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Arbuscular mycorrhizal fungi potentiate the root system and the quality of goldenberry fruits

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Abstract: The lack of information on the horticultural performance of goldenberry (*Physalis peruviana* L.) is one of the factors that limits the expansion of the crop. Still, aiming to establish a sustainable management for this culture, inoculation with arbuscular mycorrhizal fungi (AMF) can be adopted. Therefore, the objective of the research was to investigate whether goldenberry plants in the absence and presence of inoculation with AMF differ in terms of horticultural performance. The four treatments studied were the absence (control) and the presence of three inoculants based on AMF (mycorrhizal community, *Glomus intraradices* and *Rhizophagus clarus*), arranged in a randomized block design, with five replications. Goldenberry plants produced in substrate enriched with AMF had a more voluminous root system and a greater amount of fine roots. Additionally, the fruits were sweeter and more flavorful when produced by plants inoculated with the mycorrhizal community and with *R. clarus*. It is concluded that mycorrhization has no effect on fruit production. However, goldenberry plants submitted to mycorrhizal biotechnology enhance the chemical quality of fruits and present a more profuse root system. *G. intraradices* is most effective in colonizing the roots of the plant host.

1. Introduction

Goldenberry (*Physalis peruviana* L., Solanaceae) is a horticultural crop native to the Andean highlands that has attracted worldwide attention due to its bioactive compounds such as carotenoids, physalins and polyphenols (Ramadan, 2011). In addition to promoting the health of consumers, these biomolecules present in fruit extracts have antifungal action against phytopathogens, such as *Botrytis cinerea* Pers., and therefore can be widely used in agriculture as a bioinput (Filippi *et al.,* 2020). Still, goldenberry stands out for its potential for intensive cultivation

(Etzbach *et al.,* 2018). A single plant can produce 300 fruits and the productivity of this horticultural crop can reach from 20 to 33 tons per hectare (Yildiz *et al.,* 2015). Despite the traditional establishment of crops in the open field (Muniz *et al.,* 2014), the goldenberry cultivation in greenhouse is increasing (Aguilar-Carpio *et al.,* 2018). This is because greenhouse cultivation can help to avoid inconveniences such as pests, diseases, rain, strong winds, hail and frost (Costa *et al.,* 2016).

Similar to the traditional cultivation of other Solanaceae, such as tomato and pepper, in order to obtain an optimal productive yield of goldenberry, producers need to use a large amount of chemical inputs, which can contaminate the agroecosystem of cultivation (Chiomento *et al.,* 2020 a). There is no doubt, therefore, that the establishment of agroecological agriculture is an important tool for sustainable food production, with environmental and socioeconomic benefits (Llano *et al.,* 2018). Thus, an alternative to minimize the inconveniences in the cultivation of goldenberry and start the establishment of sustainable management in this horticultural culture corresponds to the use of inoculants based on arbuscular mycorrhizal fungi (AMF). As there is a limitation regarding the availability of commercial AMF-based inoculants available in Brazil (Trentin *et al.,* 2022), this reduces the use of mycorrhizal biotechnology in the production of goldenberry due to the lack of knowledge of this bioinput by the producers. The limitation regarding the availability of commercial inoculants is mainly due to the high cost linked to the production technology of this bioinput.

AMF (phylum Glomeromycota), a ubiquitous group of soil microorganisms, establish symbiotic associations with more than 70% of vascular plants (Brundrett and Tedersoo, 2018). The literature reporting the association between mycorrhiza and goldenberry is scarce. For example, under saline conditions, AMF increased fruit growth rate (Miranda *et al.,* 2011) and improved berry unsaturated fatty acid concentration in response to heavy metal stress (Hristozkova *et al.,* 2017). Under water stress, arbuscular mycorrhiza increased root dry matter accumulation and improved attributes related to plant gas exchange (Reyes *et al.,* 2019). In non-stressful environments, it was found that goldenberry plants subjected to mycorrhizal biotechnology produced less acidic and tastier fruits (Chiomento *et al.,* 2020 a). This scarcity of information demands more research to fill the existing gaps regarding the morpho-horticultural performance of goldenberry and their interactive effects with mycorrhizas.

