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AMF associated with minor millet crops in rainfed areas : Plant growth Performance and Mycorrhizal dependency

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The mutual relationship between the plant and mycorrhizal fungi has opened a new area of research in the interest of plant health in adverse soil conditions. An investigation was carried out to record AMF diversity in the rhizosphere of different minor millets viz. *Eleusine coracana, Panicum miliaceum* and *Paspalum scrobiculatum* growing in the rainfed areas of Bihar and Jharkhand states. Pot experiments were conducted in order to evaluate the inoculation effect of mycorrhiza on plant growth performance at four different levels of phosphorus in soil.

Key words : Mycorrhiza, minor millets, mycorrhizal dependency

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are a group of fungi, those establish symbiotic association with majority of vascular plants by penetrating their roots and form characteristic intracellular structures 'arbuscules'. These fungi help the plants in uptaking of diffusion-limited nutrients, such as phosphorus, copper and zinc, consequently enhancing the plant growth and yield, in turn, takes carbohydrates from the root cortex. Thus, survival and better growth performance of many plants, particularly in nutrient and water stress condition, depend on their mycorrhizal association. This mutual relationship between the plant and the fungus has opened a new area of research in the interest of plant health in adverse soil conditions. Though, for its rational exploitation a serious attention is required to determine mycorrhizal inoculation effect and dependency for a corp in soil of different fertility levels.

Some of the areas adjoining Bihar and Jharkhand states fall under rain dependent upland zone where water does not stand consequently the soil become dry with high temperature. Therefore, the farmers in this region usually prefer dry farming of minor millets viz. finger, proso, kodo, barnyard, foxtail millets both as food and fodder. Application of arbuscular mycorrhizal fungi has been reported to be highly effective in such condition to improve crop productivity (Hayman, 1980 ; Ravekar and Tilak, 1988 ; Sharma *et al.*, 1987 ; Tilak, 1993 ; Ross, 1970).

Keeping these in view, the present investigation has been carried out to record AMF diversity in the rhizosphere of different minor millets viz. finger, proso, kodo millets growing in this region. Pot experiments have been conducted in order to evaluate the inoculation effect of mycorrhiza on plant growth performance and to determine mycorrhizal dependency of a crop at four different phosphorus levels in soil.

MATERIALS AND METHODS

Eight sites at the border of Bihar and Jharkhand were randomly selected for regular survey of crop fields of finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*) and kodo millet (*Paspalum scrobiculatum*) for the collection of rhizosphere soil and root samples. Wet-sieving and decantation method (Gerdeman and Nicolson, 1963) was followed for screening of AM spores, where spore population was determined in terms of number of spores per 10 g of dry soil. Root colonization developed by AM fungi was detected by staining the roots with trypan blue (Philips and Hayman, 1970) after KOH (10%) treatment for

softening of tissues. Per cent root colonization was determined as the number of colonized roots per one hundred segments observed. Species of AM fungi were identified on the basis of their morphological characters of intraradical (hyphae) and extraradical (spores) structures as described in the Manual of Schenck and Perez (1990).

To understand AMF effect on plant growth performance, experiment was conducted in earthen pots (21 cm diam) containing non sterilized soil of rainfed areas, the physicochemical profile of which is as pH = 6.7 ; P = 0.2 mg/100 g ; K = 27 mg/100 g ; N = 3 mg/100 g) and indigenous AM spore population was 2 spore/10 g soil.

