

Genetic Diversity and Character Association for Yield and Yield Related Traits in Soybean (*Glycine Max L.*) Genotypes

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ABSTRACT

The objectives of the study were to evaluate genetic diversity, heritability and genetic advance of traits in soybean genotypes and to estimate the magnitudes of associations among the different traits. The experiment was carried out at Uke Research Site, Western Ethiopia during 2018 main cropping season. The materials consisted of 100 soybean genotypes and was laid out in 10*10 simple lattice design. Agronomic traits were collected and statistical analysis was carried out using GLM procedure of SAS (SAS, 2004). The results showed that there were highly significant differences among the genotypes for all the traits except for number of primary branches per plant and number of nodules per plant. High genotypic and phenotypic coefficients of variations (greater than 15%) were observed for number of primary branches per plant, number of pods per plant, biological yield and seed yield. Higher values of broad sense heritability estimates (greater than 60%) coupled with higher values of GAM (greater than 20%) were observed for days to 50% emergence, grain filling period, biological yield, and seed yield indicating the possibility of the traits controlled by additive gene types and phenotypic selection for the traits could be useful. Both at genotypic and phenotypic levels, seed yield was highly and positively correlated with days to 95% maturity, plant height, number of primary branches per plant, number of pods per plant, biological yield and harvest index. Hence, indirect selection using these traits might improve the seed yield in present soybean populations. D2 statistics showed that the genotypes were clustered in to 10 diverse groups, indicating further genetic diversity in the genotypes. The principal component analysis revealed that the first four PCA have accounted for 61.96% of the total variation among the genotypes. In conclusion, there is sufficient genetic variability among the soybean genotypes which could be used for selection based on phenotypes or use for parental stocks for crossing program.

Keywords: Cluster; Correlation; Genetic advance; Genotype; Heritability; Soybean

INTRODUCTION

Soybean (*Glycine max L.*) is known as golden bean and one of the versatile crops in the world. It occupies an important position among grain legumes for its economic benefits. Seeds of soybean are used as food for humans, feed for animals and raw materials for industries. It contributes to soil fertility improvement through symbiotic nitrogen fixation [1]. Soybean is considered as the king of beans, due to its high protein and oil content in the seed, which is approximately 40% and 20%, respectively on dry weight basis, the protein in soybean has complete essential amino acid composition more than most other foods crops [2]. Soybean oil is the largest component of the world's edible oils with more than 30 % share and soybean is also used as the ingredient for more than 50% of

the world's high protein meal [3]. Soybean is known for its wide adaptability coupled with its higher productivity per unit area compared to other grain legumes. It could be grown from sea level up to 2200 m and with annual rainfall as low as 500-700 mm, but it performs best between 1300 and 1800 meter above sea level with annual rainfall of 900-1300 mm, and average annual temperature between 20-25°C and soil pH of 5.5 to 7 [4]. Globally, soybean production constitutes more than 6% of all total arable land in the world and has the highest percentage increase in area under production than any other crop [4]. According to FAO (2016) the total world soybean production was 320.15 million metric ton. Brazil, USA and Argentina are the three major producers in the world while South Africa, Nigeria and Zambia are the leading producers in Africa.

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Ethiopia has favorable climatic and soil conditions for soybean production and it grows well in several parts of the country i.e., which includes: western Eastern and southern Ethiopia regions [5]. In general, the low to mid altitude maize production areas of the country are believed to be suitable for soybean production [6]. In Ethiopia, soybean has increasing trends in production and productivity over the last decade. A total of 38,072.7 ha of land were covered by soybean (CSA, 2017) and ranks first in yield per ha⁻¹ among pulse and oil and 5th in area coverage among oil crops grown in the country. In the year 2017 the national average productivity of soybean was 2.27 tha⁻¹ (CSA, 2017), however, in research fields productivity of the crop, is up to 3 tha⁻¹ [7]. Currently, soybean is one of the focus subsectors supported by Ethiopian government and non-governmental organization. In the recent years several soybean processing plants have been set up in different parts of the country, which began demanding varieties with high grain nutritional compositions viz., oil and protein. The major production constraints that have been responsible for low productivity of the crop in the country includes: narrow genetic base/low yield; poor soil fertility and foliar diseases, which includes: Asian soybean rust caused (*Phakospora pachyrhizi*) and common bacterial blight (*Pseudomonas savastanoi* pv. Soja) [8].

