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Influence of arbuscular mycorrhizal hyphal length on simulation of P influx with the mechanistic model

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The objective of the present investigation was to quantify the total hyphal length of maize and groundnut and its influence on simulation of P influx. The field experiments were carried out with three P levels i.e., P - 0 (no P), P - 50 (50 mg P kg $^{-1}$ soil) and P - 400 (400 mg P kg^{-1} soil) . Test crops were maize and groundnut. Four harvests were made to cover whole growing season and at each harvest hyphal length was measured. Application of P fertilizer reduced hyphal length by 30 ‒ 50% in maize and 25 ‒ 50% in groundnut. At P-0, hyphal length of maize crop was higher (5.4 mg^{-1}) than groundnut (3.8 mg^{-1}). To assess the influence of hyphal length on calculated P influx, mechanistic model was used. The model was employed first to calculate the P influx with root hairs of both the crops and then substituting root hairs by arbuscular mycorrhizal (AM) hyphae. When root hairs were substituted by AM hyphae the calculated influx increased by a factor of 1.5 to 2 for plots receiving no P. Concentration profile around the root cylinder at P - 0 showed that hyphae was able to decrease the soil solution concentration more than the root hairs.

Key words: Maize, Groundnut, Arbuscular mycorrhiza, Hyphae, P influx.

INTRODUCTION

The contribution and potential of mycorrhizal hyphae (Rousseau et al., 1994) on P influx to crops is enhanced in soils with low available P (Tinker et al., 1992 and Jakobson et al., 2001). In plants with well-established infection, each centimeter of root length is associated with 0.5 - 1.5 m of extra-radical hyphae (Harley, 1989). Hence, the status of the extra-radical mycelium development in the soil appears to be a major determinant of the efficiency of arbuscular mycorrhizae (AM) fungi to P uptake. The aim of this experiment was to quantify the arbuscular mycorrhizal hyphal length of maize and groundnut and to assess the influence of AM hyphae on predicted P influx by employing mechanistic nutrient uptake model.

MATERIALS AND METHODS

The hyphal length and its contribution to P uptake were measured with maize and groundnut crop on an Alfisol (with 14 - 16% clay, organic carbon, 0.35% and pH 5.31). Treatment consisted of three fertilizer P levels i.e., P - 0 (no P), P - 50 (50 mg P kg^{-1} soil) and P – 400 (400 mg P kg^{-1} soil) in the form of single super phosphate. Four harvests were made for each crop over the whole growth period and at each harvest, AM hyphal length, soil solution P concentration, shoot yield, shoot P content and root parameters were determined for the calculation of P influx by model. P influx was calculated as

P influx (In): $In = 2(U_2-U_1)/((t_2-t_1)(RL_2+RL_1)$

Where, In = P influx (mol cm^{-1}s^{-1}), U = P uptake (mol m^{-2}), RL = root length (cm m^{-2}), t_1 and t_2 = time (s).

To estimate total hyphal length modified gridline intersect method (Sylvia, 1988) was employed. Soil cores of known volume were collected at random, thoroughly mixed, sub samples (10 g) removed and suspended in water and passed through a sieve with 250 µm openings. The filtrate was blended for 15 s and a portion (25 ml) passed through a membrane with pores < 5 µm diameter. The membrane was briefly flooded with a tryptan blue solution (0.5 g of tryptan blue+500 ml of deionized water+170 ml of Lactic acid+330 ml of glycerine) and rinsed with deionized water. The membrane was cut to fit on a microscope slide and observed at 100X through an eyepiece whipple disc that had a 10 by 10 lined intersect grid, formed by 11 horizontal and 11 vertical lines intercrossed perpendicularly, was incorporated into the lens (×10) of a compound microscope. The hyphae on the membrane filters were viewed at ×100 counting the intersections between the hyphae and the gridlines. Proportions of the hyphae belonging to AM fungi from those

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Table 1. Hyphal length (m g^{-1}) of maize and groundnut at no P (P - 0), 50 mg P kg^{-1} (P - 50) and 400 mg P kg^{-1} (P - 400) application to the soil.

P levels (mg kg^{-1} soil)	Maize				Groundnut			
	DAS*							
	25	47	81	124	30	50	68	112
0	2.42	4.8	5.4	3.82	2.45	3.87	2.68	2.25
50	1.6	3.15	3.68	2.85	1.79	2.83	2.51	2.14
400	1.45	2.27	2.65	1.65	1.3	2.0	1.48	0.98
SEm±	0.13	0.13	0.10	0.11	0.09	0.10	0.10	0.07
LSD (0.05)	0.41	0.39	0.30	0.35	0.27	0.30	0.31	0.22

*DAS: Days after sowing.

of saprophytic fungi in soil were difficult to distinguish. AM hyphae was identified on the basis of its morphological characteristics i.e., coarse aseptate hyphae with distinct angular projections. The hyphae lengths were estimated over 25 fields of view along two transects forming a double-cross on the membrane from four replicate membrane filters for each extraction technique. The total length of hyphae was estimated by using the following equation (Tennant, 1975):

$$R = (\pi \times A \times n)/(2 \times H)$$

Where, R = hyphal length; A = total area in which roots are distributed; n = no of intersections between roots and scribed lines, and H = total length of scribed lines.

