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Nodulation Study in Some Varieties of Frenchbean Crop (*Phaseolus vulgaris* L.)

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ABSTRACT : In the present fuel subsidized agriculture practices, leguminous plants play an unique role by fixing nitrogen through symbiotic association with *Rhizobium* or *Bradyrhizobium.* Among the legumes, French bean or *Phaseolus vulgaris* L. Which are commonly known as Rajmash are used profusely by the common people as an alternative diet of protein. It is very nutritious and contain 22.9% of protein, 1.2% of fat, 60.6% of carbohydrate and a large number of minerals like calcium (260 mg/100g of seed), phosphorus (101 mg/100g of seed) and iron (5.8 mg/100g of seed). The genus *Phaseolus* belongs to the family leguminaceae, subfamily Papilionaceae with diploid chromosome number 2n = 22. *Phaseolus vulgaris* has a wide range of acceptability for different species of *Rhizobium.* It can be nodulated by *Rhizobium leguminosarum* bv. *phaseoli, Rhizobium tropici* or rhizobium etli.

Five varieties of *Phaseolus vulgaris* viz Cirpir-50, Local, Anupam, Selection 9 and Kalingpong were studied in respect of their nodular characters and yield in response to (*i*) mixed culture of *Rhizobium* strains,(*ii*) *Rhizobium* + PSB and (*iii*) *Rhizobium* +PSB +KM treatments. Moreover, genetic parameters of variation and correlation coefficient of yield contributing characters with nodular characters were also estimated. The main objective of these estimations is to understand the association of biological nitrogen fixing characters with yield contributing characters. Among these three treatments, a considerable increase in expression of all the characters was observed in case of *Rhizobium* + PSB treatment. The variety Anupam showed the maximum values in respect of almost all nodular characters and yield. So it indicates that though the *Rhizobium* alone has significant effect in all nodular and yield attributing characters but the addition of PSB augments this effect to the highest level. A strong correlation was observed between the fresh nodular weight and fresh root weight. Similarly, positive correlation was observed between branch number and yield and number of nodules with yield which indicates a positive influence of nitrogen substances towards yield. The positive correlation between leghaemoglobin content of nodule with the nodule number, fresh nodular weight and yield indicates that leghaemoglobin content plays a positive role towards yield.

Keywords : *Phaseolus, Rhizobium,* PSB, KM, Nodulation, leghaemoglobin.

INTRODUCTION

In the present fuel subsidized agriculture practices, leguminous plants play an unique role by fixing nitrogen through symbiotic association with *Rhizobium* or *Bradyrhizobium*. Among the legumes, French bean or *Phaseolus vulgaris* L. Which are commonly known as Rajmash are used profusely by the common people as an alternative diet of protein. It is very nutritious and contain 22.9% of protein, 1.2% of fat, 60.6% of carbohydrate and a large number of minerals like calcium (260 mg/100g of seed), phosphorus (101 mg/100g of seed) and iron (5.8 mg/100g of seed).

It has been known that nod ABC genes of the bacterial strain determines the host spectrum. It has been shown that the sterile *Rhizobiunm* culture filtrates are capable of inducing different types of reaction in the root system, especially the characteristic root hair deformation (Dinarie *et al.*, 1992). These deformation are not seen in case of the culture filtrates of mutant strains altered in the common nod ABC genes. The chemical structure of the nod factor has been established in all the species of *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*. All of them produce nod factors of the lipo-chito oligosaccharidic type, consisting of an oligochitene skeleton of four or five N-acetyl Glucosamin residues, the acetyl group of the Glucosamine residue at the non-reducing and being replaced by a fatty acid chain (Carlson *et al.*, 1994). The length and degree of unsaturation of these fatty acid as well the nature of other substitute present at the end of the oligochitene skeleton vary considerably depending on the species.

