ARBUSCULAR MYCORRHIZAL FUNGI FROM DRY ENVIRONMENTS AS BEST OPTIONS TO PRESERVE PHOTOSYNTHETIC PARAMETERS OF SOLANUM LYCOPERSICUM L. PLANTS UNDER LIMITED IRRIGATION

MENA-ECHEVARRÍA, A.¹ – RAMÍREZ-TOBIAS, H. M.^{2*} – MÉNDEZ-CORTÉS, H.² – ROJAS-VELÁZQUEZ, A. N.² – RAMOS-CRUZ, C. M.¹ – HIPÓLITO-PIEDRAS, R. P.²

¹General Terán Experimental Field, National Institute of Forestry and Livestock Agricultural Research, Km. 31 Highway Montemorelos-China, General Terán, Nuevo León 67400, Mexico

²Faculty of Agronomy and Veterinary Medicine, Autonomous University of San Luis Potosí, Km. 14.5 Highway San Luis Potosí, Matehuala, Ejido Palma de la Cruz, Soledad de Graciano Sánchez, San Luis Potosí 78321, Apdo. Postal 32, Mexico

> *Corresponding author e-mail: hugo.ramirez@uaslp.mx

(Received 21st May 2024; accepted 25th Sep 2024)

Abstract. The efficiency of arbuscular mycorrhizal fungi (AMF) in photosynthesis will depend on the AMF species used. AMF species from two different environments, inoculated as single species or in consortium, and their influence on photosynthesis in tomato plants subjected to different irrigation regimes were evaluated. The mycorrhizal inocula were: humid environment (*Glomus* sp. and the -C1- consortium) and semi-arid environment (*Claroideoglomus etunicatum* and the -C2- consortium), and three irrigation doses were applied (70%, 85% and 100%). A 15% reduction of the irrigation dose affected colonization with *C. etunicatum*, and with the species of the humid environment it was lower with the 100% irrigation dose. The mycorrhizal consortium C2 maintained stable colonization levels at all three irrigation doses. Glomus sp. and *Claroideoglomus etunicatum* showed increases in net photosynthesis (P_N), stomatal conductance (g_s), transpiration (*E*), water use efficiency (WUE), non-photochemical quenching (q_N) and electron transport rate (ETR) with decreasing irrigation dose. The C2 mycorrhizal consortium was stable with 15% less irrigation. Simple species maintain stable photosynthesis despite water scarcity and AMF from semi-arid environments are more efficient in maintaining gas exchange under water reductions. **Keywords:** *chlorophyll fluorescence*, *gas exchange*, *mycorrhizae*, *tomato*, *water*

Introduction

The rhizosphere is home to many microorganisms that compete for space and nutrients. The interactions that occur between plants, microorganisms and the environment have a direct impact on the growth and development of plant species (Rodriguez et al., 2019; Qu et al., 2020). In this environment, pathogenic microorganisms that cause serious plant diseases can be found (Bhatt et al., 2022). The following pathogenic species have been identified as causes of crop disease: *Phytophthora capsici, Rhizoctonia solani, Fusarium solani, Fusarium oxysporum, Pythium* spp., *Aspergillus flavus, Botrytis cinerea*, and *Aphanomyces cochlioides* (Pal et al., 2006). There are also beneficial microorganisms that can perform different functions such as nitrogen fixation, phosphate solubilisation, plant growth promotion and biocontrol (Qu et al., 2020). There are also some microorganisms such as arbuscular mycorrhizal fungi (AMF) that can enhance the process of photosynthesis, as well as increase crop productivity (Mathur et al., 2019). AMF stimulate physiological, genetic, and molecular mechanisms, mainly

when plants are under stress (Rouphael et al., 2015). In turn, mycorrhizal symbiosis favors stomatal regulation and optimizes Water Use Efficiency (WUE) when there is water deficit (Augé et al., 2015). AMF reduce peroxidative damage due to increased activity of antioxidant enzymes (Chang et al., 2018; Duc et al., 2018) and induce morphophysiological changes in some organs and tissues of mycorrhizal plants, making them tolerant to water stress (Begum et al., 2019). Other authors report that mycorrhizal symbiosis could regulate a variety of physio-biochemical processes in plants such as, increased proline accumulation (Yooyongwech et al., 2015); increased growth and photosynthesis due to the regulation of the antioxidant system (Li et al., 2019) and increased levels of glutathione, one of the main antioxidant metabolites used by plants to tolerate various biotic and abiotic stresses (Rani, 2016).

In the tomato-AMF interaction, increases in crop productivity have been reported due to increased nutrient uptake, which has a positive impact on fruit quality and yield (Chitarra et al., 2016; Bakr et al., 2017; Volpe et al., 2018). However, the effectiveness of the symbiosis will depend on the AMF species, its host and host growth conditions (Tedersoo et al., 2020). These aspects must be considered for effective establishment of mycorrhizal symbiosis. Similarly, the efficacy of mycorrhizal inoculants may vary depending on the type of inoculum (Grümberg et al., 2014). Some authors have reported that AMF consortia isolated from desert soils can stimulate growth in plants under water and salt stress (Herrera-Corrales et al., 2014), and humid environments have a higher diversity of AMF species, which is associated with the great diversity of plants that cohabit these environments (Álvarez-Sánchez et al., 2017). On the other hand, there is great interest in the use of mixed native AMF inocula, since it has been reported that inoculation with mixed AMF consortia would have a greater buffering capacity against water stress than a single fungal inoculum (Armada Rodríguez, 2016). Considering that information on the physiology of mycorrhizal tomato plants under water stress is scarce, the aim of this research was to evaluate AMF species from two different environments, inoculated as single species or in consortium, and their influence on photosynthesis in tomato (Solanum lycopersicum L.) plants subjected to different irrigation regimes. The above, under the hypothesis that AMF inoculation will maintain photosynthetic parameters and water use efficiency in tomato plants when substrate moisture is restricted and in dependence on the AMF species inoculated singly or in consortium.

