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Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses

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Summary

Arbuscular mycorrhizal (AM) symbiosis can confer increased host resistance to drought stress, although the effect is unpredictable. Since AM symbiosis also frequently increases host resistance to salinity stress, and since drought and salinity stress are often linked in drying soils, we speculated that the AM influence on plant drought response may be partially the result of AM influence on salinity stress. We tested the hypothesis that AM-induced effects on drought responses would be more pronounced when plants of comparable size are exposed to drought in salinized soils. In two greenhouse experiments, several water relations characteristics were measured in sorghum plants colonized by *Glomus intraradices* (*Gi*), *Gigaspora margarita* (*Gm*) or a mixture of AM species, during a sustained drought following exposure to salinity treatments (NaCl stress, osmotic stress via concentrated macronutrients, or soil leaching). The presence of excess salt in soils widened the difference in drought responses between AM and nonAM plants in just two instances. Days required for plants to reach stomatal closure were similar for *Gi* and nonAM plants exposed to drought alone, but with exposure to combined NaCl and drought stress, stomates of *Gi* plants remained open 17—22% longer than in nonAM plants. Promotion of stomatal conductance by *Gm* occurred with exposure to NaCl/drought stress but not with drought alone or with soil leaching before drought. In other instances, however, the addition of salt tended to nullify an AM-induced change in drought response. Our

Abbreviations: AM arbuscular mycorrhizal; AZ species assemblage of AM fungi from a semi-arid grassland in Arizona; *Gi Glomus intraradices*; *Gm Glomus margarita*; Ψ_π osmotic potential; Ψ_π^{100} osmotic potential at full turgor; g_s stomatal conductance; Ψ water potential

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findings confirm that AM fungi can alter host response to drought but do not lend much support to the idea that AM-induced salt resistance might help explain why AM plants can be more resilient to drought stress than their nonAM counterparts.

Introduction

Arbuscular mycorrhizal (AM) symbiosis is often alleged to improve plant resistance to drought stress. Several studies have demonstrated this under varying experimental conditions (e.g. Subramanian and Charest, 1998; Ruiz-Lozano and Azcón, 2000; Porcel et al., 2003), while others revealed little or no AM enhancement of resistance (e.g. Hetrick et al., 1987; Simpson and Daft, 1991). There are reports of AM-induced increases in physiological drought tolerance, involving both increased dehydration avoidance and dehydration tolerance (Allen and Boosalis, 1983; Davies et al., 1993). Most experiments examining AM effects on drought resistance have shown that when the symbiosis improves host drought resistance, it does so by aiding drought avoidance (Augé, 2001). The AM influence on plants in drying soils remains unpredictable and uncertain, particularly in soils with adequate phosphorus.

AM symbiosis has frequently increased resilience of host plants to salinity stress, perhaps with greater consistency than to drought stress. Growth in saline soils was increased by inoculation with *Glomus* spp, with AM plants having increased phosphate and decreased Na concentrations in shoots compared to uninoculated controls (Pfeiffer and Bloss, 1987; Giri and Mukerji, 2004). Salt resistance was improved by AM colonization in maize (Feng et al., 2002), mung bean (Jindal et al., 1993) and clover (Ben Khaled et al., 2003), with the AM effect correlated with improved osmoregulation or proline accumulation. AM colonization also improved NaCl resistance in tomato, with extent of improvement related to salt sensitivity of the cultivar (Al-Karaki, 2000; Al-Karaki et al., 2001). AM improvement of salt resistance has usually been associated with AM-induced increases in P acquisition and plant growth, although two of three AM fungi tested were able to protect cucumber plants from NaCl stress compared to similarly sized nonAM plants (Rosendahl and Rosendahl, 1991). Alfalfa was also more effectively protected against salinity stress by AM symbiosis than by P supplementation (Azcón and El-Atrash, 1997), and the improvement of NaCl resistance in lettuce induced by several AM fungi was not attributed to nutrition (Ruiz-Lozano et al., 1996).

Since solutes can concentrate in the soil solution just outside roots as soil dries (Stirzaker and Passioura, 1996), and since AM symbiosis often increases plant resistance to salinity stress, we speculated that the amount of salt in drying soil may be one experimental factor that could explain why AM fungi increased drought resistance in some studies but not in others, i.e. perhaps AM effects on drought resistance are linked to AM effects on salt resistance; in those reports where AM symbiosis did improve drought resistance, AM fungi may have helped to overcome plant susceptibility to an osmotic or NaCl stress that developed as soil dried. Osmotic potential (Ψ_π) and NaCl concentration of soil are typically not reported in drought studies, so it is difficult to examine the mycorrhizal literature for such trends.

We conducted experiments to test whether AM effects on physiological drought resistance would be more pronounced under saline conditions. We exposed sorghum to various salinity treatments just prior to exposing them to drought. Plants must often endure both drought and salinity stress in arid and semi-arid regions, and it would be useful to know if AM symbiosis can increase resistance to these combined stresses.

