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ORIGINAL ARTICLE

Effects of inoculation with mycorrhizae and the benefits of bacteria on physicochemical and microbiological properties of soil, growth, productivity and quality of table grapes grown under Mediterranean climate conditions

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Abstract

Excessive use of chemical fertilizers, in agriculture, has negative impacts on water, soil and affects the environment and health. In recent decades, researchers have been interested in the natural benefits of natural microorganisms and how they could be a good alternative to the use of chemical fertilizers. The aim of this study was to investigate the effect of soil inoculation with strains of mycorrhizae and beneficial bacteria on soil properties and productivity of table grapes. Field trials were conducted on a commercial table grape production farm (*Vitis vinifiera* cv. Mousca), located in northeastern Morocco. Twelve-year-old plants were used. Control plants were not inoculated (T1). The prototype plants were inoculated with 1.2×10^4 of *Glomus iranicum* var. *tenuihypharum*/100 g (T2), a mixture of 1/2 concentration of *Glomus iranicum* var. *tenuihypharum* and 1/2 concentration of *Pseudomonas putida* (T3) and 1×10^8 CFU · g^{-1} of *Pseudomonas putida* (T4). The inoculations were realized twice; the first inoculation was completed on July 19, 2019 while the second inoculation on February 21, 2020. Soil analyses were carried out, both physicochemical (pH, electrical conductivity (EC), salinity, % of dry matter) and microbiological properties (total flora, fungi and actinobacteria). Plant growth (length of the plant, number and diameter of sticks, number of clusters per tree, number of nodes per stick, distance between nodes and bud burst), yield and fruit quality (number of berries per cluster, cluster weight, cluster length and width, pH, Brix degrees, acidity, EC and % dry matter) were measured. Results showed slight trends regarding the effects of treatments on the physicochemical and microbiological properties of the soil, plant growth and fruit quality. The number of clusters was significantly higher in *Glomus* (T2) *Pseudomonas* (T4) and *Glomus* than in control treatments.

Keywords: *Glomus iranicum*, fertilizer, nutrition, *Pseudomonas putida*, symbiosis, yield

Introduction

During the last decades, the world population has known considerable growth accompanied by an increased need for food products. Since the birth of intensive agriculture, the massive use of fertilizers and plant protection products has taken place to maximize agricultural production (Aktar *et al.* 2009). High productivity is linked to chemical inputs, in particular fertilizers which enrich the soil with essential nutrients for plant growth, and increase yield and productivity (Yousaf *et al.* 2017). However, the excessive use

of chemicals causes serious problems for the environment and human health (Childers *et al.* 2011; Riah *et al.* 2014). This intensive use influences the quality of soils and aquatic environments if the needs of crops are largely exceeded. According to Shaver and Chapin (1986), using fertilizer showed a large accumulation of heavy metals in plants. In addition, the presence of cadmium in certain fertilizers at high concentrations is of great concern because of the toxicity of this metal, and its ability to accumulate in plant tissues (Atafa *et al.* 2010). Also, excessive application of chemical fertilizers leads to the accumulation of nitrate in plant products (Ye *et al.* 2020), which is harmful to human health (Hmelak Gorenjak 2013). Other effects have been demonstrated by a multitude of studies (Criss and Davisson 2004; Farhadinejad *et al.* 2014). Consequently, there is a need to develop strategies in order to optimize soil fertility and biological diversity, by creating forms of agro-systems that respect natural and ecological processes and ensure reasonable crop productivity. This challenge has led researchers to look for alternatives, such as the use of natural organic fertilizers and substitutes for chemical fertilizers (Geng *et al.* 2019). This can still be done when the properties of the soil allow it. However, this substitution remains limited because of the increase in production costs due to the competitiveness of chemical fertilizer costs. However, scientists want to keep a small portion of chemicals in the fertilization input for crops but use natural soil stimulants based on beneficial microorganisms to stimulate humification and mineralization of the soils in order to improve plant assimilation of nutrients (Halpern *et al.* 2015; Popko *et al.* 2018). This presents a good alternative since it establishes a sustainable agriculture which uses bio-fertilizers instead of chemical fertilizers.

