MAPPING THE QTL FOR YIELD TRAITS IN BITTER GOURD

(*Momordica charantia* L.)

By

LAVALE SHIVAJI AJINATH

(2018-21-044)

CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

COLLEGE OF AGRICULTURE, VELLANIKKARA

THRISSUR – 680656

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

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CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

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THRISSUR – 680656

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2022

DECLARATION

I hereby declare that the thesis entitled **"Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not 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: 16.03.2022

Lavale Shivaji Ajinath

(2018-21-044)

CERTIFICATE

Certified that the thesis entitled **"Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)"** is a record of research work done independently by **Mr. Lavale Shivaji Ajinath (2018-21-044)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

Vellanikkara

Date: 16.03.2022

Dr. Deepu Mathew
(Chairperson, Advisory committee)
Professor,
Centre for Plant Biotechnology and Molecular Biology
College of Agriculture,
Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Lavale Shivaji Ajinath (2018-21-044),** a candidate for the degree of **Doctor of Philosophy in Agriculture** with major field in **Plant Biotechnology,** agree that the thesis entitled **"Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)"** may be submitted by **Mr. Lavale Shivaji Ajinath,** in partial fulfilment of the requirement for the degree.

16/3/2022

Dr. Deepu Mathew
(Chairperson, Advisory committee)
Professor,
Centre for Plant Biotechnology and
Molecular Biology
College of Agriculture, Vellanikkara

16/3/22

Dr. Abida P. S.
Professor and Head,
Department of Plant Biotechnology,
College of Agriculture,
Vellanikkara

16/3/2022

Dr. T. Pradeepkumar
Professor and Head,
Director of Planning,
Department of Vegetable Science,
College of Agriculture,
Vellanikkara

16/3/22

Dr. Jiji Joseph
Professor and Head,
Department of Plant Breeding
and Genetics,
College of Agriculture,
Vellanikkara

16.3.22

Dr. Joseph John K.
Principal Scientist,
NBPGR-RS, Thrissur

16/3/22

Dr. Asha Devi A.
(External examiner)
Principal Scientist,
ICAR-CTCRI
Sreekariyam, Thiruvananthapuram

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Lavale Shivaji Ajinath

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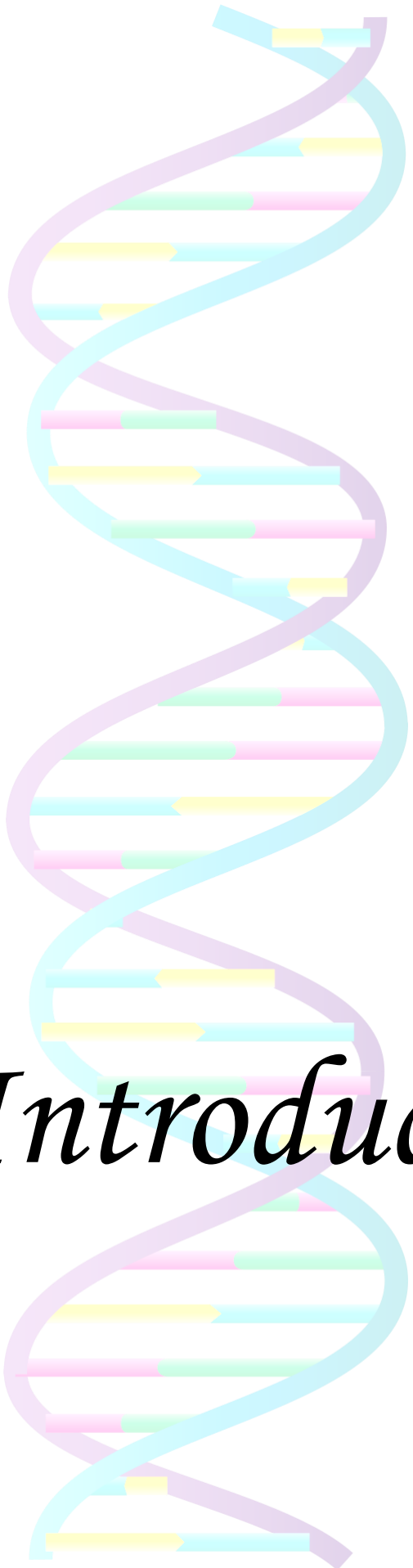


Introduction

1. INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is an important commercial cucurbit belonging to the family Cucurbitaceae. It is originated in tropical Africa and later spread to Asia and other parts of the world. Among sixty species, of which 47 are found in Africa and 13 in south-east Asia (Schaefer and Renner, 2010), *M. charantia* is widely cultivated. This species is distributed in China, Malaysia, India and tropical Africa.

The fruits of bitter gourd are rich in β-carotene, vitamin C, folic acid (vitamin B9), magnesium, phosphorus and potassium (Dhillon *et al*., 2017). Besides it is known to contain substantial medicinal compounds, which are important against diabetes (Chen *et al*., 2003). It also contains hypoglycaemic compounds, anticarcinogenic and hypercholesterolemic compounds, charantin, and momorcharin, and compounds exhibiting anti-HIV activity and momordicoside A and B (Okabe *et al*., 1980). Bitter gourd seed oil contains high value potential nutraceuticals such as lipids, mainly α-eleostearic acid and considerable levels of phytosterols (Yoshime *et al*., 2016).

Most of the cucurbitaceous vegetables including bitter gourd are usually cultivated in relatively small areas for local consumption and do not enter the production statistics in a significant way. India is considered as the primary centre of diversity of bitter gourd and China as the secondary centre of diversity (Grubben, 1977). In India, it is grown throughout the country as rainy and summer season vegetable. India produces about 10.83 lakh metric tonnes of bitter gourd from an

area of 0.96 lakh hectare (Mallikarjunarao *et al*., 2018). The leading states under bitter gourd cultivation in India include Chattisgarh, Telangana, Andhra Pradesh, Orissa, Madhya Pradesh, Uttar Pradesh, Bihar, Tamil Nadu, Haryana, Maharashtra, Gujarat, Kerala, etc. In India, there is a wide range of variability available in bitter gourd and hence, there is a vast opportunity for its genetic improvement (Behera *et al*., 2010). The consumer preferences vary from region to region with respect to fruit colour, fruit length, fruit diameter, fruit shape, fruit size and tubercles (Dey *et al.*, 2010). Dark green long fruited types (15-40 cm) are preferred in north India, while medium long fruited types (12-20 cm) are common in south India, whereas short fruited types are in high demand in eastern parts of India (Mishra *et al*., 2015).

Breeders intend to breed bitter gourd for higher yield, earliness of harvesting, gynoecy, fruit size, fruit shape, fruit color, disease and pest resistance *etc*. (Rao, 2021). Earliness and fruit yield is directly controlled by some of the flower and fruit related traits and are the major traits which will directly help the farmers to take the produce early in the market with high quantity to fetch better profits. Conventional breeding procedures like selection of desired plant in a large population is a laborious and challenging task. Selection of plant at full maturity at every generation takes a very long time for varietal improvement. Moreover, the introgression of desired traits from multiple parents becomes highly difficult in traditional breeding. Marker assisted selection (MAS) of desired recombinants from a population always assures the presence of favourable alleles and fast recovery of recurrent parent genome in the cultivar under improvement.

Use of molecular markers for the aid of selection requires full proof knowledge of genomic locations governing a trait and tightly linked. The success of MAS always depends on high-quality genetic map and mapping of quantitative trait loci (QTL) for the target traits. Genetic maps have been constructed for the cucurbits such as cucumber (Wang *et al.*, 2005; Sun *et al.*, 2006; Yuan *et al.*, 2008; Miao *et al.*, 2011; Zhang *et al.*, 2012) and melon (Harel-Beja *et al.*, 2010; Yuste-Lisbona *et al.*, 2010; Diaz *et al.*, 2011). In bitter gourd, limited investigations have been done to identify the loci governing yield related traits using dominant makers such as AFLP (Kole *et al.*, 2012; Wang and Xiang, 2013) and co-dominant markers such as SNPs (Matsumura *et al.*, 2014; Cui *et al.*, 2018; Rao *et al.*, 2018; Rao *et al*., 2021). A considerable number of microsatellite markers are reported (Wang *et al.*, 2010; Guo *et al.*, 2012; Ji *et al.*, 2012; Saxena *et al.*, 2015; Dhillon *et al.*, 2016), but a genetic map accommodating these valuable microsatellite markers is yet to be reported. A systematic study to map microsatellites in bitter gourd genome will also help in locating the yield and other important traits in near future.

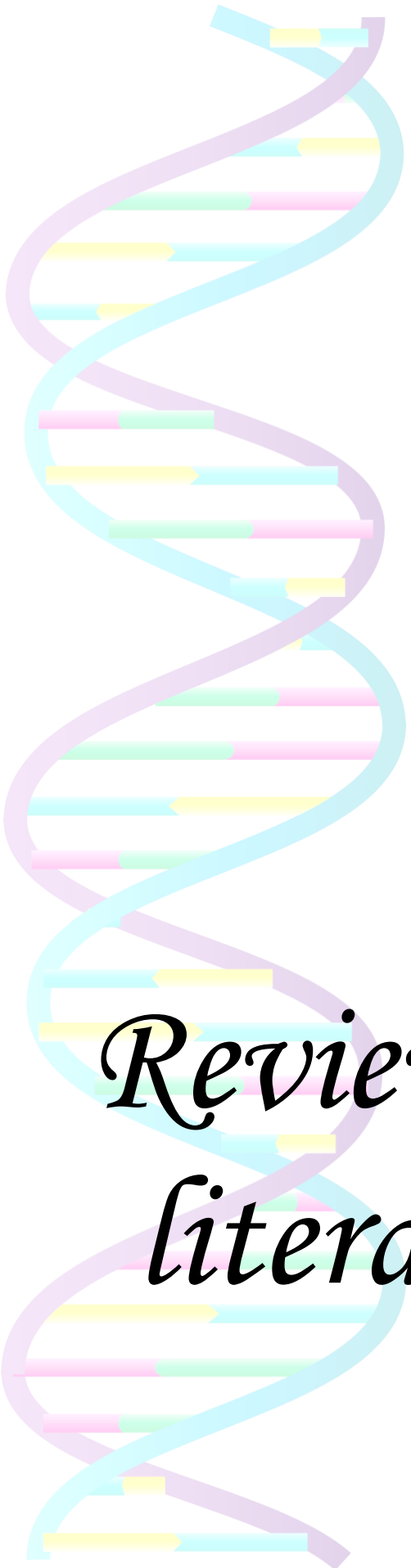
With this background, the present study entitled 'Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)' was taken up with the objective to map the quantitative trait loci and to develop a chromosome-wise fine map for yield traits in bitter gourds with following underlying experiments:

i. Development of an $F_{2:3}$ mapping population representing good variability for the yield related traits
ii. Study of parental polymorphism using microsatellite markers
iii. Phenotyping of mapping population for yield-related traits

iv. Genotyping the mapping population using polymorphic microsatellite markers

v. Construction of linkage map

vi. Identification of QTL associated with yield related traits



Review of literature

2. REVIEW OF LITERATURE

The investigations on "Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)" was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Agriculture, Kerala Agricultural University, Thrissur, with the objective to map the quantitative trait loci and to develop a chromosome-wise maps for yield traits in bitter gourd. A detailed review on the available literature in this line is presented below.

2.1 IMPORTANCE OF BITTER GOURD

Bitter gourd (*Momordica charantia*; 2n = 2x = 22) is a tropical and subtropical vine belonging to family Cucurbitaceae, and widely cultivated in Asia, Africa, and the Caribbean, for its edible and medicinal fruits (Grover and Yadav, 2004; Marr *et al*., 2004; Van Wyk, 2015). Immature fruits and seeds of bitter gourd, rich in β-carotene, vitamin C, folic acid (vitamin B9), magnesium, phosphorus and potassium, are consumed together (Dhillon *et al*., 2017; Yuwai *et al*., 1991). It is helpful against diabetes prevailing in India, China and Central America (Chen *et al*., 2003). It also contains hypoglycaemic compounds (Jayasooriya *et al*., 2000); anticarcinogenic and hypercholesterolemic compounds (Ahmed *et al*., 2001; Ganguly *et al*., 2000); charantin (Yeh *et al*., 2003); momorcharin (Leung *et al*., 1997); compounds exhibiting anti-HIV activity (Lee *et al*., 1995) and momordicoside A and B (Okabe *et al*., 1980). It also possess antimicrobial (Yeşilada *et al*., 1999), antiviral (Nerurkar *et al*., 2006), antiulcerogenic (Gurbuz *et*

al., 2000), steroidal (Grover and Yadav, 2004) and antitumor (Fang and Ng, 2011) properties. Bitter gourd seed oil contains high value potential nutraceuticals such as lipids, mainly α-eleostearic acid and considerable levels of phytosterols (Yoshime *et al*., 2016). It is known that seed lipid of bitter gourd contains more than 50 per cent conjugated linolenic acids (CLN), and it remarkably inhibits the development of AOM-induced colonic aberrant crypt foci (ACF) (Kohno *et al*., 2002). Small but distinct amount of CLN are also found in the flesh of bitter gourd (Suzuki *et al*., 2001). The seed coat is considered diacritical in the taxonomy of the *Momordica* genus (Aguoru and Okoli, 2009).

The genus *Momordica* is originated in tropical Africa and later spread to Asia and other parts of the world (Schaefer *et al*., 2009; Schaefer and Renner, 2010). It comprises 60 species, of which 47 are found in Africa and 13 in south-east Asia (De Wilde and Duyfies, 2002). A cultivated species *M. charantia* consists of two botanical varieties *viz*., *M. charantia* var. *muricata*, a wild variety with small and round fruits having markedly sculptured seeds and *M. charantia* var. *charantia*, which produces large fusiform fruits (Chakravarty, 1990).

2.2 BITTER GOURD GENOME

A monoecious inbred line, OHB3-1, was used for the first *de novo* sequencing of bitter gourd genome (Urasaki *et al*., 2017). Different types of libraries, including libraries of paired-end PCR-free and mate-pair with different sizes, were prepared and 37 Gb of sequence data was used in *de novo* assembly, which was approximately 110 times that of the estimated genome size of 339 Mb (Urasaki *et al*., 2015). ALLPATH-LG assembler (Gnerre *et al*., 2011), which is

expected to develop high-quality assembly, was used for this genome assembly. The total length of the assembled scaffolds of OHB3-1 genome was 285.5 Mb, which comprised 1029 scaffolds, corresponding to approximately 84% of the previously estimated genome size (339 Mb).

Using Illumina Hiseq2000 genome analyzer, Rasheed *et al*. (2020) have sequenced and assembled 162,471 bp of the chloroplast genome of *M. charantia* (GenBank accession number MG019415), which included a pair of invert repeat (IR) regions of 29,671 bp each, a large single copy region (LSC) of 76,932 bp and a small single copy region (SSC) of 26,197 bp. With 36.7% GC content, chloroplast genome had 147 genes including 31 tRNA genes, 4 rRNA genes, 27 duplicated genes and 85 protein coding genes.

2.3 MOLECULAR MARKERS IN BITTER GOURD

There are three types of genetic markers: morphological markers, biochemical markers and DNA markers. Morphological markers are usually visually characterized phenotypic characters such as flower color, seed shape, growth habits or pigmentation. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining (Tanksley and Rick, 1980; Tanksley, 1983). The major disadvantages of these two markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995).

Molecular markers are stretch of DNA sequence showing polymorphism between different individuals without any ambiguity (Jiang, 2013). These markers are unlimited in number and are not affected by environmental factors and

developmental stages of the plant (Winter and Kahl, 1995). The differences that distinguish one plant from another are encoded in the plant's genetic material, the deoxyribonucleic acid (DNA). They are used to 'flag' the position of a particular gene or the inheritance of a particular characteristic. In a given cross, the characteristic/ trait of interest should stay linked with the molecular markers. Thus, individuals can be selected based on the presence or absence of molecular marker, since the marker indicates the desired characteristic (Winter and Kahl, 1995).

DNA marker systems are classified as hybridization based marker systems and polymerase chain reaction (PCR) based marker systems. In the first category are RFLPs which are visualized by hybridization of restriction enzyme digested DNA to a labelled probe of known sequence or origin. However, RFLP markers are not preferred nowadays due to complexity of protocols. PCR based markers, which utilize the technique of polymerase chain reaction are the most useful markers due to their simplicity, robustness and speed of assay. The suitability of a marker is determined by several considerations such as ease of assay, ability to discriminate between individuals, the frequency of occurrence of the marker (abundance) and more importantly the type of marker: Co-dominant or Dominant (Winter and Kahl, 1995).

DNA marker systems including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs), have been developed in various crop plants. Among them, SSR markers are used in a variety of applications in plant genetics and breeding

because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage. SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variation (Winter and Kahl, 1995).

Use of molecular markers in bitter gourd breeding is still at an introductory phase. Marker systems such as RAPD (Behera *et al*., 2008; Dey *et al*., 2006), AFLP (Behera *et al*., 2008; Gaikwad *et al*., 2008; Kole *et al*., 2009a), SSR (Dhillon *et al*., 2016; Kole *et al*., 2009a) and inter-simple sequence repeat (ISSR) (Behera *et al*., 2008) have been initially used for genetic diversity and population structure analysis in this crop.

Molecular markers like RAPD, AFLP and ISSR are helpful for genetic diversity analysis, however, they are less preferred in marker assisted selection (MAS) because of its dominant nature and poor reproducibility. Being co-dominant, multi-allelic, highly reproducible and abundant in number throughout the genome, microsatellites or SSR markers holds a great importance among breeders for their use in MAS (Walter and Epperson, 2001). Although the initial cost of SSR marker development is high, once developed, it is highly repeatable and, consequently, easily transferred across laboratories (Maughan *et al*., 1995). Based on the suitability in various genetic analyses, SSR markers provide accurate results with a minimum number of loci. Mapping consists of placing a QTL in a given marker interval. Use of more markers in any mapping programme results in smaller

average interval size and thus higher map resolution. Bitter gourd genome is less explored for mining this very important marker system.

Wang *et al*. (2010) and Guo *et al*. (2012) have isolated 16 and 10 microsatellites, respectively, adopting Fast Isolation by AFLP of Sequences Containing Repeats (FIASCO) method. Ji *et al*. (2012) developed 11 polymorphic *M. charantia* microsatellite loci by scanning the SSR-enriched genomic libraries and examined the genetic diversity of 55 Chinese *M. charantia* germplasm cultivars at these loci. High polymorphism information content (PIC) value of 0.744 with an average of 0.572 suggested that the tested *M. charantia* germplasm has a relatively high genetic diversity at these 11 microsatellite loci. Some of these markers were found to be transferable to eight other cucurbit species based on transferability testing.

Saxena *et al*. (2015) designed unique primer-pairs for 160 microsatellite loci using the SSR-enriched genomic libraries, and 151 loci were amplified. Markers at 40 loci (78.4 %) were transferable to six species, *viz*. *M. cymbalaria*, *M. subangulata* subsp. *renigera*, *M. balsamina*, *M. dioca*, *M. cochinchinesis*, and *M. sahyadrica*. These reported microsatellite markers can be efficiently utilized for construction of fine maps for the desired traits. Subsequently, Dhillon *et al*. (2016) have characterized 114 bitter melon accessions with 50 polymorphic microsatellites. Cui *et al*. (2017) developed 21 SSR markers using the genomes of the bitter gourd lines ‘Dali-11’ and ‘OHB3-1’. A collection of 211 bitter gourd lines from all over the world were genotyped with these 21 SSR markers. A model-based clustering method and phylogenetic analysis indicated a clear separation among the

geographic groups suggesting the potential value of these markers in bitter gourd research. All these studies contributed to a total of 247 microsatellites; however, to date none have been mapped on the *Momordica* genome. A greater number of markers are necessary for the development of a high-density or saturated genetic map and marker-assisted selection (Tang *et al*., 2007).

With the genome resources currently available for bitter gourd, it is now possible to detect genome-wide insertion-deletion (InDel) polymorphisms among bitter gourd populations, which guides the efficient development of InDel markers. Cui *et al*. (2021) have recently identified 2502 InDel markers by mapping paired-end, clean reads of genome sequence of 61 Chinese bitter gourd accessions on the 'Dali-11' reference genome. Of which, 2466 InDel were further validated for their amplification in two bitter gourd lines and 164 were mapped using 113 F_2 individuals.

2.4 IMPORTANT TRAITS IN BITTER GOURD

The bitter gourd is generally bred for higher yield, earliness of harvesting, gynoecy (high female to male ratio of flowers, resulting in the high number of fruits per plant), fruit size (medium-sized fruits 10–15 cm long), fruit shape, fruit surface (smooth surface and continuous smooth ribs or ridges are preferred in many places), fruit color, less mature seeds, moderate bitterness, disease resistance (to important diseases like powdery mildew, downy mildew, mosaic), insect resistance (to important insect pests like red pumpkin beetle and fruit fly) (Rao, 2021).

2.5 GENETICS OF YIELD AND RELATED TRAITS IN BITTER GOURD

There are multiple flower-, fruit-, seed- and vine-related traits which directly or indirectly affects the yield of bitter gourd. The knowledge of genetics of a particular trait facilitates the breeder to design the strategy to improve the trait. Different types of gene actions are reported for all the yield related traits in various studies with populations having different genetic background.

Earliness is reported to be under the control of dominant gene effect (Shrivastava and Premnath, 1972). Days to first female flower is controlled under additive (Pal *et al*., 1983; Singh and Ram, 2003) and duplicate epistasis (Mishra *et al*., 2015; Rao, 2017). First female flower node has duplicate epistasis type of gene action (Mishra *et al*., 2015; Rao, 2017). Fruit length and diameter are controlled by dominant and dominant × dominant (Sirohi and Choudhary, 1980), non-additive (Pal *et al*., 1983; Matoria and Khandelwal, 1999; Sharma and Bhutani, 2000), additive (Pornsuriya and Pornsuriya, 2009), additive and non-additive (Gopalakrishnan, 1986; Dalamu *et al*., 2012), and duplicate epistasis gene actions (Mishra *et al*., 2015; Rao, 2017).

Number of fruits per plant was revealed to be governed by additive and additive × additive (Sirohi and Choudhary, 1980; Pal *et al*., 1983), non-additive (Matoria and Khandelwal, 1999), additive and non-additive (Gopalakrishnan, 1986; Dalamu *et al*., 2012), and duplicate epistasis gene action (Rao, 2017). Days to maturity of fruit is governed by non-additive (Pal *et al*., 1983), and duplicate epistasis type of gene action (Mishra *et al*., 2015; Rao, 2017). Flesh thickness is reported to be under additive and dominant gene action (Singh and Ram, 2003)

whereas fruit weight is under additive (Gopalakrishnan, 1986), non-additive (Pal *et al*., 1983; Sharma and Bhutani, 2000), and duplicate epistasis gene action (Rao, 2017). Fruit yield per plant was governed by complementary epistasis, dominant and dominant × dominant (Sirohi and Choudhary, 1980), non-additive (Pal *et al*., 1983; Matoria and Khandelwal, 1999), dominant (Patel *et al*., 2005) and duplicate epistasis gene actions (Mishra *et al*., 2015; Rao, 2017).

2.6 QTL MAPPING FOR YIELD TRAITS IN BITTER GOURD

Breeders always seek to breed bitter gourd varieties for traits such as early maturity and high yield. However, limited investigations have been done to identify loci governing yield related traits. The fruit-related traits such as fruit length and diameter, flesh thickness, and flower-related traits like first female flower node, female to male flower ratio, greatly affect the early yield and the total yield of bitter gourd. Molecular mapping in consideration with these traits are helpful in tapping the yield trait loci.

MAS utilises genomic tools such as molecular markers to derive quick and effective selection of the desired traits and significantly speed up the breeding process. The success of MAS depends on high-quality genetic linkage map and genetic mapping of QTL for the target traits. Fine mapping of QTL increases the efficiency of foreground selection in introgression programs through MAS because the genomic region that has to be controlled is smaller. This will reduce the number of individuals that is required and the genotyping cost. In addition, introgression of a smaller genomic region helps to eliminate unwanted genes that are located around the target QTL. For MAS to be effective, the target QTL must be free from any

undesirable linkage. The large size of the regions encompassing QTL and the likely presence of undesirable linked genes make it essential to fine map such regions to facilitate their precise introgression and to identify candidate genes within these QTL. Further, fine mapping will help to clone the genes residing at the target QTL.

The time required for generating a mapping population as a RIL is higher (more than seven seasons). Developing DH population is handicapped by lack of standardized tissue culture techniques for most of the crop species. For majority of crop species, the rapid and most preferred approach is to develop an F_2 mapping population for early linkage mapping and preliminary QTL analysis (Clarke *et al*., 1995; Gardiner *et al*., 1993; Harushima *et al*., 1998), especially in species with limited information on molecular markers (Feng *et al*., 2012; Levi *et al*., 2003).

Genetic maps have been constructed for cucurbits such as cucumber (Wang *et al*., 2005; Sun *et al*., 2006; Yuan *et al*., 2008; Miao *et al*., 2011; Zhang *et al*., 2012) and melon (Harel-Beja *et al*., 2010; Yuste-Lisbona *et al*., 2010; Diaz *et al*., 2011). Very limited attempts have been made in genetic research of bitter gourd.

In the very first attempt of QTL mapping in bitter gourd, Kole *et al*. (2012) used 108 AFLP markers to genotype 146 F_2 progenies derived from an inter-botanical variety cross between Taiwan White (*M. charantia* var. *charantia*) and CBM12 (*M. charantia* var. *muricata*). Twelve QTL controlling five polygenic fruit traits including length, diameter, weight, number of fruits, and yield were detected on five linkage groups that individually explained 11.1 to 39.7 per cent of the corresponding total phenotypic variance.

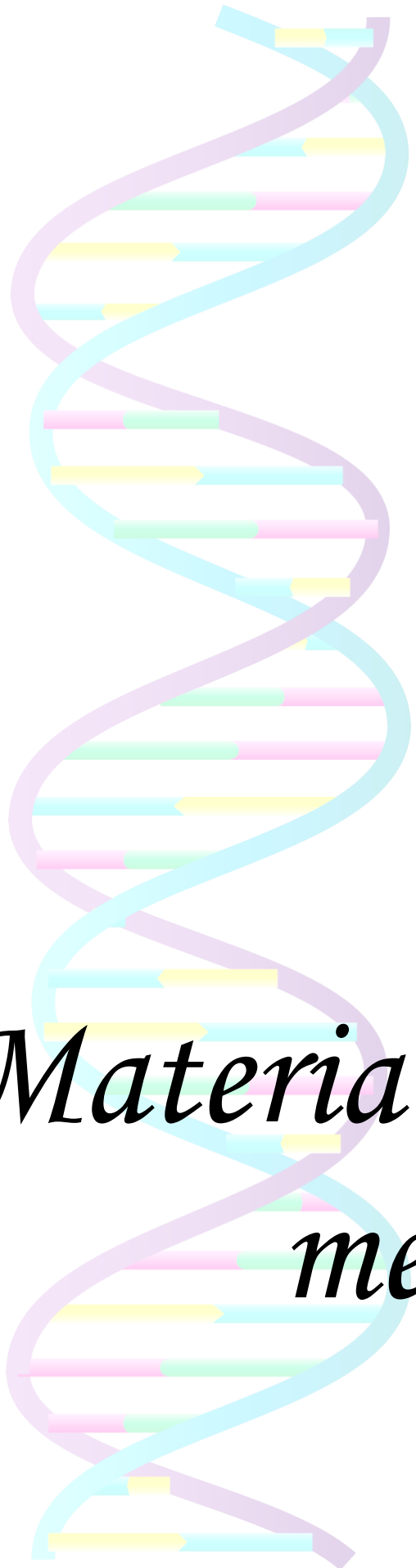
The SSR, EST-SSR and AFLP markers were also employed to map QTL for traits such as female flower ratios and first female flower node, fruit length, fruit diameter, flesh thickness, fruit shape, fruit pedicel length, and fruit length pedicel ratios, fruit weight, fruit numbers per plant, and yield per plant, stem diameter and internodes length (Wang and Xiang, 2013). Linkage map spanning a distance of 1009.5 cM was constructed using an $F_{2:3}$ consisting 144 lines derived from cross between two cultivated inbred lines gynoecia Z-1-4 and 189-4-1. A total of 43 QTL with 5.1–33.1 per cent phenotypic variance, were identified on nine chromosomes for thirteen horticulture traits.

Matsumura *et al*. (2014) mapped gynoecy in bitter gourd using RAD-Seq analysis using 48 F_2 individuals derived from cross between a gynoecious line OHB61-5 and a monoecious line OHB95-1A. A total of 552 markers were employed to construct a linkage map encompassing 1821 cM distance. One putative gynoecious locus (*Mcgy*) was mapped with several SNPs (Matsumura *et al*., 2014). Using Genotyping-by-Sequencing (GBS) technology four traits *viz*., gynoecy, sex ratio, node and days at first female flower appearance were mapped (Rao *et al*., 2018). A total of 2013 SNPs were employed to derive a linkage map covering 2329.2 cM distance. An F_2 mapping consisting 90 individuals was phenotypically evaluated and 22 QTLs were identified. The gynoecious (*gy-1*) locus is flanked by markers TP_54865 and TP_54890 on LG 12. Cui *et al*. (2018) mapped sex expression, fruit epidermal structure and immature fruit color in F_2 mapping population comprising 423 individuals derived from the gynoecious line K44 and the monoecious line Dali-11. The linkage map was obtained using 1009 SNPs

covering 2203.95 cM distance in 11 linkage groups. QTLs were identified for each of the trait with phenotypic variation ranging from 11.20 to 86.10 per cent.

Rao *et al*. (2021) have recently mapped six major yield-contributing traits such as fruit length, fruit diameter, fruit weight, fruit flesh thickness, number of fruits per plant and yield per plant. Using 2013 SNPs derived from GBS of an $F_{2:3}$ mapping population generated from the cross DBGy-201 × Pusa Do Mausami, a linkage map was generated. A total of 19 QTLs were mapped for the yield traits with phenotypic variation ranging from 0.09 to 32.65 per cent.

Mapping QTL for yield traits requires careful selection of parental lines contrasting for fruit and yield related traits. *M. charantia* consists of two varieties, *M. charantia* var. *charantia*, which produces large fusiform fruits, and *M. charantia* var. *muricata*, a wild variety with small and round fruits (Chakravarthy, 1990). These two botanical varieties differ contrastingly with regard to shape, size (Chakravarty, 1990), and many other qualitative and quantitative traits (Kole *et al*., 2009a, 2009b, 2010). *M. charantia* is efficiently crossable with *M. muricata* and high fruit set (97.12%) was observed in these interspecific crosses. Hybrids produced were also found to be fertile (85.4% fertile pollen) which suggests use of *M. muricata* in crossing programs with *M. charantia* (Bharathi, 2010). Moreover, interspecific crosses generate a wide variability in the population giving scope for identification of novel loci for economically important traits.



Materials and methods

3. MATERIALS AND METHODS

The study entitled “Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)” was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Agriculture, Kerala Agricultural University, Thrissur, during October, 2018 to December, 2021. Details regarding the experimental materials used and methodology adopted for various experiments are presented in this chapter.

3.1 PARENTAL LINES

Inclusion of phenotypically and genotypically distinct parents in generation of mapping population is a prerequisite for successful QTL mapping experiments. Priyanka (*Momordica charantia* var. *charantia*), a high yielding variety released from Kerala Agricultural University, Thrissur, was used as the female parent in this study. The seeds of this variety were procured from KAU-Agricultural Research Station, Thiruvalla. It bears greenish white, spindle shaped large fruits yielding up to 29 t/ha. It is resistant to powdery mildew disease and recommended for acid alluvial soils of Kerala.

A wild bitter gourd accession IC634896 (*M. charantia* var. *muricata*) was used as the male parent. The seeds for this accession were procured from Regional Station of ICAR-National Bureau of Plant Genetic Resources, Thrissur. This accession bears small, round and dark green fruits (Plate 1).

3.2 GENOMIC DNA EXTRACTION FROM THE LEAVES

Genomic DNA was extracted by following the Cetyl trimethylammonium bromide (CTAB) method (Dellaporta *et al*., 1983). DNA was extracted from individual plants of the parental lines. Leaves of three to four-week old seedlings were taken from the nethouse and stored at -20 °C till further use. DNA was extracted by following the procedure:

Frozen leaf sample was ground into a fine powder in liquid nitrogen in sterile mortar and pestle. Extraction buffer was added to this finely powdered sample. The contents were mixed well, transferred to 2 ml microcentrifuge tube and incubated at 65 °C for one hour, with occasional mixing by gentle inversions. After incubation, the contents were spun for 5 minute at 8000 rpm. About 750 µl of supernatant was transferred to fresh 1.5 ml microcentrifuge tube and the remaining was discarded. About 750 µl of Chloroform: Isoamyl alcohol (24:1) was added to this supernatant. The contents were mixed thoroughly and centrifuged for 10 minute at 13000 rpm. This step was repeated one more time. The aqueous phase was extracted and transferred to fresh 1.5 ml microcentrifuge tube and equal volume of isopropanol was added and mixed by gentle inversion and incubated at -20 °C overnight. After overnight incubation, the tubes were centrifuged at 10000 rpm for 10 minutes and the supernatant was gently decanted. DNA pellet was washed with 50 µl of 70 per cent ethanol and tubes were kept inverted till the pellet was air dried completely. Pellet was dissolved in TE buffer (40-50 µl) and stored at -20 °C.

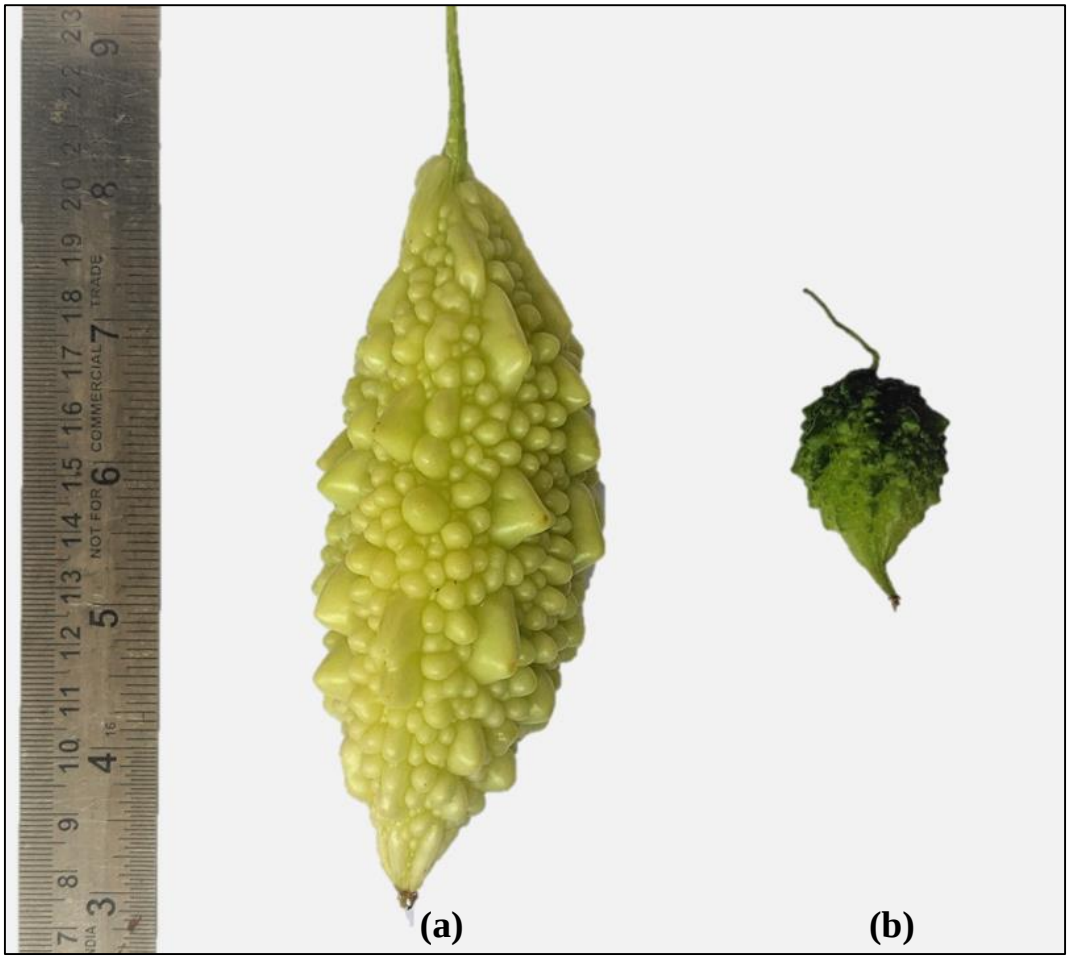


Plate 1. Difference in fruit characters in parental lines

(a) Priyanka, (b) IC634896

3.2.1 Purification of extracted genomic DNA

The DNA samples were treated with 2 µl RNase A solution (1 mg/ml) per 40 µl of TE buffer and the tubes were incubated at 37 °C in a water bath for one hour. After incubation, temperature was increased to 65 °C for 10-15 minutes to denature the RNase A. Equal volume (~40 µl) of chloroform:isoamyl alcohol (24:1) was added and the contents were mixed thoroughly and centrifuged at 11000 rpm for 5 minutes. Supernatant was extracted and transferred to a fresh sterile 1.5 ml micro-centrifuge tube and equal volume of isopropanol was added. Contents were mixed by gentle inversions and the tubes were kept at -20 °C for two hours. The tubes were centrifuged at 10000 rpm for 10 minutes and supernatant was gently decanted. DNA pellet was washed with 50 µl of 70 per cent ethanol, air dried completely and dissolved in TE buffer and stored at -20 °C.

3.2.2 Quantification of DNA

The amount of DNA in each sample was quantified from the absorbance at 260 nm and 280 nm in a NanoDrop spectrophotometer (ND-1000 V3.5.2, NanoDrop Technologies Inc., USA). Initialization of the instrument was done with autoclaved distilled water. The instrument was set blank with 2.0 µl TE buffer. The quantity of DNA was measured by loading 1.0 µl DNA sample on NanoDrop spectrophotometer pedestal. The DNA quantity in ng/µl and OD value for each sample were noted. The ratio between the readings at 260 and 280 nm (OD_{260}/OD_{280}) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have 260 / 280 nm OD ratio between 1.7 and 1.8 (Sambrook

and Russel, 2001). DNA concentrations computed using the OD values were used to dilute the DNA samples to the working concentrations of 50 ng/µl. Amount of stock DNA solution to be taken for dilution was calculated using following formula, $M_1V_1 = M_2V_2$ where M_1 is stock DNA concentration (for example, 1000 ng/µl), V_1 is volume of stock to be diluted, M_2 is concentration of working solution (50 ng/µl) and V_2 is volume of working solution to be prepared (100 µl). Then the volume of stock to be diluted (V_1) can be calculated as

$$(1000 \text{ ng/µl})\ V_1 = (50 \text{ ng/µl})\ (100 \text{ µl})$$

$$V_1 = (50 \text{ ng/µl})\ (100 \text{ µl}) / (1000 \text{ ng/µl})$$

$$V_1 = 5 \text{ µl}$$

The appropriate volume from the stock was transferred to 1.5 ml microcentrifuge tube and the volume was made to 100 µl using TE buffer. The DNA working solutions were stored at -20 °C till further use.

3.2.3 DNA quality check by agarose gel electrophoresis

The gel casting tray was cleaned with distilled water and open ends were sealed with a tape. The comb was then positioned parallel to open edges about 2 mm above the surface of tray. Agarose (1.2 g) was added to 150 ml 1X TAE buffer and dissolved by melting. The solution was then allowed to cool and when the temperature reached nearly 55 °C, 7.5 µl of ethidium bromide was added as a staining agent. Then the solution was poured into the gel casting tray and allowed to solidify. After setting, the gel was placed in the electrophoresis unit with wells towards the cathode and tank was filled with 1X TAE buffer just enough to cover the surface of the gel.

The DNA sample was pipetted onto a parafilm and mixed well with 2 µl of 6X loading dye. DNA samples were loaded in individual wells. The electrodes were connected to power supply and electrophoresis was carried out at 80 volts for 1-1.5 hours till the dye migrates to the end of the gel. The DNA was visualized and documented using a gel documentation system (Biorad Gel Doc XR^{+}).

3.3 PARENTAL POLYMORPHISM STUDY USING SSR MARKERS

A clear polymorphism between parental lines is a prerequisite in undertaking gene/QTL mapping programme. Initial screening of the parents was carried out for polymorphism study. Genomic DNA from both the parentals were subjected to PCR amplification with a set of 450 microsatellite markers (Annexure I) to screen them for polymorphism. Separation and visualization of PCR products was done on agarose gel (5.0 %) with ethidium bromide staining.

3.3.1 Microsatellite primers from literature

An extensive literature survey was done to collect the available microsatellites in *Momordica* genome. Among the 450 microsatellite markers initially used for parental polymorphism study, 247 belonged to *Momordica* genome and 203 belonged to related genomes. These included 127 EST-SSRs from *Luffa cylindrica* (Wu *et al*., 2016), 23 SSRs from *Cucumis melo* (Chiba *et al*., 2003), 10 EST-SSRs from *Cucumis melo* (Kong *et al*., 2007), seven EST-SSRs from *Cucumis sativus* (Kong *et al*., 2006), three EST-SSRs from Chinese cabbage (Xin *et al*., 2006), six SSRs from *Capsicum annuum* (Minamiyama *et al*., 2006), six SSRs from bell pepper (Lee *et al*., 2004), and 21 SSRs from other genomes (Wang

and Xiang, 2013). The microsatellite primers from all the sources other than *Luffa cylindrica* were already reported to be polymorphic in bitter gourd (Wang and Xiang, 2013). Details of 450 microsatellite markers are given in Annexure I.

3.3.2 Mining of microsatellites through *Momordica* genome analysis

Draft genome sequence of bitter gourd was used for SSR mining. The scaffold level genome sequence of OHB-3 cultivar (Matsumura *et al*., 2020) was retrieved from genomic resources of the National Center for Biotechnology Information (NCBI). The genome sequences assembly with accession numbers of GCA_013281855.1 (Size 303 Mbp) was downloaded in FASTA format from NCBI.

Microsatellites were identified using Genome wide Microsatellite Analysing Tool (GMATo) (Wang *et al*., 2013). The contigs were screened for mono-, di-, tri-, tetra-, penta-, hexa-, septa-, and octa-nucleotide repeat motifs using a criteria summarized in Table 1. The sequences containing microsatellites were used to design primers using online program Primer3 (version 0.4.0) (Untergasser *et al*., 2012). The primer designing was performed using corresponding sequences with at least 50 bp on both sides of the SSR repeats. The parameters considered for primer designing were, 18–24 bp primer length and optimum GC contents of approximately 40-50%. Seventy five designed primer pairs were labelled as 'KAUBG_n' where n is serial number. These primers were synthesized by Sigma-Aldrich Pvt Ltd. (India), at 0.05 µmole scale.

Table 1. Summary of SSR loci identified by GMATo in the *Momordica* genome using different parameter combinations

GMATo parameters		Number of microsatellites found	Motifs identified
Motif length range (bp)	**Minimum number of repeats**		
1-10	50	38	Mono-, Di-
3-10	30	5	Tri-
4-10	20	1	Septa-
4-10	10	25	Tetra-, Penta-, Hexa-, Septa-
8-10	5	6	Octa-

3.3.3 PCR amplification

PCR amplification of 525 microsatellites was done using forward and reverse primer pairs. PCR reaction mixture was prepared as master mix for both the parental DNAs in a single microcentrifuge tube. Then it was distributed to all 0.2 ml PCR tubes and 1 µl of respective DNA template was added. Short spin was given to mix template DNA with all reaction components and then tubes were loaded in a thermal cycler.

Contents of PCR reaction mixture

Reagents	**Volume (µl)**
Taq assay buffer (10X)	2.0
dNTPs (2.5 mM each)	1.5
Forward primer (10 mM)	1.0
Reverse primer (10 mM)	1.0
Taq DNA Polymerase (3U/ µl)	0.3
Template (DNA 50 ng/µl)	1.0
Sterile distilled water	13.2
Total	20.0

(dNTPs, Assay buffer and *Taq* DNA polymerase were obtained from GeNei Laboratories Pvt Ltd., Primers were synthesized from Sigma Aldrich Pvt. Ltd.).

Polymerase chain reaction was carried out in thermal cycler (ProFlex, Life Technologies) to amplify each marker using the programme given below.

Sl. No.	Reaction step	Temperature (°C)	Time (min.)
1	Initial denaturation	95.0	2.0
2	Denaturation	95.0	0:30
3	Annealing	55.0	0:45
4	Primer extension	72.0	2.0
5	Repeat (Step 2-4)	36 cycles	
6	Final extension	72.0	10.0
7	Hold at	4.0	

PCR products were electrophoresed on 5.0 per cent agarose gel. Samples were mixed with 3 µl 6X bromophenol blue dye, loaded into the wells and the electrophoresis was carried out at 80 volts until the dye reached the end of the gel. After the electrophoresis, products were visualized and documented in a gel documentation system (Biorad Gel Doc XR^{+}).

3.4 GENERATION OF MAPPING POPULATION

The parent material used in the crossing program included a released variety Priyanka (*M. charantia* var. *charantia*) and a wild accession IC634896 (*M. charantia* var. *muricata*). Seeds were treated with hot water (50 °C for 3 h) followed

by 60 ppm Gibberellic Acid (GA_3) for 12 hrs. Subsequently the seeds were treated with the mixture of Zinc sulphate (1.0 %), Potassium nitrate (2.0 %) and Potassium chloride (1.0 %) for 1 h. Seed coat was carefully punctured using forceps before sowing, without damaging the embryo. Seeds were sown in polybags and after 10 days of germination, the seedlings were transplanted to earthen pots containing potting mixture (2 parts soil: 1 parts sand: 1 part FYM).

Hybridization was carried out during October to December, 2019 under net house conditions. One day prior to pollination, the chosen flowers from the female parent were emasculated and the fully matured, unopened male and female flower buds were covered with butter paper bag. Next day between 6.00 and 9.00 am, pollination was done manually. The pollinated flowers were labelled and covered with butter paper bags again to avoid pollen contamination. Both direct and reciprocal crosses were made between the parental lines. The seeds were harvested from ripened fruits and dried under shade.

After treating the seeds with hot water (50 °C for 3 h) followed by 60 ppm Gibberellic Acid (GA_3) for 12 hrs, and with the mixture of Zinc sulphate (1.0 %), Potassium nitrate (2.0 %) and Potassium chloride (1.0 %) for 1 h the F_1 seeds were sown in polybags. Seedlings were transplanted in pits of 60 × 60 × 30 cm size, with one plant per pit. The plants were raised during February to April, 2021 with the spacing of 2 × 2 m between the plants. Pandals have been erected to support the plants when plants started vining and recommended practices were followed as per the Package of Practices of Kerala Agricultural University (KAU, 2016). Hybridity of F_1 plants was confirmed using a polymorphic microsatellite marker McSSR62.

