Different levels of nitrogen and phosphorus with or absence of VAM fungal inoculum on rice (*Oryza sativa* L)

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Abstract: Pot experiment was conducted at BINA headquarter, Mymensingh, Bangladesh to evaluate the response of different levels of nitrogen and phosphorus containing fertilizers with or without VAM fungal on rice. The treatment combinations were N_0P_0, N_0P_{20}, N_0P_{40}, $N_{100}P_0$, $N_{100}P_{20}$, $N_{100}P_{40}$, $N_{200}P_0$, $N_{200}P_{20}$ & $N_{200}P_{40}$ with or without VAM fungal inoculum. Experiment was laid out in a completely randomized design with four replications. Results on root and shoot dry weights were significantly affected by the treatments at different growth stages of plant. At 40 and 95 DAT, maximum shoot dry weight (9.75 and 24.33 g hill^{-1}) and root dry weight (2.95 and 3.23 g hill^{-1}) were observed in $N_{200}P_{40}$ + VAM fungal treatment. The minimum shoot and root dry weight was found in N_0P_{20} treatment. The maximum root infection (74.33%) was observed in $N_{200}P_0$ + VAM which was statistically similar to $N_{100}P_0$ + VAM and $N_{100}P_{20}$ + VAM. The highest plant height, number of tillers per hill, panicle length, filled grain per panicle and 1000 seed weight was obtained from $N_{200}P_{40}$ + VAM. Unfilled grain had negative and significant correlation with number of tiller, panicle length and filled grain of rice suggesting it lower number of unfilled grain helped to increase rice yield. Number of tiller per hill showed positive and significant correlation with grain yield of rice in $N_{200}P_{40}$ + VAM treatment. Maximum grain yield (4.45 t ha^{-1}) was observed in $N_{200}P_{40}$ + VAM which was statistically similar to $N_{200}P_{20}$ + VAM treatment. In conclusion, application of VAM inoculum may be stimulated growth and yield of rice as compared to uninoculated plant.

Key words: VAM. Mycorrhiza, rice, different levels of N and P, root infection, yield.

Introduction

Rice (*Oryza sativa* L) is the second most important cereal in the world after wheat and the principal crop in Asia, serving as food for about 50 per cent of the world's population (Sass and Cicerone, 2002). It is predicted that 50 to 60 per cent increase in rice production will be required to meet demand from population growth by 2025 (Zhang and Wang, 2005). Bangladesh, though ranks 4th in the world both in acreage and production of rice, but it ranks 39th in respect of yield. The average yield is quite low (3.44 t ha^{-1}) compared to other rice growing countries such as China, Korea, Japan and USA, where per hectare yield is 6.26, 6.23, 6.58 and 7.37 t ha^{-1}, respectively (FAO, 2003). Phosphorus deficiency is one of the most limiting factors in rice production and annualy large amounts of P fertilizers are being used in rice fields in the world (Itao *et al*., 1999). The available phosphorus status in most of the areas of Bangladesh soils is generally quite low due to it fixation. Application of phosphatic fertilizer is essential to obtain better yield of crops. Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi with the roots of higher plants. Vesicular arbuscular mycorrhiza (VAM) have been shown to improve productivity in soils of low fertility and are particularly important for increasing the uptake of slowly diffusing ion such as PO^{3-}_4 (Jacobsen *et al*., 1992). On the other hand, nitrogen fertilizer use efficiency is very low due to its leaching, volatilization and run off loss during crop production. Mycorrhizal fungi can also improve absorption of N from NH_4^+-N mineral fertilizers and transporting it to the host plant (Johansen *et al*., 1993). A few research works have been done on the crop species such as clover (Bi *et al*., 2003), sorghum (Bagayoko *et al*., 2000) and wheat (Hawkins and George, 1999), but there is little experimental evidences on the role of mycorrhizal colonization on rice growth (Purakayastha and Chhonkar, 2001; Gao *et al*., 2007). With this view, it is essential to determine the effect of different levels of nitrogen and phosphorus with and without VAM inoculum on root and shoot development at different growth stages, mycorrhizal root infection, yield contributing parameters and yield of rice.

