Growth and phosphorus uptake by cassava in P-deficient soil in response to mycorrhizal inoculation

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ABSTRACT: Phosphorus (P) is one of the most difficult nutrients for plants to acquire because of its low content in the soil solution. Cassava (Manihot esculenta Crantz) has a thick and sparse absorbent root system; therefore, it is dependent on its association with arbuscular mycorrhizal fungi (AMF) for P acquisition from the soil. Thus, inoculation of cassava with AMF can improve the development of this root crop. This study evaluated the effects of soil disinfection (disinfected vs. natural) and the spore rates of Rhizophagus clarus inoculation (0, 50, 100 and 200 spores per plant) in greenhouse conditions on the initial growth, yield, P acquisition, and P use efficiency of cassava, as well as to evaluate the contribution of the native AMF to P acquisition from the soil. For cassava production in P-deficient soil, inoculation with Rhizophagus clarus significantly increased cassava growth, P uptake, and storage root yield only when the soil was disinfected. When the soil is not disinfected, native AMF contributes up to 86 % of the P taken up by cassava. However, high spore rates of Rhizophagus clarus in natural soil cause detrimental consequences for native AMF by reducing the colonization of the absorbent roots. Therefore, for cassava grown in natural soil under greenhouse conditions, a rate of 50 spores per plant of Rhizophagus clarus is sufficient to promote a 14.5 % increase in the yield of fresh storage roots. A management strategy that favors the native AMF multiplication in the soil is a potential strategy to improve P uptake and yield of cassava in P-deficient soils.

Keywords: Rhizophagus clarus, root colonization, native arbuscular mycorrhizal fungi, phosphorus nutrition.
INTRODUCTION

Phosphorus (P) is necessary for plant growth, but it is one of the most difficult nutrients to acquire from the soil (Aliyu et al., 2019). Phosphate fertilizers are derived from non-renewable phosphate rock deposits that have become an increasingly limited resource; the increasing demand for phosphate rock and fertilizers has excessively increased prices in a short time, leading to shortages of phosphate fertilizers in some countries (Alewell et al., 2020). Furthermore, the high cost of conventional soluble sources of fertilizers ensures that many small farmers do not have access to these types of fertilizers and therefore, are unable to adequately manage soil fertility (Pádua, 2012). Therefore, it is necessary to adopt management techniques to support agricultural production using less mineral P.

In the cassava crop (Manihot esculenta Crantz), P fertilization is considered essential for improving cassava storage root yield (Peña Venegas et al., 2021). However, as cassava has thick absorbent roots and few root hairs, it has a low root surface area for nutrient uptake (Silveira et al., 2015); therefore, it is one of the crops most dependent on the association with arbuscular mycorrhizal fungi (AMF) (Balota et al., 1999; Silveira et al., 2015; De Bauw et al., 2021). Arbuscular mycorrhizal fungi occur naturally in soils, and mutualism between plants and AMF occurs in 72% of terrestrial plant species (Brundrett and Tedersoo, 2018). These fungi form vesicles, arbuscles, and hyphae in the roots as well as spores and hyphae in the rhizosphere (Begum et al., 2019; Fossalunga and Novero, 2019); in this symbiosis, AMFs are supplied with sugars and lipids by the plants (Peña Venegas et al., 2021). Hyphal network formation by AMF with plant roots significantly increases root access beyond the P depletion zone, exploiting a greater soil volume than is possible through the roots alone as well as improving P uptake (Begum et al., 2019; Fossalunga and Novero, 2019; Peña Venegas et al., 2021).

Studies on AMF in cassava crops, especially in African and South American countries (Fagbola et al., 1998; Balota et al., 1999; Okon et al., 2010; Straker et al., 2010; Ceballos et al., 2013; Heberle et al., 2015; Begoude et al., 2016; De Bauw et al., 2021; Peña Venegas et al., 2021) have shown that there is a diversity of AMF species associated with the root system of cassava, predominantly within the genera Acaulospora, Entrophospora, Rhizophagus, Glomus, Gigaspora and Scutellospora (Balota et al., 1999; Straker et al., 2010; Begoude et al., 2016; Chukwuka et al., 2017). However, some studies indicated variability in the response of cassava to mycorrhizal inoculation depending on the cassava variety and the AMF strain in the inoculant (Aliyu et al., 2019; Peña Venegas et al., 2021).

