp) characterization of OsSULTR3;6. (A) Transient expression of an YFP::OsSULTR3;6 fusion proteins in *Nicotiana benthamiana* shows the transporter is primarily localized at the plasma membrane. (B) Plasma membrane localization of YFP::OsSULTR3;6 in *Xenopus*. Cell Mask^{TM} Deep Red was used as Plasma Membrane stain for colocalization purposes. (C) Resting membrane potentials and (D-E) electrogenic transport in *Xenopus* oocytes in ND96 solution (pH 7.5 or 5.5) injected with water (control) or 25 ng of OsSULTR3;6 cRNA (n = 6-8 each). (D) Examples of currents elicited in response to holding potentials ranging from 0 to -150 mV (in 10-mV increments inter-episode holding potential of -20 mV for 10 s). (E) Mean current/voltage (I/V) relationships from recordings like those shown in D.

CONCLUSIONS

OsSULTR3;6 is a membrane transporter that mediates the movement of net charge. These observations align with published work on sulfate uptake mechanisms in plants and individual SULTR isoforms in heterologous systems, in which sulfate uptake is enhanced at lower extracellular pH (Lass and Ullrich-Eberius, 1984; Wang et al., 2021). However, the identification of distinct functional roles among SULTR3 isoforms requires additional experimentation before applying a blanket *in planta* function to members grouped by sequence identity. Additional experimentation using OsSULTR3 sub-group members in heterologous systems to determine substrate selectivity is required, alongside phenotyping of OsSULTR3 mutant rice lines to contextualize findings from non-native systems.

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TRACING DIURNAL CA^{2+} FRUIT DELIVERY IN TOMATO PLANTS

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Keywords: Calcium; Strontium; Tomato fruit

INTRODUCTION

Calcium (Ca^{2+}) is an essential plant macronutrient involved in stomatal movement, intracellular signalling, cell wall integrity, stress and disorder relief (Thor, 2019). Ca^{2+} enters the plant's root system primarily via the mass flow of water and is delivered to different plant organs primarily via the transpirational flow. Due to this, highly transpiring plant organs such as leaves are characterised by high levels of largely immobile Ca^{2+} (Kumar et al., 2015). In comparison, low Ca^{2+} levels characterise low-transpiring organs such as fruits, leading to arrested development, disorders (e.g., blossom-end rot, bitter pit), and pathogen susceptibility, especially under stressful conditions (Parvin et al., 2019). Characterising Ca^{2+} uptake, delivery and distribution in the plants is difficult considering that it is hard to distinguish between Ca^{2+} already present in the plant and the newly uptaken Ca^{2+}. Luckily, pathways of Ca^{2+} uptake and transport in plants, to a large degree, are not specific only to Ca^{2+}, which led to the establishment of the "Sr^{2+} as a tracer for Ca^{2+}" paradigm. Which led to Sr^{2+} being reliably used as a Ca^{2+} tracer in different plant organs and plant species (Song et al., 2018); however, not much data is available for tomato plants (*Solanum lycopersicum* L.), if any. Motivated by this, we conducted several experiments to test the validity of Sr^{2+} as a tracer for Ca^{2+} in tomato plants using short and long-term trials. We subsequently used the Sr^{2+} to characterise Ca^{2+} uptake, delivery and distribution in tomato plant organs, specifically focusing on tomato Ca^{2+} fruit delivery under varying and stressful environmental conditions, operating under the hypothesis that the nighttime might offer stress relief and certain benefits for fruit Ca^{2+} delivery under stressful conditions during the day.

METHODS

Long-term Sr^{2+} trials

To establish the "Sr^{2+} as a tracer for Ca^{2+}" paradigm, tomato plants (*Solanum lycopersicum* L.) were grown in a net house with perlite substrate and irrigated three times a day with a full fertigation solution. Five to six weeks after planting, three treatments were applied: Ca^{2+} nutrition (4mM $CaCl_2$) ("+Ca"); Sr^{2+} nutrition (4mM $SrCl_2$) ("+Sr") and no Ca^{2+} and no Sr^{2+} ("-Ca/-Sr") (**Fig 1** c). Plants were treated for 24 days, after which they were harvested, and tomato fruits were sent to EDX spectroscopy (Energy-dispersive X-ray spectroscopy) to observe and compare the spatial cell wall distribution of Ca^{2+} and Sr^{2+} expressed as a weight percentage of selected elements (**Fig 1** c).

Diurnal Sr^{2+}/Ca^{2+} transport

To characterise the diurnal transport of Sr^{2+}/Ca^{2+} to tomato fruit, tomato plants were grown in growing rooms (hydroponics and perlite) under different humidity and temperature levels, designed to manipulate the evapotranspiration (ET) rate. Here we present the data from one representative experiment conducted at eight levels of VPD by manipulating room temperature (ranging from 17 ºC to 38.5 ºC). The plants were treated with 4 mM $SrCl_2$ for 12h during the day or night. After which, the Sr^{2+}concentration of the shoot and the fruit was determined, and the per cent of Sr^{2+} delivered to the fruit, compared to the total Sr^{2+} uptake, was calculated (**Fig 1** a and b).

RESULTS AND DISCUSSION

Our results from the long term Sr^{2+} exposure, obtained with the EDX spectroscopy, show that the relative cell wall weight of Ca^{2+} in the tomatoes which did not receive Ca^{2+} after the fifth week of growth ("-Ca/-Sr" and "+Sr") was more than 50% lower compared to the group which did ("+Ca") (**Fig 1** c). While in the group "+Sr", which received Sr^{2+} instead of Ca^{2+} after the fifth week of growth, combined weights of Ca^{2+} and Sr^{2+} were equal to the group which continued to receive full fertilisation ("+Ca") (**Fig 1** c). These results showed that Ca^{2+} and Sr^{2+} were deposited in tomato fruits at the same rate and in the same place (cell wall), confirming the

"Sr^{2+} as a tracer for Ca^{2+}" paradigm in tomato plants (Jovanovi? et al., 2021). Additionally, by utilising the paradigm, we showed that under environmental conditions which promote evapotranspiration (increasing temperature and daytime), the concentration of Ca^{2+} found in tomato fruits dramatically decreases (**Fig 1** a) and that the most significant share of the Ca^{2+} is delivered to the fruits during the night (**Fig 1** b).

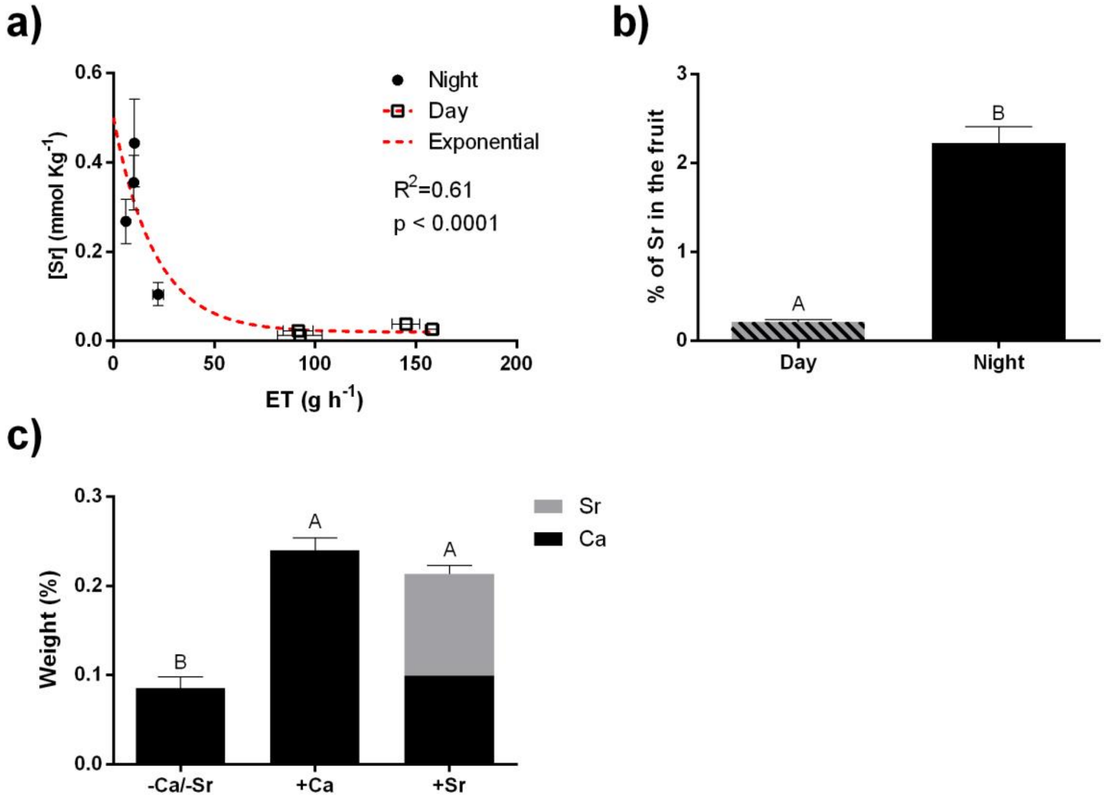

Fig 1 Daily Sr^{2+} concentrations in the fruit after exposure to 4 mM $SrCl_2$ as a function of plant evapotranspiration (ET) (a); Per cent of Sr^{2+} in the fruit after exposure to 4 mM $SrCl_2$ in the day and night; (c) EDX spectroscopy of the tomato fruit after long-term exposure to 4mM Sr^{2+}, Ca^{2+} or full fertilisation

CONCLUSIONS

These findings highlight the centrality of environmental conditions to fruit Ca^{2+} accumulation. Hence we speculate that the projected climate change and rising day, and specifically, night temperatures, are expected to impair the share of Ca^{2+} delivered to the fruits, aggravate physiological disorders and inflict substantial economic damage. Moreover, these findings provide us with the specific time frame (the night) that could be targeted to mitigate the possible adverse effects of climate change on nutrient delivery. Our findings also confirm that Sr^{2+} can be used as a reliable tracer of Ca^{2+} in tomato plants.

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DIFFERENT PHOSPHORUS-ACQUISITION STRATEGIES AND HIGH PHOSPHORUS-USE EFFICIENCY IN *ADENANTHOS* SPECIES (PROTEACEAE) IN SOUTH-WESTERN AUSTRALIA

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Keywords: Phosphorus acquisition strategies; Phosphorus-use efficiency; *Adenanthos* species

INTRODUCTION

In extremely low-phosphorus (P) environments, most Proteaceae exude large amounts of carboxylates into the rhizosphere through cluster roots, which mobilises inorganic P and concomittently leads to a relatively high leaf manganese concentration ([Mn]) (Lambers et al., 2021). Therefore, leaf [Mn] is a proxy for carboxylate concentrations in the rhizosphere (Lambers et al., 2021). However, *Adenanthos cygnorum* does not produce functional cluster roots. Instead, based on leaf [Mn], we found that *A. cygnorum* growing next to *Banksia attenuata* (Proteaceae) in severely P-impoverished soil was faciliated in acquiring P, most likely by this carboxylate-releasing neighbour. On soil with more available P, phosphatases released into soil by *A. cygnorum* roots hydrolysed organic P into inorganic P without significant carboxylate exudation (Shen et al., in review). *Adenanthos cygnorum* also has a photosynthetic P-use efficiency (PPUE) as high as most other Proteaceae growing in the same region (Shen et al., in review). Based on the P-acquisition strategies and high P-use efficiency of *A. cygnorum*, we aimed to understand the P-acquisition strategies and P-use efficiency of other *Adenanthos* species in south-western Australia.

METHODS

We selected *A. meisneri*, *A. barbiger*, *A. cuneatus* and *A. sericeus* as the target species. A *Banksia* species at the same location was used as a positive reference, as these species produce robust cluster roots that release carbxylates into soil to mobilise soil bound P and Mn. *Xanthorrhoea preissii* (Xanthorrhoeaceae) was used as a negative reference, as they do not produce cluster roots to exude carboxylates. At locations where *X. preissii* was not found, we used young leaves from the target plant as a negative reference. We determined leaf [Mn] and [P] for all plants and the soil [P] and pH under the *Adenanthos* plants. Photosynthetic rates and leaf nitrogen concentrations ([N]) were measured for *Adenanthos*. Where possible, we also determined cluster root carboxylate and phosphatase exudation for the *Adenanthos* species.

RESULTS and discussion

We found three distinct P-acquisition strategies in *Adenanthos* species. First, P-mining strategies for *A. meisneri*, *A. cuneatus* and *A. sericeus* which exhibited higher leaf [Mn] than their negative references, presumably by releasing carboxylates into soil. Second, facilitation of P acquisition by P-mobilising neighbours for *A. cygnorum* (Shen et al., in review). Third, because *A. barbiger* is one of the first plants to resprout after a fire event and does not express either of the two P-acquisition strategies that we found for the other *Adenanthos* species, it likely depends on P released during a fire (Lambers et al., in review). Thus, all *Adenanthos* species in the present study acquired P from soil, without depending on facilitation, unlike *A. cygnorum*.

Leaf [P] of the targeted *Adenanthos* species were similar to that of their reference *Banksia* species, but higher than that of *A. cygnorum* (Shen et al., in review), though very low compared with crops (Epstein and Bloom, 2005). They used P and N efficiently with a high PPUE and photosynthetic N-use efficiency (PNUE) compared with crops.

CONCLUSIONS

Unlike most genera in Proteaceae which are only known to exhibit a single P-acquisition strategy, three P acquisition strategies were found in the five *Adenanthos* species, including *A. cygnorum*, we have so far examined. *Adenanthos* species also had low leaf [P] and [N], but high PPUE and PNUE, as is the case for most other Proteaceae when compared with crops. This knowledge provides a better understanding of the diversity of nutrient-acquisition and nutrient-use strategies in severely P-impoverished environments.

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EFFECT OF NITROGEN NUTRITION ON UPTAKE AND TRANSLOCATION OF ZN FROM SOIL AND FOLIAR ZN FERTILIZER IN MAIZE AND WHEAT

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Keywords: Agronomic Biofortification; Zn, N, Fe; Wheat, Maize

INTRODUCTION

Success in agronomic biofortification of cereals (e.g. wheat, rice and maize) is highly variable (Rehman et al., 2021). In cereal grains, major portion of Zn is believed to be confined in the form of protein-Zn-phytate complexes (Rodriguez et al., 2022). Adequate Nitrogen (N) increases the levels of metal-chelating nitrogenous compounds, hence; facilitates the Zn and Fe uptake and transport to the grain (Kutman et al. 2011).

However, knowledge about the aspects improving root and leaf absorption of Zn from soil and foliar Zn fertilizers in wheat and maize is vital to enhance the nutritional value of these important cereal crops (Hui et al., 2022). Moreover, considering the fact that maize is very sensitive to Zn deficiency, any influence of N fertilization to improving Zn nutrition of maize will be beneficial for yield as well as nutritional quality of grains (Butail et al., 2022).

Current study was performed to investigate the effect of nitrogen (N) supply on uptake and translocation of Zn in maize and wheat during vegetative stage from soil and foliar Zn fertilization.

METHODS

In first experiment, maize and wheat plants grown with sufficient (2 mg Zn kg^{-1}) soil Zn, were supplied with low (100 mg N kg^{-1}) or adequate N (200 mg N kg^{-1}) in soil. Plants were harvested at two developmental stages for elemental analysis. In second experiment, maize and wheat plants were grown in marginal (0.5 mg Zn kg^{-1}) soil Zn at three different N rates viz: low (50 mg N kg^{-1} soil), medium (100 mg N kg^{-1} soil) and adequate (200 mg N kg^{-1} soil). At 3-4 leaf stage plants were sprayed with 0.25% (w/v) $ZnSO_4.7H_2O$ and fresh young un-sprayed leaves were analyzed for Zn and N concentration.

RESULTS AND DISCUSSION

In first experiment, improving N supply significantly enhanced the biomass production as well as absorption and shoot accumulation of Zn and Fe in both maize and wheat but more significantly in wheat (Table 1).

Results suggested that increased N nutrition enhance the Zn (also Fe) uptake from soil and helps in translocation to the young plant tissues in both wheat and maize but more efficiently in wheat (Results of second experiments are not shown here).

Crop	Soil N (mg kg^{-1})	Dry matter (g $plant^{-1}$)	N (%)	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Maize	100	2.89 ± 0.48	0.99 ± 0.09	5.18 ± 0.13	37.5 ± 1.41
Maize	200	4.51 ± 0.34	1.78 ± 0.20	6.64 ± 0.31	46.2 ± 5.50
Wheat	100	1.88 ± 0.06	1.84 ± 0.27	7.64 ± 0.88	37.1 ± 3.49
Wheat	200	2.21 ± 0.12	2.84 ± 0.14	12.7 ± 0.77	44.4 ± 1.93

Table 1. Shoot dry matter production, N, Zn, and Fe concentration in shoots of 11-weeks-old maize and wheat plants grown with low (100 mg N kg-1 soil) and adequate (200 mg N kg-1 soil) N supply.

Foliar application of Zn have a little effect on improving the grain Zn concentration as compared to wheat. Maize has less capacity to uptake and translocate Zn from foliar Zn application as compared to wheat (Rehman et al., 2021). Higher uptake and translocation of Zn in wheat compared to maize was also visualized and demonstrated by using Zinpyr-1 fluorescent dye (Fig 1).

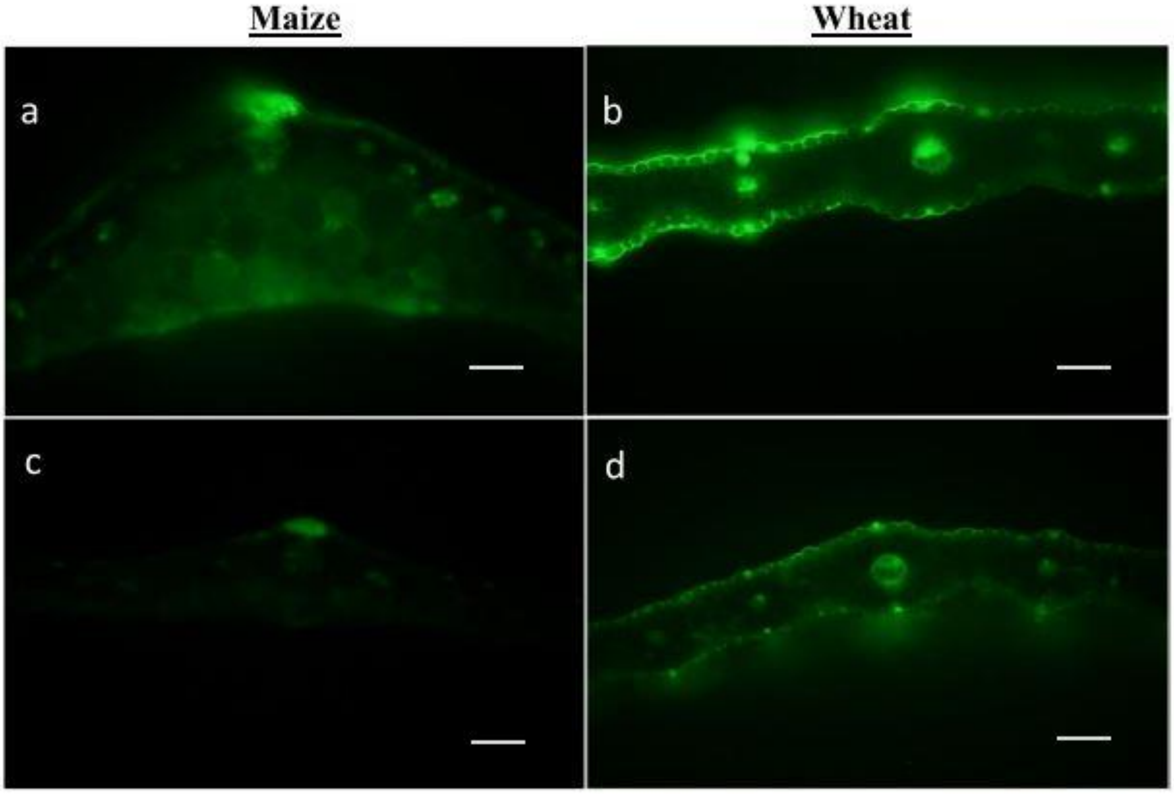

Fig. 1. Cross-sections (10x) of: (a) Zn-treated old maize leaf, (b) Zn- treated old wheat leaf, (c) upper younger leaf of Zn- treated maize, (d) upper younger leaf of Zn- treated wheat. Intensity of green fluorescence indicates binding of Zinpyr-1 with the Zn in leaf tissue. Scale bars = 100 µm (Rehman et al., 2021)

Therefore, current study was designed to assess that whether N nutrition would have a better effect on plant Zn status in maize or not. Results from current study will provide an insight about the role of N nutrition in the uptake and translocation of foliarly applied Zn in maize and wheat.

CONCLUSIONS

As compared to maize, wheat has more potential to accumulate Zn and Fe in grain when there are sufficient amounts of Zn and N available in soil or on foliage (Foliar spray). Therefore, improving N nutrition, could be a very helpful tool for agronomic biofortification of Zn and Fe in cereal crops particularly in wheat.

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A NOVEL PATHWAY OF PLANT PHOSPHORUS UPTAKE FROM DUST DEPOSITED ON LEAVES

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Keywords: Phosphorus uptake; Dust; Anatomic adjustment

INTRODUCTION

Phosphorus (P) limitation is prevalent worldwide, primarily because most soil P has low bioavailability. Desert dust plays a crucial role in shaping the function and structure of Eastern Mediterranean ecosystems by continuously supplying nutrients to the soil. In P poor ecosystems, deposition of P-rich desert dust is recognized as a major component of the P cycle. The acknowledged paradigm is that plants acquire P deposited in soil primarily via their roots. We hypothesized that plants that evolved in ecosystems downwind to deserts developed foliar chemical and structural traits that elevate dust capture and solubilization. This study examined the effects of desert dust on Mediterranean plants grown under contrasting P availability levels. We tested whether and to what extent plants acquire P directly from dust deposited on their leaves and what are the uptake mechanisms of insoluble P.

METHODS

Either phosphate-rich dust or apatite powder was applied to P sufficient and P deficient chickpea, maize, and wheat plants grown in a greenhouse and was compared to plants that received inert silica powder. Structural modifications associated with P limitation were measured by binocular and scanning electron microscopy (SEM). Leaf surface pH was measured by a flat pH electrode. Leaf dust holding capacity was estimated by applying 1 g dust on pre-weighted leaves. For leaf surface metabolites, 1 g of fresh leaves was sampled randomly from +P and -P chickpea and maize plants. Leaves were washed with 2 ml of distilled water and methanol (50:50) for 20 seconds. The dissolved metabolites were then introduced to a Gas Chromatograph (GC) and detected by a mass spectrometer.

RESULTS AND DISCUSSION

Phosphorus content in dust was found to be in the range of 4,120 to 1,930 mg kg^{-1}, substantially higher than P concentration in most soils (usually in the range of 300-800 mg kg^{-1}). Foliar application of dust nearly doubled the growth of P stressed chickpea and wheat, two crops originating near the Syrian desert- but not in maize, a plant from Meso-America. A representative image of chickpea plants following foliar application of desert dust or synthetic apatite compared with positive or negative controls is presented in Fig. 1.

P starvations induced morphological and chemical modifications in the leaf surface: trichome and stomatal density increased in response to P starvation, elevating leaf dust holding capacity by 3.5 fold, contributed mainly by the high capacity of young, starved leaves (Fig. 2). In response to P deficiency, chickpea plants enhanced the secretion of organic acids that accelerated solubilization of mineral P with low bioavailability (Gross et al., 2021). In addition to P, dust also enriched plants with other micro and macronutrients such as K and Fe (not presented).

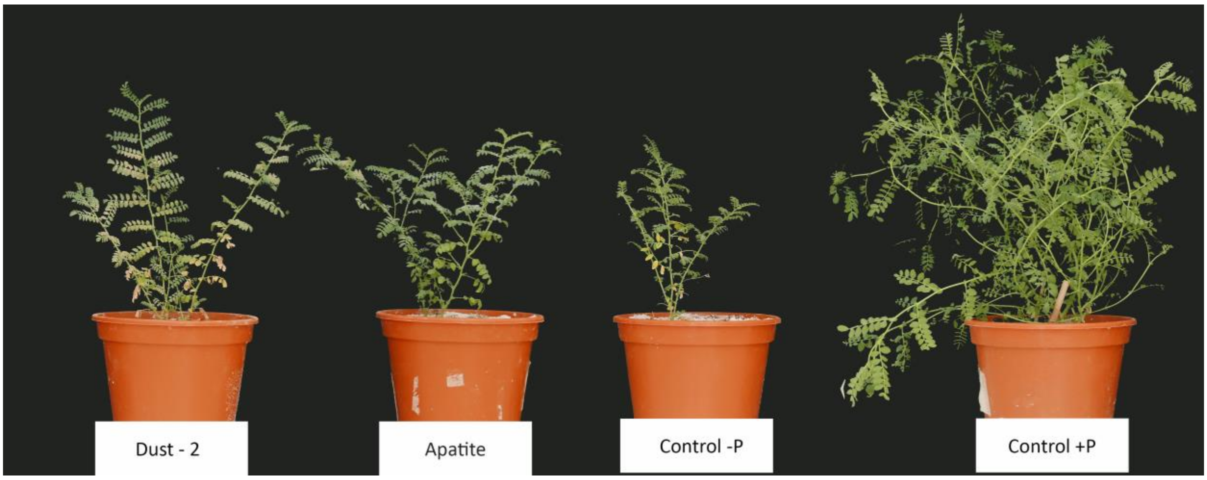

Fig. 1. Visual effect of foliar application of apatite or dust to P depleted chickpea compared with negative control (-P) and positive control (+P).

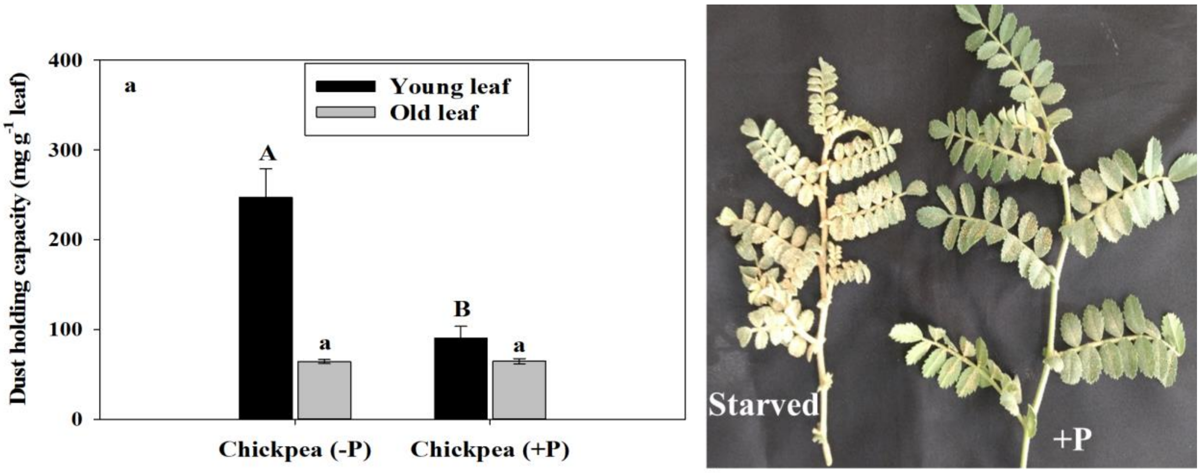

Fig. 2. Dust holding capacity of young or mature chickpea leaves as affected by P availability during the growth. Bars represent averages ±SD of five plants. Different letters within P level indicates significant difference.

CONCLUSIONS

Our results suggest that some plant species developed chemical and structural traits enabling plants in dusty regions to acclimate to nutrient-poor conditions via utilizing an alternative foliar acquisition pathway. These traits are somewhat comparable to the well-established root-system responses to P limitation, e.g., acidification, secretion of organic acids, and extended surface area. These traits enable a significant increase in dust capture and P solubilization.

As both P limitation and dust deposition are projected to increase due to global environmental changes, plants adopting dust traits will gain a competitive advantage over other species. Thus, we propose that future exploitation of these traits may assist in the development of a more effective P management strategy.

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NITRATE IS THE RIGHT NUTRITION SOURCE FOR PRUNUS TREE SPECIES

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Keywords: almond; apricot; ammonium

INTRODUCTION

The production of California almonds covers 650,000 hectares and is valued at over $9 billion/year. Due to the high value of this crop, the production intensity, and the high nitrogen removal in the harvested nuts, careful attention is given to proper fertilization practices. Additionally, the presence of nitrate-enriched groundwater in the region causes regulatory scrutiny to encourage proper nitrogen management practices.

The use of high-frequency (weekly), low nitrogen application rate fertigation for almonds has shown excellent results for farmers and is being widely adopted. Many farmers commonly apply fertilizer containing 50% urea-N, 25% ammonium-N, and 25% nitrate-N. This practice ensures that a large fraction of the plant-available N in the rootzone is present as ammonium throughout the growing season. The use of high frequency, low-N application fertigation also reduces nitrous oxide emissions from the soil, especially when a nitrate-based fertilizer is used (Wolff et al., 2017).

To verify potential plant-growth benefits associated with N fertilizer forms, a greenhouse hydroponic study was conducted to measure almond (*Prunus dulcis* var Supareil) sapling growth and physiological response to different NH_4^+:NO_3^- proportions. A hydroponic system was used because unlike soil, nitrogen and other inputs can be carefully monitored. A second follow-up study was conducted in a similar manner to study the response of apricot saplings (*Prunus armeniaca*) to different NH_4^+:NO_3^- proportions in a different species in the same botanical family.

METHODS

ALMOND: The study was conducted in a glass greenhouse at Tennessee State University, Nashville, TN, USA. In brief, almond saplings were first potted in 2-L plastic containers filled with biochar as growing substrate and pruned to a uniform height (30 cm). Five ammonium to nitrate ratios (NH_4^+:NO_3^-; 100:0, 75:25, 50:50, 25:75 and 0:100) were prepared by modifying Hoagland solution to provide a total N concentration of 7.5 mM at pH 5.7. Saplings were later transferred into 8-L containers. Experimental units were arranged in completely randomized design with five replications. Continuous aeration was provided and nutrient solutions were changed weekly. Shoot growth rate, shoot height, internodal length, number to total leaves, leaf area, fresh and dry weight of shoot and leaves, whole plant weight, tissue nutrient concentrations, and the length of the root elongation zone were measured. Water uptake, stomatal conductance (g_{sw}), fluorescence of chlorophyll *a* in photosystem II (PS II) and leaf water potential were measured on weekly basis.

APRICOT: A follow-up experiment was conducted on apricots using the same five ratios of NH_4^+:NO_3^- (100:0, 75:25, 50:50, 25:75 and 0:100) with the nutrient solution maintained at pH 5.8 or 6.8.

RESULTS

All the growth parameters of almond were significantly influenced by the NH_4^+:NO_3^- ratio. Saplings supplied with 100% NO_3^--nitrogen (0:100) had significantly more biomass production, higher shoot, internodal length, leaf and shoot fresh and dry weight, number of leaves and leaf area (Table 1). However, there was no significant difference in most of these parameters among the remaining treatments (25:75, 50:50, 75:25 and 100:0).

Table.1. Growth parameters of almond saplings in response to NH_4:NO_3 nutrition

$NH_4^+:NO_3^-$	SH (cm)	IL (cm)	WWC (g)	TSFW (g)	TLFW (g)	TSDW (g)	TLDW (g)	TNL#	TLA (cm^2)	REZ (cm)
0:100	28.75[a]	1.46[a]	6.41[a]	4.05[a]	14.81[a]	1.08[a]	3.76[a]	22.10[a]	846.33[a]	11.40[a]
25:75	7.40[b]	0.77[b]	-8.06[b]	1.23[b]	4.80[b]	0.34[b]	1.32[b]	12.70[b]	146.98[b]	3.90[b]
50:50	7.31[b]	0.72[b]	-3.75[b]	0.89[b]	3.45[b]	0.26[b]	0.92[b]	11.70[b]	90.04[b]	1.84[c]
75:25	7.82[b]	0.79[b]	-3.69[b]	1.08[b]	6.45[b]	0.30[b]	1.68[b]	12.10[b]	247.16[b]	1.54[c]
100:0	6.11[b]	0.69[b]	-6.78[b]	1.21[b]	3.92[b]	0.34[b]	1.08[b]	11.60[b]	121.63[b]	1.42[c]
Significance	<0.0001	<0.0001	<0.0001	0.0017	0.0003	0.0005	0.0006	0.0002	<0.0001	<0.0001

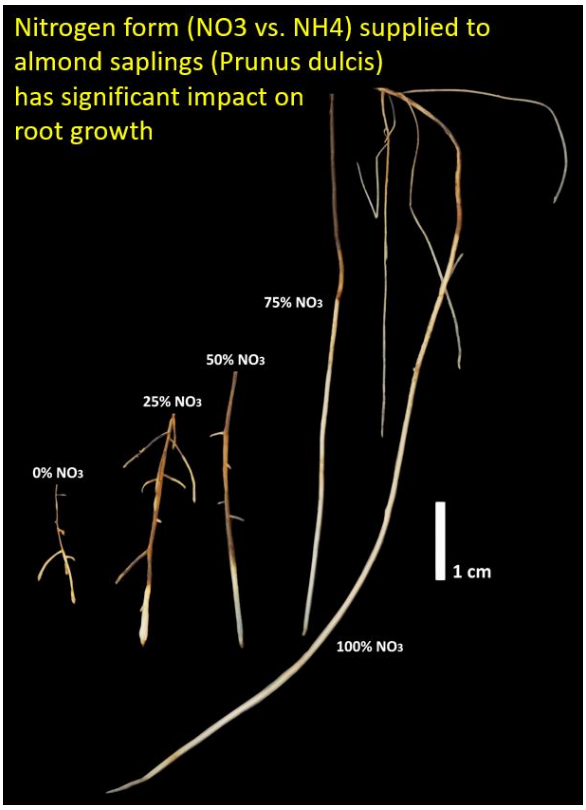
Note: SH= Shoot Height, IL=Internodal length, WWC= Weight change in a week and (-) denotes weight loss, TSFW=Tissue shoots fresh weight, TLFW=Tissue leaf fresh wight, TSDW=Tissue shoots dry weight, TLDW= Tissue leaf dry weight, TNL= Total number of leaves above 5 cm mark, TLA= Tissue leaf area, REZ=Root Elongation Zone Length. Different superscript letters denote statistically significant differences ($p<0.05$) between treatments by Tukey HSD.

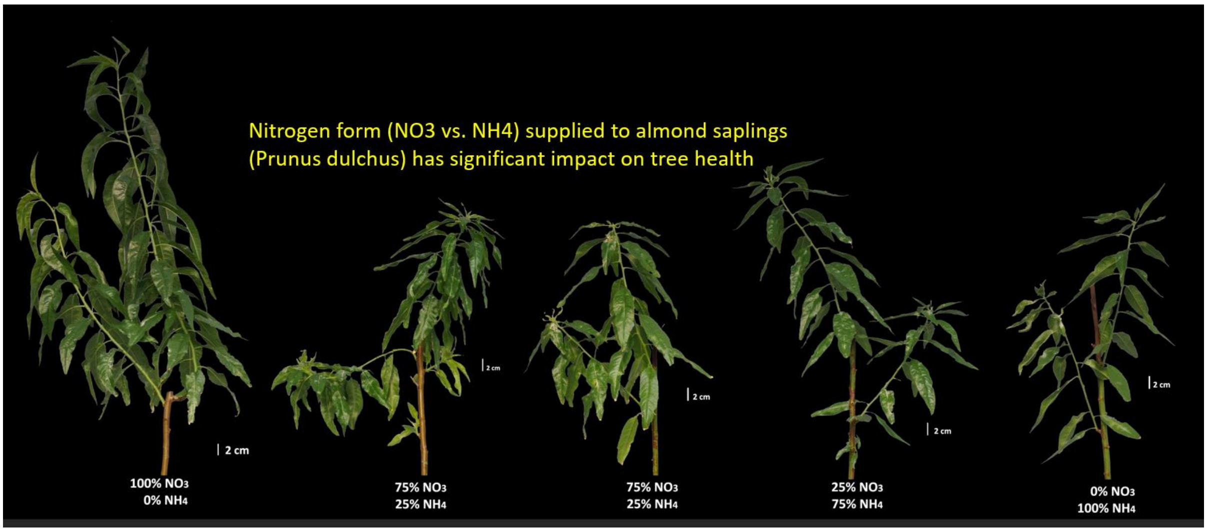
Similar to the almond response, apricots supplied with 0% NH_4^+:100% NO_3^- had significantly higher whole plant biomass, shoot height, dry weight (shoot, leaf, and root), root length, root elongation zone length, water uptake, and stomatal conductance, irrespective of solution pH.

CONCLUSIONS

The growth and physiological response of both almonds and apricots was significantly improved by nitrate-only nutrition. This large positive response needs further investigation in field situations to document the potential yield and tree health benefits derived from nitrate nutrition.

REFERENCE Wolff et al., 2017 Effects of drip fertigation frequency and N-source on soil N2O production in almonds. http://dx.doi.org/10.1016/j.agee.2016.08.001


Nitrogen form (NO3 vs. NH4) supplied to almond saplings (Prunus dulcis) has significant impact on root growth
75% NO3
50% NO3
25% NO3
0% NO3
1 cm
100% NO3


Nitrogen form (NO3 vs. NH4) supplied to almond saplings (Prunus dulchus) has significant impact on tree health
2 cm
100% NO3
0% NH4
75% NO3
25% NH4
75% NO3
25% NH4
25% NO3
75% NH4
0% NO3
100% NH4

FOLIAR NITROGEN FERTILIZATION AS A COMPLEMENT TO SOIL-APPLIED NITROGEN: A TOOL TO IMPROVE THE SUSTAINABILITY OF SUGARCANE

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Keywords: Nitrogen fertilizer recovery by sugarcane leaves; Nitrogen redistribution; Sugarcane yield

INTRODUCTION

The world population and the concentration of greenhouse gases in the atmosphere are increasing; so, crops such as sugarcane, which have dual-purpose and can be used both for food (e.g., sugar) and renewable fuel (e.g., ethanol) production, have become important worldwide (Formann et al., 2020). Nitrogen (N) fertilization is important in sugarcane since N is the second most mineral essential element required by the plant. N-fertilizer is commonly applied to the soil, showing low N-fertilizer recovery by the plant (~26%), remaining in the soil, where nitrogen can be lost to the environment causing and intensifying environmental problems, respectively, the eutrophication of water bodies and the emission of greenhouse gases (i.e., nitrous oxide) (Otto et al., 2016). Foliar N fertilization is an agricultural practice that can bring benefits to sustainability in sugarcane agrosystems, which should be able to provide a reduction in the N-fertilizer rate applied to the soil by synchronizing the period of greatest plant requirement for N with the N-fertilizer application. To prove this hypothesis, studies were carried out in greenhouse conditions and in the field to evaluate the N-fertilizer recovery by the sugarcane leaves (NRP), the sugarcane biomass production, and the agronomic efficiency.

METHODS

Sugarcane variety RB855156 was used in both experiments because it is responsive to N fertilization and it is harvested at the beginning of the sugarcane harvest season in southeastern Brazil, the main sugarcane production region (Castro et al., 2019).

Greenhouse experiment

The pre-sprouted sugarcane seedlings were transplanted into pots with 15 kg of soil, followed by soil nutrients fertilization to prevent plant nutritional deficiency. The irrigation of the experimental units was performed daily. 95 days after sugarcane seedlings transplanting, foliar N fertilization was carried out using ^{15}N-urea (solutions with 8, 16, 24, and 32 % of N, plus a control treatment, without foliar N fertilization), depositing 1 µL drops on the three youngest fully expanded leaves (totaling 875 µL of N-solution per plant). Plants were evaluated at 0.5, 1.5, 5, and 20 days after foliar N fertilization (DAFNf) to assess the rate of uptake and redistribution of ^{15}N-urea. The design used was randomized blocks with four replications.

Field experiment

The experiment was carried out in a commercial sugarcane area at Sales Oliveira, São Paulo State, Brazil across three following sugarcane growing seasons (2nd, 3rd, and 4th sugarcane ratoon). The experimental design was randomized block, with four replications and fifteen treatments, as follows: 0 + 0, 40 + 0, 60 + 0, 80 + 0, 120 + 0, 160 + 0, 0 + 12, 0 + 24, 0 + 36, 40 + 12, 40 + 24, 40 + 36, 60 + 12, 60 + 24, and 60 + 36 kg ha^{-1} N applied, respectively, to the soil (ammonium nitrate), and to the leaves (urea). The volume of N-urea solution sprayed was 60 L ha^{-1} with 20 % N. N-urea rates greater than 12 kg ha^{-1} N were split into 2 or 3 application times, spaced, approximately, 1 month apart. The plots had 8 rows of sugarcane spaced at 1.5 m and 15 m in length. Stalk productivity, in the three years, and the N-urea recovery by the plant, in the 2nd year, were evaluated in the central area of each plot.

RESULTS AND DISCUSSION

Greenhouse condition experiment

The highest NRP was obtained at 5 DAFNf; N-concentration in solution down-regulated NRP (69, 61, 50, and 58 % NRP, respectively, in treatments 8, 16, 24, and 32 % N). The main sink of N-urea was the stalk, followed by the not expanded leaves (NEL), and the roots.

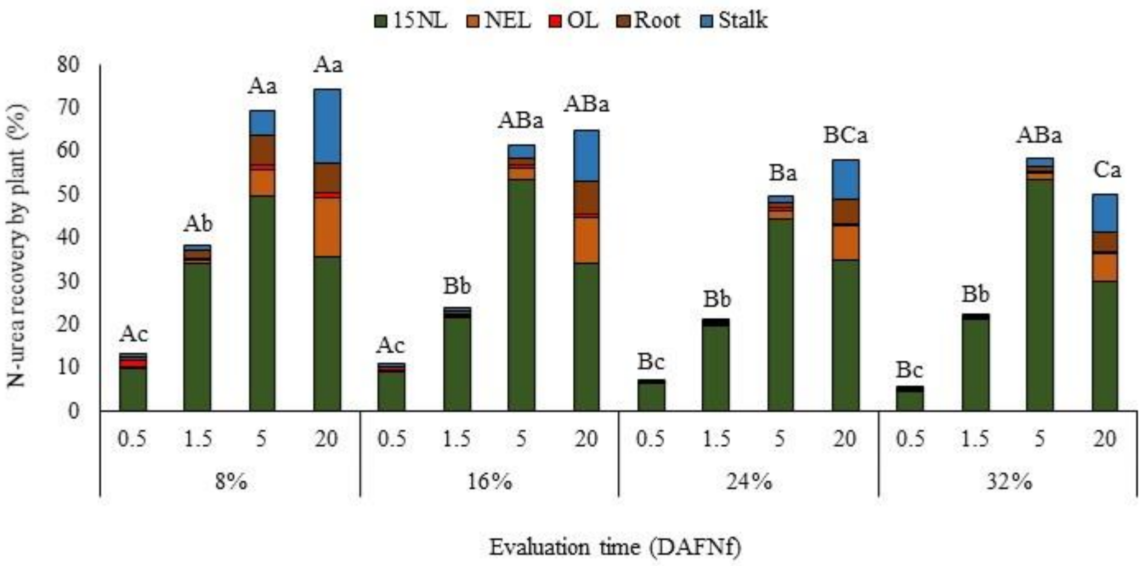

Fig. 1. N-urea recovery by each plant tissue sampled at 0.5, 1.5, 5, and 20 DAFNf with increasing N-concentration viz 8, 16, 24, and 32 % N. Small and capital letters indicate the difference between, respectively, evaluation time at each treatment and treatments at each evaluation time, according to LSD's post-hoc test ($p < 0.05$).

Field experiment

Among the highest stalk yields obtained, summing the three years, are the treatments 120 + 0 kg ha^{-1} N (383 Mg ha^{-1} stalk) and 40 + 36 kg ha^{-1} N (328 Mg ha^{-1} stalk), considered statistically equal. All the treatments in which N-fertilizer was applied to the soil presented positive agronomic efficiency in the 3rd and 4th sugarcane ratoon, showing that the site is responsive to N fertilization. The N-urea recovery by plant did not differ between treatments, with an average of 44 % NRP.

CONCLUSIONS

The foliar N fertilization increases the N-fertilizer recovery by sugarcane; foliar N fertilization as a complement to the soil N fertilization reduces the N-fertilizer rate, maintaining sugarcane stalk yield throughout subsequent growing seasons.

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USING NANOBUBBLES IN AGRICULTURE - WHAT DO WE KNOW?

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Keywords: Nutrients; Oxygen; Uptake

Aeration of irrigation water with nanobubbles (NB-water; <1 µm in diameter) emerges as a new promising method to improve soil aeration. Nanobubbles have unique physical properties, which include long-term stability in water, high gas transfer efficiency, and a negatively charged surface at the liquid-air interface.

In recent years, irrigation with NB-water (i.e., air or oxygen NB) has emerged as a new method to alleviate hypoxic conditions in the rhizosphere. In our studies, we aimed to investigate the effects of hydroponics and drip irrigation with oxygen NB (ONB) aerated waters of different qualities [i.e., fresh (0.4 dS/m), secondary TWW[(a)] (1.3 dS/m), saline (3 dS/m)] on (a) oxygen availability in the root zone and its mass balance (i.e., amount of oxygen applied and consumed), (b) plant resilience and yield, (c) nutrient uptake, and (d) greenhouse gas emissions.

The introduction of ONB into the irrigation waters managed to substantially increase the dissolved oxygen (DO) concentrations from ~6 mg/L to >30 mg/L. In all tested soils (sandy to clayey), irrigation with ONB water significantly increased the oxygen concentration in the soil air (p<0.05), yet the increase or decrease did exceed ±1% of the absolute oxygen concentration. Overall, ONB contributed between 1% - 5% of the oxygen consumed in the rhizosphere. Nevertheless, drip irrigation with NB-water positively impacted the growth rate, the chlorophyll and carotenoid concentrations in the leaves of lettuce (*Lactuca sativa*), and reduced the membrane leakage and the osmotic potential. Furthermore, irrigation with ONB water improved the lettuce's recovery rate from heat stress, especially when irrigated with water of poor quality. Drip irrigation with ONB-water was also accompanied by reduced emissions of nitrous oxide (N_2O).

Aeration of water with ONB water in nutrient film technique (NFT) hydroponic systems significantly increased the yields of Coriander (*C. sativum*), arugula (*Eruca sativa*), and lettuce by 15 - 30%, and impacted nutrient uptake.

All of our findings suggest that the long-term stability and high gas transfer of ONB in water may be utilized in on-surface and subsurface drip irrigation to improve soil aeration. Further study is needed to understand the direct effects of ONB water on plants.

ROOT SHALLOWNESS ENHANCES PHOSPHORUS ACQUISITION IN SUGARCANE

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Keywords: sugarcane; root shallowness; phosphorus acquisition

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important sugar and biofuel feedstock crop that provides 80% of the world's sugar and 40% of its ethanol, and mainly grows in tropical and subtropical acidic soil regions. The availability of phosphorus (P) profoundly affects plant metabolism and growth, while the concentration of available P is far from sufficient to meet the growth needs of plant in most cultivated land especially in acid soil, making P deficiency acts one of the primary limitations of high yield of sugarcane. Change of the root system architecture (RSA) is a vital mechanism for crop low P adaption, while the RSA of sugarcane has been little-studied because of its complex root system. In this study, the response of RSA under low P stress and its substantial effect on topsoil foraging, P acquisition, and biomass was investigated using three sugarcane genotypes.

METHODS

The change of the root length density (RLD) and its relationship with P acquisition were investigated in a P-efficient sugarcane genotype ROC22 (R22) and two P-inefficient genotypes Yunzhe 03-103 (YZ) and Japan 2 (JP) under low P (LP, 5 mg kg^{-1} P_2O_5) and normal P (NP, 200 mg kg^{-1} P_2O_5) conditions. The sugarcanes were planted in a root observation room and the planting troughs were filled with the severely P-deficient soil. The root length and section area of each section was analyzed with WinRHIZO and the RLD of each section was calculated from (root length/section area). The shoot and cane were collected and weighed after washing with deionized water, drying at 105 °C for 1 h and then at 65 °C for 3 days. The shoot samples were digested with 30% H_2O_2 and 98% H_2SO_4 after crushing and sieving. The P concentration in the digested solution was measured measured with the molybdenum blue colorimetry method. The P accumulation was calculated from shoot biomass and P concentration. The relative coefficient was defined as % of a certain trait in the LP condition relative to the NP condtion.

RESULTS AND DISCUSSION

The sugarcane RSA was changed under P deficiency, R22 had an earlier response than YZ and JP and presented an obvious feature of root shallowness. Compared with the normal P condition, the shallow RLD was increased by 112% in R22 under P deficiency (Fig. C) while decreased by 26% in YZ (Fig. 1A), and not modified in JP (Fig. 1B). Meanwhile, R22 exhibited a shallower root distribution than YZ and JP under P deficiency and the ratio of shallow RLD to total RLD in R22 was greatest in three genotypes (Fig. 1F). As a result, R22 had a 17% and 16% greater relative shoot P concentration (Fig. 2A), a 47% and 56% higher relative shoot P accumulation than YZ and JP (Fig. 2B), which thereby increased the relative shoot biomass by 36% and 33% (Fig. 2C), and the relative cane weight by 31% and 36% (Fig. 2D), compared with YZ and JP under P deficiency, respectively. Further, the relative shoot P concentration(Fig. 2E), P accumulation(Fig. 2F), shoot biomass(Fig. 2G) and cane weight (Fig. 2H) were positively correlated with the relative shallow RLD. The findings demonstrate that root shallowness facilitates P acquisition and plant growth in sugarcane.