Hence, introduction/collection of germplasm and assessment of genetic variability is a basic step in any crop improvement programme. Seed yield being a complex character controlled by polygenes influenced by environment, is interrelated to a number of yield contributing characters and also. Assessing the genetic variations in the existing germplasm is necessary to design the breeding program to get the desired yield level. The tools for genetic variations indirect measurement through estimating genetic parameters like Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), heritability and genetic

advance. In the absence of sufficient genetic variability in the existing genetic materials selection for desirable attributes cannot be realized. Therefore, the objectives of study were to estimate genotypic and phenotypic variances, heritability and expected genetic advance in the soybean genotypes; and to estimate the genotypic and phenotypic association among characters.

MATERIALS AND METHODS

Site description

The field experiment was conducted at Uke, East wollega, Western Ethiopia in 2018 cropping season. The Uke site is located 365km from Addis Ababa. The Site is located at 8° 11'52" and 10° 94'44" north latitude and 36° 97'51" and 37° 11' 52" East longitude, and at the altitude of 1500-1700 masl above sea level, with a prevalence of lowlands. The place has a mean maximum and minimum temperature is 31°C and 16°C with 1400 mm and 1200 mm rainfall respectively. The pH of the soil is acidic with red color of Nitosol a dominant soil type in the western Ethiopia (Guto gida Agricultural Bureau 2016, unpublished).

Experimental materials and design

A total of 100 genotypes (97 genotypes and 3 varieties) of soybean were obtained from Jimma Agricultural Research Center, Ethiopia and used for the study (Table 1). The experiment was laid out in 10*10 simple lattice design. Each genotype in the plot was grown in two rows of 4m lengths. A spacing of 60cm between rows and 5cm between plants was used. The crop was sown 8th of July, 2018 and NPS fertilizer was applied at the rate of 120 kg per ha at sowing time and all other recommended agronomic management practices were applied uniformly to all plots.

Table 1: List of soybean genotypes used in the study.

S/No	Accession	S/No	Accession	S/No	Accession
1	T29-15-T63-16-SA1	35	T47-15-T122-16-SA1	69	T6-15-T2-16-SA2
2	T23-15-T45-16-SA1	36	T46-15-T119-16-SA1	70	T27-15-T52-16-SB1
3	T73-15-T228-16-SC1	37	T70-15-T220-16-SG1	71	T27-15-T51-16-SA1
4	T73-15-T231-16-SF1	38	T27-15-T57-16-SG1	72	T55-15-T159-16-SA1
5	T45-15-T116-SC2	39	T24-15-T46-16-A2	73	T15-15-T20-16-SH1
6	T45-15-T155-16-SA2	40	T72-15-T225-16-SA1	74	Nyala
7	T15-15-T18-16-SF1	41	T50-15-T141-16-SC1	75	T35-15-T80-16-SE1
8	T35-15-T77-16-SB1	42	T35-15-T78-16-SC1	76	T48-15-T128-16-SA1
9	T74-15-T239-16-SE2	43	T27-15-T57-16-SG2	77	T44-15-T106-16-SD1
10	T63-15-T183-16-SB1	44	T54-15-T156-16-SB1	78	T25-15-T47-16-SA2
11	T16-15-T23-16-SC1	45	T49-15-T132-16-SA1	79	T45-15-T116-16-SA1
12	T6-15-T223-16-SB	46	T16-15-T27-16-SG3	80	T15-15-T16-16-SD1
13	T35-15-T79-16-SD1	47	T44-15-T107-16-SE1	81	T33-15-T68-16-SA1
14	T66-15-T193-16-SB1	48	T44-15-T111-16-SI1	82	Afgat
15	T53-15-T153-16-SC1	49	T52-15-T147-16-SA1	83	T28-15-T62-16-SD1
16	T52-15-T149-16-SC1	50	T70-15-T219-16-SF2	84	T34-15-T70-16-SA1
17	T55-15-T160-16-SB2	51	T25-15-T47-16-SA1	85	T19-15-T39-16-SD1
18	T74-15-T240-16-SF2	52	T49-15-T134-16-SC1	86	T56-15-T163-16-SA1
19	T56-15-T164-16-SB1	53	T25-15-T48-16-SB1	87	T26-15-T50-16-SB1
20	T50-15-T139-16-SB2	54	T52-15-T148-16-SB1	88	T27-15-T58-16-SH1
21	T42-15-T97-16-SA1	55	T33-15-T69-16-SB1	89	T24-15-T46-16-SA1
22	T44-15-T111-16-SI2	56	T29-15-T64-16-SB1	90	T56-15-T165-16-SC1
23	T54-15-T155-16-SA1	57	T39-15-T92-16-SC2	91	T27-15-T51-16-SA2

