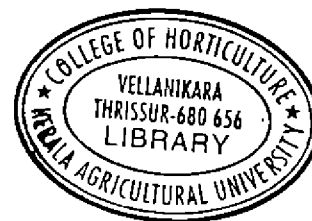# QTL MAPPING FOR YIELD TRAITS IN VEGETABLE COWPEA

By

ASHWIN VARGHEESE

(2015-11-001)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

(PLANT BIOTECHNOLOGY)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

COLLEGE OF HORTICULTURE

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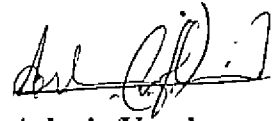
KERALA , INDIA

2017

DECLARATION

I hereby declare that the thesis entitled "**QTL mapping for yield traits in vegetable cowpea**" is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.
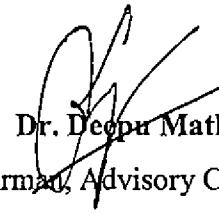
Vellanikkara

Date: 5· 07· 2017

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CERTIFICATE

Certified that the thesis entitled **"QTL mapping for yield traits in vegetable cowpea"** is a record of research work done independently by **Mr. Ashwin Vargheese (2015-11-001)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship, fellowship to him.

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And so comes the time to look back on the path traversed during the endeavour and to remember the faces and spirits behind the action with a sense of gratitude. Nothing of significance can be accomplished without the acts of assistance, words of encouragement and gestures of help from other members of the society.

*First and foremost, I bow my head before the **God** who enabled me to successfully complete the thesis work.*

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Ashwin Vargheese
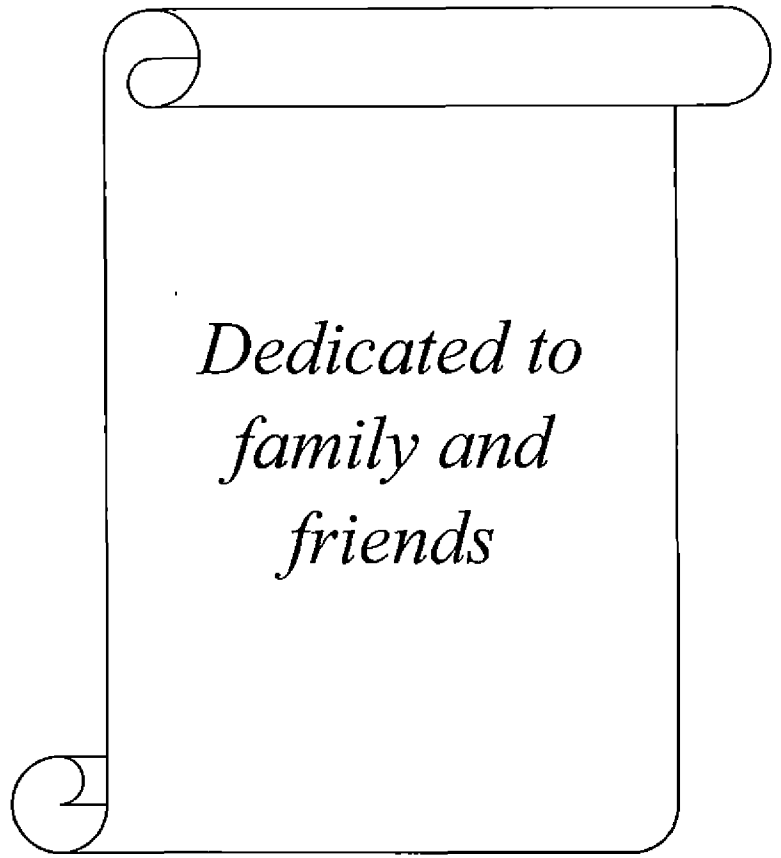
*Dedicated to
family and
friends*

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ABBREVIATIONS

AFLP	:	Amplified fragment length polymorphism
ANOVA	:	Analysis of Variance
BC	:	Backcross
bp	:	Basepair
BLUPs	:	Best Linear Unbiased Predictors
BSA	:	Bulk Segregant Analysis
cm	:	centimetre
cM	:	centimorgan
CPBMB	:	Centre for Plant Biotechnology and Molecular Biology
CTAB	:	Cetyltrimethyl Ammonium Bromide
cDNA	:	complementary DNA
CIM	:	Composite Interval Mapping
CMV	:	Cowpea Mosaic Virus
cv.	:	Cultivar
DTFF	:	Days Taken For First Flowering
°C	:	Degree Celsius
dNTP	:	Deoxynucleotide triphosphate
DNA	:	Deoxyribonucleic acid
DHL	:	Double Haploid Line
EDTA	:	Ethylene Diamine Tetra Acetic Acid
EST	:	Expressed Sequence Tag
FAO	:	Food and Agriculture Organisation
GOI	:	Government of India
g	:	gram
ha	:	hectare
HEGS	:	High Efficiency Genomic Sequencing
ICIM	:	Inclusive Composite Interval Mapping
IPW	:	Individual Pod weight
ISSR	:	Inter Simple Sequence Repeats
IL	:	Introgression Line

KAU	:	Kerala Agriculture University
kg	:	Kilo gram
LG	:	Linkage Group
LOD	:	Loagarithm of Odds
μl	:	microlitre
ml	:	millilitre
ng	:	Nanogram
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
PVP	:	Polyvinylpyrrolidone
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RIL	:	Recombinant Inbred Line
RARS	:	Regional Agricultural Research Station
RFLP	:	Restriction Fragment Length Polymorphism
REMAP	:	Retrotransposon Microsatellite Amplified PolymorphismB
RNA	:	Ribonucleic Acid
rpm	:	Rotations per minute
STR	:	Short Tandem Repeat
SIM	:	Simple Interval Mappinng
SSR	:	Simple Sequence Repeat
SMA	:	Single Marker Analysis
ssp.	:	Subspecies
TDPY	:	Total Dry Pod Yield
TAE	:	Tris Acetic acid EDTA
TE	:	Tris EDTA
UV	:	Ultraviolet

Introduction

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most cultivated pulse crops in the semi arid tropics of Asia, Africa, Southern Europe, and other parts of the world. Cowpea is either used for vegetable purpose or for fodder purpose. Attractive, succulent, tender pods are cooked as vegetable alone where as the nutritious grains could be used along with other vegetables as a rich protein source for the poor. Fodder type cowpeas serve both purposes, they provide grains for human consumption and the tender leaves are used as fodder for cattle. According to the FAO (2013), the crop is cultivated in an estimated area of 12.5 million hectares worldwide. In India, this crop is cultivated in an area of 0.5 million hectares with an average productivity of 600 to 750 kg grains ha^{-1}. States like Karnataka, Kerala, Tamil Nadu and Madhya Pradesh are few of the major producers of this crop (GOI, 2013). In India, cowpea is exclusively used as a kharif crop grown widely in the humid tropics. Recently the demand for the crop has increased as the food habits of people are inclining towards rich, natural source of nutrition. Cowpea pods contain 22-24 % protein, 0.08-0.11% calcium, 0.005% iron and essential amino acids like lysine, leucine and phenylalanine (GOI, 2016). Because of rich protein content, it is often used as a meat substitute by vegetarians. Even though dual purpose varieties give good pod yield and fodder, because of its one time harvest problem, farmers prefer vegetable cowpea varieties as they allow up to four to five pickings. Additionally, a crop of cowpea fixes up to 240 kg ha^{-1} atmospheric nitrogen and leaves about 60-70 kg nitrogen for next crops (Aikins and Afuakwa, 2008).
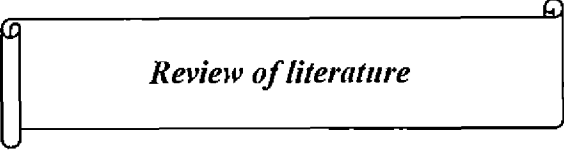
Two major domesticated cowpea types, the pole type [*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis*] and the semi-trailing type [*Vigna unguiculata* (L.) Walp. ssp. *cylindrica*] are both used for vegetable purpose. Cowpea breeding for higher yield has been one of the important research objectives since late nineties. Few classical breeding works have been done in that regard (Blade, 1991; Ohler and Mitchell, 1996; Singh and Sharma, 1996; Umaharan *et al*, 1997; Singh *et al*, 1997; Kitch *et al.*, 1998; Singh *et al*, 2003). However, the research outcomes were not stable and took relatively much longer time to reach farmers level. Because selecting plants for yield traits based on mere phenotypic variation is very difficult and vague as yield is a polygenic trait and is the final outcome of interaction of multiple correlated component traits. The main problem with selecting plants based on only phenotype was the high level of variation observed from generation to generation. The genotypic variance did not actually account for the total

phenotypic variation in field in case of quantitative traits, and moreover, traditional breeding methods would require the crop to go through complete life cycle which consumed many years.

Discovery of molecular markers, fuelled multiple studies trying to answer the long asked question of how to select plants from a population when the trait is governed by multiple genes. Molecular markers, acting analogous to the signboards on a road, give precise information regarding the position of a particular gene on the chromosome. Codominant markers give the added bonus to differentiate between the different allelic forms of a single gene. Hence with the advent of molecular markers, determining the relative position of a gene with respect to a marker, and knowing the allelic condition of that particular gene was made possible.

A linkage map is like a roadmap of chromosomes of a plant derived from two different parents. These maps depict the relative position of molecular markers along the chromosome of the plant. Using linkage map, QTL hotspots, which are the regions on chromosomes harbouring genes responsible for particular quantitative trait could be mapped on the chromosomes. Once the QTLs are mapped along with the linked molecular markers, it is then called a QTL map. Identification of QTL hotspots for the genes governing a trait gives invaluable information in marker assisted breeding.

Though few noteworthy works have been done with regard to mapping QTLs for individual plant traits in cowpea like resistance to *Thrips tabaci* and *Frankliniella schultzei*, drought stress-induced premature senescence and maturity, flowering time, pod length and seed weight (Maughan *et al.*, 1996; Kelly *et al.*, 2003; Muchero *et al.*, 2009; Muchero *et al.*, 2010; Andargie *et al.*, 2011; Kongjaimun *et al.*, 2012), there still remains dearth of information regarding markers associated with different genes contributing towards yield and genes involved in yield contributing pathways. Hence the present study "QTL mapping for yield traits in vegetable cowpea" was carried out with the objective of mapping the SSR markers and identifying the quantitative trait loci for yield components in the genome of vegetable cowpea.

Review of literature

2. Review of Literature

Cowpea (*Vigna unguiculata* L. Walp.) with chromosome number of (2n=2x=22) and genome size of approximately 620 Mb, belongs to Phaseoleae tribe of Leguminosae family. As the plant was an important source of hay for cows, the name cowpea was used (Timko *et al.*, 2007). Because of its ability to restore soil fertility, and as an option in crop rotation, cowpea is a vital component of farming systems (Sanginga *et al.*, 2003). As a cost effective source of dietary protein, early maturing cowpea varieties shorten the hungry period preceding the harvest of current season's crop among farming communities (Aliyu and Wachap, 2014).

2.1 Types of cowpeas

Vegetable type cowpeas are used for human consumption either as grains or as fresh pods and the fodder purpose or dual purpose cowpeas are used both as grains and fodder for livestock (Timko *et al.*, 2007). Vegetable type cowpeas are the main source of protein, vitamins and minerals in the developing countries. Addition of even small amounts of cowpea in the daily diet along with other starchy food is said to maintain the nutritional requirement of the individual (Singh *et al.*, 1997).

The grain type yields around 2 tonnes ha^{-1} with vegetable type yielding upto 18 tonnes ha^{-1}. It allows three to four pickings with continuous fresh green pod supply till six to seven weeks after the first picking. Because of its constant supply, fresh green pods and protein rich grains, vegetable type has great consumer acceptability when compared to fodder purpose cowpea varieties. Even though fodder purpose cowpeas yield around 0.6 tonnes ha^{-1} of grains and 30 tonnes ha^{-1} of fodder, since it is a one time harvest crop, farmers prefer vegetable type more (Singh *et al.*, 2003).

2.2 Yield and yield related traits in cowpea

Yield in cowpea is a quantitative trait governed by multiple genes with multiple traits contributing to the final yield (Asins, 2002). Li *et al.* (1997) reported that three traits, 1000 kernel weight, grains per panicle and grain weight per panicle show high level of epistatic interactions affecting the overall grain yield in rice. Traits like days to flowering, days to maturity, individual pod weight, number of grains per pod, green pod length, number of pods per plant and grain yield are considered to be the components of yield in cowpea (Santos *et al.*, 2014). Almeida *et al.* (2014) stated that number of grains per pod

and green pod length contribute highest towards the total productivity. Romanus *et al.* (2008) proved that green pod length has highly significant positive correlation to the number of grains per pod and that longer pods help accommodate more grains, thereby increasing overall yield. Oladejo *et al.* (2011) reported that grain yield was positively correlated with all morphological traits and traits like pod weight, days taken for first flowering and pod length could be used for indirect selection for plants with improved yield in cowpea.

Shimelis and Shiringani (2010) reported that with a heritability of 50 per cent for number of days to first flowering, 66 per cent for days to maturity and 53 per cent for productive branches per plant, these traits could be effectively used for direct phenotypic selection for improved cowpea variety development. Peksen (2004) found highly significant positive correlations between the traits, individual pod weight, number of branches per plant, average pod length and number of pods per plant. Umaharan *et al.* (1997) reported that average pod weight, total dry pod yield and total number of pods per plant could be used for vegetable cowpea improvement breeding programmes as these characters show highly significant positive correlation to total productivity.

Law *et al.* (1978) suggested to go for selection of plant height for yield improvement rather than selecting for yield itself as they found it more efficient because of the trait's highly positive correlation to total yield in wheat. Anjos *et al.* (1992) reported that resistance to Cowpea Mosaic Virus (CMV) is an important factor contributing to the total yield in cowpea. Carmi and Shalhevet (1983) reported that, reduction in plant growth rate and differences in yield have got more to do with the root growth rather than availability of water or fertilizers, thereby proving that root growth is an important factor deciding the total yield in cotton.

2.3 Breeding vegetable cowpea for higher yield

Because of their exceptional ability to grow in harsh conditions and use as nutritious vegetable many works across the world has been taken up for improving the yield potential of vegetable cowpea. Mehta and Zaveri (1997) reported that the mean performance of F_3 population developed by single seed descent method with respect to yield in cowpea was better when compared to single plant selection. Many interspecific cross attempts were made to improve yield in vegetable cowpea like cross between *Vigna vexillata* and *Vigna*

unguiculata (Gomathinayagam *et al.,* 1998) and between *Vigna radiata* and *Vigna unguiculata* (Tyagi and Chawla, 1999).

Distant hybridisation was found to be quite successful in incorporating yield traits to cultivated varieties like in cross between C 152 (cv.) and DWDCC 016 (landrace) which resulted in increased number of pods per plant (Hegde and Mishra, 2009). Popelka *et al.* (2004) suggested genetic engineering technology as an efficient way for introgressing genes governing yield traits.

2.4 QTL mapping in MAS

It has been a long time since the world has obtained the genetic blueprint of a plant. The lab rat for the plant science, *Arabidopsis thaliana,* which joined the club of organisms having every gene sequenced, paved way for the sequencing of agriculturally important crops like rice, wheat, maize etc., (The Arabidopsis Genome Initiative, 2000). This huge wealth of genetic information had a profound impact on evolutionary biology, by elucidating the most endured problem of lack of clarity regarding the genetic basis of complex traits in evolution and molecular biology (Mauricio, 2001). These complex traits are generally governed by polygenes. "Polygenes" as defined by the classical quantitative geneticists are the ones whose inheritance is largely controlled by multiple number of genes which are said to have approximately equal effect and is affected by environmental variations (Mather, 1941). Most of the traits which are of agricultural importance such as yield, quality and disease resistance are governed by many genes and are called quantitative traits. The genetic locus, which is a specific region inside the genome that contains genes controlling a particular trait is called a quantitative trait locus (QTL) (Collard *et al.,* 2005).

The identification of QTLs with the help of mere phenotypic evaluation is impossible. However, the advent of molecular markers in the 1980s made it possible to detect QTLs by characterising the quantitative trait (Weeks, 1995). These molecular markers helped to a great extent in construction of linkage maps which is one of the prerequisites for QTL analysis. QTL mapping is defined as the process of constructing linkage maps and carrying on QTL analysis to identify genomic regions associated with the trait (McCouch *et al.,* 1997)

2.5 Molecular markers in QTL mapping

Molecular markers, also called as DNA markers, unlike morphological and biochemical markers, reveal sites of variation at DNA level. These markers are selectively neutral as their presence is usually localised to the non-coding regions of DNA. Their unlimited number and developmental stage independence makes them the most widely used candidates when it comes to construction of linkage maps and QTL analysis (Belaj *et al.*, 2003).

Advantages and disadvantages of most commonly used molecular markers for QTL analysis are presented in Table 2.1

2.5.1 Microsatellite markers

Microsatellites, the term first coined by Litt and Lutty in 1989, are the stretches of DNA consisting of tandem repeating mono- to penta-nucleotide units that are arranged throughout the genomes of most eukaryotic and few prokaryotic species. They are also called as simple sequence repeats (SSRs) and short tandem repeats (STRs). Microsatellites are amplified by PCR primer pairs present in the conserved flanking region of the particular SSR locus. Together, these primer pairs amplify the specific microsatellite locus. The resultant PCR products will have variability in size with respect to the number of repeated DNA units in that microsatellite allele(s). So, these SSR markers can be further used to detect high level of length polymorphism present as a result of varying number short tandem units. These PCR products are usually run on a polyacrylamide gel and are observed for polymorphism under UV transilluminator (Vieira *et al.*, 2016)

2.5.1.1 Development of SSR markers

According to (Senan *et al.*, 2014), the development of SSR markers can be briefly divided into the following categories.

2.5.1.1.1 Getting information about the nucleotide sequences in which SSRs occur

Sequence data is the prime requisite for microsatellite detection. Until recently, screening of size-fractionated genomic DNA or EST (expressed sequence tag) libraries remained the only option to find out the sequences concealing SSR motifs (Zane *et al.*, 2002). Being present in the more constrained regions of the genome, the SSRs obtained from ESTs are often proven to show less variability than their genomic SSR counterparts. Their amplicon sizes is also reported to vary from the expected size because of the presence

Table 2.1 Advantages and disadvantages of most commonly used molecular markers for QTL analysis

Sl. No.	Co-dominant /Dominant	Advantages	Disadvantages
1.	Restriction Fragment Length polymorphism (RFLP) (Beckmann and Soller, 1986)		
	Co-dominant	-Highly reproducible -Transferable across population -Robust and reliable -Locus specific	-High quality and quantity of DNA required -Radiolabeled probes required -Time consuming, laborious and expensive -Limited polymorphism -Not amenable for automation
2.	Microsatellite or Simple Sequence Repeats (SSRs) (Somers et al., 2004)		
	Co-dominant	-Highly reproducible -Transferable across population -Robust and reliable -Locus specific -Amenable for automation -Technically simple	-High development cost -Primer development is highly time consuming and laborious -Usually polyacrylamide gel electrophoresis is required which is again laborious and time consuming
3.	Express Sequence Tag-SSR (EST-SSR) (Cato et al., 2001)		
	Co-dominant	-Highly reproducible, robust and reliable -High degree of sequence conservation -Transportable across the pedigree and species -Enable transfer of linkage information between species	-Marker development is limited to species for which sequencing information already exists
4.	Amplified Fragment Length Polymorphism (AFLP) (Chalmers et al., 2001)		
Dominant		-Highly reproducible -Highly polymorphic -Used for any organisms without sequence information -Provide good genome coverage	-High quality and quantity of DNA required -Complicated methadology

5. Randomly Amplified Polymorphic DNA (RAPD) (Harun-Or-Rashid *et al.*, 2016)		
Dominant	-Quick, simple and inexpensive -Small quantity of DNA enough	-Non-reproducibility -Generally not transferable
6. Inter Simple Sequence Repeats (ISSRs) (Dirlewanger *et al.*, 1998)		
Dominant	-Highly polymorphic -Simple	-Non-reproducibility -Generally not transferable

of introns in flanking regions which often go undetected (Gupta *et al.*, 2003).

However the mere disadvantages of EST-SSRs are masked by several important advantages over genomic SSRs, such as their ability to detect variation in the expressed region of the genome which is often of great importance to studies related to marker-trait associations. Their development is of no cost as they are obtained from EST databases and once these EST-SSR markers are developed, they usually work across different species making it highly transferable as the possibility of expressed genomic region being conserved across related species is very high (Varshney *et al.*, 2005)

When it comes to the cost and time required, conventional laboratory methods involving cloning, cDNA library construction or sequencing by Sanger method remains inefficient regardless of whether genomic or EST sequences are used for SSR detection (Squirrell *et al.*, 2003). To overcome this problem, nowadays, next-generation sequencing techniques are being used to detect sequences concealing SSR motifs which reduced the cost by two to five times as well as significantly reducing the time consumed (Santana *et al.*, 2009). Once the sequence is obtained, it can be analysed for regions harbouring SSR motifs using softwares like MSAT-COMMANDER (Faircloth, 2008) or QDD (Meglécz *et al.*, 2010).

2.5.1.1.2 Designing primers complementary to the region flanking the SSR

Once the sequences concealing repeat motifs are identified, the next step is to choose suitable primers. In order to keep the quality of SSRs high, stringent selection is important. Usually primer pairs which got the capability to amplify fragments of distinct sizes are chosen (Varshney *et al.*, 2006). Many computer programs are available which along with identifying SSRs, also design primers for multiplex (Rachlin *et al.*, 2005). Most of them search for appropriate primer pair combinations for multiplex PCR and are capable of handling large set of data simultaneously. In order to ensure successful co-amplification in multiplex PCR, care is taken to exclude primers with potential primer-dimer interactions (Van *et al.*, 2010).

2.5.1.1.3 Polymorphism detection among individuals

Once the identified SSR primers are screened, the PCR products are run on gels and polymorphism is detected (Creste *et al.*, 2001).

2.5.1.2 Development and use of SSR markers in Legumes and *Vigna*

Mishra *et al.* (2012) developed 577 EST derived SSR primer pairs in *Pisum sativum* and found that the markers had high percentage of transferability among related legume species. Choudhary *et al.* (2009) developed 246 EST derived SSR primer pairs in chickpea and reported high transferability (mean 82.6 per cent) across *Cicer* species and legume genera. They used these markers to assess intraspecific variability among 30 chickpea accessions. Dutta *et al.* (2011) developed and validated 550 genic-SSR primer pairs using deep transcriptome sequencing data of pigeon pea. Upon testing the primers for their ability to exhibit polymorphism across eight pigeon pea varieties, 71 SSRs showed clear polymorphism. These polymorphic SSRs used for analysing genetic diversity across 22 pigeon pea varieties and eight wild species showed distinct separation in a Neighbour-joining dendrogram.

Wang *et al.* (2004) developed 50 SSR primer pairs in azuki bean by constructing an (AG)n-SSR enriched library which resulted in 116 fold enrichment when compared to non-enriched library. They suggested that the construction of specific SSR repeat motif enriched library could give SSR primers with high amplification percentage (98). Gupta and Gopalakrishna (2010) developed 803 SSR primer pairs using Unigene sequences in cowpea out of which 102 SSRs were characterised. These SSRs showed high rate (88 per cent) of transferability across *Vigna* species. Kelly *et al.* (2003) by identifying around 30 markers linked to 17 different disease resistance genes and QTL opened up new horizon of opportunities for improvement of cowpea and bean with the help of Marker Assisted Selection (MAS).

2.5.1.3 Use of SSR markers in QTL analysis

Using maize F₂ population comprising of 450 plants derived by crossing high oil inbred By804 and normal inbred B73 and 158 polymorphic SSR markers, Song et al. (2004) have constructed a 1759.1 cM long linkage map for the kernel oil concentration, falling in 12 linkage groups. *Brassica napus* genetic map consisting of 19 linkage groups spanning 46.2 to 276.5 cM was constructed with 240 SSR markers using an F2 population (Piquemal *et al.*, 2005). A QTL map with 13 linkage groups attributing to the trait grain protein content in bread wheat was constructed using 171 polymorphic SSR markers (Prasad *et al.*, 2003).

Using PCR based markers such as SSR, SRAP, RAPD and REMAP (Retrotransposon Microsatellite Amplified Polymorphism), He *et al.* (2006) have mapped QTLs responsible for the economic traits in cotton. They found SSRs very efficient in detecting polymorphism and useful as anchors to couple linkage maps.

In a study to identify the QTLs for *Fusarium* head blight resistance in wheat, SSR markers were found capable to explain greater proportion of phenotypic variation when compared to other marker systems like AFLP and RFLP (Anderson *et al.*, 2001). Similarly, during an attempt to identify QTLs for agronomic traits in soybean genetic map using different markers like RFLP, SSR and ESTs, Zhang *et al.* (2004) have reported that ESTs and SSR markers are highly efficient in genetic analysis of different plant species. Using SSR markers, Shen *et al.* (2005) have developed a QTL map with 17 significant and 22 suggestive QTLs for fibre qualities in upland cotton.

In a study conducted by Andargie *et al.* (2011), using 202 polymorphic SSR markers, a genetic linkage map with 11 linkage groups covering 677cM was constructed. With a mapping population of 159 recombinant inbred line (RIL) individuals obtained from crossing the wild and cultivated type cowpea, they found four QTLs with significance values of $P = 0.05$ controlling fibre layer thickness which controls pod shattering. Since two QTLs LG1 and LG10 control both seed size and pod shattering, they suggest that the SSR markers used in this study can be directly used for the rapid elimination of wild phenotype traits as these markers are developed from coding regions.

Tanksley and Nelson (1996) have suggested advanced backcross QTL analysis as a new method to simultaneously identify and transfer important QTLs from less explored germplasm to the elite breeding cultivars. In this method, negative selection was carried out throughout the mapping population development and QTL analysis was done only when the population reached BC_2 or BC_3 stage which removed the identification of false QTLs and reduced the effect of epistatic interactions among alleles from wild parent. High Efficiency Genome Scanning (HEGS) system which reduced the time required was employed by Hori *et al.* (2003). For the three traits (plant height, spike exertion length and 1,000-kernel weight) considered for the study, they generated a high density linkage map consisting of 1172 loci distributed across 1595.7 cM genetic map. With multiple studies successfully cloning the QTL to around 2 cM distance accuracy, Price (2006) has proven that QTLs are accurate and are of great value to genetic studies in crop plants.

2.6 QTL analysis methods

QTL analysis is carried out by first developing a linkage map and then analysing it along with morphological and genotypic data for QTL identification.

2.6.1 Linkage map development

The whole science of QTL mapping is based on the principle that says genes and the linked markers segregate through chromosomal recombination (often called as crossing-over) occurring during sexual reproduction (meiosis) rendering their analysis in the progeny. Linkage map acts as a road map of the chromosomes obtained from two different parents indicating the relative position and distances between genetic markers across chromosomes (Ritter and Salamini,1996). Linkage maps are of great help in identifying chromosomal locations harbouring genes and in QTL mapping.

