

# Performance of the root system of tomato plants inoculated with arbuscular mycorrhizal fungi and submitted to the grafting technique

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## Abstract

Information about the combined use of arbuscular mycorrhizal fungi (AMF) in grafted horticultural crops are scarce, as is the case of tomato. Therefore, we investigated if the association between AMF and the grafting technique modifies the performance of the root system of tomato plants grown on substrate. The treatments, outlined in a two-factorial scheme, were absence of inoculation and two inoculants of AMF (*Rhizophagus clarus* and mycorrhizal community) inserted in grafted and non-grafted tomato plants. The experiment was designed entirely at random, with five replications. The evaluations in the root system of the plants were carried out at 30 and 120 days after transplantation (DAT). Grafted plants evaluated at 30 DAT showed greater mycorrhizal colonization when cultivated with *R. clarus*. However, in the 120 DAT evaluation, the greatest mycorrhizal colonization was observed in non-grafted plants produced with the mycorrhizal community. At 120 DAT, the plants produced with the mycorrhizal community showed a more developed root system in relation to non-mycorrhized plants. The root system of plants non-grafted at 120 DAT was more robust when compared to grafted plants. In conclusion, the AMF-grafting interface interferes in the mycorrhizal colonization of the root system of tomato plants. The grafting technique does not improve the development of the root system. The inoculation of tomato plants with the mycorrhizal community enhances the development of roots at 120 DAT.

**Keywords:** arbuscular mycorrhiza, propagation, roots, *Solanum lycopersicum* L.

## Introduction

Tomato (*Solanum lycopersicum* L.) is one of the main horticultural crops produced in many parts of the world (Al-Karaki, 2006). The tomato production in Brazil (4.1 million tons) is still concentrated in the conventional cultivation system (in the soil, in open sky), with low productivity (65.1 t ha<sup>-1</sup>) when compared to the countries considered the biggest producers, such as the United States of America (90.2 t ha<sup>-1</sup>) and Spain (86.1 t ha<sup>-1</sup>). These data show the Brazilian challenge to introduce new technologies to reduce the distance of productivity among the most developed countries and to improve the performance of the culture. As a result, in the Southern Brazil most producers are migrating from traditional cultivation on soil to substrate cultivation (container).

The substrate cultivation profile (hydroponics) is characterized by requiring many chemical fertilizers (Andrade et al., 2017). Still, one of the factors that limits

the production of tomato plants is their susceptibility to phytopathogens, especially those that inhabit the growth medium and, therefore, the use of biocides is frequent during the cultivation of this horticultural crop. For these and other reasons, soilless technology needs high investment, which is sometimes not viable for tomato producers. The use of arbuscular mycorrhizal fungi (AMF) and/or the grafting technique can be a less expensive and a sustainable solution to combat the adverse effects of monoculture, instead of costly soilless culture (Vosátka et al., 2012). Thus, for tomato producers to introduce these technologies during cultivation and at the same time enhance sustainable agriculture, the following question arises: how the association between AMF and grafting technique affects the performance of the root system of tomato plants grown on substrate?

The grafting technique is commonly used in horticultural crops of fruits, including solanaceous crops

(Ombódi et al., 2019), with the objective of improving plant tolerance to biotic (Lee, 2007) and abiotic stresses (Rouphael et al., 2017). The introduced plant (graft) has the function of improving the agronomic and qualitative characteristics of the fruits, while the host plant (rootstock) relieves biotic and abiotic stresses, improving the sustenance in the growth medium, the water/nutrient supply and disease resistance. In addition to grafting, the use of mycorrhizae in agroecosystems is receiving increasing attention. The AMF (phylum Glomeromycota) establish symbiotic associations with 80% of the terrestrial flora (Berruti et al., 2016). The symbiotic process is coordinated by molecular signals between AMF and host plants (Kamel et al., 2017). Once symbiosis is established, there is a bidirectional flow between symbionts: the fungus provides nutrients to the plant, which allocates carbohydrates to the fungus (Garcia et al., 2016). In tomato culture, AMF benefit root biomass (Ronga et al., 2019), increase the tolerance of plants inserted in stressful environments (Kumar et al., 2015) and improve fruit production (Al-Karaki, 2006).

