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Ajeet Kumar, Diwakar Paswan. Response of pea (*Pisum sativum* L.) to Rhizobium inoculation and nitrogen application in soils of North East alluvial plains of Bihar. *AN ASIAN JOURNAL OF SOIL SCIENCE*, 2015, 10 (2), pp.252-258. <10.15740/HAS/AJSS/10.2/252-258>. <hal-04676715>

**HAL Id: hal-04676715**

**<https://hal.science/hal-04676715v1>**

Submitted on 3 Sep 2024

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**Research Article**

DOI : 10.15740/HAS/AJSS/10.2/252-258

# Response of pea (*Pisum sativum* L.) to *Rhizobium* inoculation and nitrogen application in soils of North East alluvial plains of Bihar

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Received : 26.06.2015; Revised : 27.10.2015; Accepted : 12.11.2015

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**Summary**

Among legumes, pea (*Pisum sativum* L.) is the second most important legume, which is widely used both in human nutrition and as fodder. Currently, pre-sowing inoculation of pea seeds is a promising treatment and is one of agronomic solutions for sustainable agriculture. As per the objective of the research, to determine the effects of *Rhizobium* inoculation and different levels of nitrogen on the yield potential and growth attributes of pea, the research was conducted in Split Plot Design with 3 replications, at Research farm of RRS, Agwanpur, Saharsa. Nitrogen application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodules, seed yield, biomass yield, harvest index, number of pods, as well as crude protein rate of seeds in both years. Plant height, number of pods, harvest index, number of nodules, crude protein rate and root dry weight were higher with application of 20 kg N ha<sup>-1</sup> while seed yield, shoot dry weight and number of branches were higher with application of 60 kg N ha<sup>-1</sup>, in the 2 seasons. The lowest values related to these parameters were obtained from the control treatment. Inoculation treatment had also significant effect on the all parameters investigated in both the years. The highest values regarding these parameters were obtained from inoculated plants, whereas the lowest values were obtained from the un-inoculated plants. Interactions of nitrogen and inoculation had significant effects on the all parameters investigated, except for number of nodules in 2009-2010 and crude protein rate of seeds in both years. The highest seed yield was obtained under treatment 20 kg N ha<sup>-1</sup> + inoculation with 1654.3 and 1625.0 kg ha<sup>-1</sup> in 2009-10 and 2010-11, respectively.

**Key words :** Pea, *Rhizobium*, Nitrogen, Nodulation, Yield**Co-authors :**

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**How to cite this article :** Kumar, Ajeet and Paswan, Diwakar (2015). Response of pea (*Pisum sativum* L.) to *Rhizobium* inoculation and nitrogen application in soils of North East alluvial plains of Bihar. *Asian J. Soil Sci.*, **10**(2): 252-258.**Introduction**

Grain legumes are widely recognized as a significant source of proteins (Duranti and Guis, 1997) and have become very important in human and domestic animal nutrition (Cummings *et al.*, 2001). Presently, the

increasing interest in low input sustainable agricultural systems on cropping farms, from an economic and environmental viewpoint opens the door for continued interest in grain legumes (White, 1989). Among legumes, pea (*Pisum sativum* L.) is the second most important

grain legume crop in the world, which is widely used in human nutrition and as fodder (Cristou, 1997). The importance of legumes to these purposes is not only due to their nitrogen (N) fixing capabilities but also due to their ability to interrupt the cycles of diseases and pests affecting other crops (Cummings *et al.*, 2001). Many authors claimed that yields could be increased by legume seed inoculation compared to nitrogen fertilizer variants (Dobereiner *et al.*, 1995). Legume inoculation is an established agricultural practice that has been used for more than a century to introduce Rhizobia into the soil (Deaker *et al.*, 2004). Inoculants are produced commercially in many countries. Their quality depends on both the number of *Rhizobia* they contain and their effectiveness in fixing N with the target host. Symbiotic effectiveness is one of the most important factors when selecting an inoculant strain (Stephens and Rask, 2000). Inoculation of annual crop legumes has produced variable results when different inoculation methods were used. Granular seed inoculation enhanced seed yield compared to peat-based seed inoculants in dry peas (*Pisum sativum* L.) and in chickpea (*Cicer arietinum* L.) (Gan *et al.*, 2005). Nitrogen is major nutrient element for grain legumes. Although, nitrogen is abundant in the atmosphere in gaseous form, plants cannot directly utilize it. Availability of nitrogen amount in soil is getting decreased by plant cultivation, increased oxidation in soil or it can also be easily washed away. Therefore, nitrogen becomes a limited factor in the plant cultivation. The main ways of the nitrogen application into soil are the mineral nitrogen fertilizer or *Rhizobium* inoculation of seeds or soil in order to obtain biological fixation of nitrogen. High cost and its dependence to moisture is the main disadvantageous of mineral fertilizer. Biological nitrogen fixation is an important nitrogen source due to the fact that it requires less energy and causes less environmental pollution.

