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QTL mapping for yield traits in vegetable cowpea

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ABSTRACT

The F_4 population comprising of 92 plants descended from the cross of semi-trailing, medium-long podded, low yielding cv. Kanakamony [*Vigna unguiculata* (L.) ssp. *cylindrica*] with pole type, long podded, high yielding cv. Sharika [*V. unguiculata.* ssp. *sesquipedalis*] was used for mapping the yield-related traits in vegetable type cowpea. Using 30 polymorphic SSR markers, a linkage map spanning 908 cM with two linkage groups (LG1 with eight markers spanning 637 cM and LG2 with five markers spanning 271 cM) was constructed. CLM0083 was an anchored marker for pod weight and total dry pod yield. For days taken for the first flowering, LG1 and LG2 had four and three hotspots, respectively. SMA confirmed that CLM0177 on LG1 and CLM0300 are anchored markers for this trait. For branch number, a hotspot was found on LG2 between CLM0200 and CLM0088. SMA confirmed the linkage of CLM0200 with this trait. For root length, a hotspot was found between CLM0244 and CLM0008 on LG1. On LG2, the hotspots were present between CLM0200 and CLM0088, and between CLM0260 and CLM0218. SMA confirmed the linkage of CLM0201, CLM0244 and CLM0300, with this trait. For plant height, a hotspot existed between markers CLM008 and CLM177 on LG1. For plant weight, a hotspot with anchored marker CLM0244 and flanking markers CLM0186 and CLM0008 was found on LG1. The region 24.25-126.86 cM on LG1, bracketed by CLM0244 and CLM0177 and with an anchored marker CLM0008, had QTL hotspots for days taken for first flowering, total dry pod yield, root length, plant height and plant weight.

Key words: *Vigna unguiculata* (L.), yield traits, linkage map, marker-assisted selection, microsatellite markers, SSR.

INTRODUCTION

Cowpea is a very important vegetable crop in the tropical and subtropical regions of the world, and it includes the pole type, long podded and high yielding *Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* and the bush or semi-trailing, medium to short podded and medium to low yielding *Vigna unguiculata* (L.) Walp. ssp. *cylindrica*. Breeding for vegetable cowpea is very much demand-driven. The commercial cultivation demands high yielding pole types with long and dark green pods, whereas short podded semi-trailing types are preferred under kitchen gardens and dry farming. Even though a sizable number of varieties are released under both types, there is still a large scope for the enhancement of pod yield and plant architecture in vegetable cowpeas.

For the marker-assisted selection of quantitative traits, Single Sequence Repeat (SSR) marker-based QTL maps are extensively used. SSR-based QTL mapping of domestication traits in vegetable cowpea was initially done by Andargie *et al*. (2). They later developed the maps for flowering time-related traits (Andargie *et al*., 3). QTL maps for pod length (Kongjaimun *et al*., 8), pod tenderness and total soluble solids (Kongjaimun *et al*., 9) were further developed. QTL maps for flowering time (González *et al*., 7), pod fiber content (Watcharatpong *et al*., 16) and horticulturally important traits (Xu *et al*., 17) have been reported. However, a comprehensive report on QTL and anchored markers for important yield and plant traits, developed on a highly diverse inter-subspecies population, is missing. This research work was carried out with the objective of finding out the QTL hotspots and markers associated with pod length, pod weight, pod number, days taken for first flowering, total dry pod yield, seeds per pod, number of branches, root length, plant height, and plant weight, in vegetable type cowpea using SSR markers segregating in an F_4 inter-subspecies population. These traits were selected because they are reported to be positively correlated to yield (Almeida *et al*., 1; Santos *et al*., 14).

