Genetic Variability for Biological Nitrogen Fixation Traits in Tropical Soybeans (*Glycine max* (L) Merr)

D. K. Ojo, J. O. Amira, and O. A. Oduwaye*
Department of plant breeding and seed Technology, University of Agriculture, Abeokuta, Nigeria
segunoa@yahoo.com

Abstract: Twenty five soybean (*Glycine max* (L) Merrill) genotypes were sown at the Teaching and Research Farm of UNAAB in Nigeria during the late planting season in year 2004 to obtain data for grain yield and seven Biological Nitrogen Fixation (BNF) traits. The data were subjected to analysis of variance (ANOVA) and were later used in the computation of heritability estimates for grain weight, days to flower, nodulation rating, days to maturity, number of nodules, nodule weight, primary root length and number of secondary roots. Varieties were observed to be genotypically diverse with respect to the traits evaluated. Large genotypic and phenotypic variations were also observed for the characters. Nodule number and nodule weight that had positive and significant association with nodulation rating, with relatively high broad-sense heritability estimates were adjudged as possible selection criteria for genetic improvement for BNF. Genotype TGx 1921-2F was identified as the only genotype that has the potentials for genetic manipulation of host plant for the improvement of soil nitrogen among the genotypes that were evaluated. [Nature and Science. 2007;5(1):69-74].

Keyword: Native rhizobia, Nodulation, Organic biological nitrogen fixation, tropical soil

Introduction

The initial breeding approach of the International Institute of Tropical Agriculture (IITA) soybean scientists in the improvement of biological nitrogen (N) fixation (BNF) to meet the demand for Nitrogen in soybean in the early 1980s was to breed for promiscuous varieties that would nodulate freely with indigenous soil rhizobia to make inoculation unnecessary (Pulver *et al*., 1982; Kueneman *et al*., 1984). Promiscuous soybean varieties are those that are compatible with and could form effective symbiotic association with a large majority of native rhizobial strains (IITA, 1983) thus, making the use of synthetic fertilizers unnecessary. Compatible lines are lines that have high number of nodules with high biomass production. Shoot dry matter yield (biomass production) in particular has been reported to have strong and positive association with soybean grain yield (Okogun and Sanginga, 2003).

Reports in the last decade (Sanginga *et al*., 1997; Okogun and Sanginga, 2003) have shown that promiscuous soybean varieties derived about 85 kg N from BNF. Positive response of soybean to inoculation (yield increase of 179%) has also been reported to occur in situations where indigenous bradyrhizobial cells were fewer than 10 cells per grain of soil or where the rhizobial populations were not effective (RENEASA, 1996). Sanginga (2003), Okogun and Sanginga (2003) have confirmed that tropical soil rhizobial strains were not effective in fixing enough biological nitrogen to sustain soybean growth and, thus, the need for a starter dose of nitrogen fertilizer or a search for more effective rhizobial strains.

Effectiveness of soil rhizobial strains is influenced by prevailing environmental conditions such as moisture (Nantakorn and Weaver, 1982) and competition for nodule sites (Harold and Fudi, 1992). Excess soil moisture of more than 10% (Osa-Afiana and Alexander, 1979) probably, affects the rhizobial population and, thus, their symbiotic activities (Okogun and Sanginga, 2003).

Efforts have been made to incorporate the promiscuous gene into elite soybean varieties developed between 1991 and the present (FAO, 1999; Ojo, 2002) and selection for promiscuity and nodulation as reflected by the number of nodules and biomass production has been attempted (Sanginga *et al*., 1997; Sanginga *et al*., 2000).

It has been reported earlier that inheritance of promiscuity in tropical soybean was conditioned by a few major genes (IITA, 1980) suggesting that the trait is qualitatively inherited.

Genetic evaluation of BNF traits, particularly, the estimation of the degree of genetic determination of those traits that have significant association with grain yield among selected tropical soybean lines could provide a fast and cost effective means of knowing the traits that can be used as good predictors of yield in this crop. This is because heritability estimates indicate how easy or difficulty it will be to produce a change in a given trait by applying selection (Graham and Welch, 1996) and the amount of grain anticipated from such a selection is best given by heritability (Borojevic, 1990). The exercise could result in an intelligent choice of the best lines and the right breeding procedure needed for their genetic improvement for effective nitrogen fixation in tropical soils without the use of synthetic fertilizers. Sanginga *et al*. (1997) have observed that the choice of breeding lines could influence the potential contribution of fixed nitrogen to farming system.

This research was intended to (1) examine genetic variability for BNF traits in selected soybean lines (2) examine possible association that exist between grain yield and BNF traits and (3) identify which of the traits that can be used as selection criteria for BNF and thus, grain yield in tropical soybeans.

Materials And Methods

Twenty five tropical soybean genotypes obtained from IITA, Ibadan, Nigeria were sown in the Teaching and Research Farm of the University of Agriculture, Abeokuta, (UNAAB), Nigeria in August 2004.

