J. Mycopathol. Res. **40**(2) : 101–105 (2002)

Nitrogenase activity and identification of superior families in groundnut (*Arachis hypogaea* L.)

S. K. BANDYOPADHYAY
Nodule Research Laboratory, Bidhan Chandra Krishi Viswavidyalaya, P.O. Mohanpur, Pin 741252, Nadia, West Bengal

A large number of scientists (Kishinevsky *et al.*, 1987; Arrendell *et al.*, 1988 and Dutta *et al.*, 1988) studied the inoculation effect of *Bradyrhizobium* sp. on nodulation and nitrogen fixation in groundnut. But a little attention has been made to identify the criteria to select better plant families. In the present investigation eighteen groundnut varieties (twelve belonging to bunch type and six to spreading runner type) were inoculated with two serologically distinct strains of *Bradyrhizobium* species both individually as well as in a mixture of two in equal proportionn. Experiments were laid in a randomized block design with three replication in *kharif* - 1998 and summer - 1999. Plants were harvested at 60 and 120 DAS and the following +characters : days to maturity, nodule number per plant, nodule weight per plant, dry matter accummulation, root weight per plant, nitrogenase activity, pod number and pod yield were considered. Analysis of variance in respect to the above mentioned characters was done to test the differences among the eighteen groundnut genotypes and three rhizobial strains along with the control. Regression co-efficient studies of these two seasons showed the positive co-efficient for yield on nitrogenase activity for all the cultural strains. But nitrogenase activity in single strain inoculation only showed strong linear and highly significant relationship with yield.

Key words : Nitrogenase activity, identification groundnut families

INTRODUCTION

India is the largest groundnut growing country accounting for 45% of the area and 55% of the production under oil seed. As such the edible oil economy in India is primarily dependent upon groundnut production. But the productivity in India is lower (100 kg/ha) than the world average. "The Technology mission on oilseed", which was developed during 1988, encourages extensive research work to make India self-sufficient in oil seed production.

Inspite of extensive research accomplishment groundnut continues to be an unpredictable legume, showing inconsistency in pod and oil yield over seasons, years and locations. Therefore, it needs to intensify research efforts in several areas of the crop. In the present investigation an attempt has been made to identify a specific character to select a superior family of groundnut under tropical and humid atmosphere of West Bengal.

MATERIALS AND METHODS

Eighteen groundnut varieties of which twelve belong to bunch type and six spreading runner type were taken in this experiment.

Two serologically distinct strains of *Bradyrhizobium* species (1) JCG-Goyespur (A) and (2) HMU-2 (B) collected from the "Microbiology Cell" of 'Nodule Research Laboratory" B.C.K.V. These strains of *Bradyrhizobium* were used both individually as well as in a mixture of two in equal proportion.

Field experiments were conducted in *kharif*, 1998 and summer, 1999 at "Inchek Farm", BCKV. The experiment was laid out in a randomized block design with three replications. After inoculation with liquid culture of *Bradyrhizobium* the seeds of each entry was shown in two in 2.5 m long rows

with a spacing of 40 cm. × 12 cm, row to plant. The plants were harvested at 60 and 120 DAS after sowing by carefully uprooting without damaging the nodulated roots as far as possible for counting and collection of nodules. The following characters : days to maturity, nodule number and weight per plant, dry matter accumulation (DMA), root weight per plant, nitrogenase activity of the nodules per plant, pod number and yield per plant were noted. Analysis of variance (ANOVA) (Panse and Sukhatme, 1985) in respect to the above mentioned characters was done to test the difference among the eighteen grounding genotypes and three rhizobial strains along with the control. Regression co-efficient studies of these two seasons were slso made to identify the differences of yield on different yield contributing characters. Moreover, correlation co-efficient studies of different characters with yield were also done.

Estimation of nitrogenase activity of the root nodules at 60 and 120 DAS were done by acetylene reduction technique (Dilworth, 1996). Analysis of acetylene-ethylene was done by gas-chromatography installed at the Soil-Science and Chemistry Laboratory, BCKV.

The formula was used in order to calculate nitrogenase activity :

$$\frac{\text{Pick height (graph)} \times \text{Vial Voume X Factor}}{\text{Hours} \times \text{Weight}} \times \text{h mole}$$

The factor taken was as 5.273.

