

Full Length Research Paper

Genetic linkage map of cowpea (*Vigna unguiculata* (L.) Walp) using SNP markers

Adetumbi J. A.*, Akinyosoye S. T., Olowolafe M. O., Oloyede-Kamiyo Q. O.
and Agbeleje O. A.

Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Moor Plantation, Ibadan, Oyo State, Nigeria.

Received 12 December, 2015; Accepted 5 April, 2016

Genetic linkage maps provide a genomic framework for quantitative trait loci identification applied in marker assisted selection breeding in crops with limited resources. It serves as a powerful tool to breeders for analysing the mode of inheritance of genes of interest and monitoring of the transmission of target genes from parents to progeny. Single nucleotide polymorphism (SNP) markers have a great influence on mapping research studies: for faster development of new varieties of crops due to its abundance and high number of polymorphism. Thus, this research work aimed at constructing genetic linkage map of cowpea using SNP markers. This will facilitate development of new varieties of cowpea. Single seed descent method of breeding was used to generate mapping population between IT-95K-193-12 and Ife-Brown. Fresh leaves from both parental lines and F1 hybrids were genotyped with 135 SNP markers. Data from 125 polymorphic SNP markers were used to construct linkage map. Results revealed 12 linkage maps with an average of 10 markers to a chromosome and average marker distance of 3.02 cM between the markers. Constructed map provides basic information that could assist in genetic improvement of cowpea, most especially in developing brown blotch resistant cowpea varieties through detection of quantitative trait loci (QTL) responsible for brown blotch resistance in cowpea when utilizing marker assisted selection method.

Key words: Cowpea, genetic linkage map, single nucleotide polymorphism (SNP) markers, brown blotch resistance.

INTRODUCTION

Genetic map refers to chromosomal linear linkage map which uses chromosome recombinant exchange rate as relative length units. It mainly consists of genetic markers and serves as a starting point to map quantitative trait loci (QTL) for target traits. Construction of a high-density

genetic linkage map is of great significance in variety development because it can be used to locate and mark the target gene to promote the application of marker-assisted breeding (Huaqiang et al., 2012). The construction of a linkage map requires a segregating

*Corresponding author. E-mail: jaadetumbi@yahoo.com.

plant population (that is, a population derived from sexual reproduction). The parents selected for the mapping population will differ in one or more traits of interest (Angaji, 2009). Consensus genetic linkage maps provide a genomic framework for quantitative trait loci identification, map-based cloning, assessment of genetic diversity, association mapping and applied breeding in marker-assisted selection schemes, among “orphan crops” with limited genomic resources such as cowpea (Muchero et al., 2009).

Cowpea feeds millions of people in the developing world with an annual world production estimated at about 4.5 million metric tons on 12 to 14 million ha. Nigeria is the largest producer of cowpea in Africa (Agboola, 1979) but despite the importance of cowpea in nutritional improvement of many families in Nigeria, yield is still very low due to various biotic factors such as insect and disease infestation. One of the suggested solutions to the problem is breeding for resistance and tolerance which has not been rapid as compared to other crops such as maize and rice. This is attributed to low genetic diversity reports in cultivated cowpea (Doebley, 1989) as well as excessive time and resources required to breed for insect and disease resistance with the aid of phenotypic selection.

Because of the multiplicity of breeding objectives in beans (Gepts, 1988), no single population would segregate for all the economic traits of interest. Thus, genes for these traits have been located on maps developed from different segregating populations (Kelly et al., 2003). When screening conditions are not ideal, indirect selection of race specific genes offers breeders a viable alternative to ensure that favourable gene combinations are present in new breeding lines. Markers, tightly linked to individual race-specific resistance genes, form the basis for effective indirect selection of major gene resistance (Kelly et al., 2003). From a breeder's perspective, linkages among resistance loci are beneficial when they are derived from an individual parental line (cis-configuration), because when crossed with susceptible parents these resistances will be inherited as a single unit and will likely remain intact in subsequent progeny (Geffroy et al., 1998; Kelly et al., 2003). When linked resistance genes are donated by different parents (trans-configuration) recombination between the loci is necessary to obtain both resistance sources in a single line (Kelly et al., 2003).