To reduce the application of chemical fertilizers inoculating the soil with beneficial microorganisms such as mycorrhizae is a good alternative (Koide and Mosse 2004). The majority of vascular plants are mycorrhizal; 72% are arbuscular mycorrhizae fungi (AMF), 2% are ectomycorrhizae (EcM), 1.5% are ericoid mycorrhizae (ErM) and 10% are orchid mycorrhizae (OrM) (Brundrett and Tedersoo 2018). The plant-fungal relationship has been the subject of many studies for evaluation of the effects of this symbiotic relationship (Neuenkamp *et al.* 2018; Ronga *et al.* 2019). The AMF are key nutrient components (Johansson and Paul 2004) which allow plants to expand their roots and eventually reach poorly available nutrients like phosphate, nitrogen and microelements, as well as water. In return, plants provide these fungi with sugars and lipids (Jiang *et al.* 2017). This symbiosis is believed to be beneficial for plant development, yield and biomass accumulation (Liu *et al.* 2018). Aguín *et al.* (2004) studied the effects of inoculation with AMF mycorrhizae on three grapevine rootstocks on morphology and growth. The results showed that inoculation of *Glomus aggregatum* into the rooting zone of vine cuttings altered root morphology and increased branching of lateral roots. In addition, when rooted cuttings were transplanted into pots, with a sufficient amount of phosphorus in the soil and in combination with AMF, a significant improvement of growth of two inoculated rootstocks was noted. On the other hand, Trouvelot *et al.* (2015) showed that AMF increases tolerance to abiotic stresses such as water stress, soil salinity, ferric chlorosis and heavy metal toxicity and also protects against biotic stresses, such as root diseases. In the same context, trials on carrot and green onion demonstrated that soil inoculation with AMF decreased plant residues of Phoxim, a popular organophosphate insecticide used against agricultural crop pests (Wang *et al.* 2011). Moreover, Ozdemir *et al.* (2010) observed that *G. mosseae* and *G. intraradices* promoted shoot and root growth as well as leaf phosphorus and zinc contents. As for EcM fungi, they can transfer nitrogen from one plant to another, increasing the use of different forms of nitrogen by plants. AMF improved the potassium nutrition of plants (Garcia and Zimmermann 2014) and it can also increase the uptake of other nutrients by plants (Birhane *et al.* 2012). However, AMFs could reduce nutrient leaching from the soil (Rodriguez-Echeverria *et al.* 2007). Bender *et al.* (2015) showed that inoculation with AMF increased nutrient uptake by plants, and reduced leaching of dissolved organic nitrogen and phosphorus. Soares and Siqueira (2008) demonstrated that phosphorus-based fertilization and inoculation of plants with AMFs significantly improved plant growth in soils polluted with heavy metals. Also, several studies have shown that AMFs increase the tolerance of plants to drought and water stress (Meddich *et al.* 2015; Boutasknit *et al.* 2020). In this context, by inoculating plants with drought tolerant AMF, a reduction of up to 42% in the water requirements of the plants could be achieved (Gianinazzi *et al.* 2010). This has led researchers to study the mechanisms by which AMF increases the tolerance of plants to drought which include: nutritional mechanisms, hormonal changes, improvement of hyphal soil, ability of hyphae to trap water in micropores, increased rate of photosynthesis in plants and accumulation of compatible osmolytes and other mechanisms (Birhane *et al.* 2012). Regarding plant defense mechanisms, AMF increases the tolerance of plants to pathogens by stimulating plants to produce secondary metabolites (Gianinazzi *et al.* 2010). These produced metabolites are known to defend plants against a wide variety of pathogens. Very few studies have reported on the effects of *G. iranicum* and *Pseudomonas putida* on table grapes grown in the south-eastern part

of the Mediterranean area of Morocco. The important thing is that growers do not use natural and beneficial microorganisms to complement plant fertilizers. The objective of this work was to study the effects of mycorrhizal and bacteria on physicochemical and microbiological properties of the soil, growth, productivity and quality of table grapes (*Vitis vinifiera*, cv. Mousca), a crop which has great economic value and uses significant amounts of chemical fertilizers.

