Bidirectional Selection for Nitrogenase Activity and Shoot Dry Weight Among Late Generation Progenies of a Virginia x Spanish Peanut Cross[1]

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ABSTRACT

Improvement of the host contribution to nitrogen fixation has been proposed as a method of increasing nitrogen fixation. Significant variability and generally high broad-sense heritability estimates (.60 ± .27 to .82 ± .26 for nitrogenase activity and .53 ± .29 to .85 ± .26 for shoot dry weight) have been reported for F_2-derived families from a cross between the virginia (*Arachis hypogaea* L. ssp. *hypogaea* var. *hypogaea*) cultivar NC 6 and the spanish (ssp. *fastigiata* Waldron *vulgaris* Harz.) breeding line 922, indicating selection for increased nigtogen fixation should be effective in this population. Lines from this population were chosen randomly from F_2-derived families selected for high and low nitrogenase activity and high and low shoot dry weight after evaluation at three dates and two locations in each of 2 years (F_5 and F_6 generations). This study's objectives were to evaluate the N_2-fixing ability of the selected lines and to evaluate the association between plant growth habit and N_2 fixation. Twenty-four lines in each of the four selection groups and the parents, NC 6 and 922, were evaluated at two sampling dates and two locations. Mean nitrogenase activity of lines selected for increased nitrogenase activity was significantly greater than the mean of the lines selected for low nitrogenase activity. Improved nitrogenase activity was associated with increased fruit weight. The fruit weight mean of the group selected for increased fruit weight. The fruit weight mean of the group selected for increased nitrogenase activity was 39% greater than the mean of the group selected for low nitrogenase activity. Mean shoot dry weight of lines selected for increased shoot dry weight was significantly greater than the mean of the lines selected for low shoot dry weight; however, the fruit weight means of these two groups did not differ.

It was hypothesized that selection for increased N_2 fixation in a population derived from a cross between virginia and spanish types would eliminate genotypes with spanish growth habit. Groups selected for high nitrogenase activity and for high shoot dry weight had longer and wider leaflets, longer cotyledonary laterals and greater main stem height than did their respective low selection groups. However, these traits chosen to characterize plant growth habit were inadequate in discriminating parental growth habits. Consequently, the data neither substantiated nor refuted the hypothesis.

Key Words: *Arachis hypogaea* L., groundnut, *Bradyrhizobium*, acetylene reduction.

Results from selection for increased N_2 fixation are generally consistent with the notion that this symbiotic process can be enhanced by improvement of the host via traditional breeding methods. Duhigg *et al.* (4) reported progeny of genotypes selected from Mesilla alfalfa (*Medicago sativa* L.) for high acetylene reduction and intermated had acetylene reduction values 82% greater

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than the original unselected population. Associated with this increase was increased top dry weight and total N. Similar results were obtained from selection within six alfalfa cultivars evaluated in the greenhouse after inoculation with *Rhizobium* (9). Progeny from crosses among selections for high acetylene reduction had acetylene reduction values twice that of progeny from crosses among low acetylene reduction selections. Acetylene reduction was positively and significantly correlated with root and top weights and nodule and fibrous root scores. Two cycles of bidirectional recurrent phenotypic selection for nitrogenase activity, top dry weight, nodule mass and fibrous root mass were effective in either or both of two alfalfa gene pools tested (10). Selection increased nitrogenase activity 61-66%. Unfortunately, no increase in nitrogen fixed was apparent when materials were evaluated in the field (5).

Common bean (*Phaseolus vulgaris* L.) lines developed by the backcross inbred line method from a cross between a low N_2-fixing commercial cultivar and a high N_2-fixing donor parent were selected for high acetylene reduction and high seed yield. Selected lines fixed more total N, had a greater percent of total N from fixation and produced more seed than the recurrent parent (2).

In red clover (*Trifolium pratense* L.). plants were selected for yield with N_2 fixation provided the sole source of nitrogen (7). After three generations of selection, crosses among high selections showed a 42-62% increase in yield.