Materials and Methods

Pot experiment was conducted at BINA headquarter, Mymensingh to evaluate the response of different levels of nitrogen and phosphorus containing fertilizers with or without VAM fungal on vegetative growth at different stages, mycorrhizal root infection, yield contributing parameters and yield of rice. Initial soil samples were randomly collected, air dried and passed through a 2 mm sieve for analyzing some important physicochemical properties. The experimental soils were sandy loam in texture containing 0.46% organic carbon, 0.06% total nitrogen, 14 ppm available phosphorus, 0.13 ppm exchangeable potassium and a pH of 6.9 in H_2O. The treatment combinations were N_0P_0, N_0P_{20}, N_0P_{40}, $N_{100}P_0$, $N_{100}P_{20}$, $N_{100}P_{40}$, $N_{200}P_0$, $N_{200}P_{20}$ & $N_{200}P_{40}$ with or without VAM inoculum. Treatments were replicated thrice and arranged in a completely randomized design. Total number of pots (54) was used in this experiment. A ten kg soils was placed into 20 × 15 × 15 cm earthen pot. Forty two kg K_2O ha^{-1} as muriate of potash and 10 kg S ha^{-1} as gypsum were applied as basal dose. According to treatments combination one third of nitrogen and full dose of phosphorus as urea and triple super phosphate were applied with basal dose of potassium and sulphur containing fertilizers, respectively. Three seedlings of *Oryza sativa* L (cv. BINAdhan 6) were grown in each pot. VAM spores were collected from rice, marigold, mungbean and peanut rhizosphere soils. This inoculum was covered with a soil layer of 2 cm in which rice seedlings were transplanted. The rest nitrogen as urea was applied in two splits at maximum vegetative phage and panicle initiation stage of rice. Cultural practice was done whenever necessary. At 40 and 95 DAT (days after transplanting), single plant was uprooted from the experimental pot. Root and shoot were separated with the help of a sharp scissors. Samples of shoot and root portions of plant at vegetative stages, grain and straw at maturity were sampled for dry weight estimation. For root infection study, fine roots were collected and washed with

water. The washed roots were cut into 1 cm segments and thoroughly mixed. This mixed sample was bleached with 10 per cent (w/v) KOH at 90^0C for 2 h and stained with trypan blue (Phillips and Hayman, 1970). The presence or absence of infection was calculated as follows:

$$\text{Root infection (\%)} = \frac{\text{Number of VAM positive segments}}{\text{Total number of segments}} \times 100$$

Shoot and root dry weight of plant were measured at 40 and 95 DAT. Data on yield contributing parameters and grain yield of rice was computed on 14 per cent moisture basis. Data were statistically analyzed and Duncan's Multiple Range was applied to examine significant differences between the treatment means (Gomez and Gomez, 1984).

Results and Discussion

The application of VAM inoculum had a positive effect on growth dynamics of rice plant. Shoot and root dry weight results of rice at 40 and 95 DAT under different treatments are presented in Table 1. Data indicated that the amount of shoot dry weight varied from 5.00 to 9.75 and 15.00 to 24.33 g plant^{-1} at 40 and 95 DAT and the VAM inoculum significantly increased the shoot dry weight of rice over control (N_0P_0). At 40 DAT, maximum shoot dry weight (9.75 g hill^{-1}) was observed in $N_{200}P_{40}$ + VAM treated plots which followed by $N_{200}P_{40}$ and $N_{200}P_{20}$ + VAM treatments. The lowest shoot dry weight was found in N_0P_0 treatment. At 95 DAT, the highest shoot dry weight (24.33 g hill^{-1}) was found in $N_{200}P_{40}$ + VAM which was statistically identical to $N_{100}P_{20}$ + VAM treatment. The minimum shoot dry weight (5.67 g hill^{-1}) showed in N_0P_0 treatment. Shoot dry weight was higher in VAM treated plots than alone use of different levels of nitrogen and phosphorus treatments including control. Root dry weight ranged from 1.00 to 2.95 and 1.75 to 3.23 g plant^{-1} at 40 and 95 DAT, respectively. At 40 DAT, maximum root dry weight (2.95 g hill^{-1}) was observed in $N_{200}P_{40}$ + VAM treated plots and followed by $N_{200}P_{20}$ and $N_{100}P_{40}$ + VAM treatments. The lowest root dry weight was found in N_0P_{20} treatment. At 95 DAT, the highest root dry weight (3.23 g hill^{-1}) was found in $N_{200}P_{40}$ + VAM which followed by $N_{100}P_{40}$ + VAM treated plots. The minimum root dry weight (1.75 g hill^{-1}) showed in N_0P_{20}. VAM with different doses of N and P treated plots performed better performance than without VAM treated nitrogen and phosphorus treated fertilizers. This may be due to the addition of VAM to all the different doses of N and P and it might have had favorable effect on phosphorus solubilization as well as effective use of nitrogenous fertilizers. Similar promoter effects of VAM on the shoot and root system, of the plants have been reported by several authors (Lionin and Medina, 2002; Fernandez *et al*., 1999). Beneficial effects of VAM on plant growth were observed after the establishment of the symbiont. This response could be close related to the mycorrhizal effectiveness as the symbiosis process involves fluxes of photosynthates to the root system. Results also reported by Debouba *et al*. (2006) showed a greater shoot and root dry matter in tomato plants preinoculated with AMF irrigated with both saline and nonsaline water. Similar shoot and root weight increases were reported in cotton (Tian *et al*., 2004) and soybean (Sharifi *et al*., 2007).

