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Short Communication

Enhanced N-Transfer from a Soybean to Maize by Vesicular Arbuscular Mycorrhizal (VAM) Fungi[1]

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ABSTRACT

Using a split-root technique, roots of soybean plants were divided between two pots. In one of the two pots, two maize plants were grown and half of those pots were inoculated with the vesicular arbuscular mycorrhizal (VAM) fungus, Glomus fasciculatus. Fifty-.two days after planting, ^{15}N-labeled ammonium sulfate was applied to the pots which contained only soybean roots. Forty-eight hours after application, significantly higher values for atom per cent ^{15}N excess were found in roots and leaves of VAM-infected maize plants as compared with the nonVAM-infected maize plants. Results indicated that VAM fungi did enhance N transfer from one plant to another.

Vesicular arbuscular mycorrhizal fungi are ubiquitous and infect plant roots of most species under a wide variety of soil conditions (8). The fungi form a symbiosis with host plants in which the plant provides carbon for VAM[2] growth and in turn the VAM fungi provide plant nutrients, especially phosphorus, from the soil solution (11). Growth responses of host plants to infection by VAM may be dramatic in nutrient-poor environments (7). Hyphae of mycorrhizae may also spread from one infected plant and enter the roots of one or more other plants (9). It has been shown that assimilates may be transported from one plant to another through VAM hyphal connections. Transfer of ^{14}C photosynthate from one plant to another was primarily through VAM hyphae rather than leakage from the roots of the donor plants (2, 6, 14). Similar results were obtained in a ^{32}P experiment where hyphal linkage between plants was the dominant factor for transferring P (3, 17).

Leguminous plants infected with both *Rhizobium* and VAM showed an increase in nodulation and N_2-fixation as compared with VAM-uninfected legumes (4, 5). The increase in total N has been explained mainly by an increase in N_2-fixation as a result of a higher P uptake through the VAM hyphae rather than increased soil N uptake (15). Although the role of VAM on N uptake and transport has been studied, the results are inconclusive. Rhodes and Gerdemann (15) stated that N translocation by VAM to the host plant would probably be of little significance, while Raven *et al.* (13) attributed a considerable role of VAM to the N nutrition of plants.

Under conditions of low N and P availability which exist in many tropical soils, the possible transfer from the host plant to another plant by VAM may well become important. We exam

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Z **Abbreviation: VAM vesicular arbuscular mycorrhizal.**

fined VAM mediated transfer of N from a soybean to maize using highly labeled (`NH4)ZSOQ and a split-root technique.

MATERIALS AND METHODS

Surface sterilized (2 min in 3% NaOCI seeds of soybeans *(Glycine max* [L.J Men) and maize (Zea *mays* L.) were germinated in vermiculite. The last 0.5 cm of the soybean tap roots were removed and the two seedlings were placed in two separate plastic elbows (pvc elbow, 21 mm o.d., 13 mm diameter hole) (16). At the same time, two pregerminated maize seedlings were planted in pot B (Fig 1).

Half of the B pots were inoculated with a VAM fungi, *Glomus fasciculatus,* maintained in a course sand pot culture with each inoculated pot receiving 30 g of inoculum which contained small

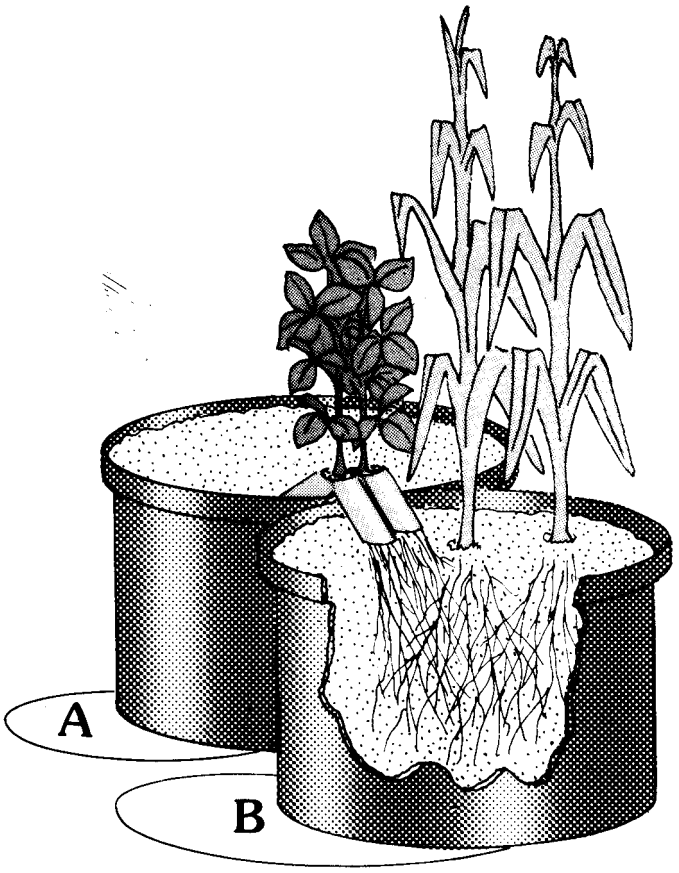

FIG. 1. A split-root system where the roots of the soybean were allowed to grow into two separated pots.

root fragments, hyphae, and approximately 25 spores per gram. The inoculum was spread evenly over the surface of sterile sand which filled one-half of 3-L pots. Sterile sand was added to fill the pots completely after inoculation. Nonmycorrhizal plants were treated with 50 ml suspensions of 30 g of inoculum filtered through Whatman No. 1 filter paper.

