Research Paper :

Combined Inoculation of *Arbuscular mycorrhizal* Fungi and Azotobacter Beneficial to (Wheat) *Triticum aestivum* L.

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SUMMARY

The effect of *Glomus macrocarpum, Glomus fasciculatum* and *Azotobacter* on wheat (*Triticum aestivum* L.) was studied in sterilized soil. Compared to un-inoculated plants, chlorophyll content, height, weight, number and area of leaves of plant and weight were maximum in plants inoculated with *G. macrocarpum, G. fasciculatum* and *Azotobactor* or with *G. macrocarpum* and *G. fasciculatum* or with only *G. macrocarpum*, or *G. fasciculatum*. The levels of root colonization was higher in all AM inoculated plants. There was significant increase in N (3.86%) and P (0.18%) in the plant treated with a combination of *Glomus macrocarpum, Glomus fasciculatum* and *Azotobacter*. The results clearly indicated that compared to individual inoculation, AM fungal species with *Azotobacter* used in combinations were more beneficial for much improved growth of wheat.

Key words : *Glomus mosseae, G. fasciculatum, Azotobacter, Triticum aestivum*, Per cent colonization

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Soil provides the matrix for the biological processes involved in nutrient cycling. Among the biological processes involved in the rhizoplane, the unique role of symbiotic bacteria and the AM fungi which ensure fixation and mobilization, and availability to nitrogen and phosphorous to plants have been well recognized (Marchner, 1995). It is well-established fact that the AM always prefer certain host exhibiting maximum symbiotic response and increase the growth and yield of crop mainly through improved uptake of nutrients (Allen, 1991). A few scientists have observed wide variation among and within different species on AM fungi in their ability to promote plant growth (Read,1996). This led to the concept of host preference by AM fungi (Mosse, 1973). Hence, it is always better to select an efficient AM fungus for a particular host-soil-climate combination to harness maximum benefits. This study was aimed to find the response of *Triticum aestivum* to inoculation of *Glomus macrocarpum*, *G. fasciculatum* and *Azotobacter* in unsterile soil either singly or in combinations. There are some microorganisms which establish symbiotic relationships with different parts of plants and may develop special structures as the site of nitrogen fixation (Lakshman, 2009).

MATERIALS AND METHODS

Present investigation was carried out in sterilized soil of polyhouse during 2006 to 2007 using aromatic plant seedling of *Triticum aestivum*. The soil used for experiment contained organic carbon 0.86%, pH 6.8, available N 0.74%, available P_2O_5 2.8 kg/ha, available 91 kg/ha, Electrical conductivity (EC) was 0.17 ohm^{-1} and native AM spore population averaging 87 spores / 50 g soil. Seedlings used in the experiment were grown on sterile soil and 30 seedlings were transplanted in earthen pots measuring 25 x 15 cm diameter. Soil-root-cultures of *Glomus macrocarpum* and *G. fasciculatum* were cultivated on maize roots using mixture of soil : sand : FYM (1:1:1). The cultures containing clamydospores (96-112 spore/50 g soil) and root segments of maize colonized by particular AM fungus were used as mycorrhizal inoculum. Application of AM inoculum was 129 g soil / plant when single species was used and 5g soil/plant when two species used in combination in soil with seedling roots. *Azotobacter* as per treatment was 10g/ plant. The experiment was arranged in a completely randomized design with five replications. Observations were recorded, 30 and 60 d after planting. Observations such as chlorophyll content, plant height, number of leaves, size of stem and fresh and dry weight of shoot were recorded. The fresh weight of plant and leaves was recorded immediately after harvest. The dry weight was determined after drying the plant at 80^0 C for 48 hrs. The

root samples of each treatment were collected, processed and stained in 0.05% cotton blue in lactophenol (Philips and Hayman, 1970). Per cent root colonization was calculated using the method of Giovanetti and Mosse (1980). The nitrogen and phosphorus contents were determined following the method of Jackson (1973). Estimation of chlorophyll was carried out using the method of Arnon (1949).

RESULTS AND DISCUSSION

There was more than 6 folds increase in total chlorophyll content 30 d after inoculation of plants with *G. macrocarpum*, *G. fasciculatum* and *Azotobacter* compared to uninoculated plants.

In inoculated plants with *G. fasciculatum* or with G. *macrocarpum* and *G. fasciculatum* also total chlorophyll content increased. However, 60 days after inoculation, the total chlorophyll content dropped gradually (Table 1) while control plants showed a continued small increase. Increase in chlorophyll content in VAM inoculated plants was reported by Devi and Reddy (2004) in groundnut and Shivaputra *et al*. (2004) in papaya. Plants treated with *G. fasciculatum* and *Azotobacter* in combination recorded rapid increase in dry weight from 30 to 60 days after inoculation. A similar increase in dry wt was observed when inoculated with *G. macrocarpum* and *G. fasciculatum* or *G. fasciculatum* or *Azotobacter* (Table 1).

