EFFECT OF INOCULATION METHODS OF *RHIZOBIUM* ON YIELD ATTRIBUTES, YIELD AND PROTEIN CONTENT IN SEED OF PEA

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ABSTRACT

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A field experiment was conducted at the experimental farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during November, 2003 to March, 2004 to evaluate different methods of *Rhizobium* inoculation on yield attributes, pod and seed yields and protein content in seed of pea (*Pisum sativum*) cv. IPSA Motorshuti-3. Seed or soil or both seed and soil were inoculated either with *Rhizobium* strain BARI RPs-2001 or BARI RPs-2002. The performances of *Rhizobium* strains BARI RPs-2001 and BARI RPs-2002 used as seed or soil inoculation were superior to uninoculated control in all the parameters of the crop. Among the treatments, seed and soil inoculation with *Rhizobium* strain BARI RPs-2002 performed best in recording number of pods/plant, number of seeds/pod, 1000- seed weight, pod and seed yields and protein content in seed of pea plants. The highest green pod yield (3.29 t/ha), green seed yield (1.90 t/ha), mature pod yield (1.03 t/ha) and mature seed yield (0.76 t/ha) were obtained by coinoculation of seed and soil with *Rhizobium* strain BARI RPs-2002 showing 53, 58, 91 and 100% increase in yield over uninoculated control, respectively. Coinoculation of both seed and soil with *Rhizobium* strain BARI RPs-2001 was similar to the effect of the treatment receiving seed and soil inoculation with *Rhizobium* strain BARI RPs-2002. There was a positive correlation among yield attributes and pod and seed yields and protein content in seed of pea. From the viewpoint of yield attributes and yield of pea, coinoculation of both seed and soil with *Rhizobium* strain BARI RPs-2002 was considered to be an effective treatment for achieving the maximum output through cultivation of pea in Shallow-Red Brown Terrace soil.

Key words: *Rhizobium*, yield attribute, protein content

INTRODUCTION

Pea (*Pisum sativm* L.) is one of the widely spread, early maturing legume crops grown during the winter seasons in Bangladesh. The green pods and mature seeds of pea are rich in protein and vitamins. As pea is short durable crop it's cultivation is highly profitable and preferable to the farmers. It can be grown in all types of soil. It grows best in the soils having pH 5.5 to 6.7. This crop like many other legumes is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with *Rhizobium*. The crop thus improves soil, economizes crop production reducing the requirement of added synthetic nitrogenous fertilizers.

Seed inoculation is the most widely used methods of inoculant application. Many researchers (Rahman *et al.*, 1994; Solaiman, 1999; Solaiman and Habibullah 1990; Solaiman and Rabbani, 2005) have reported the beneficial effects of inoculation of grain legumes. Research report showed that *Rhizobium* inoculated plants added 80 kg N/ha and average yield was increased in pea plants over uninoculated control (Micanovic *et al.*, 1996). A significant increase in pod yield was obtained by *Rhizobium* inoculation of peas (Feng *et al.*, 1997). It was observed that seed yield and protein content was increased by 1200-170 kg/ha 18-25%, respectively with *Rhizobium* inoculation (Tolkachev *et al.*, 1994). Direct application of peat powder inoculants to seed was the most common until an easier method of seed application was introduced with liquid inoculants. The inoculant can be applied to the soil as a solid or as a liquid form. A solid inoculant or soil implant is produced by coating a solid granulated material with peat inoculant with an adhesive. Liquid inoculant method is very effective which increases protein content in seed of pea. A peat culture of *rhizobia* is suspended in water which can be applied by a gravity-flow applicator or sprayer. Considering these views in mind, the present study was undertaken to assess the effect of inoculation methods of *Rhizobium* on yield attributes, yield and protein content in seed of pea.

MATERIALS AND METHODS

A field experiment was conducted during the Rabi season covering the period of November, 2003 to March, 2004 at the experimental farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur with a view to find out the effect of inoculation methods of *Rhizobium* on yield attributes, yield and protein content in seed of pea. The soil of the experimental farm was Shallow-Red Brown Terrace soil under Madhupur Tract (AEZ No. 28). It was of clay loam texture and contained 0.73% organic carbon, 12.05

