

Mycorrhizal dependence of citrus rootstock

Priscila Batista Miranda¹, Andrea Hentz de Mello², Katia Cristina Kupper³

Abstract - Plant growth promoting microorganisms, such as mycorrhizal fungi, have been widely used in agriculture because they help plants in the absorption of nutrients and water, influencing their growth. The objective of this work was to verify the effect of the mycorrhizal association of *Glomus clarum* and *Glomus etunicatum* on the initial growth of the rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin. The experimental design was completely randomized with a 3 x 4 factorial arrangement, with five replications, corresponding to three rootstocks, four managements: mineral fertilization, inoculation with *G. clarum* (IGC), inoculation with *G. etunicatum* (IGE) and Control. The seedlings were produced in greenhouse, using as substrate the vermicompost earthworm, distributed in polyethylene bags, depositing three seeds of each rootstock / bag. The inoculation with mycorrhizal fungi was carried out at the time of sowing (45 spores of each species / bag). The mineral fertilization was divided into two phases: phosphorus application before sowing (40g of single superphosphate / bag); nitrogen application (1.7 g dissolved in 40 ml of water), divided in eight applications of 5 ml of the solution, via irrigation water, manually and weekly; and micronutrients (B, Cu, Fe, Mn and Zn), sprayed manually every two weeks. The following parameters were evaluated: height, stem diameter, number of leaves, shoot dry mass, colonization and mycorrhizal dependence. There was a significant interaction between the rootstocks and treatments applied in this study for most of the evaluated variables. Rangpur lime and Swingle citrumelo obtained higher height and the management that conferred higher height to the rootstocks was the mineral fertilization and IGE. The largest diameter of the stem was reached by Swingle citrumelo and the management that provided the largest diameter was the inoculation with *G. etunicatum*. Rangpur lime presented moderate mycorrhizal dependence for *G. clarum* and marginal for *G. etunicatum*. Mycorrhizal colonization did not show a significant interaction between rootstocks and the management with inoculation. The mycorrhizal association of *G. clarum* and *G. etunicatum* was not presented as the best management for the growth of Rangpur lime, Swingle citrumelo and Sunki mandarin, when compared to the mineral fertilization. However, the inoculation of mycorrhizal fungi provided a positive effect on the uptake of some nutrients by the rootstocks, especially K, Mg, N and P.

Index terms: Mycorrhizal Association, Rangpur lime, Swingle citrumelo, Sunki mandarin.

Dependência micorrízica de porta-enxertos de citros

Resumo – Os microrganismos promotores de crescimento de plantas, como os fungos micorrízicos, têm sido muito utilizados na agricultura, por auxiliarem os vegetais na absorção de nutrientes e de água, influenciando no crescimento destes. Objetivou-se com este trabalho verificar os efeitos da associação micorrízica de *Glomus clarum* e *Glomus etunicatum* no crescimento inicial dos porta-enxertos Limão-cravo, Citrumelo swingle e Tangerina sunki. O delineamento experimental foi o inteiramente casualizado, em arranjo fatorial 3 x 4, com cinco repetições, correspondendo a três porta-enxertos, quatro manejos, sendo os manejos: adubação mineral (ADB), inoculação com *Glomus clarum* (IGC), inoculação com *Glomus etunicatum* (IGE) e Controle. As mudas foram produzidas em estufa, utilizando como substrato vermicomposto de minhoca, distribuído em sacos de polietileno, depositando-se três sementes de cada porta-enxerto/saco. A inoculação com os fungos micorrízicos foi realizada no momento da semeadura (45 esporos de cada espécie/saco). A adubação mineral foi dividida em dois momentos: aplicação de fósforo antes da semeadura (40g de superfosfato simples/saco); aplicação de nitrogênio (1,7g dissolvidos em 40 mL de água), parcelado em oito aplicações de 5 mL da solução, via água de irrigação, manualmente, de forma semanal; e micronutrientes (B, Cu, Fe, Mn e Zn), aplicados com pulverizador manual, quinzenalmente. Foram avaliados altura, diâmetro do caule, número de folhas, massa seca da parte aérea e colonização, e dependência micorrízica. Verificou-se interação significativa entre os porta-enxertos e os manejos aplicados neste estudo, para a maioria das variáveis avaliadas. Limão-cravo e Citrumelo swingle obtiveram maior altura, e o manejo que conferiu maior altura, aos porta-enxertos foram o ADB e o IGE. O maior diâmetro do caule foi atingido pelo Citrumelo swingle, e o manejo que proporcionou maior diâmetro foi o de IGE. Limão-cravo apresentou dependência micorrízica moderada para *Glomus clarum* e marginal para *G. etunicatum*. A colonização micorrízica não apresentou interação significativa entre os porta-enxertos e os manejos com inoculação. A associação micorrízica de *G. clarum* e *G. etunicatum* não se apresentou como o melhor manejo para o crescimento das plantas de Limão-cravo, Citrumelo swingle e Tangerina sunki, quando comparado com a adubação mineral. No entanto, a inoculação de fungos micorrízicos proporcionou um efeito positivo na absorção de alguns nutrientes pelos porta-enxertos, especialmente, de K, Mg, N e P.

