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Short Communication

Mycorrhizal impact on osmotic adjustment in *Ocimum basilicum* during a lethal drying episode

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Summary

Arbuscular mycorrhizal symbiosis can improve drought avoidance capabilities of plants, but the actual dehydration tolerance of mycorrhizal and nonmycorrhizal foliage has not been compared. In this study, leaves of mycorrhizal *Ocimum basilicum* plants developed lower, full-turgor Ψ_π than leaves of nonmycorrhizal plants during a lethal drought, indicative of greater active osmotic adjustment. This did not translate into increased drought resistance, as lethal leaf Ψ, lethal soil Ψ_m and length of the lethal drought episode were not affected by the symbiosis.

Key words: arbuscular mycorrhiza – basil – dehydration tolerance – drought – water relations

Abbreviations: AM arbuscular mycorrhizal. – Ψ_m matric potential. – NH nonmycorrhizal high phosphorus. – NL nonmycorrhizal low phosphorus. – Ψ_π osmotic potential. – [P] phosphorus concentration. – Ψ water potential

Introduction

Colonization of roots by arbuscular mycorrhizal (AM) fungi can affect the water relations and drought resistance of host plants. Often, mycorrhizal improvement of drought resistance occurs via drought avoidance (Reid 1979, Augé 2001). Foliage is less affected by drought in AM plants than in nonAM counterparts because AM plants have dehydrated less during the drought episode. Improved drought avoidance has often been associated with mycorrhizal growth enhancement, linked to improved acquisition of nutrients (e.g. Sylvia et al. 1993, Subramanian and Charest 1999).

Conversely, ability of leaves themselves to tolerate drought has rarely been compared in AM and nonAM plants. Foliar drought tolerance has been characterized by ability to osmotically adjust and to withstand desiccation during a lethal drying episode, by the extent to which osmotic potential (Ψ_π) and water potential (Ψ) decline before leaves die (Ludlow 1989). In a few instances leaves of AM plants have shown more osmotic adjustment than nonAM plants in the face of similar, non-lethal drought pressure (e.g. Allen and Boosalis 1983,

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Augé et al. 1986, Davies et al. 1993). However, neither osmotic adjustment during a lethal drought nor lethal leaf Ψ have been examined in relation to AM symbiosis.

Our objective was to determine if AM symbiosis could modify lethal leaf Ψ, lethal soil Ψ_m, osmotic adjustment, or length of a lethal drought episode in a plant having little native drought resistance.

Materials and Methods

Thirty basil (*Ocimum basilicum* L. cv. Italian Large Leaf Cal Select) plants were grown from seed in pasteurized silica sand and calcined montmorillonite clay (2 : 1, v/v), in 1.25 L plastic pots arranged in a completely randomized design. The medium in 10 pots was mixed with AM pot culture at 4 parts fresh medium to 1 part pot culture (v/v). Fresh medium was mixed with nonAM pot culture at the same rate in another 20 pots to maintain similar soil-water retention properties among treatments. AM and nonAM pot cultures were 21-week-old sorghum plants grown on the medium described above, with AM cultures colonized by *Glomus intraradices* Schenck and Smith isolate WV114.

With each watering, plants received a liquid macro- and micro-nutrient fertilizer at 14.3 mmol/L N (Champion 15N–0P–15K Alkaline Plus, Chilean Nitrate Co., Norfolk, VA). In an attempt to standardize phosphorus nutrition and control for plant size (i.e. produce at least one group of nonAM plants similar in size to AM plants), phosphorus was applied weekly as 1.0 mmol/L KH_2PO_4 to AM plants and 1.5 and 3.0 mmol/L KH_2PO_4 to two groups of nonAM plants (designated NL for nonAM low phosphorus and NH for nonAM high phosphorus). The experiment was conducted in a glasshouse in Knoxville, TN, USA, and plants were adequately watered until the drying treatment began. Daytime glasshouse temperatures ranged from 24 to 33 °C during the day and stayed near 20 °C at night. PPF, measured every 10 s during the drying period, ranged from 1.9 to 6.5 mol $m^{-2} d^{-1}$ and averaged 4.6 mol $m^{-2} d^{-1}$.

Fourteen weeks after germination, plants were transplanted into 2.5 L pots. At 17 weeks, 6 plants of each treatment were watered to field capacity and then subjected to a continuous soil drying episode by withholding water from pots. On the last day plants were watered (subsequently referred to as day 0), one recently expanded, unshaded leaf from each plant was collected between 0830 and 1030 h EST for measurement of initial (pre-drought) Ψ_π at full-turgor using vapor pressure osmometry as described before (Augé et al. 1998). Lethal, post-drought Ψ_π at full turgor was measured on two recently expanded, unshaded leaves per plant. Leaf osmotic adjustment during the drying episode was assessed as pre-drought Ψ_π at full turgor – post-drought Ψ_π at full turgor. This procedure for estimating osmotic adjustment integrates both phenologically-induced and drought-induced solute changes.