Materials and Methods

Experimental conditions

The experiment was conducted at the Faculty of Agronomy and Veterinary of the Autonomous University of San Luis Potosi, Mexico. Tomato plants var. Río Grande were used under a completely randomized experimental design with a 4x3 factorial arrangement, being the factors to be studied the type of AMF inoculum and the irrigation dose. The plants were grown in a greenhouse under semi-controlled conditions, irrigation and fertilization were applied manually, and the frequency of irrigation was established according to the physiological needs of the crop. Tezontle gravel with a grain size of 5 to 8 mm was used as substrate. Seedlings were transplanted in black polyethylene bags of 37 cm x 37 cm with a capacity of 11 L (*Figure 1*.).

Mena-Echevarría et al.: Arbuscular mycorrhizal fungi from dry environments as best options to preserve photosynthetic parameters of *Solanum lycopersicum* L. plants under limited irrigation - 345 -



Figure 1. Experimental conditions in which tomato plants were established

The type of AMF inoculum depended on the composition of the species (*Table 1.*) and on the places from species were collected. They were found at the following geographical coordinates and nominated as next. Humid climate, $21^{\circ} 23'$ N and $98^{\circ} 59'$ W and at an altitude of 600 to 900 m., municipality of Xilitla. Semi-dry climate: $22^{\circ} 27'$ N and $100^{\circ} 42'$ W and at an altitude of 1700. Villa Hidalgo. Both places are in the state of San Luis Potosí, Mexico.

Inoculum type	Origin and predominant vegetation				
	Humid climate (humid forest vegetation)	Semi-arid climate (mesquite vegetation)			
Simple	Glomus sp.	Claroideoglomus etunicatum			
Consortium	C1 Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüssler Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler Glomus sp. Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck	C2 Acaulospora morrowiae Spain & N.C. Schenck Claroideoglomus etunicatum Glomus macrocarpum Tul. &C. Tul.			

Table 1. Composition of the inocula according to AMF species and their origin

The AMF species present in the collected soils were propagated on sorghum and maize plants (Mena Echevarría et al., 2021), using the trap culture method (Brundrett et al., 1996). Four inocula were prepared with the AMF species that produced the highest number of individuals after establishment of the trap cultures. AMF spores were isolated by the "wet sieving and decanting" method proposed by Gerdemann and Nicolson (1963) and the "Shannon-Weaver" method (Magurran, 2004) was used to determine the abundance of AMF species. AMF spores were inoculated individually or in consortium, for the elaboration of the different types of inocula, approximately 100 spores were collected; in the specific case of the consortia, the same proportion of AMF species selected in each environment was applied by the "Shannon-Weaver" method until completing the quantity of 100 spores per inoculum. The types of AMF inocula are mentioned in the paper as follows: *Glomus* sp., *C. etunicatum*, mycorrhizal consortium C1 humid environment and mycorrhizal consortium C2 semi-arid environment. The dose

of irrigation water applied to the plants was established as follows: [100% (830.2±53 ml per day); 85% (706±45.1 ml per day) and 70% (581.8±37.2 ml per day)]. Water doses were determined using the "container capacity" method proposed by Martínez and Roca (2011).

Description of studied variables

Mycorrhizal parameters

The mycorrhizal variables analyzed were the percentage and intensity of colonization at the conclusion of the experiment. The percentage of colonization quantifies the presence of mycorrhizal structures inside the root, while the intensity evaluates the number of mycorrhizal structures formed inside the root. The mycorrhizal parameters were read on 10 fragments of fine roots, each approximately 1 cm in length. These were stained with Parker Quink ink at 0.25% (Yon et al., 2015). The calculation was performed using the method proposed by Trouvelot et al. (1986), with equations (1 and 2). Roots exhibiting mycorrhizal structures (intraradical hyphae, arbuscules, and vesicles) were classified as colonized roots.

%colonization =
$$\frac{\Sigma B}{\Sigma Z} \times 100$$
 (Eq.1)

intensity of colonization
$$=\frac{\Sigma A}{\Sigma Z}$$
 (Eq.2)

where:

 Σ B: total number of roots with mycorrhizal structures,

 ΣZ : total of 100 roots evaluated,

 Σ A: result of multiplying mycorrhizal roots by a constant established for an evaluation level.

Level	0	1	2	3	4	5
Constant	0	1	2.5	15.5	35.5	47.5

Photosynthetic parameters

Photosynthetic efficiency was assessed by leaf gas exchange and chlorophyll fluorescence. A portable photosynthesis measurement system (LI-6400; LI-COR Inc., Lincoln, NE, USA), equipped with a gas analyzer in the infrared spectrum connected to a leaf chamber fluorometer (LCF) (6400-40B, 2 cm² leaf area, Licor Bioscience, Inc. Lincoln, NE, USA) was used. Five tomato plants per treatment, randomly selected, were analyzed. Readings were taken on the fully developed leaf. The measurements were taken between 11:00 and 17:00 h. For the measurement of the gas exchange parameters: net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E), the reference CO₂ and the circulating air flow rate within the system were set at 400 ppm and 300 μ mol s⁻¹, respectively. They were measured at 38 days after planting (DAP). The water-use efficiency (WUE) was calculated from $(= P_N/E)$ (Poni et al., 2009). Chlorophyll fluorescence parameters, maximal quantum yield of PSII photochemistry (F_v / F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), nonphotochemical quenching (q_N) , photochemical quenching (q_P) and electron transport rate (ETR) was measured at 41 DAP. The PAR inside the measurement chamber was set at 850 [μ mol(photon) m⁻² s⁻¹] after estimating this radiation level in the measurement area with the PAR sensor of the

same equipment (Buchanan et al., 2015). The F_0 ' values used for the calculation were obtained using a programmed sequence in the photosynthesis measurement system, in which a dark period with far-red light preferentially excites PSI and forces electron drainage from PSII causing all PSII centres to oxidise (

Figure 2.).



