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Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico

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Abstract

Selecting indigenous mycorrhizal fungi that enhance plant water status is important in Mexico for sustainable production systems of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis). To determine mycorrhizal enhancement of drought resistance, plants were either non-inoculated (NonAMF), or inoculated with arbuscular mycorrhizal fungi (AMF): *Glomus fasciculatum* and a mixed *Glomus* spp. from Mexico (ZAC-19). Plants were then exposed to a 20-day drought cycle. To equalize growth and minimize tissue-P differences, NonAMF plants received higher P than AMF plants. Drought reduced leaf water potential (Ψ_{leaf}), tissue relative water content (RWC), stomatal conductance (g_s), whole plant transpiration ($\text{mg H}_2\text{O m}^{-2} \text{s}^{-1}$), leaf transpirational surface area and plant biomass. Only plants colonized with ZAC-19 had enhanced drought resistance, as indicated by higher Ψ_{leaf} and fewest plants with visible wilting during peak drought stress. A higher root/shoot ratio occurred with ZAC-19 plants (despite equal total plant biomass among droughted plants), which may have also contributed to drought resistance. Drought enhanced arbuscule formation and hyphae development of ZAC-19, while reducing colonization of *G. fasciculatum*. Tissue P was not a contributing factor to drought resistance. AMF did not enhance water-use efficiency (WUE) as-determined gravimetrically on a whole plant basis (g carbon/kg H_2O) or by carbon isotope discrimination (Δ). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arbuscular mycorrhiza; Carbon isotope discrimination; Drought resistance; Water-use efficiency

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) can mitigate plant response to water stress (Davies et al., 1996; Nelson, 1987). Possible mechanisms for improved drought resistance of AMF plants include increased root hydraulic conductivity (Duan et al., 1996), changes in stomatal regulation, which may be influenced by ABA levels in xylem sap (Duan et al., 1996), enhanced water uptake due to extraradical hyphae (Davies et al., 1992; Hardie, 1985), osmotic adjustment that promotes turgor maintenance even at low tissue water potential (Augé et al., 1986a) and changes in cell wall elasticity (Augé et al., 1987). Regardless of how drought resistance develops, it should increase plant hydration and turgor, which in turn should promote stomatal conductance (g_s) and photosynthetic flux (A) (Kramer and Boyer, 1995). According to Fitter (1988), the influence of AMF on plant–water relations may be a secondary consequence of enhanced host P nutrition, although these effects are inconsistent. Increased tissue P concentration in AMF plants are associated with improved water relations in onion (Nelson, 1987). Conversely, AMF improved drought resistance of roses (Augé et al., 1986a), geraniums (Sweatt and Davies, 1984) and bell pepper plants (Davies et al., 1993) differentially fertilized to equalize tissue P concentration. Consequently, the involvement of P in drought resistance of AMF plants is controversial.

Chile ancho is a rich and important source of vitamin C and together with the essential amino acids supplied by corn tortillas and beans constitutes a very staple diet in Mexico that can be supplemented with meat and poultry. The more important production zones of Chile ancho are in the semi-arid valleys at the center of Mexico—in the states of Guanajuato, San Luis Potosí, Durango, Aguascalientes and Zacatecas (Lobore and Pozo, 1982). The availability of sufficient good quality irrigation water limits the production of Chile ancho in these semi-arid regions. Hence, there is interest in selecting indigenous AMF that enhance drought resistance.

The objectives of this research were: (1) to determine the influence of AMF in alleviating drought stress effects of Chile ancho pepper plants based on plant morphological response to drought, plant–water relations, and gas exchange characteristics; (2) to evaluate the influence of drought on AMF development of arbuscules, vesicles, and hyphae formation. A goal of this research is to demonstrate the usefulness of AMF to increase drought resistance in sustainable production systems for this important agricultural crop in Mexico.