Different research works have been going on since long to unveil variation in nodulation in relation to yield attributing characters. Eight varieties of *Phaseolus vulgaris* inoculated with 13 strains of *Rhizobium phaseoli* was studied and analysis of variance the number of nodules and nodular fresh weight was found to be significantly controlled both by the host variety and bacterial strains (Bandyopadhyay, 1988). Hungria and Ruschel in 1989 studied four different cultivars of *Phaseolus vulgaris* L. inoculated with three strains of *Rhizobium leguminosarum biovar phaseoli* in green house condition studying

nitrogenase activity, H_2 evolution, relative efficiency of nodules, respiration rate of nodulated roots and detached roots and dry weight and total nitrogen of plants and found that cultivar × strain interaction had an effect on all the parameters. Genotypic variation of N_2 fixation in *Phaseolus vulgaris* L. in response to iron deficiency was also studied by Krouma *et al.*, in 2006. Iniquez *et al.*, (2004) recorded the significant yield increase in *Phaseolus vulgaris* obtained by inoculation with a trifolitoxin producing strains of *Rhizobium legeminosarum* bv. *phaseoli*. The symbiotic and competitive performance of two highly effective *Rhizobia* nodulating *Phaseolus vulgaris* was studied in silky loam and clay soil by Moawad *et al.*, in 2004. The polyphasic stage of *Rhizobium* also observed by Moschetti *et al.*, (2005). They isolated 27 new strains of *Rhizobium* from *Vicia*, *Lathyrus* and *Pisum* from different agro ecological areas and all *Rhizobial* isolates (except one) were presumptively identified as *Rhizobium leguminosarum* bv. *viciae*. The compatibility of phosphate solubilizing bacteria [(PSB1), *Bacillus megaterium*, and PSB2, *Bacillus Polymyxa*)] and plant growth promoting *Rhizobacteria* (*Pseudomonas fluorescens*) with *Rhizoboum* were studied by Prasad *et al.*, (2002). Nodulation, plant dry weight and yield did not significantly vary with PSB-1 or PGRP + *Rhizobium* + PSB-1 resulted in greater phosphorus uptake than *Rhizobium* alone. PSB1 + *Rhizobium* also increase the number of nodules, *Rhizobium* + PSB-1 + PGPR was superior in terms of plant dry weight, grain yield and nitrogen and phosphorus uptake. Chandra and Parkeek in 2002 observed the effect of *Rhizobium, Azotobacter, Chroococcum* and *Bacillus* sp. on *Vigna mungo, Rhizobium,* Phosphate solubilizing bacteria, (*Bacillus megaterium*) and *Rhizosphere* bacteria (RB viz. *Azotobacter chroococcum, Azospirillum* sp., *Bacillus* sp. and *Pseudomenas fluorescens*) on lentil. They inoculated to seeds singly or in combination. *Rhizobium* strain increase the number and dry weight of nodules, plant dry weight and grain yield in both crops. Gunasekaran *et al.*, 2004 studied the synergism between *Rhizobium*, plant growth promoting *Rhizobacteria* (PGPR) and Phosphate Solubilizing bacteria (PSB) in black gram. Combination of all the three organisms (*Rhizobium*, PGPR and PSB) were recorded the maximum nodule, plant biomass and grain yield even when compared to the inoculation with *Rhizobium* alone. Wolyn *et al.*, 1989 while studying the role of lateral root nodule in N_2 fixation and their relationship between total shoot nitrogen and several traits which influence or control nitrogen fixation in *Phaseolus vulgaris*. They measured acetylene reduction value, specific nodule activity, *leghaemoglobin* concentration, total *laghaemoglobin* and nodule mass. There was significant variation among lines and cultivars for all traits measured. Moris *et al.*, in 2005 studied the symbiotic interaction between *Rhizobium* etli and *Phaseolus vulgaris* and identified the presence of rel4 gene in the strains of *Rhizobium* etli. Wilkinson and Iyer (1993) studied the nodulation specificity by *Rhizobium* with an interest of identification of the nature of some of the chemical signals that are exchanged between bacterium and plant during the symbiosis process. These are thought to determine nodulation specificity.

