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Influence of Fertilizer Levels and Mycorrhiza on Root Colonization, Root Attributes and Yield of Hybrid Maize

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A Field experiment was conducted at Agricultural Research Station, Bhavanisagar during kharif 2009 to study the influence of mycorrhizal inoculation and fertilizer levels on root growth and grain yield of hybrid maize under irrigated condition. The experiment was laid out in a factorial randomized block design with four replications. Four fertilizer levels *viz*., 200:100:100, 150:100:100, 200:75:100 and 150:75:100 NPK kg ha^{-1} were the treatments under factor 'A'. Two mycorrhizal treatments viz., no inoculation of mycorrhiza (control) (M^{-}) and inoculation of mycorrhiza (M^{+}) were included under factor 'B'. The results revealed that the root parameters such as root length, root volume, root dry mass, root- shoot ratio and root colonization were higher under 200:100:100 NPK kg ha^{-1} and inoculation of mycorrhiza. Regarding the treatment combinations, application of 150:75:100 NPK kg ha^{-1} along with mycorrhizal inoculation recorded significantly better root parameters. With regard to the yield, 200:100:100 NPK kg ha^{-1} recorded the highest grain and stover yield (6,494 and 9,894 kg ha^{-1}, respectively). Among the mycorrhiza, mycorrhizal inoculated treatments recorded the highest grain and stover yield (6,736 and 10,041 kg ha^{-1}, respectively). Regarding the treatment combinations, application of 150:75:100 NPK kg ha^{-1} along with mycorrhizal inoculation recorded the highest grain and stover yield.

Key words: Hybrid maize, *Glomus intraradices,* nitrogen, phosphorus, root morphology.

Maize (*Zea mays* L.) is one of the most versatile crops and can be grown in diverse environmental conditions and has diversified uses as human food and animal feed. Besides its use as food and fodder, maize is now gaining importance on account of its potential uses in manufacturing of starch, resins, syrups, ethanol, etc. It has got immense potential and is therefore called as "miracle crop" and also "queen of cereals". Maize, being a C4 plant is an efficient converter of absorbed nutrients into food.

The productivity of any crop is the ultimate result of its growth and development. Plant population, inorganic and organic fertilization are the important prime factors that determine the yield of maize crop. Among the plant nutrients, primary nutrients such as, nitrogen, phosphorus and potassium play a crucial role in deciding the growth and yield. The nitrogen use efficiency can be improved with the use of hybrids, soil application of arbuscular mycorrhiza and application of fertilizers coinciding with peak need by the crop.

Arbuscular mycorrhiza fungi are considered as obligate symbionts to crop plants for better utilization of P and other essential elements. AM fungal association is probably the most ancient and wide spread association involving the plants (Simon *et al.,* 1993). This association is characterized by fungal acquired nutrients through the external hyphae extending from the root surface into the soil by the plants (Mosse *et al*., 1981).

The readily available form of N to any crop plant is NO_3- which is highly labile in soil solution and thus the role of mycorrhiza is insignificant. Conversely, drought stress impedes the mobility of NO_3- ions in soils due to its low concentration and diffusion rate (Azcon *et al*., 1996). Under such environmental condition, AM fungi may play a crucial role in transporting N from the soil to the root surface, thereby contributing to plant growth and nutrition.

Phosphorus is an indispensable nutrient element for plants. Without adequate P, rates of various processes will be depressed and growth and development cannot continue at a normal rate. Phosphorus is generally available in small quantities in soil solution because most of inorganic phosphate ions are bound to soil colloids or fixed as iron aluminium PO_4 (Larsen, 1967). Nearly 98 percent of the Indian soils are deficient in P and about 10-15 percent of P from soil is utilized by plants (Gaur, 1982). Moreover, less than 15-25 percent of P from PO_4 fertilizer applied to soil is normally available to plants and a large quantity of P remains unavailable due to its fixation (Singh and Singh,

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1992). Under such circumstances AM fungi can be effectively utilized to enhance the P mobilization.