2. Materials and methods

2.1. Cultural conditions

This study was conducted under glasshouse conditions at the CINVESTAV Plant Biology Institute, Irapuato, Mexico. Seeds of *Capsicum annuum* L. (Chile ancho cv. San Luis) were sown in a medium of pasteurized 1 peat:1 perlite (v/v), on 16 September, 1993, and transplanted 4 weeks later into 1.7-l pots containing 1 coarse sand:1 low P sandy loam soil (v/v) with a textural analysis of 77% sand, 9% clay and 14% silt.

The sandy loam soil was collected from an agricultural production site in Irapuato, Guanajuato, Mexico. The soil contained 0.26% organic matter and in $\mu\text{g g}^{-1}$: P (7), K (125), Mg (136), Fe (8), Mn (5), Zn (3) and Cu (6). The container medium was previously steam pasteurized during two consecutive days for 5 h per day at 80 °C. To avoid problems of different sized plants and differences in transpirational surfaces in this drought study, non-colonized (NonAMF) plants were fertilized with twice the level of P than colonized (AMF) plants. During the first 4.5 weeks of establishment, plants were irrigated as needed and fertilized weekly with 200 ml per pot of modified Long Ashton nutrient solution (LANS; Hewitt, 1966) to supply 44 $\mu\text{g P ml}^{-1}$ (full strength P) to NonAMF plants and 22 $\mu\text{g P ml}^{-1}$ to AMF plants. Fertilization was continued through the initial 2 weeks of the drought cycle and then discontinued (last 8 days of experiment). Potted plants were maintained in the greenhouse under an average minimum/maximum temperature of 14/34 °C and an average maximum high PPFD of 1052 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Photoperiod ranged from 10.8 to 9.5 h, and the greenhouse was illuminated with incandescent lights for 2 h middle night interruption to maintain long-day conditions.

The mycorrhizal treatments were: ZAC-19—a mixed isolate of *Glomus albidum*, *Glomus claroides* and *Glomus diaphanum* (Chamizo et al., 1998), a pure isolate of *Glomus fasciculatum* (Gerdemann and Trappe, 1974) and a non-inoculated control (NonAMF). The ZAC-19 isolate was collected from a non-irrigated, low nutrient and low organic matter soil used for commercial bean production in the State of Zacatecas, Mexico with an annual precipitation of 450 mm. The sandy-loam soil had a pH of 5.4, 16 $\mu\text{g g}^{-1}$ P and 1.1% organic matter (Contreras and Ferrera-Cerrato, 1989). The isolate of *G. fasciculatum* originated from Oregon (Gerdemann and Trappe, 1974) and is reported to enhance plant growth and nutrient uptake (J.M. Trappe, USDA/ARS, Corvallis, OR, personal communication). Plants were inoculated with an average of 2980 spores of *G. fasciculatum* and 250 spores the ZAC-19 isolate (including colonized root segments of onion and sorghum host plants used for isolate multiplication). The inocula were banded in the middle of the container.

After the initial 4.5-week establishment period in the greenhouse, half the plants were exposed to a 20-day drought cycle (19 November 1993–8 December 1993) that was regulated by monitoring daily evaporative loss and irrigating containers to replenish 70% of the daily water evaporated. Non-droughted plants were irrigated as needed. The surfaces of the container media was covered with poly bags which were wrapped around the base of the stems so that water loss occurred only through transpirational surfaces of the plant. Parameters monitored during the drought cycle included % soil moisture content, whole plant transpiration, leaf water potential (Ψ_{leaf}), relative water content (RWC) and stomatal conductance (g_s). After peak drought stress was achieved (day 20), plants were rehydrated and the experiment terminated and all plants harvested the following day.

