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Identification of Genomic Region Governing Yield Related Characters in Soybean, *Glycine max* (L.) Merrill Using SNP Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author OFA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OFA managed the analyses of the study. Authors OFA, ACO and BOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to identify the quantitative trait locus (QTL) associated with seed yield related characters in soybean using F_2 population. A population of 63 F_2 plants were genotyped by 32 SNP markers. Nine QTLs were found to be associated with seed yield related characters (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 were found located on the linkage group A1 (chromosome 5). The QTLs for DTF and DTM identified in this study could be regarded as stable QTLs because of their detection in the two years. However, two novel QTLs for days to flowering (DTF) and total pod weight (TPW) on linkage group A1 were identified in the present study.

Keywords: Genomic region; yield related characters; soybean; SNP.

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1. INTRODUCTION

In soybean, Glycine *max* seed yield as in other crops is an extremely complex character being the result of the expression and association of several plant growth components which is dependent on many variables. To increase the genetic yield potential, maximum utilization of the desirable characters for synthesizing of any ideal genotype is essential in selection for higher yield [1,2]. The complex interrelationship between the yield contributing characters usually shows a complex chain of interacting relationship [3,4]. Selection for seed yield which is a polygenic character often leads to changes in other characters. Hence, knowledge of the relationship between seed yield and other characters is desirable to be able to choose the appropriate selection breeding program [5,6].

There have been several reports of the application of molecular marker to plant improvement [7,8]. The selection methods were largely enhanced using molecular marker and the success of marker assisted (MAS) selection depend on the degree of association among the characters of interest. The greater the association between the marker and the gene controlling the character of interest the higher the selection efficiency [9].

The application of molecular marker techniques for QTL analysis has proved to be a useful powerful genetic approach to dissect complex trait [10]. Molecular markers which are associated with QTL are available in the soybean genome and have been used extensively for mapping QTL and construction of linkage maps [11]. Many breeding companies have in the past two decades to varying degrees started using markers to increase the effectiveness in breeding and to significantly shorten the development time of varieties. As a result of this development, plant geneticist consider molecular marker assisted selection a useful additional tool in plant breeding programmes to make selection more efficient [12,13,14].

With the help of molecular marker, disease resistance in soybean varieties have been developed [15]; resistance to cereal diseases in maize [16]; drought tolerance in maize [17] and resistance to shattering markers have been developed in soybean by [18].

Until recent, a few of QTLs for important agronomic traits in soybean have been identified, which include seed weight [19,20], days to flowering and maturity and seed filing period [21, 22,23] and number of pods per plant [24]. The aim of this study is to identify the QTLs determining seed yield and its related traits in soybean. These genomic regions could provide a basis of mapping genes responsible for seed yield with the ultimate aim of increasing soybean yield.

2. MATERIALS AND METHODS

2.1 Plant Materials

The 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.

2.2 Study Location and Research Design

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 Randomised Complete Block Design (RCBD) with three replications in 2014 and 2015 cropping seasons. A single row plot was adopted of length 3 m by 0.6 m maintaining 15 plants per plot with a spacing of 60 cm by 20 cm between and within rows.

2.3 Data Collection

Data were collected on ten competitive mid-plants on the following yield related characters; days to flowering (days), days to maturity (days), number of pods per plant (NPP), total pod weight (g), 100-seed weight (g) and seed yield per plant (g). Days to flowering was recorded as number of days from planting to when 50% of the plants in the plot were flowering; days to maturity was counted from planting date when 95% of the pods on each plot had reached their matured brown colour; number of pods per plant was obtained by counting all the pods on the selected plants at maturity; total pod weight was obtained by weighing the pods from each of the selected plants; 100-seed weight was estimated as the weight of one hundred well dried, clean and randomly selected seeds of each of the selected plant; seed yield was estimated by measuring the weight of all the seeds from each of the selected plants after threshing and removing all the chaffs and dirt.

