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Arbuscular mycorrhiza potentiates the quality of fruits but does not influence the precocity of goldenberry plants

A micorriza arbuscular potencializa a qualidade de frutos mas não influencia a precocidade de plantas de fisális

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ABSTRACT
The alternative to minimize the impacts to the agroecosystem and to establish a sustainable management for the goldenberry cultivation (*Physalis peruviana* L.) corresponds to the use of inoculants based on arbuscular mycorrhizal fungi (AMF). However, this biotechnological tool is lacking and unknown to producers. The objective of the research was to investigate whether goldenberry plants in the absence and presence of inoculation with AMF differ in their horticultural potential. The treatments were absence of inoculation (control) and three mycorrhizal inoculants, arranged in a randomized block design, with five replications. The phyllochron, mycorrhizal colonization and fruit quality were evaluated. Non-mycorrhized plants had a higher leaf appearance rate and, therefore, a lower phyllochron value. Mycorrhizal colonization was greater in roots of plants produced with *Glomus intraradices*. Less acid and more tasty fruits were produced by plants inoculated with AMF, regardless of the fungal treatment used. In conclusion, goldenberry plants in the absence and presence of inoculation with AMF have different horticultural potential. Plants devoid of arbuscular mycorrhiza are earlier to start flowering. The fungal species *G. intraradices* is more effective in colonizing the roots of the plant host. Plants submitted to mycorrhizal biotechnology potentiate the chemical quality of berries.

Keywords: *Physalis peruviana* L., arbuscular mycorrhizal fungi, phyllochron, flavor.

RESUMO
A alternativa para minimizar os impactos ao agroecossistema e estabelecer um manejo sustentável para a cultura do fisális (*Physalis peruviana* L.) corresponde ao uso de inoculantes à base de fungos micorrízicos arbusculares (FMA). Porém, essa ferramenta biotecnológica é carente e desconhecida aos produtores. O objetivo da pesquisa foi investigar se plantas de fisális na ausência e presença de inoculação com FMA diferem quanto ao seu potencial hortícola. Os tratamentos foram ausência de inoculação (testemunha) e três inoculantes micorrízicos, dispostos no delineamento em blocos casualizados, com cinco repetições. Foram avaliados o filocrono, a colonização micorrízica e a qualidade de frutos. Plantas não micorrizadas tiveram maior taxa de emissão de folhas e, assim, menor valor de filocrono. A colonização micorrízica foi maior em raízes das plantas produzidas com *Glomus intraradices*. Frutos menos ácidos e mais saborosos foram produzidos por plantas inoculadas com FMA, independente do tratamento fúngico usado. Em conclusão, plantas de fisális na ausência e presença de inoculação com FMA têm desempenho hortícola distinto. Plantas desprovidas da micorriza arbuscular são mais precoces para iniciar a floração. A espécie fúngica *G. intraradices* é mais eficaz em colonizar as raízes do hospedeiro vegetal. Plantas submetidas à biotecnologia micorrízica potencializam a qualidade química das bagas.

Palavras-chave: *Physalis peruviana* L., fungos micorrízicos arbusculares, filocrono, sabor.

1 INTRODUCTION

Goldenberry (*Physalis peruviana* L.) is a horticultural crop widespread in the international market mainly for its nutritional value (ETZBACH et al., 2018). The worldwide interest in the consumption of goldenberry has increased during the last decade due to its organoleptic properties, nutritional composition and bioactive compounds that provide health benefits and reduce the risks

of diseases such as cancer, hepatitis and rheumatism (OLIVARES-TENORIO et al., 2016). These aspects represent an emerging market of increasing economic importance (TORRES-OSSANDÓN et al., 2018).

Due to this growing market, studies are needed to develop more profitable cultivation technologies, since the lack of information on the horticultural performance of goldenberry is one of the factors that limit the expansion of the crop. Still, considering that the traditional cultivation of other Solanaceae requires great use of biocides and chemical fertilizers, such as tomatoes and peppers, this can cause the contamination of the agroecosystem. The alternative to minimize these obstacles and establish a sustainable management for the goldenberry cultivation corresponds to the use of inoculants based on arbuscular mycorrhizal fungi (AMF). However, this biotechnological tool is lacking and unknown to producers. In Brazil, this occurs mainly due to the high cost involved in the registration of mycorrhizal inoculants (SILVA et al., 2020).