MATERIALS AND METHODS

An F_2 mapping population was developed at the Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Thrissur, by crossing the semi-trailing, medium-long podded, low-yielding cv. Kanakamony [*Vigna unguiculata* (L.) ssp. *cylindrica*] with pole type, long podded, high yielding cv. Sharika [*V. unguiculata* ssp. *sesquipedalis*] (Pradhan *et al*., 13). This population was forwarded to F_4 through single seed descent method, and for mapping, 500 plants were raised following standard package of practices (Kumar *et al*., 10). This population has been

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evaluated for the morphological traits under study, and based on the clustering pattern developed from the unweighted hierarchical clustering (Mathew *et al*., 11), 92 plants representing the variability for the traits were selected as members of the mapping population for this study. Traits including pod length, pod weight, pod number, days taken for first flowering, total dry pod yield, seeds per pod, number of branches, root length, plant height, and plant weight were recorded from all 92 plants in the mapping population. This data was subjected to Box-Cox power transformation and the transformed values were used to derive BLUP (Best Linear Unbiased Prediction) for each trait in each plant, using R codes. R codes for used for Box-Cox transformation and generation of BLUP values are presented at https://doi.org/10.13140/RG.2.2.31048.74243.

One hundred cowpea-specific SSR primer sets (Xu *et al*., 18) were screened for polymorphism among the parents (Sharika and Kanakamony). Thirty successful primer sets were used to assess the allelic distribution in each locus in the mapping population (Table 1). DNA from all the 92 plants were isolated by CTAB method (Doyle and Doyle, 6) and PCR amplified

Table 1. Details of polymorphic primer sets used for genotyping the mapping population.

S. No.	Marker ID	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temp. (°C)
1	CLM0007	ACAGGTTCCTTGTGAAGCAC	GCCATACGCAACTCAGCTAT	55
2	CLM0008	CGGTTCTAGTGCCACCAA	GAAACCGGCACTGGAAAC	51
3	CLM0031	CGCTTTTGTAGGATTGGAAC	TTAGCATGGGAGAGTTTTCG	53
4	CLM0050	CTTCTCTCCGTCAAGTGGAA	AGCAGACAACCACAGATGCT	55
5	CLM0063	CATCCACCACATCAAAATCA	CCCAATTGAAGTCCTTGATG	55
6	CLM0066	AACCCAGCATACCTGCATAA	CTCGCCAATGATTCTGAGAT	53
7	CLM0068	AATGTTTGGACTGGTCAGGA	GAGGACAAGTCAGGAAGCAA	54
8	CLM0077	AAAGCGGAAAAAGTTTGGAT	AGCACTCTGCACACAAATCA	56
9	CLM0083	GGCGACGTCTTTCCATATTA	TGGAATCGATGTTGTGATTG	55
10	CLM0085	CACAACTGTGATTTGCTCGAT	TCGGAAACAGGTTCACCTAC	55
11	CLM0088	TCGTCGGTCTTCATAAAAATG	AACGCTTCGATTATCTGCAC	53
12	CLM0101	TGTCTTTGCAGGTTGTTTCA	GCTACATGGTGATGCCACTT	53
13	CLM0119	GAGATGTTGAGATGGTGGCT	CCTTGGTCATTGAACCTCTC	56
14	CLM0151	TGCTTGAGTGTCACTTGAATG	TCGCAAAGAGAGGAATATCG	54
15	CLM0156	GGGCTTCCTAGGTCACAAAT	CCATTCTCTTCGGTTAGTTATT	55
16	CLM0168	TGAGAGGACCAAATTACTCCA	TCACCATTCTAAGAAACAAGTGA	57
17	CLM0177	AATTGGGTTGTAAAGTGAGATTT	CGAAAGTGGTTTGCGTATTT	54
18	CLM0186	TTTGAACTCATATAAAGCACTTG	GATCCTTCTTCCCTCTCTCG	57
19	CLM0195	AGGCATGATGTGTGGAGTTT	TTTCTCACGTTGTTTAGCCTT	55
20	CLM0200	AATTTGATCGCCTAACGACA	TCAAACGTATATGCGTAAATAAT	52
21	CLM0201	CCAAAACAAACACCAACCTC	GAGACCTGCGATCAGAACAT	54
22	CLM0218	TTTCCGATTTGCGATTTTTA	CGACCAGTGACAAATGAACC	51
23	CLM0244	GTGGAGTTCAGTGGCAAAGT	CCAAAATCGCATGTAGTTCC	54
24	CLM0251	CTTTTCATGGGAATTGTTGG	TGAACTTTCCAAGGAACTCG	52
25	CLM0260	TCGATCAAATTTTCCTCTGC	TGCCACCATCTTTCATTTCT	51
26	CLM0279	TGCAAAACGTGAAAGCAATA	ACAAGGAGACCAAGGAGCTT	52
27	CLM0287	TTGGGTCATTAACTCCTTTCC	ACGGCAAGCATGAACAATAG	55
28	CLM0292	GAGAGACGTGATGGAGAGGA	TCAATGATCGTATAAAGCCTCA	57
29	CLM0300	TTTTGTTGGTTGAGCATCTG	GGTGTTCAATGTCAGGAATAACA	56
30	CLM0322	ACTGAACAGCAAGGACGTTT	TGTGTTTCCAGTGCAAGAAT	54

