

Physiological and Productive Response of Two Varieties of Tomato (*Solanum lycopersicon*) to the Application of *Glomus iranicum* in the Region of Extremadura

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Abstract: Two varieties of tomato plants, H 1015 (1) and H 3402 (2) from two adjoining commercial farms located in San Benito, Extremadura and cultivated in the same soil and fertirrigation conditions, were inoculated with the arbuscular mycorrhizal fungus *Glomus iranicum* variety *Tenuihypharum*, (AMF) from the commercial product Mycogrowth. The applied treatments to the plants were: control 1, AMF 1, control 2 and AMF 2. The physiological indicators and productive responses of the plants from the four treatments were studied. The percentage of mycorrhization, soil moisture, the growth of dry aerial biomass, leaf water potential (Ψ leaf), stomatal conductance (gs), photosynthetic rate (Pn) and performance of its components rate were evaluated 81 days after transplantation. The (Ψ leaf) and (Pn) were measured twice (10.00 and 13.00 h). The results showed that symbiosis between tomato plants and the AMF was successful. Plants of both varieties treated with AMF, had better physiological performance, increased leaf water potential, increased gas exchange (stomatal conductance and photosynthetic rate), and showed improvements in growth, quality and productivity at plot level. The positive effects of this fungus show that it could result of great importance for reducing the use of chemical fertilizers, at least partially, in the future.

Keywords: Horticultural, Mycogrowth, Water Relations, Gas Exchange, Performance

1. Introduction

Tomato is one of the most important horticultural crops in the world, the second in volume of production, with more than 164 million tons produced annually [1]. Spain is considered one of the main producers of tomato in Europe, since it represents 23% of the total value of production in the sector [2].

In Extremadura, *Solanum lycopersicum* L tomato is

without doubt the most important horticultural crop. The fact that this autonomous community is the leading producer of tomatoes within Spain explains why, of the thirty canning companies in the region, fifteen are engaged in the transformation of this vegetable. In addition, this region is an extremely important agricultural area, which has been transformed from an area of dry land. However, estimates of rain and water distribution are increasingly uncertain in areas affected by drought, where climate change will be even more evident in the future [3].

In this context, the increasingly frequent periods of drought suffered in Extremadura may significantly affect crop yields and limit areas suited to planting.

Modern agriculture requires new approaches, in terms of sustainability, backed by research, innovation and technology, with a view to reducing the environmental impact of the intensive practices associated with agricultural production. In this sense, the efficient management of biological processes that constitute the soil-plant system, demand greater efforts to improve the introduction of bio-fertilizers as basic elements in agroecosystems [4].

In the agricultural context, arbuscular mycorrhizal fungi (AMF) represent a group of soil microorganisms that establish symbiosis with an extensive group of plant species.

The application of AMF in agriculture has focused on the idea of using nowhere amounts of chemical inputs, to enhance safety [5], as well as to preserve forest ecosystems [6]. Among the main benefits from this association are the direct effects on mineral nutrition, especially in the absorption of phosphorus [7], the induction of tolerance against abiotic stress conditions (drought and salinity) [8, 9], its contribution to the stability of soil aggregates [10] and the protection given to crops against pathogens. Symbiosis with AMF is known to improve the performance of the plant and affect changes water relations, both in terms of good irrigation and water stress [11, 12]. The fine hyphae of the AMF can explore pores in the soil that are inaccessible to roots, so they reach water sources which are otherwise inaccessible to the plant. In addition, they facilitate the formation of stable aggregates in water and they can influence in the properties of soil moisture retention [11].

However, for the success of the fungus-plant interaction, attention must be paid to the choice of the inoculum, the choice of the plant and growing conditions. According to [13], there are three factors that determine the success of inoculation and the persistence of AMF in soils: the compatibility of the introduced species and the conditions imposed, the load capacity in the field and abundance (the amount of inoculum and the degree to which the particular system supports AMF populations), and other effects such as the influence of the weather and competition in the establishment of alternative stable communities [14].

There are several studies on the implementation of AMF in tomato, but few results in terms of the practical application of AMF in agricultural production and in field conditions, where practices such as the use of fungicides and tilling can negatively affect arbuscular mycorrhizal associations [15, 16].

