

# Improved nitrogen uptake and transport from $^{15}\text{N}$ -labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions

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## SUMMARY

The significance of the external mycelium of arbuscular mycorrhiza for uptake and transport of N from  $^{15}\text{N}$ -labelled nitrate in benefiting plant nutrition, was evaluated under either well-irrigated or water-stressed conditions. Plants of lettuce (*Lactuca sativa* L.) were grown under controlled conditions in a neutral agricultural soil/sand mix either non-mycorrhizal or in association with the arbuscular-mycorrhizal fungus, *Glomus fasciculatum* (Taxter *sensu* Gerd.) Gerd. and Trappe. The pots comprised a two-compartment system, where a fine nylon mesh screen prevented the development of roots in a hyphal compartment.  $^{15}\text{NO}_3^-$  was applied to this hyphal compartment where access of the root was not possible. At harvest, the  $^{15}\text{N}$  enrichment in plant tissues was the same for both mycorrhizal and non-mycorrhizal plants under optimal water supply conditions. However, under water-stressed conditions, where the mass flow and diffusion of  $\text{NO}_3^-$  ions to the roots can be affected, the  $^{15}\text{N}$  enrichment was four times higher in mycorrhizal than in non-mycorrhizal plants. This provides evidence of hyphal transport of N from a nitrate source, supporting the view that arbuscular mycorrhiza can be important for the N-nutrition of plants in relatively dry agricultural soil where nitrate is actually the predominant nitrogen form.

Key words: Arbuscular mycorrhiza, nitrate uptake,  $^{15}\text{N}$ -labelled fertilizers, drought stress.

## INTRODUCTION

The external mycelium in arbuscular mycorrhiza plays a critical role in the transport of slowly-diffusing plant nutrients from the soil to the root cell, thereby contributing efficiently to plant growth and nutrition. This is the case with phosphate and ammonium (Barea, 1991). The uptake of [ $^{32}\text{P}$ ] phosphate by mycorrhizal hyphae can occur from distant zones which are otherwise not accessible to the root itself (Bolan, 1991; Jakobsen, Abbott & Robson, 1992). The use of [ $^{15}\text{N}$ ] ammonium has also established that, as in the case with phosphate, the external hyphae in arbuscular mycorrhiza can derive  $^{15}\text{N}$  from labelled ammonium salts (Ames *et al.*, 1983; Johansen, Jakobsen & Jensen, 1991). This fact is of interest in many climax communities where the primary form of N available to plants is ammonium (Stribley & Read, 1980; Bowen & Smith, 1981). Conversely, nitrate ions, which are the predominant N-form in many agricultural soils, are readily

mobile; therefore, arbuscular mycorrhiza may be of less importance for N-nutrition in these soils (Smith *et al.*, 1985; Barea, Azcón-Aguilar & Azcón, 1987; Harley, 1989). However, owing to the great demand for N by plants and considering that certain ecophysiological situations, such as a drought stress, may interfere with the mobility of  $\text{NO}_3^-$  to the root surface, the effect of these mycorrhiza could be relevant in water-deficient soil (Smith *et al.*, 1985). Since water-deficiency is a relative common situation, the role of mycorrhiza on  $\text{NO}_3^-$  uptake in dry conditions needs to be tested. This is the main objective of this study. The experimental approach used to monitor such activity of the arbuscular mycorrhizal mycelium was based on the feasibility of applying  $^{15}\text{N}$ -labelled  $\text{NO}_3^-$ , and on the use of an appropriate experimental system (Fig. 1). It is well-established that  $^{15}\text{N}$ -labelled fertilizers enable calculation of the enrichment ( $^{15}\text{N}/^{14}\text{N}$  ratio) of the N-pools used by plants (Zapata, 1990). Therefore, the  $^{15}\text{N}$  tracer technique allows investigation of a

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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  $P = 0.001$ .

## RESULTS AND DISCUSSION

The results, summarized in Table 1, show that under non-limited water supply conditions, the biomass and total N and P content in the plant material were significantly increased in the mycorrhizal treatment. In spite of this, the atomic excess ( $^{15}\text{N}$ ) in plant tissues was the same for mycorrhizal and non-mycorrhizal plants. This means that these

**Table 1.** Shoot dry weight and nutrient content ( $\text{mg pot}^{-1}$ ), atom percent  $^{15}\text{N}$  excess and the percentage of mycorrhizal root length in lettuce plants grown under well-irrigated conditions

Parameter	Non-mycorrhizal plants	Mycorrhizal plants
Dry matter yield	1180b	2260 <sup>a</sup>
P content	2.65b	5.85a
N content	51b	98a
$^{15}\text{N}$ atomic excess in plant tissues (%)	0.55a	0.55a
Degree of mycorrhizal colonization (%)	—	75

For each parameter mean values (five replicates) not sharing a letter in common differ significantly ( $P < 0.001$ ).

