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Inoculation of Arbuscular Mycorrhizal Fungi Isolated from Peach Orchards and Vineyards in Aldrighi Peach Rootstock Plants

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Abstract

Arbuscular mycorrhizal fungi species (AMFs) present intraspecific differences in the ability to stimulate plant growth and depend on genetic and environmental factors involved among the biotic agents. This work aimed to evaluate the influence of the AMFs *Glomus clarum* and *G. etunicatum* isolated from peach orchards and vineyard on the vegetative growth, content of nutrients and carbohidrate contents on Aldrighi peach rootstocks plants. The experiment was conducted in a greenhouse, with a split-plot experiment, with 15 plants per plots and four repetitions. The plants inoculated with AMFs had higher responses in height, diameter, leaf area, fresh and dry biomass of shoots and content of reserves, due to the increased absorption of nitrogen, phosphorus and potassium in excess of non-inoculated plants. Data were submitted to analysis of variance using the Statistical Analysis System (SAS) program and the means were compared using Duncan's test at a 5% significance level. Simple correlation analyzes were performed using Pearson's correlation coefficient (r) with maximum values represented by r = 1 and r = -1. All AMF isolates benefited the Aldrighi peach rootstock plants, accelerating vegetative development and increasing macronutrient content. The study showed a correlation between root colonization and the increase in plant development parameters. The symbiosis efficiency was higher with isolates from peach orchards, when compared with isolates from grapevine orchards.

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Introduction

The efficiency of AMFs is represented by their ability to stimulate plant growth and depends on genetics[1] and edaphoclimatic factors involving both.[2,3] Differences between AMFs have been reported in promoting the growth and development of plant species.[1] Thus, a cultivar may have low susceptibility to a particular AMFs and an excellent

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response when inoculated with another species.[4] This response can be interpreted as specificity[4] or functional compatibility between the host and the fungus.[5]

This functional specificity is related to the role that plant hormones, especially abscisic acid (ABA), play during the establishment of symbiosis. ABA contributes to the susceptibility or not of the plant species to AMF infection, due to its important role, related to the development of a complete and functional arbuscle.[6]

The increased absorption of nutrients and the balance of the nutritional status of plants promoted by the action of AMFs can influence plant growth, in addition to promoting increased tolerance to abiotic stresses.[7] Thus, if, on the one hand, the hyphae and the external mycelium increase the soil's ability to exploit the soil, promoting greater absorption of nutrients,[8] on the other hand, they favor it in the water-plant relationship, allowing greater resistance of plants to conditions of water deficit, as a result of a series of changes that occur in its physiology.[9]

The dynamics of AMFs populations present in an area is essential to understand their influence on the conditions of establishment, development and maintenance of a plant population in this location.[10] In addition, the diversity, density and infectivity potential of AMF propagules in the soil are indirectly related to the climatic and edaphic conditions of each ecosystem[11,5] and directly to the fungus physiology[12,13,4] with mycorrhizal colonization being zica linked to the genotype of the plant and the fungus, as well as to the environment. In this aspect, the autochthonous AMFs of a given region, which are already adapted to the edaphoclimatic conditions of the same, tend to confer greater response potential when inoculated into plants to be cultivated in that region.[8] Thus, the correct interpretation of the local dynamics of autochthonous AMFs in an area is fundamental, which are already adapted to the soil and climate conditions of the same, tend to confer a greater response potential when inoculated in plants to be cultivated in this region.[9,14]

The objective of this work was to evaluate the efficiency of isolates of the AMFs *Glomus clarum* and *G. etunicatum* from Central Depression peach orchards and Mountain range Gaucha vineyards, on vegetative growth, content of macronutrients and reserve substances of Aldrighi peach rootstocks plants.

Materials e Methods

The experiment was carried out in a shade screen (50% shading) located at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), county of Eldorado do Sul, Rio Grande do Sul state, Brazil, located at latitude 30°05' south and longitude 51°39' west, over a period of three hundred thirty (330) days.

