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Testing of strains of *Rhizobium phaseoli* on French bean (*Phaseolus vulgaris*) under greenhouse and field conditions

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ABSTRACT

Seven selected strains of *Rhizobium phaseoli* (from the USDA *Rhizobium* Culture Collection, Beltsville) were tested with 'Bean-Lot 610 E' French bean (*Phaseolus vulgaris* L.) (from Idaho) at Beltsville in 1989. Later, in a preliminary field trial at Varanasi in early 1990, 6 of these strains were applied as inoculant to the seedlings of 2 bean varieties — American 'Bean-Lot 610 E' and Indian 'HUR 15'. Indian variety showed better nodulation than the American one. Strain differences could also be noted. During 1990–91, 4 of the 6 rhizobial strains along with 2 fresh isolates from the preliminary field trial were studied in a statistically designed field experiment with basally and uniformly applied N, P and K @ 23, 21 and 26 kg/ha respectively. Three of the culture treatments and 1 top-dressing of N @ 46 kg/ha (N_{46}) without inoculum were significantly superior to the control. The USDA 2680 strain was the best strain, which differed significantly even from the N_{64} as top-dress treatment in enhancing the pod and grain yields. Increase in grain yield due to this strain over the control was 200%.

Rhizobia, specific to the introduced temperate beans of the *Phaseolus* group, are virtually absent from Indian soils, and inoculation with specific strains of *Rhizobium phaseoli* is necessary to overcome one of the limitations in the establishment of kidney bean or French bean or *rajmash (P. vulgaris* L.) in India (Guar and Subba Rao, 1984). In absence of effective nodulation, successful planting of kidney bean is presently possible through heavy applications of fertilizer nitrogen. This study is aimed at finding out suitable strains of french-bean *Rhizobium,* so that fertilizer N could be saved for economic cultivation of kidney bean through rhizobial inoculation in India.

MATERIALS AND METHODS

Leonard jar testing

Seven strains (Table 1) of *R. phaseoli* (*R. leguminosarum* biovar *phaseoli*) from the Beltsville *Rhizobium* Culture Collection (Keyser and Richard, 1987) were chosen for Leonard jar testing on 'Bean-Lot 610 E_1' (Tendrette Bushy from Idaho) French bean at the Nitrogen Fixation and Soybean Genetics Laboratory, Beltsville. Three seeds were planted in each jar containing vermiculite and pearlite (2 : 1) and nutrient solution on 12 October 1989. Inoculum @ 2 ml/3 seeds was used and the treatments including the uninoculated control were replicated thrice. The jars, kept in a

Table 1. *Rhizobium phaseoli* strains used in Leonard jar testing

Accession number of strain	Host plant	Geographical source	Year	Comments
USDA 2667	*P. vulgaris*	Washington	1948	Recommended
USDA 2669	*P. vulgaris*			Recommended
USDA 2674	*P. vulgaris*	Brazil		Recommended
USDA 2676	*P. vulgaris*	Columbia	1972	
USDA 2680	*P. vulgaris*	Idaho		
USDA 3317	*P. lunatus*			
USDA 3359	*P. lunatus*			

glasshouse, were irrigated with nutrient solution as and when required. Plants were uprooted 40 days after planting for assessing the nodulation, dry-matter production and acetylene reduction.

Preliminary field trial

The above strains in lyophilized condition were brought to the Soil Microbiology Laboratory, Banaras Hindu University in December 1989 and later rejuvinated. In early 1990, Indian var. 'HUR 15' (V_1) and Idaho var. 'Bean-Lot-610-E_1' (V_2) of French bean were raised, each in 2 blocks, and 7 treatments (no inoculum, USDA 2667, USDA 2669, USDA 2674, USDA 2680 and USDA 3317) were randomly allocated to the 7 lines (2.5 m long) in all the blocks. Ten-day-old roth cultures of different strains were used @ 1 ml/plant to inoculate the 15-day-old seedlings with the help of sterilized pipettes at the site of stem emergence in soil. Three plants from each line were randomly uprooted 45 days after planting for nodule observation.French isolates of *Rhizobium* from the healthy nodules of other plants were also made.