Materials and Methods

Experimentation site

The field experiments were conducted on a production farm of 35 ha located in Sector 2, El Garet, Al Aaroui, Morocco (latitude: 34°56'10.8"N and longitude: 3°00'19"W). This region is characterized by a Mediterranean climate with an average annual temperature of 17.3°C and an average precipitation of 500 mm. The experiments were carried out on 12-year-old table grape trees (*V. vinifera*) (Fig. 1). The field plots covered 8 ha of the farm and production is intended for the local market. The cultivar was Muscat of Italy. Crops were irrigated and fertilized with a drip irrigation system. Production was managed according to the table grape production practices in Morocco. The planting density was 2,000 plants · ha^{-1} and "Pergola" was the structure used for the plantations.

Treatments

Table grape plants were inoculated with microorganisms (fungi and bacteria). This inoculation was carried out above the root system. Two solutions were used:

- solution (A) contained 1.2×10^4 CFU · 100 g^{-1} of *G. iranicum* var. *tenuihypharum*;
- solution (B) contained 1×10^8 CFU · g^{-1} of *P. putida.*

We used the following treatments:

- T1 = control (without inoculation);
- T2 = *G. iranicum* var. *tenuihypharum* (or solution A);
- T3 = *G. iranicum* var. *tenuihypharum* + *P. putida* (or mixture of 50% concentration of solution A and 50% of concentration of solution B);
- T4 = *P. putida* or solution B.

The soil inoculation of plants with microorganisms was completed in two steps spaced by 7 months; the first inoculation was carried out on July 19, 2019 and the second inoculation was completed on February 21, 2020. The method of inoculation of plants with the solution product was done in the form of an injection of the product into the ground near the roots of trees or just under the dripper. The dose used for the injection was 1.5 mg of product diluted in 15 ml of water for each tree. This represents a dose of 3 kg · ha^{-1} (each hectare contained 2,000 trees). Except in the case of (T3), 0.75 mg of the solution product A and 0.75 mg of the solution product B were used and diluted in 15 ml of water for each tree.

Experimental plan

The area of field trees was subdivided into four plots of 2 ha. Each plot presented the repetition of the other plot. Each plot contained four rows where each row presented the repetition of the other row. Each row included the four treatments (T1, T2, T3, and T4). In each treatment, five Poteau (which contained two plants for each Poteau) were used. A total of 640 plants were used, where 480 plants were inoculated (T2, T3, T4). The overall formula used to determine the number of plants used is described as the following formulate:

Fig. 1. Photos of table grape parcels (cv. Muscat) grown with "Pergola" system

$$4\ \text{Treatments} \times \frac{2\ \text{Plants}}{\text{Poteau}} \times \frac{5\ \text{Poteau}}{\text{Row}} \times \times \frac{4\ \text{Rows}}{\text{Parcels}} \times 4\ \text{Parcels} = 640\ \text{plants}. \quad \text{(Eq. 1)}$$

Soil analysis

Soil samples were taken 3 months after the first inoculation. About 700 g to 1 kg were taken from the root area of each plant, 10 to 20 cm from the ground. Then, the samples were kept cool (at 4°C) for physicochemical and microbiological measurements (Reuter 2008). For soil moisture, a quantity of soil was placed in Petri dishes and heated for 24 h at 105°C. The difference in weights gives the water content removed by evaporation. The soil moisture percentage was determined using the formula:

$$\%\,\text{Moisture} = = \frac{\text{Wet soil weight} - \text{Dry soil weight}}{\text{Dry soil weight}} \times 100. \quad \text{(Eq. 2)}$$

For the pH, 5 g of dry soil was mixed in a beaker with 25 ml of distilled water. The mixture was stirred until it was completely combined. The mixture was allowed to stand until a supernatant layer formed. A calibrated pH meter probe was immersed in the supernatant layer to take the pH reading. For EC, a mixture of 1/5 soil sample and 4/5 distilled water was used. After stirring for a few minutes, a conductivity meter electrode was immersed into the mixture to take the reading in $\mu S \cdot cm^{-1}$. Conductivity and pH readings were generally recorded at a constant temperature of 20–25°C. The soil salinity was calculated based on the EC of the sample, and a KCl solution according to the following formula:

$$S = \frac{A \times 35}{B}, \quad \text{(Eq. 3)}$$

where: S – salinity of the sample; A – conductivity of the sample [$\mu S \cdot cm^{-1}$]; B – conductivity of KCl solution: 1,409 [$\mu S \cdot cm^{-1}$] at 25°C; 35 – salinity reference.

