Peanut Science (1980) 7, 101-105

Quantitative Genetic Aspects of Nitrogen Fixation in Peanuts (*Arachis hypogaea* L.)[1, 2]

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ABSTRACT

Manipulation of the host genotype has been proposed as a method of increasing biological nitrogen fixation by rhizobia in symbiosis with the peanut (*Arachis hypogaea* L.). The F_1 generation of a diallel cross of 10 South American cultivars was evaluated in the greenhouse in an analysis of gene action for traits related to nitrogen fixation. The parents represented five secondary centers of diversity and effects in the diallel model were partitioned into among- and within-center components. Variation of center effects was significant for several characters but was smaller in magnitude than within-center variation. Specific combining abilities were significant and accounted for more variability than general combining abilities for nodule number, nodule mass, specific nitrogenase activity, shoot weight, and total nitrogen, indicating non-additive types of gene action. Maternal effects were observed for the same characters. The parents with the highest general combining abilities (GCA's) for nitrogen fixation were both fastigiate types, while Virginia-type parents had generally low GCA's. Correlations between parental and GCA effects were nonsignificant for all traits, so simple evaluation of lines for nitrogen-fixing capacity may not identify superior parents for use in breeding programs.

Key Words: Diallel cross, nitrogenase activity, nodulation, cytoplasmic effects.

Peanuts (*Arachis hypogaea* L.) share with other legumes the ability to enter a symbiotic relationship with bacteria of the genus *Rhizobium* Frank. In peanuts, the contribution of biological fixation to the plant's total nitrogen requirement has not been completely quantified, but there are indications that peanut rhizobia may be able to fix enough atmospheric nitrogen to reduce or eliminate the response to subsequent nitrogen fertilization (15).

The three major factors influencing symbiotic nitrogen fixation are the rhizobial strain, the genotype of the host plant, and elements of the external environment. Of these, it appears that the host genotype may be the one most amenable to change (2, 10). Differences in nodulation among peanut genotypes in the field were first reported by Duggar (4, 5). Burton (1) found variation in nitrogen accumulation among peanut cultivars grown in the greenhouse with biological fixation as the sole source of nitrogen. Similar results have been obtained for nodulation and fixation rate in several studies at North Carolina State University (16). All these studies suggested that the host genotype might be manipulated to increase nodulation and nitrogen fixation, but investigations of the genetics of the observed variation were lacking.

If the ability to form a more effective efficient symbiotic relationship is controlled by genes with largely additive or additive types of epistatic effects, then it should be possible to increase biological nitrogen fixation in the peanut through conventional plant breeding methods. It was the purpose of the present study to assess the variation in nitrogen-fixing ability in a sample of diverse peanut cultivars and to identify the relative importance of additive and nonadditive effects in hybrid progenies derived from crosses of such cultivars.

Materials and Methods

Parental Material

A sample of parental cultivars was chosen from germplasm originally collected from South America (Table 1). Each of the five South American secondary centers of diversity described by Krapovickas (11, 12) was represented in the sample initially, as well as the tertiary center (Center VI) postulated by Gregory and Gregory (6). Center III, the secondary center in the region of Rondonia and northwest Mato Grosso, Brazil, was deleted from the study because of the extremely late flowering and maturation of its representative, *Arachis hypogaea* ssp. *hypogaea* var. *nambyquarae* (Hoehne) Burkart.

Cultivars designated "A", "B", or "C" were described by Parker *et al.* (14); the remainder were described by Layrisse (13). The parental sample included representatives of the taxa ssp. *hypogaea* var. *hypogaea*, ssp. *fastifiata* Waldron var. *fastigiata* and ssp. *fastigiata* var. *vulgaris* Harz, as well as some intermediate types.

Experimental Design

The 10 parental lines were crossed in full diallel. Pregerminated embryos of the 90 F_1 hybrid progenies and 10 parents were planted in the greenhouse on October 19, 1977 in a sterilized medium of washed sand and vermiculite in 10.2-cm diameter pots. Each experimental unit consisted of a single potted plant. Four replicates were planted in a randomized complete block (RCB) design, but because of seed shortage and some germination failures, none of the replicates were complete. Nitragin® peat-based peanut inoculant (Lot LX 579) was applied to the growth medium at planting and again 14 days later. The plants were fertilized four times at 7- to 9-day intervals with 50 ml of Bond's stock salt solution, providing no nitrogenous salts. Plants of the first through fourth replications were harvested at 42, 43, 48, and 49 days after planting, respectively.

