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Influence of Vesicular Arbuscular Mycorrhizal Fungi and Applied Phosphorus on Root Colonization in Wheat and Plant Nutrient Dynamics in a Phosphorus-Deficient Acid Alfisol of Western Himalayas

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*Vesicular arbuscular mycorrhizal (VAM) fungi symbiosis confers benefits directly to the host plant's growth and yield through acquisition of phosphorus and other macro- and micronutrients, especially from phosphorus (P)–deficient acidic soils. The inoculation of three VAM cultures [viz., local culture (*Glomus mosseae*), VAM culture from Indian Agricultural Research Institute (IARI), New Delhi (*Glomus mosseae*), and a culture from the Centre for Mycorrhizal Research, Energy Research Institute (TERI), New Delhi (*Glomus intraradices*)] along with P fertilization in wheat in a P-deficient acidic alfisol improved the root colonization by 16–24% while grain and straw yields increased by 12.6–15.7% and 13.4–15.4%, respectively, over the control. Uptake of nitrogen (N), P, potassium (K), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) was also improved with VAM inoculation over control, but the magnitude of uptake was significantly greater only in the cases of P, Fe, Zn, and Cu. Inoculation of wheat with three VAM cultures in combination with increasing inorganic P application from 50% to 75% of the recommended* P_2O_5 *dose to wheat through the targeted yield concept following the soil-test crop response (STCR) precision model resulted in consistent and significant improvement in grain and straw yield, macronutrient (NPK) uptake, and micronutrient (Fe, Mn, Zn, Cu) uptake in wheat though root colonization did not improve at* P_2O_5 *doses beyond 50% of the recommended dose. The VAM cultures alone or in combination with increasing P levels from 50% to 75%* P_2O_5 *dose resulted in reduction of diethylenetriaminepentaacetic acid (DTPA)–extractable micronutrient (Fe, Mn, Zn, Cu) contents in P-deficient acidic soil over the control and initial fertility status, although micronutrient contents were relatively greater in VAM-supplied plots alone or in combination with 50% to 75%* P_2O_5 *dose over sole application of 100%* P_2O_5 *dose, thereby indicating the positive role of VAM in nutrient mobilization and nutrient dynamics in the soil–plant system. There was significant improvement in available N and P status in soil with VAM inoculation coupled with increasing P levels upto 75%* P_2O_5 *dose, although the greatest P buildup was obtained with sole application of 100%* P_2O_5 *dose. The TERI VAM culture (*Glomus intraradices*) showed its superiority*

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*over the other two cultures (*Glomus mosseae*) in terms of crop yield and nutrient uptake in wheat though the differences were nonsignificant among the VAM cultures alone or at each P level. Overall, it was inferred that use of VA-mycorrhizal fungi is beneficial under low soil P or in low input (nutrient)–intensive agroecosystems.*

Keywords *Glomus mosseae*, *Glomus intraradices*, micronutrient, nutrient dynamics, phosphorus, vesicular arbuscular mycorrhizae

Introduction

Phosphorus (P) is an important plant nutrient, which constitutes 0.2% of a plant's dry weight (Schactman, Reid, and Ayling 1998). Low availability of phosphorus (P) in soils limits plant uptake. The vesicular arbuscular mycorrhizal (VAM) fungi symbiosis confers benefits directly to the host plant growth and development through the acquisition of phosphate and other mineral nutrients from the soil by the fungal hyphae (Harrier and Watson 2003). A number of field studies carried out in various parts of the world, tropical as well as temperate regions, have shown significant beneficial effects of VAM fungi inoculation in maize, wheat, soybean, cotton, barley, oats, white clover, lucerne, cowpea, potato, peach, and citrus (Carling, Roncadori, and Hussay 1989; Vejsadava, Drinkryl, and Vancura 1990; Karagiannidis and Hadjisavva 1998; Singh and Kapoor 1999; Kelly et al. 2001). These effects are mainly attributed to the fact that the VAM hyphal network in soil matrix increases the mobilization and absorption of plant nutrients from the rhizosphere and outside the rhizosphere in the soil system (Harrier and Watson 2003), allowing an increase in the uptake of macronutrients (especially P) and micronutrients by the crops (Liu, Shen, and Qiu 1994; Bagayoko et al. 2000; Khan, Ahmad, and Ayub 2003). Quite high crop response and nutrient uptake by VAM are reported from P-deficient soils (Solaiman and Hirata 1997; Liu, Zhang, and Liao 2001). The soils of wet temperate Himalayas are acidic in nature and have high P-fixing power because of excessive presence of iron (Fe) and aluminum (Al) ions, resulting in low P availability for crop production (Sharma, Verma, and Bhumbla 1980). There is very limited information on the influence of VAM fungi with or without P in wheat on crop productivity and macro- and micronutrient dynamics in plants and P-deficient Himalayan acidic Alfisols. Wheat is grown under rainfed conditions in the Himalayan region. Since the VAM inoculated plants can withstand the water stress conditions (Harrier and Watson 2003), in the present study, we have investigated the influence of VAM fungi and applied P on crop productivity as well as macro- and micronutrient uptake in rainfed wheat and their availability in P-deficient acidic alfisols in the western Himalayas.