Materials and methods

Experiment 1: *Glomus intraradices*, NaCl and osmotic stress

Plant materials and culture

One hundred and five 2.8-L plastic pots were seeded with *Sorghum bicolor* L. cv Dekalb DK40Y on 6 February 2002, with 5 seeds per pot. Potting medium was autoclaved silica sand (commercial medium grade, No. 1962-51, Quikrete, Atlanta, GA, USA). Thirty-five pots received pot culture colonized by *Glomus intraradices* Schenck and Smith INVAM isolate UT143 (*Gi*) and 70 pots received nonAM pot culture. AM and nonAM pot cultures were established on sorghum in a loam:sand (1:1) mixture and were 9 months old at the time of inoculation. 100–150 g of either AM or nonAM pot culture were banded beneath seeds. In addition to using nonAM pot cultures grown under similar conditions as AM pot culture, similar soil microbial

populations were encouraged in AM and nonAM pots by also applying water filtrates of AM inoculum to nonAM pots (44-μm sieve).

Plants were fertilized weekly with water soluble fertilizer at 5.6 mM N (Peters, N:P:K = 15:0:5, Scotts-Sierra Horticultural Product Co., Marysville, OH, USA) and biweekly with a micronutrient solution at 0.02 mM Fe (Microplex, Miller Chemical and Fertilizer Co., Hanover, PA, USA). Phosphorus was supplied once per week as 0.8 mM KH_2PO_4 to AM plants and one group of nonAM plants and as 1.6 mM KH_2PO_4 to a second group of nonAM plants. Plants were grown in a glasshouse in Knoxville, TN, with temperature maintained at 25–29 °C/18–23 °C (day/night) under natural light. The glasshouse was covered between May and October with 55% shade cloth to aid in temperature control. Plants were watered as needed prior to stress applications.

The AM and nonAM soil treatments were allowed to establish for 10 months before initiating the stress treatments, following the procedures of Augé et al., 2004b. To renew shoot growth during the soil establishment phase, plants were sheared three times (1 May, 17 June, 16 October 2002) and pots reseeded twice (16 August, 12 November 2002; 5 seeds per pot) during the 10-month establishment period. High-pressure sodium lamps (400 W) were installed 80 cm above the pots (16 h day^{-1}) to supplement low natural light during the winter months.

Leaves from the final shearing and crowns were collected for each pot and dry weights determined, to assess which nonAM treatment group was closest in size to the AM plants. AM and nonAM pots given low P were selected for further study because they had similar shoot dry weight (4.68 ± 0.16 and 4.65 ± 0.11 g pot^{-1}, respectively).

Drought and salinity treatments

When plants in the final planting were 10 weeks old, and tests confirmed that AM plants were colonized and nonAM plants were free of colonization, stress treatments were applied. Soils were exposed to three salinity stress treatments just prior to initiating the drought treatment: a concentrated macronutrient solution, a NaCl solution or distilled water. The drought treatment consisted of allowing plants to dry, with the last day that plants were watered designated as "day 0" (27 January 2003).

The first salinity stress applications were applied 7 d before withholding water (day-7). A macronutrient solution was used to lower soil Ψ_π and induce osmotic stress. It was composed of 40 mM $MgSO_4$, 90 mM $Ca(NO_3)_2$, 1.6 mM KH_2PO_4, 62 mM KNO_3 and 19 mM NH_4NO_3 (Augé et al., 1992). The solution was adjusted to a water potential (Ψ) of −0.4 MPa for the first application on day −7 and −0.8 MPa for the second application on day 0. Plants were watered with tap water once (day −4) between the first and second applications of the salt solutions. The second salinity stress treatment was NaCl solutions, applied at 40 mM for the first application on day −7 and 80 mM for the second application on day 0. Ψ of the 40 mM and 80 mM NaCl solutions were −0.19 and −0.37 MPa, respectively. The third salinity stress treatment was a distilled water control. For each application, 200 mL of distilled water, NaCl or macronutrient solution was applied to pots. Water was withheld from all pots after the second application. The more dilute first application was applied 1 week prior to the full application, followed by one irrigation 3 d later with tap water, to ease the plants into the salinity stress.

Plant, soil and solution measurements

Ψ of solutions was measured with a chilled mirror dewpoint hygrometer (WP4, Decagon Devices Inc., Pullman WA, USA) calibrated with NaCl solutions. Soil electrical conductivity (EC) of each pot was measured with an EC meter (AR 20, Fisher Scientific Inc., Pittsburgh, PA, USA) after calibration (0.01 N KCl solution) by the 1:5 soil/water suspension method (Rayment and Higginson, 1992).

Beginning on day 3 of the drought stress period, stomatal conductance (g_s) of plants in each pot was measured each day at 1300 EST on abaxial leaf surfaces with an automatic-cycling porometer (AP4, Delta-T Devices, Cambridge, England). Three leaves per pot were measured each day, at the distil end of the largest, unshaded leaves. Daily measurements continued until the average g_s for the foliage in a particular pot declined to below 10 mmol m^{-2} s^{-1}. This point was defined as the stomatal closure point, as stomates of sorghum leaves tend not to close completely. Stomatal conductance was also measured 2–3 times per week prior to application of salt treatment solutions.

Lethal leaf Ψ was measured with thermocouple psychrometers (TruPsi, Decagon Devices Inc.) as described before (Augé et al., 2001a, b). Sampling was performed between 1030 and 1130 h EST.

For measurement of initial (pre-stress) Ψ_π, one leaf from each pot in each treatment was excised on day-7. The leaves were cut into two halves. The proximal half was immediately sealed in a syringe and plunged into liquid N_2 for measurement of Ψ_π using a vapor pressure osmometer (model 5500XR, Wescor Inc., Logan UT, USA). The base of the distal half was submerged in distilled water in a covered beaker and rehydrated in a refrigerator (4 °C)

overnight for measurement of Ψ_π at full turgor (Ψ_π^{100}) as described before (Chapman and Augé, 1994).