In Brazil there is only one commercial AMF inoculant available to farmers. The commercial scale production of this bioinput has high costs linked to the inoculum production technology, such as the establishment of cultures of AMF species and transport, handling and development of the carrier substrate (Schlemper and Stürmer, 2014). To avoid some of these costs, *on-farm* production of inoculants is used, with indigenous or exotic AMF isolates, in which the technology can be easily transferred to farmers (Douds Junior *et al.,* 2012). The process of obtaining the *on-farm* inoculant can be started using AMF infective propagules, such as spores, hyphae and parts of colonized roots (Douds Junior *et al.,* 2010).

Therefore, based on the hypothesis that mycorrhizal biotechnology enhances plant host growth and improves fruit chemical quality, here we investigate whether the horticultural performance of goldenberry is influenced by the use of AMF-based *on-farm* inoculants.

2. Materials and Methods

Plant material

The research was carried out in Passo Fundo (28° 15’ 46” S, 52° 24’ 24” W), Rio Grande do Sul (RS), Brazil, in greenhouses, from August (winter) 2018 to July (winter) 2019 .

A commercial tray with goldenberry fruits at maturation stage 5 was purchased (ICONTEC, 1998). In August 2018, seeds from three randomly chosen fruits were selected, transferred to paper towels and kept at room temperature until dry. Subsequently, these seeds were germinated in plastic gerbox boxes containing blotting paper and 0.1 molar (M) potassium nitrate (KNO_3) solution. The boxes were stored in a biochemical oxygen demand (BOD) oven, at 25°C±1°C, until the plants were obtained for the production of seedlings, which constituted the plant material for the research. The steps for obtaining the plants are shown in figure 1.

Experimental design

The four treatments studied were the absence (control) and the presence of three inoculants based on AMF [mycorrhizal community, *Glomus intraradices* N.C. Schenck & G.S. Mr. and *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C.

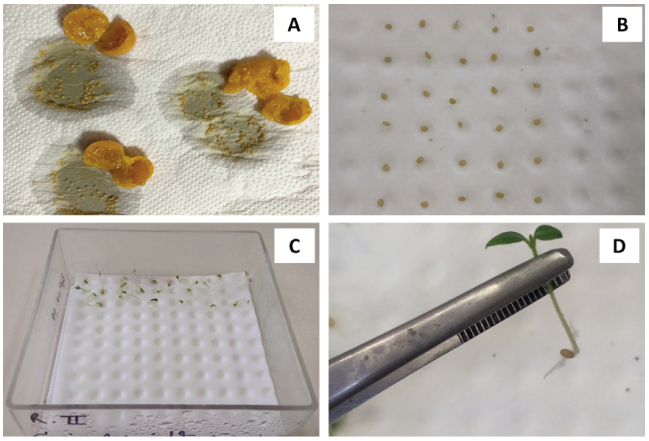

Fig. 1 - Obtaining goldenberry plants. (A) Separation of seeds from fruits. (B) Selection of seeds. (C) Germination in gerbox. (D) Plants produced.

Walker & A. Schüßler], arranged in a randomized block design with five replications. Each plot consisted of three goldenberry plants.

The AMF community used came from the croptrap of agricultural soil collected at a reference site for strawberry cultivation in the municipality of São José do Hortêncio (29° 29' 33" S, 51° 12' 24" W), Rio Grande do Sul State, Brazil (Chiomento *et al.,* 2019 a), composed of ten fungal species according to the classification of Glomeromycota proposed by Redecker *et al.* (2013): *Acaulospora foveata* Trappe & Janos, *Claroideoglomus* aff. *luteum*, *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler, *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, *Funneliformis* aff. *geosporum*, *Funneliformis* aff. *mosseae*, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Glomus* aff. *versiforme*, *Glomus* sp. (caesaris like) and *Glomus* sp2. The isolate *G. intraradices* came from the commercial product MYKE® PRO and the isolate *R. clarus* was obtained from the International Collection of Glomeromycota Culture (CICG).