Table 1. Effect of different doses of N and P containing fertilizers with presence or absence of VAM on rice

Treatment	Shoot dry weight (g hill^{-1})		Root dry weight (g hill^{-1})		Root infection (%)
	40 DAT	95 DAT	40 DAT	95 DAT	
N_0P_0	5.00	15.00	1.33	1.80	26.33
N_0P_{20}	5.33	15.33	1.00	1.75	33.00
N_0P_{40}	6.33	16.00	1.40	1.85	29.67
$N_{100}P_0$	6.33	16.33	1.60	2.13	55.00
$N_{100}P_{20}$	8.80	22.67	1.77	2.82	46.33
$N_{100}P_{40}$	8.67	22.33	1.80	2.89	38.00
$N_{200}P_0$	7.67	19.00	1.55	2.56	57.33
$N_{200}P_{20}$	9.00	22.67	1.85	2.90	45.00
$N_{200}P_{40}$	9.67	23.00	1.90	2.95	39.00
N_0P_0 + VAM	5.67	15.67	1.56	1.95	55.00
N_0P_{20} + VAM	6.00	16.00	1.75	1.87	56.00
N_0P_{40} + VAM	6.67	17.00	1.85	1.90	46.33
$N_{100}P_0$ + VAM	6.50	21.33	2.13	2.33	72.00
$N_{100}P_{20}$ + VAM	9.60	24.20	2.82	2.95	68.00
$N_{100}P_{40}$ + VAM	9.13	24.00	2.89	3.00	52.00
$N_{200}P_0$ + VAM	8.20	19.67	2.56	2.65	74.00
$N_{200}P_{20}$ + VAM	9.65	23.00	2.90	2.98	66.00
$N_{200}P_{40}$ + VAM	9.75	24.33	2.95	3.23	56.00
$LSD_{0.05}$	0.27	0.97	1.69	0.97	3.72

Root infection by VAM was observed significantly difference among the treatments (Table 1). Root infection results ranged from 26.33 to 74.33 per cent in N_0P_0 and $N_{200}P_0$ treatments. The maximum root infection (74.33%) was observed in $N_{200}P_0$ + VAM which was statistically similar to $N_{100}P_0$ + VAM and $N_{100}P_{20}$ + VAM treatments. The minimum root infection (26.33%) was found in N_0P_0 treatment. Root infection was higher in VAM treated plots than without VAM treated plots. These results also indicate that VAM had positive effect on per cent root infection. The results of these studies allow us to infer that higher dose of nitrogen fertilizer without or less amount of phosphatic fertilizer with VAM is helpful in augmenting

the higher root infection of rice. Similar findings have been reported by Olalde *et al*. (1994).

