

# Selection Among Early Generation Peanut Progeny for Enhanced Nitrogen Fixation<sup>1</sup>

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## ABSTRACT

Recurrent selection provides an established methodology for host improvement which should be applicable to increasing N<sub>2</sub> fixation. This study was conducted to evaluate response to three cycles of phenotypic selection for fresh plant weight, nodule number and dry weight, and acetylene reduction when individual peanut (*Arachis hypogaea* L.) plants were evaluated in the greenhouse using a mixture of four *Bradyrhizobium* strains as inoculum. The F<sub>2</sub> generation derived from a single cross, Florigiant x CES 101, constituted the base population. In each of three cycles the 20 superior individuals were selected and randomly intermated to generate the subsequent cycle for selection. The 20 selections from each cycle were bulked and evaluated with the original parents in two greenhouse trials to evaluate response to selection. No variability for fresh plant weight, nodule number or dry weight, or acetylene reduction was detected among cycles. Realized heritability estimates were 0.01±0.02, 0.05±0.03, -0.06±0.06, and 0.31±0.32 for fresh plant weight, nodule number, nodule dry weight, and acetylene reduction, respectively. Agronomic traits of the same entries were evaluated in one field study. No variability for these traits was detected among cycles. Insufficient control of environmental variation was suggested as a possible cause of lack of response to selection.

Key Words: *Arachis hypogaea* L., *Bradyrhizobium*, recurrent selection, realized heritability, acetylene reduction, groundnut.

Recurrent selection encompasses such a variety of breeding procedures that the only common aspect is the cyclical process of selecting and recombining superior individuals. Population improvement *per se* or population improvement prior to extraction of inbred lines may be the goal. In either case, improvement via increased frequency of favorable alleles is sought while maintaining genetic variability in the population.

Recurrent selection methods may be divided into two types: phenotypic and genotypic (35). Phenotypic recurrent selection includes all recurrent selection methods in which the phenotype of the selection unit is the basis of selection. Control of microclimatic variation is essential to the success of a phenotypic recurrent selection procedure (16).

Although first proposed for use in cross-pollinated crops, recurrent selection has been extended to use in self-pollinators. The number of pollinations required during the recombination phase and the length of time between cycles were initially seen as obstacles to its use in self-pollinators. Compton (14) suggested a procedure incorporating single seed descent to maintain maximum variability while allowing a manageable

number of hybridizations. Wynne (43) suggested an adaptation of Compton's procedure which was used successfully to increase yield in peanut (*Arachis hypogaea* L.) (30). When available, male sterility may be manipulated to reduce the labor required to intermate selections (8,17).

Use of recurrent selection procedures in autogamous species appears to be successful although the conclusion may be biased by the tendency to publish positive rather than negative results (35). Successful recurrent selection programs have been reported for the following self-pollinated crops: barley (*Hordeum vulgare* L.) (13), common bean (*Phaseolus vulgaris* L.) (40), cotton (*Gossypium hirsutum* L.) (29), peanut (18,30), oats (*Avena sativa* L.) (24,34), soybean [*Glycine max* (L.) Merrill] (7,10,11,23,28,37,38,42), tobacco (*Nicotiana tabacum* L.) (19,26), and wheat (*Triticum aestivum* L.) (5,12,13,25,27).

This paper reports the results of phenotypic recurrent selection for enhanced nitrogen fixation in peanut. The objectives of this study were to a) measure response to three cycles of phenotypic recurrent selection when selection for increased fresh plant weight, nodule number and dry weight, and acetylene reduction was practiced; b) estimate realized heritability for each of these traits; and c) evaluate the correlated response of selected agronomic traits.

## Materials and Methods

The peanut cultivars Florigiant and CES 101 were chosen as parents because of their differing N<sub>2</sub>-fixing abilities (1). F<sub>1</sub> seed resulting from this single cross were planted 9 May 1980 at the Central Crops Research Station, Clayton, NC. Two hundred resulting F<sub>2</sub> seed formed the base population (C<sub>0</sub>) for this study. The F<sub>2</sub> individuals and the original parents were screened in the greenhouse at North Carolina State University, Raleigh, NC. Seed were germinated in sterile vermiculite. When radicles had emerged approximately 1-2 cm, seeds were transplanted to modified Leonard jars (45) and inoculated. At planting, the reservoir of each jar was filled with approximately 500 mL of a nutrient solution containing Bond's salt mixture and micronutrients (9).

