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Genetic Analysis Of Seed Yield And Its Contributing Characters In F2 Population Of Soybean Genotypes, *Glycine Max* (L) Merrill Using Morphological and SNP Markers

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ABSTRACT

This study was carried out on the genetic analysis of seed yield and its contributing characters in F_2 population of soybean genotypes, Glycine max (l). Merrill genotypes using morphological and SNP markers. The aim of this study is to determine the extent of genetic variability for seed yield and its contributing characters among the F_2 population of soybean genotypes and to determine quantitative trait loci (QTL) associated with seed yield in the soybean genotypes with a view of being utilized in soybean breeding programme. The field experiment was laid out in a randomized complete block design (RCBD) with three replications. Heritability in the broad sense ranged from 40.00% to 99.97% for number of seeds and number of pods respectively. The genetic advance mean ranged between 10.03 and 130.17 for days to maturity and seed yield respectively. Nine QTLs were identified (3 QTLs for days to flowering (DTF), 3 QTLs for days to maturity (DTM), 2 QTLs for total pod weight (TPW) and 1 QTL for seed yield (SYP)) and located on the linkage group A1 (chromosome 5). However, two stable QTLs for days to flowering (DTF) and days to maturity (DTM) and two novel QTLs for days to flowering (DTF) and total pod weight (TPW) were identified in the present study.

Keywords

Genetic analysis, Seed yield, Contributing characters, Soybean, Morphological, SNP.

Introduction

Soybean, *Glycine max* (L.) Merrill is a member of the family Fabaceae [1]. It is one of the most important leguminous seed crop among the oil crop plants [2]. The United States of America currently leads in soybean production accounting for more than 35% of the total soybean production worldwide [3]. Nigeria is the largest producer in Africa [4]. It adapts to a fairly wide range and array of climatic, soil and growth conditions although performs best in rainfed land, [5]. Soybean is used directly as food, oil production, soymilk and soy protein powder. The benefits are related to their excellent protein contents, high levels of essential fatty acids, numerous vitamins and minerals [6].

Genetic improvement of any crop depends upon the nature and magnitude of interrelationships of heritable and non- heritable variation in yield and its major contributing characters [7]. Evaluation of genotypes for the extent of variability is the first step in any crop improvement program [8]. The presence of genetic variability in a gene pool is the pre requisite of a breeding program [9]. Understanding of the magnitude of genetic variability and its components is key and very important in planning any breeding program [10,11]. Selection which is the retention of desired genotypes and elimination of undesirable ones is a major process in breeding programmes [12]. The level of success in breeding depends on the quantity of heritable constituents expressed in a phenotype [13]. The magnitude of genetic variability present in a particular population of any crop species is key to any crop improvement strategy to be exploited by plant breeders for yield improvement [14]. Estimates of genetic variability and heritability are useful in planning and evaluating the breeding program [15].

However, Johnson *et al.,* [16] and Hamza et al., (2020), stated that heritability estimates together with genetic advance are more important than heritability alone in predicting the resulting effect of selection.

The heritability of a character determines the extent to which it is transmitted from one generation to the next and it is a valuable tool when used in conjunction with other genetic components in predicting genetic gain [17]. High heritability values indicate that the dependence of phenotypic expression reflect the genotype's ability to transmit the genes to the offspring [18]. The higher the heritability estimates the easy the selection criteria [19]. High heritability estimates indicate less environmental influence in the observed variation [20]. However, it has been shown that heritability alone has no practical importance without genetic advance [21]. Genetic advance shows the degree of gene obtained in a character under a particular selection process and also indicates the magnitude of the expected genetic gain from one cycle of selection [22,23]. High genetic advance coupled with high heritability estimates offer the most suitable conditions for selection [20]; hence, traits with higher range of genetic variability and high heritability would be effective for selection to improve seed yield and quality in soybean [24]. Several breeding programmes have used molecular techniques, aiming at marker assisted selection [25]. The use of molecular markers presents several advantages over morphological markers or conventional breeding, in the sense that it allows for section to be carried out at the seedling stage thus, reducing the time required before the phenotype of an individual plant is known and it requires only small portion of the breeding materials. Molecular marker breeding technique is not also affected by the environment, hence, allowing the selection to be performed under any environmental condition. It also facilitates the identification and selection of traits that are controlled by multiple or individual QTLs present in the same individual simultaneously which may not be possible in phenotypic selection due to the fact that a particular gene may mask the effect of other genes [26].

