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Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions

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Abstract This study examined the uptake of nitrogen by external hyphae of an arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck & Smith) and its impact on physiological responses in maize plants subjected to well-watered or drought-stressed conditions. Plants were grown in compartmented boxes divided by a nylon mesh (40 μm) into a root compartment and a hyphal compartment. Maize plants (*Zea mays* cv. 'Tuxpeño sequia' selection cycle C0) were exposed to 2 weeks of drought 56 days after sowing. A ^{15}N tracer was applied as $K^{15}NO_3$ to the hyphal compartment at a distance of 5 cm from the root compartment. Root and shoot samples were then analyzed for ^{15}N atom % excess (APE), glutamine synthetase (GS) activity, protein concentration and nutritional status. Evapotranspiration rate and stomatal resistance were monitored daily to determine the degree of drought stress. The APE values for AM shoots and roots were 32% and 33% higher than non-AM shoots and roots, respectively, under drought conditions. This provides clear evidence that the external mycelium of AM fungus transports considerable amounts of $^{15}NO_3^-$ to the host plant under drought conditions. Drought-stressed AM roots had 28% higher GS activity, possibly as a consequence of higher hyphal acquisition of NO_3^- ions. Mycorrhizal colonization significantly increased the host plant P status regardless of soil moisture regime. In addition, the N status of drought-stressed AM shoots and roots was slightly higher than stressed non-AM shoots and roots. The improved nutritional status may assist AM plants to exploit available soil moisture more efficiently and to maintain higher leaf relative water content under moderate drought conditions.

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Introduction

The symbiotic association between arbuscular mycorrhizal (AM) fungi and roots makes a significant contribution to plant growth and nutrition (Smith and Read 1997). The major effect on nutrition is believed to result from hyphal transport of immobile mineral ions. Hyphae growing beyond the rhizosphere soil increase the absorptive surface of the root (George et al. 1995). Thus activities of AM mycelium in soil result in greater efficiency of nutrient absorption (Smith and Gianinazzi-Pearson 1988). This process is particularly important for slowly diffusing mineral ions such as P (Jakobsen et al. 1992).

Few studies have been carried out on the role of AM symbiosis in N nutrition of crops. Radioisotopic studies have revealed that external mycelium can utilize soil inorganic N very efficiently (Ames et al. 1983; Frey and Schüepp 1992; Johansen et al. 1993, 1994) and transport it 10–30 cm through the soil. Thus, AM plants have access to forms of N unavailable to non-AM plants (Azcón-Aguilar et al. 1993; Tobar et al. 1994a, b) and disruption of the hyphal network has implications for N acquisition. Johansen et al. (1993) showed that external hyphae of *Glomus intraradices* absorb both NO_3^- and NH_4^+ from the growth medium and translocate them to plants. Active uptake of NO_3^- by external mycelium of *G. intraradices* in monoxenic culture has also been reported (Bago et al. 1996).

It is widely believed that NO_3^- ions are the predominant form of N in many agricultural soils. Being highly mobile they reduce the importance of AM fungi in such soils (Barea et al. 1987; Harley 1989). However, NO_3^- mobility is severely restricted by drought due to its low concentration and diffusion rate (Azcón et al. 1996). Thus, under such conditions, the role of mycorrhizae in NO_3^- transport to the root surface may be significant.

Tobar et al. (1994a, b) provided evidence of hyphal transport of N from a NO_3^- source, supporting the view that AM fungi can be important for N nutrition of plants in dry soils. Cliquet and Stewart (1993) reported that nitrate reductase (NR) and glutamine synthetase (GS) activities in roots and shoots of maize plants increase when colonized with *G. fasciculatum*. This indicates that NO_3^- mobilized from soil by an AM fungus could be transferred directly to the root cells for further reduction and assimilation. Recently, we showed that AM colonization of maize stimulated activities of key enzymes involved in N assimilation such as NR, GS and glutamate synthase (GOGAT), especially under drought conditions (Subramanian and Charest 1998). These metabolic modifications and improved nutritional status appear to enable the host plant to withstand water-deficit and recover rapidly when irrigation is restored.