Cultivation procedures

We applied the treatments (AMF) in two stages: 1) in the acclimatization of the seedlings; 2) in transplanting to the place of cultivation. Thus, of the total amount of mycorrhizal inoculant used (10 g), we applied 5 g in the acclimatization of the seedlings and 5 g at the time of transplanting.

In September (spring) of 2018, thirty days after the germination of goldenberry seeds, the plants obtained (Fig. 1D) were acclimatized in 72-cell polystyrene trays, filled with the sterilized Horta 2® substrate (120°C for 20 minutes) and with treatments related to mycorrhization (1/2 of the total amount), with the purpose of seedling production. Horta 2® is composed of pine bark, vermiculite, acidity correctors and fertilizers (nitrogen, phosphorus and potassium) in amounts not supplied by the manufacturer. A 500 g sample of the substrate was analyzed to obtain its physical (Brazil, 2007) and chemical (MAPA, 2014) attributes (Table 1).

The trays were kept on metal benches, 1.2 m from the ground surface, in a greenhouse (90 m^2), installed in the northeast-southeast direction, with a semicircular roof. The galvanized steel structure is covered with a low-density polyethylene film with anti-ultraviolet additive (150 micron thickness) and the sides are covered with an anti-aphid screen. The irrigation used during acclimatization was with sprinklers (1.8 $L.min^{-1}$ per unit), in the mechanized system. The irrigation regime consisted of activating the sprinklers seven times a day, with total wetness of 14 minutes. The water depth supplied to the seedlings was 7.8 $mm.day^{-1}$.

In December (summer) 2018, after three months of acclimatization, the seedlings were transplanted

Table 1 - Physical and chemical properties of the Horta 2® substrate

Substrate	Physical properties						
	D (Kg m^{-3})	TP ($m^3.m^{-3}$)	AE ($m^3.m^{-3}$)	RAW ($m^3.m^{-3}$)	BW ($m^3.m^{-3}$)	RW ($m^3.m^{-3}$)	
Horta 2 ®	241	0.837	0.303	0.149	0.020	0.365	
	Chemical properties						
	N % ($m.m^{-1}$)	P_2O_5 ($m.m^{-1}$)	K_2O % ($m.m^{-1}$)	OC % ($m.m^{-1}$)	pH	EC % ($mS.cm^{-1}$)	CEC ($mmol_c.kg^{-1}$)
	0.36	0.39	0.00	12.60	6.1	0.45	278.60

(z) D= density; TP= total porosity; AE= aeration space; RAW= easily available water; BW= buffer water; RW= remaining water.

(w) N= nitrogen; P_2O_5 = phosphorus pentoxide; K_2O= potassium oxide; OC= organic carbon; pH= hydrogen potential; EC= electric conductivity; CEC= cation exchange capacity.

into pots (3.6 L), filled with sterilized Horta 2® (120°C for 20 minutes) and complemented with the other part of the treatments related to mycorrhization (1/2 of the total amount). The pots were kept in beds covered with mulching, in a greenhouse (430 m^2), with a semicircular roof, installed in the northeast-southeast direction. The galvanized steel structure was covered with a low-density polyethylene film (150 microns thick) and with an anti-ultraviolet additive.

Localized irrigation was carried out using drip rods (2.4 $L.h^{-1}$ per unit), in the mechanized system. The irrigation regime consisted of activating the dripping rods six times a day, with total wetting for six minutes. The nutrient solutions supplied to the plants, fortnightly, were made according to Furlani and Fernandes Júnior (2004), but with a 50% reduction in phosphorus supply. Through a mini meteorological station, we verified that the average general temperature recorded during the experiment was 25.66°C.