Termos para indexação: Associação micorrízica, Limão-cravo, Citrumelo swingle, Tangerina sunki.

Corresponding author:

katia@ccsm.br

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¹Master in Agroecology and Rural Development Program, Universidade Federal de São Carlos, Araras, SP. E-mail: engagro.priscila@outlook.com

²Doctor of Instituto de Estudos em Desenvolvimento Agrário e Regional, Universidade Federal do Sul e Sudeste do Pará. E-mail: andreahtz@unifesspa.edu.br

³Doctor and professor of Agroecology and Rural Development Program, Universidade Federal de São Carlos, Araras, SP. and researcher of Centro Apta Citros Sylvio Moreira/IAC, Cordeirópolis-SP. E-mail: katia@ccsm.br

Introduction

Citriculture has been highlighted as an important activity of the Brazilian agricultural sector, accounting for 60% of the world's orange juice production (BRASIL, 2016). The national production is 15,952,253 tons and the state of São Paulo is the main producer, with 11,642,000 tons, corresponding to approximately 73% of the total orange produced in the country (IBGE, 2016).

Rangpur lime (*C. limonia* Osb.) is the most widely used rootstock for the production of citrus seedlings in Brazil (KOLLER; SOPRANO, 2013). Its characteristics such as high vigor, tolerance to water stress, easy seed acquisition, great vigor in the nursery, good establishment of seedlings, rapid growth, high and early production, make this rootstock to be used in about 80% of citrus orchards (BASTOS et al., 2014).

Sunki mandarin has high vigor, good fruit yield, resistance to tristeza (caused by *Citrus tristeza virus*), decline and citrus sudden death, being also tolerant to drought (BASSANEZI et al., 2002; POMPEU JUNIOR, 2005). Swingle citrumelo rootstock is considered tolerant to tristeza virus, gummosis, nematode, cold (BASTOS et al., 2014) and citrus sudden death (KUPPER et al., 2016). Such characteristics make the use of these rootstocks relevant for the production of citrus seedlings.

Several studies have been carried out in order to incorporate the use of biological inputs in agriculture, such as the use of plant growth promoting microorganisms (FLORES-AYALAS et al., 2003; LIMA et al., MIRANDA et al., 2016). However, most of these studies use microorganisms as a complementary input to reduce the addition of mineral fertilizers, especially phosphorus, in the processes of seedling production. Thus, studies should be carried out in order to evaluate the isolated effect of these microorganisms and the dependence of plants on them.