2.4 Statistical Analysis

Phenotypic correlation among the characters were obtained following the method of [25]. The analysis of variance was conducted using individual plot means for each year and combined across the years. The broad sense heritability was calculated using the method of [26].

$$\text{Heritability} = \sigma^2 g / \sigma^2 ph$$

$$\text{Genetic variance} = \sigma 2g = \frac{MSG-MSE}{r}$$

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

Where:

MSG and MSE are genotypic and error mean squares respectively,
r is the number of replications, $\sigma^2 e$ is the environmental variance

2.5 DNA Extraction and SNP Analysis

Total genomic DNA was extracted using the modified mini preparation protocol described by [27] 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℃) plant extraction buffer(PEB) [containing 637.5 ml of double distilled water (ddH_20), 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 $Nacl_2$ and 62.5 ml 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℃ 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 (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℃. 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 [28,29,30]. 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℃ for 4 min followed by 45 cycles of 94℃ for 20 s, 56℃ for 30 s, 72℃ for 1 min, and then followed by 3 min at 72℃. 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℃ for 20 minutes followed by 5 minutes at 85℃ to deactivate the enzyme – called SAP procedure in iPLEX.

2.6 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℃ for 30 s followed by 40 cycles of 94℃ for 5 s, then followed 5 cycles of 52℃ for 5 s, and 80℃

for 5 s within the 40 cycles, then 72℃ 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).

2.7 QTL Detection

The QTL analysis was performed following composite interval mapping (CIM) method [31] using the software WINQTL CART. Vsn 2.5 [32]. An initial minimum logarithm of odd (LOD) score of 2.0 was set to determine the presence of a QTL in a genomic region. 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.

Plates 1, 2 and 3 show some of the laboratory activities in the course of this study.

Plate 1. DNA isolation of the leaves of the F2 plants

Plate 2. DNA quantification using spectrophotometry

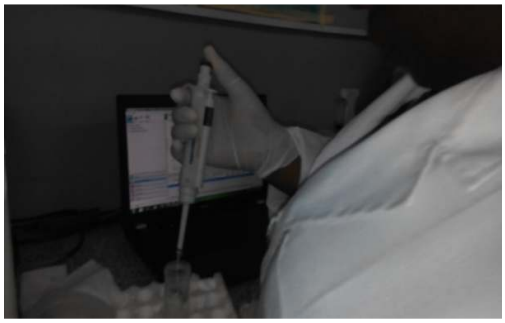

Plate 3. Staining of gel and loading of amplicon

3. RESULTS

Genetic parameter data for seed yield related characters is presented in Table 1. All the characters recorded low coefficient of variation ranging from 0.36% (NPP) to 4.41% (DTF). Though the heritability estimates for all the characters were high ranging from 67% (100 SW) to 100% (NPP, TPW and SYP) in this population. The genetic advance as percent of mean ranged from 1.13% (NPP) to 10.29% (100-SW). Heritability alone may be misleading during selection. Hence, heritability and genetic advance as percent of mean together should be taken into consideration for selection.

The estimates of phenotypic coefficient of correlation is presented in Table 2. Coefficient of correlation ranged from -0.09 to 0.99. Positive and significant correlation were detected between DTF and DTM; TPW and NPP; 100-SW and NPP and TPW; SYP and NPP, TPW and 100-SW.

3.1 QTL Analysis

All QTLs identified in this study are listed in Table 3. Using composite interval mapping (CIM) 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 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 BARC-028793- 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 through composite interval mapping in individual year demonstrated 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.

4. DISCUSSION

The high broad sense heritability estimates in this study for DTF, NPP and 100 SW corroborates the findings of [21,19]. High heritability indicates less environmental influence in the observed variation [34]. However, high estimates of heritability percentage do not necessarily provide high values of genetic advance [35]. Heritability estimates together with genetic advance are more simportant than heritability alone to predict the resulting effect of selecting the best individuals [36,37].

The significant positive correlation of number of pods and 100 - seed weight and seed yield is consistent with the findings of [38]. A character which had high degree of positive and significant correlation coefficient would be a very effective tool to improve seed yield. From this study, the positive and significant correlation recorded between seed yield and number of pods, 100 - seed weight and total pod weight, is an indication that every significant increase in any of these characters would lead to an appreciable increase in seed yield. In this regard utmost attention may be given to these characters during selection for yield improvement.

Remarkable progress has been made in the construction of soybean genetic maps and QTL mapping of important agronomic characters [39]. The absence of QTL for NPP is contrary to the findings of [24] who reported that NPP has been found to be located on linkage group A1. Likewise the absence of QTLs for 100 SW recorded in this study is contrary to the earlier research report that QTL for 100 SW was located on linkage group D1a and L [40,41].