AM mycorrhizae are involved in P nutrition of maize and an understanding of their functioning will assist us in modifying management practices to maximize economic returns through increased fertilizer efficiency. Despite the fact that AM fungal colonization promotes P or N nutrition of host plants independently, the interaction between P and N has been rarely studied in the maize-mycorrhizal system. We hypothesized that mycorrhizal colonization modifies the antagonistic interactions between P and N, which favorably improves the host plant nutritional status besides alleviating P-induced Zn deficiency. To test this hypothesis, an experiment was conducted to estimate root morphological attributes, root growth and grain yield of AM fungus-inoculated and uninoculated maize plants under differentially fertilized P and N.

Materials and Methods

Field experiment was conducted at Agricultural Research Station, Bhavanisagar during *kharif* 2009 to study the influence of mycorrhizal inoculation and fertilizer levels on the root parameters and yield of hybrid maize under irrigated condition. The experiment was laid out in a factorial randomized block design with four replications. Four fertilizer levels *viz*., 200:100:100, 150:100:100, 200:75:100 and 150:75:100 NPK kg ha^{-1} were the treatments under factor 'A'. Two mycorrhizal treatments viz., no inoculation of mycorrhiza (control) (M^-) and inoculation of mycorrhiza (M^+) were included under factor 'B'. The soil of the experimental field was red sandy loam in texture belonging to Typic Paleustalfs. The nutrient status of soil during start of the experiment was low in available nitrogen (229.6 kg ha^{-1}), medium in available phosphorus (20.2 kg ha^{-1}) and medium in available potassium (268.2 kg ha^{-1}). Maize hybrid, COH (M) 5, a high yielding single cross hybrid released by Tamil Nadu Agricultural University, Coimbatore was chosen for the study.

Seeds of maize hybrids were sown on the side of the ridges by adopting a spacing of 75 x 20 cm along with vermiculite based mycorrhizal inoculum at a depth of 5 cm below the seeds. The mycorrhizal inoculum (Glomus intraradices TNAU-03-08) used in this study was purchased from the Department of Microbiology, Tamil Nadu Agricultural University. This strain was cultured in maize plants and propagules comprised of infected root bits and spores were blended in sterile vermiculite. The inoculum with the spore density of 200 spores g^{-1} was applied as a thin layer beneath the seeds prior to sowing @ 100 kg ha^{-1}. Seeds were dibbled at the rate of one seed $hill^{-1}$.

Well decomposed farm yard manure at the rate of 12.5 t ha^{-1} was applied uniformly over the field before last ploughing. $ZnSO_4$ @ 37.5 kg ha^{-1} was applied uniformly as basal to all the plots. As per the treatment schedule, nitrogen was applied in three splits *viz*., 25: 50: 25 per cent as basal, 25 and 45 DAS, respectively. The entire dose of phosphorus was applied basally. The potassium was applied in two equal split doses *viz.*, basal and at 45 DAS. The N, P and K fertilizers were applied in the form of urea (46 % N), single super phosphate (16 % P_2O_5) and muriate of potash (60 % K_2O), respectively.

Mycorrhizal colonization, plant and root analyses

The root and shoot samples were collected at 45 and 60 DAS. The root architecture of mycorrhizal and nonmycorrhizal plants was observed in terms of root length, root volume, and root drymass. Inoculated and uninoculated maize plant roots were washed thoroughly with water and cut into 1-cm segments, bleached with 2.5% KOH, acidified in 1 M HCl, and stained in 0.05% tryphan blue solution (tryphan blue 0.5 g, glycerol 500 ml, HCL (1%) 50 ml, and distilled water 450 ml) and destained before mounting on slides. One hundred root segments per treatment were examined for the presence of arbuscules, external hyphae and spores at 55 and 75 DAS.

Root length was measured from the base of the root to the tip of the primary root. To record the root volume, water was poured into a clean measuring cylinder (nearly three fourth of its volume) and the level of water noted. A string was attached to the root and lowered into the water and the new level of water was noted. The difference in the above two readings was calculated and expressed as root volume in cm^3 $plant^{-1}$.

Plants from soil were removed and washed off any loose soil. The roots after drying were separated from the top (cut at soil line). The root and top for each plant was weighed separately and recorded (Dry weight for roots/dry weight for top of plant = root/ shoot ratio). The root/shoot ratio was calculated for each treatment. Roots were dried at 70°C for 48 h, weighed, and reported as root dry mass.