There have been several reports of the application of molecular marker to crop improvement [27]. The selection methods were largely enhanced by the use of molecular marker and the success of marker assisted selection depend on the degree of association among the characteristics of interest. The greater the association, between the marker and the gene controlling the character of interest, the higher the selection efficiency [11]. The application of molecular marker techniques for quantitative trait loci (QTL) analysis has proved to be a useful powerful genetic approach to dissect complex trait [28]. Molecular marker which are associated with QTL are available in the soybean genome and have been used extensively for mapping QTL and construction of linkage maps [29]. A number of breeding companies have in the past two decades to varying degrees started using markers to increase the effectiveness in breeding and therefore plant breeders and geneticists consider molecular markers useful additional tool in plant breeding programmes to make selection more efficient [30]. The aim of this study is to determine the extent of genetic variability for seed yield and its contributing characters among soybean genotypes and to determine quantitative trait loci (QTL) associated with seed yield and its contributing characters in the soybean genotypes with a view of being utilized in soybean breeding programme.

Materials And Methods

The plant populations used in this study was developed from crosses made from seven soybean genotypes following diallel analysis. The seven soybean genotypes were obtained from the soybean germplasm collection of international institute of tropical agriculture (IITA) Ibadan, Oyo- state Nigeria. A total of 63 F_2 plants derived from the seven parents were sown in the field of teaching and research farm of the Federal University of Technology Akure, Ondo- State, Nigeria in a Randomized Complete Block Design (RCBD) with three replications in 2014 and 2015 cropping seasons. A single row plot was adopted with an intra and inter row spacing of 60cm by 20cm. a maximum of fifteen (15) plants were maintained per plot. Data were collected on ten competitive mid-plants on the following agronomic and yield related characters; days to flowering (days), plant height at flowering (cm) days to maturity (days), plant height at maturity (cm), number of pods per plant (NPP), number of seeds per pod (NSP), pod length (PL)(cm) total pod weight (g) and seed yield per plant (g).

Statistical Analysis

Analysis of variance was conducted using individual plot means for each year and combined across years 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 (Hbs) and genetic parameters were detected through variance component method (Larik *et al.,* 1987) as follow:- Genetic variance= σ^2g = MSG-MSE / r ; Phenotypic variance = $\sigma^2ph = \sigma^2g + \sigma^2e$ Heritability = σ^2g / σ^2ph 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 = (σ^2ph / X) x 100 The genotypic coefficient of variation (GCV) was calculated as: GCV = (σ^2g / X) x 100 Where X = Grand mean of all genotypes.

QTL Detection

The QTL analysis was performed following composite interval mapping (CIM) method [31] using the software WINQTL CART. Vsn 2.5 [32]. A total of 1000 permutations [33] were performed on each character with a significant level of 0.05 for getting genome wide critical threshold value for the experiment. The QTL was considered significant when its LOD score was found higher than the threshold value in at least one of the two years or the average of both years.