There is a need to better understand the benefits that AMF can provide for cassava crops. Although cassava benefited from the AMF inoculation in disinfected soils under controlled conditions (Howeler et al., 1982), inoculation of AMF does not always improve the P uptake or cassava performance in non-disinfected soils (Howeler et al., 1982; Ceballos et al., 2013; Heberle et al., 2015). There are studies indicating that management practices that increase the abundance of native AMF in the soil may be more efficient than the inoculation of exotic AMF (Verbruggen et al., 2013). However, it is not in all situations that cassava growth or yield increase due to the previous adoption of management practices that favor the native AMF multiplication in the soil (Heberle et al., 2015; De Bauw et al., 2021). For example, the practice of fallow can increase the native AMF population in terms of richness and diversity and not improve the cassava yield (De Bauw et al., 2021). In contrast, field studies showed that cassava response to AMF inoculation is not necessarily restricted to conditions of low P availability (Peña Venegas et al., 2021); it is complex and depends on factors such as cassava variety, soil heterogeneity and the fungal strains of the inoculant (Aliyu et al., 2019; Peña Venegas et al., 2021). Thus, a better understanding of the influence of inoculation of exotic AMF on the P acquisition capacity of cassava and its effects on plant growth and storage root yield should assist in the future application of this management practice.
This study aimed to evaluate the effect of *Rhizophagus clarus* (Syn. *Glomus clarum*) inoculation on the initial growth and storage root yield of cassava with the following specific objectives: i) to evaluate the effect of *Rhizophagus clarus* inoculation on the P acquisition capacity, P use efficiency, and cassava performance when the native AMF are, or are not eliminated; ii) to evaluate the contribution of native AMF to the cassava crop; and iii) to provide baseline data on the relationship between spore rates of *Rhizophagus clarus* inoculated per plant and the productive performance of cassava in greenhouse conditions.

**MATERIALS AND METHODS**

**Site description, experimental design, and treatments**

The experiment was carried out in 38 dm$^3$ pots in a greenhouse at the Center for Tropical Roots and Starches (CERAT) of the São Paulo State University, Botucatu-SP, southeastern Brazil (22° 50’ 36” S; 48° 25’ 32” W and 740 m a.s.l.), between October 2018 and May 2019. The greenhouse, covered with a clear 150 µm-thick plastic, was 4.8 m high, 7 m wide, 25 m long, and closed on the sides with a screen. Artificial light or shade nets were not used. During cassava growth, the air temperature inside the greenhouse varied as 14.6–42.7 °C. The relative humidity inside the greenhouse was not monitored.

The experimental design used was randomized blocks in a 2 × 4 factorial scheme with four replications. The treatments were represented by two soil types (disinfected and natural) combined with four inoculation contents of *Rhizophagus clarus* (0, 50, 100 and 200 spores per plant). Each plot contained one pot with one cassava plant. The *Rhizophagus clarus* inoculum was obtained from the Embrapa Agrobiologia collection and was previously multiplied in palisade grass. For inoculum multiplication, *Urochloa brizantha* (syn. *Brachiaria brizantha*) seeds were disinfected with sodium hypochlorite (10 %) and pre-germinated in a sterilized substrate. Five days after germination, five seedlings were transplanted to 4 kg plastic pots containing sterile soil, and 1 g of *Rhizophagus clarus* inoculum was deposited per pot below the roots of the seedlings. Host plants were grown in the greenhouse according to particular crop recommendations and were harvested after 100 days. The inoculum obtained and used in the present study contained 12 spores per gram and other propagules.

**Soil preparation, cassava planting and management**

The soil used was from the 0.00-0.20 m layer of a *Latossolo Vermelho Distroférrico* (Santos et al., 2018), which corresponding to a Hapludox (Soil Survey Staff, 2014) with low P availability. The soil was previously corrected with dolomitic limestone (241 mg dm$^{-3}$) and fertilized with 30, 7.0, 92.5, 0.78, and 0.60 mg dm$^{-3}$ of N, P, K, B and Zn, respectively. The fertilizer sources used were urea (45 % N), triple superphosphate (45 % P$_2$O$_5$), potassium chloride (60 % K$_2$O), borax (11 % B) and zinc sulfate (20 % Zn). After correction and fertilization, the soil was divided into two parts, one of which was disinfected in an autoclave for 45 min at 1 atm. The two soils were then sampled and analyzed, since the initial P availability was considered low for cassava (Table 1; Lorenzi et al., 1997).