Two most dominant AMF, collected from the rhizosphere of these crops, *Glomus fasciculatum* and *Gigaspora margarita* were multiplied on maize roots and used as AM inoculum to evaluate their synergistic effect on plant growth. The top soil in each pot up to a depth of 6 cm was mixed with 10 g (5 g of each species) of root base having AM inoculum of 100% colonization. Ten surface sterilized seeds (2% NaOCI) of *E. coracana, P. miliaceum & P. scrobiculatum* were sown in separate pots, those were thinned up to 6 seedlings of uniform size in each pot. Pot soilds were mixed with four levels of additional phosphorous i.e. 0 mg/ 100 g, 0.5 mg/100 g and 1.0 mg/100 g. Three replicates were maintained for each treatment. Control set for each P-level was kept without adding any AM inoculum. The plants were provided same environmental conditions under green house. Intensity of root colonization by AMF partner was checked after 30 days of emergence of seedlings. Plant growth parameters were measured after 100 days in terms of dry matter (g / plant) grain yield (g / plant) and P-content in plant tissues by Vandamolyb-dophosphoric yellow colour method (Jackson, 1973). Mycorrhizal dependency (MD) of crops was evaluated with formula MD = [(N-NM)/NM] × 100 (Menge *et al.*, 1978).

RESULTS AND DISCUSSION

All the three crops under study exhibited symbiotic relationship with AM fungi (Table 1). Sensitivity of graminaceous plants towards the AM colonization has also been confirmed by many workers (Doss *et al.*, 1998 ; Doss and Bagyaraj 1990, Roy *et al* 1997, Roy and Rashmi, 2002). Variation in spore population from 80-200 g^{-10} dry soil and root colonization from 65-100% was recorded in case of *E. coracana,* whereas it was 10-70^{-10} and 70-150^{-10} with 75-100% & 60-80% root colonization in case of *P. scrobiculatum* and *P. miliaceum* respectively. In all the cases every root segment was considered mycorrhizal if any of the three structurs i.e. vesicles, arbuscules and typical hyphae were present. Arbuscules were invariably present densely and seldom less in number which indicated strong symbiotic relationship with the crops. Spore population was not always correlated with per cent root colonization. It might be explanined as the spore population and root colonization are the two entirely different growth phase, which may be

Table 1 : Spore population and root colonization by AM fungi in association with the three minor millet crops.

Sites	Crops	Spore population (no. of spores/ 10g soil)	Root colonization (%)	Intraradical structures		
				Hyphae	Arbuscules	Vesicles
Angwali	Pm	120	75	+++	++	++
	Ps	70	100	++	++	+++
	Ec	90	70	+++	++	++
Daldali	Pm	10	70	+++	++	++
	Ps	20	75	+++	++	+++
	Ec	45	85	+++	++	++
Sitkia	Pm	13	70	++	+	+
	Ps	11	80	+++	++	++
	Ec	21	92	+++	++	++
Lobhnada	Pm	77	75	++	+++	++
	Ps	59	80	++	++	+
	Ec	79	87	+++	+++	++
Sunderpahari	Pm	150	80	+++	+	++
	Ps	40	100	+++	+	++
	Ec	140	100	+++	++	+++
Bhaljorh	Pm	80	75	+++	++	++
	Ps	56	80	+++	+	+
	Ec	200	95	+++	++	+
Rajapokhar	Pm	98	70	+++	+	++
	Ps	76	70	+++	++	++
	Ec	100	75	+++	+++	+++
Mahadeograh	Pm	70	60	+++	+	++
	Ps	60	75	++	+	+
	Ec	180	100	+++	++	++

Pm = *Panicum miliaceum* ; Ps = *Paspalum scrobiculatum ;* Ec = *Eleusine coracana ;* + = less present ; ++ = present ; +++ = densely present.

governed by the fungal own requirement. Thus it is expected that the no. of spores in rhizosphere zone would not be always directly proportional to the root infection and *vice versa*. Furthermore, the benefit from AMF must be evaluated on the basis of root colonization and arbuscules formation as it promises the compatibility of both the partner in AM symbosis. Variation in spore population and root colonization was recorded for the same crop growing in different sites which might be due to differences in edaphic conditions. Further, it was also noticed that at the same sites the spore population and root colonization varied for different crops. This might be corroborated with the fact that every plant differs in their rhizosphere effect due to differences lie in the nature of organic compounds released by the root in its surrounding soil which influence the microbial activities of that particular zone. Amongst the three test crops *E. coracana* harboured maximum number of AMF species as well as their population density comparatively.

