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Measurement of Arbuscular Mycorrhizal Hyphal Length and Prediction of P Influx by a Mechanistic Model

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Abstract: When P is deficient in soil solution, the critical root parameter controlling its uptake is its surface area. Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of the root. Modified gridline intersect method was employed for extracting hyphae from the soil with the following 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) as single super phosphate. Test crop was maize. Four harvests were made to cover whole growing season and at each harvest, hyphal length was determined. To assess the importance of mycorrhizal hyphae, nutrient uptake model (NST3.0 Version) was used. P 0 recorded maximum hyphal length and it was significantly higher as compared to other P application levels. When root hairs are included in the model, the measured influx on an average was nearly fifteen times higher than the predicted influx at all the harvests interval. The predicted influx further increased by a factor of 1.5 to 2 when root hairs are substituted by hyphal length. Concentration profile around the root cylinder at P 0, which was calculated by the model shows that hyphae was able to decrease the solution concentration at root surface more than root hairs. The results shows that mycorrhizal hyphae may make a significant contribution to P influx, but other factors like P solubilization by root exudates may be even more important.

Key words: Maize · influx · arbuscular mycorrhiza · root exudates · nutrient uptake model

INTRODUCTION

Hyphae of Arbuscular Mycorrhizal (AM) fungi have the potential to greatly increase the absorbing surface area of the root besides root hairs when phosphorus is deficient in soil solution [1, 2]. Mycorrhizal roots due to their extramatrical hyphae that are capable of absorbing and translocating nutrients, can explore more soil volume than the non-mycorrhizal roots and thus increase the supply of slowly diffusing ions, such as phosphate to the plant [3]. The extra-radical hyphae formed by AM fungi extend from the roots (from 27 mm to 70 mm) into the surrounding soil beyond the P depletion zone, which generally develops around plant roots because P uptake by the plant is normally faster than P diffusion towards the roots [4]. Most studies to investigate the significance of AM hyphae for P uptake has been done in pots with sterilized and AM-re inoculated soil. Few studies regarding measurement of hyphal length in the soil have been carried out under field condition. Hence, the status of the extra-radical mycelium development in the field soil appears to be a major determinant of the efficiency of AM fungi to P uptake. The aim of this experiment was to quantify the native arbuscular mycorrhizal hyphal length by modified gridline intersect method [5, 6] of maize crop grown under field condition and to assess the importance of mycorrhizal hyphae for predicting P influx using a mechanistic model [7].

MATERIALS AND METHODS

We measured the hyphal length and its contribution to P uptake with maize crop on soil (with 14-16 % clay, organic carbon, 0.35 % and pH (H_2O) 5.31.) with the following three levels:P 0, P 50 and P 400 mg kg^{-1} soil as single super phosphate. The total hyphae lengths were estimated by following method.

AM hyphal length: To estimate total hyphae length modified gridline intersect method [5] 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 is blended for 15 s and a

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portion (25 mL) passed through a membrane with pores <5 μm diam. The membrane is briefly flooded with a tryptan blue solution (0.5 g of tryptan blue + 500 mL of deionised water + 170 mL of Lactic acid + 330 mL of glycerine) and rinsed with deionised water. The membrane cut to fit on a microscope slide and observed at 100 X through an eyepiece whipple disc that has a 10 by 10 lined intersect grid. The total length of the hyphae was estimated by gridline intersect method [8] using the following equation:

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

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

Hyphae were counted only if showing characterstics described as typical for AM fungal hypahe, such as dichotomous branching, abundant angular projections and absence of septa [9].

Description of the model: The model calculates transport of P towards the root by diffusion and mass flow, taking the sorption of P to the soil matrix into account [10]. Uptake of P is described by a Michealis- Menten kinetics. The model is based on the transport equation of Nye and Mariot [11] extended by a term A to take uptake by root hairs into account [7]:

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

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. The sink term A for root hair uptake uses a steady-state approach that allows the use of Michealis-Menten kinetics also for uptake by root hairs [7].

