Global Advanced Research Journal of Food Science and Technology (ISSN: 2315-5098) Vol. 3(5) pp. 135-140, October 2014
Available online http://garj.org/garjfst/index.htm

Full Length Research Paper

Genetic Variability and Character Association of Twenty Soybean (*Glycine Max* L. Merrill) Genotypes for Biological Nitrogen Fixation and Related Traits in Pawe District Metekel Zone Northwestern Ethiopia

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Accepted 01 October 2014

The study was conducted to evaluate the genetic variability of soybean genotypes and genotypic correlation among BNF and related traits. Genotypes were observed to be genotypically diverse with respect to the traits evaluated. Large genotypic and phenotypic variations were also observed for the traits. Number of nodules per plant showed significant positive genotypic correlation with both seed yield per plant (p< 0.05, 0.456) and seed yield per plot (p< 0.05, 0.674). Effective number of nodules per plant showed positive and significant association with days to flowering (p< 0.05. 0.63) and days to maturity (p< 0.05, 0.631), and highly significant association with number of nodules per plant (p< 0.01, 0.993) and at phenotypic level with dry weight of nodules per plant (p< 0.01, 0.505). Among the tested genotypes, TGX-1987-40F and TGX-1987-9F were found significantly superior in BNF and related traits. Therefore, from this study it can be suggested to use at least one of the genotypes as parent in the hybridization work for a future genetic improvement of biological nitrogen fixation.

Keywords: genetic variability, genotypic correlation and BNF related traits

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the most important crop due to its good chemical compositions. Nutritional value of soybean lies in its protein (40 - 42%) and oil contents (18 - 22%) and is free from cholesterol making it highly desirable in the human die (Faisal *et al*, 2006). Soybean is the most important among the entire leguminous crop to improve the soil nutrient status (McNeil, 2010).

Symbiotic nitrogen fixation by soybean plays a key role in supplying nitrogen for agricultural systems. In symbiotic associations with *Bradyrhizobium japonicum* soybean can fix up to 200 kg N per hectare per year (Siczek *et al*, 2009).This reduces the need for expensive and often environmentally harmful because of leaching nitrogen fertilization.

Reports indicate the variability of genotypes in influencing the nitrogen fixation activity of microbes (Farnia *et al*., 2005). Plant breeders are continuously searching for new sources of useful genetic variation that will positively impact their breeding programs. Mruthunjaya and Mahadevappa (1995) reported that the success of crop improvement program depends on the

definition and assembly of the required genetic variations and selection for yield through high heritable traits, excluding the environmental components.

Positive response of soybean to inoculation (yield increase of 179%) has been reported by Reneasa (1996). An increase in nodulation and seed yield is possible only when seed is inoculated with effective and adequate number of Rhizobia (Albareda *et al*., 2009). Functioning nodules on well-nodulated soybeans will provide up to 50 to 60 percent or more of the nitrogen needed by the plant (Kansas State University, 2012). Improvement of soybean for high N_2 fixation is the best way to enhance soybean production particularly for N deficient growing areas. In addition, study of genotypic correlation with BNF and related traits help to formulate effective breeding program. Therefore, the present study was conducted to evaluate the genetic variability of soybean genotypes and genotypic correlations of BNF and related traits.

MATERIAL AND METHODS

The study was conducted under rain-fed conditions during the year 2012 main cropping season in Pawe District (11^0 18' N and 036^0 24' E) of Metekel Zone of the Benishangul Gumuz National Regional State, North-western Ethiopia. The area is located about 570 km away from Addis Ababa. The altitude of the study area ranges between 1000 to1200 meters above sea level. The specific soil type of the site is well drained Nitisol with the pH value ranging from 5.3 to 5.5. Besides, Pawe is well known for its high and torrential rainfall with a unimodal pattern that extends from May to October. The area receives an intensive rainfall amounting to 1586 mm. The mean annual maximum and minimum temperatures are 32.6 and 16.5 ^{0}C, respectively.

$\frac{ms_g - ms_e}{r}$

Twenty soybean genotypes were sown in mid June, 2012. The experiment was arranged as a Randomize Complete Block Design (RCBD) in three replications. Materials were planted each on a 3.6 m x 4 m plot size having 6 rows including 2 borders. Spacing between rows, plants, plots and replications were 60 cm, 5 cm, 60 cm and 1.5 m respectively. All the recommended agronomic practices were followed during the course of.

Triple super phosphate was applied at the rate of 100kg/ha during sowing time. No nitrogen fertilizer was applied. The genotypes were inoculated by TSBF 1495 rhizobium stains using slurry method **(anonymous**, 2012).