The objective of this study was to evaluate the physiological and productive response of two varieties of tomato to the application of the product Mycogrowth as inoculant agent of the fungus *Glomus iranicum* var. *Tenuihypharum*, which is of recognized importance for crops and soil. For this, we measured the soil moisture content, the percentage of root colonization, water relations and gas exchange, as well as dry biomass, yield, plant production and fruit quality.

2. Materials and Methods

2.1. Experimental Conditions

The experiment was carried out on the farms "La Cigüeña" (farm 1) and "Las Puerkas" (farm 2) belonging to the companies TEPRO and TRANSA, respectively (San Benito, Extremadura) (38°57'20.0"N 5°52'50.0"W) between the months of April and September 2015. The soil of the two farms is classified as Entisol, sub-order Orthents, group xerothent. They are thin soils, formed by recently eroded surfaces forming an association of xerochrept and haploxeralf. On farm 1, the domain of the sedimentary formations is sand and clay, while farm 2, the domain is slate, sandstone and quartzite.

During the experiment, climatic data were gathered by a nearby meteorological station. The average temperature was 16.24°C, the average RH 61.99%. The reference evapotranspiration was 3.62 mm/day. Rainfall was the maximum in September (90.4 mm).

On farm 1, transplantation was carried out on the 29 April, 2015, using variety 1 of commercial tomato H 1015 (variety 1). On farm 2, transplantation was performed on May 7, 2015 and the variety used was H 3402 (variety 2). The growing cycles were 115 and 122 days, respectively. The two varieties behave very similarly and are listed as multipurpose varieties with high yield potential and high °Brix (5.3). These varieties are very common on commercial farms and are well adapted to the climate (arid conditions, as well as wet), soil and water conditions.

Plots with an area of two hectares were selected on each farm, with similar soil conditions and fertigation systems. The plots had a plantation framework of 0.23 m between plants and 1.52 m between lines (28,600 plants ha⁻¹). Irrigation was carried out by irrigation tubes located at 20 cm depth with drippers 30 cm apart and a flow rate of 1 L·h⁻¹. The dose and frequency of irrigation, as well as all cultural practices were similar in all the plots.

Thirty days after transplant (DAT) and on each farm, one of the hectares was inoculated with the commercial product Mycogrowth (3 kg ha⁻¹). Mycogrowth is a product, which contains the fungus *Glomus iranicum* var. *Tenuihypharum* as an active ingredient, in a clay mineral substrate, with a concentration of 1.2 x 10⁴ propagules in 100 ml. of substrate (depending on the most probable number method). The application was made, following the manufacturer's instructions, through the irrigation system.

Therefore, the farm 1 consisted in one hectare of H 1015 variety (variety 1) without mycorrhizae (control 1) and one hectare of H 1015 variety with micorrhizae (AMF 1), while the farm 2 consisted in one hectare of H 3402 variety (variety 2) without micorrhizae (control 2) and one hectare of H 3402 variety with micorrhizae (AMF 2). In short, the treatments were four: control 1, AMF 1, control 2 and AMF 2.

2.2. Moisture in the Soil and Percentage of Mycorrhization

At 81 DAT, the percentage of mycorrhization and soil

moisture was determined. The soil moisture was determined for each treatment by using the gravimetric method, selecting three sampling points in each treatment and taking samples with a corkscrew drill at 25 cm of depth. Each sample was placed in tared filters, dried in a forced draught stove at 110 °C for 24 hours, cooled in a desiccator and weighed until constant weight. The percentage of humidity was calculated using the formula:

$$\% \text{ of Moisture} = \frac{\text{fresh mass} - \text{dry mass}}{\text{dry mass}} \times 100 \quad (1)$$

To determine the percentage of mycorrhization, approximately 250 mg of secondary roots in 10 plants per treatment were taken, at a depth of 0 - 20 cm, which were carefully washed with deionized water, dried in an oven at 70°C until constant mass and then stained and clarified according to the methodology described by [17, 18,]. Mycorrhizal colonization was evaluated in a dissecting microscope (Carl Zeiss, Stemi 2000-C/50x) using the intercepts method, developed by [19].