24	T28-15-T61-16-SC1	58	T44-15-T108-16-SF1	92	T76-15-T241-16-SC1
25	T16-15-T28-16-SH1	59	T27-15-T51-16-SA3	93	T39-15-T96-16-SG1
26	T27-15-T58-16-SH2	60	T37-15-T81-16-SA1	94	T47-15-T124-16-SC1
27	T19-15-T38-16-SC1	61	T53-15-T152-16-SB1	95	T51-15-T142-16-SA1
28	T52-15-T147-16-SA2	62	T16-15-T31-16-SK1	96	T47-15-T122-16-SA2
29	T73-15-T230-16-SE1	63	T71-15-T222-16-SA1	97	T51-15-T146-16-SE1
30	T74-15-T240-16-SF1	64	T27-15-T53-16-SC1	98	T57-15-T167-16-SB1
31	T71-15-T224-16-SC1	65	T62-15-T181-16-SA1	99	T55-15-T161-16-SC3
32	T75-15-T240-16-SA1	66	T74-15-T236-16-SB1	100	T50-15-T139-16-SA1
33	T54-15-T157-16-SC1	67	Clarck 63 K		
34	T74-15-T239-16-SE1	68	T67-15-T203-16-SG2		

Data collected

The following data were recorded: Days to 50% emergence, days to 50% flowering; days to 90% maturity; days to grain filling period; Plant height, number of primary branches per plant; number of nodules per plant; number of pods per plant; number of seeds per pod; hundred seed weight, biological yield, seed yield and harvest index.

Statistical data analysis: For statistical data analysis the following model is used:

$$Y_{ijk} = \mu + G_i + R_j + B_k + E_{ijk}$$

Where, Y_{ijk} is the performance of genotype i th in replication j th and block k th; μ is the general mean for each trait; G_i is the effect of i th genotype; R_j is the effect of j th replication; B_k is the effect of k th block; and E_{ijk} is the experimental error associated with i th genotype in j th replication and k th block. The data were subjected to analysis of variance using GLM procedures of the SAS.

Cluster analysis: Clustering of genotypes into different sets were carried out by average linkage clustering method. The proper numbers of clusters were determined by following the approach suggested by Copper and Milligan (1988) by looking into three statistics namely Pseudo F, Pseudo t^2 and cubic clustering criteria. The points where local peaks of the CCC and pseudo F statistic join with small values of the pseudo- t^2 statistic followed by a larger pseudo- t^2 for the next cluster fashion.

Genetic divergence analysis: A measure of a group distance based on multiple traits was given by generalized Mahalanobis D^2 statistics for quantitative characters in matrix notation, the distance between any two groups was estimated from the following relationship:

$$D_{ij}^2 = (X_i - X_j)' S^{-1} (X_i - X_j)$$

Where: D_{ij}^2 = the squared distance between case i and j ; X_i and X_j = vectors of the values of cases i th and j th genotypes; S^{-1} = the inverse of pooled variance covariance matrix within groups. Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of χ^2 (chi-square) and tested against the tabulated χ^2 values at $p-1$ degree of freedom at 1% and 5% probability level, where P = number of characters used for clustering genotypes.