Inoculum consisted of a mixture of four *Bradyrhizobium* strains: TAL1000, CB756, 3C4b20, and 176A22. Strains had been chosen for good performance on a variety of peanut cultivars. To prepare the inoculum, each strain was grown separately in yeast extract mannitol broth (33) to approximately stationary phase. Immediately prior to inoculation, equal volumes of each strain were mixed thoroughly. Each seed was then drenched with 10 mL of inoculum and covered with the planting medium. Plants were grown for approximately 60 days. Deionized water was added to the reservoir as needed.

At harvest, roots were detached at the hypocotyl and placed in glass jars for the measurement of acetylene reduction (22). After extraction of gas samples, nodules were removed from individual roots by hand, counted, dried, and weighed. Also at harvest, fresh plant weight (excluding roots) was measured. Vegetative cuttings were then taken from each plant and placed under intermittent mist in a sand bench to allow rooting.

Selection of individual genotypes from the base population was based on a principal components analysis using the correlation matrix for the following traits: fresh plant weight, nodule number and dry weight, and acetylene reduction. The first principal component assigned approximately equal weightings to each of the four variables and accounted for approximately 70% of the total variability in the

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base population. Single trait selection based on a simple ranking of a given trait would have been possible, but the single trait which would have been most effective in improving the overall  $N_2$ -fixing ability of the plant was unclear. The best combination of traits, based on phenotypic values, was indicated by the first principal component. Thus, the first principal component score was used as the selection criterion. The 200 individuals in the base population were partitioned into two sets (*e.g.*, 100 Leonard jars per set). Ten individuals per set were selected.

Cuttings from the selected individuals (parents of  $C_1$ ) were then used to generate 100 randomly paired matings. Each individual was used as a male five times and as a female five times. Five crosses failed to produce seed; thus, 95  $F_1$  constituted the next cycle for selection ( $C_1$ ). Evaluation of  $C_1$  proceeded as described above with one exception. Individuals in  $C_1$  were partitioned into five sets of 19 individuals. CES 101 was added to each set as a check. Fresh plant weight, nodule number and dry weight, and acetylene reduction observations were expressed as deviations from set means. These deviations were then used in the principal components analysis. Cuttings of the 20 individuals selected on the basis of the first principal component score (parents of  $C_2$ ) were randomly intermated with the restriction of no half-sib matings. One hundred progeny from these matings constituted the final cycle of selection,  $C_2$ . This cycle was evaluated in the greenhouse, and 20 individuals (parents of  $C_3$ ) were selected following the procedure outlined for  $C_1$ .

Realized heritability estimates for each trait were obtained by regressing cumulative response on cumulative selection differential (21). In each base population observations were expressed as deviations from the mean of CES 101, then realized heritability estimates and their standard errors were calculated.

Parents of  $C_1$ ,  $C_2$ , and  $C_3$  were evaluated in field and greenhouse studies to assess selection response. To insure adequate seed number and similar seed age and quality, selfed seed from each selection was increased in a winter nursery in Thailand. An equal number of seed from each parent was bulked within each cycle. This bulked seed—representing parents of  $C_1$ ,  $C_2$ , and  $C_3$ —was used to evaluate progress from selection.

Five entries (Florigiant, CES 101, and bulked parents of  $C_1$ ,  $C_2$ , and  $C_3$ ) were evaluated in the greenhouse using modified Leonard jars. Preparation of inoculum and the inoculation procedure were the same as that used during the selection process. Entries were evaluated in two separate tests. The first test was planted September 1984 and the second, May 1985. Each test was planted as a randomized complete block design with 12 replications. Each plot consisted of four Leonard jars. Each test was analyzed separately. Data were then combined over tests. In the combined analysis, test and replications were considered random effects, and entries were considered fixed effects.

Agronomic performance of the five entries was evaluated in 1985 at the Peanut Belt Research Station, Lewiston, NC and the Upper Coastal Plain Research Station, Rocky Mount, NC. A randomized complete block design with 10 replications was used at each location. Each plot consisted of two 28-seed rows with 25.4 cm within row and 91 cm between row spacings. Planting and harvesting dates were 12 May and 10 October, respectively, at Lewiston and 14 May and 25 September, respectively, at Rocky Mount. After plot yield was measured, fruit length (cm), fruit weight (g), seed weight (g), and seed count were obtained from a 20-pod sample drawn at random from each plot. These variables were used to calculate seed size (100 times the ratio of seed weight to seed count) and meat content (100 times the ratio of seed weight to fruit weight).

