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A SNP Genetic Linkage Map Based on F2 Population Genotyping in Soybean, *Glycine max* (L.) Merrill

O. F. Adewusi[1*], A. C. Odiyi[1] and B. O. Akinyele[1]

[1]Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria.

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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Original Research Article

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ABSTRACT

This study reports a low density genetic linkage map based on 7 x 7 F_2 population of soybean, *Glycine max* (L.) Merrill and constructed with single nucleotide polymorphism (SNP) markers. 50 SNP markers were used to screen the DNA samples of the soybean out of which only 32 were polymorphic with the samples. These 32 SNP markers were mapped using the Mapchart of WINQTL CART. Vsn 2.5 and the SNPs were distributed on 13 LGs (linkage groups) among the 20 chromosomes of the soybean genome. The total map length was just 2211.46cM with an average marker density of 905.86cM. This SNP based genetic linkage map of soybean could be used to map quantitative trait loci (QTL) for important agronomic characters in soybean.

Keywords: Soybean; F2 population; genotyping; linkage map; SNP markers.

**Corresponding author: E-mail: ofadewusi@gmail.com;*

1. INTRODUCTION

Soybean has 20 pairs of chromosomes (2*n* = 40) and 20 linkage groups (LGs) assigned to them [1]. To cover the whole soybean genome for the purpose of genome-wide analysis, a large number of molecular markers are imperative [2,3]. Molecular markers have been extensively used for the identification and authentication of plant taxonomy and these markers are not influenced by age, physiological condition of sample and environmental factors [4,5]. The efficiency of DNA based marker in discriminating closely related varieties and even individuals of same species is very high. They have proved their utility in various fields such as genetic diversity, genomic fingerprinting and mapping, population genetics, taxonomic studies and plant breeding programs [6,7].

The application of molecular genetic mapping technique has allowed development of a detailed soybean genetic map in short time [8]. In the early 1990's, studies were conducted to identify molecular markers associated with quantitative trait loci. Several soybean genetic maps which have been published already include hundreds of DNA-based markers [9]. Markers from these maps have been used for the identification of QTLs for agronomically important traits including disease resistance [10]. Genetic linkage maps are important genomic tools for identifying quantitative trait loci (QTL) and candidate genes to enhance marker-assisted selection (MAS) in crop improvement programs [11]. In the past few years, SNP markers have been widely used for assembling linkage maps due to having much variation or diversity [12,13]. Soybean scientists have constructed genetic linkage maps by using molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). The first soybean genetic linkage map was constructed by [14] using RFLP markers. The soybean genetic linkage maps developed with RFLPs, AFLPs, RAPDs, and SSRs were expanded to include SNP markers in a soybean transcript map constructed by [15], who were the first to report a soybean genetic linkage map using SNP markers. SNPs are now projected to become the most useful of genetic markers, especially for the construction of dense maps [16,17]. High-throughput SNP genotyping is widely used in plant genomics studies such as genome wide association [18,19,20], comparative genomics [21] and genetic linkage maps construction [22].

Furthermore, millions of SNPs have been generated in Soybean [23], Arabidopsis [24], Rice [25] and other crops [26,27] in order to enhance studies on marker assisted breeding or selection. The objective of this study was to construct a SNP- based genetic linkage map that could be used for quantitative trait locus (QTL) detection of desired agronomic character.

2. MATERIALS AND METHODS

2.1 Plant Materials

In this study, 63 F_2 population derived from a 7 x 7 diallel cross of soybean was used. Seeds of 7 soybean genotypes were obtained from the soybean germplasm collection of International Institute of Tropical Agriculture (IITA), Ibadan Oyo State, Nigeria. The F_1s were generated in 2014 and advanced to F_2 generation in 2015. Three plants from each of the 21 crosses were used for the analysis. Two seeds of each of the F_2s were sown in pots filled with top soil and at two weeks after planting, the young leaves were collected from the seedlings and DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) protocol of [28].