Nitrogen-fixing ability was measured by the acetylene reduction assay of nitrogenase activity (8). Immediately upon harvest, the root system of each plant was detached from the shoot by clipping the hypocotyl at its junction with the mainstem. The severed root was placed in a .473 L (1 pt) Kerr® self-sealing mason jar whose lid was fitted with a 7 x 11 mm screen serum bottle stopper anchored in Dow-Corning® silicone rubber bath-

[1]Paper No. 6020 of the Journal Series of the North Carolina Agricultural Research Service. This investigation was supported in part by grant USDA-SEA-CR 616-15-192 and 701-15-24 under A.I.D. PASA AG/TAG 610-0-76.

[2]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

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Table 1. Representation of South American centers of diversity in parental cultivars.

Center of diversity	Parent	Identity
Guarani region (I)	I_1	PI 262000 ("C_2")
	I_2	PI 261954
Goias and Minas Gerais, Brazil (II)	II_1	PI 275708
	II_2	PI 275744
Bolivia (IV)	IV_1	PI 262090 ("B_1")
	IV_2	NC Ac 16145
Peru (V)	V_1	PI 275751 ("A_1")
	V_2	NC Ac 17090 ("A_2")
Northeast Brazil (VI)	VI_1	NC Ac 17143
	VI_2	NC Ac 17144

tub caulk. Thirty-five cubic centimeters of the atmosphere in each jar was replaced with acetylene using a syringe inserted through the serum bottle stopper, providing approximately .074 atm of acetylene in each jar. After 3 hours of incubation at room temperature, two 1-ml atmospheric samples were removed from each jar. Duplicate samples were taken to insure against loss of a single sample; only one sample per jar was analyzed. The same order was maintained in harvest, acetylation, and sample removal in order to provide uniformity in the treatment of each plot. Ethylene (C_2H_4) content of the atmospheric sample was measured with a Varian® 940 gas chromatograph equipped with a hydrogen flame detector and a stainless steel column 244 cm long and 3.175 mm in diameter, packed with Poropack® N. Activity was expressed as μmoles of ethylene evolved per plant per hour.

In addition to nitrogenase activity, the following characters were measured for each F_1 plant: number of nodules, shoot dry weight (g), nitrogen content of the shoot (% by weight) determined by Kjeldahl analysis, and dry weight per nodule (mg) measured as the mean of 10 randomly selected nodules. Other variables were determined arithmetically from measured characters: nodule mass (mg) as the product of nodule number and dry weight, specific nitrogenase activity (μmoles of C_2H_4 evolved/g hr) as 1000 times the quotient of nitrogenase activity divided by nodule mass, and total nitrogen in the shoot (mg) as the product of shoot dry weight and nitrogen content.

Statistical Analysis

Diallel analysis was performed for each character using a modification of Griffing's Method 3, Model I (7) with the extensions described by Cockerham and Weir (3) pertaining to maternal and reciprocal effects. For a hybrid, the linear model for their design was:

$$Y_{i(jk)(\ell m)} = \mu + b_i + g^*_{(jk)} + g^*_{(\ell m)} + s^*_{(jk)(\ell m)} + m^*_{(jk)} - m^*_{(\ell m)} + r^*_{(jk)(\ell m)} + \epsilon_{i(jk)(\ell m)}$$

where the subscript (jk) referred to the k^{th} of w parents from the j^{th} of c centers of diversity. The model included an overall mean (μ) and the effects of blocks (b), general combining ability (g*) and specific combining ability (s*), maternal (m*), paternal (-m*), and specific reciprocal (r*) effects, and random error (ϵ). For a parent, the corresponding linear model was

$$Y_{i(jk)(jk)} = \mu' + b_i + p^*_{(jk)} + \epsilon_{i(jk)(jk)}$$

where μ' was the overall mean for parents and $p^*_{(jk)}$ the effect of the jk^{th} parent. In the fixed effects (Model I) analysis, the constraints placed on the parameters were:

