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Original Article

Effects of Inoculation with *Rhizobium* and Arbuscular Mycorrhiza and Phosphorus on Growth, Yield and Nutrient Uptake by Pea Grown in Soil

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The experiment was conducted in pot at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh to study the response of pea (*Pisum sativum*) cv. IPSA Motorshuti-3 to dual inoculation with *Rhizobium* (R) and Arbuscular Mycorrhiza (AM) in presence and absence of phosphorus (P) on root colonization, spore population, nodulation, nitrogenase activity, growth, yield, and nutrient uptake by shoot. The performance of *Rhizobium* inoculant alone was superior to control in all the parameters of the crop studied. Among the treatments dual inoculation with *Rhizobium* and Arbuscular Mycorrhiza in combination with 25 kg P ha^{-1} performed best in recording per cent root colonization, number and dry weight of nodules, dry weight of shoots, nitrogenase activity, number of pods/plant, number of seeds/pod, pod and seed yields, and nutrient uptake by pea. This treatment combination recorded green pod yield of 12.0 g/plant (140 % increase over control) and mature pod yield of 7.0 g/plant (146% increase over control). The effect of this treatment was however similar to the effect of dual inoculation with R and AM in combination with 12.5 kg P ha^{-1} in most of the parameters. From the view point of nodulation, nitrogenase activity, growth, and yield of pea, dual inoculation with *Rhizobium* inoculant and arbuscular mycorrhiza in combination with 25 kg P ha^{-1} was considered to be the best for the supply of balanced combination of nutrients for achieving the maximum output through cultivation of pea in Shallow Red Brown Terrace soil of Bangladesh.

Keywords: Pea, *Rhizobium*, Arbuscular mycorrhiza, Phosphorus

Introduction

Pea (*Pisum sativm* L.) is the most important pulse crop for diet and is also used in preparing a variety of snack foods, sweets and condiments. Nutritionally, pea is relatively free from various antinutritional factors, has a high protein digestibility, and is richer in phosphorus and calcium than other pulses. The leguminous plants respond well particularly to mycorrhizal infection, which indirectly increases the possibilities of atmospheric N_2 fixation through improved P uptake [1]. Inoculation of legumes with AM fungi can stimulate nodulation and nitrogen fixation[2]. Most legumes are associated with nitrogen fixing organisms and AM fungi[3]. This double symbiosis enables legumes to accumulate large amount of nutrients even under sub-optimal soil conditions. Legumes have less extensive root systems and are dependent on colonization by native AM fungi for their nutritional needs[4].

Several studies are available on the interaction between AM fungi and rhizobia for soybean[5], greengram[6], and chickpea[7]. A synergistic effect of dual inoculation with AM fungi and bradyrhizobia/rhizobia on growth and nutrition in legumes has been demonstrated[8]. Mycorrhizal infection might help pea to obtain the required phosphorus for nodulation. A pot experiment was conducted[9] and found that dual inoculation with P fertilizers recorded the highest nodule number and nodule weight both at pre-flowering and 50% flowering stages of chickpea. But no attempt has so far been made on the dual inoculation of AM and *Rhizobium* on pea. The present experiment was carried out to assess the role of AM fungi, *Rhizobium* and phosphorus on the growth, yield and nutrient uptake by pea.

Materials and Methods

A pot experiment was carried out at the premises of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh to study the effect of dual inoculation of AM and *Rhizobium*, and phosphorus on pea variety IPSA Motorshuti-3, a newly released variety as the test crop. The soil was silty clay loam having organic carbon 0.94%, pH 6.5, CEC 15.5 meq/100 g soil, total nitrogen 0.07%, available P 12 ppm, available K 0.32 meq/100 g soil, exchangeable Ca 6.5 meq/100 g soil, exchangeable K 7.44 meq/100 g soil, exchangeable Mg 3.30 meq/100 g soil, exchangeable Na 0.76 meq/100 g soil. The number of viable *Rhizobium* was 4.1 x 10^5/g soil. Standard methods were followed to determine the above properties. Eight kilogram sterilized soil was put in each earthen pot. Phosphorus (P) at the rate of 12.5