DNA Extraction And SNP Analysis

Total genomic DNA was extracted using the modified mini preparation protocol described by [34] as follows: Approximately 200 mg (0.2 g) of lyophilized leaf sample was ground into fine

powder. To each tube 70 0ul of hot (65°C) plant extraction buffer(PEB) [containing 637.5 ml of double distilled water (ddH20), 100 ml of 1 M Tris-HCl (pH 8.0), 100 ml of 0.5 M ethylene diamine tetra acetic acid (EDTA) (pH 8.0), 100 ml of 5 M Nacl2 and 62.5 ml of 20% sodium dodecyl sulphate (SDS)] was added. One percent beta 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 minutes with occasional mixing to homogenize the samples. After 20 minutes, 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 (CH3COOK) 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 1.5 ml eppendorf tubes using wider bore pipette tips (1000 µl) and making sure debris were not taken along with the supernatant. 700 µl chloroform isoamylalcohol was added to the supernatant and spun at 10,000 rpm for 10 minutes. The supernatant was carefully discarded and the DNA pellets were washed and air dried completely. After drying, 60 µl of 1×TE [10 mM Tris-HCL (pH 8.0), 1 mM EDTA (pH 8.0)] was added to the pellets, followed by 2 µl of 10ng/ml Rnase to remove the RNA. The DNA was measured using Nanodrop ND – 1000 UV-Vis Spectrophotometer.

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 [21,35,36]. 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 MgCl2, 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°C for 4 min followed by 45 cycles of 94°C for 20 s, 56°C for 30 s, 72°C for 1 min, and then followed by 3 min at 72°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°C for 20 minutes followed by 5 minutes at 85°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 7 µM, 9 µM, 11 µM and 14 µM according to extension primer mass from smallest to largest. This four-tier system was used for Oligo validation and peak optimisation on the Maldi-Tof. Then, the iPLEX extend was carried out with a final concentration of between 0.625 and 1.5l µM for each extension primer, depending on the mass of the probe, iPLEX termination mix (Agena Biosciences) and 1.35 µM iPLEX enzyme (Agena Biosciences) and conducted a two-step cycles program; 94°C for 30 s followed by 40 cycles of 94°C for 5 s, then followed 5 cycles of 52°C for 5 s, and 80°C for 5 s within the 40 cycles, then 72°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).

Result

Table 1: The Names And Source Of Soybeans, Glycine Max Genotypes.

Parental No	Genotype Name	Source
1	TGx 1835 – 40E	International Institute
2	TGx 1990 – 55F	of Tropical Agriculture
3	TGx 1990 – 3F	(IITA) Ibadan, Oyo, State Nigeria
4	TGx 1990 – 37F	
5	TGx 1989 – 21F	
6	TGx 1830 – 20 E	
7	TGx 1990 – 57F	

The estimates of the genetic components of the characters under study are presented in Table 3. 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 whereas the highest estimates of GCV were recorded in Number of Pods per Plant (581.73) and in Seed yield per plant (1676.20) in the first and second year respectively. The Hb estimates were very high for almost all the characters under study being highest in number of pods per plant (99.97) and in seed yield per plant (99.96) in the first and second year respectively. The highest GA estimates was recorded in number of pods per plant (58.57; 94.47) in the first and second year respectively.

The QTLs identified in this study are listed in Table 4. A total of 9 QTLs were detected across the characters in both years with single QTLs explaining between 1 to 47% of the phenotypic variations. The identified QTLs were all located on linkage group A1. 3 QTLs were found each for days to flowering and days to maturity, 2 QTLs for total pod weight and 1 QTL for seed yield. No QTL was found associated with number of pods per plant and 100seed weight in either of the two years. The largest QTL was found in BARC028793- 06015 with a LOD score of 5.25 explaining 6% of the phenotypic variation. No QTLs were found in 2014 for total pod weight and seed yield. The QTL analysis for individual year showed that 2 QTLs were detected in 2014 and 4 QTLs in 2015. 2 stable QTLs including one locus each for days to flowering and days to maturity were mapped in both years.

