International Journal of Biotechnology Research Vol. 6(1), pp. 001-012, March, 2020
Available online at http://academeresearchjournals.org/journal/ijbr
ISSN 2328-3505 ©2020 Academe Research Journals

Full Length Research Paper

Genetic variability and molecular characterization of F2 population of soybean, *Glycine max* (l.) Merrill genotypes using SNP markers

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Accepted 11 January, 2020

This study was carried out on the genetic variability and molecular characterization of F2 population of soybean, *Glycine max* (l.) Merrill genotypes using SNP markers. The aim of the study was to assess the magnitude of genetic variability, heritability and genetic advance and estimating the genetic diversity among the population using SNP markers. The field experiment was laid out in a randomized complete block design (RCBD) with three replications. The mean performance and genetic components of yield and its related characters were estimated as well as the genetic diversity among the F2 populations using SNP markers. The results showed that TGx1835–40E x TGx1830–20E(A), TGx1830–20E x TGx1990–57F(B) and TGx1990 – 3F x TGx1990–37F(B) recorded the shortest days to flowering while TGx1835–40E x TGx1830–20E(A) and TGx1835–40E x TGx1830–20E(B) recorded the highest mean values for seed yield. Heritability in the brad sense ranged from 40.00% to 99.97% for number of seeds and number of pods respectively. The difference between PCV% and GCV% ranged between 0.18 and 15.87 for number of pods and number of seeds respectively. The genetic advance mean ranged from 10.03 to 130.17 for days to maturity and seed yield respectively. At the molecular level, SNP markers were used to assess the extent of polymorphism among the F2 populations. The markers showed remarkable genetic diversity.

Key words: Genetic variability, molecular characterization, F2 population, soybean genotypes, SNP markers

INTRODUCTION

Soybean, *Glycine max* (L.) Merrill belongs to the family Fabaceae. It is the most important leguminous seed crop among the oil crop plants, which accounted for 56% of global oil production in the international market in 2011. In the international trade markets, it is ranked number one in world oil production among the major crops such as cotton seed, peanut, sunflower seed, rape seed, coconut and palm kernel. (Soy Stats, 2012). Globally, soybean production constitutes 6% of all total arable land in the world and has the highest percentage increase in area under production than any other crop (Hartman *et al.,* 2011). According to FAO (2018), total world production at 2016 was 336,894,085 million metric tonnes. The five major world producing countries are U.S.A (117,208,380 million metric tonnes), Brazil (96,296,714 million metric tonnes), Argentina (58,799,258 million metric tonnes), China (11,963,244 million metric tonnes) and India (14,008,000 million metric tonnes). Nigeria is the leading producer in West Africa with 588,201 metric tonnes with 45% of the total production coming from Benue State (FAO, 2018).

Soybean is grown primarily for the production of seed and has several uses in the food and industrial sectors. It

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represents one of the major sources of edible vegetable oil and proteins for livestock feed. The demand for soybean as a raw material for the oil and poultry industries has been on the ascendency over the years. Its nutritional benefits for the consumer have been well documented (Asafo-Adjei *et al*., 2007; Mebrahtu, 2008).The importance of soybean worldwide is due mostly to its high oil and protein components, which are approximately 20 and 40 percent of seed concentrations, respectively. The great proportion of the world's soybean seed, about 85%, is crushed to produce soybean oil and meal. Soybean meal, as a by-product of soybean processing, is primarily used as protein source for livestock. However, a small amount of the meal, about 2%, is also used in preparing food use products such as soy flours, beverages, and toppings (Soyatech, LLC, 2011). Consumption of foods containing soybean and soybean constituents has been associated with reduced heart disease risk factors, reduced osteoporosis, alleviation of menopausal symptoms, reduced cancer risk and reduced diabetes. It also help people to stay lean (reduced obesity) (Maskarinec *et al.,* 2008). Isoflavone compounds found in soybean, especially genistein help to stay lean by causing us to produce fewer and smaller fat cells (Naaz *et al.,* 2003). Soybean works in the prevention of heart problems and stroke by lowering cholesterol (AHA, 2000; Desroches *et al.,* 2004; Teixeira *et al.,* 2000; Rosell *et al.,* 2004).

Genetic improvement of crop species is necessary to enhance their economic traits such as yield, resistance to abiotic and biotic stresses, etc. and thus forms the ultimate goal of plant breeding. Over the last three decades a new class of markers, namely, molecular markers or DNA markers has been introduced. They possess unique advantages over the phenotypic and biochemical markers. They look directly at the basic level of variation, i.e. DNA level giving direct insight into the genetic makeup, screen the whole genome and reveal variations in both coding and non-coding regions and hence offer large extent of polymorphism. DNA markers are highly amenable to automation and once automated, they can be used as efficient selection tools by the plant breeders and contribute in Marker Assisted Selection (MAS). The very powerful PCR – based have emerged which are very fast, reliable and require minimal amount f tissue for investigation. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP) and Simple Sequence Repeats (SSR) have large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis and evaluation of genetic diversity within and between species and populations (Guo-Liang , 2013).

DNA markers have now become the marker of choice for breeders in their crop genetic studies, and have revolutionized the practical applications of plant biotechnology (Kumar *et al*., 2009). The rapid advancement in molecular marker technology has provided new classes of genetic markers at the DNA level. It plays a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programs (Kasha, 1999). These developments have stimulated new interest in exploring the applications of genetic markers in plant breeding and allow breeders to dissect complex traits without having to measure the phenotype, thus reducing the need for extensive field-testing over time and space.

In recent times, single nucleotide polymorphism (SNP) markers have become available, and because of their huge numbers and the ability to genotype without using electrophoretic gel systems, soybean breeders and geneticists have moved quickly to use SNP markers in their work (Zhu *et al.,* 2003; Choi *et al*., 2007; Brown and Caligari, 2008). The efficiency of DNA based marker is so high to discriminate closely related varieties and even individuals of same species. They have proved their utility in various fields such as genetic diversity, genomic fingerprinting and mapping, population genetics, taxonomic studies and plant breeding programs.

DNA markers have specific biological function. The genetic markers have differences with respect to copy number, polymorphic profile, locus specificity level of reproducibility, technical requirement and financial value (Kumar *et al.,* 2009). The objectives of this study is to (i) assess the magnitude of genetic variability, heritability and genetic advance from selection of important characters in soybean and (ii) to evaluate the genetic diversity among the genotypes using molecular markers.

MATERIALS AND METHODS

The experimental materials for the present study consisted of seven genotypes collected from the soybean germplasm collection of the international institute of tropical agriculture, Ibadan, Oyo – State , Nigeria. The experiment was carried out in phases. The first phase was the generation of the F_1s from the crossing of the parental lines. The F_1 seeds were later planted to generate the F_2 generations through self-pollination. The second phase of the experiment was the molecular analysis using SNP markers. The field experiment was carried out on the Teaching and Research Farm of the Federal University Of Technology, Akure, Ondo – State, Nigeria in year 2014 and 2015 respectively. The experiment was laid out in a randomized complete block design (RCBD) with three replications. A single row plot was adopted. Fifteen plants were maintained per plot with an inter and intra row spacing of 60cm and 20cm respectively. Standard agronomic and plant protection treatment were carried out uniformly across the plots for the duration of the experiment. Data were collected on

ten competitive mid – plants on the following agronomic characters: plant height at flowering (PHTF), days to flowering (DTF), number of branches per plant (NBP), plant height at maturity (PHTM), days to maturity (DTM), number of pods per plant (NPP), number of seeds per pod (NSP), pod length per plant (PL) and seed yield per plant (SYP).

