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Induced genetic variability in *Rhizobium leguminosarum* for nitrogen fixation parameters in *Vicia faba* L.

R.S. Shukla, C.B. Singh and J.N. Dubey

Department of Plant Breeding and Genetics, J.N. Agricultural University, Jabalpur-482 004 (M.P.), India

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Summary. The objective of this work was to know the behaviour and variability of *Rhizobium leguminosarum* after irradiation. The induced variation was tested under greenhouse conditions on the variety JV 3 of broad beans (*Vicia faba*) in six replications. Induced genetic variabilty was observed for strain, parent and mutant versus parent. Out of 24 irradiated strains, strain 93-32 performed better with a greater number of nodules and higher dry weight of nodules per plant and biological yield. Environment played an important role in the expression of characters observed. High heritability and genetic advance of these traits indicated that the nitrogen fixation ability of Rhizobium can easily be improved by selection.

Key words: *Rhizobium* – Irradiation – Nitrogen fixation – *Vicia*

Introduction

Legumes are important in crop rotation due to their ability to fix nitrogen. To achieve the objective of high nitrogen fixation in *Vicia faba*, it is imperative to screen characters among the genotypes contributing to high seed yield. This should be possible once the extent of genetic variation is thoroughly assessed with the help of genetic parameters. It is also well understood that total variation in the population is the result of a combination of genotypic and environmental effects. The proportion of variation due to each determinant is of importance, since the amount of genetic variation is critical in achieving genetic gain from phenotypic expression. The genetic part is measured by heritability in the broad sense. Hence, attempts have been made to carry out the induced genetic variability, after gamma irradiation, of *Rhizobium leguminosarum*.

Materials and methods

Rhizobium leguminosarum specialized on *Vicia faba* was obtained from germplasm collections of the Department of Plant Breeding and Genetics, J. N. Agricultural University, Jabalpur. Nodules were sterilized (Vincent 1970), crushed and preserved in a refrigerator at 4 °C. Isolation was done under aseptic conditions. Thirty selected colonies were controlled by biochemical tests as to purity, i.e. YEMA, glucose peptone with bromocresol purple blue (Date 1969) and nile blue sulphate (Janima et al. 1968) and a gram staining test.

The *Rhizobium* are isolated from nodules of the host plant *Vicia faba*. Three sets of culture tubes containing (a) yeast mannitol agar medium (YEMA), (b) bromocresol purple blue medium and (c) nile blue sulphate medium were prepared. The inoculation of 30 isolates was done in three sets of tubes and these were replicated three times. In this way, there were 90 tubes in replicates. *Rhizobia* did not reduce the intensity of colour of nile blue and bromocresol purple blue and showed good growth in yeast mannitol agar media. Only strains showing good growth were selected and selected strains were subjected to a gram staining test to determine gram-positive and gram-negative reaction of *Rhizobia*. On the basis of the above test, 24 pure strains were identified for further study.

Of 30 isolates, 24 were pure, and of these, 10 isolates were randomly selected for screening in the greenhouse on the basis of symbiotic properties. Of these 10 isolates, 3 efficient *Rhizobium* strains – 083, 093 and 0103 – were treated at BARC, Bombay with three doses of gamma rays, viz. 10, 20 and 30 Kr. Of the irradiated broth of the strain, 0.1 ml was plated on the YEMA plates, except for 20 Kr treatment of the 083, which was lost in transit. Five colonies were randomly selected on the basis of their shape. Small and large colonies were transferred to separate culture tubes containing YEMA broth. Three isolates from each of eight treatments were finally removed for examination.

Altogether, 24 stable mutants (colonies) and three parents were tested in the greenhouse for symbiotic properties using six replications. Lionard jars were filled with nitrogen-free sand, which was sterilized at 121 °C for 5 h. Four JV-3 seeds were sterilized in rectified spirit for 5 min, followed by 5–6 min of washing, after being and presoaked for 18 h in distilled water; they were then planted in each jar. After germination, inoculation of each culture was made by syringe and the inoculation

Table 1. Analysis of variance for various observations of *Rhizobium* strains in variety JV-3

