Specificity of French Bean (*Phaseolus vulgaris* L.) Genotypes and *Rhizobium phaseoli* Strains to Establish Symbiotic N-fixation in Inceptisols of Varanasi, Uttar Pradesh, India

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Abstract

A pot experiment consisted of 15 treatment combinations of 5 genotypes of *Phaseolus vulgaris* L. (G_1=HUR 15, G_2= IC 14919, G_3=DARL 1136, G_4=DARL 1434 and G_5=PI 175822) and 2 strains of *Rhizobium leguminosarum* biovar *phaseoli* along with a control (I_0=Control, I_1=USDA 2667 and I_2=USDA 2676) was carried out in triplicates under factorial randomized block design (FCRBD) during *rabi* season of 2006-07 at Banaras Hindu University, Varanasi (Uttar Pradesh). Among the genotypes, G_2 (IC 14919) revealed superiority in nodulation and plant growth at flowering stage with higher grain yield (3.06 g plant^{-1}), N content (3.50%) and its uptake (0.016 mg plant $^{-1}$) at par values with G_5. The *Rhizobium* strain USDA 2676 was comparatively superior for higher nodulation and nitrogen fixation with each of the genotypes. However, the most effective *Rhizobium* strain *Phaseolus vulgaris* genotypes specificity was seen in between USDA 2676 and G_2 with maximum grain yield (3.56 g plant^{-1}), N content in grain (3.67%) and its uptake /grain (0.018 mg plant^{-1}) followed by strain USDA 2676 and G_5. The results indicate that the success of symbiotic N- fixation in French bean (common bean) is highly dependent on strain-genotypes specificity and thus, it should be further tested in field conditions to popularize its cultivation at low fertilizer N input in eastern part of Uttar Pradesh, India.

1. Introduction

French bean (*Phaseolus vulgaris* L.) is an important legume containing 21.1% grain protein, 69.9% carbohydrate, 1.5% fat besides 381 g Ca, 42.5 mg phosphorus and 12.4 mg iron 100 g^{-1} of edible parts. Globally it occupies 28.2 mha area with an annual production of 18.95 Mt. In India, it is traditionally grown in hilly tracts of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Bihar, Orissa and some parts of Maharashtra state in India in *kharif* season (Ali and Kushwaha, 1987). It was introduced in 1980s as *rabi* legume in eastern part of Uttar Pradesh. Due to absence of appropriate kind of *Rhizobia* in soils of this plain or due to poor nodulation, French bean (common bean) is cultivated by application of 120 kg N ha^{-1} under irrigated condition. Therefore, introduction of effective *Rhizobium* strains was essential to induce nodulation and N-fixation in this crop in the plains of eastern Uttar Pradesh. The effectiveness of *Rhizobium* is very much concern to the strain-legume genotype specificity. Hence the present research work was undertaken to select effective *Rhizobium* strain for promising genotypes of common bean based on strain-genotype specificity in Varanasi district of Uttar Pradesh state in India.

2. Materials and Methods

The experiment was carried out in pot culture with 15 treatment combinations of 5 genotypes (G_1=HUR 15, G_2=IC 14924, G_3=DARL 1136, G_4=DARL 1434, G_5=PI 175822) of common bean (*Phaseolus vulgaris* L.) and 2 *Rhizobium phaseoli* strains along with a control (I_0=Control, I_1=USDA 2667, I_2=USDA 2676) in two sets of three replications under factorial randomized block design (FCRBD) during *rabi* season of 2006-07 at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The 1st set was used for nodulation study at flowering stage and the 2nd set was observed up to harvesting for yield, N-content and nutrient uptake. Ninety earthen pots of diameter 12 inches and height 15 inches were selected and each of them was filled with mixture of 10 kg soil, 8.7 g urea @ 23 kg ha^{-1}, 56 g SSP @ 21 kg ha^{-1}, 9.7 g MOP @ 26 kg ha^{-1}. Healthy and counted seeds (10 numbers) for each pot were separately inoculated into small beakers with 5 ml of sticker solution and 5 ml of desired broth of *Rhizobium* culture. After soaking in shade for 30 minutes, seeds were sown as per treatment in different pots at specific depth. Five plants were maintained in each pot for the study. The initial properties of the experimental soil was clay loam in texture with pH 7.6, EC 0.265 dS m^{-1}, organic carbon 4.9 g kg^{-1}, available N 215 kg^{-1}, available P 11.2 kg^{-1} and available K 214 kg^{-1}.