Arbuscular mycorrhizal fungi (AMFs) belonging to phylum Glomeromycota stand out as growth promoting microorganisms, which associate with the roots of plants (SCHÜBLER et al., 2001). Arbuscular mycorrhizae are nutritional mutual associations, where the plant supplies the fungus with energy for its growth and maintenance via photosynthetic products, and the fungus provides the plant with better absorption of nutrients and water (BERBARA et al., 2006).

Numerous studies have been carried out in order to prove the efficiency of mycorrhization in several crops, evidencing the increment in the development of plants inoculated with AMFs, to the detriment of those not inoculated, presenting significant gains in growth and absorption of nutrients, especially phosphorus, due to the increase of the area of root absorption, with their extension by AMFs (BALOTA et al., 2011, FERREIRA et al., 2015, GARCIA et al., 2016).

In view of the above, this work aimed to verify the effects of mycorrhizal association of *Glomus clarum* Nicol. & Schenck and *Glomus etunicatum* Becker & Gerd. on the initial growth of Rangpur lime (*Citrus limonia* Osbeck), Swingle citrumelo (*Citrus paradisi* Macf x *Poncirus trifoliata* (L) Raf.) and Sunki mandarin (*Citrus sunki* Hort. ex Tan.) rootstocks.

Material and Methods

The experiment was carried out from March to October 2016 at the greenhouse of the Faculty of Agrarian Sciences of Marabá (FCAM), Federal University of Southern and Southeastern Pará (Unifesspa), located in the municipality of Marabá – PA, Brazil.

This study had a completely randomized design in a 3 x 4 factorial scheme with 5 replicates, corresponding to 3 rootstocks (Rangpur lime, Swingle citrumelo and Sunki mandarin) and 4 managements (mineral fertilization - MF, inoculation with *Glomus clarum* - IGC, inoculation with *Glomus etunicatum* - IGE and Control, without fertilization and inoculation).

Spores, obtained from FCAM / Unifesspa's Inoculum Bank, were extracted and separated at the laboratory by means of the wet sieving technique (GERDEMANN; NICOLSON, 1963) and centrifugation in water and 40% sucrose (JENKINS, 1964). After extraction, spores were inoculated in plastic pots of black coloration, with no drainage layer, and capacity of 1 kg, containing autoclaved sand as substrate, in which *Brachiaria brizantha* was sown. Each AMF species contained three pots as trap cultivation, which remained for 120 days in benches inside a greenhouse with plastic cover and lateral anti-aphid screen.

The substrate used for seedling production was *Eisenia foetida* vermicompost, whose characteristics, after chemical analysis were: pH (water) = 6.6; organic matter = 2.9dag / kg; cation exchange capacity = 8.9cmol / dm³; Mg = 0.7cmol / dm³; Ca = 5.1cmol / dm³; P = 158mg / dm³; K = 650mg / dm³; S = 25mg / dm³; Cu = 3.2mg / dm³; Fe = 236 mg / dm³; Mn = 48mg / dm³; Zn = 9.5mg / dm³; B = 0.47mg / dm³. The substrate was autoclaved for 20 minutes at pressure of 1 atm or 1 kgf / cm², three times, in order to eliminate organisms that could compete with AMFs used, interfering in the results.

For seedling production, rootstock seeds were firstly prepared, washed in running water and immersed in water heated at 50°C for 10 minutes. The seed coat was also manually removed. Subsequently, seeds were sown on the substrate at a depth of approximately 3 cm in black polyethylene bags with capacity of 50 cm³, depositing three seeds of each rootstock per bag. Pots were kept on benches throughout the experimental period. Before sowing, the substrate was treated with the different isolates

of mycorrhizal fungi and / or mineral fertilization.

The two managements with inoculation consisted of depositing 45 spores of each AMF species in a hole made at the center of the substrate of approximately 3cm in depth and 3cm in diameter.