Correlation coefficients: Correlation analyses were also done with proc corr procedures of SAS for genotypic and phenotypic correlation coefficients.

RESULTS AND DISCUSSION

The analysis of variance showed that genotypes were significantly

($p < 0.01$) different for days to 50% emergence, days to 50% flowering, days to 95% maturity, grain filling period, plant height, number of pods per plant, hundred seed weight, harvest index, biomass yield and seed yield. According to the studies by [9,10] genotypes were significantly different for number of pods per plant, days to 50% flowering, days to 95% maturity and seed yield; plant height, harvest index and seed yield.

Mean, ranges, genotypic and phenotypic variances

Mean values for mean, range, and other genetic parameters are presented. For phenological parameters days to 95% maturity followed by grain filling period and days to 50% flowering had high mean ranges while days to emergence had low mean range. From vegetative parameters higher mean ranges were observed for plant height and number of nodules per plant while number of primary branches had low mean range. From yield and related parameters biological yield followed by seed yield and number of pods per plant had higher mean ranges while hundred seed weight, harvest index, and number of seed per pod had lower mean ranges. From the results both the yield and its component and other parameters are variable in the genotypes which could be helpful for the future breeding activities. For days to 50% flowering, the genotype T67-15-T203-16-SG2 was the earliest (47.9 days) while genotype T74-15-T236-16-SB1 was the latest in flowering (63.5 days). Days to 95% maturity ranged from 94.5 days (T16-15-T28-16-SH1) to 125.0 days (T63-15-T183-16-SB1).

About forty two percent of the genotypes were earlier in maturity than the released varieties AFGAT. Similar trends of variability in phenology were also reported in soybean i.e., 49 to 64 for days to 50% flowering, and a range of 96 to 116 days for maturity [11]. The range of grain filling period was from 40.0 for the genotype T33-15-T68-16-SA1 to 69.0 for T35-15-T79-16-SD1 days with mean value of

63.5 days. The genotype T62-15-T181-16-SA1 had the highest plant height (92.17 cm). Abush reported a range of 43.3 - 77.5 cm plant height in various soybean genotypes. Therefore, when breeding for higher plant height is preferred the genotype should be considered.

Number of primary branches ranged from 1.9 for T74-15-T240-16-SF1 to 8.4 for T29-15-T63-16-SA1 with mean value of 5.2; number of nodules per plant ranged from 11.0 for T33-15-T69-16-SB1 to

42.0 for T46-15-T119-16-SA1. Number of seeds per pod ranged from 2.0-2.6 with mean value of 5.16; hundred seed weight ranged from 12.3g for T27-15-T53-16-SC1 to 18.3 g for T39-15-T92-16-SC2. The genotype T51-15-T146-16-SE1 had the highest number of pods per plant (78.00) indicating it could be the preferable genotype in breeding for high seed yield per plant. The present result is in agreement with Shankar (2014) who reported that high number of

Pods per plant (19.20 to 53.93) with a general mean of (48.82) in soybean genotypes.

The highest seed yield (2897 Kg ha⁻¹) was recorded from the genotype T51-15-T146-16-SE1 followed by T48-15-T128-16-SA1 (2,574 kg ha⁻¹) which were higher than the grand mean of the genotypes studied (1579 kg ha⁻¹). While low yield (883 kg ha⁻¹) was obtained from the genotype T16-15-T23-16-SC1. About 41 percent of the genotypes gave above the grand mean for seed yield. If the breeding objective is to improve seed yield, genotypes with high yield in this study need further evaluation over locations and year for genotype by environment stability studies. The range and mean values for different agronomic traits in the present study suggests there existence of sufficient variability among the tested genotypes for the majority of the characters including biological yield, harvest index, seed yield, hundred seed weight and number of pods per plant among others.