Materials and Methods

Field studies were conducted with wheat (winter season 2001–2002) in a P-deficient acidic silty clay loam soil (Table 1) at Palampur, India (32° 6′ N latitude and 76° 3′ E longitude, 1291 m above mean sea level). The experimental design employed was a randomized block design replicated three times. Treatments were absolute control, farmers' practice [nitrogen (N) at 30 kg ha^{-1}], and three VAM fungi cultures: VAM_L (local VAM culture *Glomus mosseae*, developed by CSK Himachal Pradesh Agricultural University, Palampur, India), VAM_T [VAM culture *Glomus intraradices* AM 1004, developed by the Centre for Mycorrhizal Research, Energy Research Institute (TERI), New Delhi, India], and VAM_I [VAM culture *Glomus mosseae*, developed by the Division of Microbiology,

Table 1
Properties and location of experimental site

Location	Western Himalayas of India (at Palampur)
Type	Acid Alfisol (Typic Hapludalf)
Sand (%)	20
Silt (%)	45
Clay (%)	34
pH (1:2.5 soil/water)	5.2
Organic carbon (g kg^{-1} soil)	3.5
Available N [$KMnO_4$-N (kg ha^{-1})]	110
Available P [Olsen P (kg ha^{-1})]	5
Available K [NH_4Ac-K (kg ha^{-1})]	278
DTPA-exchangeable micronutrients (ppm)	
Fe	12.00
Mn	14.40
Zn	0.41
Cu	1.52

Notes. The methods used were as follows: sand, silt, and clay by pipette (Piper 1966); organic C by the method of Walkley and Black (1934); available N by the method of Subbiah and Asija (1956); available P by the method of Olsen et al. (1954); available K by the method of AOAC (1970); and micronutrients by the DTPA method (Lindsay and Norvell 1978).

Indian Agricultural Research Institute (IARI), New Delhi, India], alone or with 50% and 75% of recommended phosphorus pentoxide (P_2O_5) dose to wheat based on targeted yield concept following the soil-test crop response (STCR) precision model (Ramamoorthy, Narasimhan, and Dinesh 1967) as well as one treatment with sole application of 100% of recommended P_2O_5 dose based on the STCR model. One hundred percent of recommended nitrogen (N) and potassium (K) fertilizers were added in 50%, 75%, and 100% of recommended P_2O_5 treatments based on the STCR model. Nitrogen, P, and K were supplied through urea (46% N), single superphosphate (16% P_2O_5), and muriate of potash (60% K_2O), respectively. Fertilizer N, P, and K were calculated per yield target of wheat following the STCR precision model using these fertilizer adjustment equations:

$$FN = 4.91\ T - 0.12\ SN$$

$$FP_2O_5 = 7.86\ T - 5.16\ SP$$

$$FK_2O = 2.44\ T - 0.187\ SK$$

where FN, FP_2O_5, and FK_2O stand for fertilizer N, P_2O_5, and K_2O in kg ha^{-1}, respectively, while T is the target grain yield of 30 q ha^{-1}. The SN, SP, and SK are available N, P, and K contents of soil in their elemental form in kg ha^{-1}. Wheat (cv. HPW-89) was sown on 20 December 2001 in 2-m^2 plots and harvested on 21 May 2002. Rainfall during the crop season was 178.6 mm, while temperature ranged between 3.9 and 29.6 °C. Plant analysis (grain and straw) was done by the standard procedures of Jackson (1967).

