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RESEARCH PAPER

Quantitative Trait Loci mapping of agronomic traits in a cowpea (Vigna unguiculata L.) bi-parental cross

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Abstract

Although Cowpea is an important food legume with multiple benefits in sub-Saharan Africa (SSA) its productivity on farmers' fields is very low due to biotic and abiotic stresses, and by the paucity of useful trait-linked genetic markers or QTLs for agronomic traits. Hence, the objective of this study was to construct genetic linkages map of cowpea and identify regions of the genome associated with agronomic traits of cowpea in the F2 population (200 progenies) developed from a cross between a cowpea line (TVu2185) and a yard-long bean line (TVu6643). Diversity Array Technology genotyping platform was used for SNP genotyping the DNA samples. The linkage map and QTL analysis were performed using Join Map® 4.1, and MapQTL® 6 QTL mapping programs, respectively. The linkage map spanned 689 cM of the cowpea genome. Major and minor QTLs (15 in total) were detected for the agronomic traits studied on 6 of the 11 LGs in cowpea. The major seed traits related QTLs were present on LG8 along with that for pod length. The highest phenotypic variance explained (PVE) by individual QTL was 31.2% for seed length followed by 21.1% for seed number per pod on LG8. The phenotypic variance explained by all QTLs per trait ranged from 10.2% for SPW to 48% for SL. The QTLs with large effect detected for pod length and seed traits indicates that the QTLs are potential candidates for marker development and marker assisted selection in cowpea.

Keywords: Cowpea, molecular mapping, Single Nucleotide Polymorphism, linkage map, yield

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] (2n=22) is an important food and forage legume which is adapted and grown in tropics and sub-tropics. It is a versatile crop cultivated between 35°N to 30°S of the equator including Africa, Asia, the middle East, southern Europe, southern USA and central and south America (Singh, 1997). This crop plays crucial role in the lives of millions of people in Africa and other developing countries as it is used as cheap source of dietary protein (17-24%) which nutritionally complements staple low-protein cereal and tuber crops, generating income to farmers, enhancing soil fertility and serving as fodder for livestock. Hence, cowpea has a key role in sustaining food security for both human being and their livestock (Boukar *et al*., 2011; Fatokun *et al*., 2002).

Cowpea production has been estimated to be nearly 6.5 million metric tons of dry cowpea grain annually on about 14.5 million hectares worldwide. More than 80% of this production comes from west and central Africa indicating that Africa takes a lion's share of the global cowpea production (Boukar, *et al*., 2016). However, its productivity is still low in Africa. An estimated potential yield of up to 2 tons per hectare in well-managed experimental stations can be obtained but globally the average yield is up to 450kg/ha. This average, in spite of its importance and wide cultivation, is lower in Africa with average yield ranging from 100 to 400 kg/ha (Singh, 2006). According to Fatokun *et al*., (2012) global cowpea production has increased in the past three decades in both

quantity of seed produced and total area of production; however, the increment in yield has resulted mainly from expansion of area under production and less from improved yield per unit area (productivity) which is partly attributable to cultivars the farmers use. Farmers' traditional cultivars are known to be well adapted to the low input conditions; however, they are generally poor in yield and highly susceptible to the major pests and diseases (Aliyu & Makinde, 2016). Developing improved varieties that give high yield per unit area and resistant to biotic and abiotic stresses using molecular techniques to accelerate cowpea improvement may help to achieve the anticipated increase in global cowpea demand because of the increasing world population.

Several agronomically important traits are governed by many genes, and hence they are called quantitative traits (or polygenic or complex traits). The parts on the genome containing genes associated with a given quantitative trait are termed as quantitative trait loci (QTLs). Identification of QTLs on the genome of an organism is very essential in accelerating improvement of crops by facilitating the marker assisted selection/breeding (MAS). Such identification of QTLs can hardly be done with conventional phenotypic evaluation alone (Sehgal *et al*, 2016). Molecular markers are utilized in construction of genetic linkage maps, and this linkage map, in turn, is utilized to identify regions on the chromosomes that contain genes governing quantitative traits. Single Nucleotide Polymorphism (SNP) marker is one of such molecular markers which 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, these markers have got a lot of applications in recent genetic mapping studies (; Mammadov *et al.*, 2012; Gupta *et al.*, 2001). Molecular markers with greater abundance in the genome of an organism have been found particularly useful for generating dense genetic linkage maps that can help breeders in their selection process. Once markers closely linked with the traits of interest have been identified, through QTL mapping, selection for the desired traits can be made indirectly by selecting for those markers. Before the advent of QTL mapping concept, analysis of quantitative traits was performed by using statistical techniques based on means, variances and covariances of relatives without actual knowledge of the number and location of genes (polygenes) underlying the traits (Semagn *et al*., 2010; Kearsey & Farquhar, 1998;). However, currently the concept of QTL mapping, the process of constructing linkage maps and analyzing QTLs so as to identify the region on the genome associated with a particular trait of interest, is implemented to study such traits (Collard *et al.*, 2005; Lander & Botstein, 1989).

Despite all the aforementioned importance of cowpea in the developing countries this crop is still underexploited. Its low productivity can be attributed to biotic (insects, diseases, parasitic weeds, and nematodes) and abiotic (drought, low soil fertility and heat) factors. Moreover, farmers producing cowpea also have limited access to improved cowpea varieties. Currently, several research works are going on under the cowpea breeding and improvement programs in order to avert these problems. Among the major goals of cowpea breeding and improvement programs is stacking of desirable agronomic traits and accelerating cowpea improvement through implementing molecular breeding techniques such as Marker Assisted Recurrent selection and Marker Assisted Backcross. In a review about current status and prospectus of molecular breeding of cowpea in sub-Saharan Africa Gedil *et al*., (2016) indicated that enriched genomic resources can provide more opportunity to accelerate genetic gain, stack traits, characterize existing diversity in cultivated cowpea and wild relatives, and pyramid favorable gene/alleles in farmer preferred varieties. However, implementation of these molecular techniques in cowpea is severely limited by the paucity of useful trait-linked genetic markers or QTLs for various agronomic traits (Timko *et al*., 2008; Fatokun *et al*., 1997). Different types of molecular markers have been developed and used in cowpea, such as Restriction Fragment Length Polymorphism (RFLP) (Menanciohautea *et al*., 1993), Random-Amplified Polymorphic DNA (RAPD) (Ba *et al*., 2004) and AFLP (Ouédraogo *et al*., 2002). But these developed markers were not sufficiently informative and

highly polymorphic, and hence SSR markers, which are highly abundant and well distributed throughout the genome, has been developed and used recently (Chen *et al*., 2017). However, it was reported that identification of polymorphic SSR markers in cowpea is difficult due to the narrow genetic variability (self-pollinating nature) of cowpea (Chen *et al*., 2017). Consequently, single nucleotide polymorphism (SNP) markers have gained an increasing importance due to their bi-allelic nature, higher frequency in the genome than SSRs and other markers, and due to their easily automated genotyping (Carvalho, *et al*., 2017). Hence, the objectives of this study were to construct genetic linkage map of cowpea and identify regions of the genome (QTLs) associated with some agronomic traits of cowpea in the F_2 population developed from a cross between a cowpea line (TVu2185) and a yard-long bean line (TVu6643).

