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Vantagens fisiológicas e nutricionais às plantas de milho inoculadas com fungos micorrízicos arbusculares

Physiological and nutritional advantages to maize plants inoculated with arbuscular mycorrhizal fungi

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Abstract: The use of arbuscular mycorrhizal fungi (AMF) for the cultivation of plants, including maize, is an important tool for food production, as it provides an increase in growth, productivity and tolerance to edaphoclimatic adversities. We studied the influence of AMF on growth and on some biochemical and physiological parameters of maize plants subjected to different doses of phosphorus (P). The P treatments used were 30 kg ha^{-1} WF, 60 kg ha^{-1} WF, 90 kg ha^{-1} WF in the presence of AMF and 90 kg ha^{-1} AF without the presence of AMF. The plants were kept in a greenhouse for 20 days. Afterwards, leaf water content, plant dry mass was evaluated, in addition to the quantification of phosphorus, carbohydrates, proline, ammonium, nitrate. The treatments, 30 kg ha^{-1} WF and 60 kg ha^{-1} WF provided the highest leaf water contents. The 90 kg ha^{-1} WF treatment showed the highest concentration of phosphorus and ammonium, while the treatment 60 kg ha^{-1} WF provided the same concentrations of P

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as 90 kg ha^{-1} AF without the presence of AMF. Proline concentration was higher in mycorrhizal plants. Arbuscular mycorrhizal fungi contribute to the growth and nutrition of maize plants at an early stage of development.

Index terms: amazon, glomeraceae, native fungi, savanna

Vantagens fisiológicas e nutricionais às plantas de milho inoculadas com fungos micorrízicos arbusculares

Resumo - A utilização dos fungos micorrízicos arbusculares (FMA) para o cultivo de plantas, incluindo o milho, é uma importante ferramenta para produção de alimentos, pois proporciona incremento no crescimento, produtividade e tolerância às adversidades edafoclimáticas. Estudamos a influência dos FMA no crescimento e em alguns parâmetros bioquímicos e fisiológicos das plantas de milho submetidas a diferentes doses de fósforo (P). Os tratamentos utilizados de P foram 30 kg ha^{-1}CF, 60 kg ha^{-1}CF, 90 kg ha^{-1} CF na presença de FMA e 90 kg ha^{-1}SF sem a presença de FMA. As plantas foram mantidas em casa de vegetação durante 20 dias. Após, avaliou-se, teor de água na folha, massa seca das plantas, além da quantificação dos teores de fósforo, prolina, amônia (NH_4^+) e nitrato (NO_3^-). Os tratamentos, 30 kg ha^{-1}CF e 60 kg ha^{-1}CF proporcionaram os maiores teores de água foliar. O tratamentos 90 kg ha^{-1}CF apresentou o maior teor de fósforo (P) e amônia (NH_4^+). A concentração de prolina foi maior nas plantas micorrizadas. Os fungos micorrízicos arbusculares contribuem para o crescimento e nutrição das plantas de milho em estágio inicial de desenvolvimento.

Termos para indexação: amazônia, glomeraceae, fungos nativos, savana

INTRODUCTION

The estimate for world maize (*Zea mays* L) production for the 2020/21 crop is 1.163 billion tons, while for the Brazilian crop it is 107 million tons (USDA, 2020). Maize is widely used in the food industry, but its productivity and initial growth are strongly influenced by edaphoclimatic factors, such as water deficit (Pavithra & Yapa 2018) and low nutritional soil availability, especially of phosphorus (P) and nitrogen (N) (Hou et al. 2021).

However, the use of arbuscular mycorrhizal fungi (AMF) during maize cultivation decreases the productivity loss and provides an increase in initial growth, as they help in the absorption of nutrients and water (Smith & Read, 2008). The benefits of these organisms in nutrient absorption were reported by Hou et al (2021) in relation to P, and Teutscherova et al. (2019) in relation to nitrogen. The water benefits were reported by Pavithra & Yapa (2018) justified by the increase in proline levels, one of the main molecules controlling cellular water potential.