The model calculates transport of P towards the root by diffusion and mass flow, taking the sorption of P to the soil matrix in to account (Claassen, 1990). Uptake of P is described by Michealis-Menten kinetics. The model is based on the transport equation of Nye and Mariott (1969) extended by a term A to take uptake by root hairs into account (Claassen and Steingrobe, 1999):

$$b\partial C_{Li} /\partial t = 1/r * \partial/\partial r * (r * D_e *b (\partial C_{Li})/\partial r + r_o * V_o * C_{Li})-A$$

Where C_{Li} (µM cm^{-3}) is the soil solution concentration, b is the Buffer power, r (cm) is the radial distance to the root axis, D_e (cm^2 s^{-1}) is the effective diffusion coefficient, V_o (cm s^{-1}) is the water flux across the root surface and r_0 (cm) is the root radius. To run the model the following soil and plant parameters were either determined or obtained from the literature: Diffusion coefficient in water (D_L, cm^2 s^{-1}), soil volumetric water content (θ, cm cm^{-3}), impedance factor (f), and buffer power (b). Plant parameters are rate of water uptake (ν_o, cm^3 cm^{-2} s^{-1}), maximum influx (I_{max}) (mol cm^{-2} s^{-1}), michaelis constant (K_m, mol cm^{-3}), minimum concentration (C_{Lmin}, mol cm^{-3}), root radius (r_0, cm), average half distance among neighbouring roots (r_1, cm), initial root length (L_0, cm m^{-2}), root growth rate (k, cm m^{-2} d^{-1}) and root hairs.

Modeling with hyphae

External hyphae similar to root hairs that is they spread radialy from the root cylinder into the soil was included in the model considering the uptake kinetics of hyphae and the distribution of the mycelium in soil around roots similar to root hairs. For knowing the distribution of the mycelium in soil around roots the total hyphae length, i.e. cm per cm^{-3} of soil was calculated as in case of roots length. Mycelium generally concentrates more close to the root similar to root hairs. From hyphae and root length per cm^3 of soil, the length of hyphae per cm of root was calculated. Assuming hyphae length per cm of root is highest near the root and decreases steady down to 0 at the distance r_1 (the average distance between neighboring roots), at a given distance how many cm of hyphae from the root was calculated and volume of the compartments as a function of distance from the root was obtained and by dividing hyphae length by the volume, hyphae length per unit volume can be calculated. From this r_{1H}, the half distance among hyphae was calculated and used for model calculations.

Symbols and formulas used for calculation of r and r_{1H} for mycorrhizal hyphae to be included in NST (3.0) model are: Lv, root length density; r_0, root radius; r_1 , half distance among centers of the root=$1/(Lv x \Delta)^{0.5}$; Δr, size of the compartment =(r1-r0)/n; HLv, hyphae per cm^3 soil; H_t, total hyphae length per cm of root=HLv/Lv; n, number of compartments; V_x, volume of compartment = $\Delta r x \Delta(2r0+\Delta(2x-1))$; H_x, cm hyphae in compartment xHx=2Ht/n*(1-(x-0.5)/n); HLv in compartment x=Hx/Vx; $r_{1H}=1/(HLv x \Delta)^{0.5}$.

Statistical analyses were carried out with MSTAT-C, standard statistical software for the comparisons between the treatments means using the F-test, standard error of means and critical differences.

RESULTS AND DISCUSSION

Table 1 shows that there was a significant ($P < 0.05$) influence of phosphorus application on hyphal length for both maize and groundnut crop. Application of P fertilizer reduced hyphal length by 30 – 50% in maize and 25 – 50% in groundnut and may be related to the inhibitory effect of P fertilization on AM-intra and extra radical colonization. Abbot et al. (1985) also reported that high P application reduced the proliferation of the external hyphae. Such a negative effect may be explained by the direct influence that P might have on changing the rhizosphere microbial population in the AM root environment (Kothari et al., 1991; Khaliq and Sanders, 1997). The estimated length of AM hyphae of maize and groundnut crop ranged bet-ween 1 to 5 mg^{-1} air-dried soil and was similar to the find-ings of Abbott et al., (1985) and Sylvia (1988). In general, hyphal length was higher for maize (5.4 mg^{-1}) than groundnut (3.8 mg^{-1}) and may be related to differences in the rate of growth of the fungus through root cortex of the crop species. Hyphal length in maize increased steeply

Table 2. Measured and calculated P influx of maize and groundnut at different harvest intervals at P - 0.