Aldrighi peach Rootstock seeds were stratified in recipients (40 cm X 28 cm X 10 cm plastic box) containing sand and placed, for a period of forty-five (45) days, in a refrigerator at 4ºC, in order to interrupt the embryo dormancy and facilitate the germination. The sand was previously autoclaved at 120°C for one hour. All seeds came from the same harvest and were left to dry in a sterile environment and forced air for 40 days after harvest. The average size of the lumps was 5 to 7 cm at the greatest length. After the stratification period, the seeds were sown in plastic boxes (40 cm X 28 cm X 10 cm), filled with autoclaved sand and kept in a greenhouse equipped with a sprinkler irrigation system. For the first sixty (60) days, the irrigation shift interval was twenty-four (24) hours at the beginning of the day. In the warmer months (November, December, January and February), the number of shifts was increased to two, twelve (12) hours apart, taken at the beginning and end of the day, returning to the twenty-four (24) hour shift.) hours in the following months until the end of the experiment (August).

When the seedlings were about five (5) cm high from the shoot, they were pricked into black plastic bags of five (5) liters, containing a substrate consisting of clayey soil of a typical dystrophic Red Argisol soil15, sand (average granulometry, between 0.6 and 1 mm) and decomposed residue of black wattle bark (1:1:1, V:VV). The substrate was previously disinfected with a 10% formaldehyde solution.

Immediately before subculture, the AMF inoculum was added to the substrate containing thirty (30) grams per plastic bag, ten (10) spores per gram of inoculum), in a layer located at the intermediate height of each container of approximately fifteen (15)

cm high. The AMFs tested were *G. clarum* (Nicol. & Schenck) and *G. etunicatum* (Becker & Gerd), whose spores were isolated from soil samples taken from Gaúcha mountain range vineyards and peach orchards in the Central Depression region, through the method of washing, decanting, sieving[16] and centrifugation.[17] The morphological identification of the species was carried out through observation under an optical microscope.[18] The multiplication of the species was carried out by monosporic cultivation, using the plant species oregano (*Origanum vulgare* Link), for the isolates from vineyards, and brachiaria (Brachiaria decumbens Stapf.), for the isolates from orchards peach. The experimental design used was randomized blocks in a split-plot scheme (the origin of the AMF constituted the main plot and the species, the sub-plot), with fifteen (15) plants per plot and four replications.

At three hundred thirty (330) days after sowing, the height of the plants was evaluated, from the stem to the apex of the main stem, and the diameter of the main stem, at the height of the stem of the plants. For this purpose, a tape measure and a caliper from the RS Baty brand were used. In addition, five (5) plants from each repetition of the treatments were used to determine the leaf area, through the use of a Li-Cor brand leaf area meter (model LI - 3000), and the fresh and dry biomass of the aerial part, by weighing and drying to constant weight. The dried samples were ground and fractions were removed from them to evaluate the macronutrient content by digestion, distillation and flame spectrophotometry of the plant tissue.[19]

From the ground samples, sub-samples of one gram each were collected, which were placed in a bag made with a special mesh for filtering food, and the weights of each bag were recorded before and after the digestion process in an aqueous solution with 5% trichloroacetic acid. (99%) and 35% methanol (99%), aiming at extracting all components of the plant tissue (carbohydrates, fats, etc.) other than fibers (cellulose, hemicellulose and lignin), as agreed as reserve substances.[20] In addition, secondary root segments, measuring one (1) cm, were collected to determine root colonization (number of infected segments.total-1 analyzed) and hyphae presence indices (0: absence; 1: weak; 2: moderate; 3: intense), vesicles and arbuscules (0: absence; 1: 1 to 50; 2: 51 to 100; 3: more than 100 structures. cm-1 of radicella).[21]

Data were submitted to analysis of variance using the Statistical Analysis System (SAS) program and the means were compared using Duncan's test at a 5% significance level. Growth parameters (height, diameter, leaf area, fresh and dry biomass), mineral nutrition (% macronutrients) and reserve substances (%) of leaves and simple stems were correlated with root colonization (%) using the correlation coefficient of Pearson (r) with maximum values represented by r = 1 and r = -1.

Results and Discussion

The AMFs accelerated the growth of Aldrighi peach Rootstock plants, inducing greater height, diameter, leaf area, fresh and dry biomass of shoot tissues, when compared with the control (Table 1).

Table 1: Height (H), diameter (D), leaf area (LA), fresh (F) and dry (S) biomass of leaves and stems of Aldrighi peach rootstocks plants inoculated with isolates of two AMFs (G. clarum and G.etunicatum), from orchards peach (OP) and vineyards (V), measured at three hundred thirty (330) days after sowing. Eldorado do Sul, RS.