Materials and methods

Experimental site

The experiment was conducted at International Institute of Tropical Agriculture, Ibadan, Nigeria (7º29'11.99''N, 3º54'2.88''E) from September 2016 to August 2018.

Plant material and development of mapping population

A cowpea (Vigna unguiculata ssp. unguiculata) line (TVu2185), and a yardlong bean (Vigna unguiculata ssp. sesquipedalis) line (TVu6643) were used as parents. They were obtained through screening of the germplasm available at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria which maintains more than 15,000 accessions of cultivated cowpea and more than 2,000 wild relatives. The two parents were selected based on several contrasting agronomic traits especially pod length and seed related traits. The two lines were crossed to generate F1, and which in turn is allowed to self-fertilize to develop F2 mapping population. F2 individuals were planted in pots in the screen house, and the resulting F2:3 seeds were sown in progeny rows in the field.

DNA extraction, quantification and quality

A newly expanded trifoliate leaf was harvested from each F2 plant four weeks after planting for DNA extraction. Total genomic DNA was extracted from leaves of 200 F2 population and 2 parental lines using Diversity Arrays technology (DArT) DNA extraction protocol (https://www.diversityarrays.com/files/DArT_DNA_isolation.pdf).

A leaf sample of approx. 1g was placed into 2ml Eppendorf tubes containing 2 steel grinding balls (size 2.4mm), and grinding was done at 1000 strokes/minute for 1 min using a GENOGRINDER 2000® instrument (BT and C Inc., New Jersey), a machine specially designed for high throughput DNA extraction. The fresh buffer solution used for DNA extraction was prepared from extraction buffer (0.35 M sorbitol, 0.1 M TrisHCl, 5 mM EDTA pH 8.0), lysis buffer (0.2 M TrisHCl, 0.05 M EDTA pH 8.0, 2 M NaC1. 2% CTAB), sarcosyl 5% (w/v), sodium disulfite 0.5% (w/v) and Polyvinylpyrrolidone (PVP) 2%. This buffer solution was pre-heated to 65ºC in water bath for dissolving, and 1ml of it was aliquoted to each tubes. This mixture was then incubated at 65ºC for 1 hour in water bath with gentle shake. After incubation the samples were cooled down for 5 minutes at room temperature (RT), and then 1 ml of chloroform: isoamyl alcohol (CIA) (24:1) mixture was added. This was mixed well for 30 minutes, and then centrifuged for 20 minutes at 10,000 x g, RT. The aqueous phase (600ml) was transferred to another tube; equal volume (600ml) of ice-cold isopropanol was added to precipitate DNA, and the tubes were inverted about 10 times, and then centrifuged again for 30 minutes at 10,000 x g at RT. The supernatant was then discarded and the pellet was washed with 2ml of 70% ethanol (EtOH). The EtOH was discarded; the pellets were air dried in the fume hood at room temperature; 10µl of RNAse enzyme was added and then dissolved in 150µl of milli-Q water.

DNA was quantified spectrophotometrically i.e. using Nanodrop (NANODROP 2000c spectrophotometer, Thermo scientific, USA). For checking the quality of the extracted

genomic DNA both gel 0.8% (w/v) agarose electrophoresis and Nanodrop were used.

Genotypic data

The DNA samples were genotyped using DArTseq genotyping by sequencing system. SNP markers with high quality of genotype calls and are heterozygous in at least one parent were retained for linkage analysis. Firstly, SNP markers polymorphic (opposite allele calls in parents) between the parents were screened. In other words, SNP data were first converted into parental genotypes for polymorphic SNPs with no missing or heterozygous genotypes for both parents. Markers with proportion of missing data points of >10% (Edae et al., 2017) were excluded from further analysis as they are not informative for linkage mapping. A total of 1,525 SNP markers that passed such filtering criteria were used for linkage mapping and further analysis.

Phenotypic data and trait analysis

The two parents together with 200 F2:3 progenies were evaluated in the field using alpha-lattice design with two replications. Each plot had 4.0m length with a spacing 0.75 cm between rows and 0.5m within rows. Phenotypic data were collected on the following traits: days to flowering (DF) - recorded as the number of days from planting to first flowering; pod length (PL) - recorded as the average length of 10 fully matured pods for each individuals/genotype; 100-seed weight (HSW) - recorded as the weight in grams of 100 seeds; pod number per plant (PN) - recorded as the total number of pods per individuals; seed number per pod (SN) - recorded as total number of seeds in a pod. Seed length (SL) and seed thickness (ST) - recorded as the average length and thickness of ten seeds per individuals were measured with electronic digital calipers. In addition, data on derived traits: pod to seed weight ratio (SPW) - calculated as the weight of 10 pod walls weight (TPWW) divided by the weight of 10 pods seed weight (TPSW). Pearson's correlation coefficient among each traits and the frequency distribution of each of these trait means was analyzed using R statistical package.

Linkage map construction

Linkage analysis of the entire markers, their subsequent grouping into respective linkage groups and construction of genetic linkage map was done using a computer program called JoinMap® 4.1 (Van Ooijen, 2006). Determining linkage groups of the markers was based on recombination frequency test statistics (of JoinMap). To calculate map distance and loci order Kosambi's mapping function (Kosambi, 1943) was used. For analyzing segregation distortion of markers significant deviation from the expected Mendelian genotypic frequencies (1:2:1 genotypic ratio for F2 population) was tested using chi-square goodness of fit test. The segregation distortion values provided as Chi-square (X2) values were used to identify distorted markers.

QTL mapping

Mapping of the quantitative trait loci was done using MapQTL® 6 (Van Ooijen, 2009). QTL mapping was performed by initially scanning the entire genome using the interval mapping. The interval mapping model was used initially to locate the putative QTL regions and to select cofactors (other markers in the surrounding with significant LOD values) for further analysis. This was followed by multiple QTL model (MQM) analysis to increase power of QTL detection. The method internally controls false discovery rates (FDR) and tests different QTL models by elimination of non-significant cofactors (Arends et al., 2014). Determination of appropriate significance threshold of the LOD value was performed by using permutation analysis with 1000 random data shuffles as described by Churchill & Doerge, (1994) to provide genome-wide 0.05 significance level using MapQTL program.