using the first primer combination and electrophoresed on a single 96-well 3.0 % agarose gel, following the protocols given by Pradeepkumar *et al*. (12). For an SSR locus, plant having the band representing the allele from Kanakamony was scored as 2, alleles from both the parents 1, allele from Sharika 0, and missing data -1. This procedure was repeated for the rest of the primer combinations, one by one.

Genotyping data on marker patterns in the mapping population was used to develop the linkage map, using ICIMapping v. 4.2 (https://isbreedingen.caas.cn/software/qtlIcimapping/294607.htm). The linkage map, along with the BLUP values of morphological data, was used for QTL mapping of each trait. Markers distributed across the linkage groups were used for additive linkage mapping (also known as Inclusive Composite Interval Mapping, ICIM) whereas all the 30 markers were used in single marker analysis (SMA). Steps involved in QTL analysis of quantitative traits segregating in normal fashion in a biparental mapping population, using ICIMapping software is presented at https://doi.org/10.13140/RG.2.2.14950.32327.

RESULTS AND DISCUSSION

Availability of strongly linked anchored markers, QTL loci and flanking markers is necessary in marker assisted selection (MAS) programmes. The population used in the mapping programmes need to have a wide genetic background. Thus, genetically diverse parents belonging to different subspecies and having diverse morphological features were used in this study. The F_1 was forwarded to F_4 to make the members of the mapping population homozygous to the possible extent, so that the allelic influences can be better understood during the mapping. However, since the F_4 population is not homozygous as in case of recombinant inbred lines (RILs), segregation is expected, and individuals with same genetic makeup cannot be reconstituted in subsequent generations. Hence, the evaluation of morphological traits over multiple seasons or generations could not be performed.

Thirty markers distinctly polymorphic between the parental lines were used in the mapping programme. Linkage mapping has generated two linkage groups spanning 908 cM, LG1 with eight markers (CLM0186, CLM0244, CLM0008, CLM0177, CLM0279, CLM0322, CLM0168 and CLM0195) distributed across 637 cM and LG2 with five markers (CLM0200, CLM0088, CLM0260, CLM0218 and CLM0077) across 271 cM. Remaining 17 markers had no linkage association. Box-Cox power transformation is used to transform the target variable to closely resemble a normal distribution in the population under study, with a common variance and additive error structure (Box and Cox, 5). This involves taking the log of a variable and raising it to some power (lambda), depending on how skewed the data is. The transformation in this study has distinctly reduced the skewness of data on all the traits in the population. BLUP is a method for estimating the random effects of a mixed model. It fits the phenotypic data better to the non-genetic effects by shrinkage effect toward the expected genetic values (Bermejo *et al*., 4). As an example, histograms of the distribution of plant weight, on transformed and BLUP values are presented in Fig. 1.