This led to the idea that the hyphal contribution of N by AM symbiosis may be considerable. We hypothesized that the external mycelium of an AM fungus can transport $^{15}NO_3^-$ from a hyphal compartment in soil to a host plant, and that this varies with the moisture status of the growth medium. Further, the hyphal contribution causes modification of N assimilation, nutritional status and other physiological responses in the host plant. To test this, we examined N acquisition, GS activity, nutritional and water status, and growth parameters of mycorrhizal maize plants grown in compartmented boxes under well-watered or drought-stressed conditions.

Materials and methods

A greenhouse experiment was conducted using freshly regenerated seeds of *Zea mays* L. cultivar 'Tuxpeño sequia' selection cycle C0 (drought-sensitive) obtained from CIMMYT, Mexico. Our previous data (Subramanian and Charest 1997) clearly demonstrated that cultivar C0 is more responsive to AM colonization than the drought-resistant cultivar C8, and hence C0 was used in this study. Plants were either well-watered (WW) or drought-stressed (DS), where irrigation was withheld for 2 weeks at 56 days after sowing (DAS) and were either without (non-AM) or with (AM) inoculation of *G. intraradices*. The four treatment combinations were replicated four times in a randomized block design.

Compartmented boxes used in this study were a modified version of those described by Frey and Schüepp (1993). Each box was built with plexiglass (8 mm thick) and divided into a root compartment (RC, 30 cm × 30 cm × 20 cm) and a hyphal compartment (HC, 30 cm × 30 cm × 10 cm) by a 40-μm nylon mesh (Thompson, Ville Mt-Royal, Québec, Canada) which was shown to restrict root growth while allowing the passage of external mycelium (Frey and Schüepp 1993). One plant was grown in each box at the centre of the RC, in a sterile pot mixture (w/w/w 1 peat moss:1 vermiculite:1 sand) at 24 °C with 14-h photoperiod, 65–70% RH, and irradiance 400–600 μmol m^{-2} sec^{-1} provided by natural light in conjunction with high-pressure sodium vapour lamps.

The RC and HC were filled with 8 and 4 l of growth mixture, respectively. At the beginning of the experiment, the pot mixture was wetted with water to saturation. Subsequently, the volume of irrigation water required for saturation was assessed from the weekly cumulative evapotranspiration (ET) rate determined gravimetrically (data not shown). During the 2 weeks of drought, the ET rate was determined daily. The mycorrhizal inoculum (*G. intraradices* Schenck & Smith; DAOM no. 181602) used in this study was provided by Premier Tech, Rivière-du-Loup, Québec, Canada. This strain incorporated in peat moss was originally isolated from the rhizosphere of *Fraxinus* sp. and subsequently cultured on *Coleus* and *Mimulus* spp.

Inoculated or non-inoculated peat moss (200 ml/100 g) was applied per box uniformly as a 1-cm layer, 5 cm below seeds in each container prior to sowing. The AM and non-AM plants were fertilized each week uniformly along with the irrigation water using a ready-made fertilizer mixture carrying 30:10:10 NPK and micronutrients (Plant-Prod, Plant Products Co. Ltd. Brampton, Ontario, Canada). All plants were fertilized with a balanced nutrient solution (each plant received 0.78 g of fertilizer mixture dissolved in 500 ml distilled H_2O per week) supplying 234 mg N, 78 mg P, and 78 mg K per plant per week. For drought-stressed plants, excessive amounts of fertilizer were applied prior to the imposition of drought stress to maintain uniformity in the fertilizer levels. One half of the AM and non-AM plants were exposed to drought by withholding irrigation for 2 weeks starting 56 DAS. The loss of substrate moisture, assessed daily using a gravimetric method, was 60–70% by the end of the drought period. To prevent algal growth and surface evaporation, and to ensure gradual depletion of substrate moisture, the boxes were covered with black polyethylene bags. Extra plants were grown under similar conditions to determine the progression of root colonization, and to ensure that symbiosis was established before plants were exposed to drought treatment.

