



RESEARCH PAPER

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Influence of *Rhizobium* inoculation on nodules, growth and yield of french beans cultivars

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Abstract

To study the influence of *Rhizobium* inoculation on nodules, growth and yield of French beans (*Phaseolus vulgaris* L.) cultivars, an experiment was conducted at National Tea and High value crops research Institute (NTHRI) Shinkari, Mansehra during 2016. Seeds of three different cultivars of French bean i.e 'Evergreen', 'Komal green' and 'Winner' were inoculated with *Rhizobium* in main plot; whereas cultivars kept in sub plots in order to evaluate various growth parameter and yield. The *Rhizobium* inoculation significantly enhanced the growth and yield parameter of French beans cultivars. Maximum germination percentage (90.01%), plant height (29.55 cm), plant spread (27.55 cm), number of nodules (27.22 plant⁻¹), number of branches (6.38 plant⁻¹), number of pods (41.83 plant⁻¹), pods length (20 cm) and yield (5939.4 kg ha⁻¹) was observed in synthetic *Rhizobium* inoculated seeds. Evergreen cultivars of French beans showed best result i.e. maximum germination percentage (89.48), plant height (28.83cm), plant spread (25.83 cm), pods length (18.95 cm), number of branches (6.38 plant⁻¹), number of pods (40.80 plant⁻¹), number of nodules (25.00 plant⁻¹) and yield (5810.8 kg ha⁻¹). All the parameter of growth and yield showing best result for synthetic *Rhizobium* inoculation of seeds while among the cultivar 'Evergreen' is best for cultivation.

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Introduction

French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable of the world. Particularly, french bean green pod is used as green vegetable (Voysset and Dessert, 1991). It was originated in Mexico and domesticated there about 8000 year ago from where it spread to the other parts of the world (Van der Maesen and Somaatmadja, 1989; Kaplan and Kaplan, 1988). Globally about 12 million metric tonnes of french bean are produced annually on an area of 611700 ha (FAO, 2014).

In Pakistan, approximately 847800 tonnes are produced on an area of 789 ha (Agriculture Statistics of Pakistan, 2014). French bean is good source of protein, iron, vitamins and other minerals. Mostly it is used as green vegetables which contain high level of vitamin A which are helpful to reduce the risk of night blindness however in some countries it is used as dry beans (Silbernagel *et al.*, 1991; Birjdar, 2006). Leguminous crop like soya bean, French bean and ground nut, a bacterium which are responsible for fixing the atmospheric nitrogen in root zone/nodule, providing nitrogen to the existing crop, increase fertility, texture and structure of the soil (Jensen and Hauggaard-Nielsen, 2003).

These bacteria are present in most of the soils vary in number, effective nodulation and nitrogen-fixation. In order to improve nodulation, an optimum *Rhizobium* population in the soil, seed of legumes should be inoculated with an efficient *Rhizobium* strain which can improve crop growth and yield of leguminous crops (Henzell, 1988). *Rhizobium* spp are nitrogen-fixing bacteria in the soil that enter into the root hairs of legumes and result in the formation of nodules, which reduces the atmospheric nitrogen into ammonia (Boivin *et al.*, 1997). However, in those areas where the nodulation process is poor, alternative source of nitrogen to legumes crops are *Rhizobium* spp inoculation. It was found the *Rhizobium* spp inoculation in mungbean significantly increased photosynthetic rate, plant height, leaf area and dry matter (Tharkur and Panwar, 1995; Ndakidemi *et al.*, 2006).

Bar and Lal (1991) reported an increase in no of nodule per plant and yield with *Rhizobium* inoculation. Similarly Pandher *et al.* (1991) found that *Rhizobium* inoculation of mong bean significantly increases nodule per plant and yield. French bean Being high nutritional value and its different types of culinary uses, it is imperative to improve the crop production and quality using inoculation and other agronomic practices, so this experiment was carried out to study to identify growth and yield responses of different cultivars of French beans to *Rhizobium* inoculation and cultivars evaluation in order to identify best French bean cultivar for District Mansehra.