Two mapping strategies, additive linkage mapping and SMA were performed. SMA is the simplest strategy, used when the linkage map is not dense with enough number of markers, considers one marker at a time and tests for its linkage to any trait (Tanksley, 15). This will be successful when a QTL and the marker are closely located, and a recombination event is ruled out. Additive linkage mapping on the other hand, is an exact approach towards QTL identification with high power of QTL detection and specificity. SMA had shown that marker CLM0083 is significantly associated with individual pod weight.

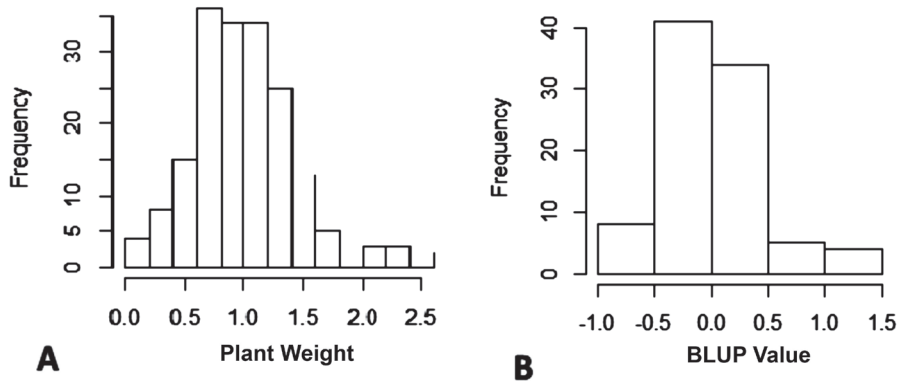

Fig. 1. Histogram showing the distribution of plant weight in the population (A) on Box-Cox transformed values (B) on BLUP values.

For pod number, a nearly significant hotspot was found on LG1 between the markers CLM008 and CLM177. For days taken for first flowering (DTFF), multiple QTL hotspots were observed (Fig. 2). Region between markers CLM0008 and CLM0279 on LG1 had two significant hotspots with LOD value of 15. Hotspots were also found between markers CLM279 and CLM322, between CLM322 and CLM168, and between CLM168 and CLM195 on LG1. On LG2, hotspots were found between markers CLM088 and CLM260, between CLM218 and CLM077, and beyond CLM077. SMA confirmed that CLM0177 on LG1 and CLM0300 are anchored markers for this trait (Fig. 3). For total dry pod yield, a nearly significant hotspot was observed between CLM0008 and CLM0177 on LG1. CLM0083 was an anchored marker for pod weight and total dry pod yield (Fig. 4). For branch number, a significant hotspot was found on LG2 between CLM0200 and CLM0088 (Fig. 5). SMA confirmed the linkage of CLM0200 with this trait. For root length, a hotspot was found between CLM0244 and CLM0008 on LG1. On LG2, hotspots were present between CLM0200 and CLM0088, and between CLM0260 and CLM0218 (Fig. 6). SMA confirmed the linkage of CLM0201, CLM0244 and CLM0300, with this trait (Fig. 7). For plant height, a significant hotspot existed between markers CLM008 and CLM177 on LG1 (Fig. 8). CLM0151 was identified as the anchored markers for this trait (Fig. 9). For plant weight, hotspot with flanking markers CLM0186 and CLM0008 on LG1