2.2. Plant water and gas exchange measurements

Predawn Ψ_{leaf} was measured with newly matured leaves using a pressure chamber (Kramer and Boyer, 1995). The % RWC was determined by fresh mass–dry mass/saturated mass–dry mass (FM–DM/SM–DM). FM was determined on leaf discs taken

from newly matured leaves at the time leaf water potentials were measured. Leaf discs were then floated on water in a closed Petri dish for 2 h to achieve saturation without water accumulating in the intracellular spaces. After determining the SM, the discs were then dried at 70 °C and DM determined. The % soil moisture was determined by taking a cork borer and inserting it into the container soil and extracting a soil core which was immediately weighed (FM) and then dried at 70 °C to gravimetrically determine the DM. Water-use efficiency (WUE; g carbon/kg H₂O) was determined by harvesting four representative plants from each treatment on day 0 of the drought stress cycle to determine their initial DM. The final DM was taken when plants were harvested at the end of the experiment. The total amount of water transpired and reapplied during the 20-day cycle was recorded. Whole plant transpiration (mg H₂O m⁻² s⁻¹) was also determined gravimetrically over the last 6 days of the drought cycle (Kramer and Boyer, 1995).

Measurements of g_s were made with an autoporometer (LI-COR 1600, LI-COR, Lincoln, NE). Measurements were made on newly matured leaves from 9:00 to 11:00 h in the glasshouse under cloudless days, with an average high PPFD of 1052 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Plants were also visually evaluated for wilting response to drought late in the afternoon of day 20 during peak drought stress by using the following criteria—(1) non-wilted: leaves and petioles turgid; (2) moderately wilted: petioles horizontal and partial wilting of leaves; (3) severely wilted: petioles with a 90° droop, severely wilted leaves curved under at margins and partial wilting of stem.

At the termination of the experiment, carbon isotope composition was measured on dried, ground leaf samples from newly expanding leaves that developed during the drought cycle ($n = 5$) using a gas isotope ratio mass spectrometer (VG Microgas 903, VG Isogas, Middlewich, UK). Carbon isotope ratios ($\delta^{13}\text{C}$) were calculated from isotope compositions, relative to the PeeDee Belemnite standard, and converted to carbon isotope discrimination (Δ , ‰) values, using atmospheric carbon dioxide values of -8‰ (Boutton, 1991).

2.3. Assessment of plant growth and development and leaf tissue elemental analysis

Final growth measurements of 13 plants per treatment ($n = 13$) were taken 53 days after transplanting, and included leaf area, shoot DM (stem, leaves, fruit), root DM, root/shoot ratio (g g⁻¹) and leaf area ratio (LAR; cm² g⁻¹). Leaves were analyzed for elemental phosphorus with an inductively coupled atomic emission spectrophotometer (3510 ICP, W.R. Grace, Fogelsville, PA). There were no differences in tissue P prior to drought treatments. On day 0 of the drought cycle, newly matured leaves from four plants each of NonAMF, ZAC-19 and *G. fasciculatum* were analyzed; leaf tissue P ranged from 2.5 to 2.8 g P kg⁻¹. At the termination of the experiment, leaf tissue was taken from newly matured leaves of 12 plants per treatment. Samples were pooled into three composite samples per treatment for analysis ($n = 4$).

2.4. Assessment of mycorrhizal development

For AMF analysis of roots, 1 cm root segments from 15 plants per treatment were sampled at harvest and pooled to assess colonization percentage through clearing and

staining of the root samples (Phillips and Hyman, 1970). Ten 1 cm stained root pieces were placed on each slide and three microscopic observations per 1 cm root piece at $40\times$ was made at the top, the middle and the bottom of each root piece. There were 15 slides per treatment ($n = 450$). The presence of arbuscules, vesicles and hyphae were recorded and the data obtained were statistically analyzed using χ^2 -analysis with a 2-test pair separation.