After the establishment of mycorrhizal symbiosis at 56 DAS, 100 mg of N (25 mg kg^{-1} HC substrate) were applied to the HC in the form of 10 atom % $K^{15}NO_3$ (Isotech Inc., A Matheson, USA Company, Miamisburg, OH). The labelled N was prepared (25 ml) with deionized H_2O and injected using a syringe at 5 cm from the RC. A 5-ml portion of this solution was injected at 5 different spots in order to distribute the label uniformly. After 2 weeks (70 DAS), plants were harvested and analyzed for nutritional and biochemical parameters.

Root colonization studies

The AM-colonized roots were stained with aniline blue (Dalpé 1993) before mounting on slides in polyvinyl alcohol-lactic acid-glycerol medium. One-hundred 1-cm root segments per treatment were examined for the presence of arbuscules, vesicles, or hyphae. The percentage AM colonization was determined at the beginning (56 DAS) and the end of the 2 weeks of drought or well-watered treatment (70 DAS).

Determination of biomass, ^{15}N, nitrogen and phosphorus content

At the end of the experiment, harvested roots and shoots were oven-dried at 70 °C for 48 h and dry masses determined. The dried samples were powdered using a wiley mill and analyzed for P, N and ^{15}N. For P concentration, dried tissues (200 mg) were digested in a $HClO_3$–H_2O_2 (v/v 7:3) mixture for 30 min in a sealed chamber under a fumehood (Subramanian and Charest 1997). The digested samples were diluted to 25 ml with distilled H_2O. P was assayed spectrophotometrically using the ascorbic method (Walsh and Beaton 1973). N was measured with an elemental analyzer (Carlo Elba, CE Instrument, EA 1110 CHNS, Milan, Italy) and ^{15}N enrichment was determined using a mass spectrometer (Finnigan Delta Plus, Bremen, Germany). The nutrient content was calculated by multiplying the mineral concentrations by the dry masses of roots or shoots. The % ^{15}N of the atmosphere served as background reference to estimate the atom % ^{15}N excess in plant material.

Biochemical analyses

Soluble proteins from roots or shoots (100 mg freeze-dried tissue) were extracted on ice using 10 ml Tris-HCl buffer (pH 7.8), centrifuged at 10 000 rpm at 4 °C for 25 min and determined according to Bradford (1976). The glutamine synthetase (EC 6.3.1.2) activity was measured from the same extract by the transferase assay (Shapiro and Stadtman 1970) as previously described (Subramanian and Charest 1998).

Physiological responses

Stomatal resistance was measured during the 2-week treatment period using a Licor steady-state porometer (LI 1600) on a fully expanded leaf between 10:00 and 12:00 (Dwyer and Stewart 1985) to assess the progressive drought effects on AM and non-AM plants. At the end of the 2 weeks, leaf relative water content (*RWC*) was estimated using the following formula (Turner 1986)

$$RWC = (FW–DW/TW–DW)\ 100$$

where *FW* is leaf fresh weight, *DW* is leaf dry weight after 24 h drying at 70 °C, and *TW* is leaf turgid weight after submergence of a fresh leaf in distilled H_2O for 4 h.

Statistical analysis

Two-way analysis of variance (ANOVA) was used (SAS Institute Inc.) for all parameters measured. Critical differences at the 5% level of significance were tested using Tukey's Studentized range (HSD) test.

Results

Mycorrhizal colonization

The mycorrhizal colonization was 50 ± 4% at the beginning (56 DAS) of the treatment and 60 ± 8% and 47 ± 5% under WW and DS conditions, respectively, at the end of the experiment (70 DAS).

Root and shoot dry masses

Mycorrhizal colonization significantly increased shoot and root dry masses, by 9.4% and 25.8%, respectively, under WW conditions only (Fig. 1A, Table 1). Drought stress significantly decreased shoot masses of AM and non-AM plants by 34.2% and 35.4%, respectively, and AM root mass by 26%.

P status

AM colonization significantly increased shoot P contents by 35.5% and 32.1% under WW and DS conditions, respectively (Fig. 1B, Table 1). Drought stress significantly decreased the P contents of AM and non-AM shoots by 57% and 54.4%, respectively. The root P content was not altered by mycorrhizal or drought treatment. Well-watered AM and non-AM plants had 2- and 2.3-fold higher P contents than the respective DS plants.