Significant variability and generally high broad-sense estimates of heritability for traits indicative of N_2 fixation have been reported for F_2-derived families from a cross between the peanut cultivar NC 6 and the breeding line 922 (1). Selections for nitrogenase activity as measured by acetylene reduction and for shoot dry weight were made based on family means averaged over three dates, two locations, and two years (F_5 and F_6 generations). The objectives of this study were to (a) evaluate the N_2-fixing ability of near homozygous lines extracted from these heterogeneous families selected for high and low nitrogenase activity and shoot dry weight, (b) assess the feasibility of continued selection in this population, and (c) evaluate the association between plant growth habit and nitrogenase activity.

Materials and Methods

Thirty F_2-derived families originating from the cross of the virginia cultivar NC 6 and the spanish breeding line 922 were evaluated at three sampling dates and two locations in each of 2 years for traits indicative of nitrogen fixation (1). Family means over dates, locations and years were used to select $F_{2,6}$ families based on the following criteria: high and low nitrogenase activity and high and low shoot dry weight (HNA, LNA, HSW, and LSW, respectively). Individual plants were chosen randomly from each selected family. Seed from these selections and seed of NC 6 and 922 were planted for increase on May 5, 1981 at the Peanut Belt Research Station, Lewiston, NC.

The 98 entries (24 $F_{6,7}$ lines/selection group, NC 6 and 922) were planted May 10, 1982 at the Peanut Belt Research Station, Lewiston, NC and May 13, 1982 at the Upper Coastal Plain Research Station, Rocky Mount, NC. Well-established populations of indigenous *Bradyrhizobium* existed at both locations. Each plot consisted of two 25-seed rows with 0.9 m between rows and 0.25 m between plants within a row. Plots were sampled approximately 100 and 130 days after planting (sampling dates 1 and 2, respectively). At each sampling date two samples of two bordered plants each were dug from each plot. Roots were removed at the hypocotyl and placed in glass jars for measurement of acetylene reduction (6). Roots were incubated 1 h at room temperature prior to removing gas samples. At sampling date 1 nodules were removed by hand from each root, counted, dried and weighed. Specific activity was calculated as acetylene reduced per gram nodule dry weight. Plant shoots, excluding fruits, were weighed without drying to obtain a measure of shoot fresh weight. Shoots from three entries per replication were then dried in a forced air dryer. Average percent moisture was calculated for each replication and used to estimate shoot dry weight.

At sampling date 2 nitrogenase activity was measured as described for date 1. Shoots, including fruit, were dried in a forced air dryer. Fruits were removed from the plants, and shoots and fruits were weighed separately. The amount of nodulation was estimated using a visual scale from 0 (no nodulation) to 9 (abundant nodulation). Each fresh root sample was scored by two independent observers and then weighed. The average of the two scores was the nodule rating for a given sample.

Data from each location and sampling date were analyzed as a randomized complete block design using the PROC GLM procedure of SAS (8). Preplanned nonorthogonal contrasts were used to compare entry means. Data were then analyzed over locations within sampling dates. Simple correlation coefficients within and between sampling dates were calculated using entry means for each trait.

Approximately 135 days after planting, morphologic traits were measured on two randomly selected plants in each plot. Leaflet length and width were measured on a random fully expanded leaflet on the main stem. Main stem height and the length of a cotyledonary lateral were also measured. The SAS procedure for principal components analysis, PROC PRINCOMP (8), was performed on the data set consisting of morphologic measurements (main stem height, length of a cotyledonary lateral, and leaflet length and width) of NC 6 and 922.