RESULTS AND DISCUSSIONS

Analysis of variance with respect to nodule, root, shoot and yield characters was done to test the differences among the eighteen groundnut genotypes and three rhizobial cultures along with the controlled one (Table 1). Variance due to genotypes, culture and genotype x culture interactions was highly significant for all the twelve characters taken under consideration. Only the genotype x culture interaction for nodule weight (60 DAS) during 1999 did not show significant effect. Wynne *et al.* (1978) reported that nodulation and nitrogen fixation differed among groundnut genotypes. But they fail to observe strain x genotype interaction for these two characters. Arunachalam *et al.* (1984) and Arrendell *et al.* (1988) suggested the selection of superior host genotypes in symbiosis with *Rhizobium* as a means of increasing N-fixation in groundnuts. In the present study the seasonal variations during *kharif*, 1998 and summer, 1999 have been reflected for different morphological, nodular and yield character which has been recorded by Nambiar and Dart (1983) and Dutta *et al.* (1988). Though the nodule number (60 DAS) and weight were found to be higher in mixed inoculum in both types of groundnut, single inoculation, particularly HMU-2 for spreading type and JCG-Goyespur for bunch type, expressed better yield performance and thereby showed greater symbiotic compatibility and preferentiality to the host (Joshi and Kulkarni, 1983; Byalebeka *et al.*, 1987).

To study the dependence of different inoculating strains on different genotypes of groundnut of both bunch and spreading types, regression co-efficient and their equations were computed and displayed in Table 2. In 1998, the regression co-efficients of yield vs. nodule number were found to be negative at both 60 and 120 DAS, for all the inoculating strains. But for nodule weight at 120 DAS, regression co-efficient was found to be positive in strain B (6.65452) and mixed strain (1.53115). The dependence between yield and DMA was found to be positive in strain B (0.14158) and in strain A (0.01385) at 60 and 120 DAS respectively. The constant i.e. 'a' value was found ot be higher (20.05381) in strain B then the rest at 120 DAS. In no cases R^2 value was significant.

But interestingly, the values of co-efficients for nitrogenase activity vs. yield were found to be positive in all the cultural strains and for strain 'B', the constant 'a' was also the highest (-39.5887). R^2 values were also highly significant in all the cases.

During 1999, dependence of yield on nodule number was found to be positive in strain 'A' at 60 DAS (0.01681), at 120 DAS (0.31463) and in mixed inoculum it only at 120 DAS (0.01231). But in case of nodule weight the positive regression co-efficient were observed in strain 'A' and "A+B" at 60 DAS and only in strain 'A' at 120 DAS

Table 1 : ANOVA of different nodular and yield contributing character of *Rhzobium* treated groundnut.

Sources of variation	D.F.	Days to maturity	Nodule number per plant		Nodule weight per plant		Dry matter accumu lation/plant		Root weight per plant		Nitrogenase activity	Pod No. per plant	Pod Yield/plant
			60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS	60 DAS		
						Kharif - 1998							
Replication	2	15.67	87.67	947.31	0.0002	0.032	25.03	161.77	0.039	0.046	0.172	19.01	19.72
Genotype	17	412.85**	2078.42**	6437.19**	0.008**	0.017**	173.17**	548.31**	0.137**	0.206**	31.47**	317.42**	103.47**
Culture	3	193.59**	3491.19**	5531.72**	0.016**	0.076**	216.54**	979.45**	0.095**	0.257**	7.83**	225.73**	93.18**
Genotype X Culture	51	11.32**	1021.16**	2256.37**	0.003**	0.011**	46.188**	273.16**	0.067**	0.044**	26.42**	76.48**	21.53**
Error	142	9.63	51.35	972.67	0.001	0.009	16.559	123.37	0.004	0.005	0.26	21.05	11.68
						Summer - 1999							
Replication	2	2.35	32.89	1048.00	0.04	0.011	0.89	138.25	0.072	0.067	0.32	8.46	36.23
Genotype	17	336.72**	1612.37**	4781.71**	0.274**	0.011**	68.44**	684.93**	0.075**	0.317**	44.33**	215.26**	66.74**
Culture	3	201.13**	5729.25**	6634.35**	0.114**	0.055**	95.91**	1187.31**	0.017**	0.423**	4.17**	310.88**	150.52**
Genotype X Culture	51	6.49**	804.91**	2521.05**	0.011**	0.009**	24.37**	188.15**	0.013**	0.095**	18.11**	23.78**	9.28**
Error	142	6.71	39.28	1092.70	0.012	0.006	1.03	179.23	0.002	0.007	0.44	11.85	8.57