Although the combined benefits of AMF and the grafting technique are possibly stronger than the unique effect of each, most studies have focused on investigating each of these tools in isolation. Therefore, information about arbuscular mycorrhiza in grafted horticultural crops are scarce (Rouphael et al., 2015), as is the case of tomato (Öztekin et al., 2013; Kumar et al., 2015).

In the horticultural industry, producers have traditionally focused on yield (Baum et al., 2015). However, as the most important benefit of the AMF corresponds to the changes that occur in the architecture and morphology of the roots, to improve access to water and nutrients (Wu et al., 2010), no study was concerned with analyzing the root system of the plants in detail. The thinner the roots of the plant symbiont, the greater the chances of colonization by AMF (Zou et al., 2017), which can result in improvements in the acquisition of

resources, as these roots, in addition to being more easily associated with AMF, are the ones that most acquire and use the resources available in the plant growth medium (McCormack et al., 2015).

Therefore, based on the hypothesis that mycorrhized and grafted tomato plants have better root growth and development, here we investigate if the association between AMF and the grafting technique modifies the performance of the root system of tomato plants grown on substrate.

## Materials and Methods

The research was developed at the Universidade de Passo Fundo (28° 15' 46" S, 52° 24' 24" W), Rio Grande do Sul (RS), Brazil, in a greenhouse, during the period of December (summer) 2016 to April (fall) 2017.

Plant material in this study corresponded to tomato seedlings. The seedlings were produced in October (spring) 2016 at the Hortimudas nursery, in the city of Nova Bassano (28° 42' 23" S, 51° 41' 21" W), RS, Brazil. The cultivar used as a graft was 'Paron', belonging to the salad group and with an indeterminate growth habit, and the rootstock chosen was the 'Schincheonggang', an indeterminate growth hybrid. The grafting technique used was a bevel cut with a tube clip. Subsequently, the seedlings (grafted and non-grafted) were acclimatized in a greenhouse in the Hortimudas nursery, of 220 m<sup>2</sup>, with a semicircular roof, installed in the north-south direction, from October to December 2016.

The treatments used in this study were absence of inoculation (control) and two inoculants of AMF [*Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler and a mycorrhizal community (Table 1)] inserted in non-grafted tomato seedlings ('Paron' cultivar only) and grafted ('Paron' cultivar as a graft and 'Schincheonggang' hybrid as rootstock). The experiment was designed entirely at random, with treatments arranged in a bifactorial scheme (3 x 2), with five replications (n = 5) of a single plant.

**Table 1.** AMF community identified from soil collected in Passo Fundo, RS, Brazil.

City	Mycorrhizal community <sup>1</sup>
Passo Fundo	<i>Acaulospora mellea</i> Spain & Schenck, <i>Acaulospora morrowiae</i> Spain & Schenck, <i>Cetranspora pellucida</i> (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd., <i>Claroideoglosum etunicatum</i> (W.N. Becker & Gerd.) C. Walker & A. Schüßler, <i>Glomus</i> sp. and <i>Septoglosum viscosum</i> (T.H. Nicolson) C. Walker, D. Redecker, Stiller & A. Schüßler

<sup>1</sup>Classification of Glomeromycota by Redecker et al. (2013).

The *R. clarus* isolate came from the International Culture Collection of Glomeromycota (CICG). The AMF community (Table 1) used came from the trap culture of soil collected in an agroecosystem grown with horticultural

crops [lettuce (*Lactuca sativa* L.), strawberry (*Fragaria X ananassa* Duch.), cucumber (*Cucumis sativus* L.), sweet bell pepper (*Capsicum annuum* L.) and tomato], in the city of Passo Fundo (28° 15' 46" S, 52° 24' 24" W), RS, Brazil.

In December 2016, after eight weeks of acclimatization in the Hortimudas nursery, the tomato seedlings produced (grafted and non-grafted) were transported to the city of Passo Fundo, RS, Brazil, and transplanted in polyethylene pots (9 L), filled with sterilized Carolina Soil II® commercial substrate (120°C for 20 minutes). At the time of implementation of the experiment, the tomato seedlings had an average stem diameter of 3.02 mm and three fully expanded leaves. For treatments inoculated with AMF, 10 g of inoculant was added in the planting pit of the seedlings at the time of transplantation (the inoculant was not mixed with the sterile substrate).