Results and Discussion

Variation among entries was significant for nitrogenase activity at both sampling dates in spite of significant entry x location interactions (Table 1). The mean of lines selected for increased nitrogenase activity, 52.5 μmoles C_2H_4/plant/h and 23.9 C_2H_4 μmoles/plant/h at dates 1 and 2, respectively, was significantly greater than the mean of lines selected for low nitrogenase activity, 32.9 and 15.8 μmoles C_2H_4/plant/h at dates 1 and 2, respectively (Table 2). Associated with improved nitrogenase activity was increased nodule number and weight, specific activity, estimated shoot dry weight and shoot fresh weigth at date 1, and increased shoot dry weight, fresh root weight, nodule rating and fruit weight at date 2.

The mean nitrogenase activity of the HNA selection group was equal to the midparent mean at dates 1 and 2. Mean fruit weight of the midparent, 98.4 g/plant, was significantly greater than that of the HNA selection group, 77.5 g/plant; however, within the HNA group the phenotypic correlation between fruit weight and nitrogenase activity was not significantly different from 0 (data not shown).

The shoot dry weight mean of the HSW selection group, 180.1 g/plant, was significantly greater than that of the LSW selection group, 127.5 g/plant (Table 2). Associated with this increase was an increase in nitrogenase activity, fresh root weight and nodule rating at date 2. The fruit weight means of the HSW and LSW groups were not different (Table 1). In addition, the mean shoot dry weight of HSW was greater than that of the midparent. This response to selection for improved shoot weight is in contrast to a study (Arrendell, S., J. C. Wynne, G. H. Elkan and T. J. Schneeweis, N. C. State Univ., unpubl. data) which indicated large year effects

Table 1. Mean squares from analyses of variance of traits indicative of nitrogen fixation and fruit weight for progeny selected for high and low nitrogenase activity and high and low shoot weight.

Source[†]	df[‡]	Sampling date 1						Sampling date 2				
		Nitrogenase activity	Estimated shoot dry wt	Shoot fresh wt	Nodule number	Nodule dry wt	Specific activity	Nitrogenase activity	Shoot dry wt	Fresh root wt	Nodule rating	Fruit wt
Entry	97	1172.5**	8444.9**	140892.5**	160481.7**	550311.3**	477.8**	310.8**	9778.7**	55.0**	5.3**	2713.9**
HNA vs LNA	1	27794.5**	75448.3**	1384378.7**	1512857.6**	9336528.8**	1300.1*	4571.2**	18808.8**	587.0**	62.2**	32272.5**
HNA vs MP	1	742.6	11922.2**	218916.2**	246211.3*	10377.5	89.5	36.6	873.6	7.0	1.9	14755.8**
HSW vs LSW	1	117811.3**	179270.3**	3197499.0**	867958.0**	7815114.1**	11.9	3915.0**	202753.1**	264.4**	60.9**	715.0
HSW vs MP	1	90.0	2736.9	48871.5	602256.9**	16791.0	74.7	11.9	12825.2**	18.0	7.9	34272.1**
HNA vs HSW	1	627.0	6470.1*	121853.4*	156660.0	107153.6	1.3	13.1	41292.7**	5.1	4.1*	8158.3**
Within HNA	23	773.8**	3721.4**	58455.5**	132180.2**	338166.8**	362.7	285.7**	8916.0**	58.7**	3.5**	1346.9**
Within LNA	23	403.0**	5236.8**	72211.3**	57233.9	183709.2	478.6	145.2	4825.3**	35.7**	3.2**	2280.0**
Within HSW	23	782.6**	4864.9**	77583.6**	164761.3**	298062.6**	468.7	286.9**	8664.7**	40.0**	4.3**	2242.1**
Within LSW	23	849.7**	5900.5**	103067.5**	108439.6**	551500.7**	392.9	206.5*	8512.9**	53.3**	3.7*	1115.3**
Entry • Location	97	200.2**	1210.1	20779.8	46707.9	136409.3	294.7	113.4**	1554.5	15.6**	0.9	471.6*
Error	423	133.9	1266.1	21442.1	49037.0	134368.4	286.0	55.4	1368.8	9.7	0.6	366.6

*,**Indicate significance at .05 and .01 levels of probability, respectively.