AMF have a biological role in the functioning and stability of ecosystems, providing better initial plant establishment (GOETTEN et al., 2016), greater protection against pathogens (JUNG et al., 2012) and greater nutrient acquisition (GÓMEZ-BELLOT et al., 2015). Few studies have investigated the use of AMF in the goldenberry cultivation. In this crop, arbuscular mycorrhiza has been shown to increase berry growth rates under saline conditions (MIRANDA et al., 2011) and to increase the concentration of unsaturated fatty acids in fruits in response to heavy metal stress (HRISTOZKOVA et al., 2017). Still, the mycorrhization of goldenberry under water stress promoted beneficial effects on the accumulation of dry root matter and improved attributes related to the gas exchange of plants (REYES et al., 2019). However, further studies are needed to fill the gaps in relation to the horticultural potential of goldenberry submitted to mycorrhizal biotechnology.

Therefore, based on the hypothesis that the use of AMF in the growing substrate increases the precocity of plants and improves the quality of goldenberry fruits, here we investigate whether goldenberry plants in the absence and presence of inoculation with AMF differ in their horticultural potential. This study provides a view of phyllochron, mycorrhizal colonization and chemical quality of berries of goldenberry plants produced on substrate enriched with mycorrhizae, in a greenhouse.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND EXPERIMENT LOCATION

The research was carried out at the University of Passo Fundo (28º 15’ 46” S, 52º 24’ 24” W), Rio Grande do Sul (RS), Brazil, in a greenhouse, from August (winter) 2019 to April (autumn) 2020.

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A commercial tray with goldenberry fruits, at maturation stage 5 (ICONTEC, 1998), was acquired. In August 2019, seeds of three fruits chosen at random were selected, transferred to paper towels and kept at room temperature (25ºC±1ºC) until they were dry. Afterwards, these seeds were germinated in gerbox plastic boxes containing blotting paper and potassium nitrate (KNO_3) solution 0.1 molar (M). The boxes were stored in a biochemical oxygen demand (BOD) oven, at 25ºC±1ºC, until the plants were obtained for the seedling production (plant material).

2.2 EXPERIMENTAL DESIGN

The treatments used in this study were absence of inoculation (control) and three inoculants of AMF [*Glomus intraradices* N.C. Schenck & G.S. Sm., *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler and mycorrhizal community (Table 1)], arranged in a randomized block design, with five replications. Each plot consisted of three goldenberry plants.

The AMF community used came from the trap-cultivation of agricultural soil collected at reference-site in strawberry cultivation in the municipality of São José do Hortêncio (29º 29' 33" S, 51º 12' 24" W), RS (CHIOMENTO et al., 2019), composed of the ten fungal species shown in Table 1. The isolate *G. intraradices* came from the commercial product MYKE® PRO and the isolate *R. clarus* was obtained from the International Glomeromycota Culture Collection (CICG).

Table 1. AMF community identified from agricultural soil collected in São José do Hortêncio, RS.

Municipality	AMF community[1]
São José do Hortêncio	*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

Source: Chiomento et al. (2019).
[1]Glomeromycota classification by Redecker et al. (2013).

2.3 CULTIVATION TECHNIQUES

We chose to apply the treatments (AMF) in two stages: 1) in seedling acclimatization; 2) when transplanting to the place of cultivation. Thus, of the total amount of mycorrhizal inoculant used (10 g), 1/2 was applied to acclimatize the seedlings and 1/2 at the time of transplanting into pots.

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In September (spring) 2019, thirty days after seed germination, the obtained plants were acclimatized in 72-cell polystyrene trays (100 $cm^3.cell^{-1}$), filled with sterilized (120ºC for 20 minutes) commercial substrate Horta 2® and with treatments related to mycorrhization (1/2 of the total amount = 5 g), with the purpose of seedling production. Horta 2® is composed of pine bark, vermiculite, acidity correctives and fertilizers (nitrogen, phosphorus and potassium) in quantities not provided by the manufacturer. A sample of 500 g of substrate was analyzed to obtain its physical and chemical attributes (Table 2).