Material and methods

In order to investigate the interactive effect of *Rhizobium* inoculation and cultivars on nodules, growth and yield of French beans (*Phaseolus vulgaris* L.), a field experiment was conducted at National Tea and High Value Research Institute (NTHRI) during 2016. The layout of the experiment was laid out in randomized complete block design with split plot arrangement. Inoculated seeds were kept in main plot and varieties evergreen, 'Komal green' and were placed in the subplot. Total experimental area was 152m². Plant to Plant and Row to Row distance was 30 and 60 cm respectively.

Nitrogen at the rate of 18 kg and phosphorus at the rate of 24 kg ha⁻¹ as urea and single super phosphate respectively were applied at the time of sowing. Cultural practices like, hoeing, irrigation weeding and pesticide application was carried out uniformly. Data regarding, germination (%), plant height (cm), Plant spread (cm), no of branches plants⁻¹, no of pods plants⁻¹, pods length (cm), yield (kg ha⁻¹) and number nodule plants⁻¹ was recorded during the growth and after the harvesting of crop.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using Statistic 9 for windows software with RCBD with split plot arrangement including cultivars in subplot while *Rhizobium* inoculation was placed in main plot. The effects of treatments were determined from the least significant differences test (LSD) at $P \leq 0.05$.

Results and discussion

Germination Percentage

Germination percentage was significantly affected by *Rhizobium* inoculation, cultivars and their interaction.

Rhizobium application to seeds increases germination percentage; as the highest germination (90.01%) was recorded in plants which were inoculated with *Rhizobium* and the lowest (85.36%) was observed in non-inoculated plants (Table. 1). In case of different cultivars, maximum (89.48%) and minimum germination (87.04%) were recorded in 'Evergreen' and respectively (Table. 1). In case of interaction effect of *Rhizobium* inoculation and cultivars, application of *Rhizobium* to seeds gave maximum germination (91.963%) in cultivar 'Evergreen' whereas non-inoculated plots resulted in minimum germination (86.00%) in cultivar 'Winner' (Fig. 1).

No of Nodules plants⁻¹

Rhizobium inoculation, cultivars and their interaction had significant effect on No of nodules plant⁻¹ (Table-1) In *Rhizobium* treatments, maximum number of nodules plant⁻¹ (27.222) were recorded in *Rhizobium* inoculated plants while minimum number of nodules plant⁻¹ (16.333) were obtained in non-inoculated plants In case of cultivars, highest number of nodules plant⁻¹ (25.00) was noted in cultivar 'Evergreen'; whereas the lowest number of nodules plant⁻¹ (18.667) was recorded in cultivar 'Winner' (Table. 1).

The interaction between *Rhizobium* and cultivars indicates (Fig. 2) that highest number of nodules plant⁻¹ (31.333) was observed in *Rhizobium* inoculated plants in cultivar 'Evergreen' as compared to lowest number of nodules plant⁻¹ (15.33) in non-inoculated plants in cultivar 'Winner'. It has been reported that *Rhizobium* inoculation enhance nitrogen fixation and synthesis of B-indole acetic acid which are growth promoting substances, which might have resulted in good nodulation of crops (Baijuka and Semu, 1998).

Plant Height (cm)

Rhizobium inoculation and cultivars had significant effect on plant height (Table. 1). In case of *Rhizobium*, taller plants (29.55cm) were observed where seeds were treated with *Rhizobium* and shorter plants of 22.22 cm were produced in plots where seeds were not treated with *Rhizobium* (Table. 1). In case of cultivars, tallest (28.83cm) and shortest (22.66cm) plants were recorded in cultivars 'Evergreen' and 'Winner' respectively. Recent study showed that adequate amount of nitrogen fixed by *Rhizobium* significantly increases number of branches per plant that resulted in more plant height (Sajid *et al.*, 2011).

Plant Spread (cm)

Rhizobium inoculation, cultivars and their interaction had significant effect on plant spread (cm) (Table. 1). In case of *Rhizobium*, maximum plant spread (27.55 cm) was recorded inoculated plants; whereas minimum plant spread (19.66 cm) was noticed in non-inoculated plants (Table. 1). In cultivars, maximum plant spread (25.83 cm) was recorded in cultivar 'Evergreen' and minimum plant spread (21.50 cm) was found in cultivar 'Winner' (Table. 1). *Rhizobium* inoculation and cultivars interaction indicates that maximum plant spread (30.00 cm) was observed in *Rhizobium* inoculated plants with cultivar 'Evergreen' whereas minimum plant spread (17.66 cm) was noticed in non-inoculated plants with cultivar 'Winner' (Fig. 3). Previous study showed that, plants spread were significantly affected by *Rhizobium* inoculation as bacteria fixed the atmospheric nitrogen enhance photosynthetic activity of plant resulted in more branches and ultimately plants spread were increased, however difference between plant spread for cultivars might be due to genetic potential of the cultivars (Brooks *et al.*, 1988).