The physiological and biochemical drifts provided to plants is directly related to the diversity of AMF that is determined by the plant species that make up the environment and the edaphoclimatic conditions of the environment, thus, the production of the inoculum using native AMF from the cultivation soil adds significant advantages (Gross et al., 2004). The Alter do Chão Savanna, Santarém-PA, Brazil is characterized by low fertility soil (Magnusson et al., 2002), naturally offering adverse abiotic conditions for plant growth and development, with low levels of phosphorus and opposite water regimes during the year, making the mutualistic AMF-plant relationship fundamental for the growth and perpetuation of plant species.

Thus, the Savanna region of Alter de Chão is ideal for AMF collect, in addition studies with native AMF in the region are non-existent, in our study we aimed to study the influence of AMF from the Savanna region of Alter do Chão, Santarém- PA, Brazil on growth and some biochemical and physiological parameters of maize plants subjected to different doses of P.

MATERIAL AND METHODS

The inoculum was produced from rhizospheric soil collected from the savanna area located at coordinates 2º28’1" S and 54º49’41" W in the region of Alter do Chão, in Santarém city, west of the state of Pará-Brazil. A mixture of 3 kg of collected soil with 3 kg of commercial substrate was performed, which was sterilized in an autoclave at 121°C for 15 min, repeated twice as suggested by Santos et al. (2021).In this mixture, maize seeds were planted, which were kept for 20 days under artificial lighting with a 12-hour photoperiod, daily irrigation to maintain field capacity at 65% and temperature at 27 °C. After this period, the plants were removed and the inoculum was considered, as well as the rest of the material present in the vases, and most of the AMF were identified as belonging to the Glomeraceae family.

The inoculum produced was used in treatments in the presence of AMF. Maize cultivation was carried out in a mixture consisting of 317 g of ferralsols, 133 g of commercial substrate and 25 g of inoculum, in treatments in the presence of AMF, whereas in the control treatment, without the presence of AMF, 317 g of ferralsols were added and 158 g of commercial substrate. Pots with a capacity of 700 mL were used and kept in a greenhouse at the Laboratory of Plant Physiology and Plant Growth at the Federal University of Western Pará. The soil and the substrate were sterilized before cultivation, under the same conditions described above, in addition to the correction of the pH added to limestone. The soils used were ferralsols and organic commercial substrate, with the following characteristics (Table I).

Table I: Chemical properties of ferralsols and organic commercial substrate

Ferralsols								
pH	P	K	Ca	Mg	Al	H+AL	SB	T
H_2O	mgdm-³		cmolc dm-³					
5	15	6	0,45	0,15	1,3	6,15	0,62	6,77
Commercial substrate								
pH	P	K	Ca	Mg	Al	H+AL	SB	T
H_2O	mgdm-³		cmolc dm-³					
5,9	49,8	354	6,6	1,43	0,01	3	8,9	8,9

P-phosphorus; K-potassium; Ca-calcium; Mg-magnesium; Al-Aluminum; H+Al-calcium acetate; SB-sum of bases; T-CTC effective. (CTC-cap.cation exchange)

Plants were subjected to treatments with different doses of phosphorus (P), 30 kg ha^{-1} WF, 60 kg ha^{-1} WF, 90 kg ha^{-1} WF in the presence of AMF, in addition to a control treatment, with 90 kg ha^{-1} AF without the presence of AMF, where WF means "with fungus" and AF means "fungus absence", simple superphosphate was used as a source of P. All treatments were fertilized with 60 kg ha^{-1} K and 80 kg ha^{-1} N, using potassium chloride and urea as sources, respectively (Silva et al., 2007). In treatments with AMF, 25 g of inoculum was used as suggested by Santos et al. (2020).

The treatments consisted of 7 replications, with the pot being considered the repetition, totaling 28 pots, with 2 maize plants remaining in each pot. Seeds were planted under the same conditions in which the inoculum was produced. The application of P and K occurred at the time the seeds were planted, and the N was applied 4 days after planting.

After 20 days of planting, the following variables were performed: leaf area index (LAI) using the formula LA= L x W x 0.75, ie, leaf length (L) and leaf width (W). To obtain the root volume, a 20 mL syringe was used, and the following formula was used, FV (final volume) - IV (initial volume), where FV is the water in the syringe (used as a standard 15 mL) plus root and IV water in the syringe (used 15 mL as standard). And the leaf water content (LWC) according to Sairam & Srivastava (2002).