Data were collected for days to flowering (DF), days to maturity (DM), above ground biomass yield (AGBY), harvest index (HI), hundred seed weight (HSW) and Seed yield per plot (SYPP). Ten random plants from each replication were subjected for number of branches per plant (NB), number of nodules (NN), dry weight of nodules (DWN), effective number of nodules (NEN), number of pods per plant (NP), number of seeds per pod (NSP), plant height (PH) and seed yield per plant (SYP).

Analysis of Variance

The data obtained for different traits was statistically analyzed using SAS version 9.0 Software. Analysis of Variance for RCBD design was done using the mean of ten sampled plants for the characters such as number of branch per plant, number of nodulation, weight of nodulation, effectiveness of nodulation, plant height, number of seeds per pod, number of pods per plant, seed yield per plant and plot mean for seed yield per plot, days to maturity, biological yields, harvest index, hundred seed weight, days to flowering. Mean comparisons among treatment means were conducted by Least Significance Difference (LSD) methods at 5% levels of significance. The RCBD design analysis of variance was used to derive variance components as structured in Table 1 (Cochran and Cox, 1957).

Estimation of phenotypic and genotypic variances

The phenotypic and genotypic variances of each trait were estimated from the RCBD analysis of variance. The expected mean squares under the assumption of random effects model was computed from linear combinations of the mean squares and the phenotypic and genotypic coefficient of variations were computed as per the methods suggested by Burton and Devane (1953).

Genotypic variance ($\sigma^2 g$) =

Environmental variance (σ^2_e) = mse

Where, msg and mse are the mean sum of squares for the genotypes and error in the analysis of variance, respectively. r is the number of replications. The phenotypic variance was estimated as the sum of the genotypic and environmental variances.

Phenotypic variance ($\sigma^2 ph$) = $\sigma^2_g + \sigma^2_e$

Estimation of genotypic and phenotypic coefficient of variability

The genotypic and phenotypic coefficients of variability were calculated according to the formulae of Singh and Chaundary (1977).

Genotypic Coefficient of Variation (GCV) = (σg/grand mean) x 100

Phenotypic Coefficient of Variation (PCV) = (σ_{ph}/grand mean) x 100

Where, σ_g and σ_{ph} are genotypic and phenotypic standard deviations, respectively.

Table 1. RCBD analysis of variance and expected mean square

Source of variation	Df	Mean square	Expected mean square
Replication	r-1	Ms_r	$\sigma^2 e + g\sigma^2_r$
Genotypes	g-1	Ms_g	$\sigma^2_e + r\sigma^2_g$
Error	(r-1)(g-1)	Ms_e	σ^2_e

Where, r = number of replications; ms_r = mean square due to replications; g = number of genotypes; ms_g = mean square due to genotypes; ms_e = mean square of error; σ^2_g, σ^2_r and σ^2_e are variances due to genotype, replication and error, respectively.

RCBD ANOVA was computed using the following model:

$Y_{ij} = \mu + r_j + g_i + \varepsilon_{ij}$

Where, Y_{ij} = the response of trait Y in the i^{th} genotype and the j^{th} replication

S = the grand mean of trait Y

r_j = the effect of the j^{th} replication

g_i = the effect of the i^{th} genotype

ε_{ij} = experimental error effect

Table 2. The mean squares for different sources of variation, the corresponding CV (%) and Coefficient of determination (R^2) for the 15 characters studied

Characters	Replication	Genotypes	Error	C.V. %	R^2
DF	1.34	48.86**	1.16	1.19	0.96
DM	0.60	126.88**	1.62	1.76	0.98
NN	67.75	367.63**	28.60	19.86	0.87
DWN	4460.64	27463.79**	2283.34	26.45	0.86
HSW	0.27	10.19**	0.95	8.77	0.84
AGBY	0.00	11.82**	1.07	23.74	0.85
HI	159.54	370.21**	65.42	21.77	0.75
NC	4.57	43.71**	6.17	26.18	0.78
NP	7.43	187.52**	22.98	25.21	0.80
SYPP	0.48	36.69**	7.29	37.71	0.72
SYP	78175.89	1499783.5**	96292.2	19.64	0.89
NB	0.16	1.12*	0.35	30.20	0.62
NEN	0.01	0.099*	0.05	16.76	0.50
PH	70.58	302.65*	84.7	16.184	0.65

*, ** indicate significant at the 0.05 and 0.01 probability levels respectively.
Where DF=Days to 50 % flowering, DM=Days to 50% maturity, NNP= Number of nodules per plant, AGBY= Above ground biomass yield, EN= Number of effective nodules per plant, DWN= Dry weight of nodules per plant, HSW= Hundred seed weight, HI= Harvest index, NB= number of branches per plant, NC= Number of clusters per plant, NP = Number of pods per plant, PH= Plant height, SYPP= Seed yield per plant and SYP= Seed yield per plot.

Coefficient of Correlation

Estimation of genotypic and phenotypic correlation coefficients were done based on the procedure of Dabholkar (1992).