Species composition of AMF recorded from rhizosphere of these crops comprised of 18 species belonging to five genera (Table 2). On the basis of observations of their morphological characters it was found that species of *Glomus* and *Gigaspora* were dominating the whole AMF population followed by *Scutellospora, sclerocysits* and *Acaulospora* sp. HIghest number of species (16 spp.) was recorded in the rhizosphere of *E. coracana* in comparison to the other two corps (Table 2).

Table 2 : Species composition of AM fungi in rhizosphere soil of three minor millets.

Crops	AM fungi
Panicum miliaceum (10 spp.)	*Glomus fasciculatum, Glomus aggregatum, Glomus caledonium, Glomus mosseae, G. geosporum, G. macrocarpum Gigaspora margarita, Scutellospora nigra, S. persica, Sclerocystis pakistanika*
Paspalum scrobiculatum (8 spp.)	*Acaulospora scrobiculata, G. fasciculatum, G. aggregatum, G. caledonium, G. mosseae, Gigaspora margarita Gi. gignatea, Scutellospora nigra*
Eleusine coracana (16 spp.)	*Acaulospora scrobiculata, A. gilmori, G. fasciculatum, G. albidus, G. geosporum, G. botryoides, G. macrocarpum, G. microaggregatum, G. caledonium, G. mosseae, Gigaspora margarita Gi. gignatea, Gi. decipense, Sclerocystis* sp. *Scutellospora nigra*

The data depicted in the table 3 clearly indicated that the plant growth profile was directly related to the P-concentration in soil as on its increased level plant also attained better growth in case of all the test crops either mycorrhizal or non-mycorrhizal. Moreover, on comparative review of data it was notice that the per cent increase in growth parameters of the mycorrhizal plant over their respective control varied at each level of phosphorus (Figs. 1, 2 & 3) which confirmed that in a mycorrhizal symbiotic system the benefit availed by the host plant from its fungal partner is greatly affected by the nutrient status of the soil where the plants are growing.

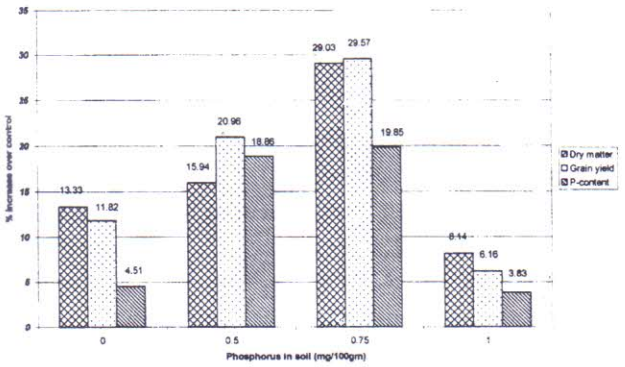

Fig. 1 : Percent increase in growth profiles of *E. coracana* incoculated with AMF at four different phosphorus levels

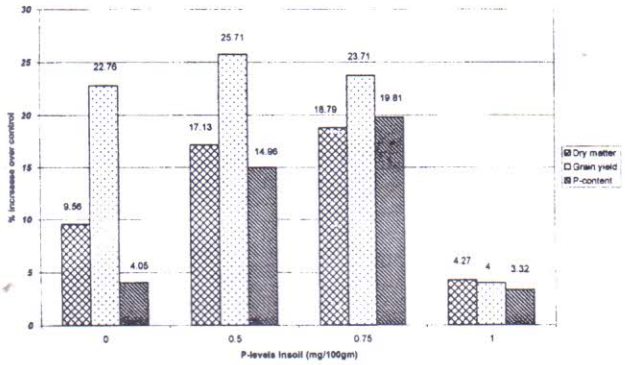

Fig. 2 : Percent increase in growth profiles of *P. milliaceum* incoculated with AMF at different phosphorus levels in soil.