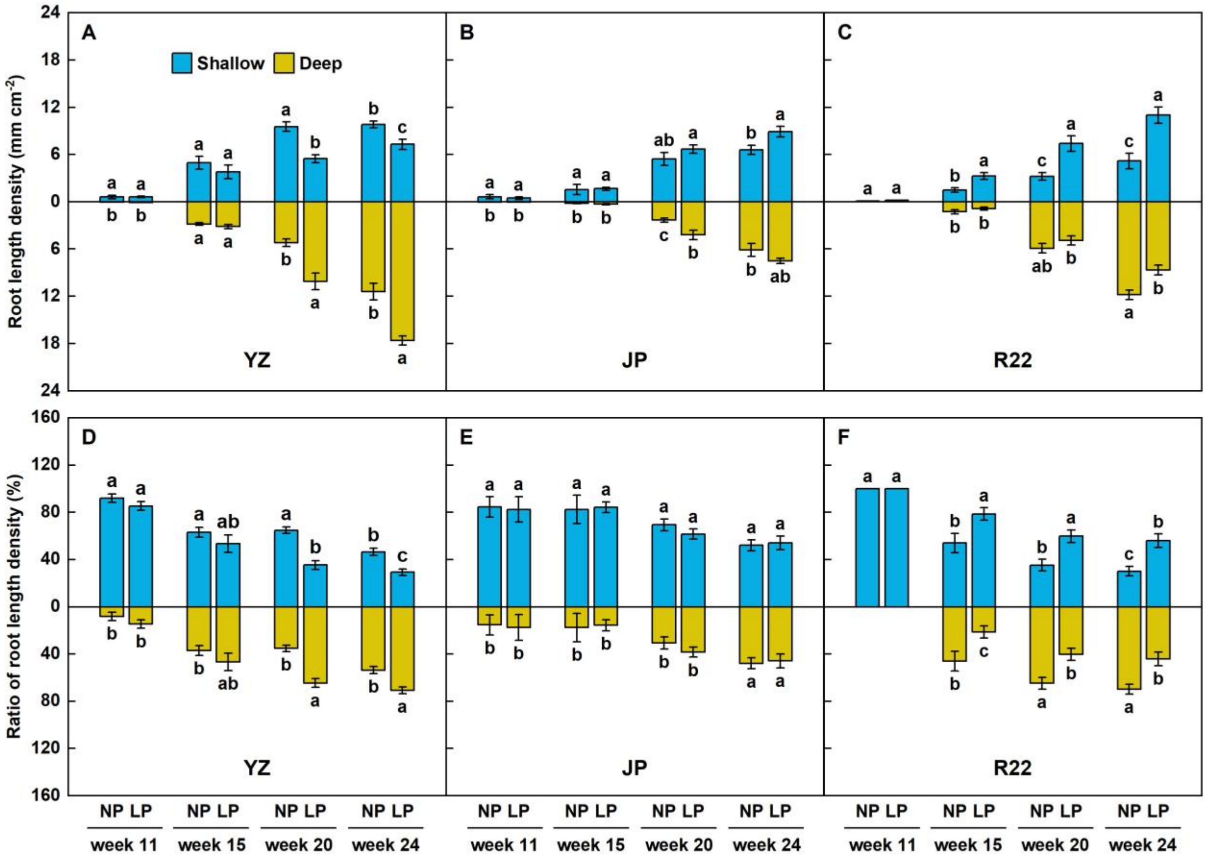

Fig. 1. Dynamic changes of shallow and deep RLD under P deficiency in three sugarcane genotypes.

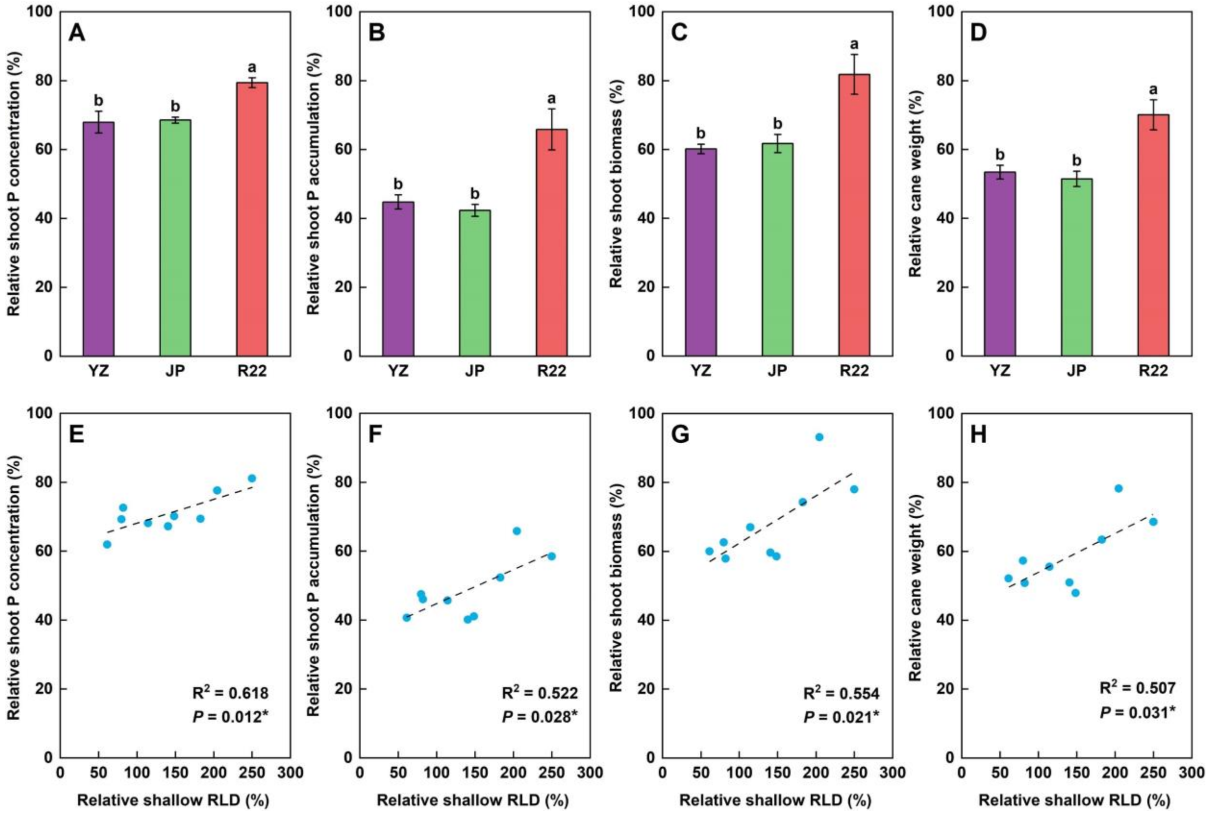

Fig. 2. The relative coefficient and correlation analysis of P acquisition and shoot growth.

CONCLUSIONS

The root shallowness was contributed to improving P acquisition and low P adaption under P deficiency in sugarcane and the shallow root distribution merits consideration as an evaluation trait for breeding P efficient sugarcane genotypes and genetic improvement.

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Financial Support

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HORMONAL REGULATION OF ROOT NITROGEN FORAGING

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Keywords: nitrogen deficiency; root system architecture; natural variation

INTRODUCTION

Plant root system architecture (RSA) responds to a limiting dose of nitrogen (N) in a dual manner (Gruber et al., 2013). While regulatory modules and hormones involve in arresting root elongation and branching under severe N deficiency (Jia et al., 2020), little is known about the regulation of RSA responses to mild N deficiency. Mild N deficiency expresses in an elongation of the primary and lateral roots, mediating a systemic foraging response and allowing the overall root system to explore a larger soil volume. This foraging response is of particular interest for N uptake efficiency and thus for more efficient fertilizer use, as it promises to decrease future N fertilization doses by maintaining yield. A presumption to integrate such knowledge in plant breeding builds on knowledge of the molecular mechanisms underlying the N foraging response.

METHODS

Aiming at identifying genetic comopnents that regulate root elongation under low N availability, we initiated a large-scale root phenotyping of 200 Arabidopsis natural accessions grown under high N (HN, 11.4 mM) or low N (LN, 0.55 mM) for 9 days, after a 7-day preculture on high N. We then scanned roots into images by Epson Expression 10000XL scanner (Seiko Epson) and performed image analysis with WinRhizo Pro version 2009c (Regent Instruments) to acquire root traits that were implemented to genome-wide association study (GWAS) to identify marker-trait associations. The uncovered significant associations were employed to prioritize candidate genes that were investigated with reverse genetics, molecular and cell biology approaches.

RESULTS and discussion

Substantial genetic variation was observed for RSA traits and their responses to low N. Notably, N deficiency induced root elongation is conserved across 200 lines and correlates significantly with shoot N accumulation specificly at low N (Fig. 1A-C), corroborating with the view that a more expanded root system can increase N uptake and help plants to better adapt to this environmental constraint. GWAS identified brassinosteroid (BR) signaling gene *BSK3* and auxin biosynthesis gene *YUC8* regulating N deficiency induced primary and lateral root elongation under low N (Fig. 1D-K). Allelic coding variants of BSK3 and YUC8 in natural accessions of *A. thaliana* determine the extent of the root foraging response to low N by differentially modulating cell elongation.

CONCLUSIONS

Low external N availability that results in mild N deficiency induces the expression of the BR co-receptor *BAK1* and several genes involved in BR biosynthesis. The activated BR signaling then activates auxin biosynthesis module composed of *TAA1* and *YUC8* together with its homologs *YUC5* and *YUC7* to generate more IAA in the apical meristem of root tips (blue area in root tip). Upon transport to the elongation zone (blue arrows), locally generated IAA enhances cell expansion (Fig. 1L).

ACKNOWLEDGEMENTS

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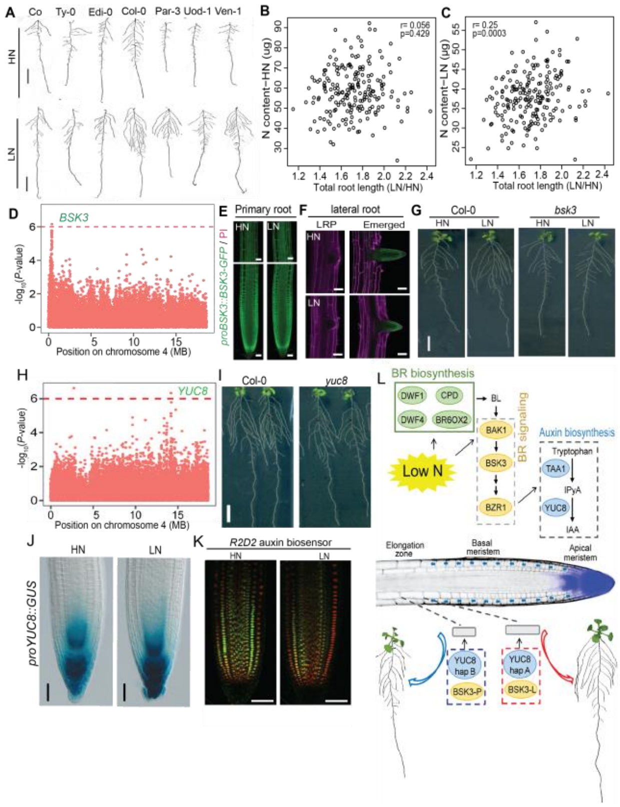

Fig. 1. A root BR-auxin hormonal regulatory module determines root foraging response to mild N deficiency. A, Representative accessions of *A. thaliana* that show weak (Co, Ty-0, Edi-0), intermediate (Col-0), and strong (Par-3, Uod-1, Ven-1) response to low N availability; B and C, Correlations of total root length (i.e. LN-to-HN ratio) with shoot N content at HN (B) and LN (C); D, Genome-wide *P* values of chromosome 4 from the GWAS for primary root length under LN; E and F, BSK3 expression pattern and protein localization in response to N availability in primary root (E) and non-emerged or emerged lateral root (F), LRP, lateral root primordia; G, Root phenotype of *bsk3* knock out mutant; H, Genome-wide *P* values of chromosome 4 from the GWAS for LN-to-HN ratio of lateral root; I, Root phenotype of *yuc8* knock out mutant under LN; J, *proYUC8-GUS* activity in the tips of lateral root at HN or LN; K, Auxin biosensor *R2D2* expression in the tips of lateral root at HN or LN; L, a working model of BR-auxin module in root foraging response to low N.

X-RAY FLUORESCENCE SPECTROMETRY: A VERSATILE TOOL TO ASSESS THE NUTRITIONAL STATUS & NUTRIENT DYNAMICS IN PLANTS

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Keywords: X-ray fluorescence spectrometry; nutrient dynamics; mechanisms of uptake & transport

INTRODUCTION

The understanding of nutrient uptake, translocation, and remobilization processes is a basic premise to support the development of new fertilizers and increase of the mineral content in crops. Since one cannot adequately manage what is not properly measured, our group has been developing X-ray fluorescence spectrometry-based methods (XRF) to determine the nutritional status of crops and trace the uptake & transport of nutrients in plants. The XRF technique relies on the excitation of atoms under X-rays irradiation, leading to the emission of energy-characteristic X-ray photons that identifies the emitting atom and reveals its concentration. Herein, we demonstrate some contributions of XRF to plant nutrition. The main constraints, technical challenges, and expected advances in the foreseeable future are also discussed.

METHODS

The variants of XRF can be grouped into "bulk" and "imaging & space-resolved" strategies. The bulk analysis aims at determining the average elemental concentration of either powdered or intact plant samples, including detached fresh leaves. It also enables real-time monitoring of the elemental transport through the petiole and stems tissues of living plants. These measurements are usually performed using 1-3 mm wide X-ray beams. On the other hand, imaging and spaced-resolved strategies comprise 2D or 3D mapping and linear scannings, usually employing X-ray beams spanning from a few mm down to submicron size ranges, which requires a precise sample positioning system.

Figure 1 presents some experimental setups used for both strategies, including lab-made customized equipment (Fig.1A), commercial handheld (Fig.1B), and benchtop micro-XRF systems (Fig.1C), as well as beamlines in large synchrotron facilities (Fig.1D). Regardless of the type, all XRF equipment share three major features: an X-ray source for excitation, an X-ray detector to identify and count the X-ray photons, and a sample environment/positioning system.

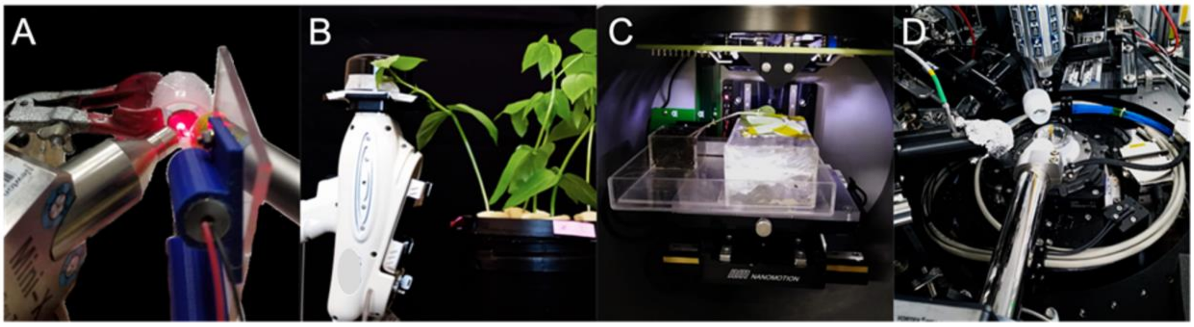

Fig. 1. X-ray fluorescence setups and geometries in laboratory and synchrotron beamline.

RESULTS AND DISCUSSION

Since X-rays are highly penetrating into the matter, under controlled doses, it can be employed as a tool to monitor plant behavior under *vivo* conditions avoiding radiation damage. Fig. 2 shows some representative data collected by lab XRF devices. Fig. 2A shows a 2D map revealing the spatial distribution of a Zn, Mn, and Fe in the embryo axis of a soybean (*Glycine max*) seed. One can notice that Mn & Fe present a double strip-like projection along the axis, anatomical investigations using visible light microscopy revelaed that this region corresponds to provascular tissues. The embryo cross sections taken 0.5 mm from the radicle tip show that Fe

& Mn displine inner and outer poles in a ring-like shape. We are currently investigating why these elements are more concentraed in such cells and if they can triger the tissue transformation during germination. Fig.2B shows the elemental content in leaves of kidney bean (*Phaseolus vulgaris*) whose growth solution was spiked with an $Mn^{2+}_{(aq)}$, $Zn^{2+}_{(aq)}$, and $Cu^{2+}_{(aq)}$ solution at the instant zero of the graph. One can observe that the counting rate for several elements in the leaf surface starts to increase less than 10 min after the spiking, whereas the uptake regime slows down near to 30 min. Furthermore, Fig.2C shows that the absorption of $CaCl_{2(aq)}$ by the leaves tomato (*Solanum lycopersicum*) follows exponential decay behavior, requiring *ca*. 24 ± 5 h to incorporate 50% of the treatment. Our studies show that the type of nutrient source, additives, air relative humidity, leaf surface attributes, and plant species stands out as the main factors controlling the nutrient uptake in plants. Finally, Fig. 2D shows the reference and predicted concentrations of K and Ca in soybean leaves recorded by portable XRF equipment in field conditions. Several strategies for transforming XRF signal into element concentration have been tested, one could highlight, for example, the multiple linear regression and partial least squares. The method has also been successfully employed for maize (*Zea mays*) and coffee (*Coffea arabica*) crops.

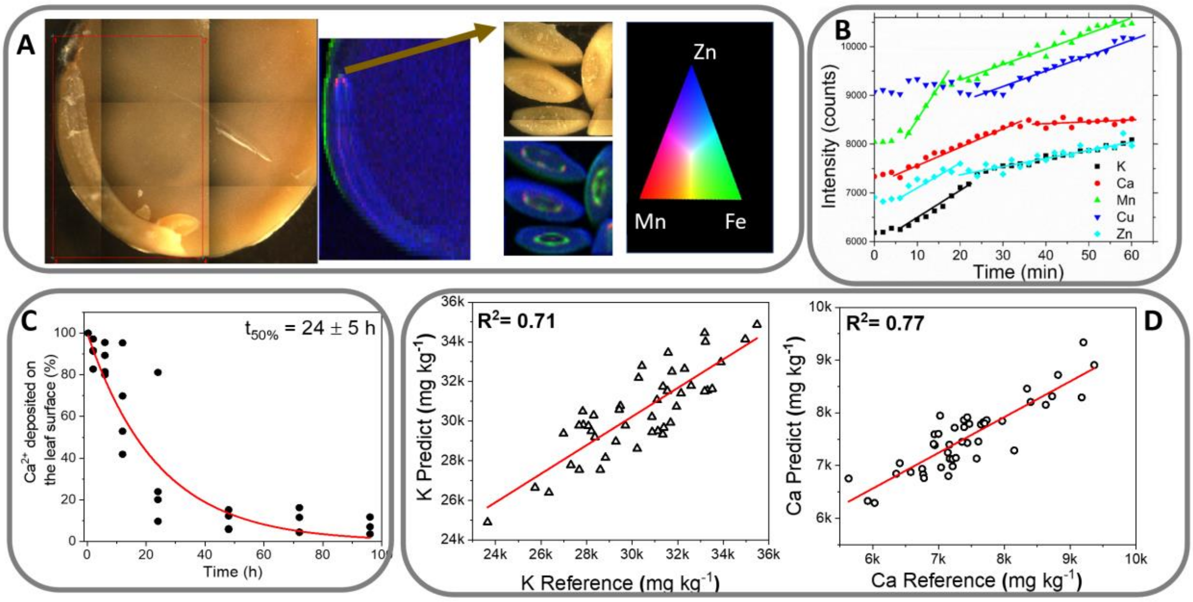

Fig. 2. Some experiments applying X-ray spectrometry to investigate plant nutrition at the Center for Nuclear Energy in Agriculture, University of São Paulo.

CONCLUSIONS

X-ray fluorescence spectrometry can unveil the average composition of elements, monitor dynamic phenomena in real-time, and depict the spatial distribution of elements in plant tissues. Compared to other techniques, such as radioisotope tracers, XRF is easier to handle and not restricted to laboratory conditions. Although XRF exhibits higher limits of detection than radioactive tracers, this drawback has been becoming less critical as larger area detectors and fast electronics have been deployed. As a future trend, we highlight the XRF can be coupled to other techniques such VIS-NIR & IRGA allowing the simultaneous detection of distinct plant responses.

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Financial Support

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SUBSTITUTION OF POTASSIUM CHLORIDE BY GRANULATED POLYHALITE FOR CROP GRAIN PRODUCTION UNDER A NO-TILL SYSTEM

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Keywords: potash; fertilizer; multi-nutrient

INTRODUCTION

No-till systems with diversified crop rotation have stood out as one of the most effective strategies to improve agricultural sustainability in tropical and subtropical regions. Brazil is a leading country regarding the adoption of the no-till system, which is used in more than 90% of the cash crop area in southern Brazil. In this region, the main cash crops grown under no-till are soybean, maize, and winter cereals (mainly wheat).

Fertilizer deliveries to the agricultural market in Brazil increased from 24.5 million tons in 2010 to 35.5 million tons in 2018 (FAO, 2021). The consumption of potassium (K) via fertilizers in Brazilian agriculture increased from 3.4 million tons in 2010 to 5.6 million tons in 2018. This means that high amounts of K fertilizers have been necessary to ensure increases in Brazilian agricultural yield.

Polyhalite ($K_2SO_4.MgSO_4.2CaSO_4.2H_2O$) is a natural alternative source of K that also contains calcium (Ca) and magnesium (Mg) in the form of sulfates in its composition. Because Brazilian soils have limited availability of K, serious problems of sulfur (S) nutritional disorders, and low levels of exchangeable Ca and Mg, polyhalite may provide several alternative solutions to solve some chronic nutrition problems associated with Brazilian agriculture. This study reports a field trial that examined the effects of substituting applications of K as potassium chloride (KCl) by granulated polyhalite on nutrition and grain yields of wheat, soybean, and maize under a no-till system.

METHODS

A field experiment was carried out in Ponta Grossa, Parana State, Brazil, on an Oxisol under a no-till system. The climate at the site is categorized as mesothermal, humid, subtropical. The average altitude is 970 m and the annual precipitation is about 1550 mm. Topsoil (0-0.2 m) had $pHCaCl_2$ 4.4; exchangeable Ca, Mg, and K contents of 17, 7, and 2.2 $mmol_c\ dm^{-3}$, respectively; P (Mehlich-1) 26.3 mg $dm^{-}3$, and SO_4-S (0.01 mol L^{-1} calcium phosphate) 6.8 mg dm^{-3}, and 205 g kg^{-1} of clay. Dolomitic lime was applied to the soil surface on June, 2018 to raise the base saturation in the toposoil to 70%.

A randomized complete block design was used, with four replications. Plot size was 8 by 6 m. Treatments consisted of various K-percent combinations using granulated polyhalite to substitute KCl at 0, 25%, 50%, 75%, and 100% in wheat, soybean, and maize crops. The crop sequence was: wheat, soybean, black oat, and maize. Black oat was grown as a cover plant without any fertilizer. Applications of K were performed on the soil surface just before sowing wheat, soybean, and maize at rates of 50 kg K ha^{-1} for wheat and 100 kg K ha^{-1} for soybean and maize. Fertilizers were applied as mono-ammonium phosphate (MAP) in the sowing and urea-N in top dressing to wheat and maize crops.

Wheat, soybean, and maize leaf samples included 30 leaves per plot that were collected during the flowering period of the crops. In wheat, the flag leaf was taken; in soybean, the third trefoil from the apex of the plants; and in maize, the leaf below and opposite to ear. Leaf concentrations of N, P, K, Ca, Mg, and S were determined. Wheat and soybean were harvested from a 10.8 m^2 and maize from a 7.2 m^2 area on each plot. Grain yield was expressed at 130 g kg^{-1} moisture. Data were analyzed using ANOVA and regression analysis. When the effect was significant at $P < 0.05$, the magnitude of the determination coefficients was used as a criterion for choosing the model.

RESULTS AND DISCUSSION

Increasing the K-percent as polyhalite in substitution for KCl increased leaf concentrations of N, Mg, and S of wheat, the Ca-leaf concentration of soybean, and leaf concentrations of N, Ca, Mg, and S of maize (Table 1). There was no change in K-leaf concentration of wheat, soybean, and maize. Thus, polyhalite and KCl were equally efficient in supplying K to the crops. Wheat and soybean grain yields were not changed by treatments. Increasing the K-percent as polyhalite in substitution for KCl linearly increased maize grain yield by up to 15.5%. The increase in maize yield could have been caused by the greater availability and uptake of Ca, Mg, and S by the plants due to use of polyhalite. The increase in N-leaf concentration of maize was probably a result of a higher S-leaf concentration with the use of polyhalite. A similar effect on leaf N and S concentrations of wheat was also observed, although wheat grain yield was not significantly changed with treatments. Since polyhalite contains S, the interaction between N and S may influence plant N uptake (Salvagiotti et al., 2009).

Table 1. Regression equations between leaf nutrient concentrations and grain yields of wheat, soybean, and maize ($\hat{y}$) and K applications at various percent combinations using granulated polyhalite in substitution for KCl (x, in % K as polyhalite) under a no-till system.

$\hat{y}$

Wheat

Soybean

Maize

Equation

$R^{2?}$

Equation

$R^{2?}$

Equation

$R^{2?}$

N (g kg^{-1})

$\hat{Y} = 37.49 + 0.288x - 0.0023x^2$

0.87*

$\hat{y}$ = ? = 49.8

-

$\hat{y} = 32.95 + 0.048x$

0.85*

P (g kg^{-1})

$\hat{y}$ = ? = 6.2

-

$\hat{y}$ = ? = 7.4

-

$\hat{y}$ = ? = 7.8

-

K (g kg^{-1})

$\hat{y}$ = ? = 33.3

-

$\hat{y}$ = ? = 28.2

-

$\hat{y}$ = ? = 31.8

-

Ca (g kg^{-1})

$\hat{y}$ = ? = 4.8

-

$\hat{y} = 7.22 + 0.006x$

0.90*

$\hat{y} = 3.28 + 0.009x$

0.90**

Mg (g kg^{-1})

$\hat{y} = 1.63 + 0.003x$

0.78**

$\hat{y}$ = ? = 3.1

-

$\hat{y} = 1.88 + 0.005x$

0.91**

S (g kg^{-1})

$\hat{y} = 2.35 + 0.010x$

0.94**

$\hat{y}$ = ? = 2.4

-

$\hat{y} = 1.62 + 0.011x - 0.00009x^2$

0.90*

Grain yield (kg ha$^{-1)}$

$\hat{y}$ = ? = 3551

-

$\hat{y}$ = ? = 3695

-

$\hat{y} = 13697.91 + 21.198x$

0.92*

? Coefficient of determination. *P < 0.05 and **P < 0.01.

When considering crop rotation, the cumulative grain yield of the wheat, soybean, and maize crops ($\hat{y}$, in kg ha^{-1}) linearly increased ($P < 0.05$) with increasing K-percent as polyhalite in substitution for KCl (x, in %): $\hat{y} = 20870.56 + 22.65x$ ($R^2 = 0.93$). The maximum cumulative grain yield would be reached with the exclusive application of polyhalite (23138 kg ha^{-1}), resulting in a 11% increase in cumulative grain yield compared to exclusive application of KCl (20871 kg ha^{-1}).

CONCLUSIONS

Polyhalite and KCl were equally efficient in supplying K to wheat, soybean, and maize under no-till.

Potassium fertilization with polyhalite, in combination with KCl or exclusively, improved N, Mg, and S nutrition of wheat and maize, and Ca nutrition of soybean and maize under no-till.

The exclusive application of granulated polyhalite under a no-till system increased maize grain yield by 15.5% and cumulative wheat, soybean, and maize grain yield by 11%.

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SOIL ACIDITY ALLEVIATION AND FATE OF NITROGEN IN A MAIZE-FORAGE GRASS INTERCROPPING

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Keywords: Liming; Gypsum; 15N-fertilizer

INTRODUCTION

Liming alone has not been effective in alleviating subsoil acidity in systems under no-till (Caires et al., 1998). However, gypsum application can raise cation concentration and decrease Al toxicity in the subsoil, favoring deep root growth (Rosolem et al., 1998). Under no-till lime is applied on the soil surface, resulting in an overdose in the first cm of the soil, leading to very high pH, and increasing nitrification. Then, nitrate can be leached deeper into the soil profile. If there are roots in this region, N use efficiency will be enhanced (Rosolem et al., 2017).

Growing maize intercropped with forage as a relay crop after soybean under no-till has been used as an effective conservation system, and the introduction of legumes and/or forage grasses with deep, vigorous roots in the system can prevent nitrate leaching. However, it is not known the fate of the N used in these systems. We hypothesized that soil acidity alleviation with gypsum associated with lime would enhance N use efficiency in a system with maize intercropped with forage grass by increasing root growth in the soil profile. The objective of this work was to determine how lime, lime+gypsum, and N fertilization affect N use and distribution in a cropping system where maize was intercropped with Guinea grass (*Megathyrsus maximus)* and grown as a relay crop after soybean.

METHODS

The experiment was conducted in Botucatu, state of São Paulo, Brazil, 22° 49' 50.94" S, 48° 25' 38.67" W, and 770 m altitude, from 2017 to 2020, in a clay Typic Hapludox. Treatments were soil acidity alleviation with limestone, limestone + gypsum, and a control (no application of correctives), in the presence or absence of 160 kg ha^{-1} of N (^{15}N enriched ammonium sulfate), applied to maize. Maize was grown intercropped with Guinea grass as a relay crop after soybean in 2017, 2018, and 2019.

RESULTS AND DISCUSSION

Most of the applied ^{15}N was found in the superficial soil layer (0-10 cm). The N derived from fertilizer in the soil profile was not affected by soil acidity alleviation (Fig 1). In the first year, approximately 50% of the fertilizer applied was found in the forage grass, on average, with the application of lime and lime + gypsum. In the third year, soil acidity alleviation resulted in a 43% greater accumulation of N by maize, on average, than in the control.

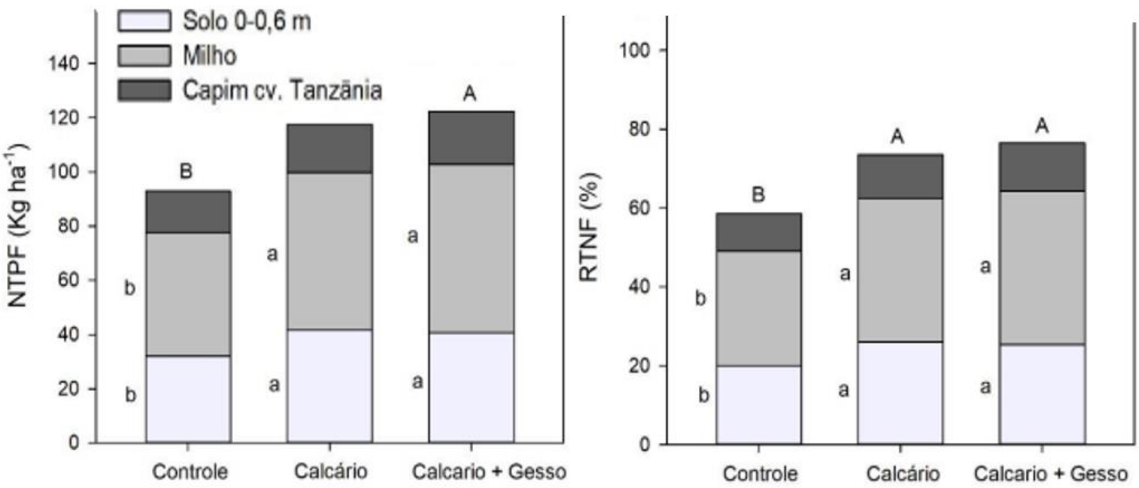

Fig 1. Nitrogen derived from fertilizer (NDFF; a) and ^{15}N-recovery (b) in the soil, maize, and forage grass affected by lime, lime + gypsum, and a control (no correctives applied).

The percentage of N recovery in maize + forage shows that the alleviation of soil acidity improved the efficiency of N uptake, which showed a higher percentage of recovery when lime and or lime + gypsum was applied, accumulating, on average, about 42% more N from the fertilizer than in the control treatment. According to Rosolem et al. (2017), roots play a crucial role in N acquisition, and it has been reported that gypsum application improves root growth in the subsoil, which can explain the increased N use efficiency observed in this work with lime and gypsum.

CONCLUSIONS

The results of this study provide important information on the effects of soil and subsoil acidity alleviation on the fate of N fertilizer in a no-till system. Correction of soil acidity with lime and gypsum increases soil N, probably by decreasing loss of the fertilizer N, and results in greater uptake of nitrogen from the fertilizer by maize intercropped with a forage grass grown as a relay crop after soybean.

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EXPLORATION OF IRON EXCESS-RESPONSIVE NOVEL CIS-REGULATORY ELEMENTS IN RICE AND SIMULATION OF PROMOTER STRUCTURES BY A BIOINFORMATICS APPROACH

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Keywords: Iron excess; bioinformatics; cis regulatary elements

Exploration of iron excess-responsive novel *cis*-regulatory elements in rice and simulation of promoter structures by bioinformatics approach

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INTRODUCTION

Iron (Fe) excess is a major constraint on crop production in flooded acidic soils, especially in rice cultivation. Under Fe excess, plants activate a complex mechanism and network regulating Fe exclusion by roots and isolation in various tissues (Aung and Masuda 2020). *Cis*-regulatory elements (CREs) are important for the precise control of gene expression. In rice, the transcription factors and CREsthat regulate Fe excess response mechanisms remain largely unknown. We previously reported comprehensive microarray analyses of several rice tissues in response to various levels of Fe excess stress (Aung *et al*. 2018). In this study, we further explored novel CREs and promoter structures in rice using bioinformatics approaches using this microarray data.

METHODS

We first performed network analyses to predict Fe excess-related CREs through the categorization of the gene expression patterns of Fe excess-responsive transcriptional regulons, and found four major expression clusters: Fe storage type, Fe chelator type, Fe uptake type, and WRKY and other co-expression type. Next, we explored CREs within these four clusters of gene expression types using a machine-learning method called microarray-associated motif analyzer (MAMA), which we previously established. The promoter region of -500 to +150 relative to Transcription Start Site (TSS) were used for searching CREs.

RESULTS and discussion

Through a comprehensive bioinformatics approach, we identified a total of 560 CRE candidates extracted by MAMA analyses and 42 important conserved sequences of CREs directly related to the Fe excess response in various rice tissues. We explored several novel cis-elements as candidate Fe excess CREs including GCWGCWGC, CGACACGC, and Myb bindinglike motifs and new roles of known CREs in the Fe excess response (**Table 1**). Novel CRE candidates exist significantly around TSS of Fe excess-reponsive genes and thought to involve gene expression regulation (**Fig 1. A,C,E**). The coverage ratio (the percentage of sequences) of these important motifs was shown in the region -500 to +150 relative to TSS in the Fe excess-induced genes in specific tissues (**Fig 1. B,D,F**). These CREs might be the binding sequences of important transcription factors that regulate Fe homeostasis in rice. Based on the presence or absence of candidate CREs using MAMA and known PLACE CREs, we found that the Boruta-XGBoost model explained expression patterns with high accuracy of about 83%. Enriched sequences of both novel MAMA CREs and known PLACE CREs led to high accuracy expression patterns. We also found new roles of known CREs in the Fe excess response, including the DCEp2 motif, IDEF1-, Zinc Finger-, WRKY-, Myb-, AP2/ ERF-, MADS- box-, bZIP and bHLH- binding sequence-containing motifs among Fe excess-responsive genes. In addition, we built a molecular model and promoter structures regulating Fe excess-responsive genes based on new finding CREs.

Table 1. Top 10 Important motifs in gene expression pattern model.

Sequence	**Importance (weight in tree model)**	**Motif Name and Annotation**
CATGCATG	100.00	IDEF1 binding
ATCGATCG	56.83	Downstream core element in plant 2 (DCEp2)
ATAATGGC	54.71	Novel : Motif extracted from Zn regulated genes
GCAGCAGC	54.05	Novel : GCWGCWGC?
CGACACGC	49.93	Novel : CGACACGC
EECCRCAH1	48.68	Myb- binding
CACCAACC		

48.68

Novel : Myb-binding like

CRTDREHVCBF2

48.22

AP2/ERF binding

GCGCGCCA

46.23

GCGC box

CTACGTGC

44.20

bZIP/bHLH

Chart, histogram Description automatically generated

Fig. 1. ?Distribution of novel CRE candidates in the upstream of Fe excess-response genes.

(A,C,E) show relative frequency (distribution) of novel motifs in Fe excess tissues compared to the average frequency in each 50 bp window within -3,000 to +2,000 bp of the TSS. Blue line shows all genes. Red line shows Fe excess-regulated genes.

(B,D,F) show coverage ratio or of the motifs (in A,C,E respectively) in the -500 bp to +150 bp area relative to the TSS. MAMA-extracted Novel CRE candidates were simulated in Fe excess newest leaves **(A, B, E, F)**, in Fe excess old leaves **(C, D)**.

CONCLUSIONS

Our findings about Fe excess-related CREs and conserved sequences will provide a comprehensive resource for discovery of genes and transcription factors involved in Fe excess-responsive pathways, clarification of the Fe excess response mechanism in rice, and future application of the promoter sequences to produce genotypes tolerant of Fe excess.

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Plant microorganism interaction and nutrient acquisition

RHIZOBACTERIUM SERRATIA SP. AND PHOSPHORUS FERTILIZATION RATES AFFECT AEROBIC RICE DEVELOPMENT

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Keywords: Bioestimulants; Beneficial Microorganisms; Upland rice

INTRODUCTION

There are still few studies under field conditions for the use of rizhobacteria in crop development, especially in rainfed places and fewer in the Cerrado region. Therefore, the objective was to determine the effect of the Serratia spp. isolated BRM32114 and doses of P at sowing fertilization on the yield components and grain yield of aerobic rice, under a no-tillage system in a Cerrado Region.

METHODS

The experiments were conducted at Capivara Experimental Station of Embrapa Rice and Beans, located at Santo Antônio de Goiás, GO, Brazil, 16º28'00"S and 49º17'00"W coordinates, and at 823 m of elevation. The climate is Tropical Savanna and is considered Aw (tropical with wet summer and dry winter) according to Köppen classification. Therefore, there are two well-defined seasons: a usually dry season from May to September (autumn/winter) and a wet season from October to April (spring/summer). The average annual rainfall is between 1500 and 1700 mm, and the average annual temperature is 22.7°C, ranging annually from 14.2 °C to 34.8 °C. The experimental soil is classified as a clay loam (kaolinitic, thermic Typic Haplorthox) acidic soil.

Trials were conducted in rainfed conditions using the rice genotype Primavera CL. Trials were arranged in a complete randomized block in a 4x2x2 factorial scheme, with four replications in different areas very close to each other. The plots had the dimension of 3.5 m x 6 m. The usable area of the plot (place where we sampled the data) was composed of the four central rows of rice, disregarding one row of each side of the plot and 0.50 m to each side of the rows in each plot. The treatments consisted of four P2O5 doses (0, 40, 80 and 120 kg ha^{-1} as triple superphosphate) with or without *Serratia* spp in two growing seasons (2015/16 and 2016/17). The strain BRM32114 was applied by seed microbiolization and sprayed at plant/soil in the field at the 7 and 15 DAS (days after sowing) in all plots with rhizobacteria treatment. In the plots with no microorganisms, we applied only water.

RESULTS and DISCUSSION

Phosphorus rates provided increases in number of panicles and grain yield. Increasing rates of P, provided increases in the number of panicles and in grain yield. This could be due to the levels of P in the soil. However, the low response of nutrients to increase P may be due to the adequate level in 2015/16 and high level in 2016/17. The number of panicle per plant was higher in treated plants. It seems that the number of formed and fertile tillers made the difference. The beginning of tiller phase, when rice plants emits the 3rd leaves does not depend on the environmental conditions. However, the emergence and development until 65 to 70 days, depend on nutritional and temperature conditions. To achieve vigorous tillering, N, P and S are necessary, together with carbohydrate (Fageria 1984). According to our data, there is a positive influence of different P rates on tillage numbers m^{-2} , until 60 days after sowing. BRM 32114 is an Indoleacetic acid (IAA) and phosphatase producer (Nascente et al., 2017), which may enhance the tillering successful ensuring a higher number of fertile tillers and formed panicles. Our results showed that using rhizobacterium *Serratia* spp. strain BRM 32114 with phosphorus allowed more increase in grain yield than using only P rates. In this sense, it is likely that the rhizobacterium BRM32114 positively interacted with P fertilization.

CONCLUSIONS

Our trial showed that phosphorus rates increased the number of panicles and grain yield of aerobic rice, and the grain yield was higher with *Serratia* inoculation than without Serratia spp.

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SOIL P MOBILIZATION AND MYCORRHIZA IN A SOYBEAN ROTATION WITH MAIZE INTERCROPPED WITH A FORAGE GRASS

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Keywords: P legacy; mycorrhiza; forage grass

INTRODUCTION

Phosphorus adsorption in weathered soils is one of the most limiting factors for crop productivity. Thus, phosphate fertilizers are applied in higher rates than those required by crops and P ends up accumulating in the soil in forms not readily available, which is called the phosphorus legacy. The introduction of cover crops able to acquire P from less labile pools such as ruzigrass (*Urochloa ruzisiensis*) was proposed as a tool to recycling this nutrient into the cropping system, recovering some of the legacy P and increasing P use efficiency (PUE). With the decay of the grass residue, the cycled phosphorus becomes bioavailable to the next crop. We hypothesized that the effect of ruzigrass on P recovery and availability could be associated with a modification on the microbial population of the soil, mainly mycorrhiza, which is also an important mechanism to increase P use efficiency (PUE). The aim of the study was to evaluate the long-term effect of a soybean-maize/ruzigrass rotation on phosphorus mobilization and on the soil mycorrhizal population, as well as on the availability of residual phosphorus.

METHODS

Three phosphorus managements were set in plots: continuous fertilization, residual fertilization and a control without P; soybean grown in the spring/summer and maize intercropped or not with ruzigrass as a relay crop were grown in subplots; and P rates were applied in sub-subplots. The continuous and residual treatments had received a total of 245 kg ha^{-1} of P up to 2017, while the control treatment received no fertilization. As of 2017, P fertilization was continued only in the continuous treatment at rates of 0, 17.5 and 35 kg ha^{-1} of P. Soil and plant samples were collected, and organic and inorganic P were analyzed as in Olsen & Sommers (1982). Mycorrhiza infection was analyzed as in Giovanethi & Mosse (1980). Soybean yields were also determined.

RESULTS AND DISCUSSION

Soybean yields were always lower in the control. However, yields were similar with annual fertilization or residual P up to the 4^{th} year, regardless of the presence or not of ruzigrass (Fig. 1). Almeida (2016) reported that ruzigrass had no effect of soybean yields despite an increase in available soil P. However, from the 5th harvest onwards, yields were decreased in residual plots. Simultaneously, soil organic P decreased, and inorganic P increased, showing the importance of organic P reserves in the soil. It has been shown that ruzigrass is able to take up non-labile P (Merlin et al., 2015), and it accumulates in organic forms (Almeida et al., 2016). Under this type of crop rotation, at least part of the P applied as fertilizer ends up in the organic pool in the soil, and the availability of this P is lower (Almeida et al. 2019). However, it can sustain yields for some time (Fig. 1), showing that the legacy P can be recovered, at least in part. These results corroborate that the mineralization of organic P by plant used in the cropping system can be important for plants in weathered soils or systems with low phosphorus addition (Carneiro et al., 2011). In treatments with low P addition, despite the lower yields, mycorrhizal colonization was important in providing P to soybean. Since mycorrhizas act to compensate for the low P content due to the high area of soil exploration, they result in greater P uptake when compared with that taken up through the roots. Furthermore, plots with ruzigrass showed higher mycorrhiza colonization by when compared to areas with fallow in the off-season. This is a concurrent mechanism to enhance soil P availability.

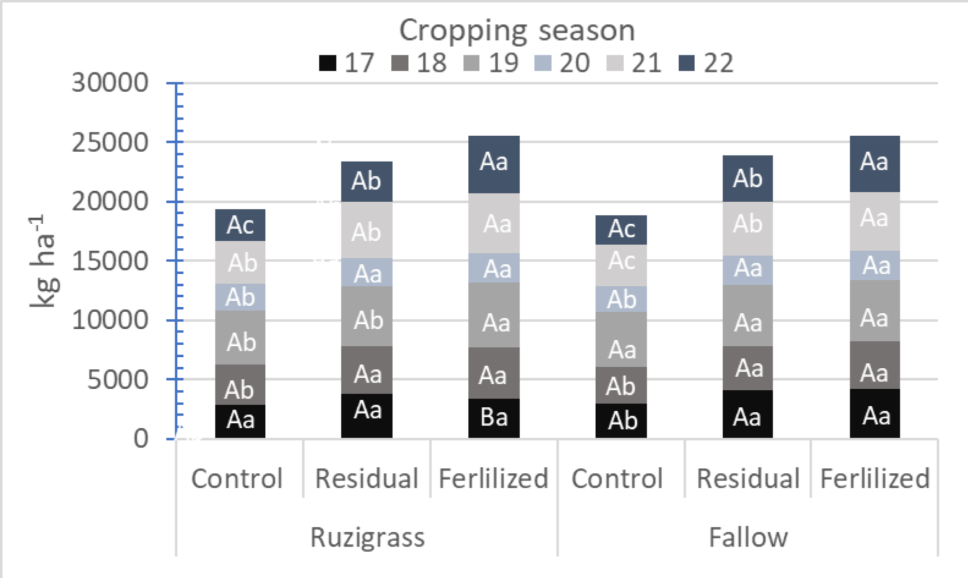

Fig. 1. Soybean yields from six cropping seasons responses to P fertilization and residual P in two intercropped system, ruzigrass intercropped or not with maize. Diferrent lower letter represent the difference between phosphorus management and capital letters represent the difference between maize intercrop management, in te columns are significant at $p < 0,05$ (Tukey).

CONCLUSIONS

The long-term cultivation of ruzigrass intercropped with maize in the soybean off-season is not effective in increasing yields and P availability for soybean. A decrease in organic P throughout the seasons without P addition shows the importance of this soil P pool. However, it is not enough to support high yields, even with the contribution of mycorrhiza. Further studies are needed to understand the role of soil microorganisms in soil P legacy recovering, and also on the critical levels of the organic P legacy.

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AGRONOMIC BIOFORTIFICATION OF COMMON BEAN WITH NANO-ZINC AND DIAZOTROPHIC BACTERIA

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Keywords: zinc use efficiency; zinc uptake; productivity

INTRODUCTION

Zinc (Zn) malnutrition or hidden hunger is a global issue affecting more than 2 billion people and is expected to be 9.7 billion by 2050, especially in developing countries (Maxfield and Crane, 2020). Biofortification of grain crops with Zn and diazotrophic bacteria is an efficient and sustainable solution to malnutrition and hidden hunger, reducing productivity losses with improving cycling and use efficiency of nutrients under climatic extremes tropical savannah (Jalal et al., 2021).

Food security and increasing production demands are the most challenging issues that lead to vulnerable agriculture inputs, Zn deficiency, and malnutrition (Praharaj et al., 2021). Biofortification of staple crops (legumes) with nano-Zn has grabbed attention to combat "hunger of the day" in a sustainable way. There is a research gap on the association of bacterial co-inoculation and nano-Zn fertilization on Zn nutrition, ZnUE, and yield of common bean. It is necessary to determine strategies to improve Zn nutritional quality and biofortification of crops for the dietary intake of the population. Therefore, we hypothesized that co-inoculations of bacteria might have synergetic relation with Zn application on grain Zn accumulation, ZnUE, and daily intake of biofortified grains in Brazil. Therefore, we proposed to assess best performing bacteria in relation to nano-Zn application on common bean grains Zn accumulation, ZnUE, and estimated Zn intake, in two consecutive growing seasons.

METHODS

Two years of field experiments with common bean were conducted at Research Farm of São Paulo State University (UNESP) - Campus de Ilha Solteira, in state of Mato Grosso do Sul, Brazil at geographical coordinates of 20° 22' S latitude, 51° 22' W longitude and an altitude of 335. The soil of experimental site is classified as Rhodic Haplustox (Soil Survey Staff, 2014), with Zn content (0.9 mg $dm^{?3}$). The experimental design was a randomized complete block in 7 x 3 factorial scheme with four replications. The treatments were consisted of seeds inoculations (No inoculation, *Rhizobium tropici, R. tropici + Azospirillum brasilense, R. tropici + Bacillus subtilis, R. tropici + Pseudomonas fluorescens, R. tropici + A. brasilense + B. subtilis, and R. tropici + A. brasilense + P. fluorescens*) and Nano-Zn oxide (50% Zn) doses (0, 1.5, 3 kg ha^{-1}). Inoculation of *R. tropici* strain SEMIA 4080 with 2×10^9 colony forming units (CFU) $g^{?1}$ at a dose of 200 g per 100 kg of seeds, *A. brasilense* strains Ab-V5 and Ab-V6 (CNPSo 2083 and CNPSo 2084 with a guarantee of 2 × 108 CFU $mL^{?1}$ respectively) at a dose of 300 mL ha^{-1}, *B. subtilis* (CCTB04 with a guarantee of 1×10^8 CFU $mL^{?1}$) and *P. fluorescens* (CCTB03 with a guarantee of 2×10^8 CFU $mL^{?1}$) were applied at a dose of 150 mL $ha^{?1}$. Zinc use efficiency and estimated intake were calculated following equations 1 and 2 (Jalal et al., 2021);

1) Zn intake= [Zn] × C, where [Zn] (g $kg^{?1}$) is Zn grain concentration in the current study and C (kg $person^{?1}$ $day^{?1}$) is the mean consumption of common bean grains per person in Brazil.