Significant increase was recorded in growth parameters, number of leaves, plant dry weight, stem diameter, fresh and dry weight of leaves (Table 2). The inoculation of *G. macrocarpum*, *G. fasciculatum* and *Azotobacter* increased plant height 55.35 cm after 40 days to 74.25 cm after 80 days Similarly in the same treatment, maximum number of functional leaves increased from 6 and 12 and leaf area from 51.20 to 90.64 cm^2. Similar findings were recorded by Krishna and Bagyaraj, 1982 in lady's finger and Indi *et al*. (1989) in brinjal recorded significant increase in height, number of leaves and overall growth of plants due to inoculation with AM fungi. It is evident from (Table 2) that root colonization 30 days after inoculation was higher in combined inoculation with *G. macrocarpum*, *G. fasciculatum* and *Azotobacter*. After 60 days, maximum root colonization was found in this treatment. Clamydospore count in rhizosphere soil 60 days after inoculation was also the highest on inoculation of wheat plants with *G. macrocarpum*, *G. fasciculatum* and *Azotobacter*. G. *fasciculatum* and *G. macrocarpum* also showed high levels. Inoculation with *G. mosseae* or *G. fasciculatum*

Table 1 : Effect of AM fungi and *Azotobacter* inoculation showing chlorophyll content and fresh and dry weight of shoots of *Triticum aestivum* L.

AM	Chl a	Chl b	Total chl	Chl a	Chl b	Total chl	Shoot fresh wt (g)		Shoot dry wt (g)	
	30 DAP			60 DAP			30 DAP		60 DAP	
Control	0.170	0.126	0.113	0.213	0.088	0.311	3.11	11.13	0.74	1.32
Gm	0.236	0.129	0.381	0.260	0.128	0.380	9.69	24.17	0.76	1.32
Gf	0.317	0.281	0.587	0.243	0.098	0.342	13.74	32.68	1.23	3.52
Gm+Gf	0.269	0.19	0.439	0.252	0.147	0.386	8.01	41.12	0.92	4.63
Gm+Gf+B.P	0.371	0.414	0.782	0.244	0.173	0.423	14.25	71.03	1.24	7.24
CD(*P=0.05*)	0.07	0.12	0.23	0.012	0.033	0.046	3.02	27.62	0.25	2.72

Gm - *Glomus macrocarpum*, Gf = *Glomus fasciulatum*, B.P=*Azotobacter chrococcum*

Table 2 : Effect of AM fungi and *Azotobacter* inoculation on leaves, plant, VAM colonization, N and P status in *Triticum aestivum* L.

AM	Leaf (no)		Leaf area		Plant height (cm)		VAM colonization (%)		N (%) in shoot/plant		P (%)in shoot/plant	
	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)
Control	3	6	30.4	35.7	49.11	46.21	-	-	2.05	3.11	0.06	0.08
Gm	5	10	28.01	51.20	44.52	59.30	49.10	73.10	3.42	3.72	0.08	0.11
Gf	5	10	34.8	77.00	51.4	71.14	61.0	62.50	3.55	3.80	0.12	0.12
Gm+Gf	7	10	30.15	84.32	46.32	74.10	61.0	78.4	3.68	4.62	0.13	0.15
Gm+Gf+B.P	7	12	35.12	91.64	55.35	74.25	67.3	84.22	3.71	3.86	0.13	0.18
C.D.(P=0.05)	1	12	3.18	22.15	5.05	13.10	31.06	41.03	0.71	0.82	0.02	0.02

Gm - *Glomus macrocarpum*, Gf = *Glomus fasciulatum*, B.P=*Azotobacter chrococcum*

Table 3 : Effect of AM fungi inoculation on leaf weight and stem size of *Triticum aestivum* L.

Treatment	Leaf fresh wt (g)		Leaf dry wt (g)		Stem size (cm)	
	(30 d)	(60 d)	(30 d)	(60 d)	(30 d)	(60 d)
Control	1.0	2.9	0.189	0.431	0.6	1.13
Gm	1.3	4.10	0.381	0.473	0.8	1.64
Gf	2.7	6.62	0.302	0.442	0.8	1.72
Gm+Gf	2.1	7.72	0.576	0.761	0.9	1.78
Gm+Gf+B.P	2.6	8.44	0.605	0.891	0.9	1.84
C.D. (P=0.05)	0.88	2.45	0.158	0.210	0.14	0.23

Gm - *Glomus macrocarpum*, Gf = *Glomus fasciulatum*, B.P=*Azotobacter chrococcum*

alone was also effective. This indicated that AM fungi used in inoculations were efficient in colonization as reported earlier in *Ablemoschus esculentus* (Krishna and Bagyaraj, 1982). In the present investigation, AM species were proved to be the best for the growth of *Triticum aestivum* when used in combination. All AM inoculated plants showed significantly higher uptake of N and P over control (Table 2). Plants inoculated with G. *macrocarpum, G. fasciculatum* and *Azotobacter* recorded significantly after 40days the max. 0.13 P% uptake (3.71% N in plant). *G. macrocarpum* and *G. fasciculatum* and *G. fasciculatum, G. fasciculatum* and *G. macrocarpum* also showed high uptake of N and P. After 80 days of inoculation, the maximum N uptake was with *G. macrocarpum, G. fasciculatum* and *Azotobacter* was 3.86% N in plant. Plants inoculated with *G. macrocarpum, G. fasciculatum* and *Azotobacter* in combination showed a max. P uptake (0.18%). Inoculation with *G. macrocarpum* and *G. fasciculatum, G. fasciculatum* or *G. macrocarpum* also led to increased P uptake.