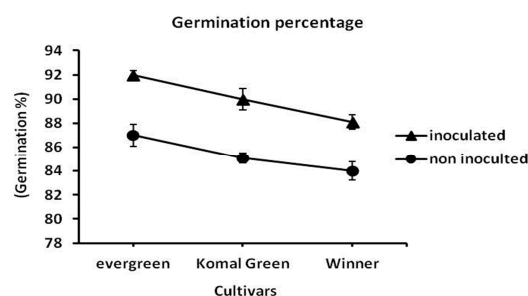


Fig. 1. Interaction effect of *Rhizobium* inoculation and cultivars on germination percentage. Vertical bars represent \pm SE of mean $P \leq 0.05$.

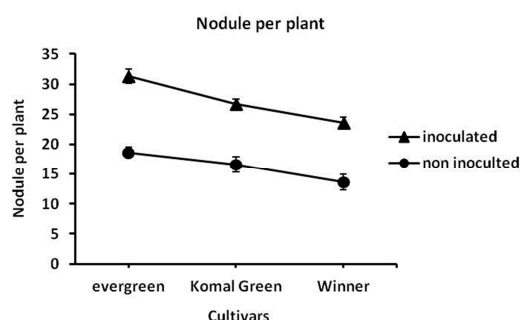


Fig. 2. Interaction effect of *Rhizobium* inoculation and cultivars on no of nodule per plant. Vertical bars represent \pm SE of mean $P \leq 0.05$.

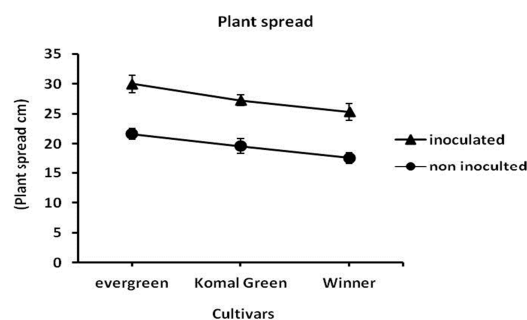


Fig. 3. Interaction effect of *Rhizobium* inoculation and cultivars on no plant spread. Vertical bars represent \pm SE of mean $P \leq 0.05$.

Table. 1. Mean value of germination percentage, No of Nodules plant⁻¹, plant height, plant spread as affected by *Rhizobium* inoculation and cultivars.

Treatments	Germination Percentage	No of Nodules plant ⁻¹	Plant Height (cm)	Plant Spread (cm)
<i>Rhizobium</i>				
Inoculated Plots	90.018 A	27.222 A	29.556 A	27.55 A
Non-inoculated plots	85.36 B	16.333 B	22.222 B	19.66 B
LSD	0.4888	0.4781	1.4342	0.4781
Varieties				
Evergreen	89.482 A	25.000 A	28.83 A	25.83 A
Komal green	87.540 B	21.667 B	26.16 B	23.50 B
Winner	86.047 C	18.667 C	22.66 C	21.50 C
LSD	0.2086	0.3138	0.7017	0.7359
Interaction				
R x V	Fig.1	Fig 2	NS	Fig. 3

Means followed by different letters for a given parameter for *Rhizobium* inoculation and cultivars significantly different at $P \leq 0.05$ (LSD test).

No of pods plant⁻¹

The treatments had significant effect on number of pods plant⁻¹ (Table. 2). In case of *Rhizobium*, maximum number of pods⁻¹ (41.83) was recorded with *Rhizobium* inoculated plants and minimum number of pods plant⁻¹ (35.55) was observed in non-inoculated plants.