Table 1. Mean and variability parameters for seed yield and component characters in F_2 population derived from 7 X 7 diallel cross of soybean over two cropping years

Character	Year	Min	Max	Mean	CV%	Heritability%	GA%
DTF	2014	33.00	48.30	40.03	4.41	81.00	4.17
	2015	45.17	64.10	52.92	1.29	99.00	3.85
DTM	2014	78.00	89.67	82.02	0.76	98.00	2.46
	2015	115.17	134.10	123.05	0.44	99.00	1.66
NPP	2014	102.00	180.70	139.00	0.36	100.00	1.48
	2015	115.00	253.50	181.95	0.91	100.00	1.13
TPW	2014	25.00	56.62	39.93	1.34	100.00	5.16
	2015	22.58	98.58	56.54	0.88	100.00	3.64
100 SW	2014	12.00	16.91	14.30	3.73	67.00	9.65
	2015	11.90	20.50	18.65	2.60	93.00	10.29
SYP	2014	16.00	39.26	28.42	1.80	100.00	7.25
	2015	11.90	86.20	41.95	1.23	100.00	4.91

DTF= Days to flowering; DTM = Days to maturity; NPP = Number of pods per plant; TPW = Total pod weight; 100 SW = 100 - seed weight; SYP = 164 Seed yield per plant

Table 2. Phenotypic correlation coefficient among yield related characters in F_2 population derived from 7 X 7 diallel 171 cross of soybean over two cropping years

Character	Year	DTF	DTM	NPP	TPW	100 SW
DTM	2014	0.99**				
	2015	0.94**				
NPP	2014	-0.28**	-0.27**			
	2015	-0.19**	-0.08			
TPW	2014	-0.32**	-0.32**	0.89**		
	2015	-0.18*	-0.08	0.96**		
100 SW	2014	0.11	0.11	0.43**	0.36**	
	2015	0.04	0.07	0.37**	0.29**	
SYP	2014	-0.36**	-0.36**	0.88**	0.92**	0.30**
	2015	-0.15*	-0.09	0.89**	0.94**	0.31**

DTF= Days to flowering; DTM = Days to maturity; NPP = Number of pods per plant; TPW = Total pod weight; 100 SW = 100 - seed weight; SYP = 174 Seed yield per plant

Table 3. QTLs associated with seed yield and its components in 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
NPP	2014	-	-	-	-	-	-	-	-
	2015	-	-	-	-	-	-	-	-
	MEAN	-	-	-	-	-	-	-	-
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
100 SW	2014	-	-	-	-	-	-	-	-
	2015	-	-	-	-	-	-	-	-
	MEAN	-	-	-	-	-	-	-	-
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, NPP = number of pods per plant, TPW= total pod weight; 100SW = 100- seed weight, SYP= seed yield

From previous studies on QTLs for days to flowering as reported in soybase [42] 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 corroborates the findings of [43]. They reported that 13 QTLs for seed yield were mapped on linkage groups A1, B2, C1, C2, J, K, L and O. The QTL for days to maturity mapped on linkage group A1 in this study is comparable to the findings of [44], 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. According to [45], QTLs for pod wall thickness and pod wall were mapped on linkage group A1. QTL for total pod weight detected on linkage group A1 in this study can then be referred to as a novel QTL. 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 indicates that considerable attention should be given to this linkage group in future soybean breeding programmes. The markers on this linkage group are expected to be simultaneously considered in marker assisted breeding.

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 [40]. Hence, it is required that QTL analysis be carried out across multiple environments. Stable and validated QTLs are more desirable to be used in marker assisted selection [46]. 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 the current study because there have been no report of such QTLs to be found located on linkage group A1. One of the novel QTLs detected in this study could be referred to as a stable QTL (DTF) due to the fact that it was detected in both years while the other novel QTL (TPW) is not a stable QTL because it was detected in only one of the years and in their mean.

5. CONCLUSION

There exist an appreciable level of variation in the F_2 population utilized for this study. Two stable (QTLs for DTF and DTM) and two novel QTLs for DTF and TPW were identified in the current study. The failure to detect any QTL for NPP and 100-SW in this study could be due to small number of population utilized for the 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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