Broad sense heritability and genetic advance

Broad sense heritability values were greater than 60% for the traits including days to 50% emergence, days to 50% flowering, days to 95% maturity, grain filling period, hundred seed weight, seed yield and biological yield, while the values were between 40 and 60% for the traits including plant height, number of pods per plant and harvest index. Broad sense heritability values were less than 40% for the traits including number of primary branches per plant, number of nodules per plant and number of seeds per pod. High values of genetic advance were recorded for biological yield and seed yield indicating that using these populations through one cycle of selection for biomass yield and seed yield advance could be made by 1466 kg and 577 kg respectively. Medium genetic advance as percent of mean (between 10 and 20%) was recorded for days to 50% flowering and days to 95% maturity, plant height, number of primary branches per plant, number of nodules per plant, hundred seed weight. Relatively high genetic advance as percent of mean (greater than 20%) were recorded for the traits including days to 50% emergence, grain filling period, number of pods per plant and seed yield. The magnitudes of heritability for most of the quantitative characters were moderate to high, except for number of primary branches per plant and number of seeds per pod. Barh reported high heritability for days to 50% flowering in soybean. In addition different researchers [12-15] reported high heritability values for days to 50% flowering. When high heritability values are coupled with high genetic advance as percent of mean for the traits it indicates the predominance of additive gene action in the expression of the traits. It also indicates that selection can improve the traits. Moreover, according to moderate heritability was observed for pods plant-1 which is in line with the present findings.

The high values of heritability as well as genetic advance as percent of mean was observed for harvest index (%) indicating the predominance of additive gene action in the expression of this trait. Sriranjani reported high heritability value coupled with high genetic advance as percent of mean for harvest index. These finding confirms characters with high heritability allow more success in selection, so that the chance of obtaining superior progenies with selected individuals is larger. Indirect selection is used to obtain more gains with characters with low heritability, selecting correlated characters. In the present study higher genetic advance as percent of the mean coupled with higher heritability values were observed for days to 50% emergence, grain filling period and seed yield indicating the predominance of additive gene action in the

expression of these traits. Low estimate of heritability with low genetic advance expressed as percentage of mean was observed for number of nodules per plant revealed the presence of non-additive gene action and influence of environment in the expression of this character. Thus, the selection would be less effective for these trait.

CONCLUSION

The results showed that the genotypes exhibited considerable variation for most of the characters studied. For phenological parameters wide mean range was observed for days to maturity followed by grain filling period and days to flowering. From yield components biological yield had the highest mean ranges and the highest mean was 5743 kg ha⁻¹ (T57-15-T167-16-SB1) and it was about three fold of the lowest mean, 2109 kg ha⁻¹ (T16-15-T23-16-SC1). Generally, higher harvest index greater than the mean (45%) were recorded for most of the genotypes indicating high potential for productivity. Seed yield had the highest mean of 2897 kg ha⁻¹ and the lowest was 1579 kg ha⁻¹. About 41% of the genotypes gave above the grand mean for seed yield which could indicate most of the genotypes are productive and needs to evaluate over location and years for genotype by environment interaction. Among the tested genotypes the most four high yielding genotypes T44-15-T107-16-SE1 (2414 kg ha⁻¹), T55-15-T159-16-SA1 (2575 kg ha⁻¹), T47-15-T124-16-SC1 (2377 kg ha⁻¹) and T51-15-T146-16-SE1 (2897 kg ha⁻¹). Higher values of H₂ (greater than 60%) were observed for days to 50% emergence, days to 50% flowering, days to 95% maturity, grain filling period, hundred seed weight, biological yield and seed yield; while between 40-60% were observed for plant height, number of nodules per plant and harvest index. Highest value for genetic advance was observed for biological yield (1466 kg) followed by seed yield (577 kg), which indicates that selection for 5% of the top genotypes would increase biological yield and seed yield by 1466 kg and 577 kg biological yield and seed yield respectively in the new population after one cycle of selection. Higher values of H₂ and GAM were observed for days to emergence, grain filling period, number of pods per plant, biological yield and seed yield which could indicate these traits might be controlled by additive genes and selection would be successful in breeding program.