A nutrient solution containing 6 g/g of Mg as $MgS0_4$-$7H_20$ 39 g/g K as $KN0_3$, 20 g/g Ca as $Ca(N0_3)2.4H_20$, 1.5 g/g P asKH_2PO_4, and 53 g/g N as $KN0_3$ (14 g/g), $Ca(N0_3)2.4H_20$ (14 g/g), and NH_4N0_3 (25 g/g) was applied five times daily at a rate of 150 ml/plant per application. A liquid micronutrient concentrate (Monterry Chemical Co.) was added at 0.125 ml/L which provided 1.55 g/g Fe, 0.6 g/g Zn, 0.6 g/g Mn, 0.4 g/g B, 0.2 g/g Cu, 0.05 g/g Mo, and 0.04 g/g Co per L. Plants were grown in a greenhouse without additional light; treatments were replicated eight times. Fifty d after planting, pots A and B were leached with deionized H_20 followed by the addition of 0.7 mmol ^{15}N/ pot as 99.99 atom% ^{15}N enriched $(NH4)_2S0_4$ to pot A. A second application of $('SNH_4)_2S0_4$ was added 24 h after the first application. Plants were harvested 48 h after the first ^{15}N application.

Soybeans and maize roots were separated as carefully as possible. Complete separation of the two root masses was not possible and therefore only roots which were still attached to the stem of the soybean or corn plants were analyzed. The rest of the root mass was discarded. Roots were thoroughly washed to remove any contaminating traces of enriched ^{15}N. All maize and soybean roots were checked for VAM infection after staining in 0.05% trypan blue in lactophenol (12). Maize leaves and roots and soybean roots were dried at 70°C until constant weight was obtained. Plants parts were ground separately in a cyclone sample mill. After grinding each sample, the mill was taken apart and thoroughly brushed and vacuum cleaned to avoid any crosscontamination between samples. Tissues were digested and analyzed for total N including NO_2 and $N0_3$ (1). Digestions were made alkaline with 13 N NaOH and steam distilled for 7 min in an all glass steam distilling apparatus. Distillates were collected in 0.02 N H_2S0_4. To avoid cross-contamination, 20 ml of ethyl alcohol was distilled between each sample. Samples were analyzed for total N using the indophenol blue method (8). The rest of the distillate was adjusted to a pH of 4, concentrated and analyzed for ^{15}N. The analyses were carried out at the Isotope Service, Inc. in Los Alamos, NM.

The atom % ^{15}N of VAM-infected and non-VAM-infected plants in pot B, but which had not received "N-labeled $(NH_4)_2S0_4$ in pot A, were used for calculating the atom % ^{15}N excess of VAM-infected and non-VAM-infected maize and soybean plants which had received ^{15}N-labeled $(NH_4)_2S0_4$-

RESULTS AND DISCUSSION

All inoculated roots of maize and soybeans were infected with VAM fungi, whereas none of the noninoculated root systems showed any infection. Table I shows the % N of maize leaves,

Table I. *Per cent N, Dry Weight and Atom per cent ^{15}N Excess of + and − VAM-infected Maize and Soybean Plants*

Plant Origin	Treatment	N	Dry Wt	^{15}N
		%	*g*	*atom % excess*
Maize leaves	−VAM	1.62a[a]	8.94a	0.0013a
	+VAM	1.40a	11.64a	0.0031b
Corn roots	−VAM	1.39a		0.0032a
	+VAM	1.17b		0.0113b
Soybean roots	−VAM	2.17a		0.1966a
(Pot B)	+VAM	2.00a		0.2128a

[a] Means followed by the same letter are not significantly different at the P = 0.05 level by means of the *t* test.

maize roots, and soybean roots which were grown in pot B of VAM-infected and non-VAM-infected plants. VAM-infected plants did show a significantly lower % N for roots as compared with non-VAM-infected maize roots. There was a similar tendency in maize leaves. However, total N in VAM-infected and non-VAM-infected maize leaves were not different because of the higher dryweight of the former. VAM infection may have stimulated growth by increasing the availability of some other factor that limited dry matter accumulation in non-VAM plants.

Leaves and roots of VAM-infected maize plants did show an atom % ^{15}N excess significantly higher at the P = 0.05 level when compared with the leaves and roots of non-VAM-infected maize. This indicates that the VAM fungi has facilitated the transfer of labeled ^{15}N from the soybean to the maize plant. In addition to soil N-derived compounds, N_2-derived compounds may also be transferred from legumes to nonlegumes. The mechanism how this occurred can be by direct, active transport through the mycelia from the donor plant to the receiving plant as has been shown for P (6) and for C (2) as well. However, it also may be possible that VAM-infected plants leak more N compounds out of the roots into the medium than non-VAM-infected plants. Subsequently, more ^{15}N-labeled material will enter the medium and become available for the corn plant.

We have established that a VAM mediated N-transfer from a legume to a nonlegume does occur. Additional experiments are needed before insight can be given about the nature and amount of N transferred from one plant to another through VAM fungi.

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