Inheritance of Symbiotic Nitrogen Fixation in Two Peanut Crosses[1]

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ABSTRACT

Symbiotic nitrogen fixation in peanut (*Arachis hypogaea* L.) may be improved by genetically manipulating the host plant. This requires an understanding of the inheritance of the traits involved in nitrogen fixation. The objectives of this study were to determine the inheritance of several N_2 fixation-related traits for two peanut crosses based on Mather and Jink's fixation-related traits for two peanut crosses based on Mather and Jink's additive-dominance model, and to determine if epistasis was important in the inheritance of these traits. A generation means analysis using parents, reciprocal F_1s and F_2s, and two back-cross generations was conducted for both crosses. Plants of different generations were grown in modified Leonard jars in the greenhouse for about 60 days at which time nodule number and dry weight, shoot dry weight, nitrogenase activity, and specific activity were measured. Means of the traits for the generations from both crosses (Robut 33-1 x NC 4 and Robut 33-1 x Argentine) showed significant differences. Reciprocal differences were found for most traits measured in the cross of Robut 33-1 x Argentine, a cross of virginia x spanish botanical types. Lack of fit of the additive-dominance model indicated significant epistasis for inheritance of nodule number, nodule weight, top dry weight, and nitrogenase activity in both crosses. Three types of digenic interactions (additive x additive, additive x dominance and dominance x dominance) were found. The presence of nonadditive genetic effects suggests that early generation selection would be ineffective.

Key Words: Groundnut, *A. hypogaea* L., *Bradyrhizobium*.

The peanut (Arachis hypogaea L.) shares with other leguminous plants the ability to fix atmospheric nitrogen through a symbiotic relationship with certain soil-borne bacteria (1). In peanut, bacteria of the genus Bradyrhizobium enter the roots where nodules form and nitrogen fixation occurs. There is evidence in peanuts that nitrogen fixation may reduce or eliminate the response to subsequent nitrogen fertilization by meeting a large portion of the plant's total nitrogen requirement (17).

Three important factors influencing symbiotic nitrogen fixation are the host plant, bacterial strain, and environment. Of these factors, the host genotype may be the easiest to alter (5,16). Burton (3) found variability in nitrogen accumulation among greenhouse-grown peanut cultivars with symbiotic nitrogen fixation as the only nitrogen source. Other authors have reported variation for traits related to N_2 fixation among cultivars, both in greenhouse and field studies (2,7,12,19,20).

Improving the nitrogen-fixing ability of peanut through breeding requires (a) sufficient genetic variation, (b) an understanding of genetic control of the traits, (c) techniques for measuring the traits, and (d) a breeding strategy to effectively use the variation (21). If the ability to form a more efficient symbiotic relationship for nitrogen fixation is controlled by genes with additive or additive types of epistatic effects, then conventional plant breeding methods for peanut could be used to increase biological nitrogen fixation.

This study was conducted to gain a clearer understanding of the genetics of nitrogen fixation in peanut. The specific objectives were to (a) determine the inheritance of several N_2 fixation related traits for two peanut crosses using Bradyrhizobium strain NC92 and (b) determine if digenic epistatic interactions were important in the inheritance of these traits.

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Materials and Methods

The inheritance of nitrogen fixation-related traits was examined in the cross of Robut 33-1 x NC 4 (Cross 1) and Robut 33-1 x Argentine (Cross 2). These parents exhibit a wide range of expression of nitrogen-fixing traits (Table 1) and also represent diversity in origin and botanical type. NC 4 is a large-seeded virginia botanical type, Robut 33-1 is a small-seeded virginia botanical type, and Argentine is a small-seeded spanish botanical type. In addition, a specificity between peanut host Robut 33-1 and B*radyrhizobium* strain NC92 has been reported to result in significant yield increases (11). Crosses and self-pollinations were made in the greenhouse from 1983-1985 to generate reciprocal F_1s, reciprocal F_2s and backcrosses of the reciprocal F_1s. (For Cross 1: P_1 = Robut 33-1, P_2 = NC 4, F_1 = Robut 33-1 x NC 4, F_1' = NC 4 x Robut 33-1, F_2 = F_1 selfed, F_2' = F_1' selfed, BCa = F_1 x Robut 33-1 and BCb = F_1 x NC 4. For Cross 2: P_1 = Robut 33-1, P_2 = Argentine, F1 = Robut 33-1 x Argentine, F_1' = Argentine x Robut 33-1, F_2 = F_1 selfed, F_2' = F_1' selfed, BCa = F_1 x Robut 33-1 and BCb = F_1 x Argentine.)