The present study would help to identify appropriate strain of *Rhizobium*, capable of inducing effective and efficient nodules and higher yield. Along with this *Rhizobium* strains, phosphate solubilizing bacteria (*Bacillus megaterium*) and some Potassium Mobilizer was added in addition to augment the efficiency of the bacterial strains by increasing their population in the rhizosphere. Results were analysed by standard biometrical method with a view to understand the intervarietal differences in response to mixed strains of *Rhizobium* with further addition of PSB and to different host in respect of root, shoot, nodule and yield characters. Measures of correlation co-efficient was used to find out the degree and direction of relationship between two or more characters to determine the component characters on which selection can be based for genetic improvement of yield. The present investigation was carried out to know the degree and direction of association among nodular and yield characters for finding out the characters which should be selected to improve yield.

The present experiment was therefore, conducted only in the laboratory condition both in Leonard jar as well as in earthen pot with a view to understand:

(*a*) The response of different local varieties to Rhizobial strains, plus PSB, plus Potassium mobilizer.

(*b*) Heritability, genetic advance as percentage of mean and Analyis of variance for different quantitative characters under different atmosphere of treatment.

(*c*) The degree and direction of these association among morphological, nodular and yield parameters.

MATERIALS AND METHODS

For the present experiment, five varieties of *Phaseolus vulgaris* L. were used. Out of five one variety (local) was collected from the seed house at Seorafuli, Hooghli, West Bengal. Other Phaseolus vulgaris varieties were collected from Sutton seed house at Kolkata. The names of the varieties are : Cirpir-50 , Local , Anupam, Selection 9 and Kalingpong.

The experiment was conducted at a green house condition of Nodule Research Laboratory, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal. Collection and compilation of data were done at Nodule Research Laboratory and Statistical Department of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia district. The sowing time of the crop is on 1st week of December, and picking of pods were completed in the 2nd week of March.

Two strains of Rhizobium leguminosarum bv. phaseoli (*i*) strain no NR-3 and (*ii*) NR-7 were used in this

experiment. They were collected from the Nodule Research laboratory B.C.K.V., Mohanpur. These strains were isolated from the nodule of Phaseolus vulgaris grown in the pot culture in complete aseptic condition. The mixture of these strains in equal proportion with a population of 108 -1010 were used.

Yeast, water, Mannitol broth for the *Rhizobium leguminosarum* bv. *phaseoli* were prepared by taking the following combination suggested by Vinsent (1970).

Constituent	*Amount*
Mannitol	10.0g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Yeast extract (Powder)	0.4g.
Water	Make up the volume to 1000ml.

The broth was then sterilized in the autoclave at 20 pound pressure for 20 minutes. The conical flask containing the broth was then cooled down and inoculated separately by two strains of *Rhizobium leguminosarum* bv. *phaseoli* (strain no NR-3 and NR-7.) They were then kept for 72 hours in an incubator. When the population of the *Rhizobium* strains were raised as 10^8-10^{10} cells/ml, it was considered as a proper inoculum. These inoculums were then used to inoculate the seedlings in the pot culture experiment conducted in the aseptic laboratory condition. All the experiments were conducted in the pot culture both in the Leonard jar as well as in the big earthen pot which were previously sterilized and were half filled with clean coarse and dry sand which was previously treated with 20% H_2SO_4 for several hours followed by repeated washing with distilled water till it attains pH 7.0 approximately. The sand were kept overnight at 150°C in a dry oven for making it almost free for organic substances. Healthy seeds were first immerged in 95% ethyl alcohol to remove air layers, specially from the area of the hilum scar. The seeds were kept agitated for 3-5 minutes in the alcohol after which they are treated 0.2% mercuric chloride solution for 2-3 minutes followed by repeated washing with distilled water. After washing the seeds were placed in sterile petri dishes in moist filter paper. These were kept for germination at room temperature (at 30°C). Each germinated seeds were aseptically transferred to a properly sterile Leonard jar and sterile earthen pod @ 3 germinated seeds/pot. The germinated seeds were dribbled inside the sand and 20 ml of (in case of Leonard jar) or 100 ml (in case of sterile earthen pot) of sterile nitrogen free nutrient solution was added to each bottle. On the next day, each pot was inoculated with 50 ml of mixed culture of *Rhizobium leguminosarum* bv. *phaseoli* and subsequently sterile distilled water was added in each pot.