Five plants from each of the pot of one set of experiment were uprooted with the help of *khurpi* 60 days after sowing (DAS). Plant roots with the lump of soil were washed in running water. Root nodules were carefully separated using forceps. Number, fresh and oven dried weight of nodules, root and shoot plant^{-1} were recorded. Harvesting of crop of another set was done at the physiological maturity. Pod and grain yield of each pot were recorded separately using electrical balance and expressed in g plant^{-1}.

Initial soil sample was analyzed for pH, EC (1:2.5 soil water suspensions), available nitrogen (alkaline permanganate), available phosphorus (Olsen's) and available potassium (NH_4OAC extractable). Nitrogen in grain and straw was analyzed using diacid mixture ($4H_2SO_4$:$1HClO_4$) as per calorimetric Nessler's reagent using standard method described by Jackson (1973).

3. Results and Discussion

3.1. Effect on nodulation at flowering stage

Among genotypes, G_5 was found significantly superior with nodule number (6) and dry weight (49.55 mg) plant^{-1} over the other treatments (Table 1). G_3 and G_4 have shown at par values in number of nodule but values of their weight were differing significantly with each other and over the other genotypes. Negligible number and dry weight of nodules by uninoculated treatments supported the view that the soils of this region are either free from native *Rhizobia* specific for nodulation or have poor nodulating capacity in common bean (Gaur and Subba Rao, 1984; Yadav and Sanoria, 1994). The *Rhizobium* strain USDA 2676 (I_2) was found most effective in nodulation with highest number (6.51) and dry weight (68.66 mg plant^{-1}) of nodules, significantly higher over the control and other inoculants. Considering the weight of nodules, the interaction of G_5 with USDA 2676 (G_5I_2) has shown highest specificity with value of 91.97 mg plant^{-1} followed by G_3I_2 and G_2I_2. The variability in nodulation in different genotypes caused by different inoculants was due to genotype-strain specificity (Park and Buttery, 1989) which is very much governed by the involvement of polysaccharides secreted by the root of French bean (Fraysse et al., 2003).

Table 1: Effect of common bean genotypes and *Rhizobium phaseoli* strains on nodulation and plant growth at flowering (40 DAS)