The plants were conducted with three stems and were tutored with the aid of wires. No biocides were used during the crop cycle. The evaluations started after the fruiting of the plants, in February (summer) of 2019. We evaluated the root system morphology and the productive yield (number and weight) and quality of fruits.

Root system morphology

At the end of the experiment, in July 2019, the plants roots were washed in water to eliminate substrate fragments. The roots were digitized by a scanner and the images obtained were analyzed by the WinRHIZO® software. The attributes evaluated were total length (TL, cm), surface area (SA, cm^2) and volume (V, cm^3). The roots were grouped by the software into different diameter classes in relation to their total length (Böhm, 1979): very thin (VT, Ø<0.5 mm), thin (TH, Ø from 0.5 to 2 mm) and thick (TK, Ø>2 mm).

To verify the infective capacity of AMF, root portions of mycorrhizal plants were prepared according to Phillips and Hayman (1970) and their percentage of mycorrhizal colonization (MC) was determined according to Trouvelot *et al.* (1986), by the equation:

$$MC(\%) = \frac{(\text{total number of fragments with mycorrhizal roots})}{(\text{total number of fragments})} \times 100 \quad (1)$$

Fruit production

From fruiting, in February 2019, the total number of fruits (TNF, number per plant) and the total production of berries (TP, grams per plant) were evaluated. In addition, the average fresh fruit mass (AFFM, grams) was evaluated. The fruits were harvested when they were in the stages of maturation between 4 and 6 (ICONTEC, 1998). The fruits were weighed on an electronic digital scale.

Chemical fruit quality

The analysis of fruit quality was performed at the end of the experiment, in July 2019. The chemical characteristics of the fruits were evaluated regarding the content of total soluble solids (TSS, %) and total titratable acidity (TTA, % of citric acid), from 20 fruits of each treatment for each repetition. The TSS content was determined in an analog refractometer, and the TTA was performed according to the norms of the Adolfo Lutz Institute (Zenebon *et al.,* 2008). To evaluate the flavor of the fruits, the TSS/TTA ratio was determined.

Data analysis

The data obtained were submitted to analysis of variance (Anova) and the averages of the treatments were compared by the Tukey test, at 5% error probability, with the aid of the Costat® program (Cohort Software, 2003).

3. Results

Root system morphology

We verified a significant effect of mycorrhizal inoculants only for the attributes MC, V and TH. *G. intraradices* had a greater ability to infect plant roots than the mycorrhizal community and *R. clarus* (Fig. 2A). The fungal structures identified inside the roots of goldenberry plants were hyphae, vesicles and arbuscules. In addition, plants inoculated with *R. clarus* had 41% and 42% greater root volume than non-mycorrhizal plants and those inoculated with the AMF community, respectively (Fig. 2B). Also, plants inoculated with the mycorrhizal community had a greater amount of fine roots (+47%) compared to the control (Fig. 2C).

Fruit production

We observed a positive effect of treatments only for the AFFM attribute. Non-mycorrhizal plants produced fruits with higher average fresh mass (+29%) compared to plants inoculated with the mycorrhizal