2) Zn use efficiency = GYF - GYW ÷ Applied Zn dose, GYF= grain with Zn and GYW = without Zn.

The data were submitted to analysis of variance (F test), the Tukey test ($p \leq 0.05$) was used for means comparison of Zn foliar fertilization whereas Scott-Knott test ($p \leq 0.05$) was used for means comparison of co-inoculations of bacteria, using ExpDes package in R software.

RESULTS and discussion

The foliar Zn application and co-inoculations of bacteria and their interactions improved grain yield, grain Zn accumulation, Zn intake, and use efficiency of common bean in 2019 and 2020 (Fig. 1). Foliar Zn spray at a dose of 1.5 kg ha^{-1} increased grain yield by 16 and 9%, grain Zn accumulation by 93 and 16%, Zn estimated consumption in Brazil by 12 and 6% as compared to without foliar spray in 2019 and 2020, respectively for 1.5 and 3 kg Zn ha^{-1}. Co-inoculation of *R. tropici* + *B. subtilis* remained the most effective which resulted in greater grain yield, Zn accumulation, estimated Zn intake and Zn use efficiency in both studied years under co-inoculations (Fig. 1). This might be due to foliar Zn functioning in translocation of Zn from vegetative to grains tissues to its availability and accumulation with better metabolism and productivity. Plants-microbe interaction has increased Zn availability in plant tissues of legume crops by solubilizing Zn insoluble complexes, including phosphate, oxides, and carbonates, and making them available for plant uptake and leading to healthy, quality and nutrients enriched bean grains (Khande et al., 2017).

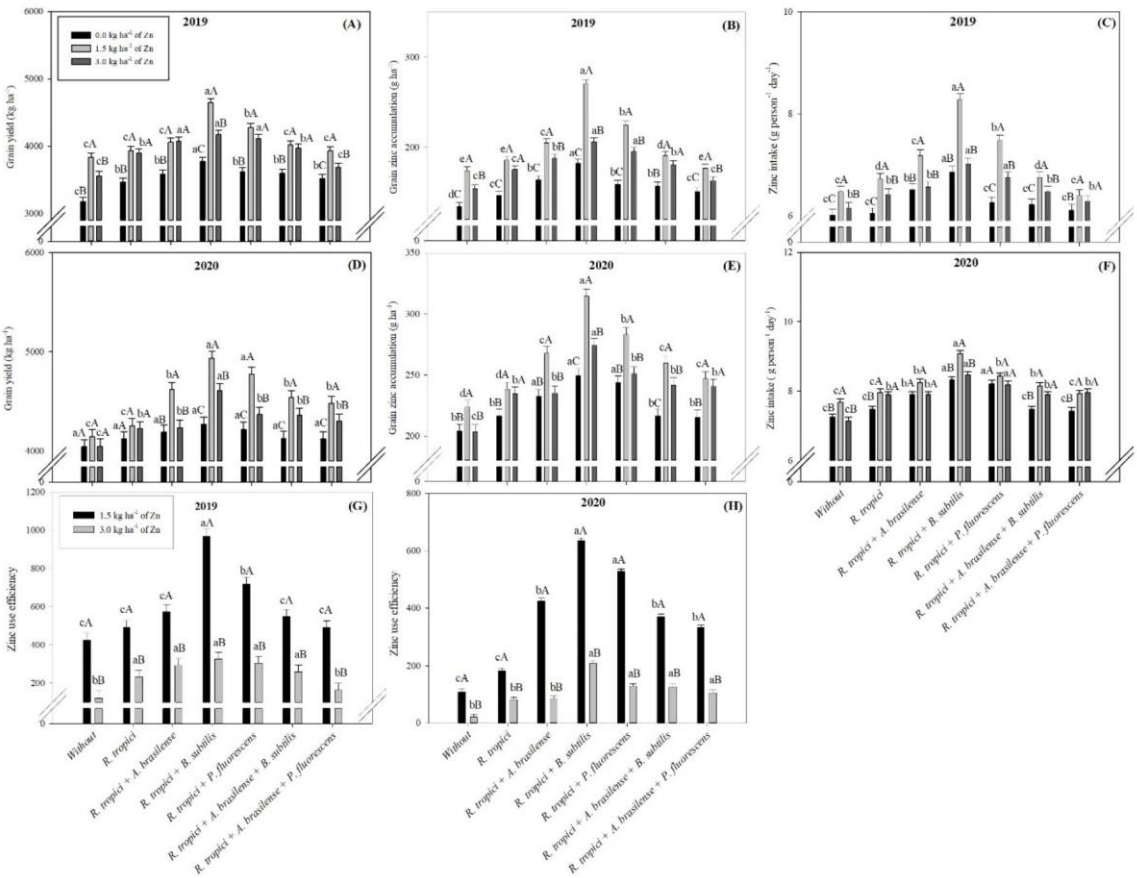

Figure 1.

Fig. 1. Influence of diazotrophic bacteria and foliar Zn doses on grain yield (A, D), grain Zn accumulation (B, E), estimated Zn intake in Brazil (C, F) and Zn use efficiency (G, H), in 2019 and 2020 respectively. Capital letters indicated differences with inoculations and small letters among foliar Zn doses.

CONCLUSIONS

Foliar Zn fertilization and co-inoculation of diazotrophic bacteria is the most feasible and sustainable approach to increased zinc use efficiency in an eco-friendly manner. It was concluded that foliar nano-Zn fertilization with co-inoculation increased grain yield and Zn nutritional status of common bean. The co-inoculation of *R. tropici* + *B. subtilis* with 1.5 kg ha^{-1} of foliar Zn application were the most effective treatments for grain yield, nutritional status, and estimated consumption of common bean, leading to improve biofortification.

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INOCULATION WITH *AZOSPIRILLUM BRASILENSE* REDUCED LEAF NITRATE ACCUMULATION AND INCREASED LEAF YIELD IN HYDROPONIC LETTUCE

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Keywords: Nitrogen metabolism; Nitrogen uptake; water use efficiency

INTRODUCTION

Lettuce is the most produced and consumed leafy vegetable in the world due to its desirable culinary characteristics in the form of salads and being the most produced vegetable in hydroponic system (Dalastra et al., 2020). The increase in nutrient concentrations of nutrients solution is increasing electrical conductivity in a hydroponic system and reducing consumption of water in agriculture in relation to soil, increasing water use efficiency (WUE) by plants, precocity and productivity of lettuce (Dalastra et al al., 2020).

The increase in electrical conductivity can provide greater use of fertilizers which in turn increase accumulation of nutrients in lettuce leaves, such as nitrate (NO_3^-) which is harmful to human health (Tabaglio et al., 2020). Inoculation with *A. brasilense* has previously reported to fix nitrogen (N) in non-legume crops (Galindo et al., 2021), stimulating activity of nitrate reductase (NR) enzyme, and managing to reduce NO_3^- concentration in plant leaves (Reis Junior et al., 2008).

The use of this inoculant via nutrient solution in hydroponics has not yet been studied, therefore objective of the study was to investigate doses of *A. brasilense* in iceberg lettuce cultivation in hydroponic system to improve N acquisition, reduce NO_3^- and increase leaves yield of lettuce.

METHODS

The Nutrient Film Technique (NFT) hydroponic system was developed fo lettuce cultivation in a greenhouse with 30% shading at Sao Paulo State University (UNESP), Ilha Solteira - SP. The experiment with NFT system was installed on an individual bench of 6m in length and 10% slope. The cultivation channels were made of PVC with a rectangular section of 8 cm wide and 4 cm high and upper perforations to accommodate plants at every 25 cm with a flow rate of 1 L min^{-1}.

The electrical conductivity was adjusted as required by plants: 1.3 dS m^{-1} (0.0 - 10 day), 1.5 dS m^{-1} (11 - 20 day) and 1.7 dS m^{-1} (21 - 30 day) while harvested at 31 days after plantation. The measurment of electrical conductivity and pH correction were performed daily. The nutrient solution was composed of different nutrients (%): 25.5 of N, 9 of P, 28 of K, 4.3 of S, 19 of Ca, 3.3 of Mg, 0.06 of B, 0.01 of Cu, 0.05 of Mn, 0.07 of Mo , 0.02 of Zn and 0.6% of Fe.

The experiment was carried out in randomized blocks with five replications. The treatments were consisted of *A. brasilense* strains AbV5 and AbV6 (guarantee of 2x108 CFU mL-1) doses [non-inoculated (control), 8, 16, 32 and 64 mL of liquid inoculant for every 100 liters of nutrient solution], applied only on the day of transplanting seedlings of lettuce cv. Angelina.

Lettuce yield was calculated (shoot fresh weight in kg X plant population m^{-2} = yield in kg m^{-2}), where plant population was 19.5 plants m^{-2}. Nitrogen concentration was analyzed according to methodology of Malavolta et al. (1997), and NO_3^- and ammonium (NH_4^+) by follwing methodology of Cataldo et al. (1975). The accumulation was obtained from dry weight in kg m^{-2} X concentration in g or mg kg^{-1}. Water use efficiency (WUE) and net photosynthesis rate (A) were quantified by IRGA.

The relative means of inoculant doses with *A. brasilense* were adjusted in regression at 5% probability.

RESULTS AND DISCUSSION

There was an increase in NH_4^+ accumulation and reduction in leaf NO_3^- accumulation with increasing doses of *A. brasilense* (Figure 1A and 1B). The highest leaf N accumulation, leaf yield, A and WUE were observed at estimated doses of 37, 44, 32 and 32 ml $100L^{-1}$ respectively (Figure 1C, 1D, 1E and 1F). Inoculation with *A. brasilense* had increased shoot N accumulation by increasing biological fixation of N, WUE and leaf chlorophyll, resulting in higher crop yields (Galindo et al., 2021). The increase in NR activity reduces NO_3^- while synthesis NH_4^+ and amino acids that improve plant leaves development (Tablaglio et al., 2020).

Figure 1. Effect of *A. brasilense* rates on leaf ammonium (NH_4^+) accumulation (A), nitrate (NO_3^-) accumulation (B), nitrogen (N) accumulation (C), leaf yield (D), photosynthetic rate "A" (E) and water use effieciency "WUE" (F) in lettuce hydroponic.

CONCLUSIONSInoculation with *A. brasilense* at doses ranging from 32 to 44 ml $100L^{-1}$ increased yield, N uptake, photosynthetic rate and WUE while reduced leaves nitrate accumulation, which is a worldwide problem.

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SOIL MICROBIAL ACTIVITY AS AFFECTED BY TIMING OF K APPLICATION, N RATE AND COVER CROPS

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Keywords: Enzyme; Glomalin; Microbial carbon

INTRODUCTION

Soil consists a living system including organisms with different activities and functions. The way we manage it directly affects its quality, interfering in the production of grains and fiber (FAO, 2015). Improving soil quality is essential to assure food, fiber and energy production. Microorganisms play an important role in building the sustainability of the agrosystem, its activities and biodiversity affect organic matter deposition and nutrient cycling. (Lei et al., 2022). However, when it comes to cotton, few studies report the effect of the interaction covering crops with fertilizers. In this case, the objective of this study was to evaluate soil microbiological acticity after cotton grown under cover crops in responde of K fertilization and nitrogen rates.

METHODS

The experiment was carried out in the field at the Experimental Farm of the University of Western São Paulo, in Presidente Bernardes-SP, in a sandy. The experimental design was a randomized block with five replications in a split-plot scheme. Cover crops were allocated in the plots: Fallow (F); Velvet Bean (VB); Ruzigrass (R); Ruzigrass + Velvet Bean (R+VB); Inoculated Ruzigrass (IR); and Inoculated ruzigrass + Velvet Bean (IR+VB). Potassium fertilization, pre-planting (on cover crops) or post-planting (on cotton), at a rate of 120 kg ha^{-1} of K (potassium chloride), were allocated in the sub-plots. Nitrogen rates, 80 and 120 kg ha^{-1} were used in two different experiments. Ruzigrass was innoculated with *Azospirillum brasiliensis* at a rate of 4 ml kg^{-1} of seed, using the commercial product AZOMAX (2.0×10^8 UFC ml^{-1}).The experiment was conducted on 2018/2019 and 2019/2020 seasons, but the microbial activity was evaluated after cotton harvest in 2019/2020. Five subsamples per plot were collected from soil depth of 0 to 10 cm. We evaluated microbial biomass carbon (MBC) (Vance et al., 1987) and nitrogen (MBN) (Brookes et al., 1985), ß-glucosidase enzyme (Tabatabai and Bremmer, 1972) and easily extractable glomalin (EEG) (Wright and Upadhyava, 1996). Treatments means were compared by LSD test ($p < 0.05$).

RESULTS and discussion

The association of inoculated ruzigrass+velvet bean increased MBC by 83% with K in pre-planting, and 80 kg of N, but when applying K in post-planting, single ruzigrass showed higher MBC (Fig. 1a). Under 120 N, velvet bean expressed higher MBC with K in pre-planting, but on post-planting K, ruzigrass resulted in higher MBC (Fig. 1b). MBN was higher under inoculated ruzigrass with K in pre-planting in the lowest N rate (80 N) (Fig. 1c), however, at 120 N ruzigrass showed a substantialy higher MBN (Fig. 1d). Applying potassium on cotton (post-planting) provided an increase of 124% (80 N) and 76% (120 N) on MBN on inoculated ruzigrass+velvet bean compared to the others treatments.

ß-glucosidase activity was higher under ruzigrass+velvet bean on pre-planting K, but applying K in post-planting resulted in higher enzyme activity on ruzigrass+velvet bean and inoculated ruzigrass on 80 N (Fig. 2a). Under 120 N velvet bean and ruzigrass+velvet bean provided an increase of ß-glucosidase activity on pre-planting K, however, on post-planting K application the association of ruzigrass+velvet bean resulted in better activity compared other cover crops. Glomalin was not affected by treatments at 80 N (Fig. 2c), but at 120 N it was 60% higher with pre-planting K on velvet bean, compared to the other cover crops (Fig. 2d).

CONCLUSIONS

Soil biological activity conservation on cotton fertilezed with higher N rates demands K application at pre-planting, and it is affected by the cover crop used.

ACKNOWLEDGEMENTS

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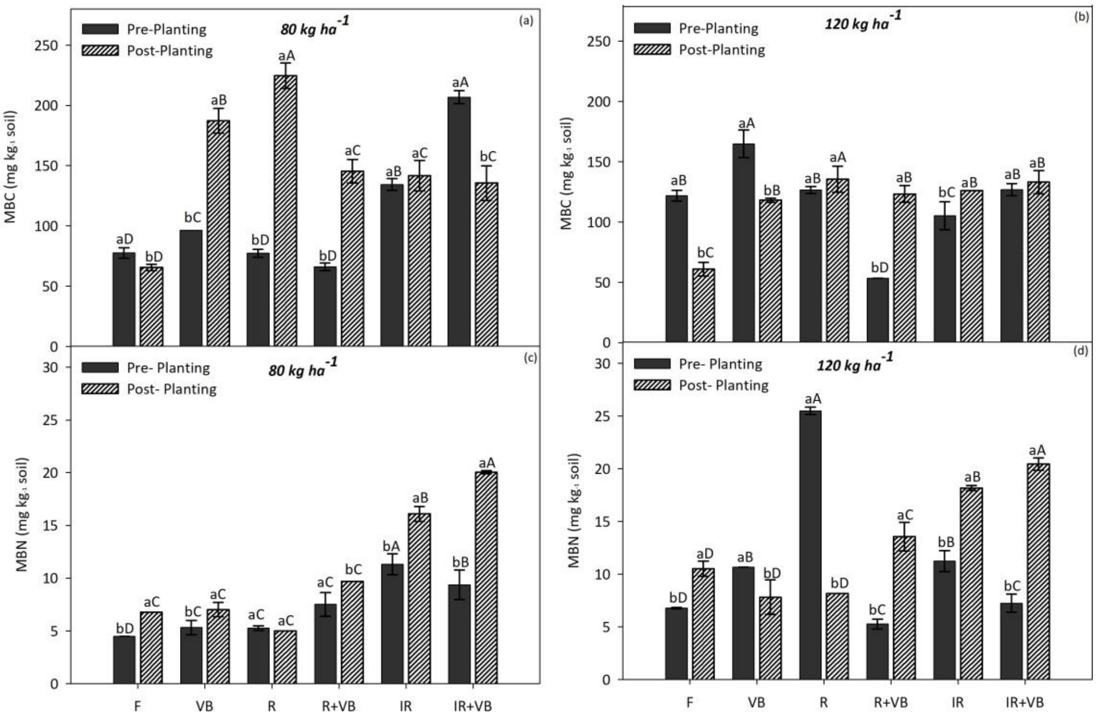

Fig. 1. Microbial biomass of carbon and nitrogen affected by cover crops, potassium application time, and nitrogen rates. Fallow (F); Velvet Bean (VB); U. ruziziensis (R); U. ruziziensis + Velvet Bean (R+VB); Inoculated U. ruziziensis (IR); and Inoculated U. ruziziensis + Velvet Bean (IR+VB). Uppercase letters compare cover crops and lowercase letters compare N doses in each cover crop (LSD test $p<0.05$).

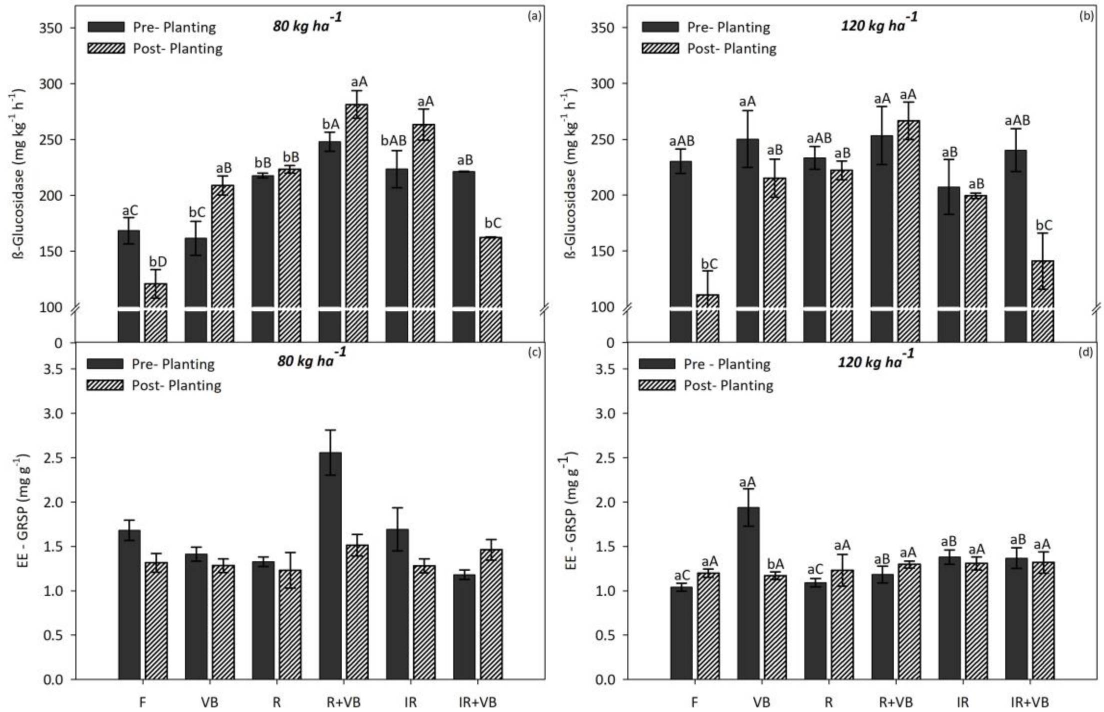

Fig. 2. Soil enzymatic activity of ß-glucosidase (ßG) and easily extractable glomalin (EEG) affected by cover crops, potassium application time, and nitrogen rates. Fallow (F); Velvet Bean (VB); U. ruziziensis (R); U. ruziziensis + Velvet Bean (R+VB); Inoculated U. ruziziensis (IR); and Inoculated U. ruziziensis + Velvet Bean (IR+VB). Uppercase letters compare cover crops and lowercase letters compare N rates in each cover crop (LSD test $p<0.05$).

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FEREMYCORRHIZA: A NATURE'S KEY TO UNLOCK THE PHOSPHORUS TREASURE TRAPPED IN SOIL

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Keywords: Feremycorrhiza; Biofertilizer; Phosphorus

INTRODUCTION

The Australian native fungus *Austroboletus occidentalis* (*Boletaceae*, Basidiomycota) establishes feremycorrhizal (FM) symbiosis, conferring significant growth and nutritional (N, P, K, Fe, Zn *etc*.) benefits to diverse host plants, including woody plants (*Eucalyptus marginata*) (Kariman et al. 2014), arbuscular mycorrhizal (AM) crops such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), as well as the non-mycorrhizal crop canola (*Brassica napus*) (Kariman et al. 2014, 2020, 2022). It is known that all currently documented *Austroboletus* species (except *A. occidentalis*) form ectomycorrhizal (ECM) symbiosis with plants, suggesting that the FM symbiosis has evolved from the ECM symbiosis as manifested in an *Austroboletus* species native to Australia within the harsh, nutrient-poor jarrah forest ecosystem. In contrast to all known mycorrhizal/endophytic associations, in FM symbiosis, fungal hyphae do not colonize plant roots (Kariman et al. 2014, 2020). Hence, functional pathways in the FM symbiosis are different from those in the conventional AM and (to some extent) ECM symbioses. A direct intraradical nutrient exchange between host plants and fungal partners occurs in AM/ECM symbioses, but not in the FM symbiosis due to lack of root colonization; however, both FM and ECM symbioses exhibit organic acid anion-mediated nutrient solubilization (Kariman et al. 2014, 2020). Therefore, the nutritional benefits of the FM symbiosis are attributed primarily to the fungal role in rhizosphere modification and nutrient solubilization/mobilization. The FM symbiotic benefits go beyond plant nutrition; the fungus was shown to induce drought tolerance in AM (wheat) and non-mycorrhizal (canola) crops through biochemical (*e.g.* enhanced levels of organic osmolytes such as proline and glycine betaine in leaves) and root anatomical modifications. Here, we briefly present the results of controlled-environment trials showing the significant growth/yield benefits of the FM fungus in different crops, as well as the *in vitro* studies on P solubilization function of the fungus and underlying mechanisms (Kariman et al. 2020).

METHODS

Controlled-environment trials were conducted to characterize the potential effects of the FM symbiosis on two AM crops (wheat and barley) as well as the non-mycorrhizal crop canola grown in a low-nutrient soil. The natural field soil (containing indigenous microbes) was mixed with the living hyphal inoculum (inoculated treatments) or autoclaved inoculum (control treatments) (10:1 v/v). During the growth period, pots were watered to field capacity (14% volumetric water content). Pots were not fertilized during the growth period in order to maintain the poor nutrient conditions. Plants were initially grown at 12/12 h light/dark and 20/15 ?C day/night temperature for 6 weeks, followed by growth at 16/8 h light/dark and 22/17 ?C day/night temperature for additional 10 weeks. Furthermore, the phosphate solubilization capacity of the FM fungus and underpinning mechanisms were investigated in *in vitro* experiments. The fungus was grown in a low P agar-based medium supplemented with different water-insoluble P compounds (Calcium phosphate as hydroxyapatite: $[Ca_{10}(PO_4)6(OH)_2]$), $FePO_4$, and $AlPO_4$) and the water-soluble P (KH_2PO_4) as positive control. Different analyses were performed, including quantification of concentrations of P and organic acid anions in media, and ^{31}P nuclear magnetic resonance (NMR) spectroscopy to determine P speciation.

RESULTS AND DISCUSSIONS

(a) After 16 weeks of growth in a low-nutrient soil, the two AM crops wheat and barley and the non-mycorrhizal crop canola inoculated with *A. occidentalis* had significantly higher shoot biomass compared to control (non-inoculated) plants. The FM symbiosis also led to significant grain yield increases in wheat (by 54%) and barley (by 37%) plants compared to the control plants, while canola plants were harvested before setting seeds due to their slow growth under nutrient deficiency conditions (Fig. 1; Kariman et al. 2020).

(b) In all crops, the FM symbiosis significantly increased the shoot nutrient content, including that of P, K and Mg. Total N accumulation (shoot + grain content) was also significantly higher in all inoculated *vs* control crops.

(c) The *in vitro* studies demonstrated the solubilization of the water-insoluble P forms including calcium phosphate (hydroxyapatite), $FePO_4$ and $AlPO_4$ by *A. occidentalis* via exudation of organic acid anions (mainly oxalate, but also citrate and fumarate).

(d) The ^{31}P nuclear magnetic resonance (NMR) spectroscopic analysis revealed the presence of similar P species (dominated by orthophosphate and long-chain inorganic polyphosphates) in the agar-based media supplemented with different P forms (KH_2PO_4, $[Ca_{10}(PO_4)6(OH)_2]$, $FePO_4$ or $AlPO_4$), indicating that all three water-insoluble P compounds were solubilized and transformed by the FM fungus in a similar way.

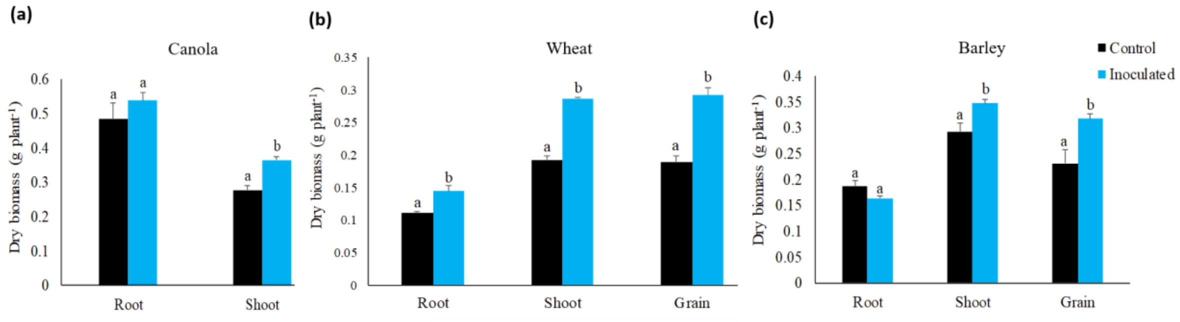

Fig. 1. Biomass responses to feremycorrhizal symbiosis in canola (a), wheat (b) and barley (c) plants grown in a low-nutrient soil with low P availability (Colwell P = 7 mg kg-1). For each parameter (shoot or root), bars with different letters are significantly different according to the Student's t-test ($p \leq 0.05$). Error bars indicate standard errors (n = 4) (Kariman et al. 2020).

CONCLUSIONS

The results demonstrated that the three important grain crops, regardless of their capacity to estalish AM symbiosis, could get the growth and nutritional benefits from the FM symbiosis. The *in vitro* trials revealed the remarkable P solubilization function of the FM fungus, which is mediated by exudation of organic acid anions. The findings reinforce the potential to exploit *A. occidentalis* as a novel fungal biofertilizer for agricultural crops, aiming to reduce the use of synthetic fertilizers and long-term sustainability of agroecosystems while maintaining economic viability.

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EVALUATION OF YEAST STRAINS WITH SYNTHESIS OF INDOLE-3-ACETIC ACID AND/OR ACC DEAMINASE ACTIVITY AND THEIR EFFECT ON PLANT GROWTH PROMOTION

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Keywords: Yeast; ACC deaminase activity; indole-3-acetic acid

Evaluation of yeast strains with synthesis of indole-3-acetic acid and/or ACC deaminase activity and their effect on plant growth promotion

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INTRODUCTION.

Plant growth promoting microorganism enhance plant development because of their metabolic activity, providing nutrients to the plants by increasing their availability and enhancing nutrient uptake. Little information is available with regards to the beneficial potential of yeasts. Several strains can produce plant hormones such as indole-3-acetic acid (IAA) (Freimoser *et al.*, 2019). It has also been reported that some yeasts present ACC deaminase activity thanks to the 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme, which metabolizes ACC (a precursor of ethylene), therefore, reducing ethylene levels while preventing early senescence of the plant (Gupta & Pandey, 2019). The main objective of this study was to evaluate plant growth using tomato seedlings subjected to the inoculation with yeast strains selected for their capacity to synthesize IAA or present ACC deaminase activity.

METHODS

IAA quantification. Twenty-three strains of yeasts were selected from the codified collection held at Pontificia Universidad Católica de Chile. Yeasts were cultured in YPD medium with or without 0,1% (w/v) L-tryptophan and incubated in a shaker for 7 days in darkness at 28°C. Later, 100 µL of the supernatant were mixed with 100 µL of Salkowski's reagent (2 mL of Fe_2Cl_3 + 98 mL of 35% $HClO_4$) in Petri dishes using triplicates for each strain. After 30 minutes of incubation at ambient temperature the colour was quantified by spectrophotometry (model Infinite M200 Pro, Tecan, Switzerland) at 530 nm.

ACC deaminase activity. The same strains selected for the method above were cultured in YPD medium for 2 days. Later, 50 µL of the saturated culture were transferred to a tube with 5 mL of YNB medium (with no nitrogen source) with or whitout 3 mM ACC and were incubated for 14 days in a shaker at 28°C. Turbidity in the culture medium, measured as OD at 630 nm, was used as indicator for positive growth in the absence of nitrogen sources other than ACC. Following, ACCD activity was quantified by determininig the concentration of α-ketobutyrate using a spectrophotometer at 540 nm.

Inoculation of tomato seedlings with selected yeast strains. Selected strains from the methods above were used to determine root growth promotion. Tomato seeds (*Solanum lycopersicum* cv. 'Attiya') were soaked in 1% sodium-hypochlorite solution for 15 minutes and washed with sterile water. Five seeds were placed in Petri dishes with MS medium and incubated for 2 days in darkness at 28°C. Later, four dishes per strain were inoculated with 50 µL containing 10^8 CFU mL^{-1} and were placed in a growth chamber for 5 days at 25°C with 16h light. The control treatment consisted in seedlings with no yeast inoculation. Plant lateral roots number and root length were measured using WinRhizo software. Here we present the results for seven strains selected for their production of IAA or ACCD activity.

RESULTS AND DISCUSSION

IAA quantification. The most AIA producing strains were selected and was evaluated their potential effect on the development of lateral roots. The lowest IAA synthesis was shown by *Debaryomyces hansenii* (*Dh-8*) (0.803 ± 0.007 µg mL^{-1}) while the highest was observed in *Rhodotorula babjevae* (*Rd-42*) (3.303 ± 0.172 µg mL^{-1}). In other studie, the addition of L-Trp increased the synthesis of IAA (Sun et al. 2014), but our results show no significant difference between with or without L-Trp.

ACC deaminase activity. Two different strains of *Solicoccozyma aeria* were the only yeasts showing ACCD activity, *Sa-75* with a final concentration of 0.38 ± 0.007 mM α-ketobutyrate and *Sa-79* producing 0.43 ± 0.006 mM. The difference between both strains is their origin, *Sd75* was isolated from Central Chile (mediterranean climate, aridisol) while *Sd79* was isolated from southern Chile (temperate climate, andisol). The activity of ACCD has been confirmed in several genres of soil bacterias (Gupta & Pandey, 2019), while in soil yeasts it has only been previously described in *Candida tropicalis* (Amprayn et al., 2012).

Inoculation of tomato seeds. The formation of lateral roots was significantly enhanced in tomato seedlings by six of the seven strains selected (Figure 1). The highest root development was achieved by *Rb-42* and *Sa-75* (Figure 2). The length of the principal root decreased in all the strains, except for *Rb-42*. Similar results have been reported in seedlings of lettuce, corn, tobacco and arabidopsis (Fernandez-San Millan, et al. 2020; Bunsangiam, et al. 2019).

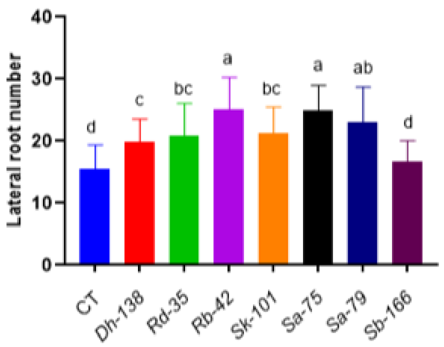

Figure 1. Number of lateral roots in tomato seedlings after 5 days of incubation with the yeasts. CT: control, *Dh-5: Debaryomyces hanseni, Rd-35: Rhodotorula dairenensis, Rb-42: Rhodotorula babjevae, Sk-101: Suhomyces kilbournensis, Sa-75: Solicoccozyma aeria, Sa-79: Solicoccozyma aeria, Sb-166: Saccharomyces bayanus*

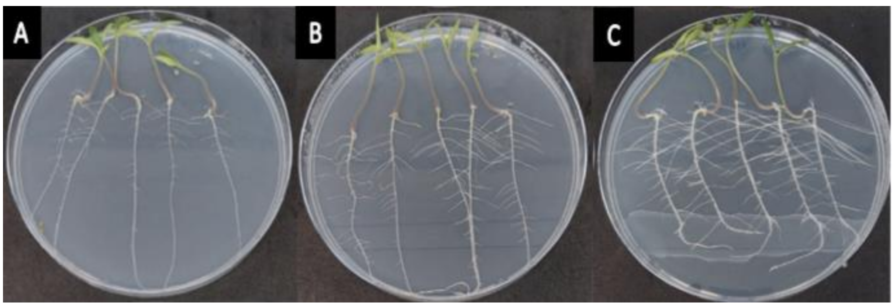

Figure 2. A: Control, B: *Rhodotorula babjevae- 42*, C: *Solicoccozyma aeria-75*

CONCLUSIONS

Two native yeast strains from Chile, *Rb-42* and *Sa-75*, arise as promising root growth promoting microorganisms thanks to their capacity to synthesis IAA or ACCD activity.

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MYCORRHIZAL SYMBIOSIS ALLEVIATES MN TOXICITY IN *EUCALYPTUS TERETICORNIS* UNDER LOW PHOSPHORUS AVAILABILITY

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Keywords: arbuscular mycorrhiza; gene expression; metal stress

Mycorrhizal symbiosis alleviates Mn toxicity in *Eucalyptus tereticornis* under low phosphorus availability

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INTRODUCTION

Tropical, highly weathered, acid soils often present two intersecting issues that can hinder plant growth: low phosphorus (P) and high manganese (Mn) availability (Alejandro et al. 2020). *Eucalyptus tereticornis* is a tree species often cultivated in such soil conditions. Under P limitation, eucalypts can mobilize P by releasing organic acids into the rhizosphere, however, acidification can further increase Mn availability (Lambers et al. 2015), leading to phytotoxicity. We investigated the effects of Mn in *E. tereticornis* under low P in soil, and the role of the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* in alleviating any Mn stress, possibly by improving P nutrition and altering the expression of Mn transporter genes in roots.

METHODS

E. tereticornis seeds were germinated in sterile vermiculite with (AM) or without (NM) *R. irregularis* inoculum. One month-old seedlings were transplanted to pots with 6 kg of soil at low P availability (5 mg kg^{-1} P) or sufficient P (15 mg kg^{-1} P), and under three Mn doses: control (no Mn addition), 75 and 150 mg kg^{-1} Mn. Plants were grown for seven months. We measured biomass, AM colonization, concentrations of Mn and P in leaves. Root total RNA was extracted to determine the effects of Mn, P and AM symbiosis on the expression of genes related to Mn transport and homeostasis, by RT-qPCR. The evaluated genes were: *IRT1*, *VIT1* and *YSL6*.

RESULTS AND DISCUSSION

Plants under low P presented a significant decrease of up to 94% in biomass production when compared to plants under sufficient P conditions, which were further affected by Mn additions (Fig 1a), confirming that low P availability exacerbates Mn toxicity. While biomass was unaffected by increasing soil Mn availability at sufficient P (data not shown). AM symbiosis improved plant growth at low P availability not by effectively decreasing Mn uptake (Figs 1a, b), but by improving P nutrition (Fig 1c). The highest Mn dose decreased foliar P concentrations only in NM plants (Fig 1c).

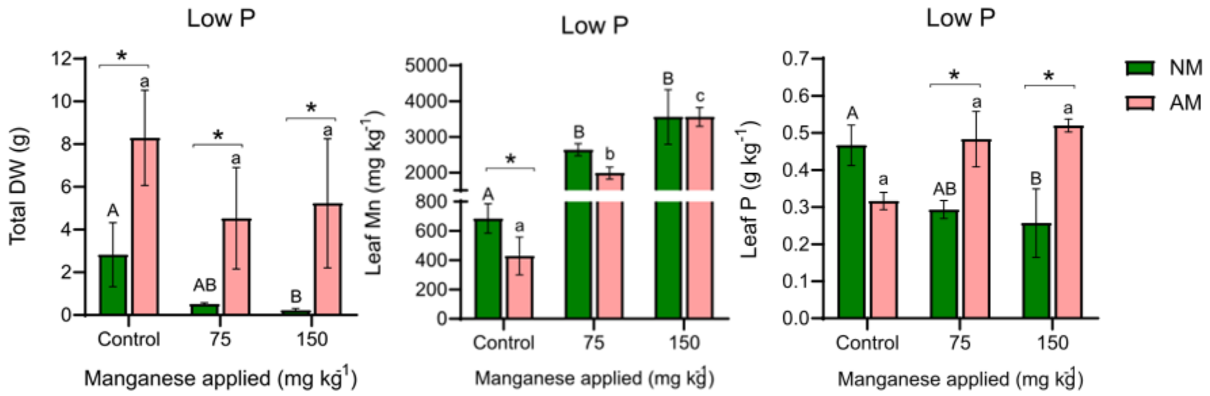

Fig. 1. Total dry weight (DW) (a), concentration of Mn (b) and P (c) in leaves of *E. tereticornis* under low P availability and three Mn doses, with (AM) or without (NM) mycorrhizal symbiosis. Capital letters compare Mn treatments within the NM group, lower-case letters compare treatments within the AM group, asterisks compare between AM and NM treatments within the same Mn treatment (Tukey, p<0.05).

AM plants had 37% less Mn in leaves than NM plants in low P soils without Mn addition. Decrease in Mn accumulation has been previously reported in AM plants (Nogueira et al. 2007), but here, under excess Mn (75 and 150), AM colonisation was low (≤ 8%) and did not affect Mn accumulation (Fig. 1b). However, even low colonisation rates have been shown to effectively alter root gene expression patterns. The expression of the putative Mn transporter *EtIRT1* was downregulated by AM symbiosis (Fig 2a), which can be related to the higher P uptake, since higher expression of Fe transporters is associated with P-starvation responses (Wang et al. 2020). *EtYSL6* codifies a Mn-nicotianamine transporter that was upregulated at excess Mn regardless of P status (Fig 2b), suggesting that Mn complexation can be a tolerance mechanism in *E. tereticornis* roots. Similarly, Mn addition upregulated the expression of the tonoplast transporter *EtVIT1* (Fig 2c), indicating vacuolar sequestration of excess Mn. These last genes were barely affected by mycorrhization, even when P was sufficient, suggesting that the AM influence goes beyond P status modulating Mn transport within root vacuoles or long distance transport from root to shoot.

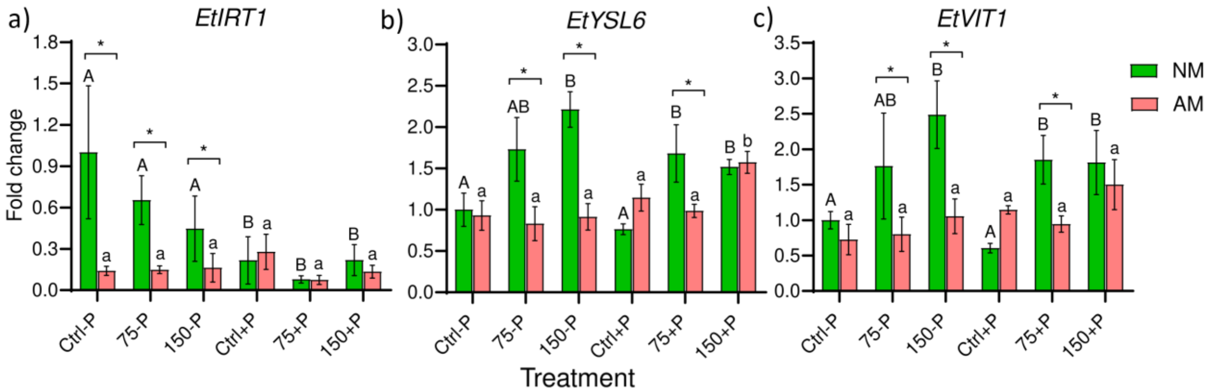

Fig. 2. Expression of three putative Mn transporters: *EtIRT1* (a), *EtYSL6* (b) and *EtVIT1* (c) in roots of *E. tereticornis* under low (-P) and sufficient (+P) soil P availabilities, and three Mn doses (Control, 75 and 150 mg kg^{-1}), with (AM) or without (NM) mycorrhizal symbiosis. Capital letters compare treatments within the NM group, lower-case letters compare treatments within the AM group and the asterisk compares between AM and NM treatments within the same Mn/P treatments (Tukey, p<0.05).

CONCLUSIONS

Low P availability exacerbated Mn toxicity in NM *E. tereticornis* plants while AM symbiosis alleviated Mn toxicity and P defficiency. Expression patterns of Mn transporters differed between NM and AM plants, which was not always related to soil P status. Upregulation of *EtYSL6* under high Mn points to nicotianamine chelation as a detoxification mechanism, as well as vacuolar sequestration carried by *EtVIT1*.

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Plant nutrition and food quality

CAN THE SUPPLEMENTAL FOLIAR APPLICATION OF POTASSIUM SULPHATE ATTENUATE THE EFFECTS OF CHLORINE ON COFFEE QUALITY?

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Keywords: Fertilizers; potassium chloride; drink

Although studies indicate that the substitution of potassium chloride (KCl) for potassium sulfate (SOP) in coffee fertilizer reduces the deleterious effects of chlorine and increases the quality of the beverage, the use of KCl still predominates in brazilian coffee production, due to its greater availability and better cost-benefit ratio (Ernani et al., 2007). Studies with some crops demonstrated that SOP foliar spray, can increase yield and quality of products (Jifon and Lester, 2009; Dkhil et al., 2011; Salim et al., 2014; Abid et al., 2016; Shen et al., 2016; Shen et al. al., 2016; Dalal et al., 2017; Solhjoo et al., 2017). However, not have studies on this technology for coffee.

The aim of this study was to evaluate the effect of supplemental foliar fertilization of potassium sulfate on the grain yield and quality of the coffee beverage.

METHODS

The experiment was carried out in Altinópolis, State of São Paulo, Brazil, in four harvests from 2017 to 2020. The variety used was Catuaí Amarelo cultivated in a Oxisol. The experimental design adopted was randomized blocks, with six treatments and five replicates. The experimental design adopted was randomized blocks, with six treatments and five replicates. The treatments aplied was this: 100% KCl (200 kg ha$^{?1}$ of K_2O to soil fertilizer); 75% KCl + 25% SOP; 50% KCl + 50% SOP; 25% KCl + 75% SOP; 100% SOP and100% KCl via soil + two foliar applications of SOP (3% K_2SO_4). Supplemental foliar spray of SOP was conducted every year in two stages, being the first application done at the end of the flowering period, and the second at the beginning of the grain filling phase. The sprays were performed using volume equivalent to 400 L ha^{-1}. In addition to K, phosphorus (P), nitrogen (N), calcium (Ca) and boron (B) were applied annually to the soil in the following doses: 20 kg ha^{-1} of P_2O_5, 250 kg ha^{-1} of N, 148 kg ha^{-1} of Ca and 2.4 kg ha^{-1} of B through mono-ammonium phosphate and ammonium nitrate + boric acid, according to Raij et al. (1997). These were the effects of the treatments on the chlorine content in the leaves, on the productivity and on the beverage quality of the coffee beans. The results were statistically analyzed using analysis of variance (ANOVA) and the means were compared by the t-student test ($P<0.05$).

RESULTS and discussion

The replacement of KCl by SOP decreased the absorption of chlorine by the coffee plant proportionally to the amounts of SOP used in the blend. The average foliar Cl content of the 4 crops studied decreased by 50% with the total replacement of KCl by SOP (Table 1).

Despite the decrease in foliar chlorine levels in all crops, the quality of the beverage only increased with the replacement of KCl application by SOP, via soil in high productivity crops. The scores on sensory tests increased by up to one point after replacing 50% of the KCl fertilization with SOP.

Supplemental foliar application of SOP did not reduce the effect of KCl application via soil on foliar Cl contents of the coffee tree, however, it increased the beverage quality grade in the 2018 and 2020 harvests, reaching a special coffee score. Potassium is essential in determining the physical and chemical characteristics of coffee beans. Silva et al. (1999; 2002) studied the effect of sources and doses of K provided by K2SO4 and KNO3, and concluded that fertilization with sources devoid of Cl provided better quality beans in arabica coffee, especially SOP.

According to Dalal et al. (2017), foliar potassium fertilization in fruit trees can promote the improvement of vegetative growth, flowering, correction of deficiency symptoms and, variably, fruit quality. The foliar application of K_2SO_4 in sweet orange cv. Jaffa, improved the qualitative characteristics, increased the average fruit weight, peel thickness, peel content, juice (%), acidity and ascorbic acid.

In addition to improving quality, foliar application of SOP increased the yield of coffee beans. Evaluating the accumulated productivity, it is verified that the application of this treatment increased the grain productivity in 9% in relation to the treatment 100% KCl via soil. The application of the highest percentages of SOP in the soil (75 and 100%) also increased coffee productivity, but the productivity gain accumulated in 4 seasons with these treatments was 3% lower than in the treatment with foliar SOP supplementation + 100 % KCl via soil.

Table 1. Effect of treatments on the chlorine content in the leaves, on the productivity and on the beverage quality of the coffee beans.

TREATMENTS	2017		2018		2019		2020		Acumulated	Average
	Yield	Drink Quality	Yield	Drink Quality	Yield	Drink Quality	Yield	Drink Quality	Yield	Cl Foliar Content*
	kg ha^{-1}	Score	kg ha^{-1}	Score	kg ha^{-1}	Score	kg ha^{-1}	Score	kg ha^{-1}	g Kg^{-1}
100%KCl	2448	78.0	4956abc	78.0	235	79.0	4796b	82.0	12435bc	1429.3a
75%KCl+25%K_2SO_4	2605	78.0	4520c	78.0	192	79.0	4907b	82.0	12223c	1267.6b
50%KCl+50%K_2SO_4	2648	78.0	4847abc	79.0	203	79.0	4765b	83.0	12463bc	1170.2b
25%KCl+75%K_2SO_4	2715	78.0	4603bc	79.0	242	79.0	5657a	83.0	13217ab	876.9c
100%K_2SO_4	2473	78.0	5031ab	79.0	221	79.0	5353ab	83.0	13079ab	680.7d
100%KCl+Foliar K_2SO_4	2720	78.0	5270a	80.0	172	79.0	5391ab	83.0	13553a	1508.1a

Effect of treatments on the chlorine content in the leaves, on the productivity and on the beverage quality of the coffee beans

CONCLUSIONS

Supplementary foliar application of potassium sulfate can attenuate the deleterious effects of chlorine on coffee, similarly to the replacement of KCl application by SOP in the soil, increasing productivity and coffee beverage quality. However, for this technology to be recommended to producers, it is necessary to carry out more studies to define doses and application times.

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HIGH ZINC DENSITY PEANUTS AS A FOOD CAN ALLEVIATE ZINC MALNUTRITION

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Keywords: high Zinc density ; peanut; food legume

High zinc density peanuts as a food can alleviate zinc malnutrition

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INTRODUCTION

Zinc is an essential nutrient for human and animal health, but nearly half of the world human population, mainly in Asia and African countries, are at the risk of its deficiency. This calls for a food-based solution to alleviate this malady. Peanuts, with 2-3 times higher Zn in their seed than the cereals, may be a solution as a food crop to ensure adequate level of Zn uptake in human (Singh et al 2017, Singh and Chaudhari, 2015). Also, it is an important food legume of Asia and Africa and, as a high-energy and protein comparatively at a low cost, it is consumed by a large populations (Singh et al 2021). Selection of high Zn containing peanut cultivars and advocating them as food may be a solution to combat the Zn malnutrition. Monitoring of micronutrient concentrations in seeds of peanut cultivars and their enhancement through genetic selection and biofortification through fertilizers, are the regular features of this Institute for the past 20 years. Here, we have summarised the result of the experiments conducted to identify the high Zn density peanut cultivars.

METHODS

A total of 230 peanut cultivars were investigated during last 15 years (70 cultivars during first five years, 110-120 cultivars during the second five and 190-230 cultivars during the last five years) grown in the field with medium black calcareous clayey soil (having 1.2-1.4 mg kg^{-1} DTPA extractable Zn) under recommended package of practices with and without application of Zn (1-2 kg ha^{-1} Zn as zinc sulphate). The seed samples of these were analyzed for Zn content using AAS and on the mean and standard deviation (SD) values of seed Zn concentration, every year the cultivars were categorized into three categories: high (Zn above mean plus SD values), low (Zn below mean minus SD values) and medium Zn density.

RESULTS and discussion

Among the 70 old peanut cultivars, mostly of small to medium seed size and investigated initially during the first five years, the seed Zn concentration ranged from 31 to 79 mg kg^{-1} with an average of 45 mg kg^{-1} without Zn. These values with application of 2 kg ha^{-1} Zn increased to 39-88 mg kg^{-1} with a mean of 51 mg kg^{-1} and the peanut cultivars having > 55 mg kg^{-1} in their seeds were categorised as high Zn cultivars.