Phenotypic observations were taken on traits like days to staminate flower, days to pistillate flower, length and breadth of fruit, length of peduncle, flesh thickness, number of fruits per plant, mean fruit weight, number of fruits per plant, yield per plant. Five F_1 plants (four with cross IC634896 × Priyanka and one with cross Priyanka × IC634896) were selfed and the ripened fruits were harvested from each plant separately, to get F_2 seeds.

Seeds harvested from single F_1 plant (Priyanka × IC634896) were treated as detailed earlier under this section and sown in polybags. Seedlings were transplanted in pits at 1 × 1 m spacing. Two hundred F_2 plants were raised in open field conditions during April to June, 2021. Individual plant was selfed to get the seeds of $F_{2:3}$ population. Seeds were extracted from ripened fruits, separately from each F_2 plant.

One seed from each F_2 plant was sown in separate polybags to raise the $F_{2:3}$ population. Thus, 200 $F_{2:3}$ plants along with parental lines were raised in open field on soil mounds with 1 × 1 m spacing during July to October, 2021. Necessary plant protection measures were adopted to ensure healthy growth of the plants.

3.5 PHENOTYPING OF MAPPING POPULATION

The observations on quantitative traits contributing to the yield and few important qualitative traits with economic significance were recorded for each plant, following the NBPGR Minimal Descriptors (Srivastava *et al*., 2001). These traits included different characters related to flowers, fruits, seeds, leaves, and vine. Details of the traits recorded are given below.

Flower related characters

1. **Days to first staminate flower**

   Recorded as number of days from sowing date to the date when first male flower opened.

2. **Days to first pistillate flower**

   Recorded as number of days from sowing date to the date when first female flower opened.

3. **Number of staminate flowers**

   Total number of male flowers produced on the plant until it was removed after completing the harvest.

4. **Number of pistillate flowers**

   Total number of female flowers produced on the plant until it was removed after completing the harvest.

5. **Sex ratio (staminate:pistillate flower)**

   Recorded as ratio of total number of male to female flowers on a plant.

6. **First female flower node**

   Recorded as the node number in which the first female flower appeared.

Fruit related characters

7. **Fruit length (cm)**

   Recorded as the mean length of five random fruits.

8. **Fruit breadth (cm)**

   Recorded as the mean breadth of five random fruits.

9. **Fruit length to breadth ratio**

Recorded as the mean length to breadth ratio of five random fruits.

10. **Peduncle length (cm)**

Recorded as the mean length of the peduncle of same five fruits.

11. **Fruit : peduncle length ratio**

Recorded as the mean ratio of the lengths of the fruits and peduncles of same five fruits.

12. **Flesh thickness (mm)**

Recorded as the mean thickness of flesh from the same five fruits.

13. **Fruit weight (g)**

Recorded as the mean weight of the same five fruits.

14. **Number of fruits per plant**

Total number of fruits harvested from a plant across all the harvests.

15. **Fruit yield per plant (g)**

Recorded as the cumulative weight of all the fruits harvested from a plant.

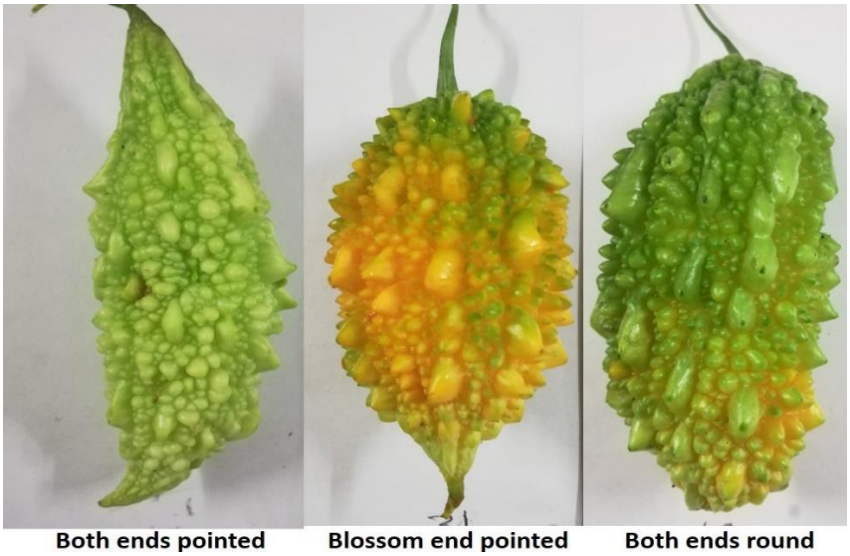
16. **Fruit ends**

This qualitative trait was recorded as both ends pointed or only blossom end pointed or both ends round (Plate 2a), following the NBPGR Minimal Descriptors.

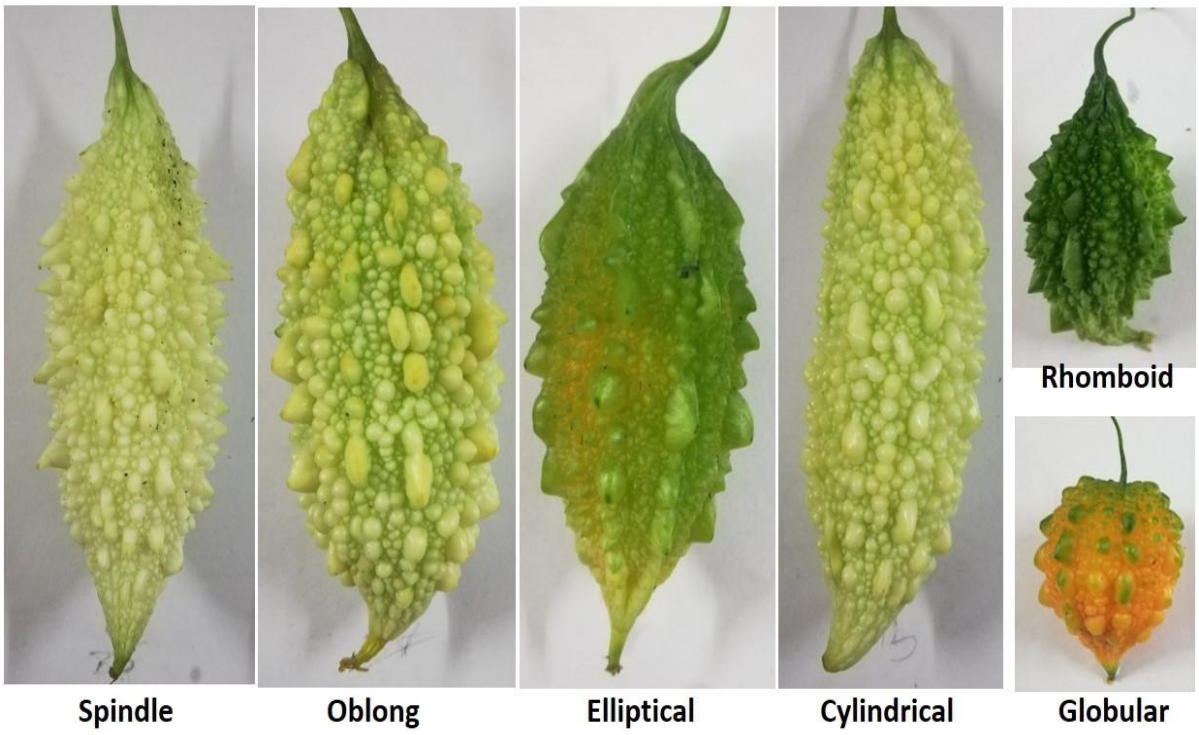
17. **Fruit colour**

This qualitative trait was recorded as light green, green, dark green and, both green and white.

18. **Fruit shape**



(a)



(b)

Plate 2. Variability in fruits of bitter gourd

a. Fruit ends, b. Fruit shape

This qualitative trait was recorded following the NBPGR Minimal Descriptors (Plate 2b).

Seed related characters

19. Number of seeds per fruit

Recorded the mean number of seeds from five randomly selected ripe fruits.

20. Seed length

Recorded the mean length of five seeds from randomly selected ripe fruits.

21. Seed breadth

Recorded the mean breadth of five seeds from randomly selected ripe fruits.

Leaf related characters

22. Leaf size (cm^2)

Mean size of five randomly selected leaves from each plant was calculated using ImageJ software (https://imagej.nih.gov/ij/download.html).

23. Leaf color

Leaf color was recorded as dark green or green or light green.

Vine related characters

24. Vine length (m)

Total length of the primary and secondary vines of each plant was recorded.

25. Internodal length (cm)

Average of lengths of five internode from fourth node was recorded.

26. Number of side branches

27. Stem thickness (mm)

Thickness was measured by taking a cross section of the vine at third node from the ground.

3.6 GENOTYPING OF $F_{2:3}$ MAPPING POPULATION

Using the square root transformed values for the 27 traits recorded, 200 $F_{2:3}$ plants in the mapping population were subjected to hierarchical clustering and based on this, a set of 90 plants was selected, such that it represented the total phenotypic variation for all the traits in initial population. Genomic DNA from these 90 plants was extracted and their genotyping was done using the microsatellite markers, which were polymorphic for the parents.

The genotypic data was coded using ABH system where A represented the homozygous allele from female parent Priyanka, B represented the homozygous allele from male parent IC634896 and H represented the heterozygous allelic status.

3.7 QTL MAPPING

Phenotypic and genotypic data of 90 $F_{2:3}$ plants were used for mapping the QTL for different yield related traits. The QTL mapping included two stages:

1. Construction of genetic linkage map,

2. Construction of QTL map.

Both of these steps were performed in IciMapping software (Version 4.2.53; Meng *et al*., 2015).

3.7.1 Construction of linkage map

3.7.1.1 *Preparation of input file*

Molecular marker amplification data was entered in *.xlsx file. The template for input files was strictly followed according to the IciMapping user manual. Care was taken to remove space between the words of marker names, trait names etc., as the software does not allow space between the words. First page of Excel file was named Generallnfo (Figure 1). In A1 of first page, the mapping population type was entered as 8 (F_3). In A2 of first page, code for mapping function as 1 (kosambi), in A3, marker space type as 2 (positions), in A4, number of markers used as 85, and in A5, number of plants in mapping population as 90, were entered. The codes for each of the above mentioned parameters were given according to the IciMapping user manual.

Second page in Excel was named Genotype and it was where the marker scores were entered (Figure 2). Details on scoring of SSR codominant maker system are given in IciMapping user manual. For SSR, marker representing the allele in first parent was given score 'A' and marker for the other allele present in second parent was given score B. Heterozygous individuals having both markers (representing both alleles) were scored H. 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.

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. Care was taken to ensure that the names and order of markers were exactly as given second page of Excel (Figure 3).

3.7.1.2 *Linkage map analysis*

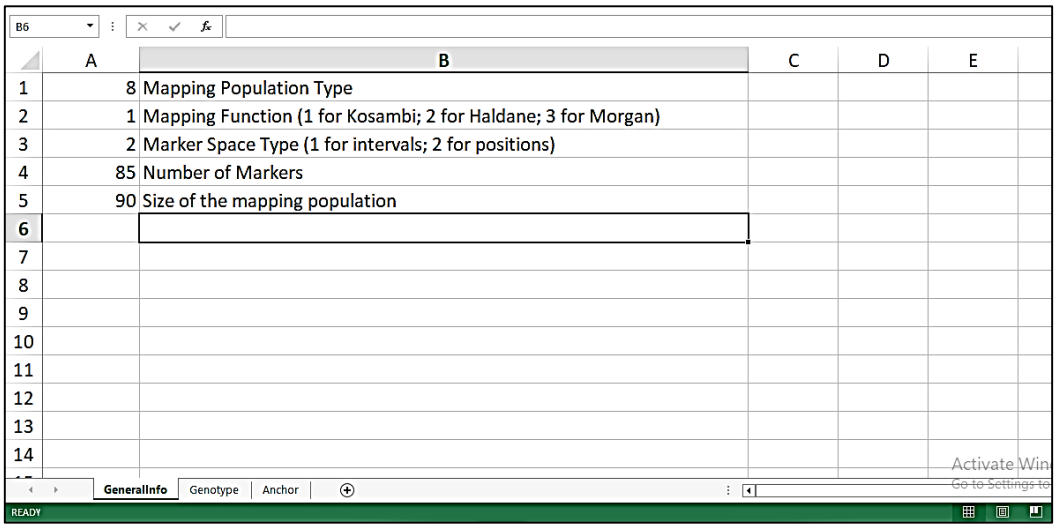
The objective of the linkage mapping prior to QTL mapping is to allocate the markers to linkage groups, when their positions on chromosomes are not known.

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 *.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 linkage 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.

3.7.2 QTL identification

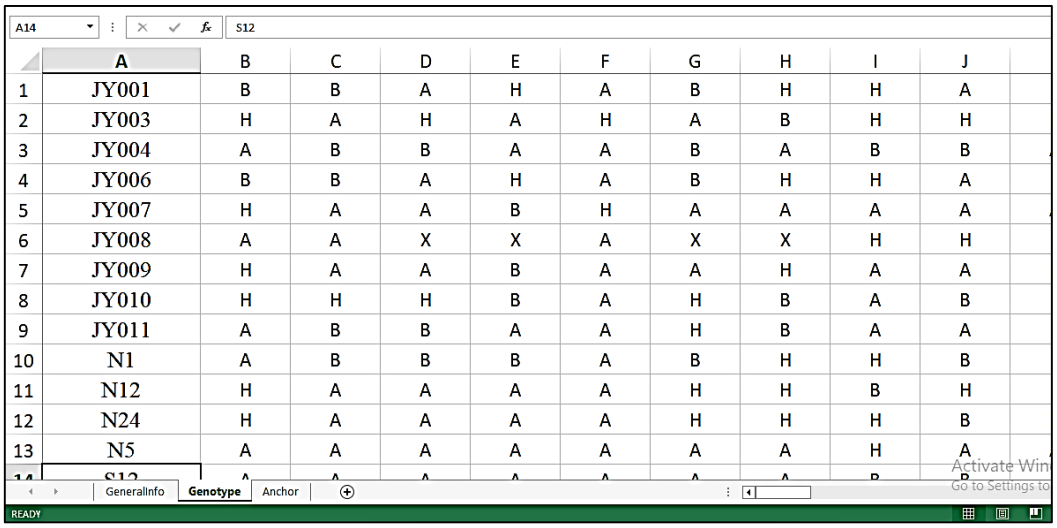
3.7.2.1 *Preparation of input file*

The input file had five parts. In the first page Generallnfo, A1 contained 'Indicator' which says whether this was a mapping study or simulation. As it is for mapping study, 1 was entered. A2 detailed the 'Population type' and for $F_{2:3}$, code



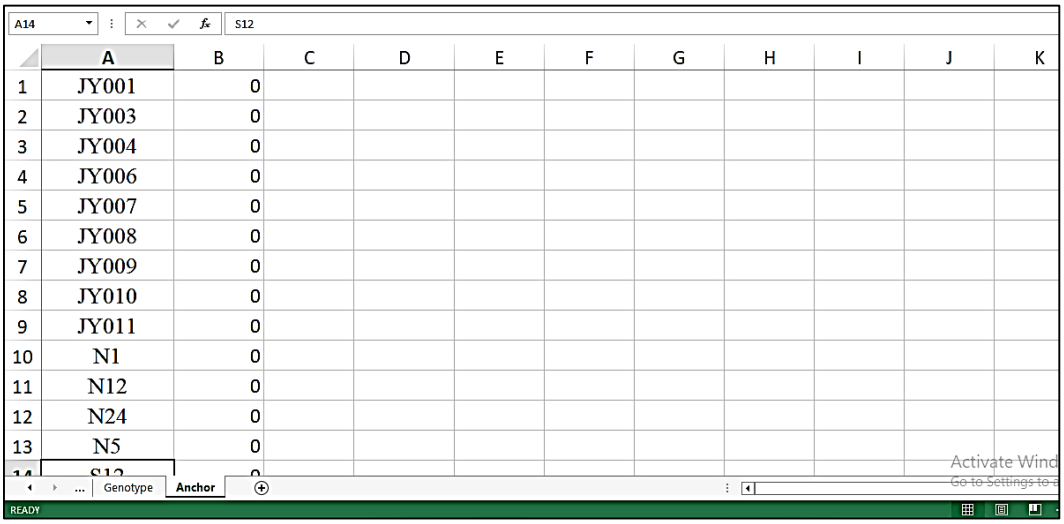
	A	B	C	D	E
1	8	Mapping Population Type			
2	1	Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)			
3	2	Marker Space Type (1 for intervals; 2 for positions)			
4	85	Number of Markers			
5	90	Size of the mapping population			
6					
7					
8					
9					
10					
11					
12					
13					
14					

Figure 1. Format of filling general information in first sheet of input file



	A	B	C	D	E	F	G	H	I	J
1	JY001	B	B	A	H	A	B	H	H	A
2	JY003	H	A	H	A	H	A	B	H	H
3	JY004	A	B	B	A	A	B	A	B	B
4	JY006	B	B	A	H	A	B	H	H	A
5	JY007	H	A	A	B	H	A	A	A	A
6	JY008	A	A	X	X	A	X	X	H	H
7	JY009	H	A	A	B	A	A	H	A	A
8	JY010	H	H	H	B	A	H	B	A	B
9	JY011	A	B	B	A	A	H	B	A	A
10	N1	A	B	B	B	A	B	H	H	B
11	N12	H	A	A	A	A	H	H	B	H
12	N24	H	A	A	A	A	H	H	H	B
13	N5	A	A	A	A	A	A	A	H	A

Figure 2. Format of filling genotype data in second sheet of input file



	A	B	C	D	E	F	G	H	I	J	K
1	JY001	0									
2	JY003	0									
3	JY004	0									
4	JY006	0									
5	JY007	0									
6	JY008	0									
7	JY009	0									
8	JY010	0									
9	JY011	0									
10	N1	0									
11	N12	0									
12	N24	0									
13	N5	0									

Figure 3. Format of filling anchor information in third sheet of input file

8 was used. A3 contained mapping function. 'Marker space type' was given as 2 (positions) in A4. As interval in terms of cM was used in the study, 1 was given. A5 contained the 'Marker space unit', where 1 was entered for 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 *Momordica charantia*, 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 (Figure 4).

Second page was named Chromosome and this contained the details on number of markers in each chromosome (Figure 5). When the chromosome details were not known, the linkage details obtained from 'Linkage analysis' was given.

Third page was named LinkageMap and this contained the details on marker positions (Figure 6). 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.

Fourth page was named Genotype and the marker type information or marker scoring was entered. 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 (Figure 7).

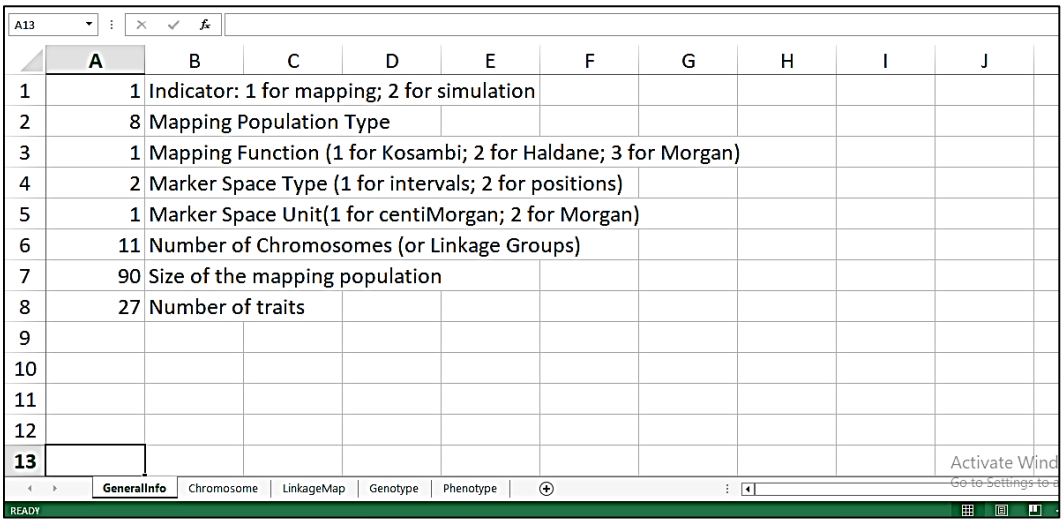
Fifth page was named Phenotype and in this page, phenotype of plants in the population was entered trait wise (Figure 8). First column represented the trait names (with no space in between word) and first row represented the expression of first trait in the members in a population. Missing values were scored as '*'.

3.7.2.2 *QTL mapping 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. Inclusive Composite Interval Mapping- Additive (ICIM-ADD) was selected as mapping method. 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.

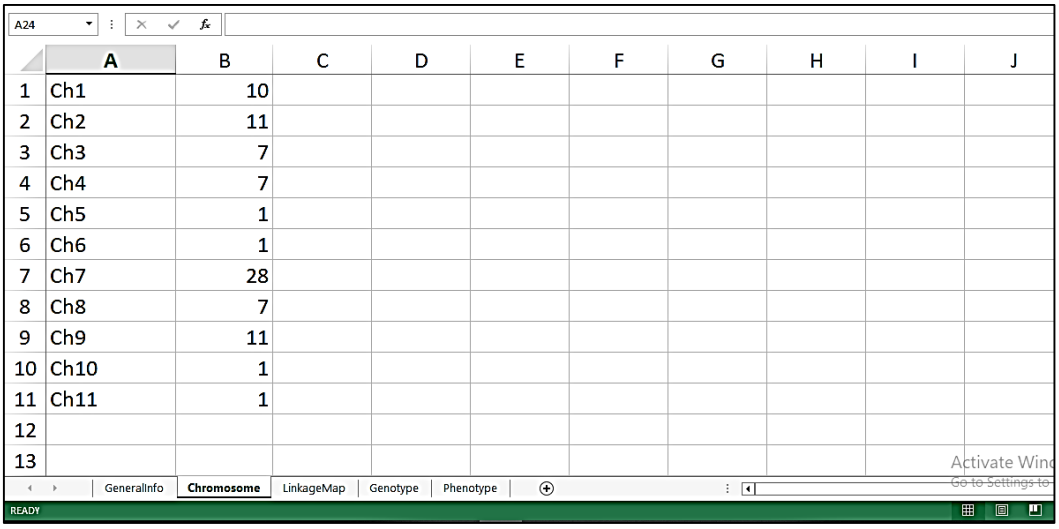
3.8 MARKER-TRAIT CO-SEGREGATION ANALYSIS

Single marker analysis (SMA) was done to identify the markers co-segregating with each trait (LOD >3.0). To validate the markers, respective alleles were PCR amplified from five each of the best and five least performing lines from the population. Amplicons were electrophoresed in 5 per cent agarose gel. The marker-trait association was considered successful when the alleles have distinguished the lines corresponding to the level of expression of the trait.



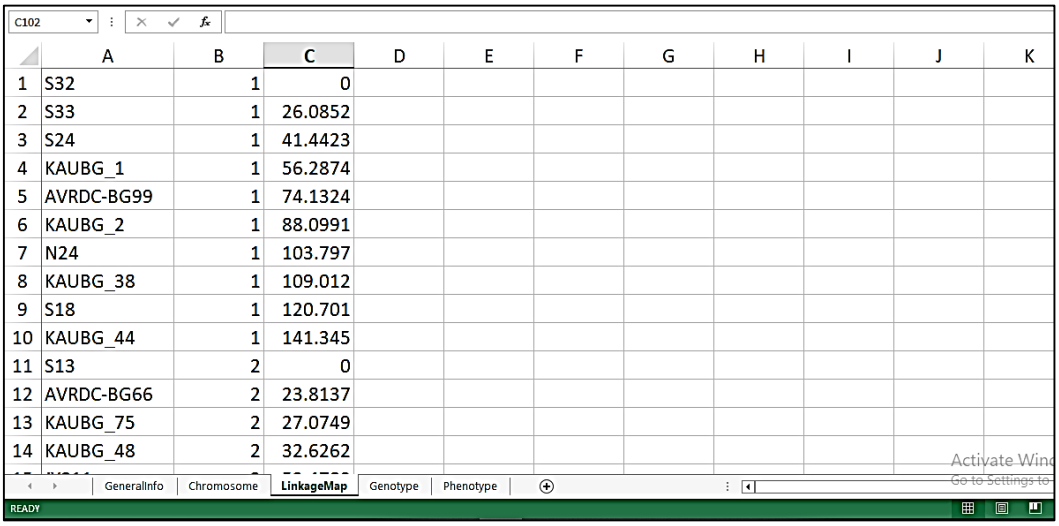
	A	B
1	1	Indicator: 1 for mapping; 2 for simulation
2	8	Mapping Population Type
3	1	Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)
4	2	Marker Space Type (1 for intervals; 2 for positions)
5	1	Marker Space Unit(1 for centiMorgan; 2 for Morgan)
6	11	Number of Chromosomes (or Linkage Groups)
7	90	Size of the mapping population
8	27	Number of traits

Figure 4. Format of filling general information in first sheet of input file



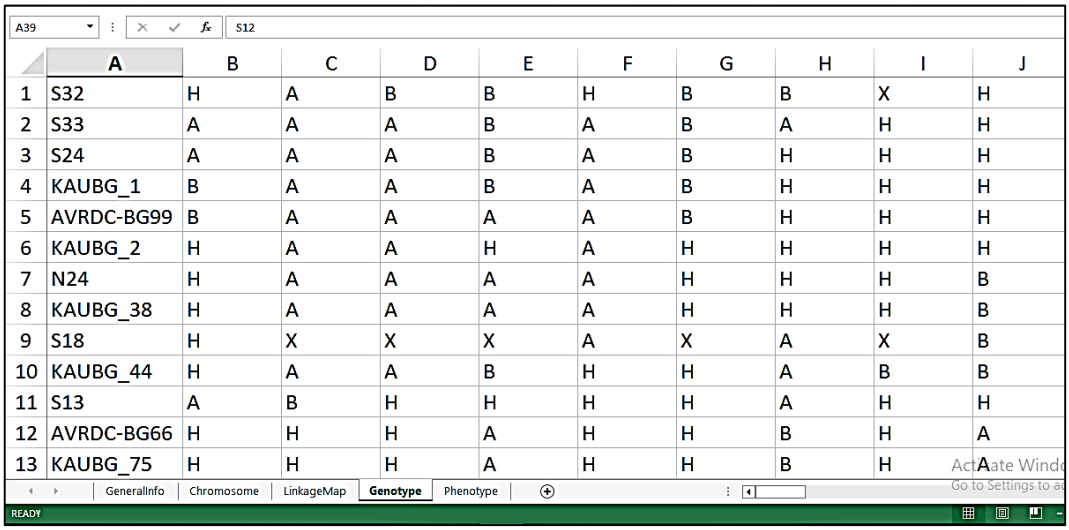
	A	B
1	Ch1	10
2	Ch2	11
3	Ch3	7
4	Ch4	7
5	Ch5	1
6	Ch6	1
7	Ch7	28
8	Ch8	7
9	Ch9	11
10	Ch10	1
11	Ch11	1

Figure 5. Format of filling chromosome information in second sheet of input file



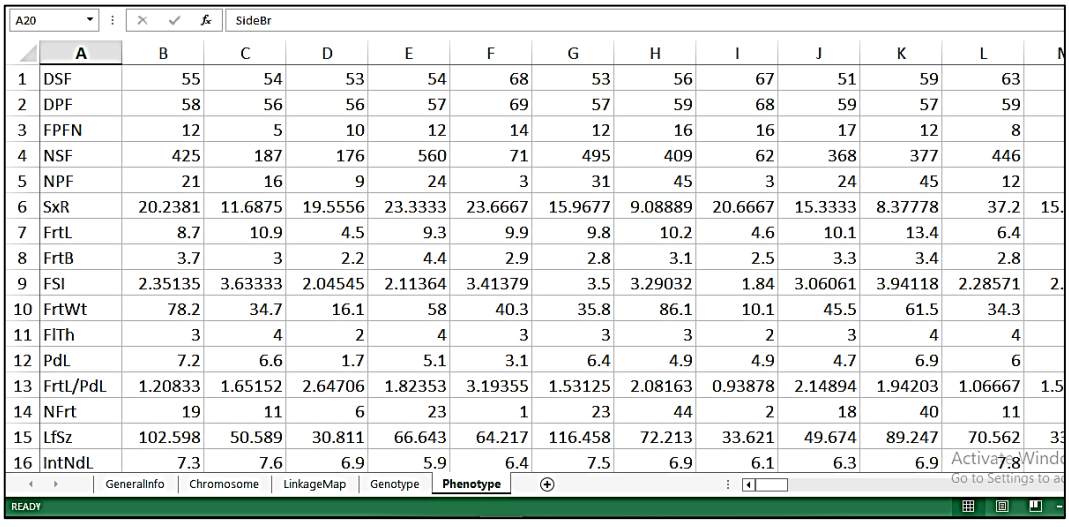
	A	B	C
1	S32	1	0
2	S33	1	26.0852
3	S24	1	41.4423
4	KAUBG_1	1	56.2874
5	AVRDC-BG99	1	74.1324
6	KAUBG_2	1	88.0991
7	N24	1	103.797
8	KAUBG_38	1	109.012
9	S18	1	120.701
10	KAUBG_44	1	141.345
11	S13	2	0
12	AVRDC-BG66	2	23.8137
13	KAUBG_75	2	27.0749
14	KAUBG_48	2	32.6262

Figure 6. Format of filling linkage map information in third sheet of input file



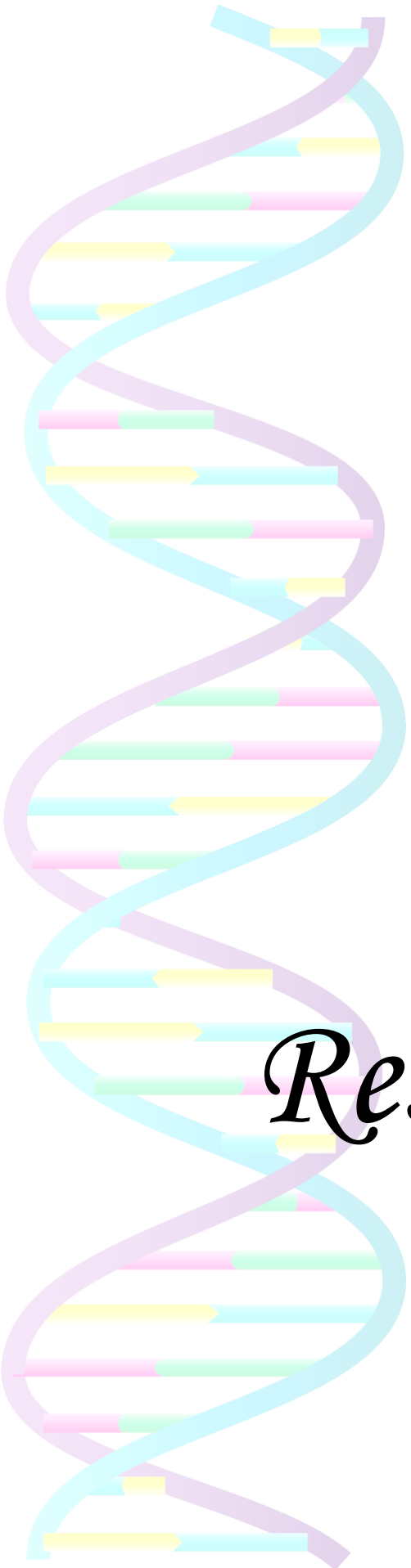
	A	B	C	D	E	F	G	H	I	J
1	S32	H	A	B	B	H	B	B	X	H
2	S33	A	A	A	B	A	B	A	H	H
3	S24	A	A	A	B	A	B	H	H	H
4	KAUBG_1	B	A	A	B	A	B	H	H	H
5	AVRDC-BG99	B	A	A	A	A	B	H	H	H
6	KAUBG_2	H	A	A	H	A	H	H	H	H
7	N24	H	A	A	A	A	H	H	H	B
8	KAUBG_38	H	A	A	A	A	H	H	H	B
9	S18	H	X	X	X	A	X	A	X	B
10	KAUBG_44	H	A	A	B	H	H	A	B	B
11	S13	A	B	H	H	H	H	A	H	H
12	AVRDC-BG66	H	H	H	A	H	H	B	H	A
13	KAUBG_75	H	H	H	A	H	H	B	H	A

Figure 7. Format of filling genotypic data in fourth sheet of input file



	A	B	C	D	E	F	G	H	I	J	K	L
1	DSF	55	54	53	54	68	53	56	67	51	59	63
2	DPF	58	56	56	57	69	57	59	68	59	57	59
3	FPFN	12	5	10	12	14	12	16	16	17	12	8
4	NSF	425	187	176	560	71	495	409	62	368	377	446
5	NPF	21	16	9	24	3	31	45	3	24	45	12
6	SxR	20.2381	11.6875	19.5556	23.3333	23.6667	15.9677	9.08889	20.6667	15.3333	8.37778	37.2
7	FrtL	8.7	10.9	4.5	9.3	9.9	9.8	10.2	4.6	10.1	13.4	6.4
8	FrtB	3.7	3	2.2	4.4	2.9	2.8	3.1	2.5	3.3	3.4	2.8
9	FSI	2.35135	3.63333	2.04545	2.11364	3.41379	3.5	3.29032	1.84	3.06061	3.94118	2.28571
10	FrtWt	78.2	34.7	16.1	58	40.3	35.8	86.1	10.1	45.5	61.5	34.3
11	FlTh	3	4	2	4	3	3	3	2	3	4	4
12	PdL	7.2	6.6	1.7	5.1	3.1	6.4	4.9	4.9	4.7	6.9	6
13	FrtL/PdL	1.20833	1.65152	2.64706	1.82353	3.19355	1.53125	2.08163	0.93878	2.14894	1.94203	1.06667
14	NFrt	19	11	6	23	1	23	44	2	18	40	11
15	LfSz	102.598	50.589	30.811	66.643	64.217	116.458	72.213	33.621	49.674	89.247	70.562
16	IntNdL	7.3	7.6	6.9	5.9	6.4	7.5	6.9	6.1	6.3	6.9	7.8

Figure 8. Format of filling phenotypic data in fifth sheet of input file



Results

4. RESULTS

The results of the study on "Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)" carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Agriculture, Kerala Agricultural University, Thrissur, during October, 2018 to December, 2021 are presented below. The study aimed to map the quantitative trait loci and chromosome-wise maps for yield traits in bitter gourd.

4.1 GENOMIC DNA EXTRACTION FROM THE LEAVES

Genomic DNA was extracted from both the parent lines *viz*. Priyanka (*Momordica charantia* var. *charantia*) and IC634896 (*M. charantia* var. *muricata*) by CTAB method (Dellaporta *et al*., 1983). The quality of DNA was checked on 0.8 per cent agarose gel. Samples yielded good quality DNA with single intact band which was amenable to PCR amplification (Plate 3). The DNA was diluted to get 50 ng/µl concentration for PCR reaction.

4.2 IDENTIFICATION OF POLYMORPHIC MICROSATELLITES BETWEEN PARENTAL LINES

4.2.1 Level of polymorphism in reported markers

A clear polymorphism between parental lines is a prerequisite in undertaking gene/QTL mapping programme. Genomic DNA from both the parents were subjected to PCR amplification with a set of 450 microsatellites (Annexure I).

Among these microsatellites 231 (51.33 %) did not show any amplification. Among the remaining microsatellites 47 (10.44 %) were polymorphic and 172 (38.22 %) were monomorphic (Table 2). Polymorphic markers amplified one to two alleles between two parents (Plate 4), with the amplicon size ranged from 80 bp to 450 bp. The number of polymorphic markers was less to generate good quality linkage map, hence *Momordica* genome was scanned for mining hyper-variable microsatellites which have to be used for further linkage mapping study.

4.2.2 Mining and validation of microsatellites through *Momordica* genome analysis

The *Momordica* genome was retrieved (Cultivar: OHB-3; Sequence accession numbers: GCA_013281855.1; Size: 303 Mbp) and scanned for identification of novel microsatellites using GMATo software. Scanning of the draft genome resulted in 75 microsatellites satisfying the identification criteria (Table 1). Details regarding marker motif, forward and reverse primer sequence and expected amplicon size are given in Table 3. These microsatellites were amplified using genomic DNA of parental lines. Among the 75 microsatellites, 69 (92 %) were successfully amplified, producing 121 alleles, validating these microsatellite for further use. The size of amplicon ranged from 125 bp to 589 bp.

4.2.3 Level of polymorphism by newly identified markers

Among the 69 validated microsatellites, 38 microsatellites (50.7 %) were polymorphic for the parental lines (Table 3). Each polymorphic marker amplified

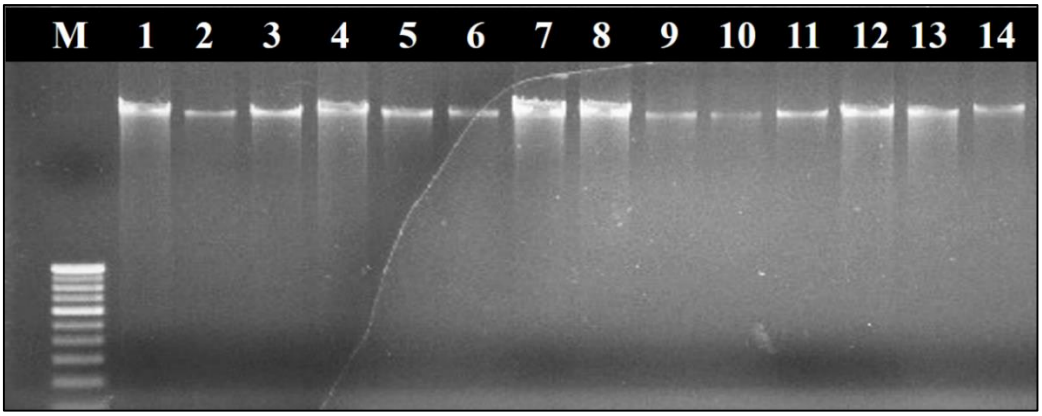


Plate 3. Genomic DNA of parental lines

M: 100 bp Ladder, 1-7: Priyanka, 8-14: IC634896

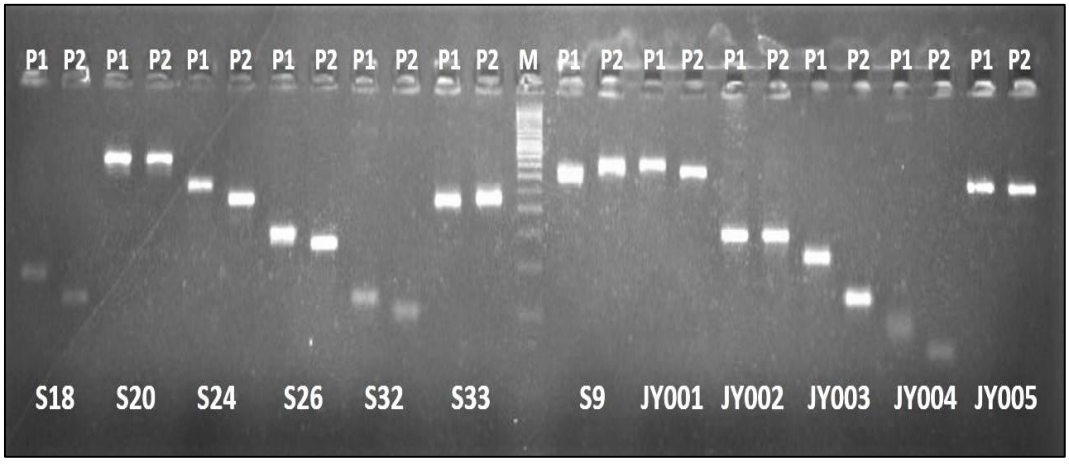


Plate 4. Amplification profile of representative SSR markers

M: 50 bp Ladder, P1: Priyanka, P2: IC63489

Table 2. Status of polymorphism for 450 microsatellites

Sl. No.	Marker	Status of Polymorphism	Sl. No.	Marker	Status of Polymorphism
1	CMMS 30-3	NP	41	JY008	P
2	CMMS4-3	NA	42	JY009	P
3	CMMS33-2	NA	43	JY010	P
4	CMMS33-1	NA	44	JY011	P
5	CMMS35-5	NA	45	cm04	NP
6	CMMS12-6	NA	46	cm09	NP
7	CMMS15-4	NA	47	cm17	NP
8	CMMS2-3	NA	48	cm23	NA
9	CMMS22-2	NA	49	cm46	NA
10	CMMS1-7	NA	50	cm47	NP
11	CMMS27-1	NA	51	cm48	NA
12	CMMS35-4	NA	52	cm50	NA
13	CMMS34-10	NA	53	cm53	NP
14	CMMS34-8	NA	54	cs01	NA
15	CMMS31-3	NA	55	cs05	NA
16	CMMS14-1	NA	56	cs13	NA
17	CMMS1-3	NA	57	cs22	NA
18	CMMS3-1	NA	58	cs37	NP
19	CMMS34-6	NA	59	cs48	NA
20	CMMS35-3	NA	60	cs50	NP
21	CMMS35-1	NA	61	p004	NA
22	CMMS12-4	NA	62	p007	NA
23	CMMS36-2	NP	63	p008	NP
24	A2	NA	64	ju2	NA
25	A47	NA	65	ju5	NA
26	C1	NA	66	ju9	NA
27	C4	NA	67	ju14	NA
28	C7	NA	68	ssrb01	NA
29	C9	NA	69	ssrb04	NA
30	C11	NA	70	ssrb05	NA
31	C17	NA	71	cams101	NA
32	C24	NA	72	cams-163	NA
33	C30	NA	73	cams-351	NA
34	JY001	P	74	cams-373	NA
35	JY002	NP	75	cams-424	NP
36	JY003	P	76	cams-885	NA
37	JY004	P	77	cm0005	NA
38	JY005	NP	78	hpms 1-5	NA
39	JY006	P	79	hpms 1-41	NA
40	JY007	P	80	hpms 1-62	NA
			81	hpms 1-173	NA

Sl. No.	Marker	Status of Polymorphism	Sl. No.	Marker	Status of Polymorphism
82	hpms 1-168	NA	124	AVRDC-BG33	NP
83	hpms 2-2h	NA	125	AVRDC-BG35	NP
84	ssr32	NA	126	AVRDC-BG37	NP
85	ssr108	NA	127	AVRDC-BG41	NP
86	ssr192	NP	128	AVRDC-BG48	NP
87	ssr82	NA	129	AVRDC-BG49	NP
88	ga-e	NA	130	AVRDC-BG50	NA
89	m8	NA	131	AVRDC-BG51	NP
90	ra2-g09	NP	132	AVRDC-BG54	NP
91	cpssr2	NP	133	AVRDC-BG55	NP
92	A	NP	134	AVRDC-BG56	NP
93	agi030	NA	135	AVRDC-BG57	NP
94	ssras46	NA	136	AVRDC-BG58	NP
95	ssra3	NA	137	AVRDC-BG59	NP
96	pbcessrr3na3	NA	138	AVRDC-BG66	P
97	j	NP	139	AVRDC-BG67	NP
98	N1	P	140	AVRDC-BG70	P
99	N12	P	141	AVRDC-BG71	P
100	N24	P	142	AVRDC-BG73	NA
101	N5	P	143	AVRDC-BG74	NA
102	N6	NP	144	AVRDC-BG75	P
103	N9	NP	145	AVRDC-BG83	NA
104	S12	P	146	AVRDC-BG85	P
105	S13	P	147	AVRDC-BG86	P
106	S15	P	148	AVRDC-BG90	NP
107	S18	P	149	AVRDC-BG92	NA
108	S20	NP	150	AVRDC-BG93	NP
109	S24	P	151	AVRDC-BG94	NP
110	S26	P	152	AVRDC-BG97	NP
111	S32	P	153	AVRDC-BG98	P
112	S33	P	154	AVRDC-BG99	P
113	S9	P	155	AVRDC-BG100	NP
114	AVRDC-BG1	NA	156	AVRDC-BG101	P
115	AVRDC-BG2	NA	157	AVRDC-BG104	P
116	AVRDC-BG3	NP	158	AVRDC-BG109	P
117	AVRDC-BG15	NA	159	AVRDC-BG111	NP
118	AVRDC-BG25	P	160	AVRDC-BG112	P
119	AVRDC-BG26	P	161	AVRDC-BG125	NP
120	AVRDC-BG27	NP	162	AVRDC-BG135	NP
121	AVRDC-BG29	NA	163	AVRDC-BG136	NP
122	AVRDC-BG30	NP	164	McSSR 1	NA
123	AVRDC-BG32	NP	165	McSSR 2	NA

Sl. No.	Marker	Status of Polymorphism	Sl. No.	Marker	Status of Polymorphism
166	McSSR 3	NA	208	McSSR 45	NP
167	McSSR 4	NP	209	McSSR 46	NP
168	McSSR 5	NP	210	McSSR 47	NP
169	McSSR 6	NA	211	McSSR 48	NP
170	McSSR 7	NA	212	McSSR 49	NP
171	McSSR 8	NP	213	McSSR 50	NA
172	McSSR 9	NA	214	McSSR 51	NP
173	McSSR 10	NP	215	McSSR 52	NP
174	McSSR 11	NA	216	McSSR 53	NP
175	McSSR 12	NA	217	McSSR 54	NP
176	McSSR 13	NP	218	McSSR 55	NP
177	McSSR 14	NP	219	McSSR 56	NP
178	McSSR 15	NP	220	McSSR 57	P
179	McSSR 16	NP	221	McSSR 58	NP
180	McSSR 17	NP	222	McSSR 59	NP
181	McSSR 18	NP	223	McSSR 60	NP
182	McSSR 19	NP	224	McSSR 61	NP
183	McSSR 20	P	225	McSSR 62	P
184	McSSR 21	NP	226	McSSR 63	NP
185	McSSR 22	NP	227	McSSR 64	NP
186	McSSR 23	NP	228	McSSR 65	NP
187	McSSR 24	NP	229	McSSR 66	P
188	McSSR 25	NP	230	McSSR 67	NP
189	McSSR 26	NP	231	McSSR 68	NP
190	McSSR 27	NP	232	McSSR 69	NA
191	McSSR 28	NP	233	McSSR 70	NA
192	McSSR 29	NP	234	McSSR 71	NA
193	McSSR 30	NP	235	McSSR 72	NP
194	McSSR 31	NP	236	McSSR 73	NP
195	McSSR 32	NP	237	McSSR 74	NA
196	McSSR 33	NP	238	McSSR 75	NA
197	McSSR 34	NP	239	McSSR 76	NP
198	McSSR 35	P	240	McSSR 77	NA
199	McSSR 36	NA	241	McSSR 78	NA
200	McSSR 37	NP	242	McSSR 79	NP
201	McSSR 38	NP	243	McSSR 80	NP
202	McSSR 39	NP	244	McSSR 81	NP
203	McSSR 40	P	245	McSSR 82	NP
204	McSSR 41	NA	246	McSSR 83	NP
205	McSSR 42	NP	247	McSSR 84	NA
206	McSSR 43	NP	248	McSSR 85	NP
207	McSSR 44	NP	249	McSSR 86	NP