To measure and calculate the water retention capacity, a quantity of soil was place in an Erlenmeyer funnel covered with filter paper. Using a graduated cylinder, a precise amount of water was measured and then introduced into the soil, until the water dripped. The amount collected was measured. The water retention capacity was determined using the following formula:

$$\text{Water retention capacity} = = \text{Initial volume} - \text{Collected volume}. \quad \text{(Eq. 4)}$$

The study of the quantitative distribution of total flora, actinobacteria and fungi was also quantified. A quantity of 10 g of the soil sample was weighed and then placed in 90 ml of physiological water. This method of microbiological analysis of the soil was based on the dilution-spreading technique: (1) collecting 1 ml of suspension using a sterile pipette; (2) flaming and closing the tube; (3) opening the 9 ml tube of diluent, flaming the opening, introducing the collected volume into it without touching; (4) flaming and closing the tube; (5) serial or successive dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. Each dilution required a 9 ml tube of diluent and a sterile 1 ml pipette. For example, the 10^{-5} dilution was carried out by taking 1 ml of the previously homogenized 10^{-4} dilution which was introduced into a tube containing 9 ml of diluent. However, for each type of microorganism a culture medium was chosen. A preparation of the "Nutrient Agar", "PDA" and "Bennett" media was made, respectively, for the total flora, fungi and for actinobacteria. The different spreading steps are presented as follows: (1) homogenizing the dilution to be taken; (2) withdrawing a precise volume using a sterile pipette and placing 0.1 ml of the dilution in the center of the agar surface; (3) spreading with a rake pipette; (4) the Petri dishes used for the cultures were placed in an oven for incubation.

Plant growth and development

Initial growth measurements were taken on September 30, 2019 (2 months after inoculation of trees). These were: the initial length of the grape tree to the intersection with the sticks, the number of sticks per tree, length of sticks, number of clusters per tree, number of nodes per stick and the distance between nodes (Fig. 2). For this, one stick was chosen per tree to measure its length and the number of nodes that held each stick. The distance between two nodes was calculated using the formula:

$$\text{Space between nodes [cm]} = = \frac{\text{Length of stick [m]}}{\text{Number of nodes}}. \quad \text{(Eq. 5)}$$

Another series of growth measurements were taken on March 13, 2020, just after the second inoculation. These were: the initial length of the tree to the intersection with the sticks, the number of sticks per tree, the number of buds opened/closed per stick and the diameter of the stick. Only one stick was selected per tree for each measurement. The diameter in the middle of each selected stick was determined.

Fruit quality

Samples of grape fruits were taken in order to evaluate their qualities in the laboratory, respectively, on September 30 and October 3, 2019. One bunch of fruit

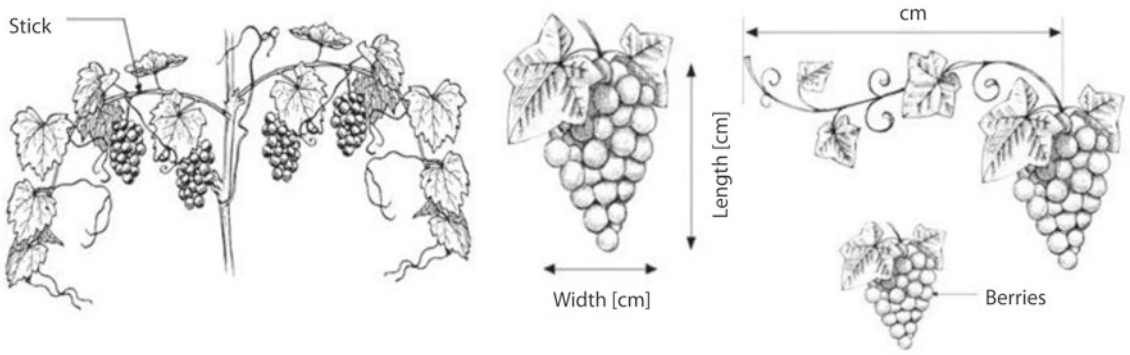

Fig. 2. Drawing illustrating part of a grape tree, showing the stick, clusters of grapes and the dimensions measured

