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GENETIC ANALYSIS OF NODULATION AND BIOLOGICAL NITROGEN FIXATION IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

A. MISRA, S. K. GHOSE* AND P. K. DAS

Department of Genetics,
Bidhan Chandra Krishi Viswa Vidyalaya, Mohanpur 741 252

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ABSTRACT

Gene action for nodulation associated with biological nitrogen fixation has been studied in groundnut. Both additive and non- additive gene interactions are prevalent for nodule number/plant, nodule dry weight/plant, average weight of nodules and nitrogenase activity of the nodules. The genotypes 'Gunajato' and ICGV-86055 were the best general combiners for nodule characters and nitrogenase activity in the nodules. The cross ICGV-86055 × EC-21989 appeared to be the promising combination for high nodule number and nitrogenase activity.

Key Words : Leguminous plant, groundnut, *Rhizobium*, symbiotic association, nodulation, biological nitrogen fixation, gene interaction

Nodules onto the roots of leguminous plants in which the atmospheric diatomic nitrogen is fixed by the symbiotic association of *Bradyrhizobium* are the established vehicles of biological nitrogen fixation. These would serve as renewable low cost alternative energy source to the use of highly expensive industrially fixed nitrogen being used in the current agricultural farming system. Nodulation *vis a vis* the fixation of atmospheric nitrogen into the highly differentiated nodules is a very complex biological mechanism which is dependent on the genetic systems of both the bacteria and the host. The present study deals with the elucidation of gene action for nodulation and diatomic nitrogen fixation in groundnut.

MATERIALS AND METHODS

The experimental materials consisted of six genotypes of groundnut (*Arachis hypogaea* L.). The genotypes were of different botanical types. They were '64-2', 'Gunajato' and 'ICGV-86055, (the virginia bunch types); 'TMV-2 (7731)', the spanish bunch type; 'EC-21989', the virginia runner type; and 'PI-314817', the valencia type.

*Corresponding author.

The *Bradyrhizobium* strain was isolated from the farm soil of the Midnapur (West) district in West Bengal. The isolated strain was named as G.Nut (M) and it was characterized by Misra [2] as superior to the strains G.NUT (P) isolated from Purulia and IGR-6 (the commercial strain isolated by NRC for Groundnut, Junagadh) under high acidic pH (5.6) both *in vitro* and in the field soil.

For the study of gene action, a 6 × 6 half diallel set of crosses was made. Crossing was done following the technique of Nigam et al. [3]. Six parents and their fifteen F_1's were grown in randomized blocks with three replications (plot size, 15m × 10m; spacing, 40 cm between rows and 30 cm between plants). Two sets of experiments were conducted. One was involved with the *Rhizobium*-charcoal mixture treatment and the other was without. Following water soaking seeds were dressed with Rhizobium-charcoal mixture (75 g/kg seed with bacterial population around 10^{-8}/ml broth). Seeds were sown in the first week of August in Anandapur farm (Midnapur West) of West Bengal, known as laterite area (pH 5.6, NPK .05%, 04%, .03%). All the recommended agronomic practices were followed. Observations were recorded on nodule number/plant, total nodule dry weight (g)/plant, average weight of nodules(mg) and nitrogenase activity of the detached nodules (nodules with roots detached from shoots).

Nitrogenase activity was measured through acetylene reduction test using G.L.C. following the method of Hardy *et al.* [4] which is based on nitrogenase catalyzed reaction of C_2H_2 to C_2H_4. Thirty days after sowing five healthy looking competitive plants were samples from each of the replications. Subsequently observations on nodule characters as mentioned were made and nitrogenase activity was measured from individual plants viz. fifteen plants (5 × 3) from three replications. Means were calculated for each observations separately. The combining ability and the genetic components were analysed according to Griffing's Method-2, Model-1 [5].

RESULTS AND DISCUSSION

The analysis of variance of combining ability (Table 1) showed that mean squares due to gca and sca were highly significant for all the characters with or without *Rhizobium* treatments which indicated that the parents and crosses differed significantly in their combining ability effects. Total nodule dry weight and nitrogenase activity in the nodules showed high heritability estimates in narrow sense with *Rhizobium* treatment which suggests that the direct selection for the improvement of these characters would be effective. Whereas, heritabilities for total number of nodules/plant and average weight of nodules are comparatively low. Direct selection with these characters would not be profitable which also supported the earlier

Table 1. ANOVA for combining ability of different nodule characters including nitrogenase activity with (Rh^+) or without (Rh^-) *Rhizobium* G.NUT (M) treatment.