Genotypic correlation coefficient (rg) = COVg (xy)/ σg (x) * σg (y)

Phenotypic correlation coefficient (rph) = COVph (xy)/ σph (x) * σph (y)

Where, COVg (xy) and COVph (xy) are the genotypic and phenotypic covariances of two variables (X and Y), respectively. σg (x) and σg (y) are the genotypic standard deviations for variables, X and Y, respectively. σph (x) and σph (y) are the phenotypic standard deviations of variables, X and Y, respectively.

The calculated phenotypic correlation value was tested for its significance using t-test:

$t = r_{ph}/SE\ (r_{ph})$

Table 3. Mean values of the studied 20 genotypes for NNP, EN and DWN characters

Genotypes	NNP	EN	DWN	Genotypes	NNP	EN	DWN
TGX-1987-40F	56a	43.47a	148hdfge	**TGX-1986-3F**	29.9cbd	25.33bac	98hg
TGX-1987-19F	35cb	28.87bac	141.33hfge	**TGX-1987-15F**	8.67g	10.67dc	160dfge
TGX-1987-37F	30.27cbd	24.53bdac	90hg	**TGX-1986-14F**	19efd	35.87a	216dfce
TGX-1987-9F	29.5cbd	40.93a	484a	**TGX-1987-62F**	27.87cebd	26.87bac	128hfg
TGX-1987-35F	24.2efd	32.27ab	226def	**TG-1987-38F**	17.07gf	16.93bdac	141.33hfge
Gishama	30.93cbd	28.13bac	252c	**AFGAT**	17.9gef	27.53bac	334b
Ethiougozlavia	22.8efd	17bdac	198dfce	**TG-1987-11F**	15.8gf	20.07bdac	154.67dfgc
TG-1987-34F	29.8cbd	24.27bdac	234.67dc	**TGX-1987-23F**	37.5b	29.8bac	162dfge
Wegayen	8.3g	8.93d	100hg	**TGX-1987-10F**	37b	27.4bac	148hgfge
TGX-1987-20F	35.9b	27.87bac	66h	**CV(%)**	**19.86**	**16.76**	**26.45**
Belesa-95	19.1ef	12.8bdc	126hfg				

Where: NNP = Number of nodules per plant, EN= Number of effective nodules per plant and DWN= Dry weight of nodules per plant
Means value followed by the same letter in the columns indicate there is no significance.

Where, r_{ph} = Phenotypic correlation; SE (r_{ph}) = Standard error of phenotypic correlation was obtained using the following formula (Sharma, 1998).

SE (rph) = $\sqrt{}$ (1-r^2ph)/(n-2)

Where, n is the number of genotypes tested, rph is phenotypic correlation coefficient. The coefficients of correlations at genotypic levels were tested for their significance by the formula described by Robertson (1959) as indicated below:

t = rgxy/Sergxy

The calculated "t" value was compared with the tabulated "t" value at (n-2) degree of freedom at 5% level of significance. Where, n is number of genotypes.

SErgxy = $\sqrt{}$(1-r^2gxy)/2h^2x.h^2y

Where,

h^2x = Heritability of trait x

h^2y = Heritability of trait y

RESULTS and DISCUSSION

Variance

The result indicated that the existence of genetic variability between the studied soybean genotypes. As Table 2 show highly significant difference among treatments at 0.01 probability level for DF, DM, NN, DWN, HSW, AGBY, HI, NC, NP, SYPP and SYP; while NB, NEN and PH result significant difference at 0.05 probability level. Anleksandra *et al* (2001) were reported comparable result for PH, NP, HI and SYP.

The mean value comparison

The estimated mean values for NNP, EN and DWN characters are presented in Table 3. The result indicated that the existence of wide variation among genotypes for studied traits. Treatment TGX-1987-40F score significantly higher mean value (56a) for NNP than the other while TGX-1987-23F, TGX-1987-20F, and TGX-1987-10F follow with the respective mean value (37.5b), (35.9b) and (37b). On the other hand Wegayen (8.3g) and TGX-1987-15F (8.67g) resulted the lowest mean value for NNP. The result indicated genotypes have wide genetic variation for biological nitrogen fixation and related characters such as NNP, EN and DWN. Based on mean performance, TGX-1987-9F, TGX-1987-35F and Gishama were significantly superior on BNF and related traits

Association of characters

Estimates of phenotypic and genotypic correlation

Table 4. Genotypic (above diagonal) and phenotypic correlation coefficients for fourteen characters studied