DNA EXTRACTION

Total genomic DNA was extracted using the modified mini preparation protocol described by Dellaporta *et al.,* (1983) as follows:Approximately 200mg (0.2g) of lyophilized leaf sample was ground into fine powder. To each tube 700ul of hot (65^{o}C) plant extraction buffer (PEB) [containing 637.5ml of double distilled water (ddH_20), 100ml of 1M Tris-HCl (pH 8.0), 100ml of 0.5M ethylene diamine tetraacetic acid (EDTA) (pH 8.0), 100ml of 5M $Nacl_2$ and 62.5ml of 20% sodium dodecyl sulphate (SDS)] was added . One percent b-mercaptoethanol was added to the pre- warmed PEB just before use. The tubes were capped and inverted gently 6-7 times to mix the sample with buffer.The solution was incubated at 65°C in water bath for 20 mins with occasional mixing to homogenize the samples. After 20 mins, samples were removed from the water bath and uncapped. The tubes were allowed to cool at room temperature for 2 minutes after which 500ul of 5M of potassium acetate (CH_3COOK) was added to each tube and recapped. The tubes were then mixed by gently inverting 6-7 times and incubated on ice for 20 minutes.After 20 minutes of incubation on ice tubes were spun at 12,000 rpm for10 minutes at 4°C. The supernatant was transferred into new 1.5ml eppendorf tubes using wider bore pipette tips (1000 µl) and making sure debris were not taken along with the supernatant. 700µl chloroform isoamylalcohol was added to the supernatant and spun at 10,000 rpm for 10 minutes. The supernatant was transferred again into a new correspondingly labeled tubes and 700µl ice-cold isopropanol was added to each tube and mixed by gently inverting the tubes 6-10 times. The tubes were allowed to stand undisturbed in a rack and stored in a freezer (-20°C) for at least 1 hour or overnight to precipitate the DNA. After 1-hour precipitation in the freezer, the tubes were centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was carefully discarded with great care to disallow the pellet from dislodging from the bottom of the tube. The tubes were allowed to drain inverted on clean paper towels for 1 hour or more.The DNA pellets were washed twice in 100µl, cold 70% ethanol for 20 minutes and air dried completely. After drying, 60µl of 1×TE [10mM Tris-HCL (pH 8.0), 1mM EDTA (pH8.0)] was added to the pellets, followed by 2µl of 10ng/ml Rnase to remove the RNA.

The solution was incubated for 40 minutes at 37°C with gentle mix at 10 minutes intervals.

SNP Analysis

SNP genotyping was done at Inqaba Biotechnical Industries (Pty) Ltd Pretoria, South Africa on the MassARRAY system from Agena Biosciences using the iPLEX reagents which included the iPLEX PCR, SAP, and iPLEX Extend following the iPLEX Gold Application Guide from Agena Biosciences (http://www.sequenom.com/Files/Genetic-Analysis—Graphics/iPLEXApplication/iPLEX-Gold-Application-Guide-v2r1) (Gabriel *et al*., 2009; Masouleh *et al.,* 2009; Pattemore and Henry, 2008). The procedure of iPLEX PCR is the same as the normal PCR. Briefly, 10 ng genomic DNA was amplified in a 5µl reaction containing 1 x HotStar Taq PCR buffer (Qiagen), 1.625 mM $MgCl_2$, 0.5 mM each dNTP, 0.1µM each PCR primer, and 0.5 U Hot Star Taq DNA polymerase (Qiagen). The reaction was incubated at 94^{o}C for 4 min followed by 45 cycles of 94^{o}C for 20 s, 56^{o}C for 30 s, 72^{o}C for 1 min, and then followed by 3 min at 72^{o}C. After iPLEX, excess dNTPs were removed from the reaction by adding 2 µl shrimp alkaline phosphatase (SAP) enzyme solution (1.53 µl water (HPLC grade), 0.17 µl SAP buffer (10x), 0.30 µl SAP enzyme (1.7 U/ µl)) into each sample well and mixed, and then incubated at 37^{o}C for 20 minutes followed by 5 minutes at 85^{o}C to deactivate the enzyme – called SAP procedure in iPLEX.

Extension Reaction

Extension Primers were synthesized at Inqaba Biotechnical Industries Pty Ltd. Pretoria South Africa. They were diluted to a stock concentration of 500 µM. This stock was split into a four-tier concentration grouping of 7µM, 9µM, 11µM and 14µM according to extension primer mass from smallest to largest. This four-tier system was used for Oligovalidation and peak optimisation on the Maldi-Tof Then, the iPLEX extend was carried out with a final concentration of between 0.625 and 1.5 l µM for each extension primer, depending on the mass of the probe, iPLEX termination mix (Agena Biosciences) and 1.35µM iPLEX enzyme (Agena Biosciences) and conducted a two-step cycles program; 94^{o}C for 30 s followed by 40 cycles of 94^{o}C for 5 s, then followed 5 cycles of 52^{o}C for 5 s, and 80^{o}C for 5 s within the 40 cycles, then 72^{o}C for 3 min in the 40 cycles. The reaction was then desalted by addition of 6 mg resin to each well followed by mixing and centrifugation to settle the contents of the tube. The extension product was spotted onto a 96- well spectrochip before being flown in the MALDI-TOF (Matrix – Assisted Laser Desorption Ionisation Time of Flight) mass spectrometer (Agena Biosciences).

Bands were detected n UV-transilluminator and photographed by Gel documentation 2000, Bi o– Rad.

Table 1. The Names and Source of Soybeans, Glycine max Genotypes

Parental No	Genotype Name	Source
1	TGx1835 – 40E	International Institute
2	TGx1990 – 55F	of Tropical Agriculture
3	TGx1990 – 3F	(IITA) Ibadan, Oyo, State Nigeria
4	TGx1990 – 37F	
5	TGx1989 – 21F	
6	TGx1830 – 20 E	
7	TGx1990 – 57F	

STATISTICAL ANALYSIS

Analysis of variance was conducted using individual plot means for each year and combined across years. Statistical analysis was computed using the GLM (General linear model of Plant Breeding tools software). Estimates of phenotypic and genotypic variance were obtained from the combined analysis for the F_2 genotypes. Broad sense heritability (H^2bs) and genetic parameters were detected through variance component method (Larik *et al.,* 1987) as follow:-

Genetic variance= $\sigma^2 g = \frac{MSG-MSE}{R}$

Phenotypic variance = $\sigma^2 ph = \sigma^2 g + \sigma^2 e$

Heritability = $\sigma^2 g / \sigma^2 ph$

Selection index (s) = K σph

Genetic Advance = hb x K x σph

Genetic advance % = GA / X x 100

Where:

MSG and MSE are genotypic and error mean squares respectively,

r is the number of replications,

X is population mean and

K is a constant = 2.06 (Kang *et al.,* 1983).

The phenotypic coefficient of variation (PCV) was calculated as :

PCV = ($\sigma^2 ph$ / X) x 100

The genotypic coefficient of variation (GCV) was calculated as :

GCV = ($\sigma^2 g$ / X) x 100

Where X = Grand mean of all genotypes

The presence and absence of bands were scored 1 or 0 respectively.

Construction of the dendogram tree was performed using the unweighted pair group method based on arithmetic mean (UPGMA) in the Minitab program version 17.