Figure 2. Reading of gas exchange variables and chlorophyll fluorescence efficiency in mycorrhized tomato plants through a portable system for photosynthesis measurements LI-6400XT (Li-Cor. Inc.)

Statistical analysis

Data were studied by analysis of variance, using *Tukey's test* ($\alpha = 0.05$) for multiple comparison of means, the number of replicates per treatment was five. Data for variables percentage and intensity of colonization, $[F_v / F_m = (F_m - F_0) / F_m]$, q_N were transformed with the equation (3) to meet the criteria for normality. To determine the performance of each AMF inoculum source with respect to irrigation doses, analysis of variance and multiple comparison of means by independent inoculum type were performed. The independent groups were *Glomus* sp. and irrigation dose (70%, 85% and 100%); *C. etunicatum* and irrigation dose (70%, 85% and 100%), respectively. All statistical analyses were performed with Minitab 15 Windows software.

$$\arcsin\left(\sqrt{\frac{x}{100}}\right)$$
 (Eq.3)

Results

Mycorrhizal parameters

The analysis of variance by independent factors showed statistical differences (P < 0.05) in the percentage of colonization for inoculum type but not for dose of irrigation, the least percentage of colonization was observed when plants were only inoculated with *Glomus* sp. On the other side, colonization intensity was not significant affected neither for inoculum type nor for dose of irrigation (*Table 2.*). However, inoculum type and dose of irrigation had a significant interaction over both variables,

percentage, and intensity of colonization (*Table 2.*). Inoculation with *Glomus* sp. promoted the lowest values of colonization percentage with the three irrigation doses, while *C. etunicatum* with the 100% irrigation dose generated the highest values of percentage (56%) and intensity of colonization (3.87%) with the 100% irrigation dose, in addition to exceeding *Glomus* sp. in colonization percentage by 45%. However, *C. etunicatum* with the 85% irrigation dose showed significantly low values in both variables. On the other hand, the mycorrhizal consortium C1 showed similar values of colonization percentage and intensity with 70% and 85% irrigation doses, but these decreased by 27% and 48% with 100% irrigation doses, respectively. As for the mycorrhizal consortium C2, it did not show statistical differences with the three irrigation doses (*Table 3.*).

Samaa of maniation	Colonization (%)			
Source of variation	Percentage	Intensity		
Inoculum type				
Glomus sp.	30.7b	1.93		
C. etunicatum	42.9a	2.34		
C1	44.7a	2.82		
C2	48.0a	2.18		
Dose of irrigation				
70%	37.8	2.20		
85%	43.2	2.12		
100%	43.6	2.63		
SE	1.60	0.15		
Inoculum type	0.000***	0.142 <i>ns</i>		
Dose of irrigation	0.313 ns	0.099 <i>ns</i>		
Interaction	0.000***	0.000***		

Table 2. Results of the analysis of variance of the percentage and intensity of root colonizationof tomato plants inoculated with different AMF and subjected to different irrigation doses

*P<0.05; ** P<0.01 and *** P<0.000. C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. SE (\pm standard error). Means with similar letters are not different according with Tukey's test (\propto =0.05)

Table 3. Average results of the combination of factors on the percentage and intensity of root
colonization of tomato plants inoculated with different AMF and subjected to different
irrigation doses

Interestion	Colonization (%)			
Interaction	Percentage	Intensity		
Glomus sp. 70%	33.0d	1.88abc		
Glomus sp. 85%	42.5bcd	2.63abc		
Glomus sp. 100%	16.5e	1.38bc		
C. etunicatum 70%	43.5abcd	3.43ab		
C. etunicatum 85%	34.5cd	1.19c		
C. etunicatum 100%	56.0a	3.87a		
C1 70%	46.8abc	2.76abc		
C1 85%	47.5ab	2.77abc		
C1 100%	34.5cd	1.50bc		
C2 70%	51.3ab	2.57abc		
C2 85%	48.5ab	1.91abc		
C2 100%	44.3abcd	2.07abc		

C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. SE (\pm standard error). Means with similar letters are not different according with Tukey's test ($\propto = 0.05$)

Photosynthetic parameters

Responses of the gas exchange variables (for P_N , g_s , E and WUE) were different due the significant interaction (P < 0.05) between the factors, inoculum type and irrigation dose (*Table 4*). Then, as irrigation dose gone down values of g_s and WUE did not change when plants were inoculated with AMF single species and with the C2 (*Figure 3.b* and *Figure 4.b*), even with *Glomus* sp. *E* increased (*Figure 3.a*) with medium and low irrigation doses.