Eight populations, including the parents of each cross, were grown in the greenhouse in the fall of 1985 using a randomized complete block design with three replications. Each replication consisted of five plants each of the P_1, P_2, F_1, F_1' generations, and 10 plants each of the F_2, F_2', BCa, and BCb generations.

Bradyrhizobium strain NC92 was used to inoculate each plot which consisted of a single plant in a modified Leonard jar assembly (20) with a medium of sand and vermiculite (1:1, v/v). Each reservoir was filled with 700 mL of Bond's stock salt (4) and 0.1 ppm Zn, 0.04 ppm Mo and 0.001 ppm Co. Each plot was inoculated with 10 mL of *Bradyrhizobium* in YEM liquid broth (15) at a cell density of 10^9 cfu/mL. Water was applied to the plants as needed during growth. Eight weeks (Cross 1) and 9 weeks (Cross 2) after planting, plots were sampled and the following traits measured: nodule number, nodule weight, plant top dry weight, and nitrogenase activity. Nitrogen fixation ability was measured by the acetylene reduction assay as described by Hardy et al. (9) and Isleib et al. (12). Specific nitrogenase activity was calculated by dividing nitrogenase activity by nodule weight.

Trait means for each generation (P_1, P_2, F_1, F_1', F_2, F_2', BCa, and BCb) were used to estimate the model parameters. For each cross, trait means for the eight generations expressed on a per-plant basis were analyzed using a general linear model procedure (10). A Waller-Duncan K-ratio T-test was used for each trait to determine whether significant differences existed between the means of the various generations. Contrasts were made for the two reciprocal crosses to determine if reciprocal effects were significant. Where reciprocals were different, they were deleted from the analysis in order to have the same cytoplasm in all generations being considered. Otherwise, reciprocal F_1s and F_2s were pooled providing six generation means for analysis. A separate analysis of variance was made using the six populations to determine whether there were differences among them for the traits measured.

The generation means analysis of Mather and Jinks (14) was used for each trait measured. Gamble's (8) notation was used in defining the parameters of the models, where

m = midparent value,
a = pooled additive effects,
d = pooled dominance effects,
aa = pooled additive x additive effects,
ad = pooled additive x dominance effects, and
dd = pooled dominance x dominance effects.

First, a three-parameter model using m, a, and d was tested. Natural log and square root transformations were made to test whether either transformation would allow the three-parameter model to fit the data. The three parameters (m, a, and d) were estimated by a weighted least square method described by Rowe and Alexander (18). The six means were weighted by the reciprocal of the corresponding variance. Cavalli's (6) joint scaling test was used to test goodness of fit of the additive-dominance model. Also, individual scaling tests as described by Mather (13) were calculated for comparison with the joint scaling test results. The three individual scaling tests are:

$$A = 2\,\overline{BC}a - \overline{P}_1 - \overline{F}_1$$
$$B = 2\,\overline{BC}b - \overline{P}_2 - \overline{F}_1$$
$$C = 4\,\overline{F}_2 - 2\,\overline{F}_1 - \overline{P}_1 - \overline{P}_2$$

where A, B, and C should be statistically not different from zero if the additive-dominance model adequately describes the means of the generations.

Epistasis was ignored in the three-parameter model. Where the additive-dominance model was inadequate (did not fit the data), a six-parameter model, including the three possible digenic interactions, was tested.