The first attempt to build a genetic map of cowpea was performed by using a population resulting from a cross between an improved genotype and its wild progenitor *Vigna unguiculata* ssp. *Dekindtiana* (Fatokun et al., 1992). The recent influx of molecular markers has improved our understanding of cowpea's genome architecture. Single nucleotide polymorphism (SNP) is abundant and produces high number of polymorphism in the genome. Due to the abundance of SNPs and development of sophisticated high-throughput SNP detection systems, it has recently been proposed that SNP markers will have a

great influence on future mapping research studies and marker assisted selection (MAS) (Rafalski, 2002; Koebner and Summers, 2003). Genomics methods have led to a renaissance of interest in both fields of enquiry. While the earliest applications of genomics were in the mapping of Mendelian loci, the emerging importance of SNPs lies in the mapping and identification of quantitative trait loci, which are loci that contribute to polygenic phenotypic variation. SNPs have been reported to provide the means to scan genomes for linkage to QTL; however, it has also been observed that the vast majority of QTL effects are almost certainly due to unidentified SNPs. For both reasons, characterization of the distribution of SNP variation is a major goal of most genome projects (Greg and Muse, 2004). Thus, this research work aimed at constructing genetic linkage map of cowpea using SNP markers with a view to accessing the usefulness of SNPs for linkage map construction in cowpea so as to facilitate development of improved cowpea varieties.

MATERIALS AND METHODS

Construction of mapping population

Single seed descent method of breeding was employed in generating mapping population. One cowpea variety known to be resistant to most fungal disease of cowpea IT-95K-193-12 and a known susceptible variety Ife-Brown (Adegbite and Amusa, 2008) selected from an earlier conducted screening trial in Institute of Agricultural Research and Training (IAR&T) screen house were used for the experiment. The two varieties were grown in the screen house of I.A.R&T. and crossed by rapid method of emasculation procedure described by Rachie et al. (1975). This was done in the morning between 6 – 8 am (when some buds had initiated and flowered). Ninety six (96) F₁ populations were obtained from the crosses. The F₁ plants were self-pollinated to obtain F₂ segregating population.

Genotyping with single nucleotide polymorphism (SNPs) markers

Both parents and F₂ progenies were grown in the screen house of IAR&T for fresh leaf tissues. Two weeks after planting, ten leaf discs were cut from the young leaf tissues of each plant and placed into the wells of a storage plate. The plate was sealed with a perforated (gas-permeable) heat seal and placed in a heavy-duty, sealed bag with desiccant to dehydrate and preserve the leaf tissue during transit to LGC genomics in United Kingdom for genotyping.

LGC genomics used a unique proprietary in-house technology (oKtopure™ protocol) to extract the total DNA. Isolated DNA was analyzed using UV spectrophotometry to estimate both the quality and quantity of the DNA, while preliminary PCR at a serial dilution was carried out and the results produced were duplicated to identify the best dilution to run the samples.

The SNP genotyping was done using KASP genotyping reactions. KASP™ is also proprietary genotyping technology of LGC™. It consists of three components namely the sample DNA, KASP assay mix and KASP master mix. KASP master mix contains two universal (FRET) fluorescent resonance energy transfer cassettes (FAM and HEX), ROX™ passive reference dye, Taq polymerase, free nucleotides and MgCl₂ in an optimized buffer solution, while

the KASP assay mix is specific to the targeted SNP and consists of two competitive, allele-specific forward primers and one common reverse primer. Each forward primer incorporates an additional tail sequence that corresponds to one of two universal FRET cassettes present in the KASP master mix (<http://www.lgcgroup.com/our-science/genomics-solutions>)

Linkage mapping construction

Data from all the SNP markers that were polymorphic were used to construct linkage map using QTL IciMapping software 3.2 (Wang et al., 2014). Ninety eight population samples (plus two parents) were used and 135 SNP markers were used to screen the population. Linkage grouping was done using the independent logarithm of difference at a LOD score (logarithm of the odds) of 3.0 as threshold for a better grouping. Recombination fractions were converted into map distances in centi-Morgans (cM) using the Kosambi (1944) mapping function. 'Group' command was used to identify linkage groups and 'Order' command was used to establish the most-likely order within each linkage group, while the orders were confirmed by permuting all adjacent markers by the 'Ripple' function.