The management with mineral fertilization was carried out in two stages. In the first, a source of phosphorus (P), in the form of single superphosphate (SS - 16% of P₂O₅) was added, homogenizing 40g of fertilizer with substrate contained in each container, prior to sowing. The second stage was carried out after 30 days from the emergence of cotyledons, in which pruning and application of nitrogen (N) and micronutrients (B, Cu, Fe, Mn and Zn) were performed. A total of 1.7 g of N, dissolved in 40 ml of water, was applied in a split – plot scheme, consisting of eight weekly applications of urea cover, via manual irrigation water. Therefore, 5 ml of this solution were applied near the root of each seedling (DECARLOS NETO et al., 2002). The micronutrients, in turn, were sprayed with a manual sprayer only on the shoots, every two weeks, according to recommendation of EMBRAPA (2003).

At 30, 60 and 90 days after the emergence of plants, the following growth parameters were evaluated: shoot height, number of leaves and stem diameter (cm). To measure height, a ruler graduated in millimeters was used and for stem diameter, a universal caliper graduated in millimeters was used.

After 90 days, plants were collected and evaluated for shoot dry mass (g), mycorrhizal dependence and colonization. In order to determine the weight of the shoot dry mass (SDM), seedlings had the leaves and branches separated from the root and this material was taken to the oven with air circulation at 65° C for 48 hours until reaching constant weight.

The mycorrhizal dependence was calculated for each management with inoculation through formula suggested by Plenchette et al. (1983):

$$MD = \frac{DBMP - DBNMP}{DBMP} \times 100$$

Where: MD = mycorrhizal dependence; DBMP = dry biomass of mycorrhizal plants; DBNMP = dry biomass of non-mycorrhized plants.

In order to classify mycorrhizal dependence, the following categories adapted from Habte and Manjunath (1991) were used: overly dependent plants (MD > 75%), highly dependent plants (50-75%), moderately dependent plants (25 - 50%), marginally dependent (< 25%) or independent (do not respond to mycorrhization).

To obtain the percentage of mycorrhizal colonization, roots were collected, washed and stained by the staining and clarification method of Phillips and Hayman (1970).

Colonization quantification was performed by the quadrant intersection method of Giovannetti and Mosse (1980).

Data were submitted to analysis of variance and means were compared by the Tukey's test at 5% probability, using the Assisat 7.7 software (SILVA; AZEVEDO, 2016).

Results and discussion

There was a significant interaction between rootstocks and managements for the shoot height variable (Table 1). The highest averages of this variable were reached by Rangpur lime and Swingle citrumelo plants (Table 2) and the management that provided the highest height to plants was mineral fertilization and inoculation with *G. etunicatum*, although the last treatment was not statistically different from *G. clarum* inoculation and control (Table 3).

Melloni et al. (2000) verified result similar to this in relation to *Glomus etunicatum* in an experiment with Rangpur lime rootstock cultivated in substrate with different P levels. The authors found that this AMF was not efficient in promoting the increase in the mean values of the evaluated variables, not differing from control. However, in the same research, they demonstrated that Rangpur lime depended on mycorrhization with *Glomus intraradices* to reach higher height, stem diameter and shoot dry matter.

There was a significant interaction between rootstocks and treatments for the stem diameter variable (Table 4). The highest mean of this variable was obtained by Swingle citrumelo (Table 5) and the management that attributed the largest diameter to the rootstocks was that in which *Glomus etunicatum* was used (Table 6).

There was no significant interaction between rootstocks and managements, when the number of leaves was evaluated (Table 7). However, the management that provided the highest average number of leaves to plants was that of mineral fertilization (Table 8).

In relation to shoot dry mass (SDM) (Figure 1), mineral fertilization was the management that resulted in higher averages for all rootstocks. Swingle citrumelo presented the highest SDM values, considering all the treatments applied.