2.3. Measurements of Plant Water Status and Gas Exchange

Leaf water potential (Ψ_{leaf}) was measured twice 81 DAT (10.00 and 13.00 h) in 10 plants per treatment. The measurement was performed according to [20], using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) into which the leaves were introduced immediately after collection and pressurized at 0.02 MPa s⁻¹ [21].

Stomatal conductance (g_s) and photosynthetic rate (P_n) were measured at the same time on the same date in sunny leaves of 10 plants per treatment, using a portable gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA).

2.4. Growth, Crop Yield and Fruit Quality

On the same day (81 DAT), the dry mass of aerial parts was determined in 10 plants per treatment. The aerial part of plants was separated from the root system. Leaves and stems were dried in a forced air oven at 75°C until constant weight (approximately 72 hours) and cooled in a desiccator. Subsequently, the dry weight was determined.

Crop yield, number of fruits and average weight per fruit was evaluated in 10 plants per treatment. The soluble solids content were evaluated by refractometers and expressed in °Brix as an indicator of internal fruit quality of the fruit, in 50 fruits per treatment.

2.5. Statistical Analysis

During the experiment, each treatment had a surface area of 1 ha, with two repetitions per treatment. The data was analysed using a one way analysis of simple variance (ANOVA) with the program SPSS version 15 for Office 2011 Mac. To measure the differences between means the Duncan multiple comparisons test we used.

3. Results

3.1. Soil Moisture and Mycorrhizal Colonization Percentage

In general, the moisture content of the soil for variety 2 (H 3402) was considerably higher than for variety 1 (H 1015) (Figure 1). In both varieties, the mycorrhizal treatments (AMF 1 and AMF 2) tended to increase the soil moisture content compared with the control treatments (control 1 and control 2), but without significant differences.

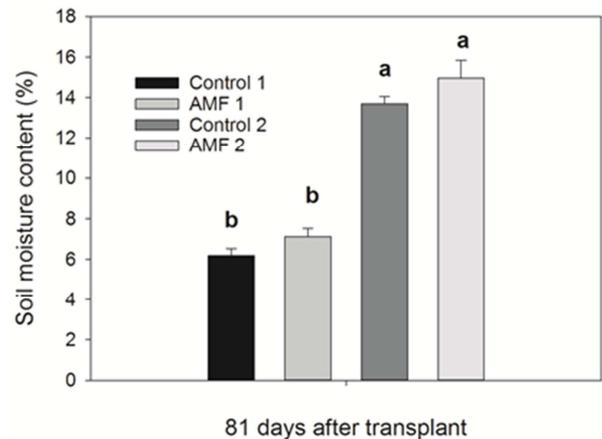


Figure 1. Moisture content of the soil of variety 1 in absence (control 1) and presence of mycorrhizal fungi (AMF 1) and of variety 2 in absence (control 2) and presence (AMF 2) of mycorrhizal fungi. The values are averages of three samples per treatment. Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

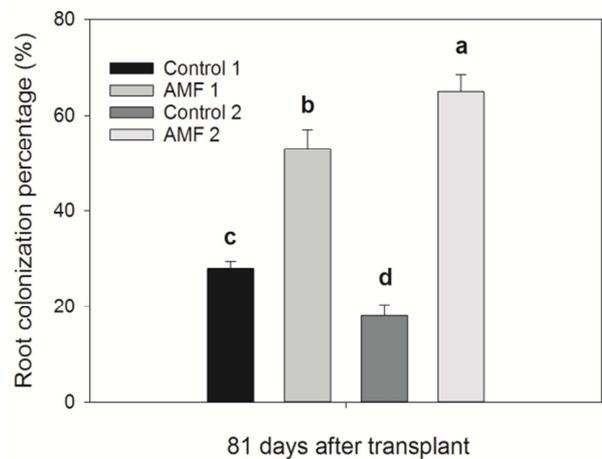


Figure 2. Percentage of colonization of roots in tomato plants of variety 1 in absence (control 1) and presence of mycorrhizal fungi (AMF 1) and variety 2 in absence (control 2) and presence (AMF 2) of mycorrhizal fungi. The values are averages of 10 plants per treatment. Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

As regards the percentage of colonization, substantial differences were observed between the treatments control 1 and AMF 1, and control 2 and AMF 2 (Figure 2). The control 1 treatment showed around 28% colonization, whereas AMF

1 reached 53% colonization.

