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Isotopic (^{15}N) Evidence of the Use of Less Available N Forms by VA Mycorrhizas

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Abstract

Allium cepa L. (onion) was grown either under mycorrhizal or non mycorrhizal controlled conditions in a soil amended with increasing amounts of assimilable phosphate. The idea was to try to get comparable mycorrhizal and non mycorrhizal, but P-compensated plants as regards both plant growth and P status. An amount of ^{15}N-labelled fertilizer was added to each pot to try to distinguish the source of N for the plant. The time-course of the development of the plants, the dry matter production and the P-biomass yield showed that the effect of vesicular-arbuscular mycorrhizal (VAM) inoculation, without P addition, was similar to a given level of added phosphate in non mycorrhizal plants. However, VAM inoculation still increased both N concentration and content in plant shoots over such P-treated, non mycorrhizal, counterparts. The isotopic composition (^{14}N/^{15}N) was always higher in VAM plants showing that VAM fungi are able to use soil N sources which are less available to non mycorrhizal plants.

Keywords: VA Mycorrhiza, ^{15}N-labelled fertilizers, N uptake, P response curves

1. Introduction

In contrast with the overwhelming quantity of information on VA mycorrhizas and phosphate nutrition, few studies have been carried out to assess the role of such plant-fungal symbiosis on nitrogen uptake by plants, an effect which is well established for other types of mycorrhizas (Stribley and Read,

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1974, 1980; Martin et al., 1987; Finlay et al., 1988, 1989, 1992; Chalot et al., 1991). However, some key conclusions have been reached concerning N acquisition by VA mycorrhizas. For example, the fungal symbionts are known to use both NO_3^- and NH_4^+ (Bowen and Smith, 1981), to assimilate ammonium, via glutamine synthetase activity (Smith et al., 1985), and to increase the N inflow to the root cells (Smith et al., 1986). The use of the isotope ^{15}N has allowed the following suggestions: (1) hyphal transport in VA mycorrhizal (VAM) symbiosis (Ames et al., 1983), (2) a differential exploitation of different forms of N in the growth medium (Ames et al., 1984), and (3) a role of the VAM mycelium in increasing N uptake from soil, by using legume species (Barea et al., 1987, 1989; Azcón and Barea, 1992).

In order to study more deeply the role of VAM in N uptake, a number of key points must be taken into account, i.e. (1) to use non N_2-fixing species as test plants to avoid the effect of the interaction between N uptake and N_2 fixation, (2) to design specific assays allowing one to distinguish between direct VAM effects and indirect, P-mediated ones, using soil as substrate, and (3) to use ^{15}N as a tracer to distinguish the source of N for VAM plants. It is obvious that (2) could be achieved by including growth response curves to added phosphate for either VAM or non mycorrhizal plants (Abbott and Robson, 1984). The idea was to get comparable mycorrhizal and non mycorrhizal, but P compensated, plants, as regards both plant growth and P status.

Accordingly an experiment was carried out by combining the use of these P response curves with the application of a ^{15}N-labelled fertilizer as a tracer. The objective was to try to determine whether VAM plants use the same available N sources as the roots, or if VA mycorrhizas can derive N from soil sources less available to non VAM plants.

2. Materials and Methods

Experimental design

The experiment was based on a randomized complete block factorial with two factors: (1) P level, i.e. four doses of soluble phosphate, and (2) VAM inoculation (versus non mycorrhizal controls), given eight treatments that were replicated five times for a total of 40 pots.

Host plant and test soil

Onion (*Allium cepa* L. cv Babosa) was the test plant. Ten-day-old uniform seedlings, obtained by germinating the seeds in sterile sand, were transplanted (one plant/pot) into pots containing 1 kg of the experimental soil, previously

supplied with the appropriate amount of soluble phosphate, and a VAM inoculum applied at transplanting. The test soil was collected from Granada Province, Spain. It was a Cryumbrept type (10.2% clay, 33.6% loam, 56.2% sand) with 3.2% organic matter; pH = 5.7; 0.25% total N and 1.3 mg P/kg soil extracted with 0.5 M $NaHCO_3$ (Olsen P). The soil was sieved (2 mm), diluted with sand (5:2, v:v), steam-sterilized (100° C for 1 hr for 3 consecutive days) and then reinoculated with a soil filtrate containing the own microbial population except VAM propagules (Barea et al., 1989). Each pot received 5 ml of such filtrate.