Sl. No.	Marker	Status of Polymorphism	Sl. No.	Marker	Status of Polymorphism
250	McSSR 87	NP	292	McSSR 129	NP
251	McSSR 88	NP	293	McSSR 130	NA
252	McSSR 89	NP	294	McSSR 131	NP
253	McSSR 90	NP	295	McSSR 132	NP
254	McSSR 91	NP	296	McSSR 133	NP
255	McSSR 92	NA	297	McSSR 134	NP
256	McSSR 93	NP	298	McSSR 135	NP
257	McSSR 94	NP	299	McSSR 136	NP
258	McSSR 95	NP	300	McSSR 137	NP
259	McSSR 96	NP	301	McSSR 138	NP
260	McSSR 97	NP	302	McSSR 139	NP
261	McSSR 98	NP	303	McSSR 140	NA
262	McSSR 99	P	304	McSSR 141	NA
263	McSSR 100	NP	305	McSSR 142	NP
264	McSSR 101	NA	306	McSSR 143	NP
265	McSSR 102	NA	307	McSSR 144	NA
266	McSSR 103	NP	308	McSSR 145	NP
267	McSSR 104	NP	309	McSSR 146	NP
268	McSSR 105	NA	310	McSSR 147	NP
269	McSSR 106	P	311	McSSR 148	NP
270	McSSR 107	NA	312	McSSR 149	NA
271	McSSR 108	NP	313	McSSR 150	P
272	McSSR 109	NP	314	McSSR 151	NP
273	McSSR 110	NP	315	McSSR 152	NP
274	McSSR 111	NA	316	McSSR 153	NA
275	McSSR 112	P	317	McSSR 154	NA
276	McSSR 113	NP	318	McSSR 155	NA
277	McSSR 114	P	319	McSSR 156	NP
278	McSSR 115	NP	320	McSSR 157	NP
279	McSSR 116	NP	321	McSSR 158	NA
280	McSSR 117	NP	322	McSSR 159	NA
281	McSSR 118	NP	323	McSSR 160	NA
282	McSSR 119	NA	324	SGJ643	NA
283	McSSR 120	NP	325	SGJ644	NA
284	McSSR 121	NA	326	SGJ646	NA
285	McSSR 122	NP	327	SGJ648	NA
286	McSSR 123	NP	328	SGJ652	NA
287	McSSR 124	NP	329	SGJ654	NA
288	McSSR 125	NA	330	SGJ659	NA
289	McSSR 126	NP	331	SGJ666	NA
290	McSSR 127	NA	332	SGJ671	NA
291	McSSR 128	NP	333	SGJ677	NA

Sl. No.	Marker	Status of Polymorphism	Sl. No.	Marker	Status of Polymorphism
334	SGJ684	NA	376	SGJ840	NA
335	SGJ689	NA	377	SGK844	NA
336	SGJ691	NA	378	SGK851	NA
337	SGJ714	NA	379	SGK857	NA
338	SGJ718	NA	380	SGK875	NA
339	SGJ722	NA	381	SGK881	NA
340	SGJ731	NA	382	SGK882	NA
341	SGJ732	NA	383	SGK884	NA
342	SGJ739	NA	384	SGK885	NA
343	SGJ740	NA	385	SGK886	NA
344	SGJ745	NA	386	SGK891	NA
345	SGJ748	NA	387	SGK892	NA
346	SGJ750	NA	388	SGK894	NA
347	SGJ753	NP	389	SGK902	NA
348	SGJ756	NA	390	SGK903	NA
349	SGJ759	NA	391	SGK906	NA
350	SGJ760	NA	392	SGK909	NA
351	SGJ764	NA	393	SGK922	NP
352	SGJ774	NA	394	SGK923	NA
353	SGJ777	NP	395	SGK938	NA
354	SGJ781	NA	396	SGK941	NA
355	SGJ784	NA	397	SGK960	NA
356	SGJ789	NA	398	SGK969	NA
357	SGJ790	NA	399	SGK972	NA
358	SGJ791	NA	400	SGK974	NA
359	SGJ792	NA	401	SGK980	NA
360	SGJ795	NA	402	SGK981	NA
361	SGJ800	NA	403	SGK984	NA
362	SGJ802	NA	404	SGK991	NA
363	SGJ803	NA	405	SGK992	NP
364	SGJ805	NA	406	SGK1005	NP
365	SGJ806	NA	407	SGK1011	NA
366	SGJ808	NA	408	SGK1017	NA
367	SGJ809	NA	409	SGK1018	NA
368	SGJ811	NA	410	SGK1022	NA
369	SGJ813	NA	411	SGK1025	NA
370	SGJ819	NA	412	SGK1029	NA
371	SGJ823	NA	413	SGK1031	NP
372	SGJ828	NA	414	SGK1032	NA
373	SGJ830	NA	415	SGK1033	NA
374	SGJ832	NA	416	SGK1034	NA
375	SGJ833	NA	417	SGK1035	NA

Sl. No.	Marker	Status of Polymorphism
418	SGK1037	NA
419	SGK1039	NA
420	SGK1041	NP
421	SGK1043	NA
422	SGK1045	NA
423	SGK1046	NP
424	SGJ647	NA
425	SGJ649	NA
426	SGJ650	NA
427	SGJ657	NA
428	SGJ663	NP
429	SGJ668	NA
430	SGJ669	NP
431	SGJ673	NA
432	SGJ675	NA
433	SGJ678	NA
434	SGJ679	NP
435	SGJ681	NA
436	SGJ685	NA
437	SGJ690	NP
438	SGJ692	NA
439	SGJ693	NA
440	SGJ696	NP
441	SGJ697	NA
442	SGJ698	NA
443	SGJ700	NP
444	SGJ705	NA
445	SGJ708	NA
446	SGJ709	NA
447	SGJ715	NA
448	SGJ726	NP
449	SGJ729	NA
450	SGJ733	NA

P: Polymorphic

NP: Not polymorphic

NA: Not amplified

Table 3. PCR primer sequences used for the amplification and validation of seventy five microsatellite markers identified in this study

Marker name	Motif	Polymorphism status	No. of alleles	Amplicon size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
KAUBG_1	$(C)_{67}$	P	2	210-220	TCCCCTACGAAACAAATGGT	GGATGGAACTCAGGTTGCTG
KAUBG_2	$(G)_{62}$	P	2	196-225	CTTGTATTGGTGTTTGTCAAGG	AGCAGCTAAGGGGACACAAA
KAUBG_3	$(CT)_{58}$	P	3	170-252	TGCGATCTGTTTGTTTCCTG	GAAATCAAGGAGAGGGAGAGG
KAUBG_4	$(AC)_{177}$	NP	1	457	CCCACTTGGAGAGAGAAAGAGA	CCAATTCCTAACCTTGTGTGTG
KAUBG_5	$(AG)_{61}$	P	2	140-194	GTGGGATTGTGAAGCGAGTT	TTCCATTGTTGTCGTAGTAAGGAG
KAUBG_6	$(C)_{73}$	NP	1	200	CCCAGGTTTGACAGGTTTTG	GGTGCCAGTTGGTGTGACTA
KAUBG_7	G64	NA	0	192	TAATTCCCGGAGCAACAGAC	CTTCCTGCCAAGGAAAGGAT
KAUBG_8	TG120	NA	0	468	TCATTAAGAATGAAGGGTTGAGA	AAAAAGTCAAAAGTTACCGTTGGT
KAUBG_9	$(G)_{52}$	NP	1	199	GTCCATAAAGCCCATCATAAGC	CCGAGCATAATGCAAGGAGT
KAUBG_10	$(CA)_{67}$	NP	1	350	GCTTAACACTCAGCTTAACACACA	TCAAACCGGTACACAGAGCA
KAUBG_11	$(TC)_{63}$	P	2	310-320	AAGCCATTGTCATTCCTCAAA	CAAGTCAGAACATAGTTCGAGTGAG
KAUBG_12	$(G)_{60}$	NP	1	183	GACAGACACGGCAACCAGTA	TGATTCAACCCGTAAATTGG
KAUBG_13	$(AG)_{58}$	P	2	140-179	AGGGTGACCAGTCGAAGAAA	GACCAACTTCGCTTCCCTTC
KAUBG_14	$(C)_{54}$	NP	1	190	CGGCTCATATTTGCTCTCAG	CGGGTTCGATTTGTGAATTT
KAUBG_15	$(TC)_{78}$	P	2	350-375	TCCATGTACCTTTCAGCTTTTG	TTGTTGCTTGTGGTACAGCAG

KAUBG_16	CT55	NA	0	189	TGATCAACCACAGCTCATCAA	AAAGAAGGTCTCTGGCATGG
KAUBG_17	$(GA)_{73}$	NP	2	200-220	AATCCCATTTCTTCGCCTTT	GCCATTAGTGGGCAATATTCA
KAUBG_18	$(C)_{52}$	P	2	155-165	TAGGGCTGGACTCATTGACC	ACTGCGGAGAAAACCATCAC
KAUBG_19	$(AG)_{51}$	NP	1	227	CGGTGTGCCCCCTACTTTTA	TTGGGTAATTTGGTCTCTCTATCC
KAUBG_20	$(CT)_{59}$	P	3	200-240	TGGGGATACCAATTACCAACTT	TCCCTCAAATCCATATTCCATC
KAUBG_21	$(AT)_{62}$	P	2	200-216	GGTTAGTTCTGGCTGGACTG	GGGAACACAGCTCCAACTCT
KAUBG_22	$(C)_{54}$	NP	1	148	CCCTCTCCCCATACAATGAC	TTGTGACATCTGCAGACAAGG
KAUBG_23	$(CA)_{73}$	P	3	500-523	TGTCCTGATATATAAGCATGTACAAA	ATTTTATGTATAACCACCCGTTG
KAUBG_24	$(C)_{66}$	NP	1	196	GTGCCTTTATGGCCTTTGTG	CCATATGCCTGGCAAACAGA
KAUBG_25	$(GA)_{51}$	P	2	235-245	AGTCGCCGAAACCCTAATTT	GGTTCGCTGCTGTTCTTTCT
KAUBG_26	$(CT)_{66}$	P	3	250-333	AAGAGCAGCAAAAGGCATTC	TCCGAAACGGGTTTACTCTG
KAUBG_27	$(G)_{52}$	NP	1	158	CGATCTGCACCTGAAAACAA	GCGATGTCAAGTATGTTGTCTGT
KAUBG_28	$(TG)_{120}$	NP	1	525	TCATTAAGAATGAAGGGTTGAGA	TCACATTAAATGCTATGTCGGTTT
KAUBG_29	$(TC)_{68}$	P	2	190-223	TGTCATGGTGTGGGATCTGT	CAGGTCGAACTTGAGCAACA
KAUBG_30	$(CT)_{50}$	P	2	200-209	GACGGAGAAAATGGGCTATTC	CTTTGGAACCCAGTGGAAGA
KAUBG_31	$(G)_{56}$	NP	1	298	TCGGATTTGTTCCAAACTCA	GAAGAGAGAATAACAGACGAGGAA
KAUBG_32	$(TC)_{50}$	P	3	150-197	TCAAGGGACAGGATCTAATCG	GGCTGCCATTAATTCTTCTAGG
KAUBG_33	$(GA)_{52}$	NP	2	248	ACACAACAACAGGAGGCAAT	CCAGCTGAATCTTGGTTGGT
KAUBG_34	AC262	NA	0	643	GGTTACAGCATTACAACTCACAAC	GCAAATTGGCTCGGAATATG
KAUBG_35	$(CA)_{216}$	NP	1	582	TCAACTCCTTGTCCCGTTTC	GTTTTGGCGGGAAATAACAC

KAUBG_36	$(CA)_{113...}(CA)_{50}$	NP	1	589	AAAGTTACCGTTTTCACACACG	ATCGGTATGGCAACCCTAGA
KAUBG_37	$(TC)_{51}$	P	2	240-280	TTCTTTTCGCCTCTCACACA	CATCGTCGACCGAGGATATT
KAUBG_38	$(AAG)_{44}$	P	2	190-221	ACCCAACCCAATTGGTAGAA	TTCCTTCTTCACTCCATCTTCC
KAUBG_39	$(TTC)_{36}$	NP	1	300	CCAAAGAAAACCCACATTCC	CCAGCATGGGAAACAAAACT
KAUBG_40	$(CTT)_{31}$	P	2	250-289	GAAGTGATTTTGCAACCTATCAAC	GATGAAGCTCTATGTTGTGTTTTG
KAUBG_41	$(AGA)_{33}$	P	2	100-158	CGGAAGCTGCTGTATGAACA	CGGAGCACAGGATCCATAAT
KAUBG_42	$(AGA)_{33}$	P	2	365-370	GATGGTGAGAATGGCGTTTT	TGGCATGATTAGGCTTTTCC
KAUBG_43	$(AAG)_{32}$	P	2	250-328	CAAAGTATAGAGACTACCTTGGAC	TTTGGGGTAGGCAGTCAAAA
KAUBG_44	$(ACAT)_{10}$	P	3	207-280	TGGTTGTTACACATACATACACAC	GTAGTTGAGTTTAAATAGAGGACA
KAUBG_45	$(AAAT)_{12}$	NP	1	125	CACATTGGCCAAGCAGAAAT	AAACCTGAATCACATCCACCA
KAUBG_46	$(TAAA)_{10}$	P	3	290-301	AAACCTAGGGTTTAAGGGCTTTT	GCAAAAATTAAATAGGGGAAGG
KAUBG_47	$(TTTA)_{10}$	P	3	230-243	ATCCGAACAAGGCAATTGAA	TGAATGTTTATTGCGGTGAA
KAUBG_48	$(TATT)_{10}$	P	2	290-310	GTTCAAATCTTCACCCCACA	GTTGCATCACCATTTGTCCA
KAUBG_49	$(CCCTT)_{10}$	NP	1	286	CAAGCAGCAGCAACAACACT	GTTTAGATGGGATGGGATGG
KAUBG_50	$(TTCT)_{13}$	NP	1	245	CTCTCTAACGGCTCTTCAACG	GACATGTGGCGAGTCAAAAA
KAUBG_51	$(GATGA)_{12}$	P	2	295-306	TCTTTTGCTCTGACGGAGGT	TGATGAGCGAGGAAGAAGGT
KAUBG_52	$(ATAC)_{12}$	P	2	200-216	CCCAAATCGAAGAACCGATA	CGTACACGCTCATTTCGTTG
KAUBG_53	$(ATGATTG)_{10}$	P	2	200-243	GGAAGTATCAGCTCCGCAAG	TGGAGTAGGTGGGATTTTGTC
KAUBG_54	$(ACGC)_{19}..(ACGC)_{11}$	P	2	200-419	ACACACACACACACGCACACGCAC	GCCACAAGAAAAATGTTGGAA
KAUBG_55	$(TATT)_{10}$	P	2	241-250	GTGTAAATCAATAAGGTAGATCAGGAC	GAACGTTGATGAACAATATCCA

KAUBG_56	$(TATT_{10}$	NP	1	300	TGGAATACTATAGGTTATCAGGGATAGA	CACCATTTCCCAGCAAAAAT
KAUBG_57	$(TGTA)_{12}(TATG)_{10}$	NP	1	180	CCCTCCCCTCCATCTTCATA	TCCTTCGACTCGACAAAATG
KAUBG_58	$(TATG)_{12}$	NP	1	253	GCATTGAAGGATGAGCAATG	TCCCCGTGTCTACGGTATTC
KAUBG_59	$(TATT)_{10}$	P	2	230-243	ATCCGAACAAGGCAATTGAA	TGAATGTTTATTGCGGTGAA
KAUBG_60	$(TTAATTT)_{10}$	P	2	290-308	GCGTGTGGAGGAAGAAGAAG	CAACTGCTCCAAGTAAAGAAAAA
KAUBG_61	$(GAGG)_{10}$	NP	1	222	CATCCCCTCCCACAGTGATA	TTGAGGAACTCCTGGCTGAT
KAUBG_62	$(GAGG)_{10}$	NP	1	335	GTGGAGATTTCAAGGCCAAA	TCTCTCTCATTGGTAGTATGCCTT
KAUBG_63	$(ATACAT)_{10}$	P	2	150-227	CCTTGTTCCGTCGAATTTATGT	GGGTGATAATTTTAACCTACTTCTTAAA
KAUBG_64	$(AATA)_{10}$	P	2	144-155	TGGATCCCTGATACTCATGTCA	CCAAAGACCAAAAGCCGAAA
KAUBG_65	$(CTTCT)_{11}$	P	2	190-208	GCTGCAATATTTGCAACAGC	TGTGGAATGCCCTCAAAATC
KAUBG_66	$(AACCCTA)_{25}$	P	3	200-320	AACCCTAAACCCTAAACCCTAAAC	CAACCTAAAGAAAGAGTCCAACTAA
KAUBG_67	$(ATGT)_{18}$	NP	1	253	GCATTGAAGGATGAGCAATG	TCCCCGTGTCTACGGTATTC
KAUBG_68	CCTAAAC12	NA	0	364	CCTAAACCCTAAACCCTAAACCCT	CCCCACTTCCTCGCTTTTT
KAUBG_69	$(TTTTAAAA)_5$	NP	3	248-260	CACTTGCCATTTCTTTAACTTTTC	CGTAAACATCACACCAATACCTT
KAUBG_70	$(TCTTCCTT)_6$	NP	1	181	TGAGCTCGCATCTACTCTTCC	TTCGTCTCCTCCTCCTTCAA
KAUBG_71	AAAATTAA5	NA	0	193	CCTAAGTCCTTGAAATAGCAATGG	CTACTAATCATTTTCTTC
KAUBG_72	$(CTCCCTCT)_5$	P	2	290-300	CCCGAATCCAAGAAAAGGAT	ATGGCAAGAAACGAAGATGG
KAUBG_73	$(AGAAAGAG)_5$	P	2	240-257	GCATGTCTCTGTCACCAGGA	ATGCCCTTTCTCTCCTACCC
KAUBG_74	$(AAGAAAAA)_5$	NP	1	209	ATCCATGAGCTCCAAACCAA	GTTACGAGGGTGGCAATCAT
KAUBG_75	$(TC)_{24}$	P	2	250-286	TCTGACACTGCCACAGGTTC	AAACAGACAAATGGGGTTGC

P: Polymorphic, NP: Not polymorphic, NA: Not amplified

one to two alleles in the parental lines (Plate 5), producing a total of 88 alleles of size ranging between 144 bp to 523 bp.

4.3 GENERATION OF MAPPING POPULATION

4.3.1 Crossing of parental lines

Direct and reciprocal crosses were made between cultivar Priyanka (*Momordica charantia* var. *charantia*) and IC634896 (*M. charantia* var. *muricata*). Hybridization was carried out under net house conditions. Completely matured seeds were harvested from the fruits that were generated through crossing. The number of flowers crossed, fruits obtained and seeds harvested from each of the cross are presented in Table 4.

4.3.2 Confirmation of hybridity in F_1 plants

The harvested seeds of both cross combinations were sown. The germinated seedlings were subjected to hybridity confirmation using a parental polymorphic microsatellite marker, McSSR62. All the germinated plantlets were hybrid as they showed a heterozygous locus for the polymorphic marker (Plate 6). Details of number of seeds germinated and number of confirmed F_1 plants are given in Table 4. Hybrids of both the crosses were showing intermediate phenotype between the parental lines for traits like seeds size, fruit length, fruit breadth, and leaf size (Table 5). Phenotypic observations of hybrid plants of the cross Priyanka × IC634896 had higher values than reciprocal cross for the traits like length and breadth of fruit, length of peduncle, flesh thickness, mean fruit weight, number of

fruits per plant, and yield per plant (Table 5). The hybrid plants of the cross Priyanka × IC634896 also flowered earlier than that of IC634896 × Priyanka.

4.3.3 Generation of $F_{2:3}$ population

The confirmed F_1 plants were further selfed to obtain the F_2 seeds during February to April, 2021. The F_2 population of 200 plants was raised in field and each plant was selfed to obtain $F_{2:3}$ seeds during April to June, 2021. Seeds were harvested separately from each F_2 plants and one seed from each plant was taken to constitute $F_{2:3}$ population of 200 plants. This mapping population was raised in field during July to October, 2021 (Plate 7) and evaluated for the yield traits.

4.4 PHENOTYPIC EVALUATION OF $F_{2:3}$ POPULATION FOR YIELD TRAITS

Mapping population was evaluated for twenty seven different traits including different characters related to flowers, fruits, seeds, leaves, and vines. Details of all the observations are given in Annexures II and III where summary of these observations is given in Table 6. Significant variation was observed for all the traits in the mapping population. The observations for almost all the investigated traits fall under normal distribution indicating polygenic nature of these traits (Figure 9).

4.4.1 Flower related traits

Days to staminate flower initiation ranged from 51 to 71 with the mean of 59.52 days in the mapping population. Days to pistillate flower initiation ranged

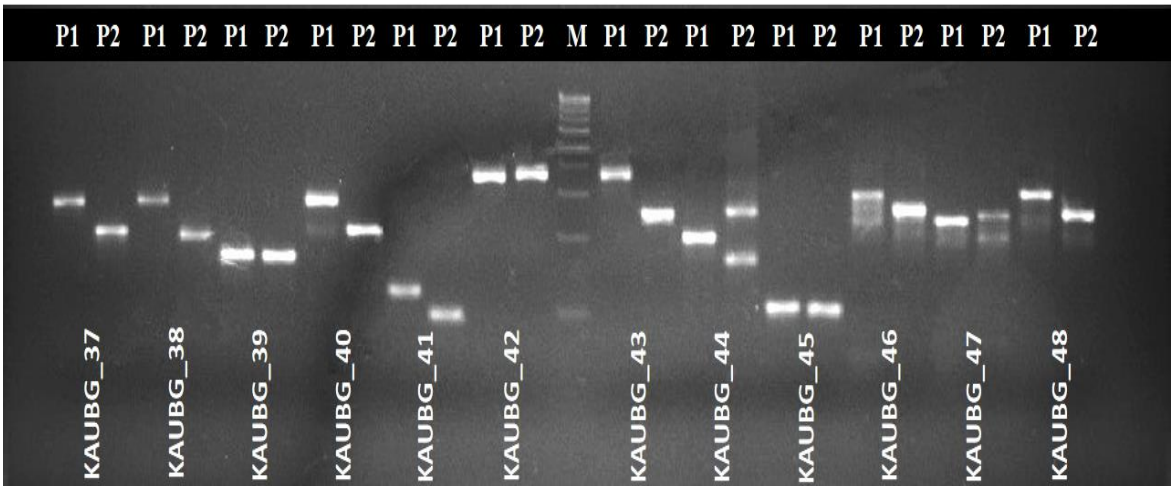


Plate 5. Amplification profile of representative newly identified microsatellite markers

M: 50 bp Ladder, P1: Priyanka, P2: IC634896

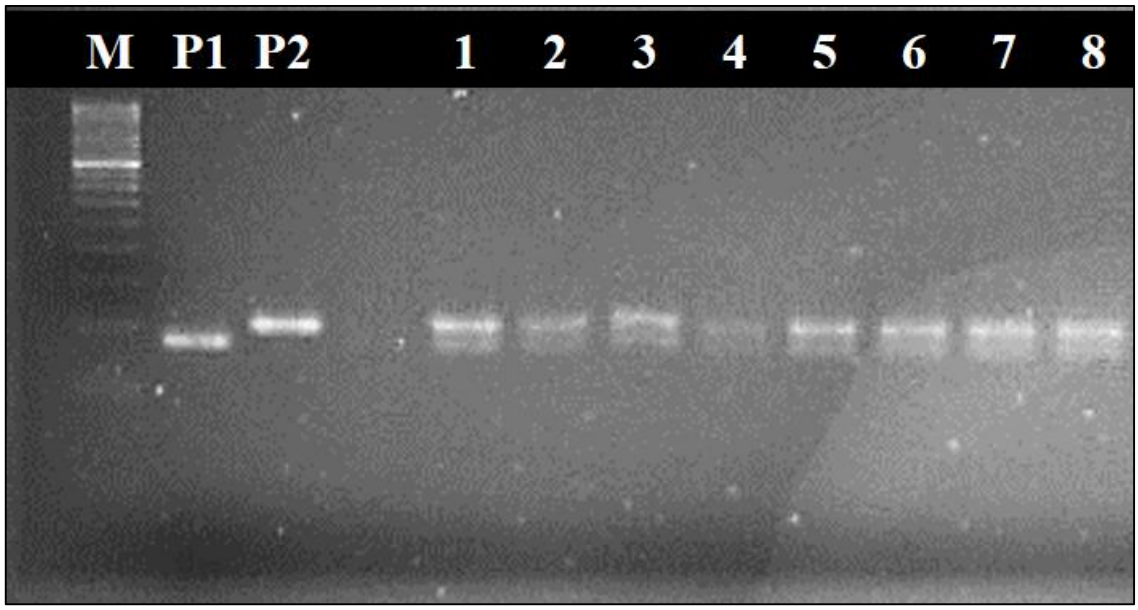


Plate 6. Agarose gel electrophoresis showing heterozygous locus at McSSR62 in F_1 plants

M: 100 bp Ladder, P1: Priyanka, P2: IC634896,

1-4: Priyanka × IC634896, 5-8: IC634896 × Priyanka

Plate 7. Evaluation of $F_{2:3}$ population under field condition

Table 4. Number of F_1 seeds obtained in this study

Cross	No. of flowers crossed	No. of fruits obtained	No. of seeds harvested	No. of seeds sown	Seeds germinated	No. of hybrids confirmed with polymorphic marker
Priyanka × IC634896	3	3	53	4	4	4
IC634896 × Priyanka	26	26	222	6	4	4

Table 5. Morphological characters of parental lines and F_1 plants

Cross	Days to staminate flower	Days to pistillate flower	Fruit length (cm)	Fruit breadth (cm)	Fruit Length /Breadth	Peduncle length (cm)	Fruit Length/ Peduncle length	Flesh thickness (mm)	Number of seeds	Fruit weight (g)	No. of Fruits / plant	Yield/ plant (g)	Leaf area (cm^2)
Priyanka	37	39	14.2	5.4	2.63	7.1	2.16	7.2	21.2	114.46	17	1042.32	130.7
IC634896	45	42	3.9	2.4	1.69	2.4	1.75	2.3	18.8	6.16	38	241.2	30.5
Priyanka × IC634896	45	42	9.8	3.8	2.57	6	1.66	5.4	26.6	47.02	80	2287.41	84.65
IC634896 × Priyanka	50	49	8.8	3.5	2.56	4.5	2.28	4.9	23.4	36.13	33	783.68	39.25

Table 6. Summary of morphological observations on 200 $F_{2:3}$ plants

Traits	Priyanka	IC634896	$F_{2:3}$ plants		
			Mean	Minimum	Maximum
Days to staminate flower	54	50	59.52	51	71
Days to pistillate flower	57	48	59.54	51	76
First pistillate flower node	16	9	14.38	3	26
Number of staminate flowers	149	327	378.3	13	1005
Number of pistillate flowers	16	48	27.75	2	85
Sex ratio	9.3	6.8	16.71	4.3	37.2
Fruit length (cm)	14.2	3.9	9.6	4	15.8
Fruit breadth (cm)	5.4	2.4	3.52	2.2	4.8
Fruit Length /Breadth	2.6	1.7	2.7	1.5	4.1
Fruit weight (g)	114.46	6.16	53.32	7.1	115
Flesh thickness (mm)	7.00	2.00	3.52	2	5
Peduncle length (cm)	7.1	2.4	5.62	1.1	11.7
Fruit Length/Peduncle length	2.0	1.6	1.93	0.8	6.6

Traits	Priyanka	IC634896	Mean	Minimum	Maximum
Number of seeds	21.2	18.3	15.88	7	27
Seed length (mm)	15.4	9.5	11.2	8.9	14.2
Seed breadth (mm)	8.2	5.1	6.3	4.1	8.6
Number of fruits per plant	14	42	24.7	1	71
Fruit yield per plant (g)	1570	321	1180	18	3597
Leaf size (cm^2)	137.2	35.32	72.7	13.7	167.5
Internodal length (cm)	8.3	6.2	6.6	4.2	9.5
Vine length (m)	3.1	2.7	3.9	1.2	6.7
Stem girth (cm)	1.9	0.6	1.3	0.5	2.5
Number of side branches	17	21	16.3	8	30

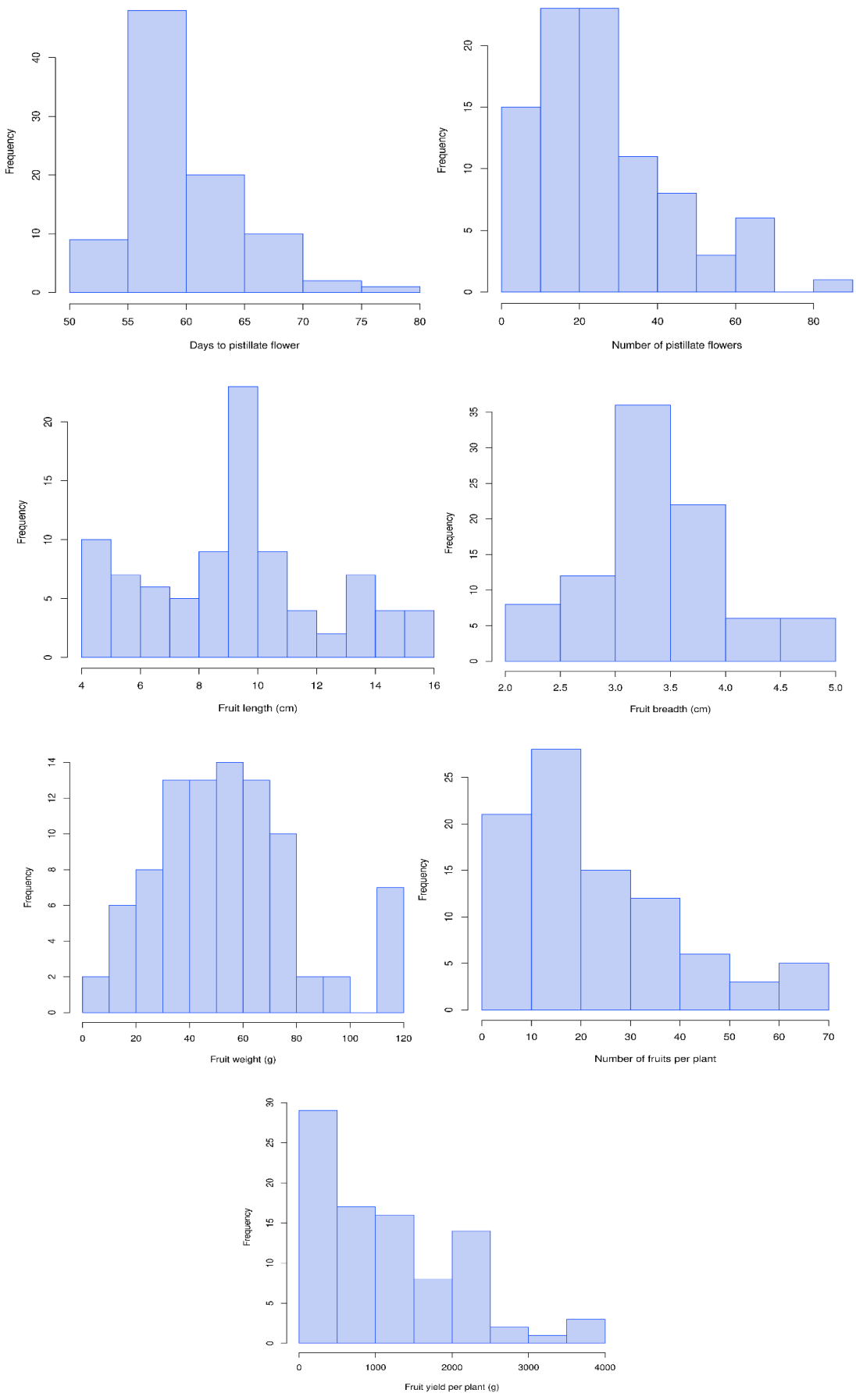


Figure 9. Frequency distribution of yield contributing traits in $F_{2:3}$ population derived from a cross between Priyanka and IC634896

from 51 to 76 with the mean of 59.54 days. IC634896 flowered earlier (50 and 48 days, respectively for male and female flowers) than Priyanka (54 and 57 days, respectively for male and female flowers). First pistillate flower node had a mean of 14.38 while ranging from 3 to 26 in mapping population. IC634896 (9^{th} node) had first female flower at lower node than Priyanka (16^{th} node). Number of staminate flower ranged from 13 to 1005 with the mean of 378.3, while IC634896 had more staminate flowers than Priyanka. IC634896 (48) had significantly more number of pistillate flowers than Priyanka (16), where it ranged from 2 to 85 in the mapping population with the mean of 27.8 flowers. Sex ratio i.e. ratio between number of staminate flowers to pistillate flowers was higher in Priyanka (9.3) than in IC634896 (6.8), whereas it ranged from 4.3 to 37.2 in the mapping population, with the mean of 16.7 (Table 6).

4.4.2 Fruit related traits

Cultivar Priyanka (14.2 cm) had longer fruits than that of IC634896 (3.9 cm) (Plate 1) while it ranged from 4.0 to 16.0 cm in the mapping population, 9.6 cm being the mean value (Plate 8). Breadth of the fruits was more in Priyanka (5.4 cm) than IC634896 (2.4 cm) where mapping population had a mean fruit breadth of 3.5 cm. The ratio between length and breadth of the fruit was ranging between 1.5 and 4.1 in mapping population while Priyanka (2.6) showed higher ratio than IC634896 (1.7). Peduncle length varied widely, ranging from 1.1 to 12.0 cm in the mapping population, with the mean of 5.6 cm. Ratio between fruit length and peduncle length ranged from 0.8 to 6.6 in mapping population where it was higher

in Priyanka (2.0) than IC634896 (1.6). Flesh thickness and fruit weight were more in Priyanka (7 mm and 114.46 g, respectively) than those in IC634896 (2.0 mm and 6.16 g, respectively). In the mapping population, flesh thickness ranged from 2.0 to 5.0 mm and fruit weight ranged from 7.1 to 115.0 g. Accession IC634896 (42) had remarkably higher number of fruits than Priyanka (14). It ranged between 1 and 71 in the mapping population where the mean was 24.7. Fruit yield in the mapping population varied between 18.0 and 3597.0 g with the mean yield of 1180.4 g. Priyanka yielded higher (1570 g) than IC634896 (321 g) (Table 6).

4.4.3 Seed related traits

Number of seeds per fruit has ranged between 7 and 27 in the mapping population while Priyanka (21.2 seeds) had more seeds than IC634896 (18.3 seeds). Priyanka had more seed length (15.4 mm) and breadth (8.2 mm) than that of IC634896 (9.5 mm and 5.1 mm, respectively). In the mapping population, seed length and breadth have ranged from 8.9 to 14.2 mm and 4.1 to 8.6 mm, respectively (Table 6; Plate 9).

4.4.4 Leaf related traits

Cultivar Priyanka had larger leaves with mean area of 137.23 cm^2 compared to IC634896 (35.32 cm^2). It ranged from 13.7 to 167.5 cm^2 in the mapping population, with the mean of 72.7 cm^2. IC634896 had leaves with darker green color compared to Priyanka (Table 6).

Plate 8. Variation for length, breadth and color of fruits in $F_{2:3}$ population derived from a cross between Priyanka and IC634896

Plate 9. Variation for length, breadth and color of seeds in $F_{2:3}$ population derived from a cross between Priyanka and IC634896

4.4.5 Vine related traits

Cultivar Priyanka had longer vines (3.1 m) than IC634896 (2.7 m). It ranged from 1.2 to 6.7 m in the mapping population, with a mean of 3.9 m. Internodal length was more in Priyanka (8.3 cm) compared to IC634896 (6.2 cm) while it ranged from 4.2 to 9.5 cm in the mapping population. Number of side branches varied from 8 to 30 in the mapping population where it was higher in IC634896 (21.0) than Priyanka (17.0). Stem thickness was more in Priyanka (1.9 cm) than IC634896 (0.6 cm) while it ranged between 0.5 to 2.5 cm in the mapping population (Table 6).

4.5 GENOTYPING OF $F_{2:3}$ MAPPING POPULATION

Initial polymorphism analysis, where 450 microsatellites from the literature were screened against parental lines, has identified 47 polymorphic markers. Subsequently, 75 additional microsatellites were identified in this study by scanning *Momordica* genome and 38 of them were polymorphic among the parents. This constituted a set of 85 polymorphic microsatellites which were ready to be used in further analysis. Phenotypic evaluation of 200 $F_{2:3}$ plants had shown significant variation for the traits studied. Using the square root transformed values for the 27 traits recorded, 200 $F_{2:3}$ plants in the mapping population were subjected to hierarchical clustering and based on this, a set of 90 plants was selected, such that it represented the phenotypic variation of entire mapping population. Details of these selected plants along with their phenotypic values are given in Table 7 and 8.

The genomic DNA was isolated from the panel of selected 90 plants and it was used for PCR amplification of 85 polymorphic microsatellites to obtain the genotypic data of the mapping population. The genotypic data was entered in Microsoft excel worksheet using ABH system where 'A' represented the homozygous allele from female parent Priyanka, 'B' represented the homozygous allele from male parent IC634896 and 'H' represented the heterozygous allelic status. The missing data was coded as 'X' (Figure 2).

4.6 CONSTRUCTION OF LINKAGE MAP

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. Linkage map was constructed using IciMapping software. The linkage map generated had 11 linkage groups (LGs) corresponding to 11 chromosomes, accommodating all the 85 markers, covering 1287.99 cM distance (Figure 10). The linkage groups were allocated to respective chromosomes by using published anchor information of microsatellites. This anchor information was included in the input file used for linkage mapping (Figure 3). Summary of the marker distribution on linkage map is given in Table 9. LG 7 (28) consisted maximum number of markers followed by LG 2 and LG 9, each having 11 markers. LG 1 had 10 markers whereas LG 3, 4 and 8 had seven markers each. LG 5, 6, 10 and 11 had only one marker each. LG 7 covered maximum map distance of 384.19 cM where LG 8 covered least map distance of 68.58 cM. The linkage group wise details of markers along with marker position and interval between them are given in Table 10.

Table 7. Morphological observations on flower and fruit related traits of 90 $F_{2:3}$ plants used for QTL mapping

Plant name	Days to staminate flower	Days to pistillate flower	First pistillate flower node	Number of staminate flowers	Number of pistillate flowers	Sex ratio	Fruit length (cm)	Fruit breadth (cm)	Fruit Length / Breadth	Fruit weight (g)	Flesh thickness (mm)	Peduncle length (cm)	Fruit Length/ Peduncle length	Number of fruits per plant	Fruit yield per plant (g)
1	55	58	12	425	21	20.2	8.7	3.7	2.4	78.2	3	7.2	1.2	19	1364
2	54	56	5	187	16	11.7	10.9	3	3.6	34.7	4	6.6	1.7	11	304
4	53	56	10	176	9	19.6	4.5	2.2	2.0	16.1	2	1.7	2.6	6	45
5	54	57	12	560	24	23.3	9.3	4.4	2.1	58	4	5.1	1.8	23	997
7	68	69	14	71	3	23.7	9.9	2.9	3.4	40.3	3	3.1	3.2	1	40
8	53	57	12	495	31	16.0	9.8	2.8	3.5	35.8	3	6.4	1.5	23	677
9	56	59	16	409	45	9.1	10.2	3.1	3.3	86.1	3	4.9	2.1	44	2737
10	67	68	16	62	3	20.7	4.6	2.5	1.8	10.1	2	4.9	0.9	2	18
12	51	59	17	368	24	15.3	10.1	3.3	3.1	45.5	3	4.7	2.1	18	733
13	59	57	12	377	45	8.4	13.4	3.4	3.9	61.5	4	6.9	1.9	40	2289
14	63	59	8	446	12	37.2	6.4	2.8	2.3	34.3	4	6	1.1	11	321
17	51	52	14	511	33	15.5	11.1	4.3	2.6	60.2	4	7.2	1.5	30	1637
18	61	63	17	182	17	10.7	13.7	3.6	3.8	62.5	3	5	2.7	13	799
19	55	55	20	451	48	9.4	9	3.4	2.6	49.3	3	6.7	1.3	40	1666
21	56	57	17	368	63	5.8	9.4	3.3	2.8	32.4	4	5.9	1.6	61	1684

22	65	56	4	371	15	24.7	10.4	3.7	2.8	73.2	3	5.2	2.0	14	1120
23	61	59	15	280	17	16.5	9.3	3.9	2.4	52.3	4	3.6	2.6	12	587
24	57	59	18	461	26	17.7	5.2	3.1	1.7	49.2	3	3.1	1.7	22	1101
25	59	61	13	416	40	10.4	12.4	3.6	3.4	77.4	3	5.3	2.3	36	2358
26	57	54	17	308	57	5.4	8.3	3.1	2.7	37.2	3	5.4	1.5	49	1474
27	56	56	17	782	37	21.1	7.3	2.9	2.5	31.4	4	5.1	1.4	37	1163
28	59	55	14	37	2	18.5	8.3	3.1	2.7	27	3	5.5	1.5	1	27
29	53	54	7	598	84	7.1	6.2	3.3	1.9	28.6	4	5.8	1.1	70	2123
30	62	57	11	374	17	22.0	5.6	3.4	1.6	20.4	3	2.4	2.3	14	211
31	55	57	12	978	46	21.3	8.7	3.6	2.4	53.6	4	11.4	0.8	44	2092
32	54	60	17	580	36	16.1	10.9	3.5	3.1	82.2	4	6.9	1.6	31	1993
33	59	61	15	391	18	21.7	7.2	3.4	2.1	32.1	3	5.2	1.4	11	421
34	59	59	9	390	65	6.0	9.4	3.5	2.7	51.4	5	6.9	1.4	65	2427
35	56	59	12	273	22	12.4	14.1	4.6	3.1	110.2	5	11.4	1.2	20	2120
36	65	59	17	132	20	6.6	4.9	3.2	1.5	17.4	3	1.4	3.5	19	297
37	58	60	23	294	22	13.4	15.2	3.7	4.1	115	5	7.5	2.0	22	3578
38	69	69	23	190	11	17.3	14.6	4.6	3.2	115	5	5.9	2.5	8	1206
39	64	76	18	392	13	30.2	6.6	3.1	2.1	25.2	3	1.1	6.0	5	118
40	63	68	13	446	12	37.2	6.8	2.6	2.6	62.6	3	4.6	1.5	7	378

41	55	60	19	416	23	18.1	9.8	3.4	2.9	48.2	4	8.2	1.2	20	664
42	61	67	24	368	21	17.5	9.2	2.7	3.4	63.6	4	5.1	1.8	18	1094
43	56	60	16	494	37	13.4	13.1	4.6	2.8	115	5	6.7	2.0	37	3578
44	57	53	14	220	32	6.9	5.6	2.8	2.0	58.4	3	1.7	3.3	31	1639
45	59	57	11	247	58	4.3	11.6	3.4	3.4	72.6	3	6.9	1.7	53	3003
46	59	60	17	187	8	23.4	4	2.2	1.8	20.4	2	3.9	1.0	8	134
47	51	53	8	598	49	12.2	7.6	3.4	2.2	44.6	3	5.9	1.3	49	1471
48	58	62	19	264	23	11.5	9.7	4	2.4	66.2	3	2.9	3.3	18	1201
49	53	56	10	588	55	10.7	10.6	3.3	3.2	76.4	3	6.5	1.6	43	2558
50	51	57	15	341	16	21.3	5.6	3.4	1.6	57.1	3	3.1	1.8	14	767
51	60	59	16	414	24	17.3	9.6	3.7	2.6	48.4	5	5.2	1.8	24	1000
52	59	61	26	280	12	23.3	8.3	3.1	2.7	56.6	3	3.1	2.7	10	621
54	59	62	13	741	63	11.8	4.7	2.3	2.0	7.6	2	4.1	1.1	60	457
56	54	53	12	320	13	24.6	7.3	2.9	2.5	42.8	3	1.1	6.6	13	510
57	70	65	15	641	29	22.1	9.3	3.3	2.8	54.4	3	7.2	1.3	22	1063
58	61	71	18	316	15	21.1	7.3	3.2	2.3	43.9	3	3.4	2.1	11	476
59	63	74	14	445	19	23.4	5.6	2.9	1.9	53.2	3	3.9	1.4	16	923
60	64	65	14	13	2	6.5	9.9	3.8	2.6	33.8	4	5.1	1.9	1	34
61	59	65	19	315	23	13.7	15.2	4.1	3.7	94.2	4	7.6	2.0	22	2050

62	63	62	18	91	3	30.3	12.4	3.8	3.3	72.9	4	5.9	2.1	3	192
63	69	64	13	143	4	35.8	11.1	3.3	3.4	72.2	2	8.9	1.2	4	289
64	69	59	12	441	17	25.9	6.4	3.1	2.1	32.3	3	2.1	3.0	14	493
65	56	61	10	192	11	17.5	10.4	3.7	2.8	40	3	4.6	2.3	10	372
66	57	65	21	208	10	20.8	6.9	3.1	2.2	28.2	3	1.9	3.6	9	261
67	59	56	19	260	36	7.2	13.4	3.8	3.5	66.4	5	4.1	3.3	35	2295
68	60	62	17	224	10	22.4	10.7	4.1	2.6	75.8	5	6.2	1.7	10	443
69	62	67	13	280	39	7.2	11.1	3.2	3.5	63.4	3	8.1	1.4	34	1922
71	68	70	16	143	5	28.6	10.6	4.6	2.3	80	5	9.1	1.2	5	421
75	59	60	17	187	8	23.4	4	2.2	1.8	20.4	2	3.9	1.0	8	134
76	59	56	19	260	36	7.2	13.4	3.8	3.5	66.4	5	4.1	3.3	35	2295
79	53	56	10	176	9	19.6	4.5	2.2	2.0	16.1	2	1.7	2.6	6	45
80	59	57	12	377	45	8.4	13.4	3.4	3.9	61.5	4	6.9	1.9	40	2289
81	67	68	16	62	3	20.7	4.6	2.5	1.8	10.1	2	4.9	0.9	2	18
84	61	63	17	182	17	10.7	13.7	3.6	3.8	62.5	3	5	2.7	13	799
87	59	62	13	741	63	11.8	4.7	2.3	2.0	7.6	2	4.1	1.1	60	457
90	56	59	12	273	22	12.4	14.1	4.6	3.1	110.2	5	11.4	1.2	20	2120
93	65	59	17	132	20	6.6	4.9	3.2	1.5	17.4	3	1.4	3.5	19	297
95	69	69	23	190	11	17.3	14.6	4.6	3.2	115	5	5.9	2.5	8	1206

