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Arbuscular mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq

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ABSTRACT

Objective. We evaluated the effect of arbuscular mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq plants with the application of *Glomus* spp. Zac- 19 (*G. claroides*, *G. diaphanum* and *G. albidum*) to assess growth under greenhouse conditions without fertilizer application.

Methodology. The evaluated treatments were inoculation with 2,4,6,8, and 10 g of inoculum, plus a control without inoculation in an experimental design of complete randomized blocks with three replications. Variables were plant height, stem diameter, number of leaves, foliar area, dry weight, and mycorrhizal colonization.

Results. A statistically significant response ($p \leq 0.01$) to inoculation was observed, registering higher growth of mycorrhizal plants. The inoculation was considered appropriate for all the levels evaluated. The outstanding treatment was 10 g of inoculum that produced plants with 110.16 cm in height for the last sampling; 38.56 leaves per plant; 1.13 cm stem diameter; 35.95 g dry weight of root; 76.88 g dry weight of the aerial part of the plant; 225.03 cm^2 of leaf area per plant and 88.87% of mycorrhizal colonization.

Conclusions. The application of 10 g per plant to the roots of *Citrus volkameriana* from the *Glomus* Zac-19 arbuscular mycorrhizal consortium promoted the production of more vigorous plants for grafting, without the application of chemical fertilizer.

Keywords: Microorganisms, Glomus spp Zac- 19 consortium

INTRODUCTION

In the state of Campeche, Mexico, it is estimated that there are 7220 ha of commercial orchards and 2101 ha of backyard orchards in citrus reconversion with the use of patterns resistant to the citrus quick decline disease virus (Citrus Tristeza virus, in Spanish VTC). The sour orange tree (*Citrus aurantium*) had been the most used grafting rootstock until the appearance of the Tristeza virus, which has led to the search for other rootstocks, such as the lemon tree *Citrus volkameriana* Tan & Tasq, which is tolerant to *Phytophtora parasitica* and VTC, tolerant to calcareous soils such as those existing in the Yucatán peninsula, Mexico (Cruz and de la Garza, 2003). Given the need to have quality rootstocks produced in the nursery, the possibility of

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using arbuscular mycorrhizal fungi (in Spanish HMA) arises because they establish a symbiotic relationship with plants (Smith and Read, 2008). Their main effect is the increased efficiency in the absorption of nutrients by the plant due to the extra radical hyphae. Many researchers have recorded an increase in the absorption of N, P, by plants and increasing in their growth (Zaidi *et al*., 2003). In addition, there is evidence that mycorrhizae-enhanced citrus plants have better growth compared to non-mycorrhizae plants (Srivastava *et al*., 2002). Thus, it becomes important the mycorrhizal effect on the production of citrus plants in nurseries; as the initial part of the citrus production process, when they will be grafted and afterwards, taken to the field. The effectiveness of HMA in the production of citrus seedlings has been demonstrated in several studies (Alarcon and Ferrera-Cerrato, 1999). Effects of mycorrhizal inoculation on *Citrus volkameriana* rootstocks were found by Haghighatnia *et al*., (2011) when registering higher growth in plants, expressed in height, stem diameter, number of leaves, dry weight and nutrient content. Authors such as González-Chávez and Ferrera-Cerrato (2000) with the use of phosphoric rock as fertilizer in sour orange tree and *Glomus* spp Zac-19 (*G. claroides*, *G. diaphanum* and *G. albidum*), registered increases in seedlings growth from 135 days after transplantation (in Spanish, ddt); the height of the inoculated plants was three times greater than the height of uninoculate plants. In a more recent study in sour orange plants (*C. aurantifolia*) inoculated with different species of mycorrhiza, Orton and Ustuner (2014) observed that after ten months the mycorrhizal plants showed increments in height, number of leaves and dry weight. Others like Navarro *et al*. (2015) obtained effects by mycorrhizal inoculation, in plant height and fresh weight of *C. macrophyla*. Similar results were obtained by Qiang-Sheng and Ying-Ning (2015) in *Citrus tangerine* with *Glomus mosseae* in plant height, stem diameter, plant dry weight and photosynthetic index. The HMA inoculation in *Citrus volkameriana* Tan & Pasq seedlings showed plants 60 cm in height and 5 mm in diameter at the base of the stem at 120 days without the addition of fertilizer or fungicides. This size was considered sufficient to perform the graft (Quisehuatl *et al*., 2015). Based on the above, this study aimed to evaluate the effect of mycorrhizal inoculation with the *Glomus* spp Zac-19 consortium on the growth of the *Citrus volkameriana* Tan & Pasq. rootstock.