Seed yield had positive and significant correlation ($p \leq 0.01$) with days to 95% maturity, plant height, number of pods per plant and biological yield both at genotypic and phenotypic levels and with harvest index and number of primary branches per plant at genotypic and phenotypic level respectively. At phenotypic level; number of pods per plant had positive and highly significant correlation with hundred seed weight. Using D2 analysis, genotypes were clustered into ten groups and cluster I and VI; CI and CVI, CIII and CX; CIV and CX; and CIV and CX had maximum inter cluster distances; which indicates they are genetically more divergent and hybridization between genotypes of divergent clusters are likely to produce wide variability with desirable segregant. In addition, the principal components analysis showed that the first four PCs contributed 61.96% of the total variation among the 100 genotypes of soybean. The traits which contributed more to PC1 include: number of primary branches per plant, number of pods per plant, harvest index, biological yield and seed yield. This implies that these traits are the major contributors for the total variation in the studied genotypes and selection of genotypes for seed yield should targets these traits. Based on the present investigation results, it can be concluded that there is adequate genetic variability for most of quantitative characters evaluated, that the genotypes with high seed yield should be selected from different clusters and crossed so

as to improve seed yield. In general, genotypes with above grand mean for seed yield performances have been selected for further over locations and years testing, and selection for other traits could also be done with the populations since ample generic variability have been deselected.

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REFERENCES

- Ojo DK, Amira JO, Oduwaye OA. Genetic Variability for Biological Nitrogen Fixation Traits in Tropical Soybeans (*Glycine max* (L) Merr). *J Nat Sci*. 2007;5(2):11-15.
- Dugie IY, Omoigui LO, Ekeleme F, Lava Kumar RP, Kamara AY. Guide to soybean production in northern Nigeria. 2009;21.
- Jayeeta PD, Ghosh PR, Choudhury. An assessment of genetic relatedness between soybean [*Glycine max* (L.) Merrill] cultivars using SSR Markers. 2014.
- Hartman GL, West ED, Herman TK. Crops that feed the World. Soybean- worldwide production, use and constraints caused by pathogens and pests. *Food Security*. 2011;8:1-13.
- Wijnands JHM, Gurmesa ND, Lute JCM, Van Loo EN. Ethiopian soybean and sunflower value chains: Opportunities and challenge. 2011.
- FAOSTAT. Area harvested, total production and productivity of crops. 2011.
- Abush Tesfaye. Genetic analysis of quantitative traits in Soybean (*Glycine max* L. Merrill) under low and high phosphorus conditions. 2012;184.
- Rodrigues B, Seram F, Noguiera APO, Hamawaki OT. Correlation between traits in soybean (*Glycine max* L.) Naturally infected with Asian rust (*Phakopsora pachyrhizi*). *Genet Mol Res*. 2015;14:17718- 17729.
- Agdew B, Getnet A. The studied genetic variability in 49 soybean genotypes at Hawassa and Gofa, southern Ethiopia. 2006.
- Iqbal Z, Arshad M, Ashraf M, Naeem R, Malik MF, Waheed A. Genetic divergence and correlation studies of soybean [*Glycine max* (L.) Merrill.] genotypes. *Pak J Bot*. 2010;42(2):971-976.
- Tadesse G, Sentayehu A. Genetic divergence analysis on some soybean (*Glycine max* L. Merrill) genotypes grown in Pawe, Ethiopia. *American- Eurasian Journal Agric Environ Sci*. 2015;15(10):1927-1933.
- Barh A, Pushpendra R, Khulbe K, Meenakshi J. A new source of genetic divergence for soybean improvement. *Afr J Agric Res*. 2014;9(1):119-124.
- Gohil VN, Pandya HM, Mehta DR. Genetic variability for seed yield and its component traits in soybean. *Agric Sci Digest*. 2006;26(1):73- 74.
- Malik MF, Afsari SQ, Muhammad A, Ghafoor A. Genetic variability of the main yield related characters in soybean. *Int J Agric Bio*. 2006;8(6):815-819.
- Abady S, Fitsum M and Zinaw D. Heritability and path coefficient analysis in soybean (*Glycine max* L. Merrill) genotypes at Pawe, North Western Ethiopia. *J EnvSci Water Resou*. 2013;2(8):270-276.