For measurements of soil Ψ, samples (about 2.5 mL) were excavated from the middle (14–17 cm from top) and bottom (27–30 cm from top) of each pot at each time period, from flaps cut in the pot, immediately sealed in sample cups and Ψ measured using the WP4 hygrometer. Flaps in pots were sealed with duct tape following sampling.

Leaf Ψ, leaf Ψ_π, leaf Ψ_π^{100} and soil Ψ were measured at the stomatal closure and lethal points as well as 1 d after application of the half- and full-strength soil solutions.

On day-10, shoot dry weight was determined for plants in five extra AM and nonAM pots. Soil from each pot was mixed and subsampled for determination of soil hyphal density, root colonization and root density. Soil hyphal density was measured as described before (Augé et al., 2001b), on 10 g subsamples. Roots were carefully excavated from another 25 g subsample of each replicate for measurement of root length using scanning equipment and imaging software (WinRhizo, Regent Instruments Inc., Quebec City, Canada). Root colonization was quantified on 100 ~0.5-cm root pieces from each pot, after clearing with 10% KOH in an autoclave at 121 °C for 15 min, staining with trypan blue for 1 h, and destaining.

On day-10, leaf P concentration of one of the largest, most recently expanded leaves of four plants from each treatment was determined spectrophotometrically using the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 700 °C for 2 h and digested in nitric acid (Chapman and Pratt, 1961).

Experiment 2: *Gigaspora margarita*, a semi-arid AM mix, and NaCl stress

Plant materials and culture

One-hundred and eight 2.8-L plastic pots were seeded with *S. bicolor* L. cv Dekalb DK40Y on 18 April 2003, with 8 seeds per pot. The potting medium was composed of autoclaved silica sand as in Experiment 1. Thirty-six pots received pot culture colonized by *Gigaspora margarita* Gerdemann & Trappe INVAM isolate 215 (*Gm*), 36 pots received pot culture colonized by a mixture of AM species originally isolated from a semiarid grassland in Arizona (AZ mix, described in Augé et al., 2003), and 36 pots received nonAM pot culture. *Gm* and the AZ-mix pot cultures were established on sorghum in a loam:sand (1:1) mixture and were 12 months old at the time of inoculation. Composition of the AZ-mix greenhouse pot culture in terms of spore abundance was 88% *Gl. intraradices*, 10% *Glomus* AZ 123, 2% *Acaulospora rehmii*.

Other cultural details were as described for Experiment 1. Phosphorus was supplied once a week as 0.8 mM KH_2PO_4 to plants in each AM treatment and to nonAM plants. After emergence, pots were thinned back to the six strongest seedlings.

Drought and salinity treatments; plant, soil and solution measurements

Drought and salinity treatments were applied as described for Experiment 1, with one modification of the salinity treatments: the macronutrient salinity treatment was replaced by a leaching treatment. In this treatment, pots were leached with distilled water (approximately 600 mL applied to each pot in three 200-mL leachings) to remove some soil solutes. The first, half-strength application of soil solutions occurred 11 June 2003 (day-7) and the second, full-strength application 18 June 2003 (day 0). Plant, soil and solution measurements were made as in Experiment 1, with two modifications: g_s measurements began on day 1, and measurements were not made at the lethal point.

Experimental design and statistical analysis

For each factorial experiment, pots were arranged in a completely randomized block design. Experiment 1 (2×3 factorial) had two mycorrhizal treatments and Experiment 2 (3×3 factorial) had three mycorrhizal treatments. Each experiment had three salinity treatments: an NaCl solution, concentrated macronutrient solution and water for Experiment 1; and an NaCl solution, water and water leaching for Experiment 2. Each level of each factor had six replicates for each experiment. ANOVA was performed using the General Linear Model procedure (SAS, Cary, NC, USA). Duncan's mean separation tests and *t* tests were used to examine differences among means.

Results

Experiment 1: *Glomus intraradices*, NaCl and osmotic stress

Shoot, root, fungal and soil EC characteristics

NonAM and *Gi* plants had similar shoot dry weights, leaf [P], and root mass and length densities (Table 1). Roots of *Gi* plants developed considerable AM colonization. NonAM plants re-

Table 1. Shoot dry weight, leaf [P], and root and fungal characteristics of representative sorghum plants of each AM treatment on day-10 in Experiments 1 and 2

	Nonmycorrhizal	*Gl. intraradices*	
Experiment 1			
Shoot dry weight ($g\,pot^{-1}$)	4.5 a	4.4 a	
Leaf [P] ($mg\,g^{-1}$ DW)	1.5 a	1.5 a	
Root mass density ($mg\,g^{-1}$ dry soil)	1.3 a	1.4 a	
Root length density ($cm\,g^{-1}$ dry soil)	13.8 a	15.6 a	
AM root colonization (%)	0 a	53 b	
Soil hyphal density ($m\,g^{-1}$ dry soil)	0.2 a	1.2 b	
Experiment 2	Nonmycorrhizal	AZ mix	*Gi. margarita*
Shoot dry weight ($g\,pot^{-1}$)	1.2 b	0.8 a	1.4 b
Leaf [P] ($mg\,g^{-1}$ DW)	1.5 a	1.8 b	1.8 ab
Root mass density ($mg\,g^{-1}$ dry soil)	0.5 a	0.3 a	0.6 a
Root length density ($cm\,g^{-1}$ dry soil)	6.1 a	6.0 a	8.6 b
AM root colonization (%)	0 a	36 c	24 b
Soil hyphal density ($m\,g^{-1}$ dry soil)	0.0 a	0.4 c	0.2 b