97	55	58	12	425	21	20.2	8.7	3.7	2.4	78.2	3	7.2	1.2	19	1364
99	57	59	18	461	26	17.7	5.2	3.1	1.7	49.2	3	3.1	1.7	22	1101
101	59	65	19	315	23	13.7	15.2	4.1	3.7	94.2	4	7.6	2.0	22	2050
103	62	57	11	374	17	22.0	5.6	3.4	1.6	20.4	3	2.4	2.3	14	211
105	55	57	12	978	46	21.3	8.7	3.6	2.4	53.6	4	11.4	0.8	44	2092
109	58	60	23	294	22	13.4	15.2	3.7	4.1	115	5	7.5	2.0	22	3578
113	55	55	20	451	48	9.4	9	3.4	2.6	49.3	3	6.7	1.3	40	1666
122	55	60	19	416	23	18.1	9.8	3.4	2.9	48.2	4	8.2	1.2	20	664
125	61	67	24	368	21	17.5	9.2	2.7	3.4	63.6	4	5.1	1.8	18	1094
135	61	59	15	280	17	16.5	9.3	3.9	2.4	52.3	4	3.6	2.6	12	587
149	70	65	15	641	29	22.1	9.3	3.3	2.8	54.4	3	7.2	1.3	22	1063
153	59	59	9	390	65	6.0	9.4	3.5	2.7	51.4	5	6.9	1.4	65	2427
163	60	59	16	414	24	17.3	9.6	3.7	2.6	48.4	5	5.2	1.8	24	1000
168	54	57	12	560	24	23.3	9.3	4.4	2.1	58	4	5.1	1.8	23	997
171	53	57	12	495	31	16.0	9.8	2.8	3.5	35.8	3	6.4	1.5	23	677
181	56	57	17	368	63	5.8	9.4	3.3	2.8	32.4	4	5.9	1.6	61	1684
189	58	62	19	264	23	11.5	9.7	4	2.4	66.2	3	2.9	3.3	18	1201
191	64	65	14	13	2	6.5	9.9	3.8	2.6	33.8	4	5.1	1.9	1	34

Table 8. Morphological observations on fruit, seed, leaf and vine related traits of 90 $F_{2:3}$ plants used for QTL mapping

Plant name	Fruit color	Fruit ends	Fruit shape	Leaf color	Number of seeds	Seed length (mm)	Seed breadth (mm)	Leaf size (cm^2)	Internodal length (cm)	Vine length (m)	Stem girth (cm)	Number of side branches
1	White	Both ends pointed	Spindle	Light green	15.1	11.7	6.9	102.6	7.3	3.53	1.4	17
2	White	Both ends pointed	Spindle	Light green	18.2	12.2	7.0	50.6	7.6	3.09	1.2	11
4	Light green	Blossom end pointed	-	Dark green	7.0	11.6	7.0	30.8	6.9	3.95	1.1	18
5	Light green	Blossom end pointed	Oblong	Light green	8.0	11.7	6.7	66.6	5.9	3.1	0.7	24
7	White	Both ends pointed	-	Dark green	10.0	11.9	7.1	64.2	6.4	1.9	0.9	14
8	White	Both ends pointed	Cylindrical	Light green	11.0	11.0	6.0	116.5	7.5	4.65	1.9	15
9	Light green	Both ends pointed	Spindle	Light green	15.0	11.0	6.6	72.2	6.9	3.55	1.5	25
10	Light green	Both ends pointed	-	Light green	8.0	10.9	6.9	33.6	6.1	2.4	0.7	19
12	White	Both ends pointed	Spindle	Light green	13.0	12.5	7.5	49.7	6.3	4.8	0.8	16
13	White	Blossom end pointed	Oblong	Light green	20.0	12.2	7.1	89.2	6.9	3.6	1.4	13
14	White	Blossom end pointed	Elliptical	Light green	10.0	11.9	7.1	70.6	7.8	4.1	1.1	11
17	White	Both ends pointed	Spindle	Dark green	13.0	11.3	7.7	33.9	6.1	4.65	1.1	18
18	Light green	Both ends pointed	Spindle	Dark green	16.0	11.7	7.4	64.2	6.4	4.9	0.8	13
19	White	Both ends pointed	Spindle	Light green	12.0	10.7	6.5	64.7	6.1	5.1	1.6	23
21	Green	Blossom end pointed	Elliptical	Light green	22.0	10.1	5.8	71.8	5.7	5.8	1.6	16

22	Light green	Both ends pointed	-	Light green	18.0	12.1	7.2	81.1	6.3	2.9	1.2	-
23	Light green	Both ends pointed	-	Light green	14.0	10.9	6.5	87.4	6.8	3	1.8	18
24	Green	Blossom end pointed	-	Dark green	10.0	9.9	5.7	43.1	4.8	3.8	0.9	-
25	Light green	Both ends pointed	Spindle	Light green	14.0	11.0	5.8	91.8	7.2	5.55	1.2	13
26	Green+white	Blossom end pointed	Cylindrical	Dark green	14.0	10.0	6.3	91.1	7.6	3.95	1.8	14
27	Green+white	Blossom end pointed	Elliptical	Dark green	9.0	10.4	6.3	59.8	6.6	5.6	2.3	23
28	White	Both ends pointed	-	Dark green	21.0	9.5	5.3	89.1	6.1	1.6	0.8	-
29	Green	Blossom end pointed	Elliptical	Dark green	19.0	9.5	5.6	71.5	5.9	4.22	1.6	23
30	Green	Blossom end pointed	Globular	Dark green	8.0	9.9	5.7	21.1	4.8	1.94	1.2	17
31	Green	Blossom end pointed	Elliptical	Dark green	17.0	11.4	7.0	77.3	6.1	5.16	1.9	30
32	Light green	Blossom end pointed	Elliptical	Dark green	16.0	12.5	8.0	87.4	6.8	4.75	2.1	20
33	Green	Blossom end pointed	-	Light green	10.0	10.1	6.1	19.5	7.1	1.62	0.5	-
34	Light green	Blossom end pointed	Spindle	Dark green	18.0	10.9	6.5	87.3	6.3	2.96	0.9	15
35	Light green	Both ends round	Cylindrical	Light green	24.0	14.2	8.6	-	8.7	4.13	1.8	13
36	Light green	Blossom end pointed	Elliptical	Dark green	14.0	9.9	6.4	16.5	5.6	1.4	0.7	12
37	Light green	Both ends pointed	Oblong	Dark green	24.0	13.2	7.7	164.5	9.4	3.5	1.2	14
38	Light green	Both ends round	Cylindrical	Light green	26.0	14.2	8.6	35.7	6.9	3.1	0.9	10
39	Light green	Blossom end pointed	Elliptical	Dark green	12.0	10.3	6.8	36.0	7.4	4	1.1	28
40	Light green	Blossom end pointed	Rhomboid	Dark green	13.0	-	-	16.2	5.8	1.5	0.5	25

41	Light green	Blossom end pointed	Spindle	Dark green	20.0	12.4	7.9	148.4	8.8	4.22	1.2	26
42	White	Both ends pointed	Spindle	Dark green	11.0	11.1	7.1	115.6	8.6	4.34	2.4	16
43	Green	Both ends round	Elliptical	Dark green	13.0	13.8	8.3	92.1	6.1	4.8	1.5	13
44	Light green	Both ends pointed	Spindle	Dark green	9.0	11.2	6.4	43.1	4.8	1.7	0.8	22
45	Green	Blossom end pointed	Cylindrical	Dark green	12.0	10.8	8.0	45.7	6.7	6.3	2.1	13
46	Light green	Blossom end pointed	Spindle	Dark green	12.0	12.6	7.0	13.7	4.6	1.25	0.5	17
47	White	Both ends pointed	Spindle	Light green	17.0	11.9	7.1	131.5	8.2	4.95	2.3	22
48	White	Blossom end pointed	Elliptical	Dark green	13.0	12.0	6.7	80.9	7.6	4.9	1.6	12
49	Light green	Blossom end pointed	Spindle	Dark green	10.0	12.1	7.2	-	7.1	2.3	1.6	21
50	Green	Both ends pointed	-	Dark green	11.0	11.0	6.0	-	5.2	-	-	-
51	Light green	Blossom end pointed	Spindle	Light green	14.0	12.2	5.8	85.7	8.8	5.05	1.6	18
52	Light green	Both ends pointed	-	Light green	13.0	10.0	6.3	-	-	-	-	-
54	Green	Both ends pointed	Rhomboid	Dark green	16.0	9.5	5.3	22.0	5.3	5.5	1.4	19
56	Light green	Blossom end pointed	Spindle	Dark green	13.0	-	-	19.4	5.9	1.2	0.9	20
57	Green	Both ends pointed	Cylindrical	Dark green	19.0	-	-	91.1	6.7	4.6	1.2	28
58	Light green	Both ends pointed	-	Dark green	15.0	-	-	23.2	4.2	1.7	0.7	24
59	Light green	Blossom end pointed	-	Dark green	18.0	9.1	4.9	-	5.3	1.65	0.7	24
60	Light green	Both ends pointed	-	Dark green	19.0	-	-	114.6	5.6	1.9	0.6	16
61	Light green	Both ends pointed	Cylindrical	Light green	24.0	11.8	7.1	109.8	6.8	5.15	1.6	15

62	Light green	Both ends pointed	-	Dark green	23.0	11.2	7.2	121.2	6.1	1.2	0.7	16
63	White	Both ends pointed	Spindle	Dark green	15.0	-	-	113.9	8.3	4.2	0.9	11
64	Green	Both ends pointed	-	Dark green	13.0	-	-	-	5.1	1.3	-	-
65	Light green	Both ends pointed	Spindle	Light green	7.0	-	-	36.5	6.1	4.1	0.9	12
66	White	Both ends pointed	Spindle	Dark green	11.0	-	-	21.8	5.9	2.15	0.7	16
67	Light green	Both ends pointed	Spindle	Light green	23.0	11.9	7.5	63.1	6.9	4.7	1.3	13
68	Light green	Both ends pointed	Elliptical	Light green	21.0	10.9	5.6	51.8	5.6	3.4	1.2	14
69	White	Both ends pointed	Spindle	Light green	19.0	-	-	117.6	7.8	5.7	1.9	10
71	Light green	Both ends pointed	Oblong	Dark green	25.0	-	-	81.2	8.4	3.2	1.2	13
75	Light green	Blossom end pointed	Spindle	Dark green	12.0	12.6	7.0	13.7	4.6	1.25	0.5	17
76	Light green	Both ends pointed	Spindle	Light green	23.0	11.9	7.5	63.1	6.9	4.7	1.3	13
79	Light green	Blossom end pointed	-	Dark green	7.0	11.6	7.0	30.8	6.9	3.95	1.1	18
80	White	Blossom end pointed	Oblong	Light green	20.0	12.2	7.1	89.2	6.9	3.6	1.4	13
81	Light green	Both ends pointed	-	Light green	8.0	10.9	6.9	33.6	6.1	2.4	0.7	19
84	Light green	Both ends pointed	Spindle	Dark green	16.0	11.7	7.4	64.2	6.4	4.9	0.8	13
87	Green	Both ends pointed	Rhomboid	Dark green	16.0	9.5	5.3	22.0	5.3	5.5	1.4	19
90	Light green	Both ends round	Cylindrical	Light green	24.0	14.2	8.6	-	8.7	4.13	1.8	13
93	Light green	Blossom end pointed	Elliptical	Dark green	14.0	9.9	6.4	16.5	5.6	1.4	0.7	12
95	Light green	Both ends round	Cylindrical	Light green	26.0	14.2	8.6	35.7	6.9	3.1	0.9	10

97	White	Both ends pointed	Spindle	Light green	15.1	11.7	6.9	102.6	7.3	3.53	1.4	17
99	Green	Blossom end pointed	-	Dark green	10.0	9.9	5.7	43.1	4.8	3.8	0.9	-
101	Light green	Both ends pointed	Cylindrical	Light green	24.0	11.8	7.1	109.8	6.8	5.15	1.6	15
103	Green	Blossom end pointed	Globular	Dark green	8.0	9.9	5.7	21.1	4.8	1.94	1.2	17
105	Green	Blossom end pointed	Elliptical	Dark green	17.0	11.4	7.0	77.3	6.1	5.16	1.9	30
109	Light green	Both ends pointed	Oblong	Dark green	24.0	13.2	7.7	164.5	9.4	3.5	1.2	14
113	White	Both ends pointed	Spindle	Light green	12.0	10.7	6.5	64.7	6.1	5.1	1.6	23
122	Light green	Blossom end pointed	Spindle	Dark green	20.0	12.4	7.9	148.4	8.8	4.22	1.2	26
125	White	Both ends pointed	Spindle	Dark green	11.0	11.1	7.1	115.6	8.6	4.34	2.4	16
135	Light green	Both ends pointed	-	Light green	14.0	10.9	6.5	87.4	6.8	3	1.8	18
149	Green	Both ends pointed	Cylindrical	Dark green	19.0	-	-	91.1	6.7	4.6	1.2	28
153	Light green	Blossom end pointed	Spindle	Dark green	18.0	10.9	6.5	87.3	6.3	2.96	0.9	15
163	Light green	Blossom end pointed	Spindle	Light green	14.0	12.2	5.8	85.7	8.8	5.05	1.6	18
168	Light green	Blossom end pointed	oblong	Light green	8.0	11.7	6.7	66.6	5.9	3.1	0.7	24
171	White	Both ends pointed	Cylindrical	Light green	11.0	11.0	6.0	116.5	7.5	4.65	1.9	15
181	Green	Blossom end pointed	Elliptical	Light green	22.0	10.1	5.8	71.8	5.7	5.8	1.6	16
189	White	Blossom end pointed	Elliptical	Dark green	13.0	12.0	6.7	80.9	7.6	4.9	1.6	12
191	Light green	Both ends pointed	-	Dark green	19.0	-	-	114.6	5.6	1.9	0.6	16

'-' Missing data

Table 9. Summary of markers distribution on linkage map

Chromosome number	Linkage group	No. of markers	Length covered (cM)
1	LG 1	10	141.3
2	LG 2	11	197.4
3	LG 3	7	106.1
4	LG 4	7	183.1
5	LG 5	1	0.0
6	LG 6	1	0.0
7	LG 7	28	384.2
8	LG 8	7	68.6
9	LG 9	11	207.3
10	LG 10	1	0.0
11	LG 11	1	0.0
Total		85	1288.0

Table 10. Linkage group wise distribution of markers on linkage map

Linkage group	Marker name	Position of marker (cM)	Interval distance between the markers (cM)
1	S32	0.0	0.0
	S33	26.1	26.1
	S24	41.4	15.4
	KAUBG_1	56.3	14.8
	AVRDC-BG99	74.1	17.8
	KAUBG_2	88.1	14.0
	N24	103.8	15.7
	KAUBG_38	109.0	5.2
	S18	120.7	11.7
	KAUBG_44	141.3	20.6
2	S13	0.0	0.0
	AVRDC-BG66	23.8	23.8
	KAUBG_75	27.1	3.3
	KAUBG_48	32.6	5.6
	JY011	52.5	19.9
	AVRDC-BG70	98.3	45.8
	KAUBG_47	127.4	29.1
	KAUBG_5	133.7	6.3
	KAUBG_3	148.3	14.6
	JY009	180.0	31.7
	KAUBG_46	197.4	17.4
3	KAUBG_53	0.0	0.0
	S9	38.1	38.1
	KAUBG_51	58.4	20.3
	McSSR150	62.3	3.9
	KAUBG_52	70.1	7.8
	AVRDC-BG86	80.7	10.6
	N1	106.1	25.5

	KAUBG_13	0.0	0.0
	JY007	54.1	54.1
	KAUBG_55	84.6	30.5
4	KAUBG_15	104.8	20.2
	KAUBG_72	118.4	13.7
	KAUBG_40	143.7	25.2
	KAUBG_11	183.1	39.4
5	KAUBG_20	0.0	0.0
6	KAUBG_59	0.0	0.0
	JY004	0.0	0.0
	S12	28.1	28.1
	McSSR40	44.4	16.3
	AVRDC-BG101	62.7	18.3
	McSSR106	83.5	20.8
	McSSR57	109.6	26.1
	McSSR66	120.8	11.2
	N5	138.6	17.8
	AVRDC-BG75	166.0	27.4
	AVRDC-BG98	175.8	9.8
	McSSR99	187.3	11.6
7	KAUBG_26	196.0	8.7
	KAUBG_73	196.9	0.9
	AVRDC-BG109	203.7	6.8
	AVRDC-BG85	211.4	7.7
	McSSR62	222.3	10.9
	AVRDC-BG104	237.1	14.8
	McSSR20	253.6	16.5
	KAUBG_63	287.2	33.6
	KAUBG_25	301.9	14.7
	JY003	313.3	11.4
	KAUBG_41	319.5	6.2
	McSSR114	333.2	13.7

	S15	340.9	7.8
	N12	342.7	1.7
	KAUBG_21	350.7	8.1
	KAUBG_60	359.8	9.0
	KAUBG_23	384.2	24.4
	KAUBG_42	0.0	0.0
	AVRDC-BG112	12.3	12.3
	McSSR112	16.8	4.5
8	KAUBG_64	41.5	24.7
	KAUBG_43	61.6	20.1
	KAUBG_29	67.2	5.6
	KAUBG_30	68.6	1.4
	JY010	0.0	0.0
	S26	25.1	25.1
	JY008	42.7	17.6
	KAUBG_32	56.7	14.0
	JY006	97.4	40.7
9	JY001	97.4	0.0
	KAUBG_65	108.0	10.6
	McSSR35	113.4	5.4
	KAUBG_66	165.2	51.8
	AVRDC-BG26	183.0	17.8
	AVRDC-BG71	207.3	24.3
10	KAUBG_37	0.0	0.0
11	AVRDC-BG25	0.0	0.0

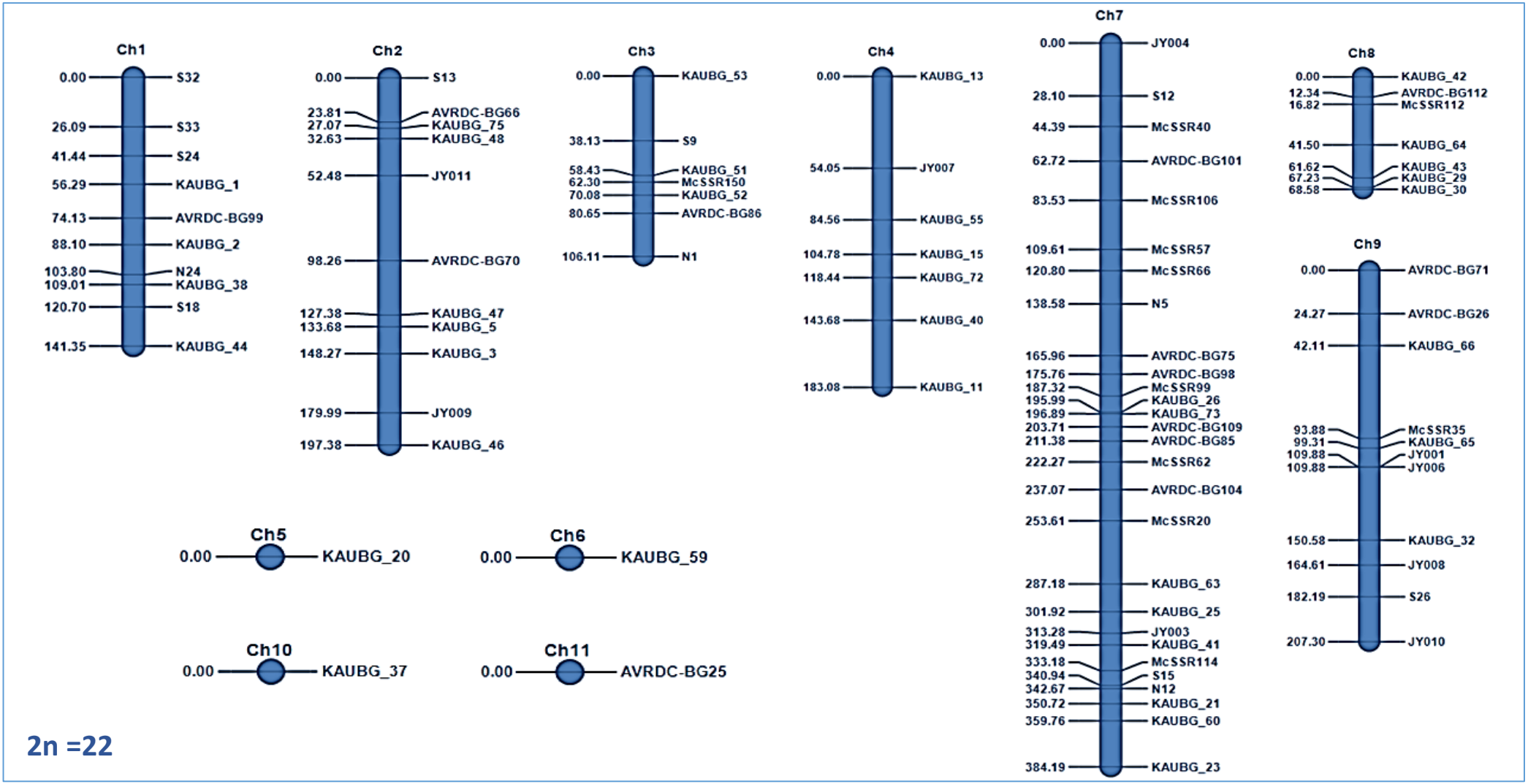


Figure 10. Linkage map of microsatellites using $F_{2:3}$ population (Priyanka × IC634896) of bitter gourd

4.7 QTL MAPPING

The linkage map obtained using molecular data of mapping population was utilized for QTL mapping. The input files were carefully prepared and software was run for identification of QTL showing LOD score of more than three. A total of 60 QTL were identified for 24 different traits on seven chromosomes. Highest number of QTL identified for a trait is 4, where the LOD value for these QTL ranged from 3.1 to 15.2. Per cent of phenotypic variation explained (PVE) by these QTL ranged from 1.8 per cent to 35.9 per cent. List of QTL identified for each trait is given in Table 11.

4.7.1 Flower related traits

Four QTLs were identified for days to staminate flower, one each on chromosome 3 and 8, and two on chromosome 7 (Figure 11; Table 11). LOD value ranged from 3.1 to 5.0 with PVE of 7.0 to 17.8 per cent. QTL qDSF-3-1 was located on chromosome 3 between marker interval KAUBG_51 to McSSR150 spanning a distance of 3.86 cM having LOD value 5.0 and PVE of 17.8 per cent. QTL qDSF-8-1 was located on chromosome 8 between marker interval KAUBG_42 to AVRDC-BG112 spanning a distance of 12.34 cM having LOD value 4.6 and PVE of 11.9 per cent. QTL qDSF-7-1 was located on chromosome 7 between marker interval AVRDC-BG109 to AVRDC-BG85 spanning a distance of 7.67 cM having LOD value 3.8 and PVE of 10.9 per cent. QTL qDSF-7-2 was located on chromosome 7 between marker interval S15 to N12 spanning a distance of 1.73 cM having LOD value 3.1 and PVE of 7.0 per cent.

Three QTL were identified for days to pistillate flower, one each on chromosome 1, 3 and 7 (Figure 12; Table 11). LOD value ranged from 3.1 to 4.1 with PVE of 9.2 to 12.1 per cent. QTL qDPF-7-1 was located on chromosome 7 between marker interval McSSR66 to N5 spanning a distance of 17.77 cM having LOD value 4.1 and PVE of 12.1 per cent. QTL qDPF-1-1 was located on chromosome 1 between marker interval KAUBG_1 to AVRDC-BG99 spanning a distance of 17.85 cM having LOD value 3.1 and PVE of 10.3 per cent. QTL qDPF-3-1 was located on chromosome 3 between marker interval KAUBG_51 to McSSR150 spanning a distance of 3.86 cM having LOD value 3.1 and PVE of 9.2 per cent.

One QTL qFPFN-2-1 was identified for first pistillate flower node on chromosome 2 between marker interval KAUBG_47 to KAUBG_5 spanning a distance of 6.31 cM having LOD value 4.1 and PVE of 18.3 per cent (Figure 13; Table 11).

Three QTL were identified for number of staminate flower, one on chromosome 7 and two on chromosome 9 (Figure 14; Table 11). LOD value ranged from 3.4 to 5.4 with PVE of 8.6 to 21.1 per cent. QTL qNSF-7-1 was located on chromosome 7 between marker interval AVRDC-BG104 to McSSR20 spanning a distance of 16.54 cM having LOD value 5.4 and PVE of 10.6 per cent. QTL qNSF-9-1 was located on chromosome 9 between marker interval JY006 to KAUBG_32 spanning a distance of 40.70 cM having LOD value 3.7 and PVE of 21.1 per cent. QTL qNSF-9-2 was located on chromosome 9 between marker interval AVRDC-

Table 11. Details of QTL identified for yield traits in bitter gourd

Name of the trait	No. of QTLs	Chromo some No.	QTL name	Left Marker	Right Marker	LOD value	Phenotypic variation explained (%)
Days to staminate flower	4	3	qDSF-3-1*	KAUBG_51	McSSR150	5.0	17.8
		8	qDSF-8-1	KAUBG_42	AVRDC-BG112	4.6	11.9
		7	qDSF-7-1	AVRDC-BG109	AVRDC-BG85	3.8	10.9
		7	qDSF-7-2	S15	N12	3.1	7.0
Days to pistillate flower	3	7	qDPF-7-1	McSSR66	N5	4.1	12.1
		1	qDPF-1-1	KAUBG_1	AVRDC-BG99	3.1	10.3
		3	qDPF-3-1*	KAUBG_51	McSSR150	3.1	9.2
First pistillate flower node	1	2	qFPFN-2-1	KAUBG_47	KAUBG_5	4.1	18.3
	3	7	qNSF-7-1	AVRDC-BG104	McSSR20	5.4	10.6

Number of staminate flowers		9	qNSF-9-2	JY006	KAUBG_32	3.7	21.1
		9	qNSF-9-1*	AVRDC-BG71	AVRDC-BG26	3.4	8.6
Number of pistillate flowers	2	2	qNPF-2-1*	JY009	KAUBG_46	7.6	26.0
		7	qNPF-7-1*	N5	AVRDC-BG75	3.5	8.7
Fruit length	1	8	qFrtL-8-1*	KAUBG_29	KAUBG_30	4.3	13.8
Fruit breadth	1	9	qFrtB-9-1*	KAUBG_66	McSSR35	6.9	21.2
Fruit Length /Breadth	1	2	qFSI-2-1	AVRDC-BG70	KAUBG_47	6.5	31.6
Fruit weight	3	2	qFrtWt-2-1*	S13	AVRDC-BG66	6.8	14.5
		9	qFrtWt-9-1*	KAUBG_66	McSSR35	4.3	18.1
		9	qFrtWt-9-2*	McSSR35	KAUBG_65	3.3	5.5
Flesh thickness	3	1	qFlTh-1-1*	KAUBG_2	N24	7.9	15.0
		2	qFlTh-2-1*	S13	AVRDC-BG66	7.9	21.3
		9	qFlTh-9-1*	KAUBG_32	JY008	4.0	9.9
	2	2	qNFrt-2-1*	JY009	KAUBG_46	7.7	27.1

Number of fruits per plant		7	qNFrt-7-1*	N5	AVRDC-BG75	3.4	6.8
Fruit yield per plant	2	8	qYldpl-8-1	McSSR112	KAUBG_64	3.5	16.9
		9	qYldpl-9-1*	KAUBG_32	JY008	3.1	5.7
Leaf size	4	2	qLfSz-2-2	KAUBG_3	JY009	4.7	13.5
		2	qLfSz-2-1*	JY011	AVRDC-BG70	4.1	10.8
		1	qLfSz-1-1*	S18	KAUBG_44	4.0	5.9
		7	qLfSz-7-1	KAUBG_73	AVRDC-BG109	3.5	3.2
Internodal length	3	9	qIntNdl-9-1*	AVRDC-BG71	AVRDC-BG26	5.2	14.8
		9	qIntNdl-9-2*	McSSR35	KAUBG_65	4.8	14.9
		7	qIntNdl-7-1	JY004	S12	3.5	9.5
Vine length	2	4	qVnL-4-1	KAUBG_13	JY007	4.5	1.8
		9	qVnL-9-1*	KAUBG_66	McSSR35	3.2	1.9
Stem girth	2	1	qStmGrth-1-1*	KAUBG_2	N24	3.4	3.4

		7	qStmGrth-7-1*	N5	AVRDC-BG75	3.3	5.6
Number of side branches	3	9	qSideBr-9-1*	AVRDC-BG71	AVRDC-BG26	8.7	17.6
		7	qSideBr-7-1	KAUBG_63	KAUBG_25	6.8	9.3
		2	qSideBr-2-1*	JY011	AVRDC-BG70	3.3	4.8
Number of seeds per fruit	2	9	qNSd-9-1*	McSSR35	KAUBG_65	4.9	12.7
		1	qNSd-1-1	N24	KAUBG_38	3.2	7.1
Seed length	4	1	qSdL-1-1*	KAUBG_2	N24	10.8	26.3
		4	qSdL-4-1*	KAUBG_15	KAUBG_72	8.6	21.0
		9	qSdL-9-1	AVRDC-BG26	KAUBG_66	4.8	8.5
		2	qSdL-2-1*	S13	AVRDC-BG66	3.3	5.6
Seed breadth	1	7	qSdB-7-1*	N5	AVRDC-BG75	4.2	16.6
Fruit color	3	9	qFrtClr-9-1*	AVRDC-BG71	AVRDC-BG26	10.0	35.0
		9	qFrtClr-9-2	KAUBG_65	JY001	7.0	20.4

		4	qFrtClr-4-1*	KAUBG_15	KAUBG_72	3.5	7.6
Fruit ends	3	7	qFrtEnds-7-1	KAUBG_41	McSSR114	8.4	22.5
		1	qFrtEnds-1-1	S32	S33	5.7	14.4
		3	qFrtEnds-3-1	McSSR150	KAUBG_52	4.9	15.3
Fruit shape	4	9	qFrtShp-9-1*	AVRDC-BG71	AVRDC-BG26	15.2	35.9
		7	qFrtShp-7-1	KAUBG_60	KAUBG_23	9.1	15.3
		8	qFrtShp-8-1*	KAUBG_29	KAUBG_30	4.7	7.6
		1	qFrtShp-1-1	S24	KAUBG_1	4.6	10.0
Leaf color	3	2	qLfClr-2-1*	S13	AVRDC-BG66	6.5	13.2
		1	qLfClr-1-1*	S18	KAUBG_44	5.3	15.4
		3	qLfClr-3-1	KAUBG_53	S9	4.0	15.8

*QTL clustered with more than one trait

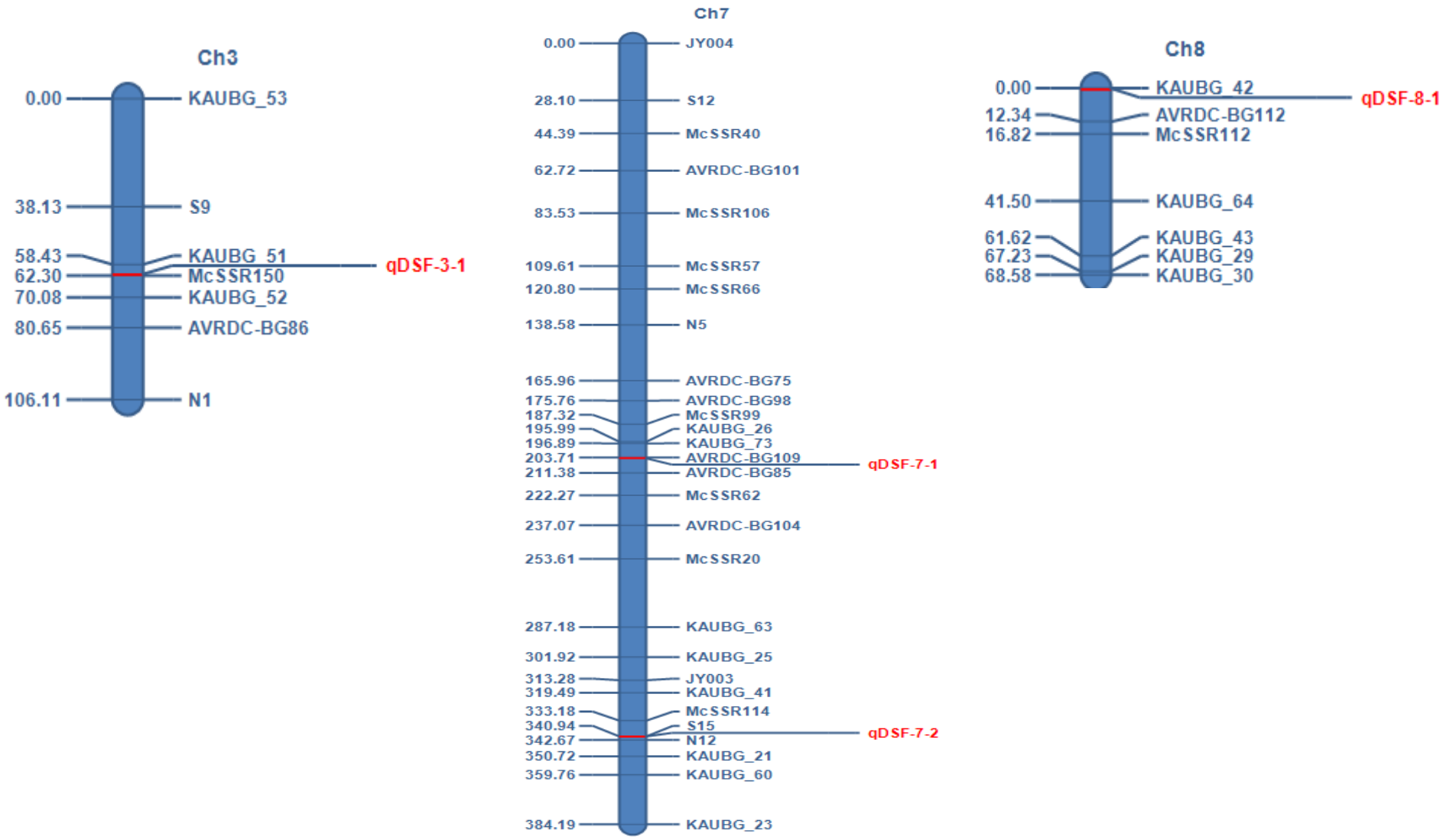


Figure 11. QTL map for days to staminate flower emergence

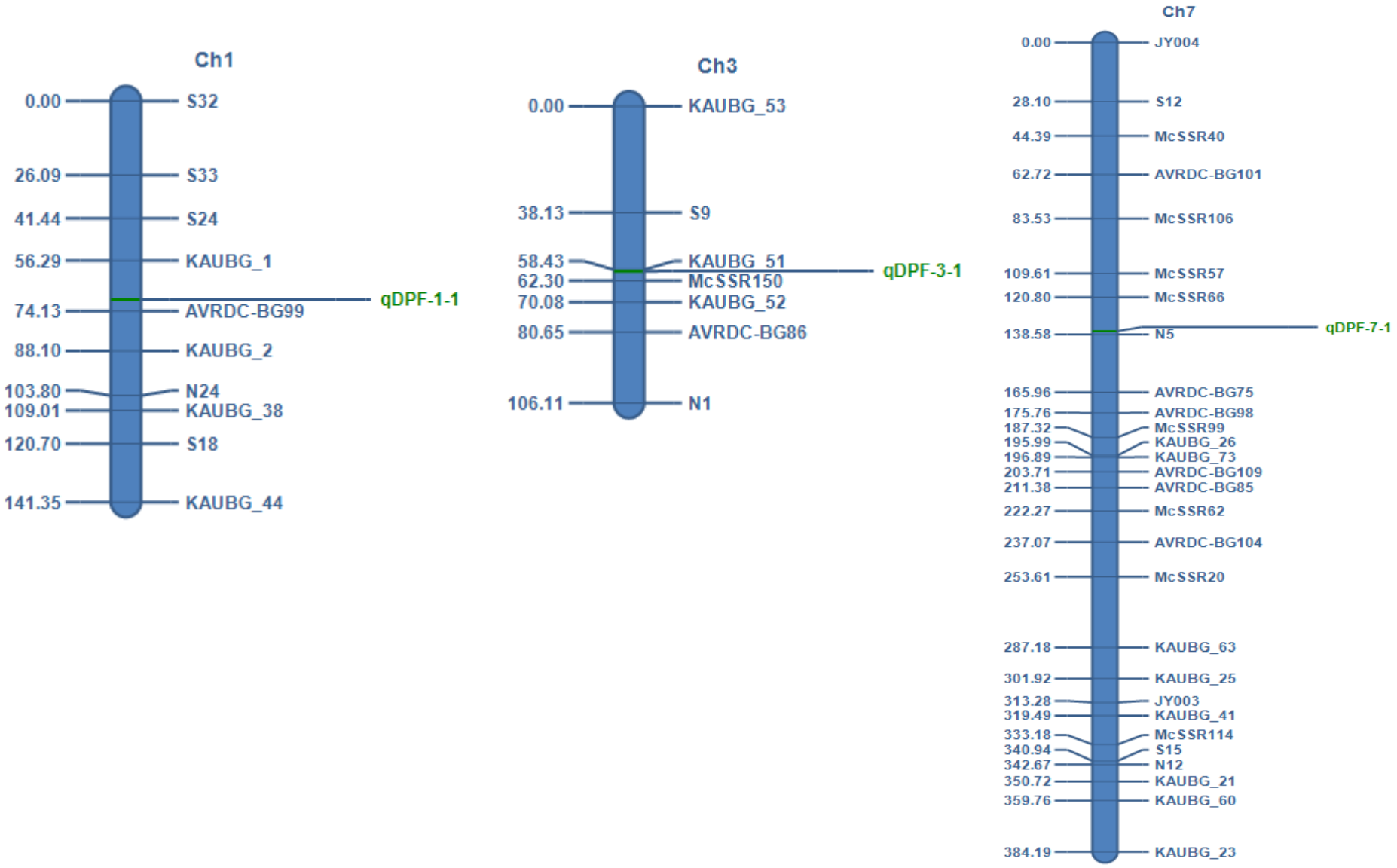


Figure 12. QTL map for days to pistillate flower emergence

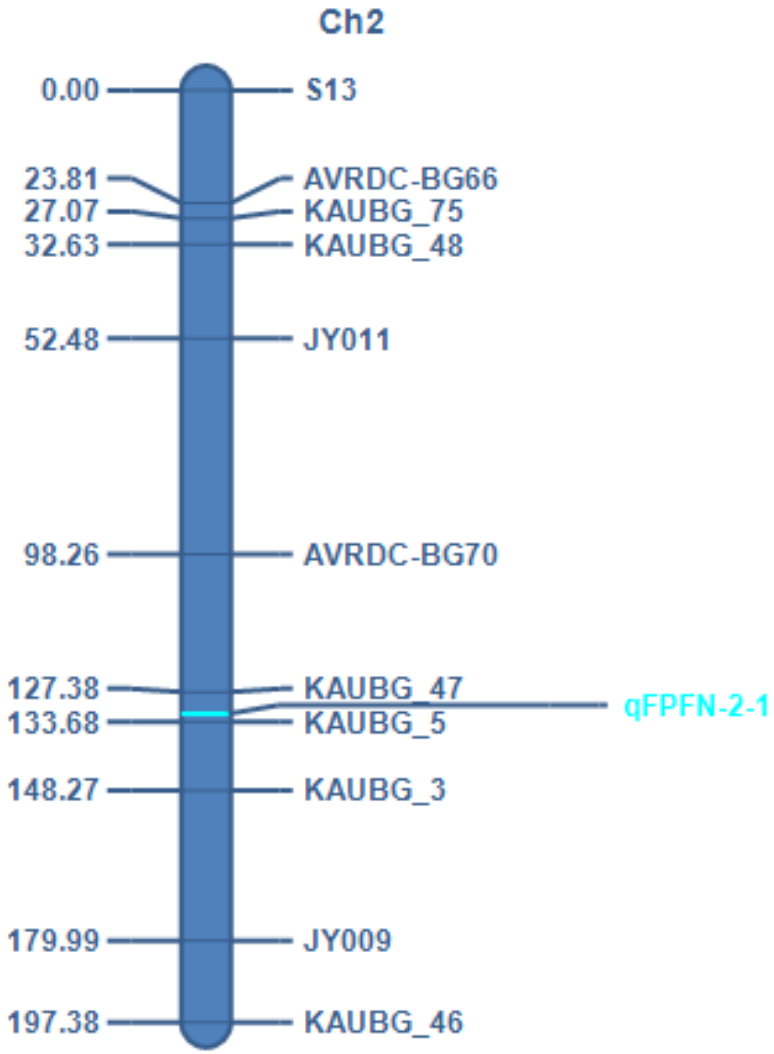


Figure 13. QTL map for first pistillate flower node

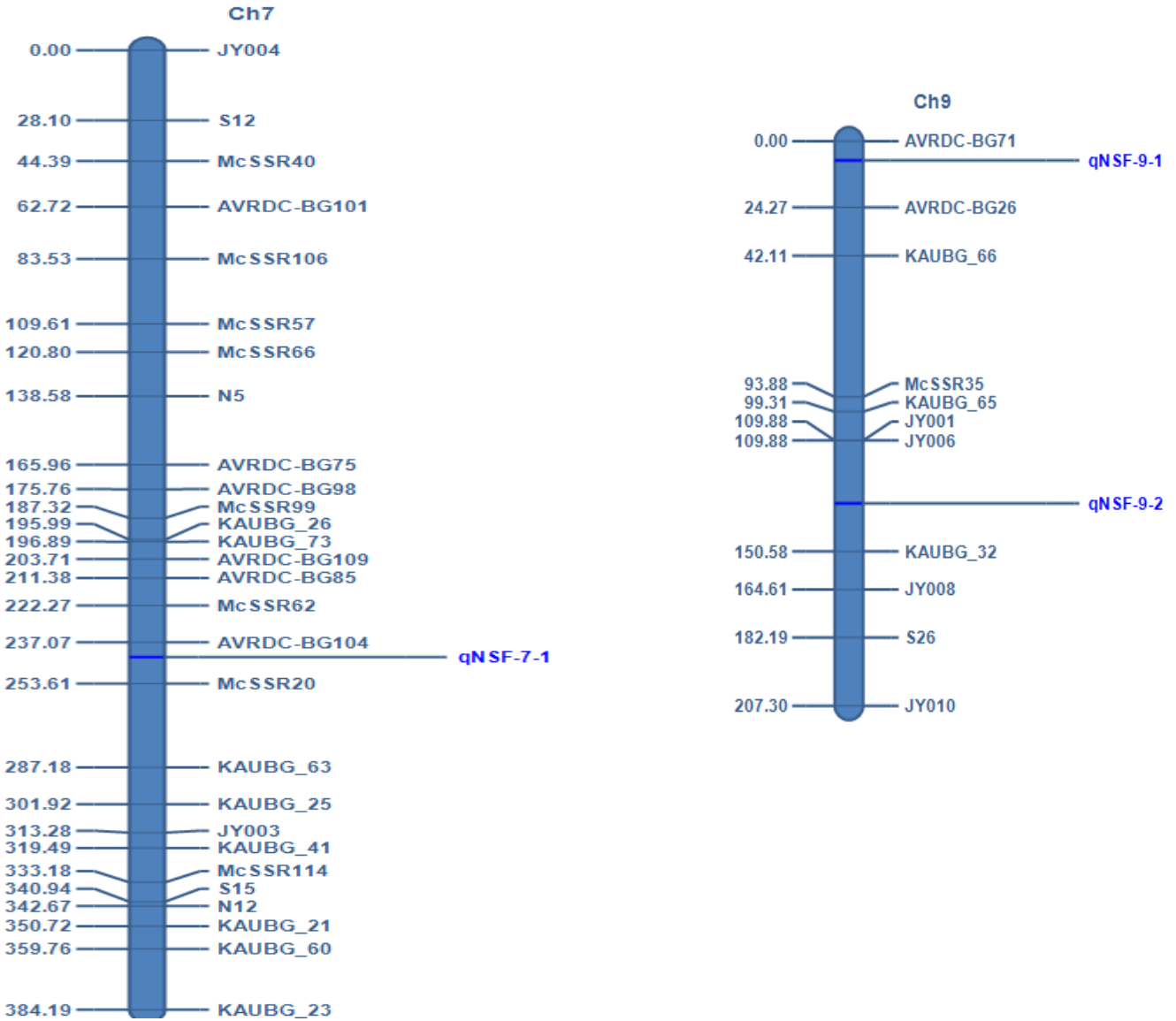


Figure 14. QTL map for number of staminate flower

BG71 to AVRDC-BG26 spanning a distance of 24.27 cM having LOD value 3.4 and PVE of 8.6 per cent.

Two QTL were identified for number of pistillate flower, one each on chromosome 2 and 7 with LOD value ranging of 7.6 and 3.5, explaining 26.0 and 8.7 per cent of phenotypic variation, respectively (Table 11; Figure 15). QTL qNPF-2-1 was located on chromosome 2 between marker interval JY009 to KAUBG_46 spanning a distance of 17.39 cM having LOD value 7.6 and PVE of 26.0 per cent. QTL qNPF-7-1 was located on chromosome 7 between marker interval N5 to AVRDC-BG75 spanning a distance of 27.38 cM having LOD value 3.5 and PVE of 8.7 per cent.

4.7.2 Fruit related traits

One QTL each was identified for fruit length, fruit breadth and ratio of fruit length and breadth (Fruit shape index) on chromosome 8, 9 and 2, with LOD value of 4.3, 6.9 and 6.5 explaining 13.8, 21.2 and 31.6 per cent of phenotypic variation, respectively (Figure 16; Table 11). QTL qFrtL-8-1 was located on chromosome 8 between marker interval KAUBG_29 to KAUBG_30 spanning a distance of 1.35 cM having LOD value 4.3 and PVE of 13.8 per cent. QTL qFrtB-9-1 was located on chromosome 9 between marker interval KAUBG_66 to McSSR35 spanning a distance of 51.77 cM having LOD value 6.9 and PVE of 21.2 per cent. QTL qFSI-2-1 was located on chromosome 2 between marker interval AVRDC-BG70 to KAUBG_47 spanning a distance of 29.12 cM having LOD value 6.5 and PVE of 31.6 per cent.