Table 1 ANOVA results for growth (mass), nutritional parameters (P and N contents), and ^{15}N atom% excess (APE), measured in shoots and roots of maize plants exposed to 2 weeks of drought or well-watered treatment (D), and with or without AM colonization (M)

Parameters	Drought (D)	Mycorrhizae (M)	D × M
Shoot dry mass	***	*	NS
Root dry mass	*	*	**
Shoot P content	***	***	*
Root P content	NS	NS	NS
APE in shoots	NS	*	NS
APE in roots	NS	*	**
Shoot N content	***	NS	NS
Root N content	***	*	NS

*$P \le 0.05$
**$P \le 0.01$
***$P \le 0.001$
NS not significant

Atom ^{15}N % excess (APE) and N status

AM colonization significantly increased APE values in shoots and roots by 32% and 33%, respectively, under DS conditions, and in WW shoots by 41% (Fig. 1C, Table 1). The APE values in shoots were significantly higher in AM than non AM plants independent of substrate water conditions. The effect of drought on APE was more pronounced in shoots than in roots. Drought-stressed AM roots had APE values comparable to WW AM or non-AM roots. Drought stress significantly decreased N contents of both shoots and roots of AM and non-AM plants (Fig. 1D, Table 1). AM shoots had significantly higher (10%) N content than non-AM shoots under DS conditions. Under WW conditions, the N content was significantly higher (26%) in AM than non-AM roots. However, the N content of DS AM roots was comparable to DS or WW non-AM roots.

Protein concentration

In general, DS shoots and roots had significantly lower soluble protein concentrations than their WW counterparts (Table 2). Mycorrhizal status had no significant effect on shoot protein concentration. Interestingly, DS and WW AM roots had significantly higher protein concentrations than DS and WW non-AM roots, by 2.5- and 1.2-fold, respectively.

GS activity

GS activity in shoots and roots was significantly affected by both drought and mycorrhizal treatment (Table 2). In shoots, AM colonization increased GS activity by 27% and 29% under WW and DS conditions, respectively. Drought-stressed AM shoots had a GS activity similar to WW non-AM shoots. In AM plants, GS activity in roots significantly increased by 28% under