Crop	Harvest Interval (DAS)	Influx (10^{-14}mol cm^{-1} s^{-1})		
		Measured	Calculated	
			+RH*	+Hyphae^
Maize	25 - 47	0.86	0.26	0.41
	47 - 81	5.65	0.21	0.30
	81 - 124	1.56	0.09	0.14
Groundnut	30 - 50	5.49	0.07	0.14
	50 - 68	5.04	0.06	0.09
	68 - 112	2.59	0.05	0.06

+RH*: Calculated influx with root hairs
+Hyphae^: Calculated influx with AM hyphae

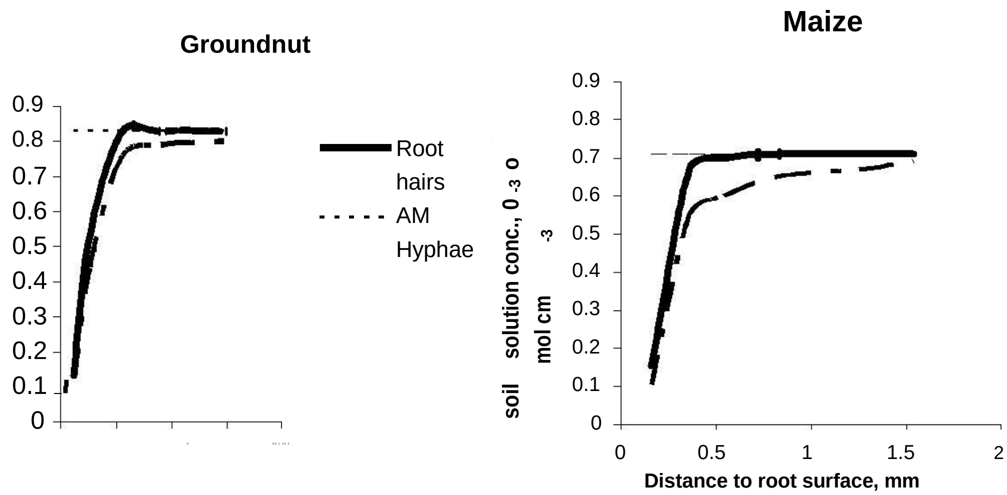

Figure 1. Calculated P concentration profiles around roots of maize and groundnut with root hairs and hyphae at P – 0 at 10 days.

between 25 to 47 days after sowing (DAS) and then moderately up to 81 DAS and thereafter it declined. For groundnut, the trend was more or less similar to that in maize where hyphal length increased up to 50 DAS and there after decreased with plant age. Sutton (1973) described AM formation process to be consisting of three phases: (1) lag phase - during which spore germination, germ tube growth and initial penetration occurs, (2) a rapid growth phase - coinciding with the development of external mycelium, multiple infection and extensive spread of the fungus within the root and (3) a stable phase - during which the proportion of mycorrhizal to non-mycorrhizal roots remains approximately constant. Bethlenfalvay and Linderman (1992) also found a peak in the quantity of external hyphae at 70 DAS, which thereafter declined in a 130 days study. In a pot culture experiment, Sylvia (1988) also reported that the range of external hyphae length (4.4 - 7.2 mg^{-1}) in 40 DAS reduced to a range of 4.1 - 5.4 mg^{-1} in 80 DAS.

Calculations on P influx were carried out first with root hairs and thereafter substituting root hairs by hyphal length. The measured influx in maize was nearly three to twenty-five times higher than the calculated influx even after inclusion of root hairs (Table 2). For groundnut, the measured influx was nearly fifty times higher than the predicted influx at all harvest intervals. When root hairs were substituted by hyphae, the calculated influx improved and increased by a factor of 1.5 to 2 for plots receiving no P.

Concentration profile around the root cylinder of maize calculated by nutrient uptake model shows that after tenth day the concentration at the root surface dropped from 0.71 to a concentration of 0.11 μM with hyphae and to a concentration of 0.15 μM with root hairs. In case of groundnut, the original soil P concentration of 0.83 μM got decreased to 0.10 μM with hyphae and 0.13 μM with root hairs (Figure 1). The results indicate that hyphae were able to decrease the solution concentration at root surface more than root hairs. Li et al. (1991) have shown that the presence of hyphae exceeds the phosphate depletion zone well beyond the roots.

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