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Combining Ability Analysis of N2-Fixation and Related Traits in Peanut[1]

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ABSTRACT

Analysis of a six parent diallel cross involving high and low nitrogen fixing peanut (*Arachis hypogaea* L.) genotypes revealed the predominant nature of non-additive genetic variance for nitrogenase activity and other traits. Germplasm line, NC Ac 2821 had the highest general combining ability for nitrogenase activity, total nitrogen, leaf area, and top weight, and therefore, it should be a good parent for use in breeding programs. Nitrogenase activity was significantly and positively correlated with nodule number, nodule mass, total nitrogen, top weight, and root weight. This evidence suggests the possibility of breeding for increased nitrogen fixation and thus yield in peanut.

Key Words: Groundnut, *Arachis hypogaea*, genetic variance, correlations.

Considerable genetic variation occurs in leguminous crops including peanut, *Arachis hypogaea* L., for the characters associated with nitrogen fixation. Several workers have shown genetic differences for nodulation, nodule mass, and nitrogenase activity among peanut cultivars (4,6,8,9,10,13,14), and efforts are being made to exploit this genetic variation to increase nitrogen fixing ability for cultivar improvement.

Earlier reports on the gene action of traits related to N2-fixation in peanut revealed predominantly non-additive genetic variance for nodule number, nodule mass, specific nitrogenase activity, shoot weight, and total nitrogen (8,14). Recently Arunachalam *et al.* (3) reported that nitrogenase activity and nodule mass were the most important characters in identifying the relative performance of a cultivar for high N2-fixation. Wynne *et al.*, (15) investigated the relative importance of the host, the *Rhizobium* strain, or the host-*Rhizobium* strain interaction for the genetic manipulation of host-*Rhizobium* symbiosis. They reported large additive genetic effects for nodule weight, and shoot weight; large non-additive genetic effects for nodule number and nitrogenase activity suggesting that these traits could be genetically exploited either by the selection of host-cultivars alone or in combinations of host-cultivars and rhizobia.

The present study was conducted to estimate the relative importance of general and specific combining ability for nitrogenase activity and other traits related to nitrogen fixation from a six parent diallel cross involving high and low N2-fixing genotypes. Correlations between traits were also measured.

Materials and Methods

Genotypes with low (MH 2, NC Ac 516, and NC Ac 1740) and high (NC Ac 490, NC Ac 2654, and NC Ac 2821) nitrogenase activity, selected on the basis of earlier observations (9,10) were crossed in full diallel. Thirty F1 progenies and six parents were grown in alfisol fields during the 1981-82 postrainy season (December - March) at the ICRISAT research center. The experimental design was a randomized complete block with four replications. The crop was sown as single row plots on ridges 75 cm apart with plants spaced 15 cm within the row. Single superphosphate was applied at the rate of 17.6 kg P/ha. The plots were not inoculated since there were abundant native *Rhizobium* populations in the soil ($10^{2.4}$ rhizobia/g dry soil). The crop was irrigated regularly and protected against pest damage.

Sixty days after sowing, twenty competitive plants from each plot were carefully dug (20-23 cm deep), the soil was removed from the roots, and the following observations were recorded:

A. Nitrogenase activity (μ moles C_2H_4/plant/hr)

Nitrogen fixing activity was measured by the acetylene reduction assay (5,11). The nodulated roots were placed in a 20 litre plastic bucket with a tight fitting lid. The container was made airtight by sealing the edges with adhesive tape. Acetylene was injected into these buckets by a syringe operated by acetylene cylinder pressure to give a final concentration of 10% v/v. The excess gas was then let out through a needle for 2-3 minutes to allow the pressure inside the container to reach equilibrium with the atmosphere. It was not possible to maintain a standard incubation temperature in the field, but variation in the bottle temperature was reduced by placing a wet jute bag over the bottles during incubation. All assays were carried out between 0930 and 1400 hr. The nitrogenase activity of all values was corrected by deducting the values obtained from a blank check.

B. Total nitrogen (mg/plant)

The nitrogen content in the plant tissue was determined as described by Technicon (Autoanalyser 11, Industrial method NC 218-72, AA 11). Shoot of 20 plants were ground to pass through 3 mm sieve, mixed well, and 20 g of the powder was ground again to pass through 1 mm sieve. Eighty mg of the powder was digested with 4 mL of selinium:

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sulphuric acid mixture, diluted to 75 mL with distilled water, and 0.33 mL was used for ammonia estimation based on Berthelot reaction (for details refer to Industrial method No. 218-72 A, AA 11, Technicon Industrial systems, Tarrytown, N. Y. 10591, USA).