Results and Discussion

Plant mycorrhizal colonization

Mycorrhizal colonization was assessed in AM treated and untreated plants at 45 and 60 DAS (Table 1). Inoculation of *Glomus intraradices* increased the percentage of colonization at both the stages even at lower levels of fertilizer application (i.e.) 150:75:100 NPK kg ha^{-1}. In contrast, the higher level P (100 kg ha^{-1}) caused a slight inhibitory effect on coloni zation.The results showed that the percentage of colonization was found to be higher (30-60%) in AM treated plants than untreated plants (20.-30%) at 60 DAS when compared to 45 DAS.

The plants in the non mycorrhizal treatment were also colonized by AM fungi, which indicated that

Table 1. Mycorrhizal colonization in maize roots as influenced by mycorrhiza, nitrogen and phosphorus levels

Fertilizer level NPK kg ha^{-1}	Arbuscules (%) 45 DAS			External mycelium (%) 45 DAS			Arbuscules (%) 60 DAS			External mycelium (%) 60 DAS		
	(M-)	(M+)	Mean	(M-)	(M+)	Mean	(M-)	(M+)	Mean	(M-)	(M+)	Mean
200:100:100	27.25	36.25	31.75	34.50	39.25	36.88	24.25	33.50	28.88	23.25	36.50	29.88
150:100:100	22.50	34.75	28.63	26.75	36.50	31.63	20.13	31.50	25.81	21.50	29.50	25.50
200:75:100	23.00	40.00	31.50	30.38	42.25	36.31	21.13	34.25	27.69	22.00	37.50	29.75
150:75:100	20.75	42.50	31.63	22.63	45.75	34.19	16.25	35.50	25.88	17.50	39.00	28.25
Mean	23.38	38.38		28.56	40.94		20.44	33.69		21.06	35.63	
	SEd	CD (P=0.05)		SEd	CD (P=0.05)		SEd	CD (P=0.05)		SEd	CD (P=0.05)	
F	1.16	2.42		1.77	3.69		1.18	2.45		1.18	2.45	
M	0.82	1.71		1.25	2.61		0.83	1.73		0.83	1.73	
F x M	1.64	3.42		2.51	5.22		1.66	3.46		1.66	3.46	

indigenous AM fungi occurred in the soils, a few of AM fungal species belonging to the genera *Glomus* and *Acaulospora* were found but at a low spore density and with a limited species. AM fungi can colonize the roots of maize, mycorrhizal colonization rates were lower relative to the mycorrhizal inocula. The reasons may be the propagules in the soils were far lower than those in mycorrhizal inocula, so the overall effect was probably lower in non mycorrhizal soil under field conditions (Wang *et al.,* 2006).

A significant increase in the proportion of mycorrhizal colonization in Arbuscular Mycorrhiza inoculated roots (AM^+) from non-inoculated (AM^-) maize plants at all the levels of P and N fertilizers were noticed. The higher proportion of mycorrhizal colonization in the AM+ maize plants was consistent across mycorrhizal structures such as arbuscules and external mycelium, and across time of sample collection (45 and 60 DAS).

The development of mycorrhizal structures such as arbuscules had significant changes at varying P and N levels, but the growth of external mycelium was decreased at higher levels of fertilizer application in AM^+ plants. Monocots like grasses with rapidly developing roots are the ideal stock plants for AM, but any host plant which is readily colonized by AM and which can be easily grown in the greenhouse can also serve as the stock plant (Ferguson and Woodhead, 1982). The reduction of mycorrhizal infection in the presence of added phosphorus is owing to a self regulatory mechanism of plant discarding the mycorrhizal fungus when its phosphorus requirement is more than that satisfied (Hayman, 1982). Similar results were reported by Mehraban *et al.* (2009) and Albert *et al.*, (2009) in sorghum.

Root morphology

Root morphological features such as length, volume and root-shoot ratio were significantly increased by mycorrhizal inoculation and N or P fertilization at 45 and 60 DAS (Table 2 and 3).