2.5. Statistical design

The $3 \text{ AMF} \times 2$ drought level factorial experiment was in a completely randomized design with each plant as a single replicate. There were 20 plants per treatment, and plants were sampled at times indicated with the data. Treatment effects were determined by using ANOVA (SAS, 1988) as indicated with the data. All growth parameter measurements at experiment termination were made on 13 plants ($n = 13$). The following numbers (n) of samples per treatment were taken at each sampling time: (a) for stomatal conductance, $n = 10$ (two leaves for each of five plants); (b) for water status determination, $n = 4$, except on day 20 (peak drought stress), when $n = 5$ for RWC, $n = 8$ for Ψ_{leaf} , $n = 5$ for % soil moisture. For whole plant transpiration, $n = 13$, and for visual evaluation of drought during peak environmental stress, $n = 19$. For mycorrhizal analysis, $n = 450$.

3. Results

By applying higher levels of P to NonAMF plants, all mycorrhizal treatments had comparable leaf area, shoot, root and total plant DM, root/shoot ratio and LAR ($\text{cm}^2 \text{g}^{-1}$) under non-droughted conditions (Table 1). Drought caused a reduction in leaf transpirational surface area and plant biomass, however, all mycorrhizal treatments had similar leaf area and plant mass. The only difference was with ZAC-19 that had the highest root/shoot ratio during drought, while maintaining similar total plant mass with NonAMF and *G. fasciculatum* colonized plants. Leaf tissue P was comparable among non-droughted mycorrhizal treatments ($1.8\text{--}2.1 \text{ g P kg}^{-1}$) (Table 1). However, droughted AMF plants had slightly higher leaf tissue P ($2.3\text{--}2.6 \text{ g P kg}^{-1}$) than NonAMF plants (2.0 g P kg^{-1}). The level of leaf tissue P was statistically similar between droughted *G. fasciculatum* and ZAC-19 plants.

During non-stress (day 1 of the drought cycle), there were no differences in % soil moisture, Ψ_{leaf} , g_s and RWC among the mycorrhizal treatments (Table 2). However, by day 14, there were reductions in % soil moisture, Ψ_{leaf} , g_s and RWC among droughted plants (Table 2). The exception was with ZAC-19 colonized plants which had comparable Ψ_{leaf} to non-droughted plants, despite a reduction in % soil moisture, g_s and RWC. During peak drought stress (day 20), greatest reductions occurred in % soil moisture, RWC, g_s and whole plant transpiration (Table 3). However, droughted ZAC-19 plants were still able to maintain a high Ψ_{leaf} and had the greatest number of plants that were only moderately wilted (Table 4). While all droughted plants showed some level of wilting, *G. fasciculatum* colonized plants had the highest number of plants that were

Table 1

Effect of mycorrhizal fungi (AMF; *G. fasciculatum*, mixed *Glomus* spp.—ZAC-19) and drought stress on growth and leaf elemental phosphorus uptake of Chile ancho pepper plants (*C. annuum* L. cv. San Luis)

AMF	Drought	Leaf area (cm ²)	Shoot DM (g)	Root DM (g)	Total plant DM (g)	Root/shoot ratio (g g ⁻¹)	LAR ^a (cm ² g ⁻¹)	P ^b (g kg ⁻¹)
Control	No	358 ± 11 ^c	5.5 ± 0.1 ^c	1.8 ± 0.1 ^c	7.3 ± 0.2 ^c	0.33 ± 0.02 ^c	49.2 ^c	1.5 ± 0.3 ^d
<i>G. fasciculatum</i>		371 ± 9	5.5 ± 0.1	2.2 ± 0.1	7.7 ± 0.2	0.39 ± 0.03	48.4	2.1 ± 0.1
ZAC-19		375 ± 11	5.2 ± 0.2	2.1 ± 0.1	7.3 ± 0.3	0.39 ± 0.02	51.8	2.1 ± 0.2
Control	Yes	313 ± 11	4.4 ± 0.2	1.7 ± 0.1	6.1 ± 0.3	0.38 ± 0.01	51.7	1.8 ± 0.2
<i>G. fasciculatum</i>		322 ± 15	4.4 ± 0.2	1.8 ± 0.1	6.2 ± 0.3	0.39 ± 0.03	52.2	2.3 ± 0.1
ZAC-19		322 ± 13	4.2 ± 0.1	1.9 ± 0.1	6.1 ± 0.2	0.45 ± 0.02	53.0	2.6 ± 0.2
<i>Significance</i>								
AMF		NS	NS	NS	NS	0.0219	NS	0.0453
Drought		0.0001	0.0001	0.0115	0.0001	NS	NS	0.0044
AMF × drought		NS	NS	NS	NS	NS	NS	NS