The increased uptake of N and P could be attributed to the increased root absorption area induced by AM through an efficient symbiosis with the host and by assimilation and translocation of N (Yao and Li, 1999). It is well known fact that P uptake by plant is improved by AM association (Shivputra *et al*., 2004). Differential ability of inoculated AM fungi stimulating P uptake was also reported in different crops *viz.*, on lady's finger (Krishna and Bagyaraj, 1982) and and garlic (Wani and Konde, 1998). There were significant differences in stem diametre fresh and dry weight of leaves following inoculations with AM fungi (Table 3). Inoculations with any AM in general increased leaves fresh and dry weight and stem size when inoculation was made with *G. macrocarpum, G. fasciculatus* and *Azotobacter* in combination. Similar studies have been conducted earlier by Lakshman, 2000 and Ramananda and Sreenivasa (2000) reported that the fresh and dry wt of *Triticum aestivum* L. inoculated with different AM fungi increased significantly. An increase in stem diam. is attributed to the increased dry matter accumulation. In conclusion, the results confirm that appropriate strains of AMF and *Azotobacter* inoculum would help in increased biomass production and nutrient uptake in *Triticum aestivum* plants.

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REFERENCES

Allen, F. (1991). *Ecophysiology of Mycorrhiza*, Oxford Press, London, 249pp.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**:l-15.

Devi, M.C. and Reddy, M.N. (2004). Effect of arbuscular mycorrhizal fungi and Rhizobium association on chlorophyll content of groundnut *(Arachis hypogaea* L.). *Mycorrhiza News,* **16**(1) :15-17.

Giovanetti, M. and Mosse, B. (1980). An evaluation of technique for measuring vesicular-arbuscular mycorrhizal infection in the plants. *New Phytol.*, **95**:69-82.

Indi, D.V., Konde, B.K., Wani, P.V. and Kale, P.N. (1989). Response of brinjal genotypes to mycorrhizal inoculation. In: *Biofertilizer Transfer Technology*- (Ed. Gangwane L.V.). Assoc. Publ. Co. New Delhi. pp. 223-229.

Jackson, M.L. (1973). *Soil Chemical Analysis.* Prentice Hall of India Pvt., New Delhi, pp.370-387.

Krishna, K.R. and Bagyaraj, D.J. (1982). Effect of vesicular arbuscular mycorrhize and soluble phosphate on *Ablemoschus esculenlus* (L.) Moench. *Plant Soil,* **64**(2) : 209-2I3.

Lakshman, H.C. (2009). Biofertilizers for sustainable plant growth. *ICAR J.*, **113** : 22-24.

Lakshman, H.C. (2000). Dual inoculation of *Rhizobium* and AM fungi improve seedling of *Pterocarpus marsupium, J. Env. Ecol. Cons.*,**171** : 205-210.

Marchner, H. (1995). *Mineral Nutrition of Higher Plants*. Academic Press, London, 198pp.

Mosse, B. (1973). Advances in the study of vesicular arbuscular mycorrhiza. *Ann. Rev. Phytopath.,* **11**:171- 196.

Phillips, J. M. and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.,* **55**:158-161.

Ramananda, T.V. and Sreenivasa, M.N. (2000). Influence of different AM fungi on growth of onion. *J. Maharashtra agric. Univ.,* **25** (l) : 93-94.

Read, J. W. (1996). *Mycorrhizal function*. Academic Press. New York. 91pp.

Sandersen, P., Raja, N.U., Gunasekaran, P. and Lakshamanan, M. (1988). Studies on nitriate reduction by VAM fungal spores. *Curr. Sci.,* **57**(2):84-85.

Shivaputra, S. S., Patil, C. P., Swamy, G. S. K. and Patil, P. B. (2004). Cumulative effect of VAM fungi and vermicompost on nitrogen, phosphorus, potassium and chlorophyll content of papaya leaf. *Mycorrhiza News*, **16**(2): 15-16.

Wani, P. V. and Konde, B. K. (1998). Response of garlic to inoculation with vesicular arbuscular mycorrhizae. *J. Maharashtra agric. Univ.,* **23** (I) : 35-38.

Yao, Q. and Li, D. (1999). Influence of VA mycorrhiza on juice components and arid colour of Citrus fruit. *J. fruit. Sc.* China, **16** : 38 - 42.

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