MATERIALS AND METHODS

The experiment was established in the nursery of the Secretaría de Desarrollo Rural del gobierno del estado de Campeche, Mexico, using clay soil, which was previously sterilized. Certified seed of *Citrus volkameriana* Tan & Pasq from Cuba, which was pretreated by applying an immersion bath in neutral water for 48 h for pre-germination and avoiding the use of vain seed. Before sowing the seeds were disinfected with 5% sodium hypochlorite for 7 min, and then rinsed with neutral water. The seeds were sown in 200-cavity plastic trays, which were given the same seed cleaning treatment. Peat moss was used as substrate and sowing was carried out on January 10, depositing a seed in each cavity of the trays at a depth of 2 cm. Subsequently, the trays were covered with black plastic in order to homogenize and accelerate germination, placing them in a cool and dry place. On the fifth day after sowing (in Spanish, dds), the black plastic was removed and the trays were arranged in terraces, waiting for the total emergence of the seedlings, an event that began at 15 dds and lasted eight days.

The transplant substrate was a mixture of pure forest soil, which was sterilized with a 456 g (1 lb) can of methyl bromide. After 7 d of fumigation and an aeration period of 3 d, the substrate was used to fill polyethylene nursery bags with a volume of 2.1 kg. On March 14 (63 dds) plants were transplanted to black polyethylene bags, also inoculating the roots of the plants with the *Glomus* spp Zac-19 consortium, with approximately 100 spores in the root of alfalfa (*Medicago sativa* L.) colonized in 72%. The plants were extracted from the trays, and a small blow was given to extract the root ball, contacting the root zone of each plant with the inoculum in the dose corresponding to each treatment.

The inoculum that did not adhere to the root was applied directly to the bottom of the bag. Then, each bag was filled with the forest soil used for transplanting the plants. Once the inoculation and transplantation were finished, the plants were irrigated. The treatments were defined by the use of five doses of mycorrhizal inoculation with the *Glomus* spp Zac-19 consortium: 2, 4, 6 8 and 10 g, plus an uninoculated control, in total six treatments, arranged in an experimental design of complete randomized blocks, with three replications. The experimental unit consisted of a group of 25 plants, with a separation of 1 m between units. The treatments were randomly assigned to each experimental unit within each block, with a separation between them of 1 m. Within each experimental unit, 16 plants were sampled throughout the term of the research. Evaluated variables were: plant height, stem diameter, number of leaves, foliar area (integrator of foliar area LI-COR model li-3100), dry weight of root, dry weight of the aerial part of the plant, and percentage of mycorrhizal colonization (Philips and Hayman, 1970). The statistical analysis of the results was carried out by means of an analysis of variance and Tukey's mean comparison test (α=0.05), with the use of the statistical program Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Plant height and stem diameter

Growth in plants of *Citrus volkameriana* was attributed to the effect of the arbuscular mycorrhizal inoculation with *Glomus* spp Zac-19, observed effect from 60 d after transplantation (ddt) in plant height, stem diameter, number of leaves per plant and leaf area (data not shown). The height of the *Citrus volkameriana* plants inoculated with 10 g of the inoculum showed larger plants from 60 ddt and up to 240 ddt, which was attributed to better plant nutrition, because these fungi improve the availability of nutrients for the plant, specially nitrogen and phosphorus (Table 1). Mycorrhizal plants showed more vigorous plants and uniform green coloration. The magnitude of the difference in the plant height for the best treatment (10 g), compared to the control treatment was 78.66 cm, with a final growth per day for the treatment of 10 g of inoculum in the order of 2.62 cm.