Expectations of the generation means are as follows:

$$\overline{P}_1 = m + a + aa$$
$$\overline{P}_2 = m - a + aa$$
$$\overline{F}_1 = m + d + dd$$
$$\overline{F}_2 = m + 1/2d + 1/4dd$$
$$\overline{BC}a = m + 1/2a + 1/2d + 1/4aa + 1/4ad + 1/4dd$$
$$\overline{BC}b = m - 1/2a + 1/2d + 1/4aa - 1/4ad + 1/4dd.$$

As pointed out by Mather and Jinks (14), six parameters are used in these expressions and only six means are available to estimate them. This allows perfect fit estimates of the six parameters to be determined.

$$m = 1/2\overline{P}_1 + 1/2\overline{P}_2 + 4\overline{F}_2 - 2\overline{BC}a$$
$$a = 1/2\overline{P}_1 - 1/2\overline{P}_2$$
$$d = 6\overline{BC}a + 6\overline{BC}b - 8\overline{F}_2 - \overline{F}_1 - 1\ 1/2\overline{P}_1 - 1\ 1/2\overline{P}_2$$
$$aa = 2\overline{BC}a + 2\overline{BC}b - 4\overline{F}_2$$
$$ad = 2\overline{BC}a - \overline{P}_1 - 2\overline{BC}b + \overline{P}_2$$
$$dd = \overline{P}_1 + \overline{P}_2 + 2\overline{F}_1 + 4\overline{F}_2 - 4\overline{BC}a - 4\overline{BC}b$$

Next, a five-parameter model was used for each cross to provide one degree of freedom so goodness of fit of the model could be tested. An added benefit of using a five-parameter model is that the remaining parameters are estimated with improved precision. In Cross 1, the [dd] component was deleted, while for Cross 2 the [ad] component was omitted because their values in the respective crosses were not different from zero for most traits.

Results and Discussion

Differences among generation means in Cross 1 were statistically significant for all traits except specific nitrogenase activity. In Cross 2, significant differences existed among generations for nodule number, nodule weight, and top dry weight. No generation mean differences were seen for nitrogenase and specific nitrogenase activity in populations from this cross.

Contrasts between reciprocal crosses showed no significant differences for either reciprocal F_1s or F_2s for any traits for Cross 1. However, for Cross 2, significant reciprocal differences were found between the reciprocal F_1s for nodule number, nodule weight, and top dry weight. Reciprocal F_2s, however, were not significantly different from each other. For the generation means analysis, means for reciprocal populations that were not different were pooled. Means from the cross with constant cytoplasm in all generations were used for traits with significantly different reciprocal cross means.

Cross 1

In Cross 1, the mean for nodule number and nodule weight of parent NC 4 was higher than that of Robut 33-1 (Table 1). The means of the F_1 hybrids were greater than the midparent for both of these traits, and the F_1 mean for nodule weight exceeded the mean of the better parent. The F_2 and BCa generation means for nodule number and nodule weight were lower than the means of the parents and F_1 generation. The BCb generation mean was higher than the means of the two parental populations. These results suggest that significant epistatic interactions are involved in the inheritance of these two traits.

The cultivar NC 4 produced more top dry matter than Robut 33-1 (Table 1). The F_1 mean for top dry weight was about equal to that of the high parent. The F_2 and BCa means for this trait were significantly lower than both parents. Again, the BCb population mean was higher than any other population, indicating an epistatic interaction governing trait expression. Means of the parents for nitrogenase activity

Table 1. Generation means and variances for nitrogen fixation-related traits in *A. hypogaea* for the crosses Robut 33-1 x NC 4 (Cross 1) and Robut 33-1 x Argentine (Cross 2).