Data from the field studies were combined over locations. Entries were considered fixed effects. All other factors were considered random effects. A log transformation of yield was used to correct heterogeneity of error variances.

## Results and Discussion

The range of phenotypic values measured in each cycle and the selection differentials are presented in Table 1. Selection did not increase mean fresh plant weight, mean nodule number, mean nodule dry weight, or mean acetylene reduction. Significant variability ( $\alpha = 0.05$ ) among cycles was not detected for any of these

traits (Table 2). Lack of response was again indicated by the regression coefficients (Table 2). The regression coefficient for each trait was not significantly different from zero indicating selection had failed to increase the mean of any of the four traits.

Table 1. Range of means and selection differentials for three cycles of selection.

	Fresh plant weight (g)	Nodule number	Nodule weight (g)	Acetylene reduced ( $C_2H_4$ plant $^{-1}$ hr $^{-1}$ )
Selection differentials:				
$C_0$	12.4	80.6	.1194	9.09
$C_1$	36.9	92.5	.1054	9.53
$C_2$	16.2	84.6	.0447	5.31
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$C_0$ means:				
Unselected	4.2-79.3	20-378	.0434-.5415	0-34.4
Selected	41.0-74.6	188-378	.2944-.5415	11.5-34.4
$C_1$ means:				
Unselected	37.1-185.7	66-481	.0924-1.0678	3.6-55.4
Selected	87.7-185.7	198-481	.1899-1.0678	19.5-55.4
$C_2$ means:				
Unselected	0-134.2	0-481	0-.5342	0-49.5
Selected	92-134.2	264-481	.3025-.5342	14.1-49.5

Table 2. Means of three cycles of selection, midparent mean, and response to selection.

Group	Traits under selection <sup>a</sup>			
	Fresh plant weight (g)	Nodule number	Nodule weight (g)	Acetylene reduced ( $\mu$ moles $C_2H_4$ plant $^{-1}$ hr $^{-1}$ )
Midparent	94.4	220.6	0.310	17.5
Parents of:				
$C_1$	83.6	219.6	0.287	16.3
$C_2$	91.2	225.1	0.298	16.3
$C_3$	85.7	219.4	0.284	15.6
Variability among cycles	NS	NS	NS	NS
b	1.1	-0.1	-0.001	-0.3
SE (b)	1.9	5.3	0.006	0.4

<sup>a</sup>Means were based on two greenhouse tests each with 12 replications and four plants/plot.

Varying levels of relationship between characters indicative of nitrogen fixation and yield have been reported for peanuts (2,31). Response to selection for increased nitrogen fixation might be expected to alter yield and related characters in populations where a positive correlation exists. In this population no variability among cycles ( $\alpha = 0.05$ ) was detected for mean fruit length or weight, mean seed weight, count or size, mean meat content, or mean yield (Table 3). A correlated response to selection was not expected in light of lack of response to direct selection.

In two separate studies, nonadditive effects have been associated with  $N_2$  fixation. Isleib *et al.* (22) reported significant specific combining abilities (SCA) which accounted for more variability than general combining abilities for nodule number and dry weight and plant dry weight. Maternal effects were also important. Phillips *et al.* (36) reported significant SCA effects for plant dry weight and significant GCA effects for nodule number and weight, plant dry weight, and nitrogenase

**Table 3. Means of three cycles of selection, midparent mean, and correlated response to selection.**

Group	Agronomic traits <sup>a</sup>						
	Fruit length (cm)	Fruit wt (g)	Seed wt (g)	Seed count (#/20 pods)	Seed size (g/100 seed)	Meat content (%)	Yield (kg/ha) <sup>b</sup>
Midparent	62.5	37.1	28.0	39.3	71.1	75.9	2952.7
Parents of:							
C <sub>1</sub>	60.9	34.1	25.7	38.3	67.2	76.0	2077.0
C <sub>2</sub>	60.1	34.4	26.0	38.5	67.7	75.9	2212.6
C <sub>3</sub>	58.9	31.9	24.6	39.5	62.7	77.3	2363.5
Variability among cycles	NS	NS	NS	NS	NS	NS	NS

<sup>a</sup>Means of the agronomic traits are based on 20 observations (two locations, 10 replicates/location).