To measure dry mass, the plants were placed in a greenhouse with forced air circulation at a temperature of 60°C until constant weight, and the shoot (DMAP) and root (RDM) were weighed separately. Dry matter of the shoot samples was used to quantify the levels of sucrose and total soluble sugars by the Antrona method (Dische, 1962), reducing sugars by the DNS method (Miller, 1959).

Root dry matter samples were used to measure proline, according to Bates et al. (1973) and P, which was extracted by Mehlich-1 after digestion in a muffle at 500°C, and the determination was carried out by an acidic solution of ammonium molybdate (Carmo et al., 2000). In addition, root and shoot dry matter samples were used to quantify ammonium (NH_4^+) according to Weatherburn, (1967) and nitrate (NO_3^-) according to Cataldo et al. (1975). Ammonium and nitrate data were expressed in accumulation, where the shoot dry mass was multiplied by the concentration obtained in the samples.

Data normality was verified by the Shapiro-Wilk test ($p>0.05$) using SigmaPlot version 12.0. The comparison between the means for the sources of variations was performed using the Scott-Knott test, at 5% probability, using the statistical program Sisvar® (Ferreira, 2011).

RESULTS

The P concentration in the soil, the 90 kg ha^{-1} WF treatment and the control showed similar means, and about 46% higher than that observed in the 30 kg ha^{-1} WF and 60 kg ha^{-1} WF treatments (Figure 1A). The 90 kg ha^{-1} WF treatment provided the highest P concentration in the root, about 36% higher than the control, which was also fertilized with 90 kg ha^{-1}. The 60 kg ha^{-1} WF and 90 kg ha^{-1} AF treatments were similar, while the 30 kg ha^{-1} WF treatment had the lowest value compared to the others (Figure 1B).

Figure 1. Soil (A) and root (B) phosphorus concentration of maize plants cultivated at different levels of phosphorus, in the presence (WF) and absence (AF) of arbuscular mycorrhizal fungi

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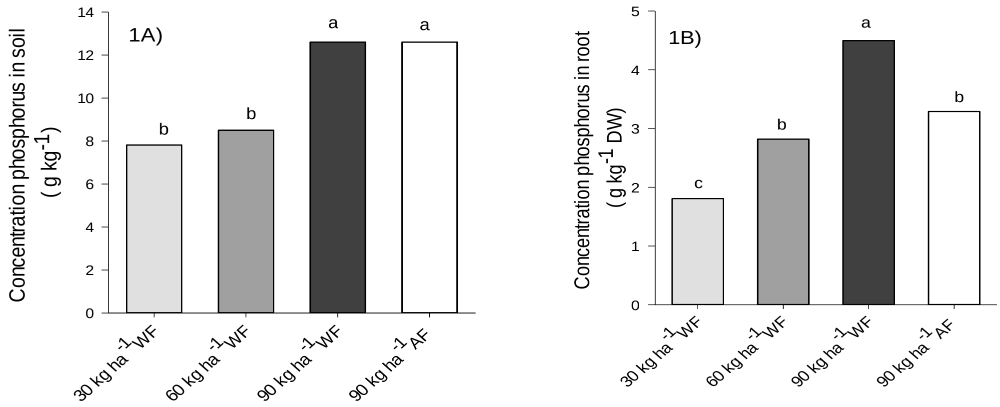


In the growth variables, the 90 kg ha^{-1} WF treatment presented the highest value in root volume (RV) in relation to the others, which presented similar values. Leaf area index (LAI) did not differ statistically ($p>0.05$) between treatments. In the variable leaf water content (LWC) the treatments of 30 kg ha^{-1} WF and 60 kg ha^{-1} WF were similar, with averages higher by about 6% than the 90 kg ha^{-1} WF treatments and the control (Table II).