Dry pea 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. When properly inoculated with an appropriate *Rhizobium* inoculant, pea can derive up to 80 per cent or more of its nitrogen through nitrogen fixation. The rest of the N must be provided from the soil or from fertilizer applications (Ali-Khan and Zimmer, 1989 and Bowren *et al.*, 1986). Seed inoculation is the most widely used methods of

inoculant application. Many researchers (Rahman *et al.*, 1994 and Solaiman and Rabbani, 2005) have reported the beneficial effects of inoculation of grain legumes.

Nodule formation and subsequent nitrogen fixation are very sensitive to external nitrogen sources, including fertilizer and available soil nitrogen. Nitrogen fertilizer applications generally inhibit biological N-fixation by *R. leguminosarum*. The inhibitory effect of N fertilizer on nodule formation results from the fertilizer's contribution to the soil N pool. Bowren *et al.* (1986) concluded that nodule formation became inhibited as soil N levels approached 40 kg ha<sup>-1</sup> and were progressively inhibited as levels exceeded this amount. This suggests that N fertilizer applications will reduce biological N-fixation by *R. leguminosarum*, except where the amount of N applied as fertilizer plus that contained in the soil is <40 kg ha<sup>-1</sup>. Small N fertilizer applications have stimulated nodule formation on pea roots in some low N environments and other pulse crops (Kauskik *et al.*, 1995). It can take 3-4 weeks after planting before nodules are fully functioning. Early plant growth may be poor in soils with low nitrogen levels and plants may appear yellow prior to the beginning of effective nitrogen fixation due to a nitrogen deficiency. This early deficiency can be overcome by adding low levels of starter nitrogen at seeding. Although, high levels of starter nitrogen may appear to help the crop overcome a nitrogen deficiency during early crop growth stages, final seed yields may not increase. Bowren *et al.* (1986) suggested that applications of 9-18 kg N ha<sup>-1</sup> enhanced pea seedling growth prior to nodule development in low N soils. The soil of the region where the study was conducted has low nitrogen and organic matter content and their availabilities are low due to the various soil and other environmental factors. Therefore, it was aimed to determine the effects of nitrogen fertilizer and *Rhizobium* inoculation on the yield and growth parameters, as well as crude protein rate of seeds in field pea.

## Resource and Research Methods

An explorative experiment was carried out in research farm of Regional Research Station, Agwanpur, (86.340 longitudes and 25.550 N latitude), Saharsa, Bihar., during *Rabi* season in the years 2009-2010 and 2010-2011. The experiment was implemented at research farm, where legumes have not be grown for at least 15 years. Pea seeds were inoculated using peat inoculant included a mixture of nodule-forming strains of

*Rhizobium leguminosarum* bv viciae specific to pea, according to the method of Somasegaran and Hoben (1994). Peat inoculant was kept in refrigerator at 4°C until use. Peat inoculant was used after cells count were adjusted to  $1 \times 10^8$  *Rhizobium* cells  $g^{-1}$  so content of peat culture was standardized by diluting peat inoculant. The most probable number (MPN) method was used for estimating viable cells of *Rhizobium* (Somasegaran and Hoben, 1994).

The soil samples were collected from experimental site and analyzed in soil testing laboratory of RRS, Agwanpur. The physico-chemical properties of the soil were analyzed, following the standard procedure Jackson (1973). The study was conducted in split-plot design with 3 replications. In order to eliminate the contamination, inoculation treatments (with and without) were randomly applied to main plots and nitrogen doses (0, 20, 40 and 60 kg N  $ha^{-1}$ ) as ammonium sulphate were randomly applied to the subplots before sowing. Plot size was 5x1.2 m. The seed was sown by hand with 25 cm row spacing in late October in both years. The seeding rate was 45 seeds  $m^{-2}$ . A basal dose of 60 kg  $ha^{-1}$  triple super phosphate was given to each plot at the time of sowing. Seeds were inoculated with *R. leguminosarum* bv viciae bacteria at the recommended rate before sowing in all plots, except for un-inoculated plots (Vincent, 1982). Application of the inoculant on the seeds was carried out by water, which contains 12 per cent sugar solution. Plots were hand-weeded 2 times when needed in each season. Plants were harvested in late June at flowering, 10 random plants were removed from each plot. Numbers of nodules as well as dry weight of root and shoot/plant were recorded. Similarly, 10 plants from each plot were removed at the harvest to determine pods number per plant as well as plant height and branches number per plant. At harvest, 2 outer rows for each plot and 50 cm from each end of the plots were left as borders and the middle 4 m of the central rows were harvested. Biomass yield and seed yield were determined after harvest. In addition to these criteria, crude protein of seeds was determined using the methods proposed by Kacar and Inal (2008).