A sample of 500 g of Carolina Soil II® substrate was analyzed to obtain its physical (Table 2) and chemical (Table 3) attributes.

**Table 2.** Physical characterization of Carolina Soil II® substrate.

Substrate	D <sup>1</sup>	TP	AS	RAW	BW	RW
	(kg m <sup>-3</sup> )		(m <sup>3</sup> m <sup>-3</sup> )			
Carolina Soil II®	104	0.914	0.337	0.260	0.034	0.283

<sup>1</sup>D: density; TP: total porosity; AS: aeration space; RAW: readily available water; BW: buffer water; RW: remaining water.

**Table 3.** Chemical characterization of Carolina Soil II® substrate.

Substrate	N <sup>1</sup>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	OC	pH	EC	CEC
	% (m m <sup>-1</sup> )					mS cm <sup>-1</sup>	mmol <sub>c</sub> kg <sup>-1</sup>
Carolina Soil II®	1.00	0.63	0.11	20.17	5.40	1.23	399.76

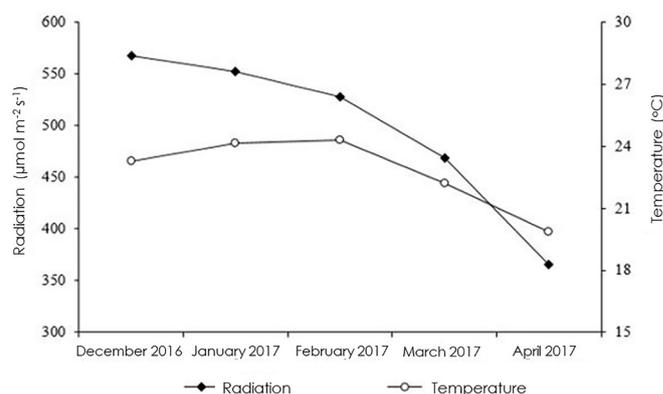
<sup>1</sup>N: nitrogen; P<sub>2</sub>O<sub>5</sub>: phosphorus pentoxide; K<sub>2</sub>O: potassium oxide; OC: organic carbon; pH: hydrogenion potential; EC: electrical conductivity; CEC: cation exchange capacity.

The pots were kept in beds covered with mulching, 0.5 x 1.0 m apart, in a greenhouse of 430 m<sup>2</sup>, with a semicircular roof, installed in the northwest-southeast direction. The galvanized steel frame is covered with low-density polyethylene film with thickness of 150 microns and antiultraviolet additive. The plants were conducted with one stem and were tutored vertically and individually with a ribbon. Drip irrigation with a flow rate of 2.4 L h<sup>-1</sup> per pot was used in the experiment. Irrigation was applied six times a day for a total of 12 minutes per day. The nutrient solution supplied to the plants (Table 4), on a monthly basis, was adapted from Benoit (1992) to maintain the K<sup>+</sup>/ (Ca<sup>2+</sup> + Mg<sup>2+</sup>) ratio needed by the tomato.

**Table 4.** Composition of the nutrient solution used in tomato cultivation.

Nutrient	Concentration (mmol L <sup>-1</sup> )
Nitrate (NO <sub>3</sub> )	15.40
Potassium (K <sup>+</sup> )	5.76
Calcium (Ca <sup>2+</sup> )	4.50
Magnesium (Mg <sup>2+</sup> )	1.25
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	1.45

During the execution of the experiment, through a meteorological mini-station, we monitored the monthly averages of photosynthetically active radiation (PAR) and air temperature inside the greenhouse (Figure 1).



**Figure 1.** Monthly averages of photosynthetically active radiation and air temperature inside the greenhouse during the conduct of the experiment. The general averages of radiation and air temperature recorded during the experiment (from December 2016 to April 2017) were 496.51 µmol m<sup>-2</sup> s<sup>-1</sup> and 22.76°C, respectively.

We performed two evaluation regarding the performance of the root system of tomato plants: 1) at 30 days after transplantation (DAT), in January (summer) 2017; 2) at 120 DAT, in April (fall) 2017. The fruit harvest started in February (summer) 2017. The average fruit production was 4.8 kg plant<sup>-1</sup> and the average lycopene content of the fruits was 8.5 µg g<sup>-1</sup>.