These results coincide with those found by Alarcón and Ferrera-Cerrato (2003) who found significant effects in this variable due to the effect of mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq, considering that HMA are nutritional enhancers (nitrogen and phosphorus), since they favor the concentration and synthesis of amino acids. The results found also coincide with what was observed by Dixon *et al*. (1989) because in their work with *Glomus fasciculatum* and *G. etuunicatum* in *Citrus janbhiri*, they observed that the use of mycorrhization caused greater growth in inoculated plants. The effect of mycorrhization in nursery plants, on plant height has been shown in various species; thus Ruiz *et al*. (2016) registered taller plants of papaya (*Carica papaya* L.), guava (*Psidium juagava* L.) and avocado (*Persea* sp.), when inoculated with different strains of arbuscular mycorrhizal fungi in two types of soil.

Results similar to those found for plant height were found for stem diameter, where the best treatment was 10 g, finding that eight months after transplantation (in November), the average diameter in the inoculated plants was 1.03 cm, while in the control plants the average diameter per plant was 0.45 cm, a difference that resulted in vigorous *C. volkameriana* plants (Table 1).

Obtained results agree with that established by Antunes and Cardoso (1991), who report that the diameter of the stem required in plants for grafting is 5 mm after six months after transplantation, a diameter exceeded in *Citrus volkameriana* plants with any of treatments with inoculation. The results found match those observed by Alarcon and Ferrera-Cerrato (2003) in *C. volkameriana* plus the inoculation with *Glomus* spp, Zac-19 to obtain at 210 ddt plants with 95% more diameter than the non-inoculated ones (45 mm). The above suggests that mycorrhizal symbiosis has a favorable

Table 1. Average height (cm) and diameter (cm) of *Citrus volkameriana* Tan & Pasq. Plants, due to the effect of mycorrhizal inoculation with *Glomus* spp. Zac-19.

Inoculum (g)	Days after transplant							
	150		180		210		240	
	AP	DT	AP	DT	AP	DT	AP	DT
0	13.743d	0.18b	21.85d	0.27c	27.81c	0.40c	29.78c	0.45c
2	21.35c	0.57a	33.86c	0.74b	60.93b	0.92ab	77.79b	1.02b
4	22.62c	0.57a	34.67c	0.74b	60.89b	0.92ab	78.84b	1.02b
6	30.99ab	0.57a	60.93a	0.73b	76.76a	0.99ab	94.10a	1.04ab
8	27.38b	0.60a	49.47b	0.69b	69.54ab	0.87b	100.34a	0.98b
10	32.02a	0.62a	61.76a	0.82a	77.75a	1.03a	102.34a	1.13a
DMSH	4.50	0.09	10.42	0.07	11.39	0.13	10.44	0.10

Treatments with the same letter are statistically equal (Tukey, α=0.05).

effect on obtaining healthy and vigorous plants in less time to graft them. Additionally, obtaining transplant plants with a larger stem diameter will offer advantages in their management and transfer from the nursery to the orchard: structurally stronger plants, capable of withstanding adverse environmental conditions, such as the action of winds and competition with other plant species for water and light.