The influence of treatments on the field pea and differences among treatments were analysed using analysis of variance procedures for Split-Plot Design in Randomised Blocks. Means of measured parameters were compared using least significance difference (LSD) at  $p < 0.05$ .

## Research Findings and Discussion

The results obtained from the present investigation as well as relevant discussion have been given in Table 1 to 7.

### Soil characteristics and climatic parameter :

The chemical composition of the soil before cropping is shown in Table 1. Soil of the experimental site had sandy loam texture, and was low in organic matter, neutral in reaction, low to medium in available N and K and medium to high in available P, lies in the recently formed alluvium of Koshi River. The soil pH was 7.2-7.8, indicating suitability of the soil for pea cultivation.

Table 1 : Physical and chemical properties of soil before planting	
Property	Value
Soil pH (1:2.5)	7.2-7.8
available N (kg/ha)	275-310
Available P (kg/ha)	15-25
Available K (kg/ha)	290-340
Carbon (%)	1.50
Nitrogen (%)	0.13
CEC (me/100 g)	6.53 (Low)
Texture	Sandy loam

The soil received rainfall 391.8 mm and 363.1 mm in 2009-2010 and 2010-2011, respectively. Annual average temperature was 10.5°C in 2009-2010 and 9.4°C in 2010-2011.

### Effect of *Rhizobium* inoculation and nitrogen application on the pea attributes :

The results indicated that the effects of nitrogen application and inoculation on the investigated parameters differed significantly between years (Table 2 and 3). In general, compared to those in 2010-2011, occurred significant increases in the values of parameters in 2009-2010 when the soil received higher amount of rainfall. Nitrogen application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodules, seed yield, biomass yield, harvest index, number of pods, as well as crude protein of seeds in both years. The lowest values related to these parameters were obtained from the control treatment. Plant height, number of pods, harvest index, number of nodules, crude protein and root dry weight were higher with application of 20 kg N  $ha^{-1}$  while seed yield, shoot dry weight and number of branches were higher with

application of 60 kg N ha<sup>-1</sup>, in the two seasons.

However, differences between 20 and 60 kg N ha<sup>-1</sup> were not significant in terms of plant height, number of pods, harvest index and number of branches (Table 2 and 3). The number of nodules differed significantly among nitrogen treatments. However, it was observed

that nodules formed also in the un-inoculated plots. Availability of the nodules in the un-inoculated treatment indicates that experimental soils contain native *Rhizobial* population. Increases in these parameters by the application of 20 kg N ha<sup>-1</sup> indicates the importance of starter nitrogen dose in the legumes for improving the

Treatments	Seed yield (kg/ha)		Biomass yield (kg/ha)		Harvest index (%)		No. of pods (plant <sup>-1</sup> )		Crude protein (% seed <sup>-1</sup> )	
	09-10	10-11	09-10	10-11	09-10	10-11	09-10	10-11	09-10	10-11
<b>Nitrogen (kg/ha)</b>										
0	963.3d	939.3d	2525.3c	2476.7c	37.40c	37.20c	3.80b	3.40c	16.70c	16.00c
20	1251.5b	1217.7b	2839.3b	2815.2b	43.50a	42.60a	5.00a	4.30a	19.60a	18.90a
40	1180.0c	1143.0c	3023.8a	2980.7a	40.80b	40.20b	4.80a	4.00b	18.10b	17.20b
60	1323.7a	1290.2a	3018.8a	2952.5a	42.1ab	41.7ab	4.70a	4.2ab	17.7bc	17.0bc
LSD (P=0.05)	46.20	47.0	73.20	66.00	2.00	2.00	0.40	0.200	1.200	1.100
<b>Inoculation with <i>Rhizobium</i></b>										
+ R	1419.9a	1388.6a	3354.5a	3331.7a	42.20a	41.8ns	5.30a	4.20a	19.30a	18.50a
- R	939.3b	906.5b	2349.3b	2300.8b	39.60b	39.0	3.90b	3.70b	16.70b	16.10b
LSD (P=0.05)	91.80	86.60	53.10	82.900	2.80	3.30	1.40	0.30	2.40	0.50
Cv (%)	26.20	26.80	20.80	21.30	8.80	8.50	21.10	13.20	10.80	8.400