RESULTS

The analysis of variance for the various characters considered for the F_2 populations is presented in Table 2. The results revealed that there exists a significant difference for all the characters except number of branch per plant. A highly significant estimates for genotype x year interaction was also recorded in Plant height at flowering, Plant height at maturity, Number of pods per plant and Seed yield per plant.

SOV= Source of Variation; DTF= Days to flowering (days); PHTF= Plant Height at Flowering (cm); NBP= Number of Branches per Plant; DTM = Days to Maturity (days); PHTM = Plant Height at Harvesting (cm); NPP = Number of Pods per Plant; NSP = Number of Seeds per Plant; PL=Pod Length per Plant (cm); SYP = Seed Yield per Plant (g).

The mean performance of the F_2 population for the various characters under study is presented in Table 3. The result revealed that the estimates of the characters were higher in the second year than the first year. In the first year, with regard to Days to flowering, the highest mean was observed in P1 X P5B (44.94 days) while the lowest value (37.55days) was recorded in P1 X P6A and P6 X P7B respectively. For Plant height at flowering and Number of branches per plant, the highest mean value were recorded in genotypes, P3 X P5C and P3 X P5B (37.57cm) and (10.51) respectively. DTM recorded the highest mean (87.70days) in P1 X P5B while the lowest value (79.11days) was recorded in P1 X P6A and P6 X P7B respectively. Highest mean value (177.90) was recorded for Number of pods per plant in P5 X P6C while the lowest mean value was recorded in P2 X P5C (103.00). Regarding Seed yield per plant, the highest mean value (38.17g) was recorded in P2X P6A while the lowest mean value (12.21g) was recorded in P4 X P7C.

In the second year, Days to flowering recorded the lowest mean value (49.11days) in P1 X P6A, P3 X P4B and P6 X P7B respectively while the highest value (64.0days) was recorded in P4 X P5B. Plant height at flowering recorded the highest mean value (68.13cm) in P1 X P4B while the lowest value (28.73cm) was recorded in P4 X P6C. The highest mean value for Number of pods per plant (242.20) was recorded in P3 X P6C while the lowest value (134.78) was recorded in P2 X P4B. The highest mean value (3.53) for pod length, was recorded in P2 X P6A while the lowest value (2.40) was recorded

Table 2. Analysis of Variance for Characters under Study in F_2 population of Soybean, Glycine max Across Two Cropping Years

SOV	Df	DTF (days)	PHTF (cm)	NBP	DTM (days)	PHTH (cm)	NPP	NSP	PL (cm)	SYP (g)
Year	1	12630.90**	6093.09**	160.74*	124579.40**	108102.30**	200137.40**	5.12**	3.78	24544.38**
Block (Year)	4	4.72	281.52**	35.10**	4.61	171.10**	6826.95**	0.65**	1.32**	697.70**
Genotype	62	32.75**	147.34**	1.65	33.05**	357.84**	682.05**	0.10**	0.26**	194.34**
Genotype x Year	62	6.58	78.53**	2.14	6.45	42.27**	369.68*	0.06	0.13	124.04**
Error	248	5.48	14.42	1.63	5.44	19.49	279.55	0.05	0.12	62.00

*,** significance at 5% and 1% level of probability respectively

in P4 X P5C. Regarding Seed yield per plant, the highest value (85.75g) was recorded in P1 X P6B while the lowest value (25.80g) was recorded in P4 X P5C.

The estimates of the genetic characters under study are presented in Table 4. The result showed that the estimates were higher in the second year than the first year. The genotypic variance ranged from 0.23 (Number of Seeds per Plant) to 808.61 (Number of Pods per Plant) and 0.22 (Number of Seeds per Plant) to 2106.18 (Number of Pods per Plant) in the first and second year respectively. The highest estimates of PCV were recorded in Number of Pods per Plant (581.91) and in Seed yield per plant. (1676.84) in the first and second year respectively. The Hb estimates were very high for almost all the characters under study being highest in Plant Height at Maturity (99.89) and (99.88) in the first and second year respectively.

MOLECULAR MARKER

The levels of polymorphism for the F2 population of Soybean by SNP markers are presented in Table 5. 32 SNP primers were used to differentiate among the F_2 population. A total of 322 bands were recorded. 214 of them were polymorphic (66.45%) and 108 were monomorphic (33.55%) . the number of amplified band per primer ranged from 3 to 15 bands a maximum number 15 bands were amplified by BARC – 030337-06857, BARC –040459 – 07745 and BARC –041267-07957 while a minimum number of 3 bands was amplified by the primer BARC –018933 – 03040. The highest polymorphism % (100%) was recorded by primer BARC – 014847 – 01910 and BARC –030337 – 06857 and lowest (0%) was recorded in BARC –018933 – 03040 and BARC –041819 – 08107.

The distribution of the polymorphic SNPs across the soybean genotypes is shown in Figure 1. From the figure, the highest number of markers were found to be associated with 41 and 42 polymorphic soybean genotypes respectively. 2 of the markers recorded no polymorphism with the soybean genotypes while 2 markers recorded 100% polymorphism with the genotypes. 25% of the markers recorded polymorphism with 62 out of the 63 Soybean genotypes. (BARC 021831-04219, BARC 024333-04850, BARC 040459-07745 and BARC 041267-07957).

A dendogram based n UPGMA cluster analysis is shown in Figure 2.The dendogram revealed that the F_2 populations were grouped into five clusters: cluster 1 (P3 X P5B), cluster 2 (P2 X P5B), cluster 3 (P4 X P6C) , cluster 4 (P1 X P3C) while the other F_2 populations were grouped under cluster 5.

Figure 2: An UPGMA cluster dendogram showing the genetic relationships among the F2 population of Soybean genotypes
P1=TGx1835 – 40E; P2=TGx1990 – 55F; P3 = TGx 1990 – 3F; P4 = TGx 1990 – 37F; P5 = TGx 1989 – 21F; P6 = TGx 1830 – 20 E; P7 = TGx 1990 – 57F

DISCUSSION

Genetic improvement of any crop depends upon the nature and extent of genetic variability available and also on the magnitude of interrelationships of heritable and non- heritable variation in yield and its major contributing characters (Nayak *et al.,* 2017; Joshi *et al*., 2018). This would ensure organized and systematic hybridization programme for creating genetic variability to be exploited for genetic improvement of the trait under consideration. Evaluation of genotypes for assessing the extent of variability is the first step in any crop improvement program (Abebe et al., 2017).

The results from this study indicated wide genetic variability among the genotypes for the different characters studied. This provides good opportunity for selection among the genotypes for the agronomic

Table 3. Mean Performance of Characters under Study for F2 population of Soybean, *Glycine max* Across Two Cropping Years