[†] MP, HNA, LNA, HSW and LSW are abbreviations for midparent, high nitrogenase activity group, low nitrogenase activity group, high shoot weight group and low shoot weight group, respectively.

[‡] Error degrees of freedom may vary due to missing values.

Table 2. Means of traits indicative of nitrogen fixation and fruit weight and ranges of means for two parental lines and their selected progeny.[†]

Entry[‡]	Sampling date 1					
	Nitrogenase activity (μmoles C_2H_4/plant/h)	Estimated shoot dry wt (g/plant)	Shoot fresh wt (g/plant)	Nodule number	Nodule dry wt (mg/plant)	Specific activity (μmoles C_2H_4/g/h)
NC 6	52.0	223.0	942.6	978.1	1343.6	44.5
922	44.1	163.2	687.1	696.9	847.1	57.4
MP	48.0	193.1	814.9	837.5	1108.8	50.9
HNA	52.5 (19.9-90.3)	175.1 (47.6-285.3)	735.5 (194.0-1175.0)	751.9 (88.5-1733.0)	1126.3 (429.2-2563.2)	52.5 (13.1-123.6)
LNA	32.9 (9.5-68.4)	142.7 (58.2-327.1)	596.8 (269.0-1125.0)	609.4 (143.0-1373.0)	765.7 (217.5-2425.7)	48.4 (15.9-104.0)
HSW	49.8 (14.2-102.7)	185.6 (80.1-326.8)	776.6 (294.0-1350.0)	707.7 (199.0-1506.0)	1087.3 (292.3-2225.3)	52.4 (20.0-176.0)
LSW	33.8 (8.5-76.0)	134.7 (53.0-314.9)	565.9 (231.5-1294.0)	597.9 (145.0-1380.0)	757.8 (175.7-2795.2)	52.0 (16.1-116.0)

Entry[‡]	Sampling date 2				
	Nitrogenase activity (μmoles C_2H_4/plant/h)	Shoot dry wt (g/plant)	Fresh root wt (g/plant)	Nodule rating	Fruit wt (g/plant)
NC 6	23.1	150.2	15.3	5.7	119.9
922	22.6	172.1	14.1	4.6	75.9
MP	22.9	160.9	14.7	5.2	98.4
HNA	23.9 (4.1-71.2)	156.5 (68.5-362.0)	14.4 (3.3-32.6)	4.9 (2.2-8.5)	77.5 (16.0-158.1)
LNA	15.8 (3.0-41.8)	140.4 (43.1-277.7)	11.6 (2.1-27.5)	4.0 (2.0-7.2)	55.8 (8.9-164.0)
HSW	23.6 (3.6-55.8)	180.1 (49.7-346.6)	14.1 (5.4-33.0)	4.7 (1.7-8.0)	66.6 (13.7-158.7)
LSW	16.0 (1.5-49.1)	127.5 (45.2-316.6)	12.2 (3.6-30.4)	3.8 (1.5-6.7)	63.4 (8.8-153.3)

[†] Levels of significance associated with specific mean comparisons are given in Table 1 and should be applied to mean comparisons in this table.

[‡] MP, HNA, LNA, HSW and LSW are abbreviations for midparent, high nitrogenase activity group, low nitrogenase activity group, high shoot weight group and low shoot weight group, respectively.

may cause erroneous conclusions to be drawn from shoot dry weight data taken in a single year on early generation progeny.

Estimated shoot dry weight (date 1) was significantly correlated (r = 0.70**) with shoot dry weight (date 2). Estimated shoot dry weight, shoot fresh weight, and nodule number and weight were significantly greater in the HSW group than LSW group at date 1. The midparent mean was greater than the mean of the HSW group for nodule number only.

With one exception, significant variability existed among lines within each of the four selection groups for the corresponding trait of selection. No significant variability for nitrogenase activity was seen in the LNA group at date 2. Significant variability for fruit weight was also measured in each of the four groups.