VAM Inoculation

Soil mixed with VAM cultures having VAM spores and fungal hyphae were used in the study. In the 3 VAM cultures (local, TERI, and IARI cultures), the spore counts were 100, 500, and 400 per 250 g air dry soil, respectively. The VAM cultures were used at 12 kg ha^{-1}. These VAM cultures were used on a spore equivalent basis taking the TERI VAM culture into consideration while using the VAM cultures. Local VAM culture was prepared by the investigating scientists (authors) themselves by raising the target crop (wheat) to maturity in pots containing 7 kg sterilized soil + 2 kg FYM and 1 kg mother culture. After harvest, the rhizosphere soil of pot as well as root biomass constituted the local VAM culture. The actual inoculation of wheat seeds with these cultures was performed by preparing a soil slurry of cultures and dipping the seeds into it for half hour followed by shade drying for making seed pallets and then sowing in the field.

Root Colonization Studies

The plants along with roots and rhizosphere soil were removed carefully from sample rows in the field plots. Then roots were washed thoroughly under running tap water. The cleaned roots were then chopped into small pieces (1 cm) and subjected to fixation, cleaning, rinsing, and bleaching in potassium hydroxide (KOH) solution following standard techniques for microscopic observations (Rajapaske and Miller 1992). In case the VAM propagules established the infection, the infected portion in rhizosphere showed the presence of VAM fructifications. On the basis of root cuttings infected and uninfected, the percentage of infectivity was worked out.

Statistical Analysis

The experimental design was a randomized block design, and the statistical analysis was done by the standard procedures suggested by Gomez and Gomez (1984).

Results and Discussion

Root Colonization by VAM Fungi

VAM fungi–inoculated roots showed root infectivity over uninnoculated plants (Figure 1). From an infectivity standpoint, both TERI and IARI VAM cultures were more efficient than local VAM culture. The inoculation of wheat with VAM cultures coupled with P fertilizer application also stimulated root infectivity in wheat roots. It was also observed that with either of three VAM cultures, the rate of increase of root colonization was greater with application of the 50% P_2O_5 dose compared to VAM cultures alone, but further increase in applied P to 75% P_2O_5 dose lowered the rate of root infectivity in local VAM culture (*Glomus mosseae*). Root infectivity did not improve in TERI (*Glomus intraradices*) and IARI (*Glomus mosseae*) VAM cultures with the application of 75% P_2O_5 compared to 50% P_2O_5 dose. It may be attributed to the fact that high soil P concentration due to high applied P doses reduced the mycorrhizal colonization in the roots (Bolan 1991; Marschner and Dell 1994), probably because of parasitic behavior of VAM fungi at high P concentrations. Therefore, the benefits of VAM fungi colonization are often observed in soils with low nutrient status, especially P (Harrier and Watson 2003).

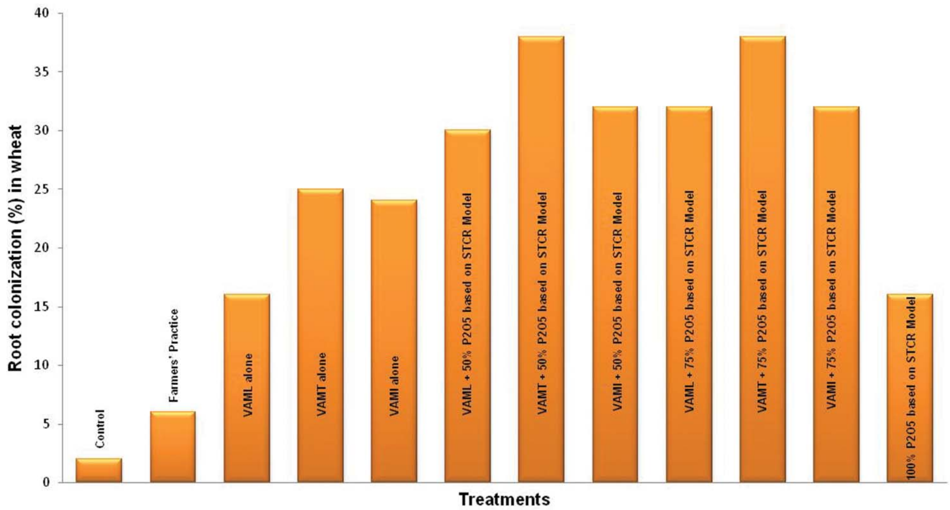

Figure 1. Effect of VA-mycorrhizal fungi and applied P on root colonization (%) in wheat at the flowering stage (color figure available online).