per tree was taken at random. The length and width of the clusters were measured for each treatment (Fig. 2). Then, these clusters were weighed using a precision balance. The number of berries per cluster was also counted. The clusters were put in a blender to obtain grape juice which was used to measure pH, EC and Brix using a pH meter, a conductivity meter and a refractometer, respectively. The grape juice obtained was also used to measure the total acidity, which is the sum of the titratable acidities, when the solution is brought to pH of 7.0 by the addition of an alkaline solution. A quantity of 5 ml of grape juice was taken and placed in a dry Erlenmeyer flask at 250 ml. Three drops of Bromothymol Blue (BBT) were added. A titration of this solution was performed using a burette and 0.1 M sodium hydroxide (NaOH) solution. When the BBT value varied from yellow to blue green, the amount of NaOH was taken to determine the titration volume with NaOH. The percentage of acidity was calculated using the following formula:

$$\%\text{Acidity} = \frac{\text{Titration volume [ml]} \times \text{Coefficient}}{\text{10 ml of juice}} \times 100. \quad \text{(Eq. 6)}$$

The coefficient used for grapes is tartaric acid: 0.0075.

The Sugar/Acidity ratio was also calculated using the following formula:

$$\frac{\text{Sugar}}{\text{Acidity ratio}} = \frac{\text{Degrees Brix}}{\%\text{Acidity}}. \quad \text{(Eq. 7)}$$

To determine the percentage of dry matter in the fruit, bunches of grapes were weighed at the start and then were placed in an oven at 90°C. for a few days. The percentage of dry matter was calculated using the following formula:

$$\%\text{Dry matter} = \frac{\text{Dry weight}}{\text{Wet weight}} \times 100. \quad \text{(Eq. 8)}$$

Statistical analysis

Statistical analyses were performed using IBM-SPSS version 21 Software. For each parameter evaluated, replicates were taken for each treatment. The mean values obtained in the treatments were compared by one-way analysis of variance (ANOVA). While the differences revealed by ANOVA were globally significant, comparisons of means were subsequently performed by Tukey's Post-hoc Test. Data that were not normally distributed were analyzed with a nonparametric test, the Kruskal-Wallis test. The significance level was $p < 0.05$.

Results

The physicochemical and microbiological properties of the soil

Table 1 shows the physicochemical properties of the soils sampled in each treatment. The pH values were 7.3, 7.1, 7.1, 7.3 for the T1, T2, T3 and T4 treatments, respectively. The ANOVA test showed no significant differences between these treatments (ANOVA, $F = 0.68$; $p > 0.05$). The same observation was noted for EC (ANOVA, $F = 0.97$; $p > 0.05$), salinity (ANOVA, $F = 0.74$; $p > 0.05$) and soil moisture (ANOVA, $F = 1.50$; $p > 0.05$) or no difference was found between treatments. For water holding capacity (WHC), the values were slightly elevated in the two treatments T2, T3 and T4 compared to the control without inoculation. The values were 5.5, 7.8, 8.3 and 6.5 ml, respectively, for T1 T2, T3 and T4, but not significantly different (ANOVA, $F = 2.69$; $p > 0.05$).

Table 2 shows the number of microorganisms (total flora, fungi and actinobacteria) found in soils sampled in T2, T3 and T4 treatments. According to the Kruskal-Wallis test, there was no statistically significant difference between the number of microorganisms and the treatments. However, the number of fungi and actinobacteria seemed to be higher in the T3 treatment than in T2 and T4 treatments.