Origin	Treatment	H	D	LA	F	S
		cm	mm	cm^2. $plant^{-1}$	g	g
OP	*G. clarum*	135.00b(1)	6.50b	653.01b	120.00b	51.00b
	G. etunicatum	158.20a	7.03a	796.90a	139.00a	62.00a
V	*G. clarum*	120.80d	6.04d	577.27d	114.00c	49.00c
	G. etunicatum	125.36c	6.22c	624.99c	115.00c	49.00c

-	Control	109.85e	5.86e	530.71e	105.00d	42.00d
C.V. (%)		7.98	7.77	7.58	7.44	6.52

(1)Means followed by distinct letters in the column differ from each other by the Duncan test ($p \leq 0,05$). Coefficient of Variation (C.V.).

The *G. etunicatum* isolate from peach orchards provided the greatest increases in all parameters of vegetative development, while the *G. clarum* isolate from the same origin promoted higher gains than the *G. clarum* and *G. etunicatum* isolates from the vineyards. The isolate *G. etunicatum* from vineyards provided plants with increments of growth parameters higher than those of *G. clarum* from the same origin, with the exception of biomass, which was similar to each other.

The fact that arbuscular mycorrhizal fungi (AMF) cause differentiated vegetative development is associated with the greater or lesser affinity that each AMFs has with the plant species used, which would be related to the existence of a functional compatibility between the host and the fungus.[12] However, the results obtained showed that the isolates of both species from the vineyards showed functional compatibility with the cultivar similar to each other, while the isolate of *G. etunicatum* from peach orchards showed greater affinity with Aldrighi peach rootstocks, showing higher results than the *G. clarum* isolate from the same origin.

The increase in growth provided by AMF may vary depending on the species used and the isolate of the same AMFs.[22] This is because the AMF native to a given environment may be more adapted to prevailing conditions, being more effective than introduced species, which coincides with the results obtained in this study. According to Chu,[23] açaí plants inoculated with the species Scutellospora gilmorei from açaí orchards, observed increases in height of 92% and 116% in diameter, when compared to the controls, also showing higher growth than plants inoculated with the same AMFs isolated from cropland.

Several authors[24,2] report that leaf area is an important parameter of vegetative development, as it defines the rate of photosynthesis performed in the plant. The plant-AMF symbiosis provides an increase in leaf area in relation to non-colonized plants[26,25,27] which may explain the observed results. Associated with the increase in leaf area, AMF colonization would also provide greater fresh biomass in inoculated plants than in non-inoculated plants, due to a higher water content, although it may show similar dry biomass.[12,28,10] However, the AMF provided better results for dry biomass than the controls, which can be attributed to the greater leaf area, which provided greater production of photo as similates and accumulation of dry biomass.

Several authors[29,27] report that sweet passion fruit seedlings inoculated with isolates of AMFs native to passion fruit orchards showed a greater increase in leaf area and greater fresh and dry biomass than isolates from the same non-native AMFs, which coincides with the data obtained in this work, since the isolates recovered from peach orchards showed greater increases in leaf area and fresh and dry biomass than the isolates of the same species recovered from soil samples from vineyards.

The plants inoculated with the AMF isolates showed higher percentages of macronutrients in the plant tissues of the shoot, with the exception of calcium (Ca) and magnesium (Mg), where all AMFs provided lower results than the control plants (Table 2).

The isolate of *G. etunicatum* from orchards peach provided the inoculated plants with the highest percentages of nitrogen (N), phosphorus (P) and potassium (K), while *G. clarum* from the same origin was superior and presented superior results to the isolates from vineyards. The isolate of *G. etunicatum* from vineyards provided the highest percentages of these macronutrients in relation to the isolate of *G. clarum* from the same source. According to several authors,[30,8] AMF induce a greater absorption of these elements, being of vital importance for plants. Through mechanisms promoted by the AMF, the hyphae and the external mycelium increase the soil's ability to exploit the root, which provides greater absorption of nutrients.[31,10]

The efficiency of hyphae is due to their small diameter and large branching in the soil, which can increase the absorption surface of the roots by up to 700%.[32] Regarding the macronutrients calcium and magnesium, some authors[6] state that they are vital for the development of plants, as they participate in the regulation of hydration and activation of enzymes, in addition to participating in photosynthesis, in the case of magnesium. However, according to several authors.[14,3] they report that AMF have the power to reduce the absorption of these elements, due to a buffer effect provided by the fungi, while others8 observe that this decrease may be due to the dilution of the concentration of these macronutrients in the tissues of the infected plants due to the increase in vegetative development, which agrees with the results obtained in this work.