Table 2. Physical and chemical characterization of Horta 2®.

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

Source: author's data.
[1]D: density; TP = total porosity; AS: aeration space; RAW: readily available water; BW: buffer water; RW: remaining water.
[2]N: nitrogen; P_2O_5: phosphorus pentoxide; K_2O: potassium oxide; OC: organic carbon; pH: potential of hydrogen; EC: electric conductivity; CEC: cation exchange capacity.

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

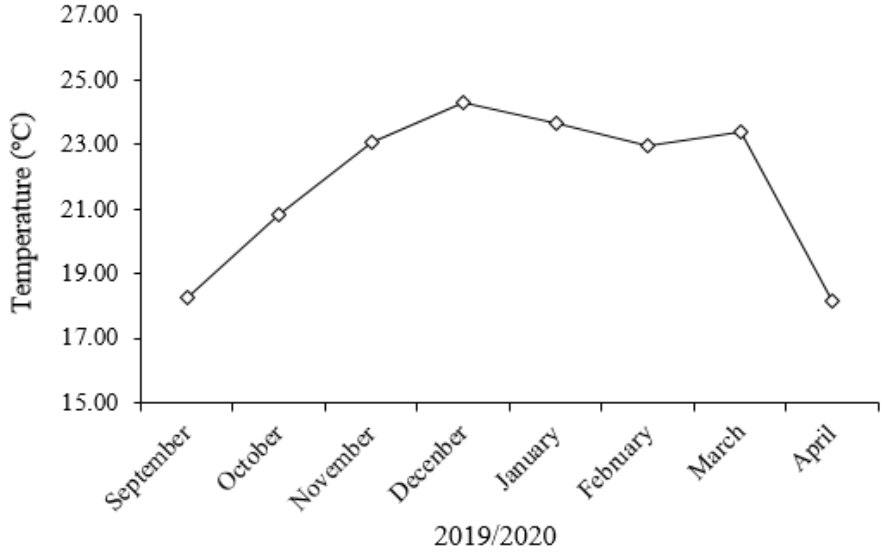
In November (spring) 2019, after two months of acclimatization, seedlings were transplanted into pots (3.6 L), filled with sterilized (120ºC for 20 minutes) commercial substrate Horta 2® and with the treatments related to mycorrhization (another 1/2 of the total amount = 5 g).

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 is covered with low density polyethylene film, with anti-UV additive and 150 microns thick. The irrigation used in the experiment was located, through drip rods, in the mechanized system, with a flow rate of 2.4 $L.h^{-1}$ per unit. The irrigation regime consisted in the activation of the dripping rods six times a day,

with a total wetness of six minutes. Nutritive solution provided to the plants, on a monthly basis, was as described by Furlani & Fernandes Júnior (2004).

During the execution of experiment, using a meteorological mini-station, the average air temperature inside the greenhouses was monitored (Figure 1). The period from September to October (spring) 2019 corresponded to the seedling acclimatization and the period from November 2019 to April 2020 covered the productive cycle of goldenberry.

Figure 1. Average monthly temperature during the experiment (September 2019 to April 2020). The general average temperature recorded during the experiment was 21.83ºC.



Plants were conducted with three stems and were tutored with wires. No biocides were used during the culture cycle. The average fruit production was 205 g.plant^{-1}.

2.4 PHYLLOCHRON

Phyllochron was evaluated by counting the number of leaves after transplantation (November 2019) until the appearance of the first flower bud [January (summer) 2020] in the different treatments studied. A new leaf was considered to be emitted when visible, approximately 1 cm long.