Treatment	Nodules plant^{-1}		Shoot plant^{-1}			Root plant^{-1}		N% in shoot
	Number	Weight (mg)	Fresh weight (g)	Dry weight (g)	Height (cm)	Fresh weight (g)	Dry weight (g)	
G_1	1.28	25.49	9.25	2.70	20.00	1.07	0.29	2.51
G_2	2.44	34.1	9.40	2.43	18.40	1.32	0.35	2.69
G_3	5.25	40.88	13.83	2.97	19.42	1.05	0.33	2.64
G_4	4.80	34.49	8.99	2.77	19.45	1.44	0.33	2.93
G_5	5.56	49.55	10.72	2.83	24.37	1.24	0.25	3.02
SEM±	0.68	3.63	1.52	0.17	1.15	0.09	0.043	0.10
CD (p=0.05)	1.97**	10.52**	4.40**	3.33**	0.281**	NS	NS	0.28**
I_0	1.32	12.26	8.23	1.83	20.32	1.13	0.24	2.53
I_1	3.77	29.79	8.92	2.77	20.35	1.39	0.33	2.62
I_2	6.51	68.66	14.16	3.38	20.82	1.54	0.49	3.10
SEM±	0.52	2.81	1.17	0.13	0.90	0.07	0.03	0.12
CD (p=0.05)	1.52**	8.15**	3.41**	0.39**	NS	0.20**	0.08**	0.36**
G_1I_0	0.22	0.88	8.21	0.96	23.75	1.08	0.24	2.09
G_1I_1	1.11	17.75	7.41	0.69	15.80	1.34	0.38	2.23
G_1I_2	2.51	57.85	12.12	1.04	21.37	0.78	0.25	2.80
G_2I_0	1.00	14.81	10.06	0.66	18.22	1.31	0.31	2.69
G_2I_1	2.77	22.31	6.05	0.73	17.35	1.47	0.41	2.37
G_2I_2	3.57	65.19	12.08	0.73	19.02	1.17	0.33	3.02
G_3I_0	0.88	4.38	8.88	0.41	20.45	0.83	0.18	2.52
G_3I_1	6.99	48.12	9.45	0.79	20.17	1.34	0.32	2.37
G_3I_2	7.88	70.13	23.15	1.85	17.67	0.98	0.49	3.03
G_4I_0	1.52	24.94	6.65	0.69	17.47	1.44	0.27	2.76
G_4I_1	2.77	20.39	10.66	0.96	22.30	1.31	0.32	2.95
G_4I_2	10.10	58.15	9.67	0.77	18.60	1.56	0.41	3.09
G_5I_0	2.99	16.29	7.35	0.71	22.67	0.99	0.20	2.21
G_5I_1	5.21	40.39	11.04	0.77	22.50	1.47	0.22	3.18
G_5I_2	8.49	91.97	13.79	0.85	27.47	1.26	0.32	3.68
SEM±	1.18	6.29	2.63	0.15	2.02	0.16	0.07	0.28
CD (p=0.05)	3.42**	18.22**	7.61**	0.43**	5.84**	0.46**	0.20**	0.81**

**Significant at 5% level of significance; NS=Non-significant; CD=Critical difference; DAS=Days after sowing

3.2. Effect on plant growth at flowering stage

Considering the genotype treatments, maximum fresh and dry weight of shoot 13.83 and 2.97 g plant^{-1}, respectively, were observed in G_3 whereas maximum height of shoot (24.37 cm plant^{-1}) of G_5 was significantly superior over all the other genotypes (Table 1). The strain USDA 2676 was found significantly superior in respect of fresh (14.16 g plant^{-1}) and dry weight (3.38 g plant^{-1}) over other inoculants. There was no significant variation in plant height due to inoculated treatments. The maximum interaction effect shown for fresh and dry shoot weight due to G_3I_2 and maximum height (27.47 cm plant^{-1}) due to G_5I_2 were significantly superior over most of the treatment combinations. The variability in increase of fresh and dry weight by different genotypes in response to inoculation over control was due to N-fixing capacity of concerned *Rhizobium phaseoli* strain as also reported by Park and Buttery (1989).

3.3. Effect of Rhizobium strains on N-content in shoot at flowering stage

The N-content in common bean genotypes varied significantly from 2.51% in G_1 to maximum 3.02% in G_5. Genotype G_4 has shown at par value (2.93%) with G_5 and both of the treatments were significantly superior over all the other genotypes (Table 1). *Rhizobial* strain USDA 2676 (I_2) recorded maximum 3.10% N in shoot significantly higher over I_0 and I_1. The interaction between the *Rhizobium* strains and common bean genotypes were significantly varying from 2.09% in G_1I_0 to maximum 3.68% in G_5I_2. These values were at par with value of 3.18% due to G_5I_1. This result confirmed the findings of Hungaria et al. (1991) and Srivastava and Ormand (1986). According to them, high N-content in shoot at flowering in common bean is the sign of establishment of symbiosis between genotypes and *Rhizobial* strains.