^a Leaf area ratio

^b Phosphorus.

^c Mean and standard error, *n* = 13.

^d Mean and standard error, *n* = 4.

Table 2
Effect of mycorrhizal fungi (AMF; *G. fasciculatum*, mixed *Glomus* spp.—ZAC-19) and drought stress on water relations of Chile ancho pepper plants (*C. annuum* L. cv. San Luis) during day 1 (non-stress) and day 14 (moderate stress) of the drought cycle^a

AMF	Drought	Day 1				Day 14			
		Soil moisture (%)	ψ_{leaf} (–MPa)	g_s (mmol m ^{–2} s ^{–1})	RWC (%)	Soil moisture (%)	ψ_{leaf} (–MPa)	g_s (mmol m ^{–2} s ^{–1})	RWC (%)
Control	No	19.3 ^b	0.2 ^b	216 ^c	95.7 ^b	19.2 ± 0.3 ^b	0.2 ± 0 ^b	194 ± 15 ^c	90.7 ± 0.7 ^b
<i>G. fasciculatum</i>		20.0	0.1	233	97.7	19.1 ± 0.5	0.2 ± 0	154 ± 14	92.9 ± 2.6
ZAC-19		20.7	0.1	202	94.2	19.9 ± 0.5	0.1 ± 0	176 ± 16	91.7 ± 0.2
Control	Yes	20.1	0.1	187	93.1	12.1 ± 1.0	0.5 ± 0.1	72 ± 15	85.3 ± 1.1
<i>G. fasciculatum</i>		19.4	0.1	219	92.7	12.5 ± 0.4	0.5 ± 0.1	75 ± 13	87.5 ± 1.5
ZAC-19		19.9	0.1	205	95.6	13.1 ± 0.3	0.2 ± 0	91 ± 20	88.6 ± 0.9
<i>Significance</i>									
AMF		NS	NS	NS	NS	NS	0.0005	NS	NS
Drought		NS	NS	NS	NS	0.0001	0.0001	0.0001	0.0007
AMF × drought		NS	NS	NS	NS	NS	0.0182	NS	NS

^a RWC: % relative water content; ψ_{leaf} : leaf water potential; g_s : stomatal conductance.

^b Mean and standard error, $n = 4$.

^c Mean and standard error, $n = 10$.

Table 3
Effect of mycorrhizal fungi (AMF; *G. fasciculatum*, mixed *Glomus* spp.—ZAC-19) and drought stress on water relations of Chile ancho pepper plants (*C. annuum* L. cv. San Luis) during peak drought stress (day 20)^a