Root length

Root length which represents the time trend of growth was recorded at different phenophases of maize.Regarding the fertilizer treatments, the highest root length (21.65 and 27.86 cm at 45 and 60 DAS,

Table 2. Root attributes of maize hybrid at 45 DAS as influenced by mycorrhiza, nitrogen and phosphorus levels

Fertilizer level NPK Kg ha^{-1}	Root length (cm)			Root volume (cc $plant^{-1}$)			Root dry mass (kg ha^{-1})			External mycelium (%) 60 DAS		
	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean
200:100:100	21.50	21.80	21.65	90.18	117.1	103.65	429.5	634.8	532.1	0.103	0.141	0.122
150:100:100	18.45	20.75	19.60	75.35	108.0	91.68	401.8	618.3	510.0	0.106	0.144	0.125
200:75:100	19.35	22.95	21.15	80.25	122.5	101.39	421.0	638.8	529.9	0.109	0.137	0.123
150:75:100	18.18	25.08	21.63	64.90	132.2	98.53	384.3	648.3	516.3	0.106	0.138	0.122
Mean	19.37	22.64		77.67	120.0		409.1	635.0		0.106	0.140	
	SEd	CD										
(P=0.05)		SEd	CD									
(P=0.05)		SEd	CD									
(P=0.05)		SEd	CD									
(P=0.05)												
F	0.61	1.20		4.05	8.43		7.62	15.86		0.003	NS	
M	0.43	0.89		2.87	5.96		5.39	11.21		0.002	0.004	
F x M	0.86	1.78		5.73	11.93		10.78	22.42		0.004	NS	

M^- - Uninoculated (control) M^+ - Inoculated with AMF (Glomus intraradices) respectively) was associated with 200:100:100 NPK kg ha^{-1} but was comparable with 200:75:100 NPK kg ha^{-1} and 150:75:100 NPK kg ha^{-1}.

Inoculation of mycorrhiza had substantial effect on root length. Mycorrhizal inoculated plants recorded higher root length (22.64 and 27.91 cm at 45 and 60 DAS, respectively), than the non mycorrhizal plants.

The interaction between fertilizer levels and mycorrhizal inoculation was significant. Increased root length was observed with 150:75:100 NPK kg ha^{-1} along with mycorrhizal inoculation (25.08 and 30.43 cm at 45 and 60 DAS, respectively) but was comparable with 200:75:100 NPK kg ha^{-1} with mycorrhizal inoculation and 200:100:100 NPK kg ha^{-1} without mycorrhizal inoculation at 60 DAS.

Root volume (cc plant -1)

In general fertilizer levels and mycorrhiza influenced root volume significantly. The root volume was found higher with fertilizer level 200:100:100 NPK kg ha-1 (103.65 and 110.9), which was closely followed by 200:75:100 NPK kg ha^{-1} and 150:75:100 NPK kg ha^{-1}. With regard to mycorrhiza, inoculated plants recorded higher root volume (120 and 129.9) than the non- inoculated plants.

The interaction effect between fertilizer levels and mycorrhiza was significant. Increased root volume was observed with treatment combination 150:75:100 NPK kg ha^{-1} (132.2 and 139.3) with mycorrhizal inoculation. This was comparable with the combination 150:100:100 NPK kg ha^{-1} (122.5 and 131.2) without inoculation at both the stages.

Root dry mass

The root dry mass increased with the age of the crop and reached the highest at harvest. The crop at 45 and 60 DAS showed a phenomenal increase in root dry mass than the other stages. Application of different levels of fertilizers showed significant influence at all the two stages. Application of 200:100:100 NPK kg ha^{-1} recorded higher root dry mass (881.1 kg ha^{-1}) than the other fertilizer levels. However, it was comparable with 200:75:100 NPK kg ha^{-1} which recorded 880.8 kg ha^{-1} (Table 2 and 3). Mycorrhizae inoculated plants showed significant increase in root dry mass than non mycorrhizal plants at 45 (635 kg ha^{-1}) and 60 (1007 kg ha^{-1}) DAS.

Among the treatment combinations, the highest root dry weight was recorded under 150:75:100 NPK kg ha^{-1} with mycorrhizal plants (648.3 and 1081.8) both at 45 and 60 DAS. However, it was comparable with 200:75:100 NPK kg ha^{-1} along with mycorrhizal inoculation and 200:100:100 NPK kg ha^{-1}with mycorrhizal inoculations.