After land preparation that involved ploughing and harrowing, seeds of each genotype were sown by drilling in four-row plots in randomized complete block design with three replications. Rows were 75 cm apart. On emergence, seedlings were thinned to a plant-to-plant spacing of 5 cm leaving a population of 266, 667 plants per hectare.

Hand weeding was employed as required. Data were collected on number of days to flowering, number of nodules at flowering, weight of fresh nodules, length of primary root, number of secondary roots and nodulating rating. At maturity data were collected for days to maturity and grain yield per plot.

Days to flowering: - this was determined as the period from date of planting to the date when 50% of the plant in the plot were at full bloom stage.

Days to maturity: - was determined as the period between date of planting and the date when plants in the plot had matured physiologically and the pods were brown in colour.

Nodulating rating: - was a rating from 1 to 5, root having very few nodules was rated 1, few nodules rated 2, moderate nodule rated 3, plenty nodules rated 4 while very plenty nodules was rated 5.

Number of nodules: - roots of ten plants were carefully dug up, packed in polythene bags together with the nodules that had become detached during digging and were transported to the laboratory where they were washed and number of nodules were counted. The mean value was then recorded.

Weight of fresh nodules: - after counting the nodules, it was weighted with a sensitive scale.

Grain yield: - grain yield in kg/plot was determined on clean dry grains of plants harvested from two middle rows of each plot.

Length of primary root: - ten plants were randomly picked from boarder rows and tap roots were measured per plant for length of primary root (cm) using a thread and meter rule. The mean length was then determined.

The number of secondary roots was also recorded as the mean number of roots of ten plants selected randomly from the boarder row.

The plot means of each character were subjected to analysis of variance (ANOVA). Simple correlation coefficients were obtained between all possible combinations of traits using Pearson correlation coefficient analysis. Also, estimates of broad-sense heritability for each character were obtained for possible use as selection criteria.

Result And Discussion

Table 1 presents the mean values of the twenty five soybean genotypes that were evaluated for eight BNF traits. Mean grain yield per plot ranged from 160 kg/plot for TGx 1923-4F to 433 kg/plot for TGx 1903-8F. Genotype TGx 1921-2F with highest nodulation rating of 3.4 matured in 87 days just as genotypes TGx 1921-1F, 1921-23F and TGx 1924-1F. Incidentally, genotype TGx 1921-2F recorded the highest number of nodules (21.0), biggest nodule weight (0.3 g/plant), very long primary root (13 cm) as well as highest number of secondary roots (10.0).

Table 1. Mean value of 25 soybean genotypes evaluated for grain weight and biological nitrogen fixation characters

S/N	Variety	GWK g/plot	DF	NR	DM	NN	NW g/plt	RL	SN
1	TG_X 1903-8F	433	40	1.7	83	7.0	0.1	9.8	10.0
2	TG_X 1908-6F	413	42	3.0	83	9.7	0.2	9.6	8.0
3	TG_X 1921-7F	353	41	3.3	81	14.0	0.2	11.0	8.0
4	TG_X 1903-4F	347	41	3.0	84	8.0	0.1	11.0	5.7
5	TG_X 1920-1F	347	42	2.0	84	2.0	0.02	12.0	6.3
6	TG_X 1924-1F	340	47	3.0	87	12.0	0.2	10.0	9.3
7	TG_X 1923-3F	327	41	3.0	84	16.0	0.2	13.0	10.0
8	TG_X 1924-4F	323	46	2.3	91	4.3	0.1	9.5	6.0
9	TG_X 1909-3F	317	42	3.0	81	10.0	0.1	13.0	8.0
10	TG_X 1903-7F	307	41	2.0	81	3.7	0.05	10.0	8.3
11	TG_X 1921-6	307	42	3.3	80	15.0	0.3	12.0	7.7
12	TG_X 1919-1F	293	42	2.3	81	4.7	0.1	11.0	7.3
13	TG_X 1922-1F	260	41	2.3	81	7.7	0.2	11.0	8.3
14	TG_X 1925-1F	259	46	2.0	87	4.7	0.0	10.0	4.7
15	TG_X 1909-2F	253	42	3.3	81	7.0	0.2	13.0	9.0
16	TG_X 1985-1D	240	40	2.3	83	6.0	0.1	11.0	6.0
17	TG_X 1921-13F	240	42	2.3	81	9.7	0.1	11.0	9.0
18	TG_X 1904-2F	220	42	2.0	84	4.0	0.1	14.0	6.0
19	TG_X 1921-1F	220	41	2.7	87	11.0	0.1	11.0	8.7
20	TG_X 1921-2F	220	42	3.4	87	21.0	0.3	13.0	10.0
21	TG_X 1904-4F	220	42	3.0	81	5.0	0.1	12.0	6.7
22	TG_X 1921-23F	200	41	2.7	81	8.0	0.1	11.0	7.0
23	TG_X 1930-20E	173	42	1.3	81	1.3	0.03	12.0	11.0
24	TG_X 1921-20F	173	44	2.0	81	14.0	0.2	13.0	7.0
25	TG_X 1923-4F	160	41	3.3	87	8.0	0.1	13.0	6.3
	Mean	227.3	42.0	2.6	83.6	8.6	0.1	11.5	7.8
	LSD	140.2	1.00	1.44	1.41	7.36	0.11	3.33	3.36

GW = Grain weight, DF = Days to flower, NR = Nodulation rating, DM = Days to maturity, NN = Nodule number, RL = Primary root length, SN = Secondary root number

Mean squares from analysis of variance for BNF traits (Table 2) showed that the twenty five soybean genotypes were highly variable with respect to grain yield, days to flowering, days to maturity, number of nodules, nodule weight and number of secondary roots to confirm the observed variations stated above.