There are three important steps in linkage map construction

2.6.1.1 Developing mapping population

A segregating population derived from crossing parents differing for one or more traits of interest is the main prerequisite for linkage map construction. Various types of plant populations used are as follows (Sehgal *et al.*, 2016)

1. Double haploid lines (DHLs): Haploid plants regenerated from pollen of F_1 plants are treated to make them diploid. Since F_1 plants are generated by meiosis, DHLs acts as the direct representative of the segregating gametes.

2. Backcross (BC) population: Obtained by backcrossing the F_1 plants to one of the parents.

3. F_2 population: Obtained by selfing the F_1 plants

4. $F_{2:3}/F_{2:4}$ lines: $F_{3/4}$ plants trace back to the same F_2 plant, so often called F_2 families.

5. Recombinant inbred lines (RILs): A population obtained by repeated selfing of F_2 plants and further selection by single seed descent.

However, the choice of type of population and the number of plants in population depends on the marker system being used for the study and also depends on the resolution of the genetic map required (Mohan *et al.*, 1997).

2.6.1.2 Identification of polymorphism

For efficient mapping of QTLs, it is very critical that DNA markers show sufficient polymorphism between two parents (Young, 1996). In case of inbreeding species, it is advised to select parents with highly contrasting traits because when compared to cross pollinated species, self-pollinated ones possess lower levels of DNA polymorphism in general (Yu and Nguyen, 1994). The choice of DNA markers depends on the availability of characterised markers in the species of study and on the feasibility in terms of time, cost and efficiency. Once the polymorphic markers are identified among parents, they are then screened through the whole mapping population to check for their ability to show polymorphism. This process is called 'marker genotyping' (Sayed *et al.*, 2002).

2.6.1.3 Linkage analysis among markers

After marker genotyping, the data is coded for each DNA marker for every individual present in the mapping population. Then linkage analysis is done using computer programs like Map- maker (Lander *et al.*, 1987), MapManager QTX (Manly *et al.*, 2001), and JoinMap (Stam, 1993). Even though linkage analysis can be performed manually when the marker and mapping population numbers are few, when it comes to handling huge number of markers and mapping population, it becomes impractical to perform the same manually. Even though finding a QTL within a whole plant genome is almost like finding a needle in a haystack, it can be made possible by grouping the haystack into small sets and then scanning them in a systematic way for the presence of QTL.

The whole QTL mapping relies on the principle of establishing an association between the genotype of markers and the phenotype (Collard *et al.*, 2005). First the entire mapping population is partitioned into different genotypic groups depending on the presence or absence of a particular marker locus with the help of molecular markers. This is determined based on the existence of significant variation between different groups with respect to the trait being measured (Young, 1996).

The marker locus being used to partition the mapping population is said to be linked to the QTL controlling the trait only when there is a significant variation between phenotypic means of the population. The assumption of linkage between a marker and QTL is strengthened if a highly significant P value is obtained. The chances of recombination occurring between marker and QTL is very less if they are situated close to each other which leads to the simultaneous inheritance of the QTL and the marker linked to it in the

progeny. Likewise, if the marker and the QTL are unlinked, they segregate independently in the progenies (Bradbury *et al.*, 2007).

2.6.2 QTL detection

Most widely used methods for detecting QTLs are:

2.6.2.1 Single-marker analysis

In single marker analysis, individual markers are picked up one at a time and possibility of the marker being a QTL or linked to a QTL is checked using statistical tests like t-tests, analysis of variance (ANOVA) and linear regression. Chen (2014) claimed that even though single marker analysis is used as a preliminary procedure for conducting more sophisticated tests (with dense markers) it is limited by two major drawbacks. The first one being the procedure's lack of power in detecting QTL. When the marker of consideration is located far away from the QTL, this method fails to relate the apparent difference in the marker genotype to the effects of different genotypes of a QTL. The second reason being the inaccuracy of QTL position even when detected. However Tanksley (1993), suggested that both of the above mentioned drawbacks could be overcome if large number of segregating DNA markers spanning the whole genome is used

2.6.2.2 Simple interval mapping

The remedy for the flaws of single marker analysis came in the form of an outstanding idea put forth by Lander and Botstein (1989), i.e, simple interval mapping. This method uses a genetic linkage map which helps to explore the presence of any loci between two markers thereby increasing both the power and accuracy of QTL mapping. In the interval mapping approach, it is unquestionably presumed that there exists at most one QTL throughout the whole genome for the trait of concern making the method most effective when the assumption is true. Martinez and Curnow (1992) pointed out that normally multiple QTLs affect a quantitative trait, the effect of which is ignored by the interval mapping approach. This lead to two main problems. One was the identification of a non-existent 'ghost' QTL as a real QTL which usually occurs when a non-QTL interval was flanked by adjacent intervals containing QTL. And the second problem as pointed out by Knott and Haley (1992), was the artificial inflation of the variance of the random error which greatly decreased the power of detecting the real QTL.

2.6.2.3 Composite interval mapping

A simple solution for interval mapping was to adjust for the effects of other QTLs during the investigation of a putative QTL. This was practically made feasible by coupling the multiple marker regression and interval mapping (Jansen, 1993). Ability to map QTLs precisely and effectively even when linked QTLs were involved, made composite interval mapping the best available method for QTL mapping (Zeng, 1994).

2.6.3 Map distance and mapping function

Kearsey and Pooni (1998) stated that the chances of Genes or markers getting transmitted together from parent to progeny is very high when they are located close to each other (tightly-linked) than located far apart. The segregating population contains a mixture of parental and recombinant genotypes. The extent of prevalence of recombinant genotypes is used for calculating the recombination fractions which helps in elucidating the genetic distances between the markers. Further, the relative order and distances between markers is determined by analysing the segregation pattern of markers. The closer the two markers located on a chromosome, the frequency of recombination will be lower. Similarly, the recombination frequency will be higher if two markers are situated further away from each other on a chromosome.

According to Ritter and Salamini (1996), those markers having a recombination frequency of 50 per cent are said to be "unlinked" and are generally presumed to be situated far apart on either the same chromosome or a different one. Frequency of recombination among genetic markers is used to measure the distance along the linkage map. Since recombination frequency and the frequency of crossing over are not linearly related, mapping functions are used to convert recombination fractions into centiMorgans (cM). Hartl and Jones (2001) stated that when map distances fall below 10 cM, even though map distance equals recombination frequency, this relation does not apply when the map distances are greater than 10 cM. Currently the most commonly used mapping functions are Kosambi mapping function and Haldane mapping function (Tan and Fornage, 2008). Kosambi mapping function assumes that recombination events influence the occurrence of adjacent recombination events (Kosambi, 1943). Whereas Haldane mapping function does not assume any interference between recombination events (Haldane, 1919).

2.7 Measuring the significance of association

According to (Lander and Kruglyak, 1995), maintaining too lax standards guarantees a snowballing of literatures claiming false positive linkages. The credibility of even the true linkages goes down when majority of the reported linkages fail to be replicated. Thus maintaining sufficiently stringent standards to ensure claiming of linkages only for those with maximum likelihood but also keeping the standards liberal enough to avoid the stillbirth of the nascent field is of paramount importance. McKusick and Edwards (1975) proposed few standards that are most commonly used in genetic linkage studies. They concluded that LOD value of minimum 3 is required to declare linkage. They also stated that, for a linkage to be called suggestive linkage, it should be statistically expected to occur once at random in a genome scan, likewise 0.05 times occurrence for significant linkage and 0.001 times occurrence for highly significant linkage. However for confirmed linkage declaration, it was set mandatory that it has to be a significant linkage from initial studies that has subsequently been confirmed with replica studies under different environmental conditions with different population having a P value of 0.01.

The linkage assertion takes its credibility from statistical analysis which is based on an observed association between two traits within families. Risch (1992) highlighted that testing the presence of linkage, depends on the distinction between two hypotheses. One is null hypothesis (no linkage) and the other one is the presence of linkage at a recombination fraction. This ratio is expressed as the logarithm of the ratio, and is called a logarithm of odds (LOD) value or LOD score. For a linkage map to be valid, usually the LOD value threshold is kept above 3 which indicates that the linkage between two markers is 1000 times more likely than no linkage.

2.8 Softwares used for QTL mapping

Multiple softwares are available for QTL mapping such as MapMaker/QTL (Lincoln *et al.*, 1993), QTL cartographer (Basten,1994), PLABQTL (Utz and Melchinger, 1996), MapManager QTX (Manly *et al.*, 2001), R/QTL (Broman *et al.*, 2002), QGene (Joehanes and Nelson, 2008). However these softwares were limited by one or the other disadvantages like, dependency on other softwares for linkage mapping, inability to analyse data for higher QTL methods like multiple interval mapping, considering only additive gene effects, inability to take into account the interactions between QTLs and failure in analysing mixed models. Moreover, as some softwares are run by codes, the user

interface seemed difficult. However Meng *et al.*, in 2015 came up with QTL ICIMapping which fulfilled most of the lacunae the earlier softwares had. QTL ICIMapping has an added advantage of considering environmental interaction, gene action, and since it has eight different working platforms, the hectic data transformation work for different tasks are minimised. This software is characterised by its highest power of QTL detection with lowest false results.

2.9 QTL mapping towards improving the agronomical traits of crop plants

Brondani *et al.* (2002) have developed a QTL map using 157 SSR markers in a BC_2F_2 population derived from distant cross of *Oryza sativa* × *Oryza glumaepatula*. From the QTL map, positive QTL effects for tillering and panicle number were observed from wild parent. Using introgression lines (ILs) obtained by crossing the green-fruited species *Lycopersicon pennellii* and the cultivated tomato (cv M82), (Eshed and Zamir, 1995), have constructed a QTL map with 16 QTLs for plant weight, 22 for green fruit weight, 11 for total yield and 14 for total soluble solids. Cui *et al.* (2011) constructed a QTL map using two RIL populations obtained from crosses between Weimai 8 and Jimai 20 (WJ) and between Weimai 8 and Yannong 19 (WY) for plant height in wheat and have mapped 10 QTLs for spike length and five QTLs for internode length both of the traits with positive correlation to plant height.

Zhang *et al.* (2004) have constructed a QTL map with 63 QTLs having highly significant LOD values for nine agronomic traits like days to flowering, days to maturity, plant height, number of nodes on main stem, lodging, number of pods per node, protein content, 100-seed weight, and plot yield using EST markers. QTL map for root hair growth components in common bean was given by Yan *et al.* (2004). Chaim *et al.* (2001) developed a QTL map consisting of 55 highly significant QTLs for fruit traits like fruit diameter, weight, pericarp thickness and pedicel diameter in *Capsicum annum*. Wang *et al.* (2010) for the first time used conditional and unconditional QTL mapping methods and mapped eight QTLs for plant height in wheat thereby explaining the complexity and the details underlying wheat plant height and development.

Kumar *et al.* (2007) identified six QTLs with pleiotropic effects for yield and yield related components in bread wheat. Weng *et al.* (2008) mapped, isolated and characterised a major QTL (*GW5*) which is associated with grain width and weight leading to higher yield in rice.

2.9.1 QTL mapping in cowpea (*Vigna* spp.)

Vaughan and Srinives (2013) reported the first ever QTL mapping for pod tenderness and total soluble solids in yardlong bean. They confirmed the QTL for pod tenderness on LG7 by a replication study using BC_1F_2 and F_3 population. Kongjaimun *et al.* (2012) in their attempt to map the QTL for the pod length of yardlong bean shed light on information which was not known for long. With a linkage map spanning 852.4 cM with 11 linkage groups, they confirmed 6 QTLs responsible for pod length using flanking markers and an F_2 population of JPS1610 x TVnu457 making them potential introgression candidates in yardlong bean and cowpea breeding. Xu *et al.* (2013) reported the QTLs and epistatic interactions for genes governing traits such as days to first flowering (FLD), nodes to first flower (NFF), leaf senescence (LS) and pod number per plant (PN) in asparagus bean. They also observed that the identified QTLs are conserved across the related legume species.

Andargie *et al.* (2013) reported five QTLs responsible for time of flower opening and three QTLs having effect on days to flower in vegetable cowpea using 202 SSR markers in an F_7 mapping population. In an inquiry into identification of QTLs for genes governing the resistance for flower bud thrips which is a major insect pest in cowpea causing an yield loss of up to 80 per cent, Omo-Ikerodah *et al.* (2008), successfully mapped five QTLs having effect on flower bud thrips resistance on linkage group 3 in RILs in cowpea.

2.10 Cultivars used in the development of mapping population
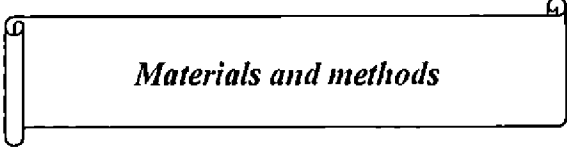
2.10.1 Kanakamany

Kanakamany, is a high yielding dual purpose variety developed in 1977 by the pure line selection of kunnamkulam at RARS, Pattambi, Kerala Agricultural University. The variety best suited for kharif and summer seasons is semi-trailing having medium long and dark green pods with an average length of 15-18 cm yielding 2.8 tonnes/ha. Being immune to anthracnose, this variety completes life cycle within 70-80 days (Kumar, 1999)

2.10.2 Sharika

Sharika, also a high yielding vegetable purpose variety developed by Kerala Agriculture University, is trailing type with long, white pods having an average pod length

of 40 cm. The variety which completes its life cycle within 80-90 days, is best suited for both kharif and rabi season. It has wide acceptance among farmers because of its long pods and ease of harvesting. But the variety's high susceptibility towards anthracnose disease is the major hurdle for crop production (Kumar, 1999).

Materials and methods

3. MATERIALS AND METHODS

The research work on QTL mapping for yield traits in vegetable cowpea was carried out at the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agriculture University, Vellanikkara, Thrissur. This chapter describes the materials used and the methodology followed in this research.

3. 1 Materials

3.1.1 Plant materials

At Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, the semi-trailing and relatively low yielding, short poded vegetable cowpea cv. Kanakamony [*Vigna unguiculata* (L.) Walp. ssp. *cylindrica*] was crossed with pole type, long poded and high yielding cv. Sharika [*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis*]. Plate 3.1 shows pictures of parents and their pods. The F_2 plants were subsequently selected for anthracnose resistance (Pradhan, 2015) and the F_3 population was further screened for the pod length. F_3 seeds with wide variability for yield and pod traits was maintained at CPBMB (Mathew, 2016). These F_3 seeds were used to raise the mapping population for phenotypic and genotypic analysis in the study.

3.1.2 Laboratory chemicals, glassware and equipment

AR grade chemicals from Merck India Ltd., Himedia and Sisco Research Laboratories (SRL) were used in this study. The constituents for PCR reaction mixture, *Taq* DNA polymerase, dNTPs, Taq buffer and primers used in this study were procured from Bangalore Genei Ltd., Sigma or Invitrogen. The plastic wares from Axygen and Tarson India Ltd. were used. The SSR primers were synthesised by Sigma Aldrich Chemicals Pvt. Ltd.

For Centrifugation, High speed refrigerated centrifuge (KUBOTA6500) was used. DNA quality and quantity estimation was done using NanoDrop® ND-1000 spectrophotometer. Agilent SureCycler 8800 PCR machine was used for the DNA amplification. Horizontal gel electrophoresis unit by Bio-Rad, USA was used for Agarose gel electrophoresis
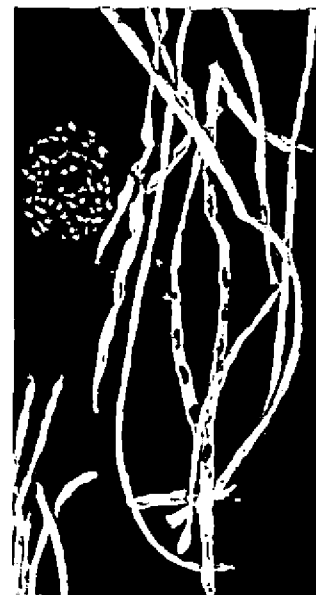
a) Semi-trailing Kanakamony

b) Pole type Sharika

c) Kanakamony pods

d) Sharika pods

Plate 3.1 Plant and pods variability among parents

3.2 Methods

3.2.1 Raising the mapping population

Seeds from 19 F_3 plants derived from the cross Sharika × Kanakamany were used for raising the F_4 mapping population. Ninety six pits were taken at a spacing of 1 m × 1 m. Two seeds each from five selected pods from each plant were sown in five pits. Two pits with 2 plants per pit were also sown for the parents, Kanakamany and Sharika (Plate 3.2.a). The basal fertilizers and FYM as per the package of practices recommendations crops of Kerala Agricultural University (KAU, 2011) were given. The plants were allowed to trail on pandal and standard crop production techniques were followed (Plate 3.2.b).

3.2.2 Morphological traits of plants considered in this study

Data of all the traits of consideration of F_4 mapping population along with parents Sharika × Kanakamany were recorded throughout the crop period.

3.2.2.1 Pod length (cm)

Pod length of all plants in the mapping population and parents were recorded by measuring the length of at least 10 pods from individual plant. The mean values for individual plants were used in the analysis.

3.2.2.2 Individual dry pod weight (g)

Individual pod weight of all the plants in the mapping population and parents were recorded by weighing at least 10 mature dried pods from individual plants and mean values were used in the analysis.

3.2.2.3 Pod number per plant

Mature pods from individual plants were harvested from time to time and the number of pods were recorded by taking the mean of 2 plants in a pit.

3.2.2.4 Days taken for first flowering (DTFF)

Total number of days taken for the emergence of first flower from the date of sowing was recorded for each plant and the mean value for two plants in each pit were used.

a) Semi-trailing Kanakamony

b) Pole type Sharika

c) Kanakamony pods

d) Sharika pods

Plate 3.1 Plant and pods variability among parents

a) F$_4$ mapping population sown in pits in field

b) F$_4$ mapping population trailed on pandal in field

Plate 3.2 Raising the F$_4$ mapping population

3.2.2.5 Total dry pod yield (g)

Dry pods from both the plants in a pit were weighed and the average weight was recorded in grams.

3.2.2.6 Grains per pod

Total number of grains in individual pods were counted for 10 pods per plant and the mean values were recorded.

3.2.2.7 Number of branches per plant

Total number of primary branches of individual plants were recorded.

3.2.2.8 Root length (cm)

Lengths of the longest roots of both the plants in a pit were taken and the mean value was recorded.

3.2.2.9 Plant height (cm)

The length of individual plant from the root initiation point to the tip of the plant was recorded for both the plants in a pit and the mean value was recorded.

3.2.2.10 Plant weight (kg)

Fresh weights of both the plants in a pit were taken and the mean value was recorded.

3.2.2.11 Response to anthracnose infection

The presence or absence of anthracnose infection was recorded in individual plants.

3.2.2.12 Response to cowpea mosaic virus (CMV) infection

The occurrence of CMV infection was also recorded in individual plants.

3.2.3 DNA isolation

For isolating DNA, the CTAB method (Doyle and Doyle, 1990) was standardised with slight modifications. The details are furnished hereunder

3.2.3.1 Reagents

I. CTAB extraction buffer (2x):

- 2 per cent CTAB (w/v)
- 100mM Tris (pH 8.0)
- 20mM EDTA (pH 8.0)
- 1.4M NaCl
- 1 per cent PVP

II. 10 per cent CTAB solution:

- 10 per cent CTAB (w/v)
- 0.7M NaCl

III. TE buffer:

- 10mM Tris (pH 8.0)
- 1mM EDTA

IV. Chloroform: Isoamyl alcohol (24:1 v/v)

V. Isopropanol (100 per cent)

VI. Ethanol, 70 per cent and 100 per cent

VII. Sterile autoclaved distilled water

3.2.3.2 Procedure

➢ Clean tender leaf sample (0.1 g) collected early in the morning before 7:00 AM was immediately kept in ice and brought to the laboratory. Samples were ground to fine powder in liquid nitrogen using pre-chilled autoclaved pestle and mortar with 15 μl β-mercapto ethanol and a pinch of polyvinyl pyrrolidone (PVP).

➢ The Homogenised sample was transferred to an autoclaved 2 ml centrifuge tube with 1 ml pre-warmed extraction buffer.

➢ The tubes were inverted few times to mix the contents and incubated at 65 °C for 20 minutes with occasional gentle inversions.

➢ After incubation, the tubes have been taken out and equal volume (1 ml) of chilled chloroform:isoamyl alcohol (24:1) was added, inverted to mix and emulsify the contents and centrifuged at 12,000 rpm for 15 minutes at 4°C.

➢ After centrifugation, the contents got separated into three distinct layers

- Aqueous top most layer - Containing DNA and RNA
- Interphase - Containing fine particles and proteins
- Lower layer – Containing chloroform and some pigments

➢ The tubes were carefully taken out from the centrifuge without disturbing the three layers and the top aqueous layer was carefully transferred to a fresh centrifuge tube. To this, 1/10th volume of 10 per cent CTAB solution and equal volume of chloroform: isoamyl alcohol (24:1) were added.

➢ The contents were mixed well with gentle inversions and centrifuged at 12,000 rpm for 15 minutes at 4 °C.

➢ After centrifugation, the tubes were taken out and the top most aqueous layer was carefully transferred to a new centrifuge tube. To this, 2 µl of RNase was added and incubated at 37 °C for 15 minutes.

➢ After incubation, equal volume of chloroform:isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 15 minutes at 4 °C.

➢ After centrifugation, the aqueous phase was carefully transferred to a fresh 1.5 ml centrifuge tube. To this, 0.6 volume of chilled isopropanol was added and the tubes were incubated at -20 °C for 2 hours.

➢ After incubation, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4 °C

➢ Then the supernatant has been discarded and to the pellet, 200 µl of 70 per cent ethanol was added and the tubes were centrifuged at 10,000 rpm for 5 minutes at 4 °C.

➢ The 100 per cent ethanol wash was repeated when the salt precipitation was high. After centrifugation, the supernatant was discarded without disturbing the pellet.

➢ The pellets were dried inside the laminar air flow until all the ethanol got evaporated and was dissolved in 70µl autoclaved distilled water.

➢ The tubes have been gently tapped to ensure complete dissolution of pellet and then the DNA samples were stored at -80 °C.

3.2.3.3 Quality and quantity checking of isolated DNA

The quality and quantity of the extracted DNA was assessed using agarose gel electrophoresis and spectrophotometer.

3.2.3.3.1 Agarose gel electrophoresis

Reagents and equipment

1. Agarose (0.8 per cent)
2. 50X TAE buffer (pH 8.0)
   - Tris buffer (1 M)
   - Glacial Acetic acid
   - 0.5M EDTA
3. Tracking/loading dye (6x)
4. Ethidium bromide (stock 10mg/ml: working concentration 0.5µg/ml)

Procedure

➢ The gel casting tray was placed appropriately in a gel caster and the movable wall was adjusted such that the gel casting tray was closed at both ends. A comb was selected depending on the number of samples to be electrophoresed and positioned on the grooves provided on the gel casting tray.

➢ Gel was prepared by adding 0.8 g of agarose in 100ml 1X TAE buffer in a glass beaker. The mixture was heated in a microwave oven till all the agarose particles were completely dissolved and a clear solution was obtained.

➢ Then the solution has been allowed to cool down to 40 to 50 ℃ and 5 µl of ethidium bromide was added and mixed well. The warm gel was then poured into the gel casting tray and left to solidify for 20 minutes at room temperature.

➢ Special care was taken to avoid any air bubbles near the wells or on the gel

➢ Once the gel was solidified, a small amount of 1X TAE was poured on top of the gel and the comb was removed carefully without breaking the gel. The 1X TAE was discarded and the gel along with the tray was kept inside the electrophoresis tank with the wells on the negative electrode side

➢ The electrophoresis tank was filled with 1X TAE sufficient enough to submerge the wells

➢ The samples to be electrophoresed were prepared by mixing 5 µl of the DNA sample with 1 µl of 6X gel loading dye. After mixing, total volume of 6 µl was loaded into individual wells.

The samples were electrophoresed at 75 volts until the gel loading dye reached two third of the gel length.

3.2.3.3.2 Gel Documentation

Documentation of the electrophoresed gel was done under UV with BioRAD Gel Doc™ XR+ gel documentation system using PDQuest™ software.

3.2.3.3.3 Quality and quantity estimation of DNA with spectrophotometer

The quantity and purity of DNA was estimated using NanoDrop ND-1000 spectrophotometer. Since the absorption maxima for nucleic acids and proteins are at 260 and 280 nm respectively, absorbances have been recorded at both the wavelengths and the purity of samples was estimated using the OD_{260}/OD_{280} ratio. The DNA sample was considered to be pure if the OD_{260}/OD_{280} value is between 1.8 and 2.0. Values above 2.0 and below 1.8 are due to contamination by RNA and protein, respectively. Then the concentration of DNA in the sample was estimated using the relation 1 OD at 260 nm = 50 ng DNA/µl

Hence, $OD_{260} \times 50$ gave the quantity of DNA (ng/µl)

Procedure

> NanoDrop spectrophotometer has been connected to the computer, ND-1000 software was initiated and 'Nucleic acid' option was selected

> The sampling arm has been opened and the pedestal was wiped with tissue paper to remove any dust particles

> Initially, 1 µl distilled autoclaved water was loaded on to the pedestal and the software was started by clicking 'OK'

> The pedestal was wiped again with tissue paper and the reading was set to zero with a blank sample

> Then 1 µl of sample was loaded on to the pedestal and 'measure' option was selected

> After the measurements, the pedestal was wiped clean with 70% ethanol using a soft laboratory wipe

3.2.4 Preparation of reaction mixture for thermal cycling

SSR markers were used for genotyping the parents and the mapping population. The reaction mixture was consisted of template DNA, reaction buffer, $MgCl_2$, *Taq* DNA polymerase, dNTPs and SSR primers. The desired number of PCR cycles, time and

temperatures for denaturation, annealing and extension were standardised based on the primers used and the conditions were programmed and saved in the thermal cycler (Model- SureCycler, Make- Aglilent).

3.2.4.1 Primer screening

The genomic DNA of both the parents Kanakamony and Sharika were diluted to a concentration of 25 ng/µl and were amplified using the SSR primers reported by Xu *et al.* (2010). Initially 100 SSR primer pairs were selected for screening among parents (Appendix III). From this, 30 primers exhibiting sufficient polymorphism were selected for genotyping the mapping population (Table 3.1).