In case of cultivars, higher number of pods plants⁻¹ (40.80) was recorded in cultivar 'Evergreen' as compared to minimum of 36.45 pods plants⁻¹ in cv winner. Interaction between *Rhizobium* inoculation and cultivars shows that greater number of pods plant⁻¹ (43.93) was recorded with *Rhizobium* inoculated plots in cultivar 'Evergreen' and minimum number of pods plants⁻¹ (33.33) was noted in non-inoculated plants at cultivar 'Winner' (Fig. Increases in pods plant⁻¹ in inoculated plants might be due to *Rhizobium* bacterium activity as more atmospheric

nitrogen were fixed by bacteria resulting in more leaves and shoots per plant which enable the plants to produce more carbohydrates to the lower parts of the plants and thus more pods per plants were produced. Gentili and Huss-Danell (2003) found that *Rhizobium* inoculation in combination with phosphorus (P) increased (67%) the no of pods plant⁻¹.

No of branches plant⁻¹

No of Branches plant⁻¹ was significantly affected by *Rhizobium* inoculation, cultivars and their interaction (Table. 2). Maximum branches plant⁻¹ (6.38). Was noticed in plots where seeds were inoculated with *Rhizobium*; whereas minimum number of branches plant⁻¹ (5.53) in non-inoculated plants. In case of cultivars, more number of branches plant⁻¹ (6.22) was recorded in cultivar 'Evergreen'; whereas less number of branches plant⁻¹ (5.70) were observed in cultivar 'Winner' (Table. 2).

In case of interaction, maximum branches plant⁻¹ (6.59) was recorded with *Rhizobium* inoculated plants in cultivar 'Evergreen'; whereas less no of branches plant⁻¹ (5.21) was observed in non-inoculated plants with cultivar 'Winner' (Fig. 5).

Several studies have shown that inoculation of legumes crops with *Rhizobium* species have positive correlation with the plant growth parameter as it enhances the nodulation, that utilize atmospheric nitrogen therefore increases photosynthesis and encourage the plant to produce more branches (Wange *et al.*, 1996; Rodriguez-Navarro *et al.*, 1999; Kyei-Boahen *et al.*, 2005).

Pods length (cm)

Rhizobium inoculation, cultivars and their interaction showed significant affect on pods length (cm) (Table. 2). In case of *Rhizobium*, longer pods (20.00 cm) were noted in *Rhizobium* inoculated plants whereas shorter pods (14.02 cm) were recorded in non-inoculated plants. Among cultivars, longer pods (18.95 cm) were noticed in cultivar 'Evergreen' and smaller pods (14.87 cm) in cv winner (Table. 2).

Interaction between *Rhizobium* and cultivars indicates that pods length (21.91cm) in cultivar 'Evergreen' was higher when seeds were treated with *Rhizobium* whereas non inoculated plants produces smaller pods (11.75 cm) in cultivar 'Winner'. The increase in pods length (cm) might be due to synthetic inoculation of *Rhizobium*, which increased.

The number of bacteria in the soil and hence longer pods are produced. However, difference among cultivars for pods length was due to the genetic potential of these cultivars. Similar results were also reported by (Jagvir *et al.*, 2004).

Yield (kg ha⁻¹)

Rhizobium inoculation, cultivars and their interaction had significantly affected yield hectare⁻¹ (Table. 2). Maximum yield (5939.4 kg ha⁻¹) was given by the plants, which were inoculated while minimum yield (5130.0 kg ha⁻¹) was there in un-inoculated plants. Among cultivars, maximum yield (5810.8 kg ha⁻¹) was recorded in cultivar 'Evergreen' and minimum yield (5260.0 kg ha⁻¹) was observed for cv winner which might be due to the genetic potential of these cultivars (Table. 2). In case of interaction among *Rhizobium* and cultivars, maximum yield (6250 kg ha⁻¹) was given by inoculated plants of cv evergreen and minimum yield was given by un-inoculated plants of cultivar 'Winner' (4866.7 kg ha⁻¹) (Fig. 7).

Maximum yield in inoculated plants may be attributed to symbiotic relationship of *Rhizobium* (bacteria) with the roots of leguminous crops, which fix the atmospheric nitrogen into roots and hence yield ha⁻¹ was increased (Ahmed *et al.*, 2009). It may also be due to more leaves and plant height, as according to the source sink relationship, more carbohydrates were produced due to more number of leaves, sank into the root zone and hence more production.