Table 2. Mean squares from analysis of variance of biological nitrogen fixation (BNF) characters in twenty five varieties of soybean

S O V	df	GW	DF	NR	DM	NN	NW	RL	SN
Replication	2	47225.65**	0.37	1.29	0.36	7.72	0.01	7.34	3.21
Variety	24	16305.72**	8.52**	1.04	25.02**	70.61**	0.02**	4.46	8.42*
Error	48	7292.32	0.37	0.77	0.74	20.08	0.01	3.84	4.19

*Significant at 5% probability level, ** Significant at 1% probability level.
SOV= source of variation, df=degree of freedom, GW= Grain weight, DF= Days to flower, NR= Nodulation rating, DM= Day to maturity, NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Observed correlation coefficients between grain yield and BNF traits as presented in Table 3 revealed that only the primary root length recorded a significant but negative correlation with grain yield. Days to flowering and days to maturity recorded non-significant and negative correlation with grain yield. However, days to flowering and days to maturity were positively and significantly correlated (0.472). Nodulation rating was equally positively and significantly associated with nodule weight (0.674) and number of nodules (0.633). Significant and positive association between two characters suggests that these characters can be improved simultaneously in a selection programme (Hayes *et al*., 1955). This is because a significant positive association shows a mutual relationship as selection for one trait would lead to selection and consequent improvement for the other traits.

Genetic manipulation of selected soybean genotypes for number of nodules, nodule weight and number of secondary roots would be an effective means of rating tropical soybean genotypes for BNF characteristics according to the current study. However, the length of primary root would not have any meaningful contribution to grain yield because both were negatively and significantly correlated.

Table 3. Correlation coefficients between Grain weight and Biological Nitrogen-Fixation characters

	GW	DF	NR	DM	NN	NW	RL	SN
GW	1.000	-0.046	0.077	-0.044	0.054	0.073	-0.561**	0.119
DF		1.000	-0.047	0.472*	0.012	0.038	-0.186	-0.239
NR			1.000	0.093	0.633**	0.674**	0.191	0.017
DM				1.000	0.063	0.040	-0.263	-0.245
NN					1.000	0.856**	0.208	0.392*
NW						1.000	0.212	0.325
RL							1.000	0.002
SN								1.000

*Significant at 5% probability level ** Significant at 1% probability level.
GW= Grain weight, DF= Days to flower, NR= Nodulation rating, DM= Day to maturity
NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Table 4 presents the phenotypic and genotypic variances and estimates of broad-sense heritability (H_B) of eight traits that were evaluated. Days to flowering and days to maturity had the largest estimates of 88 and 92%, respectively and thus were least affected by the effects of the environment. Nodule number and nodule weight recorded average estimates of 46 and 47% respectively, whereas nodulation rating, primary root length and number of secondary roots had H_B estimates that were ridiculously low (5 – 25%)

Table 4. Phenotypic and genotypic variance and heritability of eight traits that were evaluated

S/N	Traits	Phenotypic variance	Genotypic variance	Heritability (H_B)
1	Grain weight	10296.77	3004.47	0.29
2	Days to flowering	3.09	2.72	0.88
3	Nodulation rating	0.86	0.09	0.11
4	Days to maturity	8.83	8.10	0.92
5	Nodule number	36.92	16.84	0.46
6	Nodule weight	0.0088	0.0041	0.47
7	Primary root length	4.05	0.21	0.05
8	Secondary root number	5.60	1.41	0.25

Very low estimates of H_B suggest large effects of the environment and consequently larger phenotypic variances. High soil moisture (Nantakorn and Weaver, 1982), competition among local strains of Rhizobia for nodule sites (Harold and Fudi, 1992) and few number of native Bradyrhizobial cells in a unit grain of soil (Okogun and Sanginga, 2003) have been reported as some of the factors that prevent effective nodulation of soybean lines.

This study shows that number of nodules and weight of nodules were positively associated with nodulation rating. Thus, the two traits could be used as selection criteria for BNF traits among tropical soybean genotypes. As genetic manipulation of host plants offers the greatest potential for the improvement of nitrogen levels in soybean (Sanginga *et al*., 1997) genotype TGx 1921-2F with highest nodulation rating, highest number of nodules, highest nodule weight as well as highest number of secondary roots could be selected for a future genetic improvement of biological nitrogen fixation.

Correspondence to:
O. A. Oduwaye
Department of plant breeding and seed Technology
University of Agriculture, Abeokuta, Nigeria
segunoa@yahoo.com

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