Table 3.1 Details of polymorphic primers used for genotyping the mapping population

Sl. No.	Marker ID.	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperature (°C)
1.	CLM0007	ACAGGTTCCTTGTGA AGCAC	GCCATACGCAACTCA GCTAT	55
2.	CLM0008	CGGTTCTAGTGCCAC CAA	GAAACCGGCACTGG AAAC	51
3.	CLM0031	CGCTTTTGTAGGATT GGAAC	TTAGCATGGGAGAG TTTTCG	53
4.	CLM0050	CTTCTCTCCGTCAAG TGGAA	AGCAGACAACCACA GATGCT	55
5.	CLM0063	CATCCACCACATCAA AATCA	CCCAATTGAAGTCCT TGATG	55
6.	CLM0066	AACCCAGCATACCTG CATAA	CTCGCCAATGATTCT GAGAT	53
7.	CLM0068	AATGTTTGGACTGGT CAGGA	GAGGACAAGTCAGG AAGCAA	54
8.	CLM0077	AAAGCGGAAAAAGT TTGGAT	AGCACTCTGCACACA AATCA	56
9.	CLM0083	GGCGACGTCTTTCCA TATTA	TGGAATCGATGTTGT GATTG	55
10.	CLM0085	CACAACTGTGATTTG CTCGAT	TCGGAAACAGGTTC ACCTAC	55
11.	CLM0088	TCGTCGGTCTTCATA AAAATG	AACGCTTCGATTATC TGCAC	53
12.	CLM0101	TGTCTTTGCAGGTTG TTTCA	GCTACATGGTGATGC CACTT	53
13.	CLM0119	GAGATGTTGAGATG GTGGCT	CCTTGGTCATTGAAC CTCTC	56
14.	CLM0151	TGCTTGAGTGTCACT TGAATG	TCGCAAAGAGAGGA ATATCG	54
15.	CLM0156	GGGCTTCCTAGGTCA CAAAT	CCATTCTCTTCGGTT AGTTATT	55

16.	CLM0168	TGAGAGGACCAAAT TACTCCA	TCACCATTCTAAGAA ACAAGTGA	57
17.	CLM0177	AATTGGGTTGTAAAG TGAGATTT	CGAAAGTGGTTTGCG TATTT	54
18.	CLM0186	TTTGAACTCATATAA AGCACTTG	GATCCTTCTTCCCTC TCTCG	57
19.	CLM0195	AGGCATGATGTGTG GAGTTT	TTTCTCACGTTGTTT AGCCTT	55
20.	CLM0200	AATTTGATCGCCTAA CGACA	TCAAACGTATATGCG TAAATAAT	52
21.	CLM0201	CCAAAACAAACACC AACCTC	GAGACCTGCGATCA GAACAT	54
22.	CLM0218	TTTCCGATTTGCGAT TTTTA	CGACCAGTGACAAA TGAACC	51
23.	CLM0244	GTGGAGTTCAGTGGC AAAGT	CCAAAATCGCATGTA GTTCC	54
24.	CLM0251	CTTTTCATGGGAATT GTTGG	TGAACTTTCCAAGGA ACTCG	52
25.	CLM0260	TCGATCAAATTTTCC TCTGC	TGCCACCATCTTTCA TTTCT	51
26.	CLM0279	TGCAAAACGTGAAA GCAATA	ACAAGGAGACCAAG GAGCTT	52
27.	CLM0287	TTGGGTCATTAACTC CTTTCC	ACGGCAAGCATGAA CAATAG	55
28.	CLM0292	GAGAGACGTGATGG AGAGGA	TCAATGATCGTATAA AGCCTCA	57
29.	CLM0300	TTTTGTTGGTTGAGC ATCTG	GGTGTTCAATGTCAG GAATAACA	56
30.	CLM0322	ACTGAACAGCAAGG ACGTTT	TGTGTTTCCAGTGCA AGAAT	54

The PCR amplified products were electrophoresed on 2 per cent agarose gel at 75 volts. A 100 bp DNA ladder (Banglore Genei) was used and ethidium bromide was used for staining. The gel profile was visualised under UV and was saved for further analysis.

Thermal cycling was carried out in 0.2 ml PCR tubes with 20 μl reaction mixture. The composition of reaction mixture used was

a)	Genomic DNA (25ng/μl)	:	1.5 μl
b)	10X *Taq* assay buffer A or B?	:	2.0 μl
c)	MgCl$_2$	:	0.7 μl
c)	dNTP mix (2.5 mM of each)	:	1.0 μl
d)	*Taq* DNA polymerase (3 Units each)	:	0.3 μl

e)	Primer (10 pM)	:	1.0 µl each of forward and reverse
f)	Autoclaved distilled water	:	12.5 µl
	Total reaction volume	:	20.0 µl

The PCR programme followed was

a)	94°C for 4 minutes	:	Initial denaturation
b)	94°C for 45 seconds	:	Denaturation
c)	50°C to 56°C for 1 minute	:	Primer annealing
d)	72°C for 2 minutes	:	Primer extension
e)	72°C for 8 minutes	:	Final extension
f)	4°C hold for infinity	:	Storage

(b, c, d) 35 Cycles

3.2.5 Genotyping the F₄ mapping population

Thirty SSR primer pairs which were successful in exhibiting polymorphism among the parents were carried on further to screen the F$_4$ mapping population. The gel profiles of individual SSR primer were carefully observed and scored (Details on scoring of SSR codominant maker system are given in Appendix-V).

3.2.6 QTL Mapping

The ICIMapping software was downloaded from http://www.isbreeding.net/software/default.aspx?type=detail&id=20. The phenotypic data used was Box-Cox transformed and from them, BLUPS (Best Linear Unbiased Predictors) were generated. The BLUPS were analysed against the genotypic data of codominant SSR markers.

QTL mapping in ICIM software using the biparental population F$_4$ involved two stages:

(i) Construction of a genetic linkage map

(ii) Construction of QTL map

3.2.6.1 Construction of genetic linkage map

3.2.6.1.1 Preparation of input file

Molecular marker data was fed in *.xls or *.xlsx file. The names of each sheet, the column for data entry etc., were strictly followed according to the ICIMapping user manual. Care was taken to exclude space between the words of page names, cultivar names, marker names, trait names etc., as this software does not allow space between the words. First page of Excel file was named GeneralInfo (Plate 3.3.a).. In A1 of first page, the mapping population type was entered In A2 of first page, code for mapping function, in A3, marker space type, in A4, number of markers used, and in A5, number of plants in mapping population, were entered (The codes for each of the above mentioned parameters are given in Appendix-IV).

Second page in Excel was named Genotype and it was where the marker scores were entered (Details on scoring of SSR codominant maker system are given in Appendix-V). For SSR, marker representing the allele in first parent was given score 2 and marker for the other allele present in second parent was given score 0. Heterozygous individuals having both markers (representing both alleles) were scored 1. In the first column of second page, marker names were given and from second column, the scoring for the individual plants were given such that the first row of second column shows the marker score for the first marker for first plant in the population and second row of second column shows the marker score for the second marker for first plant and so on (Plate 3.3.b).

The third page of input file was named Anchor. This information had shown the anchoring information of the markers to a particular chromosome. If the SSR position is unknown, 0 was given, and if the chromosome number was known, 1 was given. Care was taken to ensure that the names and order of markers were exactly as given in page 2 (Plate 3.4.a).

	A	B	C	D
1	8 Mapping Population Type			
2	1 Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)			
3	1 Marker Space Type (1 for intervals; 2 for positions)			
4	6 Number of Markers			
5	92 Size of the mapping population			

GeneralInfo / Genotype / Anchor

a) Format of filling generalinfo in first page

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	CLM0119	0	2	2	2	0	0	2	2	2	2	2	2	0	2	2	2
2	CLM0300	0	2	2	2	0	2	2	2	2	2	2	2	0	2	0	1
3	CLM0168	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	CLM0186	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
5	CLM0292	2	0	2	2	2	1	1	1	1	2	0	0	0	2	-1	2
6	CLM0077	2	0	2	2	2	2	2	2	2	2	0	0	0	0	0	2

GeneralInfo / Genotype / Anchor

b) Format of filling genotype data in second page

Plate 3.3 Example format for input file preparation for linkage map

a) Format of filling anchor information in third page

Plate 3.4 Example format for input file preparation for linkage map

3.2.6.1.2 Analysis

The objective of the linkage mapping prior to QTL mapping is to allocate the markers to linkage groups, when their positions on chromosomes are not known. When the location of each marker is elucidated by sequencing or is retrievable from databases such as MaizeGDB, PlantGDB, GRAMENE etc., linkage mapping could be skipped and directly proceeded to QTL mapping.

New Project function was selected from the File drop down menu in the software. Project name was given and the path of the file within which the project was supposed to be saved was selected. In the newly opened box, '*.map (linkage map construction)' option was selected. In the next opened box, input file was selected by selecting the *.xls or *.xlsx options from the drop down menu near File name. The software then opened the file and marker summary was displayed. The options at the bottom of the opened box like, 'Grouping', 'Ordering', 'Ripping', 'Outputting' were used to generate linikage map. In the output folder 'Results', six independent files with different file extensions were saved. These files contained details on distance and grouping of markers. This information was used for QTL mapping.

3.2.6.2 QTL mapping

3.2.6.2.1 Preparation of input file

The input file had five parts. In the first page GeneralInfo, A1 contained 'Indicator' which says whether this was a mapping study or simulation and for mapping study, 1 was entered. A2 detailed the 'Population type' and for F_4, code 8 was used. A3 contained mapping function. A4 had 'Marker space type'. As interval in terms of cM was used in the study, 1 was given. However, when exact positions are known by sequencing, 2 may be chosen. A5 contained the 'Marker space unit', where 1 shows the measure of marker space in cM. In A6, chromosome number was given. When there is no information on position of markers on each chromosome, the number of linkage groups has to be given here). For *Vigna*, chromosome number was given 2n=2x=22. When the chromosome number is known and if the number of linkage groups generated by linkage analysis is less than that, number of linkage groups has to be entered. The underlying principle is that under no circumstances, number of linkage groups will be more than the number of chromosomes. A7 detailed population size, and A8 had information on number of traits phenotyped (Plate 3.5.a) (Details on all the eight parameters used for the general information defining of a linkage mapping population is given in Appendix VI).

Second page was named Chromosome and this contained the details on number of markers in each chromosome. When the chromosome details were not known, the linkage details obtained from 'Linkage analysis' was given (Plate 3.5.b).

Third page was named LinkageMap and this contained the details on marker positions. In column 1, markers were listed in the same order and names. In column 2, the chromosome or linkage group number and in column 3, the position or distance (distance in cM obtained from linkage analysis) were entered. It was cross verified that the chromosome number and number of markers in each were same in both pages 2 and 3 (Plate 3.6.a).

Fourth page was named Genotype and in this page, the marker type information or marker scoring was entered as done for the second page of input file for linkage analysis. Since the marker order had changed after the linkage analysis, care was taken to ensure that the markers were entered as per the marker order in page 3 of this file (Plate 3.6.b).

Fifth page was named Phenotype and in this page, phenotype of plants in the population was entered trait wise. First column represented the trait names (with no space in between word) and first row represented the expression of first trait in the members in a population. For missing values, -100 was given (Plate 3.7.a).

3.2.6.2.2 Analysis

ICIM software was opened and New Project option was selected from the drop down File menu. The mapping method and LOD were directed using the interactive window at the bottom of the display window. If the markers used in the study are not linked or the number of markers used are limited, single marker analysis (SMA) is considered to be the best option. Once these parameters were set, Start QTL Mapping option was selected from the drop down Task menu. After the mapping was completed, the Figures Tab gave maps for each type of analysis. The results were saved in seven files in a folder named 'Results' within the project file that was mentioned in the beginning of analysis. These files were converted to *.xls files by simply changing the extension of file.

a) Format for filling generalinfo in first page

The first spreadsheet shows:

Cell	Value	Description
1	1	Indicator (1 mapping study, 2 simulation)
2	8	Population type (8 for F3population)
3	1	Mapping function (1 Kosambi, 2 Haldane, 3 Morgan)
4	1	Marker space type (1 Interval, 2 Positions). When distance from linkage analysis is used, as we do now, 1 is the choice but if the position is known by sequencing, 2 is used
5	1	Marker space unit (1 cM, 2 Morgan)
6	4	Chromosome number
7	92	Population size
8	12	Number of traits phenotyped

b) Format for filling chromosome information in second page

The second spreadsheet shows:

A	B
Ch1	3
Ch2	2
Ch3	1
Ch4	1

Plate 3.5 Example format for input file preparation for QTL mapping

a) Format for filling linkagemap information in third page

b) Format for filing genotype information in fourth page

Plate 3.6 Example format for input file preparation for QTL mapping

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	PodLength	26	18.4	23.7	23.9	23.5	17.5	18.27	27.3	21.02	16.07	16.45	17.45	16.8	19.38	22.16	24.75	29.25
2	IndividualPodWeight	2.01	1.96	2.36	2.39	1.82	1.35	1.4	2.08	1.61	0.95	1.56	2.31	2.32	1.6	1.32	1.2	2.26
3	PodNumber	56.5	12	58.5	46.5	58.5	7	26.5	31.5	73	23	51	43	47	56.5	18.5	21.5	25.5
4	DTFF	38	38	38	38	38	38	38	32	38	32	32	32	32	32	33	33	35
5	TotalDryPodYield	113.565	62.72	119.38	111.135	106.47	9.485	37.345	65.52	117.53	22.01	79.56	99.33	109.04	146.9	24.42	25.8	57.63
6	GrainsperPod	16.8	10.7	12.2	12.1	12.6	11.83	8.55	17.2	12.52	8.6	13.8	16.9	15.5	18.55	14.5	13.16	16.4
7	Branchnumber	4	6	4	6	5	7.5	8	4.5	10	5.5	4	6	5	5.5	6	6	4
8	Rootlength	48	54	47	60	59	47.5	43	64	110	56	36.5	35	38.5	30.5	78.5	70.5	52.5
9	PlantHeight	510	570	520	610	590	340	370	570	770	450	420	430	510	580	410	600	540
10	PlantWeight	0.906	1.1585	0.873	1.205	1.1945	0.8175	0.6455	1.0163	2.487	1.24	0.661	0.7675	0.8323	0.597	0.71	0.815	0.968
11	Anthracnose	99	99	0	99	0	99	99	0	0	0	0	0	0	0	0	0	0
12	CMV	99	99	99	99	99	99	0	99	99	99	99	99	99	99	0	99	99

a) Format for filling phenotype information in fifth page

Plate 3.7 Example format for input file preparation for QTL mapping

Results

4. Results

The results of the study on "QTL mapping for yield traits in vegetable cowpea" which was carried out at the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agriculture University, Vellanikkara, Thrissur, are presented in this chapter.

4.1 Genotyping the mapping population

4.1.1 Isolation, purification and quantification of DNA

The genomic DNA of all the 92 plants in the mapping population and the parents, Kanakamony and Sharika were isolated. A modified new protocol designed based on the standard CTAB method (Doyle and Doyle, 1990) was used for the DNA isolation (Plate 4.1.a).

4.1.2 Quality checking using agarose gel electrophoresis

The quality and quantity of individual DNA samples were analysed through electrophoresis on 0.8% agarose gel. DNA samples have shown, intact, clear single high intensity band with no RNA or protein contamination (Plate 4.1.b).

4.1.3 Quality and quantity estimation using NanoDrop spectrophotometer

The results of the NanoDrop® spectrophotometer analysis had shown that all the DNA samples have a UV absorbance ratio ($A_{260}/_{280}$) in the range of 1.80 to 1.94. Based on the readings, the DNA samples were graded as excellent. The quantities of DNA in the isolated samples were in the range of 24.69 to 54.89 µg/100mg leaf sample.

4.1.4 Screening of microsatellite primer sets

A total of 100 SSR primer pairs were screened across the parents to check their ability to exhibit polymorphism. Thirty primer sets which showed distinct polymorphism among the parents were selected to genotype the entire mapping population. Polymorphism shown by few primer sets is presented in Plate 4.2.
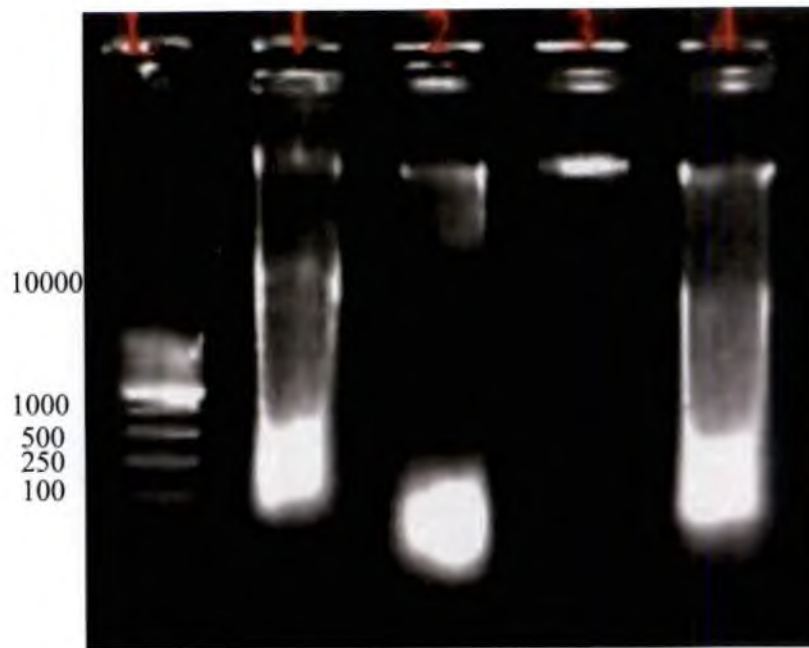
a) DNA isolation protocol standardisation

L : Ladder (1kb)

1: DNA sample from CTAB method (Doyle and Doyle, 1990)

2: DNA sample from modified CTAB method (RNase treatment during initial 65°C incubation)

3: DNA sample from protocol standardised for the current study (complete protocol detailed in materials and methods)

4: DNA sample from modified CTAB method (RNase treatment after completion of DNA extraction)
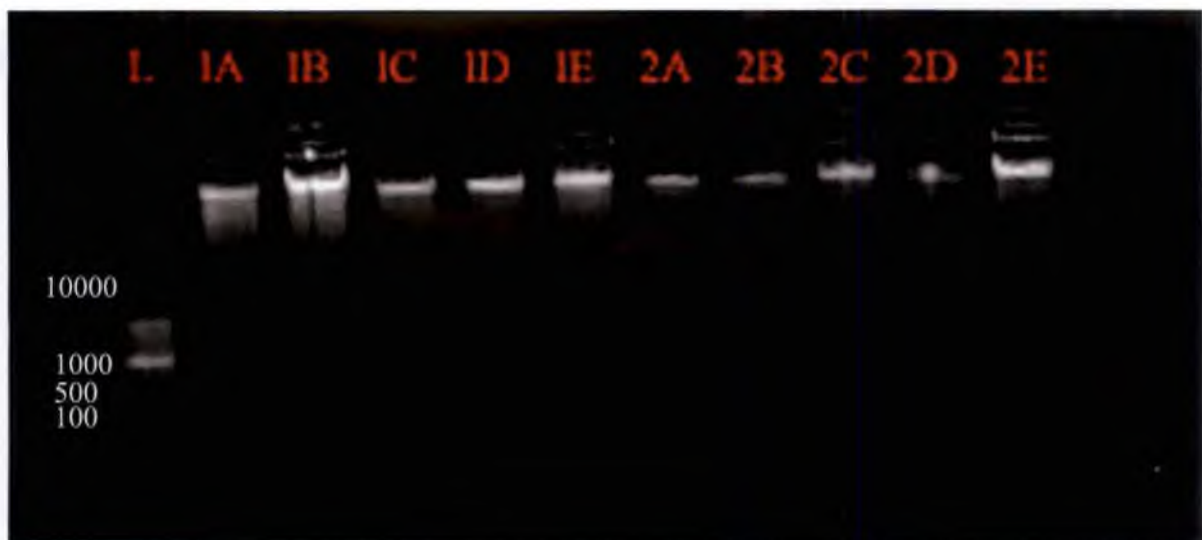
b) DNA isolation of mapping population

L: Ladder (100bp)

Progeny DNA samples: 1A, 1B, 1C, 1D, 1E, 2A, 2B, 2C, 2D, 2E

Plate 4.1 DNA extraction protocol standardisation and progeny DNA isolation
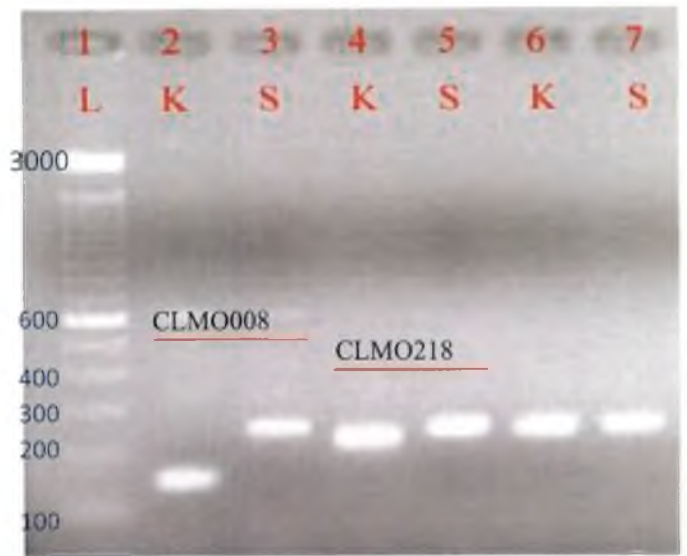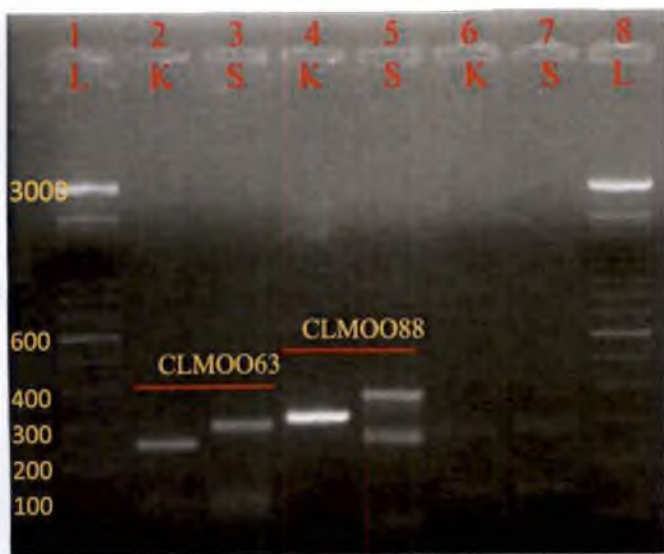
L: Ladder (100bp)

K: Kanakamony

S: Sharika

Polymorphic SSR primers: CLM0063, CLM0088, CLM0008, CLM0218, CLM0168, CLM0195, CLM0200, CLM0260, CLM0300

Plate 4.2 SSR markers showing polymorphism among parents

4.1.5 Genotyping the mapping population

All the 30 SSR markers which showed polymorphism among parents were screened individually with each of the 92 DNA samples of mapping population. The bands were scored according to the scoring pattern given in Appendix IV. Gel pictures of screening of few markers are given in (Plates 4.3 and 4.4). The scoring data of all the 30 markers are given in Appendix VI.

4.2 Morphological evaluation of F_4 mapping population for the traits under study

Data from all the plants of the mapping population and parents were collected for 12 traits under study. The complete data is given in Table 4.1.

4.3 Transformation of raw morphological data

Since the QTL analysis incorporates strong statistical tools, the raw morphological data was transformed into a normalized data using Box-Cox Transformation method and Best Linear Unbiased Predictors (BLUPs). Complete transformed data is given in Appendix VII.

The individual trait morphological data were analysed for distribution pattern among population using R software and the results showed significant difference between raw data and the Box-Cox transformed ones. Transformation power for each trait was estimated using R software (Plate 4.5.a). Images depicting the difference in data distribution after Box-Cox transformation for traits anthracnose resistance, cowpea mosaic virus, and pod length is given in Plate 4.5.b.

a) CLM0077 marker characterisation using mapping population

b) CLM0083 marker characterisation using mapping population

L: Ladder (100bp)

F_4 mapping population: 1A, 1B, 1C, 1D, 1E, 2A, 2B, 2C, 2D, 2E, 3A

K: Kanakamony (Scored as 2)

S: Sharika (Scored as 0)

Plant with alleles from both the parents scored as 1

Plate 4.3 Marker characterisation using mapping population
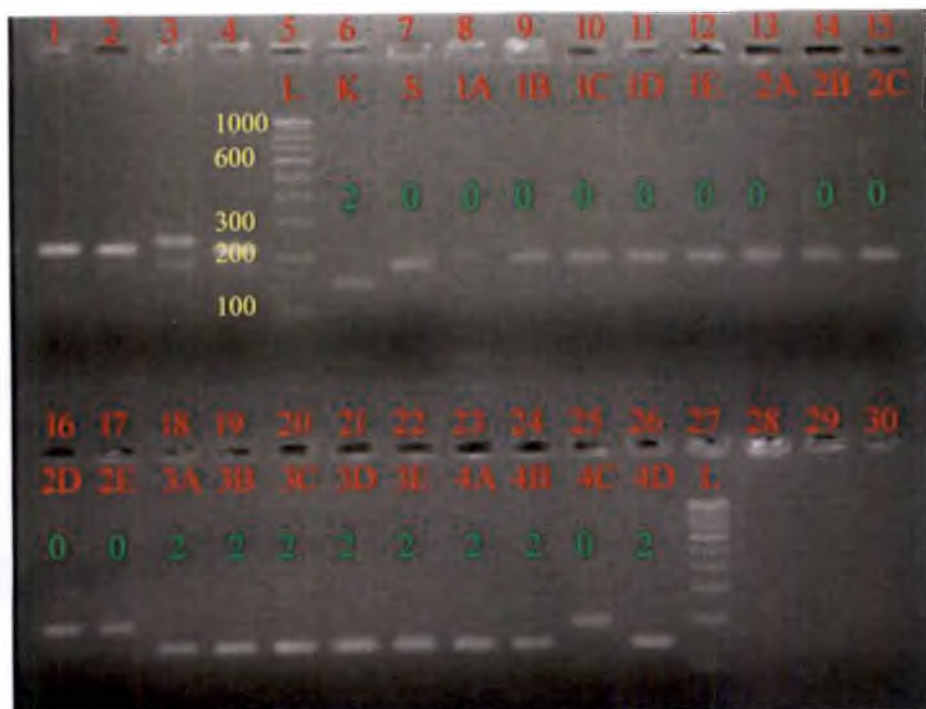
a) CLM007 marker characterisation using mapping population
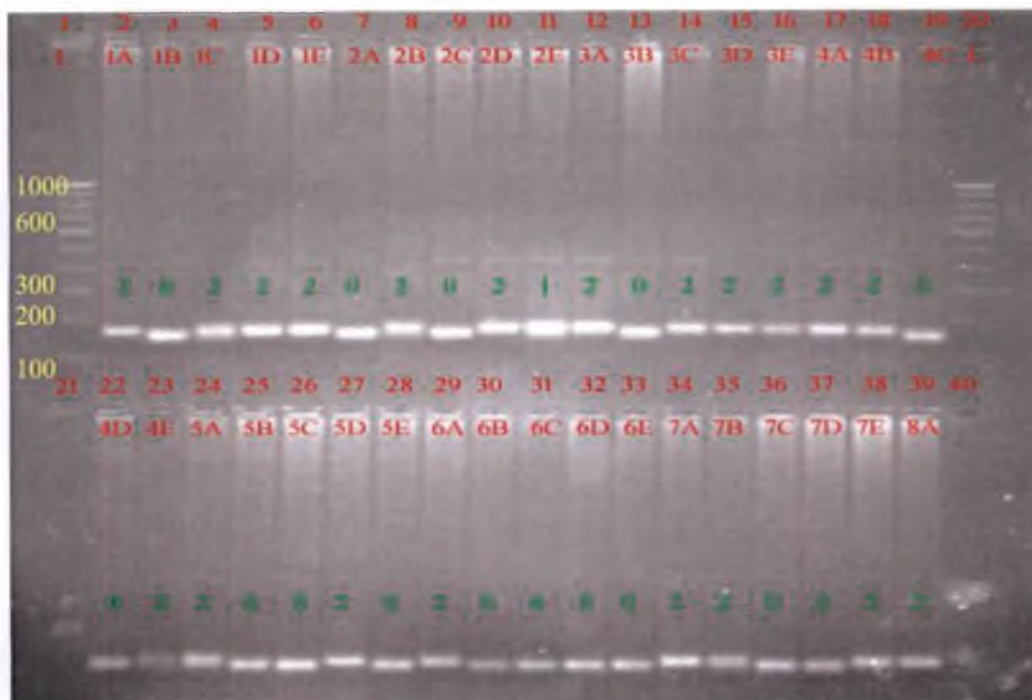
b) CLM0119 marker characterisation using mapping population

der (100bp)

ping population: 1A to 8A

nakamony (Scored as 2)

rika (Scored as 0)

with alleles from both the parents scored as 1

Plate 4.4 Marker characterisation using mapping population

Table 4.1 Morphological observations of mapping population for twelve traits considered