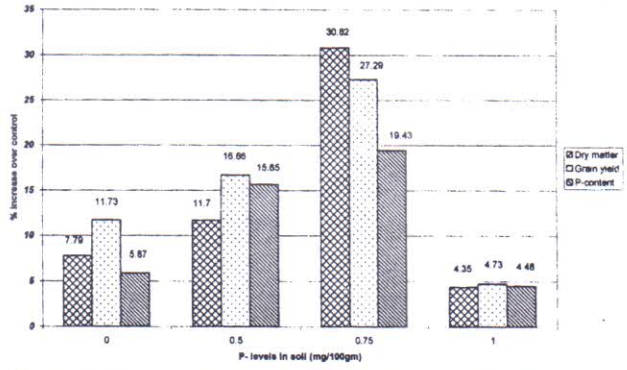

Fig. 3 : Percent increase in growth profiles of *P. scrobiculatum* incoculated with AMF at four different phosphorus levels in soil.

In the present study a gradual increase in degree of dependency i.e. from 9.56 to 18.79 % at 0 P-level to 0.75 P – level was recorded in case of *P. miliaceum.* Further incrase in the P in soil (1 P level) concentration caused significant reduction in plant's dependency on mycorrhiza as indicated by a sharp (4.27%) decline (Fig. 4) In case of *P. scrobiculatum* and *E. coracana* mycorrhizal dependency at 0 P-level was noted 7.9% and 13.33% respectively, those were slightly increased up to 11.7% and 17.13% at 0.5 P-level respectively. At 0.75 P level sharp increase was exhibited by both the crops and further increase in P in soil showed reduction in dependency on mycorrhiza up to 4.14% and 4.35% respectively.

Thus, the findings made it clear that all the three test crops showed varied degree of dependency on mycorrhiza for their growth, moreover, all of them showed maximum mycorrhizal dependency at the level of 0.75 mg P/100 g in soil. A comparative view of the data indicated that amongst all, under the same environmental and edaphic conditions, *P. scrobiculatum* exhibited maximum dependency on mycorrhiza i.e. 30.83 % which was slightly decreased up to 29.03% in case of *E. coracana,* whereas, *P. miliaceum* showed comparatively lesser degree of dependency. The increased growth performance of plant may directly be correlated with the P content in plant tissues (Table 3) which is regulated by increased P-uptake by a plant. At different P-levels in soil better performance of mycorrhizal plant may be correlated with the ability of AM fungi to increase the rate of P-uptake in plants. This is attributed by a number of factors including an extensive network of hyphae extended from the root enabling the plant roots to explore a greater volume of soil thereby overcoming limitations imposed by the slow diffusion of P in the soil (Smith and Read, 1997). The present result may also be supported with the fact that the influx of P in mycorrhizal root can be 3 to 5 times higher than in the non mycorrhizal roots (Smith and Read, 1997).

The reduction in degree of mycorrhizal dependency of plant at the highest P level (1.0 mg/100g) was found to have direct correlation with the lowest intensity of root colonization (Table 3) by the fungal partner. In the present investigation mycorrhizal dependency of plant was always found minimum at the higher P concentration in soil, which indicated that additional phosphorus beyond level of 0.75 mg/100 g soil reduces the degree of

Table 3 : Effect of mycorrhiza inoculation on the plant growth of three minor millets under four different soil P levels in soil.