Generation	N[a]	Nodule number		Nodule weight g/plant		Top dry weight g/plant		Nitrogenase activity μM C_2H_4/pl./hr		Specific activity μM C_2H_4/g nod. dry wt/hr	
		$\bar{X}$	$V_{\bar{X}}$	$\bar{X}$	$V_{\bar{X}}$	$\bar{X}$	$V_{\bar{X}}$	$\bar{X}$	$V_{\bar{X}}$	$\bar{X}$	$V_{\bar{X}}$
					Cross 1						
P_1 (Robut 33-1)	15	157.4	127.5	0.2371	0.0003	7.4	0.2	19.78	2.62	84.09	15.72
P_2 (NC 4)	15	177.7	72.7	0.2780	0.0002	8.0	0.2	22.34	1.32	82.46	21.17
F_1	30	170.5	80.3	0.2968	0.0002	8.1	0.1	22.99	0.92	79.88	8.60
F_2	60	145.7	63.1	0.2180	0.0001	5.7	0.1	17.64	0.44	82.83	6.15
BCa	30	130.5	105.1	0.2063	0.0001	5.7	0.1	18.33	0.55	91.87	11.60
BCb	30	195.0	167.1	0.2931	0.0002	8.5	0.1	22.59	1.14	78.92	10.70
					Cross 2						
P_1 (Robut 33-1)	15	166.1	104.4	0.3112	0.0001	10.4	0.2	16.07	2.06	52.04	20.49
P_2 (Argentine)	15	182.2	34.4	0.2605	0.0001	11.4	0.4	15.95	1.58	61.64	24.75
F_1	15	200.8	81.8	0.3321	0.0002	14.8	0.2	16.40	1.08	49.77	7.07
F_2	30	140.2	44.0	0.2670	0.0001	9.7	0.2	17.26	0.69	67.72	13.00
BCa	30	175.9	115.2	0.3175	0.0001	11.8	0.2	17.61	0.49	57.56	9.73
BCb	30	193.1	145.6	0.3013	0.0001	13.5	0.2	16.89	0.93	57.28	12.03

[a]N = number of plants of the different generations used in obtaining trait means.

showed that NC 4 reduced more acetylene than Robut 33-1. The F_1 mean for nitrogenase activity exceeded the high parent, while the F_2 population mean was significantly less than the mean of the low parent. The BCa population mean for nitrogenase activity also was low, while the BCb mean exceeded the midparent value. Here again epistasis was indicated. No significant differences for specific nitrogenase activity between generations were found in this study. Arrendell et al. (2) also found little variation for this trait.

Generation Means Analysis

Mather and Jinks' (14) three-parameter model assumes that the genes involved are independent of each other (or epistasis is negligible), i.e., exhibit simple autosomal inheritance. The three-parameter model provides a formulation of the mean phenotypes of the six generations in terms of the midparent, m, which depends on the general conditions of the observations, the additive component [a] and the dominance component [d], where:

[a] = the sum over loci of all a's which measure the departure of each homozygote from the midparent, m, and

[d] = the sum over loci of all d's which measure the departure of the heterozygote from the midparent, m (13).

Results of the joint scaling tests and the individual scaling tests, as well as parameter estimates for Cross 1, based on the additive-dominance model for the five dinitrogen fixation traits indicated that the additive dominance model was not adequate for any trait which had significant generation mean differences (Table 2). For the four traits, two or more individual scaling tests were different from zero. confirming the joint scaling test results in indicating important epistasis in the inheritance of nodule number, nodule weight, top dry weight, and nitrogenase activity in Cross 1. Data transformations were made to adjust the scale to determine if this would help fit the data to the model. Both natural log and square root transformations failed to improve the fit of the additive-dominance model.

Based on the joint scaling tests, significant epistatic interactions were found to affect nodule number, nodule weight, top dry weight, and nitrogenase activity on the populations derived from the cross of Robut 33-1 x NC 4 (Table 2).

Using a six-parameter model and the six means to estimate these parameters resulted in a perfect fit estimation. However, as Mather and Jinks (14) pointed out, the three degrees of freedom used in the additive-dominance model

Table 2. Genetic parameter estimates for two populations[a] using the joint-scaling test and individual scaling tests based on a three-parameter model.