The experiment was conducted in the pot following the same method done in the field experiment. The composition of the nitrogen free nutrient solution used for pot culture as prescribed by Bhaduri (1951). The design of the experiment was Completely Randomized Block Design (CRD) with three replications and there were 15 pots for each treatment. As the whole experiment was done in the green house condition, special care. There were three replications for each quantitative characters. In each variety there were three treatments and one control. The treatments were,

1. *Rhizobium* 2. *Rhizobium* + Phosphate solubilizing Bacteria (PSB) 3. *Rhizobium* + Phosphate solubilizing Bacteria + Potassium Mobilizer (KM).

The morphological data were taken for the following characters :

Shoot length (cm), Root length (cm), No. of branches/ plant, No. of nodules/plant Fresh weight of shoot/plant (g), Fresh weight of root/plant (g), Dry weight of shoot/plant (g), Dry weight of root/plant (g), Fresh weight of nodule/ plant (g), Yield (pods/plant).

Observation of biochemical trait was also taken which was Leghaemoglobin content (mg per g). Leghaemoglobin content was estimated in the nodule Research laboratory, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur. The estimation was done by the method described by Procter (1963).

For this biochemical test the material was the nodules of each variety treated with *Rhizobium*. The nodules are taken from the root portion of the crop 45 days after sowing (DAS).The estimation was done by the method described by Procter (1963).Statistical analysis that is comparison of treatment means were carried out by Duncan's Multiple Range Test. (DMRT) - According to Gomez and Gomez (1984).

RESULTS AND DISSCUSSION

The experiment was conducted with five varieties of *Phaseolus vulgaris* with a view to know the response of these varieties to mixed culture of *Rhizobium leguminosarum* bv. *phaseoli* alone compared with *Rhizobium* + PSB and *Rhizobium* + PSB + KM. Moreover genetic parameters of variation and the correlation coefficients of yield contributing characters with nodular characters were estimated to understand the degree and direction of association of biological nitrogen fixing characters with yield contributing characters. The phenomena of varietal difference has been attributed by different authors, both the morphological character and yield contributing character in *Phaseolus vulgaris.* (Vierie *et al*., 2005) showed that the difference of behaviour between different genotype of *Phaseolus vulvaris* in relation to seed inoculation and nitrogen fixation. The variety Anupam (Va. Anupam) showed the maximum values in respect of shoot length, root length, fresh and dry weight

of shoot, fresh and dry weight of root, the number of nodules and fresh weight of nodules. The lowest value was observed in case of Va. Selection-9.

There was considerable increase in the expression of almost all characters with *Rhizobium* + PSB treatment in *Phaseolus vulgaris*. L. So, most favourable condition for the expression of nodular characters was mixed treatment of *Rhizobium* with PSB. This treatment shows very good result in variety Anupam in respect of almost all morphological characters nodular characters and yield attributing characters.

High genetic coefficient of variation for nodule number per plant was the indication that improvement of nodule number was possible through selection in the genotypes taken for investigation. High genetic advance as percentage of mean along with high heritability was recorded for almost all the characters except, fresh root weight and leghaemoglobin content in *Phaseolus vulgaris*. This indicated the significance of additive gene action for these characters. The strong correlation between leghaemoglobin content of the nodule with the nodule number and fresh nodular weight and consequently with the yield indicates that leghaemoglobin content plays a positive role towards yield.