Phosphorus Concentration (mg/100 g)	Crops	RC (%)	Total dry matter (g plant-1)			Grain yield (g plant-1)			P-content (mg 100 g)		
			NM	M	%I	NM	M	%I	NM	M	%I
No phosphorus	Pm	70	1.150	1.260	*9.56*	0.224	0.275	*22.76*	40.46	42.10	*4.05*
	Ps	75	2.310	2.490	*7.79*	0.554	0.619	*11.73*	49.16	52.05	*5.87*
	Ec	69	4.500	5.100	*13.33*	1.125	1.258	*11.82*	50.09	52.35	*4.51*
0.5	Pm	85	1.447	1.695	*17.13*	0.280	0.352	*25.71*	41.23	47.40	*14.96*
	Ps	84	2.836	3.168	*11.70*	0.894	1.043	*16.66*	41.59	48.10	*15.65*
	Ec	79	6.900	8.000	*15.94*	1.984	2.400	*20.96*	42.19	50.15	*18.86*
0.75	Pm	85	1.681	1.997	*18.79*	0.447	0.553	*23.71*	46.89	56.18	*19.81*
	Ps	82	3.585	4.690	*30.82*	1.535	1.954	*27.29*	48.52	57.95	*19.43*
	Ec	74	8.510	10.981	*29.03*	2.512	3.255	*29.57*	47.54	56.98	*19.85*
1.0	Pm	65	2.105	2.195	*4.27*	0.450	0.468	*4.00*	55.89	57.75	*3.32*
	Ps	65	4.895	5.108	*4.35*	2.110	2.210	*4.73*	56.58	59.12	*4.48*
	Ec	59	11.050	11.950	*8.14*	3.550	3.769	*6.16*	57.08	59.27	*3.83*

RC = Root colonization (after 30 days of seedling emergence) ; NM = nonmycorrhizal ; M = Mycorrhizal ; %I = percent increase over control ; m = *Panicum miliaceum* ; Ps = *Paspalum scrobiculatum* ; Ec = *Eleusine coracana*

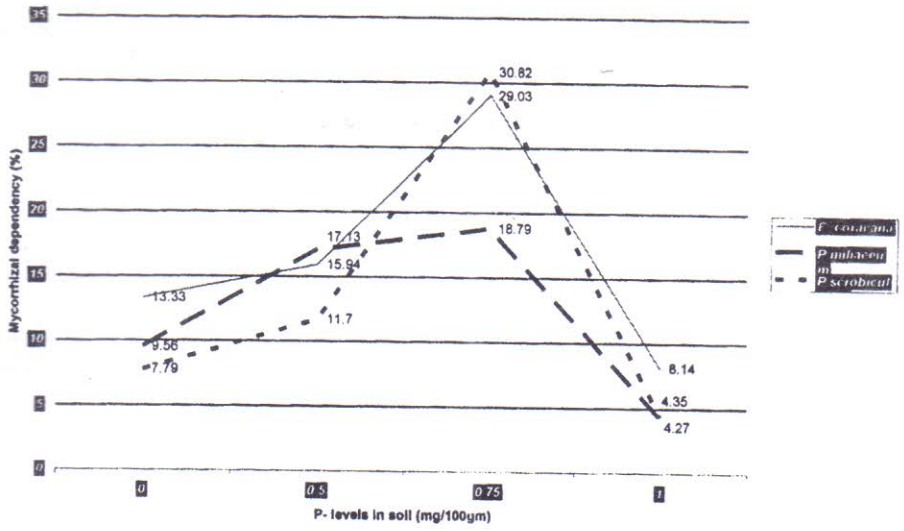

Fig. 4 : Degree of mycorrhizal dependency of three minor millet crops at four different phosphorus levels

mycorrhizal colonization vis a vis mycorrhizal dependency of plant (Fig. 4). Low level of colonization in plants growing with high P-status is due to the specific signal from the plant regulating the activity of fungus. It mycorrhizal plant demand of phosphorus may regulate the activity of P transporter in the fungal partner (Smith, 2002). It might be due to adequate quantity of phosphorus present in the soil surrounding rootlets.

The findings get support from the reports of Hall *et al.*, (1977) and Mosse (1973) regarding the suppression and ultimately death of the fungal partner at the highest P-concentration in soil. It has also been reported that addition of excess soluble phosphorus eliminates the beneficial effect of mycorrhiza (Hall, 1976 ; Mosse, 1977).