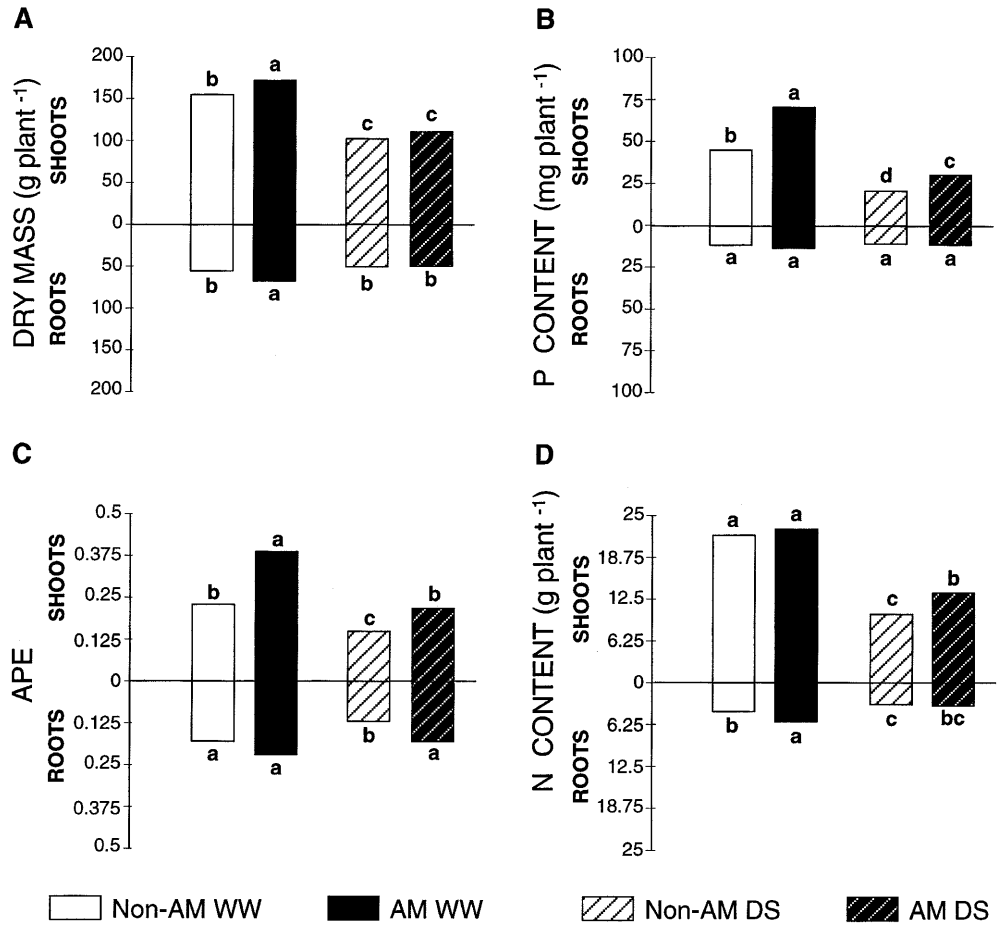

Fig. 1A Dry mass, **B** phosphorus content, **C** atom ^{15}N % excess (APE), and **D** nitrogen content in shoots (top) and roots (bottom) of maize plants with (*AM*) or without (*non-AM*) arbuscular mycorrhizal colonization under well-watered (*WW*) and drought-stressed (*DS*) conditions ($n=4$). Bars with different letters are significantly different at the 5% level according to Tukey's HSD test. For each parameter, all the data for shoots and roots have been analyzed separately. The values for roots below the horizontal line are positive

drought conditions. This mycorrhizal response was not observed in WW AM roots. Drought-stressed non-AM roots had significantly lower GS activity than the other treatments. Generally, GS specific activities in shoots and roots were significantly higher under WW than DS conditions, with or without AM association (Table 2). AM shoots had significantly higher GS specific activities than non-AM shoots under DS and WW conditions by 35.9% and 25.5%, respectively. Under both substrate moisture conditions, GS specific activities were significantly higher in non-AM than AM roots.

Physiological responses

Drought stress significantly decreased the leaf RWC, but to a much lesser extent in AM (6%) than non-AM (24%) plants (Table 3). The leaf RWC was significantly higher by 18% in AM than non-AM plants under DS conditions, but similar under WW conditions. ET rates were significantly higher in WW than DS plants, but AM colonization had no effect on ET (Table 3). The ANOVA indicated a drought effect on SR values but the Tukey's HSD test revealed no differences among the treatments (Table 3).

Discussion

Mycorrhizal colonization by *G. intraradices* improved nutritional status and N assimilation in maize plants exposed to moderate drought stress. The compartmented box system approach provided clear evidence that the external mycelium of the fungus played a direct role in the transport of the NO_3^- form of N, especially under drought conditions. This resulted in improved host plant nutritional status and an increase in GS activity. The increased capacity for N acquisition and assimilation may enable the host plant to sustain moderate drought stress conditions. The present results corroborate other reports (Ames et al.1983; Frey and Schüepp 1993; Tobar et al. 1994a, b) indicating that the external mycelium of AM fungi actively assists host plants to enhance N uptake and translocation when water availability is limited.

The mobility of NO_3^- ions is severely restricted under drought conditions by low concentration and diffu-

Table 2 Means ($n=8$) and standard errors (parentheses) of protein concentration (mg g^{-1} dry mass), glutamine synthetase (GS) activity (μmol g^{-1} dry mass h^{-1}) and specific GS activity (μmol mg^{-1} protein) in shoots and roots of maize plants with (AM) or without (non-AM) arbuscular mycorrhizal colonization, under well-watered (WW) or drought-stressed (DS) conditions. Different letters in a column indicate significant differences ($P \leq 0.05$) using Tukey's HSD Test