Three QTL were identified for weight of the fruit, one on chromosome 2 and two on chromosome 9 (Figure 17; Table 11). LOD values ranged from 3.3 to 6.8 with PVE of 5.5 to 18.1 per cent. QTL qFrtWt-2-1 was located on chromosome 2 between marker interval S13 to AVRDC-BG66 spanning a distance of 23.81 cM having LOD value 6.8 and PVE of 14.5 per cent. QTL qFrtWt-9-1 was located on chromosome 9 between marker interval KAUBG_66 to McSSR35 spanning a distance of 51.77 cM having LOD value 4.3 and PVE of 18.1 per cent. QTL qFrtWt-9-2 was located on chromosome 9 between marker interval McSSR35 to KAUBG_65 spanning a distance of 5.43 cM having LOD value 3.3 and PVE of 5.5 per cent.

Three QTL were identified for flesh thickness, one each on chromosome 1, 2, and 9 with LOD value ranging from 4.0 to 7.9, explaining 9.9 to 21.3 per cent of phenotypic variation (Figure 18; Table 11). QTL qFlTh-1-1 was located on chromosome 1 between marker interval KAUBG_2 to N24 spanning a distance of 15.70 cM having LOD value 7.9 and PVE of 15.0 per cent. QTL qFlTh-2-1 was located on chromosome 2 between marker interval S13 to AVRDC-BG66 spanning a distance of 23.81 cM having LOD value 7.9 and PVE of 21.3 per cent. QTL qFlTh-9-1 was located on chromosome 9 between marker interval KAUBG_32 to JY008 spanning a distance of 14.04 cM having LOD value 4.0 and PVE of 9.9 per cent.

Two QTL were identified for number of fruits per plant, one each on chromosome 2 (qNFrt-2-1) and 7 (qNFrt-7-1) with LOD value 7.7 and 3.4,

Figure 15. QTL map for number of pistillate flowers

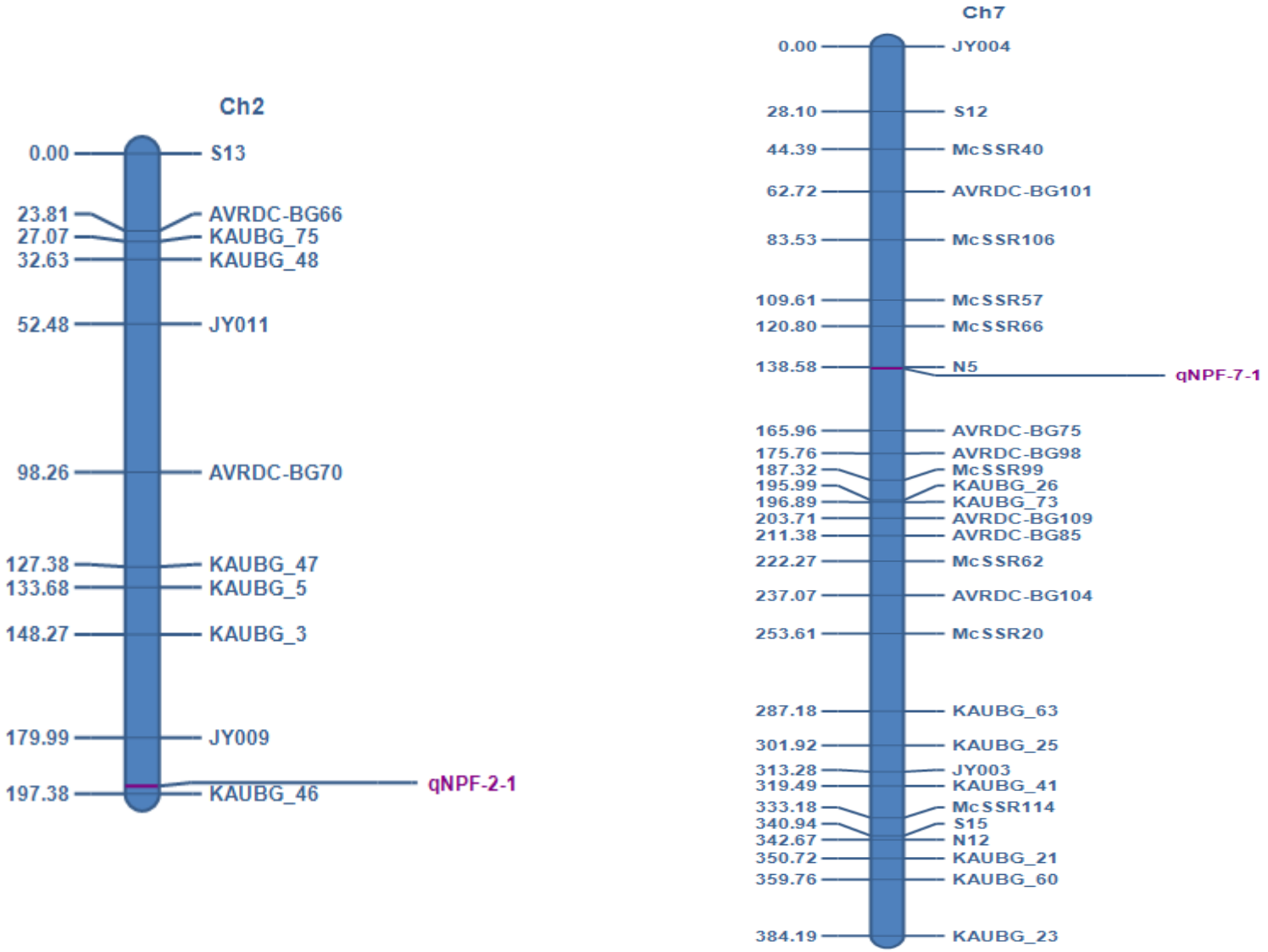


Figure 16. QTL map for fruit length, fruit breadth and fruit shape index

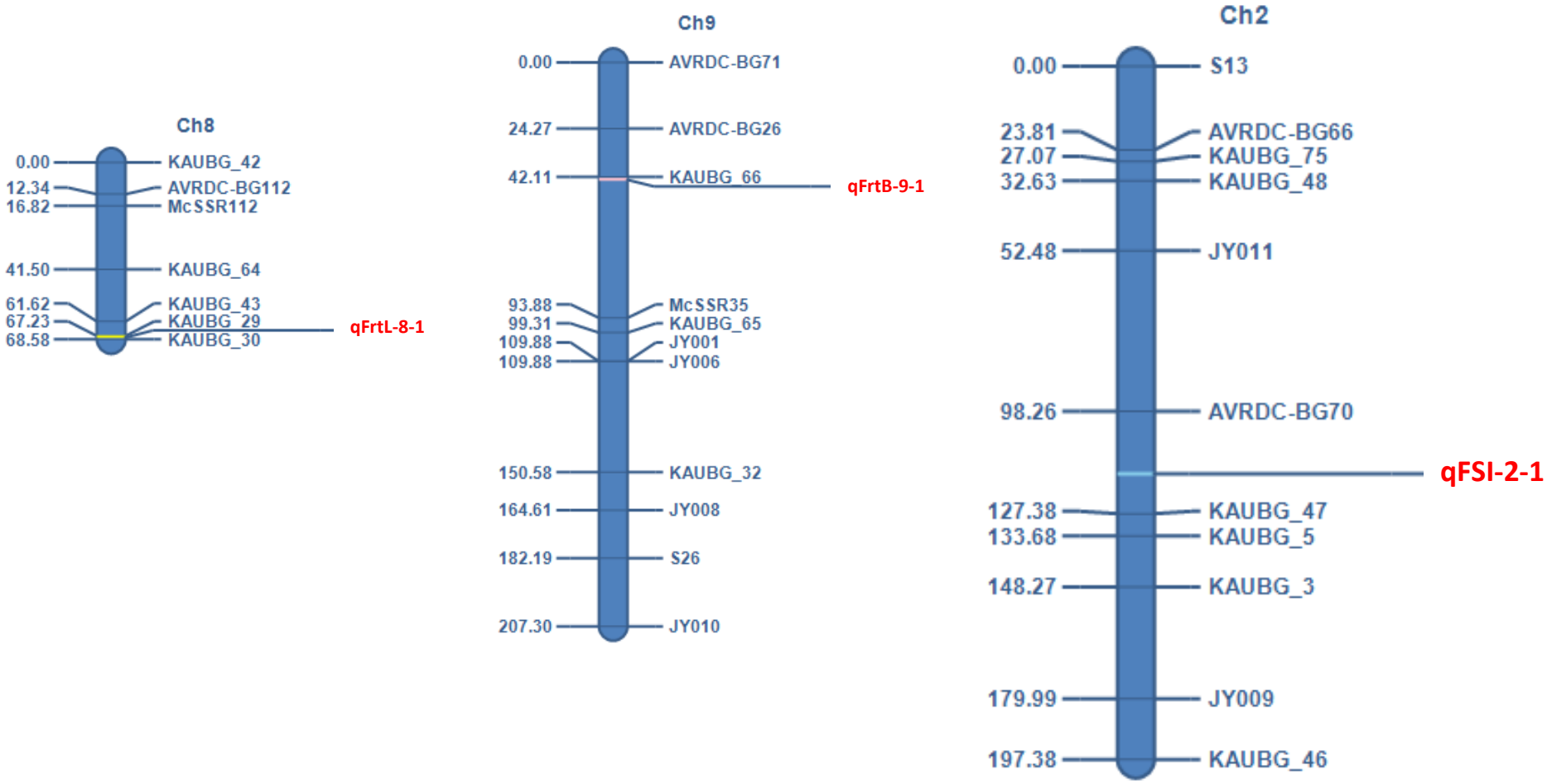


Figure 17. QTL map for fruit weight

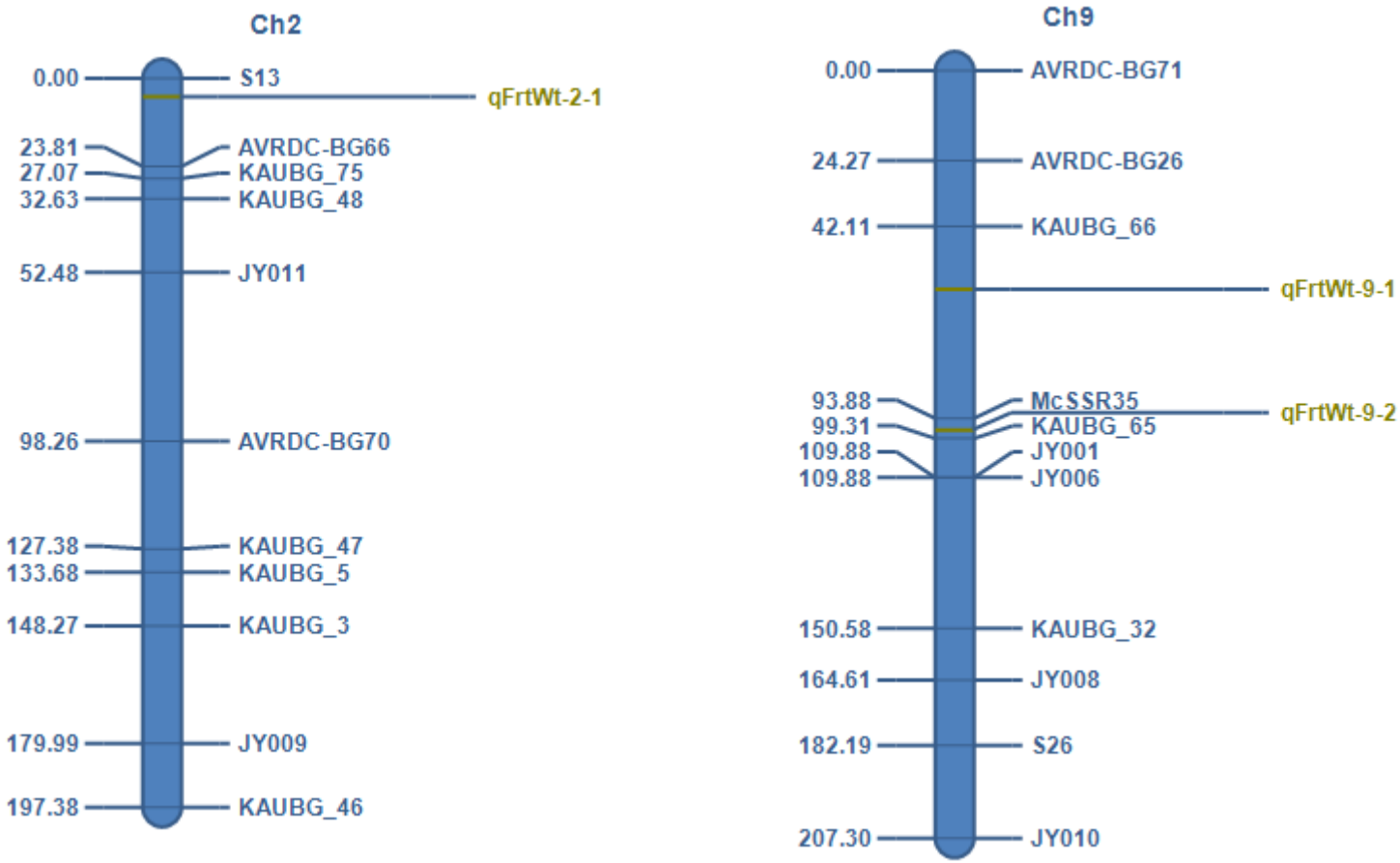
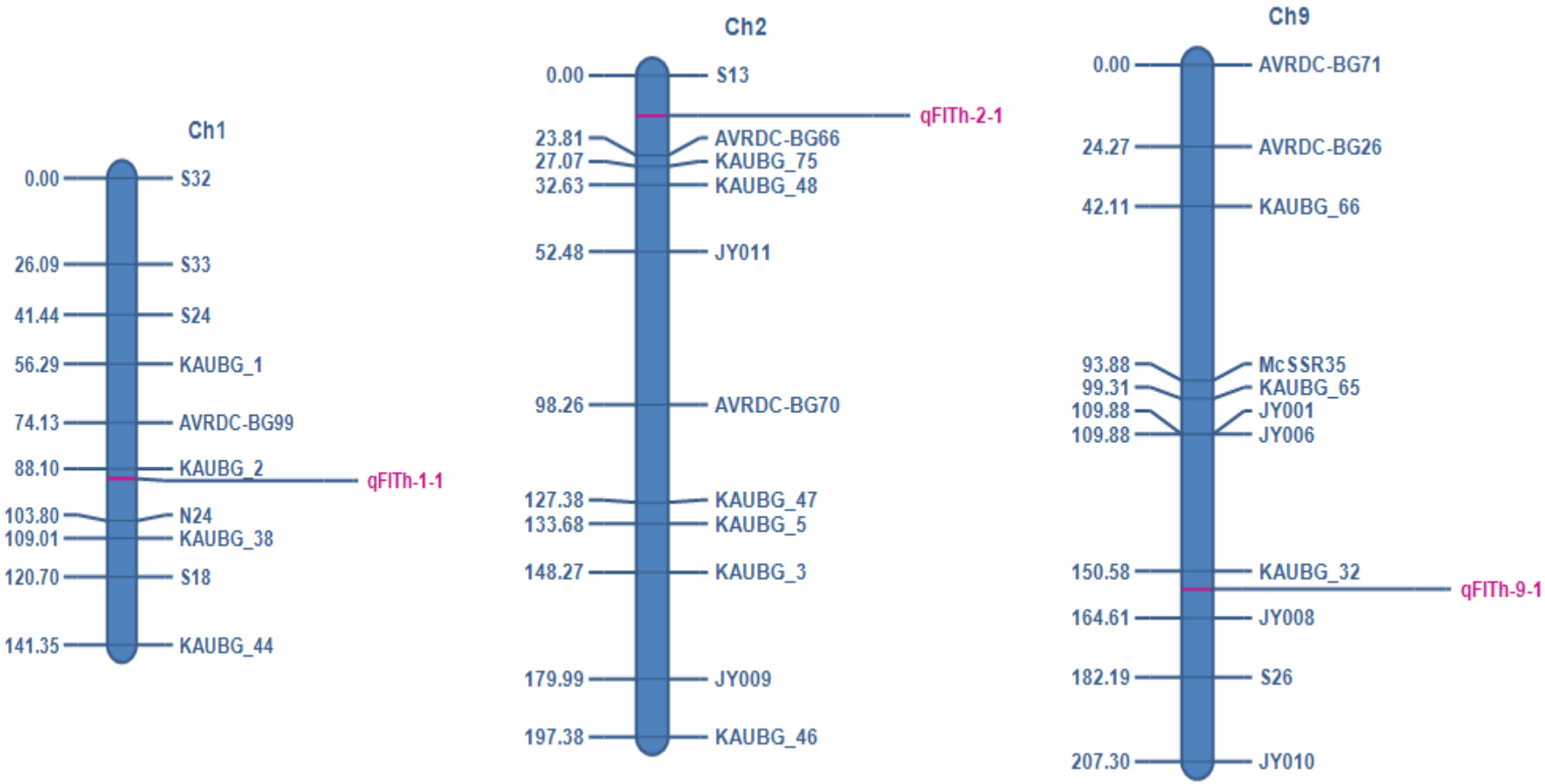


Figure 18. QTL map for flesh thickness



explaining 27.1 and 6.8 per cent of phenotypic variation, and spanning a distance of 17.39 cM (marker interval JY009 to KAUBG_46) and 27.38 cM (marker interval N5 to AVRDC-BG75), respectively (Figure 19; Table 11).

Two QTL were identified for fruit yield per plant, one each on chromosome 8 (qYldpl-8-1) and 9 (qYldpl-9-1) spanning a distance of 24.68 cM (marker interval McSSR112 to KAUBG_64) and 14.01 cM (KAUBG_32 to JY008), with LOD values 3.5 and 3.1, explaining 16.9 and 5.7 per cent of phenotypic variation, respectively (Figure 19; Table 11).

Three QTL each for fruit color and fruit ends, and four QTL for fruit shape were identified seven different chromosomes (Figure 20, 21, 22; Table 11). LOD value for these QTL was ranging from 3.5 to 15.2 with PVE per cent ranging between 7.6 and 35.9 (Table 11). QTL qFrtClr-9-1 was located on chromosome 9 between marker interval AVRDC-BG71 to AVRDC-BG26 spanning a distance of 24.27 cM having LOD value 10.0 and PVE of 35.0 per cent. QTL qFrtClr-9-2 was located on chromosome 9 between marker interval KAUBG_65 to JY001 spanning a distance of 10.57 cM having LOD value 7.0 and PVE of 20.4 per cent. QTL qFrtClr-4-1 was located on chromosome 4 between marker interval KAUBG_15 to KAUBG_72 spanning a distance of 13.66 cM having LOD value 3.5 and PVE of 7.6 per cent.

QTL qFrtEnds-7-1 was located on chromosome 7 between marker interval KAUBG_41 to McSSR114 spanning a distance of 13.68 cM having LOD value 8.4 and PVE of 22.5 per cent. QTL qFrtEnds-1-1 was located on chromosome

1 between marker interval S32 to S33 spanning a distance of 26.09 cM having LOD value 5.7 and PVE of 14.4 per cent. QTL qFrtEnds-3-1 was located on chromosome 3 between marker interval McSSR150 to KAUBG_52 spanning a distance of 7.79 cM having LOD value 4.9 and PVE of 15.3 per cent.

QTL qFrtShp-9-1 was located on chromosome 9 between marker interval AVRDC-BG71 to AVRDC-BG26 spanning a distance of 24.27 cM having LOD value 15.2 and PVE of 35.9 per cent. QTL qFrtShp-7-1 was located on chromosome 7 between marker interval KAUBG_60 to KAUBG_23 spanning a distance of 24.43 cM having LOD value 9.1 and PVE of 15.3 per cent. QTL qFrtShp-8-1 was located on chromosome 8 between marker interval KAUBG_29 to KAUBG_30 spanning a distance of 1.35 cM having LOD value 4.7 and PVE of 7.6 per cent. QTL qFrtShp-1-1 was located on chromosome 1 between marker interval S24 to KAUBG_1 spanning a distance of 14.85 cM having LOD value 4.6 and PVE of 10.0 per cent.

4.7.3 Seed related traits

Two QTL were identified for number of seeds per fruit, one each on chromosome 1 (qNSd-1-1) and 9 (qNSd-9-1) spanning a distance of 5.22 cM (marker interval N24 to KAUBG_38) and 5.43 cM (McSSR35 to KAUBG_65), with LOD value 3.2 and 4.9, explaining 7.1 and 12.7 per cent of phenotypic variation, respectively (Figure 23; Table 11).

Four QTL with LOD scores between 3.3 and 10.8 were identified for seed length explaining 5.6 to 26.3 per cent of phenotypic variation (Figure 24; Table

Figure 19. QTL map for number of fruits and yield per plant

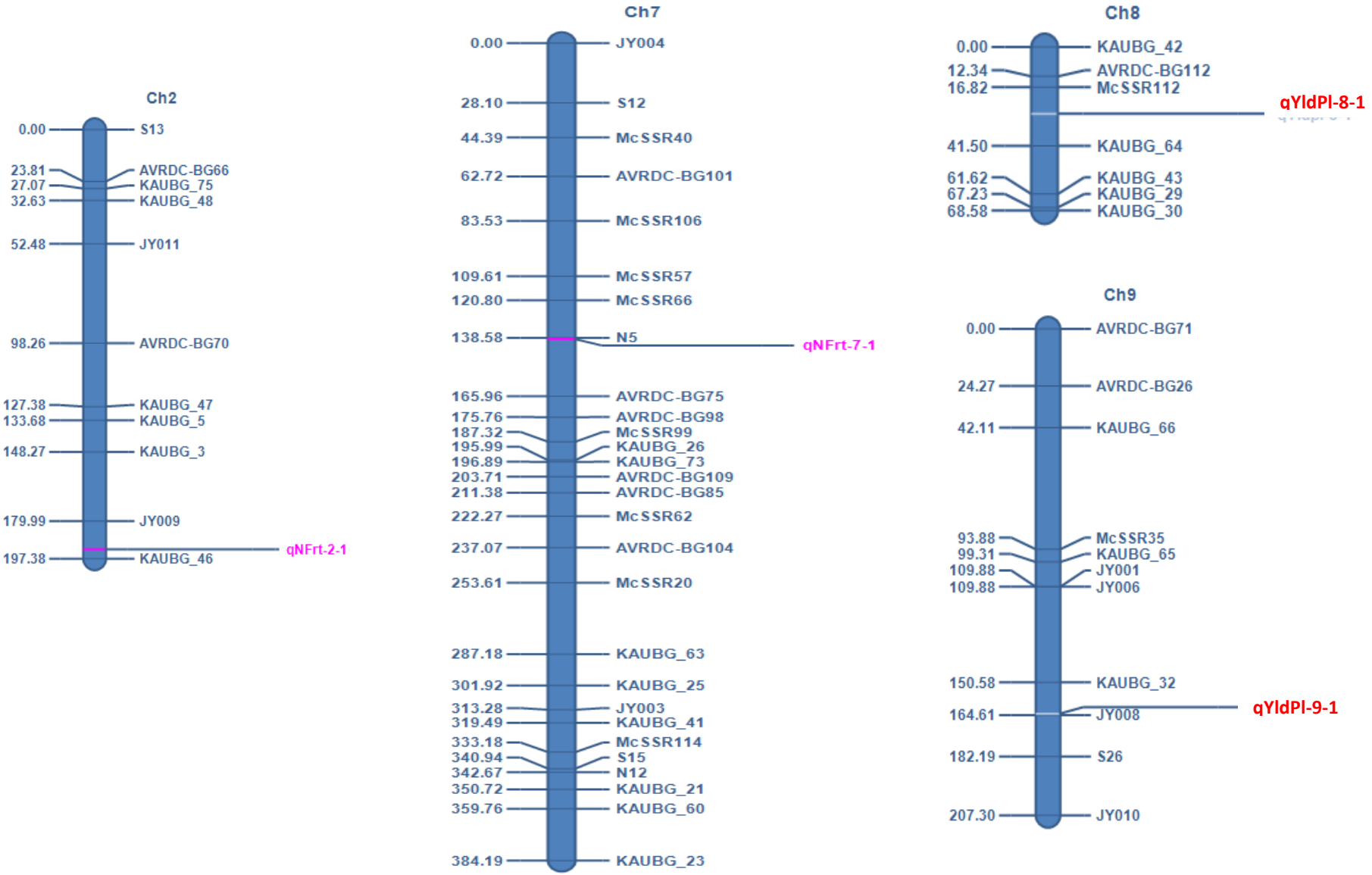


Figure 20. QTL map for fruit color

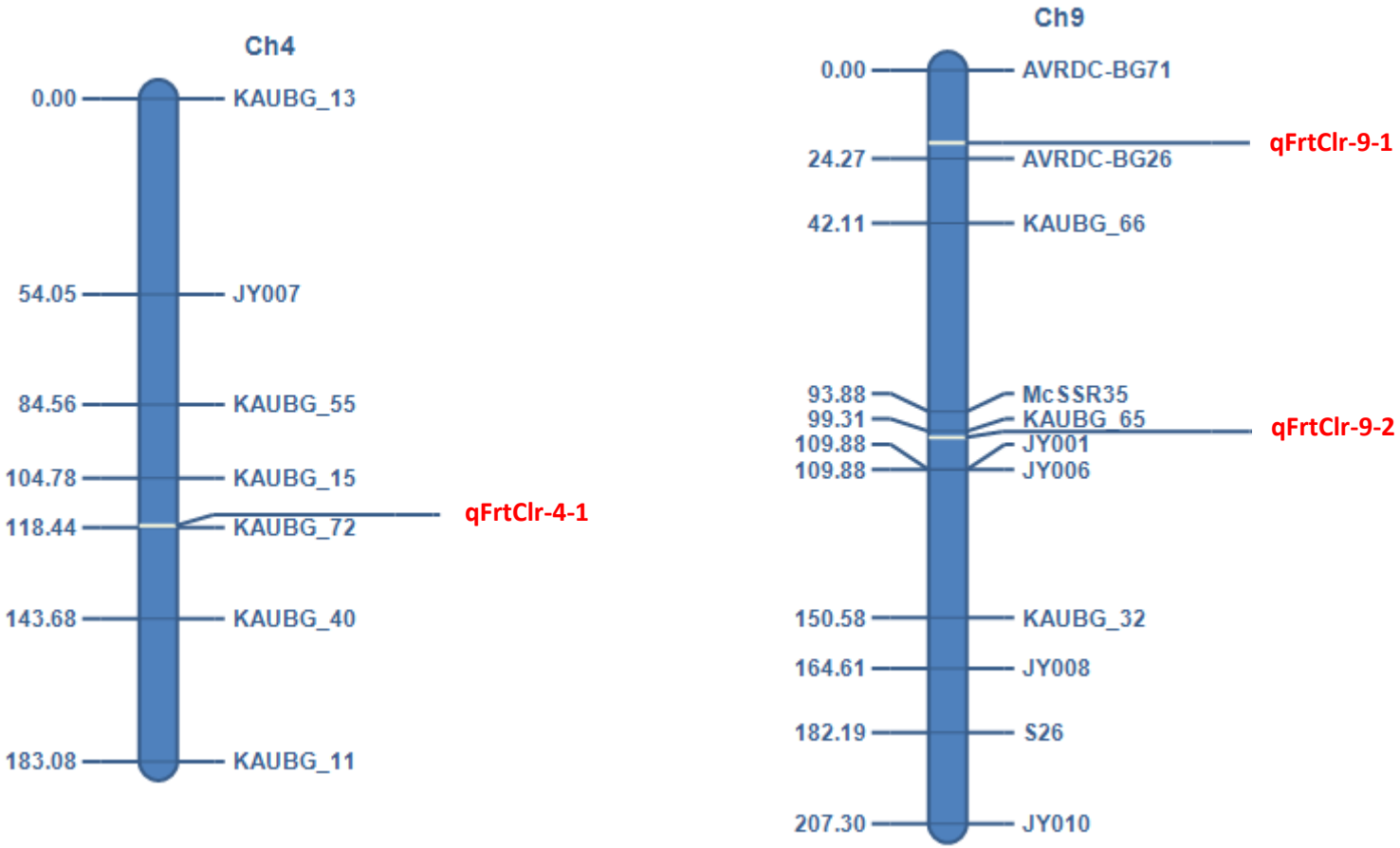


Figure 21. QTL map for fruit ends

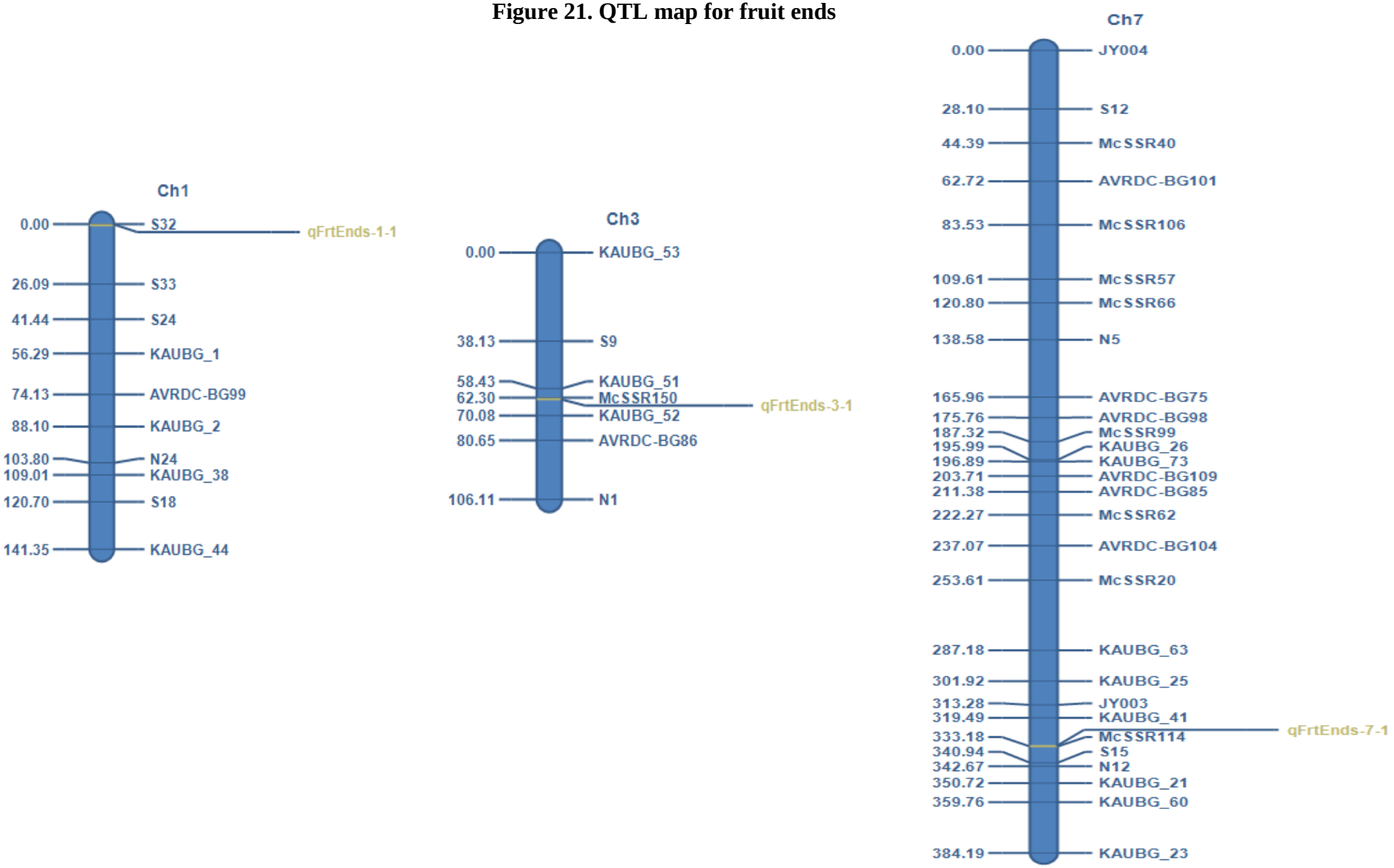


Figure 22. QTL map for fruit shape

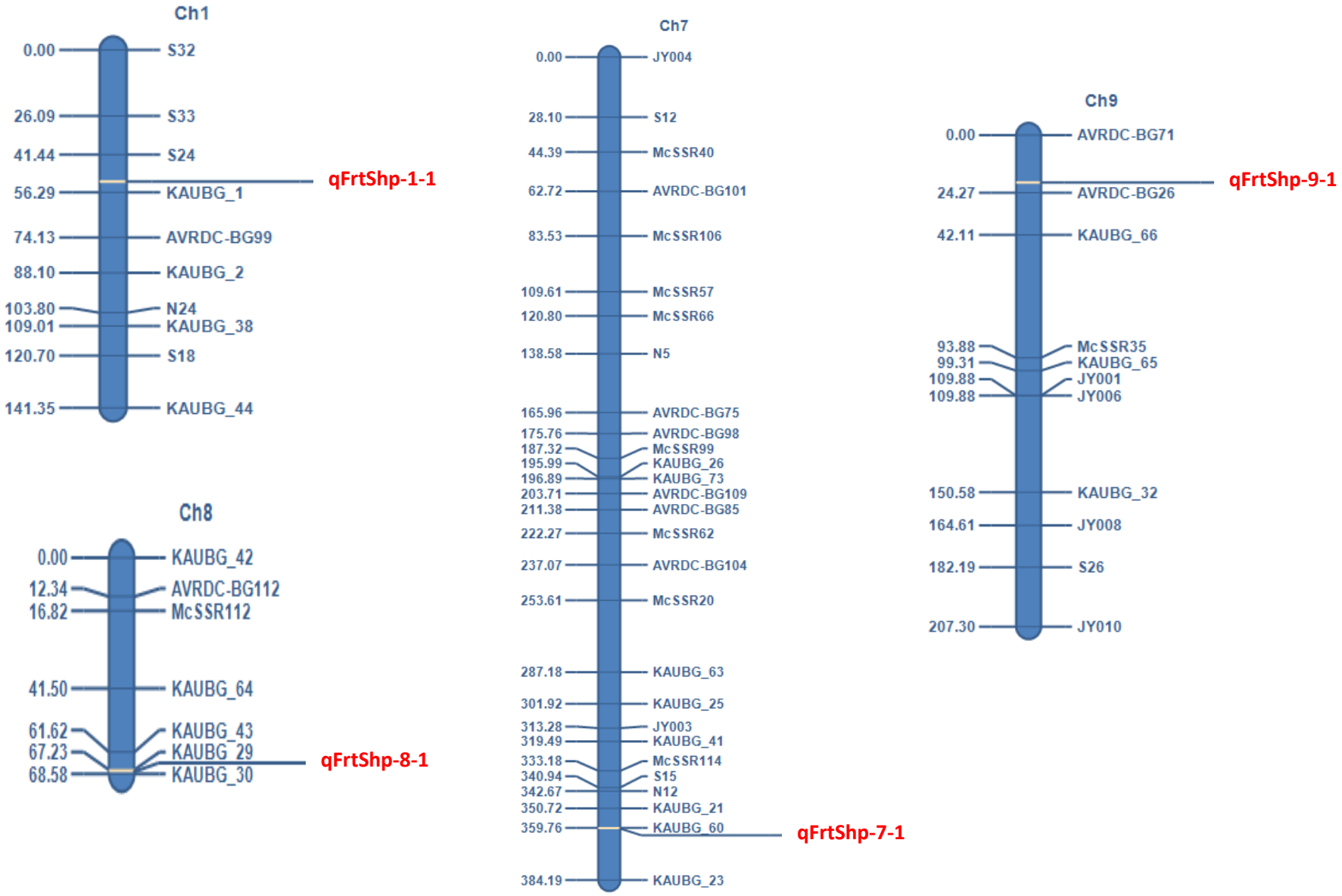


Figure 23. QTL map for number of seeds

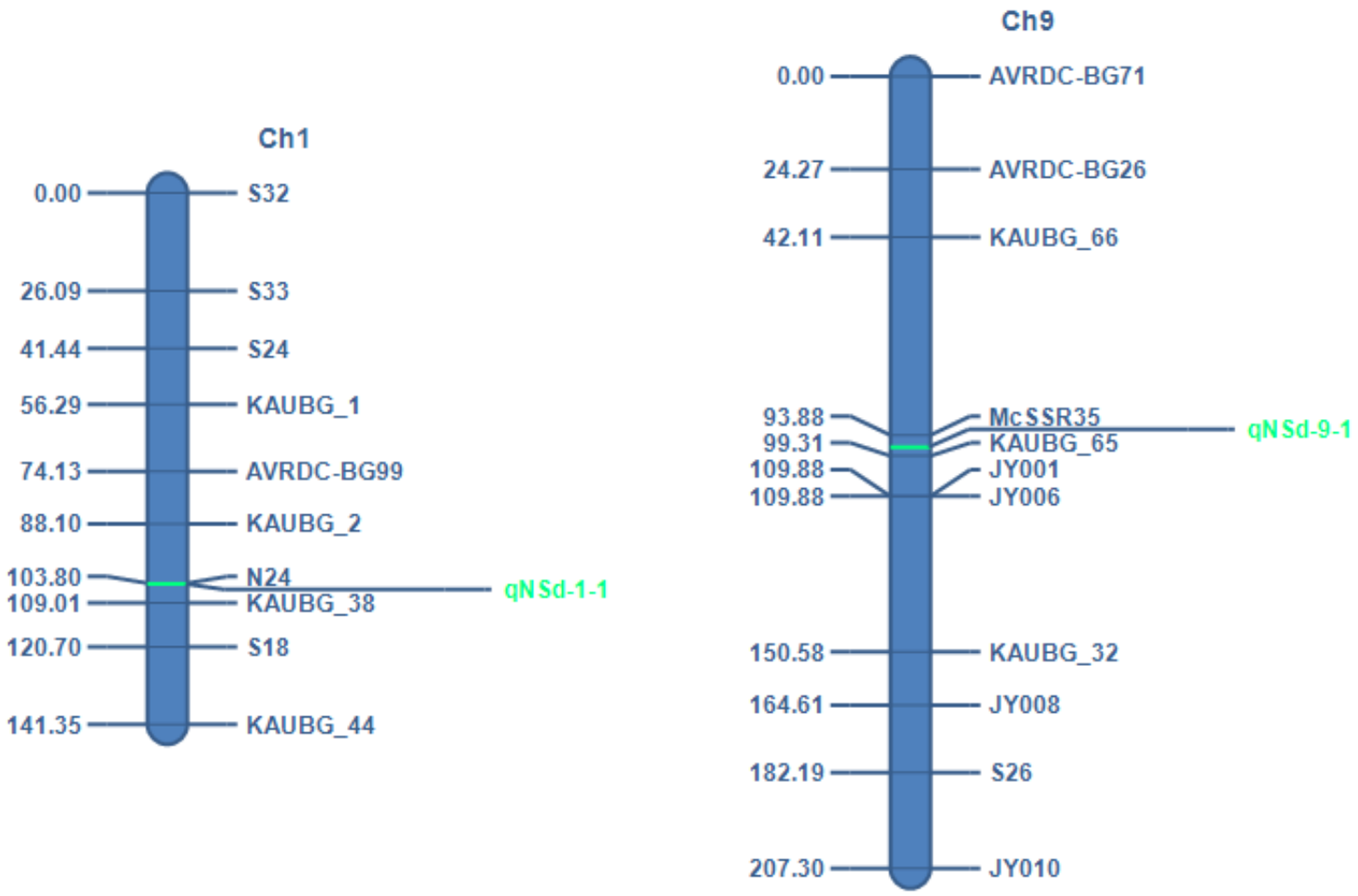
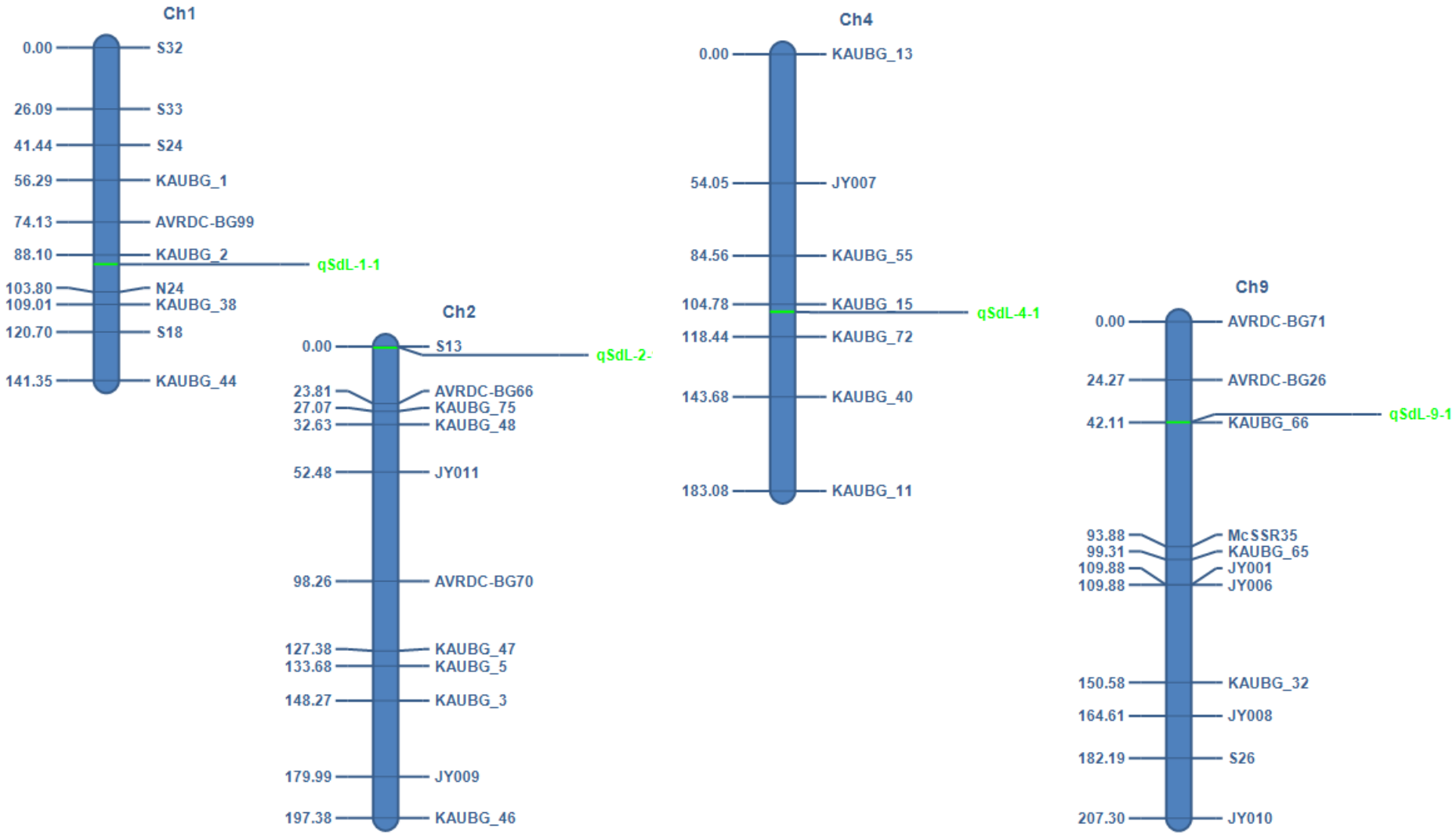


Figure 24. QTL map for seed length



11). QTL qSdL-1-1 was located on chromosome 1 between marker interval KAUBG_2 to N24 spanning a distance of 15.70 cM having LOD value 10.8 and PVE of 26.3 per cent. QTL qSdL-4-1 was located on chromosome 4 between marker interval KAUBG_15 to KAUBG_72 spanning a distance of 13.66 cM having LOD value 8.6 and PVE of 21.0 per cent. QTL qSdL-9-1 was located on chromosome 9 between marker interval AVRDC-BG26 to KAUBG_66 spanning a distance of 17.83 cM having LOD value 4.8 and PVE of 8.5 per cent. QTL qSdL-2-1 was located on chromosome 2 between marker interval S13 to AVRDC-BG66 spanning a distance of 23.81 cM having LOD value 3.3 and PVE of 5.6 per cent.

Only one QTL for seed breadth, qSdB-7-1 was located on chromosome 7 between marker interval N5 to AVRDC-BG75 spanning a distance of 27.38 cM having LOD value 4.2 and PVE of 16.6 per cent (Figure 25; Table 11).

4.7.4 Leaf related traits

Four QTL were identified for leaf size with LOD value ranging from 3.5 to 4.7 and PVE ranging from 3.2 to 13.5 per cent. QTL qLfSz-2-1 was located on chromosome 2 between marker interval KAUBG_3 to JY009 spanning a distance of 31.72 cM having LOD value 4.7 and PVE of 13.5 per cent (Figure 26; Table 11). QTL qLfSz-2-2 was located on chromosome 2 between marker interval JY011 to AVRDC-BG70 spanning a distance of 45.78 cM having LOD value 4.1 and PVE of 10.8 per cent. QTL qLfSz-1-1 was located on chromosome 1 between marker interval S18 to KAUBG_44 spanning a distance of 20.64 cM having LOD value 4.0 and PVE of 5.9 per cent. QTL qLfSz-7-1 was located on chromosome 7 between

marker interval KAUBG_73 to AVRDC-BG109 spanning a distance of 6.81 cM having LOD value 3.5 and PVE of 3.2 per cent.

Three QTL were identified for leaf color with LOD value ranging from 4.0 to 6.5 and PVE ranging from 13.2 to 15.8 per cent (Figure 27; Table 11). QTL qLfClr-2-1 was located on chromosome 2 between marker interval S13 to AVRDC-BG66 spanning a distance of 23.81 cM having LOD value 6.5 and PVE of 13.2 per cent. QTL qLfClr-1-1 was located on chromosome 1 between marker interval S18 to KAUBG_44 spanning a distance of 20.64 cM having LOD value 5.3 and PVE of 15.4 per cent. QTL qLfClr-3-1 was located on chromosome 3 between marker interval KAUBG_53 to S9 spanning a distance of 38.13 cM having LOD value 4.0 and PVE of 15.8 per cent.

4.7.5 Vine related traits

Three QTL each were identified for internodal length and number of side branches with LOD value ranging from 3.5 to 5.2, and 3.3 to 8.7, respectively. QTL qIntNdl-9-1 was located on chromosome 9 between marker interval AVRDC-BG71 to AVRDC-BG26 spanning a distance of 24.27 cM having LOD value 5.2 and PVE of 14.8 per cent (Figure 28; Table 11). QTL qIntNdl-9-2 was located on chromosome 9 between marker interval McSSR35 to KAUBG_65 spanning a distance of 5.43 cM having LOD value 4.8 and PVE of 14.9 per cent. QTL qIntNdl-7-1 was located on chromosome 7 between marker interval JY004 to S12 spanning a distance of 28.10 cM having LOD value 3.5 and PVE of 9.5 per cent.

