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The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae

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Abstract. The ability of the external mycelium in arbuscular mycorrhiza for N uptake and transport was studied. The contribution of the fungal symbiont to N acquisition by plants was studied mainly under water-stressed conditions using ^{15}N. Lettuce (*Lactuca sativa* L) was the host for two isolates of the arbuscular mycorrhizal fungi *Glomus mosseae* and *G. fasciculatum*. The experimental pots had two soil compartments separated by a fine mesh screen (60 μm). The root system was restricted to one of these compartments, while the fungal mycelium was able to cross the screen and colonize the soil in the hyphal compartment. A trace amount of $^{15}NH_4^+$ was applied to the hyphal compartment 1 week before harvest. Under water-stressed conditions both endophytes increased the ^{15}N enrichment of plant tissues; this was negligible in nonmycorrhizal control plants. This indicates a direct effect of arbuscular mycorrhizal fungi on N acquisition in relatively dry soils. *G. mosseae* had more effect on N uptake and *G. fasciculatum* on P uptake under the water-limited conditions tested, but both fungi improved plant biomass production relative to nonmycorrhizal plants to a similar extent.

Key words: Arbuscular mycorrhiza – Hyphal N uptake – ^{15}N-labelled fertilizer – Drought stress

Introduction

The use of ^{15}N-based methods has demonstrated the role of arbuscular mycorrhizal (AM) symbiosis in plant acquisition of N compounds from soil (Barea et al. 1991, 1992). Such a mycorrhizal effect is probably based on the well-established activity of external mycelium in the uptake and translocation of slowly diffusing ions (nutrients), particularly ammonium in the case of N acquisition (Harley 1989).

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Experimental systems with spatial separation of root and hyphal uptake zones are being used to ascertain the hyphal contribution to nutrient acquisition by AM plants (Schüepp et al. 1987; Li et al. 1991), in particular for ammonium uptake using ^{15}N (Johansen et al. 1992). These authors have shown a direct effect of external mycelium on ^{15}N transport, which actually produced a depletion of inorganic soil N, with a significant hyphal immobilization of the N acquired. However, under their experimental conditions, Johansen et al. (1992) did not find any benefit of this mycorrhizal activity for plant growth. These findings need to be expanded to other soil/plant/AM fungi combinations, and the effect of different environmental conditions must be tested. Water supply is of particular interest as it is well known that drought stress impedes the movement of nutrients to the root surface, with a striking effect on slow-diffusing ions like ammonium (Barea 1991).

A compartmental system providing different uptake zones for root and hyphae, and application of $^{15}NH_4^+$ can be used to ascertain fungal effects in N acquisition by AM plants in a given ecophysiological situation. The objective of this report was to study mycorrhizal activity on N uptake under water-stressed conditions and effects on plant growth. A neutral agricultural soil and two AM fungi (*Glomus mosseae* and *G. fasciculatum*) were tested.

Materials and methods

Experimental system and design

The plant containers used as a culture system are shown in Fig. 1. The container was a plastic-lined 1.2-l pot divided into two compartments separated by a 60-μm nylon mesh; this mesh retains the roots but allows hyphae to pass. The "root compartment" was filled with 400 g of the soil substrate in which the plants (one per pot) were growth either mycorrhizal or nonmycorrhizal. The "hyphal compartment" contained 600 g of the soil substrate which the AM external mycelium could colonize.

The experiment cosisted of one noninoculated treatment and two inoculated with either *G. mosseae* or *G. fasciculatum*. The

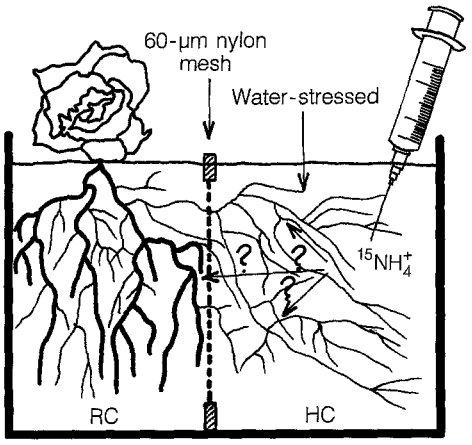

Fig. 1. The experimental system. *RC*, Root compartment; *HC*, hyphal compartment

three treatments were replicated five times to give a total of 15 completely randomly arranged pots. The experiment was carried out at either 80% or 100% of the water holding capacity (whc) of the soil.