Plant Name	Morphological Traits											
	Pod length (cm)	Individual pod weight (g)	Pod number	DTFF	Total dry pod yield (g)	Grains per pod	No. of branches	Root length (cm)	Plant height (cm)	Plant weight (kg)	Anthracnose	CMV
1A	24.75	0.68	72.5	38	49.3	13.33	3.5	35	810	1.174	NO	NO
1B	21.99	1.16	74.5	38	86.42	13	5	39.5	550	1.1675	NO	NO
1C	26.8	1.67	40.5	32	67.635	16.66	5.5	43.5	480	0.7325	NO	NO
1D	22.74	1.21	31.5	32	38.115	12.88	5.5	46.5	610	1.223	NO	NO
1E	23	1.86	27	38	50.22	13.8	4.5	20	410	0.1095	YES	YES
2A	21.1	1.97	89.5	32	176.315	17.1	5.5	37	690	1.148	YES	YES
2B	21	2.21	51.5	32	113.985	12.33	5	34.5	630	1.294	NO	NO
2C	18.61	1.99	52	33	103.765	14.55	5.5	28	650	1.47	YES	YES
2D	17.94	1.86	32	33	59.73	14.66	4.5	29	615	0.9715	YES	YES
2E	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100
3A	20.33	1.53	29.5	38	45.23	9.33	4	57.5	560	1.591	YES	NO
3B	-100	-100	-100	38	-100	-100	4	44	510	0.87	YES	NO
3C	23	1.3	2.5	38	3.25	7	5	85.5	500	1.2275	YES	NO
3D	25	1.63	22	38	35.93	10.5	7	26.5	460	0.383	YES	YES
3E	32.15	0.86	23	38	19.93	15.2	6	38.5	810	1.0455	YES	NO
4A	24	2.54	43.5	38	110.66	15.1	6.5	58	500	1.069	YES	NO

4B	24.2	1.92	35.5	38	68.16	13	6	42	550	1.02	YES	NO
4C	20.68	0.87	14.5	38	12.685	11	8	32.5	360	0.86	YES	NO
4D	22.94	1.68	23	38	38.84	14.55	6	45.5	480	1.256	YES	NO
4E	22.15	1.69	37	38	62.53	12.4	5	43	430	1.492	YES	YES
5A	21.7	1.02	44	38	44.88	9.3	5.5	42	410	1.2445	YES	NO
5B	18.83	1.33	7	38	9.355	10.66	5	23	490	1.1635	NO	NO
5C	19.32	0.97	3.5	38	3.485	10.99	4	29	450	0.99	NO	NO
5D	21.65	0.89	38	38	33.88	13.03	6	46	380	0.79	YES	NO
5E	25.8	1.23	77.5	38	95.325	15	5	52	590	1.1505	YES	NO
6A	19.35	1.59	39	38	62.01	14.1	6.5	46.5	440	0.895	YES	YES
6B	19.92	0.7	30	38	21.08	12.57	8	59	580	0.65	YES	YES
6C	21.5	1.59	35	38	55.76	14.83	7	129	760	2.31	NO	NO
6D	21.55	1.62	26.5	38	42.93	15.3	9.5	93	420	2.2625	NO	NO
7A	18.8	1.87	41	34	76.75	11.6	11	54	380	0.735	YES	NO
7B	20.9	1.26	12	38	15.12	12.6	8.5	42.5	420	0.5365	YES	NO
7C	22.05	1.62	56	35	90.9	14.44	7	38	360	0.303	YES	NO
7D	23.5	1.35	17	36	22.95	14.33	6.5	51.5	630	0.8775	YES	YES
7E	19.93	1.91	33	38	63.15	12.87	5.5	42	580	1.478	YES	NO
8A	20.15	1.61	16.5	38	26.63	15.2	4.5	35.5	440	0.1755	NO	NO
8B	18.55	1.64	14	38	23.04	11.8	4.5	59.5	550	1.3835	YES	NO
8C	19.35	1.34	23	38	30.82	10.1	7.5	40	560	0.972	NO	YES

8D	19	1.04	28	38	29.33	14	7.5	58	550	0.542	YES	YES
8E	25.5	1.73	8	38	13.86	9.66	7	65	620	1.184	YES	NO
9A	20.5	2.18	51.5	38	112.27	14.2	5	64	510	0.755	YES	YES
9B	23.85	2.64	53	38	139.92	16.6	5	63	480	0.5655	YES	YES
9C	22.75	0.85	44.5	38	37.825	13.7	6	34	420	0.5995	YES	YES
9D	18.5	1.57	53.5	38	83.995	12.1	5	61	370	0.737	YES	YES
9E	19.8	1.51	75	38	113.25	11.2	5	51	470	1.599	YES	NO
10A	26	2.01	56.5	38	113.565	16.8	4	48	510	0.906	NO	NO
10B	18.4	1.96	32	38	62.72	10.7	6	54	570	1.1585	NO	NO
10C	23.7	2.36	50.5	38	119.38	12.2	4	47	520	0.873	YES	NO
10D	23.9	2.39	46.5	38	111.135	12.1	6	60	610	1.205	NO	NO
10E	23.5	1.82	58.5	38	106.47	12.6	5	59	590	1.1945	YES	NO
11A	17.5	1.35	7	38	9.485	11.83	7.5	47.5	340	0.8175	NO	NO
11B	18.27	1.4	26.5	38	37.345	8.55	8	43	370	0.6455	NO	YES
11C	27.3	2.08	31.5	32	65.52	17.2	4.5	64	570	1.0165	YES	NO
11D	21.02	1.61	73	38	117.53	12.52	10	110	770	2.487	YES	NO
11E	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100
12A	16.07	0.95	23	32	22.01	8.6	5.5	56	450	1.24	YES	NO
12B	16.45	1.56	51	32	79.56	13.6	4	36.5	420	0.661	YES	NO
12C	17.45	2.31	43	32	99.33	16.9	6	35	430	0.7675	YES	NO
12D	16.8	2.32	47	32	109.04	15.5	5	38.5	510	0.8325	YES	NO

12E	19.38	2.6	56.5	32	146.9	18.55	5.5	30.5	580	0.597	YES	NO
15A	22.16	1.32	18.5	33	24.42	14.5	6	78.5	410	0.71	YES	YES
15B	24.75	1.2	21.5	33	25.8	13.16	6	70.5	600	0.815	YES	NO
15C	29.25	2.26	25.5	35	57.63	16.4	4	52.5	540	0.966	YES	NO
15D	19.08	1.26	11.5	38	14.565	10.66	4	43.5	450	0.69	YES	NO
15E	16.5	1.08	8	36	8.715	9.6	6	56.5	460	1.31	YES	YES
16A	-100	-100	-100	38	-100	-100	7.5	45.5	300	0.35	YES	NO
16B	20.7	1.23	39	38	48.085	15.5	5	64.5	480	0.705	YES	NO
16C	18.5	1.09	30	38	32.97	12.25	6	62	395	1.756	YES	YES
16D	14	0.82	13	38	10.72	9	3	42	475	1.23	YES	NO
16E	19	1.11	16	38	17.8	9.66	6	58	420	1.432	YES	NO
17A	25.1	2.02	26.5	38	53.53	14.7	5.5	30.5	410	0.65	YES	NO
17B	21.2	1.18	32	38	37.76	13.21	7	38	430	0.85	YES	NO
17C	22	1.31	28	38	36.68	14.2	8	38	420	1.62	NO	NO
17D	20.33	1.34	39	37	52.26	11.6	8	72.5	440	1.235	NO	NO
17E	27	2.11	25.5	38	53.805	15.4	5.5	51	485	1.35	YES	NO
18A	17.6	2.46	54.5	32	134.07	16.7	4	30.5	425	0.6	YES	NO
18B	17.31	2.07	56	35	116.07	16.45	6	29	318	0.75	YES	NO
18C	15.5	1.25	45	38	56.68	13.66	4	29	310	0.7	YES	YES
18D	16.2	2.36	29	32	68.44	15.3	4.5	35.5	410	0.6	YES	YES
19A	19	1.04	11	38	11.53	13	2	21	220	2.05	YES	NO

19B	26.75	1.25	2	38	2.51	16	6	28	150	0.274	YES	NO
19C	26.21	1.7	28	32	47.6	16.14	6	28	430	0.23	YES	NO
19D	19.2	0.93	9	38	8.37	11.2	5	45	370	0.62	YES	NO
20A	20.21	1.14	48	38	54.72	12.28	5.5	34.5	490	1.1805	YES	NO
20B	24.14	2	36	38	72	14.42	3	31	350	1.23	YES	NO
20C	18.5	0.91	16	38	14.56	11	8.5	48.22	610	1.51	YES	NO
20D	24.37	1.97	26.5	38	52.205	15.5	5.5	36.5	520	0.45	YES	NO
20E	18.95	1.56	27	38	42.12	10.7	10	48	450	1.97	YES	NO
21A	20.02	2.07	43.5	32	90.045	12.7	6	37	450	0.835	NO	NO
21B	19.8	2.12	17	32	36.04	15.3	6.5	63.5	420	1.09	NO	NO
21C	22.45	2.5	21	33	52.5	17.8	5	33.5	480	0.925	NO	NO
21D	19.45	2	17.5	32	35	14	4.5	31	490	1.01	NO	NO
21E	19.2	2.12	14.5	33	30.74	13	5	34	490	0.965	NO	NO
k	15.8	0.76	44	38	33.44	12.8	4	36	330	1.12	NO	NO
S	31.12	3.04	28	32	85.12	13.54	4	63	400	0.75	YES	YES
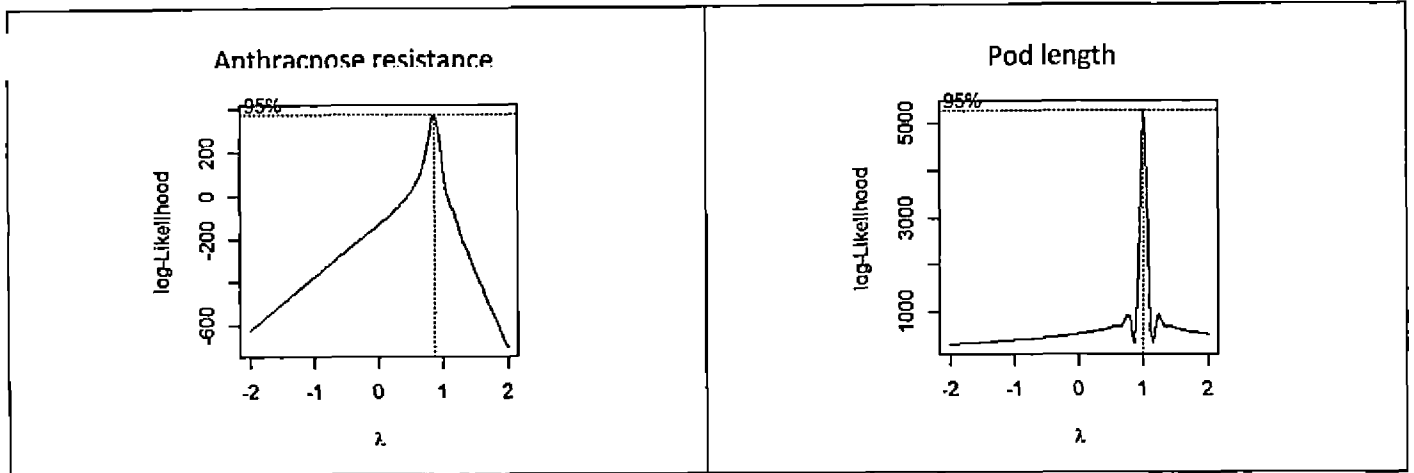
a) Estimation of transformation power in Box-Cox transformation for the traits anthracnose resistance and pod length
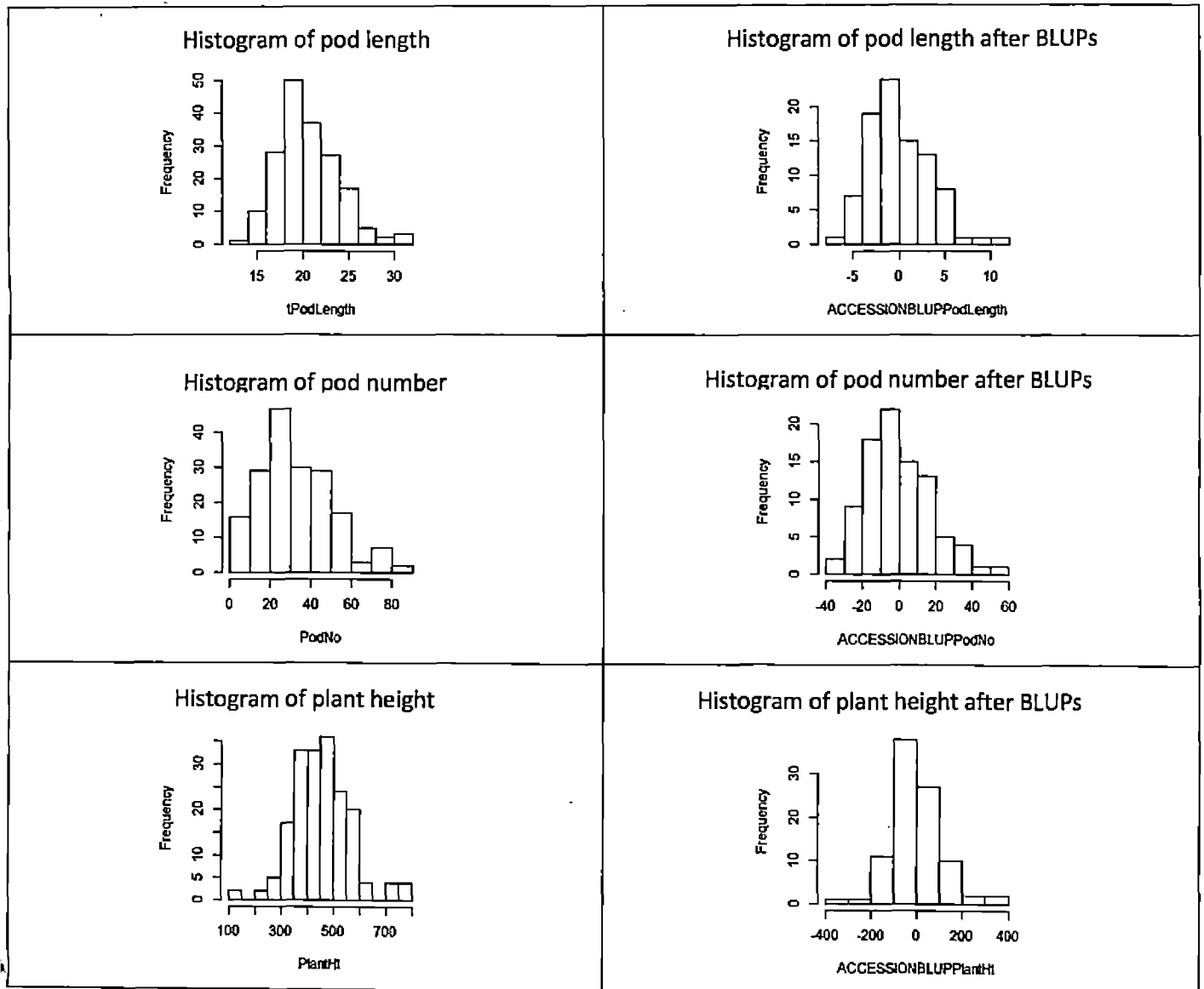
b) Distribution of traits in the population before and after the generation of BLUPs

Plate 4.5 Difference in data distribution before and after transformation

4.4 Linkage map construction

Linkage mapping has generated two linkage groups. Linkage group 1 had eight markers distributed across its length of 637 cM and Linkage group 2 had five markers distributed along a 271 cM length. Remaining 17 SSR markers were distributed across 17 groups (group 3 to 19) with each group harbouring one marker. Hence a linkage map on groups 1 and 2 with a total length of 908 cM was obtained (Plate 4.6).

Linkage group 1 showed eight linked SSR markers, namely CLM0186, CLM0244, CLM0008, CLM0177, CLM0279, CLM0322, CLM0168, and CLM0195. Linkage group 2 showed five linked SSR markers namely CLM0200, CLM0088, CLM0260, CLM0218, CLM0077. Plate 4.7 shows all the 13 markers and their respective significance towards each of the 12 traits under study.

4.5 QTL mapping

Since linkage was observed in two groups, 13 markers distributed across these two groups were used for additive linkage mapping. Simultaneously, all the 30 markers were analysed by single marker analysis method. The results of QTL mapping are as follows

4.5.1 Pod length

For trait pod length, no significant QTL hotspots were found on Linkage group 1. Even though there appears to be a hotspot in between markers CLM0088 and CLM0260 on Linkage group 2, because of the lower LOD values, the hotspot is not significant.

4.5.2 Individual pod weight

Additive linkage mapping showed no significant QTL hotspots on both the linkage groups. However, single marker analysis shows marker CLM0083 significantly associated with trait individual pod weight.
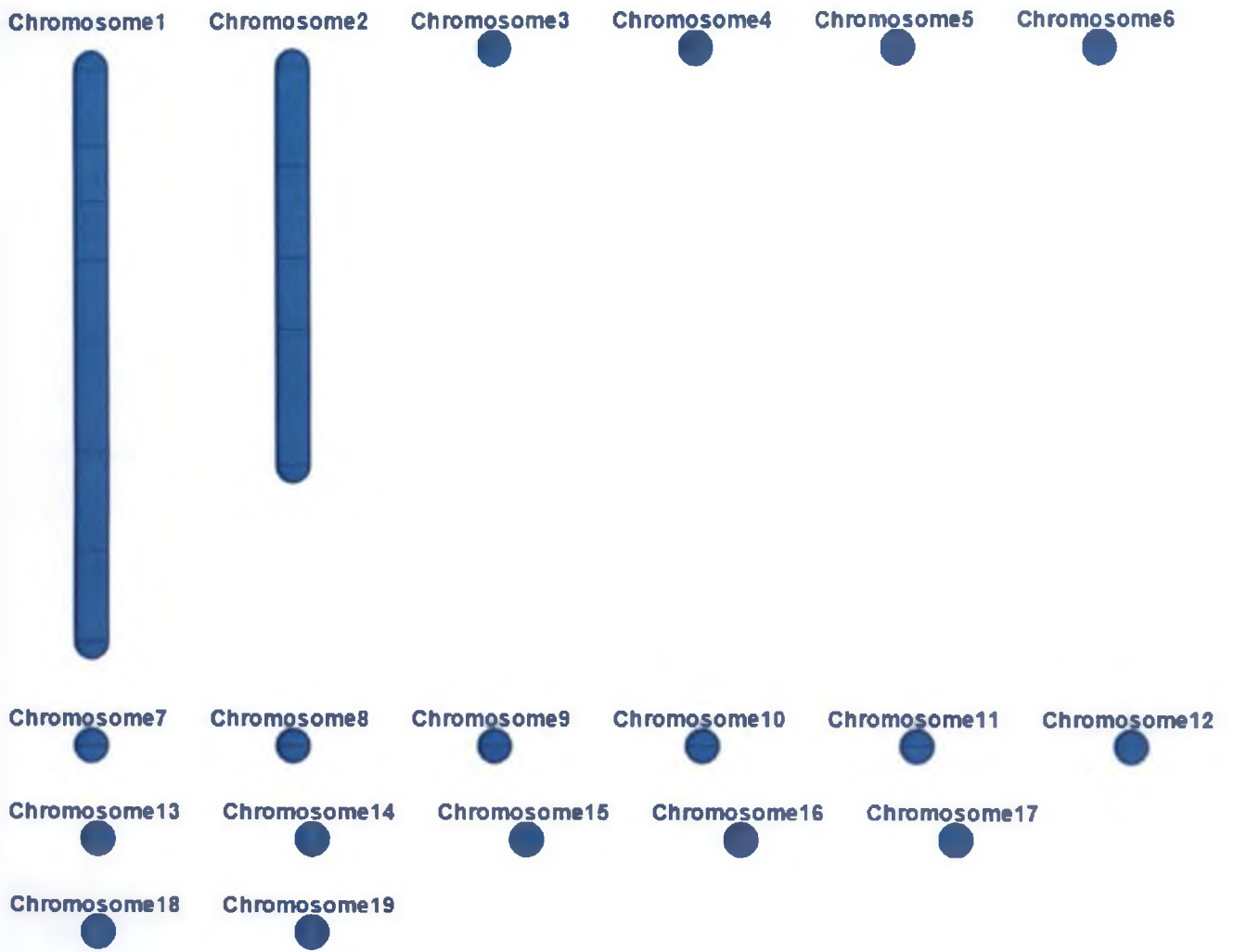
Plate 4.6 Linkage map with linkage groups
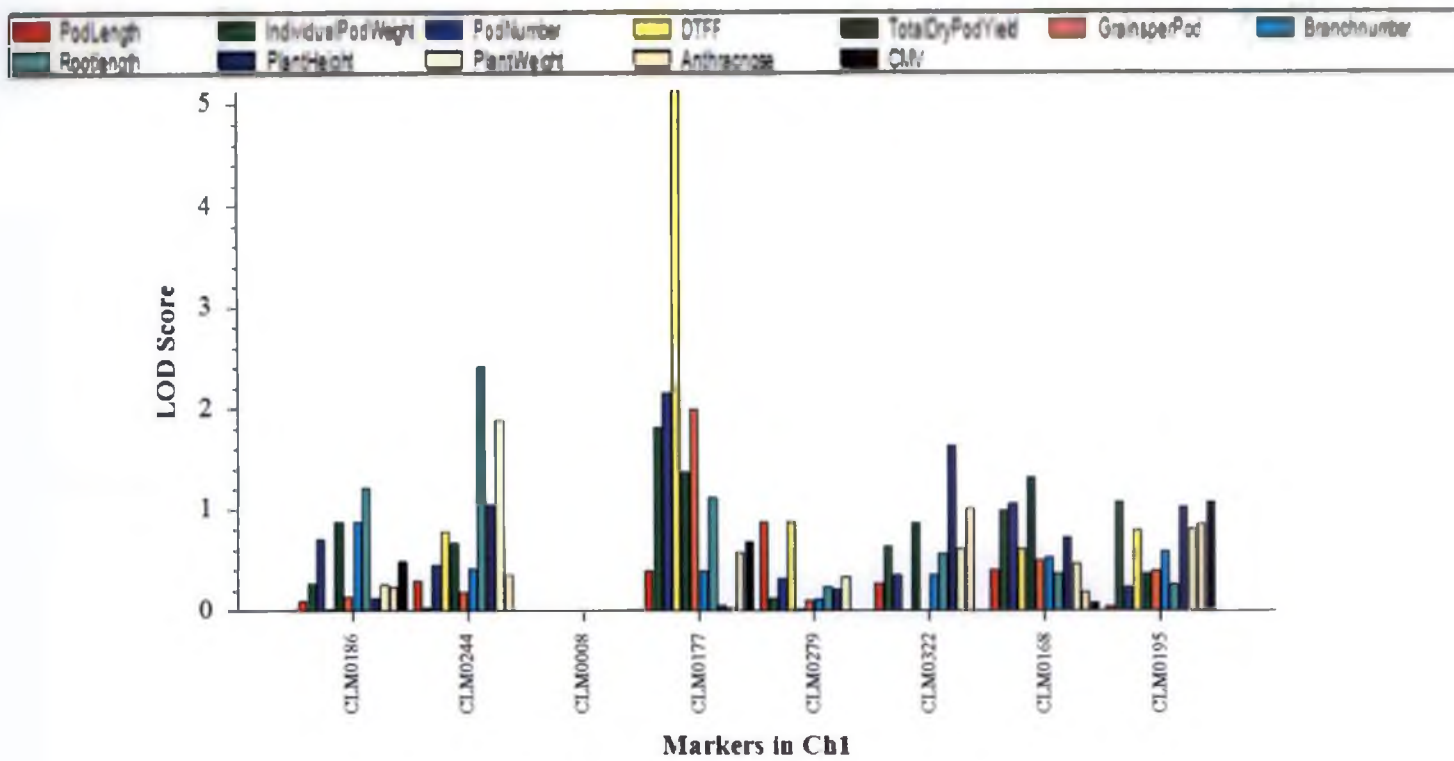
a) Linkage of markers on linkage group 1 with the traits under study
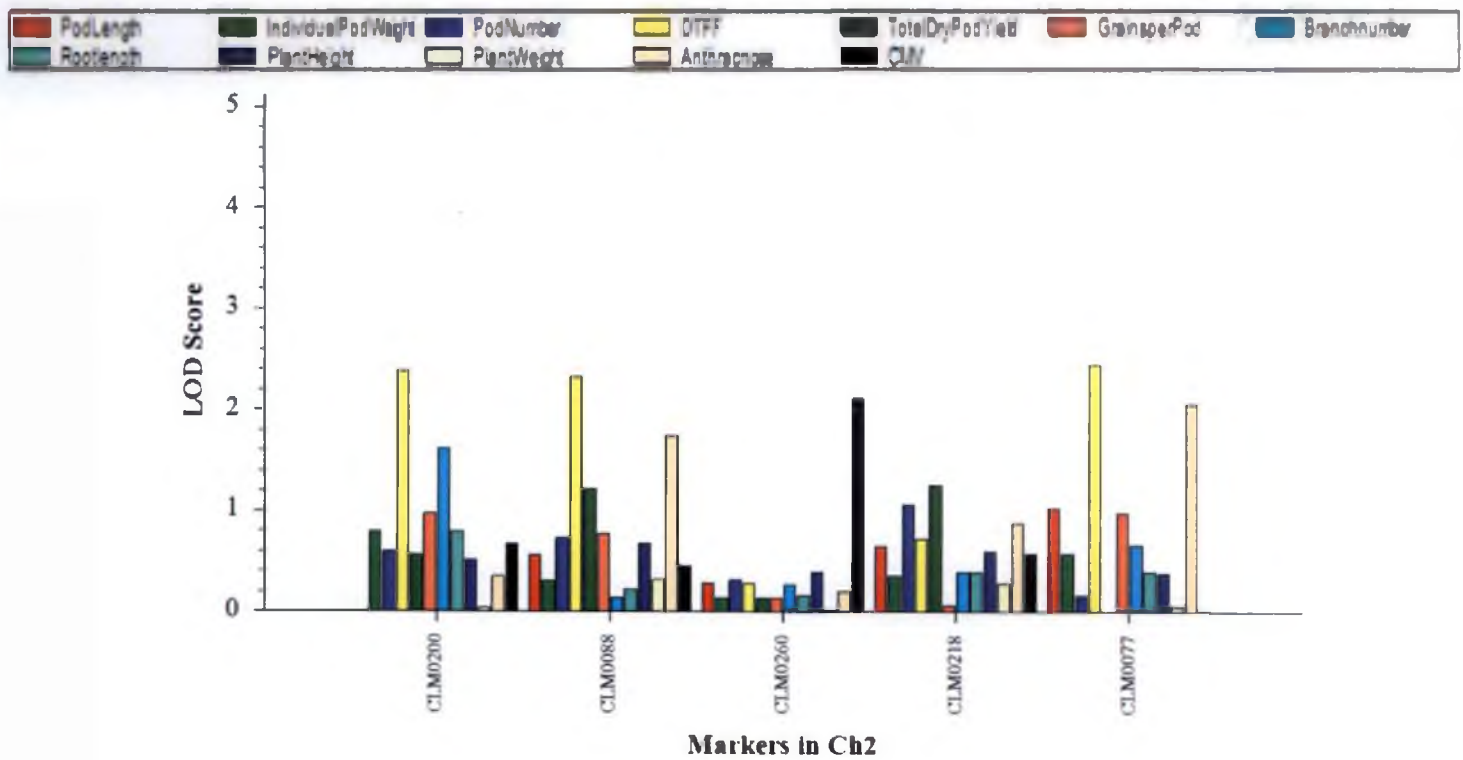
b) Linkage of markers on linkage group 2 with traits under study

Plate 4.7 Significances of linkage for thirteen linked markers with respect to traits

4.5.3 Pod number

No significant QTL hotspots were found for pod number. However, a nearly significant hotspot is found on linkage group 1 between the markers CLM0008 and CLM0177. Since the LOD value is less than 3 and single marker analysis also did not show any significant association of markers CLM0008 and CLM0177 to any of the traits under study, no hotspots were identified.