AMF	Drought	Soil moisture (%)	ψ_{leaf} (–MPa)	g_s (mmol m ^{–2} s ^{–1})	RWC (%)	Whole plant transpiration ^b (mg H ₂ O m ^{–2} s ^{–1})	WUE (10 ^{–3} g kg ^{–1})	Δ (‰)
Control	No	17.0 ± 1.4 ^c	0.1 ± 0 ^d	268 ± 14 ^e	92.6 ± 1.4 ^c	48.2 ± 1.4 ^f	5.34 ^f	18.9 ^c
<i>G. fasciculatum</i>		18.3 ± 0.4	0.1 ± 0	257 ± 13	88.9 ± 1.1	48.1 ± 1.4	5.29	18.7
ZAC-19		18.8 ± 0.2	0.1 ± 0	233 ± 19	93.0 ± 1.9	46.7 ± 2.1	5.09	18.8
Control	Yes	3.9 ± 0.1	0.7 ± 0.1	57 ± 7	80.2 ± 3.4	30.1 ± 1.7	5.55	19.1
<i>G. fasciculatum</i>		3.8 ± 0.1	0.8 ± 0.1	46 ± 4	79.3 ± 1.0	27.1 ± 1.6	5.68	19.2
ZAC-19		4.1 ± 0.4	0.2 ± 0	51 ± 4	84.4 ± 1.9	25.4 ± 1.9	5.58	19.2
<i>Significance</i>								
AMF		NS	0.0001	NS	NS	NS	NS	NS
Drought		0.0001	0.0001	0.0001	0.0001	0.0001	NS	NS
AMF × drought		NS	0.0001	NS	NS	NS	NS	NS

^a RWC: % relative water content; WUE: water-use efficiency; ψ_{leaf} : leaf water potential; g_s : stomatal conductance; Δ : carbon isotope discrimination.
^b The whole plant transpiration was determined over the last 6 days of the drought stress cycle.
^c Mean and standard error, $n = 5$.
^d Mean and standard error, $n = 8$.
^e Mean and standard error, $n = 10$.
^f Mean and standard error, $n = 13$.

Table 4

Effect of mycorrhizal fungi (AMF; *G. fasciculatum*, mixed *Glomus* spp.—ZAC-19) and drought stress on visible wilting of Chile ancho pepper plants (*C. annuum* L. cv. San Luis) during peak environmental stress (day 20)

VAM	Drought	% Plants not wilted	% Plants moderately wilted	% Plants severely wilted
Control	No	100 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
<i>G. fasciculatum</i>		100 ± 0	0 ± 0	0 ± 0
ZAC-19		100 ± 0	0 ± 0	0 ± 0
Control	Yes	0 ± 0	47 ± 12	53 ± 12
<i>G. fasciculatum</i>		0 ± 0	26 ± 10	74 ± 10
ZAC-19		0 ± 0	79 ± 10	21 ± 11
<i>Significance</i>				
AMF		NS	0.0026	0.0089
Drought		0.0001	0.0001	0.0001
AMF × drought		NS	0.0026	0.0089

^a Mean and standard error, *n* = 19.

severely wilted (Table 4). There were no differences in WUE as determined on the whole plant level (g kg^{-1}) or by carbon isotope discrimination (Δ) among colonized plants (Table 3). However, there was a trend in higher WUE with droughted plants.

Drought enhanced arbuscule formation and hyphae development of ZAC-19, while reducing mycorrhizal colonization of *G. fasciculatum* (Table 5). Arbuscule formation and vesicle development of ZAC-19 was nearly twice that of *G. fasciculatum* during

Table 5

Effect of mycorrhizal fungi (AMF; *G. fasciculatum*, mixed *Glomus* spp.—ZAC-19) and drought stress on mycorrhizal colonization of Chile ancho pepper plants (*C. annuum* L. cv. San Luis) as indicated by % arbuscule and vesicle formation and total roots with hyphae, vesicles and/or arbuscules

	<i>G. fasciculatum</i>	ZAC-19	<i>G. fasciculatum</i>	ZAC-19	Pr > <i>F</i>
% Arbuscule formation					
Drought					
No	56.4a	37.5b	56.4a	37.5b	0.0001
Yes	21.6b	43.0a	21.6b	43.0a	0.0001
Pr > <i>F</i>	0.0001	0.04			
% Vesicle formation					
Drought					
No	81.4a	29.9	81.4a	29.9b	0.0001
Yes	42.8b	26.1	42.8a	26.1b	0.0001
Pr > <i>F</i>	0.0001	NS			
% Total root colonization					
Drought					
No	90.2a	51.9b	90.2a	51.9b	0.0001
Yes	62.7b	59.9a	62.7a	59.9a	0.0001
Pr > <i>F</i>	0.0001	0.003			

drought, which was the reverse under non-droughted conditions. No colonization occurred with NonAMF plants (data not presented).