Inoculation with *G. etunicatum* favored the growth of rootstocks, considering the height and stem diameter variables, unlike *G. clarum*, which did not promote the growth of plants in any of the variables analyzed. Back et al. (2016), working with 'Kumquateiro' [*Fortunella hindsii* (L.) Swingle] and 'Fepagro C37 Reck' citrange [*P. trifoliata* (L.) Raf. *C. sinensis* (L.) Osbeck.] citrus rootstocks inoculated with three different species of mycorrhizal fungi, found that *G. etunicatum* was efficient in accelerating the growth of 'Fepagro C37 Reck'. This result corroborates those obtained in this study related

to the ability of *G. etunicatum* to promote the growth of citrus rootstocks.

Considering that AMFs benefit better species when they are found on substrates with low P levels (MOREIRA SOUZA; CARDOZO, 2002), this behavior, in relation to *G. clarum*, may be related to the fertility level presented by the substrate at the beginning of seedling production, which had a considerable P content (158 mg / dm³), and pH close to neutrality (6.6).

Likewise, Balota et al. (2011), studying acerola (*Malpighia emarginata* DC) associated with AMFs and phosphorus addition, showed that the increased incorporation of P in the soil allowed significant increase in the biomass of plants and reduced the root colonization and sporulation of AMFs, and the high mycorrhizal dependence of this plant occurred in soil with low P level (7 mg / kg⁻¹). Machineski et al. (2011) observed higher number of *G. clarum* spores at P level of 20 mg kg⁻¹, and 60 mg kg⁻¹ for *G. margarita*, favoring increases in the dry mass of castor bean at lower P levels in the soil.

In relation to mycorrhizal dependence, Rangpur lime presented a dependence of 33.11% when inoculated with *Glomus clarum* and 14.88% when inoculated with *Glomus etunicatum* (Table 9). According to the classification of Habte and Manjunath (1991), these values express moderate mycorrhizal dependence (25-50%) for *G. clarum* and marginal (<25%) for *G. etunicatum*, while the other rootstocks presented marginal dependence to these mycorrhizal fungi. However, these values allow affirming that the rootstocks presented low mycorrhizal dependence, and that the substrate fertility was sufficient to promote greater growth of plants.

Thus, mycorrhizal fungi do not have host specificities, that is, a plant species can form a mycorrhizal association with the most diverse species of AMFs, but the results of this symbiosis can vary according to the fungus-soil-plant interaction (CAVALCANTE et al., 2009), which depends on the need of nutrient uptake by the host and their available amounts, especially P, in the soil (HIPPLER, MOREIRA, 2013; SENNA et al., 2014),

as well as water content, pH, salinity, temperature, among other environmental factors (MEHROTA, 2005).

In relation to the percentage of mycorrhizal colonization, there was no significant interaction between rootstocks and inoculation with *Glomus clarum* and *Glomus etunicatum* (Table 10). Nunes et al. (2006) evaluated the mycorrhizal colonization of citrus rootstocks and found that at higher P levels, the colonization rate decreased for some rootstocks. Similarly, in non-fertilized soil with low P levels, the effects of mycorrhization were more satisfactory for *Annona squamosa* L., Annonaceae (COELHO et al., 2012) in non-fertilized soil with low P content. These results may explain that the low colonization rate was due to the high amount of P in the substrate.

The results obtained in this study allow concluding that each plant species may have different interactions, when associated with several AMFs, even when submitted to the same environmental conditions. According to Melo et al. (2016), citrus varieties can affect the amount of AMFs and P solubilizers in the rhizosphere. According to the authors, the six citrus varieties evaluated in their research (Pera D9, Rubi, Pineapple, Pera D12, Page and Pera C21) were all colonized by mycorrhizal fungi, but the Page variety had higher number of glomerospores and Pineapple, higher number of colony forming units (CFU / mL) of total fungi.

In this study, rootstocks had little dependence on mycorrhizal fungi, but high on substrate fertility. However, several studies have shown that there is a significant compatibility of citrus in association with AMFs (MELLONI et al., 2000; DECARLOS NETO et al., 2002; NUNES et al., 2006).