$$\sum_i^b b_i = 0,$$

$$\sum_{jk}^{cw} g^*_{(jk)} = 0,$$

$$\sum_{(\ell m)\neq(jk)}^{cw-1} s^*_{(jk)(\ell m)} = \sum_{(jk)\neq(\ell m)}^{cw-1} s^*_{(jk)(\ell m)} = 0,$$

$$\sum_{(jk)}^{cw} m^*_{(jk)} = 0,$$

$$\sum_{(\ell m)\neq(jk)}^{cw-1} r^*_{(jk)(\ell m)} = \sum_{(jk)\neq(\ell m)}^{cw-1} r^*_{(jk)(\ell m)} = 0, \text{ and}$$

$$\sum_{jk}^{cw} p^*_{(jk)} = 0.$$

Because of the stratified nature of the parental sample, the diallel model was refined to take into account the contributions of centers of diversity and parents within centers to the variation among genotypes. Each genotypic effect in the original model was partitioned into two components, a "center" effect and a "within-center" effect or deviation from the center effect. For example, $g^*_{(jk)}$ was defined as the sum of G_j, the average general combining ability (GCA) effect associated with parents from the j^{th} center of diversity, and g_{jk} the deviation of the GCA of the k^{th} parent of the j^{th} center from the average center effect. The refined model for a hybrid progeny was:

$$Y_{i(jk)(\ell m)} = \mu + b_i + (G_j+g_{jk}) + (G_\ell+g_{\ell m}) + (S_{j\ell}+s_{(jk)(\ell m)}) + (M_j+m_{jk}) - (M_\ell+m_{\ell m}) + (R_{j\ell}+r_{(jk)(\ell m)}) + \epsilon_{i(jk)(\ell m)},$$

while that for a parent was:

$$Y_{i(jk)(jk)} = \mu' + b_i + (P_j+p_{jk}) + \epsilon_{i(jk)(jk)}$$

The numeric constraints in the original model were preserved by imposing the following constraints in the refined model:

$$\sum_j^c G_j = 0, \quad \sum_k^w g_{jk} = 0,$$

$$(w-1)S_{jj} + w\sum_{\ell\neq j}^{c-1} S_{j\ell} = 0, \quad \sum_{(\ell m)\neq(jk)}^{cw-1} s_{(jk)(\ell m)} = 0,$$

$$\sum_j^c M_j = 0, \quad \sum_k^w m_{jk} = 0,$$

$$\sum_{\ell\neq j}^{c-1} R_{j\ell} = 0, \quad \sum_{(\ell m)\neq(jk)}^{cw-1} r_{(jk)(\ell m)} = 0,$$

$$\sum_j^c P_j = 0, \text{ and } \sum_k^w p_{jk} = 0.$$

Additional constraints were placed on the deviations from average center specific combining ability (SCA) and reciprocal effects:

$$\sum_{m>k}^{1/2w(w-1)} s_{(jk)(jm)} = 0 \text{ (where } w = 2,\ s_{jljl} = 0),$$

$$\sum_k^w \sum_m^w s_{(jk)(\ell m)} = 0, \text{ and}$$

$$\sum_k^w \sum_m^w r_{(jk)(\ell m)} = 0.$$

This design was analogous to Griffing's Method 1, Model I (7) with respect to center effects.

Because the data were unbalanced for all traits, regression methods were applied in the analysis of variance. A design matrix was constructed using the given constraints in the parameterization to allocate the degrees of freedom for effects in the simple model to their component effects in the refined model. The general linear models (GLM) procedure from the SAS 79 package of statistical programs (9) was used to compute sequential and partial sums of squares for all effects and to estimate the various parameters for both the simple and refined models.

Results and Discussion

Sequential and partial mean squares differed only trivially for all characters, so only sequential mean squares are reported (Table 2). No significant variation was detected among parental lines for any trait except nitrogen content (%) where the variation arose from differences among lines within centers. Despite this lack of variation among parents, differences among hybrid progenies were found to be significant for all characters.

Specific nitrogenase activity was the only trait for which variation among GCA effects was nonsignificant. The within-centers component of GCA was variable for all the remaining traits, while the among-centers component was variable for all but nitrogenase activity and total nitrogen. GCA accounted for no more than 36% of the variation among hybrid progenies for any trait, reaching as low as 8% for specific activity and generally falling below 30%. In every case, the among centers component of the GCA sum of squares, whether significant or not, accounted for less of the variation among crosses than did the within-centers component. For all traits except specific activity, the among-centers portion of the GCA sum of squares was less than half the within-centers portion. This indicates that in screening exotic lines for GCA for these characters, little emphasis needs to be placed on sampling any particular center of diversity as the preponderance of the variation occurs among lines within centers rather than among center means.