Results

Variability for phenotypic traits among the parents and individuals in the F2 population

Considerable variation in mean values between the two parents and among the individuals in F2 population (Table 1) were recorded for traits including pod length, days to flowering, 100 seed weight, pod number and seed length. However, there was no much difference between the parents for seed number per pod and seed thickness. Frequency distribution of all the traits in the F2 generation showed continuous variation (Figure 1). However, the distributions were skewed towards the lower values for days to flower, peduncle number per plant and pods per plant whereas hundred seed weight, seed length and seed thickness followed almost normal distribution. Ranges of mean values of the F2 population exceeded the mean values of the parents. For PL, about, 78.5% of the F2 progenies had mean values below the mid parent (i.e. closer to the short poded parent). For days to flowering, 85% of the F2 individuals had values lower than the early flowering parent while almost none of them exceeded the late flowering parent. For pod length, none of the F2 plants had longer pods than the parent with long pods (yardlong bean/sesquipedalis subspecies) nor shorter than that of the parent with short pod (cowpea/unguiculata ssp.). For HSW, 11.5% and 20% of the population had mean values less than the small seeded parent and higher than the large seeded parent, respectively. For seed length 20% had seeds shorter than the short parent while none of them produced seeds longer than the seeds of the long-seeded parent. The two parents produced almost similar number of seeds per pod. However, 38% of the progenies produced seed number lower than the parent with lower SN while 52% exceeded the high seeded parent. Similarly, for seed thickness the parents were not so different; however, 53.5% had seed thickness lower than the parent with low seed thickness while 27% had higher seed thickness than the better parent. For peduncle number, 25% of the population had peduncle numbers higher than the better parent while 19% had peduncle number lower than the other parent. For Pod number, 62% of the progenies higher values than the better parent whereas only 3% had pod number lower than the poor parent. There was significant (P=0.01) correlation among the agronomic traits evaluated in this study (Table 2). Positive and significant phenotypic correlations were observed between some of the measured traits such as between PL and SL (0.42), PL and HSW (0.41), SL and ST (0.56), HSW and ST (0.68), and HSW and SL (0.7) (Table 2). There were also significant negative correlations between SN and SL (-0.42), SN and SPW (-0.45), and PN and DF (-0.2).

Polymorphism and informativeness of the SNP markers

DArTseq genotyping generated a total of 3,103 SNP markers, out of which 1,627 (52%) were polymorphic. The SNP genotyping results showed very high call rates which had an average of 98%. The polymorphic information content (PIC) values of the markers ranged from 0.02 to 0.5, with an average of 0.39. Based on chi-square goodness of fit test, 79% of the polymorphic markers segregated according to 1:2:1 Mendel's segregation ratio for F_2 population while the remaining 21% of the markers showed significant deviation (P=0.05) from the expected ratio.

Genetic linkage map of cowpea F2 population

Six hundred and seventy-two SNP markers out of 1,525 polymorphic SNP markers were mapped into 11 (haploid chromosome number of cowpea) linkage groups (LG) (Table 3; Figure 2). The linkage group designation was based on the chromosome number the markers are located which was retrieved from Phytozome using markers' sequences. Hence, each of the markers in the linkage groups corresponds to their respective chromosome number, or in other words, the 11 LGs in this linkage map correspond to the eleven

Table 1. Comparison of parental means, and ranges, mean and standard deviation of F_2 progenies

		Parents		**F_2 Progenies**			
Traits	Abbreviation	P1 (TVu2185)	P2 (TVu6643)	Min	Max	Mean	STDev
Pod length (cm)	PL	9.9	39.6	11.60	41.70	21.86	4.66
100_seed weight (g)	HSW	11.3	17.9	6.90	26.90	15.45	3.34
Pod number per plant	PN	8.2	2.6	1.00	23.00	9.87	4.68
Seed number per pod	SN	7.0	7.2	1.00	14.00	7.69	2.55
Peduncle number	PeN	9.6	4.2	1.00	17.00	7.57	3.48
Seed length (mm)	SL	7.8	12.5	7.60	13.20	10.73	1.09
Seed thickness (mm)	ST	4.5	4.6	2.80	5.60	4.41	0.40
Days to flowering	DF	84.6	61.2	43.00	96.00	55.72	7.87
Seed pod weight ratio	SPW	0.2	0.6	0.10	1.30	0.45	0.18

TVu2185 is a cowpea line; TVu6643 is a yard-long bean line. Min, Max Mean STDev indicates the minimum, maximum, mean and standard deviation of a trait for F2 progenies. Total number of observations, N=200.