Table 2. Effect of different doses of N and P containing fertilizers with presence or absence of VAM on rice

Treatment	Plant height (cm)	No. of tillers hill^{-1}	Panicle length (cm)	Filled grain panicle^{-1}	Unfilled grain panicle^{-1}	1000 seed wt. (g)
N_0P_0	81.00	4.33	16.00	68.33	55.33	19.75
N_0P_{20}	83.67	5.00	16.10	75.20	43.00	20.00
N_0P_{40}	84.67	6.33	16.50	85.33	40.00	20.22
$N_{100}P_0$	96.33	7.67	17.33	95.22	33.67	20.13
$N_{100}P_{20}$	97.67	12.00	20.20	108.00	22.33	21.17
$N_{100}P_{40}$	99.67	11.00	21.00	112.33	18.67	21.70
$N_{200}P_0$	101.00	11.00	20.00	110.22	33.67	21.00
$N_{200}P_{20}$	101.50	12.33	21.33	115.00	22.33	22.10
$N_{200}P_{40}$	103.00	12.67	21.40	121.33	18.33	22.39
N_0P_0 + VAM	84.33	6.50	17.20	88.67	53.67	19.82
N_0P_{20} + VAM	85.00	6.33	17.90	118.00	39.00	20.10
N_0P_{40} + VAM	85.67	8.00	18.20	155.00	36.33	20.45
$N_{100}P_0$ + VAM	90.00	9.00	21.00	165.25	31.67	20.30
$N_{100}P_{20}$ + VAM	102.67	13.33	21.50	170.33	18.67	21.50
$N_{100}P_{40}$ + VAM	102.67	13.00	20.80	175.00	14.33	22.32
$N_{200}P_0$ + VAM	102.00	12.00	21.22	165.33	28.67	21.50
$N_{200}P_{20}$ + VAM	103.33	13.33	22.00	170.00	19.33	24.45
$N_{200}P_{40}$ + VAM	105.00	13.67	22.66	180.00	16.00	24.55
$LSD_{0.05}$	6.05	1.94	3.67	6.40	4.36	2.32

Results on yield contributing parameters are presented (Table 2). Plant height, number of tillers per hill, panicle length, filled grain per panicle and 1000 seed weight ranged from 81.00 to 105.00 cm, 4.33 to 13.67, 16.00 to 22.66 cm, 68.33 to 180.00, 19.75 to 24.55 were found in N_0P_0 and $N_{200}P_{40}$ + VAM, respectively. Higher dose of nitrogen performed better results than lower dose of nitrogen. Different levels of nitrogen and phosphorus in combination with VAM showed higher results than different levels of nitrogen and phosphorus without VAM treatments. These research findings are in conformity of the other researcher's results (Johansen *et al*., 1993; Jacobsen *et al*., 1992). The highest plant height, number of tillers per hill, panicle length, filled grain per panicle and 1000 seed weight was observed in $N_{200}P_{40}$ + VAM. On the other hand, maximum unfilled grain was found in N_0P_0 treatment. Highest plant height result was statistically similar to $N_{200}P_{20}$ + VAM, $N_{200}P_0$ + VAM and $N_{100}P_{20}$ + VAM treatments. Maximum panicle length and 1000 seed weight results were statistically similar to $N_{200}P_{20}$ treatment.

Table 3. Correlation coefficient among grain yield and yield contributing parameters of rice

Parameters	Grain yield	Plant height	No. of tiller	Panicle length	Filled grain	Unfilled grain	1000 seed weight
Grain yield	-	0.202^{NS}	0.953^{**}	0.894^{**}	0.643^{**}	-0.938^{**}	0.869^{**}
Plant height		-	0.191^{NS}	0.145^{NS}	0.320^{*}	-0.201^{NS}	0.183^{NS}
No. of tiller			-	0.949^{**}	0.725^{**}	-0.922^{**}	0.831^{**}
Panicle length				-	0.770^{**}	-0.879^{**}	0.781
Filled grain					-	-0.688^{**}	0.653
Unfilled grain						-	-0.792
1000 seed weight							-

n=54; *, **-indicated significant at 5 and 1% level of significance; NS-nonsignificant.