* Significant at 5% level, ** Significant at 1% level

Table 2 : Regression equation (Y = a + bx) of different nodular, root and yield characters in *Rhizobium* treated groundnut.

Characters	Year	A	(R^2)	B	(R^2)	A+B	(R^2)	C
Yield vs.								
(i) Nodule number (60DAS)		Y = 16.83291 - 0.01542 x (0.011)		Y = 17.04152-0.00826 x (0.003)		Y = 14.40651 - 0.00174 x (0.000)		Y = 19.41411 - 0.07630 x (0.200)
(ii) Nodule wt. "	1	Y = 20.14528 - 28.9031 x (0.041)		Y = 21.14203 - 27.6533 x (0.144)		Y = 25.74944 - 72.2323 x (0.324)		Y = 25.06700 - 76.7161 x (0.5641)
(iii) DMA 1 "	9	Y = 18.58158 - 0.18295 x (0.041)		Y = 13.44180 + 0.14158 x (0.053)		Y = 15.63602 - 0.07535 x (0.011)		Y = 18.52101 - 0.32251 x (0.102)
(iv) Nitrogenase	8							
activity "	8	Y = -76.3310 + 1.59666 x (0.697**)		Y = 39.5887 + 0.96696 x (0.545 **)		Y = 60.9406 + 1.33268 x (0.674**)		Y = 71.2359 + 1.54158 x (0.674**)
(v) Nodule No. (120 DAS)		Y = 18.71802 - 0.01244x(0.055)		Y = 16.73977 - 0.00185 x (0.001)		Y = 18.92790 - 0.01558 x (0.109)		Y = 17.32303 - 0.01568 x (0.061)
(vi) Nodule wt. "		Y = 18.85397 - 12.6069x(0.023)		Y = 14.65877 + 6.65452 x (0.014)		Y = 13.79547 + 1.53115 x (0.001)		Y = 19.05559 - 23.2576 x (0.061)
(vi) DMA 1 "		Y = 14.68961 + 0.01385x(0.001)		Y = 20.05381 - 0.06968 x (0.067)		Y = 17.93036 - 0.06888 x (0.029)		Y = 19.48529 - 0.11909 x (0.0144)
Yeild vs.								
(i) Nodule No. (60DAS)		Y = 14.69299 + 0.01881 x (0.005)		Y = 0.281519 - 0.05172 x (0.102)		Y = 15.91564) - 0.01269 x (0.0233)		Y = 14.32663 - 0.01276 x (0.002)
(ii) Nodule wt. "	1	Y = 14.65276 + 6.62335 x (0.002)		Y = 22.14528 - 35.7899 x (0.122)		Y = 13.65037 + 11.91402 x (0.013)		Y = 14.13917 - 3.01820 x (0.001)
(iii) DMA 1 (*) "	9	Y = 16.66405-0.08496 x (0.004)		Y = 15.25450 + 0.16064 x (0.022)		Y = 15.86400 - 0.05060 x (0.007)		Y = 11.07762 + 0.235775 x (0.001)
(iv) Nitrogenase	8							
activity	9	Y = -78.6782 + 1.644237 x(0.715**)		Y = 58.4101 + 1.319056 x (0.772)**		Y = 18.0355 + 0.585586 x (0.154)		Y = 38.1983 + 0.93914 x (0.363)
(v) Nudule. 120DAS		Y = 14.2184 + 0.31463 x (0.006)		Y = 16.02682 - 0.60838 x (0.023)		Y = 13.78244 + 0.012131 x (0.013)		Y = 16.64861 – 0.02888 x (0.006)
(vi) Nudule wt.		Y = 14.90654 + 1.793811 x (0.001)		Y = 20.02027 - 6.78376 x (0.010)		Y = 20.81862 + 16.1787 x (0.206)		Y = 16.64661 - 0.02833 x (0.036)
(vii) DMA 1		Y = 13.64239 + 0.02933 x (0.011)		Y = 20.02027 - 0.03941 x (0.010)		Y = 11.03603 + 0.06965 x (0.107)		Y = 15.74838 - 0.03794 x (0.041)
Root weight (6ODAS) vs.								
(viii) Nodule No,. (6ODAS)		Y = 0.33024 + 0.0000376 x (0.010)		Y = 0.39285 - 0.03941 x (0.004)		Y = 0.36877 + 0.000305 x (0.002)		Y = 0.459373 - 0.00322 x (0.215)
(ix) Nodule wt. "		Y = 0.30523 + 0.33699 x (0.024)		Y = 0.23164 - 0.03941 x (0.205)		Y = 0.50357 - 0.91516 x (0.059)		Y = 0.30911 + 0.10070 x (0.0021)
(x) DMA 1 "		Y = 0.297494 + 0.00371 x (0.032)		Y = 0.27473 - 0.03941 x (0.098)		Y = 0.303017 + 0.006143 x (0.084)		Y = 0.176943 + 0.01253 x (0.4782)
(xi) Nitrogenase activity "		Y = -0.4010 + 0.00678 x (0.053)		Y = 1.62978-0.03941x(0.220)		Y = -0.24299 + 0.011102 x (0.043)		Y = 0.49756 + 0.01478 x (0.174)