(meq/100g dry soil) CEC, 0.074% total N, 12.05 ppm available phosphorus and had a pH 6.4. The number of *Rhizobium* per g of soil was 5.9×10^4. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The unit plot size was 2.4m×2.4m. There were eight treatment combinations in the experiment which were uninoculated control (T_1), seed inoculation with *Rhizobium* strain BARI RPs-2001 (T_2), soil inoculation with *Rhizobium* strain BARI RPs-2001 (T_3), seed + soil inoculation with *Rhizobium* strain BARI RPs-2001 (T_4), seed inoculation with *Rhizobium* strain BARI RPs-2002 (T_5), soil inoculation with *Rhizobium* strain BARI RPs-2002 (T_6), seed + soil inoculation with *Rhizobium* strain BARI RPs-2002 (T_7) and 50 kg N/ha (T_8). At the time of final land preparation a basal dose of 60 kg P_2O_5, 50 kg K_2O and 20 kg S/ha was applied to each plot. Nitrogen at the rate of 50 kg /ha was used in the form of urea as per treatment. Pea (var. IPSA motorshuti-3) was used as the test crop for the experiment. The study was carried out with the *Rhizobium* inoculant containing strains viz. BARI RPs-2001 and BARI RPs-2002. The inoculants were collected from the Soil Science Division of the Bangladesh Agricultural Research Institute (BARI). Rhizobial population of the inoculants containing BARI RPs-2001 and BARI RPs-2002 were 1.85 x 10^8cell/g and 4.75 x 10^8cell/g, respectively as estimated following the method of Miles and Misra (1938). Seeds were taken in vinyl bags and then gum arabic solution was added to the seeds to make them sticky. Inoculants were used for inoculating seeds at the rate of 20g/kg of seed. The seeds were mixed with the inoculants thoroughly. After inoculation, the seeds were air-dried by spreading them on a polyethylene paper placed on the table. Liquid inoculant was prepared by making a suspension of peat based inoculant in distilled water and then 210g inoculant was suspended in 600 L water. Soil was inoculated with liquid inoculant at the rate of 3000 L/ha where necessary. Seeds were sown in each plot at the rate of 35kg/ha. Pods were harvested at two different stages of maturity. During harvesting stage the number of pods/plant and number of seeds/pod were recorded from five randomly selected plants from each plot. Green pod was harvested and estimated from the yield of plants grown in half portion of each plot. The rest half of the crop of each plot was kept to allow the pods to get maturity. Then the matured pods were harvested and estimated from the yield of plants grown in the remaining half portion of each plot. Thousand seed weight was estimated from both green and mature seeds. The seeds were first air-dried and then oven-dried at 65^0C for 72 hours. Then seeds were powdered by a grinding machine for nitrogen determination. Total nitrogen content in seed was determined by Micro-Kjeldahl method following Salicylic-H_2SO_4 digestion (Yamakawa, 1993) through colorimetric method. N was measured using double beam spectrophotometer (Model No. 200-20, Hitachi, Japan) at 625 nm wave length. The crude protein content was estimated by multiplying the N% determined with a factor of 6.25. The recorded data on various characters of the crop were statistically analyzed to find out the significance of variation resulting from the experimental treatments. For this purpose, analysis of variance was worked out for each character of the crop. The differences between treatment means were compared by Duncan's Multiple Range test (DMRT).

RESULTS AND DISCUSSION

Number of pods per plant

Plant receiving 50 kg N/ha produced the highest number of pods per plant (17.50) which was statistically identical with seed and soil inoculation either with BARI RPs-2001 or BARI RPs-2002 and soil inoculation with BARI RPs-2002 (Table 1). The lowest number of pods/plant (9.25) was noted in uninoculated control. The contribution of biologically fixed N on increasing the pod number was remarkable. This result was in agreement with the findings of Solaiman (1999) who conducted experiment with chickpea and reported that *Rhizobium* inoculant significantly increased number of pods compared to uninoculated control. Evans *et al.* (1993) obtained similar result in pea. Feng *et al.* (1997) reported that number of pods of pea increased due to *Rhizobium* inoculation. Rabbani *et al.* (2005) also found that the number of pods per plant was increased with *Rhizobium* inoculant in combination with 25 kg P and 1.5 kg Mo/ha.

Pod length

The main effect of different methods of *Rhizobium* inoculation on pod length was found significant (Table 1). Plant receiving the treatment, soil inoculation with *Rhizobium* strain BARI RPs-2001 recorded the highest pod length per plant (6.22 cm) which was statistically similar to the treatments of soil inoculation with *Rhizobium* strain BARI RPs-2002 and seed and soil inoculation with *Rhizobium* strain BARI RPs-2002. However, there was no significant difference among the treatments of seed and soil inoculation with *Rhizobium* strain BARI RPs-2001, seed inoculation with *Rhizobium* strain BARI RPs-2002 and seed inoculation with *Rhizobium* strain BARI RPs-2001 but their effects were superior to uninoculated control. The lowest pod length per plant (5.04 cm) was noted in treatment under uninoculated control condition. From this finding it is clear that inoculation of *Rhizobium* strain increased the pod length per plant. Rabbani *et al.* (2005) found similar result in pea.