2.2 SNP Genotyping and Genetic Map Assembling

The SNP genotyping was performed at the Inqaba Biotechnical Laboratory in South Africa on the mass array system using the IPLEX reagents. The assay procedure encompasses DNA amplification, extension reaction, staining and imaging following the procedure of [29,30, 31]. The SNP map is constructed using the Mapchart program of the WINQTL CART. Vsn. 2.5 [32].

3. RESULTS AND DISCUSSION

A total of 50 SNP markers were used to genotype the F_2 population out of which only 32 SNP markers were polymorphic while 18 markers were monomorphic and they were discarded. For each of the SNP markers, the genotyping data represent the following possible genotypes; AA, GG, CC.TT, AG, CG, CT, AT, TG and GT. These 32 SNPs were analysed using the Mapchart of WINQTL CART. 2.5 based on the markers distances. The 32 SNPs were distributed on 13 linkage groups. The linkage

groups were numbered 1 to 19 based on the assigned chromosome number of soybean (1). The basic information on the linkage groups is presented in Table 1.

The current map spanned 2211.46cM with an average marker density of 905.86cM (Table 1). The genetic length of the linkage groups (LGs) ranged from 3.08 cM (chr 6/LGC2) to 697.93 cM (chr 8/LGA2). The most covered marker linkage group was chromosome 8 (linkage group A2) that had 11 SNPs with an average marker density of 63.45cM. In contrast, linkage groups D1a, N, C2, M, B1, B2, E, D2, G and L each had the least number of SNP marker of 1.

Fig. 1 shows the SNP based soybean genetic linkage map. The SNP markers and their distances in cM are shown on the chromosomes. The chromosomes were drawn using Mapchart of WinQTLCart.V2.5. The SNP markers and distances in centiMorgans are shown with each marker's name on the right side of the chromosomes while their respective map distance is shown on the left side. The SNP markers were distributed on 13 chromosomes out of the 20 chromosomes in soybean genome. Most of the markers were located on chromosome 5 (9 markers) and chromosome 8 (11 markers) respectively. Chromosome 8 had a map interval distance of 9.10cM to 145.5 cM with an average map distance of 63.46Cm. Whereas a map interval distance of 39.40 cM to 100.10 cM with an average map distance of 77.16cM was recorded in chromosome 5.

3.1 Discussion

As earlier indicated, genetic linkage maps have been constructed using molecular markers such as RFLPs, RAPD, AFLPs, SSR and recently SNPs. In the recent times, the use of SNP markers have become more pronounced and projected to be the most useful marker due to its attribute of great deal of variation or diversity. [16] also opined that SNP markers have become the most useful in terms of construction of genetic linkage maps because of its distinguishing feature of variation. The current study reports a genetic map covering a length of 2211.46cM with the relative positions of the markers found consistent with *Glycine max* consensus map 4.0 [33]. The SNP markers were distributed on 13 linkage groups. The linkage groups were numbered from 1 to 19 based on the assigned chromosome number of soybean [1]. Eleven (11) markers were located on chromosome 8 being the highest covering approximately 697.93cM followed by chromosome 5 which had 9 markers covering 694.14cM. The remaining chromosomes have one SNP each with the exception of chromosome 16 which had 2 markers covering 108.23cM. The first genetic map in soybean constructed on 150 RFLP encompassed about 1,500cM [14]. Subsequently, different markers have been used in constructing genetic map, in soybean covering about 2,500 cM by combining markers and maps which include, RFLPs [34], AFLPs [35], SSR [36] and SNP [37].