To verify the mycorrhizal colonization rate, at 30 DAT and 120 DAT root portions of tomato plants were prepared according to Phillips & Hayman (1970) and their percentages of mycorrhizal colonization (MC, %) were determined according to Trouvelot et al. (1986), by the equation:

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

To evaluate the root system morphology at 30 DAT (January 2017) and 120 DAT (April 2017), the roots were collected and washed in water to eliminate the substrate fragments. Roots were scanned and images obtained analyzed by WinRHIZO® software. The evaluated attributes were total root length (TL, cm), surface area (SA, cm<sup>2</sup>) and root volume (RV, cm<sup>3</sup>). The roots were grouped by software in different diameter classes in relation to their total length (Böhm, 1979): very thin roots (VTR, cm: Ø < 0.5 mm), fine roots (FR, cm: Ø from 0.5 to 2.0 mm) and thick roots (TR, cm: Ø > 2.0 mm).

Data were subjected to analysis of variance (Anova) and treatment means separated by the Tukey test, at 5% probability error, using the Costat® program.

## Results

We observed an interactive effect of AMF and grafting on the mycorrhizal colonization of the root system of plants at 30 and 120 DAT (Table 5). Grafted plants evaluated at 30 DAT showed greater mycorrhizal colonization when they were grown with the fungal

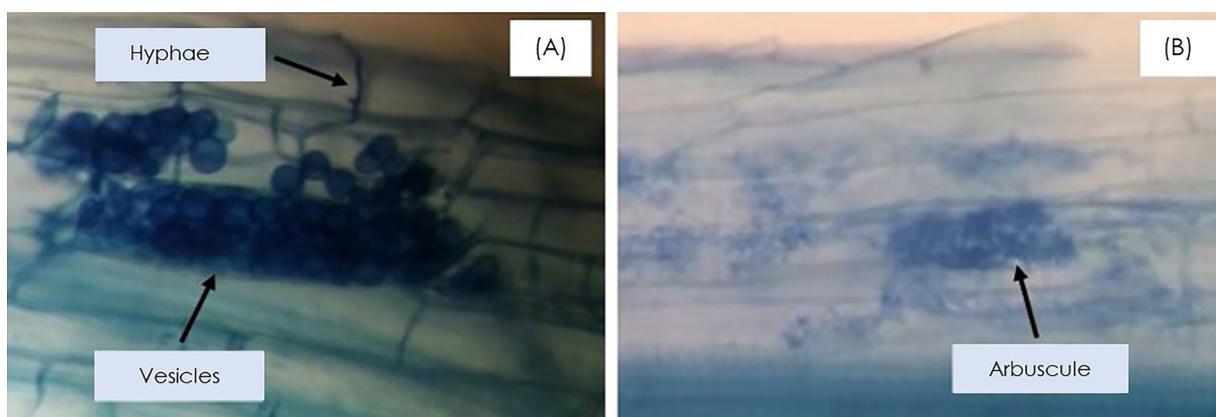
species *R. clarus* (Table 5). However, when we performed the evaluation at 120 DAT, the greatest mycorrhizal colonization was observed in non-grafted plants produced with the mycorrhizal community (Table 5).

The fungal structures formed inside the roots of tomato plants were hyphae, vesicles and arbuscules (Figure 2).

**Table 5.** Mycorrhizal colonization rate (%) in roots of tomato plants with and without grafting.

Mycorrhization	30 DAT <sup>1</sup>		120 DAT	
	Grafting		Grafting	
	With	Without	With	Without
Control	00.00±0.0 Ab	00.00±0.0 Ab	00.00±0.0 Ab	00.00±0.0 Ab
Community	24.00±2.0 Aab	12.00±1.0 Ab	22.00±3.0 Ba	66.00±3.0 Aa
<i>R. clarus</i>	58.00±4.0 Aa	36.00±3.0 Ba	14.00±1.0 Aab	22.00±2.0 Ab
Average	22.30		20.60	
CV (%) <sup>2</sup>	16.70		13.80	

Data presented as mean ± standard deviation. Means followed by the same capital letter in the row and lowercase in the column do not differ significantly by the Tukey test (p<0.05, n = 5). <sup>1</sup>30 DAT: evaluation performed 30 days after transplanting of seedlings; 120 DAT: evaluation performed 120 days after transplanting of seedlings. <sup>2</sup>Coefficient of variation.