Source of	PN	$g_{ m s}$	Ε	WUE		
variation	[µmol m ⁻² s ⁻¹]	$[mol(H_2O) m^{-2} s^{-1}]$	$[mmol(H_2O) m^{-2} s^{-1}]$	$[mol(CO_2) mol(H_2O)^{-1}]$		
Inoculum type						
Glomus sp.	13.8c	0.4997b	6.81c	2.13a		
C. etunicatum	16.35a	0.6957a	9.62 ^a	1.73b		
C1	15.51ab	0.5989ab	8.52b	1.83ab		
C2	16.68a	0.5873b	8.85ab	1.88ab		
Dose of irrigation						
70%	15.59b	0.5967ab	8.68a	1.99		
85%	16.54a	0.6549a	8.89a	1.89		
100%	14.63c	0.5346b	7.77b	1.80		
SE	0.5	0.05	0.4	0.05		
Results of ANAVA						
Inoculum type	0.000***	0.000***	0.000***	0.005**		
Dose of irrigation	0.000***	0.003**	0.001**	0.144 ns		
Interaction	0.000***	0.001**	0.000***	0.001**		

Table 4. Results of the analysis of variance of gas exchange parameters recorded at 38 DAP in tomato plants inoculated with different AMF and subjected to different doses of irrigation

*P<0.05; ** P<0.01 and *** P<0.000. C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. SE (\pm standard error). Means with similar letters are not different according with Tukey's test (\propto =0.05)

The decrease on the irrigation doses did not alter P_N when *Glomus* sp. was inoculated but the inocula from semi-dry procedence, single or consortium, promoted low P_N values at 100% irrigation and high values 85% and 70%. In the case of plants inoculated with C1, values of P_N were similar at irrigation doses of 100% and 85% but they dropped at 70% dose (*Figure 3.a*). However, with C1 g_s and WUE presented maximum values at doses of 85% but minimum ones at doses of 70% and 100% (*Figure 3.b* and *Figure 4.b*). The same happened with *E* when inoculum was C2. Transpiration responses were opposite between inocula coming from wet environments, as irrigation rates decreased from 100% to 70%, *E* went up with *Glomus* sp. but went down with C1 (*Figure 4.a*)

Responses of q_N and ETR to the change on the irrigation doses were different according to inoculum type since significant interaction between type of inoculum and irrigation dose was observed. Likewise, F_v/F_m , Φ_{PSII} and q_P were not affected by the experimental treatments (*Table 5.*).

Mena-Echevarría et al.: Arbuscular mycorrhizal fungi from dry environments as best options to preserve photosynthetic parameters of *Solanum lycopersicum* L. plants under limited irrigation - 350 -

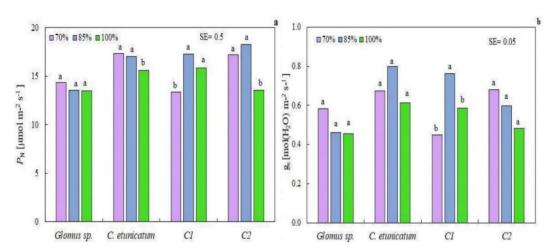


Figure 3. Effect of decreasing the irrigation dose on the $P_N(a)$ and $g_s(b)$ at 38 DAP in tomato plants inoculated with mycorrhizal spores of: Glomus sp.; C. etunicatum; C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. Irrigation dose: 70%; 85% and 100%. SE: indicates standard error. Similar letters represent the absence of statistical differences between the averages by independent groups according to the Tukey's test P < 0.05

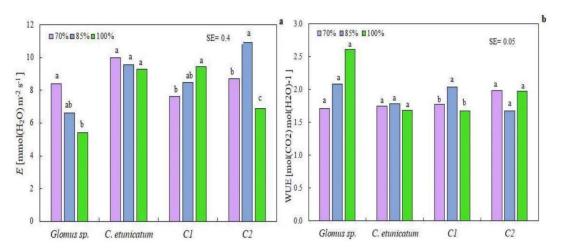


Figure 4. Effect of decreasing irrigation dose on E (a) and WUE (b) at 38 DAP in tomato plants inoculated with mycorrhizal spores of: Glomus sp.; C. etunicatum; C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. Irrigation dose: 70%; 85% and 100%. SE: indicates standard error. Similar letters represent the absence of statistical differences between the averages by independent groups according to the Tukey's test P<0.05

Glomus sp. increased q_N at the 85% and 100% irrigation rates and was 9.3% higher at the 85% irrigation rate than at the 70% irrigation rate. The inocula *C. etunicatum* and the mycorrhizal consortium C1 showed similar q_N values. On the other hand, inoculum C2 did not reflect differences with the 70% and 100% irrigation doses in q_N and was 8.5% higher with the 100% irrigation dose with respect to the 85% irrigation dose (*Figure 5.a*).

For ETR, the *Glomus* sp. inocula, *C. etunicatum* and the mycorrhizal consortium C1 showed no differences according to the three irrigation doses; however, ETR decreased by 33% with the C2 consortium when the 85% irrigation dose was used. In general, the

different inocula showed the highest values of qN and ETR at the 85% irrigation dose, except for the mycorrhizal consortium C2, which showed the lowest values at the medium dose in both variables (*Figure 5.b*).

Table 5. Results of the analysis of variance of the chlorophyll fluorescence parameters at 41 DAP in tomato plants inoculated with different AMF and subjected to different doses of irrigation

Source of variation	F _v /F _m	Φ _{PSII}	qр	qN	ETR	
Inoculum type						
Glomus sp.	0.824	0.295	0.76	0.931	111.12a	
C. etunicatum	0.789	0.291	0.769	0.911	111.66a	
C1	0.817	0.265	0.762	0.941	103.07ab	
C2	0.784	0.281	0.764	0.917	94.91b	
Dose of irrigation						
70%	0.783	0.293	0.767	0.908	113.45a	
85%	0.802	0.272	0.749	0.934	96.88b	
100%	0.826	0.284	0.776	0.934	105.24ab	
SE	0.025	0.022	0.036	0.026	5.55	
Results of ANAVA						
Inoculum type	0.144	0.377	0.992	0.484	0.002**	
Dose of irrigation	0.063	0.408	0.559	0.293	0.001**	
Interaction	0.575	0.436	0.460	0.026*	0.005**	