4. Discussion

Only Chile ancho pepper plants colonized with the Mexican ZAC-19 isolate had enhanced drought resistance, as indicated by a higher Ψ_{leaf} and fewer plants showing visible wilting during peak drought. By differentially fertilizing so that higher P was applied to NonAMF plants, equal tissue mass and evaporative surfaces were obtained among mycorrhizal treatments at each drought level (droughted and non-droughted). A major problem in interpreting most AMF–water relations studies is that the mycorrhizal plants typically have a greater mass than NonAMF plants (Fitter, 1988; Nelson, 1987). With larger mass, AMF plants should have greater transpiring surfaces, and if their roots are confined in a container, such plants should desiccate more quickly and have lower Ψ_{leaf} during drought compared to smaller-sized controls (Sweatt and Davies, 1984). In our study, Chile ancho plants had equivalent leaf area, LAR and shoot mass among all treatments due to manipulation of P fertilization. Thus the greater drought resistance of ZAC-19 plants was not confounded by differences in transpirational area, LAR or shoot mass.

Droughted ZAC-19 plants had a higher root/shoot ratio (despite similar total plant biomass and leaf area) than other treatments. The root/shoot ratio may be increased, decreased (Hardie and Layton, 1991) or is unaffected (Augé et al., 1986b) by AMF. A high root/shoot ratio is a frequent response to drought and can enhance drought resistance (Kramer and Boyer, 1995).

Although mycorrhiza can promote P uptake, and P uptake is generally reduced under drought (Kramer and Boyer, 1995), it is not clear how increased P could improve water relations or drought resistance. Despite the 2-fold higher levels of P applied to NonAMF Chile ancho plants, they still had somewhat lower tissue P compared with AMF plants. *G. fasciculatum* was ineffective in enhancing drought resistance, yet there were no differences in tissue P between this isolate and ZAC-19. This suggests that levels of tissue P were not a contributing factor to drought resistance in Chile ancho. In a study with bell pepper (*C. annuum*), improved water relations of AMF plants could not be attributed to P nutrition, which was higher in NonAMF plants (Davies et al., 1993). The P levels of droughted Chile ancho plants were in the range of recommended for commercial production of pepper (Maynard and Hochmuth, 1997).

With our experiment, drought slightly increased leaf tissue P of all treatments. In part, this may have been due to reduced carbon availability for growth under drought conditions (Davies et al., 1993). Sometimes leaves are very conservative in their P concentration (Smith and Read, 1997). Drought reduced the leaf surface area equally among all mycorrhizal Chile ancho treatments. By producing smaller leaf areas, droughted plants were able to maintain tissue P concentrations and reduce transpirational losses. The slight increase in P between droughted vs. non-droughted plants may also be attributed to fertilization being discontinued during the last 8 days of the drought cycle and recovery. This may have caused a slight dilution in tissue P of the non-droughted plants which continued to grow.

The relationship between whole plant transpiration (E), g_s , AMF and leaf P is complex and controversial even for well-watered plants. AMF are reported to increase E and leaf P (Augé, 1989). However, Fitter (1988) did not show increased g_s with increasing leaf tissue P of AMF plants, and Graham and Syvertsen (1984) working with citrus reported that AMF did not affect E . While drought reduced g_s and E in our study with Chile ancho, there were no differences among mycorrhizal treatments.