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)	TPW (g)	SYP (g)
Year	1	12630.90**	6093.09**	160.74*	124579.40**	108102.30**	200137.40**	5.12**	3.78	42194.04**	24544.38**
Block (Year)	4	4.72	281.52**	35.10**	4.61	171.10**	6826.95**	0.65**	1.32**	2735.13**	697.70**
Genotype	62	32.75**	147.34**	1.65	33.05**	357.84**	682.05**	0.10**	0.26**	279.86**	194.34**
Genotype x Year	62	6.58	78.53**	2.14	6.45	42.27**	369.68*	0.06	0.13	156.36**	124.04**
Error	248	5.48	14.42	1.63	5.44	19.49	279.55	0.05	0.12	90.95	62.00

*,** significance at 5% and 1% level of probability respectively.
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); PHTH = Plant Height at Harvesting (cm); NPP = Number of Pods per Plant; NSP = Number of Seeds per Plant; PL=Pod Length per Plant (cm); TPW= Total Pod Weight (g) SYP = Seed Yield per Plant (g).

Table 3: Estimation of genetic components of Characters under Study in F_2 population of Soybean, Glycine max Across Two Cropping Years.

Character	Year	σ2g	σ2p	PCV%	GCV%	Hb%	GA	GAM%
DTF	1	16.37	19.48	48.66	40.89	84.30	7.63	19.07
	2	40.13	40.60	76.72	75.83	98.84	12.97	24.51
PHTF	1	49.50	54.48	165.14	150.15	90.86	13.81	41.87
	2	240.58	245.71	685.96	671.64	97.91	31.63	88.29
NBP	1	2.15	2.66	27.85	22.51	80.83	2.71	28.42
	2	11.39	11.68	110.71	107.96	97.52	6.87	65.12
DTM	1	16.13	16.72	20.39	19.91	97.67	8.23	10.03
	2	43.14	43.44	36.30	35.06	99.31	13.48	10.96
PHTH	1	211.19	211.42	347.50	347.12	99.89	29.92	49.18
	2	226.67	226.95	252.87	252.56	99.88	30.99	39.52
NPP	1	808.61	808.86	581.91	581.73	99.97	58.57	42.14
	2	2106.18	2108.89	1159.05	1157.56	99.87	94.47	51.92
NSP	1	0.23	0.33	13.20	9.20	69.70	0.82	32.74
	2	0.22	0.55	26.44	10.58	40.00	0.61	29.32
PL	1	0.54	0.74	24.34	17.76	72.97	1.29	42.53
	2	0.63	0.86	32.70	23.95	73.26	1.41	53.36
TPW	1	63.15	63.95	168.02	165.92	98.75	16.27	42.75
	2	113.70	113.90	199.86	199.51	99.82	21.95	38.52
SYP	1	83.33	83.58	294.12	293.21	99.69	18.77	66.04
	2	703.50	703.77	1676.84	1676.20	99.96	54.63	130.17

σ^2g = Genetic variance; σ^2ph = Phenotypic variance; Hb = Heritability; PCV% = phenotypic coefficient of variation; GCV% = genotypic coefficient of variation; 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); TPW= Total Pod Weight (g); SYP = Seed Yield per Plant (g).

Table 4: QTLs associated with seed yield and its components in F_2 population of Soybean Genotypes.

Character	Year	QTL	LG/ CHR NO.	Marker	Position (cM)	LOD	Additive effect	Dominance effect	PVE%
DTF	2014	1	5	BARC- 028793- 06015	46.95	5.22	1.47	1.44	1.00
	2015	2	5	BARC- 01365 - 00437	95.40	4.88	1.38	0.69	1.00
	MEAN	3	5	BARC- 030337-06857	92.45	3.81	1.35	1.23	3.00
DTM	2014	4	5	BARC- 028793- 06015	46.95	5.25	1.46	1.43	6.00
	2015	5	5	BARC- 030337-06857	92.45	5.17	0.79	-0.77	2.00
	MEAN	6	5	BARC- 030337-06857	92.45	3.59	0.75	-0.73	4.00
TPW	2014	-	-						
	2015	7	5	BARC- 028793- 06015	46.95	3.48	36.90	-35.80	47.00
	MEAN	8	5	BARC- 028793- 06015	46.95	3.36	36.90	-35.80	47.00
SYP	2014	-	-	-	-	-	-	-	-
	2015	9	5	BARC- 030337-06857	95.40	4.02	42.50	-21.20	46.00
	MEAN	-	-	-	-	-	-	-	-

QTL=Quantitative trait loci, LG= linkage group, LOD=logarithm of odd, PVE% = Phenotypic variation explained; DTF=days to flowering, DTM= days to maturity, TPW= total pod weight; SYP= seed yield.