RESULTS AND DISCUSSION

Genetic linkage map based on SNP markers

All the SNP markers used in this study were co-dominant. Out of 135 markers used for genotyping, only 125 SNP were polymorphic with parental lines and hybrids. Twelve (12) linkage maps were generated at a LOD score of 3.5 and recombination fraction of 1.0 as threshold for declaring linkage. A LOD score of >3 is an indication that the null hypothesis that the likelihood of a certain recombination fraction (r) versus the likelihood of no linkage ($r = 0.5$) and a ratio of likelihoods of 1,000 to 1 is rejected (Haldane and Smith, 1947). Each linkage map represents number of chromosomes possessed by cowpea genome. The first linkage map had 23 SNP markers (Figure 1) which covered 72.02 cM of the genome, followed by eighth linkage with 21 SNP markers and covered 41.98 cM. The fourth linkage map is the next with 20 SNP markers, which covered 101.05 cM, while the remaining linkage maps had between 2 to 9 SNP markers (Figure 1 and Table 1).

Polymorphic 125 SNP markers detected in this study, covered 378.1 cM of the whole genome. There was an average of 10 markers to a chromosome with an average marker distance of 3.02 cM between the markers (Table 1). Similar result was reported by Ubi et al. (2000) and Menendez et al. (1997) in their studies with each reporting 12 linkage groups for cowpea.

Ogundiwin et al. (2005) reported the detection of 15 linkage groups for *Vigna vexillata*, a wild relative of cowpea while Ouédraogo et al. (2002) constructed a genetic linkage map for cowpea (*V. unguiculata* L. Walp.) based on the segregation of various molecular markers and biological resistance traits in a population of 94 recombinant inbred lines (RILs) derived from the cross

Table 1. Features of the genetic linkage map of the 125 markers segregating among mapping population derived from the cross Ife-brown x IT-95K-193-12.

Chromosome ID	Number of SNP markers	Length (cM)
1	23	72.02
2	8	9.99
3	2	0.00
4	20	101.05
5	8	21.42
6	7	20.64
7	5	14.37
8	21	41.98
9	9	26.47
10	5	27.46
11	9	12.03
12	8	30.67

between 'IT84S-2049' and '524B'. A set of 242 molecular markers, mostly amplified fragment length polymorphism (AFLP) and 181 random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and biochemical markers were used. Eleven (11) linkage groups (LGs) were generated spanning a total of 2670 cM, with an average distance of 6.43 cM between markers. Omo-Ikerodah et al. (2007) reported that a set of 92 recombinant inbred lines (RILs) were generated from a cross between susceptible and resistant lines of cowpea to flower bud thrips. One hundred and thirty nine markers [134 amplified fragment length polymorphism (AFLP) and 5 cowpea derived microsatellites] were used to construct a linkage map using this set of RILs. The linkage map spanned 1620 cM of the cowpea genome and markers were distributed in 11 linkage groups with an average distance between adjacent markers of 9.6 cM.

Linkage relationship among markers with a recombination frequency of less than 50% LOD or LOD score of 3 or greater indicates that a gene and markers are linked. Any two markers with a LOD higher than the threshold, or recombination frequency lower than the threshold and/or genetic distance lower than the threshold were reported to be linked together (Van Oojien et al., 2002; Wang et al., 2014). Sturtevant (1913) proposed that crossing-over was a random event and that if the assumption is correct, there is an equal chance of it occurring at any position along a pair of lined-up chromatids. This implies that two genes that are close together will be separated by crossover less frequently than two genes that are more distant from one another. Markers are mapped relative to one another on chromosomes and used as signposts against which genes of interest that are linked with marker are mapped (Boopathi, 2013).