Mycorrhizal inoculation and phosphate treatments

The test soil was divided into four batches. These were: P_0 = untreated control; P_1, P_2 and P_3 treatments of different doses of KH_2PO_4 (mg/kg of soil) as follows: $P_1 = 125$, $P_2 = 250$, $P_3 = 375$. These experimental soils were held at 19–25° C, with suitable watering, for 2 weeks to equilibrate before transplanting. Ten replicate pots were prepared from each one of the P levels and half of these were inoculated with VAM propagules. This VAM inoculum (20 g) was applied to each of the corresponding pots and dispersed throughout the experimental soil. Non mycorrhizal treatments also received 20 g of this inoculum, that was obviously sterilized accordingly. The inoculum was from a thoroughly homogenized soil sample of the VAM fungus *Glomus fasciculatum* (Taxter sensu Gerd.) Gerd. and Trappe grown in stock culture with *Medicago sativa* L. The isolate of *G. fasciculatum* came from INRA-Dijon France. The inoculum consisted of spores, mycelium, and mycorrhizal root fragments.

Growth conditions

The plants were grown in a greenhouse under a day/night cycle of 16/8 hr, 21/15° C, 50% relative humidity. A photosynthetic photon flux density of 600–700 μmol $m^{-2}sec^{-1}$ was applied as supplementary light. During the assay, plants were fertilized (20 ml/week/pot) with Long Ashton nutrient solution (Hewitt, 1952) lacking N and P. Throughout the experiment, the pots were weighed every day and water loss to reach field capacity was replaced by top watering.

^{15}N-labelled fertilizer application

After 10 days of plant growth, each pot was given a solution of $(NH_4)_2SO_4$ with 2% ^{15}N atom excess (20 mg N/pot).

Measurements

After a growth period of 9 weeks the plants were harvested. Shoot dry weight was recorded after drying at 70°C. Shoot P and N concentrations were measured after Kjeldahl digestion (for N) or by the molybdenum blue (for P) procedures (Lachica et al., 1973). Shoot N isotope composition was determined by mass spectrometry, as described by Fiedler and Proksch (1975) at the FAO/IAEA Agricultural Biotechnology Laboratory, Seibersdorf, Austria. The roots were carefully washed and the percentage of mycorrhizal root length was estimated by microscopic examination of stained samples (Phillips and Hayman, 1970), using the grid-line intersect method of Giovannetti and Mosse (1980).

The data of % ^{15}N atom excess allowed calculating the percentage of N derived from the labelled fertilizer (% NdFert), by the following equation (Danso et al., 1983):

$$\% \text{ NdfFert} = \frac{\% \ ^{15}\text{N atom excess in plant sample}}{\% \ ^{15}\text{N atom excess in fertilizer}} \times 100$$

This, in turn, allowed calculating the % NdfSoil (100 - % NdFert) and, therefore the isotopic composition of the N acquired by the plant during the growing period, once the total N yield in plants was determined.

For a statistical analysis of the data ANOVA followed by Fisher's LSD ($P< 0.05$) tests were done. In the case of the percentage of mycorrhizal root length, the data were subjected to an arc-sine square-root transformation in order to homogenize the variance.

3. Results

After 9 weeks of plant growth, the percentage of root length that was in fact mycorrhizal, was calculated and the values were: P_0 = 78a; P_1 = 81a; P_2 = 72a; P_3 = 83a ($P< 0.05$).