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and 25 kg P_2O_5/ha was applied as triple super phosphate. Twenty grams arbuscular mycorrhiza in the form of root, spore, soil etc. was applied. Potassium (K) at the rate of 35 kg K_2O/ha as muriate of potash, Sulphur (S) at the rate of 20 kg/ha as gypsum and Molybdenum (Mo) at the rate of 1 kg/ha as ammonium molybdate were applied as basal dose. The experiment was laid out in a complete randomized design (CRD) with 3 replications. There were 7 treatment combinations, viz T_1 Control, T_2 *Rhizobium* (R), T_3 Arbuscular Mycorrhiza (AM), T_4 (R + AM), T_5 (R + 25 kg P/ ha), T_6 (R+ AM+ 12.5 kg P/ ha), and T_7 (R+ AM+ 25 kg P/ ha). Four sets of pot arrangements were made for evaluation of vegetative, flowering, pod filling, and harvesting stages. Earlier soils of a seed bed were mixed with AM inoculum and sorghum (*Sorghum vulgare L*) plant was grown it. The plants were allowed to grow up to vegetative stage and then harvested. Roots of sorghum with rhizosphere soil were used as AM inoculum for the pot experiment. A layer of inoculum (2 g) was placed in pots as per treatments filled with sterilized soil and was covered with a thin soil layer (2 cm) in which seeds were sown. *Rhizobium* strain Rps-2001 was used for preparation of inoculum using sterilized peat as carrier material. Counts of viable rhizobia present in the inoculum were taken following the Drop Plate method[10]. The number of viable cells was $1.5x10^9$/g of inoculum. Seeds of pea were surface sterilized using methyl alcohol and mercuric chloride before inoculation. Then 1.12 g *Rhizobium* inoculum was mixed with 56 g seeds with the help of gum arabic as sticking agent. Four seeds were sown in each pot. Pots were irrigated up to saturation to allow the soil and inoculum to settle down in the pots. After germination of seeds, two healthy seedlings were allowed to grow per pot. Pots were watered whenever necessary to maintain field moisture condition. Intercultural operations were done when necessary. The plants were observed daily to record any change of growth. The plants were free from insects and diseases. At the time of data collection the plants were carefully uprooted with minimum disturbance of roots. The roots were washed with tap water. Roots and shoots were separated with the help of a sharp scissors. Nodules were separated from the roots and then nodule number and weight were recorded and the roots were preserved for determination of per cent root colonization after necessary processing. The root pieces (2 cm) were stained following the method of Koske and Gemma[11] with some modifications[12]. The percentage of AM root colonization was estimated by root slide technique and the spore population was determined following the wet sieving and decanting method[13]. Shoots and nodules were dried in an oven for 72 hours at 70^0C. The oven-dried plant shoot was ground in a grinding machine (Wiley Pulverizer, Type 1029-8, Yoshida Seisakusho Co. Ltd). Total N content in the shoot was determined by ashing the plant material using salicylic acid modified kjeldahl method following sulfuric acid digestion and then colorimetric assay. Colorimetric determination of phosphorus using molybdivanado phosphoric acid was done to assess phosphorus content in shoot. Nitrogen and phosphorus uptake by shoot were calculated from the data on dry matter yield and nitrogen and phosphorus contents in shoot material of the crop. Nitrogenase activity was determined by acetylene reduction assay. All data were analyzed using MSTAT-5 programme.

Results and Discussion

Per cent root colonization

The effects of different combinations of *Rhizobium*, AM and P on root colonization are presented in Table 1. The highest root colonization was found in the treatment T_7 at every stage of growth. At vegetative stage, the highest root colonization (80%) was found in T_7 which was significantly higher over other treatments but statistically similar to T_6 and T_4 and. At flowering stage, the highest root colonization (83.3%) was also found in the treatment T_7. The effect of this treatment was similar to the effects of the treatments T_6 and T_4 but superior to T_5, T_3, T_2 and control. At pod filling stage, the root colonization was higher than any other stage in each treatment and the highest was found in treatment T_7 whose effect was however, similar to T_6 and T_4 but superior to rest of the treatments. The lowest colonization was found in control condition in every stage of growth. Per cent root colonization was increased significantly by dual inoculation than single inoculation. Similar results were reported by several researchers[5,14] who reported increased root colonization in soybean by dual inoculation of AM and *Rhizobium*. In this study, colonization percentage of AM was higher with dual inoculation.