Positive significant association was observed for number of nodules/plant with nodule fresh weight/plant. Number of pods/plant (yield) showed positive significant correlation with number of nodule/plant, branch number/ plant, fresh nodule weight per plant, fresh shoot weight/ plant and dry weight of root and shoot per plant. However nodular characters exhibited positive and significant association with shoot length, number of nodules, and fresh weight of shoot and root. Yield characters showed negative and non significant correlation with shoot length and root length. So, from this observations it can be concluded that, yield can be increased in *Phaseolus vulgaris* by increasing the branch number and nodule number and nodule weight/ plant along with increase fresh weight of root and shoot and also dry weight of root and shoot per plant.

From these above observations it may be suggested that there is an ample scope of improving the yield of *Phaseolus vulgaris* with these present variety available in the market. We find that there is no reason to produce variability of the cultivar by using breeding or mutation experiment. We have to search better *Rhizobial* strains which can produce more number of effective nodules in the plant. Moreover, it is to be looked into that these exotic strains should do better in competition against the native *Rhizobial* strains present in the soil. If we find that some native strain have more competitive ability than the inoculating strain, we should isolate them and should study their biochemical activity, nitrogen fixing efficiency and wide range of host acceptability.

Table 1: Correlation between 10 morphological and yield contributing characters of *Phaseolus vulgaris* L. when inoculated with *Rhizobium leguminosarum* bv. *phaseoli*, PSB and KM.

	Shoot length (cm)	***Root length (cm)***	***Branch number per plant***	***Nodule number per plant***	***Fresh shoot weight (g)***	***Fresh root weight (g)***	***Fresh nodule weight (g)***	***Dry shoot weight (g)***	***Dry root weight (g)***	***Yield (pod per plant)***
Shoot length (cm)	1.00				*Bold correlation coefficients are significant at the 0.01 level (two tailed)*					
Root length (cm)	0.13	1.00								
Branch number per plant	–0.05	–0.19	1.00							
Nodule number per plant	**0.42**	0.07	0.05	1.00						
Fresh shoot weight (g)	0.18	0.18	0.16	0.25	1.00					
Fresh root weight (g)	–0.11	0.37	0.03	0.05	**0.71**	1.00				
Fresh nodule weight (g)	**0.48**	0.03	0.08	**0.87**	**0.54**	**0.31**	1.00			
Dry shoot weight (g)	**0.39**	0.19	0.14	0.24	**0.95**	**0.65**	**0.55**	1.00		
Dry root weight (g)	–0.09	**0.40**	0.02	0.14	**0.75**	**0.94**	**0.40**	**0.71**	1.00	
Yield (pod per plant)	–0.02	–0.07	**0.29**	**0.39**	**0.53**	**0.46**	**0.49**	**0.46**	**0.46**	1.00

From the Table 1 it can be concluded that There was a significant correlation at 5% level between fresh weight of root and fresh weight of nodule, number of branches with yield. Significant correlation at 1% level was obtained between shoot length and nodular number, fresh weight of root and shoot. Fresh weight of nodule showed significant correlation at 1% level with shoot length fresh shoot weight and number of nodules. Dry shoot weight showed significant correlation with fresh root weight and fresh nodule weight. Dry root weight showed significant correlation at 1% level with fresh and dry shoot weight and fresh nodular weight. Significant correlation of pod yield was observed with all the nodular characters and fresh and dry root and shoot weight and branch number. But a negative correlation was observed between the yields and shoot and root length. The number of branches showed negative value with root and shoot length.

The Analysis of Variance of all morphological and yield contributing characters in five varieties of *Phaseolus vulgaris* (Table 2) inoculated with *Rhizobiun leguminosarum* bv *phaseoli* showed that there was a significant variation between the varieties in respect of all morphological characters like number of nodules, fresh weight of nodule and pod yield except root length. The variety × treatment interaction also evident in all the cases except in root length. The maximum interaction between these varieties and the treatment was observed in case of number of nodules. Fresh shoot weight and number of pods per plant also showed significant interaction between the host and also with variety × treatment interaction.

Table 2: Analysis of variance of morphological and yield contributing characters in 5 varieties of *Phaseolus vulgaris* L. inoculated with *R. leguminosarum* bv. *phaseoli*.