Source of variation	*df*	Mean squares						
		No. of nodules/ plant	Dry nodule weight/plant	Dry shoot weight/plant	Dry root weight/plant	Grain yield/plant	Biological yield/plant	Harvest index
Replications	2	0.0113	20.5110	9,612.50	23,085.65	0.2378	1.9956	210.1050
Strains	27	0.2627[b]	214.8077[b]	58,278.00[b]	29,843.90[b]	0.1329[b]	0.3060	171.2077[b]
Mutants	23	0.0787[a]	204.5869[a]	81,484.95[a]	18,655.72	0.1169[a]	0.1496	178.5457[b]
Parents	2	0.0223[a]	14.1111	111,365.35[a]	61,918.11[a]	0.0089	0.2620	30.2022
Mutant vs parent	1	0.0698[a]	414.0800[b]	34,249.00	268.40	6.0060[b]	0.0861	60.5708
Mutant vs control	1	5.4329	–	13,201.33[b]	41,462.41	0.8335[a]	2.0341[b]	364.3140[a]
Error	54	0.0118	27.0551	12,263.83	12,700.95	0.0626	0.1843	72.7937

[a] Significant at 5% level
[b] Significant at 1% level

Table 2. Genetic parameter of variation for various observations of *Rhizobium* strain in variety JV 3. 6^2g – phenotypic variance, 6^2g – genotypic variance, PCV – phenotypic coefficient of variation, GCV – genotypic coefficient of variation, H^2 – heritability in broad sense and G.A. – genetic advance

Character	Mean	Range		Variance		Coefficient of variation		H^2	G.A.	G.A. as % of mean
		Min.	Max.	6^2g	6^2g	PCV	GCV			
No. of nodules/plant	1.61	0.30	2.06	0.09	0.08	18.63	17.56	0.88	0.54	33.54
Dry nodule weight/plant (g)	18.04	10.00	39.33	98.65	71.6	54.49	55.05	0.72	14.73	81.65
Dry shoot weight//plant (g)	422.11	222.66	694.00	31,689.83	19,426.00	42.17	33.01	0.61	233.69	52.99
Dry root weight/plant (g)	341.39	198.33	534.66	22,648.91	9,946.96	44.08	29.21	0.43	133.30	39.04
Grain yield/plant (g)	1.04	0.53	1.36	0.10	0.04	30.40	19.23	0.40	0.26	25.00
Biological yield/plant (g)	2.34	1.55	2.87	0.28	0.10	22.61	13.51	0.35	0.38	16.23
Harvest index	44.77	31.11	62.82	129.85	57.06	25.45	16.87	0.43	10.09	22.53

was repeated after 5 days. The jars were irrigated with sterilized quarter-strength Jansens nutrient solution (Jansen 1942).

After 40 days, observations on the number of nodules plant, dry weight of the nodules (g) and dry weight of roots and shoots (g) were recorded on three plants of each strain in three of the replications. The other three replications, each again including three plants, were left until seed formation and measured for total shoot weight (g), biological yield (g), harvest index and seed yield/plant (g). The data presented for the number of nodules per plant in Table 2 are transformed data. The data were statistically analysed for induced genetic variability, heritability and genetic advance, according to methods developed by Burton (1952) and Johnson et al. (1955), respectively.

Results and discussion

The extent of induced genetic variability and interrelationships of heritable and nonheritable variation for nodulation would decide the extent of change in symbiotic activity. Significant induced genetic variability was observed for strains, parents and mutants versus parents in all characters except biological yield per plant (Table 1). The genotypic variance for all the characters was lower than the phenotypic variance, indicating a substantial effect of environment on the expression of the strains concerned (Table 2). A wide range of genotypic coefficients of variation was observed, which varied from 13.51 for biological yield per plant to 55.05 for dry weight of nodules per plant. The difference in genotypic and phenotypic variances for biological yield and grain yield was very small, indicating a high genetic control. The irradiated strains demonstrated that a clear variation was induced. High heritability was observed for number of nodules per plant, dry weight of nodules per plant and dry weight of shoots per plant. The results indicate that nitrogen fixation ability of broad beans can be improved with impact on the seed yield and soil fertility. Although parameters directly involved in controlling nitrogen fixation were not observed, the induced genetic variability in all parameters, viz. number of nodules per plant, dry weight of nodules per plant, dry weight of shoots per plant, dry weight of roots per plant and biological yield per plant was found to be significant (Table 1). This suggests a further scope for selection of efficient *Rhizobia* strains for better nitrogen fixation ability. On the basis of this finding, strain 93-32 has been identified as an efficient strain with better nitrogen-fixing ability.

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