	Proteins		GS activity		Specific activity	
	Shoots	*Roots*	*Shoots*	*Roots*	*Shoots*	*Roots*
Non-AM WW	37.6[a]	4.1[c]	129.1[b]	81.9[a]	3.5[b]	21.3[a]
	(2.3)	(0.4)	(8.8)	(2.2)	(0.2)	(2.1)
Non-AM DS	32.9[b]	3.4[d]	81.7[c]	56.4[b]	2.5[c]	16.4[b]
	(1.9)	(0.2)	(6.3)	(7.1)	(0.2)	(2.7)
AM WW	38.1[a]	4.8[b]	176.7[a]	78.8[a]	4.7[a]	16.7[b]
	(0.8)	(0.2)	(17.7)	(6.9)	(0.5)	(1.6)
AM DS	29.7[b]	8.6[a]	115.2[b]	78.2[a]	3.9[ab]	9.1[c]
	(1.8)	(0.2)	(9.2)	(3.9)	(0.2)	(0.3)
ANOVA: D (drought treatment), M (mycorrhizal treatment), D × M (interaction)						
D	***	***	***	***	*	***
M	NS	***	***	***	***	***
D × M	NS	***	NS	***	NS	NS

*$P \leq 0.05$ **$P \leq 0.01$ ***$P \leq 0.001$ NS not significant

Table 3 Means ($n=4$) and standard errors (parentheses) for leaf relative water content (RWC), cumulative evapotranspiration rate (ET), and stomatal resistance (SR) of maize plants with (AM) or without (non-AM) arbuscular mycorrhizal colonization, under well-watered (WW) or drought-stressed (DS) conditions. Different letters in a column indicate significant differences ($P \leq 0.05$) using Tukey's HSD Test

	RWC *(%)*	ET *(kg per pot)*	SR *(s^{-1} cm^{-1})*
Non-AM WW	83.0[a]	15.2[a]	2.2[a]
	(3.3)	(1.4)	(0.3)
Non-AM DS	62.9[c]	9.0[b]	2.8[a]
	(4.0)	(0.7)	(0.3)
AM WW	81.9[a]	13.7[a]	2.2[a]
	(1.3)	(0.9)	(0.2)
AM DS	76.8[b]	8.8[b]	2.9[a]
	(2.5)	(1.2)	(0.1)
ANOVA: D (drought treatment), (mycorrhizal treatment), D × M (interaction)			
D	***	***	*
M	NS	NS	NS
D × M	*	NS	NS

*$P \leq 0.05$
**$P \leq 0.01$
***$P \leq 0.001$
NS not significant

sion rates (Azcón et al. 1996). Under such conditions, the ^{15}N in plant tissues derived from the labelled $K^{15}NO_3$ was higher by 33% in AM than non-AM roots. These data also indicate that external mycelium plays a critical role in uptake and transport of NO_3^- ions, which may be of importance to the N nutrition of plants in arid and semi-arid agricultural soils. Our data correspond to the findings of Tobar et al. (1994a, b), who reported enhanced rate ^{15}N enrichment in mycorrhizal lettuce (*Lactuca sativa* L.) plants colonized by *G. fasciculatum*, especially under drought conditions. Because the root masses produced by drought-stressed AM and non-AM plants were similar, the mycorrhizal effect is not due to differences in root size. The higher ^{15}N enrichment in mycorrhizal roots might be attributable to accumulation of ^{15}N tracer in hyphae inside the roots (Johansen et al. 1993). However, in our study, we also detected higher ^{15}N concentrations in mycorrhizal shoots than roots, which indicates that AM association is important for the transportation of N from roots to shoots. Even under WW conditions, ^{15}N enrichment was higher in AM than non-AM shoots. Nevertheless, lack of a difference in root ^{15}N enrichment suggests that AM symbiosis is less important in N acquisition when plants are well-watered than under drought stress.