			Repl					*Treat*			
Variable	*SS*	*df*	*MS*	*F*	*Sig.*	*SS*	*df*	*MS*	*F*	*Sig.*	
1.	72.70	2	36.35	0.89	0.42	12137.40	3	4045.80	98.85	0.00	**
2.	252.40	2	126.20	7.36	0.00	129.60	3	43.20	2.52	0.07	
3.	5.70	2	2.85	2.56	0.09	5.40	3	1.80	1.62	0.20	
4.	145.73	2	72.87	3.83	0.03	7104.60	3	2368.20	124.48	0.00	**
5.	2.23	2	1.12	0.34	0.71	1235.65	3	411.88	125.79	0.00	**
6.	1.09	2	0.55	7.66	0.00	6.00	3	2.00	28.09	0.00	**
7.	0.01	2	0.00	4.93	0.01	0.18	3	0.06	83.74	0.00	**
8.	0.08	2	0.04	0.36	0.70	60.43	3	20.14	173.15	0.00	**
9.	0.15	2	0.08	7.98	0.00	0.74	3	0.25	26.07	0.00	**
10.	8.03	2	4.02	1.83	0.17	207.52	3	69.17	31.56	0.00	**
			Var					*Treat * Var*			
Variable	*SS*	*df*	*MS*	*F*	*Sig.*	*SS*	*df*	*MS*	*F*	*Sig.*	
1.	37639.50	4	9409.88	229.91	0.00**	57266.10	12	4772.18	116.60	0.00	**
2.	116.10	4	29.03	1.69	0.17	267.90	12	22.33	1.30	0.26	
3.	14.10	4	3.53	3.17	0.024*	29.10	12	2.43	2.18	0.03	*
4.	9939.77	4	2484.94	130.62	0.00**	22400.90	12	1866.74	98.12	0.00	**
5.	705.90	4	176.48	53.90	0.00**	1607.40	12	133.95	40.91	0.00	**
6.	2.66	4	0.67	9.34	0.00**	7.00	12	0.58	8.20	0.00	**
7.	0.46	4	0.12	162.26	0.00**	0.67	12	0.06	78.75	0.00	**
8.	12.76	4	3.19	27.43	0.00**	77.63	12	6.47	55.61	0.00	**
9.	0.53	4	0.13	14.06	0.00**	0.94	12	0.08	8.26	0.00	**
10.	295.57	4	73.89	33.71	0.00**	76.57	12	6.38	2.91	0.01	**

	Error			*Total*		*SE (m)*			*CD (0.05)*		
Variable	*SS*	*df*	*MS*	*SS*	*df*	*tr*	*var*	*tr*var*	*tr*	*var*	*tr*var*
1.	1555.30	38	40.93	108671.00	59	1.65	1.85	3.69	4.73	5.29	10.57
2.	651.60	38	17.15	1417.60	59	1.07	1.20	2.39	NS	NS	NS
3.	42.30	38	1.11	96.60	59	0.27	0.30	0.61	NS	0.87	1.74
4.	722.93	38	19.03	40313.93	59	1.13	1.26	2.52	3.22	3.60	7.21
5.	124.43	38	3.27	3675.60	59	0.47	0.52	1.04	1.34	1.50	2.99
6.	2.70	38	0.07	19.46	59	0.07	0.08	0.15	0.20	0.22	0.44
7.	0.03	38	0.00	1.34	59	0.01	0.01	0.02	0.02	0.02	0.04
8.	4.42	38	0.12	155.32	59	0.09	0.10	0.20	0.25	0.28	0.56
9.	0.36	38	0.01	2.73	59	0.03	0.03	0.06	0.07	0.08	0.16
10.	83.30	38	2.19	670.98	59	0.38	0.43	0.85	1.09	1.22	2.45

1 = Shoot length, 2 = Root length, 3 = Branch number, 4 = Nodule number, 5 = Fresh shoot weight, 6 = Fresh root weight, = Fresh nodule weight, 8 = Dry shoot weight, 9 = Dry root weight, 10 = Yield.

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