* DMA - Dry matter accumulation, * - Significant at 5% level, ** - Significant at 1% level

Rhizobium cultures : A=JCG - Gayeshpur, B = HMU - 2 ; A + B = Equal mixture of A and B, C = Control

Table 3 : Phenotypic correlations of yield and some nodule characters in *Rhizobium* treated groundnut during summer, 1999.

Characters	Days to maturity	Pod number / plant	Pod yield / plant (g)	Dry matter production / pl	Nodule number / plant 60 DAS	Dry nodule weight / plant 60 DAS	Nodule number / plant 120 DAS	Dry nodule weight / plant 120 DAS
Nitrogenase activity	0.697** (0.766**)	0.312 (0.272)	0.570* (0.689**)	0.203 (0.119)	0.508 (0.538 *)	0.057 (0.287)	0.012 (0.382)	0.060 (0.598*)
Dry nodule weight/plant (120 DAS)	0.076 (0.583*)	0.017 (0.621 *)	0.166 (0.622 *)	0.165 (0.480)	0.326 (0.744 **)	0.226 (0.360)	0.127 (0.900 **)	
Nodule number / plant (120 DAS)	0.358 (0.479)	-0.340 (0.605*)	0.656 (0.628)	0.089 (0.113)	0.088 (0.748 **)	0.094 (0.329)		
Dry nodule weight/plant (60 DAS)	0.526 (0.809 **)	0.124 (0.754 **)	0.131 (0.699**)	-0.119 (0.058)	0.667 ** (0.681**)			
Nodule number /plant (60 DAS)	0.676** (0.442)	0.020 (0.674**)	0.057 (0.775**)	0.130 (0.171)				

*, ** : Significant at 5% and 1% level respectively
Figures in the parenthesis indicate the value of spreading types.

(1.79381). In strain 'B' and control the dependence of yield on DMA was positive at 60 DAS (0.16064 and 0.235575 respectively). At 120 DAS strain A and the mixed inoculum gave the positive values (0.02933 and 0.0696). As found in many cases cultural strain 'B' gave the highest value of constant 'a' (20.02027) at 120 DAS without any significant R^2 value.

Similar to the *kharif*, 1998, the summer 1999 crop also reflected high and significant effect of regression co-efficient (b) and R^2 values between nitrogenase activity and yield but it was true in case of single culture inoculum only. Though, as a single inoculum, JCG-Goyespur and also the mixed inoculum mostly expressed positive 'b' values, but in no cases (except yield vs nitrogenase activity) R^2 values were significant. As such nitrogenase activity, not the nodule number and weight, should considered as basis criteria for selection of an effective *Rhizobium* strains as well as effective groundnut genotypes. This observation is corroborative with the previous report of Arrendell *et al.* (1985).