Plant growth was negligible in non mycorrhizal, non P-added plants (Fig. 1). The dry matter yield and the content of P in the test plant increased with the level of P added in both VAM and non VAM plants (Fig. 1). For these two parameters, the plant response to VAM inoculation, without P addition (P_0), was equal to the response of 250 mg/kg of phosphate (P_2) in non VAM plants (Fig. 1). Evidence for such similar effects was corroborated by the data recorded in Fig. 2. These data, in fact, correspond to the time-course development of the test plant. Therefore, comparisons between these two treatments (i.e. VAM inoculation in P_0 and P_2-treated, but non VAM plants) concerning

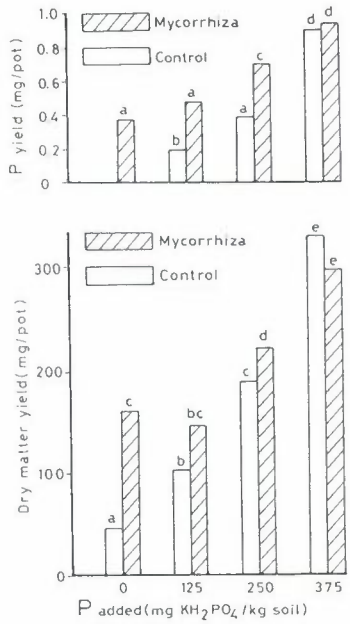

Figure 1. Dry matter and P-biomass yield in the shoots of mycorrhizal and non symbiotic nine-week-old onion plants given increasing amounts of P fertilizers. For each parameter, mean values (five replicates) not sharing a letter in common, differ significantly ($P< 0.05$). Non mycorrhizal, P_0 plants were not analyzed for P because the negligible values of dry matter yield.

N acquisition are particularly important. In this context, data summarized in Table 1 show that, in spite of such similar effects produced by these two treatments, both the concentration and total N content in plant shoots were higher in mycorrhizal plants. The calculation of the relative contribution of the two N sources is recorded in Table 2. These data indicate that the increased N acquisition in VAM plants, at P_0 level, in comparison with that induced by the addition of 250 mg/kg of phosphate to non mycorrhizal plants (P_{250}), can be accounted for by an increased uptake of N from soil sources. The $^{14}N/^{15}N$ ratio was always higher for VAM plants than for non mycorrhizal ones, regardless of the soil P level.

4. Discussion

It is evident that plant roots became mycorrhizal to a degree that was high enough to produce appropriate VAM responses. The levels of phosphate applied did not affect the percentage of the mycorrhizal root length, as was also

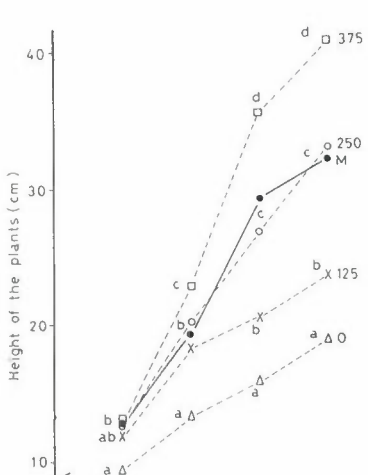

Figure 2. Time-course development of mycorrhizal (M), P_0 or non symbiotic, but P-amended, nine-week-onion plants (the P level, in mg/kg, is indicated). For each recording time, mean values (five replicates) not sharing a letter in common, differ significantly ($P< 0.05$).