GENO	YEAR	DTF	PHTF	NBP	DTM	PHTM	NPP	NSP	P.L	SYD
1 X 2A	1	44.36 k-t	27.46k	9.03abc	87.03gh	57.61c	115.57hij	2.08ab	2.46ab	22.89g-k
	2	57.86 a-f	32.79g-k	10.41abc	127.89a-f	84.13ab	165.82d	2.34ab	2.65ab	34.71d-k
1 X 2B	1	39.39t	33.41g-k	8.71bc	81.72h	68.76bc	142.49g	2.18ab	2.92a	29.26d-k
	2	50.86f-r	35.06f-k	9.37abc	120.85f	89.03ab	213.38a	2.49ab	3.02a	63.03abc
1 X 2C	1	39.21t	32.53g-k	10.21abc	81.04h	67.30bc	143.53g	2.28ab	2.76a	27.50f-k
	2	51.64c-k	40.34d-k	10.98abc	121.64c-f	87.07ab	194.67b	2.49ab	2.99a	47.49a-j
1 X 3A	1	41.54st	30.32ijk	9.37abc	83.75h	77.72b	134.39gh	2.38ab	2.28b	27.02f-k
	2	53.78b-h	41.71d-k	11.36ab	123.79b-f	101.27a	160.26d-f	2.44ab	2.97a	28.52f-k
1 X 3B	1	44.78k-t	23.98k	9.09abc	87.51gh	59.00	117.52hij	2.18ab	2.43ab	20.74ijk
	2	55.92a-g	30.32ijk	9.44abc	125.94a-f	85.92ab	161.70d-f	2.29ab	2.65ab	30.38d-k
1 X 3C	1	40.04st	31.50h-k	7.91c	82.00h	52.83cd	103.96ijk	2.17ab	2.49ab	17.16ijk
	2	52.03c-l	32.79g-k	9.44abc	122.03c-f	86.33ab	173.96cd	2.28ab	2.54ab	28.50f-k
1 X 4A	1	38.30t	31.50h-k	7.08c	79.98h	62.59bc	144.83fg	2.60a	3.00a	30.72d-k
	2	55.92a-g	38.28d-k	9.37abc	125.94a-f	86.94ab	191.50b	2.78a	3.23a	65.01abc
1 X 4B	1	40.54st	30.67ijk	9.66abc	82.59h	56.82c	140.24g	2.54a	2.66ab	29.41e-k
	2	52.61c-l	68.13a	9.77abc	122.61c-f	90.00ab	189.90b	2.68a	3.14a	47.57a-j
1 X 4C	1	41.15 st	27.94jk	10.21abc	83.29h	61.66bc	172.03cd	2.63a	2.89a	36.81d-k
	2	51.71c-k	32.68g-k	13.11a	121.05c-f	83.88ab	205.39a	2.78a	3.31a	60.44abc
1 X 5A	1	44.29k-t	33.85g-k	9.09abc	86.94gh	65.09bc	124.89h	2.34ab	2.39ab	23.23g-k
	2	57.65a-f	42.46d-k	9.93abc	127.68a-f	100.89a	169.76d	2.58a	2.60ab	33.97d-k
1 X 5B	1	44.94k-t	32.68g-k	9.39abc	87.70gh	73.27b	136.05gh	2.41ab	2.55ab	26.56f-k
	2	56.68a-g	50.37ab	10.98abc	126.70a-f	110.15a	178.31c	2.68a	2.94a	39.13c-k
1 X 5C	1	40.41st	39.36d-k	9.62abc	82.43h	78.14b	135.07gh	2.33ab	2.54ab	23.65g-k
	2	52.32c-l	43.14d-k	10.98abc	122.32c-f	97.35a	173.91cd	2.38ab	2.68ab	32.77d-k
1 X 6A	1	37.55t	27.62k	8.70bc	79.11h	59.60c	149.51fg	2.65a	2.73a	33.21d-k
	2	49.11f-r	32.08g-k	9.50abc	119.09f	81.30ab	189.36b	2.88a	3.34a	47.50a-j
1 X 6B	1	39.83t	32.31g-k	9.92abc	81.77h	58.26c	148.00fg	2.62a	3.18a	32.70d-k
	2	50.77f-r	36.49f-k	14.19a	120.76ef	90.00ab	228.58a	2.78a	3.26a	85.75a
1 X 6C	1	38.38t	32.42g-k	9.24abc	80.08h	55.13c	143.96g	2.44ab	2.82a	26.95f-k
	2	50.08f-r	36.21f-k	10.67abc	120.07ef	80.76ab	235.60a	2.58a	3.02a	64.27abc
1 X 7A	1	38.96t	31.20 h-k	9.17abc	80.75h	54.43cd	137.32gh	2.56a	2.33ab	24.11g-k
	2	49.60f-r	31.90 h-k	10.98abc	119.58f	84.29ab	154.71f	2.68a	3.11a	29.35e-k
1 X 7B	1	39.38t	32.10 g-k	9.80abc	81.24h	62.19bc	157.70ef	2.58a	2.72a	35.39d-k
	2	51.71d-t	36.15 f-k	9.97abc	121.05c-f	86.33ab	189.93b	2.78a	3.26a	49.15a-j
1 X 7C	1	39.21t	31.67 h-k	9.76abc	81.04h	47.46	118.10hij	2.25ab	2.63ab	20.90ijk
	2	51.71d-t	34.74 f-k	9.77abc	121.05c-f	85.11ab	175.17c	2.38ab	2.83a	35.09d-k
2 X 3A	1	42.29st	29.25jk	8.03bc	84.62gh	61.20bc	134.39gh	2.54a	2.68ab	29.85e-k
	2	61.64a	32.69g-k	8.50bc	131.56ab	89.40ab	183.59bc	2.68a	3.21a	45.96a-k
2 X 3B	1	40.87st	26.25k	8.43bc	82.97gh	56.62c	121.03h	2.43ab	2.70a	24.63g-k
	2	59.81a-d	45.99d-k	9.44abc	129.85ab	101.27a	175.47c	2.58a	2.92a	47.82a-j
2 X 3C	1	42.53st	26.78k	9.90abc	84.91gh	55.22c	140.15g	2.41ab	2.44ab	22.89ijk
	2	61.75a	44.57d-k	13.18a	131.80ab	90.00ab	161.53d-f	2.58a	2.82a	27.12f-k
2 X 4A	1	42.62st	29.36jk	9.30abc	85.00gh	62.08bc	147.85fg	2.59a	2.61ab	32.25d-k
	2	55.04a-g	36.15 f-k	10.70abc	125.06a-f	72.41b	177.42c	2.68a	3.22a	39.72c-k
2 X 4B	1	38.79t	25.56k	9.70abc	80.56h	58.41c	116.70hij	2.41ab	2.10b	17.26ijk
	2	52.03c-l	31.02h-k	10.98abc	122.03c-f	82.66ab	134.78gh	2.48ab	2.65ab	24.57g-k
2 X 4C	1	40.12st	33.41g-k	10.84abc	82.10h	63.59bc	157.12ef	2.53a	2.88a	30.52d-k
	2	51.06d-m	35.06f-k	11.93ab	121.70c-f	94.90ab	220.25a	2.68a	3.16a	52.89a-h
2 X 5A	1	40.96st	33.29g-k	9.03abc	83.07gh	66.37bc	140.24g	2.46ab	2.31ab	26.60f-k
	2	53.37b-h	33.85g-k	11.45ab	123.38b-f	90.62ab	162.15d-f	2.58ab	3.00a	26.91f-k
2 X 5B	1	40.37st	31.20h-k	9.23abc	82.39h	76.33b	142.39g	2.47ab	2.57ab	28.09e-k
	2	52.42c-l	46.00d-k	12.21ab	122.42c-f	100.30a	180.44bc	2.58a	2.89a	40.61c-k
2 X 5C	1	41.06st	35.03f-k	9.31abc	83.19gh	66.41bc	103.00ijk	2.21ab	2.70a	18.18ijk
	2	52.72c-l	52.84ab	13.00a	122.72c-f	104.70a	191.61b	2.48ab	2.71a	44.27b-k
2 X 6A	1	38.21t	28.20k	8.98bc	79.88h	56.42c	154.88ef	2.73a	2.91a	38.17c-k
	2	49.87f-r	34.03g-k	9.84abc	119.86f	83.47ab	201.92a	2.08ab	3.53a	58.00a-d
2 X 6B	1	38.84t	30.85jk	9.76abc	80.62h	54.25cd	147.86fg	2.70a	3.11a	34.68d-k
	2	51.06d-m	31.30h-k	15.29a	121.05c-f	82.38ab	232.67a	2.88a	3.39a	84.77a
2 X 6C	1	39.21t	28.54k	9.90abc	81.00h	58.61c	126.69h	2.45ab	3.11a	26.84f-k
	2	51.06d-m	33.64g-k	12.59ab	121.05c-f	86.58ab	251.68a	2.48ab	3.13a	68.64ab