Table 1. Physicochemical proprieties of the soil measured for each treatment

Properties	T1	T2	T3	T4
pH	7,3 ± 0,3	7,1 ± 0,1	7,1 ± 0,3	7,3 ± 0,2
EC [mS/cm]	4,6 ± 1,2	5,6 ± 1,0	5,6 ± 1,1	4,8 ± 1,0
Salinity	0,114 ± 0,03	0,121 ± 0,01	0,139 ± 0,03	0,123 ± 0,02
Dry weight [g]	35,1 ± 2,5	34,4 ± 1,6	33,6 ± 0,2	33,3 ± 0,4
Humidity [%]	18,7 ± 0,4	19,0 ± 0,9	19,2 ± 0,7	20,0 ± 1,4
WHC [ml]	5,5 ± 1,0	7,8 ± 2,2	8,3 ± 1,3	6,5 ± 1,3

EC – electrical conductivity; WHC – water holding capacity of the soil

T1 = Control (without treatment); T2 = *Glomus iranicum* var. *tenuihypharum*; T3 = *Glomus iranicum* var. *tenuihypharum* + *Pseudomonas putida*; T4 = *Pseudomonas putida*. Data are an average of four repetitions ± standard deviation. Soil was sampled for each treatment on November 25, 2019. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020

Table 2. Enumeration of total flora, fungi and actinobacteria in the soils sampled for each treatment (CFU · g^{-1} of dry soil)

Treatments	T2	T3	T4
Total flora	$1{,}69 \times 10^6$	$2{,}03 \times 10^6$	$2{,}27 \times 10^6$
Fungi	*	$2{,}64 \times 10^3$	$1{,}26 \times 10^3$
Actinobacteria	$6{,}36 \times 10^3$	$9{,}37 \times 10^3$	$4{,}42 \times 10^3$

*missing data

T2 = *Glomus iranicum* var. *tenuihypharum*; T3 = *Glomus iranicum* var. *tenuihypharum* + *Pseudomonas putida*; T4 = *Pseudomonas putida*. Microbiological analyses and dilutions were performed on January 27, 2020. Data are an average of four repetitions ± standard deviation. Soil was sampled for each treatment on November 25, 2019. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020

Growth measurements

The initial measurements taken after 73 days of the first inoculation (Tab. 3) indicated that the number of sticks was the same in the T1, T2 and T4 treatments. The values were 4, 4 and 3 sticks per tree, respectively. Regarding the length of the sticks, no difference was noted. The length was 2.82, 2.58 and 2.54 m, respectively, for T1, T2 and T4. In addition, no difference was noted in terms of number and space between nodes for each treatment. After 21 days of the second inoculation, or 7 months after the first inoculation, the growth parameters of the plants were measured and the results showed that no differences were observed between the T1, T2, T3 and T4 treatments for the plant length, number of sticks/plant, number of total buds per stick, number of closed and open buds per stick (Tab. 4). The stick diameters of the four treatments were also similar and the values were 9.04, 10.06, 8.37 and 9.07 mm, respectively, for the T1, T2, T3 and T4 treatments.

Fruit yield and quality

For the number of clusters per tree, the values were 23, 28, 25 and 27 clusters/tree, respectively, for T1, T2, T3 and T4 (Tab. 5). The ANOVA test showed significant differences between the treatments; the Tukey Post-hoc test showed that the treatment with *G. iranicum* and *P. putida* was significantly different from the control and slightly different when compared to the co-inoculation of these two microorganisms, which testifies to the probable presence of a synergistic effect.

For the parameters of the quality of the grape fruits taken in the T1, T2 and T4 treatments, on two occasions, i.e., on September 30, 2019 and October 3, 2019, the results (Tab. 6) showed that the number of berries per cluster was the same for the first sampling, while it was higher in the T1 than in the T2 and T4 treatments, in the second sampling. The values were, respectively, 109, 74 and 68 berries per cluster for the T1, T2 and T4 treatments. The cluster weights were the same for the first sampling, while they were slightly higher in the T1 than in the T2 and T4 treatments, in the second sampling. The values were 739, 452 and 555 g berries per cluster, respectively, for the T1, T2 and T4 treatments. For the length and width of the clusters, they were the same in all treatments and for the two samples (S1 and S2). The pH was the same for all the treatments and in the two samples. The Brix degree was lower in T2 than in T1 and T4 during the first sampling. The values were 17.5, 15.5 and 17.6, respectively, for T1, T2 and T4. Acidity was the same for all treatments and for both samples. The Sugar/Acidity ratio was lower in T2 than in T1 and T4 during the first sampling. The values were 39.1, 26.7 and 39.7, respectively, for T1, T2 and T4. For EC, it was lower in T1 than in T2 and T4. On the other hand, it was lower in T4 during the second sampling. The % of the dry matter of the fruits was the same for all the treatments.