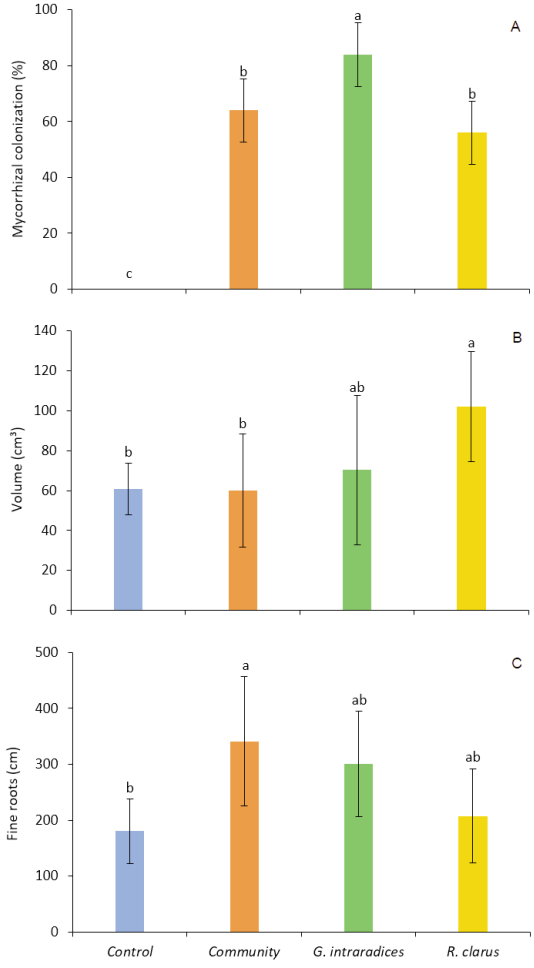

Fig. 2 - Root system morphology of goldenberry plants in the presence and absence of AMF inoculation. (A) Mycorrhizal colonization (%). (B) Root volume (cm³). (C) Amount of fine roots (cm). Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (p≤0.05).

community, but did not differ from plants mycorrhizal with *G. intraradices* and *R. clarus* (Fig. 3).

Chemical fruit quality

Mycorrhizal inoculants influenced TSS and TSS/TTA attributes (Fig. 4). Sweeter (Fig. 4A) and tastier (Fig. 4B) fruits were produced by plants inoculated with the mycorrhizal community and with the isolate *R. clarus*.

4. Discussion and Conclusions

Here, we show that goldenberry plants in the absence and presence of AMF inoculation differed in horticultural performance. In the first productive

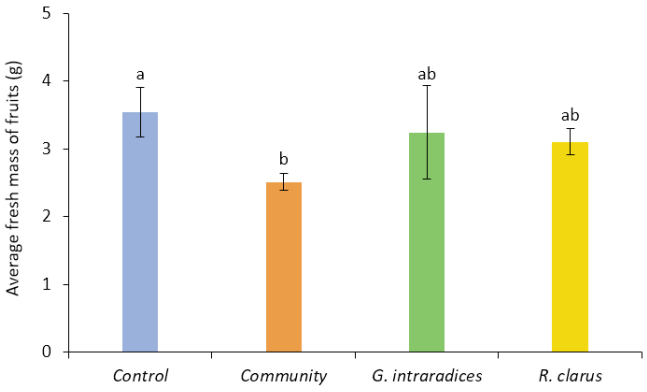

Fig. 3 - Average fresh fruit mass (grams) of goldenberry plants in the presence and absence of AMF inoculation. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (p≤0.05).

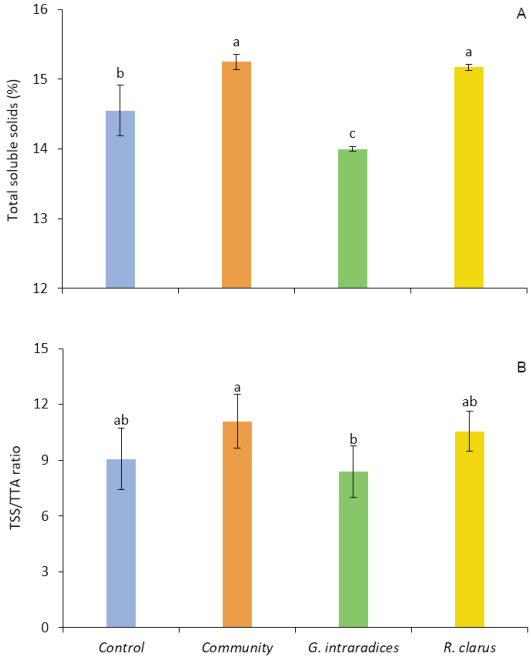

Fig. 4 - Chemical quality of goldenberry fruits in the presence and absence of AMF inoculation. (A) Total soluble solids (%). (B) Fruit flavor. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (p≤0.05).

cycle of the plants, we did not observe the effect of mycorrhization on the production of berries. We believe that in goldenberry the fungal species used require a period of more than one year to benefit the fruit yield, as occurs in strawberry (*Fragaria* X *ananassa* Duch.) cultivation (Robinson-Boyer *et al.*, 2016). However, goldenberry plants inoculated with AMF showed a more profuse root system.