Nitrogen content of fruit was determined similarly, and total nitrogen in the plant was calculated by adding nitrogen content in shoot and fruit.

C. Nodule number and mass (g/plant)

The number of nodules on the roots was counted after separating them manually from the roots. The weight of the nodules was recorded after drying at 80 C for 24 hr.

D. Leaf area (cm^2/plant)

Leaf area was measured using a LI-COR model 3100 leaf area meter. All leaves were removed manually, their fresh weight taken, and a subsample of 25g was used to measure leaf area.

E. Top and root dry weight (g/plant)

Weight of both tops and roots of the 20 bulked plants per replicate were determined after being dried at 80 C for 24 hr.

The combining ability analysis was carried out on plot means following model 1, method 1 of Griffing (7). Genotypic variance and covariance for computing phenotypic and genotypic correlations were calculated following Al-Jibouri *et al.* (1).

Results and Discussion

Significant differences were observed among parents and crosses for all traits (Table 1). The mean over all the crosses was greater than the overall parental mean for each character.

Table 1. Mean performance of genotypes for nitrogenase activity and other traits in peanut.

Genotype	Branching habit	Nitrogenase activity (µmoles C_2H_4/ plant/hr)	Total nitrogen (mg/ plant)	Nodule number/ plant	Nodule mass (gm/ plant)	Leaf area (cm^2/ plant)	Top wt. (gm/ plant)	Root wt.(gm/ plant)
MH 2	Sequential	27	262	129	0.13	633	6.6	0.55
NC Ac 1740	"	41	309	188	0.18	730	8.2	0.52
NC Ac 516	"	48	283	118	0.15	888	8.4	0.44
NC Ac 490	"	89	428	184	0.28	970	12.1	0.76
NC Ac 2654	"	87	446	171	0.22	1222	12.2	0.67
NC Ac 2821	Alternate	85	557	177	0.23	943	14.8	0.65
		± 8.0	± 30.6	± 14.8	±0.16	± 77.3	±0.87	±0.045
Mean over all parents		63	381	161	0.22	898	10.4	0.60
		± 3.3	± 12.5	± 6.1	±0.006	± 31.6	±0.35	±0.018
Mean over all crosses		94	493	239	0.28	1260	13.7	0.82
		± 1.5	± 5.6	± 2.7	±0.003	± 14.1	±0.16	±0.008

The mean squares due to general combining ability (GCA) and specific combining ability (SCA) were highly significant for all the traits, except the GCA mean square for nodule number (Table 2). Predominant SCA variance was however observed for all the characters. Similar results for nodule number, nodule mass, specific nitrogenase activity, shoot weight, and total nitrogen were previously reported (8).

Table 2. Mean squares and components of general and specific combining ability variance for nitrogenase activity and other traits in peanut.

Sources	d.f.	Nitrogenase activity	Total nitrogen	Nodule number	Nodule mass	Leaf area	Top weight	Root weight
GCA	5	2334**	45506**	1606	0.004**	177444**	30.7**	0.034**
SCA	15	778**	9418**	4885**	0.003**	88453**	8.7**	0.022**
REC	15	578*	3514	5841**	0.003**	64894**	3.9	0.035**
a) Maternal	5	365	6770	4097**	0.001	43725	3.3	0.020*
b) Non-maternal	10	684**	1885	6712**	0.004**	80878**	4.3	0.042**
Error	105	258	3743	879	0.001	23894	3.0	0.008
Var (GCA)		172	3480	60	0.0002	12795	2.3	0.002
Var (SCA)		520	5675	4006	0.0028	64559	5.7	0.014

*,** - Significant at 0.05 and 0.01 probability level.
GCA - General combining ability
SCA - Specific combining ability
REC - Reciprocal effects

Reciprocal effects were significant for nitrogenase activity, nodule number, nodule mass, leaf area, and root weight (Table 2). Partitioning of the reciprocal differences into maternal and non-maternal components indicated the importance of both for nodule number and root weight. The maternal component for other traits was nonsignificant. However, the significant non-maternal components for nitrogenase activity, nodule mass, and leaf area could be due to interaction of nuclear and extranuclear factors. Isleib *et. al.* (8) found significant maternal effects for nodule number, nodule mass, shoot weight, and total nitrogen and nonsignificant non-maternal effects only for nitrogenase activity and total nitrogen.