Table 1. Analytical data of the nitrogen status in mycorrhizal and non-mycorrhizal nine-week-old onion plants (shoots) given increasing amounts of P fertilizer

P treatment (mg KH_2PO_4/kg soil)	Total N (%)	Total N (mg/plant)	^{15}N atom excess (%)
Non mycorrhizal plants			
0*	–	–	–
125	2.33a	2.45^{a}	0.523^{a}
250	**2.69^{a}**	**4.82^{b}**	**0.501^{a}**
375	3.16^{bc}	10.50^{c}	0.470^{a}
Mycorrhizal plants			
0	**3.60^{c}**	**5.86^{d}**	**0.419^{b}**
125	3.28^{c}	4.66^{b}	0.415^{b}
250	3.16^{bc}	7.05^{d}	0.408^{b}
375	3.05^{bc}	8.85^{c}	0.430^{ab}

For each parameter, mean values (five replicates) not sharing a letter differ significantly ($P< 0.05$), regardless of their mycorrhizal status. Data in bold correspond to matched treatments concerning their effects on plant growth and P status. (*) Non mycorrhizal, P_0 plants were not analyzed for N, because of the negligible values of dry matter yield.

Table 2. Source of nitrogen (isotopic composition) for mycorrhizal and non-mycorrhizal nine-week-old onion plants (shoots) given increasing amounts of P fertilizer

P treatment (mg KH_2PO_4/kg soil)	NdfSoil (mg/plant)	NdfFert (mg/plant)	NdfSoil/ NdfFert (ratio)
Non mycorrhizal plants			
0*	–	–	–
125	1.81^{a}	0.64^{a}	2.85^{a}
250	$\mathbf{3.61^{b}}$	$\mathbf{1.20^{b}}$	$\mathbf{3.00^{a}}$
375	8.03^{c}	2.47^{c}	3.20^{a}
Mycorrhizal plants			
0	$\mathbf{4.67^{d}}$	$\mathbf{1.18^{b}}$	$\mathbf{3.96^{b}}$
125	3.71^{b}	0.96^{ab}	3.87^{b}
250	5.64^{e}	1.41^{d}	4.00^{b}
375	6.95^{f}	1.90^{e}	3.68^{b}

For each parameter, mean values (five replicates) not sharing a letter differ significantly ($P< 0.05$), regardless of their mycorrhizal status. Data in bold correspond to matched treatments concerning their plant growth and P status. (*) Non mycorrhizal, P_0 plants were not analyzed for N, because of the negligible values of dry matter yield.

found for these phosphate levels in a comparable experimental situation (Baath and Spokes, 1989).

The relationships between plant development, biomass production and the nutrient concentration and content can be discussed on the basis of the statements by Jarrel and Beverley (1981). Accordingly, and concerning the P status, it can be concluded that VAM inoculation produced results similar to those provoked by the addition of 250 mg of phosphate per kg of soil to non mycorrhizal plants. However, VAM inoculation still increased the N acquisition over such non mycorrhizal comparable counterparts. In spite of this, and concerning plant growth, P_0 mycorrhizal plants apparently did not benefit from the extra N, related to P_{250} non mycorrhizal plants. However, the increased N accumulation in P_0 mycorrhizal plants, that was obvious at the chosen harvest time, could be beneficial in later stages of plant growth.

The use of ^{15}N allowed us, in addition, to partition the source of N for mycorrhizal and non mycorrhizal plants (Zapata, 1990). The results show that the P treatments did not actually change the $^{14}N/^{15}N$, as could be expected, but this was done by VAM inoculation. The conclusion is that VAM mycorrhiza increased the "apparent soil N pool size," suggesting that mycorrhizal plants

are using available N forms more efficiently, or that they can derive N from sources less available to non mycorrhizal plants. A question is the form of N used by VAM to improve N uptake. Since the experimental soil was acidic, NH_4^+ is probably the predominant N form, which is also the less mobile inorganic N form in soils (Smith and Bowen, 1981). The possibility that VAM acquire N from organic sources, at least by a microbiota-mediated activity, has also been suggested for VAM (Ames et al., 1983), a fact which was described for other types of mycorrhizas (Abuzinadah and Read, 1986; Abuzinadah et al., 1986; Stribley and Read, 1980; Finlay et al., 1992).

In conclusion, it appears that VA mycorrhizas could be involved contributing to an overall tightening of the nitrogen cycle, as it was long before proposed for other types of mycorrhizas (Abuzinadah et al., 1986).

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