Subsequently several large seeded cultivars with attractive colours were released in India and by adding these 110-120 peanut cultivars were studied in the next phase. The mean seed Zn content of these cultivars ranged from 21 to 81 mg kg^{-1} with a mean value of 43 mg kg^{-1} which with application of Zn increased to 24-87 mg kg^{-1} with a mean of 48 mg kg^{-1}. Here the peanut cultivars above 51 mg kg^{-1} were categorised as high Zn cultivars.

With advancement of breeding technologies, several specific character and biofortified peanut cultivars with very attractive colours and seed size and shapes were released and hence in the next phase 190-230 cultivars were studied, by adding all the latest released one. The mean seed Zn content of these cultivars ranged from 19 to 82 mg kg^{-1} with a mean value of 42 mg kg^{-1} which with application of Zn increased to 26-90 mg kg^{-1} with a mean of 47 mg kg^{-1}. Here the peanut cultivars above 50 mg kg^{-1} were categorised as high Zn cultivars.

Finally the peanut cultivars showing consistantly more than 50 mg kg^{-1} were listed as high Zn cultivars (Table 1).

Table 1. Peanut cultivars with their seed Zn content more than 50 mg kg^{-1}

BAU 13, CO 1, CO 2, CO 3, CSMG 884, DH 8, Gangapuri, GG 7, GG 8, GG 20, GJG 31, ICG (FDRS) 4, ICGS 76, ICGV 86590, Jawan, JL 24, Jyoti, Kadiri 5, Kopargaon 3, KRG1, MH 1, OG 52-1, R 9251, SB XI, SG 84, TAG 24, TG 32, Tirupati 1, Tirupati 3, Tirupati 4, TMV 2, TMV 13, VRI 2, VRI (GN) 16,

The study reveals that when grown under standard package of practices, though most of the cultivar particularly of large seeded one responded to the zinc fertilizers, 34 cultivars out of 230 showed stability in their seed Zn with more than 50 mg kg^{-1}. Of these mostly were either of small seeded (CO 1, CO 2, CO 3, GG 8, Jyoti, Kadiri 5, Kopargaon 3, KRG 1, SB XI, TAG 24, TG 32, Tirupati 1, Tirupati 3, Tirupati 4, TMV 2), or of medium seed size (DH 8, Gangapuri, GJG 31, ICG (FDRS) 4, ICGV 86590, Jawan, JL 24, MH 1, OG 52-1, R 9251, SG 84, TMV 13, VRI 2, VRI GN 16) and only a few of them were of large seeded one (BAU 13, CSMG 884, GG 7, GG 20, ICGS 76) one.

Out of these, 10 cultivars (GG 7, VRI (GN) 6, OG 52-1, GG 20, CSMG 884, ICGV 86590, ICGS 76, GJG 31, TAG 24 and VRI 2) are inherently high yielding too with a potential of producing more than 5 t ha^{-1} pod yield under balanced nutrition condition. Also most of them are under commercial cultivation in a localised pocket.

There is a need to spread cultivation of these high Zn density peanut cultivars and further consumption as a food in Asian and African countries to alleviate the prdominant Zn malnutrition of the population.

CONCLUSIONS

Peanut seeds are a good source of Zn with large variations among cultivars. The identified high Zn-density peanut cultivars need extensive cultivation and consumptions as food to combat the Zn malnutrition in Asian ans African countries. Further monitoring of micronutrient concentrations in seeds of peanut cultivars and their enhancement should be the regular features of Asian and African countries where Zn deficiency is more.

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NUTRITION AND YIELD OF ARABICA COFFEE AS AFFECTED BY PHOSPHOGYPSUM AND ORGANIC COMPOST APPLICATION AND CONGO GRASS INTERCROPPING

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Keywords: *Coffea arabica*; phosphogypsum; leaf diagnoses

INTRODUCTION

Arabica coffee (*Coffea arabica*) is a perennial plant, and a good development of its root system guarantees the stability of production, for this you can use phosphogypsum, this product has the characteristic of improving soil fertility by neutralizing Al^{3+} toxic in depth and carry nutrients to deep layers such as Ca^{2+}, Mg^{2+} and K^{+} (van Raij, 2008). Other practices can be adopted, such as the use of organic compost, which are easily found in the coffee growing regions of Brazil and the use of ground cover plants, covering the soil between the coffee tree rows can avoid the increase in temperature, in addition to nutrient cycling after cutting. These practices are beneficial to the coffee plant and can increase its productivity. The objective of this study is to evaluate the effects of the application of phosphogypsum and organic compost, as well as the intercrop cultivation of congo grass in plant nutrition and bean yield of Arabica coffee crop.

METHODS

The experiment has been conducted since February 2017, in a medium-textured Acrisol, cultivated with Arabica coffee, cv. Catuaí IAC 99, planted in the field in February 2016, located in the municipality of Manduri-SP. The design used is in randomized blocks, in a 3 × 2 × 2 factorial scheme and three replications. The treatments consist of a combination of three phosphogypsum rates [control - without application, recommended rate (1,200 kg ha^{-1}) according to van Raij et al. (1997) and high rate (40,000 kg ha^{-1})], applying annually or not organic compost (10,000 kg ha^{-1}) and the presence or absence of congo grass (*Urochloa ruziziensis*) cultivation between the rows.

In January of each year (2018-2019), leaves of the third or fourth node of plagiotropic branches (20 random leaves from the middle third of the plant per plot) were collected to evaluate the plant nutritional status (Malavolta et al., 1997). In July of each year (2018-2019), the coffee fruits from eight plants in the useful area of each plot were harvested. After the harvest, the fruits were dried and the yields of the hulled green beans per area (kg ha^{-1}).

RESULTS AND DISCUSSION

Leaf P, K, Ca, Mg, and S concentrations were influenced by the treatments in the two years of evaluation. Leaf P concentration was influenced by the triple interaction (phosphogypsum x compost x congo grass) in 2018. In the interaction, it was observed that in the treatment with the application of a high rate of phosphogypsum provided a higher P concentration, regardless of organic compost and cultivation of congo grass between the rows. In 2019, there was only an effect of the phosphogypsum × congo grass interaction on P concentration in coffee leaves, and it was observed that the treatments without application of phosphogypsum promoted higher levels of P in the leaves when congo grass was not cultivated between the rows.

There was an increase in the leaf K concentration with the application of the highest rate of phosphogypsum in both years, this fact may have occurred due to the increased exploration of the root system through the benefits of the application of phosphogypsum. It is worth mentioning that in 2018 the application of organic compost also increased the K concentration in the leaves, likely greater availability of this nutrient from the compost.

Leaf Ca concentration was influenced by the triple interaction (phospohogypsum x compost x congo grass) in 2018. In the interaction, it was observed that in the treatment with application of a high rate of phosphogypsum and with the cultivation of congo grass between the rows, when organic compost was applied, the Ca concentration was higher than when the compost was not applied, this is due to the fact that the organic compost has significant levels of Ca in its composition. In 2019, the leaf Ca concentration is not influenced by the phosphogypsum factor, but by the compost × congo grass interaction. In the interaction, it was observed that there was a significant difference in the treatment without organic compost, with higher levels of Ca in the leaves when congo grass was grown between the rows.

The application of a high rate of phosphogypsum reduced the leaf Mg concentration and the highest levels were observed in the treatment without phosphogypsum application in both years. There was an increase only in the second year when compost was applied in the absence of congo grass cultivation between the rows. The high rate of phosphogypsum may have carried the Mg to deeper layers of the soil, which the root system did not have good absorption.

The S concentration in coffee leaves was influenced by the phosphogypsum × compost interaction in both years, in which the highest S concentration was observed with the application of a high rate of phosphogypsum in the absence of compost application. In 2019, there was also the interaction of phosphogypsum x congo grass, where the highest levels were observed with the application of the high rate of phosphogypsum in the absence of the cultivation of congo grass.

In the 2018 harvest, the hulled green-bean yield was influenced by the three factors studied (phosphogypsum × compost × congo grass). In the triple interaction, the highest hulled green-bean yield (972,2 kg ha^{-1}) was observed in the treatment with the application of a high rate of phosphogypsum in the absence of the application of organic compost, providing an increase of 202% when there was cultivation of congo grass between the rows. In the 2019, there was an influence of the factors phosphhogypsum and congo grass, alone, the highest hulled green-bean yield (1274,6 kg ha^{-1}) was observed for the treatment with application of the high rate of phosphgypsum, about 259% more than treatment without phosphogypsum application, and the highest hulled green-bean yield (1084,2 kg ha^{-1}) of the treatment with the cultivation of congo grass between the rows provided an increase of 147% more than the treatment without the cultivation.

CONCLUSIONS

The application of a high rate of phosphogypsum (40,000 kg ha^{-1}) promoted higher P, Ca, and S concentration and reduction Mg concentration in the coffee plant diagnosis leaf, as well as providing the highest hulled green-bean yield in the two years. The application of organic compost promoted an increase in the levels of K in the coffee leaves, while the cultivation of congo grass between the rows resulted in an increase in coffee yield.

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'ROCHA' PEAR MINERAL COMPOSITION RELATION WITH QUALITY TRAITS

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Keywords: Fruit quality; Rocha pear; Orchard managment

INTRODUCTION

Orchard's variability is mainly affected by two preharvest factors: cultural practices and climate conditions. In the case of 'Rocha' pear, a native cultivar from Portugal with PDO, it is produced by different producers in the region that athough inflencuced by different factot try to guarantee the expected quality[1]. However, even at the same geographic region, dissimilarities between orchards exist. It is unclear whether differences are due to the producer's practices and/or biological responses to the abiotic factors, making the orchard management difficult.

Although this appears to be a bewildering complex of variables, Bramlage (1993) suggested that mineral composition at harvest accounts for most fruit variability between orchards. It is known that nutrients required are in the soil and supply by fertigation and foliar application[3]. In this way, fruit mineral characterization at harvest can give some information on how orchard factors interact with nutrients and consequently explain some physicochemical and ripening differences[4]. The objective of this study was to investigate differences in mineral composition of three 'Rocha' pear orchards from one of the most representative PDO locations and relate it with quality and ripening traits.

METHODS

A data set with fruit from three different orchards located in the "West Region" of Portugal (Cadaval and Bombarral) across 7 d of ripening was used and characterized. The orchards were installed on clay-loam soils, and pears were harvested at the optimal harvest period. Fruit quality was characterized by their firmness, skin colour, soluble solids content (SSC), sugars and organic acids, titratable acidity (TA), ethylene and esters production and respiration. Macro and micronutrients were also determined. Data collected from the three orchards across ripening were subjected to principal component analysis (PCA) to highlight which parameters are differentiating the orchards within the same geographic region and under the same climate conditions, and their interactions with minerals composition at harvest.

RESULTS AND DISCUSSION

A PCA for 0 and 7 d was done separately to exclude the influence of shelf-life. A clear differentiation between the three orchards is observed in figure 1, which is maintained across ripening. Mainly pears from O3 revealed a clear separation along PC1 from the other two orchards. It is evident that variables such as the emission of esters, SSC, sucrose, malic acid, and nutrients N, P, Cu and K showed the main influence for this differentiation. We can observe that the higher esters production is connected to higher content of Cu, K, P. Besides, O3 demonstrated to have lower sugar content. The higher esters and lower sugar content are associated with the over maturity of the fruit[3] and, thus, lower quality. This connection can reveal a different nutrition program applied to O3, which affected the postharvest secondary metabolism and, consequently, the quality of the pear, mainly in terms of higher unpleasant aroma release and lower sugars content. Moreover, although it is the same location, soil texture can be different between orchards and influence the uptake of nutrients from the soil. We can also realize that respiration is associated with Zn and affects pear's ripening from O1.

CONCLUSIONS

This research shows that some minerals are connected to the ripening and quality responses of 'Rocha' pear. This allows us to conclude that quality variation between orchards from the same location at harvest exists and can be due to several factors that producers should consider. Knowing the relation of specific minerals with quality parameters can help producers manage their orchards more efficiently to meet consumers' requests.

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SILICON (SI) ACCUMULATES IN THE ENDODERMIS OF BARLEY ROOTS AND THEREBY IMPROVES SALT TOLERANCE

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Keywords: Silicon; LaserAblation; Saltstress

Silicon (Si) accumulates in the endodermis of barley roots and thereby improves salt tolerance

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INTRODUCTION

Salinity is a world-wide problem, which greatly depresses agricultural yields. Salt stress has two main components viz. osmotic stress and stress from Na^+ toxicity. Barley plants are only moderately tolerant to salt stress and are negatively affected at concentrations above 50 mM NaCl. Silicon (Si) is coined as a beneficial element to barley and other grasses, as it helps plants to withstand abiotic stresses like salt stress. The beneficial effects have been demonstrated many times, but there is a lack of understanding of why and how Si exerts its beneficial effects during salt stress (Thorne et al. 2020). In this study, we employed multi-element bioimaging and advanced microscopy to provide insights into how Si alleviates salt stress.

METHODS

Barley plants (cv. *Irina*) were exposed to NaCl concentrations in the range 50-150 mM for 7 days, after either pretreatment with Si metaslilicate given to the roots for 7 days, or no pretreatment with Si prior to Na treatment. The Si treatment was continued also during the Na treatment. All plants were grown in a hydroponic culture. Transpiration, stomatal conductance and photosynthesis were measured with a portable photosynthesis instrument (CIRAS 3). The development of suberization was quantified with fluorol yellow (FY) staining and confocal microscopy (Chen et al. 2019) and total Na-concentrations were measured by Inductively Coupled Plasma Mass Spectromety (ICP-MS). Cross sections from the mature root zone of both seminal and nodal roots were also analyzed by Laser Ablation ICP-MS, according to Persson et al. (2016) (Fig. 1).

RESULTS and discussion

Measurements of biomass production, transpiration, stomatal conductance and photosynthesis all showed indicated that Si alleviated the negative effects of salt stress. Indeed, plants treated with Si contained less Na^+ than plants not treated with Si, both in their shoots, nodal and seminal roots. The suberization of the roots, which typically increase in response to NaCl stress, was however not significantly higher in +Si plants compared to -Si plants, and therefore additional explanations to why NaCl uptake and translocation was suppressed in plants given Si, were sought after.

By use of LA-ICP-MS bioimaging, we show that Si specifically accumulated in the endodermis where Si deposition had the same pattern of development as suberin (Fig. 1). Furthermore, plants with a silicified endodermis suppressed the radial inwards movement of Na^+ ions more efficiently than -Si plants, which resulted in significantly lower Na concentrations in the stele (Fig. 1, bottom graph). We also observed that Si suppressed the apoplastic by-pass flow in root tips, which likely further supressed the shoot Na concentration.

Conclusion

Si accumulates specifically in the endodermis, where it reinforces the already suberized endodermal cells. This process enabled the roots to supress the amount of Na^+ ions reaching the stele for upwards translocation to the shoots. This makes +Si plants better able to reduce the uptake and translocation of Na than -Si plants.

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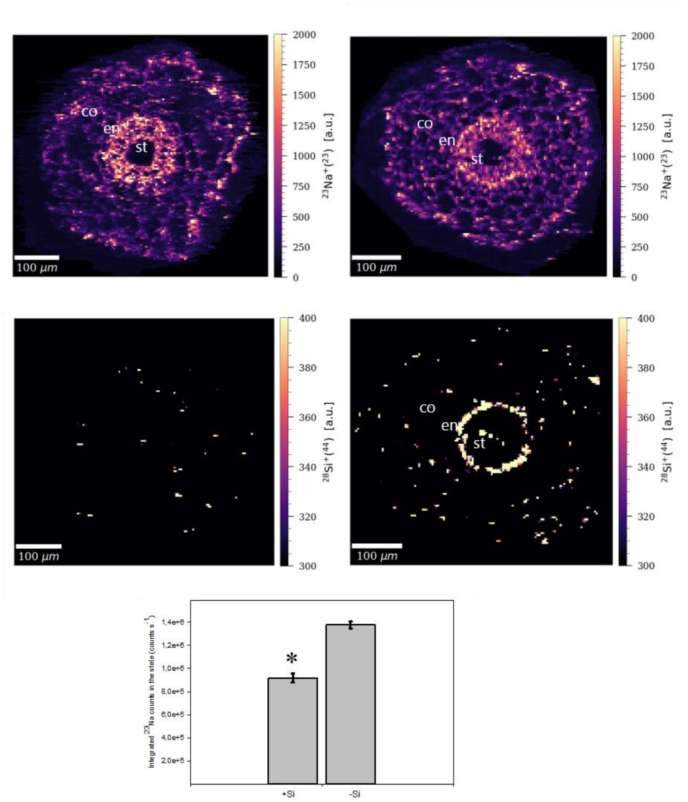

FIG. 1. Cross-sections from the mature, suberized root zone of 5-weeks old barley seminal roots, either pretreated with (images to the right) or without Si (images to the left) added to the hydroponic culture. Samples were analyzed by LA-ICP-MS, where the 23Na (upper images) and 28Si (lower images) signals were collected. All plants were treated with 100 mM NaCl for 7 days. All signals represent counts per second (a.u.) and the 23Na and 28Si signals are presented in the same scale. The 28Si signal in the Si-treated plant (lower right) correlates perfectly with the suberized endodermis, whereas it is absent in the -Si plant (lower left). The graph (bottom) shows the integrated counts of 23Na inside of the endodermis, thus respresenting the mean Na-signal in the stele of +Si (left bar) and -Si plants (right bar), respectively (n=3). Scalebars are 100 µm; en=endodermis, st=stele, co=cortex.

PHOSPHORUS AND SELENIUM INTERACTION IN AGRONOMIC BIOFORTIFICATION OF COWPEA PLANTS

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Keywords: *Vigna unguiculata*; Chlorophyll; Sucrose

INTRODUCTION

Selenium (Se) is an essential nutrient for human health (Ekuma et al. 2021). However, available Se is scarce in most soils and, thus, the edible parts of crops usually have low Se concentrations (Lanza and Reis 2021). Among the elements related to hidden hunger, Se deficiency is the third most common nutrient deficiency in humans and affects about 1 billion people around the world (Schiavon and Pilon-Smits 2017). Agronomic biofortification is a proven strategy to combat hidden hunger. The approach consists of enriching edible parts of crops with nutrients, such as Se, aiming to increase these nutrients in the human diet (White andBroadley 2009). Selenium is absorbed and transported in plants by phosphorus (P) transporters when applied as selenate (White andBroadley 2009). Thus, the interaction between P and Se application can decrease Se uptake by plants.

Cowpea (*Vigna unguiculata* (L.) Walp) is resistant to abiotic and biotic stress. Cowpea seeds are one of the most important food in low income countries (Manzeke et al. 2017). Previous reports demonstrated that, under adequate Se application, cowpea can accumulate Se at safe concentrations in seeds. Selenium application also increases leaf chlorphyll and sucrose content in cowpea leaves under adequate P supply (Silva et al. 2020). Thus, the interaction of Se with P in cowpea plants could be a useful line of investigation in trying to optimize Se biofortification in this crop. Thus, this study aimed to evaluate the effect of Se and P interaction on cowpea on yield, Se accumulation in cowpea seeds, as well as the leaf and sucrose content cowpea leaves.

METHODS

The experiment was carried out under greenhouse conditions at Federal Institute of Mato Grosso do Sul (IFMS), Nova Andradina, Mato Grosso do Sul State, Brazil. Two genotypes of cowpea (BRS XiqueXique and BRS Tumucumaque) were cultivated in response to five Se doses: 0, 5, 10, 20 and 40 g ha^{-1} combinated with two P supply: Adequate (30 mg dm^{-3}) and Low (1 mg dm^{-3}). The experimental design was completely randomized, with five replications for each genotype totaling 100 pots. The soil was collected from IFMS experimental farm and sieved 4 mm mesh to fill 5 kg pots.The soil was classified as Oxisol.The pots filled with soil were kept at incubation for 30 days before sowing and the experiment was carried out for 90 days after sowing. Harvest was performed across a series of days for each genotype according to the pod's maturity. The plant material was separated into three parts: grains, leaves + stems and roots. The separated material was dried in an oven at 65 ?C for 72 h to a constant mass to measure the dry weight (DW) $plant^{-1}$ of grains. Then, the material was homogenized in a Wiley mill for further nutrient analysis, leaf chorophyll (Lichtenthaler 1987) and sucrose analysis (Bielesk and Turner 1966). The variance analysis (F test) was performed. When differences were observed among treatments, a Tukey test at 5% probability was used to compare the means. Analysis was performed in the R software (version 3.5.1).

RESULTS AND DISCUSSION

Plants grown with adequate P supply had higher chlorophyll and sucrose content in the leaf. In addition, plants grown with adequate P demonstrate an effect of Se doses on Se accumulation in grains. This was not observed in plants grown in low P supply (Tab 1). Although P and Se are non-congeners, both are absorbed by plants when present in the anionic form in the soil, and they have similar ionic radii and physical and chemical properties. Hence, competition with oxyacid anions in soil is expected to affect the absorption and accumulation of Se by plants. However, current literature reports on the relationship between plant Se uptake and P levels and acquisition have shown conflicting results. The phosphate ion can significantly reduce Se adsorption from the soil by competition, and increasing Se concentraion in soil-solution (Zhou et al., 2015). Nie et al. (2020) also demonstrated that all Se fractions (total, organic, inorganic) in the grain of winter wheat were significantly decreased under increasing P application rates. Therefore, in agricultural production, it is very important to avoid excessive use of P fertilizer, so that a balance is achieved between higher yield and appropriate grain Se concentration. Proper P management is crucial to grain Se concentration control.

Table 1. Grain Yield, Grain Se, Chlorophyll and Sacrose concentration of cowpea plants in response to Se and P combination. Different letters indicate difference between means according to tukey test ($p \leq 0.05$).

CONCLUSIONS

The interaction between Se and P application did not impair the growth or yield of cowpea. However high levels of P application can increase Se accumulation in grains of cowpea. Thus, the supply of P should be performed carefully in crops cultivated for agronomic biofortification of seeds with Se. The wide variation in seed quality indicate that Se and P interaction in the plant could be complex, and further studies should be performed to investigate how Se and P can regulate the accumulation of sacrose en chlorohyll.

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EFFECTS OF MYCORRHIZAL COLONIZATION AND VARIED PHOSPHOROUS SUPPLY ON CADMIUM ACCUMULATION IN AEROBIC RICE PLANTS

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Keywords: Cadmium; Rice; Mycorrhizal fungi

INTRODUCTION

Cadmium (Cd) is a heavy metal and may exert harmful effects on environment and humans depending on its concentrations. The release of Cd into the environment occurs due to natural and anthropogenic activities such as industrial and agricultural practices including application of phosphorus (P) fertilizers (McLaughlin et al., 2021). Cadmium is found in phosphate (P) fertilizers as a contaminant, and therefore use of P fertilizers with high Cd is often associated with increased Cd accumulation in soils and plants. However, several previous and recent studies showed that Cd accumulation in plants treated with P fertilization is independent of Cd content of the P fertilizers applied (McLaughlin et al., 1995; Grant et al., 2002; Yazici et al., 2021). It was shown that diminished mycorrhizal root colonization by increasing P fertilization plays a key role in P-fertilization related Cd accumulation in plants as discussed by Yazici et al. (2021). It is known that mycorrhizal fungi is an important barrier against transfer of Cd from soil in the shoot parts of plants.

Rice-based foods are known to be a major dietary source of Cd exposure for the non-smoking people (Shi et al., 2020; Zhao and Wang, 2020). Therefore, it is important to study the factors affecting Cd accumulation in rice plants by paying particular attention to P fertilization and root mycorrhizal activity.

METHODS

A pot experiment was performed at the greenhouse of Sabanci University to study the effects of P fertilization and root mycorrhizal activity on shoot Cd concentrations of rice (*Oryza sativa* L. cv. Rekor CL) grown aerobically. The experiment was conducted on a calcareous soil (pH:7.8), fertilized with three P rates (20, 60, 180 mg P kg^{-1}) in the form of diammonium phosphate (DAP) containing 28 mg Cd kg^{-1}. In order to investigate the role of mycorrhizal activity, the experiment was realized in native and sterilized soil and half of both sterilized and unsterilized soils was inoculated with a mycorrhizal fungus. The study was set up as a complete factorial design that was randomized with five replicates per treatment. Plants were harvested on day 32 after sowing and analyzed for shoot dry weight and shoot concentrations of Cd, Zn and P.

RESULTS AND DISCUSSION

The effects of soil sterilization, different rates of P fertilizer, and mycorrhizal inoculum were presented in Table 1. Generally, the dry matter production of plants showed a reduction with the increase in P supply compared to low P supply. At each treatment, increasing P supply increased shoot concentration and accumulation of Cd. Soil sterilization promoted shoot Cd concentration and accumulation, especially in case of lowest P supply. By contrast, soil sterilization reduced shoot Zn concentration, but did not affect shoot P (Table 1).

Most probably, the increases in shoot Cd accumulation both by soil sterilization and by increasing P fertilization is related to suppression of root mycorrhizal colonization. When the plants were inoculated with mycorrhizae, there was a particular reduction in shoot Cd accumulation, especially at the lowest P treatment (with potentially high root mycorrhizal colonization). Based on these results,

Table 1. Effect of increasing P supply in the form of DAP on dry weight, shoot Cd concentration and content (i.e., accumulation), and shoot concentration of Zn and P in 32 days-old rice plants grown aerobically in native and sterilized soil with and without mycorrhizal inoculation.

Mycorrhizae	Soil	P	DW	Cd	Cd content	Zn	P

		(mg kg^{-1})	(mg plant^{-1})	(µg kg^{-1})	(ng plant^{-1})	(mg kg^{-1})	(%)
No	Unsterilized	20	209	6.32	1.29	5.00	0.08
		60	132	13.42	1.75	4.73	0.17
		180	160	20.61	3.33	5.08	0.27
	Sterilized	20	197	15.45	3.04	4.33	0.08
		60	171	23.13	3.98	3.90	0.16
		180	138	38.54	5.40	3.84	0.26
Yes	Unsterilized	20	199	3.78	0.75	8.50	0.15
		60	216	3.33	0.71	7.15	0.16
		180	167	11.79	1.95	5.73	0.19
	Sterilized	20	142	3.30	0.46	7.82	0.13
		60	162	5.35	0.86	6.39	0.18
		180	123	16.03	1.99	4.77	0.24

it can be suggested that mycorrhizae play a key role in reducing Cd transfer from soil in the shoot part of rice plants grown aerobically. Whether the soil was sterilized or unsterilized, the addition of mycorrhizae also enhanced shoot Zn concentration. Mycorrhizal fungi is known with its higher Cd retention capacity in roots and improves root Zn uptake. Since Zn and Cd are antagonistic during root uptake, we suggest that mycorrhizae reduce shoot Cd accumulation by immobilizing Cd in soil and by increasing root Zn uptake from soil.

CONCLUSIONS

The present study highlights the significance of root mycorrhizal activity in limiting Cd transfer from soils in the above-ground parts of the rice plants cultivated under aerobic conditions. Maintaining high root mycorrhizal colonization in aerobically grown rice would be an important strategy to reducing Cd accumulation in shoots and grains.

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NUTRITION AND NUTRIENT REMOVAL OF INDUSTRIAL POTATO AS A FUNCTION OF POTASSIUM SOURCES AND MANAGEMENT

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Keywords: Potassium chloride; *Solanum tuberosum*; Sustainable

INTRODUCTION

Sustainable and high productivity cropping systems require efficient and well-structured nutrient management strategies, aiming to establish adequate recommendations, as well as the definition of the best sources, rate, and application times. In Brazil, potassium chloride (KCl) is the main source of potassium (K) in potato cultivation. However, the application of high and continuous rate of KCl in the soil can cause damage, mainly to the photosynthetic process, with consequences in tuber yield and quality. Alternatives have been sought to improve the management and/or replacement of KCl by other sources with the absence or lower content of chlorine (Cl), as potassium sulfate (K_2SO_4), double potassium and magnesium sulfate (double sulfate, $K_2SO_4.2MgSO_4$), potassium and magnesium polysulfate [polysulfate, $K_2Ca_2Mg(SO_4)_4.2H_2O$], phonolite, and controlled-release KCl. Given these possibilities, the objective of this work was to evaluate the nutrition and removal of nutrients by the potato cultivar Asterix as a function of the sources and managements of K fertilization.

METHODS

The work was carried out in randomized blocks, in a area of potato production located in Perdizes-MG. The soil had low K content (<1,5 $mmol_c\ dm^{-3}$). The experiment was carried out in randomized blocks, with 15 treatments (Table 1) and four replications. The following evaluations were evaluated: foliar diagnosis (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn), tuber yield, and the removal of nutrients. Plant tissue analyses were made based in methods reported by Malavolta et al. (1997). Data were submitted to analysis of variance and compared by LSD tests at 5% probability.

Table 1. Treatments of the study, with sources, rate, and application time of potassium fertilization in the potato crop.

Treatments	Products	K_2O rate	Application time	
			Banded	Broadcast
		Rate K_2O ($kg\ ha^{-1}$) / Products		
1	Control	0	0	0
2	KCl banded	350	350	0
3	KCl banded	235	235	0
4	KCl split	350	245	105
5	KCl split	235	130	105
6	KCl + double sulfate	350	245 KCl	105 double sulfate
7	KCl + double sulfate	235	130 KCl	105 double sulfate
8	KCl + polysulfate	350	245 KCl	105 polysulfate

9	KCl + polysulfate	235	130 KCl	105 polysulfate
10	KCl + K_2SO_4	350	245 KCl	105 K_2SO_4
11	KCl + K_2SO_4	235	130 KCl	105 K_2SO_4
12	Phonolite + KCl	350	350	0
13	Phonolite + KCl	235	235	0
14	Controled-release KCl	350	350	0
15	Controled-release KCl	235	235	0

RESULTS AND DISCUSSION

There was no significant difference between the management of potassium fertilization regarding the leaf concentrations of N, P, Ca, Mg, S, Cu, Fe, Mg, and Zn in relation to the control. Leaf concentrations of P, Ca, S, and Mn were within the range considered adequate (Lorenzi et al., 1997); however, the leaf concentration of N , Mg, Cu, Fe, and Zn were superior to the adequate range. Leaf K concentration was significantly influenced by the K treatments. The use of KCl at a rate of 350 kg K_2O ha^{-1} in split application increased the leaf K concetration in relation to the control, split-applied KCl + K_2SO_4 (235 K_2O) and Phonolite + KCl (235 K_2O). Leaf K concentrations in all K management were within the range considered adequate for the crop.

At harvest, a significant difference was detected between treatments in terms of K sources and management, with a tendency to increase from 21.3, 18.51 to 16.51% in tuber yield for treatments T10, T6, and T8 in relation to control, respectively. The highest marketable tuber yield values were also observed in treatments T10, T6, and T8; however, without difference from the control treatment.

The removal of nutrients were not significantly influenced by the studied sources and methods of K fertilization. However, treatments T10, T2, and T8 tended to increase the K removal by tubers. Although not statistically superior, fertilization with 350 kg K_2O ha^{-1}, on average, contributed to an increase both in tuber yields and K removal.

CONCLUSIONS

Leaf K concetration was increased with the application of a rate of 350 kg K_2O ha^{-1}. The use KCl + K_2SO_4 (350 kg K_2O ha^{-1}), KCl + double sulfate (350 kg K_2O ha^{-1}), and KCl + polisulfato (350 kg K_2O ha^{-1}) tended to increase tuber yield. The removal nutrients by potato tubers were not influenced by sources and managements of K fertilizer.

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LIMESTONE AND PHOSPHOGYPSUM COMBINED INCREASED THE PERFORMANCE OF EUCALYPTUS IN THE BRAZILIAN CERRADO

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Keywords: nutrients use efficiency; soil amendments; sustainability

INTRODUCTION

Highly weathered soils frequently show a chemical barrier for root system development due to low Ca availability, high Al activity and overall low fertility (Antonangelo et al. 2017; Bordron et al. 2019). Eucalypt is known for being well adapted to such conditions, and soil ammendments are usually added to provide Ca and Mg, but not really to ameliorate chemical conditions (Barros and Novais 1999). Given the effectiveness that lime (L) and phosphogypsum (G) have been showing in tropical conditions in terms of improving root system development and overall crop productivity, resilience, and efficiency (Caires et al., 2004), the aim of this work was to test the influence of L and G on the efficiency and productivity of eucalypt planted forests in Brazil.

METHODS

Two field experiments in commercial eucalypt plantations were carried out over 7 years (full cycle) in the Brazilian Cerrado, one in Três Marias-MG (sandy soil - 20% clay) and the other in Curvelo-MG (clay soil - 62% clay). The 9 treatments applied in each location can be grouped into three: Group 1 - three treatments receiving only NPK (control treatments); Group 2 - four treatments receiving NPK +L; and Group 3 - two treatments receiving NPK +L and +G combined.

RESULTS and discussion

The application of +L and +L+G increased eucalypt productivity, with +L+G showing the best results and increasing productivity by up to 58% compared to NPK only (Fig. 1).

Chart, bar chart Description automatically generated

Fig. 1. Wood volume at the end of the cycle (7 years - m^3 ha^{-1}). NI = non-incorporated; I = incorporated; Ba = banded application (70 cm wide); Br = broadcasted in the whole area.

The use of +L in eucalypt forests increased the absorption of all nutrients. As it is a source of Ca and Mg, it increased the content of both nutrients (Fig. 2a,b), but also S (Fig. 2c). By adding +G we are keeping the same amount of Ca as in +L but decreasing Mg application and bringing an aditional nutrient, S, which is increasing the absorption of all the other nutrients even further. It is interesting to notice that while Ca and Mg are stored mainly in the bark, branches and leaves, S is stored mostly in the wood (Fig. 2a,b,c), which has implications for the management of residues and nutrition of planted forests. The NUE of Ca, Mg, and S confirmed that +L+G increases plant's use efficiencies of nutrients (Fig. 2d). Regarding N, P and K, +L increased its contents by 30.2, 23.7, and 30.0%, respectively, while +L+G increased it by 54.4, 51.8, and 54.2% against the controls (Fig. 3).

Fig. 2. Content (kg ha^{-1}) of Ca (a), Mg (b), and S (c) in the different components of the tree at the end of the cycle and Ca, Mg, and S use efficiency (%).

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Fig. 3. Content (kg ha^{-1}) of N, P e K in the whole shoot at the end of the cycle.

CONCLUSIONS

Lime and phosphogypsum are important drivers of the productivity of eucalypt planted forests, increasing plants efficiency to explore better the environment, particularly nutrients use efficiency.

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INOCULATION WITH *BACILLUS SUBTILIS* REDUCE LEAF NITRATE ACCUMULATION AND INCREASE LETTUCE LEAF YIELD IN HYDROPONICS

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Keywords: Nutrient accumulation; Nitrogen; Plant growth-promoting bacteria

INTRODUCTION

Lettuce is the leafy vegetable most produced in hydroponic system and consumed in the world, as it has desirable culinary characteristics for consumption "in natura" in the form of salads. The increase of nutrient concentrations in the nutrient solution, mensured by electrical conductivity, in a hydroponic system reduces the consumption of water in agriculture in relation to the soil, increases the water use efficiency by plants, the precocity and productivity of lettuce (Dalastra et al., 2020). However, the major problem with hydroponic crops is the high accumulation of nitrate in vegetables.

Bacillus subtilis has the ability to promote plant-growth by increasing plant hormone concentrations in plants, producing siderophores and antibiotics (Angelina et al., 2020). Also we belive that the *B. subtilis* can stimule the activity of nitrate reductase (NR) enzyme, and managing to reduce NO_3^- accumulation in plant leaves. However, the use of this beneficial bacterium applied via nutrient solution in hydroponics has not yet been studied, therefore the objective of this research was to investigate the effect of inoculant doses containing *B. subtilis* in the accumulation of NO_3^-, N, P and K, and in the productivity of crisphead lettuce leaves grown in a hydroponic system.

METHODS

The Nutrient Film Technique (NFT) hydroponic cultivation system for lettuce was developed in a greenhouse with 30% shading at Universidade Estadual Paulista - (UNESP), Ilha Solteira, state of São Paulo, Brazil. The experiment was installed under individual benches of six meters in length with a 10% slope. The cultivation channels are made of PVC with a rectangular section (8 cm wide and 4 cm high). The plants were accommodated every 25 cm, with a flow rate of 1 L min^{-1}. The experiment was carried out in randomized blocks with five replications, the treatments consisted of inoculant doses containing *Bacillus subtilis* strain CCTB04 (guarantee of $2x10^8$ CFU mL^{-1}), at doses of 0 (non-inoculated control), 7.8, 15.6, 31.2 and 62.4. mL of liquid inoculant for every 100 liters of nutrient solution, supplied only on the day of transplanting crisphead lettuce seedlings cv. Angelina. The electrical conductivity was adjusted as required by the plants: 1.3 dS m^{-1} (0-10 day), 1.5 dS m^{-1} (11-20 day) and 1.7 dS m^{-1} (21-30 day), at 31 days was harvest was carried out. Electrical conductivity adjustment and pH correction were performed daily. The nutrient solution used was a combined hydrogood fert + calcium nitrate + Fe 6% in the proportion (1:0.74:0.03).

Lettuce yield was based on the equation: shoot fresh mass in kg X plant population m^{-2} = yield in kg m^{-2}, with a plant population of 19.5 plants m^{-2}. The concentrations of N, P and K were determined based on the methodology of Malavolta et al. (1997), and of NO_3^- and NH_4^+ based on the methodology of Cataldo et al. (1975), the accumulation was obtained from the dry mass in kg m^{-2} X concentration in g kg^{-1} of N, P and K.

The means relative to doses of inoculant with *B. subtilis* were adjusted in the form of polynomial regression at 5% probability.

RESULTS and discussion There was a linear increase in leaf NH_4^+ accumulation and linear reduction in leaf NO_3^- accumulation with increasing doses of inoculant containing *B. subtilis* (Figures 1A and 1B). The increase in NR activity reduces NO_3^- while synthesis NH_4^+ and aminoacids that improve plant leaves development (Tablaglio et al., 2020). The highest leaf N, P and K accumulation, and crisphead lettuce leaf yield were observed at estimated doses of 27.0, 24.5, 17.4 and 33.6 ml $100L^{-1}$, respectively (Figures 1C, 1D, 1E and 1F). *Bacillus subtilis* has multiple growth-promoting characteristics, including the ability to grow at higher levels of alkaline stress and pH, and the ability to solubilize phosphorus, so it can efficiently colonize salt-affected roots and increase plant nutrition (AHMAD et al., 2018).

Figure 1. Effect of inoculant doses containing *B. subtilis* on leaf ammonium (NH_4^+) accumulation (A), nitrate (NO_3^-) accumulation (B), nitrogen (N) accumulation (C), phosphorus (P) accumulation (D), potassium (K) accumulation (E), and leaf yield (F) of crisphead lettuce grown in a NFT hydroponic system.

CONCLUSIONS Inoculation with *B. subtilis* increased leaf yield of crisphead lettuce up to dose 33.6 ml $100L^{-1}$, has a positive effect on leaf N, P and K accumulation up to an optimal dose, and reduced linearly leaf nitrate accumulation, which is a worldwide problem.

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THE INFLUENCE OF VERMICOMPOST ON PLANTS AGRONOMIC PERFORMANCES - A REVIEW

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Keywords: Vermicompost; plants; growth, quality and plant protection

INTRODUCTION

Vermicompost tea is a liquid vermicompost solution made by combining vermicompost with water and fermenting it for a set period of time. There are two kinds of compost tea: aerated compost tea and nonaerated compost tea. Nutrients and microorganisms are extracted from compost while it is steeped in water. The presence of microorganisms converts insoluble nutrients into soluble forms, which in turn promotes a diverse range of organisms in vermicompost tea during the brewing process (Yatoo et al., 2021)

Both methods (aerated and non-aerated) of producing vermicompost tea involve brewing matured compost in water for a specific period of time and requiring filtration before application to plants. Regardless of the brewing method used, the de-chlorination of water is critical to enhancing microbe growth and multiplication, as the presence of chlorine and chloramines can inhibit growth and propagation. This is easily accomplished by leaving tap water in the brewing container overnight or by aerating it for 20 to 120 minutes (Yatoo et al., 2021).

Aerated tea is made by aerating well-matured compost suspended in water for 12 to 24 hours. Aside from active aeration and compost source, the final biochemical characteristics of vermicompost tea are often dependent on a number of factors and are often supplemented with nutrients, microbial inoculants, and oxygen to enhance its biological activity, whereas nonaerated tea is made by suspending compost or a bag of compost in a bucket of water for 14 days so that nutrients and anaerobic microorganisms are extracted, which are then used to enhance growth and strength in crops (Yatoo et al., 2021).

The aim of this review article is to describe the effect of vermicompost tea on plants growth, yield, quality and plant protection.

PLANT PRODUCTION

All VC teas increased plant production in the same way, and this effect was most noticeable when organic fertilization was used (Sarma et al., 2010). Vermicompost teas are increasing the yield of the plant production. According to a meta-analysis, adding the vermicompost to soil increases commercial crop production by 26 percent, overall biomass by 13 percent, and root and shoot biomass by 57 and 78 percent, respectively (Yatoo et al., 2021).

CHEMICAL CONTENT

All VC (vermicompost) teas increased mineral nutrients, and total carotenoids in the same way, and this effect was most noticeable when organic fertilization was used. Organic fertilisation resulted in higher antioxidant activity and total phenolics in plants than synthetic fertilisation (Sarma et al., 2010).

DISEASE AND PEST MANAGEMENT

In the last two decades, the liquid solution of vermicompost, i.e., vermicompost tea, has been widely used for the management of plant diseases and pests. Vermicompost tea, also known as organic biofertilizer, contains microbes, nutrients, and plant growth promoters, and its use improves seed germination, growth and yield enhancement, and plant disease suppression (Yatoo et al., 2021).

It is thought that nutrients and microbes are transferred from vermicast into a liquid solution known as vermicompost tea, making it more useful. Vermicompost teas also have significant pest control potential due to the presence of phenolic substances that render plant tissues unpalatable (Yatoo et al., 2021).

Scientists tested different types of compost tea (aerated VC tea (ACTV), non-aerated VC tea (NCTV), aerated compost tea (ACTC), and non-aerated compost tea (NCTV) for suppression of the foot rot disease of rice caused by Fusarium moniliforme, and the results were compared with carbendazim treatment). Among the compost teas, ACTV produced the most healthy seedlings. Treatment of rice seeds with compost tea revealed the highest efficiency of ACTV in reducing the number of affected seeds in comparison to the field trial experiment. The use of compost tea also increased the percentage of seeds that germinated, with ACTV having the greatest effect (Sarma et al., 2010).

Extracts made from composted cow manure, composted pine bark, organic farm compost, or composted yard waste were applied as foliar sprays to tomato transplants and resulted in a moderate but statistically significant reduction in the severity of bacterial spots caused by Xanthomonas vesi catoria. Extracts prepared from composted cow manure significantly reduced the population of X. vesicatoria in infected leaves. Foliar sprays with the most effective compost extracts provided control comparable to that of the plant activator acibenzolar-S-methyl (Sarma et al., 2010).

Scientists used concentrated VC tea against CMM in vitro as well as on young tomato seedlings inoculated with the pathogen in a greenhouse setting. They discovered that VC tea could prevent the occurrence of bacterial canker on tomato plants caused by CMM in greenhouse conditions (Sarma et al., 2010).

Vermicompost tea can also coat leaf surfaces, reducing available sites for pathogen infection, or increasing microbial diversity, which can kill harmful pathogens (Yatoo et al., 2021).

Diseases and pests can be managed successfully without affecting human health or the environment by using vermicompost tea as eco-friendly organic amendments and as a replacement for inorganic pesticides and fungicides, and chemical-free food can be provided to humanity in the future (Yatoo et al., 2021).

CONCLUSIONS

As a result, these organic solutions are regarded as viable alternatives to chemical pesticides and fungicides, and they should be used more frequently to prevent disease and ensure food security and safety.

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CLOSING THE GAP IN CACAO NITROGEN NUTRITION NEEDS

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Keywords: Theobroma cacao L; Nitrogen nutrition; Crop Nutrition management

In recent years, cacao (*Theobroma Cacao L.*) yield has decreased substantially around the world. In the short and medium-term, sustainable production of cacao should rely on improved nutrition and control of pathogens and insect pests. The causal relations between these various factors, yields, and fertilizer response, as well as the requirements of cacao for different nutrients, are poorly understood due to a sparse and narrow research base.

Based on the above, in January 2021, we started a 4-year lysimeter experiment with the objective to obtain the seasonal water demand (i.e., crop coefficients) and nitrogen (N) uptake response curves of cacao trees grown from seeds of the variety CCN51. Among other parameters, we are monitoring vegetative growth, time to jorquetting, flowering, and pod development. Nitrogen was chosen since it is regularly the crop-limiting factor. K, P, Ca, Mg and trace elements are provided equally. The experimental setup allows us to determine the relations between N uptake, evapotranspiration and yield through an automated fertigation and drainage collecting system. It also enables us to characterize the seasonal trend in leaf N concentration and its relation to yield and assess the relationship between N allocation to the developing pods and Cherelle wilt.

One year into the project, significant effects of the N treatments (10 - 150 mg-N L^{-1}) on flowering and vegetative growth parameters such as the number of branches and flower cushions are already observed. Thus far, continuous fertigation with 75 mg-N L^{-1} has the best results. The relations between the N concentrations in the irrigation waters and those in the drainage water show periods with excessive N loads (i.e., in the 75 and 150 mg-N L^{-1} treatments), which might have a negative effect on the plants growth and reproduction.

REDUCED MYCORRHIZAL ACTIVITY IS A KEY FACTOR RESPONSIBLE FOR ACCUMULATION OF CADMIUM IN WHEAT PLANTS UNDER ELEVATED PHOSPHORUS FERTILIZATION

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Keywords: Phosphorus fertilization; Plant Cadmium; Arbuscular Mycorrhizae

INTRODUCTION

Cadmium (Cd) is readily transferred from soil to plant-based foods and build-up to the levels exceeding the safety limits. Several plant genetic, environmental and soil management factors are involved in soil solubility and root uptake of Cd (McLaughlin et al., 2021). Among these factors, probably phosphorus (P) fertilization is most widely studied and discussed factor in the Cd accumulation in plants.

As a contaminant Cd exists in most of the phosphorus (P) fertilizers and therefore P fertilizers often considered as an important source of Cd in plants and cultivated soils. However, increased plant Cd concentrations under high P fertilization are poorly correlated with the Cd content of the P fertilizers applied (Grant et al. 2002; Jiao et al. 2004). Recent studies suggested that by reducing root mycorrhizal colonization, increasing P fertilization promotes root uptake and shoot transport of Cd in plants (Yazici et al., 2021). Mycorrhizal fungi have a very high Cd immobilizing capacity (Joner et al., 2000) and therefore its abundancy and activity in soils appear to be of great importance in preventing Cd transfer from soils in the shoot parts of plants. This study was designed to investigate further the role of mycorrhizal fungi in Cd accumulation of plants by increasing P fertilization.

METHODS

Wheat, a mycorrhizal plant species, was grown under greenhouse conditions in native and sterilized soils inoculated with an active and inactive (autoclaved) mycorrhizal fungi. Phosphorus fertilizers (diammonium phosphate: DAP) with a high (40 mg Cd kg^{-1}) and trace amount (0.11 Cd mg kg^{-1}) were applied at different rates, and the experimental plants were analysed for concentrations of Cd and other elements during anthesis stage. Cadmium concentration of the plant samples collected (i.e., shoot and spikes) were analysed by using Inductively Coupled Plasma (ICP) Optical Emission Spectrometry (OES) and Mass Spectroscopy (MS) after digestion in 30% (v/v) premium-grade H_2O_2 and 65% (v/v) premium-grade HNO_3 in microwave system.

Results and discussions

In wheat, P fertilizers markedly increased shoot and spike Cd concentrations as P supply was enhanced, irrespective of their Cd concentration. Soil sterilization resulted in a strong increasing effect on Cd concentration of wheat plants, especially at lower P rates and clearly reduced the P fertilization associated enhancement in Cd concentration in shoots and spikes of wheat plants, probably by eliminating possible effects of mycorrhizae on Cd accumulation in shoots (Table 1). Indeed, inoculating the sterilized soil with active mycorrhizal fungi substantially reduced shoot Cd concentrations in wheat (Table 1). Accordingly, using inactivated mycorrhizal fungi attenuated the ability of inoculum to reduce Cd accumulation by wheat plants. Root mycorrhizal colonization was significantly reduced under high P supply as compared to low P supply in case of both native soil as well as in sterilized soil inoculated with exogenous mycorrhizae (data not shown).

These results highlight a particular role of arbuscular mycorrhizal fungi (AMF) in reducing Cd accumulation in plants grown in non-contaminated field soils. The possible mechanisms behind the

Table 1: Effect of P supply in form of diammonium phosphate (DAP) different in Cd contents on Cd concentrations of shoots and spikes of 68 days old wheat plants grown in unsterilized and sterilized (with and without mycorrhiza) soil. DAP fertilizers used had trace (0.11 mg Cd kg^{-1}) and high amounts (40 mg Cd kg^{-1}) of Cd.

Soil Treatment	P supply	Cd concentration			
		Shoot		Spike	
		Cd-trace DAP	High Cd DAP	Cd-trace DAP	High Cd DAP
	(mg kg^{-1})	(μg kg^{-1})			
Unsterilized	20	99	108	68	70
	60	131	153	94	95
	180	186	242	122	167
Sterilized	20	221	230	115	113
	60	205	233	125	160
	180	250	299	175	204
Sterilized + Active mycorrhizae	20	80	81	43	57
	60	106	125	65	72
	180	130	166	81	96
Sterilized + Inactive mycorrhiza	20	190	193	78	84
	60	187	242	106	138
	180	213	289	140	180

protective role of AMF in preventing Cd transfer from soil in the above-ground parts of plants have been discussed by Yazici et al. (2021). The results suggest that the factors diminishing root mycorrhizal activity such as P fertilization (irrespective of its Cd content) will increase Cd accumulation in mycorrhizal plants.