In a genetic sense, the GCA of a parent is a function both of its additive gene effects and its average dominance effect in combination with the other parents. Therefore, the test of GCA is not equivalent to a test for additive gene action unless there are no dominance interactions.

The presence of dominance may be detected in part by testing the significance in SCA effects which are functions solely of dominance interactions. These effects were significant for all traits except nitrogenase activity and nitrogen content. Average dominance effects cannot be ruled out on the basis of this test, but it appears that dominance was not as great for these two characters as for the others. Of the remaining traits, only nodule number and mass exhibited significant variation among the average SCA effects of crosses of parents from specific combinations of centers of diversity. For nodule mass, this "among-centers" component of SCA was significant, while the within-centers component was not, perhaps indicating some within-center homogeneity of the genetic system controlling nodule mass. It must be remembered, however, that the within-center components of GCA effects for nodule mass were variable. For specific activity, shoot weight, and total nitrogen, the variation in SCA was due to heterogeneity of the within-centers components. In general, the variability within centers was much larger than that among centers. Genetically, this evidence, taken with the significance of the within-centers component of GCA, points to variation of the genetic

Table 2. Mean squares from the diallel analysis of characters indicative of nitrogen-fixing ability.

Source	df	Nodule number	Nodule mass (mg)	Nitrogenase activity ($\mu M\ C_2H_4$/plant hr)	Specific activity ($\mu M\ C_2H_4$ g/hr)	Shoot weight (g)	Nitrogen content (%)	Total nitrogen (mg/plant)
Total	381							
Blocks	3	4742**	64505**	73.85**	6657**	7.85**	0.5965**	5780**
Genotypes	98	1477**	10030**	6.47**	759**	1.92**	0.1418**	2190**
Parents	9	726	8543	6.09	493	0.60	0.1508	534
Among centers	4	816	7792	6.51	1021	0.26	0.0230	168
Within centers	5	655	9143	5.75	71	0.88	0.2475*	826
Hybrid progenies	88	1562**	10132**	6.58**	784**	2.08**	0.1423**	2378**
GCA	9	4216**	26950**	17.10**	610	3.72**	0.4910**	3327**
Among centers	4	2931**	17548**	4.33	499	1.91**	0.2770*	1247
Within centers	5	5243**	34472**	27.32**	699	5.17**	0.6622**	4810**
SCA	35	1533**	8263**	4.68	806*	1.97**	0.0699	2252**
Among centers	10	1927**	10787*	7.07	812	0.94	0.0484	1063
Within centers	25	1376**	7253	3.72	804*	2.39**	0.0785	2727**
Maternal	9	1433*	16796**	3.74	1520**	2.32**	0.2197**	3674**
Among centers	4	1630	25103**	6.66	2140**	3.99**	0.0612	5328**
Within centers	5	1276	10150	1.41	1024	1.00	0.3465**	2350**
Reciprocal	35	941	5964	6.50*	619	1.69	0.1052	1954**
Among centers	6	186	1208	9.51*	787	0.71	0.0550	820
Within centers	29	1098*	6933	5.88	584	1.90	0.1156	2188**
Parents vs hybrids	1	723	14423	0.08	949	0.27	0.0143	479
Error	280	742	4825	3.97	482	0.53	0.0845	619

*,**Denote significance at the 5 and 1% levels, respectively.

systems within centers of diversity. While each center comprises a morphologically distinct set of botanical types, it appears that the variability among types within a center is more important than that among centers at least for characters reflecting nitrogen-fixing ability.

Differences between reciprocal crosses were detected for all traits. Maternal effects were significant for all but nitrogenase activity. Of these traits, only nitrogen content and total nitrogen had significant variability among the within-center components of maternal effects, while the among-centers component was the main source of variability for nitrogenase activity, specific activity, and shoot weight. This result is reflected in the relative magnitudes of the sums of squares for among and within-centers components. For maternal effects, the among-center sum of squares was as large as or larger than the within-centers sum of squares for all traits except nitrogen content. Maternal effects are generally attributed to differences in heritable extranuclear factors, such as mitochondrial and chloroplast DNA. The importance of the among-centers maternal effect shows that there are more or less distinct cytoplasms to be found in different centers, at least for the sample of parents used in this study.