3.4. Effect of Rhizobium stains on yield attributes of common bean genotypes

The number of pods ranged from 3.15 to 3.38 plant^{-1} in genotype G_3 and G_5, respectively, and there was no significant variation among the genotypes (Table 2). Both the strains USDA 2676 and USDA 2667 have shown at par values of pods. The interaction of strain USDA 2676 with genotype G_1 has shown maximum 3.80 pods plant^{-1}, significantly higher over some of the other treatment combinations. The number of grains pod^{-1} and plant^{-1} ranged from 2.94 to 3.28 and 9.20 to 11.15, respectively, and they varied significantly among the genotype treatments. The maximum number of grain (3.28 grains pod^{-1}) and its weight (11.15 g plant^{-1}) were recorded in to G_5 significantly superior over G_1. The *Rhizobial* strain USDA 2676 has shown higher interaction with values of 3.71 and 3.62 grains pod^{-1} in G_2 and G_5, respectively. Considering the number of grain plant^{-1} the G_5 and G_2 have shown higher interaction with values of 13.26 and 13.18 grains plant^{-1} when inoculated with USDA 2667 and USDA 2676 strains of *Rhizobium phaseoli,* respectively. The maximum weight of grain 3.06 g plant^{-1} in G_2 was significantly superior over all other genotypes followed by

Table 2: Effect of common bean genotypes and *Rhizobium phaseoli* strains specificity on yield attributing characters at harvest

Treatment	Pods plant^{-1} (number)	Grains pod^{-1} (number)	Grains plant^{-1} (number)	Grain weight plant^{-1} (g)	N% in grain	N% in straw	N uptake by grain (mg plant^{-1})	N uptake by straw (mg plant^{-1})
G_1	3.18	2.83	9.20	2.66	3.35	1.69	0.013	0.196
G_2	3.29	3.25	10.85	3.06	3.50	1.81	0.016	0.190
G_3	3.15	2.95	9.38	2.62	3.38	1.78	0.012	0.359
G_4	3.22	2.94	9.67	2.25	3.33	176	0.010	0.184
G_5	3.38	3.28	11.15	2.71	3.43	1.43	0.014	0.140
SEM±	0.15	0.12	0.62	0.07	0.03	0.06	0.007	0.025
CD (p=0.05)	NS	0.34**	1.79**	0.22**	0.11**	0.18**	0.020**	0.072**
I_0	2.75	2.60	7.25	2.39	3.12	1.06	0.012	0.108
I_1	3.39	3.06	10.44	2.62	3.55	1.96	0.011	0.214
I_2	3.59	3.47	12.43	2.97	3.62	2.06	0.029	0.259
SEM±	0.11	0.09	0.48	0.05	0.03	0.05	0.005	0.019
CD (p=0.05)	0.33**	0.27**	1.39**	0.17**	0.08**	0.14**	0.014**	0.050**
G_1I_0	2.40	2.44	5.83	2.70	3.01	1.05	0.014	0.087
G_1I_1	3.34	2.67	8.94	2.48	3.55	2.03	0.015	0.166
G_1I_2	3.80	3.39	12.84	2.78	3.49	2.00	0.018	0.254
G_2I_0	2.78	2.66	7.39	2.51	3.30	1.10	0.012	0.090
G_2I_1	3.56	3.39	12.00	3.12	3.53	2.14	0.013	0.227
G_2I_2	3.55	3.71	13.18	3.56	3.67	2.30	0.018	0.335
G_3I_0	2.70	3.10	8.487	2.39	3.26	1.05	0.013	0.188
G_3I_1	3.18	2.65	8.44	2.54	3.41	2.05	0.014	0.287
G_3I_2	3.57	3.10	11.07	2.95	3.48	2.24	0.018	0.301
G_4I_0	2.97	2.36	7.24	1.73	2.96	1.04	0.009	0.091
G_4I_1	3.24	2.96	9.587	2.25	3.47	1.93	0.010	0.238
G_4I_2	3.47	3.52	12.19	2.76	3.56	2.19	0.012	0.222
G_5I_0	2.92	2.45	7.30	2.61	3.10	1.06	0.013	0.084
G_5I_1	3.66	3.62	13.26	2.71	3.66	1.66	0.014	0.149
G_5I_2	3.56	3.62	12.88	2.81	3.55	1.57	0.018	0.185
SEM±	0.26	0.21	1.07	0.05	0.06	0.11	0.001	0.044
CD (p=0.05)	0.75**	0.61**	3.09**	0.17**	0.19**	0.31**	0.003**	0.127**