Source	df	Mean squares							
		Nodule number/plant		Total nodule dry wt./plant		Average wt. of nodules		Nitrogenase activity	
		Rh^+	(Rh–)	Rh^+	(Rh–)	Rh^+	(Rh–)		
Gca	5	71.0**	(64.3)**	1229.4**	(765.5)**	0.85	(1.15)**	413.0	(417.0)**
Sca	15	19.9**	(18.1)**	91.3**	(133.1)**	0.22**	(0.29)*	41.2**	(64.5)**
Error	40	3.4	(3.6)	16.6	(16.4)	0.04	(0.04)	1.0	(3.5)
h^2 (ns)%		46.4	(45.6)	76.8	(58.8)	47.74	(48.25)	71.4	(61.55)

*, ** Significant at P = .05 and .01 respectively

findings of Wynne *et al.* [6]. The gca values (Table 2) for total number of nodules/plant were found to be consistently positive and high for 'Gunajato', 'ICGV-86055' and 'EC-21989' with or without *Rhizobium* treatments. But for total nodule dry weight/plant and average weight of nodules highly positive gca values were recorded for '64-2', 'Gunajato' and 'ICGV-86055'. When these three characters were considered together, 'Gunajato' and 'ICGV-86055' exhibited consistently high gca effects for both with or without *Rhizobium* treatments. These two would be the best general combiner for nodule characters. On the other hand, 'ICGV-86055', 'EC-21989 and 'Gunajato' showed high positive gca estimates for nitrogenase activity. Thus it becomes evident from the overall trend that for both nodule characters and nitrogenase activity 'Gunajato' and 'ICGV-86055' stand out from the rest.

Six cross combinations, 64-2 × TMV 2(7731); 64-2 × Gunajato; 64-2 × PI-314817; TMV-2 (7731) × ICGV-86055; PI-314817 × EC-21989; ICGV-86055 × EC- 21989 showed high and positive sca estimates for total number of nodules/plant (Table 3). Of them ICGV-86055 × EC-21989 recorded maximum sca estimate involving parents with high × high gca effects, indicating a additive × additive gene interactions for this character. For total dry weight/plant only two crosses 64-2 × TMV 2 (7731) and Gunajato × PI-314817 recorded consistently high positive sca estimates both of which involved good × poor general combiners indicating additive × dominance gene interaction. For nitrogenase activity, out of fifteen cross combinations, only four viz, 64-2 × EC-21989; TMV-2 (7731) × Gunajato; TMV-2 (7731) × PI-314817; and ICGV-86055 × EC-21989 exhibited consistently high positive sca estimates with *Rhizobium* application

Table 2. Estimates of gca effects for different nodule characters including nitrogenase activity in the nodules with (Rh^+) or without (Rh^-) *Rhizobium* (G.NUT (M) treatment

Genotypes	Nodule number/plant		Total nodule dry wt./plant. (g)		Average wt. of nodules (mg)		Nitrogenase activity	
	Rh^+	(Rh^-)	Rh^+	(Rh^-)	Rh^+	(Rh^-)	Rh^+	(Rh^-)
6-2(VB)	-2.09**	(-1.50)*	7.91**	(7.91)**	0.38**	(0.37)	-11.29**	(-12.40)**
Gunajato (VB)	2.56**	(2.62)**	15.83**	(12.08)**	0.26**	(0.20)**	0.25	(1.53)*
ICGV 86055(VB)	1.98**	(2.09)**	8.75**	(4.16)	0.09	(0.03)	7.95**	(7.99)**
PI314817 (VL)	-4.97**	(-4.83)**	-12.49**	(-12.50)*	0.00	(-0.01)	-1.87**	(-1.68)**
TMV-2 (7731) (SB)	0.36	(-0.20)	-13.33**	(-9.58)**	-0.41**	(0.32)*	-0.92**	(-1.39)*
EC 21989 (VR)	2.51**	(1.87)*	-6.66**	(-2.08)	-0.41**	(0.27)	7.02**	(6.00)**
SE (gi)	0.59	(0.61)	1.31	(1.31)	0.06	(0.06)	0.32	(0.60)