Number of spore per 100 g soil

The highest numbers of spore population at vegetative (27.2/ 100 g soil), flowering (31.0/100 g soil) and pod filling (33.1/100 g soil) stages were obtained in T_7 (Table 1). At vegetative stage, the effect of this treatment was statistically similar to T_6 but superior to all other treatments. Similar results were found at flowering stage. There was no significant difference among the treatments T_5, T_2 and control as these treatments were free from AM at all the growth stages of the crop. Treatment T_4 showed higher number of spore than T_2. Similar results were reported by several workers [14]. Dual inoculation was more effective when P fertilizer was added. Time was also a factor for increasing number of spore. The number of spore increased at pod filling stage than the other two stages due to multiplication of AM spore.

Nodulation

At vegetative stage, the highest number of nodule (31.0/plant) was obtained in T_7, which was significantly higher than other treatments (Table 2). The lowest number of nodule was obtained in control. At flowering stage, the highest number of nodule (33.8/plant) was recorded in T_7, but the effect of this treatment was statistically similar to T_6 and superior to other treatments. Among the different combinations of *Rhizobium*, AM and P, the lowest number of nodule was obtained with arbuscular myorrhiza (T_3). The highest number of nodule was always recorded with T_7 followed by T_6 at flowering stage and T_5 at pod filling stage. The effect of T_6 was statistically similar to the effect of T_5. The highest number of nodule in T_7 may be attributed to greater availability of P, which is crucial for

Table 1. Per cent root colonization and spore number in rhizosphere soil at different growth stages of pea

Treatment	Root colonization (%)			No. of spore per 100 g soil		
	Vegetative stage	Flowering stage	Pod filling stage	Vegetative stage	Flowering stage	Pod filling stage
T_1. Control (-RAMP)	43.3d	46.7e	56.7d	5.2c	7.7c	9.0e
T_2. *Rhizobium*(*R*)	50.3c	55.0d	60.0cd	8.7c	10.0c	13.0d
T_3. AM	65.0b	70.3bc	69.7b	16.0b	18.0b	17.0c
T_4. *R*+AM	73.3ab	76.3ab	83.30a	17.0b	19.4b	18.0c
T_5. *R*+25 kg Pha^{-1}	56.7c	66.7cd	65.0c	7.0c	11.4c	13.1d
T_6 *R*+AM+12.5 kg Pha^{-1}	73.5ab	76.4ab	86.7a	24.7a	28.0a	30.1b
T_7. *R*+AM+25 kg Pha^{-1}	80.0a	83.3a	90.3a	27.2a	31.0a	33.1a
CV%	4.48	4.22	3.47	13.35	13.17	4.92

Values followed by a common letter in the same column are not significantly different at 5% level.

Table 2. Effect of *Rhizobium* inoculant, arbuscular mycorrhiza and phosphorus and their combinations on number, dry weight and nitrogenase activity at different growth stages of pea