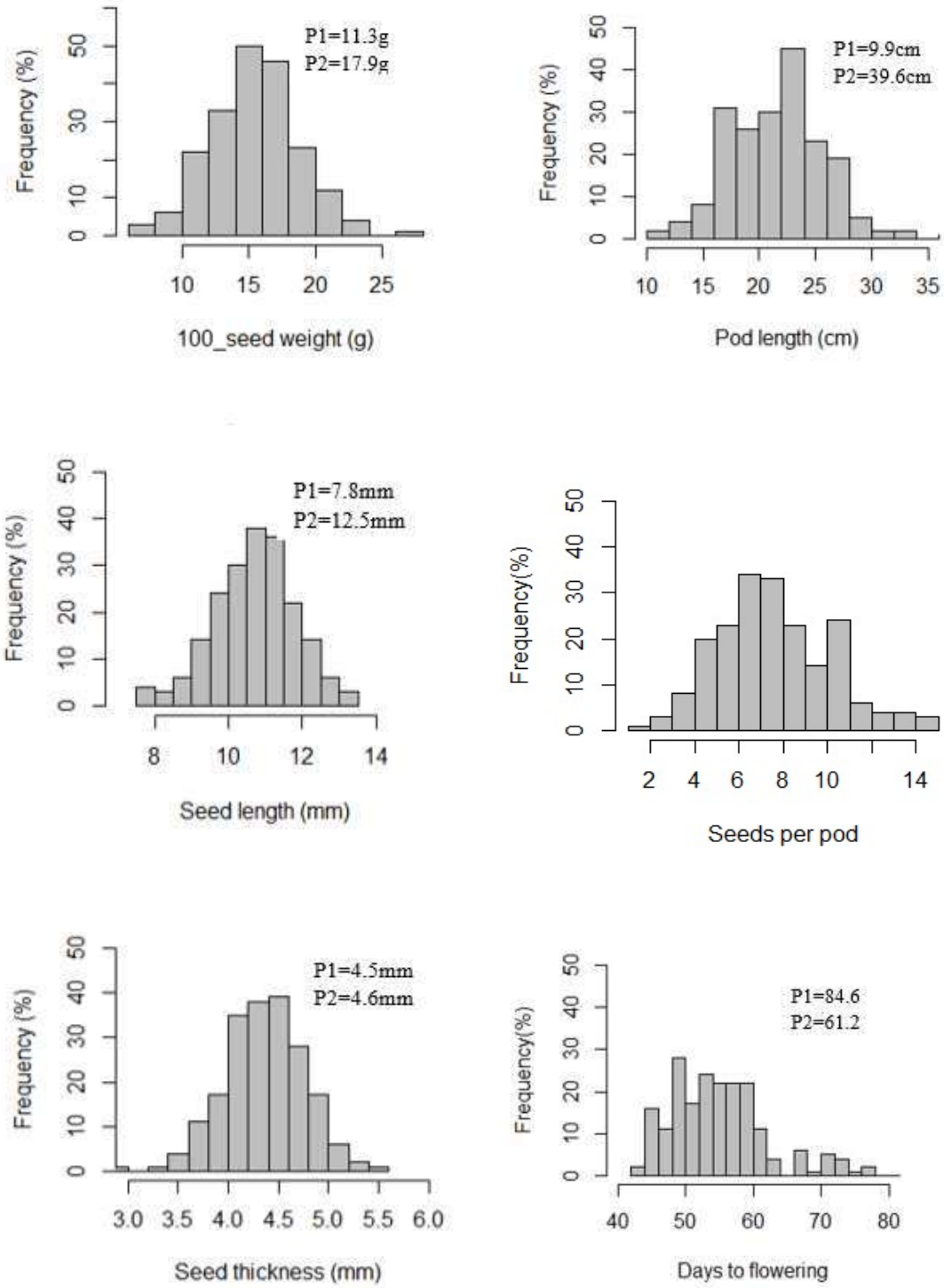
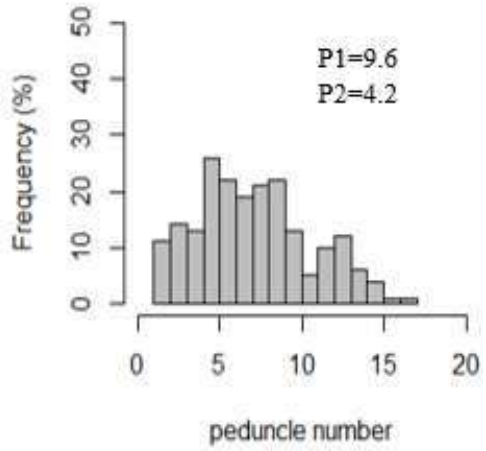


Figure 1. Histogram showing distribution of traits' means of cowpea F2 progenies. Frequency represents number or proportion of F2 progenies.



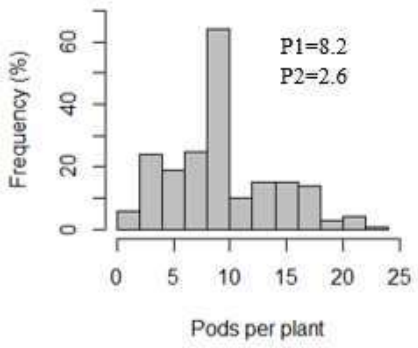


Figure 1 continued

chromosomes per haploid cowpea genome. The linkage map spanned 689cM of the cowpea genome. The size of linkage groups ranged from 34.79 cM for LG10 to 115.14 cM for LG7, with an average of 62.64 cM. The average distance between adjacent markers (marker density) ranged from 0.75 cM for LG8 to 2.03 cM for LG1 with an average distance of 1.19 cM.

Quantitative trait loci (qtls) mapped

QTLs were detected for all the agronomic traits studied in this work except for days to flowering (Figure 4.3). Generally, 15 QTLs were detected for 8 agronomic traits on six linkage groups (LGs): 3, 5, 7, 8, 9 and 10 (Table 4.4). The number of QTLs detected ranged from one for SN, PN and PeN to four for PL. The highest phenotypic variance explained (PVE) by individual QTL was 31.2% for seed length on LG8 followed by seed number per pod at 21.1 on LG8. Pod length showed the lowest PVE by a QTL at 7.8cM on LG%. However, pooled PVE by QTLs per trait ranged from 10.2% for SPW to 48% for SL. Eleven of these QTLs showed major effects accounting for >10% phenotypic variation.

Two major QTLs were detected on LG3 & LG8, and two minor ones on LG5 & LG10 for pod length all accounting for a total of 47% of the phenotypic variation in the trait. One major QTL was detected for each of PeN, SN and PN on LG8 at map positions 31.1cM, 33.6 and 38.4cM, respectively. For HSW one major and one minor QTL on LG8 and LG10, respectively, were detected explaining a total of 28.4% of phenotypic variation for the trait. For SL, two major QTLs were detected on LG7 and LG8 explaining 48% of the variation for the trait in the F2 population. For seed thickness, 2 major QTLs on LG8 & LG10 and a minor QTL on LG9 were detected which explained 39% of the variation for the trait. For seed size (HSW), QTLs were detected on LG8 and LG10. The QTLs on LG 3, 5 and 8 for PL had positive additive effect whereas QTL on LG10 had negative additive effect which can be seen from their additive effects (Table 4). The QTLs detected for SN, PN, PeN and SPW had negative allelic contribution to the traits. For HSW and ST, QTLs from LG8 had positive effect whereas QTLs from LG10 had negative allelic contribution for the traits.

Table 2. Pearson's correlation coefficient values between agronomic traits of cowpea.

	DF	PL	SN	PeN	PN	HSW	SL	ST	SPW
DF									
PL	-0.01								
SN	0.17*	0.26**							
PeN	-0.02	-0.01	0.1						
PN	0.2**	-0.07	0.07	0.63**					
HSW	0.09	0.41**	-0.17*	0.03	0				
SL	0.15*	0.42**	0.42**	-0.08	-0.11	0.7**			
ST	0.04	0.33**	-0.16*	0.16*	0.16*	0.68**	0.56**		
SPW	0.16*	0.32**	0.45**	-0.14*	0.19**	0.25**	0.4**	0.16*	

*, ** indicates significance at P=0.05 and P=0.01, respectively. DF=days to flowering; PL=pod length; SN=seed number per pod; PeN=peduncle number; PN=pod number per plant; HSW=hundred seed weight; SL=seed length; ST=seed thickness, and SPW=seed pod weight ratio.