Table 2: Content of macronutrients (%) and reserve substances (RS) (%) of leaves and stems of Aldrighi peach rootstocks plants inoculated with isolates of AMFs (G. clarum and G. etunicatum), from peach orch- ards (OP) and vineyards (V), measured at three hundred thirty (330) days after sowing. Eldorado do Sul, RS.

Origin	Treatment	N	P	K	Ca	Mg	RS
		% Aerial part					
OP	*G. clarum*	3.08b(1)	0.20b	2.50b	1.11b	0.41b	36.83a
	G. etunicatum	3.36a	0.24a	2.72a	1.10b	0.40b	40.81a
V	*G. clarum*	2.40d	0.13d	1.88d	1.10d	0.40b	25.05b
	G. etunicatum	2.68c	0.16c	2.13c	1.11b	0.40b	26.53b
-	Control	2.04d	0.10e	1.59e	1.75a	0.59a	19.43c
C.V. (%)		6.71	5.11	5.41	5.53	2.76	2.76

(1)Means followed by distinct letters in the column differ from each other by the Duncan test ($p \leq 0,05$). Coefficient of Variation (C.V.).

Regarding the reserve substances (carbohydrates, fats, fatty acids, etc.), it was observed that the plants inoculated with AMF presented the highest contents in the shoot tissues, higher than the control plants. As reported by Scatena & Scremin-Dias,[33] plants that have greater height and leaf area have greater capacity to capture light and produce photoassimilates, which allows for a more intense flow of carbohydrates towards the roots. A part of these carbohydrates would be used by AMFs in their nutrition and accumulation in reserve structures (vesicles), and the rest would be accumulated in the plant's storage tissues, in the form of reserve substances.[32]

All AMF isolates were efficient in root colonization of Aldrighi peach rootstocks plants, presenting high rates, above 90% (Table 3).

Table 3: Root colonization and presence of colonization structures (hyphae, vesicles and arbuscules) in Aldrighi peach rootstocks plants roots inoculated with isolates of AMFs (*G. clarum* and *G. etunicatum*), from orchards peach (OP) and vineyards (V), measured at three hundred thirty (330) days after sowing. Eldorado do Sul, RS.

Origin	Treatment	Colonization	Index of structures presence of AMF		
		(%)	Hyphae(2)	Vesicles(3)	Arbuscules(3)
OP	*G. clarum*	91.00a(1)	2.44b	2.36b	1.84b
	G. etunicatum	92.00a	2.57a	2.70a	2.90a
V	*G. clarum*	90.00a	1.74c	1.77c	1.86c

	G. etunicatum	90.50a	1.77c	1.77c	1.88c
-	Control	0.00b	0.00d	0.00d	0.00d
C.V. (%)		2.78	2.78	5.25	4.11

(1)Means followed by the same letter, in the column, do not differ from each other by Duncan's test at the 5% significance level. (2)AMF hyphae presence index, according to Nemec (1992): 0: absence of structures; 1: weak presence; 2: moderate presence; 3: intense presence. (3)Index of presence of AMF vesicles or arbuscules, according to Nemec[21]: 0: absence of structures; 1: 1 to 50 frames; 2: 51 to 100 structures; 3: more than 100 structures per centimeter of radicella.

The index colonization of roots with structures colonization (hyphae, vesicles and arbuscules) was considered moderate to intense for AMFs from orchards peach, and from weak to moderate for those from vineyards. Plants inoculated with isolates of *G. etunicatum* from peach orchards had the highest rates, while G. clarum, from the same source, was superior to species from vineyards, which, in turn, had statistically similar rates. The results of root colonization indicate that the symbiosis between the *G. etunicatum* isolate from peach orchards with Aldrighi peach rootstocks presents greater efficiency than the others, under the conditions in which the experiment was carried out.

The analysis of the association degree between the different growth parameters and mineral nutrition of Aldrighi peach rootstocks plants with root colonization by AMF isolates showed that there was a correlation between them (Table 4).