Char.	DF	DM	AGBY	HSW	HI	NB	CLU	NOD	EN	DWN	NNP	PH	SYPP	SYP
DF		0.304	0.358	-0.492*	-0.608*	-0.26	-0.265	0.55*	0.63*	0.029	0.288	0.278	-0.06	0.058
DM	0.301*		0.576*	-0.254	0.052	0.148	0.263	0.468*	0.631*	0.549*	0.242	0.723*	0.581*	0.599*
AGBY	0.341*	0.533**		-0.170	-0.175	0.265	0.263	0.242	0.425	0.403	0.708*	0.486*	0.350	0.298
HSW	-0.3*	-0.154	-0.032		-0.039	0.006	0.046	0.2	-0.09	-0.213	-0.032	0.157	0.005	0.049
HI	-0.527*	0.047	-0.223	-0.045		-0.122	0.085	-0.062	-0.092	-0.213	-0.306	-0.517	0.638**	0.02
NB	-0.148	0.085	0.22	0.008	0.122		0.136	0.381	0.38	0.456*	0.667*	0.414	0.53*	0.71*
CLU	-0.143	0.18	0.25	0.05	0.044	0.207		-0.18	-0.14	-0.006	0.196	-0.014	0.163	0.287
NP	0.478*	0.426*	0.196	0.33	-0.016	0.169	0.042		0.993**	0.724*	0.513*	0.50*	0.176	0.38
EN	0.390*	0.369*	0.385*	0.033	-0.052	0.18	0.05	0.991**		0.722*	0.49*	0.475*	0.16	0.386
DWN	0.026	0.293*	0.402*	-0.047	-0.11	0.402*	0.04	0.48**	0.505**		0.43	0.46*	0.434	0.406
NNP	0.207	0.201	0.634**	0.067	-0.062	0.711**	0.27*	0.215	0.227	0.269*		0.345	0.456*	0.674*
PH	0.116	0.556**	0.389*	0.246	-0.137	0.4*	0.087	0.41*	0.406*	0.39*	0.449*		0.227	0.52*
SYPP	-0.081	0.371*	0.496**	0.098	0.619**	0.108	0.087	0.116	0.077	0.088	0.114	0.05		0.564*
SYP	0.055	0.566**	0.846**	0.16	0.14	0.61**	0.375*	0.373*	0.363*	0.464*	0.613**	0.42**	0.372*	

*, ** indicate significant at the 0.05 and 0.01 probability levels respectively.

Where: Char. = Characters, DF=Days to 50 % flowering, DM=Days to 50% maturity, NNP= Number of nodules per plant, AGBY= Above ground biomass yield, EN= Number of effective nodules per plant, DWN= Dry weight of nodules per plant, HSW= Hundred seed weight, HI= Harvest index, NB= number of branches per plant, NC= Number of clusters per plant, NP = Number of pods per plant, PH= Plant height, SYPP= Seed yield per plant, SYP= Seed yield per plot.

coefficients between each pair of characters are presented in table 4. The results showed that, in most cases, the genotypic correlation coefficients were higher than the phenotypic correlation coefficients which indicated the inherent association among various characters independent of environmental influence. Higher value of genotypic correlation coefficients than the phenotypic correlation coefficients between the pair of characters have been reported in Johnson *et al*., (1955) and Sharma *et al*., (1998). Significant and positive association between two characters suggests that these characters can be improved simultaneously in a selection program (Hayes et al., 1955).

Number of nodules per plant had positive and significant at both genotypic correlations with days to flowering and days to maturity. At phenotypic level, days to flowering and above ground biomass per plot were observed to be positive and significant correlation with number of nodules per plant.

Effective number of nodules per plant showed positive and significant association with days to flowering and days to maturity, and highly significant association with number of nodules per plant and dry weight of nodules per plant. At

phenotypic level effective number of nodules per plant showed positive and significant with days to flowering and above ground biomass yield per plot, and highly significant association with number of nodules per plant. Effective number of nodules showed positive genotypic and phenotypic correlation with both seed yield per plant and seed yield per plot.

This result indicates soybean genotypes that characterized by late flowering and maturing, tend to produce more effective number of nodules per plant and then this also leads to produce more seed yield per plant as well as seed yield per plot.

CONCLUSION AND RECOMMENDATION

The analysis of variance showed the presence of highly significant ($p<0.01$) differences among the tested genotypes for most of the characters, indicating the existence of variability among the tested genotypes for these characters. Number of nodules per plant showed significant positive genotypic correlation with both seed yield per plant and seed yield per plot. Effective number of nodules per plant showed positive and significant association with days to flowering and days to maturity, and highly significant association with number of nodules per plant and dry weight of nodules per plant. At phenotypic level effective number of nodules per plant showed positive and significant with days to flowering and above ground biomass yield per plot, and highly significant association with number of nodules per plant. Among the tested genotypes, TGX-1987-40F and TGX-1987-9F were found significantly superior in BNF and related traits. Therefore, from this study it can be suggested to use at least one of the genotypes as parent in the hybridization work for a future genetic improvement of biological nitrogen fixation.

ACKNOWLEDGEMENT

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