*P<0.05; ** P<0.01 and *** P<0.000. C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. SE (\pm standard error). Means with similar letters are not different according with Tukey's test (\propto =0.05)

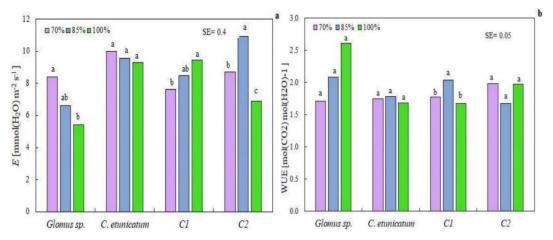


Figure 5. Effect of decreasing irrigation dose on chlorophyll fluorescence parameters at 41 DAP $q_N(a)$ and ETR (b) in tomato plants inoculated with mycorrhizal spores of: Glomus sp.; C. etunicatum; C1: mycorrhizal consortium from humid climate; C2: mycorrhizal consortium from semi-dry climate. Irrigation dose: 70%; 85% and 100%. SE: indicates standard error. Similar letters represent the absence of statistical differences between the averages by independent groups according to the Tukey's test P<0.05

Discussion

Mycorrhizal variables

The single species inoculation showed a contrasting behavior in terms of the number of colonized roots and the presence of hyphae, arbuscules and vesicles, *Glomus* sp. was very efficient with the medium irrigation dose (85%), while *C etunicatum* was more efficient with the extreme irrigation doses (70% and 100%). The ecosystem from which these AMF species originate may be the cause of this response, since there is a great difference between the characteristics of a humid ecosystem and an arid ecosystem, in terms of soil type and vegetation, so that the species that inhabit them must create different mechanisms of adaptation to these environments. However, the efficiency of AMF may not always depend on the environment they come from, but also on the synergism that can occur when working with AMF consortia. In this case, efficiency may be conditioned by the species that make up the consortium and the role played by each of them in this association.

In our results, the mycorrhizal consortium C1, which comes from a humid ecosystem, showed a greater number of colonized roots and presence of hyphae, arbuscules and vesicles with the lowest irrigation dose (70%). It appears that certain AMF may be favored in certain specific situations, and they demonstrate this by provoking transformations inside and outside the root system. The increased presence of intra- and extraradical mycorrhizal structures in the host is related to the abundance of mycorrhizal species found in the soil, although there are also strategies used by individual species for effective colonisation (Barceló et al., 2020). Lidoy et al. (2023), report that AMF species have different colonization strategies in the face of host stress, and this is reflected in symbiosis. These authors found in tomato subjected to salt stress, that it affected differently when inoculated with F. mosseae and R. irregularis species, promoting colonization with the former and restricting it with the latter. On the other hand, Orine et al. (2022), in water-stressed tomato inoculated with the individual species F. mosseae, *R. irregularis* and the union of both species; found that colonization depended on AMF species and their interaction with drought. However, under a normal irrigation regime, *R. irregularis* showed higher root colonization, while inoculation with both species was lower. Which shows that functional differences in symbiosis will depend on the functional diversity between different combinations of plant-fungal genotypes (Hart et al., 2018; Kokkoris et al., 2019).

Photosynthetic parameters

Results show that reduction of irrigation does not cause reduction on gas exchange variables (P_N , g_s , E and WUE) when plants were inoculated with single AMF species, and the C2 consortium. However, plants inoculated with the C1 consortium decreased their gas exchange when irrigation was reduced by up to 30%. This suggests that the differences in response of the consortia are due to the origin of the AMF species that make up the consortium, with those from an arid environment performing better (*Figure 3.* and *Figure 4.*). On the other hand, comparing mycorrhizal colonization of roots and gas exchange in plants inoculated with the consortia, this increased colonization when the irrigation dose was reduced by 15%. The increase in P_N , g_s , E and WUE in plants inoculated with the different AMF inoculums under water deficit may be related to the abundance of extraradical AMF mycelia, which penetrate deeply into the substrate

and provide the plant with the necessary moisture to maintain efficient gas exchange under limited water supply. As for the plants inoculated with *Glomus* sp. and *C. etunicatum*, in addition to the abundant presence of AMF hyphae in the substrate, these species were better adapted to the trap culture in which they were propagated, suggesting that *Glomus* sp. and *C etunicatum* have different adaptation mechanisms that may provide greater protection to the plant they colonize. Our results confirm the hypothesis that AMF inoculation can maintain photosynthesis in tomato plants when substrate moisture is restricted, and that this response depend on the behavior of the AMF species involved and on the way the species interact, in consortium or individually. However, it was also showed that growth and yield are more sensitive to decreases in water availability than to the inoculum type (Mena-Echevarría et al., 2024) then is necessary to deep into the mechanisms that link the benefits of inoculation with the efficiency of photosynthesis and the assignation of assimilates.

As for the efficiency of non-photochemical quenching (q_N) in plants inoculated with Glomus sp. and the C2 consortium, it decreased when irrigation was reduced by 30% and 15%, respectively. It is known that when q_N decreases in plants subjected to water stress, the photoprotective capacity is impaired and the adaptive capacity of the plant is reduced. Similarly, a marked decrease in ETR was observed in plants inoculated with the C2 consortium when irrigation was reduced by 15%, indicating that fluorescence efficiency may have been affected, since q_N and ETR are related to protective mechanisms in the plant, an increase in which reduces the formation of reactive oxygen species (ROS). However, it is important to note that when the irrigation dose was reduced by 30%, plants inoculated with the C2 consortium showed adequate values in these two variables. What happened to the efficiency in the energy centers of PSII and PSI in plants inoculated with the C2 consortium when the irrigation dose was reduced by only 15%, if the plants maintained a stable gas exchange and reflected a high level of colonization in the roots? This is a question we cannot answer with this assay, as a more extensive study is needed to learn more about the behavior of this consortium. We know that water deficit affects the efficiency of Φ_{PSII} by reducing the transport of electrons from PSII to PSI, thereby reducing the photosynthetic capacity of the plant. Our studies were limited to a short period of time and to a situation of mild water stress, so we cannot be sure if there was really damage to the photosynthetic apparatus in plants inoculated with the C2 consortium, since the rest of the photosynthetic variables showed adequate functioning.