<sup>b</sup>The log transformation of yield was used in the analysis and tests of significance. The untransformed means are presented here for simplicity.

activity. With the presence of SCA or maternal effects, little correlation between early and late generation performance would be expected. Two generations of selfing occurred between selection of parents from the base populations and evaluation of parental bulks in the study reported here. Dissipation of nonadditive effects could have occurred during these two generations of inbreeding. To assess whether apparent lack of progress as measured in the parental bulks could be attributed to dissipation of nonadditive effects, realized heritability estimates were obtained from the base populations, C<sub>0</sub>, C<sub>1</sub>, and C<sub>2</sub>. Realized heritability estimates for nodule number and dry weight and fresh plant weight were small (Table 4). The estimate for acetylene reduction was moderate but no larger than its associated standard error. The lack of response measured in the parental bulks cannot be associated with the inbreeding process. Neither parental bulks nor the base populations indicated a gain. It is doubtful, therefore, that nonadditive effects alone were responsible for the lack of response.

**Table 4. Estimates of realized heritability.**

	b <sub>c</sub>	SE(b <sub>c</sub> )
Fresh plant weight (g)	0.01	0.02
Nodule number	0.05	0.03
Nodule weight (g)	-0.06	0.06
Acetylene reduction ( $\mu\text{moles C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$ )	0.31	0.32

Improvement via recurrent selection is dependent on increasing the frequency of favorable alleles in the population. To do this, adequate genetic variability must exist and genetically superior individuals must be identified. A population of late generation lines generated from the cross of Florigiant and CES 101 responded to selection for high and low acetylene reduction (1). Significant correlated responses for nodule number and dry plant weight were also reported. Lack of selection response in this early generation population generated from the same single cross could have been due, therefore, to a large environmental variance rather than a lack of genetic variance. Selection in later gener-

ation allows for replication of lines within an environment and testing in more than one environment. Heritability is, therefore, increased due to a more accurate measure of the individual's genetic capacity. Breeding methods utilizing segregating populations of lines have been suggested as appropriate for enhancement of N<sub>2</sub> fixation of self-pollinators (4,44).

Multi-trait selection is expected to be less effective than single trait selection. Selection on the first principal component score is expected to generate slow gains relative to selection on a given trait. Little initial response may be associated with multi-trait selection, although some response would be expected after three cycles if selection were effective. No response and no indication of a trend toward a response was measured. It seems unlikely, therefore, that selection on the principal component score was the reason for lack of response.

Host variability for fixation has been well demonstrated in peanuts (1,15,22,32,36). Significant SCA effects (22,36) and maternal effects (22) have been reported. When lines were replicated and tested in more than one environment, heritabilities tended to be moderate to high (1,2). Selection for increased N<sub>2</sub> fixation has been successful in peanut (3) and bean (4); however, in each of these cases population structure allowed replication of the selection unit. Phenotypic recurrent selection for N<sub>2</sub> fixation has been successful in alfalfa when plants were selected and evaluated in the greenhouse (39,41) but ineffective when selections were evaluated in the field (6,20).

Selection for increased plant weight, nodule number and dry weight, and acetylene reduction was not successful in this population. Failure, if due to insufficient control of environmental variation, supports the recommendation of other researchers (4,44). Population structure should allow for replication of the selection unit when the breeding objective is increased N<sub>2</sub> fixation.