Table. 2. Mean value of No of branches plant⁻¹, No of pods plant⁻¹, Pods Length (cm), Yield (kg ha⁻¹) as affected by *Rhizobium* inoculation and cultivars.

Treatments	No of branches plant ⁻¹	No of pods plant ⁻¹	Pods Length (cm)	Yield (kg ha ⁻¹)
<i>Rhizobium</i>				
Inoculated Plots	6.38 A	41.83 A	20.00 A	5939.4 A
Non-inoculated plots	5.53 B	35.55 B	14.02 B	5130.0 B
LSD	0.1052	0.8311	0.3734	97.126
Varieties				
Evergreen	6.22 A	40.80 A	18.95 A	5810.8 A
Komal green	5.97 B	38.83 B	17.20 B	5533.3 B
Winner	5.70 C	36.45 C	14.87 C	5260.0 C
LSD	0.0160	0.8117	0.1947	49.018
Interaction				
R x V	Fig. 4	Fig. 5	Fig.6	Fig.7

Means followed by different letters for a given parameter for *Rhizobium* inoculation and cultivars significantly different at $P \leq 0.05$ (LSD test).

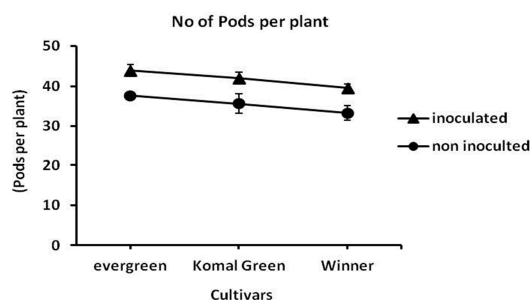


Fig. 4. Interaction effect of *Rhizobium* inoculation and cultivars on no of pods per plant. Vertical bars represent \pm SE of mean $P \leq 0$.

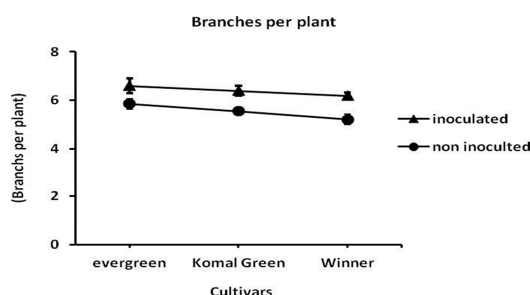


Fig. 5. Interaction effect of *Rhizobium* inoculation and cultivars on no of branches per plant. Vertical bars represent \pm SE of mean $P \leq 0.05$.

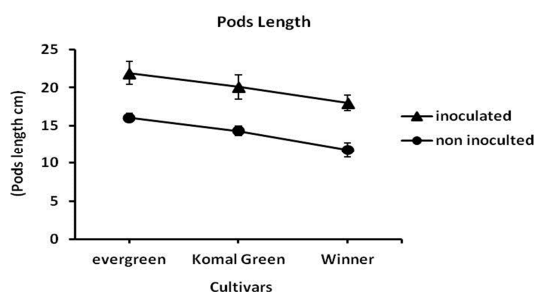


Fig. 6. Interaction effect of *Rhizobium* inoculation and cultivars on pods length. Vertical bars represent \pm SE of mean $P \leq 0.05$.

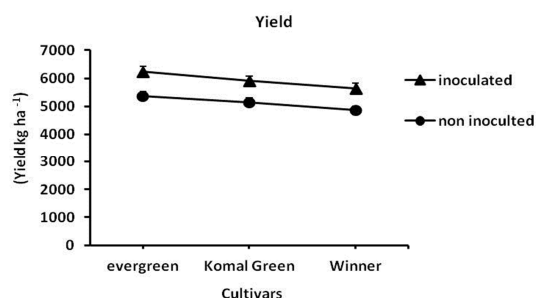


Fig. 7. Interaction effect of *Rhizobium* inoculation and cultivars on yield. Vertical bars represent \pm SE of mean $P \leq 0.05$.

Conclusion and recommendation

The study shows that there was a significant of synthetic inoculation of *Rhizobium* on the yield and other growth parameter of French beans. From present study it has been concluded that inoculation of *Rhizobium* to French beans has significantly increased plant height, yield and yielding components.

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