** Significant at 5% level of significance; NS=Non-significant; CD=Critical difference

2.71 g plant^{-1} in G_5. *Rhizobium* strain USDA 2676 (I_2) recorded highest weight of grain 2.97 g plant^{-1} significantly higher over uninoculated (I_0) treatment (2.39 g plant^{-1}) and with USDA 2667. The strain USDA 2676 has shown higher interaction with value of 3.56 g plant^{-1} grain weight with genotype G_2. The significant increase in weight of French bean genotypes might be due to greater accumulation of plant metabolites in the grain at pod filling stage as also reported by Burris (1988), and Sparrow and Ham 1983. Therefore, pod and grain yield of common bean in present study increased in strain USDA 2676 and to some extent in USDA 2667.

3.5. Effect of Rhizobium strains on N and its uptake by common bean genotypes

The value of N-content 3.50% in grain of G_2 was significantly higher over G_1, G_3 and G_4 genotypes. Maximum N-content 3.62% in USDA 2676 was significantly higher over uninoculated treatment (3.12%) and at par with strain USDA 2667 (Table 2). The interactions between the strains and genotypes were found to be significant with maximum value of 3.67% recorded in G_2I_1 followed by 3.66, 3.56 and 3.55% due to G_5I_1, G_4I_2 and G_5I_2, respectively. *Rhizobium* strain USDA 2676 gave maximum N-content (2.06%) and it was significantly higher over uninoculated treatment (1.06%). The higher uptake of N in grain (0.016 mg plant^{-1}) due to genotype G_2 was significantly higher over rest of the genotypes with an exception of G_5 (0.014 mg plant^{-1}). The inoculation of USDA 2676 has shown highest uptake (0.029 mg plant^{-1}) and it was significantly higher over control. The interaction between strain USDA 2676 and genotype G_1, G_2, G_3 and G_5 with same values 0.018 mg plant^{-1} for each have shown significant superiority over rest of treatment combinations except G_1I_1. In case of straw, the interaction effect varied significantly from 0.087 to maximum 0.335 mg plant^{-1} in G_1I_0 and G_1I_2 combinations, respectively. The strain USDA 2676 in combination to G_1 has shown maximum (0.0335 mg plant^{-1}) and significant uptake of N by straw over rest of the treatment combinations. This interaction shows very good symbiotic establishment because of genetic control and symbiotic functions between *Rhizobium* strain USDA 2676 and G_1 (Skorupska et al., 2006). The significant accumulation of N in grain and straw was due to transport of fixed N from nodules, leaves and stem to pod at pod filling stage (Dubois and Burris, 1986) and therefore, greater value of N-content and uptake by grain and straw of common bean. The high rates of differences of N accumulation by grains due to symbiosis in soybean (43%) and in French bean (56 to 78%) have been reported by Piha and Munns (1987).

4. Conclusion

The most effective *Rhizobium* strain and common bean genotype specificity was found between strain USDA 2676 and G_2 (IC 14919) for the establishment of symbiosis on the basis of grain yield (3.56 g plant^{-1}), N content (3.67% N in grain and 2.30% N in straw), nitrogen uptake (0.018 mg plant^{-1} by grain and 0.335 mg plant^{-1} by straw) followed by effective specificity between strain USDA 2676 and genotype G_5 (PI 175822). This research work needs confirmation in field conditions prior to dissemination of this technology to the local farmers.

5. Further Research

The basic problem in establishment of symbiosis between French bean and *Rhizobium* is non-survibality of strains brought from temperate regions in the soils of tropical and sub-tropical climatic conditions. Therefore, future research is needed to characterize the introduced *Rhizobial* strains for their requirement of temperature, pH, antagonistic and synergistic relationship with other beneficial rhizomicrobes in laboratory conditions followed by field testing to find actual time of sowing, varietals response and nutritional requirements.

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