REFERENCES

Doss, D. D., Bagyaraj, D. J. and Shyamsunder, J. 1988. Anatomical changes in the roots and leaves of finger millet colonized by VA mycorrhiza. *Ind. J. Microbiol.* **28**(4) :276-280.

Doss, D. D. and Bagyaraj, D. J. 1990. Anatomical observation on roots of finger millet colonized by VA mycorrhiza. *Curr. Sci.* **59**(8) : 423-424.

Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal *Endogone* Spp. extracted from soil by Wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46** : 235-244.

Hall, I. R. 1976. Response of *Coprosoma robusta* to different froms of endomycorrhizal inoculum. *Trans Br Mycol Soc.* **67**, 402-411.

Hall, I. R., Scott, R. S. and Johnston, P. D. 1977. Effect of vesicular arbuscular mycorrhiza on response of Grasslands-Huia and 'Tama' white clovers to phosphorus. NZJ *Agric Res.* **20** : 349-355.

Hayman, D. S. 1980. Mycorrhizae and crop production. *Nature* : **287** : 487-488.

Jackson, M. L. 1973. *Soil Chemical Analysis.* Prentice Hall of India (Pvt.) Ltd. New Delhi.

Menge, J. A., Johnson E. L. V. and Platt, R. G. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytol.* **81** : 553-559.

Mosse, B. 1973. Plant growth response to vesicular arbuscular mycorrhiza. IV In : Soil given additional phosphate. *New Phytol.* **72** : 127-136.

Mosse, B. 1977. The role of mycorrhizae in legume nutrition on marginal soil. Exploiting the Legume–*Rhizobium* Symbiosis in tropical Agriculture. College of tropical agriculture miscellaneous publications **145** : 275-292.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasites and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55** : 15-161.

Ravekar, K. P. and Tilak KVBR 1988. Relative efficiency of different VAM on soyabean (*Glycine max*) under varying levels of phosphorus. In : *Mycorrhizae for Green Asia.* Mahadevan A, Raman N, Natrajan K. eds : Proc. of the First Asian Conference on Mycorrhiza : Madras, Univ. of Madras pp 162-165.

Ross, J. P. 1970. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soyabeans. *Phytopathology.* **61** : 1400-1403.

Roy, A. K., Rashmi, K. and Singh H. N. P. 1997. Vesicular-Arbuscular mycorrhiza (VAM)–association with Kodo Millet : a possibility of its cultivation in Rainfed Areas. *Int. Sorgi Mill. New sletter.* **38** : 145-146.

Roy, A. K. and Rashmi, K. 2002. Mycorrhizal association with traditional minor millets and their exploitation for sustainable agriculture in upland, In. *Bioinoculants for Sustainable Agriculture and Forestry* (Eds. S. M. Reddy, S. R. Reddy, M. A. Singarachary, G. Girisham) Sci. Pub, Jodhpur pp. 61-66.

Schenck, N. C. and Perez, Y. 1990. *Manual for identification of VA mycorrhizal fungi*. 3rd Edn. Synergistic Publishers, Gainesville, Florida.

Sharma, M. P., Gaur, A., Bhatia, N. P. and Adholeya, A. 1987. Responses of *Acacia nilotica* to indigenous vesicular-arbuscular mycorrhizal fungi inoculation and single superphosphate fertilization in degraded wasteland Soil. In : *Mycorrhizae : Bofertilizer for future,* (Ed A. Adholeya and S. Singh)

Smith, S. E. and Read, D. J. 1997 ; *Mycorrhizal symbiosis.* Academic Press. San Diago, California.

Smith, F. W. 2002. The phosphate uptake Mechanism. *Plant and Soil.* **245** : 105-114.

Tilak, K.V.B.R. 1993. Association effect of vesicular-arbuscular mycorrhizae with nitrogen fixer. *Proc. of Ind. Nat. Sci. Acad.* **B59** (3 & 4) 325-332.

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