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ALTERNATIVE FOR NITROGEN SUPPLY VIA FOLIAR AND FERTIGATION TO POTATO CROP

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Keywords: *Solanum tuberosum*; nitrogen fertilization; tuber yield

INTRODUCTION

The second element most taken up by potato (*Solanum tuberosum* L.) is nitrogen (N). This element interferes with the balance between shoot growth and tuber growth, and in tropical regions, it is the nutrient that most limits tuber yield. Due to its shallow and poorly developed root system, when compared to other crops, the potato has a low efficiency of N uptake and requires high rates of this nutrient for its adequate availability during the crop growth cycle. An alternative is to split the application of N during the potato growing season, when the nutrient applied via soil. Also, the application of this element can be made via foliar or fertigation, however, there is little information about these ways of supplying N to the potato crop. In this context, a field experiment was carried out with the objective of evaluating the effect of N supply via foliar and fertigation, using a solution of urea plus K-Tionic®, on plant growth, N uptake, and tuber yield of potato crop.

METHODS

The experiment was carried out in a commercial area, located in the municipality of Paranapanema-SP, Brazil (latitude 23º32'25"S, longitude 48o41'17"W and altitude of 667 m), in a clayey Latosol, pH($CaCl_2$) of 5.1 and 57% base saturation. The cultivar Agata was planted in August 2021, using a spacing of 0.90 m between rows and 0.32 m between seed tubers. All treatments received 1740 kg ha^{-1} of the fertilizer N-P_2O_5-K_2O 03-24-11 + 6% Ca + 2.2% Mg + 6.6% S in the planting furrow. An application of 83 kg ha^{-1} of potassium chloride (KCl, 60% K_2O) was carried out beforehilling. The experimental design was in randomized blocks, with six treatments and four replications. The treatments consisted of: T1 - control (no topdressed N application, with a total of 52 kg N ha^{-1}); T2 - 45 kg N ha^{-1} applied via soil at hilling, 15, and 30 days after hilling (DAH) (total = 187 kg N ha^{-1}); T3 - 11.0 kg N ha^{-1} applied via foliar applied at hilling, 15, 30, and 50 DAH (total = 96 kg N ha^{-1}); T4 - 15.8 kg N ha^{-1} applied via foliar at hilling, 15, 30, and 50 DAH (total = 115 kg N ha^{-1}); T5 - 23.7 kg N ha^{-1} applied via fertigation at hilling, 15, and 30 DAH (total = 123 kg N ha^{-1}), and T6 - 11.0 kg N ha^{-1} applied via fertigation at hilling, 15, 30, and 50 DAH (total = 96 kg N ha^{-1}). The supply of N via soil was made using the ammonium nitrate as source. The N350 [solution with 35% urea or 15.8% N (m/v) + 1.5% K-Tionic® (v/v)] was used to supply N via foliar and fertigation. Foliar application of N was carried out using a backpack sprayer, with a CO_2 pressurizer, at a constant pressure of 2.0 kgf cm^{-2}, with Teejet spray tips, model TTi110.02, and 200 L ha^{-1}of spray volume. To simulate fertigation a watering can was used applying a volume of ~3mm of solution (water + N350). Irrigation was performed by a central pivot system, according to the technical recommendations for the crop in the region and criteria adopted by thefarmer, aiming to meet the water needs of the soil-plant system, throughout the crop gorwth cycle.

At 83 days after emergence (DAE), four plants were collected per plot to determine the dry matter (DM) in each part of the plant and N accumulation. The shoot and root materials used to determine the DM were ground and subjected to determination of total N concentration (Malavolta et al., 1997). The amount of N accumulated was obtained by multiplying the N concentration and DM in each part of the plant, with subsequent summation. Potato plants were desiccated at 85 DAE and the tubers were harvested at 93 DAE. Total and >45 mm tuber yields were measured. Agronomic efficiency (AE) was calculated as the increase in tuber yield gained over the control (without topdressed N application) in relation to applied N (except N applied in the planting furrow).

The data obtained were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Applications of 15.8 kg N ha^{-1} (100 L ha^{-1} of N350) via foliar provided slight symptoms of phytotoxicity (burning of the edges) in the potato crop leaves. Tuber and total plant DM (at 83 DAE) and tuber yield (at 93 DAE) were increased with the treatment T4 (4 applications of 15.8 kg N ha^{-1} via foliar), which differed significantly from the control (Table 1). The highest accumulations of N in the potato plant were obtained with the treatments T3 (4 applications of 11.0 kg N ha^{-1} via foliar), T4, and T6 (4 applications of 11.0 kg N ha^{-1} via fertigation) and the lowest values were verified in the control. The application of treatments T3, T4, and T5 (3 applications of 23.7 kg N ha^{-1} via fertigation) provided higher yields of potato tubers >45 mm in diameter, compared to the control, and also values of EA much higher than the treatment T2 (3 application of 45 kg N ha^{-1} via soil). These results show that the split application of N via foliar or fertigation is a viable alternative for the supply this element for potato plants, increasing tuber yield with lower N rates.

Table 1. Dry matter of shoot, tubers, and total, total N uptake, total and >45 mm tuber yield and agronomic efficiency (EA) of potato cv. Agata, depending on the forms and times of application of N.

Treatments(1)	Dry matter (kg ha^{-1})			N uptake (kg ha^{-1})	Tuber yield (Mg ha^{-1})		EA
	Shoot	Tubers	Total		Total	>45 mm	kg kg^{-1}
T1-Control	426.2a(2)	3,929b	4,355b	69.2b	33.6b	28.7b	-
T2-3x-Soil	494.1a	4,117ab	4,611ab	89.0ab	36.6ab	32.4ab	22
T3-4x-11,0kg-Foliar	501.1a	5,163ab	5,664ab	98.7a	40.4ab	36.9a	155
T4-4x-15,8kg-Foliar	471.0a	5,512a	5,983a	95.0a	43.5a	38.9a	157
T5-3x-23,7kg-Fert	409.7a	5,032ab	5,441ab	79.2ab	41.8ab	37.8a	116
T6-4x-11,0-Fert	473.3a	5,094ab	5,567ab	99.4a	37.4ab	33.7ab	85
F probability	0.448	0.015	0.028	0.007	0.025	0.006	-
CV (%)	15.9	13.0	13.0	12.1	10.0	9.7	-

(1) Soil: application with solid fertilizer (ammonium nitrate); Foliar: foliar application of N by spraying with 200 L ha^{-1} of solution; Fert: application simulating fertigation, with a volume of ~3mm. (2) Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability.

CONCLUSIONS

The application of N via foliar or fertigation, using the solution of urea plus K-Tionic® (N350), provided N efficiently and in a viable form to potato crop, promoting growth, N uptake and tuber yield similar to the application of this element via soil with solid fertilizer, with lower rates of N, which resulted in greater agronomic efficiency of the applied N.

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QUANTITATIVE AND QUALITATIVE ANALYSIS OF USDA SOYBEAN (GLYCINE MAX. L) GERMPLASM

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Keywords: USDA; *Glycine max.* L; Nutrition

Soybean (*Glycine max*. L Merril) is the most important crop and seed legume in the world which shares 25% in edible oil production and contribute 2/3rd of protein concentration for livestock and poultry in all over the world. It is used as main ingredient in more than 400 food products. Being leguminous crop, it is used as soil fertility restorer in maize, wheat, rice and cotton cropping systems. Use of soybean in animal feed is increasing, indicating that the demand for soybean will further increase. Soybean is also a good source of oil and its use for meeting the edible oil requirement will also add its increased demand in future.

Soybean crop is generally produced annually but Pakistan is among the few countries with the opportunity to harvest two crops (spring and autumn) in one year. Pakistan is spending over 500 million USD annually for the soybean import to meet its requirements. However, in Pakistan soybean production faces many challenges. Being highly photo-sensitive, soybean is not well adapted in Pakistani conditions and its cultivation faces many problems including: low germination, more vegetative growth, less flowering, less pod formation, less seed setting, improper seed filling, pod shattering, low yield and production of non-viable seeds.

Efforts to increase soybean production in Pakistan started in mid 1960s. Early success was reported, and soybean production was increased, but due to unavailability of industry, this success was not sustained. However, due to rapid increase in soybean demand, it is required to concentrate on soybean production in Pakistan. For this purpose, We have import the diverse soybean germplasm from different sources in Pakistan and analyze its qualitative and quantitative performance. Highly suitable soybean lines possessing early maturity, determinate growth, insect and pest resistance, high yielding characters and high oil and protein contents have identified. From this collection elite genotypes have selected and subjected to seed increase. Therefore, selected germplasm can be used for highly sustainable soybean breeding program in Pakistan.

MINERALS IN CORN GRAINS IN PRODUCTION SYSTEMS WITH BASALT POWDER AND UROCHLOA RUZIZIENSIS

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Keywords: Fertilizer managment; Forage; Remineralizers

INTRODUCTION

In 2020, Brazil produced more than 103.9 million tons of corn, with a production value of more than 73.9 billion reais (IBGE, 2021). The crop is one of the main agricultural commodities exported by Brazil, and is characterized by its various forms of use.

The integration of grain-producing crops combined with forage species, such as Urochloa ruzizienses, for example, is a practice widely used as an alternative for soil recharge in the off-season, and it is considered one of the best alternatives for the sustainability of tropical agricultural systems (BORGHI et al., 2013). This is due to the cycling of nutrients from the straw decomposition and the protection that these crops can provide to the surface layer of the soil (SANTOS et al., 2008).

The use of rock powder in agriculture appears as an alternative for employment in agriculture, with potential gains in economic and ecological terms, due to the low cost of the beneficiation process, which consists of grinding the rocks and applying them directly to the soil. This material is characterized by the gradual release of minerals that make up the rock, thus reducing leaching losses and favoring a long-term action of the applied input (HANISCH et at., 2013).

Every crop removed from the soil, also removes nutrients derived from the geological minerals present within the soil, and these need to be replaced, either by returning composted crop residues, manures etc, or by adding artificial fertilizers (Castellanos-Navarrete et al., 2015). Thus, this study aimed to evaluate the effect of off-season corn intercropped with Urochloa ruzizienses and the use of basalt rock powder, on the mineral content of corn grains.

METHODS

The experiment was conducted in the year of 2019. The local climate is classified as a Cwa type, defined as high-altitude tropical, with a rainy season in the summer and a dry season in the winter. The soil is classified as a clayey Distroferric Red Latosol type (SANTOS et al., 2018)

The crop succession system was adopted, alternating 300 thousand plants.ha-1 of soybean (Nov/Mar of 2020/2021), and 65 thousand plants.ha-1 of corn (Mar/Jul of 2021). The soil was prepared by raising the base saturation to 80%. The plots with U. ruziziensis had their seeds distributed along the planting row, at a density of 5 kg ha-1.

The design used was randomized blocks (4 blocks) in a 4 (fertilization) x 2 factorial scheme (with/without forage). Fertilizations were separated as the following ones: I) control (without fertilization), II) basalt powder (PB), III) monoammonium phosphate (MAP) + potassium chloride (KCl) and IV) MAP + KCl + PB. Basalt powder was applied in the experiment installation at a dosage of 5.0 t ha-1. Treatments with MAP and KCl were applied in each of the soybean and corn crop cycles.

The following minerals were evaluated according to the methodology proposed by Malavolta et al. (1997): phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and boron. (B). The evaluated grains were related to the winter crop of the year 2021.

The data were submitted to the analysis of variance and means were compared by Tukey's test at 5% probability, using the statistical analyses software AgroEstat®.

RESULTS and discussion

The fertilizer management did not differ statisticaly ($p > 0.05$) the mineral content of corn grains (Table 1). Differently from what we found in our data (not yet published) for the soybean crop, in which the fertilization affected the content of phosphorus, potassium, magnesium in the soybean grain.

Hanisch et al. (2013) found that the levels of Zn, Cu, Fe, Mn and P in the soil increased as the basalt powder doses increased, in the evaluations after 14 months of application, with the increase in phosphorus content ceasing in the subsequent evaluations (21 and 42 months after application).

The increase of nutrients in the soil does not necessarily mean an increase in plant tissue, as the entry and concentration of most nutrients is controlled by membrane proteins. In addition, there may be competition between minerals for transport sites in the absorption. Once absorbed, long-distance transport and mobility and metabolism of minerals can cause them to accumulate in different organs (TAIZ et al., 2017).

Table 1. Mineral contents in corn grains.

Manejo		P	K	Ca	Mg	S	Cu	Zn	Mn	Fe	B
		g kg-1					mg kg-1				
C	W.	0,47	0,067	0,0048	0,14	0,15	0,0028	0,061	0,0063	0,020	0,17
	W.o.	0,45	0,060	0,0038	0,13	0,17	0,0025	0,055	0,0055	0,029	0,18
PB	W.	0,42	0,065	0,0038	0,13	0,15	0,0027	0,059	0,0050	0,021	0,18
	W.o	0,51	0,065	0,0035	0,15	0,15	0,0028	0,061	0,0085	0,023	0,18
MAP+ KCl	W.	0,55	0,065	0,0050	0,14	0,15	0,0033	0,057	0,0078	0,021	0,17
	W.o	0,55	0,066	0,0033	0,15	0,15	0,0035	0,065	0,0055	0,022	0,18
MAP+KCl + PB	W.	0,49	0,059	0,0048	0,11	0,16	0,0028	0,062	0,0068	0,020	0,17
	W.o	0,58	0,068	0,0048	0,15	0,16	0,0030	0,070	0,0083	0,024	0,17

C: Control; PB: basalt powder; MAP: monoammonium phosphat; KCl: chloride; W.: with; W.o.: without.

CONCLUSIONS

The fertilizer management and intercropping with Urochloa ruzizienses grass did not promote differences in the levels of minerals present in corn grains. Basalt powder did not provide mineral gain in the grains, however it is a more economically viable source, of national origin and that requires less movement of machines, as it is applied less frequently.

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MINERAL CONTENTS IN GRAINS OF SOYBEAN UNDER BASALT POWDER FERTILIZATION MANAGEMENT

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Keywords: Nutrients; remineralizers; rock dust

INTRODUCTION

Soybean is an important source of vegetable protein and stands out among the main national agricultural commodities. In the last three decades, it has been the agricultural crop that has grown the most in Brazil. Latest harvest data shows that the production has increased by 8.5% (or 10.6 million tons), when compared to the previous harvest (CONAB, 2021).

Currently, agricultural inputs such as fertilizers and pesticides play an active role in production costs. The average share of rational soy fertilizers showed an increasing trend over the years (CONAB, 2021). Brazil is the fourth largest consumer of fertilizers in the world. The amount of NPK (nitrogen, phosphorus, potassium) fertilizers used in the country grew at an annual rate of 5.4%, with more than 50% of them being imported (EMBRAPA, 2020).

The use of rock powder, or remineralizers (RMs), is a technique that consists of using finely powdered rocks that are applied to the soil, in order to restore its fertility through the minerals that are gradually released (THEODORO et al., 2012). Thus, it can be a viable alternative in economic and ecological terms, due to the low cost of the beneficiation process and reduction of leaching losses (HANISCH et at., 2013).

To improve the nutritional quality of agricultural crops becomes increasingly important, as nutritional deficits are a serious public health problem, affecting more than half of the world's population, mainly in developing countries (WHO, 2018). Thus, the objective of this work was to evaluate the effect of basalt powder on soybean minerals contents.

METHODS

The experiment was conducted in the year of 2019. The local climate is classified as a Cwa type, defined as high-altitude tropical, with a rainy season in the summer and a dry season in the winter. The soil is classified as a clayey Distroferric Red Latosol type (SANTOS et al., 2018).

The soybean cultivation took place from November to March of 2020/2021. The soil was corrected in the 0 to 20 and 20 to 40 cm layers to raise the base saturation to 80%.

The design used was randomized blocks, with 8 blocks and 4 treatments. The treatments consisted of: i) control (without fertilization), ii) basalt powder (BP), iii) monoammonium phosphate (MAP) + potassium chloride (KCl) and iv) BP + MAP + KCl. The plots were implanted with 25m² (5m x 5m) and spacing of 1 m between the plots.

In the treatments involving the use of MAP + KCl, these fertilizers are applied in each crop cycle, depending on soil analysis and nutrient extraction by the crop. For BP, the application will be carried out every 3 years, at a dose of 5.0 t.ha1 The first application was carried out at the experiment installation (2019), when the BP was incorporated to the soil, in the layer from 0 to 20 cm deep.

The following minerals were evaluated according to the methodology proposed by Malavolta et al. (1997): phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn)

The data were submitted to the analysis of variance and means were compared by Tukey's test at 5% probability, using the statistical analyses software AgroEstat®.

RESULTS and discussion

The fertilization management promoted differences for the phosphorus, potassium, magnesium and iron contents (Table 1). Overall, the treatment iii (MAP + KCl) showed the best results (Table 1). It is known that ammonium monophosphate contains a high content of phosphorus (above 50%), in addition to nitrogen (N) in the ammoniacal form. Thus, it may have contributed to the higher levels of P and Fe, since the reactions of N in the soil can acidify it, increasing the content of Fe2+, the more soluble form. The study by Hanisch et al (2013) showed that despite having calcium and magnesium in its composition, the basalt powder incorporated into the soil did not increase the levels of these minerals.

It is important to highlight that even at higher levels in the soil, most nutrients have their entry into plants regulated by membrane proteins (EPSTEIN and BLOOM, 2006).

Table 1. Mineral contents in soybeans.

Manejo	P	K	Ca	Mg	S	Cu	Zn	Mn	Fe
	g kg-1					mg kg-1			
C	0,69b	0,19ab	0,016	0,17ab	0,43	0,009	0,11	0,027	0,053ab
PB	0,63b	0,19b	0,017	0,16b	0,46	0,015	0,11	0,028	0,047b
MAP + KCl	0,87a	0,20a	0,015	0,19a	0,43	0,009	0,11	0,029	0,060a
MAP + KCl + PB	0,88a	0,20ab	0,017	0,19a	0,42	0,010	0,10	0,027	0,047b

C: control; PB: basalt powder; MAP: monoammonium phosphate; KCl: potassium chloride. Different letters in the same column are significantly different by the Tukey test ($p < 0.05$).

CONCLUSIONS

Fertilization management promoted differences in the levels of phosphorus, potassium, magnesium and iron present in soybeans. Overall, the treatment iii (MAP + KCl) presented the highest mineral contents. Treatment ii (BP) did not differ from the control for any of the minerals.

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SOYBEAN NUTRIENT ACCUMULATION MARCH IN MEDIUM AND HIGH POTENTIAL AREA IN POCONÉ - MATO GROSSO

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Keywords: Productive potential; recommend fertilization; accumulation of macro

INTRODUCTION

Soybean production in Brazil is vital for the economy, however, given the restrictions on the expansion of land use, it is strategic to maintain the productive capacity of the soil to expand the food supply (JÓNSSON et al., 2016). Productivity growth allows for an increase in food supply and soil quality is the main factor for an increase in production area. Within this context, there are areas in Brazil that have greater production potential, characterized as high productivity areas, these produce above the average of the state of Mato Grosso (MT) and therefore must be studied and monitored.

The dynamics of nutrient absorption by plants is a factor that influences the yield of a crop in a given region or country, thus, the nutrient accumulation curve expresses the need for nutrients at each stage of plant development (ZOBIOLE et al., 2010), being an important tool to recommend fertilization. Therefore, this work aimed to verify the accumulation of nutrients in the parts of soybean plants grown in two areas of commercial agriculture, one of medium productivity (AMP) and another of high productivity (AAP), in the municipality of Poconé-MT.

METHODS

The present study was carried out at Fazenda Lagoa Dourada, located in the municipality of Poconé, in the state of Mato Grosso, in two areas: one considered high (>70 sc/ha) and the second medium (<70 sc/ha).) productivity. . The chemical and physical characterization of the soil of the two zones was carried out and the management for the sowing of the cultivar BMX Ultra 75i77RS Ipro was carried out with a cycle of 120 days with the same parameters. To determine the dry matter, 5 whole plants were collected per plot in the following growth stages: V7, R4, R5.1, R6, R7 and R8. The parts of the plant were separated into leaves, stems and pods, washed and placed in duly identified paper bags, then sent to the forced air circulation oven at 65 ºC until constant weight and subsequently the samples were ground. The nutritional chemical analysis of the samples was carried out in the Commercial Laboratory, according to the method described by EMBRAPA (2009), with sulfuric digestion for N and nitric-perchloric digestion for P, K, Ca, Mg, S, Cu, Mn, Zn.

RESULTS AND DICUSSION

For both areas, the main organ responsible for the accumulation of nutrients were the leaves, especially nitrogen, calcium and boron. The maximum accumulation of most of the macro and micronutrients in leaves and stems occurs mainly at 66 DAE, the beginning of grain development, from this stage the nutrients are exported to the grains in both zones. These results are in agreement with the studies carried out by Oliveira et al. (2014), in which the leaf was the main organ responsible for the supply of nutrients. However, in the AAP (high productivity area) the highest nutrient stocks were present, with the exception of Mn. The maximum accumulation of nutrients in the grain in the PMA occurred at 86 DAE for P, K, Ca and B, at 97 DAE for S and at 120 DAE for N, Mg, Cu, Mn and Zn. And for AAP it was presented at 97 DAE for N, K, Ca, Mg, S, B, Cu and Zn and at 120 DAE for P and Mn. It is observed that the AAP had the maximum accumulation of nutrients before the end of the cycle, unlike the AMP. The macronutrient demand in the AAP followed the following order of nutrient accumulation: N > K > Ca > Mg > P = S, with total average amounts of: 234; 187;

57; 29; 17 = 17 kg ha-1, respectively. In AMP, the accumulated was: N > K > Ca > Mg > S > P, changing the levels of P and S, with the amounts: 175; 127; 33; 18; 12; 11 kg ha-1, respectively. Values ??found by OLIVEIRA JUNIOR et al. (2014) corroborate those of this study, where the total accumulations followed the same: N > K > Ca > Mg > P > S, with the total amounts: 250; 105; fifty; 32; 22 and 14 kg ha-1, respectively. The order of NPK demand corroborates the data of Trigolo et al. (2015) who analyzed three cultivars and in all of them the order of total accumulation and export was: N > K > P and, in addition, the magnitude of the accumulated values ??varied with the productivity of the cultivars, in the same as in the present study, where higher productivity, higher extraction and higher export of nutrients. This behavior highlights the importance of adequate nutritional management to obtain high yields. In AMP, the order of micronutrient absorption was: Mn > B > Zn > Cu, with the total average amounts: 412; 323; 245; 62 g ha-1, respectively For the AAP, the order of total accumulation was different, being: B > Mn > Zn > Cu, with the total quantities: 390; 304; 281; 67 g ha-1 respectively. In the work developed by Oliveira Junior et al. (2014), with these micronutrients and in soybeans with indeterminate growth, obtained an absorption order of: Mn > Zn = B > Cu, with the total average quantities: 565; 237; 229 and 65 g/ha, respectively.

CONCLUSIONS

Nutrient uptake and partitioning in soybean crops differed in the high and medium yield potential zone. The chemical condition of the soil combined with the climatic condition in the two production environments directly interfered with the nutritional content accumulated in the plant.

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INTERACTION BETWEEN SULFATE AND SELENATE IN TETRAPLOID WHEAT (*TRITICUM TURGIDUM* L.) GENOTYPES

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Keywords: selenium; sulfate; wheat

INTRODUCTION

Selenium (Se) is a micronutrient essential to metabolism in humans and Se deficiency is estimated to be a nutritional disorder affecting over 1 billion people of the World's population. Since the main Se source is represented by vegetables-derived foods, enhancing Se concentration in plants might be a crucial approach for increasing the daily Se intake by human population. However, in plants, Se is only considered a beneficial trace element (Kaur et al., 2014; Hasanuzzaman et al., 2014) and Se content in plant tissues is closely related to its availability in the soil (Floor and Román-Ross, 2012).

Plants mainly take up Se as selenate (SeO_4^{2-}), being the most soluble and bioavailable form in alkaline and well-oxidized soils. Selenate uptake occurs through the root sulfate transport system, because of their chemical similarity (Gupta and Gupta, 2017; Schiavon and Pilon-Smits, 2017). From our current understanding, root uptake systems include high-affinity sulfate transporters, whose expression is controlled by the plant S status and by S availability in the growth medium.

Aims of this study are 1) to characterize the interaction between Se and S during root uptake process, by measuring the expression of genes coding for high affinity sulfate transporters and 2) to explore the possibility to increase plant capability to take up Se by modulating S availability in the growth medium.

METHODS

We selected as model plants different tetraploid wheat genotypes, two cultivated genotypes, such as Svevo (*Triticum turgidum* ssp. *durum*) and Kamut (*Triticum turgidum* ssp. *turanicum*), and two ancient ones, such as Turanicum 21 (*Triticum turgidum* ssp. *turanicum*) and Etrusco (*Triticum turgidum* ssp. *turanicum*).

Plants were cultivated hydroponically for 20 days in presence of two sulfate levels, adequate (S=1.2 mM) and limiting (L=0.06 mM) and three selenate levels (0, 10, 50 mM).

RESULTS and discussion

Plants grew healthily under all treatments, without any significant symptoms of damage during the experimental period, but showed significant differences in biomass yield due to the different treatments. In particular, Kamut plants showed better growth and biomass production, at both shoot and root level, under all condition as compared to the other genotypes.

Leaf greenness (SPAD) changed with the nutritional treatments (Fig. 1). Low S availability without Se (L0) slightly but significantly reduced chlorophyll content by 14, 15, 6 and 10% in Svevo, Tur-21, Kamut and Etrusco, respectively (Fig. 1). Leaf greenness progressively decreased in low-S (L) Kamut plants with increasing Se concentration in the growth medium (compared to control, by 9% and by 15% with 10 and 50 mM Se, respectively), whereas in all the other genotypes SPAD values slightly increased with the addition of 10 mM Se to low-S medium (by 4, 10 and 6% in in Svevo, Tur-21 and Etrusco, respectively), to later decrease towards the control (L0) values by adding 50 mM Se (Fig. 1). With regard to the normal-S condition, SPAD values showed progressive decrease with increasing Se concentration in the nutrient solution for all the genotypes except Etrusco, in which chlorophyll content was not affected by Se treatment (Fig. 2). The highest inhibitory effects on SPAD value were observed in Svevo and Kamut plants following addition of 50 mM Se (compared to control, by 13 and 14%, respectively) (Fig. 1).

Interestingly, Se accumulation in shoots was higher when S was limiting in the nutrient solution. In addition, our findings clearly show the differential expression of genes encoding the two high-affinity transporters (TdSultr1.1 and TdSultr1.3), which are involved in the primary uptake of sulfate from the rhizosphere.

Financial Support

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INFLUENCE OF PLANTING LOCATION AND GROWING SEASON ON THE NUTRITIONAL QUALITY OF LUPIN

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Keywords: Lupin; mineral; nutrition

INTRODUCTION

Lupin is a highly nutritious legume crop, particularly in terms of protein, and certain accessions can accumulate up to 40% of this macronutrient. They are also rich sources of zinc (Zn), magnesium (Mg), iron (Fe), potassium (K) and calcium (Ca) along with vitamin A, B and E (Bryant et al., 2022). Nutritional quality has often been studied in germplam accessions, but environmental effects has often been neglected. This research seeks to quantify the nutrient accumulation variations that could result in lupins when altering the crop location and over two different growing seasons.

METHODS

Seeds were harvested in two growing seasons (season 1, 2017-2018, and 2, 2018-2019) and from two locations (Córdoba, Loc 1 and Huelva, Loc 2, both in Spain). Protein concentration was determined using the Bradford method (Bradford, 1976) and the Pierce Coomassie Plus Assay Kit (Thermo Fisher Scientific, Massachusetts, USA). Mineral concentration was determined following microwave-assisted digestion through inductively coupled plasma - optical emission spectrometry (ICP-OES, Optima 7000 DV, PerkinElmer, USA).

RESULTS AND DISCUSSION

Results show that in season 1 grains had higher protein accumulation in Loc 1 (Figure 1.A), which was also the location where the crop yield was reduced. This aligns with previous research that shows a direct relation between lower yields and higher protein content in lupin seeds (Reckling et al., 2018). The low protein levels found in season 2 at both locations could also be related to the specific climateric conditions that were experienced during this season, resulting in a warmer year (+2Cº) with less overall rainfall than season 1 (-1,266 mm) (*data not shown*). Insuficcient mineral availability in the soil can also impact protein uptake. E.g., lower levels of sulphur and P specifically, can have a direct impact on the N content of legume shoots (Claro-Cortes et al., 2002). In season 1, P was higher in Loc 2 (Figure 1.B), while Mn, K and Zn had similar levels in both locations (Figure 1, C and D). The significant decrease in P accumulation in season 2 is attributted to drought stress. Several studies have shown that climate alterations may disturb the nutrient accumulation in major crops. In particular, drought stress has shown to limit P accumulation and reduced P translocation to the seed in soybean (Jin et al., 2015). Lastly, K accumulation was statistically similar on season 1 on both locations, displaying only a statistically difference during the second season in location 2 (Figure 1E), which could be a result of K fixation to some particles of clay that could be present in the soil at that specific season, conditioning the plant's ability to absorb the mineral (Solangi et al., 2019).

Finally, the levels of Fe, Mg and Ca did not show significant variation on their nutrient accumulation amongst seasons and locations (*data not shown*), a result that aligns with the expected resilience in mineral uptake of lupins that have been harvested in different environments (Ruiz-López et al., 2019).

CONCLUSIONS

In this study, nutrient accumulation of protein and minerals was more variable between seasons that between locations, this is mostly associated with the distinct climateric conditions of the two seasons. This was expected since temperature and water availability can influence greately the outcome of any given crop, even when locations for both trials were far from eachother, and soil quality was likely different between locations. When comparing the growing seasons, conditions of growing season 1 (2017-2018) seemed to provide a more advantageous context for nutrient accumulation. Connecting the water availability, changes in temperature, amongst others, with the final outcome.

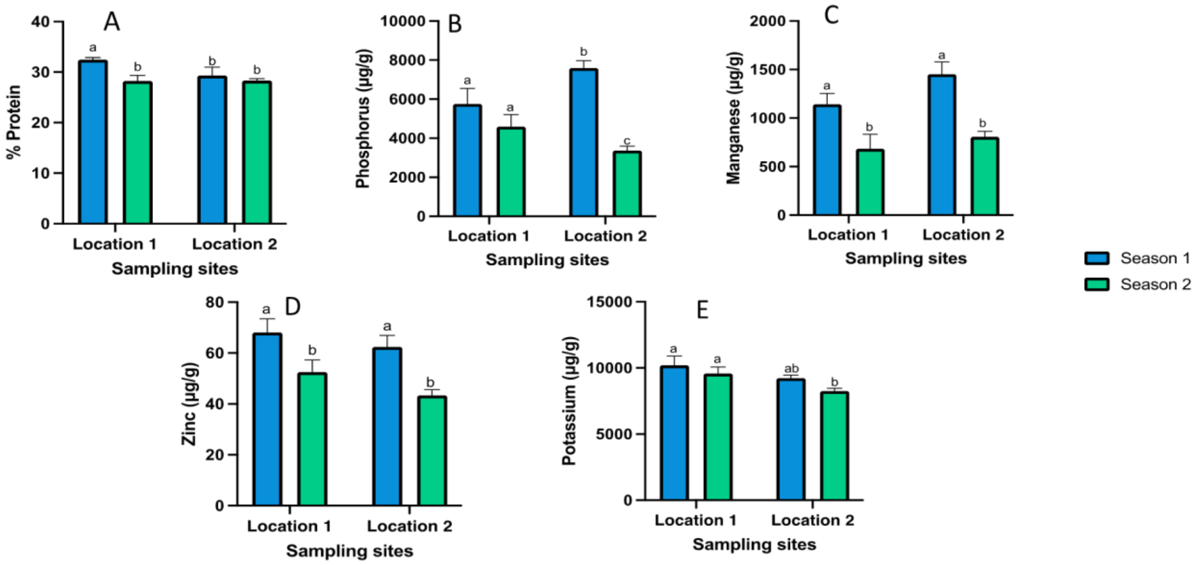

Fig. 1. Results of the effect of growing season and location on nutritional composition of lupin grains for protein (A), phosphorus (B), manganese (C), zinc (D) and potassium (E).

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EFFICIENCY IN THE INTERACTION OF NITROGEN AND ZINC FOR BIOFORTIFICATION WITH ZINC IN RICE

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Keywords: Biofortification; Micronutrient; Nutritional Quality

INTRODUCTION

The increase in malnutrition rates in the world, added to the low concentrations of minerals and vitamins in improved crops, reinforces the need of improve de amount of to develop more research aimed to increase the nutritional quality of foods, such as biofortification. The agronomic biofortification of upland rice may sustainably supply with micronutrient zinc (Zn) the population food widely consumed in the world. These agronomic practices aim the fertilization to increase the contentes of minerals and vitamins in the edible parts of a crop (Moraes et al., 2012),

The interaction between nitrogen (N) and Zn has been shown to be a promising agronomic strategy for wheat biofortification with Zn. There are evidences that, increasing N supply, the concentration of Zn in wheat grain will be improved. This and can be verified in the works of Kutman et al. (201 0) and Erenoglu et al. (2011). The same authors reported that N participates in several physiological processes that maintain Zn homeotase in the plant, such as: absorption, translocation and accumulation of the element. Based on the importance of conciliated N and Zn fertilization for plants, this work objective was to evaluate the efficiency of n and zn interaction in the plant nutrition aiming the productivity and biofortification with Zn in upland rice cultivars.

METHODS

The experiment was installed under field conditions in Sinop - MT in the year 2017, in the Brazilian Midwest and Northern Mato Grosso and predominant soil Yellow Latosol. According to the Köppen classification this region has an Aw climate and the average annual air temperature is 25 °C. The rainfall regime presents a prolonged dry season and a four-month wet season from December to March. The fertilization and cultural management were standardized for the entire experiment based on chemical and physical analysis of the soil in the 0-10 and 10-20 cm depths.

The experimental design was entirely randomized blocks, in a triple factorial scheme 5 x 2 x 2, represented by five rice genotypes (BRS Sertaneja, BRS Esmeralda, AN Cambará, BRS Caravera and BRS Carisma), two doses of Zn (0 and 10 kg ha^{-1}) and two doses of N (50 and 200 kg ha^{-1}), with 4 repetitions, totaling 80 plots. Ten plants per plot were collected at physiological maturity stage - maximum accumulation of dry mass. The material was dried in a forced circulation oven at 60°C until constant weight. Afterwards, the total dry mass of the aerial part, plant tissue analysis and nutrient accumulation were determined. The experimental data were submitted to variance analysis (F test). When they got certain level of significance, they were compared using the Tukey test at 5% probability level, using the SISVAR software (FERREIRA, 2011).

RESULTS AND DISCUSSION

There was a positive interaction between of the three variable to the average Zn contents in the aerial part of the rice (Fig.01). The cultivar BRS Caravera obtained the highest average content under high level N and Zn supply, a difference of 117.39% of the cultivar BRS Esmeralda that in the same treatment obtained 59.5 mg kg^{-1} . The cultivar BRS Sertaneja obtained 64.28 mg kg^{-1} of Zn in its aerial part in the treatment of low dose of N and application of Zn, 23.85% higher than that found in the cultivar BRS Caravera with 51.9 mg kg-1.

The mobility of Zn in plants is not great. Normally, the roots contain much more Zn than the aboveground part, especially if the plants are growing in soils rich in Zn, as it occurs in the present work (MENGEL; KIRKBY, 2001), but the plant tissues (stem and leaves) represent an important source of Zn reserves, and these are effectively remobilized to edible parts, such as grains (KUTMAN et al. 2010). Therefore, the maintenance of high amounts of Zn in plant tissues will contribute to the increase of Zn concentrations in grains, as it has been shown in Kutman et al studies.

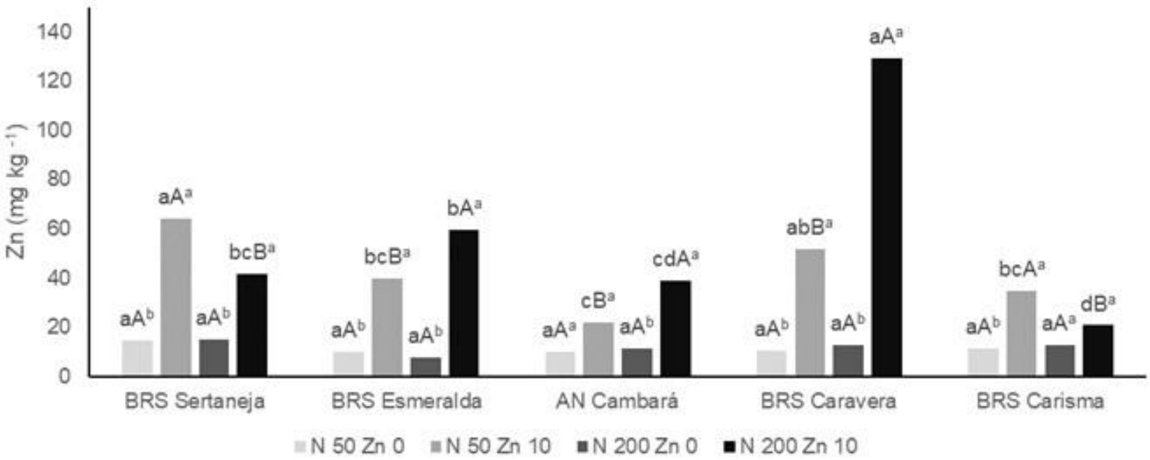

Fig. 1. Average Zn contents in the aerial part of the upland rice cultivars. Same lower case letters within cultivars, upper case letters within N doses, and superscript letters within Zn doses do not differ by Tukey's test at 5% probability level.

Fig. 1. Average Zn contents in the aerial part of the upland rice cultivars.

Same lower case letters within cultivars, upper case letters within N doses, and superscript letters within Zn doses do not differ by Tukey's test at 5% probability level.

CONCLUSIONS

The high N supply combined with Zn application promoted better physiological and nutritional quality of rice plants, which may favor productivity and grain biofortification.

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LENTIL (*LENS CULINARIS L.*) INTRASPECIFIC NUTRITIONAL VARIABILITY AND DEVELOPMENT OF A LENTIL-BASED SNACK

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Keywords: Lentil; Mineral; Protein

INTRODUCTION

Lentils (*Lens culinaris L.*) are among the oldest crops cultivated by humans. They are excellent sources of minerals, protein/amino acids, fatty acids, fibers, carbohydrates, and phytochemicals. Compared to other legumes, lentils have a faster preparation time, low phytic acid content, high arginine, and total phenolic content of antioxidant flavonoids. Besides, their low glycemic index helps avoid peaks in blood glucose, improving metabolic control. Although lentils have these benefits, their consumption in Portugal is lower than desirable, highlighting the need for greater promotion of this legume and the development of new value-added lentil-based snacks.

METHODS

Four *Lens culinaris L*. genotypes with representative seed hull colors were used. The green lentil CDC Kermit (LK) was provided by the University of Saskatchewan, Canada, and was used for its high polyphenol levels. The other three were obtained commercially and were named "Brown" (LB), "Green" (LG), and "Red" (LR).

Mineral concentration analyses

Seed samples were analyzed for seven minerals [potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and manganese (Mn)] following the procedure described by Santos et al. (2020).

Protein percentage

The percentage of protein was determined using the Dumas Nitrogen Analyzer (Dumatec 8000 Nitrogen/Protein Analyzer) after previous seed pulverization.

Development of a lentil-based snack

Several trials were performed to optimize a muffin formulation with different amounts of lentil flour (100, 50, and 25 %) mixed with oatmeal flour. The best formulation was obtained with 50 % of each flour. The control muffin was composed of 100 % of oatmeal flour.

Statistical analyses

Mean comparisons were performed by ANOVA ($p < 0.05$) on GraphPad Software (GraphPad Holdings, LLC, California, USA).

RESULTS AND DISCUSSION

(a) Mineral concentration analysis

No significant differences were observed in P, Mg, and Ca concentrations between genotypes, while K concentration was higher in LG and LR (Table 1). In terms of micronutrients, LG had the highest concentration of Fe and Mn, while LB was the variety with the highest Zn (Table 1). The mineral concentration obtained is in the range of values in studies carried out with different lentil varieties (Benayad and Aboussaleh, 2021).

(b) Protein percentage

The percentage of protein varied significantly between genotypes (Table 1). The varieties LB and LR had about 10 % highest protein (Table 1), although all varieties presented values between 19-22 %. This highlights the importance of this legume as an excellent alternative protein source. These results are according to the literature (Hamid et al., 2019).

Table 1-Mineral concentration and protein percentage of genotypes "Kermit" (LK), "Brown" (LB), "Green" (LG), and "Red" (LR). Values represent mean ± SEM. Different letters indicate significant differences (*p* < 0.05)

	LK	LB	LG	LR
Potassium (K) $mg.g^{-1}$	10.52 ± 0.04^{ab}	9.44 ± 0.02^{b}	11.92 ± 0.41^{a}	11.94 ± 0.02^{a}
Phosphor (P) $mg.g^{-1}$	3.89 ± 0.04	4.71 ± 0.03	5.78 ± 0.13	5.56 ± 0.08
Magnesium (Mg) $mg.g^{-1}$	1.35 ± 0.03	1.31 ± 0.02	1.51 ± 0.06	1.10 ± 0.03
Calcium (Ca) $mg.g^{-1}$	0.70 ± 0.01	0.93 ± 0.01	0.80 ± 0.02	0.35 ± 0.00
Iron (Fe) $\mu g.g^{-1}$	82.96 ± 1.64^{b}	100.91 ± 2.01^{a}	102.22 ± 2.06^{a}	75.19 ± 0.98^{c}
Zinc (Zn) $\mu g.g^{-1}$	43.42 ± 0.12^{d}	64.99 ± 0.14^{a}	52.03 ± 0.78^{c}	60.63 ± 0.88^{b}
Manganese (Mn) $\mu g.g^{-1}$	12.12 ± 0.17^{b}	16.87 ± 0.12^{a}	17.15 ± 0.28^{a}	15.11 ± 0.11^{a}
Protein %	19.72 ± 0.15^{b}	21.89 ± 0.14^{a}	19.16 ± 0.11^{c}	21.53 ± 0.04^{a}

(c) Development of a lentil-based snack

Muffins were developed with lentil/oatmeal flour and oatmeal flour (control) (Figure 1).

Figure 1- Muffins made with lentil/oatmeal flour (left) and oatmeal flour (right).

CONCLUSIONS

Considering the nutritional profile of the different lentil genotypes analyzed, it is possible to choose the appropriate, with better quality, for new food product development.

In a way to incorporate these lentil qualities into the human diet, we developed a snack in which commercial oatmeal flour was partially substituted with lentil flour, promoting the use of this legume grain.

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Plant nutrition, stress tolerance and climate change

SOIL CARBON AVAILABILITY AFFECTS NITROGEN TRANSFORMATIONS UNDER IRRIGATED LUCERNE

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Keywords: gas exchange; nitroux oxide; carbonic gas

INTRODUCTION

Identifying management practices that lead to reductions in N_2O emissions while maintaining or enhancing soil C is a priority. Here we investigate linkages between soil C availability, N transformations and N_2O emissions for irrigated lucerne in New Zealand.

METHODS

We performed a preliminary field experiment to measure the direct effects of the addition of sucrose to soil on C availability, microbial activity, soil mineral N concentration (NO3- and NH4+) and the rates of N2O emissions as well as AOB abundance as an indicator of microbially-driven N transformations. Unlike earlier studies where the fate of added C was not determined, we made independent measurements of C availability and its effects on microbial activity and N transformations.

Measurements were made over late summer (February and March 2019) at the Ashley Dene Research and Development Station, Lincoln, New Zealand (latitude 43.40° S, longitude 172.20° E, elevation 35 m above sea level). Irrigation was applied at a rate of approximately 3 mm day-1 over the course of the study. Soils are an excessively drained, very stony Balmoral silty loam, Udic Haplustept with mean bulk density 1380 kg m-3 and a shallow topsoil (0.2 m depth) stone content of 38%. The experimental design was a randomised block with four replicates of two treatments comprising sucrose addition of 3 Mg ha-1 (equivalent to 1.27 Mg C ha-1) and a control with no sucrose addition. Each plot was 2 × 2 m with a 0.5 m wide buffer strip separating plots and the layout was orientated in a north-south direction to minimise differences in incident irradiance. Sucrose dissolved in 10 L water was added two times during summer (31 January and 15 February 2019) and measurements of soil temperature (Ts), gravimetric soil water content (Ws), soil pH, carbon availability index (Ic), soil respiration rate (Rs), cold (Cc) and hot (Ch) water extractable carbon, soil ammonium (NH4+) and nitrate (NO3-) concentrations and N2O emissions were made on the day (1 February) or two days (17 February) following the treatments and every three days subsequently for a period of 36 days. The same amount of water with no sucrose was added to the control plots.

RESULTS and discussion

In summary, we have suggested that applying sucrose as a readily available C substrate increased Cc, Ic and Rs but the decrease in N_2O emissions relative to the control treatment was not significant in the aerobic conditions, once irrigation did not supply too much water to the soil to provide anaerobic conditions. This is despite large reductions in soil NH4+ and NO3- concentrations resulting from the treatment that were persistent over the study period. We attribute the decrease in NO3- in the soils treated with sucrose to an increase in microbial biomass associated with increasing Rs and enhanced nitrogen immobilisation, consistent with previous studies (Talbot et al., 2019). Additional replication may have yielded a statistically significant decrease in N2O emissions with increased C availability. However, the lack of a difference between the treatments on bacterial amoA gene copy abundance confirms that the effect of C availability on N transformations was attributable to N immobilisation rather than an effect on microbial nitrification.

CONCLUSIONS

In conclusion, we could suggest that there is a direct link between increased C availability and N immobilisation. This is likely to be an important mechanism for reducing mineral N concentration and leaching losses from the soil. However, the regulation of AOB activity and N2O emissions in the field conditions during our study over 36 days was attributable to environmental drivers other than C availability.

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INFLUENCE OF THE IMBALANCED SUPPLY OF P AND K ON THEIR ALLOCATION IN YOUNG EUCALYPT PLANTS.

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Keywords: Eucalypt; Nutrient crosstalk; Nutrient allocation

INTRODUCTION

Eucalypts show high adaptability to various climates and soil types, being the woody angiosperm with the largest planted area in the world. Brazil is one of the world´s leading producers of eucalypt pulpwoods, with 5.3 million hectares (IBÁ, 2019).

The rapid biomass production requires a high photosynthetic carbon fixing capacity which is directly dependent on sufficient nutrient supply, mainly potassium (K) and phosphorus (P) (Laclau et al., 2010; Warren, 2011; Garcia and Zimmermann, 2014).

The objective of this work was to evaluate the allocation of P or K according to the supply of these two elements and the interaction effects when one is supplied at high and the other at low requirements levels in *Eucalyptus tereticornis* and *E. grandis*.

METHODS

Seeds of *E. tereticornis* and *E. grandis* were germinated on a commercial substrate and after two months, they were transplanted into 290 cm^3 plastic pots containing a mixture of vermiculite and sand (1:4, v/v). For six months, once a week, each pot received 20 mL of modified Hoagland solutions (Hoagland and Arnon (1950) containing K and P at different rates. K and P treatments were: LP: low phosphate (2 mg L^{-1}); SP: sufficient phosphate (12 mg L^{-1}); HP: high phosphate (19 mg L^{-1}); LK: low potassium (27.3 mg L^{-1}); SK: sufficient potassium (58.5 mg L^{-1}) and HK: high potassium (101.4 mg L^{-1}) (Raij et al., 1997). Each treatment had five biological replicates.

After six months of treatment, the plants were collected and separated into roots, stems, and leaves. Biomass production was determined after drying and in each plant organ. The concentration of P and K present in the plant material was determined by ICP-OES analysis after nitro-perchloric acid digestion.

RESULTS AND DISCUSSION

In general, both species had a similar pattern in the distribution of P and K in the three organs, as shown in Fig. 1. In *E. grandis*, P and K imbalances had not significant changes in foliar and stem mass production, however, root mass was increased by higher K supply whe P supply was not low. In *E. tereticornis* K was preferentially allocated to stems and leaves under low P supply, with low contents in the roots. Under sufficient P supply, K content in roots increased significantly. Interestingly, at SP treatments, both species showed higher K contents in the roots than in the stems and similar to those on leaves, especially in *E. tereticornis* (Figs. 1A, 1B). In *E. grandis* the greatest the supply of P, the higher was K accumulation in the leaves (Fig. 1A). *E. tereticornis* tended to store greater contents of K when compared to *E. grandis*.

In both species, P allocation to the stems became greater at the highest P supply (Figs. 1C, 1D). In *E. grandis*, at a high P supply, the stem P contents increased with the highest K supply. However, for *E. tereticornis* at high P supply, P contents were higher in stems of plants that received the low K levels. Curiously, for both species, compared to LP e HP, roots of plants at SP accumulated more K. This, however, was not observed for P accumulation, which increased as the supply of P increased. Thus, it seems that P and K have a different interaction in roots compared with leaves and stem.