Reciprocal effects were observed for nodule number, nitrogenase activity, and total G nitrogen. The among-centers component was significant only for nitrogenase activity. Even for this character, the among-centers sum of squares was much smaller than the within-centers. These effects are generally attributed to interactions between nuclear and extranuclear factors.

Even though GCA effects accounted for relatively little of the variation among hybrids, they are still of interest to the breeder. One would simply expect the heritability of the measured traits to be low and progress from selection to be slow. An examination of GCA effects (Table 3) identifies a few lines with some potential as parents in a breeding program designed to increase nitrogen fixation in the peanut. Parent II_1, transmitted to its progeny increased nodule number and mass, nitrogenase activity, shoot weight and total nitrogen. This parent (ssp. *fastigiata* var. *fastigiata*) was from the center of diversity in Goias and Minas Gerais, states of Brazil. Parent IV_2 (ssp. *fastigiata* var. *fastigiata*) from northeastern Brazil, had a similar effect on its progeny. It is interesting to note that these two lines, both fastigiate types, were indicated as superior parents when other studies (16) have shown Virginia types (ssp. *hypogaea* var. *hypogaea*) to be better nitrogen-fixing genotypes. The two representatives of ssp. *hypogaea* in this study, parents II_2 and IV_1, made a poor showing in most characters, although IV_1 had the highest GCA for specific activity. It is also appropriate to mention that while the mean square for GCA for nitrogen content was highly significant, the effects themselves were very small. The mean nitrogen content for all genotypes was 3.6% and the GCA effects were on the order of 0.1%, or 2.7% of the mean. As a result, very slow gains from selection for increased nitrogen content could be expected.

Correlation between parental and GCA effects was not significant for any trait (Table 3). While this result might stem from the small size of the parental sample, it would seem that the performance of an inbred line cannot be used as a predictor of that line's capacity to transmit its attributes to its progeny. Such a phenomenon could be expected when there is appreciable dominance or other nonadditive genetic variation for a trait. In this study, all but two traits exhibited significant nonadditive variation as evidenced by the test of SCA effects. For these, SCA effects accounted for much more of the variation than did GCA effects.

Table 3. Parental (p*) and GCA (g*) effects with their average standard errors and correlations (r).

Parent	Nodule number		Nodule mass (g)		Nitrogenase activity (μM C_2H_4/plant hr)		Specific activity (μM C_2H_4 g/hr)		Shoot weight (g)		Nitrogen content (%)		Total nitrogen (mg/plant)	
	p*	g*	p*	g*	p*	g*	p*	g*	p*	g*	p*	g*	p*	g*
I_1	0.6	2.3	59.9	9.1	-1.3	0.3	-11.8	-2.9	-0.2	0.1	-0.2	0.0	-11.6	6.0
I_2	-10.1	-8.3	-17.4	-3.2	-0.5	-0.4	-6.5	-2.7	-0.2	-0.2	0.2	0.0	0.2	-6.0
II_1	0.2	11.4	22.2	31.8	1.0	0.8	2.8	-1.4	-0.4	0.5	0.0	-0.1	-14.6	15.5
II_2	2.4	-2.5	-6.3	-10.9	0.6	-0.5	12.7	0.5	0.1	-0.2	0.1	-0.0	6.1	-6.8
IV_1	0.2	-3.3	-11.0	-20.4	-1.2	-0.2	-13.1	6.9	0.1	-0.2	-0.0	0.0	3.0	-6.0
IV_2	33.2	8.3	80.3	11.7	-0.7	-0.1	-17.2	-5.3	0.0	0.1	0.1	0.1	3.5	4.0
V_1	0.9	-9.0	-15.6	-10.5	2.7	0.4	9.0	6.0	0.8	0.0	-0.2	-0.0	23.0	-1.1
V_2	-7.8	-5.2	-62.3	-27.4	-0.7	-0.6	8.3	2.7	-0.5	-0.3	0.0	0.1	-15.7	-6.7
VI_1	-19.1	-9.0	-60.9	-16.4	-0.6	-0.6	10.8	-0.8	-0.2	-0.2	0.3	0.1	2.7	-4.4
VI_2	-0.4	15.4	-20.0	36.1	0.7	0.9	5.0	-3.0	0.4	0.3	-0.3	-0.2	3.4	5.6
Std. error	12.9	3.4	33.0	8.7	1.0	0.3	10.4	2.8	0.4	0.1	0.1	0.0	11.8	3.1
r	0.6		0.5		0.5		0.3		0.0		0.6		-0.3	