External hyphae similar to root hairs i.e. they spread radialy from the root cylinder into the soil have been included in the model with no chemical mobilization calculations considering two aspects: 1) The uptake kinetics of hyphae and 2) the distribution of the mycelium in soil around roots. It is rerasonable to assume it to be of Michaelis-Menten type and Km and Imax are similar to those of plants. The P influx of AM hyphae was $4x10^{-16}$ $molcm^{-1}s^{-1}$, assuming a radius of $5x10^{-4}$ cm gives an influx of $1.2x10^{-13}$ $molcm^{-2}$ s^{-1}. 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. 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:

Lv: Root length density
r_0: Root radius
r_1: Half distance among centers of the root=$1/(Lv x \pi)^{0.5}$
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 =$\Delta r x \pi(2r0+\Delta(2x-1))$
H_x: cm hyphae in compartment xHx=2Ht/n*(1-(x-0.5)/n)
HLv: In compartment x=Hx/Vx
$r_{1H}=1/(HLv x \pi)^{0.5}$

Different plant parameters were determined as follows:
Root length:

$$RL = \frac{11}{14} \times N \times G$$

Where,

RL = Root length;
N = Sum of horizontal and vertical crossings;
G = Length of the grid unit (1 cm, in this case);

P uptake:

U = W x shoot P / 3100

Wwhere,

U = P uptake (mol m^{-2}),
W = Shoot dry weight (g m^{-2}),
shoot P = P concentration of shoot (%)

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 = Time (seconds)

subscripts 1 and 2 refers to current and previous harvests

RESULTS AND DISCUSSION

The estimated length of AM hyphae in rhizosphere soil ranged between 1 to 5 m g^{-1} air-dried soil and was similar to the findings of Abbott *et al.* [12] and Sylvia [5]. Miller *et al.* [13] have reported higher values of hyphal length of which a small proportion may belong to AM. As can be seen from the data presented in Table 1 that there was significant influence of phosphorus application on hyphal length. Application of P fertilizer reduced the soil hyphal length by 30-50% compared to P 0. Abbot *et al.* [12] also found that high P application could reduce proliferation of the external hyphae.

Hyphal length of maize increased at a slow rate during the initial establishment phase of AM infection and then rapidly increased, reaching a peak value of about 5.4 m g^{-1} at 81 days after sowing and thereafter decreased. For the same corresponding time period fungi of the native AM community colonized from 8 to 51%. Bethlenfavay *et al.* [14] also found that the quantity of external hyphae was highest at 70 DAS which thereafter declined in a 130 days study. In a pot culture experiment Sylvia [5] also reported that the range of external hyphae length was 4.4-7.2 m g^{-1} in 40 DAS and which was reduced to a range of 4.1-5.4 m g^{-1} in 80 DAS.

The different soil, plant and hyphal parameters used to evaluate nutrient uptake model for simulating P influx was given in table 2.Calculations on P influx were carried out first with root hairs and thereafter-substituting root hairs by hyphal length because the hyphae spread radialy from root cylinder into the soil.

The measured influx in maize was nearly three (between 1st-2nd interval) to twenty-five times (between 2nd–3rd and 3rd-4th interval) than the calculated influx (Table 3). When root hairs were substituted by hyphae,

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

	Days after Sowing (DAS)			
P levels (mg kg^{-1} soil)	25 DAS	47 DAS	81 DAS	124 DAS
0	2.42	4.80	5.40	3.82
50	1.60	3.15	3.68	2.85
400	1.45	2.27	2.65	1.65
SEm±	0.13	0.13	0.10	0.11
LSD(0.05)	0.41	0.39	0.30	0.35

DAS, Days After Sowing

Table 2: Plant and soil parameters of maize used for nutrient uptake model calculations at P 0 and different harvest interval

	P 0		
Plant parameters	I-II	II-III	III-IV
L_0 (cm m^{-2})	7820	85800	325000
r_0 (10^{-2} cm)	0.0156	0.0143	0.0136
r_1 (10^{-2} cm)	0.25	0.25	0.25
k (cm $m^{-2}d^{-1}$)	3546	7026	-446
v_0 (10^{-7} cm)	$7.5x10^{-7}$	$7.5x10^{-7}$	$7.5x10^{-7}$
Imax (µmol $cm^{-2}s^{-1}$ 10^{-6})	$2.3x10^{-6}$	$2.22x10^{-6}$	$0.746x10^{-6}$
Imax for root hairs	$1.82x10^{-6}$	$1.76x10^{-6}$	$0.593x10^{-6}$
Km (10^{-3} µmol cm^{-3})	$2.4x10^{-3}$	$2.4x10^{-3}$	$2.4x10^{-3}$
Cmin (10^{-3} µmol cm^{-3})	$0.2x10^{-3}$	$0.2x10^{-3}$	$0.2x10^{-3}$
n	40	40	40
Soil parameters			
C_{li}(10^{-3} µmol cm^{-3})	$1.01x10^{-3}$	$0.65x10^{-3}$	$0.65x10^{-3}$
De (10^{-5} cm^2 s^{-1})	$8.9x10^{-6}$	$8.9x10^{-6}$	$8.9x10^{-6}$
b	391	465	595
f	0.18	0.18	0.18
a	1	1	1
c	0	0	0
θ	0.22	0.22	0.22
t(d)	22	34	43