Values for AM root colonization represent total colonization levels and indicate presence of hyphae, arbuscules and/or vesicles. $n = 5$ for each parameter except leaf [P], where $n = 4$. Within rows, means followed by different letters are significantly different ($P \leqslant 0.05$).

mained nonmycorrhizal. Soil in *Gi* pots had about seven times more hyphal length than soil in nonAM pots.

Soil EC averaged $0.20\,dS\,m^{-1}$ in each of the six treatment groups 2 d prior to application of the first saline treatment. Application of the half-strength solutions on day-7 caused soil EC to increase about 3-fold for plants given NaCl and about 6- to 8-fold in plants given the macronutrient solution, relative to control plants given distilled water. Application of the full-strength solutions on day 0 caused soil EC to increase about 4- to 5-fold for plants given NaCl and about 20- to 30-fold in plants given the macronutrient solution, relative to control plants given distilled water.

Plant water relations

Stomatal conductance was higher in *Gi* than in nonAM plants before drought was initiated (Fig. 1). Averaged over all replicates of all treatments on all measurement days before day 0, mean pre-drought g_s of *Gi* and nonAM plants was $265\,mmol\,m^{-2}\,s^{-1}$ and $213\,mmol\,m^{-2}\,s^{-1}$, respectively (significantly different, $P = 0.02$). Average g_s of *Gi* plants during the drying period was higher than that of nonAM plants, by 32% with drought stress alone ($P = 0.03$), by 38% with NaCl/drought stress ($P = 0.007$), and by 51% with osmotic/drought stress ($P = 0.006$) (Fig. 2). Plants in individual pots reached stomatal closure between days 9 and 12.

AM symbiosis did not affect leaf Ψ 1 d after exposure to the half-strength (first application) and full-strength (second application) solutions in any of the three salinity treatments (Table 2). Leaf Ψ at stomatal closure was 1.2 MPa higher in *Gi* than in nonAM plants exposed to drought stress alone. Leaf Ψ at stomatal closure was similar in *Gi* and nonAM plants exposed to NaCl/drought stress and to osmotic/drought stress. Leaf Ψ at the lethal point was similar in *Gi* and nonAM plants exposed to each of the soil solutions. Plants in individual pots reached the lethal point between days 14 and 25.

AM symbiosis did not affect leaf Ψ_π in any of the three salinity treatments 1 d after exposure to the half-strength and full-strength solutions or at stomatal closure (Table 3). Leaf Ψ_π at the lethal point was 0.70 MPa higher in *Gi* than in nonAM plants exposed to osmotic/drought stress. Leaf Ψ_π at the lethal point was similar in *Gi* and nonAM plants exposed to NaCl/drought stress.

Leaf Ψ_π^{100} were measured 1 d after application of the first and second salinity treatment solutions, and at the lethal point. AM symbiosis had no effect on leaf Ψ_π^{100} on any of these days nor on osmotic adjustment (data not shown).

NonAM plants reached stomatal closure 2 d sooner than *Gi* plants when exposed to drought in the absence of salinity (Table 4). With exposure to either the NaCl or the macronutrient solutions during drought, nonAM and *Gi* plants reached stomatal closure at the same time. Plants remained alive longer when droughted with exposure to either salt solution relative to plants exposed to drought alone: 18–23 d vs. 14–15 d. NonAM and *Gi*