It is well known that plant growth and photosynthesis are increased when plants are mycorrhized because AMF-plant symbiosis improves water status; with a positive effect on relative water content (Chen et al., 2018). In our results, AMF native to semi-arid environments provides greater drought resistance by incorporating water and nutrients more efficiently into dry soils. Huang et al. (2020) suggest that native AMF should be considered as a biological tool to improve drought tolerance. A better adaptation of AMF from dryer ecosystems could explain best performance into less water substrate. However, photosynthesis responses when AMF come from drier environments must be investigated in order to be best explained. A possible reason for the differences found in plant gas exchange between the C1 and C2 mycorrhizal consortia may be related to the synergism present in each of the consortia and the origin of the species. It is thought that in a mycorrhizal consortium, each species involved has a specific function and may influence plant response to water deficit stress differently, although this is an issue under study. Similarly, variations in the hormonal balance (ABA, strigolactones and jasmonic acid) is another fundamental mechanism in the development and functionality of the

extensive network of explorative hyphae that are the structures in charge of transporting nutrients and water into the plant, these hormonal changes may depend on the AMF species and the plant host (Bernardo et al., 2019).

Different irrigation doses affected the functionality between species for the parameters q_N and ETR. In summary, we can suggest that the mycorrhizal consortium C1 and C. etunicatum can maintain chlorophyll fluorescence efficiency when managed at different irrigation regimes. Moustakas et al. (2020) reported increases in Φ_{PSII} due to a higher efficiency of the open PSII centres in utilising the absorbed light in Salvia fruticosa mycorrhized plants with Rhizophagus irregularis. According to these authors, an increase in ETR contributes to increased photosynthesis and plant growth. On the other hand, Mathur et al. (2019) in *Triticum aestivum* plants mycorrhized with a native consortium (Rhizophagus intraradices; Funneliformis mosseae; F. geosporum) under different irrigation regimes found that water stress-induced damage to PSII and PSI structure and function was alleviated by mycorrhizal colonization. Volpe et al. (2018) have reported finding differences in tolerance to water deficit in mycorrhizal plants, depending on the source of AMF, inoculum composition and host plant species. Mycorrhization is justified by the balance between costs and benefits, where the higher carbohydrate cost for plants in AMF symbiosis is balanced by the increase in their photosynthetic capacity (Romero-Munar et al., 2017). Our results suggest that AMF species do not respond equally when different irrigation regimes are applied; and confirm that inoculation with AMF can induce positive or negative responses in the efficiency of the plant photosynthetic apparatus, even more so if there is a water deficit.

Conclusion

Single species inoculation of AMF keeps up stable photosynthesis when plants are subjected to water reductions and AMF from drier environments maintain the most. Inoculation of AMF as consortia also help to sustain photosynthetic activity when water shortage but not as with single species inoculation. In the consortia, mycorrhizal colonization increased when the irrigation dose was reduced by 30%, while gas exchange in the plants was more stable when the irrigation dose was reduced by 15%. The C1 consortium from the wet environment decreased gas exchange when irrigation was reduced by 30%, whereas the C2 consortium from the dry environment decreased non-photochemical quenching and electron transport rate when irrigation was reduced by 15%. This suggests that the differences in the response of the consortia are due to the origin of the AMF species in the consortium.

Acknowledgements. To the Faculty of Agronomy and Veterinary Medicine of the Autonomous University of San Luis Potosí and the National Council of Science and Technology for the scholarship granted with the number 785163, which supported the first author on obtaining the grade of doctor. To University of California Riverside for providing facilities to second author during reviewing and correcting this manuscript.

