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THE ROLE OF VAM- RHIZOBIUM INTERACTION IN GROWTH OF GREEN GRAM (*VIGNA RADIATA* VAR. TARM-1) AT DIFFERENT PHOSPHATE LEVELS

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Abstract

An Experiment was conducted to study the effect of dual inoculation of *Rhizobium* (R) and Vesicular arbuscular mycorrhiza fungi (VAM) *Glomus-mossae* (G) on colonization, growth, yield and nutrient uptake on *Vigna radiata* (var. TARM-1) at different levels of phosphate applied at the rate of 0(P1), 30(P2), 60(P3), kg.ha^{-1} as single superphosphate, Nitrogen and Potassium was applied at recommended levels as urea (20 kg.ha^{-1}), and muriate of potash (20 kg.ha^{-1}). *Vigna radaita* was grown in nonfumigated soil either with or without *G.mossae.* However, all the seeds were treated with *Rhizobium*. Maximum root colonization (95%) was observed in the *Glomus Rhizobium* Phosphate (GRP2), whereas the least was noted *Rhizobium* Phosphate (RP3-22%). Higher dose of phosphorous did not affect the colonization in inoculated plots. Dual inoculation significantly increased the plant dry weight, chlorophyll and grain yield. Dual inoculated plants showed higher nodule number/weight, Nitrogen and Phosphate content.

Key words: *Rhizobium, VAM fungi, Vigna radaita, inoculation, root colonization.*

Introduction

Green gram is cultivated as a 'Kharif' crop and also as a 'Rabi' crop in Uttar Pradesh. In the agricultural system of the tropical countries, the dependence of cultivated plants on VAM is high, low Phosphate availability is one of the most critical factors affecting agricultural production. The leguminous plants respond particularly well to mycorrhizal infection which indirectly increases the possibilities of atmospheric N2-fixation through improved Phosphorous uptake (Bagyaraj *et al.* 1987). The supply of phosphorous is necessary for satisfactory nodulation and symbiotic nitrogen fixation. VAM greatly improve phosphate supply to the host plant which resulted in increase of nodulation and N_2 fixation in legumes. Many tropical legumes are infact highly mycorrhizal dependant (Adholeya *et al.,* 1988).

The most interesting feature of the legumes is their ability to form mutualistic association with certain microbes. Two type of micro-organisms which are found associated with roots of these plants include fungi (mycorrhiza) and bacteria (*Rhizobium*). These root symbionts are known to have synergistic effect on the growth and productivity of leguminous plants. The tripartite relation of *Rhizobium*-legume host-mycorrhiza helps the plant with two major elements i.e. nitrogen and phosphorous. The major beneficial effects associated with the use of mycorrhiza include: better absorption of moisture and nutrients from soil, reduction of pathogenic root infections, improvement in the soil structure. These effects, in turn results in increased survival rates at out planting as well as enhancing growth of plants with reduced fertilizer inputs. Mycorrhizal fungi are biological alternative supplement to expensive chemical fertilizers and pesticides for improving productivity of plants. Both these symbionts are active in root critical cells and it can be assumed that the presence of one symbiont will affect the activity of the other.

The present work was undertaken to study the response of Green gram (*Vigna radiata*) to VAM (*Glomus mossae*) inoculation on colonization, growth, nutrient uptake and yield with different levels of phosphate fertilizers in native soil.

Materials and Methods

The experiment was conducted in the botanical garden of Pt. J.N.P.G. College, Banda in June/July- Dec. 2001. The experimental design was randomized block with four replications of three treatments and their respective controls.

Basic Physico-chemical properties of the soil as shown below-

Properties	Value
pH	7.6
Conductivity	0.35
N ckg ha^{-1}	237.0
P_2O_5 (kgh^{-1})	5.61
K_2O (Kg ha^{-1})	335.0
Organic C (%)	0.30

Spores were isolated from soil samples following wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). Live spores of *G. mosseae* were identified and selected under a binocular stereo microscopic. About twenty spores of *G. mosseae* are surface sterilized by immersing them in 2 present (w/v) chloramines-T and 200 ppm streptomycin (Hi Media- India) for about 15 minutes followed by successive washing in sterile distilled water until the sterilent was removed. These spores were used to infect *Vigna radaita* and *Zea mays* seedlings grown in sterilized soil. The root system was checked microscopically for uniform infection by these VAM fungi at different time interval (after each 5 days). The root systems of uniformly well infected seedling together with adhering soil were finely chopped and used as the starter inoculum to scale up the bulk inoculum production by infecting fresh seedlings raised in sterile soil inoculated with 5 to 10% of starter inoculum as a layer approximately two inches below the soil level. This root based inocula was stored in polyethene bags for use in field inoculation. Inocula containing 225 spores 10 g^{-1} soil were applied (50 kg. ha-1) by spreading in furrows (5 cm deep) at the time of sowing. P was applied as single super phosphate at the rate of 0, 30, and 60 (recommended) kg ha-1 at the time of sowing. N and K was applied at recommended doses as urea (20 kg ha^{-1}) and muriate of potash (20 kg ha^{-1}).