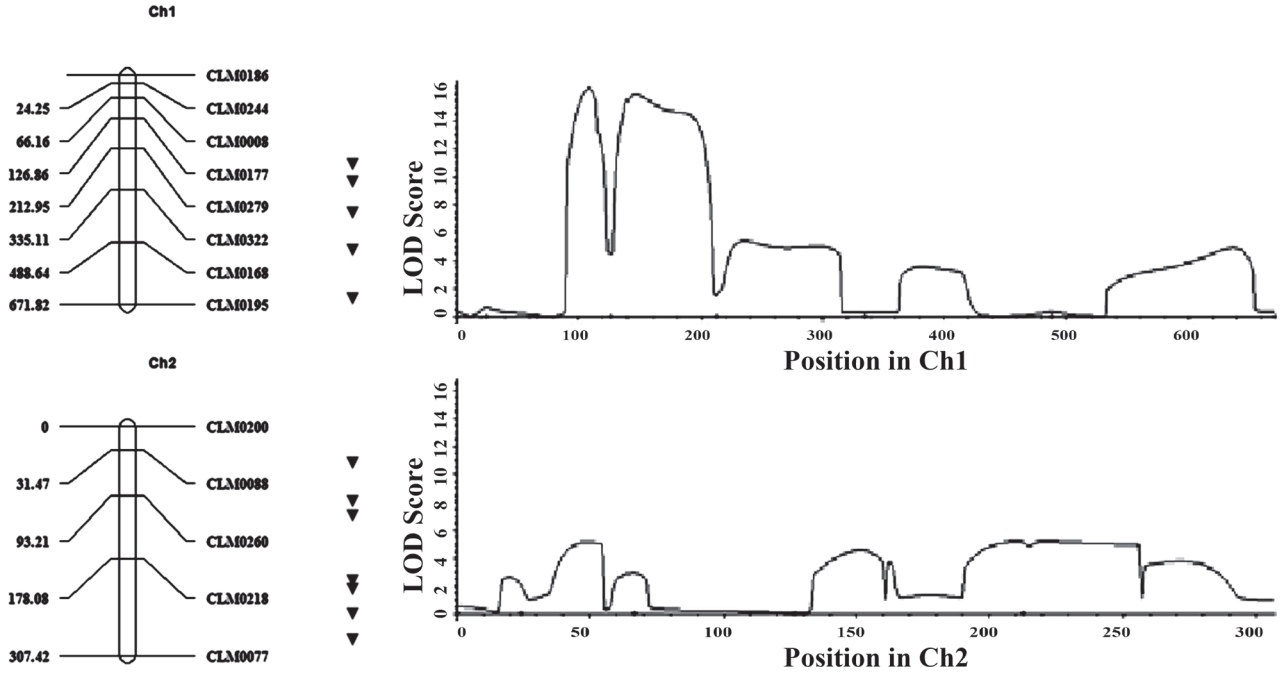

Fig. 2. Hotspots identified for the trait days taken for the first flowering, in LG1 and LG2.

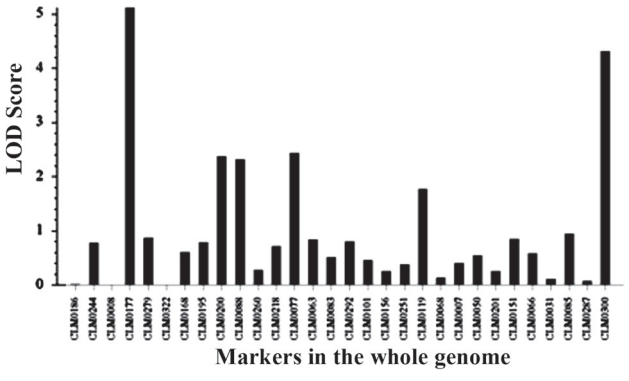

Fig. 3. Single marker analysis for the trait days taken for the first flowering, showing the anchored makers CLM0177 and CLM0300.

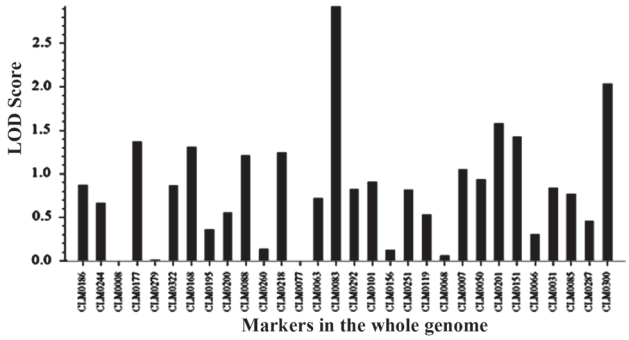

Fig. 4. Single marker analysis for the trait total dry pod yield, showing the anchored maker CLM0083.