Table 3. Distribution of SNP markers over the 11 linkage groups of cowpea

LG	**No. of markers mapped**	**Map length (cM)**	**Aver. marker density (cM)**	**Intervals >5cM**
1	34	66.95	2.03	1
2	58	73.37	1.29	3
3	66	88.22	1.66	4
4	42	35.67	0.87	-
5	41	57.30	1.43	2
6	50	39.34	0.80	2
7	97	115.14	1.20	2
8	55	40.24	0.75	-
9	54	74.52	1.41	2
10	94	34.79	0.81	-
11	81	63.52	0.79	-
Total	**672**	**689.07**	**-**	
Average		**62.64**	**1.19**	

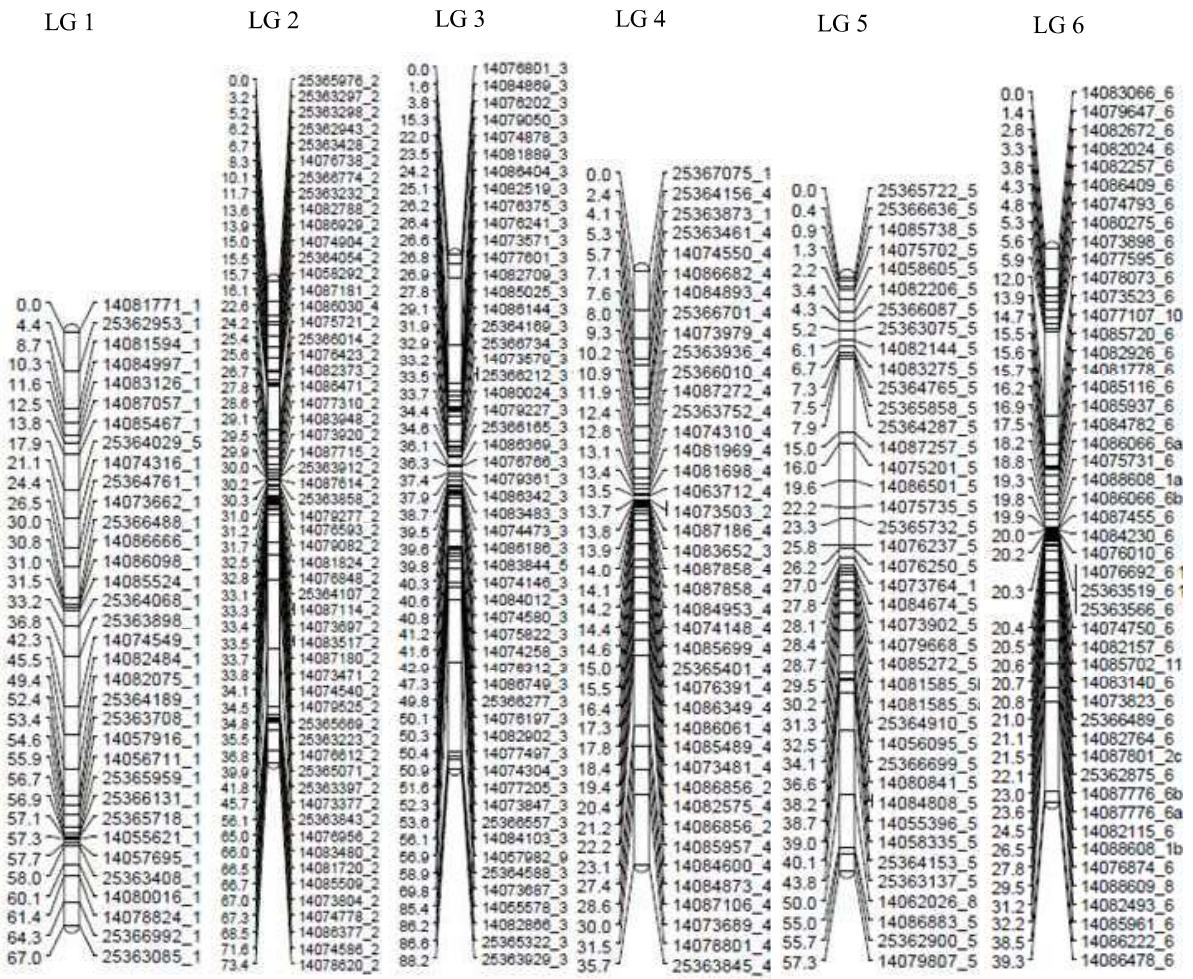


Figure 1. Genetic linkage map of Cowpea F_2 population developed from bi-parental cross between a cowpea line (Tvu2185) (*Vigna unguiculata* ssp. *Unguiculata*) and a yardlong bean line (TVu6643) (*V. unguiculata* ssp. *sesquipedalis*) based on SNP markers. Marker names are indicated on the right side, and map length (cM) on the left side of each linkage group (LG).