Crop Yields

Sole application of either of the three VAM cultures (VAM_L, VAM_T, and VAM_I) increased the grain and straw yields of wheat by 12.6–15.7% and 13.4–15.4% over the control, thus indicating that VAM fungi helps in improving crop yields, probably due to mobilization and uptake of plant nutrients by the crop (Harrier and Watson 2003; Karagiannidis and Hadjisavva 1998). Application of either of the three VAM fungi along with increasing fertilizer P doses from 50% to 75% of recommended P_2O_5 dose resulted in consistent and significant improvements in grain and straw yield of wheat, though the yield magnitude with any of the three VAM cultures was at par with each other at each P level with superiority of TERI culture (*Glomus intraradices*) over the other two cultures. The greatest grain and straw yields were observed with the sole application of 100% P_2O_5 dose, though the yield increase with this 25% greater P_2O_5 dose was not so dramatic as 75% P_2O_5 dose coupled with VAM cultures. Besides this, all the three VAM cultures along with 75% P_2O_5 produced acceptable targeted crop yields although less than the sole 100% P_2O_5 dose. Overall, it was inferred that VAM fungi can play an important role in P fertilizer economy under low soil P or low applied P conditions, which may be attributed to VAM symbiosis effects (Harrier and Watson 2003) and inorganic P fertilization as well (Suri and Puri 1997).

Nutrient Dynamics

Results in Table 2 reveal that sole application of either of the three VAM cultures resulted in an improvement in NPK and micronutrient uptake [Fe, manganese (Mn), zinc (Zn), copper (Cu)] over the control, though magnitude of the uptake was significantly greater only in cases of P, Fe, Zn, and Cu over control (Liu, Shen, and Qiu 1994). Inoculation of wheat with any of the three VAM cultures along with increasing P levels from 50% to 75% of recommended P_2O_5 dose resulted in consistent and significant improvement in N, P, K, Fe, Mn, Zn, and Cu uptake in wheat crop. This probably may be attributed to mobilization and

Table 2
Effect of VA-mycorrhizal fungi cultures and applied P on grain and straw yields as well as nutrient uptake in rainfed wheat (winter season 2001–2002)

Treatments	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})	Total NPK uptake (kg ha^{-1})			Total micronutrient uptake (g ha^{-1})			
			N	P	K	Fe	Mn	Zn	Cu
Control	11.52	16.11	22.8	3.9	11.5	367.3	43.3	75.8	67.9
Farmers' practice	15.14	20.89	30.7	5.4	15.5	488.0	60.3	100.9	90.9
VAM_L alone	12.97	18.59	26.0	5.0	13.5	436.4	44.6	87.9	81.6
VAM_T alone	13.33	18.37	26.3	5.1	13.8	438.9	55.3	90.0	82.8
VAM_I alone	13.19	18.27	26.3	5.1	13.3	435.1	54.7	88.4	82.1
VAM_L + 50% P_2O_5 based on STCR model	22.58	31.22	49.5	9.8	25.1	757.2	97.1	168.7	147.5
VAM_T + 50% P_2O_5 based on STCR model	23.75	32.70	51.9	11.0	26.5	795.8	102.1	177.9	155.0
VAM_I + 50% P_2O_5 based on STCR model	23.74	31.42	50.7	10.5	25.5	770.7	99.3	171.4	150.9
VAM_L + 75% P_2O_5 based on STCR model	30.01	39.82	64.6	14.6	34.7	991.9	130.8	233.1	193.6
VAM_T + 75% P_2O_5 based on STCR model	31.04	40.87	66.8	15.6	36.3	1021.9	136.7	245.6	199.4
VAM_I + 75% P_2O_5 based on STCR model	29.94	40.51	64.5	14.8	35.0	1005.4	132.1	239.8	195.1
100% P_2O_5 based on STCR model	34.85	45.50	76.8	18.1	41.7	1157.9	159.4	294.4	226.3
CD ($P = 0.05$)	2.83	2.68	4.4	1.1	2.3	54.3	11.5	11.8	10.8

absorption of nutrients as well as greater crop yields of wheat, which in turn raised plant nutrient uptake of respective nutrients. Overall VAM_T (*Glomus intraradices*) was superior in terms of primary and micronutrient uptake though it remained at par with other two VAM cultures at each P level. The significantly greatest magnitude of nutrient uptake was observed at the 100% P_2O_5 application rate.