Table 3. Cont'd

2 X 7A	1	39.87t	33.15g-k	8.71bc	81.72h	60.00c	120.64h	2.40ab	2.62ab	26.83f-k
	2	51.83c-k	37.44f-k	9.30abc	121.73c-f	93.92ab	159.97ef	2.58a	2.91a	33.25d-k
2 X 7B	1	39.54t	27.48k	10.57abc	81.43h	59.41c	162.97d-f	2.59a	2.49ab	35.88d-k
	2	51.45d-t	46.94d-k	11.74ab	121.44c-f	102.74a	175.66c	2.68a	2.88a	37.48c-k
2 X 7C	1	39.63t	28.28k	9.97abc	81.52h	57.21c	139.27g	2.44ab	2.83a	25.62f-k
	2	51.06d-m	38.28d-k	12.46ab	121.05c-f	83.01ab	205.94a	2.58a	2.88a	43.64b-k
3 X 4A	1	39.04t	23.66k	8.16bc	80.85h	57.81c	137.42gh	2.54a	2.48ab	29.85e-k
	2	57.57a-f	29.61jk	8.24bc	127.60a-f	79.72b	165.53d	2.68a	3.02a	34.27d-k
3 X 4B	1	38.63t	26.78k	8.56bc	80.37h	47.26	125.32gh	2.30ab	2.63ab	21.69ijk
	2	49.11f-r	31.26h-k	8.62bc	119.09f	85.11ab	211.72a	2.48ab	2.92a	59.32a-d
3 X 4C	1	39.21t	31.90h-k	9.84abc	81.04h	55.82c	145.80fg	2.44ab	2.55ab	24.09g-k
	2	51.06d-m	35.62f-k	11.19ab	121.05c-f	86.74ab	173.65cd	2.58a	3.27a	31.99d-k
3 X 5A	1	42.78st	35.62f-k	9.57abc	85.20gh	75.53b	118.88hij	2.43ab	2.43ab	23.59g-k
	2	55.24a-g	44.57d-k	11.45ab	125.25a-f	104.70a	163.87d-f	2.58a	2.47ab	31.38d-k
3 X 5B	1	42.20st	35.98f-k	10.51abc	84.52gh	85.09ab	158.19ef	2.57a	2.47ab	30.07e-k
	2	54.73 b-h	42.46d-k	9.09abc	124.74a-f	97.08a	167.70d	2.68a	2.57ab	32.58d-k
3 X 5C	1	41.12st	37.57f-k	10.04abc	82.97gh	73.94b	122.88h	2.21ab	2.21b	20.26ijk
	2	53.22 b-h	49.85a-g	11.29ab	123.22b-f	103.61a	203.33a	2.38ab	2.77a	42.44c-k
3 X 6A	1	38.05t	31.50g-k	9.17abc	79.69h	61.60c	150.29f	2.63a	2.64ab	33.87d-k
	2	49.84f-r	42.17d-k	12.52ab	119.83f	88.78ab	183.95bc	2.68a	2.75a	53.02a-g
3 X 6B	1	37.71t	29.43jk	8.90bc	79.30h	57.81c	147.46fg	2.64a	2.64ab	32.36d-k
	2	49.31f-r	32.69g-k	11.93ab	119.29f	87.07ab	211.91a	2.78a	2.93a	65.99abc
3 X 6C	1	39.21t	31.90g-k	9.97abc	81.04h	59.21c	121.61h	2.48ab	2.48ab	27.25f-k
	2	51.06d-m	32.88g-k	11.93ab	130.93ab	83.04ab	242.20a	2.68a	3.18a	63.34abc
3 X 7A	1	39.54t	31.11g-k	8.98bc	81.43h	61.40c	152.63f	2.63a	2.44ab	27.30f-k
	2	51.48d-m	44.57d-k	10.11abc	121.48c-f	98.17a	159.48ef	2.78a	2.63ab	33.54d-k
3 X 7B	1	40.87st	35.62f-k	10.04abc	82.97h	52.63cd	136.05gh	2.44ab	2.44ab	25.76f-k
	2	53.00b-h	37.44f-k	10.51abc	123.00b-f	94.90ab	165.43d	2.58a	2.51ab	27.40f-k
3 X 7C	1	38.30t	30.31ijk	8.97bc	79.98h	65.78bc	161.04d-f	2.65a	2.55ab	36.06c-k
	2	50.38f-r	35.44f-k	10.03abc	120.36f	88.54ab	166.78d	2.78a	2.65ab	37.82c-k
4 X 5A	1	43.12l-t	34.91f-k	8.14bc	85.58gh	72.94b	132.15gh	2.50a	2.50ab	28.00f-k
	2	55.62a-g	46.47a-g	9.23abc	125.64b-f	103.23a	178.78c	2.78a	2.58ab	41.54c-k
4 X 5B	1	44.28h-t	32.08f-k	8.90bc	86.93gh	67.57bc	140.44g	2.47ab	2.47ab	29.07d-k
	2	64.80 a	50.74ab	10.04abc	133.86a	104.21a	174.10cd	2.58a	2.54ab	38.78c-k
4 X 5C	1	42.72st	37.00f-k	9.84abc	85.12gh	69.85bc	125.87h	2.25ab	2.25b	19.80ijk
	2	56.16 a-g	45.75d-k	14.05a	126.18b-f	98.58a	157.63ef	2.48ab	2.40ab	25.80f-k
4 X 6A	1	37.71t	26.35k	7.04c	79.32h	50.64cd	120.35h	2.48ab	2.48ab	26.07f-k
	2	45.39h-t	30.58ijk	9.44abc	115.35f	76.12b	158.44ef	2.58a	2.63ab	34.59d-k
4 X 6B	1	38.38t	28.25k	9.39abc	80.08h	53.92cd	137.57gh	2.50a	2.27b	26.06f-k
	2	50.47 d-m	29.36jk	11.93ab	120.46f	81.19ab	163.87d-f	2.68a	2.50ab	27.63f-k
4 X 6C	1	38.30t	23.41	6.25cd	79.98h	40.89	103.37ijk	2.20ab	2.20b	18.88ijk
	2	51.06d-m	28.73k	9.10abc	121.05d-f	85.11ab	192.72b	2.28ab	2.85a	33.88d-k
4 X 7A	1	37.96t	26.51k	8.70bc	79.59h	61.80c	146.49fg	2.66a	2.66ab	35.17d-k
	2	49.60f-r	32.97g-k	10.13abc	119.58f	84.62ab	177.42c	2.88a	2.74a	49.75a-g
4 X 7B	1	40.04st	29.04jk	9.31abc	82.00h	56.69c	148.52fg	2.57a	2.57ab	31.64e-k
	2	51.74c-l	30.79jk	11.93ab	121.73d-f	86.58ab	198.08ab	2.68a	2.80a	58.08a-d
4 X 7C	1	38.38t	35.06f-k	10.03abc	80.08h	59.80c	122.55h	2.60a	2.02bc	12.21kl
	2	50.08d-m	36.33f-k	10.44abc	120.07f	85.11ab	156.05ef	2.78a	2.60ab	32.85e-k
5 X 6A	1	38.79t	28.89k	8.83bc	80.56h	53.43cd	132.73gh	2.44ab	2.44ab	25.70f-k
	2	57.38a-f	35.09f-k	9.47abc	127.40b-f	82.17ab	165.62d	2.58a	2.57ab	40.60c-k
5 X 6B	1	39.38t	34.83f-k	9.93abc	81.24h	56.82c	148.15fg	2.57a	2.57ab	30.92f-k
	2	58.08a-d	44.57d-k	10.24abc	127.89b-f	97.63a	194.12b	2.68a	2.79a	52.85a-g
5 X 6C	1	39.21t	31.37g-k	10.11abc	81.04h	56.32c	177.90c	2.69a	2.64ab	37.15c-k
	2	57.86a-f	35.27f-k	12.34ab	127.89b-f	87.28ab	183.73bc	2.88a	2.69ab	39.74c-k
5 X 7A	1	38.38t	30.70ijk	9.02abc	80.08h	48.72	130.30gh	2.58a	2.55ab	28.71f-k
	2	50.08d-m	36.65f-k	9.19abc	120.07f	89.46ab	165.22d	2.78a	2.58ab	39.31c-k
5 X 7B	1	39.38t	31.29g-k	9.84abc	81.24h	53.83cd	156.63ef	2.64a	2.40ab	28.00f-k
	2	51.27 c-l	37.71f-k	10.45abc	121.26d-f	93.27a	165.11d	2.78a	2.64ab	33.50e-k
5 X 7C	1	40.12st	31.26g-k	9.43abc	82.02h	54.07cd	132.36gh	2.48ab	2.48ab	28.00f-k
	2	52.68c-l	35.34f-k	9.91abc	122.68d-f	89.82ab	181.89bc	2.68a	2.61ab	35.22c-k
6 X 7A	1	37.88t	25.56k	8.16bc	79.50h	54.83cd	131.37gh	2.53a	2.31ab	24.72f-k