Treatment	Number of nodule per plant			Dry weight of nodule (mg)/plant			Nitrogenase activity (µ mol C_2H_4/plant/ hr)		
	Vegetative stage	Flowering stage	Pod Filling stage	Vegetative stage	Flowering stage	Pod Filling stage	Vegetative stage	Flowering stage	Pod filling stage
T_1. Control (-RAMP)	5.0g	17.8e	11.0d	66.7f	35.0e	55.0c	0.149c	0.210c	0.196d
T_2. *Rhizobium*(*R*)	17.7e	28.3c	20.5b	100.0c	66.7c	65.0b	0.248ab	0.278ab	0.281b
T_3. AM	14.5f	21.5d	14.0d	60.0e	53.3d	55.0c	0.227b	0.231bc	0.233c
T_4. *R*+AM	23.7c	28.3c	22.7b	116.7b	78.3ab	70.0b	0.248ab	0.279ab	0.286b
T_5. *R*+25 kg Pha^{-1}	23.2d	30.0b	23.2ab	160.8a	78.3ab	71.7b	0.280ab	0.280ab	0.286b
T_6.*R*+AM+12.5 kg Pha^{-1}	27.0b	33.2a	23.5ab	170.7a	82.2a	72.0b	0.298a	0.300a	0.288a
T_7. *R*+AM+25 kg Pha^{-1}	31.0a	33.8a	25.5a	175.0a	83.2a	75.0a	0.302a	0.305a	0.300a
CV%	0.06	2.38	0.01	1.67	5.15	4.28	8.59	8.54	3.07

Values followed by a common letter in the same column are not significantly different at 5% level

nodulation[15]. Further, dual inoculation (R+AM) produced significantly higher nodule number as compared to single inoculation at every stage. These results are in good agreement with other workers[16,] who reported increased nodulation due to dual inoculation as compared to single inoculation. At vegetative stage every treatment produced lower number of nodules as compared to flowering stage indicating that at early stage of growth AM was not fully capable of extracting required P for nodulation. Like nodule number, the highest nodule weight was also recorded with dual inoculation along with phosphorus (T_7) which was significantly higher over all other treatments at every stage. Similar results were also reported by other worker[17]. Treatments T_6, T_5, T_4 and T_2 alone recorded nodule weights of 72.0, 71.7, 70.0 and 65.0 mg/plant, respectively at pod filling stage, which was significantly higher than the treatment T_3.

Nitrogenase activity

Nitrogenase activity of root nodule bacteria of pea was significantly influenced by different combinations of *Rhizobium* inoculant, AM and P at all the growth stages (Table 2). Among the treatments, dual inoculation along with added phosphorus (T_7) scored the highest nitrogenase activity at all the growth stages of the crop. The highest nitrogenase activity of 0.302, 0.305 and 0.300 µmol C_2H_4/plant/hr was recorded by the treatment T_7 at vegetative, flowering and pod filling stages, respectively which was statistically similar to T_6, T_5, T_4 and T_2 except pod filling stage. The lowest nitrogenase activity was recorded in control at all the growth stages of the crop. Dual inoculation with added phosphorus showed the highest nitrogenase activity and with the increases of phosphatic fertilizer, activity of root nodule bacteria increased. Significantly higher nitrogenase activity due to inoculation of chickpea with *Rhizobium* was also repoted[18]. It was observed in pea that *Rhizobium leguminosarum* increased nitrogenase activity[19,20].

Plant height

At vegetative stage, the highest plant height (26.5 cm/plant) was recorded by T_7 treatment which was statistically similar to T_6, T_5 and T_4 (Table 3). At flowering stage, although the maximum plant

height (74 cm) was recorded in T_7 but no significant difference was observed with T_6. At pod filling stage, the similar trend was found. At harvesting stage, all treatments were statistically similar except T_1 and T_3. Data revealed with few exceptions that dual inoculation along with P (R+AM+P) showed significant increase in plant height compared to AM or *Rhizobium* alone or in combination of these two. The maximum plant height in greengram by the dual inoculation of AM+ *Rhizobium* along with 50% recommended nitrogen and phosphorus fertilize was reported[6].

Dry weight of shoot

The highest shoot dry weights of 363, 201 and 248 mg/plant were recorded in T_7 at vegetative, flowering and harvesting stages, respectively. Treatment T_6 recorded the highest dry weight of shoot of 298 mg/plant at pod filling stage (Table 3). The lowest dry weight of shoot was recorded in control at all growth stages of the crop. Dual inoculation (R+AM) increased shoot weight compared to plants inoculated with individual endophyte (R or AM). Similar results of two herbaceous legumes were reported[14]. It was reported that dual inoculation of chickpea with *Rhizobium* and AM in presence of N and P increased dry weight of shoot compared to single inoculation[7].