REFERENCES

- [1] Álvarez-Sánchez, F., Sánchez-Gallen, I., Cuevas, L., Oro, L., Meli, P. (2017): Diversidad, abundancia y variación estacional en la comunidad de hongos micorrizógenos arbusculares en la selva Lacandona, Chiapas, México. Scientia Fungorum 45: 37-51. https://doi.org/10.33885/sf.2017.0.1166.
- [2] Armada Rodríguez, E. (2016): Efectos de microorganismos rizosféricos autóctonos (bacterias y hongos micorrízico arbusculares) sobre la tolerancia de las plantas al déficit hídrico en zonas semiáridas: Mecanismos implicados. Universidad de Granada. http://hdl.handle.net/10481/41123.
- [3] Augé, R. M., Toler, H. D., Saxton, A. M. (2015): Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: A meta-analysis. – Mycorrhiza 25(1): 13-24. https://doi.org/10.1007/s00572-014-0585-4.
- [4] Bakr, J., Daood, H., Pék, Z., Prof. Dr. Helyes, L., Posta, K. (2017): Yield and quality of mycorrhized processing tomato under water scarcity. – Applied Ecology and Environmental Research 15: 401-413. https://doi.org/10.15666/aeer/1501_401413.
- [5] Barceló, M., van Bodegom, P. M., Tedersoo, L., den Haan, N., Veen, G. F. C., Ostonen, I., Trimbos, K., Soudzilovskaia, N. A. (2020): The abundance of arbuscular mycorrhiza in soils is linked to the total length of roots colonized at ecosystem level. – PloS One 15(9): e0237256. https://doi.org/10.1371/journal.pone.0237256.
- [6] Begum, N., Ahanger, M. A., Su, Y., Lei, Y., Mustafa, N. S. A., Ahmad, P., Zhang, L. (2019): Improved Drought Tolerance by AMF Inoculation in Maize (*Zea mays*) Involves Physiological and Biochemical Implications. – Plants (Basel, Switzerland) 8(12): 579. https://doi.org/10.3390/plants8120579.
- [7] Bernardo, L., Carletti, P., Badeck, F. W., Rizza, F., Morcia, C., Ghizzoni, R., Rouphael, Y., Colla, G., Terzi, V., Lucini, L. (2019): Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. – Plant Physiology and Biochemistry 137: 203-212. https://doi.org/10.1016/j.plaphy.2019.02.007.
- [8] Bhatt, U., Singh, H., Kumar, D., Strasser, R., Soni, V. (2022): Severe leaf-vein infestation upregulates antioxidant and photosynthetic activities in the lamina of Ficus religiosa. Acta Physiologiae Plantarum 44: 15. https://doi.org/10.1007/s11738-021-03348-5.
- [9] Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N. (1996): Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research. https://doi.org/10.13140/2.1.4880.5444.
- [10] Buchanan, B. B., Gruissem, W., Jones, R. L. (2015): Biochemistry & Molecular Biology of Plants. – 2nd ed. John Wiley & Sons, Hoboken, p. 1280. https://www.pelergo.com/book/2752817/biochemistry-and-molecular-biology-of-plantspdf.
- [11] Chang, W., Sui, X., Fan, X.-X., Jia, T.-T., Song, F.-Q. (2018): Arbuscular Mycorrhizal Symbiosis Modulates Antioxidant Response and Ion Distribution in Salt-Stressed Elaeagnus angustifolia Seedlings. – Frontiers in Microbiology 9: 652. https://doi.org/10.3389/fmicb.2018.00652.
- [12] Chen, M., Arato, M., Borghi, L., Nouri, E., Reinhardt, D. (2018): Beneficial Services of Arbuscular Mycorrhizal Fungi - From Ecology to Application. – Frontiers in Plant Science 9: 1270. https://doi.org/10.3389/fpls.2018.01270.
- [13] Chitarra, W., Pagliarani, C., Maserti, B., Lumini, E., Siciliano, I., Cascone, P., Schubert, A., Gambino, G., Balestrini, R., Guerrieri, E. (2016): Insights on the Impact of Arbuscular Mycorrhizal Symbiosis on Tomato Tolerance to Water Stress. – Plant Physiology 171: 1009-1023. https://doi.org/10.1104/pp.16.00307.
- [14] Duc, N., Csintalan, Z., Posta, K. (2018): Arbuscular mycorrhizal fungi mitigate negative effects of combined drought and heat stress on tomato plants. Plant Physiology and Biochemistry 132: 297-307. https://doi.org/10.1016/j.plaphy.2018.09.011.

- [15] Gerdemann, J. W., Nicolson, T. H. (1963): Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. – Transactions of the British Mycological Society 46(2): 235-244. https://doi.org/10.1016/S0007-1536(63)80079-0.
- [16] Grümberg, B., Urcelay, C., Shroeder, M., Vargas Gil, S., Luna, C. (2014): The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean.
 Biology and Fertility of Soils 51: 1-10. https://doi.org/10.1007/s00374-014-0942-7.
- [17] Hart, M. M., Antunes, P. M., Chaudhary, V. B., Abbott, L. K. (2018): Fungal inoculants in the field: Is the reward greater than the risk? – Functional Ecology 32(1): 126-135. https://doi.org/10.1111/1365-2435.12976.
- [18] Herrera-Corrales, L. C., Ospina-Alzate, D. F., Ocampo-Jiménez, O. (2014): Efecto de gremios de hongos micorrícicos arbusculares aislados de un ambiente desértico sobre el crecimiento de frijol Phaseolus vulgaris bajo una condición de déficit hídrico. – Actualidades Biológicas 36(100): 63-72. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0304-35842014000100008&lng=en&tlng=es.
- [19] Huang, G.-M., Ying-Ning, Z., Wu, Q.-S., Xu, Y.-J., Kuca, K. (2020): Mycorrhizal roles in plant growth, gas exchange, root morphology, and nutrient uptake of walnuts. – Plant, Soil and Environment 66: 295-302. https://doi.org/10.17221/240/2020-PSE.
- [20] Kokkoris, V., Li, Y., Hamel, C., Hanson, K., Hart, M. (2019): Site specificity in establishment of a commercial arbuscular mycorrhizal fungal inoculant. – The Science of the Total Environment 660: 1135-1143. https://doi.org/10.1016/j.scitotenv.2019.01.100.
- [21] Li, J., Meng, B., Chai, H., Yang, X., Song, W., Li, S., Lu, A., Zhang, T., Sun, W. (2019): Arbuscular Mycorrhizal Fungi Alleviate Drought Stress in C(3) (*Leymus chinensis*) and C(4) (*Hemarthria altissima*) Grasses via Altering Antioxidant Enzyme Activities and Photosynthesis. – Frontiers in Plant Science 10: 499. https://doi.org/10.3389/fpls.2019.00499.
- [22] Lidoy, J., López-García, Á., Amate, C., García, J. M., Flors, V., García-Garrido, J. M., Azcón-Aguilar, C., López-Raez, J. A., Pozo, M. J. (2023): Regulation of mycorrhizal colonization under stress in tomato depends on symbiotic efficiency. – Environmental and Experimental Botany 215: 105479. https://doi.org/10.1016/j.envexpbot.2023.105479.
- [23] Magurran, A. E. (2004): Measuring Biological Diversity. 1st ed. Cornwall.
- [24] Martínez, P., Roca, D. (2011): Sustratos para el cultivo sin suelo. Materiales, propiedades y manejo, pp. 37-77.
- [25] Mathur, S., Tomar, R., Jajoo, A. (2019): Arbuscular Mycorrhizal fungi (AMF) protects photosynthetic apparatus of wheat under drought stress. – Photosynthesis Research 139: 227-238. https://doi.org/10.1007/s11120-018-0538-4.
- [26] Mena-Echevarría, A., Méndez Cortes, H., Ramírez Tobías, H. M., Rojas Velázquez, Á. N. (2021): Comparación de dos suelos para la producción de inoculantes micorrízicos en San Luis Potosí, México. – Scientia Fungorum 51(0): e1315. https://doi.org/10.33885/sf.2021.51.1315.
- [27] Mena-Echevarría, A., Ramírez-Tobias, H. M., Méndez-Cortés, H., Rojas-Velázquez, Á. N., López-Palacios, C., Hipólito-Piedras, R. P. (2024): The Origin and Type of Inoculum Determine the Effect of Arbuscular Mycorrhizal Fungi on Tomato under Different Irrigation Regimes. Agronomy 14(8): 1687. https://doi.org/10.3390/agronomy14081687.
- [28] Moustakas, M., Bayçu, G., Sperdouli, I., Eroğlu, H., Eleftheriou, E. P. (2020): Arbuscular Mycorrhizal Symbiosis Enhances Photosynthesis in the Medicinal Herb Salvia fruticosa by Improving Photosystem II Photochemistry. – Plants (Basel, Switzerland) 9(8): 962. https://doi.org/10.3390/plants9080962.
- [29] Orine, D., Defossez, E., Vergara, F., Uthe, H., Dam, N., Rasmann, S. (2022): Arbuscular mycorrhizal fungi prevent the negative effect of drought and modulate the growth-defence trade-off in tomato plants. – Journal of Sustainable Agriculture and Environment 1: 177-190. https://doi.org/10.1002/sae2.12018190.