GS activity in AM plants increased by 30% under drought conditions, which may be attributed to the hyphal transport of N in the form of NO_3^- or NH_4^+ (Johansen et al. 1993, 1994; Tobar et al. 1994a, b). In the present study, we estimated the hyphal transport of N to maize plants as 33%, which is close to the value (30%) reported by Frey and Schüepp (1993). The hyphal contribution of N may vary with the functional compatibility of AM fungal isolates (Frey and Schüepp 1993) or the levels or forms of N application (Johansen et al. 1993). The increase in GS activity in mycorrhizal roots may be due partly to the presence of a GS-GOGAT system in the mycorrhizal fungus itself. Johansen et al. (1996) indicated that both NO_3^- and NH_4^+ forms of N are assimilated into the free amino acid pool of AM mycelium. These forms of N or increased N uptake may stimulate enzymes involved in N assimilation in the host plant. Recently, Subramanian and Charest (1998) reported that AM-colonized maize plants have enhanced NR, GS, and GOGAT activities under drought conditions. Conversely, Faure et al. (1998) ruled out any significant contribution of AM fungal tissue to NO_3^- reduction, because of the small fungus biomass relative to that of host plant roots.

In this study, we showed that the host plant P nutritional status was improved by mycorrhizal association. The enhanced P status of AM plants may have altered the activities of N-assimilating enzymes especially GS, which requires ATP (Lea et al. 1990). Oliver et al. (1983) demonstrated increased NR activity in AM-colonized white clover, attributed to an indirect P-mediated mechanism. On the other hand, the higher GS activity found in *G. mosseae*-colonized roots of *Allium cepa* L. than in the controls was a direct contribution of the fungus enzyme. In contrast, Azcón and Tobar (1998) showed an increase in GS activities in roots and shoots of *A. cepa* colonized by *G. fasciculatum*, regardless of P status. Our data, in conjunction with others, suggest that an AM association contributes to the increase in N enzyme activities. This may be a consequence of a direct contribution from the external mycelium and an indirect effect of improved host plant P status. AM colonization also assisted the host plant to maintain higher protein concentrations in roots regardless of moisture regimes, but the mycorrhizal response was more pronounced when plants were exposed to moderate drought conditions. This is consistent with our earlier findings (Subramanian and Charest 1998). Arines et al. (1993) detected a two- to six-fold increase in soluble protein concentration in mycorrhizal clover roots. Other studies have identified endomycorrhizins (AM-inducible proteins) in host plant species (Dumas et al. 1990; Simoneau et al. 1994). These inducible proteins may play a vital role in host-plant drought tolerance.

In addition to N nutrition, AM colonization conferred a higher P status under drought conditions. Numerous greenhouse and field experiments have shown conclusively that plants colonized by AM fungi are much more efficient in taking up soil P than non-AM plants, particularly under drought conditions (Nelsen and Safir 1982; Bethlenfalvay et al. 1988; Fitter 1988; Sylvia et al. 1993; Subramanian and Charest 1997). Improved P nutrition by AM fungi during water deficit has been postulated as a potential mechanism for enhancing host-plant drought tolerance.

There were clear effects of AM association on physiological responses. Despite 2 weeks of withheld irrigation, leaf RWC remained higher in AM than non-AM plants. This higher leaf RWC may be associated with an indirect effect of improved P status of AM plants (Fitter 1988). Subramanian et al. (1997) reported that improved nutritional status may assist AM plants to exploit available soil moisture and maintain higher leaf RWC under moderate drought conditions. In contrast, others have reported that improvement of water status by mycorrhizal fungi during drought was independent of host plant P status (Davies et al. 1993). It is likely that the external mycelium facilitated direct water uptake and transport of water by mycorrhizal roots (Hardie 1985; Faber et al. 1991). Even when plants were drought-stressed for 2 weeks, the ET rates and SR did not differ between AM and non-AM plants. Thus, mycorrhizal colonization of maize had no apparent effect on stomatal sensitivity to soil water status in our experiment. Similarly, in wheat and safflower, colonization by *G. etunicatum* did not alter the intrinsic hydraulic properties of the soil-plant system (Bryla and Duniway 1997).

In summary, using a comparmented box system, we have found that the external mycelium of *G. intraradices* may play a direct role in the uptake and translocation of the NO_3^- form of N, especially under water-deficit conditions. Our data suggest a positive relationship between N hyphal contribution and metabolic/nutritional status of the host plant. These changes may assist the host plant to withstand drought conditions.

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