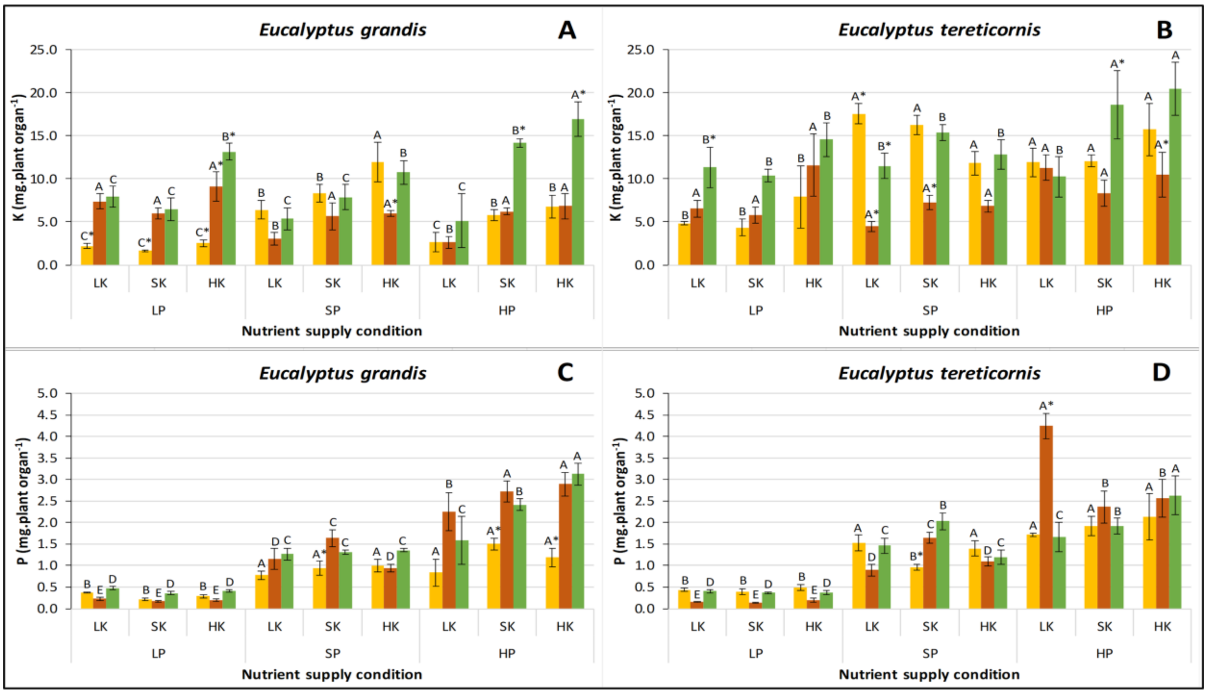

Fig 1. Contents of K (A and B) and P (C and D) in roots (yellow); stem (brown); leaves (green) in *E. grandis* and *E. tereticornis* receiving nutrient solutions with different K and P rates (LP: low phosphate; SP: sufficient phosphate; HP: high phosphate; LK: low potassium; SK: sufficient potassium and HK: high potassium). Values are averages of 5 replicates, and bars represent ± standard error. Different letters indicate statistical significance among the treatments in the same organ. Asterisk represents significant differences within the same supply of nutrients between plant organs by the Scott Knott test ($p < 0.05$).

CONCLUSIONS

These results indicate the importance of stems as P storage organs in young eucalypt plants, mainly when K supply is low. K supply influenced P accumulation mainly when plants received high P levels. The availability of P in the nutrient solution exerted a strong influence on foliar K contents, and this was more pronounced at high P supplies. The imbalance of P and K supply caused significant changes in their allocation in the plants, highlighting the importance of K in leaves and root functioning and the role of stems as P storage sites in eucalypts.

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SILICON ADDED TO BORON FOLIAR SPRAY REDUCES REACTIVE OXIGEN SPECIES IN COTTON PLANTS UNDER BORON DEFICIENCY AND TOXICITY

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Keywords: Beneficial element; Nutritional disorder; oxidative stress

INTRODUCTION

The role played by boron (B) and silicon (Si) in the antioxidant system in plant leaves has been extensively explored and discussed but their effect in B-defficient cotton leaves are stil unknown. This raises an important question regarding the possibility of increasing the efficiency of B foliar sprayings in order to mitigate the harmful effects of B-deficiency or B-toxicity by including Si in the B-spray solution, although these elements (B and Si) must be chemically compatible. Therefore, the risk of Si polymerization in different nutrient solutions should also be taken into consideration, once this is the main disadvantage of using Si in the B-spray solution. In this scenario, it is important to test the hypothesis that:a) deficiency and toxicity of B increases oxidative stress, with a consequent decrease in cotton plant development; and b) Si addition to B solutions for foliar application, without polymerization, reduces the oxidative stress in cotton plants. In this context, a study was conducted to evaluate the effect of adding Si to different foliar B-spray concentrations regarding the oxidative stress on cotton plants under B-nutritional disorder.

METHODS

Cotton seeds were sown into pots containing sand previously washed.. A complete nutrient solution, with moderate B- deficiency was applied to the plants. The experiment was carried out under a completely randomized block design in a factorial scheme 5 x 2 + 1 with: five foliar B concentrations (0.0; 0.5; 1.0; 1.5 and 2.5 g L^{-1}), absence and presence of Si (1.00 g L^{-1}); and one control treatment with no micronutrient deficiency, with four replicates for each treatment. Foliar sprays started with cotton plants in the reproductive stage B1, with four foliar applications four days apart. Cotton leaves were collected three weeks after foliar applications. In order to assess the oxidative stress, malondialdehyde (MDA) (Alexieva et al., 2001) and hydrogen peroxide (H_2O_2)(Gratão et al., 2012) concentrations were determined. The data obtained were submitted to analysis of variance (F-test), and when significant, adjusted to polynomial regression.

RESULTS and discussion

H_2O_2 concentration (Figure 1a) declined as a function of increased leaf B accumulation, with a minimum point at B concentrations of 1.37 and 1.49 g L^{-1} in the presence and absence of Si, respectively, (Figure 2a); and B concentrations of 1.66 and 1.5 g L^{-1} in the presence and absence of Si, respectively, for MDA (Figure 1b). Boron deficiency stood out in H_2O_2 (Figure 2a) and MDA production (Figure 1b) when compared to its toxicity. In the 0.0 g L^{-1} of B treatment, 539.1 and 767.3 μmol g^{-1} of H_2O_2 was produced in the presence and absence of Si, respectively. These values represent an increase of 15 and 38% in H_2O_2 produced by plants grown under B toxicity (2.5 g L^{-1}), which obtained 467.3 and 555.4 μmol g^{-1}, in the presence and absence of Si, respectively. For the treatment with 0.0 g L^{-1} of B, MDA concentration was 4.2 and 5.1 g g^{-1}, representing an increase of 50 and 30% compared to the 2.5 g L^{-1} of B treatment, which produced 2.8 and 3.9 g g^{-1} of MDA in the presence and absence of Si, respectively (Figure 1b).

Fig. 1. Hydrogen peroxide (H_2O_2) (a) and malondialdeide (MDA) (b) content in B-deficient cotton plants as a function of different leaf boron (B) and silicon (Si) concentrations. Different letters indicate differences due to the preesence of Si at the same B concentration.

Nutritional disorders are related to several losses in plant metabolism, and this effect occurs due to an increase in ROS in cotton plants. This effect is known for B-toxicity, which can leads to oxidative damage to cells. The adequate supply of B attenuates oxidative stress by first stimulating stress defense mechanisms. Micronutrient deficiency, in turn, reduces the antioxidant system by decreasing the metabolic pathways for the biosynthesis of antioxidant compounds or by using the antioxidant system for other metabolic pathways.The addition of Si to the borate solution contributed to reduce oxidative damage in cotton leaves. It is important to note that for Si to have a beneficial effect, there was no polymerization in the Si+B solutions used for foliar application, which is very important, as polymerization is a limiting factor in the foliar application of Si. Our results showed that adding Si to the B solution reduced H_2O_2 and MDA production. This beneficial effect of Si is known by its role in increasing the activity of the nonenzymatic defense system.

CONCLUSIONS

In conclusion, adding Si to a B solution for foliar spraying in cotton plants reduces H_2O_2 and MDA concentration, mitigating the oxidative stress in cotton plants under B-deficiency or toxicity.

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FOLIAR APPLICATIONS OF NICKEL IN MAIZE (*ZEA MAYS* L.) PLANTS INCREASE PHOTOSYNTHESIS

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Keywords: gas exchange; Micronutrient; Plant Nutrition

INTRODUCTION

Nickel (Ni) is known as heavy metal. In plant nutritional terms it was the last element to be considered as essential, and recently inserted in the Brazilian Fertilizer Legislation. Even though generating benefits for crops, Ni fertilization management needs to be adequate, as the excess of this micronutrient can cause symptoms of toxicity and reduce gas exchanges as well as the carboxylation efficiency. In view of the above, it is pertinent to test the hypothesis that folair applications of Ni can promote an increase in gas exchange in maize plants. However, in high concentration it impairs this variable. In this context, this study was carried out to evaluate the effects of foliar applications with increasing concentrations of Ni on maize plants, to verify the effects of Ni on gas exchange and carboxylation efficiency.

METHODS

The experiment was carried out usinh a completely randomized blocks design (DBC) in a single quantitative factor, 4 blocks and 5 doses of Nickel: 0; 20; 40; 80 and 160 g ha^{-1} of Ni, divided into two applications, with 4 repetitions. Ni sulfate (NiSO) was used as a source of Ni, with foliar applications of Ni being performed 5 days after nitrogen fertilization using a backpack sprayer. On the first fully developed leaf with visible sheath, gas exchange analyzes were carried out, at the VT phenological stage (leaf bolting), using a portable photosynthesis equipment (Infrared Gas Analyzer - IRGA). The physiological variables evaluated were: photosynthesis (A, µmol CO_2 m^{-2} s^{-1}), and instantaneous carboxylation efficiency (ICE). The data obtained were submitted to analysis of variance (F test) and, when significant, adjusted to a polynomial regression.

RESULTS and discussion

When measuring gas exchange, different Ni doses applied to the crop had a significant effect. Photosynthesis, and the instantaneous efficiency in carboxylation, for instance, showed a higher performance at the dose of 80 g ha^{-1} of Ni, with a maximum point at the dose of *A* of 84.10 g ha^{-1} of Ni (figure 1a), and ICE 82 .50 g ha^{-1} of Ni (figure 1b); with values of 31.98; and 0.27, respectively. Therefore, this dose showed an increase of up to (46.10% *A*) and (86.49% ICE) in relation to the control maize plants (0 g ha^{-1} of Ni).

Our results suggest that maize plants have the highest rates of photosynthesis at the rate of 80 g ha^{-1} of Ni. In this way, the significant result of Ni applications to crop infers strong evidence of a greater metabolic activity, which may contribute to greater plant development.

Fig. 1. Photosynthesis (*A*) (µmol CO2 m^{-2} s^{-1}) (a) and instantaneous carboxylation efficiency (*ICE*) (µmol CO2 m^{-2} s^{-1}) (b) of maize plants grown at doses of 0, 20, 40, 80 and 160 g ha^{-1} of Ni divided into two applications.

Kumar et al. (2018) showed that Ni, when in adequate concentrations in leaves, enhances urea hydrolysis, providing an increase in chlorophyll content and an increase in photosynthesis. On the other hand, dosages higher than the adequate tend to decrease photosynthesis and, consequently, result in a decline in photosynthetic pigments. It has been shown in the literature that the excess dosagee of this micronutrient causes harmful effects on physiological/biochemical processes in plants, such as photosynthesis (SHAHZAD et al., 2018). The instantaneous efficiency of carboxylation is inextricably related to the intracellular concentration of CO_2 and the rate of CO_2 assimilation.

CONCLUSIONS

The increase in Ni concentration, through foliar applications in maize plants, up to a concentration of 80 g ha^{-1} divided into two applications, resulted in an increase in gas exchange (photosynthesis and instantaneous efficiency in carboxylation).

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TECHNOLOGY IN FERTILIZERS PROMOTES SOYBEAN STRESS TOLERANCE AND GRAIN YIELD INCREASE

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Keywords: Temperature stress; Balanced nutrition; Phosphorus fertilizers

INTRODUCTION

Crop production is determined by different biotic and abiotic factors, which may affect plant adaptability in some level with consequent yield reduction. Temperature and drought stresses are the main climate factors that affect plant growth and development, inducing morphological, physiological, and biological changes in plants (Fahad et al., 2017). There are many known adaptation strategies that plants can develop to increase climate stress tolerance, however solid evidence shows that plant nutrition strongly enhances plant tolerance to environmental stresses (Waraich et al. 2011). When associated, low soil fertility and environmental stresses are the main responsible factors for severe crop production losses around the world (Cakmak, 2006). The objective of this study was to evaluate the influence of technologies in fertilizers to enhance nutrition and tolerance to temperature stress in soybean.

METHODS

A field experiment was performed near Santa Rosa del Monday, eastern Paraguay during 2020-2021 growing season. The experiment design was a randomized block with five treatments and four replications. Individual plot size was 2.5 by 5 m and soybean row spacing was 0.5 m. Treatments consisted of P fertilizers with different compositions and production technologies: T1- Control; T2- MAP (monoammonium phosphate) [(11-52-0-0), (N-P_2O_5-K_2O-S)]; T3- SSP (simple superphosphate) [(0-20-0-12), (N-P_2O_5-K_2O-S)]; T4- TSP (triple superphosphate) [(0-46-0-0), (N-P_2O_5-K_2O-S)]; T5- MicroEssentials® (monoammonium phosphate with sulfur in elemental and sulfate forms [(10-46-0-9), (N-P_2O_5-K_2O-S)]. Treatments were applied at a rate of 60 kg P_2O_5 ha^{-1}. Thus, T2 and T5 received additional 13 kg N ha^{-1}, and T3 and T5, 33 and 12 kg S ha^{-1}, respectively. Broadcast MOP (muriate of potash) was applied as source of potash at the rate of 75 kg K_2O ha^{-1} in all treatments. Soybean variety was M5947 IPRO (Monsoy) at a seeding rate of 270,000 seeds ha^{-1}.

Sampling and analyses

Phosphorus fertilizer treatments were evaluated for effects on plant shoot dry weight, N, P and S uptake, and foliar temperature at R1 soybean growth stage. The thermographic data were obtained by a FLIR model E4 Thermovisor. Grain yield was measured at harvest. The effects of P fertilizers technologies on shoot dry weight, nutrients uptake, temperature stress and grain yield were determined by Anova. When significant, multiple comparisons of means among treatments, including the control treatment, were conducted by the T-test, at $P<0.05$.

RESULTS AND DISCUSSION

There was significant effect of the P fertilizer sources on soybean shoot dry weight and S uptake (Table 1). Treatment T5 presented greater shoot dry weight than T4, and greater S uptake than T2 and T4. The presence of sulfur in the elemental and sulfate forms could have promoted an increase in the efficiency and use of S by the plant, increasing plant growth. Despite no statistical difference, T5 also showed greater N and P uptake, and grain yield, when compared to the other treatments.

Table 1. Plant shoot dry weight, N, P and S uptake, foliar temperature, and grain yield of soybean submitted to P fertilizers with different compositions and production technologies.

Treatment	Shoot Dry weight[1]	N Uptake[1]	P Uptake[1]	S Uptake[1]	Temperature[1]	Grain Yield
	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})	(°C)	(kg ha^{-1})
T1	3324,6 ab	103,1^{ns}	10,4 ^{ns}	7,1 ab	31,70^{ns}	5383,3^{ns}
T2	3205,2 ab	94,2	9,4	6,2 b	31,28	5819,4
T3	3732,4 ab	111,4	10,9	7,4 ab	31,53	5830,6
T4	3036,1 b	89,2	9,0	6,0 b	30,83	5865,3
T5	4035,3 a	118,9	12,3	8,2 a	30,23	5959,7

[1]R1 soybean growth stage. $P<0.05$. ^{ns} not significant.

Foliar temperature was affected by P fertilizer sources (Figure 1). Environmental stresses can promote an increase in foliar temperature in response to a reduction in evapotranspiration rates in the leaves. In the present study, soybean foliar temperature was higher under limited nutrition and reached lower values when more balanced nutrition was provided (N-P_2O_5-K_2O-S). Furthermore, soybean grain yield showed negative correlation with foliar temperature increase (Figure 1). Thus, drastic yield penalties can be expected under restricted soil fertility environment.

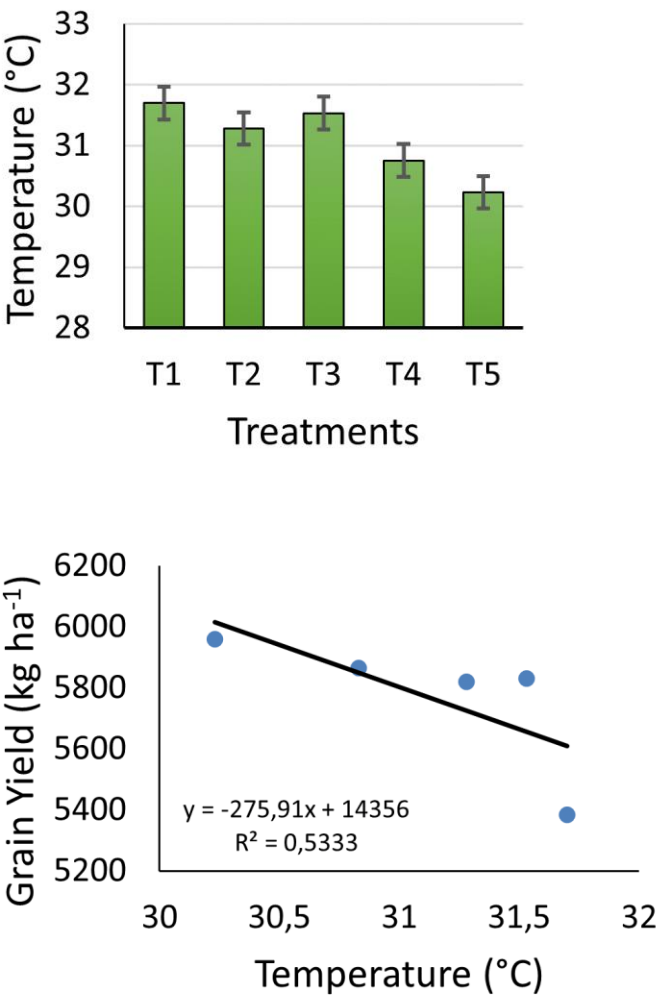

Fig. 1. Foliar temperature by fertilizer treatment (left) and the correlation of foliar temperature with soybean grain yield (right).

CONCLUSIONS

Technology and composition in fertilizers can enhance plant tolerance under environmental stresses. In this study, the P fertilizer source which promoted more balanced nutrition (with elemental and sulfate technology of release) increased the efficiency and use of S by the plant, increasing plant growth. Furthermore, lower foliar temperatures were found in this treatment, which allows to conclude that technology in fertilizers can promotes soybean stress tolerance and grain yield increase.

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ALUMINIUM EFFECTS ON GROUNDNUT (*ARACHIS HYPOGAEA* L.) SEED GERMINATION EFFECT OF ALUMINIUM ON SEED GERMINATION AND PRIMARY ROOT ELONGATION OF SEVENTEEN GROUNDNUT (*ARACHIS HYPOGEAE* L.) CULTIVARS

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Keywords: *Arachis hypogaea* L.; alumimium toxicity; seed germination

INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is preferably grown on sandy soils, which are generally prone to soil acidity and aluminium (Al) toxicity (van Rossum et al, 1994). Seed germination and seedling establishment are the most vulnerable plant growth stages to soil acidity and Al toxicity. One option to combat soil acidity and Al toxicity is to use cultivars that are tolerant to these stresses. The present study examined the effects of Al concentration in nutrient solution on seed germination and primary root growth of 17 groundnut lines.

METHODS

Seeds of 17 groundnut lines (10 seeds per rep x 4 reps) were inserted in holes drilled in 5 mm thick polystyrene sheets with the point of radicle facing down, and germinated whilie floating on top of airated nutrient solutions in 18 L basin dishes at five logarithmically spaced Al solution concentrations treatments (0, 5.7, 14.14, 53.18, 200 µM). The pH of the nutrient solution was mentained at 3.5.

Seed germination was monitored for 10 days and the experiment terminated following which the final seed germination count was taken, and the length of the primary root was measured. Citritical solution Al concenttrations for toxicity on primary root growth were intrapolated from regressions relationships between root length and solution Al concentration at 90 % of optimal growth.

RESULTS and discussion

Aluminium within the concentration range tested (0 to 200 µM) was inquensequential to seed germination of 11 of the 17 lines tested (Table 1). In the other six lines, Al inhibited seed germination in a concentration-dependent manner. Nonetheless only in two lines was germination completely inhibited by the highest Al concentration (200 µM) tested (Table 1).

Table 1. The effect of Al concentration on the seed germination percent of 17groundnut genotypes

Groundnut line

Seed Germination (%)

0 µM

5.7 µM

15 µM

53.13 µM

200 µM

MEAN

Akwa

100.00

100.00

100.00

73.33

00.00

74.67

Anel

100.00

100.00

100.00

53.33

46.67

80.00

ARC-Opal 1

100.00

73.33

100.00

53.33

0.00

57.33

CBRR 4

100.00

100.00

100.00

100.00

100.00

100.00

Eratisade

100.00

100.00

100.00

100.00

100.00

100.00

Gile 4

100.00

100.00

100.00

100.00

100.00

100.00

ICG 2240

100.00

100.00

100.00

100.00

100.00

100.00

ICG 1147

100.00

100.00

73.33

60.00

60.00

78.67

I CG 1697

100.00

100.00

73.33

66.67

46.67

77.33

ICG 2716

100.00

100.00

100.00

100.00

100.00

100.00

ICG 99529

100.00

100.00

100.00

60.00

66.67

85.33

Inkanyezi

100.00

100.00

100.00

100.00

100.00

100.00

Isigwingwi

100.00

100.00

100.00

100.00

100.00

100.00

JL 24

100.00

100.00

100.00

100.00

100.00

100.00

Manguzi-Red

100.00

100.00

100.00

100.00

100.00

100.00

Pan 3

100.00

100.00

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100.00

Mwenje

100.00

100.00

100.00

100.00

100.00

100.00

Mean

100.00

98.43

95.22

100.00

86.27

77.65

CV% = 4.9

$L.S.D_{0.05}$ Groundnut lines = 3.255; Al treatments = 1.76; Groundnut line x treatment =7.278.

The elongation growth of the primary root after germination was highly sensitive to Al toxicity. Increasing Al concentration in solution inhibited the growth of the primary root in all the lines, but to different extends among the 17 lines tested. This was reflected in the wide range of the ciritical solution Al concentration values for toxicity extending from 3.93 to 25.07 µM (Table 2), which confirmed appreciable diversity in the tolerance of the lines to Al toxicity.

Table 2. Critical Al concentrations for toxicity at 90 % maximum of primary root growth in 17 groundnut lines.

Cultivar	**Critical Al concentration for toxicity (µM)**	**Cultivar**	**Critical Al concentration for toxicity (µM)**
Akwa	10.79	ICG 2716	25.07
Anel	15.62	ICG 99529	12.44
ARC-Opal 1	3.93	Inkanyezi	12.22
CBRR4	22.45	iSingwingwi	

4.00

Eratisade

19.09

JL24

13.38

Gile 4

18.34

Manguzi Red

12.63

ICG 2240

13.25

Pan 3

27.37

ICG 1147

11.78

Mwenje

33.72

ICG 1697

8.88

CONCLUSIONS

The results from this study showed that Al under acidic conditions may affect plant establishment in two ways, either by preventing the seed to germinate in some lines or by inhibiting primary root growth following germination. However, in a majority of the lines, seed germination per se was relatively insensitive to Al toxicity compared with primary root growth elongation. In a minority of the lines (35%) seed germination was inhibited with each incremental Al concentration between 3.7 to 200 μM. Whilst the extension growth of the primary root in all the 17 groundnut lines tested was significantly and negatively affected by increasing Al concentration, there were marked differences between the lines in the extent to which they were affected. There was a wide range (3.93 μM to 33.72 μM) in the critical solution Al concentrations for toxicity among the lines tested, which indicated high diversity in the tolerance of the groundnut lines to Al toxicity. The cultivar which showed the most tolerance to Al can be good candidates for use in breeding programmes to create varieties that are tolerant or resistant to Al toxicity.

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UNCOVERING THE MOLECULAR BASIS OF PHOTOSYNTHESIS REGULATION BY NUTRIENT SIGNALS

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Keywords: Combinatorial Nutrient stress; Photosynthesis; Retrograde signaling

Plants rely on a constant supply of macro- and micronutrients to perform photosynthesis. For example, iron is an important micronutrient for photosynthesis, which is supported by longstanding observations that link iron deficiency and chlorosis. However, in the late 1970s, DeKock et al. (1979) reported the development of chlorotic leaves under high-phosphorus conditions despite replete iron levels, challenging the causal connection between iron concentration and chlorosis. More recently, we showed that iron-induced chlorosis in monocots is phosphorus-dependent, in which plants grown under combined iron and phosphorus deficiency display an intriguing "stay green" phenotype. These observations highlight a gap in our understanding of the interdependent effects of nutrient availability on photosynthesis regulation, and raise the question: how do plants integrate nutrient cues to control photosynthesis? Our results show that iron-induced chlorosis is phosphorus-dependent in both monocots and eudicots. By combining genome-wide gene expression changes and genome-wide association studies with molecular physiology in Arabidopsis thaliana, we identified a signaling pathway involved in the regulation of photosynthesis under combined iron and phosphorus stresses. This newly identified mechanism includes genes coding for chloroplastic (PHT4;4) and nuclear (bZIP58) proteins that prevent the repression of the core set of photosynthesis genes and associated chlorosis under iron and phosphorus deficiency. Furthermore, we demonstrated the existence of ROS-mediated chloroplastic retrograde signaling pathway to adapt photosynthesis to nutrient availability.

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INTERACTIVE EFFECTS OF CLIMATE CHANGE, NITROGEN AND ZINC NUTRITION ON GROWTH AND YIELD PERFORMANCE IN WHEAT

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Keywords: climate change; nitrogen ; zinc

Interactive effects of climate change, nitrogen and zinc nutrition on growth and yield performance in wheat

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INTRODUCTION

Sustaining global crop yields is key to address food security and requires a solid input of macro (e.g. nitrogen: N) and micro (e.g. zinc: Zn) nutrients. However, recent changes in the world's climate due to anthropogenic activities (IPCC, 2013) are progressively hindering the nutrition, and thus final yield of many crop species cultivated around the globe (Asif et al., 2017). This study evaluated the interactive effects of predicted climate change, N and Zn nutrition on the performance of bread wheat as a model staple food crop.

METHODS

Bread wheat (*T. aestivum* cv. Ceyhan-99) was cultivated in soil fertilized with adequate or low N and Zn in pots under ambient climate (ambient CO_2 and temperature) or predicted climate (700 µmol CO_2 mol$^{?1}$ and 3°C temperature rise) conditions in dedicated plant growth chambers. Plants were harvested at full maturity and grain yield, and yield attributes along with Zn and N status of grains were determined.

RESULTS and discussion

Plant growth rate was significantly accelerated under predicted climate (PC) conditions causing early onset of successive growth stages and maturity. Under both PC and ambient climate (AC), adequate N and Zn supply significantly improved straw and grain yield through increarsed number of spikes plant^{-1} and grains spike^{-1}. However, PC treatment significantly reduced straw and grain yield (Figure 1) due to reduced spikes plant^{-1}, particularly in plants supplied with adequate N. Adequate Zn and PC treatments were significant only under adequate N supply. Adequate N increased both grain protein and Zn, particularly under adequate Zn treatment. Although the PC treatment did not influence the grain protein concentration, grain protein yield (i.e. total mass of protein in whole grains of a single plant) was severely reduced under PC conditions.

Grain yield $HSD_{0.05}$ (C, N, Zn, CxN, CxZn, NxZn, CxNxZn) = (0.21***, 0.21***, 0.21***, 0.39***, n.s., 0.39***, 0.66*)

Figure 1: Wheat (*Triticum aestivum* cv. Ceyhan-99) grain yield as affected by climate (open bars: ambient climate, solid bars: predicted climate), N and Zn treatments. Bars not showing the same letter are significantly different from each other (Tukey's HSD, p≤0.05). All values are means ± SD (n = 4).

CONCLUSIONS

Here we demonstrated a dramatic reduction in maturation period of wheat plants cultured under a PC senario with an elevated CO_2 and a raised temperature regime. Although supplied with ample fertilization (with N, Zn and other mineral nutrients), plants under a PC senario produced lower straw and grain yield. While the PC and adequate Zn treatments enhanced main spike gain yield and grains spike^{-1}, PC declined overall grain yield, particularly due to severe reduction in spikes plant^{-1}. Our results conclude that sustaining a higher number of spikes per plant and ensuring an adequate N and Zn nutrition are essential to exploit elevated atmospheric CO_2 levels as well as to minimize the adverse effects of rising temperatures on yield and quality of wheat.

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ROLE OF PURPLE ACID PHOSPHATASE EXPRESSION ON PHOSPHATE UTILIZATION EFFICIENCY IN RYEGRASS PLANTS

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Keywords: Purple acid phosphatase; Phosphate utlization efficiency; Intracellular phosphatase activity

INTRODUCTION

Phosphorus (P) is an essential macronutrient for plant growth, development and high pasture production. The development of P-efficient pastures grown under P deficient conditions is essential for sustainable agriculture practices. The P utilization efficiency (PUE) is the capacity to produce a large amount of biomass (fruits, forage or grains) per unit of P absorbed due to an efficient uptake, remobilization, recycling and translocation of P. In this context, purple acid phosphatase (PAP) is an important plant acid phosphatase (APase), which can improve phosphate utilization through P scavenging processes and P remobilization in plant cells and tissues. The major intracellular and secreted APase in plants are encoded by PAP genes (Tran et al., 2010) However, little is known about the functions of intracellular PAP in ryegrass plants. This study is aimed to analyze the APase activity and the expression of *PAP1* gene in two ryegrass (*Lolium perenne* L.) cultivars with contrasting PUE efficiency grown under normal and deficit P conditions.

METHODS

Two ryegrass cultivars with contrasting PUE efficiency (cv. Ansa, PUE inefficient and cv. 24 Seven, PUE efficient) were hydroponically grown with low (10 µM) and optimal (100 µM) P doses for 21 days. The PUE was determined according to Lopez-Arredondo et al. (2014). At harvest, the intracellular APase activity was determined as described by Starnes et al. (2008) and *PAP1* gene expression was analyzed by quantitative Real-Time PCR on a qPCR Step One Plus. Gene-specific primers for *PAP1* was obtained from Venkatachalam et al. (2009) and housekeeping genes *LpeEF1α* (h) and *LpeEF1α* (s) were used as endogenous control. The normalized values were subjected to a $2^{?\Delta\Delta Ct}$ method.

RESULTS AND DISCUSSION

Our results showed that under both P conditions the shoot APase activity was similar between cultivars (Table 1). In contrast, the gene expression of *PAP1* was upregulated by P deficiency in roots and shoots of both cultivars, being *PAP1* expression higher in the PUE -inefficient cultivar Ansa than in the PUE efficient cultivar 24 seven (Fig. 1). Similar results have been reported by Yan et al. (2001), which found that APase activity in shoot of beans was not associated with either P acquisition or its use efficiency. Differential responses regarding the APase activity and *PAP1* expression, and their relationship with PUE, could be due to APase activity might not be a critical index for PUE in ryegrass. Moreover, the APase activity could be encoded by other *PAP* genes not explored in ryegrass yet. Further research is needed to identify the roles of intracellular APase activity in ryegrass roots.

Table 1. Phosphorus utilization efficiency (PUE) and phosphatase activity in shoots of ryegrass 24 Seven cultivar (PUE Efficient) and Ansa cultivar (PUE inefficient) under different P treatments. -P: 10µM, +P: 100µM. Date are the means ± SD of three replicates. Different letters indicate statistically significant differences (Tukey's HSD at $P \leq 0.05$) between cultivars for the same P treatment.

Cultivar	P Treatments	PUE	Phosphatase activity (µmol PNP min^{-1} mg^{-1} FW)

24 Seven	-P	1.3±0.04a	14.2±0.7a
	+P	0.3±0.02a	12.9±0.8a
Ansa	-P	0.5±0.04b	13.6±1.9a
	+P	0.2±0.03b	12.2±2.6a

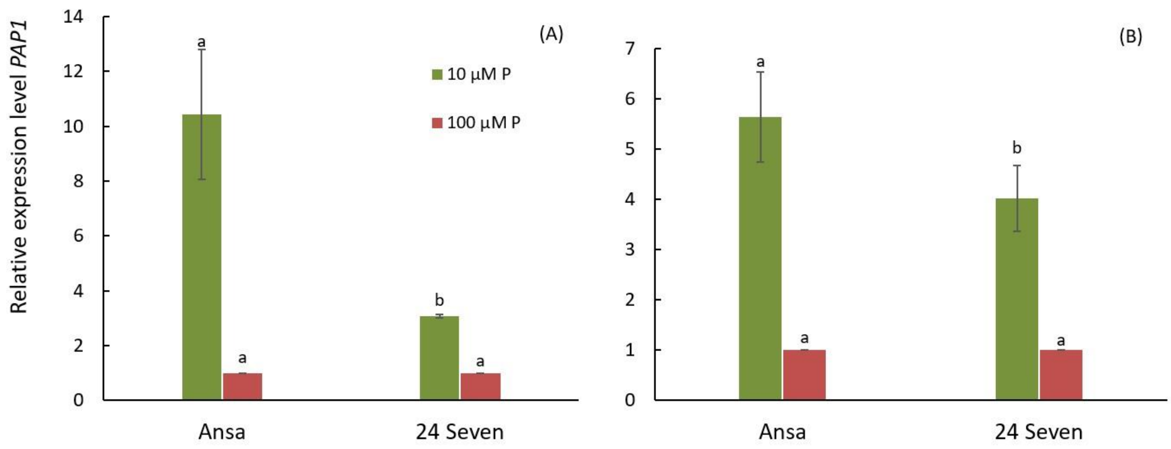

Figure 1. Relative expression level of purple acid phosphatase *PAP1* in shoots (A) and roots (B) from Ansa and 24 Seven cultivars grown in nutrient solution with different P treatments. The expression levels were normalized in relation to eEF1α(h) or eEF1α(s) gene expression. Data are the means ± SD of three replicates. Different letters indicate statistically significant differences (Tukey's HSD at $P\leq 0.05$) between cultivars for the same P treatment.

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REFLECTIVE PARTICLE FILMS IMPROVE PHYSIOLOGICAL RESPONSES AND TISSUE ANATOMY OF CITRUS TREES AFFECTING NUTRITIONAL STATUS

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Keywords: Abiotic stress; Climate change; Sunlight

High irradiance and air temperature observed during extreme weather conditions affect tree crops and impact yield and quality of fruits. Moreover, flowering and fruit set of *Citrus* are likely impaired by excess UV radiation, which reduces carbon assimilation, increases production of reactive oxygen species (ROS), damaging cell integrity and leaf photosynthetic apparatus . Coating particle films sprayed to tree canopy have been offered to minimize crop losses, given the possibility of modulating leaf temperature under adverse climate changes, even though the extent of leaf protection achieved is not well characterized. We evaluated the use of two protective films of trees on leaf photosynthesis and cell anatomy compared to others exposed to varying day light. Sweet orange [*Citrus sinensis* (L.) Osb., cv. Valência] trees were grown under full sun light sprayed or not (control) with kaolin (30 g L^{-1}) or calcium carbonate (30 g L^{-1}), as well under reduced irradiance under aluminum shade cloth 50% or anti-UV transparent plastic. The leaves was evaluated on light and transmission microscopy, and concentration and distribution of mineral elements were determined by chemical analyses of total nutrients, EDX and XRF. Gas exchange and efficiency of the photosynthetic apparatus were also determined. Reflective leaf coating particles can improve plant photosynthesis of treated trees compared to full sunlight control ones, even though calcium carbonate had more senescence clues. Shading imposed by aluminum cloth, as well anti-UV treatments, reduces net photosyntesis preserving the celular structure. Leaf concentration of K was reduced by particle coating films, which likely affected Mg and B. Further investigation is needed to demonstrate if leaf transpiration or other indirect effects of coatings changed nutrient uptake and transport in trees.

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MAINTAINING PHOSPHORUS UPTAKE DURING THE GRAIN FILLING PERIOD IS CRUCIAL FOR SOYBEAN TOLERANCE TO HIGH TEMPERATURE STRESS

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Keywords: Climate changes; Nutrition; Yield

INTRODUCTION

Extreme events of heat waves have affected the sustainability of agricultural systems, including the production of grain crops (IPCC, 2021). In this regard, soybean might be particularly affected, since it is grown during the summer in tropical, subtropical and semiarid regions, when the occurrence of air temperatures above the optimum for plant development, i.e. heat stress (HS), is most likely to occur. Although the incidence of HS at any phenological stage is detrimental for final soybean yield performance, the reproductive period is considered to be the most sensitive to this abiotic stress. Moreover, for field-grown plants, such a limiting condition might be associated with other abiotic stresses. For instance, HS impairs the capacity of the plants in sustaining nutrient acquisition during critical periods of plant development and peak of nutrient demand, leading to nutritional disorders. Regarding soybean plants, the effect of HS on post-flowering phosphorus (P) uptake might be relevant for yield formation, as a minor fraction of this nutrient is absorbed during the vegetative phase (Gaspar et al., 2017). Nevertheless, the basis governing crop yield performance under combined occurrence of HS and P deficiency during the grain-filling period on remains to be completely elucidated. We aimed, hence, to study the effects of HS on plant P accumulation and partitioning in soybean plants.

METHODS

The experiment was carried out in a greenhouse, where the average minimum and maximum air temperatures were 18°C and 35°C, respectively, and the relative humidity ranged from 52 to 89%. The soybean variety used was NA 5909 RG and the nutrient solution was prepared according to crop recommendations until flowering (R1). Then, the plants were subjected to the varying concentration of P in the fertigation solution: 20 µmol L^{-1} of P (P_{20}), 100 µmol L^{-1} of P (P_{100}) and 500 µmol L^{-1} of P (P_{500}). Plants remained in the greenhouse until the phenological stage R5.1, following the transference to two growth chambers. One of the growth chambers was adjusted to the control thermal regime (CT) (28/18 °C) and the other to the stress thermal regime (HS)(42/28.5 °C). These conditions were imposed during the phenological stages R5.1 and R8.

The evaluations were performed in two stages: before the imposition of the heat treatment (R5) and at physiological harvest (R8). At both sampling times, dry mass (DM) production of plants organs was estimated. P concentration in plant tissues was performed according to the methods described in Bataglia et al. (1983). Based on DM production and nutrient concentration in the different plant parts, nutrient accumulation was calculated. P harvest indexes (PHI, %) was obtained as the ratio between the grain P accumulation (grain-PAc) to its total accumulation in the plants. Shoot P uptake (SPUp) during grain filling was estimated as the difference between shoot P content at R5 and R8. Contribution of P remobilization (CPRem) to total grain P content was calculated as the ratio of remobilized P and accumulation of the nutrient in the grains.

The obtained data were subjected to a two-way analysis of variance (ANOVA). Tukey's multiple range test ($p < 0.05$) was used to evaluate the interactive and main effects of the factors of study imposed during the post-flowering period (P supply in the rooting medium versus air temperature).

RESULTS AND DISCUSSION

HS reduced GDM by 68% (P_{20}) and 56% (P_{100}), when compared to plants under CT; however, plants grown at P_{500} did not show any variation for GDM (Table 1). Grain P concentration was higher under HS than CT for P-deficient plants. It was also observed that HS decreased grain-PAc and SPUp (Table 1). In contrast to P_{500}, P-deficient treatments under HS showed a higher CPRem, compared to CT: approximately 75% in P_{20} and 69% in P_{100}.

Our results revealed that HS during grain filling compromises P uptake by soybean plants under limited supply of the nutrient, most likely due to damages on the functioning of the roots (Huang & Xu 2000). Furthermore, despite the high potential for P remobilization from vegetative structures, this process was not sufficient to compensate for the decrease in the ability of plants to sustain the allocation of P in grains, which, in turn, contributed also a decline in PHI. Accordingly, as there is positive relationship between grain yield and accumulation of the nutrient by the plants (Gaspar., 2017), it should be emphasized, for improved crop performance under more severe events of heat stress, the importance of favoring plant P acquisition during the grain filling period.

Table 1. Grain Dry mass (GDM), shoot P uptake (SPUp), grain P concentration (P-grain), grain P accumulation (grain PAc), P harvest index (PHI) and contribution of P remobilization to grains (CPRem).

P treatments	Thermal Regime	GDM g pot $^{-1}$	Shoot P Uptake mg pod $^{-1}$	P-grain g kg $^{-1}$	Grain-PAc mg pot $^{-1}$	PHI %	CPRem %
P_{20}	CT	28.7 Ac	50.12 Ab	2.5Ab	75.3 Ac	61.7 Ac	13.7 Ba
	HS	9.3 Bc	41.57 Bb	3.8 Ba	34.2 Bc	34.9 Bc	61.1 Aa
P_{100}	CT	38.6 Ab	57.58 Ab	2.6 Aab	106.2 Ab	67.3 Ab	12.5 Ba
	HS	17.0 Bb	42.07 Bb	3.8 Ba	63.5 Bb	54.7 Bb	59.7 Aa
P_{500}	CT	64.2 Aa	67.24 Aa	3.0 Aa	186.0 Aa	77.7 Aa	18.4 Aa
	HS	63.7 Aa	70.57 Aa	3.0 Ab	191.3 Ba	77.4 Aa	16.3 Ab

Averages followed by the same letter did not differ significantly by Tukey's test ($p < 0.05$); upper case letters were used for comparison between thermal regimes being control thermal (CT), stress thermal (HS) and lower cases letters for comparison among fertigation P concentrations.

CONCLUSIONS

Heat stress is detrimental for P uptake by soybean plants facing limited availability of the nutrient, contributing to impairments on grain yield under the combination of these abiotic stresses. Accordingly, we might argue that the maintenance of the capacity of phosphorus uptake during the grain filling period plays a critical role for soybean yield stability in a scenario of climatic changes.

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THE USE OF SUPPLEMENTAL LIGHT CAN IMPROVE THE GROWTH AND DEVELOPMENT OF STRAWBERRY PLANTS IN SALINITY AND ALKALINITY STRESS

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Keywords: Fragaria × ananassa; LED light; Abiotic stress

The use of supplemental light can improve the growth and development of strawberry plants in salinity and alkalinity stress

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Introduction

Abiotic stresses disrupt the growth, physiology and function of plants. It has always been tried to reduce the effects of these stresses on plants and the use of different spectrums of complementary light can be considered. Therefore, light is an important source of energy and an effective factor for growth, flowering, fruiting and photosynthesis in plants (Cho, 2008). LED radiation affects the nutrients and quality of strawberries. Plant growth and physiology are strongly influenced by the light spectrum, which affects plant morphology, growth and development (Johkan et al., 2010). We expect that the results of this experiment will improve the functional properties of complementary light.

METHODS

Plant material and growth conditions. This experiment was conducted in the Vali-e-Asr University of rafsanjan. We prepared rooted strawberry plants (*Fragaria × ananassa* Duch, cv. Camarosa) from a nursery in Karaj, Iran. Plants were treated by two stress levels, including control, alkalinity, salinity, and five different light conditions (light spectrum) including monochromatic blue, monochromatic red, dichromatic blue/red (1:3), white/yellow (1:1), and only ambient light.

Vegetative, and Elemental analysis. After drying the samples in the oven, their dry weight was recorded. Petiole length was measured with a ruler. Leaf area was measured with 202 m-CI leaf area. Leaf and root Na and K concentrations were measured using flame photometry. Leaf and root iron were measured by atomic absorption spectrophotometer. Ca and Mg concentrations were measured by EDTA titration.

Results

Vegetative characteristics, and Elemental analysis. Salinity and alkalinity stress caused significant reduction in dry weight under all light conditions. Under alkaline stress, red light caused the least decrease in dry weight of leaf, crown, and root (Table 1). Salinity and alkalinity stress increased Na content in shoot. The lowest Na contents in shoot were observed under blue/red and white/yellow light, respectively. Plants treated with red light had the highest amount of shoot K. The highest Ca, Mg and Fe content in shoot were observed under blue/red light (Table 1).

Table 1. Interaction of five levels of light spectrum and three levels of stress treatment on Vegetative characteristics nutrient element concentration of strawberry cv. Camarosa.

Discussion

The first response of the plant to salinity stress is leaf area limitation and low growth. Some light spectra have been shown to help plants become more resistant to biological and abiotic stresses (Kreslavski et al., 2013). has been reported that shoot dry weight increased under blue/red light (Wang et al., 2016). This increase was attributed to the effect of blue/red light on leaf number and leaf area.

In this study, the elements Fe, Ca, K, and Mg were reduced under alkaline stress. Plant uptake and accumulation of K under salt stress increased plant resistance. Bicarbonate ions interfere with the uptake and transport of essential plant nutrients (Marschner, 1995).

Conclusions

Our results showed that plants adopt different strategies against abiotic stress depending on light quality. The results showed that blue and red light spectra affect the absorption of elements and photosynthetic apparatus of plants. Therefore, they improve vegetative and reproductive growth and increase plant resistance to stress.

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SILICON MITIGATES THE EFFECTS OF LATE WATER DEFICIT OF CONTRASTING SUGARCANE CULTIVARS IN DROUGHT TOLERANCE

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Keywords: silicate; drought stress; Saccharum spp

INTRODUCTION

This study aimed to determine whether silicon (Si) mitigates the effects of water deficit at ripening phase of contrasting sugarcane cultivars in drought tolerance.

METHODS

The experiment was conducted in pots (20 L) with sandy soil (2.8 mg kg^{-1} Si in 0.01 mol L^{-1} $CaCl_2$) under greenhouse conditions. A randomized factorial design with 2 sugarcane cultivars (RB86-7515=drought-tolerant;RB85-5536=drought-sensitive), 2 Si rates (equivalent to 0 and 1000 kg ha^{-1} Si, Si- and Si+) as silicate (10.5 g kg^{-1} Si), and absence (well-watered, WW) or presence of water deficit (WD) at ripening phase was used. All nutrients were applied in soil. Sugarcane was transplanting at 10/21/20 into pots. Soil was maintained at 100% soil field capacity (FC) until 08/26/20, when occurred water deficit (55% FC) for 28 days. After harvest (09/23/20), dry biomass, Si in plant, soil chemical analysis, and total sugars were evaluated.

RESULTS AND DISCUSSION

There was influence ($p<0.05$) of Si in chemical analysis of soil samples collected after sugarcane harvest. TThe levels of Si in 0.01 $CaCl_2$ mol L^{-1}, Ca, Mg, cation exchange capacity (CEC), and basis saturation increased from 3.8 , 11.1, 2.2, 24.4, 62.1 in the control to 7.4 mg kg^{-1} Si, 12.5 $mmol_c$ dm^{-3} Ca, 3.1 $mmol_c$ dm^{-3} g Mg, 26.3 % CEC and 67.6 BS % with Si, respectively. Additionally, biomass and Si uptake were influenced ($p<0.05$) independently. The water deficit decreased both biomass and Si uptake in the leaf, and increased root biomass (Fig.1 A, D). RB86-7515 provided superior stalk, leaf, and straw biomass due to good development in all types of soils, but Si uptake increased only in leaf and straw (Figs. 1B, E). RB85-5536 showed higher biomass, and Si uptake in root (Fig. 1 B, E). Finally, Si decreased stalk biomass,but increased total sugar in stalks and reduced straw biomass.

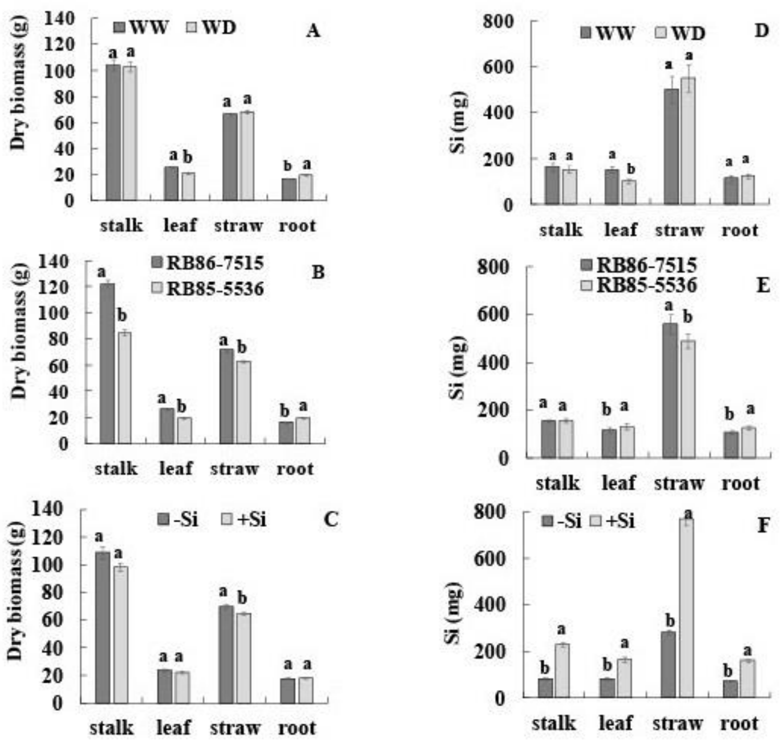

Fig. 1. Dry biomass and Si uptake of stalk, leaf, straw, and root of sugarcane cultivars grown under well-watered (WW) or water deficit (WD) conditions and with or without Si

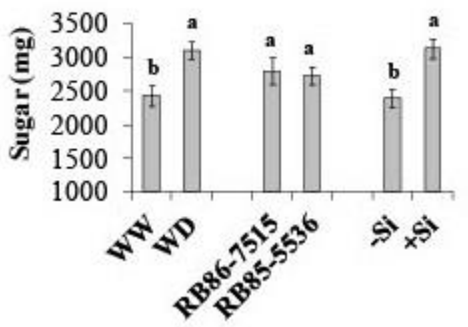

Fig. 2. Total sugar of cultivars without (WW) and with water deficit (WD) with or without Si.