The greatest reduction occurred on day 20 of the drought cycle in % soil moisture, RWC, g_s and whole plant transpiration among all treatments (Table 3). The visual wilting data during peak environmental stress support that within a treatment, some plants exhibited variation in drought stress. All AMF plants showed some level of wilting, with *G. fasciculatum* colonized plants having the highest number of plants that were severely wilted (Table 4). However, ZAC-19 had the greatest drought resistance, as indicated by the greatest number of plants showing only moderate wilting and highest Ψ_{leaf} .

WUE is thought to be related to plant performance and natural selection for plants in arid habitats, based on general expectation that increased WUE is associated with decreased carbon gain and biomass accumulation (Kramer and Boyer, 1995). Donovan and Ehleringer (1994) have demonstrated that increased WUE is not necessarily associated with decreased carbon gain. Using carbon isotope discrimination (Δ) to determine integrated WUE, we found no differences in Δ among mycorrhizal treatments. WUE at the whole plant level is the biomass accumulated per total water consumed. Plants are thought to be under selective pressure for WUE in water-limited environments. On a whole plant basis (g carbon/kg H₂O), there was a trend in higher WUE among droughted plants, but no difference in WUE among mycorrhizal treatments. There may be no selective advantage for AMF plants to have higher WUE than NonAMF plants. There is a greater carbon drain to the root system of AMF plants (Shaobing et al., 1993) that requires higher photosynthetic rates (A) to maintain the symbiosis. Under nutritional or drought stress, AMF plants frequently maintain both higher A and g_s than NonAMF plants (Augé, 1989; Davies et al., 1993). If AMF plants can photosynthesize longer by avoiding or tolerating drought because of greater access to soil water via extraradical hyphae that better maintains contact between the plant root system and the soil water continuum or by greater osmotic adjustment, then there may be no advantage in conserving water. Duan et al. (1996) reported that AMF plants have lower ABA in the xylem sap that contributes to higher g_s .

Both total colonization and arbuscule formation increased with ZAC-19 during drought, whereas there was decrease in colonization with *G. fasciculatum*. We were unable to determine extraradical hyphae because of harvesting problems at the termination of the experiment. However, in a study on P-stress (Davies et al., 2000), extraradical hyphae formation was greater in ZAC-19 than *G. fasciculatum* at low P. It is reasonable to assume that ZAC-19 also had greater extraradical hyphae formation under drought conditions, since it had the fewest plants experiencing wilting, maintained the highest Ψ_{leaf} and had a trend in higher RWC. In a study with bell peppers, drought promoted greater extraradical hyphae development of *G. deserticola* that contributed to greater water uptake (Davies et al., 1993). Greater hydraulic conductance has also been reported with AMF plants (Hardie, 1985). If AMF hyphae explore the soil volume in a manner analogous to increasing root density, then AMF roots could have higher water potentials than would occur in NonAMF roots, and this should promote higher Ψ_{leaf} and less droughted plants.

Contrary to a study on P-stress (Davies et al., 2000), where both AMF isolates enhanced reproductive and vegetative growth under reduced P conditions, only the Mexican ZAC-19 isolate, composed of *G. albidum*, *G. claroides* and *G. diaphanum* enhanced plant drought resistance. The ZAC-19 isolate was selected from an arid region in Mexico, which enhanced its ability to impart drought resistance of the host plant species. Drought stimulated an increase in arbuscules and hyphae development. Conversely drought caused reductions in all mycorrhizal parameters with *G. fasciculatum*, which originated from the more humid US Pacific Northwest (Gerdemann and Trappe, 1974). It is not clear how greater arbuscular formation of ZAC-19 would contribute to increased plant drought resistance. However, high levels of arbuscules are indicative of active symbiosis (Smith and Read, 1997).

The implication of these two studies is that ZAC-19 can potentially be incorporated into Chile ancho seedling transplant systems to enhance P-uptake and to promote drought resistance in production fields in Mexico, and other areas with sustainable agricultural systems where drought and low or unavailable soil P must be managed. A few days of higher Ψ_{leaf} during critical flowering/fruit period could lead to higher yields.

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