Therefore, it could be concluded that the fertility of the substrate to be used, especially with respect to phosphorus, should be an important factor to be considered when studying the mycorrhizal dependence of plant species, since its maximum or minimum amounts has influence proportionally inverse to the mycorrhization.

Table 1. Analysis of Variance for the height of rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin submitted to the management with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control at 90 days of evaluation.

SV	DF	SS	AS	F
Rootstocks	2	152.79433	76.39717	14.0238**
Managements	4	131.36733	43.78911	8.0381**
Rootstocks x Managements	6	156.69767	26.11628	4.7940**
Treatments	11	440.85933	40.07812	7.3569**
Residue	48	261.48800	5.44767	
Total	59	702.34733		

** Significantly at 1% of probability (p < 0.01) SV = source of variation; DF = degree of freedom; SS = sum of squares; AS = average square; F = F test.

Table 2. Averages of the height variable for rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin, submitted to the management with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control at 90 days.

Rootstocks	Averages (cm)
Rangpur lime	11.62a*
Swingle citrumelo	11.99a
Sunki mandarin	8.43b

(*) Means followed by the same letter in the column are not significantly different according to Tukey's test at 5% of probability.

Table 3. Averages of the height provided by the managements with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control, applied to the rootstocks, at 90 days of evaluation.

Managements	Averages (cm)
Mineral fertilization	13.00a*
<i>Glomus clarum</i>	9.08b
<i>Glomus etunicatum</i>	10.82ab
Control	9.79b

(*) Means followed by the same letter in the column are not significantly different according to Tukey's test at 5% of probability.

Table 4. Analysis of Variance for stem diameter (cm) of the rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin submitted to the management with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control at 90 days.

SV	DF	SS	AS	F
Rootstocks	2	0.09433	0.04717	40.42.86**
Managements	4	0.05000	0.01667	14.2857**
Rootstocks x Managements	6	0.02300	0.00383	3.2857**
Treatments	11	0.16733	0.01521	13.0390**
Residue	48	0.05600	0.00117	
Total	59	0.22333		

** Significantly at 1% of probability ($p < 0.01$) SV = source of variation; DF = degree of freedom; SS = sum of squares; AS = average square; F = F test.

Table 5. Averages of the stem diameter variable for rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin, submitted to the management with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control at 90 days.

Rootstocks	Averages (cm)
Rangpur lime	0.29b*
Swingle citrumelo	0.32a
Sunki mandarin	0.23c

(*) Means followed by the same letter in the column are not significantly different according to Tukey's test at 5% of probability.

Table 6. Averages of the stem diameter provided by the managements with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control, applied to the rootstocks, at 90 days of evaluation.

Manejos	Averages (cm)
Mineral fertilization	0.26b*
<i>Glomus clarum</i>	0.26b
<i>Glomus etunicatum</i>	0.33a
Control	0.26b

(*) Means followed by the same letter in the column are not significantly different according to Tukey's test at 5% of probability.

Table 7. Analysis of Variance for number of leaves of the rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin submitted to the management with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control at 90 days.

SV	DF	SS	AS	F
Rootstocks	2	28.03333	14.01667	0.8397ns
Managements	4	1213.93333	202.64444	24.2423 **
Rootstocks x Managements	6	152.76667	25.46111	1.5254 ns
Treatments	11	1394.73333	126.79394	7.5962
Residue	48	801.20000	16.69167	
Total	59	2195.93333		

** Significantly at 1% of probability ($p < 0.01$); ns = not significant ($p \geq 0.05$); SV = source of variation; DF = degree of freedom; SS = sum of squares; AS = average square; F = F test.

Table 8. Averages of the number of leaves provided by the managements with mineral fertilization, inoculation with *Glomus clarum*, inoculation with *Glomus etunicatum* and control, applied to the rootstocks, at 90 days of evaluation.