4.5.4 Days taken for flowering

For trait days taken for first flowering, multiple QTL hotspots were observed (Plate 4.8.a). Region between markers CLM0008 and CLM0278 on linkage group 1 had two significant hotspots with LOD value of 15. CLM0177, an anchored marker for this trait was also found. Single marker analysis also showed that marker CLM0177 is tightly linked with the trait (Plate 4.8.b). CLM0300 on group 19 was also found to have significant linkage with this trait (Plate 4.8.b) Hotspots were also found between markers CLM0278 and CLM0322, between CLM0322 and CLM0168 and between CLM0168 and CLM0195 on linkage group 1. On linkage group 2, hotspots were found between markers CLM088 and CLM0260, between CLM0218 and CLM0077, and beyond CLM0077 (Plate 4.8.a).

4.5.5 Total dry pod yield

A nearly significant QTL hotspot exists between markers CLM0008 and CLM0177 on linkage group 1 (Plate 4.9.a). But single marker analysis showed mere association with the trait (Plate 4.9.b)

.4.5.6 Grains per pod

No significant hotspots were observed for this trait on both the chromosomes.

4.5.7 Branch number

A significant QTL hotspot was observed for this trait on linkage group 2 between markers CLM0200 and CLM0088 (Plate 4.10.a). Single marker analysis also showed good significance for marker CLM0200 (Plate 4.10.b).
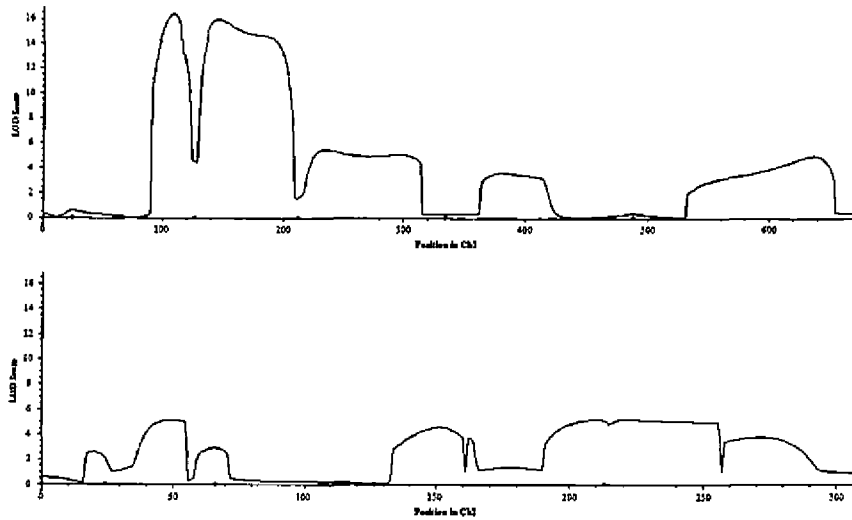
a) QTL hotspots after additive linkage analysis
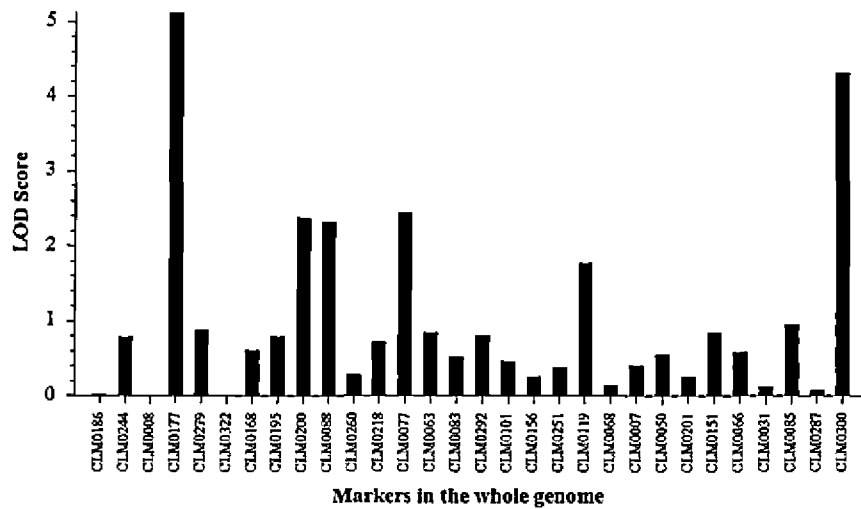
b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.8 QTL mapping Results for Days Taken For Flowering

a) QTL hotspots after additive linkage analysis

b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.9 QTL mapping Results for Total Dry Pod Yield

a) QTL hotspots after additive linkage analysis

b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.10 QTL mapping Results for Branch number
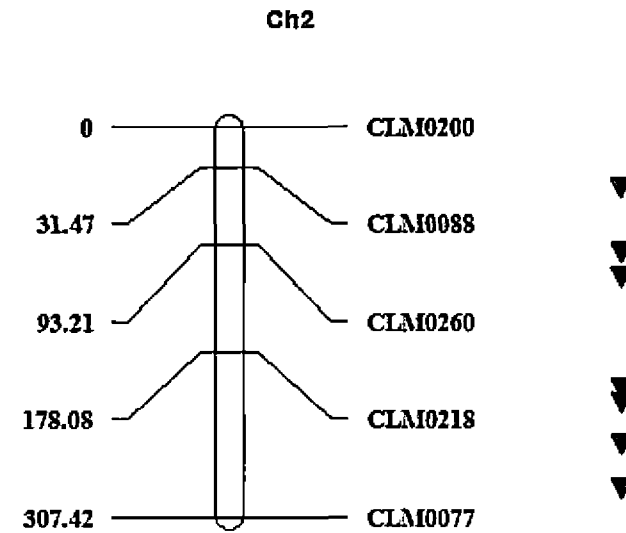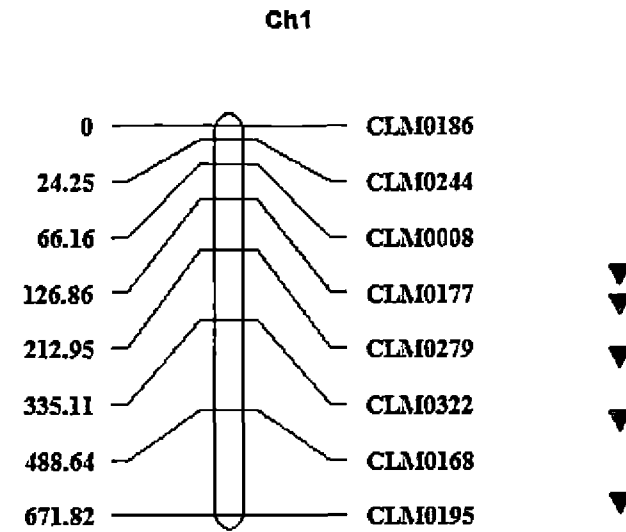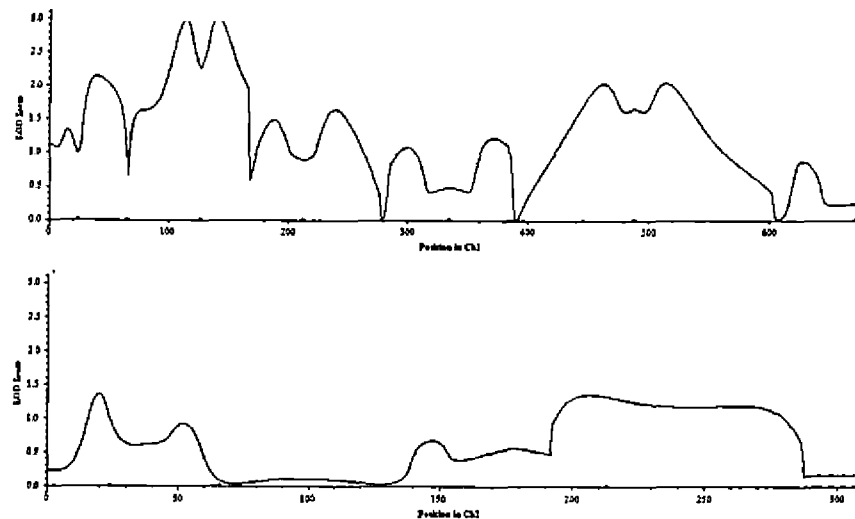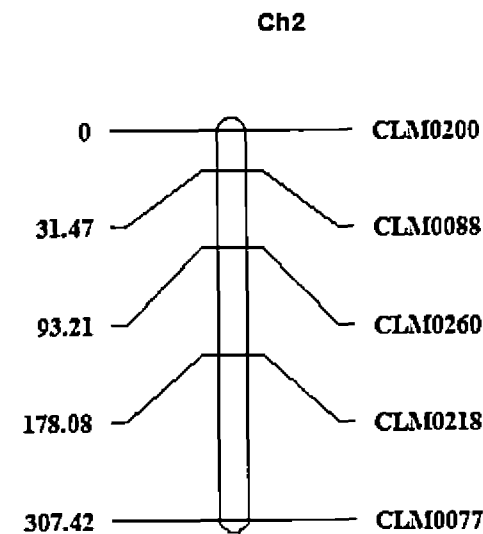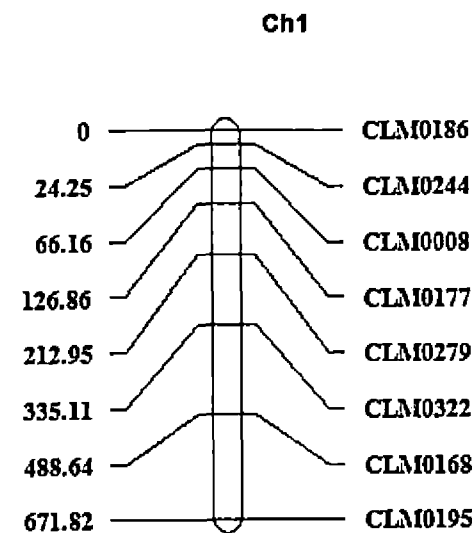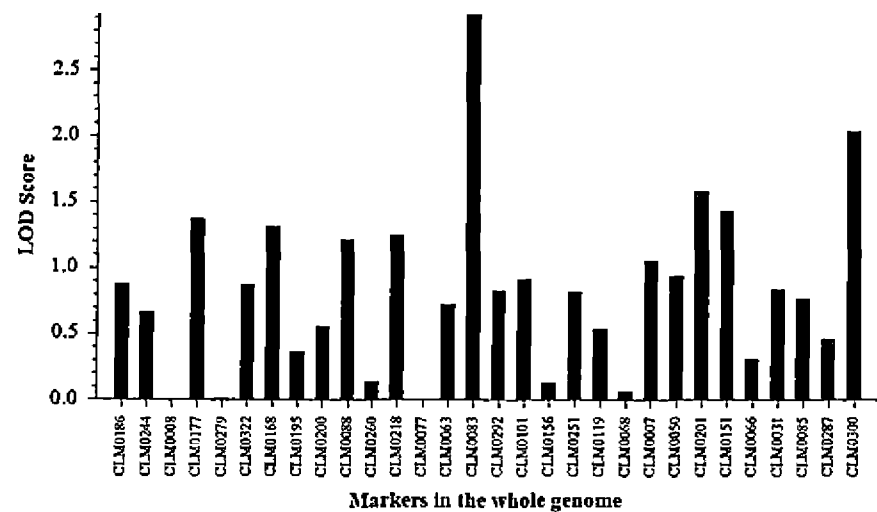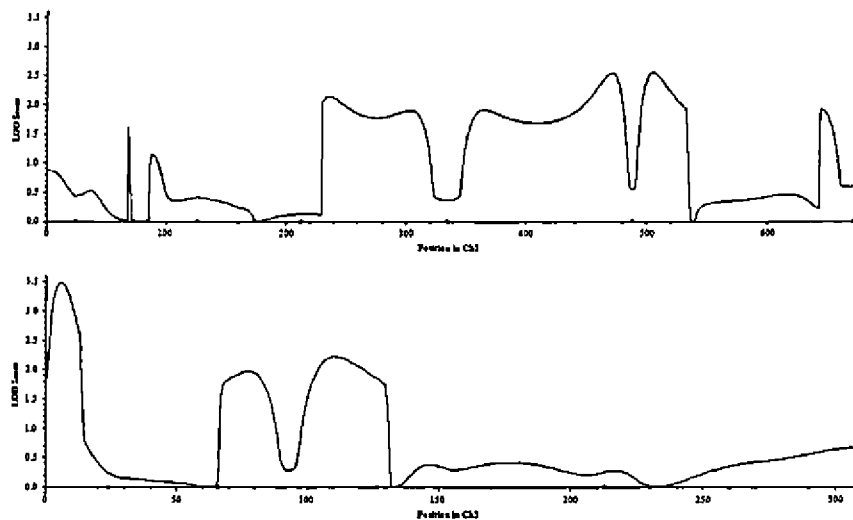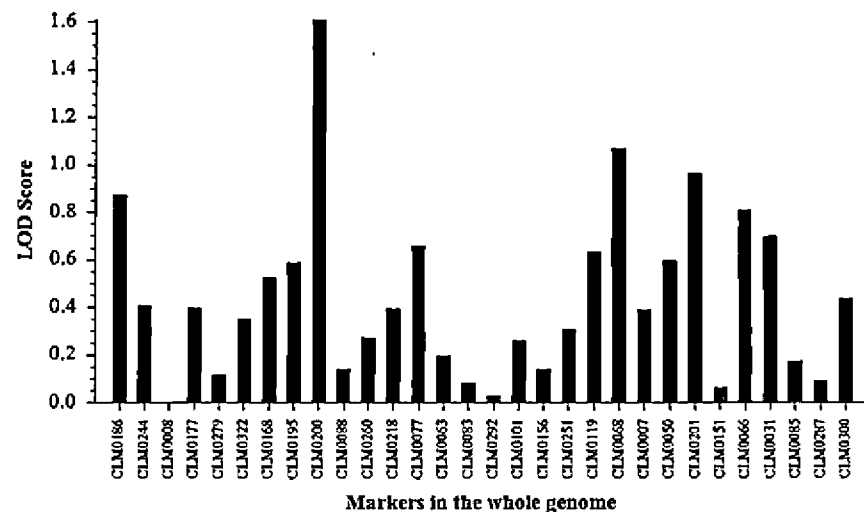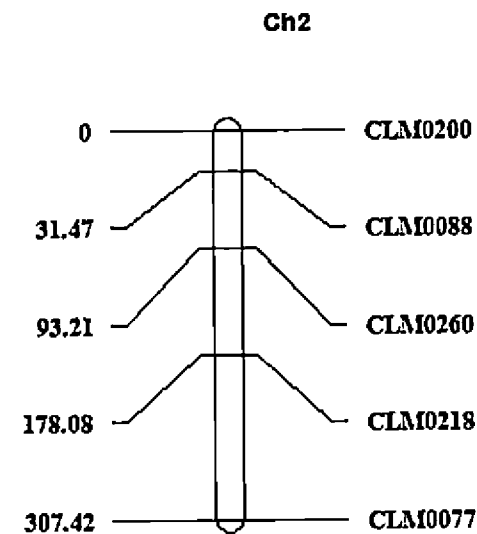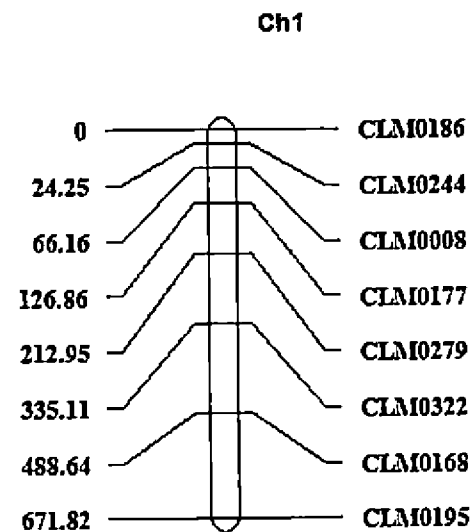
4.5.8 Root length

A significant hotspot lies between markers CLM0244 and CLM0008 on linkage group 1 (Plate 4.11.a). Single marker analysis also showed good linkage of CLM0244 with this trait. On linkage group 2, two hotspots exist between markers CLM0200 and CLM0088 and between markers CLM0260 and CLM0218. However single marker analysis showed no significant association of these markers with this trait (Plate 4.11.b).

4.5.9 Plant height

A significant hotspot exists between markers CLM0008 and CLM0177 on linkage group 1 for the trait (Plate 4.12.a). But the single marker analysis had shown no significant association of markers to the trait (Plate 4.12.b).

4.5.10 Plant weight

A QTL hotspot with an anchored marker CLM0244 and two flanking markers, CLM0186 to the left and CLM0008 to the right was observed for the trait (Plate 4.13.a). Single marker analysis also showed near significant association of marker CLM0244 with the trait (Plate 4.13.b).

4.5.11 Anthracnose resistance

Since the disease resistance was scored as only highly resistance and highly susceptible like for a dominant trait, results from additive linkage map which gave too many hotspots were not considered. Single marker analysis also did not show any significant marker association for the trait.

4.5.12 Cowpea Mosaic Virus resistance

Since the disease resistance was scored as only highly resistance and highly susceptible like for a dominant trait, results from additive linkage map which gave too many hotspots were not considered. Single marker analysis also did not show any significant marker association for the trait

a) QTL hotspots after additive linkage analysis
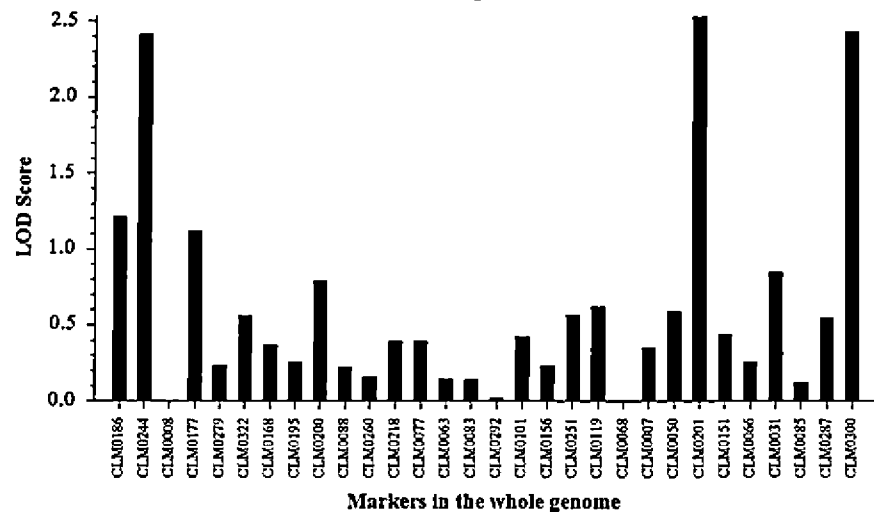
b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.11 QTL mapping Results for Root length

Ch1

CLM0186 — 0
CLM0244 — 24.25
CLM0008 — 66.16
CLM0177 — 126.86
CLM0279 — 212.95
CLM0322 — 335.11
CLM0168 — 488.64
CLM0195 — 671.82

a) QTL hotspots after additive linkage analysis

Ch2

CLM0200 — 0
CLM0088 — 31.47
CLM0260 — 93.21
CLM0218 — 178.08
CLM0077 — 307.42

b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.12 QTL mapping Results for Plant Height