## Literature Cited

- Arrendell, S. 1987. Breeding for increased nitrogen fixation in peanut (*Arachis hypogaea* L.). PhD diss., North Carolina State Univ., Raleigh.
- Arrendell, S., J. C. Wynne, G. H. Elkan and T. G. Isleib. 1985. Variation for nitrogen fixation among progenies of a virginia x spanish cross. *Crop Sci.* 25:865-869.
- Arrendell, S., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. 1986. Bidirectional selection for nitrogenase activity and shoot dry weight among late generation progenies of a virginia x spanish peanut cross. *Peanut Sci.* 13:86-89.
- Attewell, J. and F. A. Bliss. 1985. Host plant characteristics of common bean lines selected using indirect measures of N<sub>2</sub> fixation, p. 3-9. in H. J. Evans, P. J. Bottomley and W. E. Newton (eds.), *Nitrogen Fixation Research Progress*. Martinus Nighoff Publ., Boston.
- Avey, D. P., H. W. Ohm, F. L. Patterson and W. E. Nyquist. 1982. Three cycles of simple recurrent selection for early heading in winter wheat. *Crop Sci.* 22:908-911.
- Barnes, D. K., G. H. Heichel, C. P. Vance, D. R. Viands and G. Hardarson. 1981. Success and problems encountered while breeding for enhanced N<sub>2</sub> fixation in alfalfa, p. 233-248. in J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains and R. C. Huffaker (eds.), *Genetic Engineering of Symbiotic Nitrogen Fixation and Conservation of Fixed Nitrogen*. Plenum Press, New York.