Table II. Growth of maize plants cultivated at different levels of P, in the presence and absence of arbuscular mycorrhizal fungi

Treatments	LAI	LWC	RV
30 kg ha^{-1} WF	0.81 a	94.47 a	0.80 b
60 kg ha^{-1} WF	0.79 a	94.97 a	0.85 b
90 kg ha^{-1} WF	0.94 a	89.32 b	1.45 a
90 kg ha^{-1} AF	0.70 a	89.23 b	0.66 b

Treatments 30 kg ha^{-1} WF, 60 kg ha^{-1} WF and 90 kg ha^{-1} WF witch the presence and 90 kg ha^{-1} AF witch absence of arbuscular mycorrhizal fungi. LAI-Leaf Area Index; LWC- leaf water content; RV-volume from root. The means followed by the same letter, in each column, are not statistically different by the Scott-not test ≤ 5%.

In relation to dry matter, the treatment of 90 kg ha^{-1} WF provided the highest values of the dry mass of shoot (DMAP) and root dry mass (RDM), about 20% and 60%, respectively, in relation to control. In the variable of total dry mass (TDM) the treatment 90 kg ha^{-1} WF and control differed statistically from the treatments of 30 kg ha^{-1} WF and 60 kg ha^{-1} WF which presented the lowest values ($p<0.05$). (Table III)

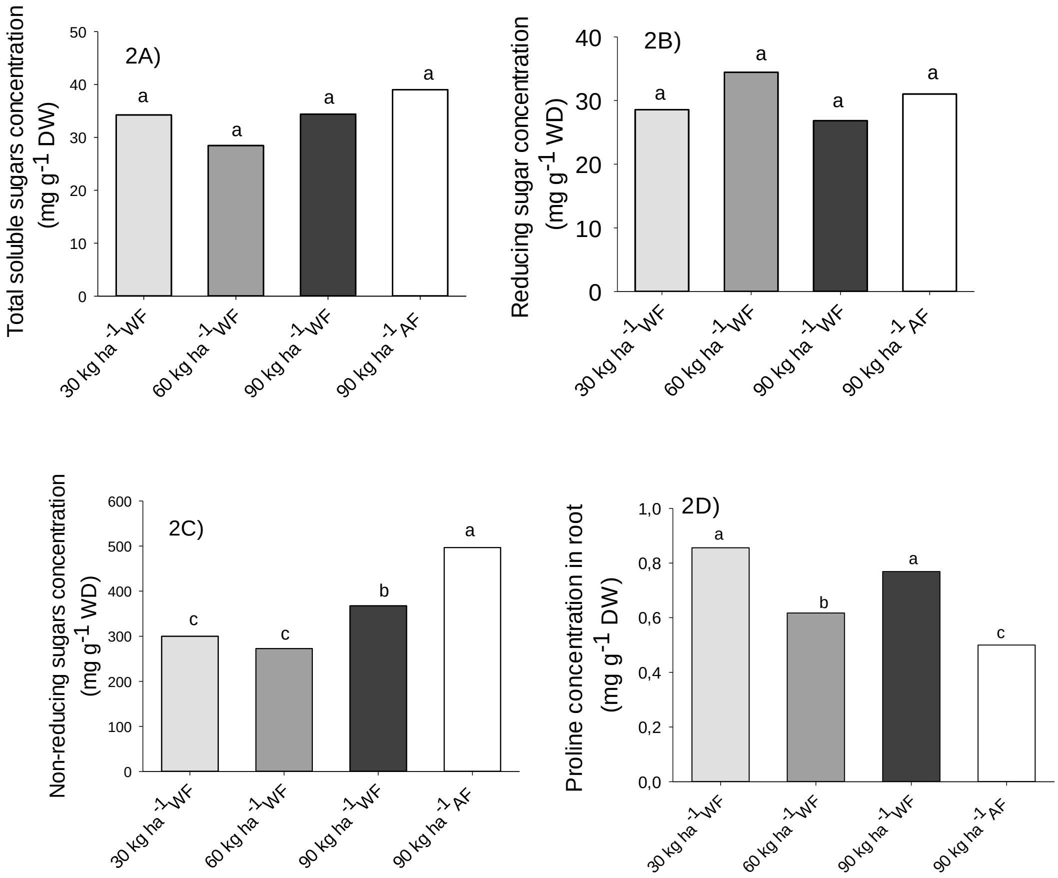
Table III. Production of dry mass of shoot (DMAP) and root system (RDM) of maize plants grown at different levels of P, in the presence and absence of arbuscular mycorrhizal fungi