*, **Significant at P = .05 and .01 respectively

or without it. The cross ICGV-86055 × EC-21989 with highest sca estimate and having good × good general combiner indicated the additive × additive gene interaction for this parameter. It is very interesting to note that the negative sca estimates of Gunajato × ICGV-86055 and 64-2 × ICGV-86055 were turned to be positive and the positive gca estimates of Gunajato was found to be negative following *Rhizobium* treatment which by and large reflects the complex nature of host-rhizobium interaction. In general, parents with high *per-se* performance exhibited very high general combining ability effects. In majority of the highest specific combinations at least one of the parents was found to be the best general combiner. The cross ICGV-86055 × EC-21989 appeared to be the acceptable combination as it recorded significantly high positive sca estimate for nitrogenase activity as also appreciably high sca effects for number of nodules/plant in the present set of experiments. Higher nodule number in association with higher nodule size would lead to higher nitrogenase activity [7, 8] meaning that development of more nodules with increased size would facilitate efficient dinitrogen fixation. Nodule number and size are known to be negatively correlated. Hence to achieve the desired goal for increasing nitrogenase activity a compromise has to be struck. However, the present analysis by and large demonstrates the possibility of scoring promising segregants exhibiting high nodule number with improved nitrogenase activity from the above cross combinations discussed.

Table 3. Estimates of sca effects for different nodule characters with (Rh^+) or without (Rh^{-1}) *Rhizobium*, G. NUT(M)

Character / Crosses	Nodule number/plant		Total nodule dry weight/ plant (g)		Average wt. of nodules (mg)		Nitrogenase activity†	
	Rh^+	Rh^{-1}	Rh^+	Rh–	Rh+	Rh–	Rh+	Rh–
64-2×TMV-2(7731)	0.81	0.94	8.27**	5.95*	0.18	–0.22	–6.72**	–3.11*
64-2×Gunajato	0.60	0.44	–4.22	–2.38	–0.21	–0.06	–8.89**	–6.04**
64-2×PI-314817	1.14	0.23	–5.893	–7.79*	–0.24	–0.31*	–3.12**	–1.37
64-2×ICGV-86055	–3.14*	–2.30	–0.47	2.20**	0.14	0.19	1.69*	–2.35*
64-2×EC-21989	–1.30	–1.13	–5.05	1.54	0.01	0.08	11.48**	0.38
TMV-2(7731)× Gunajato	–2.18	–2.51	–22.97**	–24.88**	–0.52**	–0.61**	4.80**	1.44
TMV-2(7731)× PI-314817	–0.64	–0.38	–4.64	–0.29	–0.18	–0.05	4.33*	1.30
TMV-2(7731)× ICGV-86055	1.06	0.40	7.44**	–3.36	0.14	0.22	2.60*	–0.57
TMV-2(7731)× EC-21989	–2.43	–3.09*	–0.47	22.69**	0.21	0.37*	–1.57*	3.92**
Gunajato× PI-314817	–0.51	–0.22	12.85**	4.70	0.53**	0.49**	2.77	–2.05
Gunajato× ICGV-86055	–4.14**	–4.43**	–5.05	–1.96	0.16	0.23	0.22	–1.29
Gunajato× EC-21989	–2.31	–1.92	–2.97	–9.04**	0.12	–0.11	–3.40**	–7.50**
PI-314817× ICGV-86055	–1.61	–1.63	–6.72*	–7.38	–0.06	–0.23	–13.24**	–13.25*
PI-314817× EC-21989	0.89	0.86	–4.64	–7.79*	–0.18	–0.24	–3.61**	–10.45**
ICGV-86055× EC-21989	2.27	2.32	–5.89*	–7.79*	–0.17	–0.32*	3.59**	2.07
SE(sij)	1.35	1.40	2.98	2.94	0.15	0.15	0.74	1.37

*, ** Significant at P = .05 & .01 respectively; †µmole ethylene $g^{-1}hr^{-1}$ fresh wt.

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