- [30] Pal, K. K., McSpadden-Gardener, B. (2006): Biological Control of Plant Pathogens. The Plant Health Instructor 25. https://doi.org/10.1094/PHI-A-2006-1117-02.
- [31] Qu, Q., Zhang, Z., Peijnenburg, W. J. G. M., Liu, W., Lu, T., Hu, B., Chen, J., Lin, Z., Qian, H. (2020): Rhizosphere Microbiome Assembly and Its Impact on Plant Growth. Journal of Agricultural and Food Chemistry 68(18): 5024-5038. https://doi.org/10.1021/acs.jafc.0c00073.
- [32] Rani, B. (2016): Effect of arbuscular mycorrhiza fungi on biochemical parameters in wheat (*Triticum aestivum* L.) under drought conditions. Environmental Science, Agricultural and Food Sciences, Biology. https://api.semanticscholar.org/CorpusID:89584447.
- [33] Rodriguez, P. A., Rothballer, M., Chowdhury, S. P., Nussbaumer, T., Gutjahr, C., Falter-Braun, P. (2019): Systems Biology of Plant-Microbiome Interactions. – Molecular Plant 12(6): 804-821. https://doi.org/10.1016/j.molp.2019.05.006.
- [34] Romero-Munar, A., Del-Saz, N. F., Ribas-Carbó, M., Flexas, J., Baraza, E., Florez-Sarasa, I., Fernie, A. R., Gulías, J. (2017): Arbuscular Mycorrhizal Symbiosis with Arundo donax Decreases Root Respiration and Increases Both Photosynthesis and Plant Biomass Accumulation. – Plant, Cell & Environment 40(7): 1115-1126. https://doi.org/10.1111/pce.12902.
- [35] Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., Pascale, S. D., Bonini, P., Colla, G. (2015): Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. – Scientia Horticulturae 196: 91-108. https://doi.org/10.1016/j.scienta.2015.09.002.
- [36] Tedersoo, L., Bahram, M., Zobel, M. (2020): How mycorrhizal associations drive plant population and community biology. – Science (New York, N.Y.) 367(6480): eaba1223. https://doi.org/10.1126/science.aba1223.
- [37] Trouvelot, A., Kough, J. L., Gianinazzi-Pearson, V. (1986): Estimation of VA mycorrhizal infection levels. Research for methods having a functional significance. Physiological and Genetical Aspects of mycorrhizae = Aspects physiologiques et genetiques des mycorhizes, Dijon, France. https://api.semanticscholar.org/CorpusID:90570584.
- [38] Volpe, V., Chitarra, W., Cascone, P., Volpe, M. G., Bartolini, P., Moneti, G., Pieraccini, G., Di Serio, C., Maserti, B., Guerrieri, E., Balestrini, R. (2018): The Association with Two Different Arbuscular Mycorrhizal Fungi Differently Affects Water Stress Tolerance in Tomato. Frontiers in Plant Science 9: 1480. https://doi.org/10.3389/fpls.2018.01480.
- [39] Yon, Y., Pérez, L., Medina, A., Mujica Perez, Y., Medina, L., Suárez, K., Mena, A. (2015): Alternativa de la técnica de tinción para determinar la colonización micorrízica. – Cultivos Tropicales 36(2): 18-21. http://dx.doi.org/10.13140/rg.2.2.10232.65287.
- [40] Yooyongwech, S., Samphumphuang, T., Tisarum, R., Theerawitaya, C., Cha-Um, S. (2015): Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. – Scientia Horticulturae 198: 107-117.