Foliar dehydration tolerance was characterized by measuring lethal leaf Ψ. Dehydration tolerance has been operationally defined as the Ψ (called the lethal Ψ) of the last surviving leaves on a plant subjected to a slow, continuous soil drying episode (Ludlow 1989). Each plant was checked daily after beginning the drying episode and lethal measurements began when fewer than six live leaves with minimal necrotic areas (less than 25 % of total leaf area) remained. Preliminary trials were conducted on extra plants to determine the visible signs of the lethal drought point for basil, by excising leaves at various levels of dehydration to ascertain which would rehydrate and which had died. Lethal leaf Ψ was measured (two leaves per plant) using thermocouple psychrometers as described before (Augé et al. 1998). Sampling was performed between 0830 and 1000 h EST. Soil matric potential (Ψ_m) was measured throughout the drying period using heat dissipation sensors as described earlier (Augé et al. 1994). Extent of soil drying required to bring plants to the lethal point was characterized as lethal soil Ψ_m.

At the start of the drought episode, four plants per treatment were randomly selected for measurements of shoot and root dry mass (oven-dried 48 h at 80 °C) and leaf area (LI-3000A, LiCor, NE, USA). Phosphorus concentration ([P]) of leaves of dried plants was determined spectrophotometrically using the vanadate-molybdate-yellow method (Chapman and Pratt 1961). Mycorrhizal colonization of roots at the end of the drying episode was determined for dried plants as described before (Augé et al. 1994). Data were evaluated with the General Linear Models Procedure (SAS, Cary, NC) using mean separations (Fisher's protected LSD) and correlation analysis (Pearson correlation coefficients).

Results and Discussion

Prior to drought, leaf area, shoot and root dry mass, and leaf [P] were similar between AM and NL basil plants, but were significantly higher in NH plants (Table 1). By the end of the lethal drought episode, leaf [P] slightly increased in each treatment, and plants inoculated with *G. intraradices* were

Table 1. Plant size, leaf P concentrations, level of mycorrhizal colonization and plant and soil water relations of basil.

	Mycorrhizal (AM)[1]	Nonmycorrhizal (NL)	Nonmycorrhizal (NH)
Leaf area (dm^2)	19.5a[2]	16.6a	26.4b
Shoot dry mass (g)	10.1a	9.9a	14.6b
Root dry mass (g)	4.6a	3.4a	10.5b
Leaf [P] – day 0 (mg g^{-1})	2.0a	1.9a	3.1b
Leaf [P] – lethal point (mg g^{-1})	2.2a	2.4a	3.5b
Hyphal colonization (%)	59a	1b	0b
Arbuscular colonization (%)	5a	0b	0b
Vesicular colonization (%)	24a	2b	0b
Lethal leaf Ψ (MPa)	−2.19a	−2.12a	−2.16a
Leaf Ψ_π at full turgor on day 0[3] (MPa)	−0.79a	−0.79a	−0.77a
Lethal leaf Ψ_π at full turgor (MPa)	−0.97a	−0.85b	−0.84b
Osmotic adjustment (MPa)	0.17a	0.05b	0.08ab
Lethal soil Ψ_m (MPa)	−3.24a	−2.82a	−2.88a
Lethal leaf Ψ – lethal soil Ψ_m (MPa)	1.05a	0.70a	0.72a
Length of lethal drought episode (d)	9.0a	8.7a	8.3a

[1] AM, NL and NH refer to mycorrhizal plants, nonmycorrhizal plants given low phosphorus fertilization and nonmycorrhizal plants given high phosphorus fertilization, respectively

[2] Means within rows followed by different letters were significantly different at $P = \leq 0.05$ (Fisher's protected LSD). Means followed by the same letters were not significantly different at $P = \leq 0.05$ (ANOVA). n = 12 for lethal Ψ, full-turgor Ψ_π and osmotic adjustment; n = 4 plant size parameters and leaf [P]; n = 6 for other parameters

[3] Day 0 refers to last day plants were watered

heavily colonized (Table 1). Little or no colonization was observed in NL or NH plants.

Leaves of AM and nonAM plants had similar Ψ_π when leaves were rehydrated to full turgor prior to drought (Table 1). However, by the end of the lethal drought episode, Ψ_π of fully rehydrated leaves was significantly lower in AM plants than in nonAM plants. This indicates that *G. intrardices* improved the ability of basil to accumulate solutes and osmotically adjust to drought. Osmotic adjustment has sometimes differed before between AM and nonAM plants in some species, such as wheat (Allen and Boosalis 1983), rose (Augé et al. 1986, 1987), pepper (Davies et al. 1993) and alfalfa (Goicoechea et al. 1997). However, many investigators have not observed this difference in these or other species (Augé and Stodola 1990, Henderson and Davies 1990, Faber et al. 1991, Bryla and Duniway 1997a, b). The moderate, mycorrhiza-induced increase in osmotic adjustment did not improve the ability of basil to resist drought.