Control 2 treatments showed 18% colonization while in AMF 2 the percentage more than tripled to reach 65% colonization.

3.2. Plant Water Status

In terms of the water status of tomato plants, at 10.00 h, the leaf water potential values were between 0.4 and 0.7 MPa (Figure 3A) for both varieties.

In addition, water potential values were less negative in the plants of both varieties to which were applied mycorrhizal fungi.

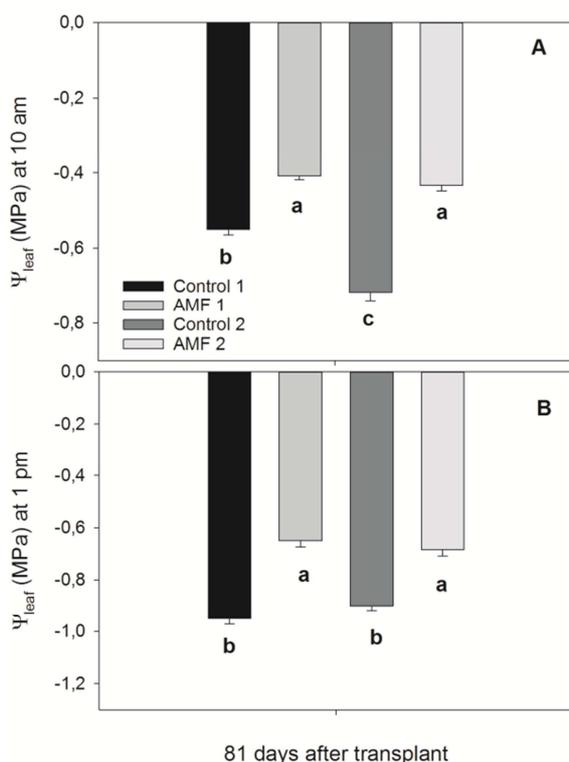


Figure 3. Leaf water potential (ψ_{leaf}) at 10.00 h (A) and 13.00 h (B) in tomato plants of variety 1 in absence (control 1) and presence (AMF 1) of mycorrhizal fungi and variety 2 in absence (control 2) and presence (AMF 2) of mycorrhizal fungi. The values are averages of 10 plants per treatment. Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

At 12.00 h, the water potential values varied between -0.95 and -0.65 MPa, the behavior of this parameter being very similar to that observed at 10.00 h, since treatments AMF 1 and AMF 2 showed a less negative water potential compared to their treatment controls (control 1 and control 2) (Figure 3B).

3.3. Gas Exchange

In general, the stomatal conductance at 10.00 h was greater in control 2 than in control 1 (Figure 4A). However, in both varieties, the mycorrhizal treatments (AMF 1 and AMF 2) increased stomatal conductance over their respective control values (control 1 and control 2), reaching 354 $\text{mmol m}^{-2} \text{s}^{-1}$ in

AMF 1 and 634 $\text{mmol m}^{-2} \text{s}^{-1}$ in AMF 2. At 13.00 h, stomatal conductance was lowest in the plants of control 1, followed by control 2 (Figure 4B).