Root-shoot ratio

The root - shoot ratio declined from 45 DAS to 60 DAS. The fertilizer levels had no significant influence on root- shoot ratio at both the stages. Among the mycorrhiza, inoculated plants (0.140 and 0.133) had higher root-shoot ratio than the non inoculated plants (0.106 and 0.107 at 45 and 60 DAS, respectively). The interaction effect was not significant.

Table 3. Root attributes of maize hybrid at 60 DAS as influenced by mycorrhiza, nitrogen and phosphorus levels

Fertilizer levels NPK kg ha^{-1}	Root length (cm)			Root volume (cc plant^{-1})			Root dry mass (kg ha^{-1})			Root - shoot ratio		
	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean	(M^-)	(M^+)	Mean
200:100:100	28.08	27.65	27.86	94.35	127.4	110.9	784.0	978.3	881.1	0.109	0.131	0.120
150:100:100	24.95	24.63	24.79	82.55	121.8	102.2	688.0	967.3	827.6	0.103	0.134	0.119
200:75:100	25.15	28.95	27.05	83.43	131.2	107.3	758.0	1003.5	880.8	0.112	0.130	0.121
150:75:100	23.40	30.43	26.91	70.58	139.3	104.9	675.0	1081.8	878.4	0.105	0.135	0.120
Mean	25.39	27.91		82.73	129.9		726.3	1007.7		0.107	0.133	
	SEd	CD										
(P=0.05)		SEd	CD									
(P=0.05)		SEd	CD									
(P=0.05)		SEd	CD									
(P=0.05)												
F	0.90	1.87		2.88	6.00		19.02	39.57		0.004	NS	
M	0.63	1.32		2.04	4.24		13.45	27.98		0.003	0.006	
F x M	1.27	2.64		4.08	8.48		26.90	55.95		0.005	NS	

The root length is an important morphological parameter involved in improving water and nutritional status of the plant. Knowledge about the root system of a crop is a prerequisite for understanding many problems connected with crop production. Root characters such as length and distribution and especially root volume help in divulging the pattern of water use and nutrient uptake by the crop.In general, the root characters such as root length, volume and its dry weight showed a gradual increase from 45 to 60 DAS.

In the case of fertilizer levels, 200:100:100 NPK kg ha^{-1} increased the root length (9.5 and 11 %), root volume (11.5 and 7.8 %) and root dry mass (4 and 6 %) in comparison with 150:100:100 NPK kg ha^{-1} level of fertilizers at both 45 and 60 DAS (Fig 1 and 2 for root length and root volume, respectively). With regard to the N and P application, the root characters such as total root length and root volume increased with increasing fertilizer levels in the present investigation. This is in corroboration with the findings of Dhurandher and Tripathi (1999). Deeper root system might play an important role not only for continuous water uptake, but also for nutrient uptake under suboptimal water availability.

With regard to mycorrhiza, the measurements of root architecture variables *viz*., root length, root volume, root dry mass and root/shoot ratio in the mycorrhizal plants were significantly higher than non-mycorrhizal (M^-) plants. The increase in root architecture measurements in mycorrhizal inoculated (M^+) plants was found in both the root samples collected at 45 and 60 DAS. The extensive root growth of AM plants might be attributed to the improved P nutrition of host plants. The enhanced supply of P by mycorrhizal symbiosis has been unequivocally demonstrated (Hetrick *et al.,* 1996; Smith and Read, 1997; Subramanian *et al*., 2006). At early stage of crop growth, AM fungal inoculated plants showed an increase in root mass while the shoot masses were similar. This may be attributed to the utilization of carbon for establishment of functional symbiosis as reported by Jakobsen and Rosendahl (1990).

Grain yield and stover yield

Among the fertilizer levels, 200:100:100 NPK kg ha^{-1} recorded the highest grain yield of 6494 kg ha^{-1} but was comparable with 200:75:100 NPK kg ha^{-1} and 150:75:100 NPK kg ha^{-1}. The increase in grain yield with 200:100:100 and 200:75:100 NPK kg ha^{-1} was 20.5 and 19.9 per cent, respectively, over the fertilizer level of 150:75:75 NPK kg ha^{-1} (Table 4). Mycorrhizal inoculation recorded higher grain yield (6736 kg ha^{-1}) than no inoculation (5869 kg ha^{-1}).