Table 3. Growth measurements taken in T1, T2 and T4, 73 days after the first inoculation

Parameters	T1	T2	T4
Number of sticks per tree	4 ± 1,0	4 ± 1,0	3 ± 0,0
Length of a sample of stick [m]	2,82 ± 0,40	2,58 ± 0,11	2,54 ± 0,15
Number of nodes per stick	44 ± 12	41 ± 2	37 ± 3
Space between nodes [cm]	6,59 ± 0,89	6,37 ± 0,06	6,85 ± 0,13

T1 = Control (without treatment); T2 = *Glomus iranicum* var. *tenuihypharum*; T3 = *Glomus iranicum* var. *tenuihypharum* + *Pseudomonas putida*; T4 = *Pseudomonas putida*. Data are an average of two repetitions ± standard deviation. Data were recorded on September 30, 2019. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020

Table 4. Plant growth parameters measured 21 days after the second inoculation

Parameters	T1	T2	T3	T4
Plant length [m]	2,59 ± 0,08	2,32 ± 0,37	2,60 ± 0,24	2,39 ± 0,04
Number of sticks per tree	3 ± 0,9	4 ± 0,5	3 ± 0,8	4 ± 0,0
Number of total buds/stick	15 ±4,2	16 ± 4,1	12 ± 3,1	11 ± 4,6
Number of open buds/stick	1 ± 0,9	2 ± 2,1	1 ± 1,9	2 ± 0,6
Number of close buds/stick	14 ± 3,6	14 ± 4,3	11 ± 5,0	9 ± 5,2
Stick diameter [mm]	9,04 ± 1,44	10,06 ± 2,56	8,37 ± 1,24	9,07 ± 0,23

T1 = Control (without treatment); T2 = *Glomus iranicum* var. *tenuihypharum*; T3 = *Glomus iranicum* var. *tenuihypharum* + *Pseudomonas putida*; T4 = *Pseudomonas putida*. Data are an average of 6 repetitions ± standard deviation. One stick was sampled and chosen per tree. Data were recorded on March 13, 2019. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020

Table 5. Number of clusters per tree, an indicator of fruit yield

Number of clusters/tree	T1	T2	T3	T4
	23 ± 6 a	28 ± 9 b	25 ± 8 ab	28 ± 7 b

T1 = Control (without treatment); T2 = *Glomus iranicum* var. *tenuihypharum*; T3 = *Glomus iranicum* var. *tenuihypharum* + *Pseudomonas putida*; T4 = *Pseudomonas putida*. Data are an average of 48 repetitions ± standard deviation. There were 2250 trees/ha and the four plots selected in the project were spread over 8 ha of production. The measurements were taken on July 9, 2020. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020. The means followed by different letters in a column are significantly different according to Tukey's test ($p < 0.05$)

Table 6. Measurements of grape fruit quality taken two times in treatments T1, T2 and T4

Parameters	September 30, 2019 (S1)			October 3, 2019 (S2)		
	T1	T2	T4	T1	T2	T4
Number of berries per cluster	91 ± 12	90 ± 9	90 ± 30	109 ± 23	74 ± 13	68 ± 3
Cluster weight [g]	606 ± 137	706 ± 28	471 ± 13	739 ± 202	452 ± 52	555 ± 57
Cluster length [cm]	27 ± 3,0	23 ± 0,6	24 ± 1,0	28 ± 2,5	28 ± 2,5	28 ± 2,5
Cluster width [cm]	13 ± 1,0	12 ± 2,6	10 ± 0,6	16 ± 2,3	16 ± 2,3	16 ± 2,3
pH	3,8 ± 0,2	4,2 ± 0,2	4,1 ± 0,1	4,0 ± 0,04	4,0 ± 0,04	4,0 ± 0,04
Degrees Brix	17,5 ± 0,0	15,5 ± 1,0	17,6 ± 1,0	16,2 ± 1,5	16,9 ± 1,1	16,5 ± 1,7
Acidity [%]	0,47 ± 0,16	0,60 ± 0,13	0,43 ± 0,07	0,34 ± 0,09	0,42 ± 0,07	0,37 ± 0,01
Ratio Sugar/Acidity	39,1 ± 11,0	26,7 ± 5,6	39,7 ± 8,3	41,4 ± 1,6	41,9 ± 6,8	44,9 ± 4,0
Electrical conductivity [mS/cm]	257 ± 9	324 ± 16	333 ± 22	298 ± 0,8	300 ± 11,8	261 ± 10,5
Dry matter [%]	19,5 ± 1,9	22,8 ± 7,8	19,0 ± 2,3	19,5 ± 0,9	19,4 ± 0,5	19,2 ± 0,8