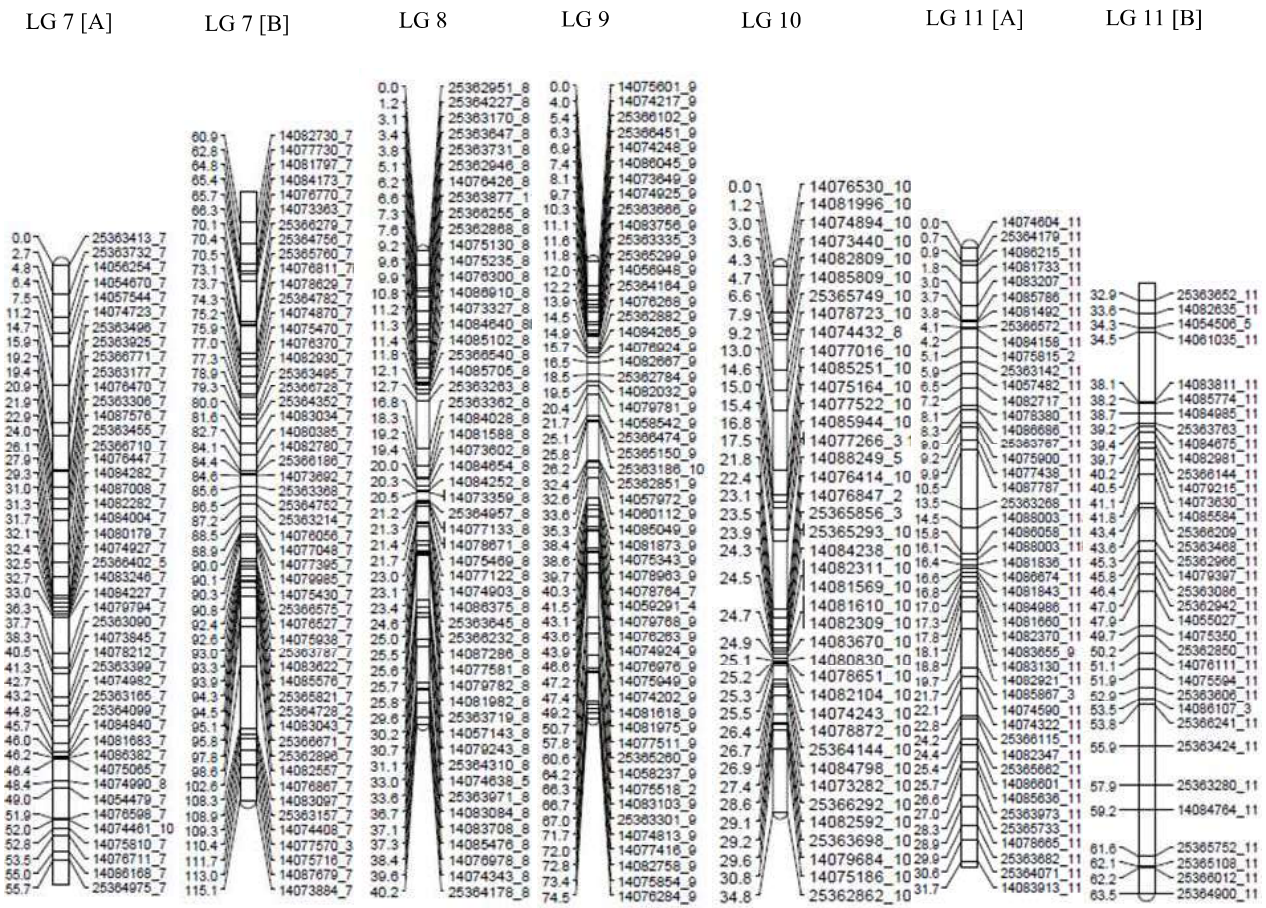


Figure 2 continued

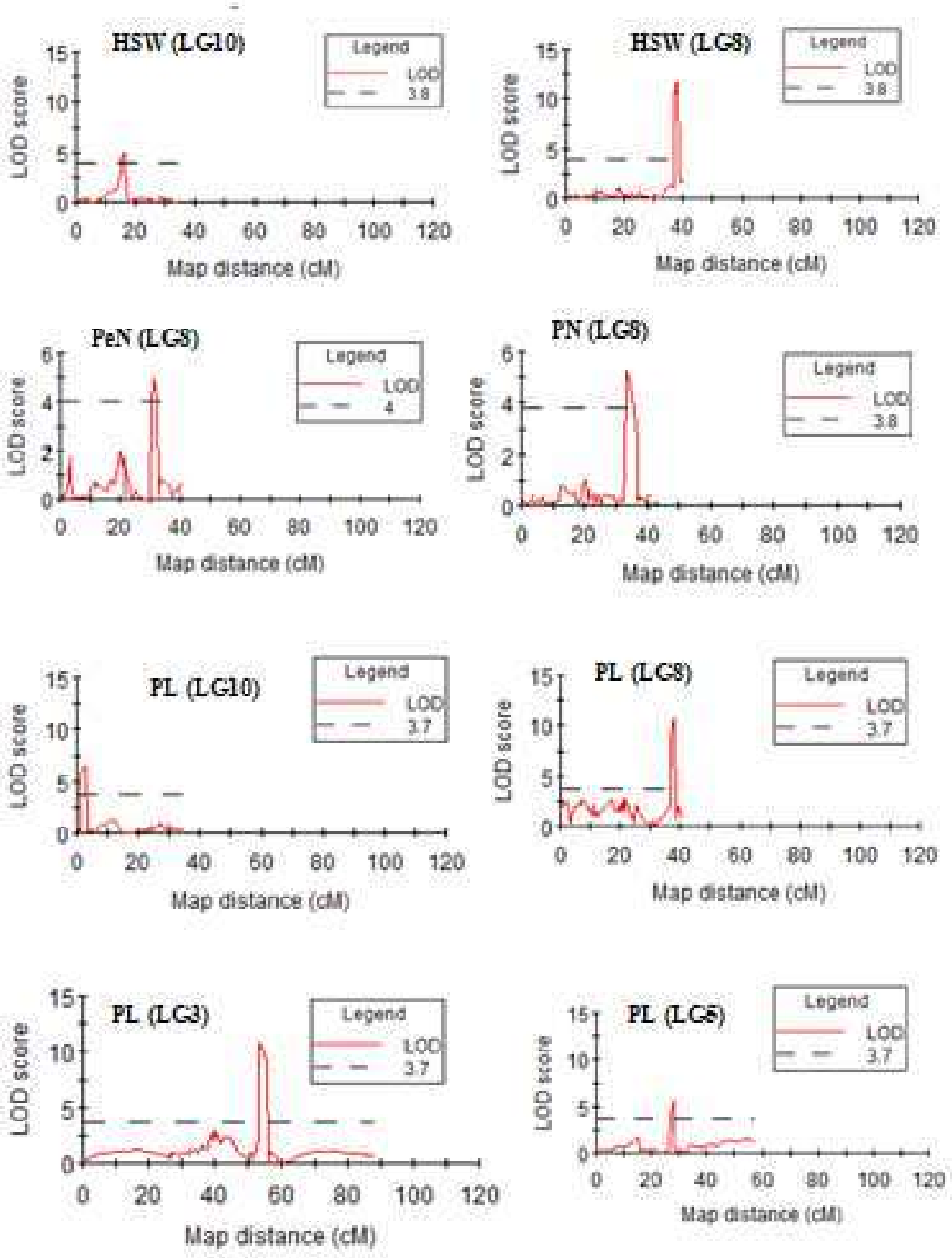


Figure 3. Chromosomal locations of detected QTLs for each agronomic traits of cowpea F2 population developed from a cross between a cowpea (Tvu2185) and a yard-long bean (TVu6643). QTL mapping was done using interval (MQM) mapping. Threshold LOD is determined for each trait separately. The genomic region where LOD peak is located indicates the position of QTL. Dashed broken lines indicate LOD threshold used to declare presence and statistical significance of the QTL