a) QTL hotspots after additive linkage analysis

Ch1

0	CLM0186
24.25	CLM0244
66.16	CLM0008
126.86	CLM0177
212.95	CLM0279
335.11	CLM0322
488.64	CLM0168
671.82	CLM0195

Ch2

0	CLM0200
31.47	CLM0088
93.21	CLM0260
178.08	CLM0218
307.42	CLM0077

b) Marker significances after Single Marker Analysis

c) QTL map on two linkage groups

Plate 4.13 QTL mapping Results for Plant Weight
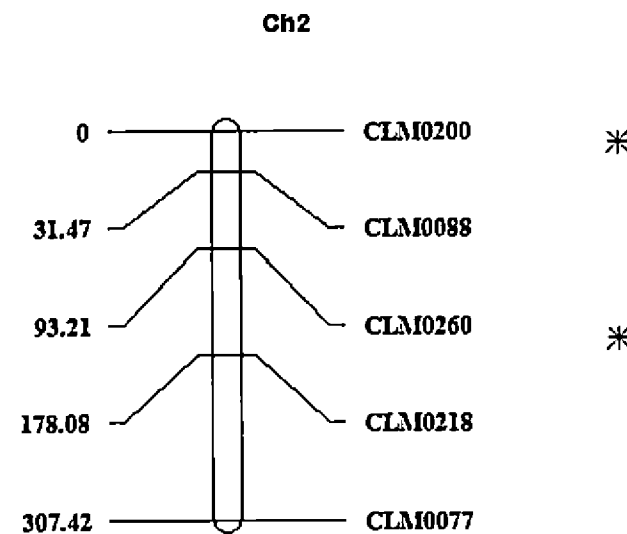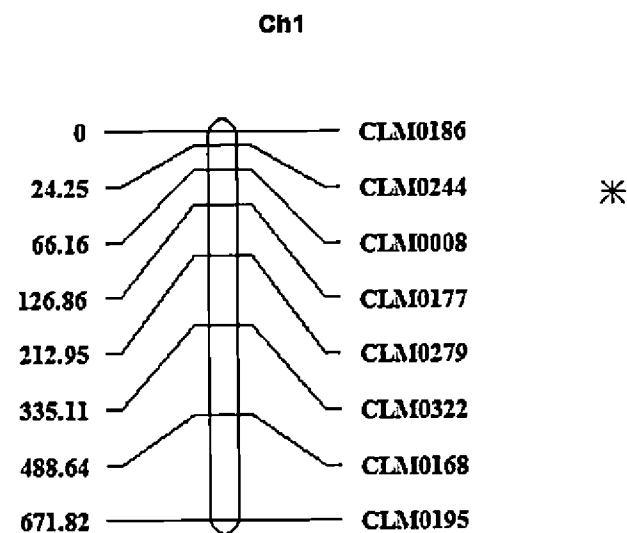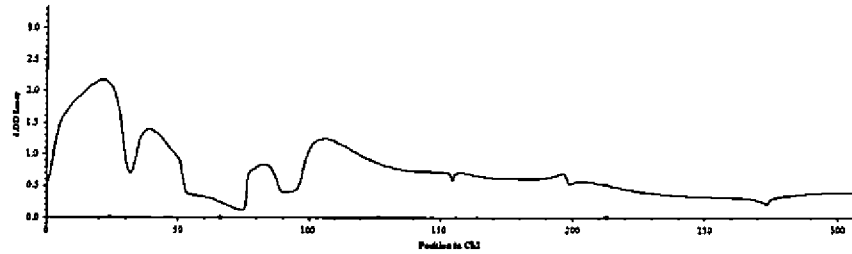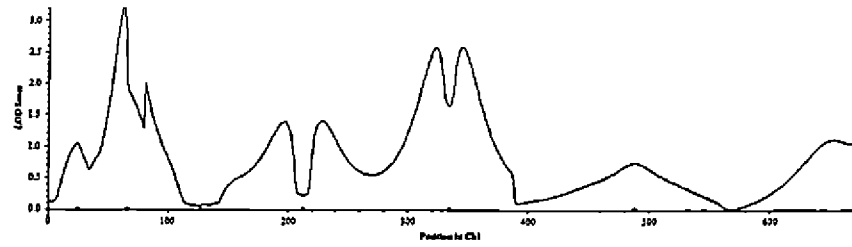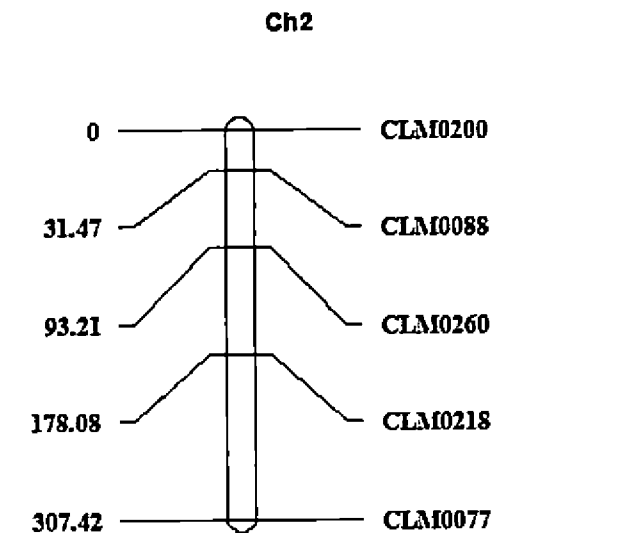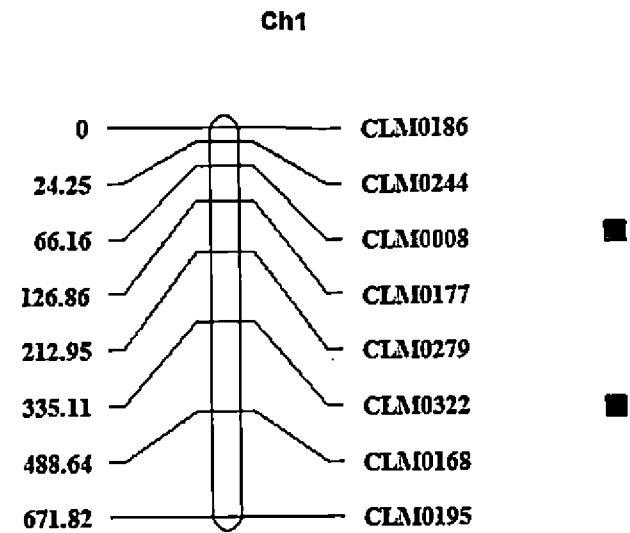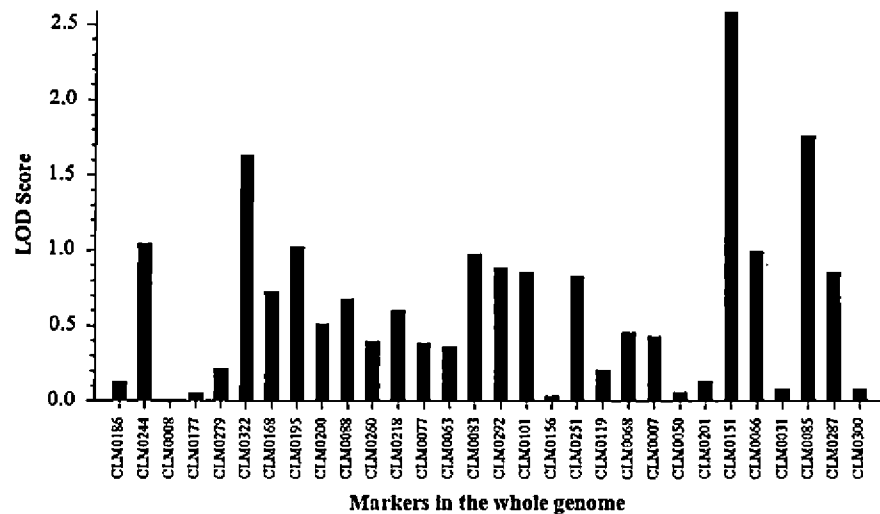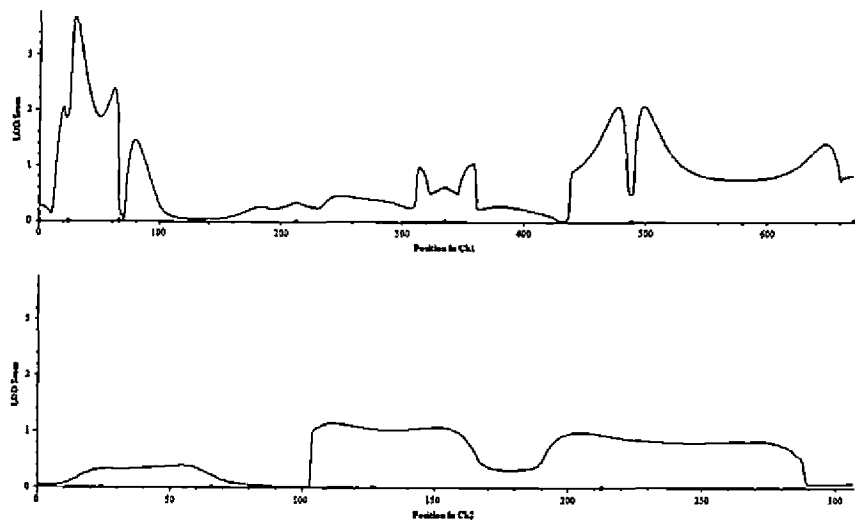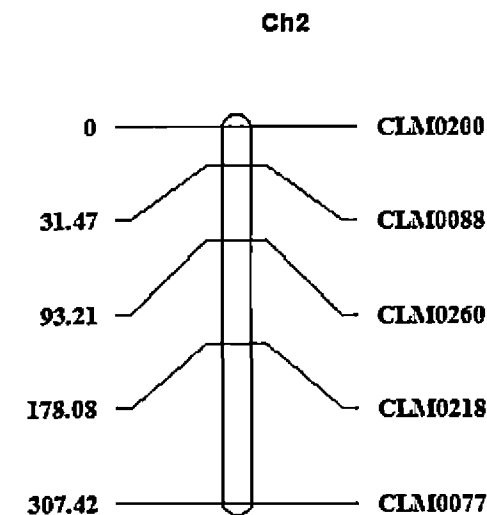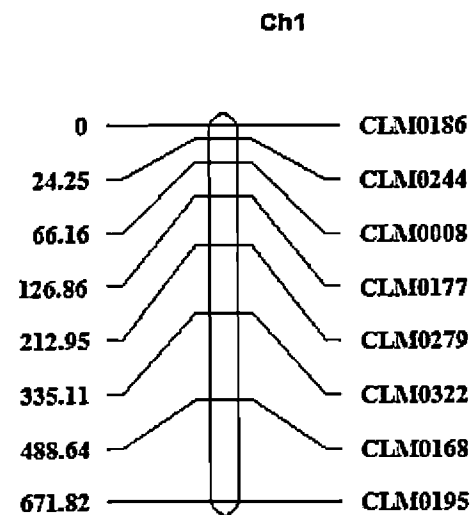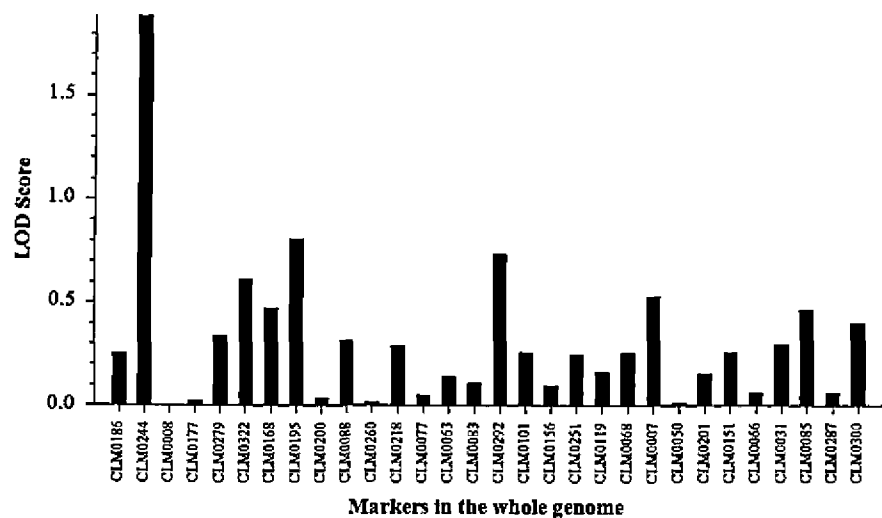
Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* L. Walp.) with a chromosome number of (2n = 2x = 22) is one of the most important pulse crop grown in India in the tropics and subtropic zones as a warm season kharif crop. The estimated area under cultivation in India is around 50,000 hectares which occupies a substantial area of cultivation in dry regions (Vidhi, 2016). Its good cooking qualities and fresh succulent pods make it a best option for vegetable purpose in Kerala (The Hindu, 2001). As it is a rich source of protein, vitamins and minerals and consumer acceptability, breeding for higher yield in cowpea remains one of the important scientific problems to be addressed.

But the main constraint in breeding for increased yield for cowpea comes in the form of nature of traits influencing the yield. Now it is understood that yield is not a single dominant character but the additive effect of multiple factors contributing a small part towards the objective (Mackay *et al.*, 2009). So, yield, a quantitative trait governed by polygenes has always been difficult to understand as the phenotypic values observed in field and the genotype were never directly correlated because of multiple factors like genotype environment interaction, pleiotrophy and epistasis (Mackay, 2001). Molecular markers played a major role in addressing this problem by helping scientists with indirect selection of plants wherein the components related to genes contributing to the yield were selected (Board *et al.*, 1997).

SSR markers played a huge role in breeding for increased yield in cowpea. Because of their specificity, ease of handling and codominant nature, these microsatellite markers became an indispensable part of genetic studies in cowpea (Wang *et al.*, 2004; Gupta and Gopalakrishna, 2010; Andargie *et al.*, 2011; Kongjaimun *et al.*, 2012).

However the earlier strategy used for identifying markers linked to a particular trait, Bulked Segregant Analysis (BSA) was found to be of no much use when selecting yield and related traits. Because BSA was proven a perfect strategy for identification of markers tightly linked to a trait, only when the trait was governed by a single gene (Wang and Paterson, 1994). However QTL mapping soon became the remedy for this problem. QTL mapping has been successfully employed in many studies to map the genes contributing to the yield (Kelly *et al.*, 2003; Blair *et al.*, 2006; Shi *et al.*, 2009; Ding *et al.*, 2012). Thus the combination of microsatellite markers and QTL mapping proved an indubitable solution to mapping genes governing quantitative traits in crop plants rendering their use in marker assisted selection

(MAS) for yield improvement (Brondani *et al.* , 2002; Prasad *et al.*, 2003; Song *et al.*, 2004; Zhang *et al.*, 2004; Shen *et al.*, 2005; Andargie *et al.*, 2013).

Two popular vegetable cowpea varieties, Sharika and Kanakamony cultivated widely in Kerala were used as parents for developing the mapping population. Sharika is a pole type variety well known among the farmers for its long, firm, attractive pods but the susceptibility of this variety to anthracnose often led to huge crop losses among farmers. On the other hand, Kanakamony, which is a semi-trailing dual purpose type was reported to be immune to anthracnose disease (Kumar, 1999). Hence the idea behind selecting the two varieties as parents was to get a progeny which has the attractive pod characters from Sharika and the immunity towards anthracnose from parent Kanakamony (Mathew, 2016). As yield and related traits are governed by polygenes, QTL mapping was decided as the strategy to identify the genes responsible for yield variability. In an attempt to address the lack of knowledge regarding the genes governing yield and related traits in cowpea, present study of "QTL mapping for yield traits in vegetable cowpea" was carried out at CPBMB, college of horticulture, KAU, Vellanikkara.

The methodology involved was, Linkage map construction and QTL mapping through Single Marker Analysis (SMA) and Additive QTL mapping. The work has involved the characterization of SSR primers for their capability to generate polymorphism, screening of mapping population under field conditions for yield traits, screening the DNA from all members in the mapping population and parents using the polymorphic SSR markers and analysis on population to develop the QTL maps for yield related traits

The results obtained for various aspects mentioned above are discussed here.

5.1 Characterization of SSR primers for their capability to generate polymorphism.

5.1.1 Genomic DNA isolation of the two parents

Initially standard CTAB protocol given by Doyle and Doyle was used for DNA extraction. But RNA and phenolic contamination was the major hurdle in getting good quality DNA (Jobes *et al.*, 1995; Pradhan, 2015). Standard DNA purification methodologies were ruled out because of two reasons. First problem was, most of the purification protocols consumed extra time and chemicals (PGRU, 2004; JGI, 2013; POL, 2011) which was not feasible as DNA of a large number of samples had to be isolated. The second problem was, after RNase treatment, even though most of the RNA contamination was removed, the DNA yield was drastically reduced. Hence a new protocol was standardized based on the standard CTAB DNA isolation protocol

and RNase treatment protocol. When the RNase treatment was incorporated into the DNA isolation process, it drastically reduced the time required for complete high quality genomic DNA extraction process. And giving a Chloroform:isoamylalcohol (24:1) wash soon after the completion of RNase treatment led to washing away of all the RNase enzyme present in the solution thereby reducing multiple chemical washes which most of the DNA purification protocols use. This gave DNA with good quantity and excellent quality without any contaminations.

5.1.2 Characterization of SSR primers among parents

The development and use of SSR primers is outlined in many of the legumes such as mung bean (Gwang *et al.*, 2006; Somta *et al.*, 2008), black gram (Gupta and Gopalakrishna, 2009; Gupta *et al.*, 2013), azuki bean (Han *et al.*, 2005), chickpea (Choudhary *et al.*, 2009; Datta *et al.*, 2010), groundnut (Varshney *et al.*, 2009), field pea and faba bean (Kaur *et al.*, 2012), cowpea (Gupta and Gopalakrishna, 2010)

Xu *et al.* (2010) developed 1010 SSR markers in cowpea by mining the unigene database and EST sequences. Since the SSR primers designed showed near 100 per cent cross species transferability and had a relatively high rate of polymorphism (32.1 per cent), these SSR primers were selected as suitable candidates for the present study. So, One hundred SSR primer pairs (Appendix III) were screened for their ability to generate polymorphism among the two parents. Out of 100 primer sets screened, 30 markers showed clear polymorphism (Table 3.1). The rest of the 70 SSR markers either failed to amplify or were monomorphic between the parents. The selected 30 polymorphic primers were used for genotyping F_4 mapping population.

5.2 Analysis on F_4 population to develop the QTL maps for yield related traits

5.2.1 Developing the mapping population

It was reported that F_2 family population ($F_{2:3}$, $F_{3:4}$, $F_{4:5}$, $F_{5:6}$, $F_{6:7}$, $F_{7:8}$) derived from a biparental cross is a good option for QTL mapping (Soller and Beckmann, 1990; Li *et al.*, 1995; Lübberstedt *et al.*, 1998; Mian *et al.*, 1998). They have the added advantage over their counterparts in being eternal and pose immense mapping possibilities because of their ability to show wide variability among plants. Hence an $F_{3:4}$ population was chosen as the mapping population for the current study. The F_3 population segregating for a wide variability of yield characters obtained from a cross between two parents, Sharika×Kanakamony, which are highly contrasting for yield traits maintained at CPBMB was used to raise the F_4 mapping population.

5.2.2 Recording the phenotypic data and data transformation

Trait observations were recorded for all the 12 traits considered for the study. Twelve traits namely pod length (cm), individual pod weight (g), pod number, days taken for flowering, total dry pod yield (g), grains per pod, number of branches, root length (cm), plant height (cm), plant weight (kg) were considered for the study as they were reported to be positively correlated to yield (Li *et al.*, 1997; Asins, 2002; Romanus *et al.*, 2008; Oladejo *et al.*, 2011; Almeida *et al.*, 2014; Santos *et al.*, 2014). However, two additional traits, anthracnose and cowpea mosaic virus resistance were also considered for experimental purpose. For QTL mapping of quantitative traits, it is mandatory that the phenotypic data is normally distributed. Because most statistical analysis packages work on two assumptions. First one being the assumption that the variables are distributed normally throughout the data and the second assumption is that the variance of variables in a range of data remains constant (Osborne, 2010). QTL mapping is no exception, as quantitative traits are the result of interactions of multiple genes, extreme variations in the data would lead to prediction of false positives. Hence, Box-Cox transformation principle was used, which analyses the raw data and determines the power to which the individual variables have to be raised in order to get the best possible normally distributed data. The morphological observation data was analysed using Box-Cox transformation and transformed with BLUPs.

5.2.3 Genotyping the mapping population and linkage map construction

All the selected 30 SSR primers which showed polymorphism among parents were used to genotype the mapping population and the bands were scored according to the method outlined in the ICIMapping software (Appendix V). A linkage map of 908 cM length consisting two linkage groups each on Chromosome 1 and Chromosome 2 was obtained. Chromosome 3 to Chromosome 17 had one marker each on individual chromosomes, unlinked. Whereas, Chromosome 18 to Chromosome 22 did not have any markers. Even though the QTL ICIMapping software assigned linkage groups to chromosomes in the present study, it was not considered as the exact chromosomal location. Because the chromosomal number was randomly assigned to the obtained linkage groups as no chromosomal location data was fed to the software. Usage of mere 30 polymorphic markers is suspected to be the reason for the contradicting 19 linkage groups obtained instead of the maximum possible haploid number of 11. Ubi *et al.* (2000) also came across a similar scenario during QTL analysis in cowpea, where they obtained 12 linkage groups and they ascertained the deviation from haploid chromosome number of 11 to the usage of only few molecular markers. The authors also claimed that

incorporating more number of markers would saturate the linkage groups and would bring the linkage group numbers equal to the haploid chromosome number.

In order to obtain a highly saturated linkage map, it is mandatory to use large number of markers so that they will be distributed across all chromosomes (Mackay, 2001; Doerge, 2002). Whereas, in the present study, only 30 polymorphic SSR primers were used which is considered too less to saturate the huge number of 22 chromosomes in cowpea. Hence, the failure to obtain a highly saturated linkage map and linkage groups on all the chromosomes is attributed to the use of less number of markers. However for a preliminary study, use of 30 polymorphic markers is considered to be sufficient to get an idea of the linkage map and probable marker locations.

5.2.4 Additive linkage mapping

Two mapping strategies were followed in the present study. Single marker analysis and additive linkage mapping. SMA, which is considered to be the simplest method of QTL mapping, considers only one marker at a time and tests for its linkage to any QTL (Tanksley, 1993). But the problem is, when a QTL and the marker are located far apart, SMA fails to establish a linkage between them, maybe because of the possibility of occurrence of a recombination event (Kearsey and Farquhar, 1998). Hence SMA is used only when the linkage map is not dense with enough number of markers (Mohan et al., 1997). Additive linkage mapping on the other hand, is a very precise approach towards QTL identification with high power of QTL detection and specificity (Li et al., 2015). Also called as Inclusive Composite Interval Mapping (ICIM), this approach makes two important assumptions. First one is that the phenotype of an individual is the summation of effects of all the QTLs affecting the trait of interest. And the second assumption is that the linked QTLs are separated by at least one marker. Because of these assumptions, ICIM gives more LOD values to a highly significant QTL and less for a non-existent QTL which makes it easy to identify the right QTL (Li et al., 2007). In the present study, since only two linkage groups were obtained which contained a total of 13 markers, single marker analysis was the only option for rest of the 17 markers. And the 13 linked markers were analysed using additive linkage mapping as it was the best proven strategy for linked markers. However all of the markers were considered for SMA, the results of which helped in cross verifying the additive linkage mapping results. Hence, if a marker showed high level of association with a particular trait in additive linkage mapping, and if the same is supplemented by the results of SMA, then that marker was declared linked to the respective trait in this study.

Going by traits, five out of twelve traits considered had significant QTL hotspots and linkage was declared as all of them had LOD values well above the universally accepted standard threshold of 3. They are as follows.

5.2.4.1 Days Taken For Flowering

For this trait, three markers were obtained, two of them acting as bracket markers (CLM0008 towards the left and CLM0278 towards the right) and one marker lying on the gene itself acting as an anchor marker (CLM0177). This combination of two bracket markers and an anchored marker would be of immense use in marker assisted selection. Since SMA has also proved that CLM0177 is tightly linked to the trait, linkage was declared. However this trait also had QTL hotspots between markers CLM0278 and CLM0322, between CLM0322 and CLM0168 and between CLM0168 and CLM0195 on linkage group 1. And between markers CLM0088 and CLM0260, between CLM0218 and CLM0077, and beyond CLM0077 on linkage group 2.

Early flowering is often associated with higher yields especially when the crop is sensitive to environmental fluctuations like high temperature and drought. Rubio *et al.* (2004) reported that early flowering in chickpea is positively correlated with total yield. Hence the combination of two bracket markers and one anchor marker for trait days taken for flowering could be used as an effective selection tool in MAS for higher yield. Early studies have also identified QTLs for early flowering in crops like rice (Yano *et al.*, 2001), maize (Chardon *et al.*, 2005), barley (Bezant *et al.*, 1996).

5.2.4.2 Plant height

A significant QTL hotspot was observed on linkage group 1 between markers CLM0244 and CLM0177. An anchor marker CLM0008 lies exactly in the middle of the QTL hotspot making the combination a perfect strategy which could be used in MAS. Bezant *et al.* (1996) reported three QTLs affecting plant height in spring barley which also had significant effects on early flowering. In the present study also it was found that QTL hotspots bracketed by markers CLM0008 and CLM0177 had effects on both plant height and early flowering.

5.2.4.3 Branch number

A significant hotspot on linkage group 2 between markers CLM0200 and CLM0088 was also supported by the results of SMA. Hence it was concluded that these two markers could be used as bracket markers during plant selection for branch number.

Branch number in legumes is a major factor contributing to total yield. Increased branching often leads to more yield and low density planting could be compensated with more number of

branches (Hedley and Ambrose, 1981). Kamai *et al.* (2014) also reported that, in cowpea, increased number of branches led to more number of peduncles thereby increasing total yield. Hence we suggest that, the above mentioned bracket markers for branch number could be used for MAS for improved yield. Wang *et al.* (2004) reported QTLs affecting lateral branch number in cucumber.

5.2.4.4 Root length

A significant hotspot was found between markers CLM0244 and CLM0008 on linkage group 1. Single marker analysis also showed good linkage of CLM0244 with the trait. Hence these markers could be used as bracket markers in MAS. Kashiwagi *et al.* (2006) reported that increased root length could be an effective contributor to yield in chickpea. Li *et al.* (2005) mapped 12 QTLs for maximum root length which were claimed to be the main contributors to drought resistance and total yield in rice. Since cowpea is a drought tolerant crop, selecting plants for increased root length could prove a best strategy to obtain higher yields. Many researchers have mapped QTLs for root length and emphasised on their importance with respect to yield in crops rice (Price and Tomos, 1997; Yadav *et al.*, 1997; Obara *et al.*, 2010) and maize (Zhu *et al.*, 2005; Hund *et al.*, 2011)

5.2.4.5 Plant Weight

A QTL hotspot with an anchored marker CLM0244 and two flanking markers, CLM0186 at 0 cM and CLM0008 at 66.16 cM was observed for the trait. Since Single marker analysis also showed near significant association of marker CLM0244 with the trait this would be a great tool for MAS. Turk and Hall (1980) attributed the increased plant weight in cowpea to the thicker and bigger leaves which showed positive correlation towards drought tolerance and thereby increasing yield.

However, eight traits namely pod length, individual pod weight, pod number, total dry pod yield, grains per pod, plant height, anthracnose, cowpea mosaic virus, either had no linked markers or the ones obtained fell way below the LOD threshold.

The abnormal results obtained for both anthracnose and cowpea mosaic virus resistance is attributed to the non-normal distribution of data. The data was non-normally distributed as no standard disease scoring scale was used and the trait character was recorded as only highly susceptible and highly resistant. However bulk segregant analysis has been used in multiple studies to map QTLs for resistance traits like draught resistance in maize (Quarrie *et al.*, 1999), leaf rust resistance in barley (Poulsen *et al.*, 1995), draught resistance (Salunkhe *et al.*, 2011) and heat tolerance (Zhang *et* al., 2009) in rice.

5.2.5 Single marker analysis

Apart from two significant QTL hotspots each having two bracket markers and an anchor marker for traits days taken for flowering and plant weight, this study has also identified two suggestive QTL hotspots with two bracket markers for branch number and root length. And the results outlined three important SSR markers

5.2.5.1 CLM0177:

It is an anchor marker for trait days taken for flowering and is a suggestive bracket marker for traits pod number, total dry pod yield and plant height.

5.2.5.2 CLM0244:

It is an anchor marker for trait plant weight and is a highly significant bracket marker for trait root length.

5.2.5.3 CLM0083:

It is an anchor marker significantly linked to traits individual pod weight and total dry pod yield, both of which are positively correlated

The region between 25 cM to 125 cM on linkage group 1 had QTL hotspots harbouring genes governing traits days taken for first flowering, total dry pod yield, root length, plant length and plant height. This entire region was bracketed by two markers, CLM0244 at 24.25 cM and CLM0177 at 126.86 cM with an anchored marker CLM0008 in between. Hence, this marker combination could be potentially used in marker assisted selection for the entire above mentioned traits.

This study concludes by giving a sketch of marker distribution on chromosomes and the linkage to yield traits in cowpea. Further research into understanding the intricacies of individual gene contribution to the trait and confirming of the linkages suggested in this study would help in efficient MAS in cowpea for increased yield.

Since QTL hotspots were identified only for five out of twelve traits considered, further studies aimed at identifying QTLs for the remaining traits which are positively correlated with yield would be of great use in MAS. Further saturating and increasing the resolution of linkage map using sufficient number of molecular markers with maximum number of mapping population would actually segment the already identified QTL hotspots into still smaller QTLs making the difference between a significant QTL and a non-significant one more apparent. And once the QTLs are confirmed through extensive replication studies at different environments, they can be cloned and thus could be used for genetic transformation of cowpea plants for higher yield.
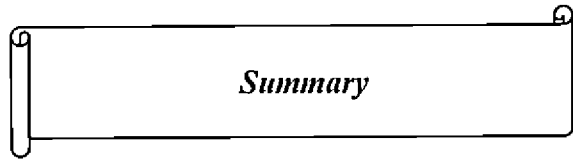
Summary
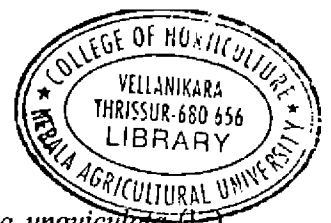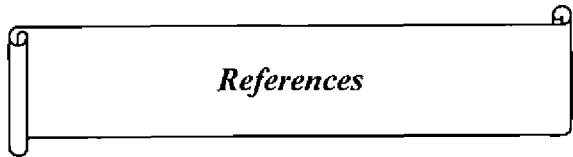
6. SUMMARY

The study on "QTL mapping for yield traits in vegetable cowpea [*Vigna unguiculata (L.)* Walp.]" was carried out at the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during the period from 2015 to 2017. The main objective of the study was to map the SSR markers and to identify the quantitative trait loci for yield components in the genome of vegetable cowpea. The semi-trailing variety Kanakamony [*Vigna unguiculata* (L.) ssp. *cylindrica*] which is immune to anthracnose and comparatively low yielding was used as the male parent. Whereas, pole type Sharika [*Vigna unguiculata* (L.) ssp. *sesquipedalis*] which is high yielding but susceptible to anthracnose disease was used as the female parent to develop the F_4 mapping population.

The summary of the work is furnished hereunder:

1. An F_4 mapping population consisting of 92 plants segregating for number of yield traits was developed using the F_3 pods available at CPBMB. Individual plants in the mapping population was carefully observed and the data was recorded for traits pod length, individual pod weight, pod number, days taken for flowering, total dry pod yield, grains per pod, branch number, root length, plant height, plant weight, anthracnose, cowpea mosaic virus.

2. DNA from all the 92 plants of F_4 mapping population along with parents was isolated using a protocol designed based on the standard CTAB DNA isolation protocol. The new protocol which incorporated RNase treatment into the DNA isolation procedure, yielded excellent DNA samples having UV absorbance ratio (A260/280) of 1.80 to 1.94 and quantity of 352.72 to 784.25 ng/µl.

3. One hundred SSR primer pairs were initially screened among the two parents to check for their ability to generate polymorphism. Out of which, the 30 clearly polymorphic SSR markers were selected, used for genotyping the mapping population and the band patterns were scored.