Number of pods per plant

Data in Table 4 revealed that the highest numbers of green and mature pod were obtained in T_7. In case of green pod, the effect of T_7 was statistically similar to T_6 and T_5 but significantly higher over other treatments. In case of mature pod the effect of T_7 was similar to T_6, T_5 and T_4 but statistically higher to T_3 and control. The lowest number of pod was found in control in both the cases. Dual inoculation in presence of P showed the best performance among the treatments (Table 4).

Number of seed per pod

Plant receiving *Rhizobium* inoculum and AM in combination with 25 kg P ha^{-1} (T_7) produced the highest number of both green and mature seed per pod (Table 4). The effect of this treatment was statistically similar to T_6 and T_5 but higher to other treatments in case of green seeds. Treatment T_7 produced statistically similar number of mature seed per pod as that of T_6, T_5 and T_4. *Rhizobium* inoculum recorded comparatively higher number of seeds than AM in both the cases. It was observed that biologically fixed N exhibited a significant effect on the number of seed per pod. Dual inoculation was more effective than single inoculation which is in agreement with other researchers[6]. The lowest number of seeds per pod was obtained with control in both the cases.

Table 3. Effect of *Rhizobium* inoculant, arbuscular mycorrhiza and phosphorus and their combinations on plant height and dry weight of shoot at different growth stages of pea

Treatment	Plant height (cm)				Dry weight of shoot per plant (mg)			
	Vegetative stage	Flowering stage	Pod filling stage	Harvesting stage	Vegetative stage	Flowering stage	Pod filling stage	Harvesting stage
T_1. Control (-RAMP)	14.7c	44.1e	60.0b	53.0b	208.0e	112.0e	192.0e	114.0e
T_2. *Rhizobium*(*R*)	21.3b	63.7bc	64.5b	60.0a	356.0ab	140.0d	256.0c	193.0c
T_3. AM	15.4c	50.8d	60.3b	55.5b	290.0d	121.2e	200.0d	141.0d
T_4. *R*+AM	24.1ab	64.0bc	66.5b	61.0a	361.0a	160.1c	272.0b	193.9c
T_5. *R*+25 kg Pha^{-1}	24.4ab	66.0b	65.2b	62.7a	360.0a	182.0b	295.0a	194.2c
T_6.*R*+AM+12.5 kg Pha^{-1}	23.4ab	68.3ab	80.2a	65.0a	362.0a	200.0a	298.0a	228.0b
T_7 .*R*+AM+25 kg Pha^{-1}	26.5a	74.0a	86.2a	66.1a	363.0a	201.0a	297.0a	248.1a
CV%	8.15	4.32	4.75	3.67	1.22	2.83	1.25	1.41

Values followed by a common letter in the same column are not significantly different at 5% level

Table 4. Effect of *Rhizobium* inoculant, arbuscular mycorrhiza and phosphorus and their combinations on number of pod/plant, number of seed/pod and pod yield per plant of pea

Treatment	Number of pods per plant		Number of seed per pod		Pod yield per plant (g)	
	Green pod	Mature pod	Green seed	Mature seed	Green seed	Mature seed
T_1. Control (-RAMP)	3.8c	1.9c	2.7d	3.0d	5.0d	2.9c
T_2. *Rhizobium*(*R*)	4.0c	6.2ab	3.1cd	4.1b	10.9b	5.3b
T_3. AM	3.8c	5.0bc	3.0d	3.4c	6.5c	3.5c
T_4. *R*+AM	4.7c	8.5ab	3.5bc	4.5ab	11.0b	6.4b
T_5. *R*+25 kg Pha^{-1}	6.0ab	6.5ab	3.8ab	4.6a	11.9a	6.4b
T_6. *R*+AM+12.5 kg Pha^{-1}	6.2ab	8.5ab	3.8ab	4.6a	11.9a	6.5b
T_7. *R*+AM+25 kg Pha^{-1}	7.0a	9.3a	4.8a	4.7a	12.0a	7.0a
CV%	7.45	11.87	4.57	4.66	4.57	4.66