Table 4: Correlation (r) between growth parameters (height, diameter, leaf area, fresh and dry biomass), mineral nutrition (% of macronutrients) and reserve substances (%) of leaves and stems of Aldrighi peach rootstocks plants and root colonization (%) by isolates of AMFs (*G. clarum* and *G. etunicatum*), from orchards peach (OP) and vineyards (V), measured at three hundred thirty (330) days after seeding. Eldorado do Sul, RS.

Parameters	r	
	Origin of AMF isolates	
	V	OP
% Colonization x height	0.70**	0.87**
% Colonization x diameter	0.93**	0.89**
% Colonization x leaf area	0.76*	0.86**
% Colonization x fresh biomass	0.84**	0.87**
% Colonization x dry biomass	0.84**	0.90**
% Colonization x % nitrogen	0.86**	0.95**
% Colonization x % Phosphorus	0.66**	0.80**
% Colonization x % Potassium	0.89**	0.92**
% Colonization x % Calcium	- 0.68**	- 0.69**
% Colonization x % Magnesium	- 0.52*	- 0.52*
% Colonization x % reserve substances	0.76**	0.83**

* and **Significant at 5% and 1% probability, respectively, by Duncan's test.

Many of the variables evaluated are positively or negatively associated with the percentage of root colonization, regardless of the AMFs used. The positive correlations were considered very significant (P<0.01) between the percentage of root colonization and plant height, stem diameter and

fresh and dry biomass of shoot tissues, in addition to the percentages of nitrogen, phosphorus, potassium and reserve substances, in plants inoculated with isolates originating from both vineyards and peach orchards, as well as the correlation between the percentage of root colonization and the leaf area of the isolates originating from peach orchards. On the other hand, positive correlations between the percentage of root colonization and leaf area of isolates originated from vineyards were considered significant ($P<0.05$).

On the other hand, the negative correlations were considered very significant between the percentage of root colonization and the percentage of calcium and significant for the percentage of magnesium, in the shoot tissues, both for the isolates from vineyards and peach orchards. This indicates that the greater the root colonization, the lower the absorption of these elements, due to a buffer effect provided by AMF,[14,8] which varies according to the species of fungus inoculated and the plant species used.[12,28]

Several authors have observed significant correlations between plant growth responses, percentage of colonization and nutrient content, as in açaí plants,[23] in sweet passion fruit plants[29,27] and in melon plants.[34] Other authors,[35] working with mangabeira plants, observed significant correlations between the percentage of colonization and the content of nutrients, especially phosphorus. However, these authors observe that there may be a negative effect of the increase in phosphorus availability in the soil on the efficiency of the mycorrhizal symbiosis, a fact not observed in this work.

In studies with acerola trees,[26] significant correlations were observed between leaf area and dry biomass for cv. Miró and height and dry biomass for cv. Barbados, however, did not observe a correlation between these parameters and the percentage of root colonization, contrary to what was observed in the present study.

The increase in photosynthetic rate of plants inoculated with AMF is directly related to the increase in leaf area, which provides an increase in vegetative growth and accumulation of fresh and dry biomass.[32] Thus, taller plants with greater leaf area have a greater capacity for photosynthesis and production of photoassimilates, providing accumulation of biomass and, therefore, a higher level of assimilated carbon.[36] This is because, according to several authors,[33,32] plants with greater height and leaf area have a greater capacity to capture light and produce photoassimilates, which allows a more intense flow of carbohydrates in the root direction, where a part would be used by the AMF in the its nutrition and accumulation in reserve structures (vesicles), and the rest would accumulate in the plant's storage tissues, in the form of reserve substances. In addition, by providing a larger stem diameter, AMF would also provide an increase in the upward flow of water and nutrients and, in the downward direction, of elaborate sap.[37,38]

Such reports coincide with the results obtained with the isolates of the AMFs tested, mainly in the case of plants inoculated with isolates from peach orchards, where the isolate of *G. etunicatum* presented the highest results in terms of height, diameter and leaf area, followed by the isolate of *G. clarum* from the same origin, with an intermediate behavior, while the isolates of the same species, originating from vineyards, showed a better behavior than the non-inoculated plants.

Conclusions

The use of AMF benefits the plants of the Aldrighi peach rootstocks, accelerating its vegetative development and improving its macronutrient content. The efficiency of symbiosis is higher with isolates from peach orchards.

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Conflict of Interest

The authors do not have any conflict of interest

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