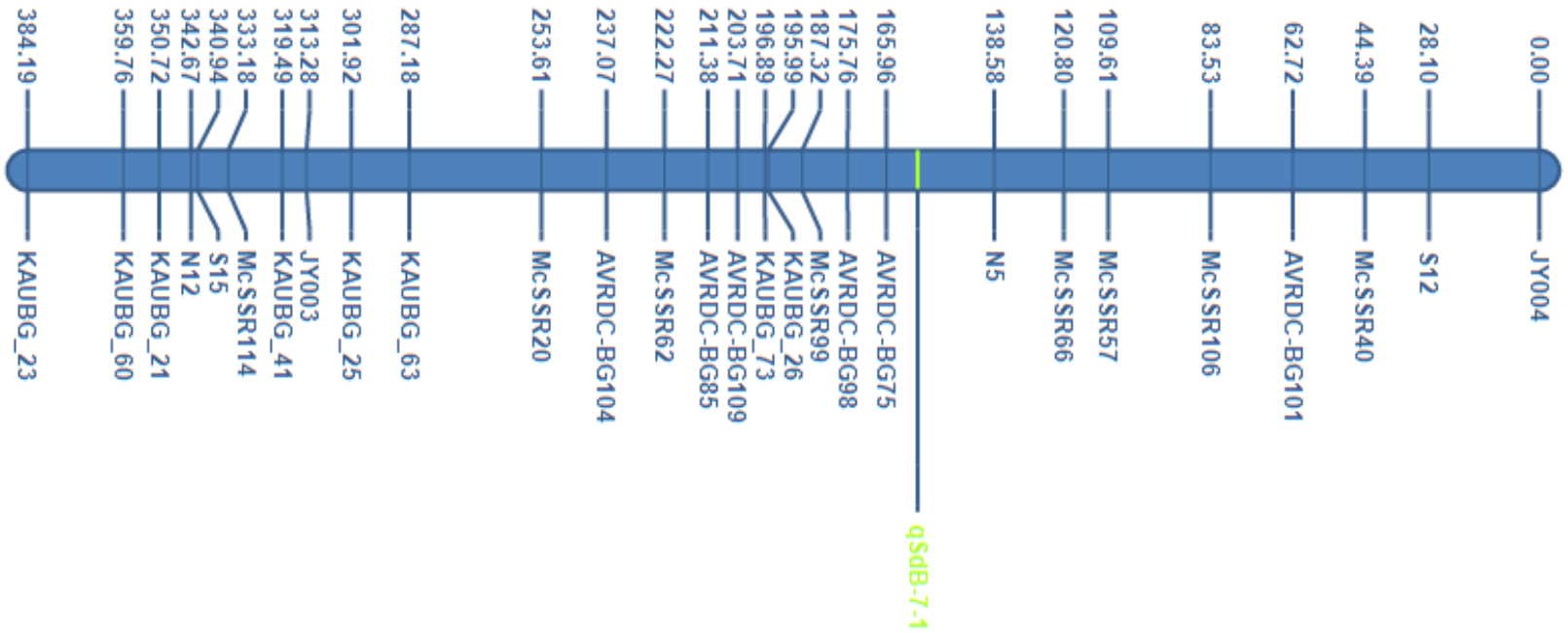


Figure 25. QTL map for seed breadth

Figure 26. QTL map for leaf size

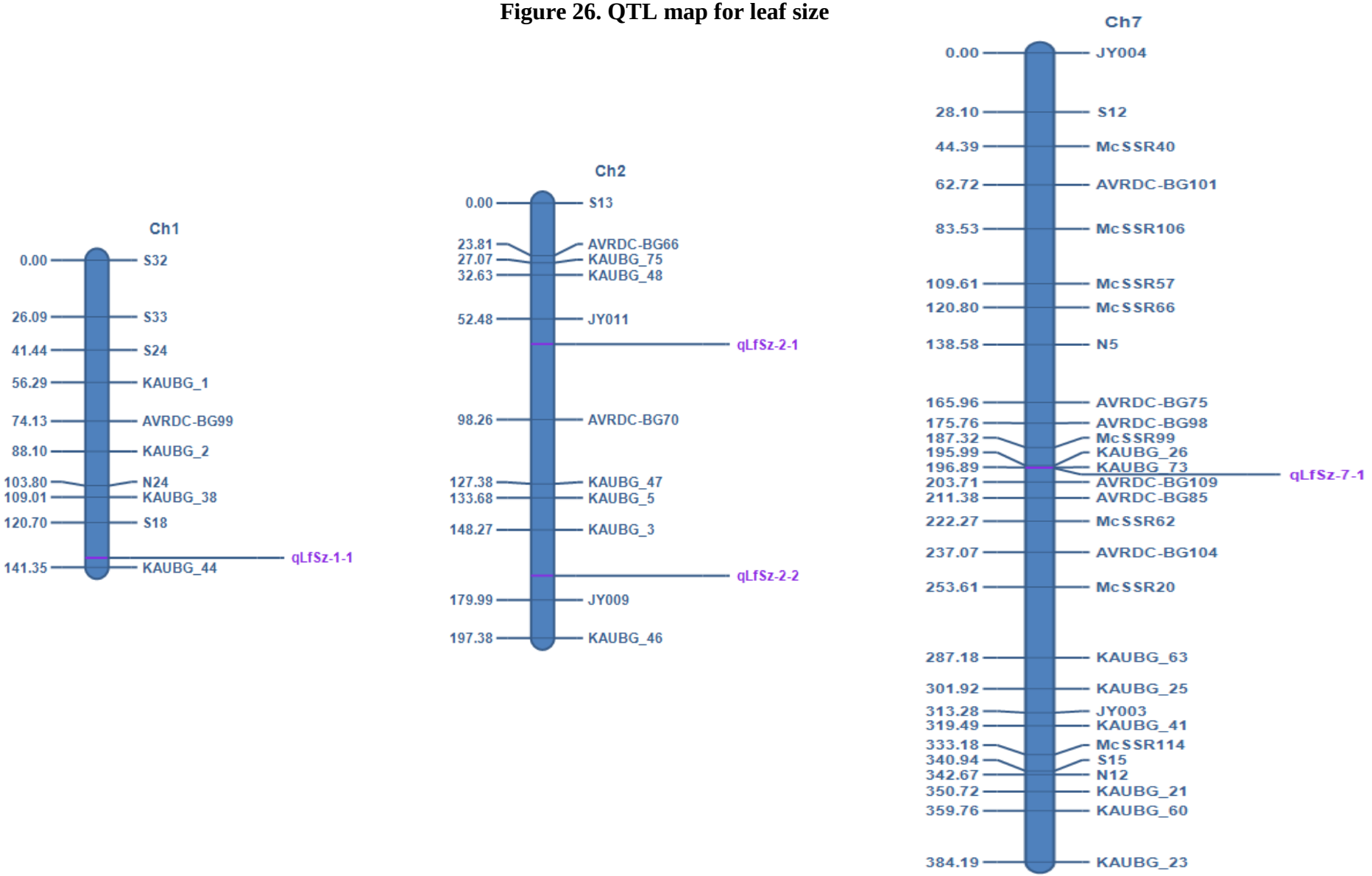


Figure 27. QTL map for leaf color

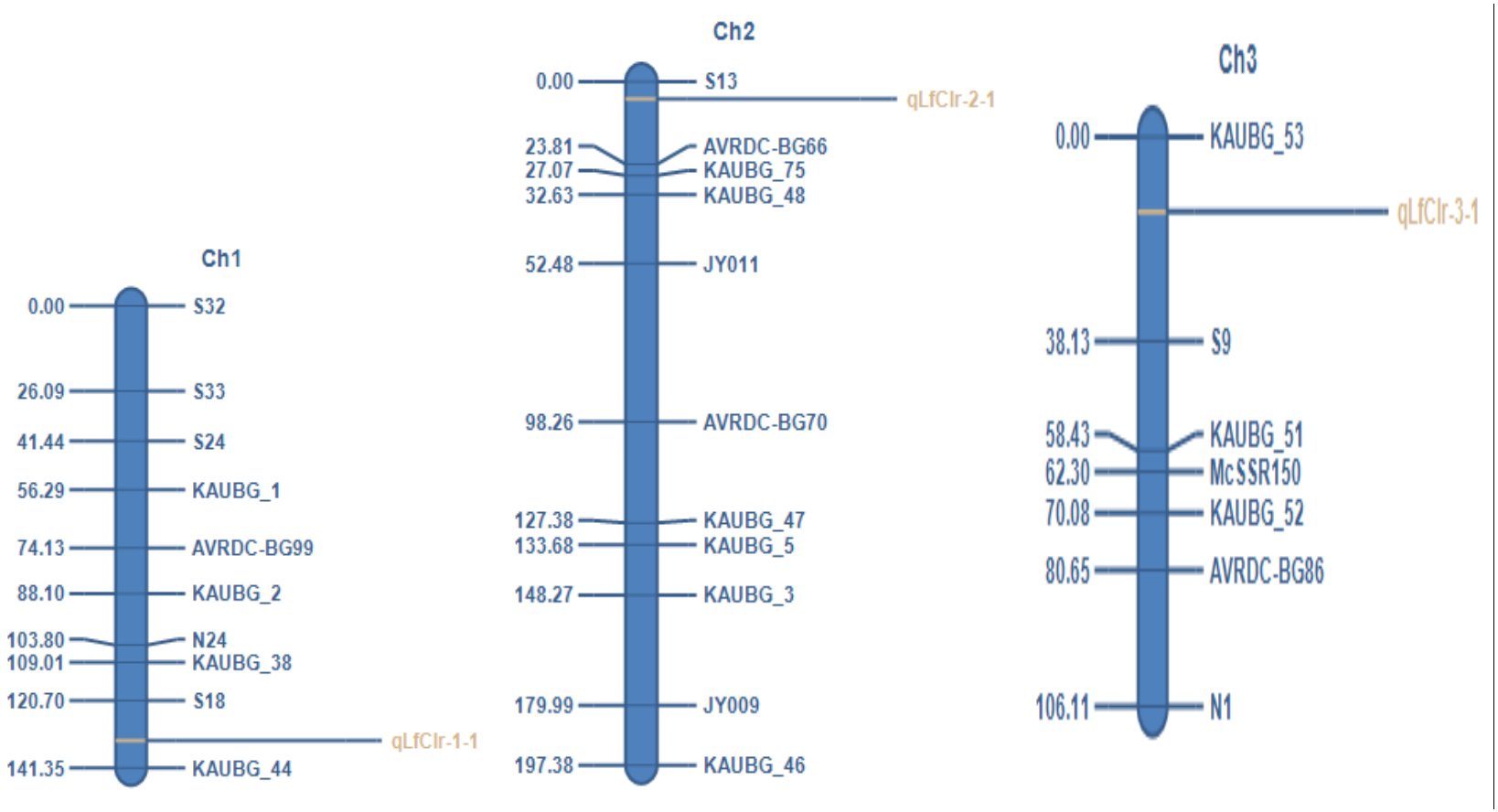
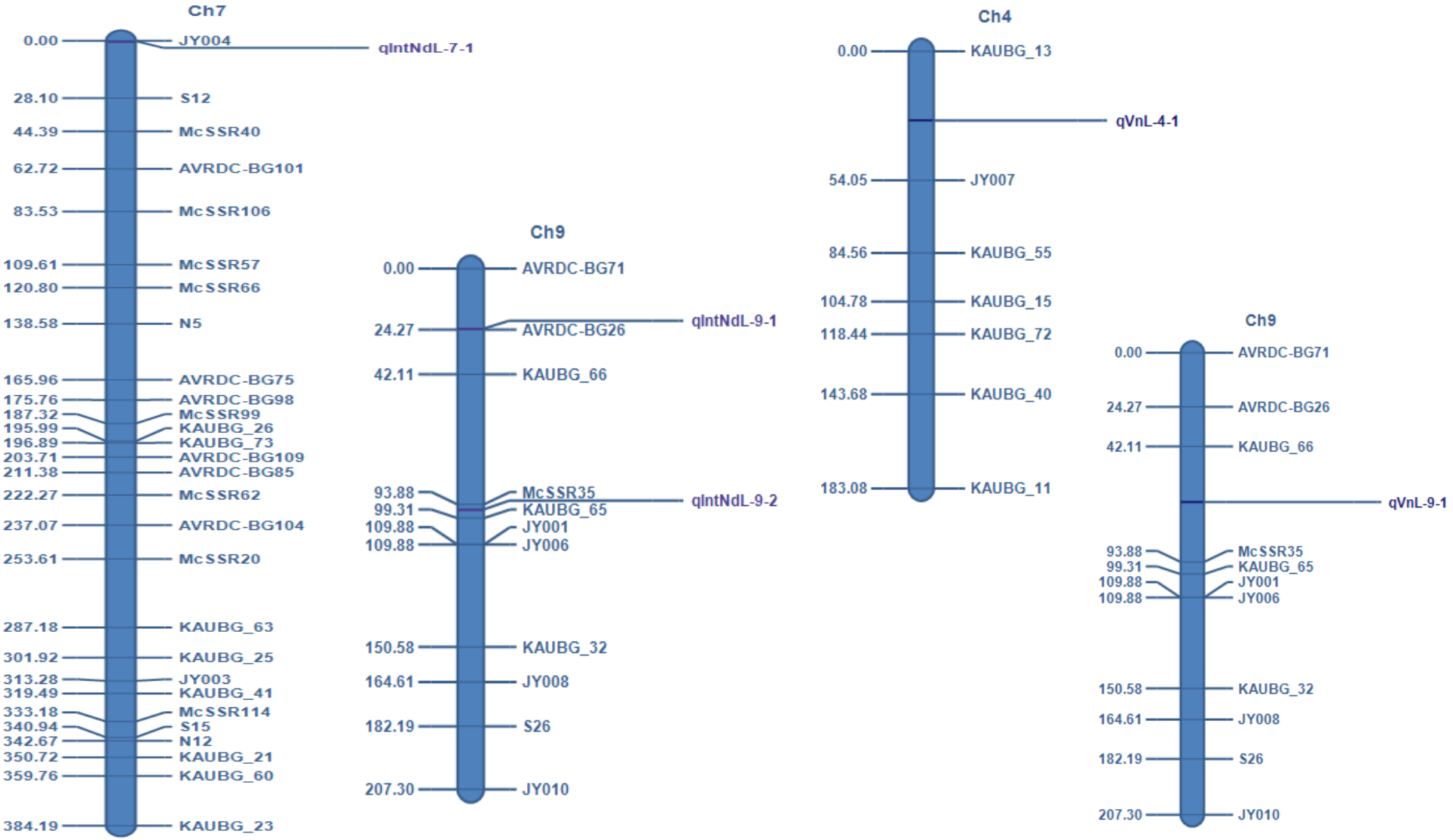


Figure 28. QTL map for internodal length and vine length



QTL qSideBr-9-1 was located on chromosome 9 between marker interval AVRDC-BG71 to AVRDC-BG26 spanning a distance of 24.27 cM having LOD value 8.7 and PVE of 17.6 per cent (Figure 30; Table 11). QTL qSideBr-7-1 was located on chromosome 7 between marker interval KAUBG_63 to KAUBG_25 spanning a distance of 14.74 cM having LOD value 6.8 and PVE of 9.3 per cent. QTL qSideBr-2-1 was located on chromosome 2 between marker interval JY011 to AVRDC-BG70 spanning a distance of 45.78 cM having LOD value 3.3 and PVE of 4.8 per cent.

Two QTL each with LOD value ranging from 3.2 to 4.5 were identified for vine length and stem thickness (Table 11; Figure 28, 29). QTL qVnL-4-1 was located on chromosome 4 between marker interval KAUBG_13 to JY007 spanning a distance of 54.05 cM having LOD value 4.5 and PVE of 1.8 per cent. QTL qVnL-9-1 was located on chromosome 9 between marker interval KAUBG_66 to McSSR35 spanning a distance of 51.77 cM having LOD value 3.2 and PVE of 1.9 per cent. QTL qStmGrth-1-1 was located on chromosome 1 between marker interval KAUBG_2 to N24 spanning a distance of 15.70 cM having LOD value 3.4 and PVE of 3.4 per cent (Figure 29; Table 11). QTL qStmGrth-7-1 was located on chromosome 7 between marker interval N5 to AVRDC-BG75 spanning a distance of 27.3 cM having LOD value 3.3 and PVE of 5.6 per cent.

4.8 CO-SEGRAGATION OF MARKERS WITH YIELD TRAITS

Single marker analysis was performed to identify markers co-segregating with the yield contributing traits. There were 129 hits for marker and trait

association with LOD value more than 3.0 and explained phenotypic variation between 11.62 to 29.34 per cent (Table 12).

Marker McSSR150 (Position 62.30 cM) on chromosome 1 was co-segregating with days to staminate flower emergence with LOD 3.41 and PVE of 15.85 per cent. Marker AVRDC-BG99 (Position 74.13 cM) on chromosome 1 was co-segregating with days to pistillate flower emergence with LOD 3.18 and PVE of 14.90 per cent. Marker KAUBG_5 (Position 133.68 cM) on chromosome 1 was co-segregating with first pistillate flower node with LOD 3.78 and PVE of 17.44 per cent. Seven markers viz. McSSR99, AVRDC-BG104, McSSR62, S24, KAUBG_11, McSSR20 and AVRDC-BG26 were co-segregating with number of staminate flowers with LOD ranging between 3.41 to 6.86 and PVE ranging between 15.96 to 29.34 per cent. Eight markers viz. KAUBG_46, McSSR99, KAUBG_11, AVRDC-BG98, AVRDC-BG26, KAUBG_72, McSSR20 and AVRDC-BG75 were co-segregating with number of pistillate flowers with LOD ranging between 3.03 to 5.75 and PVE ranging between 14.31 to 25.25 per cent (Table 12).

Three markers viz. AVRDC-BG26, KAUBG_47 and S33 were co-segregating with fruit length with LOD ranging between 3.08 to 3.38 and PVE ranging between 14.46 to 15.72 per cent. Three markers viz. AVRDC-BG66, S13 and S33 were co-segregating with fruit breadth with LOD ranging between 4.0 to 5.11 and PVE ranging between 18.34 to 22.80 per cent. There were seven markers co-segregating with ratio between fruit length and fruit breadth with LOD value ranging from 3.11 to 5.06 and PVE ranging between 14.57 to 22.60 per cent. Four

Figure 29. QTL map for stem thickness

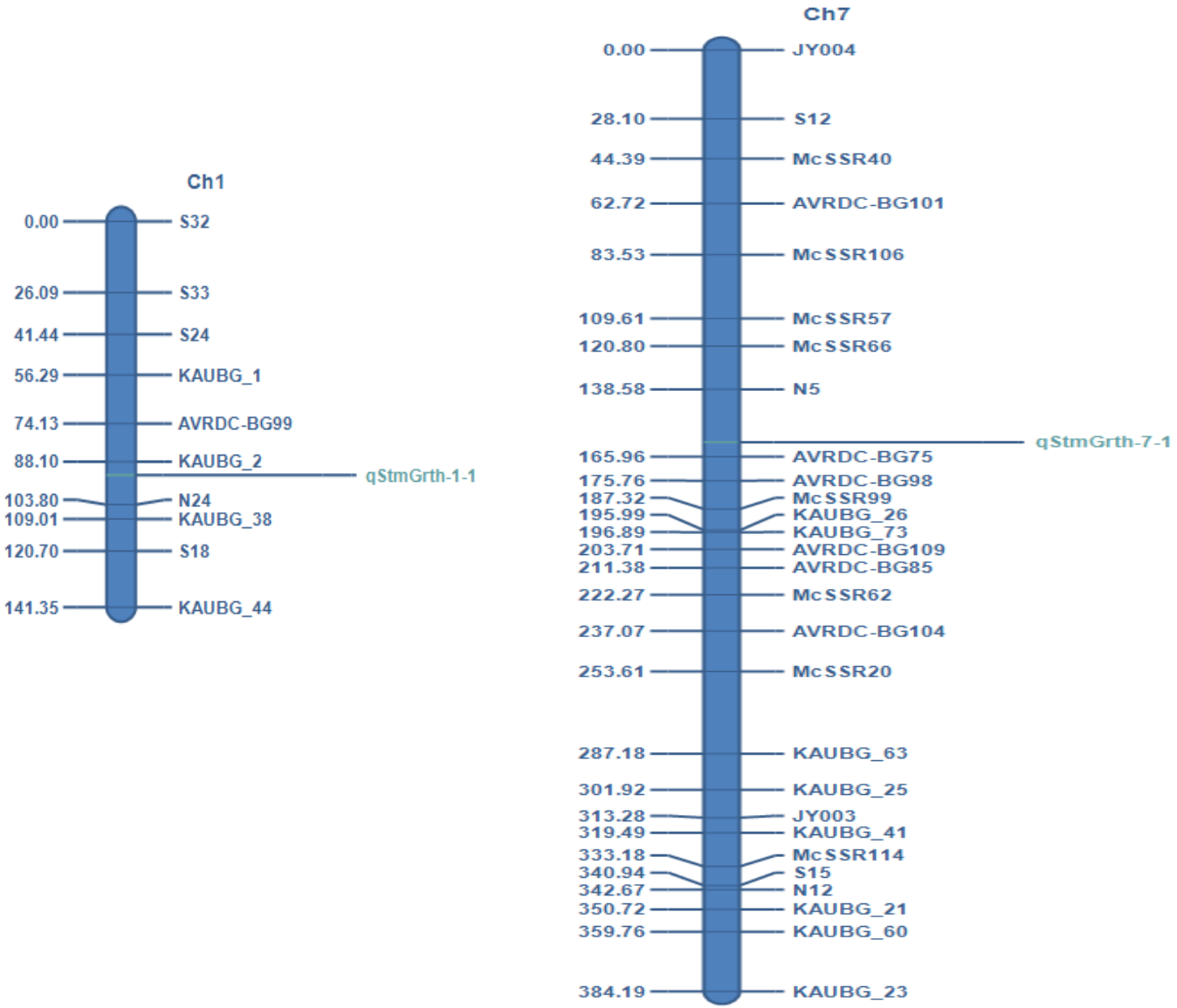


Figure 30. QTL map for number of side branch

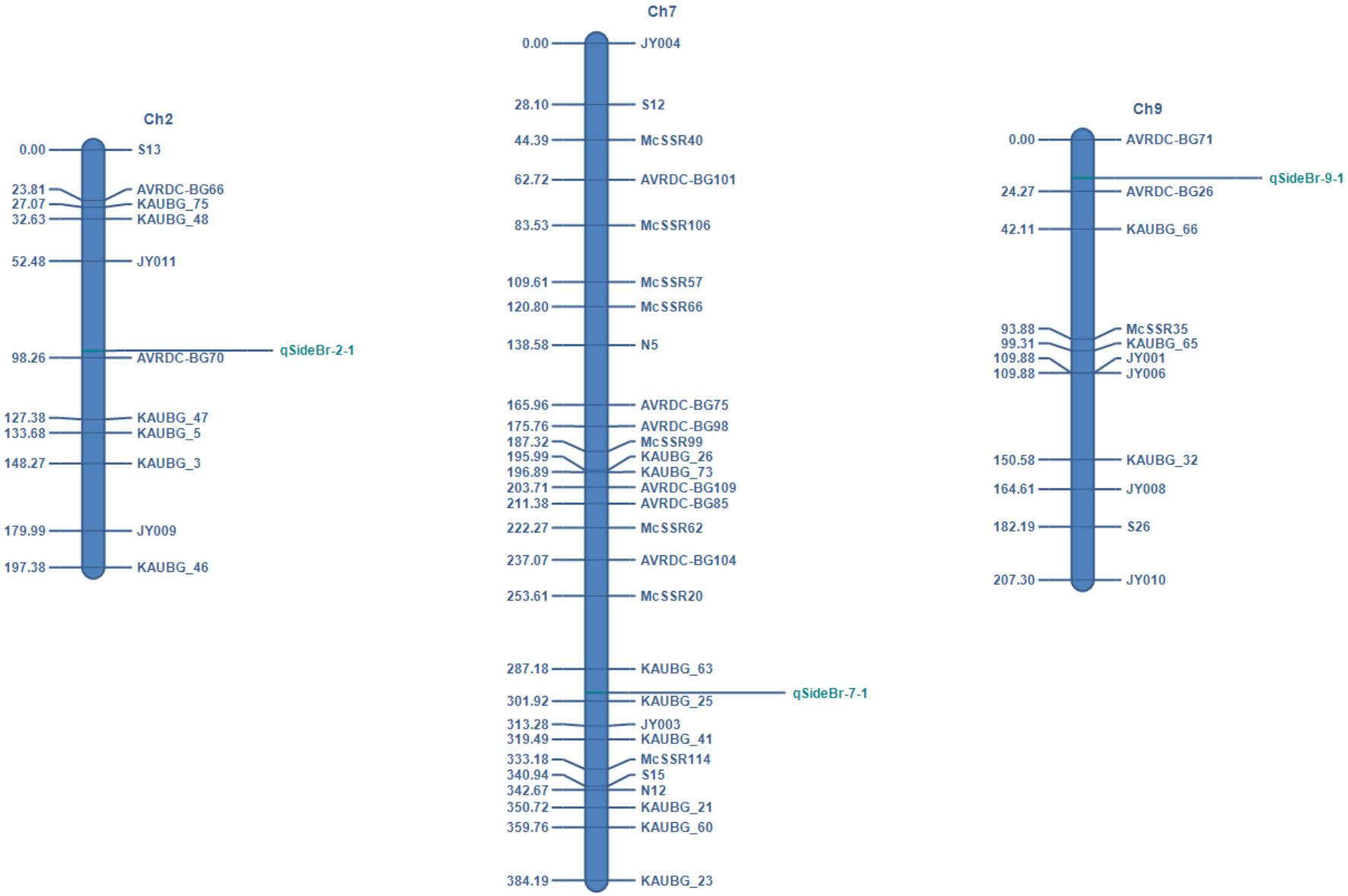


Table 12. Markers co-segregating with different yield traits

Name of the trait	Number of markers	Chromosome number	Marker name	LOD value	Phenotypic variation explained (%)
Days to staminate flower	1	3	McSSR150	3.41	15.85
Days to pistillate flower	1	1	AVRDC-BG99	3.18	14.90
First pistillate flower node	1	2	KAUBG_5	3.78	17.44
Number of staminate flowers	7	7	McSSR99	6.86	29.34
		7	AVRDC-BG104	5.39	23.92
		7	McSSR62	5.18	23.14
		1	S24	4.37	19.85
		4	KAUBG_11	4.31	19.64
		7	McSSR20	3.72	17.21
		9	AVRDC-BG26	3.41	15.96
Number of pistillate flowers	8	2	KAUBG_46	5.75	25.25
		7	McSSR99	4.85	21.81
		4	KAUBG_11	4.53	20.51
		7	AVRDC-BG98	3.76	17.38
		9	AVRDC-BG26	3.62	16.79
		4	KAUBG_72	3.57	16.61
		7	McSSR20	3.49	16.20
		7	AVRDC-BG75	3.03	14.31
Fruit length	3	9	AVRDC-BG26	3.38	15.72
		2	KAUBG_47	3.27	15.26

		1	S33	3.08	14.46
Fruit breadth	3	2	AVRDC-BG66	5.11	22.80
		2	S13	4.48	20.28
		1	S33	4.00	18.34
Ratio of fruit length to fruit breadth	7	2	KAUBG_47	5.06	22.60
		7	KAUBG_73	3.70	17.08
		7	AVRDC-BG104	3.68	17.02
		7	KAUBG_26	3.65	16.88
		9	AVRDC-BG26	3.59	16.61
		2	KAUBG_5	3.29	15.35
		3	McSSR150	3.11	14.57
Fruit weight	4	2	S13	6.35	27.47
		7	KAUBG_73	3.21	15.02
		7	KAUBG_26	3.13	14.64
		9	KAUBG_65	3.12	14.65
Flesh thickness	1	9	McSSR35	3.08	14.47
Peduncle length	1	9	AVRDC-BG26	3.08	14.46
Fruit length	1	9	AVRDC-BG26	3.08	14.46
Number of fruits	8	2	KAUBG_46	5.54	24.44
		7	McSSR99	4.84	21.75
		4	KAUBG_11	4.14	18.92
		7	AVRDC-BG98	3.75	17.35
		9	AVRDC-BG26	3.75	17.35
		7	AVRDC-BG109	3.33	15.57
		4	KAUBG_72	3.31	15.49
		7	McSSR20	3.21	15.04
Leaf size	1	3	AVRDC-BG86	3.25	13.99

Internodal length	2	9	AVRDC-BG26	3.76	17.11
		7	KAUBG_26	3.36	15.47
Number of seeds	1	3	McSSR150	3.20	14.96
Seed length	20	9	KAUBG_65	5.98	22.30
		1	KAUBG_2	5.74	21.51
		9	McSSR35	5.61	21.11
		1	KAUBG_38	5.57	20.98
		7	KAUBG_26	5.57	20.96
		7	KAUBG_73	4.73	18.18
		9	AVRDC-BG26	4.70	18.06
		7	AVRDC-BG109	4.53	17.49
		3	AVRDC-BG86	4.04	15.81
		2	S13	3.91	15.35
		7	KAUBG_63	3.77	14.86
		1	AVRDC-BG99	3.70	14.61
		7	KAUBG_41	3.39	13.49
		1	S33	3.38	13.45
		2	KAUBG_46	3.26	13.01
		2	AVRDC-BG66	3.22	12.90
		7	AVRDC-BG98	3.19	12.74
		7	AVRDC-BG75	3.19	12.73
		9	JY001	3.15	12.59
		9	JY006	3.15	12.59
Seed breadth	17	7	KAUBG_73	5.18	19.69
		1	KAUBG_2	5.17	19.65
		9	McSSR35	4.55	17.59
		9	KAUBG_65	4.52	17.49
		9	AVRDC-BG26	4.52	17.44

		7	AVRDC-BG109	4.32	16.79
		7	KAUBG_26	4.24	16.49
		1	AVRDC-BG99	4.13	16.13
		7	KAUBG_41	3.63	14.37
		7	AVRDC-BG98	3.61	14.27
		7	McSSR62	3.48	13.78
		3	AVRDC-BG86	3.35	13.33
		1	S33	3.28	13.10
		9	JY001	3.20	12.80
		9	JY006	3.20	12.80
		7	AVRDC-BG75	3.10	12.43
		8	McSSR112	3.07	12.30
Number of side branches	4	7	McSSR62	4.11	17.11
		7	AVRDC-BG104	3.59	15.15
		9	KAUBG_32	3.15	13.42
		7	McSSR99	3.01	12.90
Stem girth	1	9	JY008	3.02	13.74
Fruit color	9	9	AVRDC-BG26	5.88	25.75
		9	KAUBG_65	5.38	23.83
		9	McSSR35	4.66	21.00
		9	JY001	4.05	18.54
		9	JY006	4.05	18.54
		7	AVRDC-BG75	3.70	17.08
		4	KAUBG_72	3.23	15.08
		9	AVRDC-BG71	3.22	15.07
		7	McSSR57	3.09	14.49
Fruit ends	11	7	McSSR114	5.36	23.74
		1	KAUBG_38	3.87	17.82

		7	S15	3.57	16.55
		9	S26	3.55	16.46
		7	N12	3.54	16.43
		1	S32	3.53	16.38
		1	KAUBG_2	3.49	16.22
		8	KAUBG_30	3.44	16.00
		3	McSSR150	3.39	15.80
		8	KAUBG_43	3.27	15.26
		8	KAUBG_29	3.07	14.43
Fruit shape	15	9	AVRDC-BG26	7.77	25.17
		8	KAUBG_43	5.56	19.00
		7	KAUBG_26	5.30	18.29
		7	AVRDC-BG98	5.04	17.46
		7	AVRDC-BG75	4.90	17.04
		7	KAUBG_73	4.42	15.57
		2	KAUBG_47	4.06	14.41
		8	KAUBG_30	4.06	14.41
		8	KAUBG_29	4.05	14.38
		2	KAUBG_46	3.70	13.24
		2	KAUBG_5	3.65	13.09
		7	McSSR99	3.55	12.78
		7	AVRDC-BG109	3.47	12.51
		7	McSSR114	3.28	11.90
		4	KAUBG_13	3.20	11.62
Leaf color	2	2	S13	4.44	20.15
		3	KAUBG_53	4.39	19.94

markers were co-segregating with fruit weight with LOD value ranging from 3.12 to 6.35 and PVE ranging between 14.65 to 27.47 per cent. One marker McSSR35 on chromosome 1 was co-segregating with flesh thickness with LOD value 3.08 and PVE of 14.47 per cent. Marker AVRDC-BG26 on chromosome 1 was co-segregating with peduncle length with LOD value 3.08 and PVE of 14.46 per cent. Same marker was co-segregating with ratio between fruit length to peduncle length with LOD value 3.08 and PVE of 14.46 per cent. There were eight markers co-segregating with number of fruits per plant with LOD value ranging from 3.21 to 5.54 and PVE ranging between 15.04 to 24.44 per cent. Nine markers were co-segregating with fruit color with LOD value ranging from 3.09 to 5.88 and PVE ranging between 14.49 to 25.75 per cent. Eleven markers were co-segregating with fruit ends with LOD value ranging from 3.07 to 5.36 and PVE ranging between 14.43 to 23.74 per cent. Fifteen markers were co-segregating with fruit shape with LOD value ranging from 3.20 to 7.77 and PVE ranging between 11.62 to 25.17 per cent (Table 12).

Marker McSSR150 on chromosome 1 was co-segregating with number of seeds with LOD value of 3.20 and PVE of 14.96 per cent. Twenty markers were co-segregating with seed length with LOD value ranging from 3.15 to 5.98 and PVE ranging between 12.59 to 22.30 per cent. There were seventeen markers co-segregating with seed breadth with LOD value ranging from 3.07 to 5.18 and PVE ranging between 12.30 to 19.69 per cent (Table 12).

Two markers viz. AVRDC-BG26 and KAUBG_26 were co-segregating with internodal length with LOD 3.76 and 3.36, and PVE of 17.11 and 15.47 per

cent, respectively. Four markers were co-segregating with number of side branches with LOD value ranging from 3.01 to 4.11 and PVE ranging between 12.90 to 17.11 per cent. Marker JY008 on chromosome 1 was co-segregating with stem thickness with LOD value of 3.02 and PVE of 13.74 per cent (Table 12).

Marker AVRDC-BG86 on chromosome 1 was co-segregated with leaf size with LOD of 3.25 and PVE of 13.99 per cent. Two markers viz. S13 and KAUBG_53 were co-segregating with leaf color with LOD value ranging from 4.39 to 4.44 and PVE ranging between 19.94 to 20.15 per cent (Table 12).

4.8.1 Validation of marker-trait co-segregation

Amplification of these markers with five best and five least performers for the associated trait has validated four marker-trait co-segregations. Marker S13 for fruit breadth (LOD 4.48, PVE% 20.20), KAUBG_5 for first pistillate flower node (LOD 3.78, PVE% 17.44), KAUBG_11 for number of pistillate flowers (LOD 4.53, PVE% 20.51), and KAUBG_11 for number of fruits per plant (LOD 4.14, PVE% 18.92) were validated.

Microsatellite marker S13 produced homozygous alleles of 280 bp and 270 bp in five plants with minimum and five plants with maximum fruit breadth, respectively (Plate 10). Marker KAUBG_5 produced homozygous alleles of 194 bp in five plants with minimum value for the first pistillate flower node. However, it produced a homozygous (140 bp) as well as heterozygous alleles (194/140 bp) in five plants with the maximum value, showing that the allele at 140 bp is dominant over that at 194 bp. Thus, for early fruiting in bitter gourd, homozygous status of

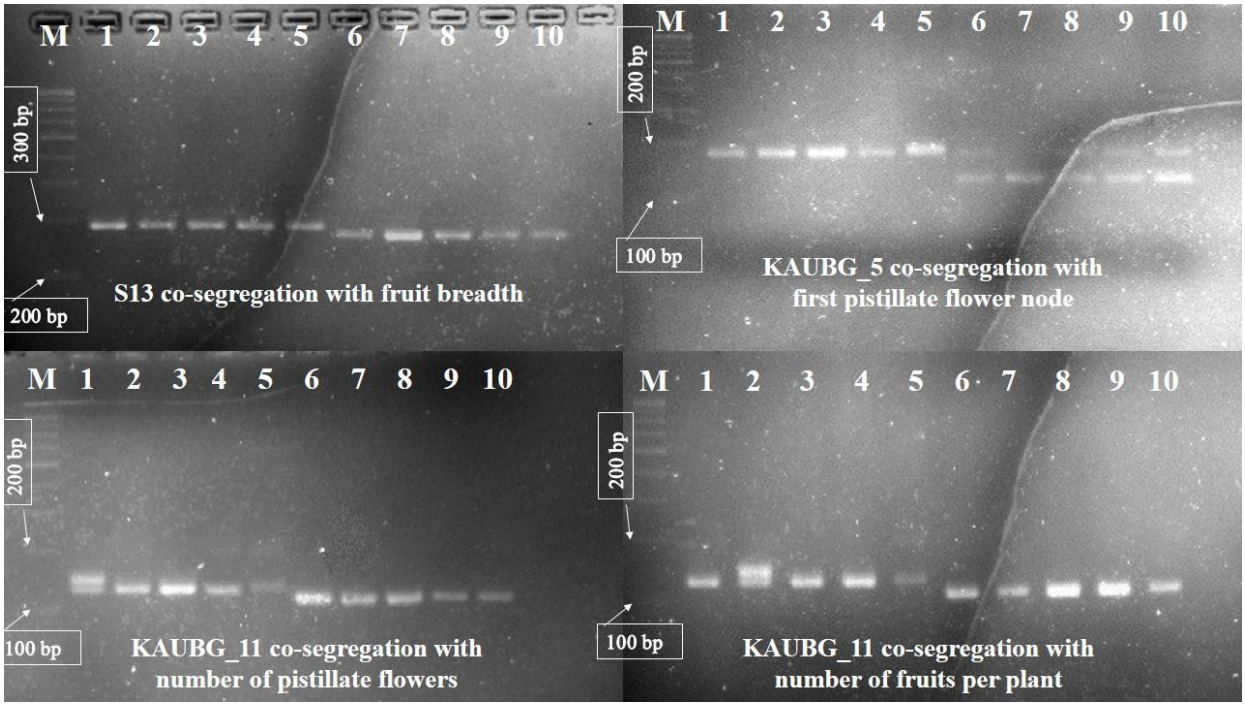


Plate 10. Validation of marker-trait co-segregation

M: 100 bp ladder,

1-5: plants with minimum value for the trait,

6-10: plants with maximum value for the trait

the recessive allele is required. Marker KAUBG_11 produced homozygous alleles (130 bp) as well as heterozygous allele (130/120 bp) in five plants with minimum number of pistillate flowers whereas the plants with maximum number of pistillate flowers had the allele at 120 bp in homozygous status. Similarly, this marker produced homozygous alleles (130 bp) as well as heterozygous allele (130/120 bp) in the plants with minimum number of fruits whereas those with maximum number of fruits generated the allele at 120 bp in homozygous status. Thus, the breeding lines generating the 120 bp marker at homozygous condition can be selected for higher fruit number and possibly the yield, at early growth stage itself.

Discussion

5. DISCUSSION

Bitter gourd (*Momordica charantia*; 2n = 2x = 22) is a tropical and subtropical vine belonging to the family Cucurbitaceae and cultivated widely in Asia, Africa, and the Caribbean for its nutritional and medicinal fruits (Grover and Yadav, 2004; Marr *et al.*, 2004; Van Wyk, 2015). Bitter gourd fruit provides a good source of phyto-nutrients such as carbohydrates, minerals, and vitamins. The yellow fruit pulp and arils are high in carotenoids, iron, phosphorous and ascorbic acid (Behera *et al.*, 2010). The pharmacological composition and properties of bitter gourd have also been widely investigated (Tan *et al.*, 2016), and the importance of bitter gourd in dietary habits is well known.

Among all the traits in bitter gourd to be addressed for its improvement, earliness and higher fruit yield are the major traits which will directly help the farmers in earning more returns through bitter gourd cultivation. Earliness and fruit yield in bitter gourd are directly controlled by other traits which include flower related traits such as early flowering, numbers of pistillate flowers and first female flower node, fruit related traits including size, weight, number per plant and flesh thickness, and vine related traits such as intermodal length, number of side branches, and vine length etc. Inclusion of molecular markers as a tool for selection of desired recombinants from a population always assures the presence of favourable alleles and fast recovery of recurrent parental genome in the cultivar under improvement. Use of molecular markers for the selection requires fool proof knowledge of the genomic locations governing a trait and the extent of linkage of

the markers with the loci. The success of marker assisted selection (MAS) always depends on high-quality genetic linkage map and genetic mapping of quantitative trait loci (QTL) for the target traits.

Fine mapping of QTL increases the efficiency of foreground selection in introgression programs through MAS by permitting the selection of the lines accommodating minimum amount of donor background. This will reduce the number of individuals that is required and the genotyping cost. In addition, introgression of a smaller genomic region helps to eliminate the linkage drag. Similar considerations also hold true for recurrent selection. For MAS to be effective, the target QTL must be free from any undesirable linkage. The large size of the regions encompassing QTL and the likely presence of undesirable linked genes make it essential to fine map such regions to facilitate their precise introgression and to identify candidate genes within these QTL. Further, fine mapping will help to clone the genes residing at the target QTL.

Genetic maps have been constructed for other cucurbits such as cucumber (Wang *et al.*, 2005; Sun *et al.*, 2006; Yuan *et al.*, 2008; Miao *et al.*, 2011; Zhang *et al.*, 2012) and melon (Harel-Beja *et al.*, 2010; Yuste-Lisbona *et al.*, 2010; Diaz *et al.*, 2011). However, genetic research in bitter gourd is still at infancy. A considerable number of microsatellite markers are reported (Wang *et al.*, 2010; Guo *et al.*, 2012; Ji *et al.*, 2012; Saxena *et al.*, 2015; Dhillon *et al.*, 2016), but a genetic map accommodating these valuable microsatellites is yet to be reported.

Breeders always seek to breed bitter gourd varieties for traits such as early maturity and high yield. Limited investigations have been made to identify the loci governing yield related traits using dominant AFLP makers (Kole *et al.*, 2012; Wang and Xiang, 2013) and co-dominant SNP markers (Matsumura *et al.*, 2014; Cui *et al.*, 2018; Rao *et al.*, 2018; Rao *et al.*, 2021). Utilizing the reported microsatellites to map the traits, which directly or indirectly affects the yield, holds a great importance in bitter gourd breeding.

5.1 GENERATION OF MAPPING POPULATION

Parental lines used for generation of mapping population included Priyanka (*Momordica charantia* var. *charantia*), a high yielding variety, with greenish white, spindle shaped large fruits, and accession IC634896 (*M. charantia* var. *muricata*), bearing small, round and dark green fruits. The parents selected for the mapping population has to be distinct for the traits under investigation so that the mapping population will accommodate all the segregating alleles (Collard *et al.*, 2005). These two lines differ contrastingly with regard to shape, size (Chakravarty, 1990), and many other qualitative and quantitative traits (Kole *et al.*, 2009a, 2009b, 2010). Cross-compatibility, high fruit set (97.12 %) and development of fertile hybrids (85.4% fertile pollen) (Bharathi, 2010), make these two subspecies highly important candidates as parental lines in generation of mapping population. Accessions belonging to *M. charantia* var. *charantia* and *M. charantia* var. *muricata* have been used to map 108 AFLP markers and five different yield traits in bitter gourd (Kole *et al.*, 2012). Moreover, interspecific crosses generate a wide

variability in the population giving scope for identification of novel loci for economically important traits.

In this study, $F_{2:3}$ population was developed for mapping yield traits in bitter gourd. Picking a type of mapping population for QTL identification depends on the knowledge of preliminary efforts of mapping, resources available and time required for its generation. If plenty of research is not conducted earlier, F_2 or $F_{2:3}$ population, being easy and fast to develop, are preferred to get the initial idea about genomic locations of the QTL. Being a mortal mapping population, $F_{2:3}$ cannot be replicated over the time and locations, however, the primary advantage of $F_{2:3}$ generation is its ability to measure the effects of additive and dominance gene actions at specific loci (Collard *et al.,* 2005). Scientists have used F_2 (Kole *et al.*, 2012; Matsumura *et al.*, 2014; Cui *et al.*, 2018; Rao *et al.*, 2018) and $F_{2:3}$ (Wang and Xiang, 2013; Rao *et al.*, 2018; Rao *et al.*, 2021) populations for mapping yield QTL in bitter gourd. Longer time required for generating RIL and lack of standardized tissue culture techniques to develop DH population makes them unconsidered for very initial QTL mapping studies (Clarke *et al.,* 1995; Gardiner *et al.*, 1993; Harushima *et al.*, 1998).

5.2 IDENTIFICATION OF POLYMORPHIC MICROSATELLITES

A set of 450 microsatellites were screened for parental polymorphism. From this set, 51.33 per cent did not show any amplification and 47 microsatellites (10.44 %) were polymorphic. This set had 182 microsatellites from other species like *L. cylindrica* (127 markers), *C. melo* (33 markers), *C. sativus* (7 markers),

chinese cabbage (3 markers), and *Capsicum annuum* (12 markers). Fifty-five markers among these were earlier been used in mapping of horticulture traits in bitter gourd where they were successfully amplified and found polymorphic (Wang and Xiang, 2013), however, only 13 primers were found cross-genera transferable, may be due to single or few nucleotide polymorphisms at the annealing site of the primer among the parents used in this study and genotypes used by Wang and Xiang (2013). Among 127 markers from *L. cylindrica*, 18 were amplified. This suggests the cross-genera transferability of these microsatellites which can be utilised as anchoring makers in synteny studies between *Momordica* and *Luffa*. The low polymorphic status of microsatellite loci in *Momordica* was previously reported by Rathod *et al.* (2019), who found only 10 (1.88%) polymorphic microsatellite markers out of 534 tested in *M. charantia* var. *charantia* and *M. charantia* var. *muricata* genotypes. Compared to that report, the current recovery rate of 10.44 per cent polymorphic markers is promising.