4. A linkage map spanning 908 cM with two linkage groups was constructed. Linkage group on Chromosome 1 had eight linked SSR markers and the one on Chromosome 2 had five linked markers. This linkage map was further used for QTL analysis.

5. QTL map was developed using ICIMapping software using both single marker analysis and additive linkage analysis strategies. Data from SMA was used to confirm the results of additive linkage mapping.

6. Two significant QTLs, each having two bracket markers and an anchor marker for traits days taken for flowering and plant weight was identified. Two suggestive QTLs with two bracket markers each for traits branch number and root length was also identified.

7. Three SSR markers, CLM0177, CLM0083 and CLM0244 were declared anchor markers for traits days taken for flowering, individual pod weight and total dry pod yield and plant weight respectively.

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Annexures

APPENDIX I

List of Laboratory Equipments used for the study

NanoDrop spectrophotometer	ND-1000	-	Thermo Scientific, USA
Cooling Centrifuge		-	KUBOTA model No. 65000, Japan
Minispin		-	Eppendorf, Germany
Horizontal electrophoresis system		-	BIO-RAD, Italy and Ge Nei, India
Agilent thermal Cycler, Veriti		-	Agilent and Applied Biosystem
Gel documentation system		-	BIO-RAD, Italy
AccuBlock™ Digital Dry bath		-	Model D110, Labnet International, Inc
Laminar air flow		-	THERMODYNE, Faridabad

Composition of reagents used for DNA isolation and purification using Doyle and Doyle (CTAB) Method

CTAB buffer (2x)

- 2 per cent CTAB (w/v)
- 100mM Tris (pH 8.0)
- 20mM EDTA (pH 8.0)
- 1.4M NaCl

10 per cent CTAB solution

- 10 per cent CTAB (w/v)
- 0.7M NaCl

TE buffer

- 10mM Tris (pH 8.0)
- 1mM EDTA

RNase stock

- RNase A   -100 mg
- Autoclaved distilled water   -1 ml

Stock was prepared by dissolving 10 mg RNase A in 1 ml water and was stored under refrigerated conditions at -20° C. The RNase A was used to prepare RNase. Ten per cent RNA solution was prepared by dissolving the same in water at 1:10 ratio. The solution was stored at -20° C for RNase treatment.

TAE Buffer (1X)

- 40Mm Tris
- 1mM EDTA
- 20Mm Glacial Acetic acid

Agarose gel composition (2 per cent)

- 2 g Agarose
- 2 ml TAE buffer
- 98 ml autoclaved distilled water

APPENDIX III

List of SSR primer sets used for screening of parents

Sl. No.	Marker ID.	Forward primer (5'-3')	Reverse primer (5'-3')	Repeat unit	Predicted product size (bp)
1.	CLM0002	ACAACAGCATCAT CCCAAGT	ATCCACAGCCTTTAT CACCA	(CAG)6	208
2.	CLM0007	ACAGGTTCCTTGTG AAGCAC	GCCATACGCAACTCA GCTAT	(CTTCCA)3	183
3.	CLM0008	CGGTTCTAGTGCCA CCAA	GAAACCGGCACTGG AAAC	(TCACCA)3	244
4.	CLM0009	AACTTCCCCGGAGT CTTCTA	GTGCGAGAAGAGAA TCGAGA	(TC)9	223
5.	CLM0010	CATTGCCTTGCATT TCTTTT	GAGTTTCTGGGACGA TCAGA	(AT)9	232
6.	CLM0014	CGTTCACCCATTTC TCATTC	CAAGATCACATCCA AGCACA	(GAT)7	225
7.	CLM0015	TGAAACGTGAAGC ATCAAAA	CTGTTGGAACTGGAG GACAC	(TGA)7	183
8.	CLM0016	AGCAACACCAAAA CACTCAAG	AGATTTGACCTAGCG CATTG	(ATT)7	234
9.	CLM0021	CCCTCAACAATTTT GTCCAC	TTTCTTTGGATGGGA TGAGA	(TGAG)6	177
10.	CLM0022	GTCCACAAATCAG ATGCACA	AGTTCCCTTCCCTTC ATGTT	(GCAATG)4	216
11.	CLM0026	GATCAGGGGTAGG AACGAAT	GGCTTTCCTCAACTG TTTCA	(AAT)8	244
12.	CLM0028	TTGCCTTGTTAGGT GAGAGC	GTGACGCGGAAAAA CTCTAA	(AGGGGC)4	150
13.	CLM0029	TGTGTGTGTTCGGT TTCTTG	GCTAGTTCCCCCTTC AGAAC	(CTT)8	232
14.	CLM0030	AGAATTGTCCCTCC CAAGAC	TCTGATAACCCCAAA AGCTG	(GAT)8	190
15.	CLM0031	CGCTTTTGTAGGAT TGGAAC	TTAGCATGGGAGAG TTTTCG	(AAG)8	249
16.	CLM0032	GAACAGCTTCCTG AACCTCA	GCTTTCATCTGCTCC AGGTA	(AT)12	169
17.	CLM0036	AAACATGATCGTG CACTCTG	AGAGTGGCAATGAG CAAAAC	(AATAT)5	248
18.	CLM0042	GAAAACAACATGG CTTCTGG	CATGGTGTTCCTGGT TGATT	(ACT)9	203
19.	CLM0050	CTTCTCTCCGTCAA GTGGAA	AGCAGACAACCACA GATGCT	(TAAC)8	178
20.	CLM0061	AACATTTTCACCAT TGATCG	CAAGCCACCAATCCT TTTAT	(TTA)12	299
21.	CLM0062	TGAAAGCTGCAAG ATTGATG	AATTTTTGTTTGCGT GCTTC	(ATG)12	242

22.	CLM0063	CATCCACCACATCA AAATCA	CCCAATTGAAGTCCT TGATG	(AG)19	195
23.	CLM0065	TCATGTCAATTTTC CCGTTT	ATATTGGGGGTGGA TTTTG	(CT)19	206
24.	CLM0066	AACCCAGCATACC TGCATAA	CTCGCCAATGATTCT GAGAT	(TA)19	244
25.	CLM0068	AATGTTGGACTGG TCAGGA	GAGGACAAGTCAGG AAGCAA	(AG)20	187
26.	CLM0077	AAAGCGGAAAAAG TTTGGAT	AGCACTCTGCACACA AATCA	(AT)23	246
27.	CLM0083	GGCGACGTCTTTCC ATATTA	TGGAATCGATGTTGT GATTG	(AT)25	195
28.	CLM0085	CACAACTGTGATTT GCTCGAT	TCGGAAACAGGTTC ACCTAC	(TA)26	291
29.	CLM0088	TCGTCGGTCTTCAT AAAAATG	AACGCTTCGATTATC TGCAC	(AG)28	188
30.	CLM0101	TGTCTTTGCAGGTT GTTTCA	GCTACATGGTGATGC CACTT	(TA)20	365
31.	CLM0102	CTCTTGTTAAACTC TTCACACCC	CCATTCATGATGCTA TACAAGC	(AT)27	350
32.	CLM0103	AAATCTAAATTGC GTCCGTG	TTTGACGTGTTTGGT AAGGTT	(AT)17	292
33.	CLM0104	GACCTTGAATTCTT CGAGCA	AGCAGCCTGTTAGTG TGAGC	(AT)36	276
34.	CLM0114	TTCCTTAGCCAAAG TGTTCC	TCAACGACAGCGTTA TCAAA	(AATAA)4	243
35.	CLM0115	TTTCCATTGCATTT ATTCCAC	TCAGGAGACAGAAT GGAAGG	(TTA)10	244
36.	CLM0119	GAGATGTTGAGAT GGTGGCT	CCTTGGTCATTGAAC CTCTC	(TA)16	192
37.	CLM0126	GGATCTCTTTGAAT CTTTGCTC	GAATGAAGGTTACG GGAGGT	(CTTC)4	199
38.	CLM0128	ATCTCTGTTAGGAG TGGCCC	CACAGCATGTTGGA ATTGTT	(TAGT)4	380
39.	CLM0130	CATGTTTCTTCAAG TTAAAGGG	GTACACACATACCCG ACAGC	(TA)29	297
40.	CLM0132	TGTTGTTGCAATGA GTGTCC	AAATTGAAGTGTGTT TCCTCAGA	(TA)44	329
41.	CLM0137	CCATCAAACCATG GTCTCTC	GAACCATAGCAAGC AAGGAA	(TCT)6	226
42.	CLM0139	GTGCCGGGTATTTA TTGTTG	TTTGTGGTGCTTATT GCACA	(ATA)13	207
43.	CLM0151	TGCTTGAGTGTCAC TTGAATG	TCGCAAAGAGAGGA ATATCG	(AT)10	372
44.	CLM0156	GGGCTTCCTAGGTC ACAAAT	CCATTCTCTTCGGTT AGTTATT	(ATT)9	400
45.	CLM0158	GGTAGGGCTACTC CCAGGTA	AGGAAAGAGAAATC ACCTCACA	(AT)22	299

46.	CLM0160	GGGTGCTTATTTGA TTGTGG	TACCCTCATTACGTT GCACA	(TA)16	351
47.	CLM0168	TGAGAGGACCAAA TTACTCCA	TCACCATTCTAAGAA ACAAGTGA	(AT)17	396
48.	CLM0172	CGCAAGGAACCTT GAGTTAT	CGTATCACATTAGTT GTGGACTG	(AT)10	399
49.	CLM0177	AATTGGGTTGTAA AGTGAGATTT	CGAAAGTGGTTTGCG TATTT	(TA)36	317
50.	CLM0185	TCAAGGTCGTGTG AGGAAGT	GTGGAGGAGAGATG ATGGTG	(CAT)8	166
51.	CLM0186	TTTGAACTCATATA AAGCACTTG	GATCCTTCTTCCCTC TCTCG	(AT)24	369
52.	CLM0187	GTGCACAACCAAT TCAATCA	CCCATGCAACATATC TACCC	(ATTA)5	375
53.	CLM0190	TGAGTGGGATTGA AAGAAGTTT	TTATCAATGGACACT CAAGGG	(AT)9	350
54.	CLM0191	TGGGATTCTTCTGC TGAGAT	TGCAAGCAAGTAAT CCCTCT	(TA)26	385
55.	CLM0192	CTGGTTCAAATATT TACAGAAA	ACGGGTTCAACATTC CAAC	(AT)15	302
56.	CLM0193	ATCAACGGTGGTT GTTTCAG	TGAGGAAACTGAAC TCAGGC	(CTG)6	142
57.	CLM0194	ATCTATCACCATTC GGCTTG	CCGAAACATTACGA GTCGAG	(TA)22	341
58.	CLM0195	AGGCATGATGTGT GGAGTTT	TTTCTCACGTTGTTT AGCCTT	(AT)19	361
59.	CLM0200	AATTTGATCGCCTA ACGACA	TCAAACGTATATGCG TAAATAAT	(TTAT)3	160
60.	CLM0201	CCAAAACAAACAC CAACCTC	GAGACCTGCGATCA GAACAT	(GAACAA)3	187
61.	CLM0215	CCGTACCTTCCTCC TTCAAT	TGCTTAGGTGTGGT GGATT	(ACC)6	155
62.	CLM0216	TCATTCAGAGCCAC CTCTTC	GATGTTGTGAGGGTG GTGAT	(CCATCA)3	186
63.	CLM0218	TTTCCGATTTGCGA TTTTA	CGACCAGTGACAAA TGAACC	(TCATGC)3	218
64.	CLM0223	TGTTGCCATTTCTT GTTGTG	GATCAAACAAAAGC CGAGAA	(TTGGTG)3	233
65.	CLM0227	CCAAGAGTGGCCT GAGTAAA	TGCAATATTCTTAGG TCTAAAACG	(GAAC)5	250
66.	CLM0228	GGATCAGCATTTTG TTCACC	TGTCATTTGCATTTT GGTTG	(TA)10	151
67.	CLM0229	ATCTTGTTTTCGCC CAAGTT	TGTCATTTGCATTTT GGTTG	(TTTA)5	198
68.	CLM0230	TCATGAGTGCACG AGTGTTT	TTCCAACAGAAGC AAGAAG	(TTC)7	199
69.	CLM0231	GTTGGACTCATACG GAAACG	TTGAAGAAACGCCA TAAAGG	(CAA)7	230

70.	CLM0232	TGCTTCGACGAACT TTTACC	CAGCTAGCGGACCA AGATAA	(AAG)7	249
71.	CLM0233	GCCTCCTCTTCTTC TTCCAC	GAACGAGTAGAACC CGTTGA	(TTC)7	197
72.	CLM0234	TTGTTTTGAACTCA ATTTTAATGAT	CATTTTCCATATTCT CACATCCT	(AT)11	226
73.	CLM0235	TGTATGGTAGAGTT CGCATCC	GCGGCAAGGCTTAA TAAACT	(AT)11	212
74.	CLM0240	CCATCGTTCACCTC AAATTC	TTGCACAGGCTTGAA CTGTA	(TATT)6	193
75.	CLM0241	CATTCACCACCACA GTTGAA	ACTCCTCACAAAGCA GTTGG	(TGT)8	235
76.	CLM0243	ACCCTCTTTGGACT CTCACC	GATTCACGCTCTGAA GGAAA	(GGA)8	229
77.	CLM0244	GTGGAGTTCAGTG GCAAAGT	CCAAAATCGCATGTA GTTCC	(TA)12	242
78.	CLM0245	TGCAGGATTCACTA GGAGGT	AGCAGGACTTATGC AAGCTG	(TCT)8	235
79.	CLM0247	CAGGAACACTTCC ACAACCT	GGGTGCGAGAATCA ATAACA	(TTC)8	235
80.	CLM0248	TGATTGGTGTTGTG ATGTCC	GGGTTCACCATTACA GATGC	(TTA)8	207
81.	CLM0251	CTTTTCATGGGAAT TGTTGG	TGAACTTTCCAAGGA ACTCG	(ATT)8	170
82.	CLM0252	AGGAAGCCCAAAA CAACTTT	TATAATGGCCAAAG GACCAA	(GA)12	245
83.	CLM0253	AAAGGAAAAGGAC ACCCAAG	AACTCTTTTGCCAT GTGCT	(AAT)8	190
84.	CLM0254	TGCATTCACAACCT GTTTTC	AGATCTATGATGGGC ACAGG	(CCA)8	246
85.	CLM0255	GGAGGCATAAAAA TGACACCT	CTCTTGGTTTGTGCA TTTCC	(GA)12	223
86.	CLM0256	TCACCACACACAA ACACACA	AGATATCAGCGTGG CAGAAC	(AAGT)6	240
87.	CLM0260	TCGATCAAATTTTC CTCTGC	TGCCACCATCTTTCA TTTCT	(CTT)8	165
88.	CLM0265	GATGTCTTCTCCCC CAAAGT	GTGGGTTCAAGAGG GAAAAT	(GATGAA)4	225
89.	CLM0269	TGCTTATGCAGCTG CTTTTT	CAAAATTTCTCTGGG GATGA	(TC)13	156
90.	CLM0273	AGCAACGAATCAA GAAAACG	ATCTCTCCGGCTATG GAATC	(AG)14	163
91.	CLM0279	TGCAAAACGTGAA AGCAATA	ACAAGGAGACCAAG GAGCTT	(AAAG)7	168
92.	CLM0281	CCTCTCTTCTCTCG CCCTAT	TTTTCTGAGCCAGGA GTTTG	(TCA)10	177
93.	CLM0287	TTGGGTCATTAACT CCTTTCC	ACGGCAAGCATGAA CAATAG	(AT)15	250

94.	CLM0291	ATGCCACTTCTCTG CTCATT	CCAGTGTTGGTTTCC TTGTC	(AT)16	248
95.	CLM0292	GAGAGACGTGATG GAGAGGA	TCAATGATCGTATAA AGCCTCA	(AT)16	188
96.	CLM0298	GGTGAGAAACGCA GAAAGAT	CATTTGCTTCCTCCC ATTTT	(AG)17	164
97.	CLM0300	TTTTGTTGGTTGAG CATCTG	GGTGTTCAATGTCAG GAATAACA	(TC)18	154
98.	CLM0304	GGGAGGGTATTCT CGTCCTA	AGCTTCCACAGTGAA GTTCG	(AT)20	169
99.	CLM0322	ACTGAACAGCAAG GACGTTT	TGTGTTTCCAGTGCA AGAAT	(TA)29	238
100	CLM0332	TGTCCTCAATTTCA ATAACAAG	CGAAACAGTTGGTC GGATAC	(TTA)12	261

APPENDIX IV

Codes used in ICIMapping for different parameters for QTL mapping

I. **Population Type:** describe the type of the population. Assuming $F_1 = P1 \times P2$, the 20 biparental populations for which ICIM can construct QTL map are:

- 1 for $P_1BC_1F_1$: the backcross population where the first parent (P_1) is used as the recurrent
- 2 for $P_2BC_1F_1$: the backcross population where the second parent (P_2) is used as the recurrent
- 3 for F_1DH: doubled haploids derived from F_1
- 4 for RIL: recombinant inbred lines derived from repeated selfing since F_1 generation
- 5 for P_1BC_1RIL: recombinant inbred lines derived from the backcross population where the first parent is used as the recurrent
- 6 for P_2BC_1RIL: recombinant inbred lines derived from the backcross population where the second parent is used as the recurrent
- 7 for F_2: the selfing generation of F_1
- 8 for F_3: the selfing generation of F_2
- 9 for $P_1BC_2F_1$: the second backcrossing where P_1 is used as the recurrent parent
- 10 for $P_2BC_2F_1$: the second backcrossing where P_2 is used as the recurrent parent
- 11 for P_1BC_2RIL: recombinant inbred lines through the repeated selfing of $P_1BC_2F_1$
- 12 for P_2BC_2RIL: recombinant inbred lines through the repeated selfing of $P_2BC_2F_1$
- 13 for $P_1BC_1F_2$: the selfing generation of $P_1BC_1F_1$
- 14 for $P_2BC_1F_2$: the selfing generation of $P_2BC_1F_1$
- 15 for $P_1BC_2F_2$: the selfing generation of $P_1BC_2F_1$
- 16 for $P_2BC_2F_2$: the selfing generation of $P_2BC_2F_1$
- 17 for P_1BC_1DH: $P_1BC_1F_1$-derived doubled haploids
- 18 for P_2BC_1DH: $P_2BC_1F_1$-derived doubled haploids
- 19 for P_1BC_2DH: $P_1BC_2F_1$-derived doubled haploids
- 20 for P_2BC_2DH: $P_2BC_2F_1$-derived doubled haploids

II. **Mapping Function:** specify the mapping function which will be used to transfer recombination frequency to mapping distance in linkage map construction.

- 1 for Kosambi mapping function
- 2 for Haldane mapping function
- 3 for Morgan mapping function

III. **Marker Space Type:** specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals

- 1 for intervals, i.e. the number behind a marker is the distance of the marker to its previous marker
- 0 is normally given for the first marker as the starting position of a chromosome or a linkage group
- 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group

IV. **Number of markers:** number of markers that need to grouped and ordered into linkage map

V. **Population Size:** number of individuals in the population

APPENDIX V

Coding of co-dominant markers

I. Coding by numbers:

- The two parental bands are coded as 2 and 0.

- If both are present in F1, then it is coded as 1.

- The coding number can be viewed as the number of parent A allele. When heterozygote is present in a population, all three numbers 2, 1, and 0 could be present. When heterozygote is absent, only numbers 2, and 0 are present.

II. Coding by letters:

- Parent A is coded as A or AA

- Parent B is coded as B or BB

- Their F1 hybrid is coded as H, AB or BA.

III. Missing values:

- Missing values of marker type are coded as -1, X, XX, *, or **.

IV. Mixed coding:

- It is recommend that either numbers or letters (not both) be used in coding a genetic population.

- Mixed coding is acceptable in QTL IciMapping software. Taking F2 population as an example, some individuals could be coded as 2, some coded as A, some coded as BA, and some coded as AA etc.

APPENDIX VI

Coding for general information of the mapping population

Indicator: This indicator lets QTL IciMapping know if a mapping study or power simulation will be conducted.

- 1 for a mapping study
- 2 for power simulation

Population Type: describe the type of the population (Refer Appendix IV)

Mapping Function: To specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.

- 1 for Kosambi mapping function.
- 2 for Haldane mapping function.
- 3 for Morgan mapping function.

Marker Space Type: To specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.

- 1 for intervals
- 0 for first marker.
- 2 for positions

Marker Space Unit: To specify the unit used in marker linkage group

- 1 for centi-Morgan (cM).
- 2 for Morgan (M). 1 M = 100 cM.

Number of Chromosomes: To specify the number of chromosomes (or linkage groups) in the mapping population.

Population Size: number of individuals in the mapping population.

Number of Traits: number of traits phenotyped in the mapping population.

Appendix VII

Morphological data after BLUPS generation

Plant Name	Morphological Traits											
	Pod Length (cm)	Individual Pod Weight (g)	Pod Number	Days Taken for First Flowering	Total Dry Pod Yield (g)	Grains per Pod	Branch number	Root length (cm)	Plant Height (cm)	Plant Weight (kg)	Anthracnose	Cowpea Mosaic Virus
1A	3.3623	-0.92091	36.9045	1.55692	-6.9829	0.04657	-2.1755	-11.571	302.395	0.16307	39.4504	12.7634
1B	0.71576	-0.44435	38.8002	1.55692	28.2342	-0.2717	-0.712	-7.2758	61.4156	0.15665	39.4504	12.7634
1C	5.32608	0.059106	6.48832	-4.173	10.4369	3.25408	-0.2252	-3.4624	-3.6423	-0.2742	39.4504	12.7634
1D	1.43525	-0.39489	-2.1043	-4.173	-17.641	-0.3875	-0.2252	-0.6047	117.112	0.21149	39.4504	12.7634
1E	1.68461	0.24619	-6.4095	1.55692	-6.1075	0.49973	-1.1992	-25.932	-68.797	-0.8972	-13.924	-40.611
2A	-0.1383	0.354404	53.0024	-4.173	112.96	3.67738	-0.2252	-9.6611	191.29	0.13737	-13.924	-40.611
2B	-0.2343	0.590287	16.9644	-4.173	54.2809	-0.9182	-0.712	-12.048	135.665	0.28161	39.4504	12.7634
2C	-2.5299	0.374072	17.44	-3.2173	44.6322	1.22254	-0.2252	-18.263	154.213	0.45527	-13.924	-40.611
2D	-3.174	0.24619	-1.6263	-3.2173	2.93319	1.32852	-1.1992	-17.306	121.751	-0.0372	-13.924	-40.611
2E	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100
3A	-0.8776	-0.07889	-4.0169	1.55692	-10.858	-3.8174	-1.687	9.85834	70.7025	0.57453	-13.924	12.7634
3B	-100	-100	-100	1.55692	-100	-100	-1.687	-2.986	24.2506	-0.1378	-13.924	12.7634
3C	1.68461	-0.30593	-100	1.55692	-100	-6.0755	-0.712	36.4073	14.9548	0.21594	-13.924	12.7634
3D	3.60187	0.019691	-11.202	1.55692	-19.726	-2.6857	1.23247	-19.699	-22.247	-0.6223	-13.924	-40.611
3E	10.4442	-0.74174	-10.243	1.55692	-35.043	1.84867	0.26108	-8.2297	302.395	0.036	-13.924	12.7634
4A	2.64345	0.914186	9.348	1.55692	51.1428	1.75236	0.74696	10.3334	14.9548	0.05925	-13.924	12.7634

4B	2.83516	0.305224	1.71735	1.55692	10.9349	-0.2717	0.26108	-4.892	61.4156	0.01077	-13.924	12.7634
4C	-0.5415	-0.73181	-18.412	1.55692	-42.016	-2.2025	2.20246	-13.959	-115.4	-0.1477	-13.924	12.7634
4D	1.62707	0.068959	-10.243	1.55692	-16.949	1.22254	0.26108	-1.5571	-3.6423	0.24409	-13.924	12.7634
4E	0.86927	0.07881	3.14931	1.55692	5.59212	-0.8506	-0.712	-3.9389	-50.17	0.47696	-13.924	-40.611
5A	0.4375	-0.58299	9.82441	1.55692	-11.191	-3.8464	-0.2252	-4.892	-68.797	0.23273	-13.924	12.7634
5B	-2.3185	-0.27629	-25.662	1.55692	-45.234	-2.531	-0.712	-23.053	5.65721	0.1527	39.4504	12.7634
5C	-1.8477	-0.63256	-100	1.55692	-100	-2.2121	-1.687	-17.306	-31.553	-0.0189	39.4504	12.7634
5D	0.38952	-0.71194	4.10363	1.55692	-21.684	-0.2428	0.26108	-1.0809	-96.753	-0.2171	-13.924	12.7634
5E	4.36834	-0.37512	41.6428	1.55692	36.6568	1.65604	-0.712	4.62961	98.5533	0.13985	-13.924	12.7634
6A	-1.8189	-0.01973	5.05769	1.55692	5.09841	0.7889	0.74696	-0.6047	-40.861	-0.113	-13.924	-40.611
6B	-1.2713	-0.90096	-3.5386	1.55692	-33.939	-0.6866	2.20246	11.2835	89.2713	-0.3562	-13.924	-40.611
6C	0.24557	-0.01973	1.23989	1.55692	-0.839	1.49229	1.23247	77.4846	256.123	1.28153	39.4504	12.7634
6D	0.29355	0.009836	-6.8883	1.55692	-13.049	1.94497	3.65517	43.5023	-59.482	1.2349	39.4504	12.7634
7A	-2.3473	0.25603	6.96508	-2.2619	19.0781	-1.6229	5.10555	6.53158	-96.753	-0.2717	-13.924	12.7634
7B	-0.3303	-0.34546	-20.823	1.55692	-39.669	-0.6576	2.68698	-4.4154	-59.482	-0.4692	-13.924	12.7634
7C	0.77333	0.009836	21.2432	-1.3068	32.4726	1.11655	1.23247	-8.7068	-115.4	-0.7024	-13.924	12.7634
7D	2.16408	-0.25654	-16.005	-0.352	-32.145	1.01055	0.74696	4.154	135.665	-0.1304	-13.924	-40.611
7E	-1.2617	0.295386	-0.6706	1.55692	6.18071	-0.3971	-0.2252	-4.892	89.2713	0.46316	-13.924	12.7634
8A	-1.0504	-2.02E-05	-16.486	1.55692	-28.618	1.84867	-1.1992	-11.093	-40.861	-0.8305	39.4504	12.7634
8B	-2.5876	0.029546	-18.894	1.55692	-32.058	-1.4298	-1.1992	11.7584	61.4156	0.36995	-13.924	12.7634
8C	-1.8189	-0.26641	-10.243	1.55692	-24.609	-3.0724	1.71763	-6.7989	70.7025	-0.0367	39.4504	-40.611
8D	-2.1551	-0.56317	-5.4522	1.55692	-26.034	0.69252	1.71763	10.3334	61.4156	-0.4637	-13.924	-40.611
8E	4.08094	0.11821	-24.692	1.55692	-40.883	-3.498	1.23247	16.9804	126.39	0.17296	-13.924	12.7634
9A	-0.7144	0.560817	16.9644	1.55692	52.6624	0.88527	-0.712	16.0313	24.2506	-0.2519	-13.924	-40.611