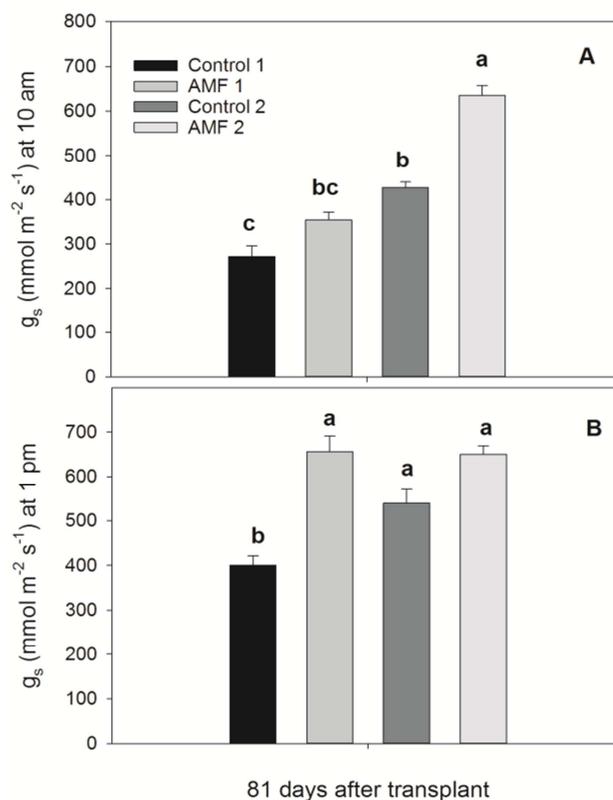


Figure 4. Stomatal conductance (g_s) at 10.00 h (A) and at 13.00 h (B) in tomato plants of variety 1 in absence (control 1) and presence of mycorrhizal fungi (AMF 1) and of variety 2 in absence (control 2) and presence of mycorrhizal fungi (AMF 2). The values are averages of 10 plants per treatment. Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

However, both treatments AMF 1 and AMF 2 produced higher values than their corresponding controls, and were very similar to each other (656 $\text{mmol m}^{-2} \text{s}^{-1}$ and 650 $\text{mmol m}^{-2} \text{s}^{-1}$ for treatments AMF 1 and AMF 2, respectively) (Figure 4B). As regards the photosynthetic rate, the behaviour was very similar to that of stomatal conductance (Figure 5A, B).

The highest photosynthetic rate at 10.00 h was found in variety 2 with values of 22 and 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the control 2 and AMF 2 treatment, respectively.

On variety 1, values were 11 and 14 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the control 1 treatment and AMF 1, respectively (Figure 5A). However, at 13.00 h, mycorrhizal treatments of both varieties (AMF 1 and AMF 2) produced a higher photosynthetic rate than that control plants (control 1 and control 2), reaching 27 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in both cases (Figure 5B).

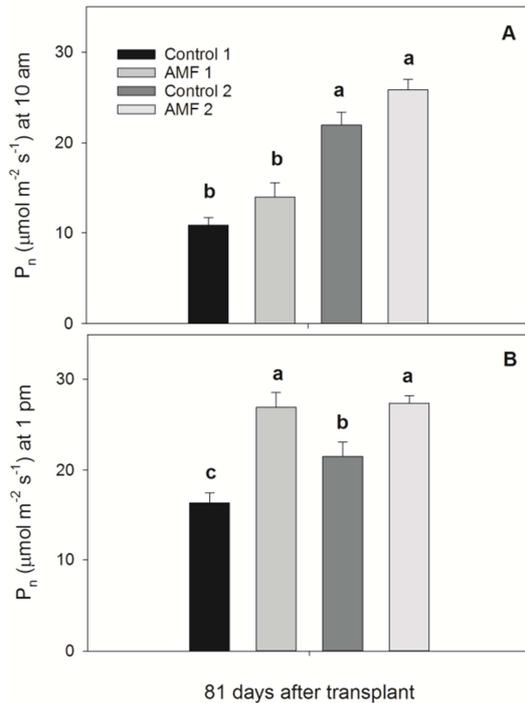


Figure 5. Net photosynthetic rate (P_n) at 10.00 h (A) and at 13.00 h (B) in tomato plants of variety 1 in absence (control 1) and presence of mycorrhizal fungi (AMF 1) and variety 2 in absence (control 2) and presence of mycorrhizal fungi (AMF 2). Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

3.4. Production, Crop Yield and Fruit Quality

In terms of growth of dry biomass of the aerial part (Figure 6A), the AMF 2 treatment reached a greater dry weight than control 2 treatment. AMF 2 treatment also showed higher values compared to the two treatments of variety 1 (control 1 and AMF 1).