CONCLUSIONS

Si fertilization increased Si uptake, sugar, and maintained green leaf during sugarcane growth, independently of water deficit, and cultivar.

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SILICON FOLIAR APLICATION TO SOYBEAN CROP PRODUCTION IN *CERRADO* (BRAZIL)

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Keywords: biotic stress; abiotic stress; root development

INTRODUCTION

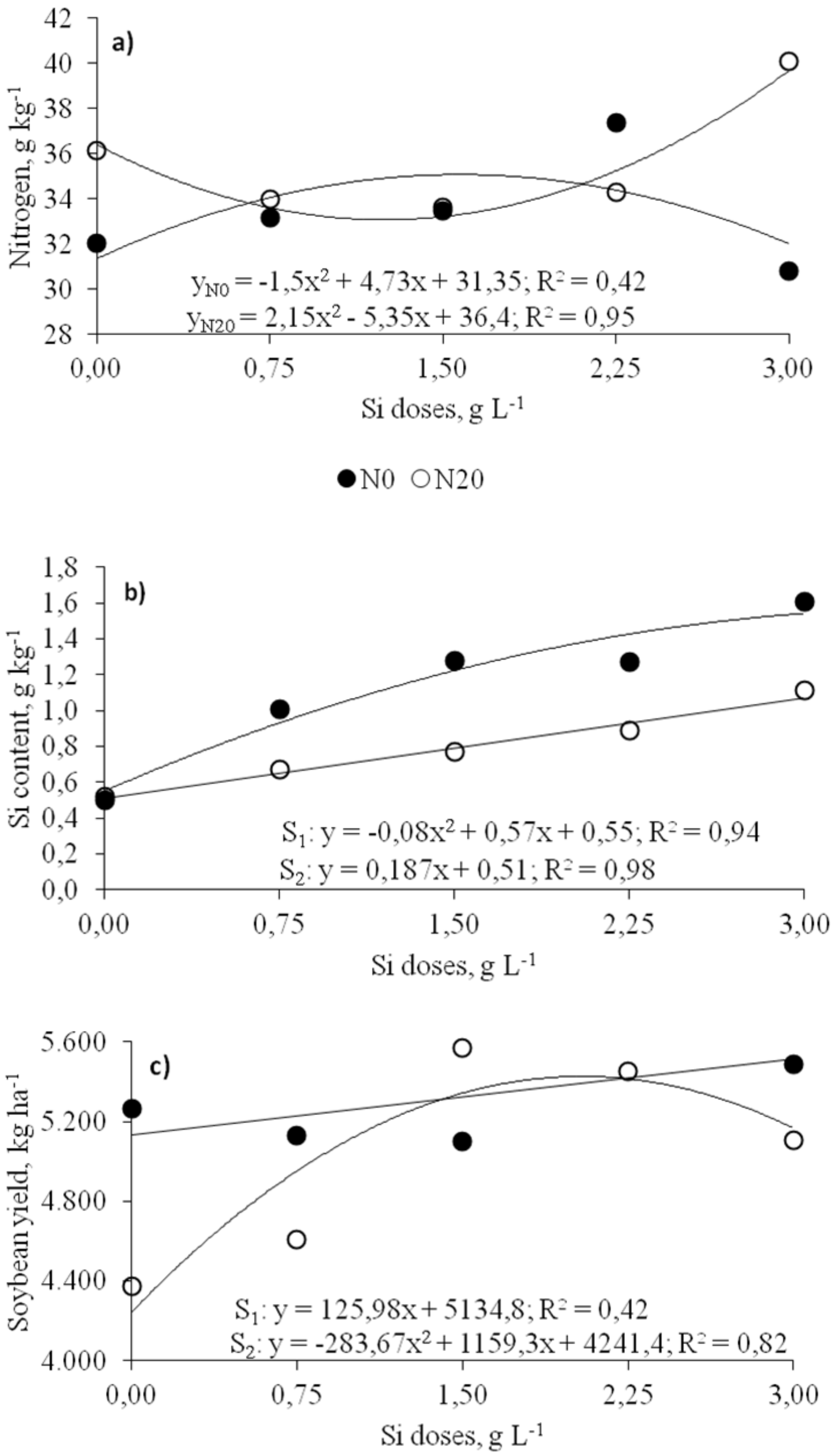
Brazil is one of the most soybean crop producers around the world. Over 40,8 millions of hectare, Brazil achieves production of 122.0 millions tons of crops per year and near to 3,000 kg ha^{-1} of productivity this year (Conab, 2022). The main problem in Brazilian soybean crop production systems is the climate risk associated with nutritional management to lead producer to high levels in term of productivity. Soybean is a legume that requires 80.0 kg of N to produce 1 ton of grains (Fagan et al., 2007). The main source to soybean in tropical conditions such as Cerrado cames from biological nitrogen fixation processus (BNF), yet there are recommendations to add in the first stage no more than 20.0 kg ha^{-1} (Sousa and Lobato, 2004). At the same time, silicon (Si) is a beneficial element to plants because it can mitigate deleterial effects caused by biotical and abiotical stress very typical in tropical systems. But, silicon, as a sourse, must to be used in very high quantity when it is directly applied in soil, and then low efficience response. In this way, it has been appeared in the market new silicon sources, and among them estabilized potassic silicate with foliar technology application usage. Finally, the aim of this study was to avaliate the effects of silicon foliar application associated with nitrogen source over soybean crop production in the especific edafoclimate conditions of Brazilian *Cerrado*.

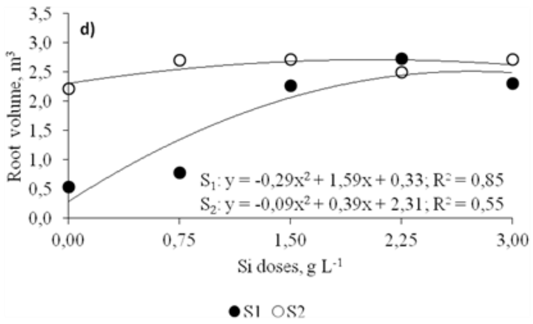
METHODS

The study was managed in-between 2019/2020 and 2020/2021 agricultural year in Universidade Federal de Goiás (EA - UFG) at 16°35"S e 49°21"W geographical coordenations and 730 m altitude. The regional climate is denominated as megathermical tropical savana (Aw), annual precipitation of 1,495 mm considering the last 36 years. This experiment was a random-factorial (5x2) and four repetitions. Treatments were composed by five silicon doses (control 0; 0.75; 1.50; 2.25 and 3.00 g L^{-1}) using cupper and potassic silicate stabilized with sorbitol 10% (Si = 107 g L^{-1}; K_2O = 34.7 g L^{-1}; Cu = 14.9 g L^{-1}; pH = 11.8), and two conditions being presence (20 kg of N ha^{-1} - urea) and absence of nitrogen source 20 days after the emergence (DAE). The fertilizations were made based on Souza and Lobato (2004). A part of the plants was seeded into acrylic columns to afterwards analysis. At the end, in the maturation phase, it was done the evaluation of roots using a root-scanner at 0,40 m of depth fixed at the columns. The harvesting was done exclusively in the middle of each plot, and humidity was corrected to 13% and transformed into kg ha^{-1}. Data were analyzed using F test and AgroEstat package.

RESULTS AND DISCUSSION

Figure 1 shows the results of nitrogen and silicon in soybean leaves, and grain productivity, root volume after treatments application. Nitrogen content in the second harvest (2020-2021) upgraded when compared with the first (2019-2020) and they do not save some direct relation with silicon applications; and the opposite when nitrogen was added in the system. Both interaction N x Si presented quadratic response being the highest performance at 1.5 g Si L^{-1} (Figure 1a). Considering silicon variation in plants both harvesting presented growing linear development achieving the maximum at the higher concentration (Figure 1b). Grain productivity performed differently to both harvesting; the first one (2019-2020) presented linear increments and the second (2020-2021) quadradic response being 2.04 g L^{-1} the most efficient result (Figure 1c). An important disclaimer is that both crop productions presented high productivity (higher than 5,000 kg ha-1). Root volume was upper to both harvesting (Figure 1d) and silicon applications (Figure 1e).





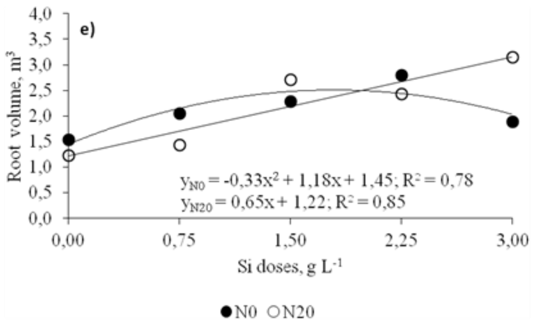


Fig. 1. Nitrogen Content (a), silicon content (b), grain productivity (c), root volume and harvesting (d), and root volume and nitrogen (e) of soybean of *Cerrado* crop system.

CONCLUSIONS

Silicon foliar application can improve soybean root development and can promote grain productivity at *Cerrado* edaphoclimatic conditions.

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INTRACANOPY LIGHTING ACCELERATES SOYBEAN GROWTH

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Keywords: *Glycine max,* soybean; Intracanopy lighting; speed breeding

INTRODUCTION

On Earth, with its nearly 7.7 billion inhabitants, a figure that is expected to climb to 9.7 billion by 2050, global climate change and the slow-release rate of new crop cultivars are raising concerns regarding global food security. Long generation spans of crops are the key limiting factor in breeding. "Speed breeding" is a method to grow plants under an extended photoperiod, higher temperatures and with supplementary LED (light emitting diode) lights to accelerate plant growth. Recently speed breeding techniques were introduced for long-day crops, but short-day plants protocol still is lacking up to date. In addition, planophile plant leaves stand perpendicular to overhead light, thus shaded inner canopy and side leaves end up less efficient in photosynthetic work. The aim of this study is to present an improved lighting system to the plants for a speed breeding protocol based on LED-based lighting systems for soybean.

METHODS

One soybean seed (*Glycine max* cv.) was sown in 7 cmx7 cmx8 cm pots including torf (NPK 1536/150/220 mg/l, BioBizz, The Holland) for all experiments. The plants were exposed to deficiencies of N, P, K, Mg. To do so, before potting the nutrient solution was depleted in one of these four nutrients, while other nutrients were kept in the fertilization were (in mg kg^{-1} soil): 250 nitrogen (N) as $Ca(NO_3)_2$, 120 N as NH_4NO_3, 100 phosphorus (P) as KH_2PO_4, 100 potassium (K) as K_2SO_4, 2 Zn as $ZnSO_4$ and 2 Fe in the form of $FeSO_4$. Watering was given 15 ml deionized water daily. The experiments were conducted within a climate-controlled chamber in 12h photoperiod, 550 µmol/m2 s PPFD light intensity. The environmental conditions were 30°C day and 29°C night and 70% relative humidity. LED modules: Full spectrum LED modules were equipped as overhead illumination. The irradiation was white (5700 K) and red (660 nm) light spectra (Fig. 1).

Fig. 1. Emission spectra of LED module type "Samsung" at 100% intensity.

The staggered harvesting dates were (55 and 65 days) in order to test different maturity periods. The harvested pods and shoot parts were dried at 45°C for 2 days before dry weight was recorded.

RESULTS AND DISCUSSION

Harvesting

Focusing on soybean we evaluated N, P, K and Mg deficiency effect on pod setting and flowering time. N and P deficient plants resulted in fastest flowering, K and Mg deficient plants showed the latest flowering with the smallest pods. The average flowering day for N deficient plants was 22 days, P deficient plants 24 days, Mg deficient plants 30 days and K deficiency plants 31 days under white-based full spectrum light regime. However the flowering time of the deficient treatment groups did not occur in a homogeneous fashion. Next, the staggered harvesting dates 56 points of day 56 and 65 of the soybean pods were evaluated according for their dry weight production and visibility. When the plants were 29 days old intracanopy lights, 100 mm diameter, energy equivalent to 400 µmol/m2 s PPFD were installed. The trial is continuing.

CONCLUSIONS

Optimizing yield and growth volume while enhancing the energy efficiency of light sources is the key success for faster plant breeding in controlled environment. The energy delivered to the inner canopy could improve the efficiency of soybean pods and shoot part. This might be through increased CO_2 assimilation, O_2 synthesis via increased photon delivery to the canopy in a reasonable amount.

THE IMPACT OF SILICON FERTILIZATION ON PLANT GROWTH AND BIOCHEMICAL ATTRIBUTES OF WHEAT CULTIVARS GROWN UNDER WATER DEFICIT STRESS

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Keywords: Silicon; Drought; Wheat

INTRODUCTION

In the framework of climate change, crop production is projected to decrease as a consequence of exacerbated environmental stresses, including water deficit derived from drought (Wang et al. 2021). In this context, the improvement of plant tolerance to drought is a crucial challenge to sustain the production and quality of massive consumption foods such as wheat. A wide range of benefits of silicon (Si) on plant metabolism has been reported under water deficit stress (Wang et al. 2021). However, the mechanisms underlying the role of Si on drought stressed plants are still unclear. This study is aimed to investigate the impact of Si fertilization on plant growth and biochemical features of two wheat cultivars grown under water deficit stress at tillering stage.

METHODS

A pot experiment using two wheat (*Triticum aestivum* L.) cultivars with differential tolerance to water deficit stress (cv. Kiron; tolerant and cv. Tukan; sensitive) was conducted. Plants were cultivated on a soil never fertilized with Si, and subjected to three irrigation regimes (well-watered, 90% of water holding capacity [WHC]; mild drought 75% WHC and severe drought 35% WHC) in combination with three Si fertilization doses (0, 500 and 1000 mg Si kg^{-1} soil; applied as Na_2SiO_3). Three weeks after imposed the experimental treatments, plants were harvested and shoot dry weight, relative water content (RWC) and water potential were evaluated. Additionally, lipid peroxidation (TBARS), free proline and total soluble sugars were determined. Experimental data were analyzed using analysis of variance (ANOVA), and differences among means were separated using Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Water deficit stress decreased shoot biomass of both wheat cultivars compared with the well-watered plants (Table 1). Low water availability also reduced RWC and water potential mainly in the sensitive cv. Tukan. Likewise, an evident increment of TBARS, proline content and soluble sugars was observed in both cultivars subjected to severe drought (Table 1; Fig. 1). In contrast, Si applied to water-deficient plants reduced the oxidative stress induced by drought, and consequently it improved shoot dry weight by about 37% and 43% in cv. Kiron and Tukan, respectively (Table 1). The water potential was also augmented by Si in cv. Kiron grown at both 75% and 35% WHC. Similarly, Si increased the water potential of cv. Tukan subjected to severe drought. On the other hand, we found that Si increased the proline levels in both cultivars exposed to severe water deficit (Fig. 1A). In addition, under 35% WHC, we found that Si decreased the sugar content in cv. Kiron, whereas it remained without changes in cv. Tukan after Si supply (Fig. 1B). Variations in the accumulation of proline and sugars as result of Si application under water deficit conditions have also been reported by Parveen et al. (2019) and Bukhari et al. (2021). Taken together, Si improved the tolerance to water deficit stress of both wheat cultivars by decreasing the oxidative damage, increasing water potential and modulating the accumulation of osmoprotectants such as proline and soluble sugars.

Table 1. Shoot dry weight (DW), relative water content (RWC), water potential, and lipid peroxidation (TBARS) of two wheat cultivars grown under different irrigation regimes and Si doses.

Cultivar	WHC	Si dose	DW (g)	RWC (%)	Water potential (Mpa)	TBARS (nmol MDA g^{-1} FW)
Kiron	90%	0	4.61 ± 0.24 a	91.11 ± 1.16 ab	-0.51 ± 0.08 a	9.99 ± 1.56 f
		500	4.58 ± 0.33 a	93.39 ± 2.97 ab	-0.82 ± 0.06 bc	10.48 ± 1.27 f
		1000	4.61 ± 0.17 a	94.08 ± 2.19 a	-0.91 ± 0.08 bcd	11.50 ± 1.92 ef
	75%	0	3.83 ± 0.01 abc	91.03 ± 3.73 ab	-1.16 ± 0.17 ef	13.12 ± 1.92 def
		500	4.21 ± 0.20 ab	92.08 ± 2.18 ab	-0.87 ± 0.09 bc	11.69 ± 0.88 ef
		1000	4.08 ± 0.28 ab	91.70 ± 1.66 ab	-0.82 ± 0.06 bc	10.18 ± 0.52 f
	35%	0	3.51 ± 0.23 bcd	85.04 ± 3.31 abcd	-1.63 ± 0.09 i	26.42 ± 1.90 a
		500	3.47 ± 0.11 bcd	79.75 ± 6.19 cde	-1.33 ± 0.04 fg	21.60 ± 0.86 b
		1000	3.97 ± 0.04 abc	87.50 ± 2.39 abc	-1.20 ± 0.08 ef	20.05 ± 1.18 bc
Tukan	90%	0	3.77 ± 0.18 abc	95.29 ± 2.52 a	-0.93 ± 0.05 cd	13.21 ± 1.25 def
		500	4.48 ± 0.16 a	92.52 ± 2.29 ab	-0.73 ± 0.04 bc	16.32 ± 1.56 cd
		1000	4.53 ± 0.41 a	93. 54 ± 2.93 ab	-0.71 ± 0.025 ab	14.83 ± 1.11 de
	75%	0	3.08 ± 0.64 cd	88.20 ± 3.93 abc	-1.20 ± 0.08 ef	21.00 ± 1.08 b
		500	3.86 ± 0.74 abc	83.66 ± 7.50 bcd	-1.08 ± 0.08 de	18.97 ± 1.36 bc
		1000	4.23 ± 0.42 ab	88.46 ± 1.37 abc	-0.85 ± 0.04 bc	19.18 ± 1.17 bc
	35%	0	2.66 ± 0.46 d	66.42 ± 6.92 f	-1.76 ± 0.04 i	26.43 ± 1.37 a
		500	3.51 ± 0.23 bcd	70.05 ± 6.14 ef	-1.58 ± 0.08 hi	21.02 ± 2.51 b
		1000	3.80 ± 0.58 abc	75.04 ± 3.44 def	-1.41 ± 0.06 gh	20.38 ± 2.01 b

Values are means ± standard deviation of four replicates. Different letters indicate statistically significant differences (p ≤ 0.05) among treatments.

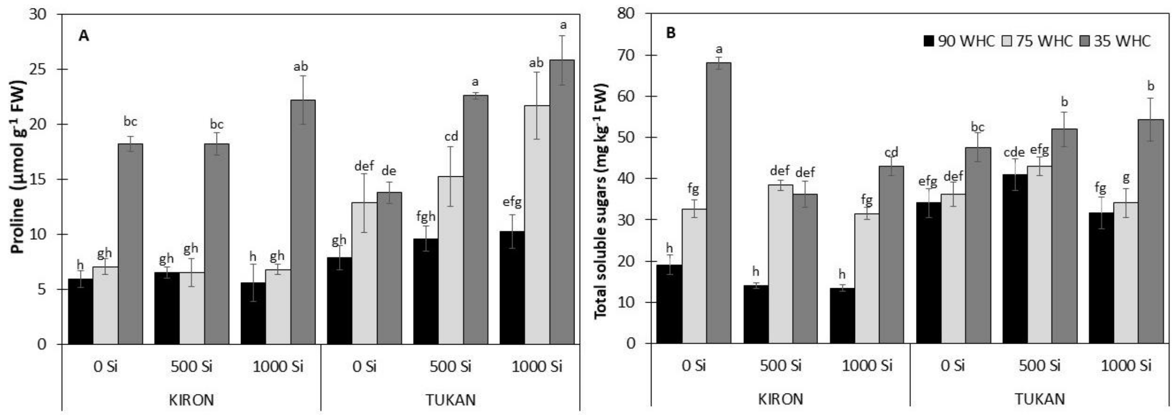

Fig. 1. Effect of silicon on proline content and soluble sugars in shoots of two wheat cultivars grown under different irrigation regimes. Values are means ± standard deviation of four replicates. Different letters indicate statistically significant differences (p ≤ 0.05) among treatments.

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INSIGHTS ON ZN DEFICIENCY AND ZN EXCESS EFFECTS ON THE ROOT APICAL MERISTEM IN ARABIDOPSIS

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Keywords: Zinc; Root Apical Meristem; RNA-Seq

Zinc, an essential cofactor to many enzymes, is an important micronutrient in plants. Crop culture on Zn deficient soils, which are widespread worldwide, is limiting productivity and quality and is considered as a concern for human nutrition. In contrast, with industrialisation, soils were contaminated in large areas with toxic metal concentrations including Zn. To address both issues of Zn deficiency and Zn excess, it is required to better understand the responses to variation in Zn supply in plants, which will enable the design of efficient biofortification or phytoremediation strategies, respectively.

Root growth is regulated at the root apex, where mitosis, as well as cell elongation and differentiation occur. Zn deficiency and excess are known to have an effect on root growth, for instance by affecting auxin production and Reactive Oxygen species (ROS) levels in the Root Apical Meristem (RAM). However, a detailed investigation on the delicately balanced regulation of the root meristematic activity by Zn is still missing.

In this study, root responses to Zn deficiency and Zn excess were examined in Arabidopsis. We re-assessed RAM activity upon both Zn deficiency and Zn excess and observed distinct effects of both treatments on cell elongation and differentiation, as well as on cell cycle itself. To better pinpoint which specific processes are affected by altered Zn nutrition, RNA-Seq datasets were generated for root tips versus differentiated roots comparing normal Zn supply to Zn excess or Zn deficiency. We observed root tip specific responses and identified potential candidate genes (e.g. transporters, hormonal response, cell-wall) for tolerance to Zn supply imbalance. Furthermore, linking these RNA-Seq datasets with Zn localization in root tissues by Laser Ablation-ICP-MS experiments and publicly available single-cell transcriptional maps provided a detailed assessment of the RAM response to Zn supply.

RESPONSE AND EFFICIENCY OF ARABICA COFFEE CULTIVARS IN THE USE OF POTASSIUM IN NUTRIENT SOLUTION

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Keywords: *Coffea arabica*; potassium doses; genetic variability

INTRODUCTION

Brazilian coffee has expanded across several regions of the country and accounts for about 35% of world coffee production (CONAB, 2022), a fact resulting from the adoption of several technologies, stood out the use of soil correctives, and use high doses of chemical fertilizers. This occurs due brazilian soils are considered acidic and of low fertility with deficiencies of bases, such as potassium (K). This element is the second most demanded nutrient by coffee plants and is involved in several important roles in plants. Among these, it is responsible for osmotic regulation and is involved in beverage quality, with an important role in sugar concentrations and polyphenol oxidase enzyme activity in fruits (Clemente et al. 2015). To achieve high yields of coffee, the country ranks second in world consumption of potassium fertilizers (GloboFert, 2022), being 90% in the form of Potassium Chloride (KCl), which makes it dependent on the importation of this compound. Currently, with the crisis in the supply of chemical fertilizers, research focusing on mineral nutrition and the identification of more efficient genetic materials in the use of nutrients has been identified as an alternative to reducing the use of these inputs. The objective of this study was to evaluate, the response and efficiency of arabica coffee cultivars in the use of potassium in nutrient solution.

METHODS

The experiment was carried out in a greenhouse, in a randomized block design, forming a 20 × 2 factorial being 20 cultivars x 2 two doses of K, being adequate (4,0 mmol. L-1) and low (1,5 mmol. L-1), with three replicates in solution nutritive. The plots consisted of two plants/pots containing 8.0 L of modified Hoagland and Arnon (1950) nutrient solution. The seeds of the cultivars were germinated in a sand bed and transplanted at the cotyledon leaf stage ("jaguar ear"). During the conduct of the experiment, the pH of the nutrient solution was adjusted to between 5.5 and 6.5 and changed based on the electrical conductivity, whenever its depletion reached 30%. After 270 days of cultivation, the plants were harvested and the plant material was dried in a forced-air circulation oven at 70 ºC until reaching constant weight. Then, the plant material was weighed to determine the total dry mass (TDM). Based on this characteristic, the cultivars were classified according to the estimate of the α parameter (CIAT 978): Efficient Responsive (RE) and Non-Responsive (ENR); Inefficient Responsive (IR), and Non-Responsive (INR).

RESULTS AND DISCUSSION

The coffee cultivars showed differences in the production capacity of TDM and in response to the addition of potassium in the cultivation medium, being distributed in all quadrants of Figura 1. Among the evaluated cultivars, ten were classified as efficient in the low concentration of K, for presented TDM values above the general average (17,65 g), therefore, with potential for cultivation environments with low fertility. Of these, 50% responded to an increase of K, because showed values of the α parameter above the general average (0.126), which also makes them promising for cultivation with the use of potassium fertilization in production systems. It was found that ten coffee cultivars were classified as inefficient in the use of K, whose TDM values are below general average when cultivated with restriction of this nutrient (Figura 1). Of these, the cultivars Paraíso MG H 419-1, San Ramon, IPR 103, Pau Brasil MG1 and Acaiá Cerrado MG 1474 were responsive to the increase in K, due increases in the TDM. The others cultivars were classified as non-responsive, probably due to the nutrient accumulation mechanisms common in old cultivars such as São Bernardo. Similar results were found in cultures with low availability of N (Moura et al., 2019) and of Zn (Pedrosa et al., 2013).

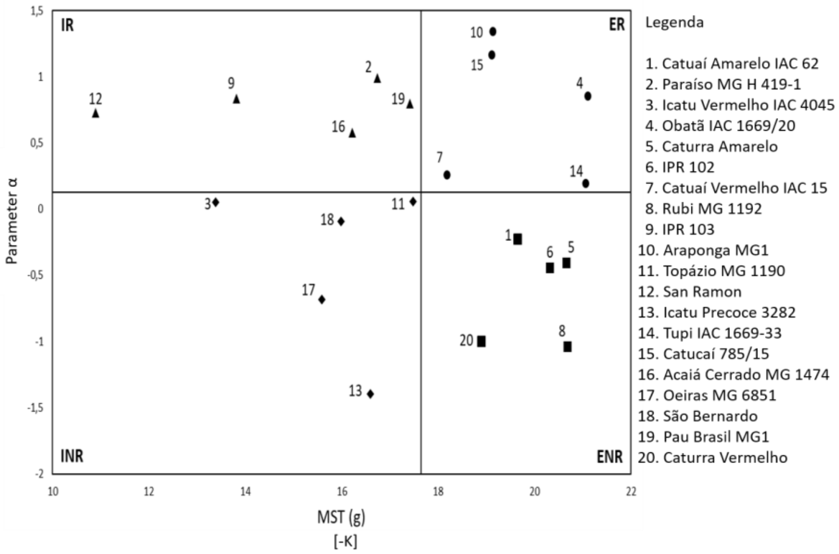

Fig. 1. Classification of 20 coffee cultivars according to nutritional efficiency and response to potassium concentrations potassium in the nutrient solution: Efficient Responsive (RE), Efficient Non-Responsive (ENR), Inefficient Responsive (IR) and Inefficient Non-Responsive (INR).

CONCLUSIONS

Genetic variability was detected among coffee cultivars concerning efficiency and response to nitrogen concentrations nutrient solution. The cultivars Obatã IAC 1669/20, Araponga MG1 andCatucaí 785/15 are the most efficient and responsive to potassium.

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ZINC NUTRITION IMPROVES STRESS TOLERANCE, PRODUCTIVITY AND GRAIN ZINC BIOAVAILABILITY IN DROUGHT-STRESSED WHEAT

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Keywords: zinc; drought; bioavailability

INTRODUCTION

Climate change and spatiotemporal shifts in agricultural droughts are threatening crop production as well as human nutrition on a global scale (IPCC 2103; FAO 2019). However, the interactive effects of drought stress and low Zn nutrition on the major biochemical and physiological functions of crops have rarely been studied. Moreover, the shifts in localization of Zn in different grain fractions and its bioavailability under drought stress and Zn deficiency, has never been studied. This study aimed to reveal whether correcting Zn nutrition can simultaneously improve ROS detoxification mechanisms, crop productivity and grain Zn bioavailability in wheat simultaneously stressed with low water and Zn availability, which is a very common growth condition particularly in arid regions and calcareous soils around the world.

METHODS

Bread wheat (*T. aestivum*) was cultured with low or adequate Zn, under well-watered and drought-stressed conditions. Changes in antioxidative and photosynthetic performance, concentrations of grain mineral nutrients, grain yield, yield components, and localization and bioavailability of Zn in the grains, were quantified. Changes in Zn bioavailability to the human gut was estimated by the [phytate]:[Zn] ratio and the trivariate model of Zn absorption.

RESULTS and discussion

Drought stress disrupted photosynthetic and antioxidative performance, plant-water relations, mineral nutrient uptake, and yield-related traits. Low Zn supply further escalated these disruptions. With adequate Zn, drought-stressed plants had higher chlorophyll density, and in well-watered plants, photosynthetic performance, indicators of plant-water relations, and biomass production were restored. Drought-stressed plants had higher leaf protein, specific superoxide dismutase activity and phenolics concentrations, lower malondialdehyde level with adequate Zn, whereas activities of ascorbate peroxidase and glutathione reductase were noted only in well-watered plants supplied with low Zn. Adequate Zn enhanced uptake of Zn, N and K, particularly under drought stress. Adequate Zn also enhanced grain yield and yield components, Zn concentration in grain fractions, and grain Zn bioavailability under well-watered or drought-stressed conditions.

CONCLUSIONS

Adequate Zn supply under drought stress increased crop performance, grain Zn concentration and bioavailability. Meeting the Zn requirement of the wheat crop is crucial to tackle drought stress and can simultaneously address food and nutrition security.

Table 1. Effect of watering regime and Zn supply on relative water content (RWC), shoot dry matter production and leaf Zn concentration at the tillering stage and grain yield, 100 grain weight, grain protein and bioavailable Zn in grain at the maturity stage of wheat.

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FOLIAR APPLICATION OF MN AND ZN IN EUCALYPTUS PLANTS AMELIORATES NEGATIVE EFFECTS OF LOW ROOT OXYGEN AVAILABILITY

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Keywords: hypoxia; micronutrients; plant stress

Flooding events are expected to increase in frequency and intesity with climate change. Consequently, yield losses caused by reduced soil oxygen availability are projected to increase in agriculture and forestry. Zinc (Zn) and manganese (Mn) play important roles in mitigating the damage caused by biotic and abiotic stresses in plants . Low Zn an Mn availability have been occurred in tropical forest soils in which flooding events are frequent. However, studies relating the application of these nutrients with reduced negative effects of hypoxia in plants are still scarce. In the present study, we conducted a pot experiment to evaluate the effect of foliar application of Mn and Znon eucalypt plants subjected or not to low oxygen availability. The experiment was conducted in greenhouse conditions. Treatments were combined in a factorial arrangement, with and without hypoxia, with and without foliar application of Zn (0.1 g/L) and Mn (0.5 g/L). The experimental design was in randomized blocks, with five replicates. Each experimental unit consisted of 1 pot, containing washed sand and 2 eucalyptus plants (*Eucalyptus urohphyla* x *Eucalyptus grandis* hybrid). In treatments with hypoxia, O_2 concentration was kept below 4 mg L^{-1}. In the treatments without hypoxia, the pots were maintained unsaturated. During the experiment gas exchange variables were measured. At the end of the of the experimental period, the content of soluble sugars and starch was quantified in plant leaves. In all the tretaments in which plants were submited to hypoxia, with and without micronutrients, plant stomatal conductance and photosintesis reduced in the first days of hypoxia. Plants that received foliar application of Mn, however, showed a considerable increase in photosynthetic rates during the stress period. The application of Zn promoted an increase in photosynthetic rates at the end of the experiment, although toxic effects of this nutrient were detected after foliar spraying. Plants that received foliar application of Mn and Mn+Zn had a higher accumulation of starch.

Financial Support

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DIFFERENTIAL RESPONSES OF KABULI AND DESI CHICKPEAS (*CICER ARIETINUM*) TO LOW WATER PROVISION AND THEIR MINERAL PROFILING

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Keywords: drought stress; underutilised crop; legumes

INTRODUCTION

Legume grains are of great importance for agriculture and the environment due to their ability to symbiotically fix atmospheric nitrogen and provide protein, minerals, vitamins, and other bioactive nutrients (Geraldo *et al*., 2022). Chickpea (*Cicer arietinum*) is one of the most consumed legumes worldwide and it has gained even more importance in recent decades. Production levels have increased, their incorporation as intercrops has been promoted, and they have been used in the formulation of novel food products (Saget *et al*., 2020). Nonetheless, the exploitation of traditional chickpea varieties, such as the Desi type (black coloured chickpea), has been overlooked, and the recovery of under-exploited traditional varieties could contribute to fostering biodiversity, and promoting environmental sustainability and diversifying diets. However, current knowledge on the nutritional profile of commercial and traditional chickpea varieties and their resilience degree to environmental stresses, such as water scarcity, is limited, thus being the focus of this work.

METHODS

Seeds of a commercial Kabuli (white) chickpea and a traditional Desi (black) genotype were analysed for mineral and protein composition (Nunes da Silva *et al*., 2022). Antioxidant capacity was determined spectrophotometrically as in Marinova *et al*. (2005). Seeds were also germinated and grown in a climate chamber under distinct water provision conditions. Water was provided three times a week, in variable amounts corresponding to 90%, 50% or 25% of the field capacity (N = 15). At pod filling, plants were analyzed for root and shoot fresh weight and number of seeds per plant.

RESULTS AND DISCUSSION

Both chickpea types had significant amounts of several macro- and micro-nutrients important for human nutrition, such as potassium (which averaged (?) 2 $mg.g^{-1}$), magnesium (? 600 $µ.g^{-1}$), zinc (? 17 $µg.g^{-1}$) and iron (? 23 $µg.g^{-1}$) (Table 1). The Kabuli chickpea was richer in boron (by 30%), while the Desi black chickpea had a higher level of antioxidant compounds (by 32%), which comprise bioactive non-nutrients important for human health (Geraldo *et al*., 2021). These results support the inclusion of chickpea in novel food formulations, including, e.g., pasta (Saget *et al*., 2020).

Table 1. Nutritional profile of Kabuli (white) and Desi (black) genotypes. Values represent the mean ± SEM and letters indicate statistically different means at *p* < 0.05.

Financial Support

ACKNOWLEDGEMENTS This research was supported by the European Union's Horizon 2020 Research and Innovation Programme through project RADIANT, Grant Agreement number 101000622, and by the project HSoil4Food co-financed by FEDER through the Northern Regional Operational Program (NORTE-01-0145-FEDER-000066). The scientific collaboration under FCT project UIDB/50016/2020 and project LEGUCON (Fundação Calouste Gulbenkian, 238442) is also acknowledged.

DISCRIMINATING FE DEFICIENT SOYBEAN PLANTS BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Keywords: bioavailability; soybean; stress conditions

INTRODUCTION

Iron deficiency chlorosis (IDC) is a serious environmental problem that affects several crops in the world. Under alkaline soils, iron (Fe) bioavailability is limited and plants suffer from leaf yellowing, stunted growth and yield losses. Finding early, non-invasive, detection methods for IDC is of great agronomic importance, as these may help farmers improve fertilization timings and avoid economic losses. Fourier transform infrared (FTIR) spectroscopy have been explored as an easy use, cheap, and environmentally friendly technique for several proposes as plant species and/or cultivars discrimination. The main goal of this work was to test the ability of FTIR spectroscopy to discriminate two groups of soybean (*Glycine max*) plants treated under different Fe stress conditions.

METHODS

Thirty *Glycine max* seeds were germinated for seven days in the dark at 25 ºC. Germinated seedlings were transferred to 5 L vessels containing hydroponic solution with 20 mM FeEDDHA (Santos et al., 2019). Plants were maintained under these conditions for two weeks, after which, they were divided in two groups - one supplied with 20 mM FeEDDHA (Fe+; n=15) and other without Fe supplementation (Fe-; n=15). For the five following weeks, three plants of each treatment were collected / week and analysed. Chlorophyll concentration was measured according to Sims and Gamon (2002). The antioxidant activity was evaluated by the ABTS and DPPH radicals scavenging assays, as described by Vilas-Boas et al. (2020). Infrared spectra acquisition was perfomed according to the methods described in Páscoa et al. (2019).

Data were analyzed with GraphPad Prism version 6. Differences among treatments were examined using 2-way ANOVA with Fisher's LSD Test. Infrared spectra were pre-processed with standard normal variate (SNV) and Savitzky-Golay filter to remove baseline drifts and further mean centered. Spectra were subsquently modelled by partial least square discriminant analysis (PLSDA) to evaluate the potential of FTIR to discriminate between Fe- and Fe+ plant samples.

RESULTS and discussion

To evaluate the stress level of plants grown under the different Fe treatments, total chlorophyll concentration and antioxidant activity was measured in the trifoliate leaves of Fe+ and Fe- plants (Table 1).

Table 1. Total chlorophylls (mmol/g FW), DPPH (mg/g) and ABTS (mg/g) concentrations in shoots of *Glycine max* plants grown under optimum conditions for two weeks and then treated either with (Fe+) or without (Fe-) ironj supplementation for the five following weeks**[1]**

		Week 1	Week 2	Week 3	Week 4	Week 5
Total chlorophylls	Fe +	0.021 ± 0.005[a]	0.020 ± 0.001	0.019 ± 0.002[a]	0.020 ± 0.001	0.024 ± 0.001[a]
	Fe -	0.010 ± 0.001[b]	0.015 ± 0.001	0.014 ± 0.001[b]	0.017 ± 0.001	0.017 ± 0.002[b]
DPPH	Fe +	0.860 ± 0.033	0.858 ± 0.026	0.808 ± 0.056[a]	0.745 ± 0.022[a]	0.734 ± 0.038[a]
	Fe -	0.867 ± 0.027	0.846 ± 0.075	0.519 ± 0.005[b]	0.491 ± 0.017[b]	0.434 ± 0.034[b]

ABTS	Fe +	0.541 ± 0.044	0.505 ± 0.079	0.595 ± 0.035[a]	0.528 ± 0.020	0.411 ± 0.092
	Fe -	0.453 ± 0.099	0.463 ± 0.054	0.339 ± 0.051[b]	0.378 ± 0.004	0.331 ± 0.046

As expected, total chlorophyll concentration was always higher in Fe+ treated plants than Fe- (Table 1). Since Fe plays a role in the biosynthesis of photosynthetic pigments, IDC has been associated with chlorophyll synthesis inhibition (Santos et al., 2019). Iron is also a main co-factor of several antioxidant enzymes. DPPH and ABTS show the radical scavenging ability of antioxidants. The results depicted in Table 1 show that, after two weeks under Fe deficiency, the antioxidant capacity of plants decreased significantly when compared to plants that were maintained under optimum conditions throughout the entire assay.

Having the physiological results confirming that the two groups of plants were under distinct stress conditions, the infrared spectra were analysed. The optimum spectral region for the discrimination between Fe+ and Fe- plants was dominated by carbohydrates functional groups vibrations enabling postulating that this class of compounds was highly affected by the Fe stress.

The PLSDA model (Figure 1A) exhibited two clusters corresponding to infrared spectra of Fe + (green) and Fe- (red) plants. The exception was noticed for spectra from week 1 for which Fe+ and Fe- infrared spectra were quite ovelap (Figure 1B- model presented in figure 1A but colored by week). This was a somehow expected results once in week 1 plants were probabiliy yet barely affected by the presence/deficiency.

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[1] Values represent means ± SEM; different letters between Fe treatments of each parameter and of each week represent statistically significant differences.

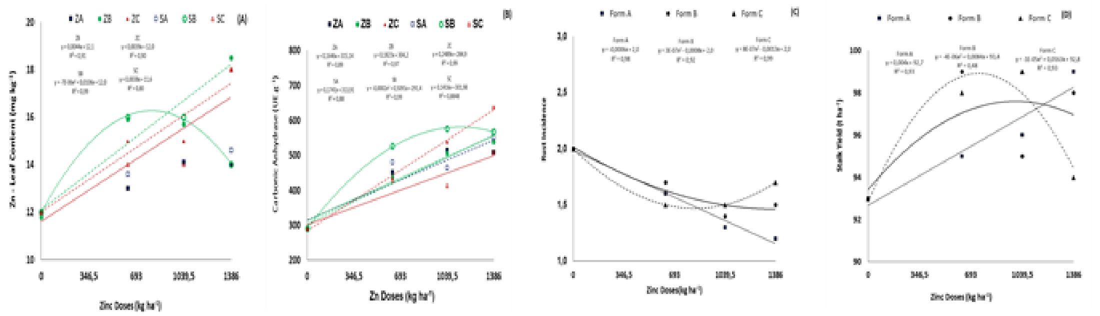

Fig. 1. Scores plot of the PLSDA regression model obtained for discrimination between Fe+ (?) and Fe- (?) plants. B: Scores plot of the PLSDA regression model obtained for discrimination between Fe+ and Fe- plants colored by week: 1st week (?); 2nd week (?); 3rd week (?); 4th week (?); 5th week (?).

CONCLUSIONS

FTIR analysis can potentially provide a rapid, easy, and non-invasive way to detect IDC. Further predictive models can help farmers deciding key factors, contributing for the implementation of sustainable agricultural practices.

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INTRASPECIFIC EFFECTS OF SHORT-TERM ELEVATED ATMOSPHERIC CO_2 IN YIELD AND NUTRITIONAL PROFILE OF *PHASEOLUS VULGARIS*

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Keywords: Common bean; Elevated CO2; Minerals

INTRODUCTION

Legumes are key contributors of essential nutrients for human health, namely iron (Fe) and zinc (Zn), but they are one of the most sensitive plant families to elevated concentrations of atmospheric CO_2 (eCO_2), a major threat to global agriculture and human nutrition. Therefore, unravelling the effects underlying eCO_2 responses on biomass yield and nutritional value is of utmost importance to anticipate potential negative effects on human nutrition and expedite mitigation strategies.

METHODS

P. vulgaris (common bean) genotypes (Logan, G1378 and Kazak) were grown in field conditions under ambient concentrations of atmospheric CO_2 (aCO_2, 400 pm) or eCO_2 (600 pm). Plant's above ground biomass of the three genotypes was collected after 1 month of exposure to aCO_2 or eCO_2. Biomass estimation considered 10 representative plants, per genotype and plot, randomly selected and uprooted. Seed samples were analyzed for important micronutrient concentrations, namely zinc (Zn), manganese (Mn), iron (Fe) and boron (B), following the procedure described by Santos *et al*. (2020). Mean comparisons were performed through analysis of variance (ANOVA) followed by Fisher's LSD test ($p < 0.05$) in GraphPad Prism version 9.0.

RESULTS AND DISCUSSSION

Exposure to eCO_2 resulted in a significant increase of plant biomass in Logan and G1378 (between 1.2- to 2.1-fold), whereas Kazak was less impacted. As CO_2 is a substrate for photosynthesis, an increase in plant growth was expected, although few studies focused on short-term exposures (Shimono *et al*., 2008).

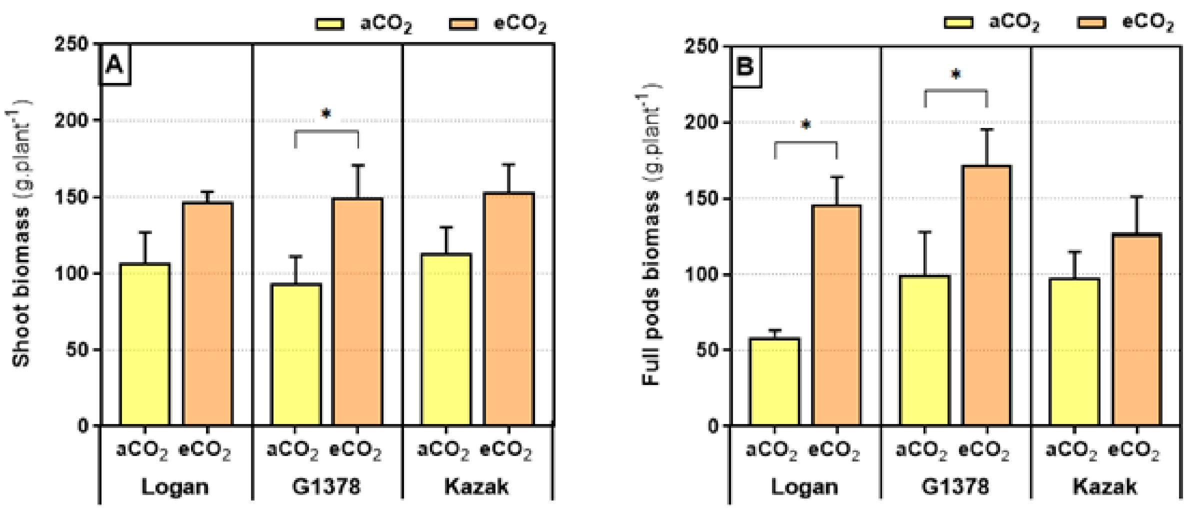

Figure 1 - Shoot (A) and full pods (B) biomass of 'Logan', 'G1378' and 'Kazak' grown under aCO_2 or eCO_2. Each value represents the average ± SEM and asterisks indicate significant differences at p<0.05

Zn and Fe accumulation in Logan was significantly reduced by eCO_2 in grains, whereas in Kazak it decreased in shoots and grains. These fluctuations in edible tissues such as the grains were also reported for wheat and barley (Loladze *et al*., 2014) and could exacerbate dietary micronutrient deficiencies in the near future (Soares *et al*., 2019). Contrarily, in G1378, Fe concentration increased in both shoots and grains. Different genotypes displayed contrasting behaviors behaviours, with Logan and G1378 appearing to be more resilient to nutritional losses caused by eCO_2.

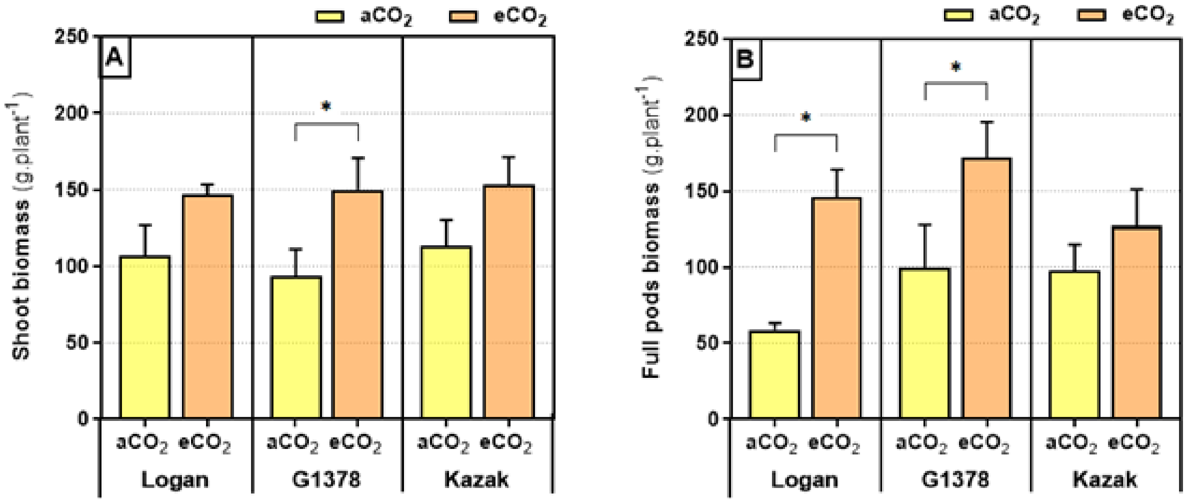

Figure 2- Leaf and grain micronutrients concentration, respectively, of 'Logan', 'G1378' and 'Kazak' grown under aCO_2 or eCO_2. Each value represents the mean ± SEM. Asterisks represent statistical differences at $p < 0.05$.

CONCLUSIONS

Elevated atmospheric CO_2 impairs common bean productivity and nutritional quality in an intraspecific manner, and genotypes such as Logan and G1378 may present higher resilience to this environmental stress.

Identifying resilient genotypes could lead to improved crops as an adaptative strategy to mitigate nutritional shortcomings under predicted eCO_2 conditions.

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MINERAL PROFILLING OF A PORTUGUESE COLLECTION OF COMMON BEAN (*PHASEOLUS VULGARIS L.*) GERMPLASM

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Keywords: Crop selection; quality of the seeds; germplasm

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most produced and consumed legume in Portugal. Recent data show an average national production of common bean of about 2397 tons, spread over about 3547 hectares of production area (FAOSTAT, 2017). As a rustic crop, beans are known to have great morphological variability and adapt to different environments and landraces and varieties vary morphologically and nutritionally. The nutritional composition of the grains can be conditioned by factors such as genotype, origin, environmental and cultivation conditions, influencing the quality of the seeds. Crop selection is generaly based on seed agronomic perfomance and the nutritional value has oftentimes been overlooked. The objective of this study was to evaluate 236 Portuguese common bean accessions from different Portuguese regions conserved in the National germplam bank for their mineral composition. Given the large diversity found in this local bean collection, understanding the intraspecific variability of mineral concentration may help in the identification of best performing accessions that can be adapted to different environmental conditions and improve the nutritional value of the seeds from farm to fork.

METHODS

Seed samples were pulverized and analyzed for six minerals [potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe) and zinc (Zn)] using ICP-OES, following the procedure described by Santos et al. (2020). Principal Component Analysis was performed using GraphPad Prism version 9.

RESULTS and discussion

One principal component analyses (PCA) was computed with the mineral composition measured in the 236 accessions of common bean (Figure 1). The first two principal components explained 67% of the total variability, with the first principal component explaining most of the variability (49%). These accessions are provenient from different regions of the Portuguese country, and it was possible to divide these accessions according to their origin in Northern, South, Centre and Madeira. The majority of accessions analysed have origin in the North of Portugal and shows more intraspecific diversity and outstanding lines when compared to seeds from other regions of the country.