Table 3. Cont'd

	2	49.60f-r	30.85ijk	10.63abc	119.58f	80.82ab	151.54f	2.68a	2.53ab	29.82e-k
6 X 7B	1	37.55t	31.20g-k	9.57abc	79.11h	63.79c	158.29ef	2.60a	2.41ab	25.84f-k
	2	49.11f-r	34.11f-k	11.17ab	119.10f	87.56ab	163.29d-f	2.78a	2.60ab	32.63e-k
6 X 7C	1	38.38t	33.16g-k	10.17abc	80.08h	62.39c	155.36ef	2.63a	2.63ab	33.13e-k
	2	50.08d-m	37.48f-k	13.15a	120.07f	86.09ab	210.06a	2.88a	2.85a	48.98a-g

Means that do not share the same letters are significantly different at 95% confidence using tukey pairwise comparison
DTF= Days to flowering (days); PHTF= Plant Height at Flowering (cm); NBP= Number of Branches per Plant; DTM = Days to Maturity (days); PHTM = Plant Height at Maturity (cm); NPP = Number of Pods per Plant; NSP = Number of Seeds per Plant; PL=Pod Length per Plant (cm); SYP = Seed Yield per Plant (g);
P1=TGx1835 – 40E; P2=TGx1990 – 55F; P3 = TGx1990 – 3F; P4 = TGx1990 – 37F; P5 = TGx1989 – 21F; P6 = TGx1830 – 20 E; P7 = TGx1990 – 57F

Table 4. Estimation of genetic components of *Characters under Study in F_2 population of Soybean, Glycine max Across Two Cropping Years*

Character	Year	σ^2g	σ^2p	PCV %	GCV%	D^2	Hb %	GA	GAM%
DTF	1	16.37	19.48	48.66	40.89	7.77	84.03	7.63	19.07
	2	40.13	40.60	76.72	75.83	0.89	98.84	12.97	24.51
PHTF	1	49.50	54.48	165.14	150.15	15.10	90.86	13.81	41.87
	2	240.58	245.71	685.96	671.64	14.32	97.91	31.63	88.29
NBP	1	2.15	2.66	27.85	22.51	5.34	80.83	2.71	28.42
	2	11.39	11.68	110.71	107.96	2.75	97.52	6.87	65.12
DTM	1	16.33	16.72	20.39	19.91	0.48	97.67	8.23	10.03
	2	43.14	43.44	35.30	35.06	0.24	99.31	13.48	10.96
PHTH	1	211.19	211.42	347.50	347.12	0.38	99.89	29.92	49.18
	2	226.67	226.95	252.87	252.56	0.31	99.88	30.99	34.52
NPP	1	808.61	808.86	581.91	581.73	0.18	99.97	58.57	42.14
	2	2106.18	2108.89	1159.05	1157.56	1.49	99.87	94.47	51.92
NSP	1	0.23	0.33	13.20	9.20	4.00	69.70	0,82	32.74
	2	0.22	0.55	26.44	10.58	15.87	40.00	0.61	29.32
PL	1	0.54	0.74	24.34	17.76	6.58	72.97	1.29	42.53
	2	0.63	0.86	32.70	23.95	8.75	73.26	1.41	53.36
SYD	1	83.33	83.59	294.12	293.21	0.91	99.69	18.77	66.04
	2	703.50	703.77	1676.84	1676.20	0.64	99.96	54.63	130.17

D^2 = The difference between phenotypic coefficient of variation (PCV%) and genotypic coefficient of variation (GCV%)
DTF= Days to flowering (days); PHTF= Plant Height at Flowering (cm); NBP= Number of Branches per Plant; DTM = Days to Maturity (days); PHTH = Plant Height at Harvesting (cm); NPP = Number of Pods per Plant; NSP = Number of Seeds per Plant; PL=Pod Length per Plant (cm); SYP = Seed Yield per Plant (g)

characters evaluated can be utilized in soybean breeding program. The significant variations among the genotypes indicate considerable genetic

variability and diversity among the F2 populations. This finding corroborates the findings of Rajkumar *et al.,* (2010) and Reni and Rao (2013). They reported that, analysis of variance revealed significant differences among the genotypes for days to flowering, plant height, number of pods, number of seeds per pod and seed yield. The significant variation observed in interaction of genotype with year (G x Y) for plant height at flowering, plant height at maturity, number of pods per plant and seed yield per plant is an indication that the F_2 populations were sensitive to variations in environmental and climatic conditions.

Mean performances serves as an important criterion in eliminating the undesirable types in a selection program. The result obtained based on the mean performance of the F_2 populations indicated that the populations differed significantly in mean values for seed yield and its component characters. This result findings is in harmony with the findings of Shanti *et al.,*(2008); El-sayed *et al*., (2005) and Nassar, (2013).The relatively high genetic coefficient of variation recorded in some of the characters studied indicated that these characters might be more genetically predominant and would be possible to achieve further improvement in them. In general, the estimates of phenotypic coefficient of variation were higher than the genotypic coefficient of variation for the characters studied.