Values followed by a common letter in the same column are not significantly different at 5% level

Table 5. Effect of *Rhizobium* inoculant, arbuscular mycorrhiza and phosphorus and their combinations on N and P uptake by shoot at different growth stages of pea

Treatment	N uptake by shoot mg/plant				P uptake by shoot mg/plant			
	Vegetative stage	Flowering stage	Pod filling stage	Harvesting stage	Vegetative stage	Flowering stage	Pod filling stage	Harvesting stage
T_1. Control (-RAMP)	3.8e	7.1g	5.0e	3.5e	1.6d	3.3f	2.2d	1.5d
T_2. *Rhizobium*(*R*)	8.5d	18.2e	14.1d	7.9d	1.7d	4.1e	3.0d	1.7d
T_3. AM	4.1e	9.4f	5.2e	4.0e	3.5c	7.1d	6.0c	3.3c
T_4. *R*+AM	10.3c	21.2c	15.2c	8.9c	4.6b	9.2c	7.4b	4.7b
T_5. *R*+25 kg Pha^{-1}	10.7c	20.0d	15.1c	9.1c	4.6b	9.1c	8.0b	4.9b
T_6. *R*+AM+12.5 kg Pha^{-1}	15.8b	31.5b	25.1b	13.7b	5.1a	10.2b	9.2a	5.1ab
T_7. *R*+AM+25 kg Pha^{-1}	17.2a	36.2a	28.1a	16.1a	5.5a	11.1a	9.6a	5.7a
CV%	2.63	10.5	2.14	4.34	4.98	3.24	5.93	7.09

Values followed by a common letter in the same column are not significantly different at 5% level

Pod yield per plant

The highest green pod yield of 12.0 g/plant was found in T_7 showing 140% higher yield over control (Table 4). The effect of this treatment was statistically similar to the treatment T_6 and T_5 but higher to other treatments. All treatments produced significantly higher pod yield over uninoculated control. The highest mature pod yield was also obtained with T_7 whose effect was statistically similar to the treatments T_6, T_5 and T_4 but higher other treatments. Treatment T_2 recorded comparatively higher pod yield than T_3. Dual inoculation along with P fertilizer recorded significantly higher pod yields compared to dual inoculation alone. The lowest mature pod yield was found in control plants.

N uptake by shoot

N uptake by shoot was influenced significantly by the single inoculation of *Rhizobium* over control in all growth stages of pea (Table 5). However, dual inoculation (R+AM) showed significant effects over single inoculation. Treatment T_7 recorded the highest amount of N uptake which was statistically higher than other treatments at all growth stages. The highest uptake of 17.2, 36.2, 28.1 and 16.1 mg/plant at vegetative, flowering, pod filling and harvesting stages, respectively were recorded in this treatment (T_7). The lowest N uptake by shoot was recorded in control. Plants receiving both the inoculants along with P fertilizer (R+AM+P) registered maximum N uptake, rather than plants, which were dual inoculated without P fertilizers (R+AM). It was reported that on severely P deficient soils, P application could led to large increase in early root growth, a prerequisite for early mycorrhizal infection and a subsequent significant contribution of AM to enhance plant growth and nitrogen uptake[22].

P uptake by shoot

The highest P uptake by shoot of 5.5, 11.1, 9.6, and 5.7 mg/plant at vegetative, flowering, pod filling and harvesting stages, respectively was found with T_7 which was statistically similar to T_6 but higher other treatments except flowering (Table 5). The results of this study clearly indicate that P uptake was higher when P fertilizer was added with dual inoculation, which was supported by other researchers[16]. The highest P uptake by mycorrhizal one than non- mycorrhizal plants was supported by many other researchers[23].

Conclusion

Dual inoculation with *Rhizobium* inoculum and arbuscular mycorrhiza in combination with 25 kg P ha^{-1} was the best in achieving maximum yield of pea in Shallow Red Brown Terrace soil of Bangladesh.

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