Discussions

The results from this study indicated the existence of wide genetic variability among the genotypes for the characters studied. This provides good opportunity for selection among the genotypes for the characters evaluated and their subsequent utilization in future soybean breeding program [37]. The significant variations among the genotypes indicate considerable genetic variability and diversity among the F_2 populations. This finding is in agreement with the findings of Langat [35] and Azam *et al.,* [38]. They reported 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 variations in environmental and climatic conditions influenced the expression of these characters [39]. In general, the estimates of phenotypic coefficient of variation were higher than the genotypic coefficient of variation for the characters studied. Heritability estimates together with genetic advance are more important than heritability alone to predict the resulting effect of selecting the best individuals [40]. High heritability with high genetic advance indicates the preponderance of additive gene action and such characters could be improved through selection (Bartaula *et al.,* 2019). The high broad sense heritability estimates in this study for Days to flowering and Number of pods per plant corroborates the findings of [41]. High heritability indicates less environmental influence in the observed variation and so there is a good scope for the improvement of these characters through direct selection [42]. Remarkable progress has been made in the construction of soybean genetic maps and QTL mapping of important agronomic characters [43]. From previous studies on QTLs for days to flowering as reported in soybase [44] indicated that the genomic regions for days to flowering are located on linkage groups C2 and B1. However, in this study, no QTL for days to flowering was found on linkage groups C2 and B1 but rather, on linkage group A1. Hence, the 3 QTLs for days to flowering linked to BARC- 028793- 06015, BARC- 01365 – 00437 and BARC-030337-06857 identified in this study could be referred to as novel QTLs for days to flowering. The QTL for seed yield mapped on linkage group A1 agrees with the findings of [45]. The QTL for days to maturity mapped on linkage group A1 in this study also corroborates the findings of [46], where it was reported that QTL for maturity were mapped on linkage groups A1, C2, F, G and M. Furthermore, to the best of our knowledge, there have been no specific QTL reported for total pod weight but there have been findings on QTL for pod wall weight and pod wall thickness [47]. Hence, the QTL for total pod weight detected on linkage group A1 in this study can then be referred to as a novel QTL [48]. Although it cannot be referred to as a stable QTL because it was not detected in both years. The co- located QTLs for days to flowering, days to maturity, seed yield and total pod weight on linkage group A1 in this study is an indication that considerable attention should be given to this linkage group in future soybean breeding programmes [48]. It should be noted that QTL analysis carried out in a single environment is likely to underestimate the number of QTLs for a particular character [49]. Hence, it is important that QTL analysis be carried out across multiple environments. Stable and validated QTLs are more desirable to be used in marker assisted selection [50]. Though some of the QTLs detected in this study were consistent with earlier detected QTLs by previous researchers, two novel QTLs (QTLs for DTF and TPW) were identified in this study due the fact that there has not been any report of such QTLs on linkage group A1. One of the novel QTLs detected in this study could be regarded as a stable QTL (DTF) due to the fact that it was detected in the two years whereas the other novel QTL (TPW) is not a stable QTL because it was detected in only one of the years.

Conclusion

It can be concluded from the study that, there was a wide genetic variability among the F_2 populations for the characters studied as indicated by the analysis of variance and the estimation of the genetic components. This will provide a good opportunity for selection among the F_2 populations for their utilization in further soybean breeding programs. Two stable (QTLs for DTF and DTM) and two novel QTLs for DTF and TPW were also identified in the current study. Further work could be carried out on the novel QTLs for days to flowering and total pod weight identified in this study for stability, validation and confirmation across multiple environments using larger population size and SNP markers.

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