**Figure 2.** AMF structures visualized in roots of tomato plants referring to hyphae (A), vesicles (A) and arbuscules (B). Observation under an optical microscope, with 400x magnification.

There was an effect of AMF and grafting, independently, on the root system morphology of the plants at 30 DAT regarding the attributes SA, RV, FR and TR (Table 6). Non-mycorrhized plants showed a more robust root system, with SA, RV and TR higher in 12%, 23% and

16%, respectively, in relation to plants grown with AMF (Table 6). The root system of non-grafted plants proved to be more profuse, with SA, RV, FR and TR higher in 19%, 24%, 22% and 30%, respectively, in relation to the grafted plants (Table 6).

**Table 6.** Effect of mycorrhization and grafting on the root system of tomato plants 30 days after transplanting of seedlings.

Mycorrhization	SA (cm <sup>2</sup> ) <sup>1</sup>	RV (cm <sup>3</sup> )	FR (cm)	TR (cm)
Control	812.00±53.6 a	26.00±2.2 a	634.00±43.3 a	231.70±10.1 a
Community	759.90±51.9 b	20.70±4.8 b	688.90±74.3 a	200.50±11.7 b
<i>R. clarus</i>	667.30±51.1 b	19.30±3.1 b	535.30±46.9 a	187.70±10.0 b
Grafting				
With	667.90±56.1 b	19.00±1.5 b	538.70±37.9 b	173.40±14.2 b
Without	831.60±44.2 a	25.00±3.7 a	696.10±47.0 a	249.30±18.1 a
Average	749.70	22.00	574.66	208.52
CV (%) <sup>2</sup>	16.10	24.40	20.09	18.60

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, n = 5). <sup>1</sup>SA: surface area; RV: root volume; FR: fine roots; TR: thick roots. <sup>2</sup>Coefficient of variation.

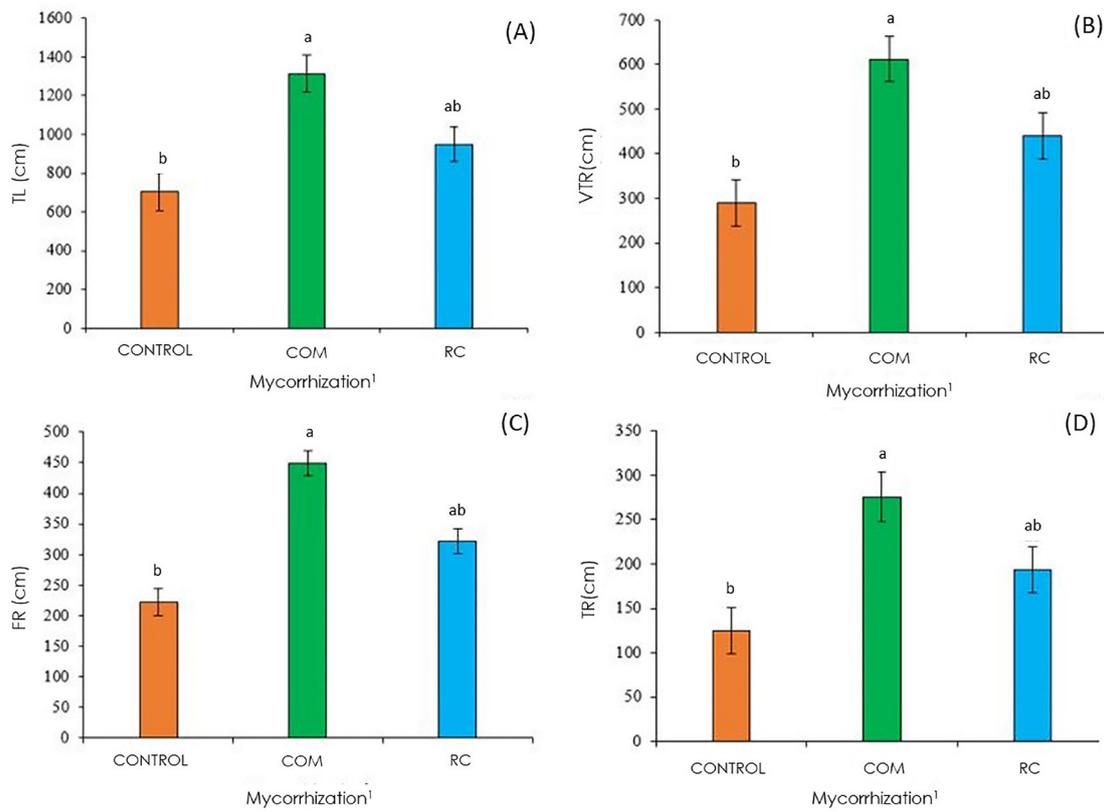
The effect of AMF and grafting also occurred independently in the root system morphology of the plants evaluated at 120 DAT regarding the attributes TL, VTR, FR and TR (Figures 3 and 4).