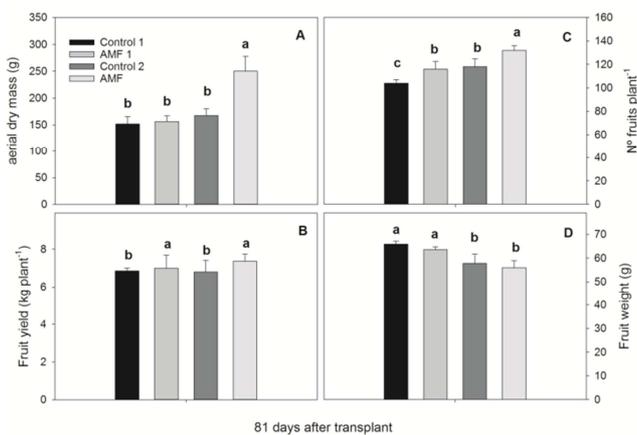


Figure 6. Aerial dry mass (A), fruit yield (B), number of fruits per plant (C) and fruit weight (D) in tomato plants of variety 1 in absence (control 1) and presence of mycorrhizal fungi (AMF 1) and of variety 2 in the absence (control 2) and presence of mycorrhizal fungi (AMF 2). Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

The fruit yield per plant, expressed as kg plant⁻¹, was

similar in the two varieties (control 1 and control 2), even though the application of mycorrhizae improved performance in both varieties (treatments AMF 1 and AMF 2) (Figure 6B).

Also, an increase was found in the inoculated treatments of both varieties (AMF 1 and AMF 2) with respect to their controls (control 1 and control 2). In terms of the average mass of fruits, the plants of control 1 had a higher average fruit weight than control 2 (Figure 6D). However, there were statistical differences among treatments for each variety, so mycorrhizae did not change the fruit weight.

The average number of fruit per plant was higher in the control treatment of variety 2 (control 2) compared to variety 1 (control 1) (Figure 6C).

The tomatoes had 5.08 °Brix in control 1 and 5.26 °Brix in control 2, while tomatoes of the AMF 1 and AMF 2 treatments showed significantly higher values (6.1 °Brix and 5.9 °Brix, respectively) (Figure 7).

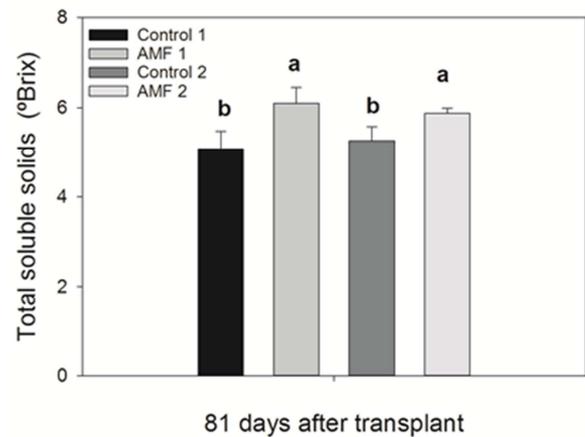


Figure 7. Total soluble solids (°Brix) in tomato plants of variety 1 in the absence (control 1) and presence of mycorrhizal fungi (AMF 1) and variety 2 in the absence (control 2) and presence of mycorrhizal fungi (AMF 2).

The values are averages of 50 fruits per treatment. Different lower case letters indicate significant differences between treatments according to Duncan 0.05 test. The vertical bars indicate standard errors.

4. Discussion

The percentage of root colonization observed in both varieties pointed to the good establishment of the fungus [22], as well as its proper development and persistence during the crop cycle [23]. In addition, the mycorrhizal dependence of this fungus species to both varieties of tomato was proved, an aspect that have been reported in previous works in this crop [24-26]. Interestingly, there was a small percentage of root colonization in control plants of both varieties, probably due to the existence of AMF that resided in the soil [27, 23].

The leaf water potential is an indicator of the water supply of the plants, and is an integrating variable of environmental, soil and plant conditions [28]. Interestingly, the variety 2 control plants showed more negative water potential at 10.00 h, coinciding with lower soil moisture. This could be due to the

slight differences between the characteristics of the soil, since sands and clays formed part of the soil characteristics of farm 1. However, the water potential values of mycorrhizal plants were very similar for both varieties, both at 10.00 h and 13.00 h. The beneficial effect of this fungus was evident from the improvement in water relations of the plants of both varieties. Numerous studies have shown that symbiosis can modify plant water relations in conditions of water stress deficit [29, 30], saline [31, 32] and also in conditions of non-stress [33, 34]. In some cases, an improvement has been found in the hydraulic conductivity of inoculated roots, where hormonal regulation plays a key role [9]. In this sense, it has been observed that plants inoculated with AMF regulate their ABA levels better and faster than non-mycorrhizal plants, allowing a more suitable balance between leaf transpiration and movement of water in the roots during periods of drought and recovery [35]. In addition, fungal exudates can promote cohesion of soil particles and increase water retention, since they have a relatively slow degradation rate compared with root exudates [36].