Furthermore, the use of the mycorrhizal community and *R. clarus* allowed to harvest fruits with better chemical quality. This indicates that the fungal species present in these two inoculants have an affinity for this horticultural crop. The use of AMF compatible with the host commonly provides more satisfactory results (Chiomento *et al.,* 2022). *G. intraradices* was more effective in colonizing the roots of the plant host. However, the mycorrhizal community tested in this study stood out in relation to the fungal isolates for generally improving the horticultural performance of goldenberry.

We verified that the root system of the mycorrhizal plants was more profuse, with greater volume (Fig. 2B) and with a greater amount of fine roots (Fig. 2C). This benefit to the roots has already been reported by Reyes *et al.* (2019), who demonstrated that mycorrhization in goldenberry under water stress increased the accumulation of root dry matter. Due to the plasticity of the roots, their characteristics can be modulated by several factors, including AMF (Hodge *et al.,* 2009). During the establishment of the association between host and fungus, many molecular signals are initiated, including an AMF (lipo-chitooligosaccharides) diffusible factor called "Myc factor", which stimulates the formation of finer roots (Oláh *et al.,* 2005), altering the morphology of the root system of plants. In addition, root modifications under mycorrhization may be related to the allocation of sugars to roots (Wu *et al.,* 2011) and hormonal regulation (Zou *et al.,* 2017), independent of symbiotic signaling (Gutjahr, 2014). The more fine roots there are in mycorrhizal plants, the better their acquisition of water and nutrients (Chiomento *et al.,* 2021), as these roots are the ones that most acquire and use the available resources in the plant growth medium (Costa *et al.,* 2019).

Differently from what was expected, the productive performance of goldenberry was higher when the plants were not mycorrhized (Fig. 3). This suggests that AMF initially demand carbon from the host for their maintenance and only later repay this benefit to the plant symbiont. These results, however, contradict the literature. For example, Miranda *et al.* (2011) reported benefits of AMF in the production of goldenberry, under limiting conditions to plants, which were subjected to abiotic stresses, which did not happen in our study. There is not always a high relationship between fungal infectivity and efficiency in promoting crop growth due to the time required to establish bidirectional flow between symbionts (Abbott and Robson, 1981), which limits the plant's response to mycorrhization (Lambais and Cardoso, 1990). Although mycorrhizal colonization is important, the percentage of root infectivity is not always correlated with the efficiency of symbiosis (Konvalinková and Jansa, 2016).

This lack of relationship between infectivity and AMF efficiency in improving crop growth may be related to the time required for the establishment of root colonization (Abbott and Robson, 1981). Under long-term, arbuscular mycorrhiza promotes more benefits to the plant host (Ortas, 2012), mainly by increasing the acquisition of water and minerals to the plant. The major function of AMF is suggested to be nutrient acquisition (Zhang *et al.,* 2019) and thus, under high nutrient conditions, AMF can shift from a net benefit to a cost for the host (Johnson *et al.,* 2015). In our study, the nutrients supplied during cultivation were not limited to goldenberry; therefore, the inoculation effect may not have been potentiated in terms of fruit yield.