At 120 DAT, the plants produced with the mycorrhizal community showed a more developed root system, with TL, VTR, FR and TR higher in 46% (Figure 3A),

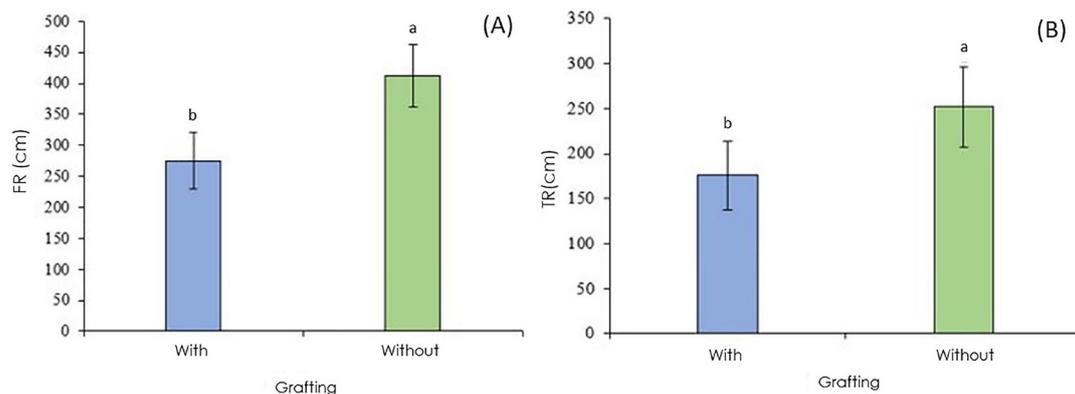
52% (Figure 3B), 50% (Figure 3C) and 54 % (Figure 3D), respectively, in relation to plants grown without AMF. Plants produced with the fungal species *R. clarus* formed an intermediate group between those grown without AMF and with the mycorrhizal community (Figure 3).

When analysing tomato plants at 120 DAT for grafting, we observed that the root system of the non-

grafted plants proved to be more robust, with FR and TR higher in 33% (Figure 4A) and 30% (Figure 4B), respectively, in relation to grafted plants.



**Figure 3.** Root system morphology of tomato plants, in the presence and absence of mycorrhization, 120 days after transplanting of seedlings. A) Total length (TL); B) very thin roots (VTR); C) fine roots (FR); D) thick roots (TR). Data presented as mean  $\pm$  standard deviation. Different letters above columns indicate significant differences by the Tukey test ( $p \leq 0.05$ ,  $n = 5$ ). <sup>1</sup>CONTROL: plants without mycorrhization; COM: mycorrhizal community; RC: *R. clarus*.



**Figure 4.** Root system morphology of tomato plants, in the presence and absence of grafting, 120 days after transplanting of seedlings. A) Fine roots (FR); B) thick roots (TR). Data presented as mean  $\pm$  standard deviation. Different letters above columns indicate significant differences by the Tukey test ( $p \leq 0.05$ ,  $n = 5$ ).

## Discussion

Here, we show that the association between AMF and the grafting technique interfered in the mycorrhizal colonization of the root system of tomato plants grown on substrate. The grafting technique, independently, did not improve the development of the root system of tomato plants. Still, in isolation, mycorrhization promoted the

main effect during tomato cultivation, potentiating the development of roots at 120 DAT when the plants were grown with the mycorrhizal community.