Number of seeds per pod

The effect of different methods of *Rhizobium* inoculation in recording the number of seeds per pod was found significant (Table 1). Plant receiving seed and soil inoculation with either BARI RPs-2001 or BARI RPs-2002 produced the highest number of seeds (5.14/pod) which was statistically similar to other treatments except 50 kg N/ha and uninoculated control. The lowest number of seeds (3.75/pod) was obtained in uninoculated control. It was observed that biologically fixed N exhibited a significant effect on the number of seeds per pod. These findings have the resemblance with the results of Rahman *et al.* (1994) who reported that *Rhizobium* inoculant produced significantly higher number of seeds per pod in chickpea. Solaiman and Rabbani (2005) observed that *Rhizobium* inoculunt alone produced more number of seeds per pod of pea than the uninoculated control.

1000-seed weight

Rhizobium inoculant alone significantly increased 1000-green and mature seed weights compared to uninoculated control (Table 1). The highest 1000-green seed (279.70 g) and 1000-mature seed (161.00 g) weights were recorded with the treatment consisting of seed and soil inoculation with BARI RPs-2002 and seed and soil inoculation with BARI RPs-2001, respectively. Treatment containing seed and soil inoculation with BARI RPs-2002 was statistically similar to other treatments with *Rhizobium* inoculation but recorded higher 1000-green seed weights compared to that receiving 50 kg N/ha and uninoculated control. Similar trend was observed in 1000-mature seed weight of the crop. The lowest 1000-green and mature seed weights were obtained in uninoculated control. This finding has the resemblance with the result of Rabbani *et al.* (2005) who obtained the highest 1000-seed weight of pea with the treatment comprising of *Rhizobium* inoculant in combination with 25 kg P and 1.5 kg Mo/ha.

Table 1. Effect of different methods of *Rhizobium* inoculation on yield attributes of pea

Treatments	Number of pods/plant	Pod length (cm)	Number of seeds/pod	1000-seed weight (g)	
				Green seed	Mature seed
Control	9.25c	5.04d	3.75b	225.90c	131.90c
Seed+BARI RPs-2001	12.25bc	5.64bc	4.29ab	254.40ab	142.10abc
Soil+BARI RPs-2001	14.45ab	6.22a	4.39ab	257.80ab	141.70abc
Seed+Soil+BARI RPs-2001	16.10a	5.70bc	5.14a	273.10ab	161.00a
Seed+BARI RPs-2002	11.82bc	5.68bc	4.72ab	255.80ab	143.80abc
Soil+BARI RPs-2002	14.40ab	5.89ab	5.07a	259.00ab	147.40abc
Seed-Soil+BARI RPs-2002	16.75a	5.88ab	5.14a	279.70a	156.00ab
N_{50}	17.50a	5.30cd	3.92b	246.10bc	136.00bc
CV (%)	14.04	5.44	14.54	6.59	10.86

Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT

Green pod yield

There was a significant effect of different treatments in recording green pod yield (Table 2). The highest green pod yield (3.29 t/ha) was found with inoculation of both seed and soil with BARI RPs-2002 which was 53% higher than that of uninoculated control. The effect of this treatment was however, statistically similar to the treatments receiving coinoculation of both seed and soil with BARI RPs-2001, soil inoculation with BARI RPs-2002 and seed inoculation with BARI RPs-2001. The lowest green pod yield (2.15 t/ha) was noted in uninoculated control. There was no significant yield variation between the treatments consisting of soil inoculation with BARI RPs-2001 and seed inoculation with BARI RPs-2002, but all the treatments produced significantly higher pod yield over uninoculated control. These findings have the resemblance with the result of Srivastava *et al.* (1998) who reported that inoculation of pea seeds with *Rhizobium leguminosarum* gave maximum pod yield of pea. Kanaujia *et al.* (1998) obtained similar result in pea crop. Maximum green pod yield of 30.78 g/plant (111% increases over uninoculated control) of pea var. BARI Motorshuti-1 was obtained when plants were inoculated with *Rhizobium* strains (Solaiman and Khondaker 2002).

Green seed yield

Green seed yield of pea was significantly influenced due to *Rhizobium* inoculant (Table 2). All the treatments produced significantly higher green seed yield compared to uninoculated control. Inoculation of both seed and soil with BARI RPs-2002 produced the maximum green seed yield (1.90 t/ha) which was 58% higher than that of uninoculated control. The effect of this treatment was however, statistically similar to other treatments except

that receiving 50 kg N/ha and uninoculated control. These results have similarity with those of Carr *et al.* (2000). They found no difference in seed yield and quality when different inoculant forms were used. In this study the lowest green seed yield (1.20 t/ha) was recorded in uninoculated control. Rabbani *et al.* (2005) found that *Rhizobium* inoculant in combination with 25 kg P and 1.5 kg Mo/ha performed best in recording with green and mature seed yields of pea.