Five of the six parents included in this study belong to ssp. *fastigiata* var. *fastigiata,* but none had good general combining ability, except NC Ac 1740 for total nitrogen, NC Ac 490 for nodule mass, and NC Ac 2654 for top weight (Table 3). NC Ac 2821 was the only parent which belongs to ssp. *hypogaea* var. *hypogaea,* and it had the best general combining ability for most traits, including high nitrogenase activity. Therefore, it should make a better parent in a breeding program under these environmental conditions for increasing N2-Fixation. These results are in contrast to those reported earlier. Isleib *et al.* (8) found that ssp. *fastigiata* var. *fastigiata* genotypes (PI 275708 and NC Ac 16145) were superior in transmitting to their progeny increased nodule number and mass, nitrogenase activity, shoot weight, and total nitrogen and the ssp *hypogaea* genotypes (PI 275744 and PI 262090) were poor combiners for most of the characters studied. Arunachalam *et al.* (2,3) also reported virginia cultivars fix more nitrogen than spanish and valencia cultivars.

Table 3. GCA effects for nitrogenase activity and other traits in peanut.

Genotype	Nitrogenase activity	Total nitrogen	Nodule number	Nodule mass	Leaf area	Top weight	Root weight
MH 2	7.8	-53.2*	-19.0	-0.020	- 86.2	-1.66*	-0.06
NC Ac 1740	-16.2*	46.8*	- 3.0	-0.021	-106.2**	-1.46*	-0.06*
NC Ac 516	- 8.9	39.1	- 5.4	-0.002	-100.0	-0.59	-0.01
NC Ac 490	3.3	- 4.4	9.9	0.024*	6.6	0.03	0.04
NC Ac 2654	7.0	39.6	11.2	0.012	96.7	1.23*	0.04
NC Ac 2821	22.5**	103.9**	6.3	0.006	189.2**	2.45**	0.05

*,** - Significant at 0.05 and 0.01 probability level

Crosses with significant SCA effects for nodule number and nodule mass were MH 2 x NC Ac 2821 and NC Ac 516 x NC Ac 2654. SCA effect for nitrogenase activity was significant only for the cross NC Ac 1740 x NC Ac 2821. Crosses with desirable SCA effects for total nitrogen and leaf area were NC Ac 516 x NC Ac 2654 and MH 2 x NC Ac 2821, respectively. Desirable crosses for top weight and root weight were NC Ac 516 x NC Ac 2654 and MH 2 x NC Ac 2821 (only for top weight). These crosses also had the high mean *per se* for these traits.

Genotypic correlation coefficients were, in general, higher than corresponding phenotypic correlations because of the removal of environmental effects (Table 4). Phenotypic correlation coefficients were significant ($P=0.01$) and positive between nitrogenase activity and total nitrogen, nodule number, nodule mass, top weight, and root weight. Similarly, total nitrogen was also significantly correlated ($P=0.01$) with nodule mass, leaf area, top weight, and root weight. Nodule number and nodule mass were significantly correlated between themselves and also with top weight and root weight. Leaf area was

significantly correlated with top weight, root weight, and nodule mass. Correlation between top weight and root weight, was also significant. Wynne *et al.* (14) also observed a high degree of correlation between nitrogenase activity and shoot weight, nodule number, nodule mass, and total nitrogen.

Table 4. Genotypic (below diagonal) and phenotypic (above diagonal) correlations for nitrogenase activity and related traits in peanut.

	Nitrogenase activity	Total nitrogen	Nodule number	Nodule mass	Leaf area	Top weight	Root weight
Nitrogenase activity	-	0.67**	0.43*	0.92**	0.18	0.58**	0.61**
Total nitrogen	0.92	-	0.15	0.84**	0.52**	0.97**	0.62**
Nodule number	0.51	0.85	-	0.54**	0.31	0.48**	0.69**
Nodule mass	0.52	0.67	0.87	-	0.56**	0.82**	0.64**
Leaf area	0.99	0.93	0.58	0.58	-	0.49**	0.40*
Top weight	0.88	0.99	0.89	0.74	0.90	-	0.58**
Root weight	0.73	0.84	0.88	0.80	0.79	0.87	-

*,** - Significant at 0.05 and 0.01 probability level.