T1 = Control (without treatments); T2 = *Glomus iranicum* var. *tenuihypharum*; T4 = *Pseudomonas putida*. Data are an average of three repetitions ± standard deviation. Fruit samples were taken per tree. Samples S1 and S2 were taken on September 30, 2019 and October 3, 2019, respectively. The first soil inoculation with the fungus and bacteria was performed on July 19, 2019. The second inoculation was performed on February 21, 2020

Discussion

The results of this work showed that T2, T3 and T4 treatments did not have an effect on pH, salinity, EC and moisture of the soil after 4 months of inoculation. This was endorsed by Li *et al.* (2012) who demonstrated that mycorrhizae did not affect soil pH; nevertheless, it decreased soil salt concentration and EC. In addition, the soil capacity for holding water had a tendency to be slightly higher in treatments with *G. iranicum* var. *tenuihypharum* and *G. iranicum* var. *tenuihypharum* + + *P. putida* than in the control without microorganisms. This may indicate that both treatments have an effect on the soil's ability to hold water. These results were supported by research work conducted by Augé *et al.* (2001), who showed that inoculation of the soil with mycorrhizae had more water-stable aggregates and significantly higher hyphal densities than soils without mycorrhizae. Also, Chen *et al.* (2020) showed that soil inoculation with microorganisms such as mycorrhizae improved soil structure, maintained its permeability and soil capacity for holding water, by consolidating small micro-aggregates into large aggregates. For actinobacteria, this value seemed to be higher for T2 than for T4 and lower for T2 than for T3. This can indicate that treatment with a mixture of *Pseudomonas* and *Glomus* increased the population of the actinobacteria. This could mean that there was probably some synergy between these microbial populations (*Glomus*, *Pseudomonas* and the actinobacteria). Thus, previous research work (Swhetal *et al.* 2010; Singh *et al.* 2013) has shown that there is synergy between *Glomus* and *Pseudomonas* which reduced root diseases and improved crop yields. Regarding the growth parameters, no effect of the treatments was recorded for growth parameters taken 73 days after the first inoculation and the growth parameters measured 21 days after the second inoculation. In contrast, Kamayestani *et al.* (2019) demonstrated that inoculation with *G. mosseae* significantly increased the growth of the table grape. Also, Luciani *et al.* (2019) used *G. iranicum* var. *tenuihypharum* sp. Nova and have shown that this strain had an impact on the development of the vine root system. A significant increase in soil volume explored by the entire root system was recorded, suggesting more efficient use of water and nutrients. For the grape yield, the number of clusters per tree was chosen as an index of yield which indicated that there was a significant increase in the number of clusters in the treatments with *Glomus* (T2) and *Pseudomonas*. (T4) compared to controls (T1) and it was slightly higher than the co-inoculation (T3). This demonstrated that the number of clusters was influenced by the fungal and bacterial treatments, especially since the number of repetitions taken for each treatment was high (48 repeats per treatment). Many research studies (Baslam *et al.* 2013; Bona *et al.* 2017; Bona *et al.* 2018) have shown the same effect of *Glomus* and many PGPRs (Plant Growth Promoting Rhizobacteria) on fruit yield. For fruit quality, different treatment effects were observed between treatments. Javanmardi *et al.* (2014) demonstrated that inoculation with *G. versiforme* increased the percentage of fruit dry matter, titratable acidity and vitamin C content compared to non-inoculated. Schubert *et al.* (2020) showed that tomato fruits from plants inoculated with mycorrhizae tended to have higher Brix than non-inoculated. Further research will be needed, in the future, with different inoculation doses, at different times of the year and with other parameters measured, in order to confirm the effects of these microorganisms on growth, productivity and quality of table grapes.

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