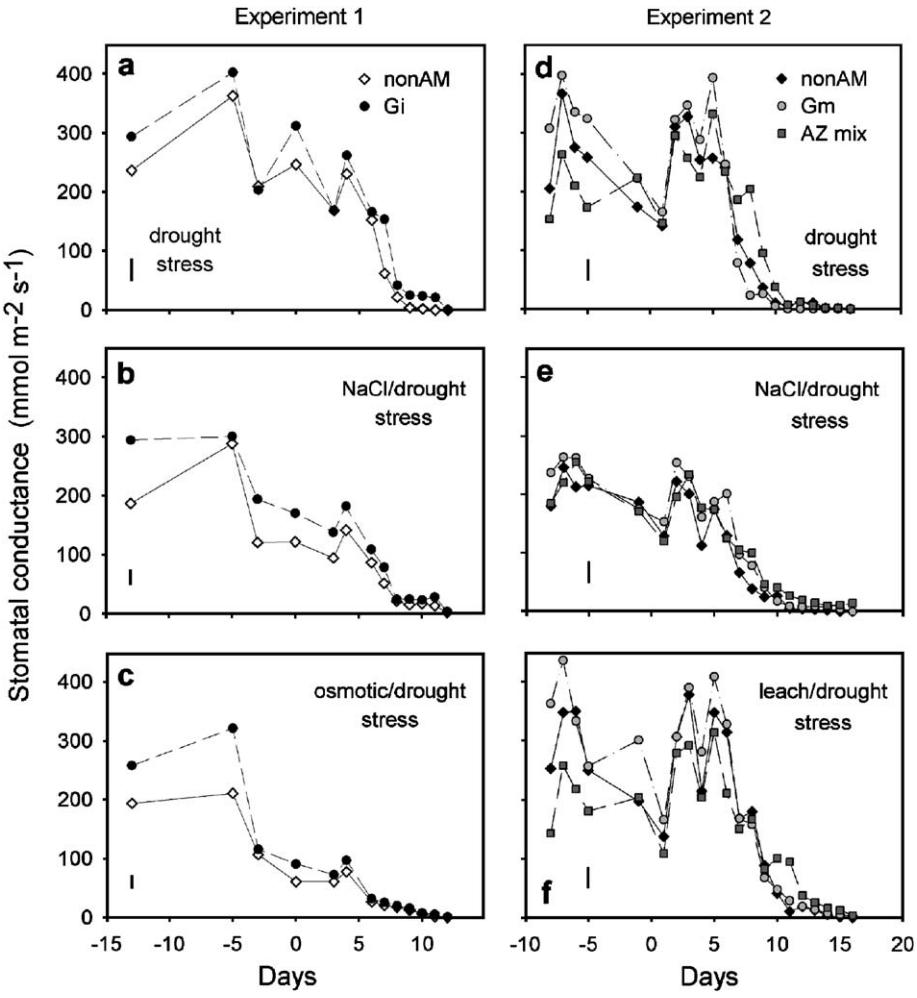

Figure 1. Stomatal conductance of sorghum plants before and following application of the soil solution treatments, for Experiment 1 (a–c) and Experiment 2 (d–f). Symbols represents the mean of 18 measurements (three leaves from 6 pots of each treatment on each day). Standard error of the means for pre-drought g_s are represented by vertical bar in each panel. Standard error of the means for g_s during the drought period were smaller than the height of a symbol.

plants reached the lethal point in the same amount of time when droughted without added salt as well as when droughted with exposure to the macronutrient solution. NonAM plants died more quickly than *Gi* plants when exposed to drought and NaCl.

Soil water relations

After the first half-strength application of soil solutions, soil Ψ was 0.05 MPa lower in *Gi* than in nonAM plants exposed to osmotic stress (Table 5). Soil Ψ was similar in *Gi* and nonAM plants exposed to NaCl after the first and second applications, as well as to osmotic stress after the second application.

Soil Ψ at the point of stomatal closure was substantially higher in *Gi* than in nonAM plants exposed to drought alone, by 2.36 MPa (Table 4). Soil Ψ was appreciably higher in *Gi* than in nonAM plants at the lethal point with exposure to NaCl/drought stress (by 1.25 MPa) and to osmotic/drought stress (by 1.77 MPa).

Experiment 2: *Gigaspora margarita*, a semi-arid AM mix, and NaCl stress

Shoot, root, fungal and soil EC characteristics

Gm and nonAM plants had similar shoot dry weights and root length densities (Table 1). Shoots of AZ-mix plants were markedly smaller than nonAM and *Gm* plants. NonAM and *Gm* plants had similar leaf [P], and AZ-mix plants had slightly higher leaf [P] than nonAM plants. The smaller AZ-mix plants had about 60% of the root mass density of nonAM plants. Plants examined in Experiment 2 were younger and smaller than those in Experiment 1 (Table 1).

Roots of *Gm* and AZ-mix plants each developed considerable AM colonization (Table 1). NonAM