Five days after soil disinfection, 0.13-m-long stem cuttings of cassava cv. IAC 576-70 harvested from the middle third of 12-month-old plants were planted in each pot. Initially, the stem cuttings were disinfected by immersion in a 70 % ethanol solution for 2 min, followed by immersion in a solution containing 20 % bleach (2.5 % sodium hypochlorite) for 10 min. After treatment, the stem cuttings were washed five times with deionized water, and each was placed in a hole of 0.10 m deep in the respective pot. *Rhizophagus clarus* inoculum was applied to the stem cuttings in the hole according to the treatments. Holes were then filled with soil. The planting occurred on October 8, 2018, and emergence began 22 days later (October 30, 2018).
At 40 days after emergence (DAE) (December 09, 2018), 40 mg dm\(^{-3}\) N was applied to all pots, using urea as the source. All treatments received manual irrigation with deionized and sterile water as necessary to maintain the soil water content close to 80 % of the maximum water retention capacity. Water applied in the irrigation was treated using an ion exchange resin deionizer coupled to a 1,000 L water box equipped with a UV lamp system to eliminate microorganisms. Cassava was grown in pots for up to 7 months after planting (MP).

### Plant sampling, measurements and analyses

At 4 MP (February 05, 2019), fully expanded leaf blades from the apex of the plants were sampled (Lorenzi et al., 1997). The samples were dried in a forced air circulation oven at 65 °C for 72 h, ground to pass through a 40-mesh stainless steel screen, and analyzed for P and Mn contents (Malavolta et al., 1997).

Cassava plants were harvested at 7 MP (May 06, 2019) and divided into shoots, planted cuttings, absorbent roots, and storage roots. Absorbent roots were sampled and analyzed for length, surface area, and mean diameter using WinRhizo software, which uses the principle proposed by Tennant (1975). To evaluate mycorrhizal colonization of the absorbent roots, we initially clarified and colorized the roots using the technique of Phillips and Hayman (1970), with adaptations by Koske and Gemma (1989) and Grace and Stribley (1991). Mycorrhizae were quantified on microscope slides using the method of McGonigle et al. (1990). Fresh storage root yield per plant was obtained by counting and weighing the storage roots of each plant. The storage root mean weight was determined as the relation between the weight and number of storage roots per plant.

All plant parts (shoot, planted cutting, absorbent roots and storage roots) were dried in a forced-air circulation oven at 65 °C for 96 h, and weighed to determine the amount of dry matter (DM) accumulated. The samples were ground to pass through a 40-mesh
stainless steel screen and analyzed for P content (Malavolta et al., 1997). The amount of P accumulated in the different plant parts was obtained by multiplying the P content by the amount of DM accumulated in each plant part (Fernandes et al., 2020). The P uptake efficiency (PUpE) was calculated as proposed by Swiader et al. (1994) (Equation 1).

\[ \text{PUpE (mg cm}^{-1}\text{)} = \frac{\text{Total amount of P taken up by the plant (mg)}}{\text{Total absorbent root length (cm)}} \]

Phosphorus use efficiency (PUE) was calculated according to Chowdhury and Zhang (2021) (Equation 2).

\[ \text{PUE (g mg}^{-1}\text{)} = \frac{\text{Total amount of plant biomass (g)}}{\text{Total amount of P taken up by plant (mg)}} \]

**Data analysis**

SISVAR statistical software was used for data analyses (Ferreira, 2011); the LSD test (p≤0.05) was applied to separate the means related to the effects of soil type (ST), while regression analysis was used to evaluate the effects of AMF inoculation rates (AMF-R). To analyze the significant ST × AMF-R interaction, the ST means were separated using Fisher’s protected LSD test (p≤0.05), and the regression equations were separately adjusted to the values of the ST treatments.