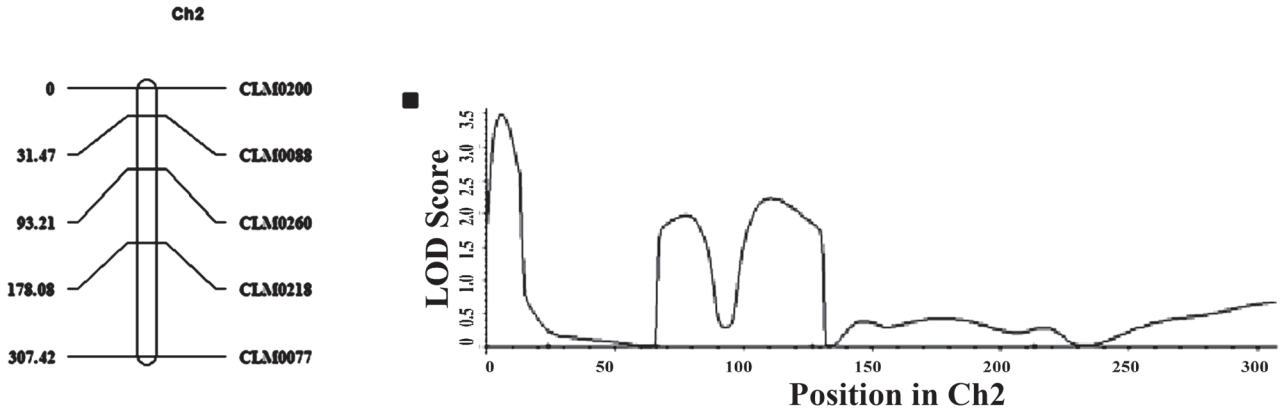

Fig. 5. Hotspots identified for the trait branch number, in LG2.