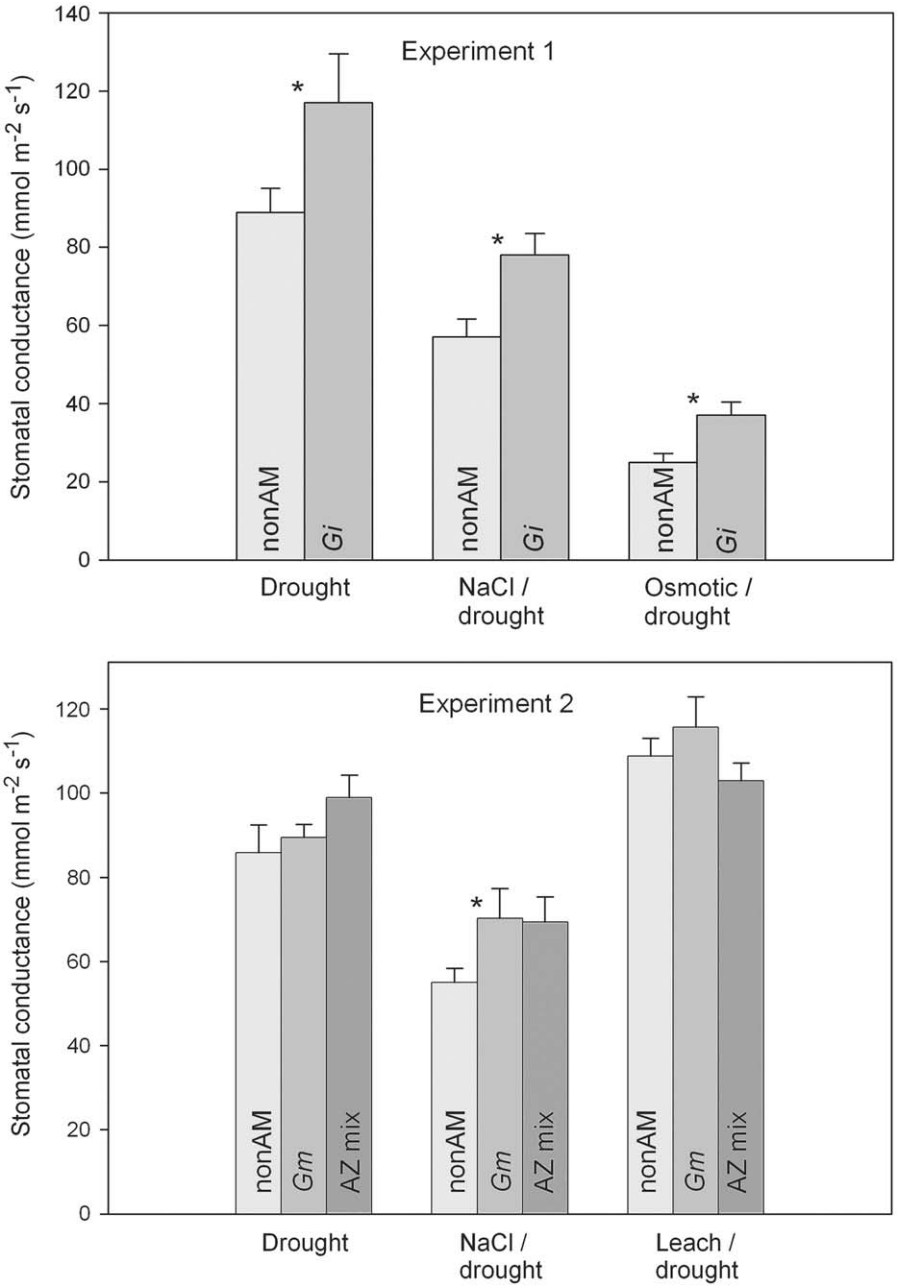

Figure 2. Average stomatal conductance within treatments during the drying episode for Experiments 1 and 2. $n = 162$ (Experiment. 1), $n = 360$ (Experiment. 2). Asterisk denotes that *Gi* or *Gm* and nonAM treatments differed significantly ($P \leqslant 0.05$). Vertical lines represent standard errors.

plants remained nonmycorrhizal. The younger plants of Experiment 2 developed less soil hyphae than in the larger and older Experiment 1 plants. Despite the smaller shoot and root mass of host plants, soil in pots with plants colonized by the AZ mix had greater hyphal length than soil in either nonAM or *Gm* pots.

Soil EC was not affected by AM symbiosis after either the first or second applications of soil solution treatments.

Plant water relations

Pre-drought g_s was affected in Experiment 2 by mycorrhizal symbiosis (Fig. 1). Averaged over all replicates of all treatments on all measurement days before day 0, mean pre-drought g_s of *Gm*, AZ-mix and nonAM plants was 296, 205 and 248 mmol m^{-2} s^{-1}, respectively, with *Gm* significantly higher than AZ ($P = 0.0001$) and nonAM ($P = 0.02$). Average g_s during the drying period was higher in *Gm* plants than in nonAM plants by

Table 2. Leaf Ψ of nonAM and AM sorghum plants during Experiments 1 and 2

Soil solution treatment	Mycorrhizal treatment	Leaf Ψ (MPa)			
		First application	Second application	Stomatal closure	Lethal point
Experiment 1					
Drought	nonAM	−0.66 ab	−1.32 a	−3.86 b	−6.88 b
	Gi	−0.52 a	−1.40 ab	−2.67 a	−7.03 b
NaCl/drought stress	nonAM	−0.84 b	−1.67 bc	−2.50 a	−5.71 a
	Gi	−0.80 b	−1.58 abc	−2.42 a	−4.36 a
Osmotic/drought stress	nonAM	−0.81 b	−1.81 c	−2.86 a	−4.79 a
	Gi	−0.83 b	−1.76 c	−2.93 a	−4.02 a
Experiment 2					
Drought	nonAM	−0.94 a	−1.00 a	−2.26 a	
	Gm	−0.99 a	−0.92 a	−2.38 a	
	AZ mix	−0.93 a	−0.85 a	−2.39 a	
NaCl/drought stress	nonAM	−0.93 a	−0.96 a	−2.48 a	
	Gm	−0.94 a	−0.87 a	−2.13 a	
	AZ mix	−0.97 a	−0.93 a	−2.66 a	
Leach/drought stress	nonAM	−0.94 a	−0.85 a	−2.61 a	
	Gm	−0.94 a	−0.84 a	−2.46 a	
	AZ mix	−0.91 a	−0.80 a	−2.03 a	

First applications, made at day −7, were a 40 mM NaCl solution (Expt. 1 and 2), distilled water (Expt. 1 and 2), −0.4 MPa macronutrient solution (Expt. 1), or three applications of distilled water to leach the soil medium (Expt. 2). Second applications, made at day 0 when drought was initiated, were an 80 mM NaCl solution (Expt. 1 and 2), distilled water (Expt. 1 and 2), −0.8 MPa macronutrient solution (Expt. 1), or three applications of distilled water to further leach the soil medium (Expt. 2). $n = 6$. Within columns, means followed by different letters are significantly different ($P \leqslant 0.05$). *Gi* = *Glomus intraradices*, *Gm* = *Gigaspora margarita*, AZ mix = a semi-arid mixture of species.