**RESULTS**

The ST × AMF-R interaction affected the P leaf content of cassava (Figure 1a). There was no significant effect of AMF-R on the leaf P content in the natural soil, but in the disinfected soil, the leaf P content was increased by 102 % until a rate of 100 spores per plant AMF. Soil disinfection reduced P content in the cassava leaves by 52 % with no inoculation of AMF, and when AMF was inoculated, the leaf P content did not differ between soils. The leaf Mn content was only affected by ST and AMF-R (Figure 1b). In the disinfected soil, the Mn content in the cassava leaves was higher than that in the natural soil, and the leaf Mn content was increased by AMF inoculation up to 130 spores per plant.

The ST × AMF-R interaction affected the length and surface area of the absorbent roots (Figures 1c and 1d). In the disinfected soil, AMF linearly increased the length by 237 % and surface area by 176 %. In the natural soil, AMF inoculation caused a curvilinear to plateau increase of 31 % in root length up to the rate of 100 spores per plant, and a quadratic effect on the root surface, with a 33 % increase up to the rate of 159 spores per plant. Length and surface area of absorbent roots were reduced by disinfecting the soil when less than 200 spores per plant AMF were inoculated.

Only the ST factor affected the diameter of the absorbent roots, which was 5.3 % less in the disinfected soil than in the natural soil (Figure 1e). Mycorrhizal colonization was affected by ST × AMF-R interaction (Figure 1f). In natural soil, AMF inoculation linearly reduced mycorrhizal colonization by 47 %. In the disinfected soil, inoculation with AMF increased mycorrhizal colonization by 1786 % up to a rate of 122 spores per plant. In the natural soil, mycorrhizal colonization was 4900 % higher with no AMF, and 24 % higher with 50 spores per plant than the disinfected soil.

The number, mean weight, and storage root yield of cassava were affected by the ST × AMF-R interaction (Figure 2). In the natural soil, AMF inoculation did not affect the storage root mean weight; however, there was an increase of 15.0 and 14.5 % in the number and yield of storage roots up to the rate of 50 spores per plant. In the disinfected soil, mycorrhizal inoculation resulted in a 4076 % increase in the storage root number up to the rate of 161 spores per plant; a 3859 % increase in the storage...
root mean weight up to the rate of 200 spores per plant; and a 24236% increase in the storage root yield up to a rate of 167 spores per plant. With no inoculation, soil disinfection reduced the number of storage roots per plant, and the mean weight and yield of storage roots were reduced by disinfecting soil at rates of less than 200 spores per plant. The amount of DM accumulated in the plant parts and whole plant, and the harvest index (HI) were affected by the ST × AMF-R interaction (Figure 3). In the natural soil, AMF inoculation did not affect the DM of shoots, storage roots, whole plants, and HI, but increased the DM of absorbent roots by 17% up to the rate of 125 spores per plant, while reducing the planted cutting DM by 12% up to the rate of 113 spores per plant. In the disinfected soil, AMF inoculation increased DM accumulation in all plant parts and increased HI. The DM of shoots, planted cutting, and absorbent roots increased
to between 127 and 142 spores per plant, while the DM of storage roots, whole plant, and HI increased up to the highest rate of AMF inoculated. Soil disinfection decreased DM of shoots when no AMF was inoculated and decreased DM of absorbent roots at rates less than 100 spores per plant. However, the DM of the storage roots, whole plant, and HI decreased significantly by disinfection of the soil at rates of less than 200 spores per plant.

Phosphorus content in the shoot and storage roots was affected by the ST × AMF-R interaction (Figures 4a and 4d). In the natural soil, AMF inoculation did not change the shoot P content, but in the storage roots, there was a 38 % increase up to 152 spores per plant. In the disinfected soil, shoot P content increased 41 % up to the rate of 132 spores per plant, and storage root P content increased 1810 % up to 135 spores per plant. Soil disinfection significantly reduced the P content in the shoots and storage roots only when no AMF was included. In the planted cuttings, P content was not affected by the treatments, but in the absorbent roots, P content was higher in the disinfected soil with no influence of AMF-R or ST × AMF-R interaction (Figures 4b and 4c).