For this, the average daily temperature (ADT) was evaluated according to the following equation, which calculates the arithmetic mean of the temperatures recorded by the meteorological mini-station every hour:

$$\text{ADT (ºC)} = \frac{(t0 + t1 + t2 + \ldots + t24)}{24} \quad (1)$$

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Then, the daily thermal sum (DTS) was calculated according to Gilmore Junior & Rogers (1958) and Arnold (1960):

$$DTS\ (^{o}C.day^{-1}) = ADT - BT \quad (2)$$

The base temperature (BT) is defined as the minimum temperature below which no leaves appear. The BT for goldenberry cultivation was considered as 6.3ºC (BETEMPS et al., 2014). DTS has been accumulated since the seedling transplant, resulting in the accumulated thermal sum (ATS), that is, ATS ($^{o}C.day^{-1}$) = Σ DTS.

Finally, a regression analysis was performed between the number of leaves and the ATS. The angular coefficient of the linear regression is considered the leaf appearance rate (leaves $^{o}C.day^{-1}$) and the phyllochron ($^{o}C\ day.leaf^{-1}$) was estimated by the inverse of the angular coefficient of the linear regression (STRECK et al., 2007).

2.5 MYCORRHIZAL COLONIZATION

To verify the infective capacity of AMF, at the end of the experiment (April 2020) root portions of mycorrhized plants were prepared according to Phillips & Hayman (1970) and their mycorrhizal colonization percentage (MCP, %) were determined according to Trouvelot et al. (1986), by the equation:

$$MCP\ (\%) = \frac{\text{total number of fragments with mycorrhizal roots}}{\text{total number of fragments}} \times 100 \quad (3)$$

2.6 CHEMICAL QUALITY OF FRUITS

The analysis of fruit quality was also done at the end of the experiment. The chemical characteristics of the fruits were evaluated regarding the content of total soluble solids (TSS), expressed in ºBrix, and total titratable acidity (TTA), expressed in % of citric acid, from 20 fruits of each treatment for each repetition. The TSS content was determined using an analog refractometer, while the TTA was performed according to the standards of the Adolfo Lutz Institute (IAL, 2008). To evaluate the fruit flavor, TSS/TTA ratio was determined.

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2.7 STATISTICAL ANALYSIS

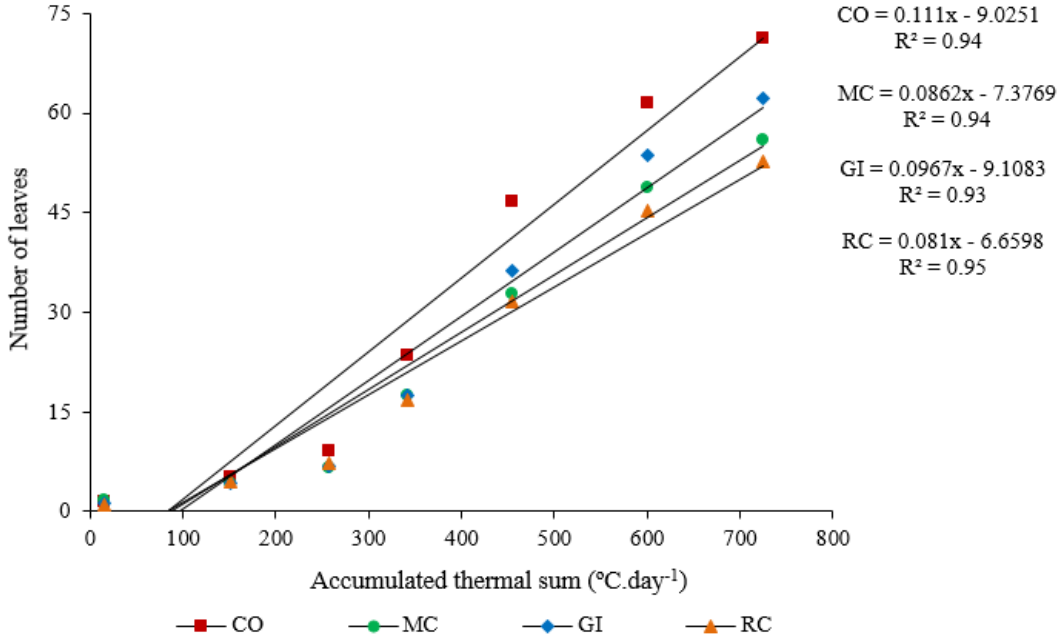
To estimate the phyllochron, linear regression was performed between the number of leaves and the ATS. The phyllochron was estimated as the inverse of the angular coefficient of the linear regression. The other data obtained were subjected to analysis of variance and the treatment means were compared using the Tukey test, at 5% probability of error, with Costat® program.