Table 2. Effect of different methods of *Rhizobium* inoculation on green pod and seed yield of pea

Treatments	Green pod yield (t/ha)	Increase over control (%)	Green seed yield (t/ha)	Increase over control (%)
Control	2.15c	-	1.20c	-
Seed+BARI RPs-2001	2.79ab	29.77	1.64ab	36.67
Soil+BARI RPs-2001	2.60bc	20.93	1.53abc	27.5
Seed+Soil+BARI RPs-2001	3.18ab	47.91	1.87a	55.83
Seed+BARI RPs-2002	2.67bc	24.19	1.66ab	38.33
Soil+BARI RPs2002	2.94ab	36.74	1.65ab	37.5
Seed+Soil+BARI RPs-2002	3.29a	53.02	1.90a	58.33
N_{50}	2.19c	1.86	1.38bc	15.00
CV (%)	13.34	-	14.48	-

Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT

Mature pod yield

There was a significant yield variation among the treatments (Table 3). The highest mature pod yield (1.03 t/ha) was found when both seed and soil were inoculated with BARI RPs-2002 giving 91% higher yield than that of uninoculated control. The effect of this treatment was statistically similar to other treatments except uninoculated control. These findings have the resemblance with the result of Daterao *et al.* (1994) who conducted experiments on groundnut, green gram and black gram and obtained highest pod yields with 20 kg N+ seed inoculation with *Rhizobium.* Potdukhe *et al.* (1998) found similar result. They reported that application of 30 kg N/ha and seed inoculation with *Rhizobium* strains IPH-195 and CCI gave the highest mature pod yield of pigeon pea over uninoculated control. In this study the lowest yield (0.54 t/ha) was noted under control. All the treatments produced significantly higher pod yield over uninoculated control. In a study with pea it was observed that var. BARI Motorshuti -1 gave maximum pod yield of 30.78 g/plant (111% increase over uninoculated control) and mature seed yield of 5.10 g/plant (86% increase over uninoculated control) when plants were inoculated with *Rhizobium* strains (Solaiman and Khondaker 2002).

Mature seed yield

Mature seed yield of pea was significantly influenced due to different treatments (Table 3). The highest yield (0.76 t/ha) was observed in receiving seed and soil inoculation with BARI RPs-2002 which recorded 100% higher yield over uninoculated control (0.38 t/ha). The effect of this treatment was statistically similar with the rest of the treatments except 50 kg N/ha and uninoculated control. All the treatments produced significantly higher seed yield over uninoculated control. This finding has the resemblance with the result of Clayton *et al.* (2005) who conducted an experiment with different methods of *Rhizobium* inoculant on pea and found of 183, 162 and 132% yield for granular, peat powder and liquid inoculant treated peas respectively over uninoculated control. Solaiman and Rabbani (2006) found that mature seed yield of pea was increased by the application of *Rhizobium* inoculant along with 5 ton compost/ha.

Protein content in seed

The effect of *Rhizobium* inoculant on protein content of both green and mature seeds of pea was significant (Table 3). Plant receiving seed and soil inoculation with BARI RPs-2002 scored the highest protein contents both in green (24.89%) and mature (17.78%) seed. The effect of this treatment was statistically similar to that of seed and soil inoculation with BARI RPs-2001 in green seed and 50 kg N/ha in mature seed. The lowest value was exhibited in uninoculated control both in green (13.19%) and mature (9.05%) seed. Solaiman and Rabbani (2005) who found that the performance of *Rhizobium* inoculant alone was superior to uninoculated control in protein content in green and mature seeds of pea.

Table 3. Effect of different methods of *Rhizobium* inoculation on mature pod, mature seed and protein content in seed of pea

Treatments	Mature pod yield (t/ha)	Increase over control (%)	Mature seed yield (t/ha)	Increase over control (%)	Protein content in green seed (%)	Protein content in mature seed (%)
Control	0.54b	-	0.38c	-	13.19d	9.05f
Seed+BARI RPs-2001	0.80ab	48.15	0.55abc	44.74	16.97c	11.55e
Soil+BARI RPs-2001	0.84ab	55.56	0.57abc	50.0	18.35c	12.58de
Seed+Soil+BARIRPs-2001	0.96a	77.78	0.73a	92.11	24.61a	14.85bc
Seed+BARI RPs-2002	0.83ab	53.70	0.60abc	57.89	17.81c	13.20cde
Soil+BARI RPs-2002	0.89a	64.81	0.64ab	68.42	21.10b	13.61cd
Seed+Soil+BARI RPs-2002	1.03a	90.74	0.76a	100	24.89a	17.78a
N_{50}	0.74ab	37.04	0.47bc	23.68	22.03b	16.42ab
CV (%)	8.15	-	9.47	-	7.4	8.39

Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT

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