Test soil

The test soil collected from Granada province (Spain) had the following characteristics: pH 7.4; organic matter 1.01%; $N\text{-}NO_3^-$ 1.5 µg/g; $N\text{-}NH_4^+$ 2.0 µg/g; assimilable P (Olsen) 6.24 µg/g; sand 54%; loam 24%; clay 22%. The soil was sieved (2 mm) and steam sterilized (100° C for 1 h for three consecutive days) and then reinoculated with a soil filtrate containing the normal microbiota without AM propagules.

Host plant and mycorrhizal inoculation

Fifteen-day-old uniform seedlings of lettuce (*Lactuca sativa* L, cv Romana) (one per pot) were transplanted into the root compartment. At transplanting, seedlings were inoculated with either *G. mosseae* or *G. fasciculatum*, or left uninoculated (nonmycorrhizal controls). Inoculation was carried out by addition of 5 g per pot of a mycorrhizal inoculum obtained from our stock culture collection and maintained for 3–6 months in polyethylene bags at 5° C. This consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. Control seedlings received the same amount of sterilized inoculum.

Nitrogen ($^{15}N/^{14}N$) applications

Plants were given a N- and P-free nutrient solution (10 ml/week/pot) (Hewitt 1952). Nitrogen was added as an unlabelled, $(NH_4)_2SO_4$ solution (Hewitt 1952) injected into the hyphal compartment (Fig. 1). One week before harvest, $^{15}(NH_4)_2SO_4$ was the N source. This ^{15}N-labelled material had 10% ^{15}N atom excess and was applied as an aqueous solution (5 ml) supplying 10 mg N/kg soil, also injected into the hyphal compartment (Fig. 1).

Growth conditions

Plants were grown under greenhouse conditions with temperatures ranging from 19 to 25° C, a 16/8 light/dark photoperiod and a relative humidity of 70–90%. A photosynthetic photon flux density of 400–700 µmol/m/s was applied as supplementary light.

Two similar experiments were carried out. In the first, the plants grew for 8 weeks in the greenhouse irrigated to 100% of the soil whc (Azcón et al. 1988). In a second experiment, irrigation was limited to 80% of whc, with the wilting point of the soil substrate taken as 70% of whc (Azcón et al. 1988), and the plants grew for 12 weeks before the $^{14}NH_4^+$ was added 1 week prior to harvest. The irrigation regimes were applied by weighing each pot and adding water to the weight calculated for the desired water regime. Throughout the experiment, the pots were weighed twice a day and water loss replaced by top watering.

Measurements

After harvest, the weights of shoots and roots were recorded and the shoot tissues were analysed for N and P (Lachica et al. 1973). The extent of root colonization by the mycorrhizal fungus was assessed by the staining method of Phillips and Hayman (1970). The percentage mycorrhizal root length was calculated by a gridline intersect technique (Giovannetti and Mosse 1980). The length of mycorrhizal hyphae in the soil of the hyphal compartment was estimated in all pots by an aqueous extraction and staining technique (Li et al. 1991). The sampling procedure allowed only qualitative (semiquantitative) measurements. The N isotopic compositions of shoots and roots were determined by mass spectrometry, as described by Fiedler and Proksch (1975), at the FAO/IAEA Agricultural Biotechnology Laboratory, Seibersdorf, Austria. The effects of the mycorrhizal treatments were detected by separate one-way analysis of variance for each parameter/experiment. Means were compared by Duncan's New Multiple Range Test at the 5% level.

Results and discussion

Figures 2 and 3 summarize the results of the two experiments (nonlimited and limited water supply, respectively). Since these two experiments were not carried out simultaneously and also because they involve plants of different ages, the results are not comparable from a quantitative point of view but they allow for interesting relative/qualitative comparisons. The scales differ for most of the parameters between Fig. 2 and Fig. 3.