Since 47 markers were insufficient to generate a dense linkage map, genome sequence of *Momordica* was scanned for the presence of microsatellites containing higher number of repeats (motifs starting from mono-nucleotide to deca-nucleotide). A total of 75 microsatellites were identified including motifs from mono-nucleotide to octa-nucleotide. Through genome-wide search, few researchers have previously identified a considerable number of microsatellites in this genus. Following the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) method, Wang *et al.* (2010) and Guo *et al.* (2012) have isolated 16 and 10 microsatellites, respectively. Similarly, by scanning the SSR-enriched genomic

libraries, Ji *et al.* (2012) and Saxena *et al.* (2015) have identified 11 and 160 microsatellites, respectively. Subsequently, Dhillon *et al.* (2016) have characterized 114 bitter gourd accessions with 50 polymorphic microsatellites contributing to the total of 247 microsatellites in this genus. Number of microsatellites identified in the current study is very high when compared to most of the earlier reports. Among 75 microsatellites, 69 were successfully amplified and 38 (50.7 %) were polymorphic which was promising than earlier report of Rathod *et al.* (2019), as well as microsatellites that were earlier reported. Failure of six primer combinations may be due to the nucleotide differences at the primer annealing site between genotypes tested and cv. OHB-3, from which the microsatellites were identified. The new set of 69 validated markers is a potential and useful addition to the list of microsatellites in bitter gourd.

5.3 PHENOTYPIC EVALUATION OF MAPPING POPULATION

Mapping population was evaluated for twenty seven traits including those related to flowers, fruits, seeds, leaves, and vine. The traits such as days to initiate the staminate and pistillate flowers, first pistillate flower node, numbers of staminate and pistillate flowers, fruit length and breadth, peduncle length, flesh thickness, fruit weight, number of fruits, vine length, internodal length, and number of side branches, are reported to be correlated with the total yield of the plant (Bhave *et al.*, 2003; Dey *et al.*, 2007; Singh and Kumar, 2008; Islam *et al.*, 2009; Singh *et al.*, 2012; Pathak *et al.*, 2014; Rani *et al.*, 2015; Yadagiri *et al.*, 2017). Mapping these traits on *Momordica* genome, ultimately leads to locating yield traits.

Significant variation was observed for all the traits in the mapping population. A panel of 90 plants was selected from 200 $F_{2:3}$ plants in such a way that it represented all the trait variation and the observations for all the traits in the selected 90 lines fall under normal distribution indicating polygenic nature of these traits. Similar frequency distribution was obtained by Cui *et al*. (2018) for flower traits like first female flower node and fruit traits like fruit warts, fruit color and fruit breadth by Rao *et al*. (2021) for yield traits such as fruit weight, fruit breadth, fruit weight, flesh thickness, number of fruits per plants and yield per plant, and by Rao *et al*. (2018) for first female flower node and sex ratio, when $F_{2:3}$ population was used for QTL mapping.

5.4 GENOTYPING AND CONSTRUCTION OF LINKAGE MAP

Mapping population consisting of 90 $F_{2:3}$ plants was genotyped using 85 polymorphic microsatellites. The amplification pattern was scored using ABH system for coding co-dominant marker. Considerable allelic variation was present in the mapping population for the microsatellites tested. A linkage map consisting eleven linkage groups covering 1287.99 cM distance was obtained. The linkage groups were allocated to respective chromosomes by using anchor information of microsatellites that were reported earlier.

The average map distance between markers was 15.15 cM. Kole *et al*. (2012) obtained linkage map spanning 3,060.7 cM consisting 11 LGs accommodating 108 AFLP markers. A map containing 12 LGs spanning 1009.5 cM 12 was derived from 194 markers including 26 EST-SSR loci, 28 SSR loci, 124

AFLP loci, and 16 SRAP loci (Wang and Xiang, 2013). A restriction site associated DNA (RAD) based genetic map of *Momordica* was developed using the F_2 population (Cui *et al*., 2018), which comprised 1009 SNP markers and spanned 2203.95 cM across 11 linkage groups. Rao *et al*. (2018) have assigned 2013 SNP markers to 20 linkage groups in high-density linkage map spanning a cumulative distance of 2329.2 cM. The average distance between markers was low (1.1 and 2.1 cM, respectively) in maps consisting of SNP markers (Rao *et al*., 2018; Cui *et al*., 2018), followed by microsatellites (Wang and Xiang, 2013) and AFLP markers (Kole *et al*., 2012). This was attributed to relatively higher abundance of SNP markers in the bitter gourd genome.

Least distance was obtained between marker interval JY006 and JY001 (0.0 cM) whereas maximum distance was found between KAUBG_13 and JY007 (54.05 cM). The mean distance between markers was 15.15 cM when all the linkage groups were considered. Least mean distance was observed on LG 7 (9.80 cM) whereas it was maximum on LG 4 (26.15 cM). With the increase in number of markers in LG the map distance between markers was reduced. In order to obtain highly saturated map, it is important to use large number of markers so that map distance between markers can be reduced (Tanksley, 1993; Mackay, 2001; Doerge, 2002; Collard *et al.*, 2005). In the current study, considerable number of microsatellites (85 out of 525 studied for initial parental polymorphism) have been used for linkage mapping, however, it might be less for saturating 11 chromosomes in bitter gourd. Hence, failure to obtain highly saturated map is attributed to use of relatively low number of markers. To this end, 2466 InDel markers recently

identified in *Momordica* genome (Cui *et al.*, 2021), would be useful in near future. However, this is the first linkage map successfully constructed using microsatellites from *Momordica* genome. Moreover, as a preliminary study like this, use of 85 polymorphic microsatellites is considered as sufficient to get an idea of the linkage map and QTLs in bitter gourd.

5.5 IDENTIFICATION OF QTL FOR YIELD TRAITS

The linkage map derived from IciMapping software along with genotypic and phenotypic data of mapping population was used to identify QTL for yield traits. Sixty QTL, including thirty seven major QTL, with LOD value ranging from 3.1 to 15.2, were identified for twenty four different traits on seven chromosomes. An LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (Collard *et al.*, 2005). The phenotypic variation explained by these QTL ranged between 1.8 and 35.9 per cent. Individual QTL is described as 'major' or 'minor' based on the proportion of the phenotypic variation explained by a QTL where major QTLs account for a relatively large amount (more than 10 %) and minor QTLs usually account for less than 10 per cent (Collard *et al.*, 2005). Number of QTL ranged from 1 to 4 for the investigated traits. One QTL each for first pistillate flower node, fruit length and breadth, fruit shape index and, seed breadth, two QTL each for number of pistillate flowers, number of fruits, vine length, yield per plant, stem girth and, number of seed, three QTL each for days to pistillate flower, number of staminate flowers, flesh thickness, fruit weight, internodal length and number of side branches, fruit color, and fruit ends, and four

QTL each for days to staminate flower, leaf size, seed length, and fruit shape were identified.

Twelve QTL for five fruit traits (Kole *et al.*, 2012), 43 QTL for thirteen traits (Wang and Xiang, 2013), 22 QTL for four traits (Rao *et al.*, 2018), three QTL for three traits Cui *et al.* (2018), 19 QTL for six traits (Rao *et al.*, 2021) were reported earlier. Most of these studies covered only fruit related traits like length, breadth, flesh thickness, weight and epidermis structure. In the present study, all the yield related traits including fruit, flower, seed, leaf and vine related traits, which can directly or indirectly contribute to yield were considered for mapping. Moreover, number of QTL identified in this study were also very high when compared to earlier reports.

A first report on QTL mapping for days to staminate flower emergence has been highlighted in this study. Totally, four QTL were identified for days to staminate flower, one each on chromosome 3 and 8, and two on chromosome 7. LOD value ranged from 3.1 to 5.0 with PVE of 7.0 to 17.8 per cent.

Three QTL were identified in present study for days to pistillate flower, one each on chromosome 1, 3 and 7. LOD value ranged from 3.1 to 4.1 with PVE of 9.2 to 12.1 per cent. Eight QTL for this trait with higher LOD (2.4 to 36.1) and PVE % (0.05 to 58.75) were reported earlier by Rao *et al.* (2018).

One QTL was identified in present study for first pistillate flower node on chromosome 2 spanning a distance of 6.31 cM having LOD value 4.1 and PVE of 18.3 per cent. This result was comparable to earlier reports by Wang and Xiang

(2013), Cui *et al.* (2018) and Rao *et al.* (2018), where three, two and five QTL were identified with LOD ranging between 2.64 to 4.71, 4.50 to 14.88 and 2.9 to 4.0, and PVE % of 12.2 to 20.6, 12.0 to 32.0 and 2.09 to 13.94, respectively.

Two QTL were identified in present study for number of pistillate flower, one each on chromosome 2 and 7 with LOD value ranging of 7.6 and 3.5, explaining 26.0 and 8.7 per cent of phenotypic variation, respectively. The LOD and PVE % was higher for two QTL identified for this trait by Cui *et al.* (2018) on chromosome 1 (LOD 7.99 and 25.12 with PVE of 21.2 and 52.8 %).

One QTL each was identified in present study for fruit length and fruit breadth on chromosome 8 and 9 with LOD value of 4.3 and 6.9 explaining 13.8 and 21.2 per cent of phenotypic variation, respectively. Multiple QTL are reported earlier for fruit length and breadth. LOD and PVE % were more in this result than that of six QTL identified earlier for fruit length and six QTL identified earlier for fruit breadth (Kole *et al.*, 2012; Wang and Xiang, 2013). LOD value and PVE % in present study were also more than that of three QTL for fruit length identified by Rao *et al.* (2021), where it was less in case of six QTL for fruit breadth.

Three QTL were identified in present study for weight of the fruit, one on chromosome 2 and two on chromosome 9. LOD values ranged from 3.3 to 6.8 with PVE of 5.5 to 18.1 per cent. LOD and PVE % were more than that of eight QTL identified earlier for fruit weight (Kole *et al.*, 2012; Wang and Xiang, 2013; Rao *et al.*, 2021).

Three QTL were identified in present study for flesh thickness, one each on chromosome 1, 2, and 9 with LOD value ranging from 4.0 to 7.9, explaining 9.9 to 21.3 per cent of phenotypic variation. LOD and PVE % were more than that of three QTL identified earlier for flesh thickness (Wang and Xiang, 2013; Rao *et al.*, 2021).

Two QTL were identified in present study for number of fruits per plant with LOD value 7.7 and 3.4, explaining 27.1 and 6.8 per cent of phenotypic variation, respectively. LOD and PVE % were comparable with that of ten QTL identified earlier for number of fruits per plant (Kole *et al.*, 2012; Wang and Xiang, 2013; Rao *et al.*, 2021).

Two QTL were identified in present study for fruit yield per plant with LOD values 3.5 and 3.1, explaining 16.9 and 5.7 per cent of phenotypic variation, respectively. LOD and PVE % were more than that of nine QTL identified earlier for fruit yield per plant (Kole *et al.*, 2012; Wang and Xiang, 2013; Rao *et al.*, 2021).

Four QTL for fruit shape were identified in the present study with LOD value ranging from 4.6 to 15.2 and PVE per cent ranging between 7.6 and 35.9. LOD and PVE % were more than that of five QTL identified earlier for fruit shape (Wang and Xiang, 2013).

Three QTL each for fruit color and fruit ends were identified in present study with LOD value ranging from 3.5 to 10 and 4.9 to 8.4, with PVE per cent ranging from 7.6 to 35.0 and 14.4 to 22.5, respectively. This is the first report of QTL mapping for fruit color and fruit ends in bitter gourd.

Three QTL were identified in current study for internodal length with LOD value ranging from 3.5 to 5.2 and PVE % of 9.5 to 14.9. LOD and PVE % were comparable with that of two QTL identified earlier for internodal length (Wang and Xiang, 2013).

Two QTL were identified for stem thickness with LOD value ranging from 3.3 to 3.4 and PVE % of 3.4 to 5.6. LOD and PVE % were lesser than that of two QTL identified earlier for stem thickness (Wang and Xiang, 2013).

Two QTL for vine length and three QTL for number of side branches were identified in current study with LOD value ranging from 3.2 to 4.5, and 3.3 to 8.7, and PVE % of 1.8 to 1.9 and 4.8 to 17.6, respectively. This is the first report of QTL mapping for these traits in bitter gourd.

Two QTL were identified for number of seeds per fruit with LOD value 3.2 and 4.9, explaining 7.1 and 12.7 per cent of phenotypic variation, respectively. Four QTL with LOD scores between 3.3 and 10.8 were identified for seed length explaining 5.6 to 26.3 per cent of phenotypic variation. One QTL for seed breadth having LOD value 4.2 and PVE of 16.6 per cent was identified in the present study. This is the first report of QTL mapping for seed traits in bitter gourd.

Four QTL were identified for leaf size with LOD value ranging from 3.5 to 4.7 and PVE ranging from 3.2 to 13.5 per cent. Three QTL were identified for leaf color with LOD value ranging from 4.0 to 6.5 and PVE ranging from 13.2 to 15.8 per cent. This is the first report of QTL mapping for leaf traits in bitter gourd.

Chromosome 1 had nine different QTLs for nine different traits and qNSd-1-1 was found with shortest marker interval of 5.22 cM (N24 to KAUBG_38). Chromosome 2 had eleven QTL for ten different traits and qFPFN-2-1 had shortest marker interval of 6.31 cM (KAUBG_47 to KAUBG_5). Four QTL were identified on chromosome 3 for four different traits and qDPF-3-1 and qDSF-3-1 were having shortest marker interval of 3.86 cM (KAUBG_51 to McSSR150) followed by qFrtEnds-3-1 with distance of 7.79 cM (McSSR150 to KAUBG_52). On chromosome 4, three QTL were found for three traits with qSdL-4-1 and qFrtClr-4-1 in same marker interval of 13.66 cM (KAUBG_15 to KAUBG_72).

Chromosome 7 had maximum number of QTL i.e. thirteen for twelve traits, qDSF-7-2 being in the shortest marker interval of 1.73 cM (S15 to N12). QTL for leaf size and days to staminate flowers landed in interval of 6.81 cM (KAUBG_73 to AVRDC-BG109) and 7.67 cM (AVRDC-BG109 to AVRDC-BG85). Four QTL were identified on chromosome 8 for four traits where QTL for fruit length and fruit shape landed in marker interval of KAUBG_29 to KAUBG_30 (1.35 cM). There were sixteen different QTL located on chromosome 9 identified for twelve different traits, with three QTL, one each for fruit weight, number of seeds and internodal length landing between shortest marker interval McSSR35 to KAUBG_65 (5.43 cM). These QTL with markers in the close distance can be efficiently used for MAS.

All the eighty five markers have been a great asset for mapping these traits in this study. Twenty seven out of thirty eight polymorphic markers (with the

series ‘KAUBG_n’, where ‘n’ is marker number) identified and validated in current study were linked to 39 different QTL reported in this study. More importantly the least map distance with these markers went up to 1.35 cM. This signifies the importance of microsatellites identified in this study.

Two QTL, flesh thickness (qFlTh-1-1) and seed length (qSdL-1-1) were found to overlap in the same marker interval on chromosome 1 (Table 13). QTL for fruit weight and flesh thickness were found overlapping with each other being in same marker interval on chromosome 2. QTL for number of pistillate flower and number of fruits landed between same marker interval on chromosome 2 and 7. QTL for fruit length and fruit shape were found in same position on chromosome 8. Flesh thickness and yield per plant were mapped on same position on chromosome 9. Fruit breadth and fruit weight were mapped between same marker interval on chromosome 9. Similar co-localisation of yield QTL was also reported by Wang and Xiang, (2013) and Kole *et al.* (2012) and they described it to be due to strong correlation between these traits. These traits are also been reported to be correlated in earlier reports (Bhave *et al.*, 2003; Dey *et al.*, 2007; Singh and Kumar, 2008; Islam *et al.*, 2009; Singh *et al.*, 2012; Pathak *et al.*, 2014; Rani *et al.*, 2015; Yadagiri *et al.*, 2017).

5.6 Co-segregation of markers with yield traits

Amplification of co-segregating markers with five best and five least performers for the associated trait has validated four marker-trait co-segregations. Marker S13 for fruit breadth (LOD 4.48, PVE% 20.20), KAUBG_5 for first

pistillate flower node (LOD 3.78, PVE% 17.44), KAUBG_11 for number of pistillate flowers (LOD 4.53, PVE% 20.51), and KAUBG_11 for number of fruits per plant (LOD 4.14, PVE% 18.92) were validated.

Verification of the 129 marker-trait co-segregations revealed by SMA has revealed four robust alleles and their genetics. Alleles of markers S13, KAUBG_5 and KAUBG_11 can be directly used for early selection for fruit breadth, first pistillate flower node (early fruiting) and number of pistillate flowers and number of fruits per plant (yield). This is the first report on the allele-level markers for direct selection for the most important yield traits in bitter gourd.

This study gives insights into the relative locations of microsatellites and major effect QTL for yield traits in *Momordica* genome. QTL with shorter marker interval (qFrtL-8-1, qDPF-3-1, qDSF-3-1, qDSF-7-1, qFrtShp-8-1) can be directly utilized in MAS for improving yield characters. Linkage observed between microsatellites identified in this study with yield traits signifies their importance in further fine mapping as well as marker assisted selection. The linkage map constructed in this study, being the first with microsatellites from *Momordica* genome, paves the path for comparative and consensus map generation with other marker types. Further, fine mapping using markers within the identified QTL hotspots can lead to possible identification and cloning of underlying genes for yield traits in bitter gourd.

Table 13. List of QTL hotspots where QTLs for multiple traits are clustered

Chromosome No.	Marker interval	Name of the traits clustered
1	KAUBG_2 to N24	Flesh thickness Seed length Stem girth
	S18 to KAUBG_44	Leaf size Leaf colour
2	JY009 to KAUBG_46	Number of pistillate flower Number of fruits per plant
	JY011 to AVRDC-BG70	Leaf size Number of side branches
	S13 to AVRDC-BG66	Fruit weight Flesh thickness Leaf colour Seed length
3	KAUBG_51 to McSSR150	Days to staminate flower Days to pistillate flower
4	KAUBG_15 to KAUBG_72	Fruit colour Seed length
7	N5 to AVRDC-BG75	Number of pistillate flower Number of fruits per plant

		Seed breadth
		Stem girth
8	KAUBG_29 to KAUBG_30	Fruit length
		Fruit shape
9	AVRDC-BG71 to AVRDC-BG26	Number of staminate flower
		Fruit colour
		Fruit shape
		Internodal length
		Number of side branches
	KAUBG_32 to JY008	Flesh thickness
		Yield per plant
	KAUBG_66 to McSSR35	Fruit breadth
		Fruit weight
		Vine length
	McSSR35 to KAUBG_65	Fruit weight
		Number of seeds
		Internodal length

Summary

6. SUMMARY

The study entitled "Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)" was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Agriculture, Kerala Agricultural University, Thrissur, during October, 2018 to December, 2021. The objective of the study was to map the quantitative trait loci associated with yield traits in bitter gourd.

- High yielding bitter gourd cultivar Priyanka (*Momordica charantia* var. *charantia*) and a wild bitter gourd accession IC634896 (*M. charantia* var. *muricata*), were used as parents in this study.
- Genomic DNA was extracted from both the parent lines by CTAB method and were subjected to PCR amplification with a set of 450 microsatellites to detect parental polymorphism. Among these 47 (10.44 %) microsatellites were polymorphic.
- Additionally, Genome wide hyper-variable microsatellites were identified using GMATo software. Seventy five microsatellites were identified and further amplified using genomic DNA of parental lines. Among the 75 microsatellites, 69 (92 %) were successfully amplified, and 38 were polymorphic between the parents. This led to the development of a set of 85 markers polymorphic between the parents.
- Direct and reciprocal crosses were made between parental lines. The F_1 plants were confirmed for hybridity using a polymorphic microsatellite marker McSSR62.
- The F_1 of the cross Priyanka × IC634896 were superior over the reciprocal cross for the traits like days to staminate and pistillate flower emergence, length

and breadth of fruit, length of peduncle, flesh thickness, number of fruits per plant, mean fruit weight, number of fruits per plant, and yield per plant.

- The confirmed F_1 plants were further selfed to obtain the F_2 seeds. The F_2 population of 200 plants was raised and each plant was selfed to obtain $F_{2:3}$ seeds. Single seed descent method was used to constitute $F_{2:3}$ population of 200 plants.
- Mapping population was evaluated for twenty seven different traits including different characters related to flowers, fruits, seeds, leaves, and vines. Significant variation was observed for all the traits in the mapping population.
- Ninety plants were selected out of 200 $F_{2:3}$ plants on the basis of morphological observation, such that all the variation from base population is represented in selected plants. Genomic DNA was isolated from these 90 plants and were subjected to amplification of 85 polymorphic microsatellites.
- The genotypic data was used to construct the linkage map using IciMapping software. The linkage map consisted 11 linkage groups (LGs) corresponding to 11 chromosomes covering 1287.99 cM distance. LG 7 (28 markers) consisted maximum number of markers followed by LG 2 and LG 9, each having 11 markers. LG 1 had 10 markers whereas LG 3, 4 and 8 had seven markers each.
- Phenotypic observations along with linkage map were used for QTL analysis using IciMapping software with Inclusive composite interval mapping (ICIM) method. A total of 60 QTL were identified for 24 different traits on seven chromosomes with LOD value ranging from 3.1 to 15.2 and per cent of phenotypic variation explained (PVE) ranging from 1.8 per cent to 35.9 per cent.

- Four QTL were identified for days to staminate flower, one each on chromosome 3 and 8, and two on chromosome 7. LOD value ranged from 3.1 to 5.0 with PVE of 7.0 to 17.8 per cent. Three QTL were identified for days to pistillate flower, one each on chromosome 1, 3 and 7. LOD value ranged from 3.1 to 4.1 with PVE of 9.2 to 12.1 per cent.
- One QTL qFPFN-2-1 was identified for first pistillate flower node on chromosome 2 between marker interval KAUBG_47 to KAUBG_5 spanning a distance of 6.31 cM having LOD value 4.1 and PVE of 18.3 per cent. Three QTL were identified for number of staminate flower, one on chromosome 7 and two on chromosome 9. LOD value ranged from 3.4 to 5.4 with PVE of 8.6 to 21.1 per cent.
- Two QTL were identified for number of pistillate flower, one each on chromosome 2 and 7 with LOD value ranging of 7.6 and 3.5, explaining 26.0 and 8.7 per cent of phenotypic variation, respectively.
- One QTL each was identified for fruit length, fruit breadth and ratio of fruit length and breadth (Fruit shape index) on chromosome 8, 9 and 2, with LOD value of 4.3, 6.9 and 6.5 explaining 13.8, 21.2 and 31.6 per cent of phenotypic variation, respectively.
- Three QTL were identified for weight of the fruit, one on chromosome 2 and two on chromosome 9. LOD values ranged from 3.3 to 6.8 with PVE of 5.5 to 18.1 per cent. Three QTL were identified for flesh thickness, one each on chromosome 1, 2, and 9 with LOD value ranging from 4.0 to 7.9, explaining 9.9 to 21.3 per cent of phenotypic variation.
- Two QTL were identified for number of fruits per plant, one each on chromosome 2 and 7 with LOD value 7.7 and 3.4, explaining 27.1 and 6.8 per cent of phenotypic variation, respectively. Two QTL were identified for fruit

yield per plant with LOD values 3.5 and 3.1, explaining 16.9 and 5.7 per cent of phenotypic variation, respectively.

- Three QTL each for fruit color and fruit ends, and four QTL for fruit shape were identified on seven different chromosomes. LOD value for these QTL ranged from 3.5 to 15.2 with PVE per cent ranging between 7.6 and 35.9.
- Two QTL were identified for number of seeds per fruit with LOD value 3.2 and 4.9, explaining 7.1 and 12.7 per cent of phenotypic variation, respectively. Four QTL with LOD scores between 3.3 and 10.8 were identified for seed length explaining 5.6 to 26.3 per cent of phenotypic variation. One QTL for seed breadth was located on chromosome 7 having LOD value 4.2 and PVE of 16.6 per cent.
- Four QTL were identified for leaf size with LOD value ranging from 3.5 to 4.7 and PVE ranging from 3.2 to 13.5 per cent. Three QTL were identified for leaf color with LOD value ranging from 4.0 to 6.5 and PVE ranging from 13.2 to 15.8 per cent.
- Three QTL each were identified for internodal length and number of side branches with LOD value ranging from 3.5 to 5.2, and 3.3 to 8.7, respectively. Two QTL each with LOD value ranging from 3.2 to 4.5 were identified for vine length and stem thickness.
- Five QTL with marker interval less than 5 cM *viz*. qFrtL-8-1, qDPF-3-1, qDSF-3-1, qDSF-7-1, qFrtShp-8-1 and QTL hotspots where QTL for multiple traits are clustered can be immediately used in MAS. Other potential QTL needs further fine mapping for application in MAS.
- Single marker analysis identified 129 marker-trait co-segregations with the yield contributing traits. These marker and trait association was having LOD

value more than 3.0 and explained phenotypic variation between 11.62 to 29.34 per cent.

- Alleles of markers S13, KAUBG_5 and KAUBG_11 can be directly used for early selection for fruit breadth, first pistillate flower node (early fruiting) and number of pistillate flowers and number of fruits per plant (yield).

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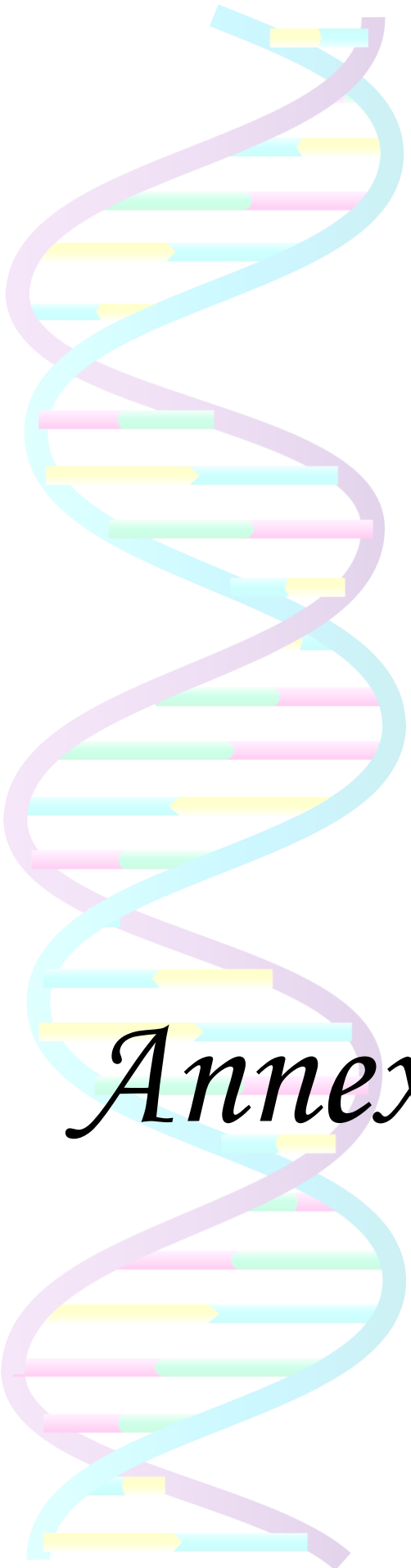

Annexures

Annexure I

List of microsatellites from literature used for parental polymorphism analysis

	Name of microsatellite	Forward primer (5' to 3')	Reverse primer (5' to 3')	Reference
1	CMMS 30-3	TTCCCACCAGCCCAACGGACACACT	GAGATACAGAAACGACGACTAACCT	Bharathi, 2010
2	CMMS4-3	ACCGAAATCATAAGGAACATAAGAG	TATGAGCTGTGTTGTGTATGAAAAC	
3	CMMS33-2	GCTACTTTTTATGGCGGCAGTGACG	ATTCGGATGATTATTCTTCGCAGTT	
4	CMMS33-1	TGTAATAGGATGACCAAGGGGAGTT	TTCAGGAGCTACAACAAGATTTCAA	
5	CMMS35-5	AACGGGATTTTGGAGGCATATTCGG	CTCCCCAGTGTATCAGCCAAATCTC	
6	CMMS12-6	AACATGATGTGTTTACCAACTTTTT	GGTTAAGGGAAAGTGAAGAGATGGT	
7	CMMS15-4	GTCCGCCATCGCCACTACAAATCAA	CTCCGTAAAACCTTCTTCCTCTCTC	
8	CMMS2-3	ATCACCCACCCCACCACTGCCAAAA	CCTTGAAAAACCACCAACATAACAC	
9	CMMS22-2	CGTTATACAAGATAGAGATAGAGAG	TTCAACTAATCCCCAAGACAAACAA	
10	CMMS1-7	CAAAAGACAAGGAGACGAAGACACC	AGACAACTGGTCGTACAGACACAGT	
11	CMMS27-1	TCCATGAATTTATCGGGACTTACCA	TTGCCTCATTACTCAACTGTATTTC	
12	CMMS35-4	ACGGATACATCGAGGAGACTTCATG	GTCAGCTTCAACCCTTTACTTTTTC	
13	CMMS34-10	GGGGTGTGAAGCTGAAGGCAAAGTC	AAAGGAAGAAGAAGAAAAAGGAGAA	
14	CMMS34-8	TTTCTTACTTTTTGGTTTGGTTCTG	GGCGCTGTGGTGAGTGTCGGGAGAG	
15	CMMS31-3	TTATGCATTAGTTCTTTACCGTTTA	CTTGTCGCAGGGTCTTTATTGTGTT	
16	CMMS14-1	CATTGCTACTATTGTCGTCGTTGCT	TTTCTTTCTTTTCCGTATCCATTTT	
17	CMMS1-3	TTGAATGATTGGAGGGAAGATAACG	CAAATATTGATGGATTTAATATATT	
18	CMMS3-1	AAATATAAGCAAACCAAAGTTGACC	CCGGGATATACGGACATACACACAC	
19	CMMS34-6	GGGGCCAGCTCAACAACCACCATAG	TGCCATCGAAGTTAGTTGAAGCTCA	
20	CMMS35-3	CGGAGAAGAAGGAAGGGTTTTAAGA	ATTCGTAGTTCATACTCTCTTTCTC	
21	CMMS35-1	CTTGGGTAAGTCTGTGGATGTTGCT	CTACGCATAACATTTTAGGCATCCA	

22	CMMS12-4	GATGCGGTGAGAAAGAGTTGAGAGA	AGAGGGAGAGAGTTTGTAAAAAAAT	
23	CMMS36-2	CCACACATTACAAACTAAACAAACA	CGATTCCGATTTGGTGTGGCGTTTT	
24	A2	GCGATGGTTGAGTGTTCC	TTCTTGTTGCCCTTGCTG	Guo *et al*., 2012
25	A47	TGGAATGGCAACTACACG	GGGGAGGCTGAAAGACTA	
26	C1	GAGATTGATGAGTCCTGAGTAA	AAAAACTACATCTCCTGTCTGA	
27	C4	TACTCTCCCTGATTCTTTATTT	TCACCAATCGCCAAATCT	
28	C7	AAAACTTGTCCTCCACCG	TGTTGAGAAGATAAAAGGATGA	
29	C9	AGTGGCGATTTTGGATAAGT	ATGAGTCCTGAGTAATTGCA	
30	C11	GAGATTGATGAGTCCTGAGTAA	AGAGAAAGAGTGAACCAAACAA	
31	C17	GAGATTGATGAGTCCTGAGTAA	TGAACTGGCTTTACATCTACCC	
32	C24	TGGCTCAGTATCGCAAGTAT	GAGGAGGAAGTTTGACCTATGA	
33	C30	CAGGGGCGATTAGATTATTC	AGGAGAAGGCTGTGTATGAA	
34	JY001	GGCTCAGAACTGGCACAG	TATCACCCATCCATTCAC	Ji *et al*., 2012
35	JY002	AAAACTGGATGGTGGAGC	GAAGAAGGTGGAGGAGATGG	
36	JY003	GTGGGTGCAATGGGTGTC	CTGCTGCTGTTGCTTCTTC	
37	JY004	GTCAACTGCCATCGGTAC	AGGGAAGAAGAAGAAGAAG	
38	JY005	TTTATAGCAAACGGCTCA	GAACATATCGCAAACCTTA	
39	JY006	TTTCCAGAGGAGCAGA	GCTCAGAACTGGCACA	
40	JY007	TCGGACGGTGGAGAAT	CACGGGCAACGAATAG	
41	JY008	CTCGAACTTTCTGCTC	TGAATTGAATTGCTCT	
42	JY009	TAAACAACAAAACCAC	CTCAGAGTCAGAGCAA	
43	JY010	TGACAGGCGCTTGGAATG	GCCCTGGCTATGGTTTCG	
44	JY011	AAGTTGGGTTTACGAGTG	TGGATGATGTAGGGTTTC	
45	cm04	CATGGCGATGTTTTCTTTCA	AAGGGAAAATTTTGGAAGTGG	Wang and Xiang, 2013
46	cm09	GTCAAAAGCATCAGCAGCAA	CAAGTTAGGCAAACCCCAAA	
47	cm17	CCTTCATCATCATCATCGTCA	GACCGGCAGTGGACATAGTT	
48	cm23	TTCTTCATTTAGGGGCACTG	AAAGGGGGCTCAACATTTTT	

49	cm46	GCTCCGGCAAACCTTTTTAT	GTGGACACGGTGATCACAAA	
50	cm47	ACTTTGAATCCTCCGCTCCT	TGCATGAGACCTTGTGGAAG	
51	cm48	TCAAACCTGATGCTGTGGAC	CAVAAAGCACACATTCCATTG	
52	cm50	TTGTTTTTGTTGGGCACTCA	TTTCAGGCTTTTTCTGATGGA	
53	cm53	CTGCCGTGAAGGAGAAGAAC	AGCCTCAATCCCCAATCTCT	
54	cs01	CCTCTGAGATGCCCTTTCTG	AGAAGGCAAAGGCAAATTCA	
55	cs05	AGGGAAAATTAGGGGCATC	CTGAATTGCAATAGGCACGA	
56	cs13	AGTCTTTTCCCACCGGTCA	TGGCTGTGACTCTGTGTGTG	
57	cs22	GCAGAACCCAATGGTGATTT	AGAAAGGAAGCTCCCCTGAG	
58	cs37	AGTGGCCAACTCTCGATGAT	TGCTTCCACTGGGTTCTTCT	
59	cs48	ATGGGAAGTTCATCGTCGTC	TCCAATCCATGGCTACACAA	
60	cs50	ACGGCTTCCATTAACACCTG	AAGCTTCAATGGCTTCCTCA	
61	p004	TGCTTGCAGAAAGACGAACA	TTCTTAGTGTCAACCAGGCG	
62	p007	TGGATTAAGACCATCCCGAG	AGAAGGAGCTCTTGTGAGCG	
63	p008	AGATTACTGGAGAAGCCGCC	AGAAGGAGCTCTTGTGAGCG	
64	ju2	TTCACATCTTCTTCATCTTCC	TTGCTATTCGTTCTCAGTCTC	
65	ju5	CCTCTTTTAATTCAAACAAGAAATCA	TTCGGACAATGGCAGTGATA	
66	ju9	CCCTACCGCTGGCTAGACTT	GCATCATGACCAACTATCAACC	
67	ju14	GCGAAGCAGTCTGAAACC	GCGAATCCGGTGAGAAAC	
68	ssrb01	TGTCTGTTTGAGTAACCCGGTA	TTGTTCAAAACCCTCACCAA	
69	ssrb04	CCTTTTGAACAATGCGACAA	TTCTTAGTGTCAACCAGGCG	
70	ssrb05	GAGGCTGAATGGATGATTTTC	CGGTTATGTTCCGGTTTGAT	
71	cams101	TGGATTGGGAGAAGATCGAC	TCAGCAATTAACATGCCAAAA	
72	cams-163	GCGTGGGAATACAATGCTAGA	TCCATATAGCCCGTGTGTGA	
73	cams-351	ACCTGCAGTTTGTTGTTGGA	CGCATGAAGCAAATGTACCA	
74	cams-373	CCTCCTACCCTATCCCCAAG	GGTTGATGGTCCATGTTCAA	
75	cams-424	TAGCAGCAGCTGATGGAGAA	CCTTCTTCTTTGCCACCTTC	

76	cams-885	AACGAAAAACAAACCCAATCA	AACGAAAAACAAACCCAATCA	
77	cm0005	CATGACCACCATGAGGATA	GATAGCCACGAGCATAGTATT	
78	hpms 1-5	CCAAACGAACCGATGAACACTC	GACAATGTTGAAAAAGGTGGAAGAC	
79	hpms 1-41	GGGTATCATCCGTTGAAAGTTAGG	CAAGAGGTATCACAACATGAGAGG	
80	hpms 1-62	CATGAGGTCTCGCATGATTTCAC	GGAGAAGGACCATGTACTGCAGAG	
81	hpms 1-173	TGCTGGGAAAGATCTCAAAAGG	ATCAAGGAAGCAAACCAATGC	
82	hpms 1-168	GCCCCGATCAATGAATTTCAAC	TGATTTTTGGGTGGAGAGAAAACC	
83	hpms 2-2h	GCAAGGATGCTTAGTTGGGTGTC	TCCCAAAATTACCTTGCAGCAC	
84	ssr32	CAACGAACATCCTCCGTTCT	TGGAAAGAAGCAGTAGCATTG	
85	ssr108	GCCATTGAAACTTGCAGAGA	TGTGTTGGATGTTTGGCACT	
86	ssr192	ATTAAATTGGGCCATGGTGA	ACAACATGGGAAGCACTTGA	
87	ssr82	TGCCTCAATCCTTCTTACCC	ACCATCGAGGCTGCATAAAG	
88	ga-e	TCTACGGCCCAGAGAAAAATG	AACACACTAACAAGGATGTGC	
89	m8	CTTATATTCATAAGCGAAGAAC	AATAACAATAGATGAATAGTCA	
90	ra2-g09	GATGAGCCTCTGGTTCAAGC	ACAGCAAGGATGTGTTGACG	
91	cpssr2	CAGCGAGCGCAACC	GCACGATCTGGCTCCTT	
92	A	AGACATGTGGGCGCATCTG	AGACGCGTGGTACCCA	
93	agi030	CCGTGTAGATACCTCTGAGGACA	GTCGTTGTCACCGGCTAAAT	
94	ssras46	AGTCGGGAATGGAACAGTTG	TGGGACGCATACACGTTAAA	
95	ssra3	CTTTCTTGGCTCGGACAAAC	GGCAGCTCGTAATAGCAAGG	
96	pbcessrr3na3	CGAGCTCCAAAGCAGATTACC	AAGAACGTGATCTCCATCGC	
97	j	CAAAGCATTGGGCCCTTGT	GGCTTTGGGACCTCCTTTCC	
98	N1	GTCTTCCAGGTTGGGAACAG	ATCTGGTTCCTCGGGAGATT	Wang *et al*., 2010
99	N12	CAGAGGGGTGGTTCCTCTTT	CCACATGGATGATCGAGAGA	
100	N24	CTCCAACTTGAGGAAAGAAAAC	AGAGCCAATTGGGGCTTTAT	
101	N5	CGTCGCTCTCACAAGAGATAAG	TTTGGTGGAAATCCCCTATT	
102	N6	GGGAATTCTCAAAGAGCCAGA	TGGCACACTCTGCATGAAAT	

103	N9	ATCCATCCCCACAAGTTGAA	CCATAAGGATATGTTTGCATGG	
104	S12	GACATCCTTCTTGCCTCTTACA	GAAACGGAACGAAACCTCA	
105	S13	TTGGTTGTGGTGCTGAGTTC	GATGTAGGGGTTGGGTTGAT	
106	S15	GGGTAGTGGAATGATGGGTT	TAGTGTTTTCGTGAGGGAGG	
107	S18	TATGGGTTTTTTCCCCCTCTT	CATCCCCACAAGTTGAAGAA	
108	S20	CCCCTTCTAATCACAACCAA	GGCCTAATTTCTGCCCTTT	
109	S24	GCTCTGCGTTTCATTCTTCA	TGAACCCTCAGACTCAAACTC	
110	S26	GAACGCCCTGTGACTTTAGC	TTTCGTCTTCCAATGAGCC	
111	S32	CTAAATCACGCAAACCCATC	GAGCAAAAGACTGAGGAAAACT	
112	S33	ATTTAGTGGGGCGGGTAGT	TGGATGAGCATGTTAGGGATC	
113	S9	TTCCCATTCACAGATCACTCC	CCACCAAATTCAAGAACCCAC	
114	AVRDC-BG1	CAAGGAACGCAGAAATCCTA	GAGGTCTGCCTCTTCCAAAA	Dhillon *et al.*, 2016
115	AVRDC-BG2	GAGCACACAGAAAATTGGGT	TGATCCACTCCCAATCTTAGC	
116	AVRDC-BG3	GACGGGTCGTTGTAAGGTTT	CCCTGGAAATCAGATGAAGG	
117	AVRDC-BG15	AGGGCTACCAAAAGCAGAAA	TACGTTGTCATTCCCAAAGC	
118	AVRDC-BG25	TCGAGATCACGATAGGGACA	CATCACGGCTACTCTTTTCG	
119	AVRDC-BG26	CAGGGACGACGATTATCTGA	GACCGTCTTTCGATTTCCA	
120	AVRDC-BG27	GGGGACCAAAGCATTGAA	CAGCTCTGTGAAAGGACCAA	
121	AVRDC-BG29	GGACATCACACCCAGCAGT	CCAAAAGCAAGAAAGAAGGG	
122	AVRDC-BG30	CAATTCTACGCGCAACTCAT	GGCTCAGAAAGGAAGCTCAC	
123	AVRDC-BG32	GGAGTTTGTGCGTATGATGG	CCGTCCGATCACCTAAAAAT	
124	AVRDC-BG33	CATTGACTGAAGTTGGCGTT	CATCCACAACTGCTCACACA	
125	AVRDC-BG35	CACCATGGCCGGTTCTTA	TTCGAAGGCTATTCAGAGGC	
126	AVRDC-BG37	ATTTCTCCATGTTCTTCCGC	AAGAGAGAGAGAAAGCGAGAGC	
127	AVRDC-BG41	TTTACCCTTCCATTACTCGC	CTTGGTGAATCTGGAGAGCA	
128	AVRDC-BG48	GCAAAAACACTGTCACCCAC	TTCGCTTCTTCCCTCTTCAT	
129	AVRDC-BG49	CTGAAGGGCAGTGTAACGAA	CACTCCCCCATTCTCAACTT	

130	AVRDC-BG50	TTAATCCAACCCGTAGGAGC	TGGCCTTTTGCTTCTTAGGT	
131	AVRDC-BG51	CCCGTTCCCTTGAAACTAAA	TTGGGTGGTGATGAAGTTGT	
132	AVRDC-BG54	GCAGTTGGTCCACCTTCATA	TTCACTAATCAGTCGCTCGG	
133	AVRDC-BG55	CCGGGGATCTTCTTCCTTTA	TCGTAGCGAAGATGTGAAGC	
134	AVRDC-BG56	ATCACCATGGACAAAACCCT	GCACCATCTTGTATCGGTTG	
135	AVRDC-BG57	GGGACAACACACACCCAAA	CCCATGGAGAAATTTCAGGT	
136	AVRDC-BG58	GGCTCCTTTTCCCAAACTCT	AGATTATGAATTCGCGGTCC	
137	AVRDC-BG59	GGGGAAAGACAAAGGTAGCA	TCGGACATTTTTGAGCAGAG	
138	AVRDC-BG66	AGAGGTCTGCCTCTTCCAAA	CAAGGAACGCAGAAATCCTA	
139	AVRDC-BG67	ACCGTGTGAACCTCTGTCAA	ACCGGTTGTGAAGTGGAAGT	
140	AVRDC-BG70	CATAAGGCCTTCCTCTGCTC	CGGGGATTCCACTCTTCTT	
141	AVRDC-BG71	TGGACTTGGAAGTGGTGAAA	TCACGACACAATCCACCTTT	
142	AVRDC-BG73	GAACGACAAAGGGAAGGAAA	CTTCTTTGCCATCATCCTCA	
143	AVRDC-BG74	AACACCTTCTGACTCCACCC	CGTTCAATCCTCTCCTCCTC	
144	AVRDC-BG75	AGACTTCCGGTACGAAAACG	TTCTCTCACATGGGAATCCA	
145	AVRDC-BG83	TATGCAGGGAAGACTGATGG	TTTTGCTGGCTAAGGTGTTG	
146	AVRDC-BG85	TGCAACCACTTGGGTTCTAA	CACGCCAGTAGCTTCAACAT	
147	AVRDC-BG86	AAGGACAGAAGCACAAACCC	TTCCCGAAGCTTCATTCTCT	
148	AVRDC-BG90	TGTCTTGGAATTGCTTCTCG	GGAGGAGAAAATGATCGGAA	
149	AVRDC-BG92	GGGGAACTATTTGCAATCCA	TGGGGAGTCATAGAACGAGA	
150	AVRDC-BG93	CCACTATGACGAATCCGTTG	TTCTTCAAGTCGCTGCTGTT	
151	AVRDC-BG94	GGGAGAACACGTTTGGATTT	ATACCCCACACAAAAAGGGA	
152	AVRDC-BG97	GGTAAAGGAGGCAAGGATGA	GGGGGTTAAGGGATTCATTT	
153	AVRDC-BG98	ACTCTTGACCGGCTCGTAGT	CCATGTTTGACGACCTTGAG	
154	AVRDC-BG99	TGAAGGCAAATGCTCCTGTA	CCTTCTGGTTGAACAAATGC	
155	AVRDC-BG100	CGTCTGTTTCTCCATCGAAT	GATCAGAACAAGAAGCGCAG	
156	AVRDC-BG101	AACCCCATATTAGACGGTG	CCAGGTTAAGCAATTTCAAG	