This PCA showed that accessions BPGV12327, BPGV00983, BGPV11615 were among the accessions with the highest Zn and Ca concentration, while BPGV12385, BPGV05839, BPGV07580 and BPGV06771 had highest K, P, Mg and Fe.

The outstanding lines with highest Zn and Ca had origin from the Northern (accession BPGV12327) and Central Region of Portugal (accessions BPGV00983 and BPGV11615), whilst the outstanding accessions with highest K, P, Mg and Fe had origin from the Northern (accessions BPGV12385, BPGV07580 and BPGV06771) and Central Region of Portugal (accession BPGV05839).

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GROUNDNUT (*ARACHIS HYPOGEA* L.) GENETIC STOCK IDENTIFIED FOR TOLERANCE TO IRON DEFICIENCY CHLOROSIS IN CALCAREAOUS SOILS OF INDIA

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Keywords: Iron deficiency chlorosis; Groundnut; Tolerance attributes

INTRODUCTION

Groundnut, a functional food, is rich in energy (564 kcal per 100g), protein (25-30%), and vital micronutrients (*viz*., iron and zinc). Besides, groundnut is also having rare phenolics namely resveratrol and ferulic acids, which are gaining worldwide attention recently, owing to their immense health benefits and plant protection attributes. India ranks second after China in production of groundnut with contribution of nearly 20% to the global production (FAOSTAT 2020). Gujarat is the largest groundnut producer in India contributing nearly 37% of the total production. However, groundnut is cultivated in Gujarat in medium black calcareous soil with high pH and low organic matter content, a condition which favors transformation of iron from ferrous (Fe^{2+}): the available form to the ferric (Fe^{3+}): the non-available form for uptake. The Fe deprived plants exhibit chlorotic symptoms due to less synthesis of chlorophyll, which under severe condition impart papery white appearance to the leaves with necrotic patches and ultimately causes plant death. The management practices for alleviating Fe-deficiency requires repeated application of $FeSO_4$ due to poor phloem mobility of Fe (Irmak et al., 2012). Therefore, Identification of genotypes tolerant to IDC for Fe-deficient conditions is of utmost importance to provide immediate solution to this long-standing problem. Thus, an experiment was undertaken to identify groundnut genotypes tolerant to IDC in calcareous soil on the basis of physiological and biochemical parameters and to validate the same by expression of the relevant transporter genes.

METHODS

The present investigation was carried out during *Kharif* seasons of the years 2018, 2019, 2020 and 2021 at the experimental farm of the ICAR- Directorate of Groundnut Research, Junagadh, Gujarat, India (Lat. 21°31´N, Long. 70°36´ E), where the soil is medium black calcareous with low organic matter (0.52%) and high pH (8-8.5). The screening of 40 advanced breeding lines (ABLs) was done in field condition in a randomized complete block design with two replications to shortlist the best performing lines, which were further studied for physiological, biochemical and molecular traits involved in imparting IDC tolerance. Genotypes, ICGV 86031 and NRCG 7472, were used as tolerant and susceptible check, respectively.

Morphophysiological & Biochemical Parameters

The field screening of 40 ABLs was done based on visual chlorotic rating (VCR) proposed by Singh and Chaudhari (1993), SPAD chlorophyll meter reading (SCMR) and yield attributes. The Chlorophyll a, b and carotenoid contents were estimated using the methodology of Hiscox and Israelstam (1979). Lipid peroxidation was determined in leaf samples as the malondialdehyde (MDA) content (Heath and Packer, 1968). Ascorbate peroxidase (APX) (EC 1.11.1.11) was assayed by recording the decrease in optical density at 290 ?m (Nakano and Asada, 1981). Peroxidase (POX) (EC 1.11.1.7) activity was measured using the method given by Castillo et al., 1984. The phenolics profiling was done using an Ion chromatograph with Acclaim 120 C 18 reverse phase column (5 µm, 4.6 x 250 mm) to separate the phenolics. The yield attributes comprised of hundred kernel weight (HKW), number of mature pods per plant (NPP), pod yield (PY), and haulm yield (HY). The expression of genes regulating the uptake of Fe under Fe-deficit conditions was also studied by qRT-PCR run on a 96 well qTOWER 3G (Analytik Jena, Germany) with 3 step cycling conditions, 5 min at 95 °C to completely denature cDNAs, followed by 40 cycles of 15s at 95 °C and 15s at 60 °C.

RESULTS AND DISCUSSION

Identification of IDC tolerant ABLs While PBS 22040 and 29192 were identified as tolerant (higher SCMR and lower VCR score and superior yield attribute) to IDC, PBS 12185 and PBS 12215 were found to be highly susceptible (higher VCR and lower SCMR). Physiological and biochemical mechanisms imparting IDC tolerance Lipid peroxidation in terms of MDA content for susceptible lines (NRCG 7472, PBS 12185, PBS 12215) ranged from 11- 13 ?M cm^{-1} while tolerant lines (PBS 22040, PBS 29192, ICGV 86031) recorded 5-9 ?M cm^{-1}. The accumulation of MDA due to ROS generation elicits a signal for activation of antioxidant enzymes to protect the biological membranes from oxidative injury (Asada 1999). The quantum of the activity of Fe containing antioxidant enzymes, ascorbate peroxidase (APX) and peroxidase (POX), are the indicators differentiating the tolerant and susceptible genotypes under Fe-deficiency (Dasgan et al., 2007). The APX recorded significantly higher activity in the PBS lines 22040 (103.9%), 29192 (39.84%) and ICGV 86031 (35.9%) with a slight increase of 4.47% in sensitive PBS lines 12215 and 12185. However, POX activity was significantly expressed in all the lines but significantly higher in tolerant lines (51- 145%) over the susceptible ones (23- 35%) as compared to NRCG 7472. The phenolics profiling revealed a significant increase of 44-61% in resveratrol content in the kernels of tolerant lines (PBS 22040 & 29192) over the susceptible check in Fe-deficient condition. However, ferulic acid content recorded an abrupt increase in kernels of PBS 22040 (378.6 ppm) and 29192 (337.6 ppm) as compared to NRCG 7472 (124.4 ppm). The possible role of resveratrol and ferulic acid in imparting IDC tolerance can be attributed to their strong antioxidant capacity, which would suppress the lipid peroxidation through better ROS scavenging. The stress condition reportedly elicits increased synthesis and accumulation of resveratrol and ferulic acid. The qRT-PCR study revealed 2-fold over-expression of *YSL 3* and 1.5-fold over-expression of *NRAMP 3* transporters in the periplasmic membrane of roots of PBS 22040 and PBS 29192 as compared to the susceptible check NRCG 7472. CONCLUSIONS
The tolerance to IDC involves a diverse and intertwined physiological, biochemical and molecular components which are activated upon Fe-deficiency in soil. The genotype, PBS 22040, has been found to be the candidate genotype as a source of IDC tolerance and would be utilized for introgressing the traits in other high yielding cultivars to reduce the yield losses due to wide spread occurrence of IDC.

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NUTRITIONAL EFFICIENCY AND RESPONSE OF ARABICA COFFEE CULTIVARS UNDER DIFFERENT NITROGEN CONCENTRATIONS IN NUTRIENT SOLUTION

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Keywords: *Coffea arabica*; nitrogen doses ; genetic variability

INTRODUCTION

Brazil is the world's largest producer and exporter of arabica coffee, responsible for 47,7 million bags of processed coffee (CONAB, 2022). Coffee cultivation, in general, presents acidic soils with low fertility, and therefore, soil pH correction and the use of large amounts of chemical fertilizers are necessary to ensure the maximum productive potential of coffee. Among the important macronutrients for plant growth and development, the nitrogen (N) is the most demanded nutrient by coffee plants, as it performs fundamental biochemical functions, such as amino acid and protein synthesis, impacting photosynthesis, formation of flower buds, in addition to the effects on the chemical composition of the fruit (Clemente et al., 2015). Brazil is the fourth largest consumer of nitrogen fertilizers in the world, which makes it dependent on the import of these compounds (GloboFert, 2021). Due to the current fertilizer supply crisis, research focusing on mineral nutrition and the identification of more efficient genetic materials in the use of nutrients has been identified as an alternative to reducing the use of these inputs. The objective of this study was to evaluate, the nutritional efficiency and response of different arabica coffee cultivars to nitrogen.

METHODS

The experiment was carried out in a greenhouse, in a randomized block design, forming a 20 × 2 factorial being 20 cultivars and 2 two doses of N (adequate 7.5 mmol. L-1 and low 1 .0 mmol. L-1) with three replicates. The plots consisted of two plants/pots containing 8.0 L of modified Hoagland and Arnon (1950) nutrient solution. The seeds of the cultivars were germinated in a sand bed and transplanted at the cotyledon leaf stage ("jaguar ear"). During the conduct of the experiment, the pH of the nutrient solution was adjusted to between 5.5 and 6.5 and changed based on the electrical conductivity, whenever its depletion reached 30%. The plants were harvested 180 days after transplantation, and the plant material was separated into the root and shoot and dried in a forced-air circulation oven at 70 ºC until reaching constant weight. Then, the plant material was weighed to determine root dry mass (RDM), shoot dry mass (SHDM), and total dry mass (TDM). Based on the TDM, the cultivars were classified according to the methodology of Fox (1978): Efficient Responsive (RE) and Non-Responsive (ENR); Inefficient Responsive (IR), and Non-Responsive (INR).

RESULTS AND DISCUSSION

The coffee cultivars were distributed into 4 groups concerning nutritional efficiency and response to nitrogen addition in the in cultivation medium (fig. 1), which indicates differences in the use and responses to the increment of the production of TDM. Among the evaluated cultivars, eleven were classified as efficient in the low concentration of N, therefore, presenting the potential for cultivation environments with low fertility. Except for the cultivars Topázio MG 1190, IPR 103 and Tupi IAC 1669-33, the other cultivars responded to the increase in N with increases in TDM, mainly SHDM about RDM. These cultivars are also promising for production systems using nitrogen fertilization. Nine cultivars were classified as inefficient in the low nitrogen concentration for presenting low production of TDM. Of these, 54% did not respond to the increase in N concentration stood out two cultivars, San Ramon and São Bernardo, which are old cultivars and probably have a nutrient accumulation mechanism. Similar results were also found at low K availability (Moura et al., 2015) and Zn (Pedrosa et al., 2013). On the other hand, the cultivars Paraíso MGH 419-1, Catuaí Vermelho IAC 15, Pau Brasil MG 1, Acaiá Cerrado MG 1474, and Caturra Vermelho were inefficient but responsive to the increase of N presented increases in the production of TDM when at greater availability of the nutrient.

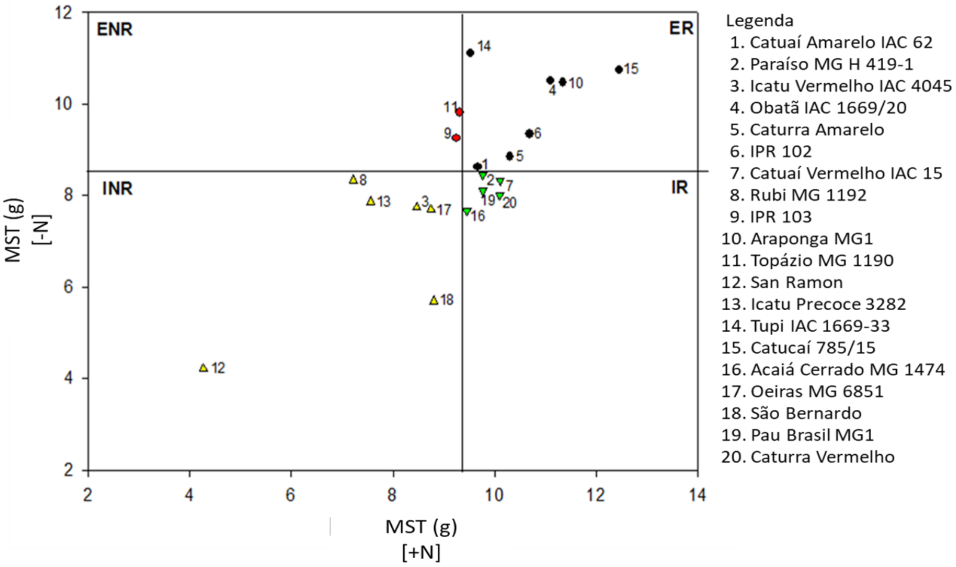

Fig. 1. Total Dry Matter (MST) of 20 coffee cultivars in response to nitrogen concentrations: adequate (+N) and low (-N), in the nutrient solution.Efficient Responsive (RE) and Non-Responsive (ENR); Inefficient Responsive (IR), and Non-Responsive (INR).

CONCLUSIONS

Genetic variability was detected among coffee cultivars concerning efficiency and response to nitrogen concentrations nutrient solution. The cultivars Obatã IAC 1669/20, Araponga MG1, Catucaí 785/15 and IPR 102 are the most efficient and responsive to nitrogen.

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The rhizosphere and plant nutrition

RHIZOSPHERE ENZYME ACTIVITY, ROOT GROWTH, AND ROOT HAIRS OF PEANUT ARE AFFECTED BY P AVAILABILITY

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Keywords: Sandy soil; P deficiency; Rhizosphere activity

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is grown mainly in sandy soils with low phosphorus availability, which limit grain yield and root growth (Naabe et al., 2021). Under low soil P availability, a strategy of peanut is to increase the density and length of root hairs or to increase root length, since some cultivars do not have root hairs (Wissuwa and Ae, 2001). However, P uptake has been reported to correlate with root length only in P-rich soils (Otani and Ae, 1996). Therefore, peanut has more than one strategy to cope with P defficiency, which is not fully understood. Thus, there is a need to evaluate how different peanut cultivars cope with low soil P availability, associating it with root growth, root hairs and enzyme activity in the rhizosphere. The aim of the study was to evaluate root growth, number of root hairs and enzyme activity in the rhizosphere of modern Brazilian peanut cultivars as affected by soil P availability.

METHODS

The study was conducted in a greenhouse in 5-liter pots with 6 kg of soil. The sandy Oxisol with 5.7 mg dm^{-3} of P (resin) was collected from a degraded pasture from the depth of 0.0 to 0.2 m. The treatments were seven peanut cultivars (*Arachis hypogaea* L.; Virginia group): IAC 886, IAC 505, IAC 503, IAC OL5, IAC OL3, Granoleico and EC-98 OL combined with two P levels: low P (5.7 mg dm^{-3} - without P fertilizer) and high P (75.8 mg dm^{-3} - application of 87 mg kg^{-1} of P, triple superphosphate).

Six seeds of peanut were germinated in each pot, and thinned to one per pot at emergence. At 45 days after emergence, the plants were harvested. Roots were washed with running water in a finemesh sieve (0.5 mm). The roots were spread in a tray, scanned, and total root length was determined. A portion of the root tips was separated to evaulate root hairs as in Vieira et al. (2007). Acid phosphatase activity was determined in the rhizospheric soil as in Alef et al. (1995).

RESULTS and discussion

The greatest root length was observed with high soil P availability for all cultivars. P application increased the root length of the cv. IAC 886 by 52% while an increase of 328% was observed for IAC OL5 (Fig. 1a). Under low soil P, Granoleico and EC-98 had the greatest root length (Fig. 1a). The greater root length of these cultivars may be a strategy to improve P acquisition (Wissuwa and Ae, 2001). With low availability of P in the soil, acid phosphatase activity in the rhizosphere was incresead by 45 % to 85 %, with a greater increase in IAC 886 and Granoleico (Fig. 1b). Interestingly, the cultivars IAC 886, IAC 503 and Granoleico also had higher root hair density under low P, similar to what was reported for acid phosphatase activity (Fig. 1 b;c). Phosphorus deficiency increased both density and length of root hairs in all cultivars. However, under low P, the cultivars IAC OL5 and IAC OL3 (modern and early-cycle cultivars) had lower root hair density and length (Fig. 1 c;d).

There was a positive relationship between acid phosphatase activity in the rhizosphere and root hairs under low soil P availability. Root hairs increase the size of the rhizosphere releasing greater amount of exudates, which increases the activity of microorganisms and enzymatic activity (Ma et al., 2018), and this is an important strategy to improve P acquisition of in soils with low P availability (Wissuwa and Ae, 2001). Additionally, the more recent, early cycle cultivars (IAC OL5 and IAC OL3) seem to be more responsive to P application and have a lower capacity to increase root hair density under P deficiency. Thus these cultivars may be less able to use less labile P forms and may be more dependent on fertilizers.

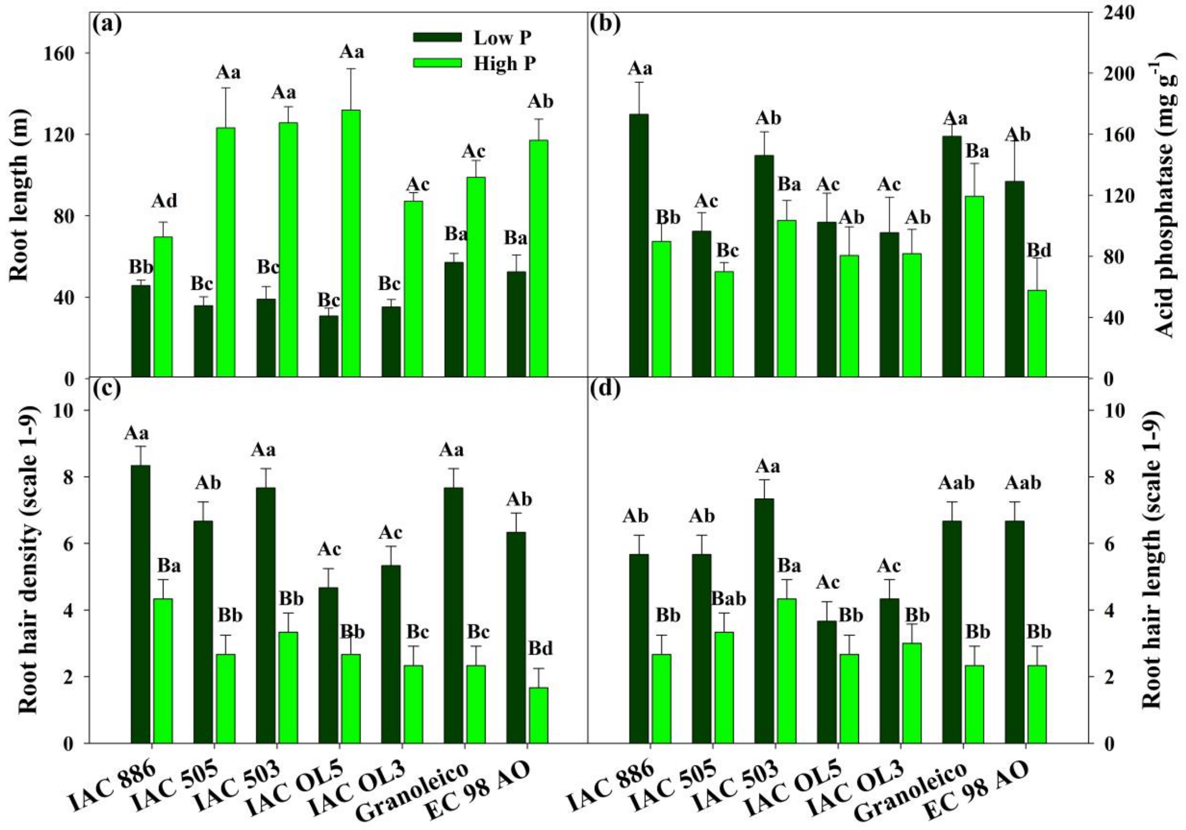

Fig. 1. Root length, acid phosphatase activity in the rhizosphere, root hair density and length in peanut cultivars grown under low and high phosphorus availability. Vertical bars represent standard errors.

CONCLUSIONS

Phosphorus deficiency limits root growth of modern peanut cultivars. Under low availability of P in the soil, there is an increase in the activity of acid phosphatase in the rhizosphere and in root hair density and length. The IAC 505, IAC 503 and IAC OL5 cultivars had greater root length with high P availability, but IAC OL5 and IAC OL3 showed a lower ability to increase root hair under P deficiency. A greater capacity for root hair formation, increasing acid phosphatase activity in the rhizosphere seems to be the main strategy of peanut adaptation to soils with low P availability.

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DEVELOPMENT OF A RHIZOMICROCOSM FOR IN-VIVO, 4D IMAGING OF RHIZOSPHERE PROCESSES AROUND AN ISOLATED ROOT

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Keywords: synchrotron X-rays; chemical imaging; tomography

Biogeochemical processes in the rhizosphere strongly affect root uptake of nutrients or toxic substances from soils. We are developing a "rhizomicrocosm" system for microscale tomographic imaging of soil physical and chemical changes in the rhizosphere around an isolated root. The system is designed for micro-X-ray fluorescence tomography (µ-XFT) chemical imaging at the CARNAÚBA beamline of the Sirius synchrotron, and micro-X-ray computed tomography (µ-XCT) physical-structural imaging at the MOGNO beamline. The rhizomicrocosm comprises a 40-mm diameter seed cup for growing a seedling, and a root guide that funnels a root into a 1- to 3-mm diameter X-ray transparent capillary filled with soil material. The system is engineered to be modular for a variety of rhizosphere studies, including for example, a shoot growth chamber for regulating light and measuring CO_2 consumption and production during photosynthesis and respiration, and a micro-dialysis soil-water sampler. Ongoing experiments include measuring rates of root growth through capillaries of varying diameter and soil particle-size fraction, and root development in the seed cup for different subsoil depths and moisture contents. Initial imaging results from a first prototype on the CARNAÚBA beamline will also be presented. Commissioning of the final rhizomicrocosm will provide a novel system for studying rhizosphere dynamics by users of the open-access Sirius synchrotron.

Financial Support

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THE FATE OF PHOTOSYNTHETICALLY-FIXED CARBON IN TROPICAL FORAGE GRASSES: A ^{14}C PULSE-LABELING STUDY

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Keywords: Rhizodeposition; Brachiaria; Carbon dioxide

INTRODUCTION

Rhizodeposition can be defined as the release of organic C through root exudates, cells from roots, and root symbionts to the soil (Oburger and Jones, 2018). Belowground C allocation and rhizodeposition from tropical forage species are unknown and studies are therefore required for deciphering C dynamics and ecological functions in the rhizosphere soil of tropical pastures. Lastly, root exudation depends on the plant species and changes according to the phenological stage. While methods to quantify exudation exclusively from live roots are highly challenging, isotope-based approaches enable to estimate rhizodeposition and partitioning of fresh assimilated C within the plant-soil-microorganism environment through the exposure of plant shoots to labeled CO_2 (generally using ^{13}C- or ^{14}C-enriched compounds) for a short time period (i.e., pulse-labeling) or throughout the experiment (i.e., continuous-labeling). Our objective is to measure the rhizodeposition and partitioning of labeled C within the plant-soil-microorganism system of two grasses of the genus *Urochloa* compared with Guinea grass (*Megathyrsus maximus*).

METHODS

The study was performed through a mesocosm experiment using a Rhodic Hapludox soil. Forage grass seeds [Guinea grass, palisade grass (*U. brizantha* cv. Marandu), and ruzigrass (*U. ruziziensis* cv. comum)] were germinated in a plastic tray and transferred to 1-L plastic pots (six seedlings per pot) 15 d after germination (DAG). For the single pulse labeling study, the pots were placed in a sealed plastic chamber and a vial containing 0.5 mL of $Na_2{}^{14}CO_3$ solution (2.5 MBq per pot) was placed within the chamber at 32 and 42 DAG. Below-ground respired $^{14}CO_2$ ($^{14}CO_2$ efflux) was captured passively by pushing two 50-mL capped polypropylene centrifuge tubes ~1 cm into the soil. The $^{14}CO_2$ was captured in traps containing 1 M NaOH. The ^{14}C activity was measured with a liquid scintillation counter.

At the end of the incubation period (6 d after labeling), plant shoots were clipped at the ground level while roots were gently separated from the soil by handpicking. Soil adhering to the roots (i.e., rhizosphere soil) was removed and sieved to pass 1-mm. Plant and soil samples were combusted in an OX400 biological oxidizer unit, with $^{14}CO_2$ trapped by Oxosol scintillator and ^{14}C determination by liquid scintillation.

All statistical analyses were performed using SAS (version 9.3, SAS Institute, Inc., Cary, NC, USA). One-way ANOVA (fixed effect models) using the GLM procedure was conducted to assess the effect of forage species at each growth stage. The F-test was used, followed by Fisher?s least significant difference (LSD) to separate means, at the 5% level of significance.

RESULTS AND DISCUSSION

Overall, no differences were observed among the forage grasses for gross ^{14}C activity in the various C pools for the two pulse-labeling events, except respiration and dissolved organic C (DOC) in the soil (Fig. 1). Plant shoot was the main sink for assimilated CO_2, with allocation varying from 45% to 65% of the total ^{14}C photosynthetically fixed (Fig. 1a). As we did not determine shoot respiration, this is very likely to be underestimated. Of the total ^{14}C fixed, Kuzyakov and Siniakina (2001) reported that 17% derived from shoot respiration. The second largest ^{14}C pool was the root, followed by soil respiration, soil, and DOC, the latter showing very low amounts of ^{14}C, less than 2% of the total ^{14}C fixed (Fig.1bcde).

The $^{14}CO_2$ efflux rate peaked at 1 and 2 d after labeling (DAL) at 32 and 42 DAG, respectively, regardless of forage grass (Fig. 1fg). The peak moments detected are similar to other studies, where $^{14}CO_2$ efflux peaked at 1 DAL (Hill et al., 2007; Sanaullah et al., 2012; Pausch et al., 2013). Moreover, the proportion of $^{14}CO_2$ efflux from the total ^{14}C recovered is similar to Sanaullah et al. (2012) and Pausch et al. (2013).

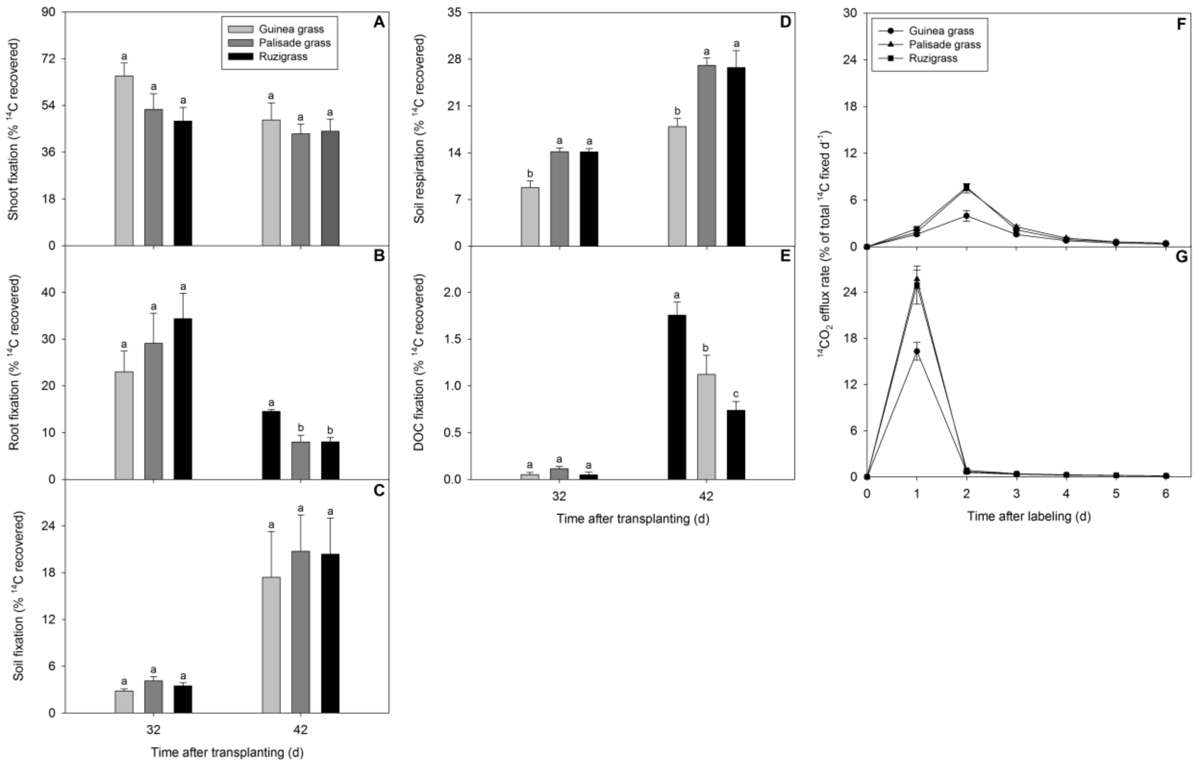

Fig. 1. Proportion of ^{14}C incorporated in the shoot (a), root (b), soil (c), soil respiration (d), and dissolved organic C (e) of three tropical forage grasses assessed at two sampling times. Total $^{14}CO_2$ efflux rate of three tropical forage grasses following 32 (e) and 42 d (f) after transplanting of three tropical forage grasses. The error bars indicate the SEM (n = 4). Common letters do not indicate differences according to the LSD-test at the 5% level of significance.

CONCLUSIONS

Overall, there is no difference among forage grasses relative to the fate of ^{14}C in the plant-soil system. Plant shoot is the main sink for photosynthetically-fixed $^{14}CO_2$, regardless of forage. Guinea has the lowest $^{14}CO_2$ efflux from below-ground respiration (root respiration and rhizomicrobial respiration) in comparison to palisade grass and ruzigrass. In contrast, no difference among forage grasses is found for the ^{14}C recovery in the various C pools.

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FUNCTIONAL CHARACTERIZATION OF FE-DEPENDENT MECHANISMS PROMOTING THE STIMULATION OF ADVENTITIOUS ROOT FORMATION IN PETUNIA CUTTINGS

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Keywords: Iron; adventitious root formation; petunia

Adventitious root (AR) formation is prerequisite for vegetative propagation of important crops and common practice to maintain the genetic identity among progenies. With increasing use of soil-free media in high-throughput propagation and growing demand for sustainable horticultural practices, a deep understanding of the role of mineral nutrition in AR formation is essential for the improvement of existing propagation protocols. Our previous work demonstrated that Fe is the most limiting nutrient for AR formation and provided compelling evidence for a unique unexploited role of Fe in stimulation of meristematic cell division, associated with its high accumulation in the nuclei of meristematic cells as performed with Perls-diaminobenzidine (DAB) staining. The Fe-dependent AR formation also revealed an improved rooting in wild petunia accession lines and the closely related Solanaceae genus tomato. Targeted RNASeq analysis of micro-dissected AR primordia in different developmental stages of root formation indicated that in the early phase of AR formation, the most significant changes in the expression of Fe-responsive genes were related to ribosome biogenesis, DNA replication, and plant hormone signal transduction, while in later stages of AR formation ribosomal proteins and sugar and amino acid metabolisms were overrepresented. A detailed analysis allowed the identification of Fe-responsive genes that will be used to functionally characterize the corresponding gene functions using CRSPR/CAS technology using anatomical and physiological approaches. Hormone and metabolite analysis showed that upon Fe treatment, changes in auxin and salicylic acid play a dominant role in regulating AR formation and correlate positively with the metabolite 2-oxoglutarate (2-OG), which in turn regulates the activity of 2-OG-dependent oxygenase's. Both, 2-OG and the 2-OG-dependent oxygenase's are involved in the synthesis and metabolism of several hormones, including auxin and salicylic acid, suggesting that Fe is indeed a limiting nutrient required for the metabolic activity in meristematic cells during AR formation at the stem base of vegetatively propagated crops.

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Leibniz Institute of Plant Genetics and Crop Plant Research

POLYPHOSPHATE HYDROLYSIS BY PLANT'S ROOTS

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Keywords: Polyphosphate; Phosphatase; PUE

INTRODUCTION

Phosphorus (P) plays a key role in many structural and biochemical processes in the plant cell. However, despite high soil P concentration (300-3,000 mg kg^{-1} soil), strong soil sorption limits its fertilization utilization efficiency (PUE), typically leaving only approximately 15-30% of the P fertilizers applied (Lynch, 2007). To overcome these limitations, in many agrosystems P is added excessively, leading to P accumulation in the upper soil layers and causing an environmental hazard (Sharpley et al., 2001). Hence, P fertilizer with superior fertilization efficiency is desirable.

Polyphosphate (poly-P) is a compound composed of two or more orthophosphates (ortho-P) units condensed to linear or cyclic chains. While various forms of poly-P are commonly applied fertilizers, ortho-P is the sole form acquired by plants. Poly-P hydrolysis typically occurs within weeks, mostly by microorganisms activities (McBeath et al., 2007). Preliminary experiments indicate that plants can efficiently hydrolyze poly-P. Thus we aimed to characterize plants' derived poly-P hydrolysis and its potential as a P uptake strategy. We hypothesized that enzymes secreted by plant roots may be efficient in the hydrolysis of complex poly-P (cyclic or long linear chain). To examine our hypothesis, we performed a series of experiments in a sterile environment.

METHODS

Sterile environment growth

Six plant species were grown in an agar medium containing all required nutrients except P. Phosphorus was added as either ortho-P (positive control), cyclic poly-P, or no-P (negative control). The plants were grown at 25°C with 16 hours of light for 5 weeks. On the harvest day, plant biomass and P concentration were recorded.

Extraction of the hydrolytic enzyme

Pepper (Capsicum annuum var. Annuum Grossum Group) roots soluble proteins were extracted with Lysis buffer (pH=8). Then total protein extracts were purified and fractionated using FPLC equipped with two columns; first separation by protein's charge, second separation by protein's size. Next, a poly-P hydrolysis assay was applied to identify the most active fraction. Finally, the selected fraction was sent for protein identification by mass spectrometry.

Overexpression and purification of the hydrolytic protein from E.coli bacteria

The identified protein sequence was used to construct a synthetic polyhistidine-tagged (His-tag) gene construct optimized for overexpression in E.coli cells. A heat-shock transformation was used to insert the plasmid into E.coli bacteria (BL21). Then, bacterial protein synthesis was induced by adding 1mM IPTG. Finally, the His-tagged proteins were purified using Ni beads.

RESULTS AND DISCUSSION

While few species had lower biomass and P uptake in the poly-P medium (e.g. Lettuce, Medicago), others showed similar biomass (e.g. Tomato, Ryegrass) in the ortho-P and poly-P medium, indicating an efficient hydrolysis mechanism (Fig 1a). Furthermore, pepper plants grew better with the poly-P fertilizer compared to the ortho-P fertilizer. Therefore, the experiments were conducted using pepper as a model plant.

We identified a specific fraction within the roots soluble proteins that exhibited the highest activity of poly-P hydrolysis. Comparison to known phosphatase enzymes revealed that this fraction has more efficient hydrolytic activity, implying that the active enzyme is different from the well-known phosphatases secreted by plants' roots. Proteomic analysis of the active fraction revealed the enzyme STH-21, an enzyme from the MLP family. The expression of this family's enzymes varied among plants species (Fujita & Inui, 2021), which can explain the differences observed in the sterile medium growth experiment. The transformation of the plasmid and the purification of the enzyme from E.coli bacteria confirmed the poly-P hydrolysis activity.

CONCLUSIONS

These observations reveal an enzymatic mechanism that allows the use of poly-P fertilizers as a P source for plants. Poly-P hydrolysis mechanism is known in microorganisms, fungi, and non-vascular plants, but, as far as we know today, has not been documented in vascular plants (Kulaev et al., 2004). This mechanism varied between plant species, so understanding those differences could lead to better utilization of P fertilizers.

Characterizing the enzyme's activity and its association with various plant species may enable to "tailor" the form of P fertilizer while combining the benefits inherent in the poly-P fertilizers in the soil and thus increasing the efficiency of P fertilizers.

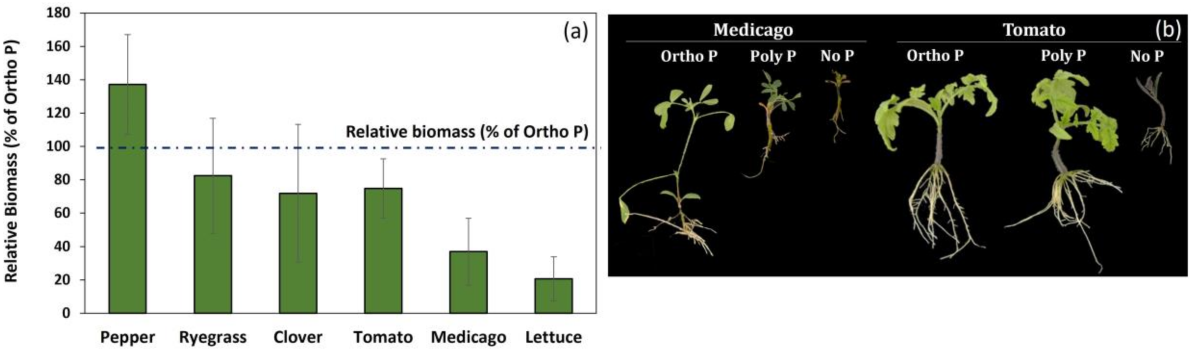

Fig 1. Biomass of various plant species grown with cyclic poly-P in a sterile medium for five weeks relative to plants' biomass grown with ortho-P, vertical lines present SD (a). *Tomato* and *Medicago* plants grown in a sterile medium with P supplied as ortho-P, poly-P on no-P at all (b).

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NUTRITROPISM OF RICE ROOTS

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Keywords: Tropism; Nutrient Gradient; root architecture

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INTRODUCTION

Plants, are autotroph and requires lights, water, and mineral nutrients for their growth. In 1880, Charles Darwin described three tropisms, i.e., phototropism, gravitropism, and hydrotropism. Light and water conditions in nature are uneven and these tropisms are important for plants to acquire light and water in the chaniging environments. Minearal nutrients in both natual and agricultural soils are unevenly distributed. In contrast there has been no experimental demonstration of tropism towards mineral nutrients. It has been well descsribed that mineral nutrients affects root architecture (Giehl and von Wiren 2014), but in most caes of such studies, nutrient gradients are not tested, rather nutient conditions are evenly changed or roots are exposed to different sections of media, each of which containing evenly distributed media to obseve their effecs on root growth. In plants, studies on the effect of chemical stimuli on the direction of root growth have been reported, but to our knowlede, these responses are likely caused by inhibition of root elongation due to the chemical applied to one side of the roots (Please see details for Yamazaki et al, 2020).

In this study we descibe tropism of rice roots responding to nutrient gradients to alter their direction of growth towards the nutrient rich regions. We used rice to test the effects of nutrient gradients and found that rice roots respond to gradients of ammonium and grow towards the source of nutrients. We termed this phenomenum as nutritropism. Nutritropism is likely to play a key role in acquisition of nutrients in soils with uneven distribution of mineral nutrients.

METHODS

Rice (*Oryza sativa* L.) seeds were germinated on plates containing growth agar medium (1/50? strength MS salts) and seven days after germination, we artificially created a nutrient gradient. A small piece of agar containing a high conecentration of nutrients, typically 10 xMS, filled in a plastic tube was placed on to the agar-solidified medium let the nutrients to diffuse around over time. In our settings, the gel inside in the tube came into contact with the external agar medium. The formation of gradients are also examined with the use of colar dye or detemining conentration of nutrients surrounding the small agar. Details of the gradient generation is described by Yamazaki et al (2020).

RESULTS AND DISCUSSION

We first tested the effects of 10 xMS on rice cultivar Taichung 65. Roots of seven days old plants were subjected to the experiments and it was found that lateral roots changes their direction of growth towards the origin of high nutrients (Fig. 1). In these experiments, lateral roots below the nutrient source (gray dot in the picture) changed the direction of elongation against gravity, strongy suggesting that the the roots are "attracted" to the nutrient souce and changed their direction of growth upwards. We termed this phenomena as nutritropism.

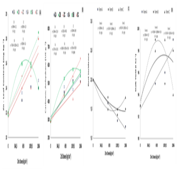

Fig. 1. Nutritropsm or rice lateral roots. Rice roots on the agar plates of seven days after germination is exposed to nutrient gradients generated from the nutrient source (gray dots in the picture). The picture was taken after 18 hours from the placement of nutrient source. (Repoduced from Yamazaki et al 2020) Bar = 1 cm

We then examined components of MS salts for nutritropism and found that NH_4^+ is required for nutritropism (Yamazaki et al 2020). The nutritropsm of lateral roots was not observed other components tested, suggesting in the case of rice lateral roots, NH_4^+ is a strong attractants of nutritropsm. In the case of Taichung 65, we found that main roots do not respond to NH_4^+ gradients in our experimental systems.

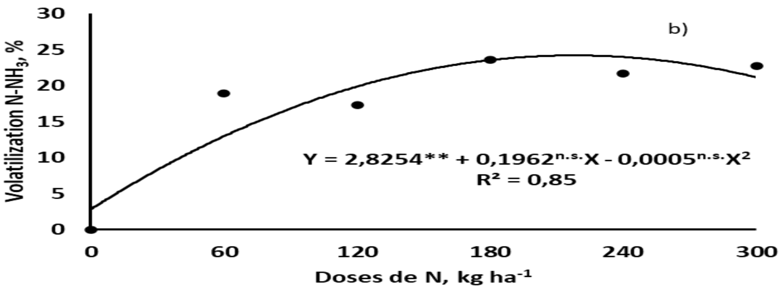

Fig. 2. Nutritropsm of rice main roots. Rice main roots are exposed to high nutrient souce sown in the gray spots in the picute and its response at the time indicated in each panel are shown. Arrow in the 0h panel demonstrates the direction of gravity. (Repoduced from Yamazaki et al 2022) Bar = 1. cm.

We then tested the variation of nutritropic response among a range of rice cultivars, with the focus on the behavior of main roots. We found cultivars exhibiting nutritropism in the main roots. An example is shown in Fig. 2. In this case, direction of elongation changed towards the nutrient source which is placed on the sideways from the direction of gravity, yet the root changed its direction of growth toward the nutrient source. We also found that in the case of nutritropism of main roots, NH_4^+ is the major attractant while phosphate has an important supportive role in manifestation of nutritropism.

CONCLUSIONS

Our study provided the first experimental evidence for nutritropsim in plants. In our experiments using rice roots, NH_4^+ is the major attractant while other nutirents also play different roles depending on the types of the roots of rice. Nutritropsim apparently gives benefits to rice in terms of acquisiton of nutrient in uneven nutrient environments and we expect aht. future studies will open a new field in both basic and agricultural sciences.

ACKNOWLEDGEMENTS

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EFFECT OF SINGLE AND COMBINED NUTRIENT DEFICIENCIES ON STABLE CARBON ISOTOPE FRACTIONATION

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Keywords: Carbon isotope ratio; Nutrient stress; Plant physiology

INTRODUCTION

The cultivation of crops such as barley, cucumber, maize, and tomato is strongly limited by soil nutrients availability (MacDonald et al., 2011; Samaranayake et al., 2012), especially nitrogen (N), phosphorus (P), and iron (Fe) (van de Wiel et al., 2016; Zuo & Zhang, 2011). Literature reports numerous studies focusing on their effect at physiological level, demonstrating how these nutrient deficiencies impair the whole plant carbon cycle, therefore modifying the plants' Stable Carbon Isotope Ratio ($\delta^{13}C$) (Cernusak et al., 2013; Tcherkez, 2010). Despite this knowledge, limited research investigated the relationship between nutrient shortages and plants' $\delta^{13}C$. Moreover, these few articles focused mainly on N deficient plants. To fill this gap of knowledge the relationship between $\delta^{13}C$ and nutrient stresses, *i.e.* phosphorus (P), iron (Fe) and combined Fe and P deficiencies, in four different plant species comprising C3 and C4 plant species, as well as mono and dicots was investigated, trying also to understand the mechanisms behind potential shifts.

METHODS

Barley, cucumber, maize, and tomato plants were germinated in the darkness for 4-10 days according to plant species, were grown in hydroponics in control conditions for one week and then for two more weeks under P, Fe, and combined P/Fe deficiencies. The $\delta^{13}C$ of the harvested, dried and grinded plants was measured by an Isotope Ratio Mass Spectrometer (IRMS). Simultaneously, plant physiological status, *i.e.* stomatal conductance, photosynthetic rate, transpiration rate, was monitored with an Infra-Red Gas Analyser (IRGA).

RESULTS and discussion

In all plant species, the $\delta^{13}C$ decreased during plant development, most probably due to a biomass dilution effect and to an initially low RuBisCO/PEPC activity ratio, with a tendency to reach a constant level towards the end of this two weeks period. Besides this common behavior, the effect of the different treatments on $\delta^{13}C$ was strictly species- and tissue-specific with only very limited variation attributable to photosynthesis type (either C3 or C4), to cotyledon number (either monocot or dicot) and/or to Fe acquisition strategy (either Strategy I or II). The tissue-specific responses highlight roots as playing a key role in the plant Δ. Combined deficiency perception was strictly species-specific but not tissue-specific: -P/-Fe condition behaved similarly to -P in tomato and cucumber but looked like -Fe in maize. Furthermore, physiological parameters showed limited correlation with $\delta^{13}C$ shifts, highlighting that the plants' $\delta^{13}C$, does not depend solely on photosynthetic Δ.

CONCLUSIONS

In conclusion, our findings highlighted the complexity of combined deficiencies perception and of Δ in plants suffering from nutrient deficiencies. Moreover, the key role of roots in the plant Δ was firstly exposed here confirming the hypothesized limitations of $\delta^{13}C$ prediction models based solely on photosynthetic Δ, *e.g.* Farquhar's model (Busch et al., 2020; Farquhar et al., 1989).

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THE EFFECT OF BIOFERTILIZERS ON ACID AND ALKALINE PHOSPHATASE ACTIVITY OF TWO CHICKPEA CULTIVARS

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Keywords: Phosphatasese; Chickpea; Biofertilizer

INTRODUCTION

Chickpea like any other legume is negatively affected by low soil phosphorus (P). In P-deficient soils, chickpea develops adaptive response mechanisms, which include changes in root morphology and the secretion of acid and alkaline phosphatase enzymes (Wang et al., 2010). The enzymes increase inorganic P acquisition from the extracellular environment (Spohn and Kuzyakov, 2013). There is limited information on phosphatase production in South Africa (Mogale et al., 2018; Moloto et al., 2021) but there are no reports on the effects of biofertilizers on rhizospheric phoshatase activity in field-grown chickpea. Therefore we investigated the effect of biofertilizers on the rhizosphere acid and alkaline phosphatase activity of two chickpea cultivars in contrasting soil types.

METHODS

Two chickpea cultivars (ACC#3 & ACC#7) were subjected to six biofertilizer treatments (zer control, Kelpak [K], Mycoroot [M], Rhizobium inoculation [R], K+R, M+R) in a field experiment at Thohoyandou (clay soil) and Syferkuil (loamy sand soil), South Africa in 2021. Rhizosphere soil was collected by digging up the root along with the attached soil. The soil was assayed for the activity of extracellular acid and alkaline phosphatase. The phosphatase activity was determined following the procedure of Tabatabai (1994).

Results and discussion

Application of Kelpak, Mycoroot and Rhizobium inoculation decreased acid phosphatase (APase) activity at Thohoyandou but K+R and M+R increased acid phophatase actvitiy suggesting a synergistic effect. In contrast to Thohoyandou, Kelpark increased APase activity at Syferkuil, and a synergistic response of APase activity to biofertilizers was also observed at Syferkuil (Table 1). Mycoroot decreased alkaline phosphatase (AlkPase) activity at Thohoyandou but M+R increased AlkPase activity which may indicate a syngerstic effect of M and R (Table 1). However, the highest AlkPase activity at Thohoyandou was observed in Kelpak treated plots. At Syferkuil in contrast, Kelpak and Mycoroot decreased AlkPase activity but the other biofertilizer treatments had no effect (Table 1). Moloto et al. (2021) similarly observed effects of a biofertilizer, Bonterra, on APase activity in silty clay loam soil.

In contrast to earlier findings (Mogale et al., 2018), APase (Syferkuil) and AlkPase (both sites) did not vary with cultivar but, there was a significant interaction between cultivar and biofertilizer(Table 1). For example K+R led to higher APase activity in ACC#3 compared to ACC#7 while it was the converse with M+R (data nt shown).

CONCLUSION

Application of biofertilizers affected extracellular acid and alkaline phosphatase activity but the effect varied with soil type. Moreover, there was syngerstic effect between Kelpak and Rhizobium inoculation, and Mycoroot and Rhizobium inoculation on APase activity. We recommend further studies to investigate the mechanisms by which the biofertilizers affect phosphatase enzyme activities in various soil types.

Table 1. The effect of biofertilizers application and genotype on rhizosphere soil acid and alkaline phosphatase activity on the rhizosphere soil.

µg ?-nitrophenol. g^{-1} Fwt.h^{-1}

Thohoyandou

Syferkuil

Biofertilizer	**APase**	**AlkPase**	**APase**	**AlkPase**
Mycoroot + Rhizobium	34.01b	49.41a	28.24b	53.36b
Kelpak + Rhizobium	40.62a	47.61ab	43.24a	59.40ab
Rhizobium inoculation	9.90e	47.91ab	8.46c	63.74ab
Control	26.72c	37.05b	9.34c	74.14a
Kelpak	10.16e	56.81a	25.95b	35.84c

Mycoroot

22.81d

22.47c

13.67c

53.52b

Genotype

ACC#3

23.75a

41.43a

21.06a

58.13a

ACC#7

24.32a

45.65a

21.90a

55.21a

P-value

Biofertilizer (B)

***

***

***

**

Genotype (G)

ns

ns

ns

ns

B*G

**

ns

ns

ns

CV (%)

6.37

23.51

24.58

25.13

Means with different letters in the same column are significantly different from each other, *P≤0.05,**P≤0.01,***P≤0.001; ns=not significant, CV=Coeffiecient of variance

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