Replicated trial

In this field trial (1990–91) 2 of the inferior strains of *R. phaseoli* from the preliminary field screening were substituted by fresh isolates of USDA 2667 and USDA 2676. Two additional uninoculated controls with top-dressing of N @ 23 and 46 kg/ha (N_{23} and N_{46}) were also included. All the 9 treatments were replicated thrice in randomized block design having plots of 4 m × 1.25 m. N, P and K @ 23, 21 and 26 kg/ha respectively were applied basal to each plot. French bean var. 'HUR 15' was sown @ 125 kg seed/ha. As per treatment, seeds of each plot were inoculated with 2.5 ml of 3-week-old broth culture and 2.5 ml sticker solution (15 g *gur* and 1.26 g sodium molybdate/100 ml water). For nodule study, 5 plants were uprooted from each plot 40 days after planting. On maturity, the pods were harvested and later grains were separated.

RESULTS AND DISCUSSION

Effect of *Rhizobium* strains was significant on plant tops of French bean in Leonard jar testing (Table 2). All the strains of *R. phaseoli* from French bean differed from the control, whereas both the strains of *R. phaseoli* from lima bean (*P. lunatus*) did not differ from the control. This shows host specificity of rhizobial strains. USDA 3317 and USDA 3359 would perhaps be more

effective on lima bean instead of French bean. Effects on root meass, nodulation and acetylene reduction were non-significant but marked differences due to most of the strains in comparison to the control were quite obvious. Maximum plant and root weights were observed in strain USDA 2676. Nodule weight and acetylene reduction were maximum in USDA 2674 and USDA 2669 respectively.

Mean values on nodule number, nodule-dry weight and grain yield for the Indian as well as American variety of French bean in the preliminary field trial (Table 3) under Indian condition indicate variability in *R. phaseoli* strains. Varanasi soils are free from rhizobial strains specific to *P vulgaris*. Nodulation of plants is a clear evidence of the establishment of the exotic rhizobial strains. Presence of nodules in the control treatment is not an indication of the presence of native rhizobia specific to French bean in soil, but rather an event of contamination from the rhizobial treatments. During morning the standing plants were inoculated by the procedure descried earlier and on the

Table 2. Leonard jar testing of *R. phaseoli* strains on French bean

Treatment	Plant tops/plant (g)	Root weight/ plant (mg)	Nodule-weight/ plant (mg)	C_2H_2 reductase plant/hr (μ moles)
Control	0.30	184	14	2.29
USDA 2667	1.29	271	33	3.62
USDA 2669	0.93	326	50	5.57
USDA 2674	1.11	332	69	5.51
USDA 2676	1.40	392	46	4.91
USDA 2680	1.02	306	45	4.91
USDA 3317	0.54	269	36	4.56
USDA 3359	0.46	343	31	4.14

Table 3. Preliminary field testing of *R. phaseoli* strains with 2 varieties of French bean

Treatment	Nodules/ plant		Nodule-dry weight/plant (mg)		Grain yield/ plant (g)	
	V_1	V_2	V_1	V_2	V_1	V_2
Control	2.5	6.3	1.2	2.7	2.1	1.1
USDA 2667	13.3	7.2	5.0	5.0	2.2	0.7
USDA 2669	3.2	2.2	3.5	0.3	2.1	1.0
USDA 2674	0.8	5.0	5.2	2.7	1.8	1.0
USDA 2676	5.7	2.8	1.2	0.2	2.7	1.1
USDA 2680	11.8	9.8	21.2	7.5	2.0	1.3
USDA 3317	1.2	0.0	0.8	0.0	1.5	2.3

V_1, 'HUR 15'; V_2, 'Bean-Lot 610 E_1'

same night there was heavy rain, leading to contamination from one row to another. Under such a situation it is difficult to draw concrete conclusion regarding efficiency of the strains. However, the data do provide clue on the poor establishment of the American variety of French bean. Moreover, nodulation appeared to be better in Indian variety of French bean. Weight of dry nodules/plant was the maximum strain USDA 2680 for both the bean varieties.