The amount of P taken up and PUpE were affected by the ST × AMF-R interaction (Figures 5a and 5b). In the natural soil, the P uptake increased by 12.6 % up to the rate of 100 spores per plant, while the PUpE decreased by 18 % up to the rate of 132 spores per plant. In the disinfected soil, the effect of AMF inoculation was a 583 % curvilinear to plateau increase in P uptake up to a rate of 100 spores per plant, and a 204 % quadratic increase in the PUpE up to the rate of 113 spores per plant. Phosphorus uptake was reduced 86 and 32 % by soil disinfection when 50 spores per plant or less were inoculated. However, PUpE decreased with soil disinfection when no AMF was...
inoculated or inoculated at a rate of 200 spores per plant. The PUE was affected only by AMF-R, and there was an 18 % reduction in the PUE up to a rate of 132 spores per plant (Figure 5c).

**DISCUSSION**

Under natural soil conditions, AMF inoculation promoted a 17 % increase in the biomass of the absorbent roots, but did not alter the initial growth of the cassava plants. However, in the disinfected soil, inoculation with AMF significantly increased the growth of cassava plants, especially storage roots. Cassava presented a positive response to AMF inoculation in disinfected soil (Howeler et al., 1982), but the benefits of AMF inoculation have not been clarified in natural soil conditions (Ceballos et al., 2013; Heberle et al., 2015) as verified in the present study. In the disinfected soil, a rate of 200 spores per plant *Rhizophagus clarus* promoted cassava growth similar to that in natural soil conditions,
Figure 4. Phosphorus content in the shoot (a), planted cutting (b), absorbent roots (c) and storage roots (d) of the cassava in response to AMF inoculation rates in natural and disinfected soils. Within each AMF inoculation rate, different lowercase letters indicate a significant difference between soil types, whereas the absence of lowercase letters indicates a non-significant difference between soil types at $p \leq 0.05$. Different uppercase letters indicate a significant difference between soil types at $p \leq 0.05$ regardless of AMF inoculation rate. * $p \leq 0.05$; ** $p \leq 0.01$.

Figure 5. Amount of P taken up (a), P uptake efficiency (PUpE) (b) and P use efficiency (PUE) (c) by the cassava in response to AMF inoculation rates in natural and disinfected soils. Within each AMF inoculation rate, different lowercase letters indicate a significant difference between soil types, whereas the absence of lowercase letters indicates a non-significant difference between soil types at $p \leq 0.05$. * $p \leq 0.05$; ** $p \leq 0.01$. 

\[ y = 1.2 - 0.0033x + 0.000012526x^2 \quad R^2 = 0.99** \]
corroborating previous studies that cassava is naturally associated with AMF (Balota et al., 1999; Straker et al., 2010; Aliyu et al., 2019) and is highly dependent on them for growth (Balota et al., 1999; De Bauw et al., 2021).

Maintenance of plant growth in the natural soil with low P availability (Table 1) even without AMF inoculation occurred because the leaf P content was sufficient (<2.0 g kg\(^{-1}\); Lorenci et al., 1997), which differs from results with the disinfected soil, where the plants only showed sufficient leaf P content from the inoculation rate of 50 spores per plant. The capacity of cassava to associate with native AMF in the soil (Balota et al., 1999; Straker et al., 2010; Aliyu et al., 2019) allows for the maintenance of high leaf content in P-deficient soils because a considerable portion of the phosphate taken up by mycorrhizal plants can be acquired via the AMF pathway (Fossalunga and Novero, 2019).

However, under low fertility, cassava can reduce the size of the plants, maintain relatively high P contents in the leaves, and photosynthesize at a similar rate to that of plants grown in highly fertile soil (Cock and Connor, 2020). In this study, soil disinfection increased the leaf Mn content, which was above the range suggested as suitable for cassava (25–100 mg kg\(^{-1}\); Lorenci et al., 1997), but leaf Mn contents remained below the content which is considered toxic to cassava (1,000 mg kg\(^{-1}\); Souza et al., 2009).

With no AMF inoculation, the mycorrhizal colonization of the roots in the natural soil was 4900 % higher than in the disinfected soil, and \textit{Rhizophagus clarus} inoculation decreased the colonization of absorbent roots, whereas the opposite occurred in the disinfected soil. This indicates a high rate of absorbent root colonization by native soil AMF and that \textit{Rhizophagus clarus} inoculation inhibited the action of these native species. Some studies indicated that plant responses to inoculation might be influenced by increases in the abundance of soil AMF (due to the adoption of alternative management) as opposed to the introduction of new strains (Verbruggen et al., 2013) since native and exotic AMF can be equally effective in increasing plant growth (Pellegrino et al., 2011), as was verified in this study. Furthermore, management practices that increase the abundance of native AMF in the soil (such as fallow) do not always increase cassava root yield (De Bauw et al., 2021).