Number of leaves and dry weight

Vegetative growth of *C. volkameriana* was favored by inoculation with *Glomus* spp. Zac-19 (Table 2). All the treatments with inoculation exceeded the average number of leaves to the treatment without inoculation in all the samplings. At 240 d, it was observed that the plants inoculated with 10 g obtained up to 77.45% higher in number of leaves compared to the control. In evaluations with different types of mycorrhizae and soil types, it was found that the inoculated plants presented 56.25% more leaves, compared to the treatment without inoculation (Ortas and Ustumer, 2014), a value lower than that found in the conditions of our research. This implies a production of citrus plants in the nursery with greater growth and vigor due to the effect of mycorrhizal inoculation, a result of the benefits attributed to a better absorption of nutrients by the plant. Pimienta-Barrios *et al*. (2009) complement this by pointing out an increase in photosynthesis, which is expressed as a better establishment in the field, thus representing an adaptive advantage.

The mycorrhizal plants had higher dry and fresh leaves weight compared to plants not inoculated, and a similar tendency was observed for the total leaf area (Table 2). On average, the inoculated plants presented 13.88 g of root dry weight and 49.12 g of dry weight of the aerial part of the plant, higher values compared to not inoculated plants of 12.26 and 10.80 g, respectively. The outstanding treatment for its effect on the dry weight of the plant and the foliar area was the mycorrhizal inoculation with 10 g of inoculant per plant, which was related to presenting plants with greater height, number of leaves and foliar area compared to the control treatment.

The higher value of fresh and dry weight of the plants is attributed to a greater assimilation of nutrients by the plants, an indirect measure of their photosynthetic activity. At evaluations with the mycorrhizal consortium *Glomus* spp. Zac-19 on *Citrus volkameriana*, Alarcon and Ferrera-Cerrato (2003) found a significant effect for dry weight on inoculated plants. Similar effects were also found by Ruiz *et al*. (2016) when evaluating six strains of HMA, in avocado, guava and papaya, where the inoculated plants showed higher fresh and dry weight compared with plants without inoculation. The use of mycorrhizal fungi to produce nursery plants should be considered as a practice to use by the plant producer, since these microorganisms deserve special attention for their positive effect on plant growth by increasing the radical absorption efficiency of nutrients (Viera *et al*., 2017). The root dry weight/dry weight ratio of the aerial part of the plants, for all the amounts of inoculum used, was on average lower in the inoculated plants (0.43) than in the uninoculated plants (1.13). These are similar results to those found in *Capsicum* by Aguilera and Gomez *et al*. (1999). These results indicate a greater distribution of carbon from the root towards the areal portion of the plant, meaning a greater foliar expression. Regarding leaf area, its increased expression in plants *C. volkameriana* was attributed to the effect of mycorrhizal inoculation, due to the fact that all the treatments inoculated presented higher average values of leaf area (190.89), compared to the control (92.51 cm^2). The increase in biomass from the expanded leaf area should be considered as a

Table 2. Average number of leaves per plant, dry weight of root and dry weight of stem and leaves per plant of inoculated plants of *Citrus volkameriana* Tan & Pasq. due to the effect of mycorrhizal inoculation with *Glomus* spp. Zac-19.

Inoculum (g)	Number of sheets (dat)				Dry weight (g)		
	150	180	210	240	raíz	Tallo y hojas	Área foliar
0	16.42d	18.17e	19.56d	21.73c	12.26c	10.80d	92.51 e
2	26.63b	24.96d	32.33bc	34.95ab	22.54b	43.81c	171.28d
4	26.48b	29.85b	34.96ab	36.97a	22.60b	56.06 b	176.43cd
6	26.43b	33.58a	35.36ab	37.31a	24.20b	61.07b	185.22bc
8	21.56c	27.41c	29.52c	32.52b	25.45b	61.78b	196.50b
10	31.76a	33.69a	36.48a	38.56a	35.97a	76.88a	225.03a
DMSH	4.42	2.24	3.23	4.07	6.16	9.87	11.30

source of photo assimilate production (Cookson *et al.*, 2005). Therefore, it is a critical variable for productivity.