However, we proved the benefit of mycorrhization on the chemical quality of the fruits through the increase in the sugar content (Fig. 4A) and the better berry flavor (Fig. 4B), as already reported for zucchini (*Cucurbita pepo* L.) (Rouphael *et al.,* 2015), strawberry (Costa *et al.,* 2020) and tomato (*Solanum lycopersicum* L.) (Sellitto *et al.,* 2019). Plants grown with AMF showed a more developed root system (Fig. 2), which allows extrarradicial hyphae to extend beyond the rhizosphere, making water acquisition more efficient (Xu *et al.,* 2017). Due to greater water availability, these plants have greater stomatal opening, which increases the rate of transpiration and, thus, there is a greater supply of carbon dioxide for photosynthesis (Vicente-Sánchez *et al.,* 2014). As a result, there is a greater production of sugars, which are the primary source of photosynthesis, and this explains the increase in the fruit sugar content and, consequently, the best flavor of the berries (Fig. 4).

The lack of effect of *G. intraradices* on berries quality can be attributed to the low effectiveness of this fungal species on goldenberry. Various factors modulate the arbuscular mycorrhiza effect on the performance of their associated plants and this includes the traits of the host and the fungi themselves (Chiomento *et al.,* 2019 a). Cultivated plants vary in their responsiveness to AMF due to their morphology (Chiomento *et al.,* 2019 b) and AMF differ in the benefits provided to the plant (Werner and Kiers, 2015).

The two genera that made up the monospecific inoculants, *Glomus* and *Rhizophagus*, have already been reported in studies of AMF diversity in goldenberry (Ramírez-Gómez *et al.,* 2019) and have also been used in applicability studies (*Rhizophagus*) in this horticultural crop (Miranda *et al.,* 2011). However, in our study, the mycorrhizal community, representing a multispecific inoculant, stood out in relation to the fungal isolates for generally improving the horticultural performance of goldenberry, mainly by increasing the amount of fine roots produced by the plants (Fig. 2C) and for benefiting fruit quality (Fig. 4). Inoculation with AMF populations, such as the fungal community used in this study, generally provides more satisfactory results due to greater compatibilities at the fungus-host interface and by increasing mutualistic effects with two or more symbionts instead of just one (Chiomento *et al.,* 2019 b). This mycorrhizal community was obtained through the trap culture technique, that is, produced *on-farm*.

A mycorrhizal inoculant rich in propagules and produced *on-farm* may be a suitable solution for large-scale inoculation of crops (agroecosystems), seedlings (nurseries) and in potting media for vegetable growers (soilless cultivation) (Douds Junior *et al.,* 2006). The use of non-sterilized growth medium (soil and/or substrate) as a component of the on-farm inoculant represents a source of propagules for other AMF present in this growth medium, which results in an inoculant with greater taxonomic diversity (Schlemper and Stürmer, 2014). This is strongly desired due to the functional diversity exhibited by mycorrhizal species for the promotion of plant growth, for example (Chiomento *et al.,* 2020 b). Furthermore, a diversified inoculant potentiates a combination between fungal isolates and an eventual host (Douds Junior *et al.,* 2006).

Therefore, the results of our research confirmed the potential of applying mycorrhizal biotechnology to goldenberry, as the work demonstrated that AMF can be a valuable tool for the cultivation of this vegetable. The complex roles of AMF in agroecosystems are just beginning to be understood (Chiomento *et al.,* 2022). In this way, a greater understanding of the application and benefits of AMF can enable their use in the sustainable production of vegetables (Trentin *et al.,* 2022).

We conclude that in the first production cycle, there is no effect of mycorrhization on the total number of fruits and total production of berries. We believe that for the goldenberry cultivation the fungal species used require a period of more than one year to benefit the fruit yield. On the other hand, goldenberry plants submitted to mycorrhizal biotechnology have a more profuse root system and produce fruits with better chemical quality. *G. intraradices* is most effective in colonizing the plant host roots. However, the mycorrhizal community stands out in relation to the fungal isolates for generally improving the horticultural performance of goldenberry. Thus, the application of this biotechnological tool in the goldenberry culture can be an alternative to spread and promote its sustainable cultivation.

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