Table 3. Leaf Ψ_π of nonAM and AM sorghum plants during Experiments 1 and 2

Soil solution treatment	Mycorrhizal treatment	Leaf Ψ_π (MPa)			
		First application	Second application	Stomatal closure	Lethal point
Experiment 1					
Drought	nonAM	−0.92 a	−1.07 a	−1.93 a	na
	Gi	−0.90 a	−0.98 a	−1.85 a	na
NaCl/drought stress	nonAM	−1.10 b	−1.47 b	−2.06 a	−3.22 a
	Gi	−1.00 ab	−1.40 b	−1.95 a	−3.01 a
Osmotic/drought stress	nonAM	−0.97 ab	−1.65 c	−2.66 b	−4.62 c
	Gi	−0.95 ab	−1.49 b	−2.60 b	−3.92 b
Experiment 2					
Drought	nonAM	−0.89 ab	−0.85 a	−1.53 a	
	Gm	−0.87 ab	−0.90 ab	−1.63 abc	
	AZ mix	−0.83 a	−0.90 ab	−1.53 a	
NaCl/drought stress	nonAM	−0.92 b	−1.05 c	−1.82 bc	
	Gm	−0.83 a	−0.89 ab	−1.78 bc	
	AZ mix	−0.89 ab	−0.91 ab	−1.85 c	
Leach/drought stress	nonAM	−0.91 b	−0.91 b	−1.61 ab	
	Gm	−0.84 a	−0.89 ab	−1.54 a	
	AZ mix	−0.86 ab	−0.93 b	−1.49 a	

$n = 6$. "na" indicates leaves were too dehydrated to provide sufficient sap for measurement. Within columns, means followed by different letters are significantly different ($P \leqslant 0.05$). *Gi* = *Glomus intraradices*, *Gm* = *Gigaspora margarita*, AZ mix = a semi-arid mixture of species.

Table 4. *Experiment 1*. Days required for plants to reach stomatal closure and the lethal point

Soil solution treatment	Mycorrhizal treatment	Days to:	
		Stomatal closure	Lethal point
Drought	nonAM	9.3 a	14.0 a
	Gl. intraradices	11.2 cd	15.0 a
NaCl/drought stress	nonAM	11.3 cd	17.8 b
	Gl. intraradices	11.8 d	20.0 c
Osmotic/drought stress	nonAM	10.0 ab	23.3 d
	Gl. intraradices	10.5 bc	22.8 d

$n = 6$. Within columns, means followed by different letters are significantly different ($P \leqslant 0.05$).

Table 5. Soil Ψ of nonAM and AM sorghum plants

Soil solution treatment	Mycorrhizal treatment	Soil Ψ (MPa)			
		First application	Second application	Stomatal closure	Lethal point
Experiment 1					
Drought	nonAM	0.00 a	−0.01 a	−5.85 c	na
	Gi	0.00 a	−0.05 a	−3.49 b	na
NaCl/drought stress	nonAM	0.00 a	−0.47 b	−1.88 a	−5.20 b
	Gi	−0.03 a	−0.37 b	−1.38 a	−3.95 a
Osmotic/drought stress	nonAM	−0.26 b	−1.00 c	−2.08 a	−5.69 b
	Gi	−0.31 c	−0.87 c	−1.87 a	−3.92 a
Experiment 2					
Drought	nonAM	0.00 a	−0.01 a	−2.74 abc	
	Gm	−0.12 a	−0.02 a	−4.78 d	
	AZ mix	−0.05 a	−0.05 a	−3.62 cd	
NaCl/drought stress	nonAM	−0.12 a	−0.21 b	−1.40 a	
	Gm	−0.07 a	−0.08 ab	−1.67 a	
	AZ mix	−0.08 a	−0.19 b	−1.91 ab	
Leach/drought stress	nonAM	−0.06 a	−0.01 a	−3.56 cd	
	Gm	−0.02 a	−0.00 a	−3.90 cd	
	AZ mix	−0.01 a	−0.09 a	−3.06 bc	

$n = 12$ (two subsamples, middle and bottom of pot, from six pots of each treatment). "na" for droughted plants at lethal point in Experiment 1 signifies soils were dry beyond the range of measurement capability of the instrument (< -40 MPa). Within columns, means followed by different letters are significantly different ($P \leqslant 0.05$). *Gi* = *Glomus intraradices*, *Gm* = *Gigaspora margarita*, AZ mix = a semi-arid mixture of species.

28% in the NaCl/drought treatment ($P = 0.05$) and similar in *Gm* and nonAM plants in the drought and leach/drought treatments ($P = 0.31$ and 0.21, respectively) (Fig. 2). Plants in individual pots reached stomatal closure between days 8 and 20.

Leaf Ψ was unaffected by AM symbiosis, 1 d after the first and second applications of the soil solutions and at stomatal closure (Table 2).

Leaf Ψ_π was 0.10–0.15 MPa higher in *Gm* than in nonAM plants after the first and second applications of the NaCl solutions and 0.07 MPa higher in *Gm* than in nonAM plants after the first soil leaching treatment (Table 3). Leaf Ψ_π at the point of stomatal closure was lowered by about 0.3 MPa in the NaCl/drought treatment relative to drought in the absence of salinity in nonAM and AZ-mix plants but was not affected in *Gm* plants. Absolute values of leaf Ψ_π at stomatal closure were similar among the three mycorrhizal treatments, with drought stress, with NaCl/drought stress or with leach/drought stress. Leaf Ψ_π^{100} was not affected by AM symbiosis.