Development of AM effectiveness

The level of mycorrhizal colonization by *G. fasciculatum* was significantly reduced in well-irrigated conditions (Fig. 2). Qualitative (semiquantitative) observations of the hyphae in the hyphal compartment showed that an abundant mycorrhizal mycelium was evident in all experimental pots, estimated as 3–5 m/g dry soil. At 100% whc, the external mycelium associated with *G. fasciculatum* mycorrhizal roots was about half that of those colonized by *G. mosseae*. The effect of AM inoculation on dry matter yield (shoots) was related to the colonization level of the AM fungi involved, as shown in Figs. 2a, c. Assuming that the root/shoot ratio reflects the degree of AM effectiveness (Smith and Gianinazzi-Pearson 1988), the data in Fig. 2b indicate low

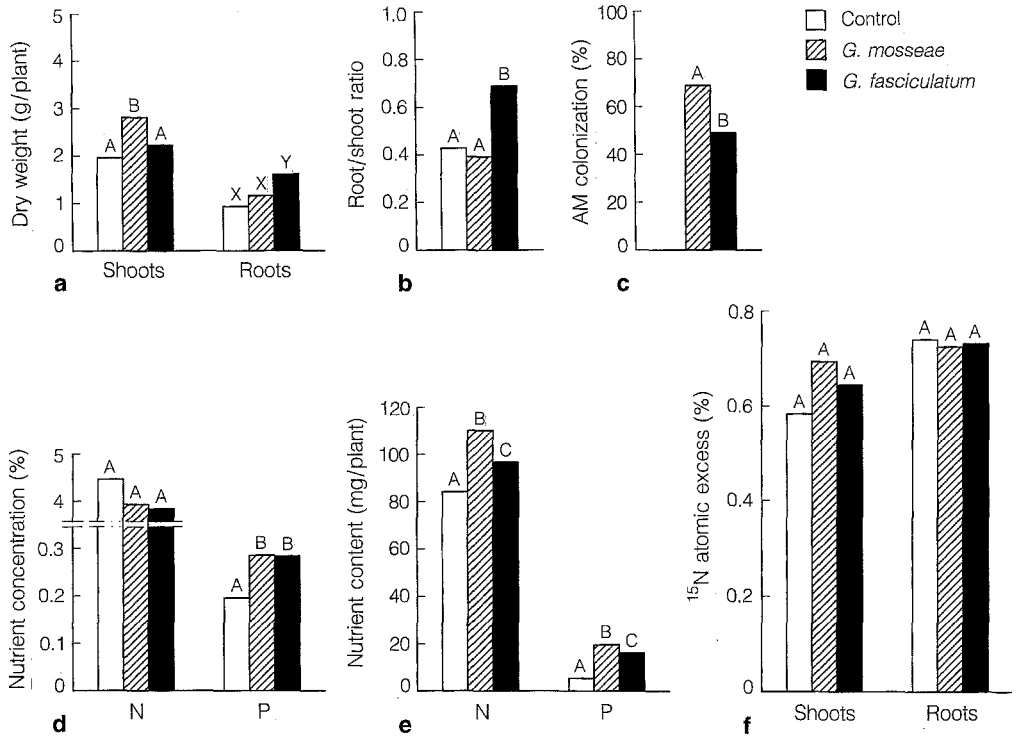

Fig. 2a–f. The effect of mycorrhizal inoculation on plant growth and nutrient acquisition under nonlimited water supply. For each parameter, the means (five replicates) with the same letter do not differ significantly at the 5% level (Duncan's New Multiple Range Test). *N*, Nitrogen; *P*, phosphorus; *AM*, arbuscular mycorrhizal

mycorrhizal activity in relation to plant biomass production under nonlimited water supply. However, plant development, biomass production and nutrient concentration and content, as affected by AM colonization, must be considered all together; this can be done following the concepts of Jarrel and Beverley (1981), who suggested that "total nutrient content" can be taken as a representative parameter of mycorrhizal effectiveness because it takes into account the well-balanced effect of nutrient acquisition/biomass production. In the case of plants growing under nonlimited water supply (Fig. 2), both endophytes appear effective in improving nutrient content (Fig. 2e), but only *G. mosseae* improved shoot dry weight (Fig. 2a).

In the case of plants growing under water-stressed conditions (Fig. 3), the two endophytes produced the same level of root colonization and about the same amount of mycelium in the hyphal compartment (3–4 mg/g dry soil); they were equally effective in improving plant growth, and resulted in a root/shoot ratio which supports the effectivity of AM symbiosis (Fig. 2). The data for nutrient acquisition under water stress (80% whc) irrigation regime (Fig. 2d, e) suggest that *G. fasciculatum* has a higher capacity for P acquisition than *G. mosseae*.

Differential effects of water content on AM fungi species in terms of both AM formation and plant response have been reported previously (Mosse et al. 1991).