At 30 DAT, mycorrhizal colonization was more effective in plants grafted and cultivated with the fungal species *R. clarus* (Table 5). However, this did not reflect a better development of the root system of these plants

(Table 6). Although mycorrhizal colonization is important, the percentage of root infectivity is not always correlated with the efficiency of symbiosis (Konvalinková & Jansa, 2016). We believe that the fungus *R. clarus* may have taken on a role as root of the plants, which were still small and had a poorly developed aerial part. In general, plants invest in the establishment of symbiosis for the benefits that can be received in this association, such as increasing the contact area of the roots with the substrate inside the pot. This is because in the early stages of plant development (30 DAT, for example) the secondary roots are still inefficient in acquiring water and nutrients.

We mostly observed that there was a reduction in mycorrhizal colonization from 30 DAT to 120 DAT (Table 5). As younger plants have cell walls with lower calcium levels, this facilitates the association with AMF, which is important for the accumulation of reserve substances necessary for the survival of plants after transplantation. The middle lamella, a layer that interfaces between the cell walls of adjacent cells, expands until the cell reaches its maturity and possibly forms secondary walls. As this layer grows, the demand for pectic compounds also increases. To promote the connection between the pectin networks and the stability of the middle lamella, calcium in its ionic form ( $\text{Ca}^{2+}$ ) is required. Thus, younger plants, with cells still expanding, have cell walls with lower calcium levels (Taiz et al., 2017). As an exception, the mycorrhizal colonization of non-grafted and cultivated plants with the AMF community increased from 30 DAT to 120 DAT (Table 5). AMF are generally nonspecific and with a very large host range. However, the greater the mycorrhizal diversity in a growth medium (soil/substrate), the greater the possibility of association with the host plant (Chiomento et al., 2019). We believe that this exception occurred due to the number of species (six, Table 1) present in the inoculum of the mycorrhizal community. Possibly some of these fungal species had greater affinity with tomato plants at this time of the evaluation (120 DAT).

Not all AMF behave the same in a given environment (Gómez-Bellot et al., 2015). Thus, host responses to mycorrhizal inoculation vary according to their phenological stage (Jiménez-Leyva et al., 2017), edaphoclimatic conditions (Halder et al., 2018) and management/agricultural practices (Velázquez et al., 2018). In addition, the relationship character of a specific microorganism and its host plant varies over time, causing a shift from mutualism to parasitism and vice versa.

Different from what we expected, the root system of plants at 30 DAT grew more when they were not mycorrhized (Table 6). This suggests that AMF

initially demand carbon from the host for maintenance and then return this benefit to the plant symbiont. It is possible that many mycorrhizal associations can change from beneficial (mutualism) to harmful (parasitism) to plants (Johnson et al., 1997), inducing negative growth responses in relation to non-mycorrhized plants (Smith & Smith, 2013), which was observed in our study (Table 6). In the case of non-mycorrhized plants, which presented a more developed radicular system (Table 6), the partition between photoassimilates occurs only between the different organs of the plants, resulting in a greater energy balance for the emission, growth and development of the roots.

On the other hand, we confirmed that the root system of plants inoculated with the mycorrhizal community at 120 DAT was more robust and profuse (Figure 3). Other researches have reported the benefit of mycorrhization to the root system of tomato plants, using biomass (Öztekin et al., 2013; Ronga et al., 2019), but without analyzing the roots in detail (using the WinRHIZO® software) as in our study [TL (cm), SA ( $\text{cm}^2$ ), RV ( $\text{cm}^3$ ), VTR (cm), FR (cm) and TR (cm)]. Due to the plasticity of the roots, their characteristics can be modulated by several factors, including the AMF (Hodge et al., 2009). During the symbiotic process, in order to occur the association between the host and the fungus, a cascade of molecular signaling is initiated, including a diffusible factor of AMF called the "Myc factor" (chitooligosaccharides), which stimulates the formation of thinner roots (Oláh et al., 2005), altering the root system morphology of the plants.

Root system changes under mycorrhization may be related to the allocation of sugars to the roots (Wu et al., 2011) and hormonal regulation (Zou et al., 2017), regardless of symbiotic signaling (Gutjahr et al., 2009). Arbuscular mycorrhiza causes morphological, nutritional and physiological changes in host plants to combat stress and improve plant growth and vigor (Alqarawi et al., 2014). However, more importantly, the AMF modify the root architecture to improve access to water and nutrients (Wu et al., 2010).