In the field trial of randomized block design, effect of treatment was significant on nodulation as well as yield of the French bean (Table 4). Very scanty nodulation in the control and N_{23} as top-dress treatments appears to be due to contamination from the inoculated plots. There was no nodulation in N_{46} as top-dress treatment, but the yields of pods and grains were increased significantly over the control. Increasing trend in the yield due to N_{23} as top-dress treatment was also apparent. USDA 2680 gave the maximum nodulation as well as yield. This treatment differed significantly from the uninoculated controls with or without additional N. USDA 2667 R and USDA 2676 were also significantly superior to the control but the latter strain appeared to be better than the former fresh isolate. The other 2 strains (USDA 2667 and USDA 2669) and fresh isolate (USDA 2676 R) were no better than the control.

French bean is inefficient in nodulation and N_2 fixation (Park and Buttery, 1989), but can utilize N from organic and inorganic sources applied as manures and fertilizers, and from symbiotically fixed N_2 in nodules. With little or no nodulation, the crop responds to large amounts of N ranging from 120 kg/ha (Ahlawat and Sharma, 1989) to 200 kg/ha (Buttery *et al.*, 1987). Although symbiotic N fixation system in the beans is unable to provide enough N for maximum grain yield, it contributes up to 90 kg N/ha which is 40–50% of the total N found in bean plants near physiological maturity (Westerman *et al.*, 1981). In the present study, uniform basal application of 23 kg N/ha dose not seem to inhibit nodulation.

Table 4. Field evaluation of rhizobial strains and fresh isolates* on nodulation and yield of Indian variety of French bean 'HUR 15'

Treatment	Nodules/plant		Yield (q/ha)	
	Number	Dry weight (mg)	Pod	Grain
Control	0.5	1.3	9.13	5.60
N_{23}	0.5	0.7	13.40	8.80
N_{46}	0.0	0.0	15.27	10.40
USDA 2667	1.3	2.7	8.93	5.80
USDA 2667*	17.0	51.3	17.67	12.26
USDA 2669	2.0	6.7	9.40	7.40
USDA 2676	22.0	77.0	19.13	13.20
USDA 2676 R*	5.3	24.7	10.80	6.80
USDA 2680	51.0	258.7	24.07	16.80
CD (P = 0.05)	14.6	7.4	4.38	3.89

N_{23}, N @ 23 kg/ha; N_{46}, N @ 46 kg/ha (both top-dressed)

Srivastava and Ormrod (1986) also reported enhanced nodulation and N fixation at a low rate of added inorganic nitrogen. The ineffectivity of certain strains might be due to their unability of establishing symbiotic relationship. Data for nodulation in all the 3 trials indicate that Leonard jar testing is just an indication of the infectiveness of the strains in the absence of competitive soil microflora but field evaluation of the strains does provide evidence for the effectiveness of the strains in presence of soil micro-organisms. The 2 promising strains (USDA 2680 and USDA 2676) and a fresh isolate (USDA 2667) may be further tested with different varieties of the bean in different agroclimatic conditions of the country.

REFERENCES

Ahlawat, I. P. S. and Sharma, R. P. 1989. Response of french bean genotypes to the soil moisture regimes and phosphate fertilizer. *Indian Journal of Agronomy* **34** (1) : 70.

Buttery, B. R., Park, S. J. and Findlay, W. I. 1987. Growth and yield of white bean (*Phaseolus vulgaris* L.) in response to nitrogen, phosphorus and potassium fertilizer and to inoculation with *Rhizobium*. *Canadian Journal of Plant Science* **67** : 425.

Gaur, Y. D. and Subba Rao, N. S. 1984. The need for *Rhizobium phaseoli* inoculation to establish American beans in India. *Current Science* **53** : 430.

Park, S. J. and Buttery, B. R. 1989. Identification and characterization of common bean (*P. vulgaris* L.) lines well nodulated in the presence of high nitrate. *Plant and Soil* **119** : 237.

Srivastava, H. S. and Ormrod, D. P. 1986. Effect of nitrogen dioxide and nitrate nutrition on nodulation, nitrogenous activity, growth and nitrogen content of bean plants. *Plant Physiology* **81** : 373.

Westerman, D. T., Kleinkopf, G. E., Porter, L. K. and Leggett, G. E. 1981. Nitrogen sources for bean seed production. *Agronomy Journal* **73** : 660.