The correlations of different nodular and yield contributing characters have been presented in Table 3. It is clearly evident that in spreading types the nodule number and weight (both at 60 and 120 DAS) were significantly and positively correlated with most of the yield contributing characters like days to maturity, pod number and yield per plant. But in case of bunch types though these relations are positive (except nodule number at 60 DAS with number/plant, r = 0.340), no statistically significant values were obtained.

The nitrogenase activity of the nodules showed positive correlation with days to maturity, pod yield/plant, nodule number/plant (60 DAS) and dry nodule weight/plant (120 DAS) in the spreading types. On the other hand in bunch type nitrogenase activity of nodules was positively and significantly correlated with only days to maturity and pod yield per plant. But in no case, negative correlation was noticed. From the correlation study it also reflects that nitrogenase activity of nodule has positive and significant correlation with only days to maturity and pod yield per plant. But in no case, negative correlation was noticed. From the correlation study it also reflects that nitrogenase activity of nodule has positive and significant correlation with yield contributing characters in both bunch and spreading types of groundnut.

Therefore, while evaluating the efficiency of a strain and to select superior genotypes of groundnut from agronomic view point, it is suggested that

nitrogenase activity of nodules rather than its number and weight per plant should be the prime consideration of the breeders. Arrendell *et al.* (1988) while working with Virginia and Spanish genotypes of groundnut, also suggested that only nitrogenase activity was required to identify superior families.

REFERENCES

Arrendell, S.; Wynne, J. C.; Elkan, G. H. and Isleib, T. G. (1985). Variation for nitrogen fixation among progenies of a Virginia X Spanish peanut cross. *Crop Sci* **25 (5)** : 865-869.

Arrendell, S.; Wynne, J. C.; Elkan, G. H. and Schneeweis, T. J. (1988). Selection among early generation peanut progeny for high and low acetylene reduction and plant weight. *Plant Breeding*, **101(3)** : 217-224.

Arunachalam, V.; Pungle, G. D.; Dutta, M.; Nambiar, P.T.C and Dart. P. J. (1984). Efficiency of nitrogenae activity and nodule mass in predicting the relative performance of genotypes assessed by a number of characters in groundnut (*Arachis hypogaea* L.) *Exptl. Agric.* **20 (4)** : 399-343.

Byalebeka, J. B.; Wynne, J. C.; Schneeweis, T. J. and Elhan, G. H. (1987). Competition between effective and non-effective bradyrhizobia nodulating peanuts. (*Arachis hypogaea* L.) *Ann App Biol*, **111(1)** : 561-569.

Dilworth, M. (1966). Acetylene reduction by nitrogen fixing preparation from *Clostridium pasteurianum. Biochem. Biophys. Acta*, **127** : 285-294.

Dutta, M.; Rao, B. S.; Nambiar, P. T. C.; Williams, J. H. and Dart, P. J. (1988). Ontogenic variation in nitrogen assimilation, nodulation and nitrogen fixation in groundnut (*Arachis hypogaea* L.) genotypes. *Ann Appl. Biol*. **112** (3) : 537-545.

Joshi, P. K. and Kulkarni, J. H. (1983). Response of groundnut to single and mixed *Rhizobium* strains. *Indian J. Agric. Sci*. **53** : 376-378.

8. Kishinevsky, B.; Strijdom. B. W. : Otto, C. J. : Lochner, H. H. and Kriel, M. M. (1987). Response to inoculation of groundnut grown under irrigationn in soil containing indigenous rhizobia, *S. Africa J. Plant & Soil* **4(2)** : 75-78.

Nambiar, P.T.C. and Dart, P. J. (1983). Factors influencing nitrogenase activity (acetylene reduction) by root-nodule of groundnut (*Arachis hypogaea* L.). *Peanut Sci*. **10** : 26-29.

Panse. V. G. and Sukhatme, P. V. (1985). *Statistical methods for Agricultural workers* Ed. ICAR Publication, New Delhi.

(Accepted for publication June 16 2002)