Interestingly, the HNA mean acetylene reduction was equal to that of the HSW at both dates. Genotypic correlations between nitrogenase activity and shoot dry weight in the base population were high (1). In this population selection for shoot dry weight may be an effective means of indirect selection for improved acetylene reduction. Although both HNA and HSW selection groups had mean fruit yields significantly lower than the midparent, the mean fruit weight of the HNA group was significantly greater than the mean of the HSW group indicating selection for improved nitrogenase activity was more effective than selection for increased shoot dry weight in maintaining plant yielding ability. The genotypic correlation between fruit weight and nitrogenase activity ($r_G = 0.57$ and 0.36 in years 1 and 2, respectively) was higher than the correlation between shoot dry weight and nitrogenase activity ($r_G = 0.13$ and 0.07 in years 1 and 2, respectively) in the base population (1).

Leaflet length and width, main stem height, and the length of a cotyledonary lateral were measured to evaluate the indirect effect of selection for improved nitrogenase activity on growth habit. Groups selected for high nitrogenase activity and for high shoot weight had longer and wider leaflets, longer cotyledonary laterals and greater main stem heights than their respective low selection groups (Table 3). Line 922 had wider leaflets and greater main stem length than did NC 6. The principal components analysis of morphologic traits indicated the first principal component accounted for 79.9% of the variation and consisted of approximately equal weightings of each of the traits. A t-test did not indicate a difference between the parental means; therefore, the principal components analysis was not used to evaluate differences in plant growth habit among the selected lines. Failure to separate the virginia and spanish parents is in contrast to earlier reports (4) where fruit weight and length, seed weight, pod yield, meat content and maturity were used to differentiate virginia and spanish types in unselected late generation progency from a cross of NC 6 and 922.

Selection for nitrogenase activity and for shoot dry weight was effective in this population as indicated by the mean difference in the high and low selection groups for traits of selection. Variability within each of the selection groups for the corresponding trait of selection and for fruit weight indicates intermating and further selection should be effective in further differentiating the high

Table 3. Means of morphologic traits, ranges of means, and levels of significance for two parental lines and their selected progeny.

Entry[+]	Means			
	Leaflet length (mm)	Leaflet width (mm)	Mainstem height (cm)	Cotyledonary lateral length (cm)
NC 6	53.3	21.1	26.0	51.2
922	52.2	22.9	30.0	46.7
MP	52.9	22.0	27.9	49.0
HNA	53.5 (37.5-73.0)	23.6 (17.0-33.5)	38.5 (14.0-61.0)	50.9 (26.0-87.5)
LNA	48.1 (32.5-63.5)	22.0 (15.0-43.0)	25.2 (9.0-59.5)	46.2 (26.0-80.5)
HSW	52.9 (37.5-69.5)	23.0 (16.5-37.0)	31.8 (16.0-53.5)	53.9 (29.0-75.5)
LSW	50.3 (38.0-64.5)	21.9 (14.0-32.0)	26.9 (14.0-41.0)	42.8 (24.0-73.5)
		Levels of significance		
NC 6 vs 922	NS	*	*	NS
HNA vs LNA	**	**	**	**
HNA vs MP	NS	**	**	NS
HSW vs LSW	**	**	**	**
HSW vs MP	NS	*	**	**
Within HNA	**	**	**	**
Within LNA	**	**	**	**
Within HSW	**	**	**	**
Within LSW	**	NS	**	**

*,**Indicate significance at .05 and .01 levels of probability, respectively.

[+] MP, HNA, LNA, HSW, and LSW are abbreviations for midparent, high nitrogenase activity group, low nitrogenase activity group, high shoot weight group, and low shoot weight group, respectively.

and low selection groups. Although groups selected for high activity and high shoot weight differed from their respective low selection groups for the four morphologic traits measured, these traits did not adequately classify the virginia and spanish growth habits. Further work must be done to evaluate the indirect effect of selection for improved nitrogenase activity on growth habit.

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