3 RESULTS

3.1 PHYLLOCHRON

In decreasing order, the leaf appearance rate was 0.111, 0.096, 0.086 and 0.081 accumulated leaves every ºC.day^{-1} for treatments related to the control (CO), *G. intraradices* (GI), mycorrhizal community (MC) and *R. clarus* (RC), respectively (Figure 2), with phyllochron of 9.01, 10.34, 11.60 and 12.35ºC day.leaf^{-1}, in the same order of treatments, to emit two consecutive leaves (Figure 2). Thus, plants grown without AMF were considered the earliest to start flowering, as they had the lowest phyllochron value, while plants grown with AMF were the latest, as they had the highest phyllochron values (Figure 2).

Figure 2. Phyllochron (ºC day.leaf^{-1}) of goldenberry plants in the absence and presence of inoculation with AMF.



Source: author's data.

[1]CO: control (non-mycorrhized plants); MC: mycorrhizal community (Table 1); GI: *G. intraradices*; RC: *R. clarus*.

3.2 MYCORRHIZAL COLONIZATION

Among the fungal treatments evaluated, it was observed that *G. intraradices* showed greater capacity to infect the roots of plants in relation to the mycorrhizal community and the fungal species

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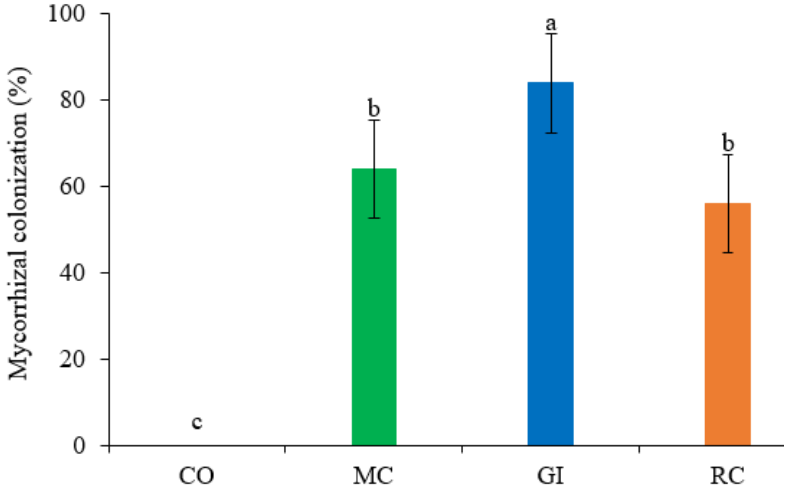
R. clarus (Figure 3). The fungal structures observed inside the roots were hyphae, mostly, vesicles and arbuscules.

Figure 3. Mycorrhizal colonization of roots of goldenberry plants in the absence and presence of inoculation with AMF.



Source: author's data.
Data presented as mean ± standard deviation. Different letters on the columns indicate difference by the Tukey's test ($p \leq 0.05$). [1]CO: control (non-mycorrhized plants); MC: mycorrhizal community (Table 1); GI: *G. intraradices*; RC: *R. clarus*.

3.3 CHEMICAL QUALITY OF FRUITS

Less acidic (Figure 4A) and tastier (Figure 4B) fruits were produced by plants inoculated with AMF. These berries showed mean values lower by 45% for TTA and higher by 57% for TSS/TTA when compared to berries produced by non-mycorrhized plants.