Treatments	DMAP	RDM	TDM
30 kg ha^{-1} WF	0.10 b	0.03 b	0.13 b
60 kg ha^{-1} WF	0.12 b	0.03 b	0.16 b
90 kg ha^{-1} WF	0.17 a	0.05 a	0.22 a
90 kg ha^{-1} AF	0.14 b	0.03 b	0.17 a

Treatments 30 kg ha^{-1} WF, 60 kg ha^{-1} WF and 90 kg ha^{-1} WF witch the presence and 90 kg ha^{-1} AF witch absence of arbuscular mycorrhizal fungi. DMAP- dry mass of shoot; RDM- root dry mass; TDM- total dry mass. The means followed by the same letter, in each column, are not statistically different by the Scott-not test ≤ 5%.

The concentrations of carbohydrates, total soluble sugar (TSS) and reducing sugar (RS) did not differ statistically ($p>0.05$), (Figure (2A e 2B). However, in non-reducing sugar (NRS) there was a variation between treatments, where the control was superior in relation to the others, while the treatments 30 kg ha^{-1} WF and 60 kg ha^{-1} WF provided the lowest concentrations and not differed statistically ($p>0.05$) from each other (Figure 2C).The influence of AMF and P doses was observed on the proline concentration, with the treatments of 30 kg ha^{-1} WF and 90 kg ha^{-1} WF statistically similar and approximately 60% higher than the control, which had the lowest value (Figure 2D).

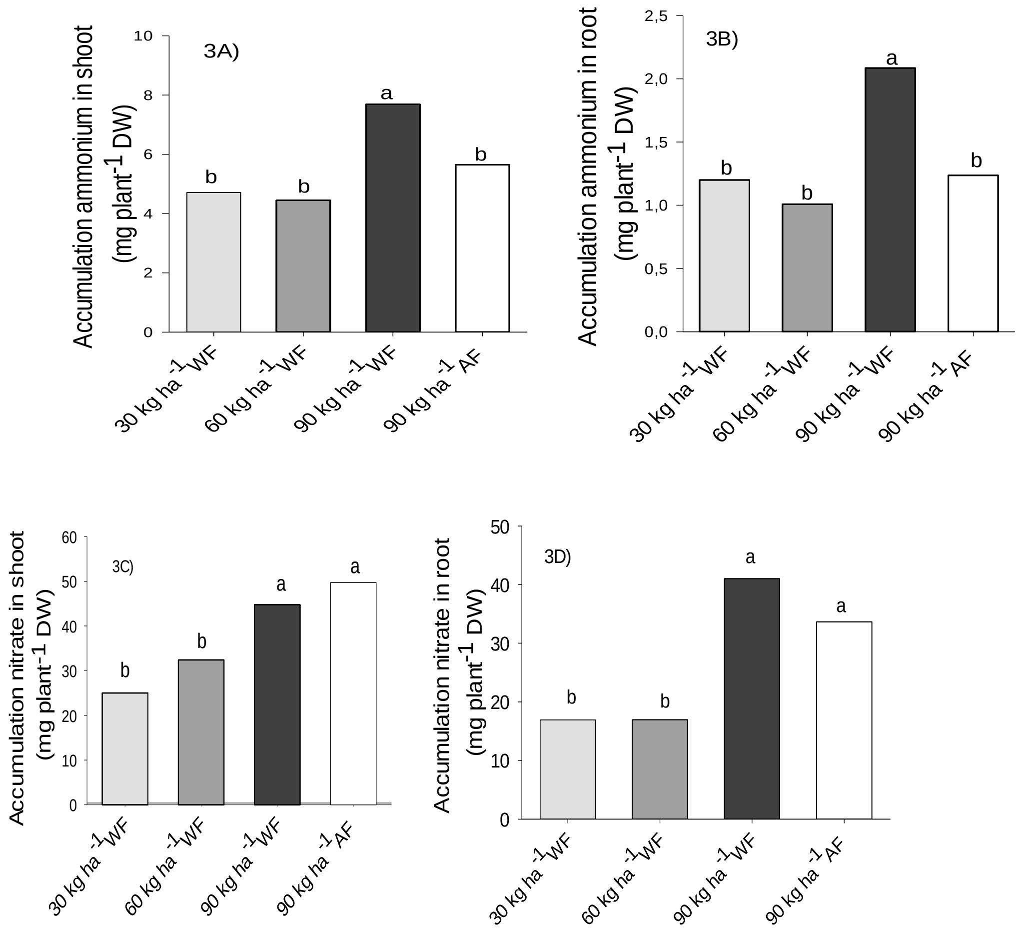
Figure 1. Carbohydrate concentrations in the shoot and proline in the root of maize plants cultivated at different levels of P, in the presence (WF) and absence (AF) of arbuscular mycorrhizal fungi.