Table 5. Levels of polymorphism for F_2 populations of Soybean, *Glycine max* by SNP- PCR analysis

PRIMER NAME	NUMBER OF BANDS	POLYMORPHIC BAND	MONOMORPHIC BAND	POLYMORPHIC %	MONOMORPHIC %
BARC-013065-00437	9.00	6.00	3.00	66.67	33.33
BARC-014847-01910	10.00	10.00	0.00	100.00	0.00
BARC-015973-02029	9.00	6.00	3.00	66.67	33.33
BARC-016485-02069	10.00	7.00	3.00	70.00	30.00
BARC-016861-02355	9.00	6.00	3.00	66.67	33.33
BARC-018933-03040	3.00	0.00	3.00	0.00	100.00
BARC-019085-03298	10.00	7.00	3.00	70.00	30.00
BARC-021329-04038	10.00	7.00	3.00	70.00	30.00
BARC-021827-04218	10.00	7.00	3.00	70.00	30.00
BARC-021831-04219	12.00	9.00	3.00	75.00	25.00
BARC-021937-04237	9.00	6.00	3.00	66.67	33.33
BARC-024043-04709	10.00	7.00	3.00	70.00	30.00
BARC-024333-04850	12.00	9.00	3.00	75.00	25.00
BARC-025961-05189	8.00	5.00	3.00	62.50	37.50
BARC-028309-05824	9.00	6.00	3.00	66.67	33.33
BARC-028793-06015	13.00	7.00	6.00	53.85	46.15
BARC-029343-06156	9.00	6.00	3.00	66.67	33.33
BARC-029859-06448	10.00	7.00	3.00	70.00	30.00
BARC-030337-06857	15.00	15.00	0.00	100.00	0.00
BARC-030735-06928	9.00	6.00	3.00	66.67	33.33
BARC-030807-06945	12.00	6.00	6.00	50.00	50.00
BARC-031701-07215	9.00	6.00	3.00	66.67	33.33
BARC-039561-07508	9.00	6.00	3.00	66.67	33.33
BARC-039593-07509	12.00	6.00	6.00	50.00	50.00
BARC-040033-07641	9.00	6.00	3.00	66.67	33.33
BARC-040075-07652	12.00	9.00	3.00	75.00	25.00
BARC-040339-07714	12.00	6.00	6.00	50.00	50.00
BARC-040459-07745	15.00	9.00	6.00	60.00	40.00
BARC-041267-07957	15.00	9.00	6.00	60.00	40.00
BARC-041819-08107	3.00	0.00	3.00	0.00	100.00
BARC-042201-08212	9.00	6.00	3.00	66.67	33.33
BARC-044047-08593	9.00	6.00	3.00	66.67	33.33
	322.00	214.00	108.00		

Heritability plays a vital role in deciding the suitability and strategy for selection of a particular character. It provides an idea of the extent of genetic control for the expression of a particular character and the reliability of phenotype in predicting its breeding value (Chopra, 2000). High heritability indicates less environmental influence in the observed variation (Mohanty, 2003). Heritability estimates together with genetic advance are more important than heritability alone to predict the resulting effect of selecting the best individuals (Hamdi *et*

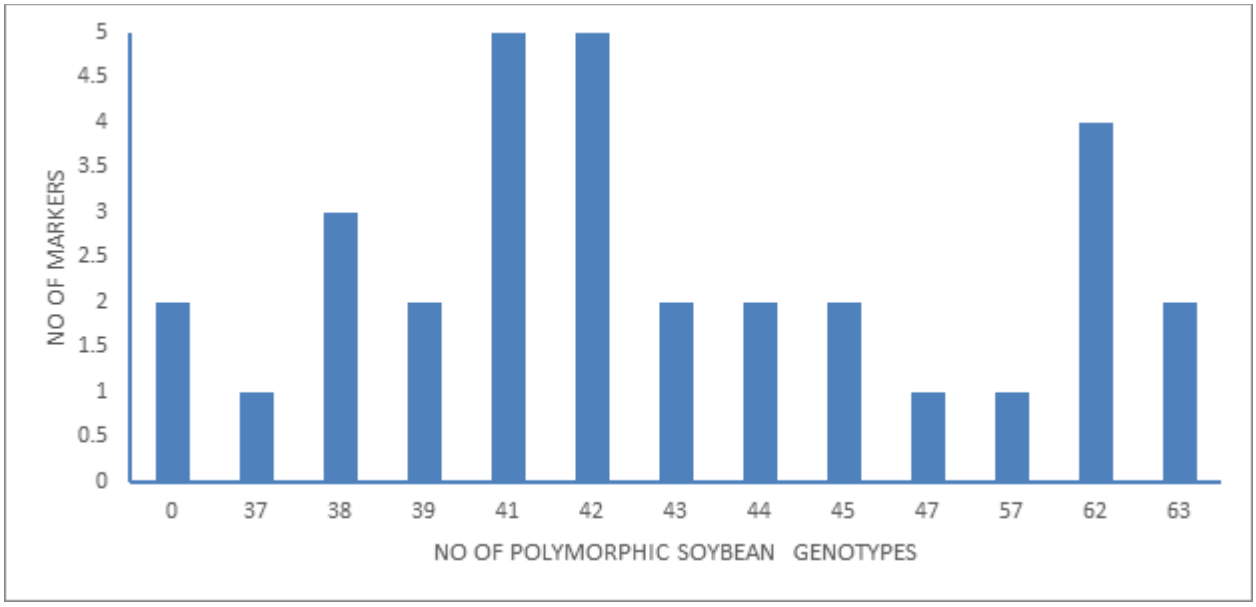

Figure 1. Distribution of Polymorphic SNPs across the Soybean Genotypes

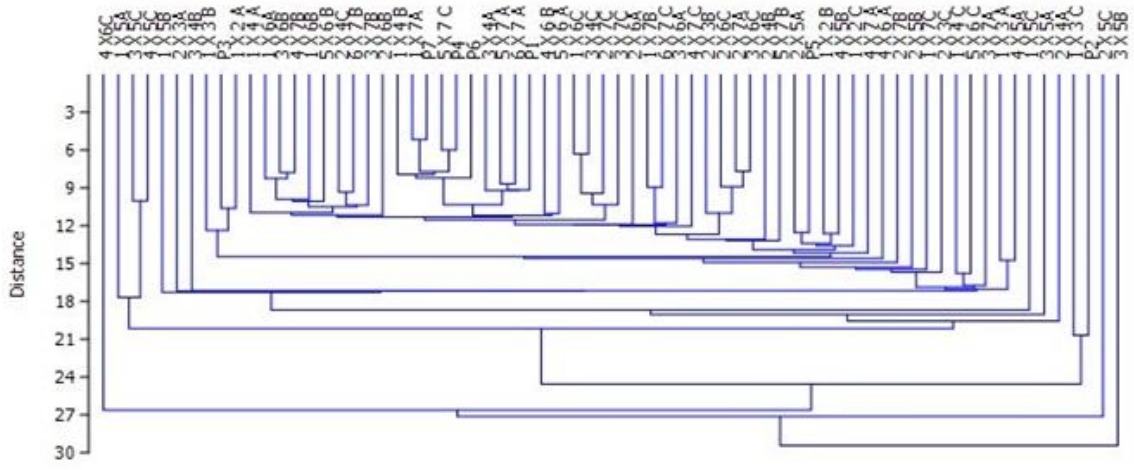

Figure 2. An UPGMA cluster dendogram showing the genetic relationships among the F2 population of Soybean genotypes

al., 2003; Milatovic *et al.,* 2010). High heritability with high genetic advance indicates the preponderance of additive gene action and such characters could be improved through selection (Tazeen *et al*., 2009). Characters with high heritability and high genetic gain is an indication that the expression of the characters are more of additive gene action and selection will be more effective based on phenotypic performance (Rashwan, 2010).