(L_0, Initial root length; r_0, Root radius; r_1, Average half distance among neighbouring roots; k, Root growth rate; v_o, Rate of water uptake; I_{max} Maximum influx; K_m, Michaelis constant; C_{min} Minimum concentration; n, Number of compartments; C_{li}, Soil solution P concentration; De, Diffusion coefficient; b, Buffer power; f, Impedance factor; a & c, are constants; θ_, Soil volumetric water content; t(d), Time interval)

Table 3: Measured and calculated P influx of maize at different harvest intervals

	Influx (10^{-14}mol cm^{-1} s^{-1})		
		Calculated P 0	
Harvest interval	Measured P 0	+RH*	+Hyphae^
1st-2nd 0.86	0.26	0.41	
2nd-3rd 5.65	0.21	0.30	
3rd-4th 1.56	0.09	0.14	

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

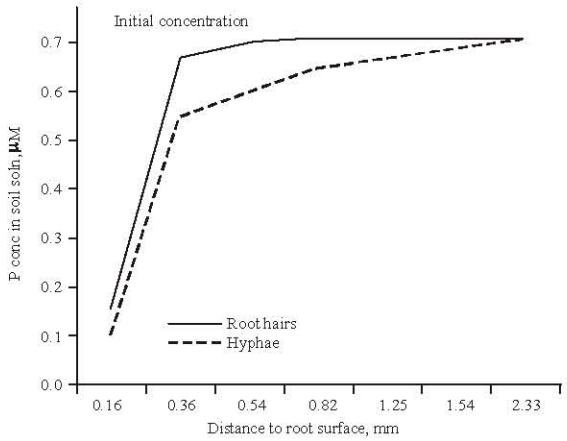

Fig. 1: Calculated P concentration profiles around roots of maize with root hairs and hyphae at P 0 at 10 days

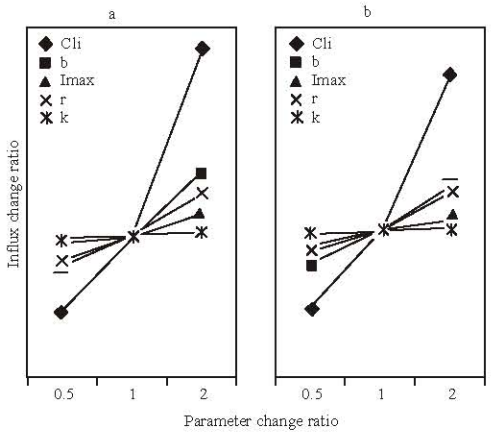

Fig. 2: Sensitivity analysis of P influx of maize at P 0 during (a) 1st-2nd and (b) 2nd-3rd harvest interval

the calculated influx increased by a factor of 1.5 to 2 for plots receiving no P. In the early growing season (first harvest interval) at low P supply (C_{Li} = 0.67 µM), the effect of mycorrhizae was rather large. At later growth stages the contribution of mycorrhiza to P influx showed a decrease. Solubilization of P by the root exudates might have played more important role than AM in the uptake of P in the later part of the growth stages. Concentration profile around the root cylinder of maize calculated by nutrient uptake model shows that after tenth day the concentration at the root surface of maize dropped from 0.71 to 0.11 µM with hypahe and to 0.15 µM with root hairs for maize at a distance of 1.6 mm (Fig. 1). The results indicate that hyphae is able to decrease the solution concentration at root surface more than root hairs. This emphasises the importance of native AM fungal hyphae in P uptake, particularly at low P supply at early growing season when root functions mainly as an absorbing organ i.e., as sink for P and in that case transport to the root is determined by the concentration in soil solution (Fig. 2a, b). Later, the root actively participates in P dynamics in the rhizosphere by excreting root exudates that influenced P solubility in soil.

It is evident from the present investigation that AM fungi may make a significant contribution to P influx of maize crop, but other factors like P solubilization by root exudates may be even more important.

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