Regarding the accumulation of NH_4^+ and NO_3^-, the 90 kg ha^{-1} WF treatment provided the highest values of NH_4^+ accumulation in both shoot and root (Figure 3A and 3B), while the other treatments did not show statistical differences ($p>0.05$). For

NO_3^-, the 90 kg ha^{-1} WF treatment and the control were superior to the other treatments both in the shoot and in the root (Figure 3C and 3D).

Figure 2. Accumulation of ammonium (NH_4^+) and nitrate (NO_3^-) in the shoot and root of maize plants grown at different levels of P, in the presence (WF) and absence (AF) ofarbuscular mycorrhizal fungi.



DISCUSSION

Plants in the 90 kg ha^{-1} WF treatment showed the best growth and nutritional results, confirming the benefits of AMF in the initial growth of maize. Plants in the 90 kg ha^{-1} WF treatment showed the greatest increase in P concentration compared to control plants (Figure 1B), even with very similar P concentration in the soil, (Figure 1B) this increase is attributed to inoculation with AMF. Hou et at. (2021) also observed higher P concentrations in *Zea mays* and *Sorghum bicolor* species cultivated in the presence of AMF.

Due to the low mobility of P in the soil, AMF hyphae are essential to reach the nutrient beyond the root depletion zone, providing a greater area available for nutrient absorption, a fact proven by the higher RV in the 90 kg ha^{-1} WF treatment plants (Table II), thus, the extra-radicular mycelium absorbs phosphate from the soil more efficiently, and transports it to the intra-radicular mycelium, which then forwards it to the plant cells, through P transporting proteins (Jansa et al, 2019).

An important point in our work is that we observed similar growth and P concentrations in the shoot of the maize from the control, that was fertilized with 90 kg ha^{-1}, witch treatment of 60 kg ha^{-1} WF, even with P values in the soil 46% higher in the control, again the AMF is efficient in P absorption and growth support. Wang et al. (2020) reported an increase in plant biomass and P uptake also at low P concentrations for maize. This fact is easily explained, as soil with low P concentration is generally related to increased mycorrhizal colonization, which reflects in efficiency gains in the absorption of P and water (Zhang et al, 2017). Benefits that begin a few days after germination, whereas infection is already observed in less than 7 days (Santos et al, 2018).

In the growth analyses, only the leaf area index variable was not sensitive to the treatments (Table II) possibly because the analyzes were carried out on plants still in the initial growth phase, when the leaves were still growing. On the other hand, the LWC that quantifies the water content in the leaves was higher in the plants of the treatments with the lowest dosages of P (Table II) meaning that, even under correct water conditions, the AMF also provided greater efficiency in water absorption, this characteristic it is important to make plants more tolerant to water deficit. Rahimi et al. (2017) also observed an increase in the relative water content of *Borago officinails* plant when cultivated in the presence of AMF.

The increase in LWC is directly related to the presence of AMF, as the lowest doses of P in the soil favor the highest percentage of infection, thus the hyphae are able to penetrate tiny pores and increase water absorption (Rahimi et al, 2017). Furthermore, the RV was strongly influenced by the treatment with a higher dose of P in the presence of AMF. This root increase is important for plants to be able to exploit a greater volume of soil and absorb more nutrients (Mathur et al, 2018).

Generally, plants associated with AMF have greater vigor and higher nutritional content, which can lead to greater biomass production (Bulgarelli., 2017). In the present work, higher DMAP and RDM were observed in the treatment with 90 kg

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ha^{-1} WF (Table III) which also presented the highest concentrations of P and ammonium. The increase in RDM in the 90 kg ha^{-1} WF treatment is reflected in the root volume, which was higher than in the other treatments.