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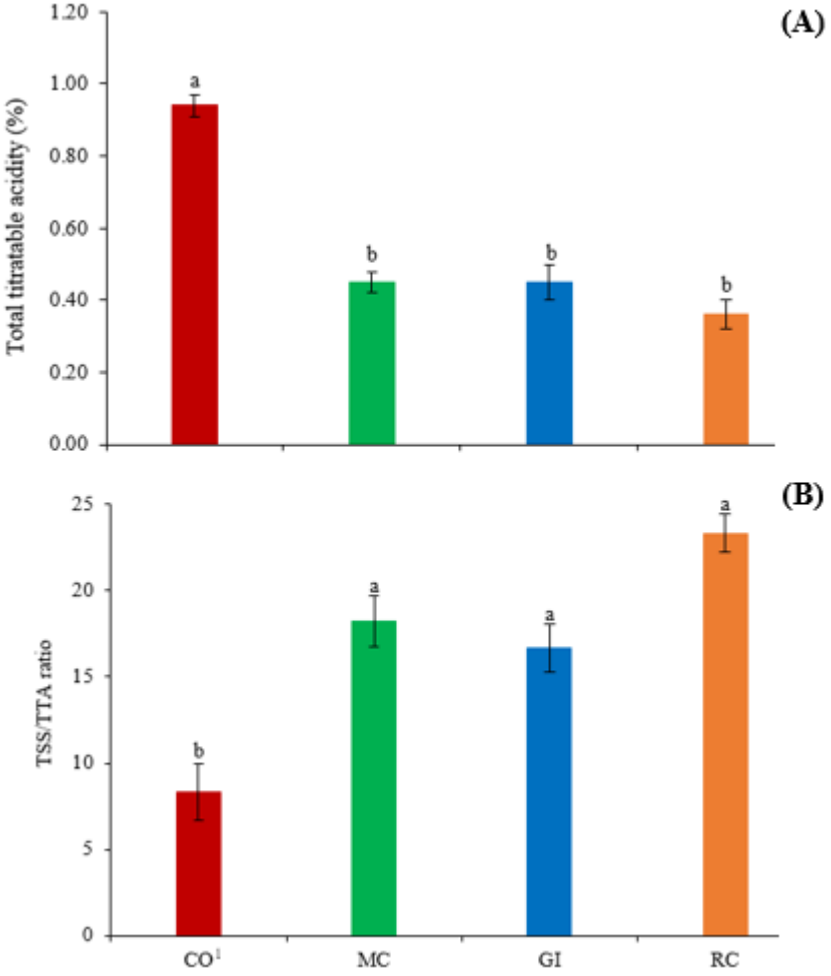
Figure 4. Chemical quality of fruits of goldenberry plants in the presence and absence of inoculation with AMF. (A) Citric acid content (%). (B) Fruit flavor.



Source: author's data.

Data presented as mean ± standard deviation. Different letters on the columns indicate difference by the Tukey's test ($p \leq 0.05$). [1]CO: control (non-mycorrhized plants); MC: mycorrhizal community (Table 1); GI: *G. intraradices*; RC: *R. clarus*.

4 DISCUSSION

Here, we show that the horticultural potential of goldenberry differed when the plants were grown in the absence and presence of inoculation with AMF. By the phyllochron, it was found that plants without arbuscular mycorrhiza were earlier to start flowering (Figure 2). However, less acidic (Figure 4A) and tastier (Figure 4B) fruits were produced by mycorrhized plants. The fungal species *G. intraradices* was more effective in colonizing the roots of plant host (Figure 3). However, analyzing the fungal treatments, this did not reflect in better fruit flavor more efficiently when the plants were inoculated with *G. intraradices* (Figure 4B). Although mycorrhizal colonization is important, the percentage of root infectivity is not always correlated with the efficiency of symbiosis (KONVALINKOVÁ & JANSA, 2016).

Information related to the phyllochron is little known, despite its importance for the models of growth and development of plants, whose responses are able to support crop management practices, sowing dates and more suitable regions for cultivation (DALMAGO et al., 2013). High phyllochron values in the plants indicate that the leaf appearance rate is lower, as the plant requires a greater number of degree-days between the appearance of two successive leaves. However, it is important that the leaf appearance rate is higher and, consequently, lower phyllochron, during the vegetative stage, to increase the number of leaves before flowering occurs, ensuring an increase in the number of fruits (ROSA et al., 2011).