^{15}N uptake and translocation

Since the $^{15}N/^{14}N$ ratio (or the ^{15}N atomic excess) is a yield-independent parameter (Zapata 1990), a comparison can be made of the AM effect at the two levels of water content tested (Figs. 2, 3). The atomic excess (% ^{15}N) in plant tissues was the same for mycorrhizal and nonmycorrhizal plants under nonlimited water supply (Fig. 2f), but was higher in mycorrhizal plants in water-stressed conditions (Fig. 3f). This indicates that a 100% whc the ^{15}N-labelled compound was able physico-chemically to reach the root surface, irrespective of mycorrhizal status; thus AM inoculation confers no additional advantage for N uptake (Fig. 2f). Conversely, in relatively dry soil (80% whc), AM fungi can be critical for N uptake (Fig. 3f). Joint consideration of the data in Fig. 3 for N concentration (d), N content (e), and ^{15}N enrichment (f) suggests that *G. mosseae* is more active in improving N acquisition from soil than *G. fasciculatum*.

The experimental system used (Fig. 1) led to the conclusion that the external mycelium in AM symbiosis plays a direct role in the uptake and translocation of N (^{15}N), corroborating the statements by Johansen et al. (1992). In addition, such AM activity was found to improve plant N nutrition and plant growth under the drought stress tested. This, together with the clear benefit that AM inoculation conferred on the P status of the plant (Fig. 3d, e), confirms the well-established effect of AM symbiosis on the acquisition of low-diffusing nutrients, particularly when water supply is limited (Barea 1991). The reported results thus support previous descriptions of AM influence on N nutrition (Barea et al. 1991, 1992), and that already demonstrated for other types of mycorrhizae (Stribley and Read 1980; Finlay et al. 1992).

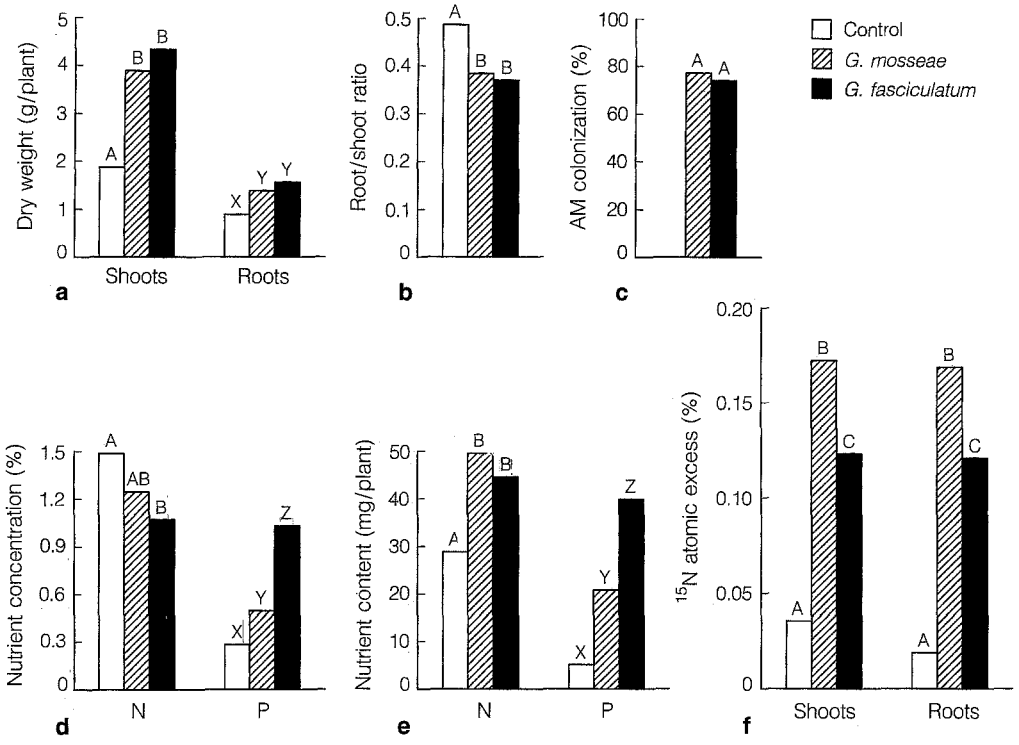

Fig. 3a–f. The effect of mycorrhizal inoculation on plant growth and nutrient acquisition under drought-stress conditions. For each parameter, the means (five replicates) with the same letter do not differ significantly at the 5% level (Duncan's New Multiple Range Test)

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