Before sowing, the soil was analyzed for the presence of VAM (Gerdemann and Nicolson, 1963). The initial number of *G. mosseae* spores in the experimental field was @20 spores 10 g^{-1} soil.

Mycorrhizal infection from harvested roots at different time intervals was estimated after staining in trypan blue (Philips and Hayman, 1970). Quantification of VAM colonization was done by morphometric techniques (Toth and Toth, 1982).

The analysis of nitrogen and phosphorous content of plant tissues was carried out by following the methods-

N % = Vol. of the titrant x 0.00007 x 104/0.5 x 5 (Kjeldhal method)

P %= C (mg.) x solution volume (ml.)/10 x aliquot (ml.) x sample wt. (gm.) (Allen 1989)

The data were evaluated statistically by Anov 'A' and students 't' test.

Results and Discussion

Table-1.a and 1.b clearly indicate the superiority of dual inoculation in almost all the parameters including percent root colonization at different P level, total chlorophyll, plant height, seed index, number of pods/plant, days of maturity, plant dry weight number of nodules and nodule dry weight, nutrient uptake (N, P) in plant tissues, and grain yield in *Vigna radaita* under field conditions.

The interaction between *Rhizobium* and VAM was synergistic for nodulation of the plant. The maximum root nodules were observed in GRP2 (80%) whereas the least was at RP1 (47%) similarly, the GRP2 plants showed maximum nodule dry weight (0.64 gm) over relative control plants.

The progress of colonization of the roots was assessed from 15 days after sowing up to harvest (90 days) at 10 day intervals. Root colonization in all the inoculated plots with VAM increased with plant growth and root colonization was observed at day after sowing = 90 (Before harvesting). In the first sampling, at 15 days there was little colonization. The fungus entered the root mainly through epidermal cells forming an appressorium and rarely through root hairs.

The VAM fungus showed three phases of development viz. a phase of slow development up to 30 days, by the end of which few roots become mycorrhizal, followed by a gradual increase in colonization up to 45 days and finally a constant phase, where there was no further increase in root colonization. Arbuscules appeared from the first sampling and showed up their presence in all the samples throughout the sampling period. The percent colonization varied with the applied P level. The maximum colonization was observed in GRP2 (94 %) whereas the least was at RP3 (22 %). There was no significant increase in colonization in GRPI over RPI. Dual inoculation significantly increased the grain yield in all the plants. The maximum grain yield was noted at GRP3 (26.8 q ha^{-1}) whereas the least was at GRPI (6.8 q ha^{-1}). (Table 1b).

Significant increase in N, P uptake was also the outcome of interaction of *Rhizobium* and VAM. This clearly indicates the synergistic role played by the VAM fungi and *Rhizobium*. The maximum P uptake was noted in the GRP3 (29.3 ug g^{-1} dry wt. plant). Whereas the least was in RP1 (10.3 mg/g dry wt. plant). Similarly the maximum N content was noted in GRP2 (348.5 mg/g) and at least was at RP1 (166.1/ug /g. wt. plant) Nodulation and N content were positively correlated.

The interaction between mycorrhiza and P fertilizer is complex. P fertilizers have varied effects on the VAM symbiosis and on the fungi themselves (Abbott and Robson 1991, Sylvia and Neal 1990). Reduction in root colonization has been reported at very high or very low availability of P (Amijee *et al.,* 1989). Such responses vary with sensitivities of the fungi to phosphorous (Schubert and Hayman, 1986). However the present findings indicate a statistically significant ($P<0.05$) increase in colonization in VAM inoculated plants independent of P doses (Table 1.a).

A significant ($p < 0.05$) increase in plant dry weight and total chlorophyll was observed in all the dual inoculated plants (Table 1.a and 1.b). However, the plants most benefited from dual inoculation were at GRP2. Our observation is in agreement with observations on other plants (Becard and piche 1989, Boyle and Pant 1989).

Principle effect of mycorrhiza on nodulation is phosphate mediated. Mycorrhiza may have other secondary effects possibly of hormonal nature (Mosse 1977). This is supported by the finding of higher p content in the mycorrhizal inoculated plants (dual inoculation). The change was highly significant ($p <0.05$). Whereas the similar pattern was observes for N uptake with respect to nodulation also (Table 1.b). GRP2 was superior over relative control. Therefore, the over all better plant growth could be attributed to enhanced absorption of N and P. It has been reported that mycorrhizal fungi could improve P uptake by roots by improving physical exploration of the soil pore space (O'keefee and Sylvia, 1993). More than twenty five percent gain in yield was recorded in GRP3 plants over relative control. Where as the gain in yield was recorded in GRP3 plants over relative control. Where as the gain was approximately 50 % and 6.1 % for GRP2 and GRPI. However no significant increase in pod number was noted in any treatment. The increase in seed index resulted in overall gain in yield.