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9B	2.49965	1.012247	18.3911	1.55692	78.7285	3.19635	-0.712	15.082	-3.6423	-0.4403	-13.924	-40.611
9C	1.44484	-0.75168	10.3008	1.55692	-17.917	0.40332	0.26108	-12.526	-59.482	-0.4064	-13.924	-40.611
9D	-2.6356	-0.03945	18.8666	1.55692	25.9391	-1.1401	-0.712	13.183	-106.08	-0.2698	-13.924	-40.611
9E	-1.3866	-0.09862	39.274	1.55692	53.5873	-2.0092	-0.712	3.67835	-12.944	0.58241	-13.924	12.7634
10A	4.55992	0.393738	21.7185	1.55692	53.8846	3.38878	-1.687	0.82341	24.2506	-0.1021	39.4504	12.7634
10B	-2.7318	0.344569	-1.6263	1.55692	5.7725	-2.4924	0.26108	6.53158	79.9877	0.14775	39.4504	12.7634
10C	2.35584	0.737573	16.013	1.55692	59.3707	-1.0436	-1.687	-0.1286	33.5445	-0.1348	-13.924	12.7634
10D	2.54758	0.767018	12.2057	1.55692	51.5911	-1.1401	0.26108	12.2333	117.112	0.19371	39.4504	12.7634
10E	2.16408	0.206822	23.6189	1.55692	47.1869	-0.6576	-0.712	11.2835	98.5533	0.18333	-13.924	12.7634
11A	-3.5971	-0.25654	-25.662	1.55692	-45.108	-1.4008	1.71763	0.34742	-134.06	-0.1899	39.4504	12.7634
11B	-2.8567	-0.20717	-6.8883	1.55692	-18.376	-4.5726	2.20246	-3.9389	-106.08	-0.3607	39.4504	-40.611
11C	5.80482	0.462553	-2.1043	-4.173	8.43014	3.77357	-1.1992	16.0313	79.9877	0.0073	-13.924	12.7634
11D	-0.2151	-2.02E-05	37.3785	1.55692	57.6256	-0.7348	4.13887	59.5634	265.38	1.4552	-13.924	12.7634
11E	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100
12A	-4.9729	-0.65239	-10.243	-4.173	-33.046	-4.5242	-0.2252	8.43284	-31.553	0.22828	-13.924	12.7634
12B	-4.6072	-0.04931	16.4888	-4.173	21.7399	0.30691	-1.687	-10.138	-59.482	-0.3453	-13.924	12.7634
12C	-3.6452	0.688489	8.87153	-4.173	40.4422	3.48498	0.26108	-11.571	-50.17	-0.2395	-13.924	12.7634
12D	-4.2704	0.698307	12.6818	-4.173	49.6135	2.13756	-0.712	-8.2297	24.2506	-0.175	-13.924	12.7634
12E	-1.79	0.973027	21.7185	-4.173	85.3	5.07158	-0.2252	-15.871	89.2713	-0.4089	-13.924	12.7634
15A	0.87887	-0.28617	-14.563	-3.2173	-30.735	1.17436	0.26108	29.7796	-68.797	-0.2966	-13.924	-40.611
15B	3.3623	-0.40478	-11.682	-3.2173	-29.413	-0.1174	0.26108	22.1978	107.834	-0.1923	-13.924	12.7634
15C	7.67105	0.639394	-7.8462	-1.3068	0.93817	3.00389	-1.687	5.10517	52.127	-0.0427	-13.924	12.7634
15D	-2.0783	-0.34546	-21.306	1.55692	-40.203	-2.531	-1.687	-3.4624	-31.553	-0.3164	-13.924	12.7634
15E	-4.559	-0.52354	-24.692	-0.352	-45.853	-3.5561	0.26108	8.90805	-22.247	0.29741	-13.924	-40.611

16A	-100	-100	-100	1.55692	-100	-100	1.71763	-1.5571	-171.42	-0.6553	-13.924	12.7634
16B	-0.5223	-0.37512	5.05769	1.55692	-8.1394	2.13756	-0.712	16.5058	-3.6423	-0.3015	-13.924	12.7634
16C	-2.6356	-0.51364	-3.5386	1.55692	-22.553	-0.9954	0.26108	14.1326	-82.772	0.73702	-13.924	-40.611
16D	-6.9667	-0.78149	-19.858	1.55692	-43.913	-4.1368	-2.6646	-4.892	-8.2929	0.21841	-13.924	12.7634
16E	-2.1551	-0.49384	-16.967	1.55692	-37.09	-3.498	0.26108	10.3334	-59.482	0.41779	-13.924	12.7634
17A	3.6977	0.403571	-6.8883	1.55692	-2.959	1.36705	-0.2252	-15.871	-68.797	-0.3562	-13.924	12.7634
17B	-0.0424	-0.42457	-1.6263	1.55692	-17.979	-0.0692	1.23247	-8.7068	-50.17	-0.1576	-13.924	12.7634
17C	0.72536	-0.29605	-5.4522	1.55692	-19.01	0.88527	2.20246	-8.7068	-59.482	0.6031	39.4504	12.7634
17D	-0.8776	-0.26641	5.05769	0.60261	-4.1668	-1.6229	2.20246	24.0941	-40.861	0.22334	39.4504	12.7634
17E	5.51759	0.492038	-7.8462	1.55692	-2.6975	2.04127	-0.2252	3.67835	1.00767	0.33689	-13.924	12.7634
18A	-3.5009	0.835708	19.8174	-4.173	73.2183	3.29257	-1.687	-15.871	-54.826	-0.4059	-13.924	12.7634
18B	-3.7798	0.452724	21.2432	-1.3068	56.2482	3.05201	0.26108	-17.306	-154.6	-0.2568	-13.924	12.7634
18C	-5.5216	-0.35534	10.7771	1.55692	0.03542	0.36476	-1.687	-17.306	-162.08	-0.3065	-13.924	-40.611
18D	-4.8478	0.737573	-4.4953	-4.173	11.2005	1.94497	-1.1992	-11.093	-68.797	-0.4059	-13.924	-40.611
19A	-2.1551	-0.56317	-21.789	1.55692	-43.131	-0.2717	-3.6457	-24.972	-246.29	1.02617	-13.924	12.7634
19B	5.2782	-0.35534	-100	1.55692	-100	2.61892	0.26108	-18.263	-312.03	-0.7315	-13.924	12.7634
19C	4.76106	0.088661	-5.4522	-4.173	-8.6011	2.75367	0.26108	-18.263	-50.17	-0.7756	-13.924	12.7634
19D	-1.963	-0.67224	-23.723	1.55692	-46.187	-2.0092	-0.712	-2.0333	-106.08	-0.386	-13.924	12.7634
20A	-0.9928	-0.46414	13.6338	1.55692	-1.8276	-0.9664	-0.2252	-12.048	5.65721	0.1695	-13.924	12.7634
20B	2.77765	0.383906	2.19474	1.55692	14.5764	1.09728	-2.6646	-15.393	-124.73	0.21841	-13.924	12.7634
20C	-2.6356	-0.69209	-16.967	1.55692	-40.208	-2.2025	2.68698	1.03284	117.112	0.4947	-13.924	12.7634
20D	2.99811	0.354404	-6.8883	1.55692	-4.2191	2.13756	-0.2252	-10.138	33.5445	-0.5554	-13.924	12.7634
20E	-2.2032	-0.04931	-6.4095	1.55692	-13.821	-2.4924	4.13887	0.82341	-31.553	0.94753	-13.924	12.7634
21A	-1.1753	0.452724	9.348	-4.173	31.6639	-0.5611	0.26108	-9.6611	-31.553	-0.1725	39.4504	12.7634

21B	-1.3866	0.501865	-16.005	-4.173	-19.621	1.94497	0.74696	15.5567	-59.482	0.08003	39.4504	12.7634
21C	1.15708	0.87495	-12.161	-3.2173	-3.9385	4.35058	-0.712	-13.003	-3.6423	-0.0833	39.4504	12.7634
21D	-1.7228	0.383906	-15.524	-4.173	-20.614	0.69252	-1.1992	-15.393	5.65721	0.00087	39.4504	12.7634
21E	-1.963	0.501865	-18.412	-3.2173	-24.685	-0.2717	-0.712	-12.526	5.65721	-0.0437	39.4504	12.7634
k	-5.2328	-0.84119	9.82441	1.55692	-22.104	-0.4646	2.68698	-10.616	-143.4	0.10969	39.4504	12.7634
S	9.45954	1.404115	-5.4522	-4.173	27.004	0.24906	-1.687	15.082	-78.113	-0.2568	-13.924	-40.611

Genotyping data for all the 30 SSR primer pairs with 94 samples

Primer No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Primer Name	CLM 0186	CLM 0244	CLM 0008	CLM 0177	CLM 0279	CLM 0322	CLM 0168	CLM 0195	CLM 0200	CLM 0088	CLM 0260	CLM 0218	CLM 0077	CLM 0063	CLM 0083
1A	2	2	0	1	2	2	0	2	0	2	2	0	2	0	2
1B	2	2	0	1	0	1	2	2	0	2	2	0	0	0	0
1C	2	2	0	1	2	1	0	1	0	1	2	0	2	1	2
1D	2	2	0	1	1	1	0	1	0	1	0	1	2	1	2
1E	2	2	0	2	0	2	0	2	0	0	2	0	2	0	2
2A	2	2	0	1	0	1	0	2	0	2	0	0	2	0	0
2B	2	2	0	1	0	2	0	2	0	2	2	0	2	1	0
2C	2	2	0	1	0	1	0	2	0	2	1	0	2	1	0
2D	2	2	0	2	0	2	0	1	0	1	2	0	2	2	0
2E	2	2	0	1	0	2	0	1	0	2	0	0	2	1	0
3A	2	2	0	2	0	1	0	0	0	2	2	0	0	2	0
3B	2	2	0	1	0	1	0	1	0	0	2	0	0	1	0
3C	2	2	0	2	0	0	0	2	2	0	2	0	0	0	2
3D	2	2	0	2	0	2	0	1	2	2	2	0	0	2	0
3E	2	2	0	2	0	0	0	2	2	0	2	0	0	2	0
4A	2	2	0	2	0	0	0	2	2	0	0	2	2	0	0
4B	2	2	0	2	0	0	0	2	2	2	0	0	2	0	0
4C	2	2	0	2	0	2	1	2	2	0	0	0	0	0	0
4D	1	2	0	2	0	2	1	2	2	0	2	0	1	0	0
4E	0	2	0	2	0	2	0	2	0	0	0	0	1	0	0
5A	2	2	0	2	0	2	-1	1	0	2	0	0	2	2	2
5B	2	2	0	2	0	2	0	1	0	2	2	1	1	2	2
5C	1	1	0	2	0	2	0	1	0	2	0	2	1	2	2
5D	0	0	0	2	0	2	0	2	2	0	2	2	1	1	2
5E	1	0	0	2	0	2	0	2	2	0	0	2	0	2	0
6A	0	0	0	2	0	2	0	2	2	0	0	2	2	2	2

6B	0	2	0	2	0	2	0	2	2	0	0	2	0	2	2
6C	0	1	0	2	0	2	2	1	2	0	0	2	2	2	2
6D	0	1	0	1	0	1	0	1	0	0	2	0	0	1	2
7A	2	2	0	2	0	1	0	1	0	0	2	0	0	2	0
7B	2	0	0	1	0	1	0	0	2	0	0	0	0	1	0
7C	2	0	0	2	0	2	0	1	2	0	0	0	0	1	0
7D	0	2	0	2	0	1	0	2	2	0	2	0	0	2	0
7E	1	2	0	1	0	1	0	2	2	0	2	0	0	2	0
8A	2	2	0	2	0	2	0	2	0	2	0	2	2	1	2
8B	2	2	0	2	0	2	0	2	2	2	0	2	2	1	2
8C	1	2	0	2	0	2	0	2	1	2	1	2	2	1	2
8D	1	2	0	2	1	1	0	2	1	2	1	2	2	1	2
8E	2	2	0	2	0	1	2	2	1	2	2	0	2	1	2
9A	2	2	0	2	0	2	2	2	2	0	0	0	0	2	0
9B	1	1	0	2	1	0	2	0	0	0	0	0	0	1	1
9C	2	2	0	2	0	2	2	2	2	0	1	1	0	2	1
9D	1	2	0	2	0	0	2	2	0	0	2	1	0	0	0
9E	2	1	0	2	0	0	2	0	2	0	2	1	0	0	0
10A	2	2	0	2	0	0	2	0	2	0	0	2	1	0	0
10B	2	2	0	2	0	0	2	0	2	0	1	1	1	2	1
10C	1	1	0	2	0	1	0	2	0	0	2	1	1	2	0
10D	1	2	0	2	0	1	0	2	2	0	2	1	1	2	0
10E	2	2	0	2	0	1	0	0	2	0	0	2	0	2	0
11A	2	2	0	2	1	2	0	0	2	2	0	2	2	0	2
11B	2	2	0	2	0	0	0	2	2	2	0	2	1	0	2
11C	1	2	0	2	1	0	0	2	2	2	0	1	1	0	2
11D	2	2	0	2	2	2	0	2	2	2	2	1	0	0	2
11E	-1	-1	-1	-1	-1	-1	2	-1	-1	-1	-1	-1	0	-1	-1
12A	1	2	0	1	1	0	2	0	2	2	2	0	0	2	1
12B	2	2	0	1	1	2	2	0	0	2	2	0	2	0	2
12C	2	2	0	1	2	0	2	0	0	2	2	0	2	0	2
12D	1	2	0	1	0	2	0	0	0	0	2	0	0	2	0

12E	1	2	0	1	0	0	2	0	0	2	0	0	1	2	1
15A	2	0	0	2	0	0	2	0	0	2	2	1	2	2	0
15B	2	0	0	2	0	2	0	0	0	2	0	1	2	1	2
15C	2	2	0	2	0	2	2	0	0	2	0	2	2	1	2
15D	2	2	0	1	0	0	0	0	0	2	0	2	1	1	2
15E	2	0	0	2	0	2	0	1	2	2	0	2	1	2	2
16A	0	2	0	2	0	0	2	1	2	2	0	2	0	2	2
16B	2	0	0	2	0	0	2	1	2	2	0	2	1	2	2
16C	1	1	2	1	2	2	1	1	0	2	0	2	1	0	2
16D	2	2	0	2	2	0	-1	2	0	2	0	2	1	2	2
16E	2	2	0	2	2	0	2	0	0	2	0	2	0	0	2
17A	2	2	0	2	2	0	0	0	0	2	0	2	0	2	2
17B	2	2	0	1	0	0	1	0	0	2	0	2	0	2	1
17C	2	0	2	2	0	2	1	2	0	2	0	2	0	2	2
17D	2	2	0	2	0	2	2	0	0	2	0	2	1	2	1
17E	2	0	0	2	0	2	0	0	0	2	0	2	0	2	0
18A	2	2	0	1	0	2	2	0	2	0	2	2	2	2	0
18B	2	0	0	1	2	0	2	0	2	0	2	2	2	0	2
18C	2	2	0	2	2	2	0	2	2	0	0	1	2	0	2
18D	2	2	0	2	2	0	2	0	0	0	2	1	2	0	2
19A	2	2	2	2	0	2	0	2	0	0	2	1	0	2	2
19B	2	0	2	1	0	2	0	0	0	0	2	0	0	2	2
19C	2	2	2	1	0	2	0	0	0	0	2	2	0	2	2
19D	1	2	0	2	0	2	0	0	2	0	2	2	0	2	2
20A	2	2	0	2	0	2	2	0	2	2	0	2	2	2	2
20B	2	2	0	0	2	2	2	0	2	2	0	2	2	2	2
20C	2	2	0	0	0	0	2	0	2	2	0	2	2	2	0
20D	0	2	0	0	0	0	2	0	0	2	0	2	2	2	0
20E	0	0	0	2	0	2	0	2	0	2	0	2	2	2	0
21A	2	0	0	2	0	2	0	2	2	2	0	2	2	0	0
21B	0	2	0	0	0	2	0	2	0	2	0	2	2	0	0
21C	2	2	2	0	0	2	0	2	0	2	0	2	2	0	0

21D	0	2	2	0	0	2	0	2	0	2	0	2	2	0	0
21E	0	0	2	0	0	2	0	0	0	2	0	2	2	0	0
k	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Primer No.	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Primer Name	CLM 0292	CLM 0101	CLM 0156	CLM 0251	CLM 0119	CLM 0068	CLM 0007	CLM 0050	CLM 0201	CLM 0151	CLM 0066	CLM 0031	CLM 0085	CLM 0287	CLM 0300
1A	2	1	2	0	0	2	2	2	2	2	2	2	0	2	0
1B	0	1	1	2	2	1	2	0	0	2	2	2	0	0	2
1C	2	1	1	1	2	1	2	0	2	2	2	2	0	1	2
1D	2	1	1	1	2	1	2	0	2	2	2	2	0	1	2
1E	2	0	1	1	0	1	2	0	2	2	0	2	2	2	0
2A	1	1	1	1	0	0	2	0	2	2	0	2	2	2	2
2B	1	1	1	0	2	2	1	0	2	2	0	2	2	2	2
2C	1	1	1	2	2	2	1	0	2	2	0	2	2	1	2
2D	1	1	1	1	2	2	2	0	2	2	0	2	2	2	2
2E	2	2	1	1	2	2	2	0	2	2	0	2	0	0	2
3A	0	2	1	0	2	2	2	0	2	0	2	0	0	0	2
3B	0	2	1	0	2	2	2	0	2	1	2	0	0	0	2
3C	0	2	0	0	0	0	0	0	2	1	2	0	0	2	0
3D	2	2	2	0	2	0	2	0	2	1	2	0	0	2	2
3E	-1	2	2	0	2	0	0	0	2	2	2	0	2	2	0
4A	2	2	2	0	2	0	0	0	2	2	2	0	0	2	1
4B	2	1	2	2	1	2	0	0	2	2	0	0	0	2	1
4C	2	1	0	2	1	0	0	2	2	0	0	0	0	2	1
4D	2	1	1	0	0	2	0	2	2	0	0	0	0	2	1
4E	2	1	0	0	2	2	0	2	2	0	0	0	0	2	1
5A	2	2	1	0	1	2	0	1	2	0	0	0	2	2	1
5B	0	0	2	0	2	2	2	2	2	1	0	0	0	0	1

Sample Names

5C	0	0	0	0	2	2	2	1	0	1	0	0	0	0	1
5D	1	0	2	0	0	2	0	2	0	0	2	1	2	0	1
5E	0	2	0	0	2	2	2	1	0	0	0	1	2	0	1
6A	2	0	0	2	2	2	0	1	0	0	2	0	2	0	1
6B	2	0	1	0	0	2	0	2	0	2	2	0	2	0	1
6C	2	0	0	0	1	2	0	0	0	2	2	0	2	0	1
6D	1	2	2	0	0	1	2	1	0	1	2	0	2	0	1
7A	0	1	1	0	0	1	1	1	0	1	0	0	0	2	1
7B	0	1	1	0	2	0	2	1	0	1	0	0	0	2	1
7C	0	1	1	0	1	0	2	1	0	2	0	2	0	2	1
7D	0	2	0	2	1	0	2	1	0	2	0	0	2	2	1
7E	0	2	2	0	1	0	2	2	0	2	0	2	2	1	1
8A	0	0	0	2	2	0	2	2	0	2	2	0	0	1	0
8B	0	0	0	2	2	0	1	2	0	2	2	2	2	2	1
8C	0	0	0	0	1	0	0	2	0	2	0	2	0	2	0
8D	0	0	2	0	1	0	2	2	0	2	0	0	1	0	1
8E	1	0	0	0	2	0	2	0	0	2	0	2	2	2	0
9A	0	-1	2	2	0	2	2	2	0	0	2	0	1	2	-1
9B	0	2	1	2	2	2	2	0	1	0	0	2	0	2	1
9C	0	0	0	2	2	2	2	0	1	0	0	2	0	2	0
9D	0	2	2	0	1	2	0	1	1	2	1	2	2	2	1
9E	0	2	2	0	2	2	0	1	1	2	2	0	2	2	1
10A	0	2	2	2	2	2	0	1	1	2	2	2	2	0	1
10B	0	0	0	1	1	2	2	1	1	1	0	2	0	2	1
10C	0	1	2	1	1	2	1	1	2	2	0	2	2	2	1
10D	0	1	0	1	2	2	2	1	2	2	0	2	2	2	1
10E	0	1	2	1	2	2	2	2	0	2	0	2	2	1	1
11A	2	1	1	1	2	0	2	2	0	2	0	0	0	0	0
11B	2	1	1	2	2	0	0	0	0	0	0	2	0	2	1
11C	0	0	0	2	2	0	0	1	0	1	0	0	0	2	1
11D	2	2	2	2	2	0	0	2	0	0	0	0	0	2	1
11E	2	-1	-1	-1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

12A	2	0	0	2	2	0	0	2	0	1	2	2	2	0	-1
12B	2	2	0	2	2	0	2	0	0	2	2	2	2	2	1
12C	2	2	0	2	2	2	0	0	2	1	2	2	2	2	1
12D	0	0	2	2	2	0	0	2	2	2	0	0	2	0	1
12E	2	0	2	0	2	0	0	2	2	2	2	0	2	2	1
15A	2	2	2	0	0	2	2	2	0	2	2	0	2	0	1
15B	2	0	2	0	2	2	2	2	1	2	0	1	2	0	1
15C	2	0	2	0	0	2	2	2	1	2	2	1	2	0	1
15D	2	2	2	0	1	2	2	2	1	1	2	1	2	0	1
15E	2	0	1	2	1	1	0	2	0	1	2	1	2	0	1
16A	2	0	2	0	2	1	2	0	0	1	2	2	0	2	0
16B	2	0	1	0	1	1	2	2	0	1	2	2	2	2	1
16C	2	0	1	0	1	2	2	2	2	1	2	2	1	2	0
16D	1	0	0	2	2	0	2·	0	0	1	1	2	0	2	0
16E	1	0	0	2	1	0	2	0	0	1	1	1	1	2	1
17A	1	2	0	2	1	0	2	2	0	2	1	1	0	0	1
17B	2	0	0	0	1	0	2	2	2	1	0	2	1	0	0
17C	0	0	0	0	2	0	2	0	2	2	0	1	0	0	1
17D	1	0	0	0	1	0	2	2	0	2	1	1	1	0	1
17E	0	2	2	0	0	2	0	2	2	2	2	0	2	0	1
18A	2	0	0	0	0	0	2	2	2	2	1	0	2	0	2
18B	0	0	0	0	2	0	0	2	2	1	0	0	0	2	2
18C	0	2	2	2	2	1	0	2	2	1	1	2	0	0	0
18D	2	2	2	2	0	1	0	0	0	1	0	0	0	2	2
19A	2	0	2	2	0	2	2	0	0	2	2	2	1	2	2
19B	2	0	2	2	2	2	2	0	0	1	2	2	0	2	0
19C	2	0	2	2	0	2	2	0	2	1	2	2	1	2	2
19D	2	2	2	0	0	2	2	2	2	1	2	2	1	2	1
20A	2	2	2	0	0	0	2	2	2	1	2	0	1	2	1
20B	2	0	2	0	0	0	2	0	2	1	2	0	2	2	1
20C	0	2	2	2	2	0	0	0	0	1	0	0	2	1	0
20D	0	2	0	2	0	2	0	0	0	1	2	0	2	1	2

20E	0	2	2	0	2	2	0	2	2	2	2	0	2	2	2
21A	0	0	1	2	0	2	0	2	2	1	0	2	2	2	2
21B	2	0	2	0	2	2	2	2	0	1	0	0	0	2	2
21C	0	0	2	0	0	2	2	2	0	2	0	0	2	0	2
21D	2	2	2	0	0	2	0	0	0	1	0	0	2	0	2
21E	2	2	2	2	0	2	2	0	0	1	0	0	2	2	2
k	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

QTL MAPPING FOR YIELD TRAITS IN VEGETABLE COWPEA

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most cultivated pulse crops in the semi-arid tropics of Asia, Africa, Southern Europe, and other parts of the world. It is used for both vegetable and fodder purpose. In India, kharif crop of vegetable cowpea is cultivated in an estimated area of 0.5 million hectares in states like Kerala, Karnataka, Tamil Nadu and Madhya Pradesh. Studies aimed at increased yield among crops were always challenged by the quantitative nature of traits. These quantitative traits are generally governed by multiple genes present in regions of the genome called quantitative trait loci (QTL). With the advent of molecular markers it is possible to localize the QTL with the help of linked markers, a process now widely known as QTL mapping. QTL mapping depicts the relative positioning of different markers on the chromosomes and their linkage to a specific trait. In cowpea, even though there has been few mapping efforts for traits such as resistance to *Thrips tabaci* and *Frankliniella schultzei*, flowering time, pod length and seed weight, an elaborate QTL map for yield and related traits is missing.

Hence, the study "QTL mapping for yield traits in vegetable cowpea" was undertaken with the objective of mapping the SSR markers and identifying the quantitative trait loci for yield components in the genome of vegetable cowpea at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, during February 2016 to June 2017.

F_3 plants maintained at CPBMB, derived from the cross of Sharika which is a pole type, long poded, high yielding but anthracnose and cowpea mosaic virus susceptible cultivar with Kanakamony which is a semi-trailing, medium-long poded, low yielding, anthracnose immune and cow pea mosaic virus resistant cultivar, were used to raise the F_4 mapping population. Morphological observation for traits pod length, individual pod weight (IPW), pod number, days taken for first flowering (DTFF), total dry pod yield (TDPY), grains per pod, branch number, root length, plant height, plant weight, and response to anthracnose and cowpea mosaic virus diseases were recorded.

High quality DNA was isolated from the parents and mapping population using the protocol standardized in this study. One hundred SSR primer pairs reported in cowpea were screened among the parental DNA for polymorphism. Thirty polymorphic primer sets were carried forward to genotype the F_4 mapping population.

The morphological and genotypic data were used to construct a linkage map using software ICIMapping. Two linkage groups, one having eight SSR markers distributed across 637 cM and another one having five SSR markers distributed across 271 cM were obtained. Two approaches, Single Marker Analysis (SMA) and Inclusive Composite Interval Mapping (ICIM) otherwise called Additive Linkage Mapping were followed for QTL mapping. LOD value threshold of 3.0 was used to determine the significance of QTL and linked markers.

Multiple QTL hotspots were observed for different traits under study. An anchored marker, CLM0083 has been identified which was significantly linked to traits individual pod weight and total dry pod yield. The region between 25 cM to 125 cM on linkage group 1 had QTL hotspots harboring genes governing traits DTFF, TDPY, root length, plant length and plant height. This entire region was bracketed by two markers, CLM0244 at 24.25 cM and CLM0177 at 126.86 cM with an anchored marker CLM0008.

This marker combination could be potentially used in marker assisted selection for the traits DTFF, TDPY, root length, plant length and plant height. Fine mapping of the QTL for these traits with large number of markers would provide more insights into the genes and hot spots involved in the yield contributing traits in cowpea.