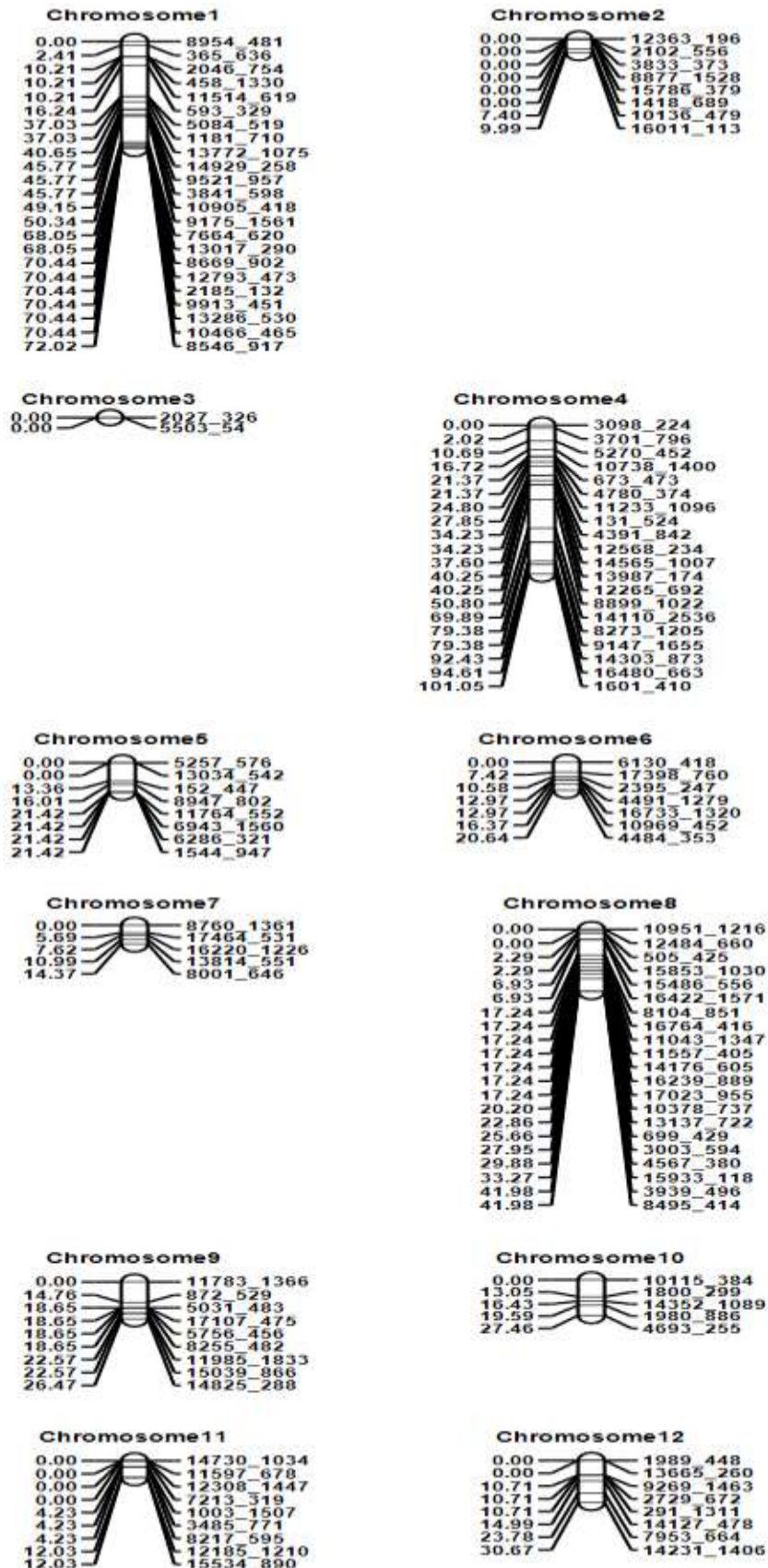


Figure 1. Genetic linkage map for chromosomes 1-12.

Conclusion

The result revealed 12 linkage maps with an average of 10 markers to a chromosome and average marker distance of 3.02 cM between the markers. The study shows that the set of SNPs markers used in this study provides baseline information to study cowpea genome with the aim of facilitating its improvement in the breeding programmes. Therefore, SNP markers could be used to construct genetic linkage map of cowpea genome which can provide basic information towards genetic improvement of cowpea in breeding programmes, most especially in developing brown blotch resistance in cowpea using marker assisted selection method. This will facilitate the development of new brown blotch resistant cowpea variety within a very short time.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The Authors acknowledged Generation Challenge Programme, Mexico for funding the genotyping of the parental lines and the progenies at LGC genomics.