Table 1. Distribution of markers and their properties on the molecular linkage groups and chromosomes of soybean

Chromosome no	MLG	No of markers	Position (cM)	Average marker density
1	D1a	1	102.96	102.96
3	N	1	75.30	75.30
5	A1	9	694.14	77.13
6	C2	1	3.08	3.08
7	M	1	17.88	17.88
8	A2	11	697.93	63.45
11	B1	1	77.01	77.01
14	B2	1	115.18	115.18
15	E	1	45.99	45.99
16	J	2	108.23	54.12
17	D2	1	124.02	124.02
18	G	1	43.22	43.22
19	L	1	102.52	102.52

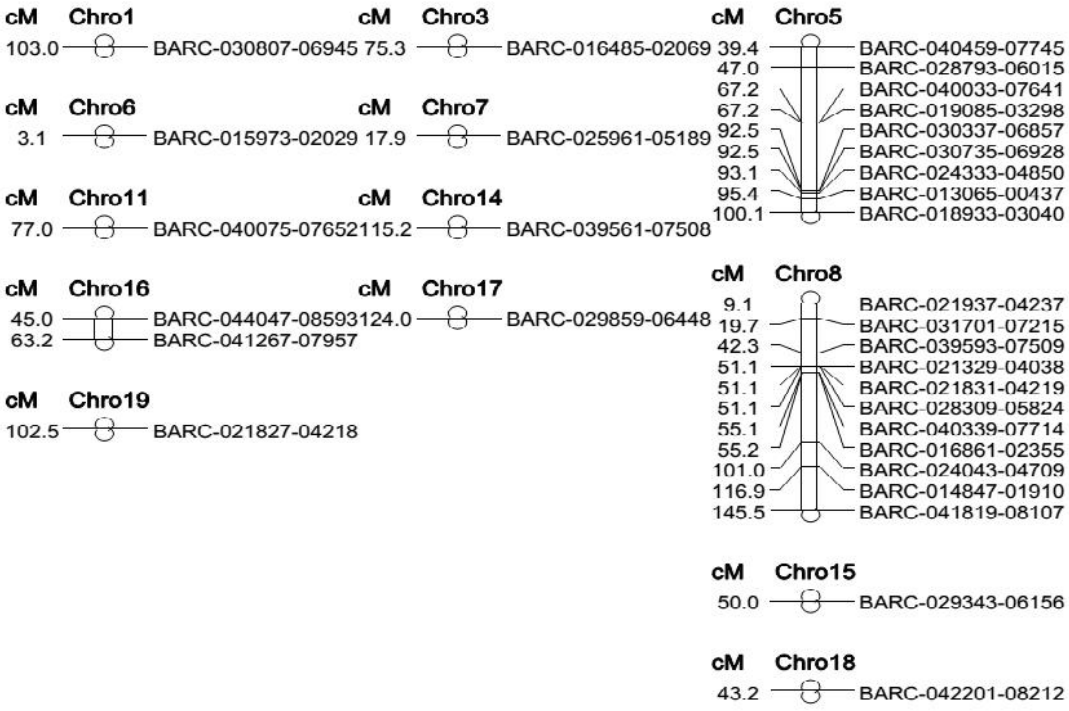

Fig. 1. SNP genetic linkage map based on 63 F_2 populations derived from 7 x 7 diallel cross in soybean*, Glycine max*

However, the genetic map constructed in this study is comparable in terms of length of map to other published maps reported earlier by [38] with map length of 2200cM; [15] with map length of 2,389 cM and [33] with map length of 2,229.40cM.

Furthermore, the genetic linkage map in the current study was constructed based on single population and few markers. However the map length (2,211.46cM) recorded by it is comparable to earlier published work of [37] with 642 SNP markers, map length 1,524.7cM with average marker distance of 2.37cM and [35] with 252 SSR markers of map length 2,200 cM with average marker distance of 8.73 cM. The genetic linkage map of the current study and that of [39] were based on a single population but with differing number of SNP markers. Although the latter work had a comparatively larger number of SNP markers (550) than the current study (50 SNP), the map length and the marker position of this study are higher than that of [39].

4. CONCLUSION

The present study showed a low density genetic linkage map of soybean (32 SNPs, total map length of 2211.46cM; average marker density of 905.86cM) based on single nucleotide polymorphisms (SNPs) markers. The availability of SNP markers attached to a specific region of the soybean genome would serve as a basis for detection of quantitative trait loci (QTLs) for agronomic characters in soybean that could be used in marker assisted selection (MAS).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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