SNP markers have proven to be a powerful tool for molecular genetic analysis and plant breeding programs to assess genetic diversity for the development of improved varieties. The grouping of the F_2 populations into the different clusters gives an insight into the genetic diversity and polymorphism and the possibility of their further use in soybean breeding programs.

CONCLUSION.

It can be concluded from the study that, there was a wide genetic variability among the F_2 populations for the different characters studied as indicated by the analysis of variance and from the result of the SNP markers

analysis. This will provide a good opportunity for selection among the F_2 populations to serve as a possibility for their utilization in further soybean breeding programs.

ACKNOWLEDGEMENT

The authors acknowledge the soybean germplasm collection and bioscience centre of International Institute of Tropical Agriculture (IITA) Ibadan, Oyo- State Nigeria, for the provision of the soybean genotypes used as parents and the DNA extraction respectively. The contributions of Daka, L. and Inqaba Biotechnical Laboratory, Pretoria South Africa, for the SNP design and the SNP analysis respectively are also acknowledged by the authors.

REFERENCES

Abebe T, Alamerew S, Tulu L (2017). Genetic variability, heritability and genetic advance for yield and its related traits in rainfed lowland rice (Oryza sativa L.) genotypes at Fogera and Pawe, Ethiopia. Adv. Crop Sci. Technol., 5(2):1-8.

AHA (2000). Heart and stroke statistical update. American Heart Association, Dallas, TX.

Asafo-Adjei B, Adu-Dapaah H, Oti-Boateng C (2007). *CSIR-CRI/CSIRSARI/MOFAFCDP* Fact Sheet: Nangbaar and Ahoto early Maturing Soybean varieties for Ghana.

Brown J, Caligari PD (2008). Breeding schemes. An Introduction to Plant Breeding. Genetika, 41(2): 34-59.

Choi IY, Hyten DL, Matukumalli LK, Song Q, Chaky JM, Quigley CV, Hwang EY (2007). A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. Genetics, 176(1): 685-696.

Chopra UL (2000) Plant Breeding: Theory and Practice 2nd ed. Oxford and IBH pub. Co. ltd, New Delhi 2000. Dellaporta SL, Wood J. and Hicks JB. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter, 1(4): 19-21. (1983).

Desroches S, Mauger JF, Ausman LM, Lichtenstein AH, Lamarche, B (2004). Soy protein favorably affects LDL size independently of isoflavones in hypercholesterolemic men and women. Journal of Nutr. 134: 574-579.

El-Sayed ZS, Solliman MM, Mokhtar SA, El-Shaboury HMG, ElHafez GAA (2005). Heterosis, Combining ability and Gene action in F_1 and F_2 diallel crosses among six Soybean genotypes. Ann. of Agric. Sci., 43(2): 454- 559.

FAOSTAT (2018). Accessed 19.11.2018.http://www.fao.org/faostat.

Guo-Liang Jiang (2013) Molecular markers and Marker assisted breeding in plants. Intech, https//doi. Org/105772 / 52583.

Hamdi A, El-Ghareib AA, Shafey SA, Ibrahim MAM (2003). Genetic variability, Heritability and expected genetic advance for earliness and seed yield from selection in lentil. Egypt Journal of Agric. Res., 81(1): 125-137.

Hartman RL, McMullen JP, Jensen KF (2011). Deciding whether to go with the flow: Evaluating the merits of flow reactors for synthesis. Angewandte Chemie International Edition, 50 (33): 7502-7519.

Joshi P, Yasin M, Sundaram P (2018). Genetic Variability, Heritability and Genetic Advance Study for Seed Yield and Yield Component Traits in a Chickpea Recombinant Inbred Line (RIL) Population. International Journal of Pure & Applied Bioscience, 6(2): 136-141.

Kumar PA, Sanjay K, Marya AN (2009). Genetic study of earliness in cowpea (*Vigna unguicalata* (L.) Walp). Indian Journal of Hort., 64:63-66.

Kumar R, Mago G, Balan V, Wyman CE (2009). Physical and chemical characterization of corn stover and poplar solids resulting from leading pretreatment technologies. Bioresource Technology, 100(17): 3948-3962.

Larik AS, Hafiz MI, AL–Saheal YA (1987).Genetic analysis of some yield parameters in barley. Journal of College Sci. King Saud Univ., 18: 129-135.

Mebrahtu T (2008). Analysis of nutritional contents in vegetable soybeans. Journal of Crop Improvement, 21(2): 157-170.

Mohanty BK (2003). Genetic variability, Heritability, Correlation and Path coefficient studies in Tomato. Indian Journal of Agricultural Research, 37:68-71.

Naaz A, Yellayi S, Zakrocymski MA., Bunick D, Doerge DR, Lubahn DB, Helferich WG, Cooke PS (2003). The soy isoflavone genistein decreases adipose deposition in mice. Endocrinol. 144: 3315-3320.

Nassar MAA (2013). Heterosis and combining ability for yield and its components in some crosses of soybean. Aust. J. Basic App. Sci., 7(1): 566-572.

Nayak NJ, Maurya PK, Maji A, Chatterjee S, Mandal AR, Chattopadhyay A (2017). Estimation of Genetic Parameters and Selection of Parents for Hybridization in Dolichos Bean (Dolichos lablab L.). Int. J. Curr. Microbiol. App. Sci., 6(12):381-95.

Rashwan AMA (2010). Estimates of some genetic parameters using six population of two cowpea hybrids. Asian Journal of Crop Sci., 2:261-266.

Rajkumar R, Kumar V, Murlidharan P, Agarwal DK. (2010). Study on genetic variability and traits interrelationship among released soybean varieties of India (*Glycine max* (L.) Merrill). Electronic Journal of Plt. Breed, 1(6): 1483 -1487.

Reni YP, Rao YK. (2013). Genetic Variability in Soybean (Glycine max (L.) Merrill). Intl. J. Plant, Anim. Environ. Sci., 3(4): 35-38.

Rosell MS, Appleby PN, Spencer EA, Key TJ (2004). Soy intake and blood cholesterol considerations: A cross-sectional study of 1033 pre and postmenopausal

women in the oxford arm of the European prospective investigation into cancer and nutrition. The American Journal of Clinical Nutrition, 80: 1391–1396.

Shanti P, Kalamkar V, Sable NH, Maheshwari JJ (2008). Study of Heterosis in Soybean. Journal of Soils and Crops, 18(1): 208-211.

Soyatech LLC (2012). Soybean genotypes (*Glycine max* L.) under moisture and normal condition. Intl. Journal of Agron. 3 (10): 447-454.

Soy Stats (2012). American Soybean Association reports. *Am. Soybean Assoc., St. Louis, MO*.

Tazeen M, Nadia K, and Farzana NN. (2009). Heritability, Phenotypic correlation and Path coefficient studies for some agronomic characters in synthetic elite lines of wheat. Journal of Food, Agriculture and Environment 7(3 and 4):278-282.

Teixeira SR, Potter SM, Weigel R, Hannum S, Erdman JW, Hasler CM (2000). Effects of feeding 4 levels of soy protein for 3 and 6 weeks on blood lipids and lipoproteins in moderately hyper – cholesterol men. Am. Journal of Clin. Nutr. 71:1077-1084.

Zhu YL, Song QJ, Hyten DL, VanTassell CP, Matukumalli LK. Grimm DR, Hyatt SM, Fickus EW, Young ND, Cregan PB (2003) Single-nucleotide polymorphisms in Soybean. Genetics, 163:1123-1134.