It is interesting to note that at no fertilization (P_0), VAM cultures have more apparent results in terms of N, P, K, Fe, Mn, Zn, and Cu uptake, but at greater P rates, the role of VAM fungi does not seem so contrasting. However, it does not mean that VAM fungi do not have any role in plant nutrient dynamics at greater P levels. It has been noted that application of any of the three VAM cultures alone as well as with increasing P levels resulted in reduction in diethylenetriaminepentaacetic acid (DTPA)–extractable micronutrient contents (Fe, Mn, Zn, Cu) in the soil over control and initial soil fertility status. On the other hand, the micronutrient status in VAM-supplied plots irrespective of the P application rates remained greater than sole application of 100% P_2O_5 dose, indicating that VAM has some role in nutrient absorption and uptake as well as its mobilization from unavailable or insoluble soil complexes.

There was consistent and significant improvement in soil N and P status with increasing P levels from no P application to 50% and 75% of recommended P_2O_5 dose along with VAM culture inoculations, but the differences among different VAM cultures were nonsignificant at each P level with respect to soil N and P content. The VAM cultures along with 50% and 75% P_2O_5 doses resulted in greater soil-available N buildup than the 100% P_2O_5 dose. Though the recommended N dose was applied in 50%, 75%, and 100% P_2O_5-supplied plots, even then 50–75% P application coupled with VAM inoculation surpassed the 100% P dose in soil N buildup, which indicates that VAM fungi have some role in N dynamics (i.e., mobilization and absorption) in soil–plant systems (Pare, Gregorich, and Nelson 1999). The sole application of three VAM cultures improved the P uptake in wheat (Table 2) but reduced the soil-available P status over the control (Table 3). Maximum available P contents were observed with the application of 100% P dose over 50% to 75% P application along with VAM cultures, which indicates that at low P soil status the VAM has a pronounced role in P dynamics but at greater P levels it did not exhibit contrasting results in terms of soil P buildup (Harrier and Watson 2003). The VAM inoculation with or without P fertilization did not affect the soil-available K status and soil pH, and the results were nonsignificant statistically. Overall application of VAM fungi cultures with or without P fertilization improved the plant uptake of N, P, K, Fe, Mn, Zn, and Cu and also exhibited contrasting effects on nutrient dynamics in the soil–plant system.

Conclusions

From this study, it can be summarized that VAM fungi at low soil P levels improved the root colonization, crop yields, macro- and micronutrient uptake, as well as nutrient dynamics in the soil–plant system. However, at greater P fertilization, VAM fungi had contemporary effect on root colonization though nutrient uptake (N, P, K, Fe, Mn, Zn, and Cu), and nutrient dynamics were significantly improved. The VAM fungi also had positive effect on crop productivity with or without applied P under the P-deficient acidic alfisol of the western Himalayas. Overall, it can be inferred that use of VAM fungi is beneficial under low soil P or in low applied-P farming situations.

Table 3
Effect of VA-mycorrhizal fungi cultures and applied P on soil nutrient status after harvest of wheat (winter season 2001–2002)

		Soil-available NPK ($kg\ ha^{-1}$)			DTPA-exchangeable micronutrients (ppm)			
Treatments	Soil pH	N	P_2O_5	K_2O	Fe	Mn	Zn	Cu
Control	5.2	89.7	4.18	270.4	11.59	14.28	0.39	1.48
Farmers' practice	5.2	98.5	4.17	268.7	11.28	14.20	0.38	1.48
VAM_L alone	5.2	87.7	4.10	268.7	11.13	14.14	0.35	1.46
VAM_T alone	5.2	85.4	4.07	267.3	10.98	14.12	0.34	1.45
VAM_I alone	5.2	86.8	4.06	268.5	10.95	14.15	0.35	1.46
VAM_L + 50% P_2O_5 based on STCR model	5.2	119.2	6.62	267.9	10.70	14.08	0.33	1.43
VAM_T + 50% P_2O_5 based on STCR model	5.3	115.1	6.04	264.5	10.65	14.16	0.32	1.43
VAM_I + 50% P_2O_5 based on STCR model	5.2	118.2	6.34	265.2	10.69	14.08	0.32	1.43
VAM_L + 75% P_2O_5 based on STCR model	5.3	113.7	7.22	269.2	10.33	13.76	0.31	1.42
VAM_T + 75% P_2O_5 based on STCR model	5.3	111.1	6.47	269.2	9.69	13.13	0.30	1.42
VAM_I + 75% P_2O_5 based on STCR model	5.3	113.7	6.49	269.1	9.99	13.18	0.32	1.42
100% P_2O_5 based on STCR model	5.3	109.7	8.07	268.1	8.95	12.73	0.30	1.40
CD ($P = 0.05$)	NS	3.9	0.13	NS	0.74	0.34	0.04	0.04
Initial status	5.2	110.0	5.0	278.0	12.00	14.40	0.41	1.52

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