+R = with Rhizobia; -R = without Rhizobia

Treatments	Root dry wt (g plant <sup>-1</sup> )		Shoot dry wt (g plant <sup>-1</sup> )		Nodules number (Plant <sup>-1</sup> )		Plant height (cm)		No. branches (Plant <sup>-1</sup> )	
	09-10	10-11	09-10	10-11	09-10	10-11	09-10	10-11	09-10	10-11
<b>Nitrogen (kg/ha)</b>										
0	0.295d	0.274d	1.490d	1.479c	13.7b	12.5c	35.50c	32.70	1.50c	1.40c
20	0.387a	0.368a	2.084b	2.071a	20.7a	19.2a	44.70a	41.50	2.13b	2.2b
40	0.339c	0.323c	1.859a	1.834b	15.3b	14.0b	41.80b	39.50	2.10b	2.00b
60	0.362b	0.339b	2.120a	2.080a	11.5c	10.5d	43.50b	40.8	2.40a	2.20a
LSD (P=0.05)	0.0090	0.0040	0.0110	0.0240	1.90	1.20	2.30	1.4	0.200	0.200
<b>Inoculation with <i>Rhizobium</i></b>										
+ R	0.361a	0.327ns	1.991a	1.960a	17.8a	16.3a	44.20a	40.90	2.30a	2.10a
- R	0.331b	0.325.	1.786b	1.772b	12.8b	11.8b	38.60b	36.30	1.90b	1.80b
LSD (P=0.05)	0.0110	0.110	0.0150	0.0260	3.50	1.00	4.60	1.9	0.300	0.300
Cv (%)	13.6	13.30	16.700	16.700	29.50	29.60	12.70	12.50	22.100	22.90

+R = with Rhizobia; -R = without Rhizobia

ns: Means in the same column are not significantly different; Means in the same column followed by the same letter are not significantly different at p<0.05

Nitrogen (kg/ha)	Inoculation with <i>Rhizobium</i>							
	Seed yield (kg/ha)		Biomass yield (kg/ha)		Harvest index (%)		No. pods (plant <sup>-1</sup> )	
	+R	-R	+R	-R	+R	-R	+R	-R
0	1267.0cd	659.7g	3121.3c	1929.3f	40.6cd	34.2e	4.3c	3.30d
20	1654.30a	848.7f	3623.0a	2056.7e	45.70a	41.3bcd	6.2a	3.9cd
40	1319.30c	1040.7e	3375.7b	2672.0d	42.7bc	39.0d	5.4b	4.10c
60	1439.00b	1208.3d	3298.3b	2739.3d	40.0cd	44.1ab	5.3b	4.20c
LSD (P=0.05)	68.900	-	96.50	-	2.700	-	0.70	-
Cv (%)	26.200	-	20.80	-	8.700	-	21.10	-

+R = with Rhizobia; -R = without Rhizobia

Means in the same parameter followed by the same letter are not significantly different at p<0.05.

efficiency of Rhizobia for biological nitrogen fixation responsible for higher growth. These findings are in conformity to the findings of Igbasan *et al.* (1996) and Azad *et al.* (1992). Inoculation treatment had also significant effect on the plant height, number of branches, number of nodules, root and shoot dry weight, number of pods, seed yield, biomass yield, harvest index and crude protein of seeds in both years (Table 2 and 3). The highest values regarding these parameters were obtained from inoculated treatment, whereas the lowest values were obtained from the un-inoculated treatment. However, differences between inoculated and un-inoculated treatments were not significant in terms of harvest index in both years and root dry weight in 2010-11.

This positive relationship of yield and growth parameters with inoculation could be related to the nitrogen fixation ability of nodules, which consequently results in increased growth and yield. Similar results were found by Jha and Singh (1996) and Idris and Sandhu (1981).