REFERENCES

- Adegbite AA, Amusa NA (2008). The major economic field diseases of cowpea in the humid agro-ecologies of South-western Nigeria. *Afr. J. Biotechnol.* 7(25):4706-4712.
- Agboola SA (1979). *An Agricultural Atlas of Nigeria*, Oxford University Press, London pp. 95-97.
- Angaji SA (2009). Single nucleotide polymorphism genotyping and its application on mapping and marker-assisted plant breeding. *Afr. J. Biotechnol.* 8(6):908-914.
- Boopathi NM (2013). *Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits*. Springer Science & Business Media.
- Doebley J (1989). Isozyme evidence and evolution of crop plants. In: Soltis D.E. & Soltis P.S. (Eds.), *Isozymes in Plant Biology*, pp. 165-191.
- Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992). Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics* 132:841-846.
- Geffroy V, Creusot F, Falquet J, Sévignac M, Adam-Blondon A-F, Bannerot H, Gepts P, Dron M (1998). A family of LRR sequences in the vicinity of the Co-2 locus for anthracnose resistance in *Phaseolus vulgaris* and its potential use in marker-assisted selection. *Theor. Appl. Genet.* 96:494-502.
- Gepts P (1988). Provisional linkage map of common bean. *Annu. Rep. Bean Improv. Coop.* 31:20-25.
- Greg G, Muse SV (2004). *A Primer of Genome Science*, Sinauer Associates Inc. Publishers. P 378.
- Haldane JBS, Smith CAB (1947). A new estimate of the linkage between the genes for colour-blindness and haemophilia in man. *Ann. Eugen.* 14:10-31.
- Huaqiang T, Manman T, Qian L, Yongpeng Z, Jia L, Huanxiu L (2012). A Review of Molecular Markers Applied in Cowpea (*Vigna unguiculata* L. Walp.) Breeding. *J. Life Sci.* 6:1190-1199.
- Kelly JD, Gepts P, Miklas PN, Coyne DP (2003). Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Agronomy & Horticulture -- Faculty Publications. Paper* 73. <http://digitalcommons.unl.edu/agronomyfacpub/73>.
- Koebner RM, Summers RW (2003). 21st century wheat breeding: selection in plots or detection in plates. *Trends Biotechnol.* 21:59-63.
- Kosambi DD (1944). The estimation of map distance from recombination values. *Ann. Eugen.* 12:172-175.
- Menendez CM, Hall AE, Gepts P (1997). A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. *Theor. Appl. Genet.* 95:1210-1217.
- Muchero W, Diop NN, Bhat PR, Fenton RD, Wanamaker S, Pottorff M, Hearne S, Cisse N, Fatokun C, Ehlers JD, Roberts PA, Close TJ (2009). A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. *Proc. Natl. Acad. Sci.* 106(43):18159-18164.
- Ogundiwin EA, Thottappilly G, AkenOva ME, Pillay M, Fatokun CA (2005). A genetic linkage map of *Vignavexillata*. *Plant Breed.* 124(4):392-398.
- Omo-Ikerodah EE, Fawole I, Fatokun CA (2007). Genetic mapping of quantitative trait loci (QTLs) with effects on resistance to flower bud thrips (*Megalurothripsjostedti*) identified in recombinant inbred lines of cowpea (*Vigna unguiculata*(L.) Walp.). *Afr. J. Biotechnol.* 7(3):263-270.
- Ouédraogo JT, Gowda BS, Jean M, Close TJ, Ehlers JD, Hall AE, Gillaspie AG, Roberts PA, Ismail AM, Bruening G, Gepts P, Timko MP, Belzile FJ (2002). An improved genetic linkage map for cowpea (*Vigna unguiculata*L.) combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. *Genome* 45:175-188.
- Rachie KO, Rawal KM, Franckowiak JD (1975). Rapid hand crossing of cowpea. *International Institute of Tropical Agriculture Technical Bulletin* 2. Ibadan, Nigeria.
- Rafalski JA (2002). Applications of single nucleotide polymorphisms in crop genetics. *Curr. Opin. Plant Biol.* 5:94-100.
- Sturtevant AH (1913). The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *J. Exp. Zool.* 14(1):43-59.
- Ubi BE, Mignouna H, Thottappilly G (2000). Construction of a genetic linkage map and QTL analysis using a recombinant inbred population derived from an intersubspecific cross of cowpea (*Vigna unguiculata*(L.) Walp.). *Breed. Sci.* 50:161-172.
- Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2002). MapQTL-4.0 software for the calculation of QTL position on genetic maps. *Plant Research International*, Wageningen.
- Wang J, Li H, Zhang L, Meng L (2014). *Users' Manual of QTL IciMapping*. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.