The statistically significant increase in growth and yield of *Vigna radaita* by dual inoculation of *Rhizobium* and VAM (*Glomus*) concomitant with increase in colonization, nodulation and nutrient uptake has led us to suggest that multilocational field trials be conducted in different agroclimatic zones for dual inoculation. However, the suitable VAM partner must be identified. This would benefit tropical farmers by lessening input in phosphate fertilizers. Trials with rock phosphate, DAP (cheaper source of P) may also be considered for future planning.

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Table 1a : Effect of plant (*Vigna radaita)* microbe (*G. mosseae*) or *Rhizobium* interaction under variable P levels on colonization, growth and nodulation.

Parameters	RP3	RP2	RP1	GRP3	GRP2	GRP1	F Value
Colonization (%)	22+1.0 a	30+1.0 a	35+1.4 a	8.5+3.8 b	94+4.5 b	35+4.5 b	*
Growth Parameters (Dry weight)							
Total Plant (g)	19+0.74 a	17.8+0.68 a	11.4+0.31 a	22.6+0.74 b	24.1+0.93 b	14.5+0.6 b	*
Shoot (S)	14.2+0.56 a	14.2+0.56 a	7.91+0.27 a	16.4+0.60 b	18.0+0.68 b	9.42+0.39 b	*
Root (R)	4.75+0.19 a	3.56+0.14 a	3.39+0.12 a	6.3+0.23 b	6.0+0.23 b	5.07+0.2 b	**
Chlorophyll (mg/gm^{-1}/leaf/wt.)	14.2+0.56 a	15.6+0.58 a	10.3+0.39 a	29.3+0.10 b	26+0.66 b	13.5+0.54 b	*
Nodulation							
Nodule number	63+2.7 a	66+2.8 a	47+1.8 a	79+3.5 b	80+3.6 b	60+2.2 b	*
Nodule dry wt. (g)	0.42+0.012 a	0.48+0.01 a	0.33+0.01 a	0.61+0.02 b	0.65+0.02 b	0.49+0.01 b	**

The data represent Mean+SE of four replication. Value not followed by identical letters in each horizontal columns are significantly different at 5% level of probability.
* = P<0.5, ** = P<0.1

RP3 = *Rhizobium* + phosphate = 60 kg ha^{-1}
RP2 = *Rhizobium* + phosphate = 30 kg ha^{-1}
RP1 = *Rhizobium* + phosphate = 0 kg ha^{-1}
GRP3 = *Glomus* + *Rhizobium* + 60 kg ha^{-1}
GRP2 = *Glomus* + *Rhizobium* + 30 kg ha^{-1}
GRP1 = *Glomus* + *Rhizobium* + 0 kg ha^{-1}

Table 1b : Effect of plant (*Vigna radaita)* microbe (*G. mosseae*) or *Rhizobium* interaction under variable P levels on grain yield and nutrient uptake.

Parameters	RP3	RP2	RP1	GRP3	GRP2	GRP1	F Value
Yield No. of							
Pods/Plant	37.0+2.0 a	31+0.9 a	22+0.8 a	36+1.8 b	33+1.5 b	26+0.89 b	
Seed Index	10.2+0.41 a	9.18+0.31 a	6.8+0.29 a	12.8+0.38 b	13.2+4.8 b	7.5+0.32 b	**
Grain Index (q/ha)	20.1+0.63 a	18.2+0.71 a	6.8+0.24 a	26.8+0.83 b	27.6+1.1 b	7.5+0.28 b	**
Nutrient Uptake (mg/gm dry wt.)							
P	20.8+0.8 a	15.6+0.5 a	10.3+0.36 a	29.3+0.93 b	26.0+0.91 b	13.5+0.5 b	
N	248.7+9.12 a	260.2+8.91 a	166.4+4.5 a	331.2+12.9 b	348.5+13.6 b	1995+6.1 b	

The data represent Mean+SE of four replication. Value not followed by identical letters in each horizontal columns are significantly different at 5% level of probability.
* = P<0.5, ** = P<0.1

RP3 = *Rhizobium* + phosphate = 60 kg ha^{-1}
RP2 = *Rhizobium* + phosphate = 30 kg ha^{-1}
RP1 = *Rhizobium* + phosphate = 0 kg ha^{-1}
GRP3 = *Glomus* + *Rhizobium* + 60 kg ha^{-1}
GRP2 = *Glomus* + *Rhizobium* + 30 kg ha^{-1}
GRP1 = *Glomus* + *Rhizobium* + 0 kg ha^{-1}

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