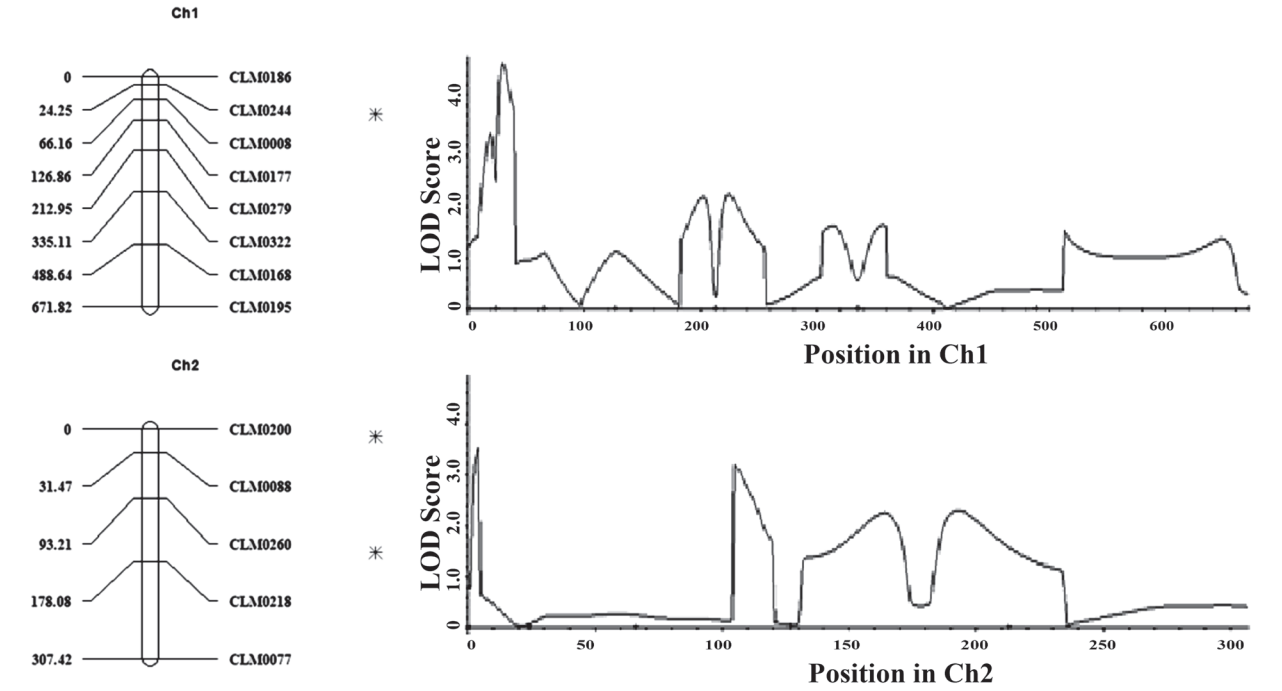

Fig. 6. Hotspots identified for the trait root length, in LG1 and LG2.

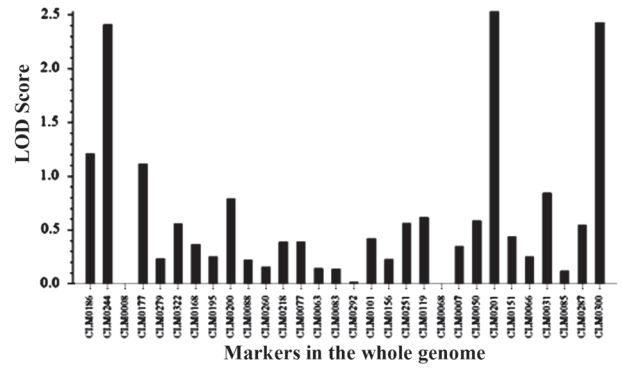

Fig. 7. Single marker analysis for the trait root length, showing the anchored makers CLM0201, CLM0244 and CLM0300.

(Fig. 10), and anchored marker CLM0244 (Fig. 11), were found. No significant hotspots or anchored markers were observed for the trait seeds per pod.

This work has identified the QTL hotspots, their respective flanking markers and anchored markers for days taken for first flowering, branch number, root length, plant height and plant weight. This is the first report on the QTL maps for branch number, root length, plant height and plant weight in vegetable-type cowpea. Additionally, a common anchored marker was identified for individual pod weight and total dry pod yield. The hotspots and flanking markers identified for pod number and dry pod yield can also be used in MAS. The identified anchored markers can be directly used as foreground markers in the

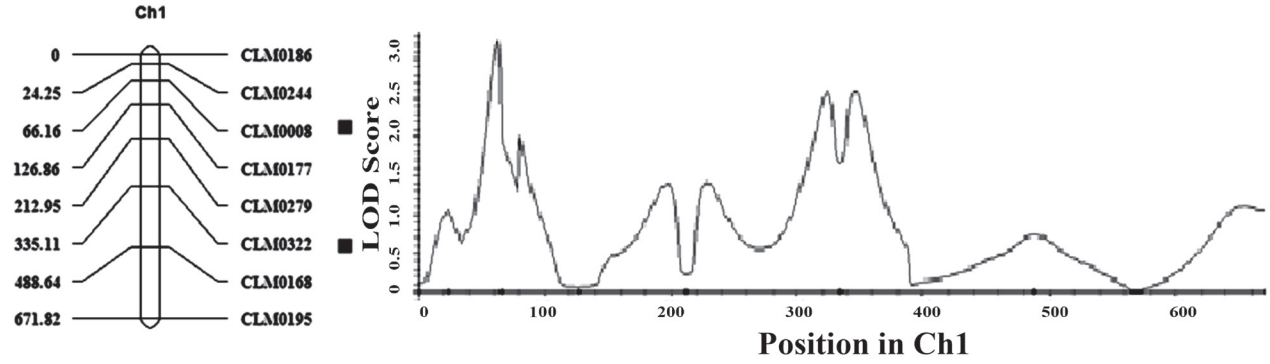

Fig. 8. Hotspot identified for the trait plant height in LG1.