Genetic parameter		Nodule number	Nodule weight g/plant	Top dry weight g/plant	Nitrogenase activity μM C_2H_4/pl./hr
			Cross 1		
m		159.0 ±6.3	0.2266 ±0.0099	6.85 ±0.27	18.725 ±0.772
a		-20.2 ±6.4	-0.0446 ±0.0098	-0.90 ±0.27	-2.719 ±0.757
d		1.4 ±11.2	0.0360 ±0.0179	-0.08 ±0.49	2.312 ±1.365
$\chi^2_{(3)}$		14.37*	31.65*	64.27*	23.26*
Individual scaling tests:	A	-66.9*±25.1	-0.1213*±0.0309	-4.04*±0.86	-6.115*±2.400
	B	41.7 ±28.7	0.0115 ±0.0348	0.91 ±0.98	-0.142 ±2.606
	C	-93.3*±39.1	-0.2367*±0.0493	-8.95*±1.35	-17.533*±3.829
			Cross 2		
m		164.8 ±5.3	0.2823 ±0.0069	10.43 ±0.32	--
a		-12.9 ±5.4	0.0232 ±0.0067	-0.67 ±0.32	--
d		14.3 ±10.3	0.0352 ±0.0150	3.57 ±0.57	--
$\chi^2_{(3)}$		33.68*	11.47*	45.16*	
Individual scaling tests:	A	-15.04 ±25.4	-0.0082 ±0.0292	-1.46*±1.03	--
	B	3.20 ±26.4	0.0099 ±0.0294	0.76 ±1.12	--
	C	-189.26*±48.1	-0.1681*±0.0553	-12.41*±2.04	--

[a]Cross 1 = Robut 33-1 x NC 4 and Cross 2 = Robut 33-1 x Argentine.
*Significant at 0.05 level.

to test the goodness of fit (X^2 statistic) of the model are now being used to estimate the three interaction parameters. No goodness of fit test is possible, but the interaction model allows identification of the type or types of interaction responsible for departure from the additive-dominance model predictions.

Results of the six-parameter model for Cross 1 indicate significant epistatic effects for all traits (Table 3). In addition, dominance effects were significant and positive for top dry weight. The [ad] component was significant but negative for nodule number, nodule weight, and top dry weight. For nodule weight, top dry weight, and nitrogenase activity the [aa] interaction component was significant and positive.

Table 3. Genetic parameter estimates for two populations[a] for N_2 fixation traits based on the six-parameter model.

Genetic parameter	Nodule number	Nodule weight g/plant	Top dry weight g/plant	Nitrogenase activity μM C_2H_4/pl./hr
		Cross 1		
m	99.4* ±46.4	0.1307**±0.0502	1.89 ±1.41	9.784* ±3.846
a	-10.1 ±7.1	-0.0205 ±0.0121	-0.31 ±0.32	-1.276 ±0.992
d	114.1 ±119.9	0.1830 ±0.1309	8.86* ±3.69	18.230 ±9.943
aa	68.1 ±45.8	0.1268* ±0.0487	5.82**±1.37	11.276**±3.716
ad	-108.6**±35.9	-0.1328**±0.0426	-4.95**±1.20	-5.973 ±3.272
dd	-43.0 ±76.7	-0.0169 ±0.0858	-2.68 ±2.43	-5.020 ±6.461
		Cross 2		
m	-3.7 ±42.2	0.1161* ±0.0550	-0.82 ±2.10	--
a	-8.1 ±5.9	0.0253**±0.0074	-0.50 ±0.70	--
d	369.7**±112.2	0.3875**±0.1325	26.59**±5.03	--
aa	177.4**±41.8	0.1698**±0.0545	11.71**±2.06	--
ad	-18.2 ±34.4	-0.0181 ±0.0348	-2.22 ±1.40	--
dd	-165.6* ±70.1	-0.1714* ±0.0846	-11.00**±3.11	--

[a]Cross 1 = Robut 33-1 x NC 4 and Cross 2 = Robut 33-1 x Argentine.
*,**Significant at 0.05 and 0.01 levels, respectively.

Mather and Jinks suggested fitting a five-parameter model by omitting one of the interaction terms to allow testing the goodness of fit of the five-parameter model by the means of a X^2 with one degree of freedom. For Cross 1, the [dd] component was not significant, so it was deleted. The X^2 values indicated that the revised model gives a very good fit for all traits (Table 4). With this model dominance effects were significant and positive for the four traits. For nodule number, nodule weight, and top dry weight, both [aa] and [ad] digenic interaction components were significant. The [aa] component was positive, while the [ad] was negative for these traits. For nitrogenase activity, [aa] was the important epistatic component.