Stomatal conductance is an efficient indicator of the rate of gas exchange and transpiration through the stomata of the leaves, since it is a function of the density, size and degree of opening of the stomata [37] and soil moisture. Stomatal control is basically conditioned by the availability of water in the rooting zone, a water reservoir that diminishes at a rate directly proportional to stomatal opening [38]. In our case, the values of g_s at 10.00 h reflected the conditions corresponding to each soil moisture content of each farm, since the lowest g_s values were found in plants of variety 1 (control 1 and AMF 1 treatments) and the highest corresponded to variety 2 (control 2 and AMF 2 treatments). Although at 13:00 h., the values of g_s in AMF 1 and AMF 2 treatments of plants were high and similar. The closer relationship between soil moisture content and the values of g_s during the morning coincides with the observations of [39, 40] working with different cultivars of the same species and in similar conditions. One of the variables most closely correlated with g_s in the morning, was soil moisture. In addition, the behavior of stomatal conductance and photosynthesis in all plants was very similar to that of Ψ_{leaf} in both varieties at 13.00 h, since the highest values of g_s and P_n always corresponded to plants with AMF. Plants inoculated with AMF often have a higher g_s and a greater ability to regulate their stomata [29, 8] than non-inoculated plants. Several authors have linked this increase in g_s with the increase of P and/or the changes that occur in the hydraulic properties of the root [41, 30], which increase the water supply to the aerial part, as is our case. On the other hand, photosynthesis (P_n) may be more related to a higher leaf nitrogen content or greater resistance to the collapse of carbon in the AM association [42].

An increase in dry biomass was observed only in the inoculated plants of variety 2 (AMF 2 treatment). This fact could be attributed mainly to the existence of the greater fungal colonization (Figure 1) on variety 2 (AMF 2 treatment) with respect to the one existing in the variety 1 (AMF 1

treatment). On the other hand, the absence of statistical differences among treatments of variety 1 (control 1 and AMF 1) on dry biomass, could be associated with the fact that variety 1 plants had reached their stage of maturity earlier, since they have a shorter cycle than variety 2 and they were in the middle of floration-fructification, when the relative rate of growth is minimal. Similar results were reported by [26] working with four species of *Glomus* in tomato. The direct effect of the AMF on growth and development of plants was probably due to the fact that the small and thin hyphae of these fungi increased the volume of absorption of the root system. This favored the water absorption of water and probably nutrients with low mobility, promoting plant growth [43].

The AMF improved yield, and the number of fruits per plant in both varieties. These results are consistent with other studies in tomato plants, where performance and the number of fruits increased with mycorrhizal inoculation [44, 45]. The possible reason for the synergistic impact of mycorrhizae in the improvement in the number of fruits is an increase in the phosphorus content in the plant which could exert a positive effect on cell division and energy storage [46]. In addition, mycorrhizal inoculation improved the quality of fruit in both varieties as a result of a greater nutritional intake on the part of the fruit [47]. The higher sugar content attained with mycorrhizal inoculation suggested that the distribution of carbohydrates in the plant was not only addressed to the AMF [48]. Finally, while plant yield in the control treatments was similar in both varieties, in variety 1, the weight of the fruits was higher and the number of fruits lower than in variety 2, a fact that is attributed to the characteristics of the two varieties.

5. Conclusions

The compatibility shown between the varieties of tomato and the fungus *Glomus iranicum* var. *Tenuihypharum* from the Mycogrowth product was excellent. Mycorrhizal symbiosis induced increases in performance, production, water relations and fruits quality in the plants of the two varieties, due to an improvement in physiological functioning, an increase in photosynthetic activity and therefore an increase in growth. The positive effects of this fungus in tomato plantations could be useful to partially reduce the use of chemical fertilizers in the future. These results open the door for the practical application of biostimulants in present day commercial farms.

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