Interactions of nitrogen and inoculation had significant effects on the seed yield, biomass yield and root and shoot dry weight, number of pods, plant height, number of branches and harvest index in both years, except for crude protein in both years. While the application of 20 kg N ha<sup>-1</sup> + inoculation gave the highest values related to all parameters, the lowest values were obtained from control without inoculation treatment. The

**Table 5 : Interaction effects of inoculation and nitrogen on plant height, number of branches, root and shoot dry weight (2009-10)**

Nitrogen (kg/ha)	Inoculation with <i>Rhizobium</i>							
	Plant height (cm)		Number of branches (plant <sup>-1</sup> )		Root dry weight (g plant <sup>-1</sup> )		Shoot dry weight (g plant <sup>-1</sup> )	
	+R	-R	+R	-R	+R	-R	+R	-R
0	38.00e	33.00f	1.70e	1.40f	0.321d	0.268e	1.509f	1.471g
20	50.70a	38.7de	2.80a	1.8de	0.439a	0.334d	2.445a	1.723e
40	42.7bc	41.0cde	2.2bc	2.0cd	0.329d	0.348c	1.883c	1.834d
60	45.30b	41.7cd	2.50b	2.40b	0.352c	0.372b	2.124b	2.115b
LSD (P=0.05)	3.400	-	0.270	-	0.0120	-	0.0150	-
Cv (%)	12.700	-	22.10	-	13.500	-	16.700	-

+R = with Rhizobia; -R = without Rhizobia

**Table 6 : Interaction effects of inoculation and nitrogen on the seed yield, biomass yield, harvest index and number of pods (2010-11)**

Nitrogen (kg/ha)	Inoculation with <i>Rhizobium</i>							
	Seed yield (kg ha <sup>-1</sup> )		Biomass yield (kg ha <sup>-1</sup> )		Harvest index (%)		Number of pods (plant <sup>-1</sup> )	
	+R	-R	+R	-R	+R	-R	+R	-R
0	1242.7c	636.0g	3086.7d	1866.7g	40.3bc	34.1d	3.6c	3.1d
20	1625.0a	810.3f	3616.7a	2013.7f	44.9a	40.3bc	4.8a	3.6c
40	1280.0c	1006.3e	3343.3b	2618.0e	42.1ab	38.4c	4.1b	4.0b
60	1406.7b	1173.7d	3200.0c	2705.0e	40.0bc	43.4a	4.3b	4.2b
LSD (P=0.05)	68.80	-	90.60	-	2.900	-	0.30	-
Cv (%)	26.80	-	21.30	-	8.500	-	13.20	-

+R = with Rhizobia; -R = without Rhizobia

**Table 7 : Interaction effects of inoculation and nitrogen on plant height, number of branches, root and shoot dry weight and nodules number (2010-11)**

Nitrogen (kg/ha)	Inoculation with <i>Rhizobium</i>									
	Plant height (cm)		No. branch (plant <sup>-1</sup> )		Root dry weight (g plant <sup>-1</sup> )		Shoot dry weight (g plant <sup>-1</sup> )		No. nodule (plant <sup>-1</sup> )	
	+R	-R	+R	-R	+R	-R	+R	-R	+R	-R
0	35.00d	30.3e	1.50e	1.2f	0.285g	0.264h	1.497g	1.460h	15.0b	10.0d
20	47.00a	36.0d	2.60a	1.7de	0.406a	0.331d	2.430a	1.712f	22.3a	16.0b
40	40.0bc	39.0c	2.1bc	1.9cd	0.302f	0.343c	1.857d	1.811e	16.0b	12.0c
60	41.70b	40.0bc	2.30b	2.2b	0.315e	0.363b	2.057c	2.104b	12.0c	9.0d
LSD (P=0.05)	1.900	-	0.280	-	0.0070	-	0.0330	-	1.50	-
Cv (%)	12.500	-	22.900	-	13.300	-	16.700	-	29.60	-

+R = with Rhizobia; -R = without Rhizobia

Means in the same parameter followed by the same letter are not significantly different at p<0.05

highest seed yield was recorded in 20 kg N ha<sup>-1</sup> + inoculation treatment with 1654.3 kg ha<sup>-1</sup> in the 1<sup>st</sup> year and 1625.0 kg ha<sup>-1</sup> in the 2<sup>nd</sup> year. These findings are in accordance with the results of Semu *et al.* (1982) and Kaya (2000).

Data, thus, presented in the above table clearly indicate the beneficial effect of artificially inoculating the effect of pea with efficient strains of *Rhizobium* along with starter dose of nitrogen application. This combination may establish more efficient bacterial-plant symbiosis over the native isolates in our soils.

This study has further, shown the necessities of producing and distributing *Rhizobium* culture for increasing the yield of pea grown in soils of North East alluvial plains of Bihar.

### Conclusion :

It is concluded that 20 kg N ha<sup>-1</sup> along-with inoculation of *Rhizobium* provided highest yield and all yield attributing components of pea. Thus, 20 kg N ha<sup>-1</sup> + inoculation treatment should be preferred to grow pea in areas where amount of nitrogen are limiting factor for biological nitrogen fixation in pea production.

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