Soil water relations

Soil Ψ was not affected by AM symbiosis after the first and second applications of the soil solution treatments (Table 5). With drought stress, soil Ψ at stomatal closure was 2 MPa lower for *Gm* than for nonAM plants. Soil Ψ at stomatal closure was unaffected by AM symbiosis in the NaCl/drought or leach/drought treatments. Average soil Ψ at stomatal closure across soil solution treatments was -3.45 MPa for *Gm* plants and -2.57 MPa for nonAM plants ($P = 0.03$).

Discussion

Our objective was to test two hypotheses, with a successful demonstration of the first a prerequisite for testing the second: (1) AM symbiosis affects plant drought response relative to nonAM plants of comparable size, and (2) the AM-induced effect on drought response is more pronounced in plants when they are exposed to drought in salinized soils. Because AZ-mix plants were considerably smaller than nonAM plants in Experiment 2, they are not considered further here in tests of the hypotheses. The discussion focuses on comparisons of AM and nonAM plants of similar size: *Gi* and nonAM plants in Experiment 1, and *Gm* and nonAM plants in Experiment 2. Plant size, regardless of mycorrhizal status of roots, can affect g_s and other plant water relations responses to drought (e.g. Ebel et al., 1996).

Hypothesis 1. Viewed as a whole, the mycorrhizal literature indicates that AM symbiosis tends to have more effect on leaf g_s than on leaf Ψ or Ψ_π (Augé, 2001). We also observed this in these experiments. Before drought was initiated, colonization by *Gi* in Experiment 1 and by *Gm* in Experiment 2 resulted in promotion of g_s by 24% and 19%, respectively, averaged over soil solution treatments on all pre-drought days. This is within the range of promotion of g_s by *Gi* and *Gm* in another experiment with adequately watered sorghum (Augé et al., 2004a). Integrating the AM effect on g_s over all soil solution treatments and days of drought, *Gi* increased g_s by 39% and *Gm* increased g_s by 17%.

AM effects on leaf Ψ, leaf Ψ_π and soil Ψ at stomatal closure or at the lethal point were more sporadic than those on g_s. Still, several *Gi* effects on these water relations parameters were observed. Hypothesis 1 was true for the following parameters and situations for *Gi* and nonAM plants: leaf Ψ at stomatal closure of plants exposed to drought stress alone; leaf Ψ_π at the lethal point of plants exposed to osmotic/drought stress; soil Ψ at stomatal closure of plants exposed to drought stress alone; soil Ψ at the lethal point of plants exposed to NaCl/drought or to osmotic/drought stress; days of drying required for plants exposed to drought alone to reach stomatal closure; days of drying required for plants exposed to NaCl/drought to reach the lethal point. *Gm* had less effect than *Gi* on host water relations responses to drought. Hypothesis 1 was true in two instances for *Gm* and nonAM plants. Soil Ψ at stomatal closure was markedly lower in *Gm* vs. nonAM plants. And relative to drought in the absence of salinity, exposure to NaCl during drought resulted in a significant decline in leaf Ψ_π at the point of stomatal closure in nonAM plants but not in *Gm* plants.

Hypothesis 2. Our second hypothesis, that the AM-induced effect on host plants during drought would be more pronounced when plants were exposed to soil drying in salinized soils, was true for just two instances. Days required for plants to reach stomatal closure were similar for *Gi* and nonAM plants exposed to drought alone, but *Gi* plants kept stomates open 2 d longer than nonAM plants with exposure to combined NaCl/drought stress. And promotion of g_s by *Gm* relative to nonAM plants occurred in the NaCl/drought stress treatment (28% promotion) but not with exposure to drought alone or to leaching of soil before drought.

The opposite of Hypothesis 2 occurred in four instances: AM effects were more evident with drought alone than with exposure to drought in salinized soils. Leaf Ψ had declined to the same extent in *Gi* and nonAM plants by the time the two salinized soils had dried sufficiently to close stomates. Yet in the unsalinized soil, stomates of *Gi* plants reached the closure point at higher leaf Ψ than nonAM plants. Similarly, *Gi* and nonAM plants reached stomatal closure at similar soil Ψ in each of the salinized soils, but *Gi* plants reached stomatal closure at much higher soil Ψ than nonAM plants in the unsalinized soil. *Gi* and nonAM plants reached stomatal closure at the same time in salinized soils, but *Gi* plants were able to maintain stomatal opening 2 d longer than nonAM plants with drought alone. *Gm* and nonAM plants reached stomatal closure at similar soil Ψ in salinized soil, but *Gm* plants reached stomatal closure in this instance at much lower soil Ψ than nonAM plants in the unsalinized soil.

We were unable to test Hypothesis 2 in two instances because leaves or soil had become so dehydrated that we could not obtain reliable

measurements: soil Ψ and leaf Ψ_π at the lethal point for plants in unsalinized soil.

Our surmise that AM-induced salinity resistance might help explain the observation that AM plants are often more resilient to drought stress than their nonAM counterparts does not appear to hold much merit. In tests with different AM symbionts and different ways of salinizing soils, the presence of excess salt in soils widened the difference in drought responses between AM and nonAM plants only occasionally. In twice as many instances, salinity stress tended to nullify an AM-induced change in drought response.

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