In this study, it was found that non-mycorrhized plants had a higher leaf appearance rate (0.111 leaves $^{o}C.day^{-1}$) and a lower phyllochron (9.01^{o}C day.$leaf^{-1}$) and, thus, were shown to be earlier in relation to flowering (Figure 2). On the other hand, mycorrhized plants, regardless of fungal treatment, were later in relation to flowering (Figure 2). This is because the fungal symbiont demands carbohydrates from the plants for their maintenance (GARCIA et al., 2016) and, therefore, the photoassimilated partition occurs between the plant organs and includes AMF. Thus, mycorrhized plants devote less energy to the emission of leaves and, consequently, have a greater phyllochron (Figure 2). In the case of non-mycorrhized plants, the photoassimilated partition occurs only between the different organs of the plants, resulting in a greater energy balance for emission of leaves and, thus, lower phyllochron (Figure 2).

According to what was expected, the benefit of mycorrhization in the chemical quality of the fruits was proven by decreasing the acidity content (Figure 4A) and better berry flavor (Figure 4B), as already reported for zucchini (*Cucurbita pepo* L.) (ROUPHAEL et al., 2015), strawberry (*Fragaria* X *ananassa* Duch.) (SINCLAIR et al., 2014) and tomato (*Solanum lycopersicum* L.) (SELLITTO et al., 2019). The fungal infection observed in the goldenberry roots (Figure 3) may have caused stress to the plants. As a defense response to colonization, the plant host can increase the activity of its secondary metabolism and thus produce more phytochemicals (LINGUA et al., 2013). These secondary metabolites, depending on the environmental conditions to which they are exposed [temperatures around 20^{o}C, for example, what was observed in this study (Figure 1)] release sugar due to its degradation (ISLAM et al., 2005) and this explains the better taste of berries (Figure 4B).

Goldenberry fruits have high levels of vitamin A, B and C (PUENTE et al., 2011) and contain macro (calcium and phosphorus) and micronutrients (iron and zinc), compounds that are necessary for the normal functioning of the body (RAMADAN, 2011). Goldenberry fruits also have levels of phenolic and carotenoid compounds (SEVERO et al., 2010), unsaturated fatty acids and

phytosterols (VALENZUELA & RONCO, 2004). The juice has a hydrogen potential (pH) approximately between 3.6 to 4.1, a range that favors the stabilization of ascorbic acid in the fruit in relation to oxidation processes, heat treatments and radiation exposure, which allows a prolongation of the presence of vitamin C during consumption. Still, the berries are rich in sugars (11 to 20 g of carbohydrates in 100 g of fresh mass) and when ripe they contain between 13 to 15ºBrix (HERRERA, 2000).

Our results confirmed the potential of applying mycorrhizal biotechnology to the goldenberry cultivations. The findings of this study may be useful to goldenberry producers who want to insert AMF into the agroecosystem as a tool to improve the quality of the berries produced, in line with environmental sustainability. The complex functions of AMF in ecosystems are just beginning to be understood (GARLAND & SCHROEDER-MORENO, 2011). Thus, a greater understanding of the application and benefits of the AMF can enable its use in the sustainable production of horticultural crops (ROBINSON-BOYER et al., 2016). Finally, these investigations are filling the gap between AMF engineering related to the phyllochron and the quality of berries produced by goldenberry plants.

5 CONCLUSION

It is concluded that goldenberry plants in the absence and presence of inoculation with AMF have different horticultural potential. Through the analysis of the phyllochron, it appears that goldenberry plants devoid of arbuscular mycorrhiza are earlier to start flowering. However, plants subjected to mycorrhizal biotechnology produce less acidic and tastier berries. The fungal species *G. intraradices* is more effective in colonizing the roots of the plant host. The application of this biotechnological tool in goldenberry can be an alternative to spread and promote the sustainable cultivation of this horticultural crop.

DECLARATION OF INTEREST

We declare that the AMF used in this study are regulated by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) of the Ministry of the Environment, Brazil, according to the registration number A198F50.

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