Table 4. Grain yield and stover yield of maize hybrid as influenced by mycorrhiza, nitrogen and phosphorus levels

Fertilizer levels NPK Kg ha^{-1}	Grain yield (kg ha^{-1})			Stover yield (kg ha^{-1})		
	Mycorrhizal Inoculation			Mycorrhizal Inoculation		
	(M-)	(M+)	Mean	(M-)	(M+)	Mean
F_1- 200:100:100	6388	6600	6494	9875	9913	9894
F_2- 150:100:100	5775	6313	6044	9025	9700	9363
F_3- 200:75:100	6151	6755	6453	9450	10025	9738
F_4- 150:75:100	5163	7275	6219	8838	10525	9681
Mean	5869	6736		9297	10041	
	SEd	CD (P=0.05)		SEd	CD (P=0.05)	
F	147.2	306.2		170.3	354.2	
M	104.1	216.5		120.4	250.5	
F x M	208.2	433.0		240.8	501	

M^- -Uninoculated (control), M^+ -Inoculated with AMF (Glomus intraradices)

The interaction between fertilizer levels and mycorrhizal inoculation on maize grain yield was significant. The highest grain yield (7275 kg ha^{-1}) was recorded under the treatment combination 150:75:100 NPK kg ha^{-1} with mycorrhizal inoculation followed by 200:75:100 NPK kg ha^{-1}. The least grain yield (5163 kg ha^{-1}) was obtained under 150:75:100 NPK kg ha^{-1} without mycorrhizal inoculation.

This increase in yield was probably due to effective utilization of applied nutrients, increased sink capacity and nutrient uptake by crop. The yield potential of maize is mainly governed by the growth and yield components. The positive and significant improvement in LAI and DMP noticed at different stages, increased yield attributes and nutrient uptake would have resulted in enhanced grain yield. The present findings are in line with the findings of Maddonni *et al*. (2006). The positive responses of hybrid maize upto 250 kg N ha^{-1} as reported by Srikanth *et al.* (2009) lend support to the present findings.

Since N is the major structural constitute of cells, as N level increased, the rate of vegetative and reproductive growth also increased in plants due to increase in assimilating surface of plants as well as total photosynthesis. In physiological terms, the grain yield of maize is largely governed by source (photosynthesis) and sinks (grain) relationship which is directly related to N. These resulted in more grain yield when N was higher.

Mycorrhiza had positive influence on grain yield of maize crop. The improved nutritional status of AM fungus-inoculated plants resulted in higher grain yields by 20% in comparison to uninoculated treatments. This yield gain in mycorrhizal plants was mainly caused by the intense flow of minerals and metabolites from the leaf to the developing kernel. The increased yields of AM fungus inoculated plants thus suggest that significant amounts of P and N were translocated from the source to the sink to support kernel development and grain yield (Subramanian and Charest, 1997).

A higher yield of maize due to mycorrhizal inoculation has been reported previously by Subramanian *et al*. (2008). A higher yield of rice due to AMF inoculation has been reported previously (Solaiman and Harita 1998).

Increasing the fertilizer levels increased the stover yield significantly. Fertilizer level of 200:100:100 NPK kg ha^{-1} recorded higher stover yield (9894 kg ha^{-1}) followed by 200:75:100 NPK kg ha^{-1}. The positive and significant improvement in LAI and DMP noticed at different stages and higher nutrient uptake due to higher dose of fertilizer would have resulted in enhanced stover yield. These results are in confirmity with the findings of Srikanth *et al.* (2009). Inoculation of mycorrhiza significantly influenced the stover yield of maize. Mycorrhizal inoculated plants recorded significantly higher (10041 kg ha^{-1}) stover yield. Mycorrhiza inoculation might have increased the stover yield of treated plants, due to increase in plant height, leaf area index and total biomass as evidenced in the present investigation.Similar results of increase in stover yield due to mycorrhizal inoculation were also reported earlier by Lauzon and Miller, (1997) in maize.

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