Total mycorrhizal colonization, colonization per vesicle, number of colonized segments and number of segments per vesicle

The fundamental aspect in all mycorrhizal inoculation study consists in determining whether the inoculated fungi establish the symbiotic association with the roots of the plants. According to the results obtained, it can be established that there are highly significant statistical differences for the variables Total colonization, Colonization per vesicle (Figure 1). It is possible to say that *C. volcameriana* is satisfactorily colonized by the *Glomus* spp Zac-19 consortium. Our percentages of mycorrhization were very good, if the report by González-Chávez and Ferrera-Cerrato (2000) is taken as a reference, from their research with sour orange, where they obtained maximum values close to 60%. These results indicate that the roots inoculation of *C. volkameriana* with 10 g of inoculum of the mycorrhizal consortium Zac-19, gave as result the root-fungus symbiosis, as it is shown from the values of total colonization and colonization per vesicle, in the order of 88.87% and 88.79%, respectively. There are higher values compared to those reported for other horticultural, fruit and forestry species such as: Tree tomato (*Solanum betaceum* Cav) 22.8% (Viera *et al.*, 2017); grasses *Brachiaria brizantha* cv. Toledo, *B. dictyoneura* cv. Llanero, *Desmodium ovalifolium* cv. Maquenque, *Panicum maximum* (CIAT 36000) and *Paspalum notatum*, 47 A 94% (Monroy *et al.*, 2013); blueberry 9.8-15% (Bautista *et al.*, (2017). In *Citrus aureantium* (Ortas and Ustuner (2014) when working with different types of soil and mycorrhiza species, they found colonization percentages from 17.33 to 50.34%, and that are lower values than 86.27% as the total colonization found in our research. Results agree with those obtained by Gonzalez-Chavez and Ferrera-Cerrato (2000), who in their research with two citrus species, they found colonization percentages from 37 to 64%. The results obtained allow establishing affinity between the *Glomus* spp Zac-19 consortium and the species evaluated in our study, suggesting that this species is susceptible to colonization by this type of endophytes, which induces a response in the growth of the inoculated lemon plants (Figure 1).

The high percentages of total colonization in the roots of the inoculated plants are expressed as a better response in variables such as plant height, number of leaves per plant, stem diameter, dry weight and foliar area, which allows us to assume that the symbiotic association with mycorrhizal fungi resulted in favorable changes on growth rates and produced biomass.

CONCLUSIONS

The management practice of mycorrhizal inoculation to the root system of *Citrus volkameriana* Tan & Pasq. plants at the time of transplantation is important because the plants presented advantages in growth compared to non-mycorrhizal ones. This gave advantages to those plants for their establishment in the orchard, in addition to a reduction in cultivation costs due to the effect of natural fertilization.

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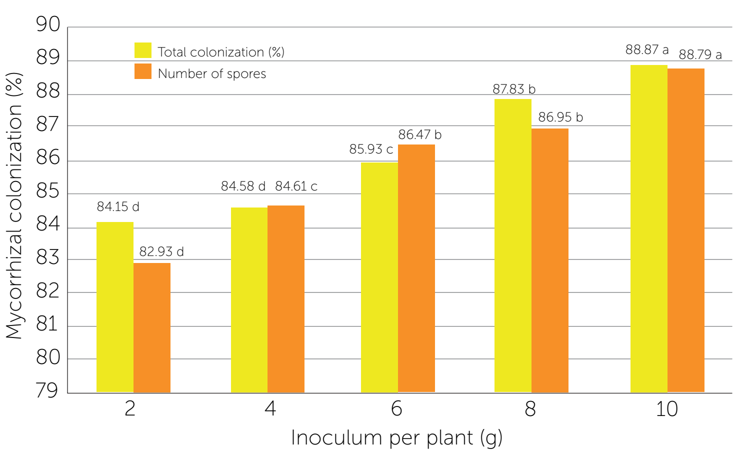

Figure 1. DMSH: 0.94 and 1.32 for total colonization and vesicle colonization, respectively. Treatments with the same letter are statistically equivalent. (Tukey, α=0.05)

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