Because of the greater importance of SCA variance for nitrogenase activity and other related traits, selection for high nitrogen fixation may not be effective in early generations, therefore, advanced generation selections derived from specific crosses should be evaluated for high N2-fixation. Recently, it has been reported that traits related to nitrogen fixation, i.e., nodule mass and nitrogenase activity accounted for more than 80% variation in seed yield in the F2 generation (2,12). These studies suggested that if genotypes with high nitrogen fixation ability (high x high) are crossed, it is possible to produce transgressive segregants combining high nitrogen fixation and yield. These observations coupled with the data presented here indicate the possibility of increasing yield by improving the N2 -fixation ability of the peanut.

Literature Cited

1. Al-Jibouri, H. A., P. A. Miller, and H. F. Robinson. 1958. Genotypic and environmental variances and covariances in an upland cotton cross of interspecific origin. Agron. J. 50:633-636.
2. Arunachalam, V., G. D. Pungle, A. Bandyopadhyay, and M. Dutta. 1984a. Breeding for increased nitrogen fixation in groundnut. p.51-61. *In* B. S. Ghai (ed.) Symbiotic Nitrogen Fixation Ludhiana, USG Publ. and Distr., India.
3. Arunachalam, V., G. D. Pungle, M. Dutta, P. T. C. Nambiar, and P. J. Dart. 1984b. Efficiency of nitrogenase activity and nodule mass in predicting the relative preformance of genotypes assessed by a number of characters in groundnut (*Arachis hypogaea*). Expl. Agric. 20:303-309.
4. Burton, J. C. 1976. Pragmatic aspects of *Rhizobium* leguminous plant association. p. 429-446. *In* W. E. Newton and C. J. Nyman (eds.) Proc. 1st International Symposium on Nitrogen Fixation, Pullman: Washington State University Press.
5. Dart, P. J., J. M. Day, and D. Harris. 1972. Assay of nitrogenase activity by acetylene reduction. p. 84-100. *In* Use of Isotopes For Study of Fertilizer Utilization by Legume Crops. Vienna: International Atomic Energy Agency/Food and Agricultural Organization.
6. Duggar, J. F. 1935. Nodulation of peanut plants as affected by variety, shelling of seed, and disinfection of seed. J. Amer. Soc. Agron. 27:286-288.
7. Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.
8. Isleib, T. G., J. C. Wynne, G. H. Elkan, and T. J. Schneeweis. 1980. Quantitative genetic aspects of nitrogen fixation in peanuts (*Arachis hypogaea* L.). Peanut Sci. 7:101-105.
9. Nambiar, P. T. C., and P. J. Dart. 1980. Studies on nitrogen fixation by groundnut at ICRISAT. p. 110-124. *In* Proc. International Workshop on Groundnut. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics.
10. Nambiar, P. T. C., P. J. Dart, S. N. Nigam, and R. W. Gibbons. 1981. Genetic manipulation of nodulation in groundnut. p. 49-55. *In* Proc. International Workshop on BNF Technology for Tropical Agriculture, Cali, Colombia: Centro Internacional de Agricultura Tropical.
11. Nambiar, P. T. C., and P. J. Dart. 1983. Factors influencing nitrogenase activity (acetylene reduction) by root nodules of groundnut, *Arachis hypogaea* L. Peanut Sci. 10:26-29.
12. Pungle, G. D. 1983. Genetic investigation on characters related to growth, biological nitrogen fixation and yield in groundnut (*Arachis hypogaea* L.). Ph. D. thesis. New Delhi, India: Indian Agricultural Research Institute.
13. Wynne, J. C., G. H. Elkan, T. J. Schneeweis, T. G. Isleib, C. M. Preston, and C. A. Meisner. 1978. Increasing nitrogen fixation of the peanut. Proc. Amer. Peanut Res. Educ. Assoc. 10:22-29.
14. Wynne, J. C., G. H. Elkan, and T. J. Schneeweis. 1980. Increasing nitrogen fixation of the groundnut by strain and host selection. p. 95-109. *In* Proc. International Workshop on Groundnut, Patancheru, India:International Crops Research Institute for the Semi-Arid Tropics.
15. Wynne, J. C., G. H. Elkan, T. G. Isleib, and T. J. Schneeweis. 1983. Effect of host plant, *Rhizobium* strain and host x strain interaction on symbiotic variablity in peanut. Peanut Sci. 10:110-114.

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