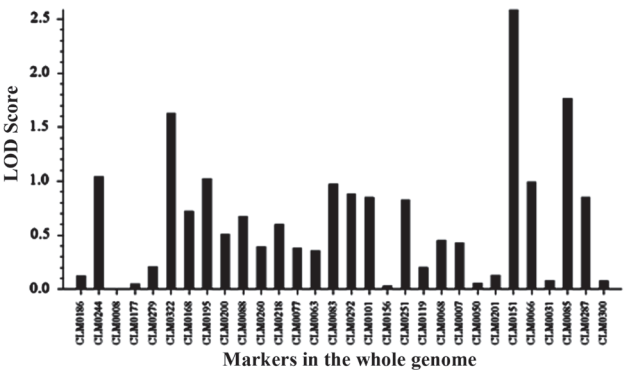

Fig. 9. Single marker analysis for the trait plant height, showing the anchored maker CLM0151.

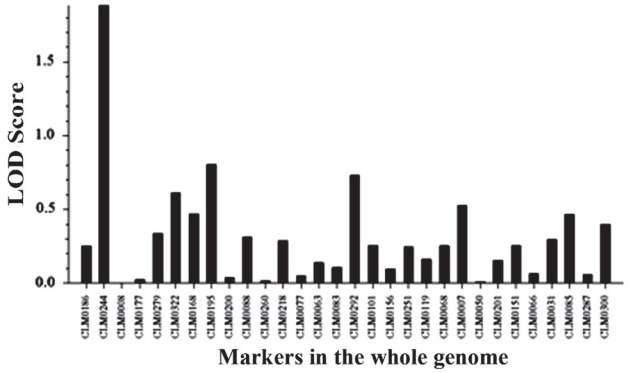

Fig. 11. Single marker analysis for the trait plant weight, showing the anchored maker CLM0244.

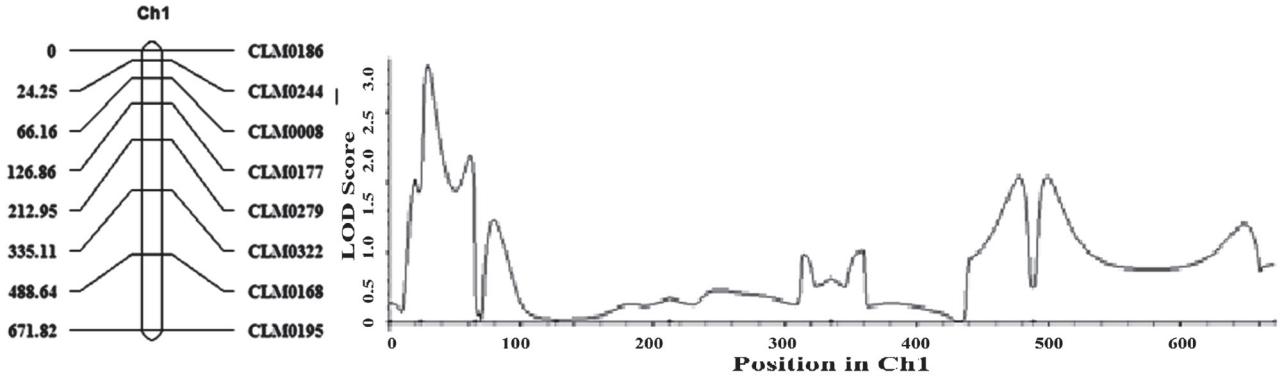

Fig. 10. Hotspot identified for the trait plant weight in LG1.

breeding programmes. Following the selection using anchored markers, flanking markers can be employed in recombinant selection to confirm that only the hotspot is transferred to the recipient line, thus to reduce any probable linkage drag.

AUTHORS' CONTRIBUTIONS

Conceptualization of research (DM, JJ, PT); Designing of the experiments (AV, DM); Execution of field/lab experiments and data collection (AV, DM); Analysis of data and interpretation (AV, DM, JJ, PT); Preparation of the manuscript (DM).

DECLARATION

Authors declare that they have no conflict of interest.

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