**Table 2.** Dry weight and nutrient content ( $\text{mg pot}^{-1}$ ), atom percent  $^{15}\text{N}$  excess and the percentage of mycorrhizal root length in lettuce plants grown under water-stressed conditions

Parameter	Non-mycorrhizal plants	Mycorrhizal plants
Dry weight		
Shoots	2230b	3660a
Roots	1640a	1530a
P content		
Shoots	1.7b	9.8a
Roots	1.2b	4.13a
N content		
Shoots	24.7b	38.4a
Roots	8.8b	13.3a
$^{15}\text{N}$ atomic excess (%)		
Shoots	0.051b	0.196a
Roots	0.031b	0.114a
Degree of mycorrhizal colonization (%)	—	74

For each parameter mean values (five replicates) not sharing a letter in common differ significantly ( $P < 0.001$ ).

two groups of plants: (i) used soil N-pools with the same  $^{15}\text{N}/^{14}\text{N}$  enrichment and (ii) the percentage of N derived from the labelled fertilizer was the same, irrespectively from the mycorrhizal status. This indicates that the movement of  $\text{NO}_3^-$  ions to root surface, and the subsequent uptake by the roots themselves, can result in uptake similar to that achieved when roots were mycorrhizal. Conversely, it is evident (Table 2) that, when movement of  $\text{NO}_3^-$  ions in the soil solution is limited by the drought stress conditions, the  $^{15}\text{N}$  in plant tissues derived from the labelled  $^{15}\text{NO}_3^-$  was significantly higher in mycorrhizal plants. Although the length of mycorrhizal hyphae in the 'hyphal compartment', was not measured using an adequate quantitative sampling design, qualitative observations showed an abundant mycorrhizal mycelium in all the experimental pots. It was estimated that the amount of this mycelium was in the range  $3\text{--}5 \text{ m g}^{-1}$  dry soil. These results: (i) provide isotopic evidence that the external mycelium in arbuscular mycorrhizas takes up and transports  $\text{NO}_3^-$  which improve plant nutrition and (ii) support the contention that arbuscular mycorrhizas are critical for N-nutrition of plants in arid and semi-arid agricultural soils. This effect cannot be accounted for by a mycorrhiza-induced change in root size, since the biomass of roots was similar in mycorrhizal and non-mycorrhizal plants, and differences in root branching were not apparent. However, total plant biomass was significantly higher in mycorrhizal than in non-mycorrhizal plants (Table 2). This difference would have affected mass flow of  $\text{NO}_3^-$ . Water potential and, consequently the mass flow, were not actually measured. However, the desired soil moisture level was controlled twice a day throughout the experiment. The marked  $^{15}\text{N}$  enrichment of mycorrhizal plants under water deficient conditions strongly indicates a hyphal transport of  $\text{NO}_3^-$  ions. Though the different ages of plants in the two experiments complicate comparisons, there are some obvious effects. The increase in P-uptake by mycorrhizae, relative to control plants, was higher under water-stressed conditions (Table 2) than in the well-irrigated soil (Table 1). This corroborates the well-known role of arbuscular mycorrhiza in the acquisition of slowly diffusing nutrients by plants, an effect which appears even more relevant in relatively dry soils (Barea, 1991). In addition, drought stress did not affect mycorrhiza formation.

It can be concluded that this study supports the theory that the external mycelium of the arbuscular mycorrhizal symbiosis is active in  $\text{NO}_3^-$  uptake in relatively dry soils. These findings are of interest, in general, but particularly for agricultural soils where  $\text{NO}_3^-$  is the predominant N form (Smith *et al.*, 1985; Barea, 1991). This paper, therefore, contributes to information on the role of arbuscular mycorrhiza on N-uptake (see Barea, 1991), an effect

which is well established for other types of mycorrhizas (Stribley & Read, 1980; Harley, 1989; Finlay, Odham & Söderström, 1989)

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