In summary, significant nonadditive genetic variation was evidenced by tests of SCA in the analyses of variance for all traits except nitrogenase activity and nitrogen content. SCA effects accounted for substantial portions of the variability among crosses for the remaining traits. The among-centers components of GCA and SCA were significant for some characters but accounted for less of the total variation than did within-center effects. This situation was reversed with respect to maternal effects where the among-center effects were more important, indicating some homogeneity of cytoplasmic factors within centers. The correlations between parental and GCA effects were nonsignificant, so that screening of lines in the greenhouse may not be an efficient means of identifying parents for inclusion in breeding programs.

Literature Cited

1. Burton, J. C. 1976. Pragmatic aspects of the *Rhizobium* leguminous plant association, pp. 429-446. *In* W. E. Newton and C. J. Nyman (eds.) Proceedings of the 1st Internat. Symp. on Nitrogen Fixation. Vol. 2. Washington State Univ. Press, Pullman.

2. Caldwell, B. E., and H. G. Vest. 1977. Genetic aspects of nodulation and dinitrogen fixation by legumes: The macrosymbiont, pp. 557-576. *In* R. W. F. Hardy and W. S. Silver (eds.) A Treatise on Dinitrogen Fixation. Section III: Biology. John Wiley and Sons, New York.

3. Cockerham, C. C., and B. S. Weir. 1977. Quadratic analyses of reciprocal crosses. Biometrics 33:187-203.

4. Duggar, J. F. 1935. The nodulation and other adaptations of certain summer legumes. J. Am. Soc. Agron. 27:32-37.

5. Duggar, J. F. 1935. Nodulation of peanut plants as affected by variety, shelling of seed and disinfection of seed. J. Am. Soc. Agron. 27:286-288.

6. Gregory, W. C., and M. P. Gregory. 1976. Groundnut, *Arachis hypogaea* (Leguminosae—Papilionatae), pp. 151-154. *In* N. W. Simmonds (ed.) Evolution of Crop Plants. Longman Inc., New York and London.

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. Hardy, R. W. F., R. C. Burns, and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5:47-81.

9. Helwig, J. T., and K. A. Council (eds.). 1979. SAS User's Guide. 1979 Edition. SAS Institute, Inc., Raleigh, NC.

10. Holl, F. B., and T. A. LaRue. 1976. Host genetics and nitrogen fixation, pp. 156-163. *In* L. D. Hill (ed.) World Soybean Research. The Interstate Printers and Publishers, Inc., Danville, IL.

11. Krapovickas, A. 1968. Origen, variabilidad y difusion del mani (*Arachis hypogaea* L.). Actas Mems XXXVII Congr. Internac. Am. 2:517-534. [English translation by J. Smartt in P. S. Ucko and G. W. Dimbleby (eds.),1969, The Domestication and Exploitation of Plants and Animals, G. Duckworth and Co., London].

12. Krapovickas, A. 1973. Evolution of the genus *Arachis*, pp. 135-151. *In* R. Moav (ed.) Agricultural Genetics. Nat. Counc. Res. Devel., Jerusalem, Israel.

13. Layrisse, A. J. 1978. Diallel cross analysis of diverse peanut (*Arachis hypogaea* L.) lines in the F_2 generation. MS thesis, North Carolina State University, Raleigh.

14. Parker, R. C., J. C. Wynne, and D. A. Emery. 1970. Combining ability estimates in *Arachis hypogaea* L. I. F_1 seedling responses in a controlled environment. Crop Sci. 10:429-432.

15. Reid, P. H., and F. R. Cox. 1973. Soil properties, mineral nutrition, and fertilization practices, pp. 271-297. *In* C. T. Wilson (ed.) Peanuts: Culture and Uses. Amer. Peanut Res. Educ. Assoc., Stillwater, OK.

16. 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.

Accepted September 30, 1980