Grain yield was significantly increased with the increasing levels of N and P in presence of VAM (Fig. 1). Grain yield varied from 2.06 to 4.45 t ha^{-1}. Maximum grain yield (4.45 t ha^{-1}) was observed in $N_{200}P_{40}$ + VAM which was statistically identical to $N_{200}P_{20}$ + VAM treatment. Minimum grain yield was found in N_0P_0 treatment. Highest grain yield was observed in $N_{200}P_{40}$ + VAM compared to other treatments which might be due to the highest production of effective tillers hill^{-1}, increased number of grain panicle^{-1} and 1000 grain yield (data not shown). The significant effects of VAM inoculum on rice was also observed by many researchers (Tu *et al*., 2006; Amani and Rao, 1996). The performance of VAM was not showed significant result in $N_{100}P_{20}$ treatment. An increase in yield in the VAM treatment was noteworthy, as a clear sign of the efficiency of this type of fungal inoculum on the plant productivity. The correlation coefficients of yield contributing parameters were worked out (Table 3) in order to evaluate their influence on rice yield. Unfilled grain had negative and significant correlation with number of tiller, panicle length and filled grain suggesting it lower number of unfilled grain was helped to increase rice yield. Number of tiller per hill showed positive and significant correlation with grain yield to indicate that increasing number of tiller per hill increased grain yield of rice.

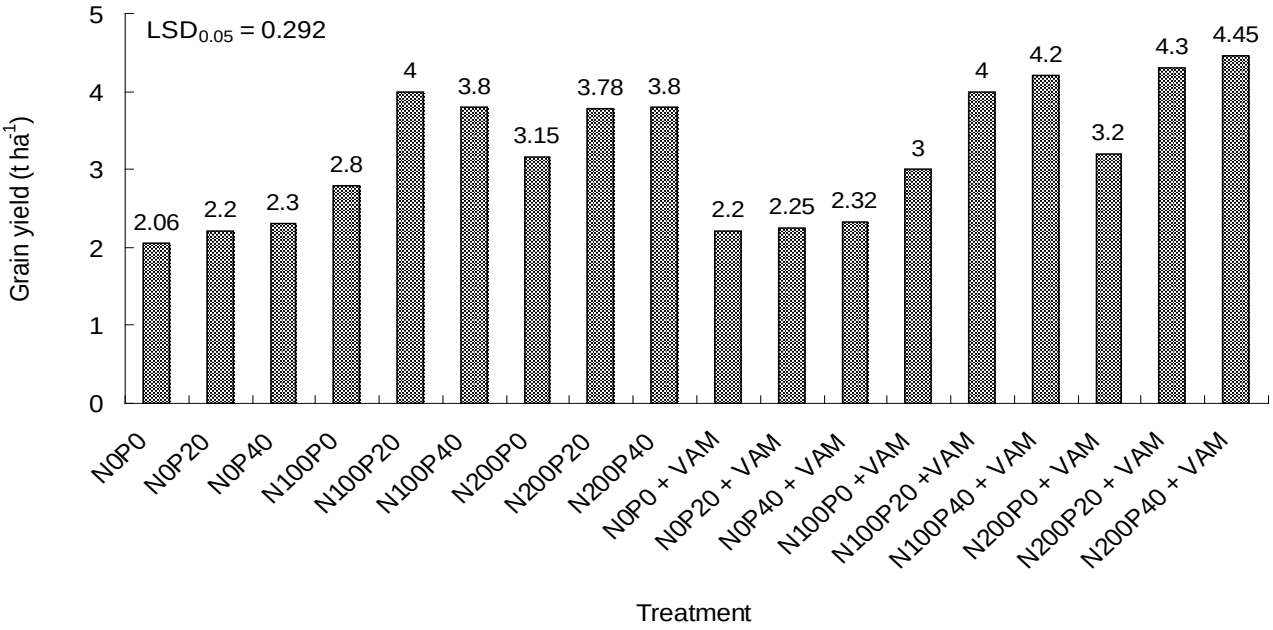

Fig. 1. Effect of different doses of N and P containing fertilizers in presence or absence of VAM on rice yield

It is clear from the results of different doses of N and P levels with VAM inoculum responded well in respect of root and shoots dry weight, root infection, yield contributing parameters and grain yield of rice.

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