Manejos	Médias
Mineral fertilization	16.80a*
<i>Glomus clarum</i>	6.46b
<i>Glomus etunicatum</i>	5.93b
Control	6.93b

(*)Means followed by the same letter in the column are not significantly different according to Tukey's test at 5% of probability.

Table 9. Mycorrhizal dependence of the rootstocks regarding the AMFs *Glomus clarum* and *Glomus etunicatum*.

Porta-enxertos	Control	<i>Glomus clarum</i>	<i>Glomus etunicatum</i>
		%	
Rangpur lime	0	33.11	14.88
Swingle citrumelo	0	3.21	7.86
Sunki mandarin	0	3.22	5.73

Table 10. Analysis of Variance for mycorrhizal colonization of the rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin, submitted to the inoculation with *Glomus clarum* and *Glomus etunicatum* at 90 days.

SV	DF	SS	AS	F
Rootstocks	2	0.20192	0.10096	2.5161 ns
Managements	1	0.00108	0.00108	0.0269 ns
Rootstocks x Managements	2	0.17192	0.08596	2.1423 ns
Treatments	5	0.37492	0.07498	1.8688 ns
Residue	24	0.96300	0.04013	
Total	29	1.33792		

*ns = not significant ($p \geq 0.05$); SV = source of variation; DF = degree of freedom; SS = sum of squares; AS = average square; F = F test.

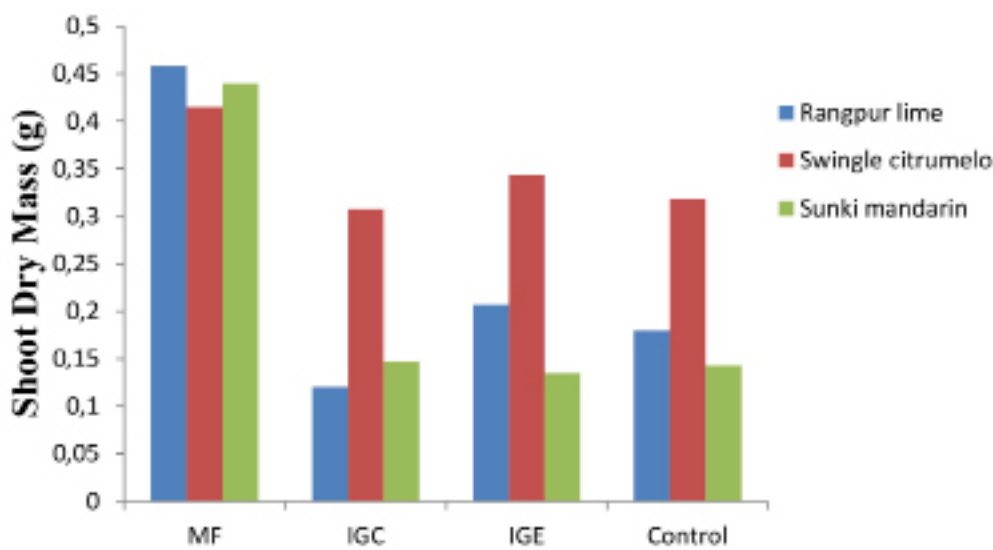


Figure 1. Shoot Dry Mass (g) of the of the rootstocks Rangpur lime, Swingle citrumelo and Sunki mandarin, submitted to the management with mineral fertilization (MF), inoculation with *Glomus clarum* (IGC), inoculation with *Glomus etunicatum* (IGE) and control at 90 days.

Conclusion

The mycorrhizal association of *Glomus clarum* and *G. etunicatum* was not presented as the best management for the growth of Rangpur lime, Swingle citrumelo and Sunki mandarin plants when compared to mineral fertilization. However, the inoculation of mycorrhizal fungi provided a positive effect on the uptake of some nutrients by rootstocks, especially K, Mg, N and P.

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