Cross 2

In the populations derived from crossing Robut 33-1 and Argentine (Cross 2), trait means also indicated epistasis (Table 1). For nodule number, Argentine had a higher mean, but for nodule weight Robut 33-1 was higher. The F_1 and two BC populations' means were greater than the midparent for both nodule number and weight. For nodule number, the F_2 population had the lowest mean while for nodule weight the F_2 population mean was between the two parents.

Table 4. Genetic parameter estimates for two peanut populations[a] for N_2 fixation traits based on five-parameter models.

Genetic parameter	Nodule number	Nodule weight g/plant	Top dry weight g/plant	Nitrogenase activity $\mu M\ C_2H_4$/pl./hr
		Cross 1		
m	123.4**±17.7	0.1397**±0.0213	3.3**±0.6	12.495**±1.617
a	-9.9 ±7.1	-0.0204 ±0.0120	-0.3 ±0.3	-1.198 ±0.987
d	48.3* ±23.8	0.1579**±0.0315	4.9**±0.9	10.719**±2.334
aa	44.9* ±19.3	0.1185**±0.0246	4.5**±0.7	8.802**±1.914
ad	-105.4**±35.4	-0.1314**±0.0420	-4.8**±1.4	-5.568 ±3.230
$\chi^2_{(1)}$	0.314	0.039	1.225	0.604
		Cross 2		
m	-1.9 ±42.1	0.1168**±0.055	-0.74 ±2.10	--
a	-9.2 ±5.5	0.0237**±0.007	-0.84* ±0.32	--
d	365.7**±112.0	0.3855**±0.132	26.35**±5.03	--
aa	175.5**±41.6	0.1687**±0.054	11.76**±2.06	--
dd	-162.9* ±72.9	-0.1701**±0.085	-10.84**±3.11	--
$\chi^2_{(1)}$	0.282	0.270	2.513	

[a]Cross 1 = Robut 33-1 x NC 4 and Cross 2 = Robut 33-1 x Argentine.

*,**Significant at 0.05 and 0.01 levels, respectively.

For top dry weight, Argentine had a higher mean than Robut 33-1. The F_1 mean exceeded the mean of the high parent. However, the F_2 population mean for top dry weight was lower than either parent. Both BC population means exceeded the mean of the high parent but were lower than the F_1 mean. For nitrogenase and specific nitrogenase activity, no differences among generations were significant.

Generation Means Analysis.

The results of Cavalli's (6) joint scaling test and the individual scaling tests indicated that the additive dominance model did not fit the data from Cross 2 for nodule number, nodule weight, or top dry weight (Table 2). Again, natural log and square root transformations did not improve the fit of the three-parameter model for Cross 2 trait means.

The six-parameter model showed that two types of epistatic interactions, [aa] and [dd], were significant for nodule number, nodule weight, and top dry weight (Table 3). The sum dominance effect, d, was significant and positive for all three traits. For nodule weight, the additive component was significant in addition to the two interactions and the dominance component. Since the [ad] interaction was not significant for any trait, this term was deleted for a five-parameter model.

The joint scaling test results for the five-parameter model for Cross 2 indicate the model fits the data very well (Table 4). For all three traits, d, [aa] and [dd] components were significant. For nodule weight and top dry weight, additive effects were significant. Dominance and [dd] components reflect the net direction of gene action and can be used to classify the major type of epistasis. For all three traits, dominance effects were positive and [dd] was negative. This situation indicates that positive alleles were dominant and the epistatic interaction was of the duplicate gene action type. Since cultivated peanut is an allotetraploid, epistatic interactions are understandable.

Other researchers have reported that differences in nodulation and N_2 fixation were heritable and influenced mainly by additive gene effects (22). This research showed contrasting findings. Epistatic effects generally were found to be more important than additive effects for most traits in the two crosses between a virginia x virginia botanical type and virginia x spanish botanical type. With improving N_2 fixation as a goal, plant breeders need to understand the inheritance of the traits affecting fixation so an appropriate breeding strategy can be formulated. These results suggest that early generation selection for improved nitrogen fixation would be ineffective.

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