One measure of plant drought resistance, whether resistance occurs via avoidance or tolerance mechanisms (Levitt 1980), is lethal soil Ψ_m: the ability of foliage to survive to low soil Ψ_m. Mycorrhizal symbiosis did not improve drought resistance of basil, as lethal soil Ψ_m was similar among AM and nonAM plants (Table 1). Mycorrhizal symbiosis also did not affect foliar dehydration tolerance, measured as lethal leaf Ψ (Table 1). Drought avoidance, the ability of plants to postpone or avoid foliar dehydration as soil dries, can be quantified as the difference between lethal leaf Ψ and lethal soil Ψ_m. Mycorrhizal symbiosis did not affect ability of basil leaves to avoid drought because lethal leaf Ψ – lethal soil Ψ_m did not differ among treatments (Table 1). Length of the lethal drying episode, another measure of drought resistance, also did not differ among treatments (Table 1). Water uptake was similar between AM and NL during the drought episode; NH plants had higher uptake, but this was expected due to their larger size (Fig. 1).

Changes in plant water relations in response to drought did not appear to be linked to plant size or leaf [P]. NH plants had much higher biomass and leaf [P] than NL plants, yet each had similar means of lethal leaf Ψ, lethal soil Ψ_m and full-turgor Ψ_π before and after the drought. Furthermore, individual plant values for water relations variables were not correlated with foliar [P] ($P \leq 0.05$).

Foliage of *O. basilicum* was very sensitive to dehydration. At near −2 MPa, its lethal leaf Ψ is at the high edge of the range of dehydration sensitivity; only reports for *Nyssa sylvatica* (Augé et al. 1998), *Phaseolus acutifolius* and *Vigna unguiculata* (Ludlow 1989) have shown higher lethal Ψ. Osmotic adjustment was also limited in basil (Table 1), which is typical of drought-sensitive plants.

When mycorrhizae benefit droughted plants, they appear to do so primarily through direct drought avoidance (Reid 1979, Augé 2001). AM symbiosis has postponed declines in leaf Ψ during drought (Huang et al. 1985, Davies et al. 1992, Dixon et al. 1994, Subramanian et al. 1995, 1997), even at similar bulk soil moisture around AM and nonAM plants (Allen and Allen 1986, Augé et al. 1987, Duan et al. 1996, Gemma et al. 1997). Leaf Ψ has also been reported to return to control levels more quickly in AM plants after relief of drought (e.g. Subramanian et al. 1997). In several reports, leaf or shoot Ψ has not differed in AM and nonAM plants during drought or during drought recovery (Levy and Krikun 1980, Nelsen and Safir 1982, Graham et al. 1987, Ramakrishnan et al. 1988, Osonubi et al. 1991, Davies et al. 1993, Osonubi 1994, Bryla and Duniway 1997a, Ebel et al. 1997, Goicoechea et al. 1997, 1998), nor has the leaf Ψ/soil Ψ relation been altered (e.g. Stahl and Smith 1984).

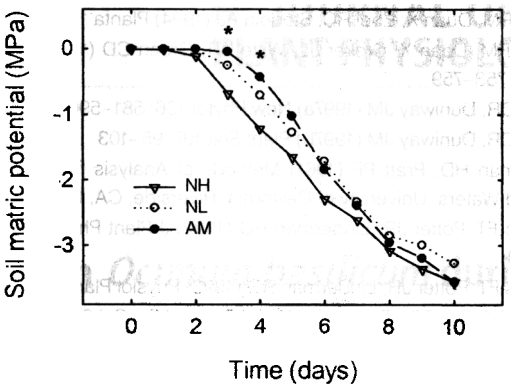

Figure 1. Decline in soil Ψ_m during a drying episode. Day 0 = last day plants were watered. Mean comparisons were performed among treatments for each day. Asterisk denotes significant difference between AM and NH plants ($P = \leq 0.05$, Fisher's protected LSD). NL plants did not differ significantly from AM or NH plants on any day. n=6.

When plant size is standardized among treatments, effects of AM symbiosis on plant drought resistance have been unpredictable and usually subtle (Augé 2001). The only effect of mycorrhizal symbiosis on water relations of basil in this study was a modest increase in osmotic adjustment. While even small osmotic changes could conceivably affect ecological fitness of a species having little capacity to withstand drought, the mycorrhiza-induced osmotic adjustment observed here did not increase the resistance of basil to lethal drought stress.

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