As a differentiator from other studies carried out on tomato plants, which used only the genera *Claroideoglossum*, *Glomus*, *Funneliformis* or *Rhizophagus* as inoculants, our results are unprecedented because the plants were also inoculated with a mycorrhizal community from soil adapted to the cultivation of horticultural crops, including tomato (Table 1). One of the factors that influences the positive effects of arbuscular mycorrhiza is the choice of fungal species (Fortuna et al., 1992).

AMF genera, species and isolates need to be

studied for their biotechnological potential to benefit the growth/development of the plant symbiont before being used commercially (Taylor & Harrier, 2001). Commercial inoculants based on AMF are usually formed by only one or a few species, which may or may not be well adapted to the conditions where they are applied (Garland & Schroeder-Moreno, 2011). Inoculation with native AMF populations, as is the case with the fungal community used in our study (Table 1), commonly provides more satisfactory results (Figure 3 and Table 5) due to fungus-host compatibility and for increasing mutualistic effects with two or more symbionts instead of just one (Koron et al., 2014).

Contrary to what we hypothesized, our results showed that grafting on tomato plants did not benefit the development of the root system of plants at 30 DAT (Table 6) and 120 DAT (Figure 4). Our results contradicted researches already carried out (Öztekin et al., 2013; Kumar et al., 2015). However, we emphasize that these studies reported the benefits of grafting or the grafting-mycorrhization interface in tomato plants under limiting conditions, generally subjecting plants to abiotic stresses (Öztekin et al., 2013; Kumar et al., 2015), which did not happen in our study. Positive or negative changes can occur in grafted plants. The role of AMF in vegetables submitted to the grafting technique is to improve nutrition and provide greater vegetative growth for rootstocks. The benefits derived from symbiosis depend on the particular combination of AMF and rootstock (Anzanello et al., 2011) and this did not reflect benefits on the roots of the plants evaluated in our work.

In this study, although in general the grafting technique did not have a significant influence on the development of tomato plant roots, the mycorrhizal association potentiated the root system of the plants. Our results are unprecedented when determining the influence of mycorrhizal biotechnology on tomato plants through an AMF community obtained from soil adapted to the cultivation of horticultural crops (Table 1). In addition, through the WinRHIZO<sup>®</sup> software, we were able to analyze in detail the root system of the plants. The findings of our study may be useful to tomato producers who want to insert the AMF, in the cultivation agroecosystem, as a biotechnological tool in favor of environmental sustainability. This is because tomato plants grown with AMF had higher amounts of finer roots, which are the most efficient in acquiring resources (water and nutrients, for example) from the plant growth medium (soil or substrate). Thus, the AMF would function as biofertilizers, which allows reducing the use of chemical

inputs during the cultivation of this horticultural crop. Finally, these investigations are filling the gap between AMF engineering related to grafting techniques in tomato cultivation.

### Conclusions

We conclude that the association between AMF and the grafting technique interferes in the mycorrhizal colonization of the root system of tomato plants grown on substrate. In younger plants (30 DAT) and grafted, the mycorrhizal colonization rate in the roots is enhanced by the fungal species *R. clarus*. However, in more advanced stages of development (120 DAT), plants non-grafted and produced with the AMF community have greater mycorrhizal colonization. On the other hand, we reject the hypothesis that mycorrhized and grafted tomato plants have better root growth. The grafting technique, by itself, does not improve the development of the root system of plants. In addition, in isolation, mycorrhization promotes the main effect during tomato cultivation. This symbiosis does not benefit the root system morphology of the plants at 30 DAT, but it enhances the development of roots at 120 DAT when the plants are grown with the mycorrhizal community. Although other studies have pointed out the positive effects of mycorrhizal symbiosis on the development of horticultural rootstocks, a greater understanding of the application and benefits of the AMF-grafting interface can contribute to improving the sustainability of the tomato cultivation system.

### Acknowledgements

To the Programa de Suporte à Pós-Graduação de Instituições Comunitárias de Ensino Particulares (PROSUC) of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to the Universidade de Passo Fundo (UPF). 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. In addition, we inform that this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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