In natural soil, AMF inoculation increased the length and surface area of the absorbent roots by 31–33 %, but this did not significantly increase P uptake, which is consistent with previous findings indicating that cassava responsiveness to inoculation is dependent on fungal genotypes, cassava varieties and native AMF communities (Aliyu et al., 2019; Peña Venegas et al., 2021). In this study, native AMF from soil contributed 86 % of P taken up by cassava when no AMF was inoculated; however, when 50 spores per plant were added, this contribution decreased to 32 and 0 % when higher rates of AMF were included. This occurred because, in natural soil, AMF inoculation reduced root colonization and PUE, and showed no benefits for plant growth. Therefore, inoculation of \textit{Rhizophagus clarus} in natural soil is not an alternative to improving cassava plants’ P uptake and growth. Perhaps the adoption of management strategies that increase the abundance of native AMF can be more effective in increasing P uptake by cassava (Verbruggen et al., 2013); however, this strategy does not always result in improved growth and yield of cassava (Heberle et al., 2015; De Bauw et al., 2021).

Interestingly, although inoculation with at least 50 spores per plant \textit{Rhizophagus clarus} reduced root colonization, it increased the yield of storage roots by 14.5 %. However, future studies need to confirm whether this beneficial effect of \textit{Rhizophagus clarus} inoculation on cassava yield is replicated for other cassava cultivars under field conditions because the inoculation response depends on the interaction between the AMF species and the host cassava variety (Peña Venegas et al., 2021). For example, in Nigeria and Colombia, inoculation with \textit{Rhizophagus manihotis} increased cassava root yield only in later harvests (Fagbola et al., 1998), whereas inoculation with \textit{Glomus deserticola} increased root colonization, P uptake, and root yield of cassava (Okon et al., 2010; Ceballos et al., 2013).
In contrast, inoculation with the same strain of *Rhizophagus irregularis* in two different cassava varieties (an improved variety and another landrace) caused a contrasting response to AMF inoculation (Peña Venegas et al., 2021). Different cassava responses to AMF inoculation also occurred when inoculants based on *Rhizophagus irregularis* were inoculated into the same cassava variety cultivated at different sites (Aliyu et al., 2019). Thus, the low response of cassava to inoculation with *Rhizophagus clarus* in the natural soil in the present study may also be related to the competition between native and inoculated AMFs (Verbruggen et al., 2013), since mycorrhizal colonization was high in the uninoculated natural soil.

The inoculation of *Rhizophagus clarus* altered the PUpE differently in the two soils, but the fact that the soil was disinfected did not change the PUE, indicating that the benefits of AMF are limited to improvements in the P acquisition from the soil and not in its further use by the plants. Arbuscular mycorrhizal fungi are very effective in helping plants uptake nutrients from deficient soils by increasing the surface absorbing capacity of the host roots and providing nutritional support for the plants even under nutrient-deficient conditions within the root cells (Begum et al., 2019; Fossalunga and Novero, 2019). The reduction in PUE caused by inoculation with *Rhizophagus clarus* indicates that there was a greater increase in P uptake than in the production of biomass from plants, especially in disinfected soil conditions.

**CONCLUSIONS**

For cassava production in P-deficient soil, inoculation with *Rhizophagus clarus* significantly increased plant growth, P uptake and storage root yield only when the soil was disinfected. In natural and P-deficient soil, native arbuscular mycorrhizal fungi (AMF) from the soil contribute up to 86 % of the P taken up by cassava. However, inoculation with high spore rates of *Rhizophagus clarus* in natural soil causes detrimental consequences for native AMF present in the soil, affecting their capacity to colonize the absorbent roots and contribute to P acquisition. The results of this study demonstrate that for cassava grown in natural soil under greenhouse conditions, inoculation with 50 spores per plant of *Rhizophagus clarus* is sufficient to promote a 14.5 % increase in the yield of fresh storage roots. A management strategy that favors the native AMF multiplication in the soil can also has the potential to improve P uptake and yield of cassava in P-deficient soils.

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