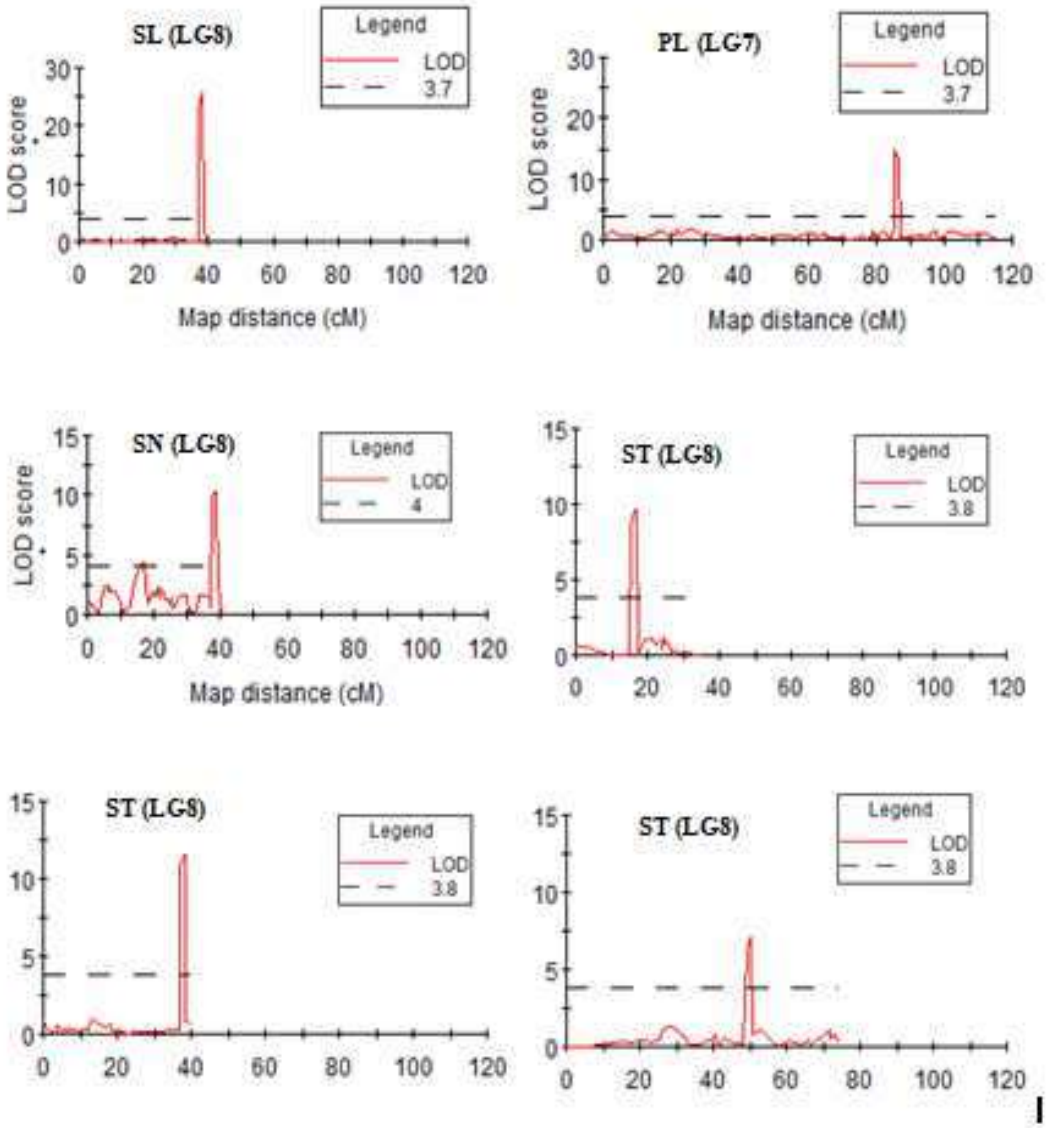


Figure 3 Continued.

Table 4. Summary of QTLs detected for agronomic traits of cowpea F2 population developed from Tvu2185 x Tv6642 crossing

Trait	QTL[a] name	LG	Locus	Map Pos. (cM)	LOD score	PVE[b] %	Additive effect
Pod length	qPL_1	3	25366557_3	53.6	10.73	15.7	2.65
	qPL_2	5	14084674_5	27.8	5.65	7.8	1.84
	qPL_3	8	14085476_8	37.3	10.08	14.6	2.28
	qPL_4	10	14074894_10	3.0	6.41	8.9	-1.47
Seed no. per pod	qSN	8	14076978_8	38.4	10.27	21.1	-1.4
100_Seed Weight	qHSW_1	10	14077522_10	15.4	4.44	7.9	-1.38
	qHSW_2	8	14083708_8	37.1	10.74	20.5	2.07
Seed length	qSL_1	7	25363368_7	85.6	14.75	16.8	0.65
	qSL_2	8	14085476_8	37.3	23.58	31.2	0.91
Pods per Plant	qPN	8	25363971_8	33.6	5.31	11.5	-1.79
Peduncle no.	qPeN	8	25364310_8	31.1	4.98	10.8	-0.47
Seed thickness	qST_1	8	14085476_8	37.3	11.05	16.3	0.22
	qST_2	9	14081618_9	49.2	6.28	8.9	-0.15
	qST_3	10	14085944_10	16.8	9.54	13.8	-0.2
Seed pod weight ratio	qSPW	8	14057143_8	30.2	5.75	10.2	-0.24

a QTLs were named by taking first 'q' for QTL and abbreviation of the trait names followed by serial number indicating QTLs. b=Phenotypic Variation Explained.

Discussion

All the agronomic traits examined in this study showed continuous variation. The continuous variation of the traits observed indicates polygenic nature of the traits and their quantitative inheritance. Substantial diversity of expression of the traits was observed between F2 segregants and the two parents. The transgressive segregation observed for traits was expected as this phenomenon is often observed in the progenies resulting from crosses involving different subspecies of a genus (DeVicente & Tanksley, 1993). The transgressive segregation observed for some traits may suggest the presence of complementary QTL alleles in the two parents. For pod length (PL), Kongjaimun et al., (2012) observed similar trend of no transgressive segregation in a cross between cultivated yard long bean and a wild cowpea as observed in this study. Although no transgressive segregation was observed for PL, the higher proportion of the F2 progenies with mean values below the mid parent (i.e. closer to the short poded parent) may suggest partial dominance of the short pods over the long pods. This result is in agreement with findings by Hazra et al. (2007) who reported predominance of additive gene effects for pod length, with short pod characteristic of cowpea (unguiculata) being partially dominant over long pods of sesquipedalis (yardlong bean). Similarly, the proportion of F2 segregants for DF may indicate partial dominance of the alleles for early maturity and short pods. This result is in agreement with the work of Ubi & Obigbesan (2001) who reported partial dominance for days to flowering in cowpea.