During the symbiotic relationship, the plant supplies carbon to the AMF, which is transported to the root via phloem in the form of sucrose (Bulgarelli, 2017). This action is reflected in the sucrose concentration in the shoot, which presented the lowest values in treatments with AMF, among them, the treatments with the lowest P values provided the lowest sucrose values (Figure 2C). This is due to the translocation of sucrose from the shoot to the root in order to meet the demand of the AMF, whereas, in treatments with lower P values, the infection is even greater, requiring more sucrose, in line with our data, and as observed by Zhang et al. (2017) that at low concentrations of P mycorrhizal colonization increases. Santos et al. (2020) observed that maize plants had their development affected under high density of AMF, justified by the greater demand for carbohydrate by AMF in symbiosis.

The highest concentrations of the proline amino acid were observed in maize plants with mycorrhizae, as observed by Pavithra & Yapa. (2018) in soybean, similarly these works demonstrate that AMF increase the tolerance of plants to water stress (Figure 2D). The greater tolerance to water stress may be due to two main characteristics: greater soil volume explored by roots and extra-radicular mycelium and/or by osmotic adjustment, with proline as a potent regulator (Smith & Read, 2008), both characteristics observed in this work. These characteristics are important to maintain cell turgor (Table II), which allows for greater growth of maize plants, mainly due to better efficiency in gas exchange.

The higher proline concentration is related to the higher activity of amino acid metabolism in plants with mycorrhizae (Hu & Chen, 2020), as in higher plants, proline biosynthesis occurs via two pathways, via glutamate or ornithine, both of which are intensified in the presence of the AMF. In the first, glutamate is synthesized from ammonium and alpha-ketoglutarate in the mitochondria by glutamate dehydrogenase (GDH) (Smith & Smith., 2011), whereas in mycorrhizal plants the ammonium accumulation were increased (Figure 3A and 3B)). In the second, amino acids can be formed via ornithine, through ornithine aminotransferase (OAT), even in the

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intraradicular mycelium cell, which is more active in mycorrhizal plants, due to the greater intensity of the urea cycle (Govindarajulu et al, 2005).

The treatment plants with 90 kg ha^{-1} WF had the highest accumulation of NH_4^+ both in the root and in the shoot, while the accumulation of NO_3^- was similar to the control plants. Teutscherova et al. (2019) report a direct and positive relationship between N and P concentrations in perennial plants and grasses, in the presence of mycorrhiza. However, we observed an increase only in the ammonium accumulation.

Inorganic ammonium nitrogen (NH_4^+) and/or nitrate (NO_3^-) are absorbed by the AM extraradicular mycelium (MER) of the soil and assimilated, finally, into arginine, which is translocated to the intraradicular mycelium, where it is broken down mainly in CO_2 and NH_4^+. The NH_4^+ is transferred to the plant cell apoplast, and is subsequently assimilated (Smith & Smith, 2011), resulting in increased amino acid and NH_4^+ accumulation. However, although it has been shown that AMF can acquire inorganic forms of N and transfer to its associated host plant, the amounts transferred vary widely, even in similar experimental systems (Hu & Chen, 2020)

The similarity in NO_3^- accumulation between the control plants and 90 kg ha^{-1} WF are due to the preference of non-mycorrhizal plants to absorb more NO_3^- than NH_4^+, thus, even with the benefits promoted by the AMF the plants of the control were able to match, simply because they preferred NO_3^- as the source of N.

CONCLUSION

Arbuscular mycorrhizal fungi native to the Savanna promoted benefits in phosphorus absorption, root volume, shoot and root dry mass, and NH_4^+ accumulation for maize plants in the 90 kg ha^{-1} WF treatment. At an intermediate dose of phosphorus 60 kg ha^{-1} in the presence of AMF equals the bigger dose 90 kg ha^{-1} absence of AMF, may reduce phosphorus application. The presence of AMF in maize cultivation was beneficial to the initial growth, mainly at the dose of 90 kg ha^{-1} which is the main dosage used in the field.

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