7. Brim, C. A. and J. W. Burton. 1979. Recurrent selection in soybeans. II. Selection for increased percent protein in seeds. *Crop Sci.* 19:494-498.
8. Brim, C. A. and C. W. Stuber. 1973. Applications of genetic male sterility to recurrent selection schemes in soybeans. *Crop Sci.* 13:528-530.
9. Burton, J. C., C. J. Martinez and R. L. Curley. 1972. Methods of testing and suggested standards for legume inoculants and preinoculated seed. Nitragin Sales Corp., Milwaukee, WI. pp. 9-10.
10. Burton, J. W. and C. A. Brim. 1981. Recurrent selection in soybeans. III. Selection for increased percent oil in seeds. *Crop Sci.* 21:31-34.
11. Burton, J. W., R. F. Wilson and C. A. Brim. 1983. Recurrent selection in soybeans. IV. Selection for increased oleic acid percentage in seed oil. *Crop Sci.* 23:744-747.
12. Busch, R. H. and K. Kofoed. 1982. Recurrent selection for kernel weight in spring wheat. *Crop Sci.* 22:568-572.
13. Byrne, I. and D. C. Rasmusson. 1974. Recurrent selection for mineral content in wheat and barley. *Euphytica* 23:241-249.
14. Compton, W. A. 1968. Recurrent selection in self-pollinated crops without extensive crossing. *Crop Sci.* 8:773.
15. Elkan, G. H., J. C. Wynne, T. J. Schneeweis and T. G. Isleib. 1980. Nodulation and nitrogenase activity of peanuts inoculated with single strain isolates of *Rhizobium*. *Peanut Sci.* 7:95-97.
16. Gardner, C. O. 1961. An evaluation of the effects of mass selection and seed irradiation with thermal neutrons on yield of corn. *Crop Sci.* 1:241-245.
17. Gilmore, E. C., Jr. 1964. Suggested method of using reciprocal recurrent selection scheme in some naturally self-pollinated species. *Crop Sci.* 4:323-325.
18. Guok, H. P., J. C. Wynne and H. T. Stalker. 1986. Recurrent selection within a population from an interspecific peanut cross. *Crop Sci.* 26:249-253.
19. Gupton, C. L. 1981. Phenotypic recurrent selection for increased leaf weight and decreased alkaloid content of burley tobacco. *Crop Sci.* 21:921-925.
20. Heichel, G. H., C. P. Vance and D. K. Barnes. 1981. Evaluating elite alfalfa lines for  $N_2$ -fixation under field conditions, pp. 217-232. in J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains and R. C. Huffaker (eds.), *Genetic Engineering of Symbiotic Nitrogen Fixation and Conservation of Fixed Nitrogen*. Plenum Press, New York.
21. Hill, W. G. 1972. Estimation of realized heritabilities from selection experiments. II. Selection in one direction. *Biometrics* 28:767-780.
22. 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.
23. Kenworthy, W. J. and C. A. Brim. 1979. Recurrent selection in soybeans. I. Seed yield. *Crop Sci.* 19:315-318.
24. Khader, F. H. and K. J. Frey. 1965. Effectiveness of recurrent selection in oat breeding. *Crop Sci.* 5:349-354.
25. Loffler, C. M., R. H. Busch and J. V. Wiersma. 1984. Recurrent selection for grain protein percentage in hard red spring wheat. *Crop Sci.* 23:1097-1101.
26. Matzinger, D. F. and E. A. Wernsman. 1968. Four cycles of mass selection in a synthetic variety of an autogamous species (*Nicotiana tabacum* L.). *Crop Sci.* 8:239-243.
27. McNeal, F. H., C. F. McGuire and M. A. Berg. 1978. Recurrent selection for grain protein content in spring wheat. *Crop Sci.* 18:779-782.
28. Miller, J. E. and W. R. Fehr. 1979. Direct and indirect recurrent selection for protein in soybeans. *Crop Sci.* 19:101-106.
29. Miller, P. A. and J. O. Rawlings. 1967. Selection for increased lint yield and correlated responses in upland cotton (*Gossypium hirsutum* L.). *Crop Sci.* 7:637-640.
30. Monteverde-Perse, E. J. 1986. Recurrent selection for fruit yield in peanut. PhD diss., North Carolina State Univ., Raleigh.
31. Nambiar, P. T. C. 1985. Response of groundnut (*Arachis hypogaea* L.) to *Rhizobium* inoculation in the field: Problems and prospects. *MIRCEN J. Appl. Microbiol. and Biotech.* 1:293-309.
32. Nigam, S. N., S. L. Dwivedi, P. T. C. Nambiar and R. W. Gibbons. 1985. Combining ability analysis of  $N_2$ -fixation and related traits in peanut. *Peanut Sci.* 12:55-57.
33. Norris, D. V. and R. A. Date. 1976. Legume bacteriology, pp. 134-174. in N. H. Shaw and W. W. Bryan (eds.), *Tropical Pasture Research—Principles and Methods*. Commonwealth Bur. Past. Field Crops Bull. No. 51.
34. Payne, T. S., D. D. Stuthman, R. L. McGraw and P. P. Bregitzer. 1986. Physiological changes associated with three cycles of recurrent selection for grain yield improvement in oats. *Crop Sci.* 26:734-736.
35. Penny, L. H., W. A. Russell, G. F. Sprague and A. R. Hallauer. 1963. Recurrent selection. N.A.S., N.R.C. Publ. 982:352-367.
36. Phillips, T. D., J. C. Wynne, T. J. Schneeweis and G. H. Elkan. 1986. Effect of *Bradyrhizobium* strain on combining ability of the host plant. *Proc. Amer. Peanut Res. Educ. Assoc., Inc.* 18:30. (Abstract).
37. Piper, T. E. and W. R. Fehr. 1987. Yield improvement in a soybean population by utilizing alternative strategies of recurrent selection. *Crop Sci.* 27:172-178.
38. Prohaska, K. R. and W. R. Fehr. 1981. Recurrent selection for resistance to iron deficiency chlorosis in soybeans. *Crop Sci.* 21:524-526.
39. Seetin, M. W. and D. K. Barnes. 1977. Variation among alfalfa genotypes for rate of acetylene reduction. *Crop Sci.* 17:783-787.
40. Sullivan, J. G. and F. A. Bliss. 1983. Recurrent mass selection for increased seed yield and seed protein percentage in the common bean (*Phaseolus vulgaris* L.) using a selection index. *J. Amer. Soc. Hort. Sci.* 108:42-46.
41. Viands, D. R., D. K. Barnes and G. H. Heichel. 1981. Nitrogen fixation in alfalfa. Responses to bidirectional selection for associated characteristics. *USDA Tech. Bull.* 1643. 24 pp.
42. Walker, A. K. and A. F. Schmitthenner. 1984. Recurrent selection for tolerance to phytophthora rot in soybeans. *Crop Sci.* 24:495-497.
43. Wynne, J. C. 1976. Use of accelerated generation increase programs in peanut breeding. An accelerated recurrent selection program. *Proc. Amer. Peanut Res. Educ. Soc.* 8:44-47.
44. Wynne, J. C., F. A. Bliss and J. C. Rosas. 1987. Principles and practice of field designs to evaluate symbiotic nitrogen fixation, pp. 371-389. in G. H. Elkan (ed.), *Symbiotic Nitrogen Fixation Technology*. Marcel Dekker, Inc., New York.
45. Wynne, J. C., G. H. Elkan, C. M. Meisner, T. J. Schneeweis and J. M. Ligon. 1980. Greenhouse evaluations of strains of *Rhizobium* for peanuts. *Agron. J.* 72:645-649.

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