The number of polymorphic markers detected between the two lines was relatively low. However, Kongjaimun et al. (2012) has reported a polymorphism level of 13% using micro-satellites and RIL mapping population of cowpea. The low level of polymorphism in cowpea can be attributed to the highly self-pollinating nature and narrow genetic base of the crop. (Li et al., 2001) have also reported low genetic diversity among cowpea lines using SSR markers. Moreover, stocks used as parents for developing the mapping population were not taxonomically distant as both of them are in the same genus Vigna and the same species unguiculata though the subspecies are different (V. unguiculata subsp. unguiculata vs V. unguiculata subsp. unguiculata sesquipedalis). Fatokun et al. (1993) indicated that the more distantly related two sexually compatible individuals are taxonomically, the higher the frequency of polymorphism detectable between them. Higher polymorphism rate would have been observed if the cross were between two different species (inter-species) of genus Vigna genus. In a QTL mapping study, Sassoum et al., (2015) crossed cultivated and wild cowpea and found 35% rates of polymorphism (using SNP markers) in the mapping population. In another mapping study using RILs developed from a cross between a breeding line and a perennial wild cowpea, only 31.6% SSR markers were found to be polymorphic between the parents (Andargie et al., 2011) though the marker type used was different.

Segregation distortion is a common phenomenon which is usually observed in wide intra- and interspecific crosses of several plants (Song et al., 2006). Distorted segregation of markers has been reported in Vigna species for genetic maps of cowpea, yard-long bean, mungbean and adzuki bean ranging from 9.7% to 30.8% (Kongjaimun et al., 2012; Nagata and Widholm, 2005). So, the segregation distortion observed in the current study is within the range that was reported for species in Vigna.

The map length obtained from this work was comparable to the linkage maps reported previously by Muchero et al., (2009) (680 cM), Ubi et al., (2000) (669.8 cM), Menendez et al., (1997) (972 cM); and Xu et al., (2011) (745 cM) regardless of the mapping population and marker types used. Also, distances between markers in the current study were mostly (97%) less than 5cM. Moreover, the average marker density is also comparable to that of consensus genetic linkage map for cowpea by Muchero et al. (2009) which had 0.73cM indicating good marker density of the linkage map. Other

studies have reported average marker distances as large as 9.9cM (Ubi et al., 2000).

The number of QTLs detected for PL in this work is comparable to that reported by Ubi et al. (2000) who detected 4 QTLs controlling the trait in a RIL population developed from a cross between cultivated cowpea (14.5 cm pod length) and wild cowpea (7.1 cm pod length). However, Kongjaimun et al. (2012) detected 7 QTLs for PL in a cross between yard long bean and cowpea. PL is the trait for which the highest number of QTLs, four on four different LGs, were detected as compared to other traits studied in this work. This can be explained by the fact that the two parents used for the cross had very huge difference in terms of the pod length (Cowpea/P1=9.9cm; yardlong bean/P2=39.6cm). Mackay & Powell (2007) indicated that the number of QTLs detected for a trait in bi-parental population depends on the number of contrasting alleles of the trait controlling loci between the two parents. The most obvious distinguishing character between cowpea and yardlong bean is the pod length and is the most important domestication trait of yardlong bean.

Detection of QTLs for seed size (HSW) on LG8 and LG10 is in agreement with the work reported by Andargie et al. (2011) where they have also detected QTLs for seed size on LG10. For days to flowering, failure to detect QTL(s) was unexpected as the two parents had considerable differences in days to flowering: 44 and 54 days to first flowering for TVu2185 and TVu6643, respectively. However, it could partly be caused by the stringent LOD threshold used (3.8) for this trait against the threshold of 2-3 used by previous studies to declare significant QTL. A putative QTL position with a LOD of 2.5 was observed on LG5. Likewise, the same stringency was applied for other traits too, and LOD values even higher than 2.5 were observed but not declared as significant QTL as they appeared below the threshold. The threshold LOD value was determined for each trait separately (mostly between 3.7 and 4) using their phenotypic data.

Although QTLs were detected on 6 linkage groups, most of them were clustered on LG8 (8 of the 15 QTLs). The major seed traits (SL, ST, HSW and SN) related QTLs were present on LG8 along with that for pod length. The genomic region from 30.2 to 38.4 cM of LG 8 is where most of the above-mentioned QTLs have mapped. QTLs for PL, HSW and ST were co-localized on LG10. Kumawat et al. (2012) reported similar observations of clustering of QTLs where they reported ten of the thirteen QTLs mapped to only two LGs in their mapping study in pigeon pea (Cajanus cajan L. Millsp.). According to Aastveit & Aastveit (1993), the clustering of QTLs within close regions on linkage groups can arise due to pleiotropic effect of a single regulatory gene/locus or due to close genetic linkage of distinct genes. The occurrence of pleiotropy could be explained in a way that certain traits are phenotypically correlated with each other due to the presence of certain genes coexisting in these QTLs. Hence, the strong positive and significant correlation observed between the agronomic traits may confirm the presence of pleiotropic effect. QTL clustering for agronomic traits have been observed in various crops (Wang, et al., 2012; Chen et al., 2007; Beattie et al., 2003). Fine mapping of these identified QTLs would provide a better understanding of whether linkage of distinct genes or pleiotropic effect of one gene are responsible for their clustering (Verma et al., 2015).

The positive additive effect observed for some traits such as PL, SL and HSW implies TVu2185 (P1) had positive allelic contribution for these trait means; however, negative additive effects were also observed for PL and HSW at few loci indicating TVu6643 (P2) also had positive effect for these two traits. The negative additive effect for the rest of the traits implies TVu6643 had positive allelic contribution to the means of these traits.

In general, detection of a few major and minor QTLs (15 in total) for eight agronomic traits (some of which are agronomic traits) distributed in only six of the eleven linkage groups in the present study agrees with the previous findings from many mapping studies that revealed domestication related traits in

cowpea are controlled by a few major genes and some minor genes and they are distributed on narrow regions on linkage groups (Kongjaimun et al., 2012; Andargie et al., 2011; Gepts, 2004).

Conclusion

Availability of genetic linkage maps for crops facilitates localization and mapping of genomic regions (QTLs) associated with one or more of the agronomic traits of interest using phenotypic data of the segregating population. Mapping of QTLs related to agronomic traits can enable dissection of their genetic control and molecular mechanism which may render the possibility to develop varieties with improved seed yield. A genetic linkage map of cowpea has been constructed using SNP markers, and the number of linkage groups coincided with the haploid number of cowpea chromosomes. In addition, several major and minor QTLs were detected for eight agronomic traits and distributed on six of the eleven linkage groups (LGs). Although QTLs were detected on six LGs, clustering of some of the QTLs in one of the LGs was observed. The fact that the same genomic region influenced more than one agronomic traits coupled with the strong and positive correlation among the traits reflects pleiotropic effect. Potential genomic regions of cowpea associated with some agronomic traits (QTLs) were identified in this study. QTLs with large effect such as qPL_1, qSN, qHSW_2 and qSL_2 are potential candidates for marker development and marker assisted selection.

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