157	AVRDC-BG104	GTAAACGGCTCTTTAGGGTT	CTCTCTCTGTTCTCTTCCTCTC	
158	AVRDC-BG109	CCCGTAAGGTTTATTGCAT	TCCTTTCCTTCTTCTTCTTC	
159	AVRDC-BG111	GAACAAGACTAATCACCCCA	CCAACCACAAGAAGAAGAAG	
160	AVRDC-BG112	ACGACGATTATCTGATTGCT	GACCGTCTTTCGATTTCC	
161	AVRDC-BG125	CGGAAGAGGCTTCGAAAT	TTCAGGCTGCTGATTTTCAC	
162	AVRDC-BG135	GCTCCTAACCATCACCCTGT	GGACACAGAATTCCAAAGCC	
163	AVRDC-BG136	TCGCAGTCTCATTTCTCAAG	AGTGGCAGAGCGTTTTACCT	
164	McSSR 1	GACAAAAACAACAACCAGAGGC	CTCCTCCTTCTTCTCTCTGCG	Saxena *et al*., 2015
165	McSSR 2	AGGGGAATAACAGAGAGGTGG	TGCTAATTTGCCTCTCGTCG	
166	McSSR 3	TTTTGTCAATTTTCCCGACG	TTTCATCTTCCTCTCGATCTCC	
167	McSSR 4	TCCCGCTTCCTCACATCTGC	GGGGTTGAAACACGAGAGTGC	
168	McSSR 5	CTTTAACTCACCTTCCACACCC	ACGATATGATCGAATGTCCACC	
169	McSSR 6	CGTGATTTTGTTTCGCCACC	TAAAACCGAAACCGAAACCC	
170	McSSR 7	AGAGAGGGAGAACGAGACGG	TTTATATGATGGGTCACTTGGC	
171	McSSR 8	TGTAGGCGTGGAGCGAGAGG	CCCTTCCTCGAATCATTCACC	
172	McSSR 9	GAAAATGGTCAGTGTGTGAGCG	GCACACGCACACTCACTGGC	
173	McSSR 10	CAATTGAGCCACCTTTTGGG	TAGCATCGATCCATGGCTCC	
174	McSSR 11	TCGTTGTTTCTCCCTCTCTCG	GCATAACACAGAATTGAGGGACC	
175	McSSR 12	CGATCTGCGAATCTTGCAGG	TCCTTCGAGGGAGAAGCACC	
176	McSSR 13	GTTCGGGATCTTCTTGCTCG	TCCCTTCTCCCCATCTCTCC	
177	McSSR 14	TTGCATGCTTTTTGGTAGAGC	GACTCATCTACCGAATCAACGG	
178	McSSR 15	GGAGGCGTCGTAAGATTCCG	ACATTTGCCAAGCGGAGAGG	
179	McSSR 16	GGCTTCCTTCAGTGAGTGCG	GTCTGTCGATGCGTCTTCGG	
180	McSSR 17	ACGAAGGCTCTCTTTCGTCG	ACGCCATGTCTGAAGAAGCG	
181	McSSR 18	TAAAGAATCGGCCAGTTCGG	GGGGTTAGAGAAAATGAGAGGC	
182	McSSR 19	GAATAGCTTTCGTCGCCTGC	CGGATATCTCCGCTTCTCTCC	
183	McSSR 20	GGAATTCAGGTGAACCTGACG	CCAGGAGGAAGAGGAACTGC	

184	McSSR 21	GAAGTTGAGGGAGGGAGAGG	TCTCTCCTCCCTCATCCTCG	
185	McSSR 22	CCATGACCGATGTAGCACTCC	TCGAACCAACCTAAACCAG	
186	McSSR 23	AGGTGGCCCTCTCTCAATCT	TATGTCGGCAGTCTCCCTCT	
187	McSSR 24	TCGGGAATTGGATTTTATGATT	GGCCTAATGTTGCAAAACCT	
188	McSSR 25	CCTTGAGGAGCCTACGTTGA	AATGGGCTCACCTTTGAGAA	
189	McSSR 26	TCCATTTTCTTTCGCAATCC	TGTTATTGGCTCCCTCTGCT	
190	McSSR 27	ATTTCCATTTCGCGATTCAG	GCCTTGTTTTCCGAAAGAGAT	
191	McSSR 28	GGAACTTTTGCTCGCATTGT	TGCCATCCACACCAGAATAA	
192	McSSR 29	TGCCATTTCGGGTTAAGAAG	CTGCGGAAAAATAGCTCGAC	
193	McSSR 30	ATTCCTAAAACGGCAGGTGA	CTTTGCTCTCTCCCGTTCC	
194	McSSR 31	CCTTGACCCTGAGATTGAGC	GTCTCTGTTGTCCGCCATCT	
195	McSSR 32	CCGATCCTTGTTTACCAACC	TCTCGAGAAACAAGTGGGCTA	
196	McSSR 33	CCCCAGTGAGGACACTGTTT	TTTTTCTTTCCCCCACTCTT	
197	McSSR 34	ACGCCAACGATATACCACCT	CCCATGGTTTGAGGTCATTC	
198	McSSR 35	TTAGCTGCTCGCTTGAGGAT	CAAGGATTCTCACATTTCCACA	
199	McSSR 36	AACGGTTGTTTTCACTCCAAA	AAGCAAAAAGATGGGGGAAA	
200	McSSR 37	CGCGAGGAGTTTTCTTCAAC	CTGCTGTGGTTCCTCCCTAC	
201	McSSR 38	CACCAGAACCGGAAGAAGAG	CAGAAGGCAGTGTTTGGTGA	
202	McSSR 39	GGAACTTTTGCTCGCATTGT	TGCCATCCACACCAGAATAA	
203	McSSR 40	AAATCTTAAGGCGCATGGAA	GGAACACACCTAAGGAGATGTCA	
204	McSSR 41	ATTCGATCGATGCTTCACTG	TTAATGATAATTACCCTGAC	
205	McSSR 42	TCCAATAAACTAAACATCCAAGG	GGGCCGTATCCATAATGTTG	
206	McSSR 43	TCACTTGGAGGAAACACAAAAA	CCCACCTCATAAAGGCATTC	
207	McSSR 44	TGGCTAGGTAAGCGTCCTGT	ACTACGGCGACGAAGAATCA	
208	McSSR 45	TGTTTCTATTCGGATCATGGTT	GAACCCTTTGTGCTGGTGTT	
209	McSSR 46	ATACCTCGAGCCAATGTTCG	ACCCCTTTCTCCCGAAGTTA	
210	McSSR 47	TTGATTTTGAATCAGCGTTGT	ATTTTGCACAAGGCCTACCA	

211	McSSR 48	TCCATTGGAATTGTTGTAACG	GGCTTTTTGGCCCTTAATCT	
212	McSSR 49	AACCTTTACAGAGCGGGTCA	TGCATTGTCCAAAATCCAAT	
213	McSSR 50	TCTTGCTTAGATCTGGACTACCG	CGATTCCCTTTTCACTCTGC	
214	McSSR 51	CCATCCACCGTTTTTGTTCT	TCTGCCATTGATGTGCTTGT	
215	McSSR 52	TGCCATTTCGGGTTAAGAAG	CTGCGGAAAAATAGCTCGAC	
216	McSSR 53	TCTGCAAAACCCAAGAAAGG	AAGTTCCCCTCAAACACCAC	
217	McSSR 54	CCATCCATATCCCAATTCCA	TCATCACAAACCTCCCTTTTTC	
218	McSSR 55	ATCCAACCAATAACCGGAAG	CTACCATTTTGGGGACGAGA	
219	McSSR 56	TGCCATACTCCCAGGAAAAG	CGGAGACCTGTGTTTTTGGT	
220	McSSR 57	TTCAGAATCCCAATCCAAGG	TGACAACCTCGTTTTCCTCTC	
221	McSSR 58	CTTGAAAGGCGCTCAAAAAG	AAGGACCCATGACGATGAAG	
222	McSSR 59	ATTCTCCGGAACCACAAGAA	GTTGGAGATAAGCGGACTCG	
223	McSSR 60	TAGTTGATGGCACGTTGCTC	GACACCCGACCTAGGAGTTG	
224	McSSR 61	TTAGGACCATTTGGGAGTGC	ACCAAAACGCATTGGAAGAC	
225	McSSR 62	GAGCTTCGAAACGACTTTCA	AAACCCAAGACCACCAACAC	
226	McSSR 63	TATGCTCAAAACCCCGATTC	ATCGGGACTAGACCAGCAAC	
227	McSSR 64	TCTGGACTACCTCAGGATCG	GGAGTCTTATGGGGGTCCTT	
228	McSSR 65	AGCACAAGGTCAGAGGGAAA	GGACTAGGAAGGTCGGAACC	
229	McSSR 66	TTCAGAATCCCAATCCAAGG	TTTCTGCCATTTTTCTTATTATT	
230	McSSR 67	TCCGCCCCTACTCAACTAAA	ATATCTCGTTACCCCCATGC	
231	McSSR 68	CTTCTCTTTGCCCCTTACGA	CAGTGCCCCACAACTATGAA	
232	McSSR 69	TGGACTAATGGTTCAAGGACCTA	GCAATCACACCATATCACATCA	
233	McSSR 70	AGATCTGGACTAGGGTAGCAAA	GCCCCTTCACTTTGTTCAAT	
234	McSSR 71	AAATAAATTAGCCGATCTTTGCAT	TCATTTCTGATCTGGAAAACCA	
235	McSSR 72	TGCAGCATCCATAGCCATAC	GGCAGTGTGATGTGATTCTGA	
236	McSSR 73	AATGGGGATATTCCCGAAAC	AATGGGAGCAAGAATTTCCA	
237	McSSR 74	GCCAAGGGAAATTGTAATACG	AAACAACGTTGATGGCAAGA	

238	McSSR 75	GAGTCCAGGTCTTGGGATTG	TCAGAGAGCACCCTTGCTAA	
239	McSSR 76	AAATTTGGGAGAGGGTAGGC	TGGGATGGGCTTATTGTTTT	
240	McSSR 77	GCTTGTGGAGCCTTTCCTAA	TGGATCAAAAACGTGGTCAA	
241	McSSR 78	AGCTGTTGGGTGGTTAGGAC	CATTGAGTTCACCGCCATTA	
242	McSSR 79	TGTGCTCGGGGTAGAAGTTT	CCGGGAAAGGGTAGAAGAAT	
243	McSSR 80	GAAGAGTTCGACCCAATGCT	CGATGGAATCTCATCATCCA	
244	McSSR 81	CGAGTGACATTGCTTCTTCG	TTCATTGGGCCTTTCGATAC	
245	McSSR 82	CGAGGAGTCACTCGATCAAA	CGCTGCCCACAGAAAATTA	
246	McSSR 83	CAAGATTTTACCATGACTGCAA	TACTGGAGGAGCAGCAATGA	
247	McSSR 84	AGAGAAAATGGTCAGTGTGTGA	CTGGACTAGCACACGCACA	
248	McSSR 85	TCCTAGGCGTAGAGGAACCA	AGTGGGAGAGAAGGGGTTTC	
249	McSSR 86	ACTCGTATGGGTGCCTTTTG	ATGTTGATTGGGCAGGAAGT	
250	McSSR 87	CCTCGGCCCTCATACTTAGA	CCCTATGCTCACGAACCAAT	
251	McSSR 88	GTTGTATGGCTCGGGTAGGA	CCCACCCCGTATAAAATCAA	
252	McSSR 89	CAAATTCCGGTCTCCAATGT	AACGCAGGTCGGATCTATCT	
253	McSSR 90	ACGTGCTCTTTCCTCCAAAA	AAATCCCGAGCACTTTACATTC	
254	McSSR 91	TGTTGATCGTCACCGAAATC	CCCATTCTTTGTTTGTTTTCTCTT	
255	McSSR 92	AGGCTCTCCAGAGCTTTCCT	TTGGAACTGAACACCCTGTG	
256	McSSR 93	TGGACTAGGAGAAGTCGTTTGA	CCCCAGTAAAAATCCCATCTT	
257	McSSR 94	CCTACATTCGACGGGACACT	TACCCCAAACACAGCAACAC	
258	McSSR 95	GTGTTGCTGTGTTTGGGGTA	GGATTATTTCCAGAACGGACA	
259	McSSR 96	GCATGCTGAATTGTGTTGGT	GTGTAACAGCCCTCGACCAT	
260	McSSR 97	CACATAAGCCGACATTACCC	TGCCTCTAAGGGTTCTTTCC	
261	McSSR 98	CCTTAGTGGCTAGGAGGAACC	GCTTTTGGACCTTCACATCC	
262	McSSR 99	TATCTGATGGTGGCGAGATG	GCACTCCCAAATGGTCCTAA	
263	McSSR 100	TTAGGACCATTTGGGAGTGC	CCAAATCGTGCTCAAACTGA	
264	McSSR 101	CTCTACGATTCCGACCGTCT	TCTTATTCTCCCCCTTCCTTTT	

265	McSSR 102	GAGGGAGAAGTGGAAGGGATA	CAATGGGATGGGGATTTTATT	
266	McSSR 103	TTCTTGCTCGGAGACAAATG	GGCCAATTCTTTCCCTTTTC	
267	McSSR 104	ACAGAGCGTAGGCTTGCTTT	ATTGGAGGGCAAGTCTGGT	
268	McSSR 105	TCGATCAGTTTTGGTCGAAAT	CCGACATTCTTTCTTGCACA	
269	McSSR 106	AAGAGCTGCTGGTGGAGAAC	CCGATGCTACATCATCAACAA	
270	McSSR 107	GAAGCACAATCACTCGTTGC	GAACGGGTGTTACCTGAGGA	
271	McSSR 108	GCAAATTTCTCATTTCCTCTTGA	ACCCACCCAGATGAATGAAT	
272	McSSR 109	GGGAATTCGATTCTCTCTCG	CCGTGTCAGGATTGGGTAAT	
273	McSSR 110	CGGGAAGGAATTGGAATGTA	TCATTGAGCGAAAGGTACGA	
274	McSSR 111	TACTATTGGCTTGGGCATGA	GAGAGGAAAAGAGGGGGAAA	
275	McSSR 112	ACCCATAGTCCAGGCTTCAA	TGTCGGCATCTACAATGGTC	
276	McSSR 113	CACGGAAACATCCGACCTAT	TTTTGGGGAATATGGGTTGA	
277	McSSR 114	TTGGTGCATTTGAAAGTTCG	CGCCCCTAAAATCATCAGAC	
278	McSSR 115	GCAATGACCCTGTTTGTTCT	CAAAGGAAGAGTGCACTTGTGT	
279	McSSR 116	TGTTTGAATGTAATGAGCCTATCC	TCCAATGCTGAATCGATGAC	
280	McSSR 117	GTCATCGATTCAGCATTGGA	GACGCAGCATGGTACTCTTTC	
281	McSSR 118	TGGCTAGGTAAGCGTCCTGT	CTACGGCGACGAAGAATCA	
282	McSSR 119	CGATAGGGCCTCATTGGTAA	ATTCCACAACAACGAAAGCA	
283	McSSR 120	AATGGGATGCCCTAATACGTT	TGTGGTCACAACCAGAAAGG	
284	McSSR 121	TGAAATTTTGAGGTTATGTTCTCG	TCTTTTTCTTATGCATGCCTTTT	
285	McSSR 122	TATCCAGGCTCCGCTTAGAA	GACAAATGCCCCAATAGCAT	
286	McSSR 123	TGGGATGTAAAAATGCATCG	GTCCATCGACTACGCCTTTC	
287	McSSR 124	GCTACCCCCTCATTTTCCTC	TCGATCACTGAGGCTGGAT	
288	McSSR 125	TGCAATTTTTATTATTCCAG	TTCGATGTAACTTTGATATACT	
289	McSSR 126	TGGACTACCTCGCACCTCTT	CATTCCAGCAGTTGGTTCAA	
290	McSSR 127	CAGAACCATCCTGTGGAACA	TGGAGCCCCTCAAGTTTTT	
291	McSSR 128	TCTGGTTCACCGCTTTAGGT	AGGGAAGTTGTGAGCATTACG	

292	McSSR 129	GATCAATTGGAGGGCAAGTC	AGGCTTGCTTTGAGCACTCT	
293	McSSR 130	TCTTTTTCATTCCCCCTTTG	GAACTGCACGGAGTTGATGA	
294	McSSR 131	GGGGGCAATGGAATACACTA	GGCGTGAATGCAAATAAAAA	
295	McSSR 132	TCTGGTTCACCGCTTTAGGT	AGGGAAGTTGTGAGCATTACG	
296	McSSR 133	CGCGTTTGTAATTCCATCAA	GCCCGCTTATTCATCTTTACA	
297	McSSR 134	GGTATCAAACCAATAACGATTCA	GCCCCTAGAGGTCGTAGAGA	
298	McSSR 135	AGGACTCACTGAGCCGAGAT	GATTCTGGCTTTCGTGCTTT	
299	McSSR 136	CGGGAAGGAATTGGAATGTA	TCATTGAGCGAAAGGTACGA	
300	McSSR 137	CCGAAATGGGTTCCTTACAA	TTTGGCAGCTAATCCTCTTGA	
301	McSSR 138	TGTGCTCCAAGAACTTCAACA	CTTATCATATTTGTCGCAAGCA	
302	McSSR 139	CCTACCCTTCTCGAGCCTAC	AGTTGTTTTTGGGTGGGATG	
303	McSSR 140	AGGACCAATGAGATGCAAAAA	TTGGTACCGTCCAATCGAA	
304	McSSR 141	TTGGTGGATAAGCACGTCAG	GAGAGCAGAGCCAAGGCTTA	
305	McSSR 142	TCCGAAGGTCTAAAGGATCG	ATTGTCAGGTGGGGAGTTTG	
306	McSSR 143	TGTTTACAGCAGCAATTCAACA	TTTTGATGGGTCCTTTTTGC	
307	McSSR 144	AGCAAACAATAGCAGCGAAA	CGTTCCACTACTAATTCAAGGAAA	
308	McSSR 145	TTACAGGCTGCCGTATTCTG	TTGATTCATTGACAGGTGCAT	
309	McSSR 146	AAGAAGGGGAGGCAAATGTT	CCAAATTGCAGTGGAAACAC	
310	McSSR 147	GAGCCCTCTTTCTCCTCGAT	CGAGATCCTTTCGATGACCT	
311	McSSR 148	CGGGAAGGAATTGGAATGTA	TCATTGAGCGAAAGGTACGA	
312	McSSR 149	TTCATTTTGAGGGGTTCAGG	TCGTGGATTTGAACTTTTATGG	
313	McSSR 150	AAGACTTGAGATTGAATCCACCA	AGAGAGGAAAACGCACCAAC	
314	McSSR 151	GACGATATCGACCGTGACCT	CATCTTTCACAATCCCTGGAG	
315	McSSR 152	CCATATTCCCCAAAAAGTGG	CGATAGGGCCTCATTGGTAA	
316	McSSR 153	GGGGGCAATGGAATACACTA	GGCGTGAATGCAAATAAAAA	
317	McSSR 154	TGCGGAAGAAAGGAAAAAGA	GTTTAGGTTCGGCCTCAATG	
318	McSSR 155	GTTGGCCATGGAATAAAGGA	GGAGATCCAAACCAAGAAGC	

319	McSSR 156	TGTAGGTCCGGGATAATCCTT	TTTACGCCCCGTAATTCTTC	
320	McSSR 157	GATCAATTGGAGGGCAAGTC	AGGCTTGCTTTGAGCACTCT	
321	McSSR 158	TCATCAACAACAACAATTCCA	TCTTGAATTGCACCGAACAC	
322	McSSR 159	ATCACGGTTGAGGGCTAATG	GTTCGATCGGCCAGAATATC	
323	McSSR 160	GATTGGAAATCGATGGAGGA	TCTTATCTTGCCCCTGCTTC	
324	SGJ643	GCCCCAAATCAGATCCTTTT	ATTCCCCCATCACTTTTTCC	Wu *et al*., 2016
325	SGJ644	GAGGAATGGAATGAAGGCAA	CTTCAAGATGTTTGGGCACC	
326	SGJ646	AATCGGGTTCTCACACGAAC	GAGGAATCCACCAAGAACCA	
327	SGJ648	AGGGAAAGGGCTCAGAGAAG	GAAAGAGTTGAATTGGGAATCA	
328	SGJ652	ACGGACCTCCTTCCATTTCT	TCCATGGGTGAGGGATTTTA	
329	SGJ654	GCTGCATGTGTGAAATCTTGA	GGGCAATGTCTAGAGCAGGA	
330	SGJ659	CTGCAAACTTCTGCCCTTTC	CTGGATACTCAGGAGGCGAC	
331	SGJ666	TGAGAGATCCCATTCCACAA	TGGAACAGTCTCTCTCTCACACA	
332	SGJ671	GAGGAGCTGAAGGGGTTTTC	ACCCTCGAAGCTCAACAACA	
333	SGJ677	GGGGTCAATTGAAGGGAAAT	AGAGAGAAGGAAAGGGGCAG	
334	SGJ684	CGCAGAAGGAACCAGAGAAC	TCCTTCTCCCTCTCTCTCTCC	
335	SGJ689	TGGAAGAGAGTGGGAAATGG	TCGAGGTGGAGAGAAGATCG	
336	SGJ691	GGAGACAACAAAAATAGAGAGAGAGA	CAAGTGGAAGAAAACCCTCG	
337	SGJ714	GTTCAATTTTCCCACATCGC	CCTGAGAATGGACAGCAACA	
338	SGJ718	TCATCAGTGGCAATATCGGA	CATGCAGCCGTACTTGAAGA	
339	SGJ722	TCCACACCAACAAAGGTGAA	ATGGCGTTGGGTATGAATGT	
340	SGJ731	GGTGTTGACCCAACGAACTC	TGGCTCGGCTCTTACTCTTC	
341	SGJ732	TTCGCCTTTAACGTACCACC	ACTGGAGAAGAAGCACGGAA	
342	SGJ739	TCATTTCATTTGTTGCTGCC	CGATTGACGGGTTCTGTTCT	
343	SGJ740	TCCCAATTCGGAAAATCAGT	ACCGATCTGAATCATCCTCG	
344	SGJ745	TCTCTGAACAAACCCCAACC	GCCGTTTTGCTGTTGATTTT	
345	SGJ748	CGATCCTCTGCATGAACTGA	GAGGAGCTGAAACAACAGGC	

346	SGJ750	GATGGCGATAGGGAATCAAA	CCATTGCCACAGAGTCTCAC	
347	SGJ753	GGATCGATTCCCTTCACGTA	CCTTGCCCTTCTGTTTTGAA	
348	SGJ756	GGGCCATTGAAGTTGGAGTA	AACGGCATTAAAATTCCCAA	
349	SGJ759	TCCGATAAAGTGATCCAGGG	CTCCTTCAATCCCCAATCAA	
350	SGJ760	TCATCGCTCTCCCTTTCTCT	CGCTTCTCTCGCTAGTCTTCA	
351	SGJ764	CGTCCTACAAATTCCCGAAA	TTTCACTCTTGGCCCGATAC	
352	SGJ774	CTGGAAAAAGGGCAAAAGAA	TGGCCCATGGTTCATCTTAT	
353	SGJ777	CACTGCCAACCAGATTCAGA	TCATCTGGGTCCTCCTGTTC	
354	SGJ781	CGCCAATGAAGCTCATGTAA	GATCCGATCGACTACCCAGA	
355	SGJ784	GGACGAATTTTGCTTTGCAT	TCCTCCCCTGCTTTACCTCT	
356	SGJ789	TCACAGTTGAAACATCCCCA	GGCTGAGAGGCAGAGAGAGA	
357	SGJ790	GGAAGGGCATTCCATCTTTT	TCTGCATCACACCGTAGAGG	
358	SGJ791	AACGAGCGAAATTCCATTTG	ACGCAGTTTGTTGACTGCAA	
359	SGJ792	TTGGTTCAGCTAAGGGCCTA	GGCTCCACTGACTAGTTGCC	
360	SGJ795	AAAGCGCCTGAATCAATCAC	CATGCCCTGGAACTGATCTT	
361	SGJ800	TGCAAAATCAGATGATTCCTAAA	TCGAGATGTGTTCTTTTGCG	
362	SGJ802	AAACAAGTGACCGATCCCAG	GGAAGGCGACAAAATCAAAA	
363	SGJ803	CAATTGATGAACAAGCCAGG	CTGGCACTAGCTGCACAAAA	
364	SGJ805	AAAGAAGAGGCCAACAGGGT	GCTCTGCCTAACTAACCCCC	
365	SGJ806	TTCCCCAAAATCAAACGAAC	CGAAGGCAAAAAGGTTGAAG	
366	SGJ808	TTCCCTAATTCTGGCTGTGG	TATCGCTGCCAGGCTAATCT	
367	SGJ809	AACAGAAGCAAGTTCGCACA	TGCCATTTCTCCATTTCCAT	
368	SGJ811	TTCAAGCAGCTGCAAAAGAG	GAGTTGGTGGACCTTGGAAA	
369	SGJ813	CCGGAAATCAAAAAGGTTCA	TCTTCAGCGCAGATTCAAGA	
370	SGJ819	GCCCATTTCCCCTTTTATGT	TGATTAACCAAACAGCAGAGGA	
371	SGJ823	CAGATCCGCAAAAATCCTGT	TCCTTCTGTTTGTGGGTTCC	
372	SGJ828	GCAATGTCAGGTTCGGGTAT	CTGCATCTGGATCCCTTGTT	

373	SGJ830	AAGGGAAGGGCTGTTTCTGT	AATGTGGGCCAATCTTTGAG	
374	SGJ832	GCAAATGTCTATGCTTGGCA	CACCAGACGCCTCGTTTATT	
375	SGJ833	AGGAAAAAGGCAAGCATTCA	CTTTCCTTTCCCTCTCGGAT	
376	SGJ840	TTTGCACCCATGAAGGTACA	TCCCCATCTCCTCTTTCCTT	
377	SGK844	TCCTCAGATGCAACAACAGC	ACTAATTGTGATCGCCCTGG	
378	SGK851	GAGCCACAACAACCTCAACA	ATGTGGTAGCGGAGGTTGAC	
379	SGK857	AGCGAAGCAACGACAGAAAT	CTTCAGTTTCTGCCTCGGTC	
380	SGK875	TGTGTCATTGTCACCCTCGT	GACAGAAGTGGCTTCCAAGG	
381	SGK881	CAGTTTCAAGAATCGCAGCA	TTCAAACCCCACCATTTCAT	
382	SGK882	TCTGGTTCGTTGTTGGTTCA	CAAGTGGAATCATGAGCAAAA	
383	SGK884	ACACTAGCGATGGGTGGTTC	TACACGCGGAACATACCAGA	
384	SGK885	GCATTTTGCAGGCTATGGTT	ACCTAACAGCCAGGATGTGG	
385	SGK886	TGTGGCCAACAGAACAGAAG	TCACGTGACATCCTTTTCCA	
386	SGK891	GCTTTTCGCTTCTTCACACC	ATTTCGCGAATCTTCCATTG	
387	SGK892	CCGTCGTTGAGGTTCAAAAT	ATGTGACTCCAAAAGGGCTG	
388	SGK894	TCTGAAAACAGAGGACCAGAAA	ACGGAATGCGTAGAATCGTC	
389	SGK902	GGCATTCGATTTGGAAAGAA	CTCAAATGCTAAAACCCCCA	
390	SGK903	CCTTCTCTGCCATCAAGAGC	CATGGCCTTCGCACTATTTT	
391	SGK906	CGACCTCAAGCCTCATCTTC	CGGCGAGTAGTCACAACAGA	
392	SGK909	CCGATCCTCGGGTACATAGA	CTTGGATTGTCCACCGTCTT	
393	SGK922	CGGGTTGGAGTCTATGCAGT	CCCTCTTGCTTTTGCTGTTC	
394	SGK923	ACCAAGTTGAATTGGATCGC	ACGACAAATGTTTTCCTCGC	
395	SGK938	CAGTTCGTGTTGGGACTGAA	GATGGCCTGATTTGCTTTTC	
396	SGK941	GTGTCACCCTCCATGCTTTT	TGTTTCTTCACATTTATGGGTGAG	
397	SGK960	TGAGAGAGGGCAGAACCACT	TTTGAAGCATGCATGAGGAG	
398	SGK969	CCTTCCGGTTTGTATCTTCG	CGAATCAGAAGCGTTGCTTT	
399	SGK972	CAACCAGACCCACCTCTGAT	TGTTTCCACCTCTCACCTCC	

400	SGK974	CGCCGTGAAATCTCTCAAAT	ACTTTCTTACGGCATCCACG	
401	SGK980	AGGTAAGAGTAAGCGCGACG	GGGACAGAGCTGAACAGGAG	
402	SGK981	AATGAAGGAGCAACAAACGG	AATTGCAGCCAAATCAGCTT	
403	SGK984	ATGGGAAGCATGGAAAGTGA	GCTCTGCCTAACTAACCCCC	
404	SGK991	GGGAGGTTGAAGACGAAACA	CCCCAAAAATAAAACAAACCAA	
405	SGK992	GGCTGATGGAGACATTTCGT	GACAAACAAGCTGAGACCCC	
406	SGK1005	GCAGAAGAGCGTCCAAGTTC	GTTTCTCTCTCCCTGCCCTT	
407	SGK1011	TGCGGAGCATCAAAATACAA	TGAATGCCCTTTCGACAAAT	
408	SGK1017	GCCCATTGAATGTCAGGTCT	TTGACTGTGGGAGGAAAAGG	
409	SGK1018	ACGAAGAGTACGAAGGCGAA	TCCTCATCCCACATGAAACA	
410	SGK1022	TTTTGTTTGAGGGGCTTCAC	TTCCTCATTTCCAAATTCCTTC	
411	SGK1025	TTCTATCGGAAAATCGGCAC	TCTTCCTCTTCGCTGCTCTC	
412	SGK1029	ACGAATTGGAGCTTTTCGTG	AATGGCGGTGAAATGAGAAG	
413	SGK1031	GCTGTTGCTGTTGCTGTTGT	CCAACAGGCCTGCTACTTTC	
414	SGK1032	CTCACCACAACGCACAGAAT	TGAGTAGCTTCCCTCCGAAA	
415	SGK1033	AATCTGGGCCTGAGAATGTG	CCCTCAGCAGCAGCTTTATC	
416	SGK1034	AAAGGGATTGGAAGGATTGG	GGATGATGGGACTTGCTCTC	
417	SGK1035	ACGAAGATGCAAACACACCA	TTTGGCTTTTTCATTGCTGA	
418	SGK1037	GGAGGCTCTCAATAAGCACG	CGATTTTAGGCTCTTCAGCG	
419	SGK1039	AGGAAGAAGAGACAACGGCA	TGAAGTCCAAGAAGCGAAGG	
420	SGK1041	AGCTTTTCTAGGAGTCCGCC	GCCATTGACGACAATTCCAT	
421	SGK1043	GCCTCGTTCGCTGTATATTC	TCGCTTCTCAATCGTGTCAG	
422	SGK1045	AACATACCAAATCGGCGAG	GCATTTCCCCTCATTCTAAC	
423	SGK1046	TCTGTTTGTGGCCATGAGAG	GCTTTGCTGATGATGTCTGG	
424	SGJ647	AGCATTTCCCGTCGTCATAC	TGCAGAGGAAGGGCTCTAAA	
425	SGJ649	AAGCTTGCTTCGATTGTTTCA	GTTAGAGCGCACAGAAAGGG	
426	SGJ650	ATCGCCATAACCCATAACCA	GGGGATAGAATGGTGGGTTT	

427	SGJ657	GAAAAGGCAAAAGGCAGAGA	ATTAAACCCCTGCCCCATAC	
428	SGJ663	GAAGAAGGAACAGAGGCGTG	CCCCCTGAAATTTCTTCTCC	
429	SGJ668	ACACGATGAAAAAGTTCGGC	AGGATTGTGATTGGACCTCG	
430	SGJ669	CACACCAAATTCAAACCCAG	CAAACCCCAAATAAACGAACA	
431	SGJ673	TCTTCCGATTTCCTCGTCTC	CAATCCAGCATCAGAAGCAA	
432	SGJ675	TGGTGTGCAAGTGGTTTCAT	TGAAGGCCAGTGTAAGCAAA	
433	SGJ678	CACGATGAATTTGCCACTCA	AAGAAAAACACGCAAATGGG	
434	SGJ679	GGTCCCCAGTCAGTCATCTC	GGCATCCCTTAAGCTCCTTC	
435	SGJ681	TTCAGCAACAGAGGCAGATG	AAACCCCATGGATTTTTCAC	
436	SGJ685	TACGATGATCTCGCCTCCTC	TCACTCCCCAAAACCACTTT	
437	SGJ690	ATCGGTCGTTGGTTGATCTC	ATCAGACAGCCACTGCTCCT	
438	SGJ692	TGGTTGATCCATACAGCGAA	GAGGGGAGGAAAGTCAGGAG	
439	SGJ693	GCCAAGGATATGAACGAACG	TCTCACACAAAGCTGGCATC	
440	SGJ696	TTGATCACTGAAATGCCTGC	CTTGCCAGATAGAAACCCCC	
441	SGJ697	GCCGATCATAACAGAGGGAA	CACATTTGGAACTCGAGGCT	
442	SGJ698	TTGGTGGGTCGCTCTGTAAT	GCTGTAGACCAAGAGGCCAG	
443	SGJ700	AACACCATTTTGAAAGGCCA	TACAAATTCCCAAATCCCCA	
444	SGJ705	CACTCGAACAATCGCGTAAA	CTTTTGAAATCCGCTCTTGC	
445	SGJ708	CCACCAGCAACTGAGAAACA	TAAGGCAAAAGCAGAAGGGA	
446	SGJ709	TCCCCTTTGCTCACAATCTC	AATGAAGCAGCGTTAAGCGT	
447	SGJ715	GTGCACACCTTGGGCTTTAT	TTGACATGGTGTCTTTTCTTGC	
448	SGJ726	AATCCTTCAACGACCATTCG	CAGGTGCATGAATTTTGGTG	
449	SGJ729	CGAACAACTTTGGTGGACCT	ATCCCCCTCCATAGCTGTTT	
450	SGJ733	GCGAGAAGTGCTCCAAGAAG	GGAGAAAAGTGATGGGGGAT	

Annexure II

Morphological observations of mapping population for Flower and fruit related traits

Plant name	Days to staminate flower	Days to pistillate flower	First pistillate flower node	Number of staminate flowers	Number of pistillate flowers	Sex ratio	Fruit length (cm)	Fruit breadth (cm)	Fruit Length /Breadth	Fruit weight (g)	Flesh thickness (mm)	Peduncle length (cm)	Fruit Length/ Peduncle length	Number of fruits per plant	Fruit yield per plant (g)
Priyanka	54	57	16	149	16	9.3	14.2	5.4	2.6	114.46	0.72	7.1	2.0	14	1570
IC634896	50	48	9	327	48	6.8	3.9	2.4	1.7	6.16	0.23	2.4	1.6	42	321
1	55	58	12	425	21	20.2	8.7	3.7	2.4	78.2	3	7.2	1.2	19	1364.0
2	54	56	5	187	16	11.7	10.9	3	3.6	34.7	4	6.6	1.7	11	304.0
3	56	57	11	452	22	20.5	9.3	3.9	2.4	77.7	3	7.5	1.2	20	1383.0
4	53	56	10	176	9	19.6	4.5	2.2	2.0	16.1	2	1.7	2.6	6	45.0
5	54	57	12	560	24	23.3	9.3	4.4	2.1	58	4	5.1	1.8	23	997.0
6	55	55	4	214	17	12.6	11.5	3.2	3.6	34.2	4	6.9	1.7	12	323.0
7	68	69	14	71	3	23.7	9.9	2.9	3.4	40.3	3	3.1	3.2	1	40.0
8	53	57	12	495	31	16.0	9.8	2.8	3.5	35.8	3	6.4	1.5	23	677.0
9	56	59	16	409	45	9.1	10.2	3.1	3.3	86.1	3	4.9	2.1	44	2737.0
10	67	68	16	62	3	20.7	4.6	2.5	1.8	10.1	2	4.9	0.9	2	18.0
11	54	55	9	203	10	20.3	5.1	2.4	2.1	15.6	2	2	2.6	7	64.0
12	51	59	17	368	24	15.3	10.1	3.3	3.1	45.5	3	4.7	2.1	18	733.0
13	59	57	12	377	45	8.4	13.4	3.4	3.9	61.5	4	6.9	1.9	40	2289.0
14	63	59	8	446	12	37.2	6.4	2.8	2.3	34.3	4	6	1.1	11	321.0
15	55	56	11	587	25	23.5	9.9	4.6	2.2	57.5	4	5.4	1.8	24	1016.0
16	69	68	13	98	4	24.5	10.5	3.1	3.4	39.8	3	3.4	3.1	2	59.0
17	51	52	14	511	33	15.5	11.1	4.3	2.6	60.2	4	7.2	1.5	30	1637.0

18	61	63	17	182	17	10.7	13.7	3.6	3.8	62.5	3	5	2.7	13	799.0
19	55	55	20	451	48	9.4	9	3.4	2.6	49.3	3	6.7	1.3	40	1666.0
20	54	56	11	522	32	16.3	10.4	3	3.5	35.3	3	6.7	1.6	24	696.0
21	56	57	17	368	63	5.8	9.4	3.3	2.8	32.4	4	5.9	1.6	61	1684.0
22	65	56	4	371	15	24.7	10.4	3.7	2.8	73.2	3	5.2	2.0	14	1120.3
23	61	59	15	280	17	16.5	9.3	3.9	2.4	52.3	4	3.6	2.6	12	586.8
24	57	59	18	461	26	17.7	5.2	3.1	1.7	49.2	3	3.1	1.7	22	1100.5
25	59	61	13	416	40	10.4	12.4	3.6	3.4	77.4	3	5.3	2.3	36	2358.0
26	57	54	17	308	57	5.4	8.3	3.1	2.7	37.2	3	5.4	1.5	49	1474.0
27	56	56	17	782	37	21.1	7.3	2.9	2.5	31.4	4	5.1	1.4	37	1163.0
28	59	55	14	37	2	18.5	8.3	3.1	2.7	27	3	5.5	1.5	1	27.0
29	53	54	7	598	84	7.1	6.2	3.3	1.9	28.6	4	5.8	1.1	70	2123.0
30	62	57	11	374	17	22.0	5.6	3.4	1.6	20.4	3	2.4	2.3	14	211.0
31	55	57	12	978	46	21.3	8.7	3.6	2.4	53.6	4	11.4	0.8	44	2092.0
32	54	60	17	580	36	16.1	10.9	3.5	3.1	82.2	4	6.9	1.6	31	1993.0
33	59	61	15	391	18	21.7	7.2	3.4	2.1	32.1	3	5.2	1.4	11	421.3
34	59	59	9	390	65	6.0	9.4	3.5	2.7	51.4	5	6.9	1.4	65	2427.0
35	56	59	12	273	22	12.4	14.1	4.6	3.1	110.2	5	11.4	1.2	20	2120.0
36	65	59	17	132	20	6.6	4.9	3.2	1.5	17.4	3	1.4	3.5	19	297.0
37	58	60	23	294	22	13.4	15.2	3.7	4.1	115	5	7.5	2.0	22	3578.0
38	69	69	23	190	11	17.3	14.6	4.6	3.2	115	5	5.9	2.5	8	1206.0
39	64	76	18	392	13	30.2	6.6	3.1	2.1	25.2	3	1.1	6.0	5	118.0
40	63	68	13	446	12	37.2	6.8	2.6	2.6	62.6	3	4.6	1.5	7	378.0
41	55	60	19	416	23	18.1	9.8	3.4	2.9	48.2	4	8.2	1.2	20	664.0
42	61	67	24	368	21	17.5	9.2	2.7	3.4	63.6	4	5.1	1.8	18	1094.0
43	56	60	16	494	37	13.4	13.1	4.6	2.8	115	5	6.7	2.0	37	3578.0
44	57	53	14	220	32	6.9	5.6	2.8	2.0	58.4	3	1.7	3.3	31	1639.0

45	59	57	11	247	58	4.3	11.6	3.4	3.4	72.6	3	6.9	1.7	53	3003.0
46	59	60	17	187	8	23.4	4	2.2	1.8	20.4	2	3.9	1.0	8	134.0
47	51	53	8	598	49	12.2	7.6	3.4	2.2	44.6	3	5.9	1.3	49	1471.0
48	58	62	19	264	23	11.5	9.7	4	2.4	66.2	3	2.9	3.3	18	1201.0
49	53	56	10	588	55	10.7	10.6	3.3	3.2	76.4	3	6.5	1.6	43	2558.0
50	51	57	15	341	16	21.3	5.6	3.4	1.6	57.1	3	3.1	1.8	14	767.3
51	60	59	16	414	24	17.3	9.6	3.7	2.6	48.4	5	5.2	1.8	24	1000.0
52	59	61	26	280	12	23.3	8.3	3.1	2.7	56.6	3	3.1	2.7	10	621.4
53	57	58	15	436	46	9.5	10.8	3.3	3.3	85.6	3	5.2	2.1	45	2756.0
54	59	62	13	741	63	11.8	4.7	2.3	2.0	7.6	2	4.1	1.1	60	457.0
55	68	67	15	89	4	22.3	5.2	2.7	1.9	9.6	2	5.2	1.0	3	37.0
56	54	53	12	320	13	24.6	7.3	2.9	2.5	42.8	3	1.1	6.6	13	510.0
57	70	65	15	641	29	22.1	9.3	3.3	2.8	54.4	3	7.2	1.3	22	1063.0
58	61	71	18	316	15	21.1	7.3	3.2	2.3	43.9	3	3.4	2.1	11	476.0
59	63	74	14	445	19	23.4	5.6	2.9	1.9	53.2	3	3.9	1.4	16	923.0
60	64	65	14	13	2	6.5	9.9	3.8	2.6	33.8	4	5.1	1.9	1	34.0
61	59	65	19	315	23	13.7	15.2	4.1	3.7	94.2	4	7.6	2.0	22	2050.0
62	63	62	18	91	3	30.3	12.4	3.8	3.3	72.9	4	5.9	2.1	3	192.0
63	69	64	13	143	4	35.8	11.1	3.3	3.4	72.2	2	8.9	1.2	4	289.0
64	69	59	12	441	17	25.9	6.4	3.1	2.1	32.3	3	2.1	3.0	14	493.0
65	56	61	10	192	11	17.5	10.4	3.7	2.8	40	3	4.6	2.3	10	372.0
66	57	65	21	208	10	20.8	6.9	3.1	2.2	28.2	3	1.9	3.6	9	261.0
67	59	56	19	260	36	7.2	13.4	3.8	3.5	66.4	5	4.1	3.3	35	2295.0
68	60	62	17	224	10	22.4	10.7	4.1	2.6	75.8	5	6.2	1.7	10	443.0
69	62	67	13	280	39	7.2	11.1	3.2	3.5	63.4	3	8.1	1.4	34	1922.0
70	52	58	16	395	25	15.8	10.7	3.5	3.1	45	3	5	2.1	19	752.0
71	68	70	16	143	5	28.6	10.6	4.6	2.3	80	5	9.1	1.2	5	421.0

72	57	58	11	300	23	13.0	14.7	4.8	3.1	109.7	5	11.7	1.3	21	2139.0
73	60	56	11	404	46	8.8	14	3.6	3.9	61	4	7.2	1.9	41	2308.0
74	64	58	7	473	13	36.4	7	3	2.3	33.8	4	6.3	1.1	12	340.0
75	59	60	17	187	8	23.4	4	2.2	1.8	20.4	2	3.9	1.0	8	134.0
76	59	56	19	260	36	7.2	13.4	3.8	3.5	66.4	5	4.1	3.3	35	2295.0
77	52	51	13	538	34	15.8	11.7	4.5	2.6	59.7	4	7.5	1.6	31	1656.0
78	62	62	16	209	18	11.6	14.3	3.8	3.8	62	3	5.3	2.7	14	818.0
79	53	56	10	176	9	19.6	4.5	2.2	2.0	16.1	2	1.7	2.6	6	45.0
80	59	57	12	377	45	8.4	13.4	3.4	3.9	61.5	4	6.9	1.9	40	2289.0
81	67	68	16	62	3	20.7	4.6	2.5	1.8	10.1	2	4.9	0.9	2	18.0
82	56	54	19	478	49	9.8	9.6	3.6	2.7	48.8	3	7	1.4	41	1685.0
83	57	56	16	395	64	6.2	10	3.5	2.9	31.9	4	6.2	1.6	62	1703.0
84	61	63	17	182	17	10.7	13.7	3.6	3.8	62.5	3	5	2.7	13	799.0
85	66	55	3	398	16	24.9	11	3.9	2.8	72.7	3	5.5	2.0	15	1139.3
86	62	58	14	307	18	17.1	9.9	4.1	2.4	51.8	4	3.9	2.5	13	605.8
87	59	62	13	741	63	11.8	4.7	2.3	2.0	7.6	2	4.1	1.1	60	457.0
88	58	58	17	488	27	18.1	5.8	3.3	1.8	48.7	3	3.4	1.7	23	1119.5
89	60	60	12	443	41	10.8	13	3.8	3.4	76.9	3	5.6	2.3	37	2377.0
90	56	59	12	273	22	12.4	14.1	4.6	3.1	110.2	5	11.4	1.2	20	2120.0
91	58	53	16	335	58	5.8	8.9	3.3	2.7	36.7	3	5.7	1.6	50	1493.0
92	57	55	16	809	38	21.3	7.9	3.1	2.5	30.9	4	5.4	1.5	38	1182.0
93	65	59	17	132	20	6.6	4.9	3.2	1.5	17.4	3	1.4	3.5	19	297.0
94	60	54	13	64	3	21.3	8.9	3.3	2.7	26.5	3	5.8	1.5	2	46.0
95	69	69	23	190	11	17.3	14.6	4.6	3.2	115	5	5.9	2.5	8	1206.0
96	54	53	6	625	85	7.4	6.8	3.5	1.9	28.1	4	6.1	1.1	71	2142.0
97	55	58	12	425	21	20.2	8.7	3.7	2.4	78.2	3	7.2	1.2	19	1364.0
98	63	56	10	401	18	22.3	6.2	3.6	1.7	19.9	3	2.7	2.3	15	230.0

99	57	59	18	461	26	17.7	5.2	3.1	1.7	49.2	3	3.1	1.7	22	1100.5
100	56	56	11	1005	47	21.4	9.3	3.8	2.4	53.1	4	11.7	0.8	45	2111.0
101	59	65	19	315	23	13.7	15.2	4.1	3.7	94.2	4	7.6	2.0	22	2050.0
102	55	59	16	607	37	16.4	11.5	3.7	3.1	81.7	4	7.2	1.6	32	2012.0
103	62	57	11	374	17	22.0	5.6	3.4	1.6	20.4	3	2.4	2.3	14	211.0
104	60	60	14	418	19	22.0	7.8	3.6	2.2	31.6	3	5.5	1.4	12	440.3
105	55	57	12	978	46	21.3	8.7	3.6	2.4	53.6	4	11.4	0.8	44	2092.0
106	60	58	8	417	66	6.3	10	3.7	2.7	50.9	5	7.2	1.4	66	2446.0
107	57	58	11	300	23	13.0	14.7	4.8	3.1	109.7	5	11.7	1.3	21	2139.0
108	66	58	16	159	21	7.6	5.5	3.4	1.6	16.9	3	1.7	3.2	20	316.0
109	58	60	23	294	22	13.4	15.2	3.7	4.1	115	5	7.5	2.0	22	3578.0
110	59	59	22	321	23	14.0	15.8	3.9	4.1	114.5	5	7.8	2.0	23	3597.0
111	70	68	22	217	12	18.1	15.2	4.8	3.2	114.5	5	6.2	2.5	9	1225.0
112	65	75	17	419	14	29.9	7.2	3.3	2.2	24.7	3	1.4	5.1	6	137.0
113	55	55	20	451	48	9.4	9	3.4	2.6	49.3	3	6.7	1.3	40	1666.0
114	62	66	23	395	22	18.0	9.8	2.9	3.4	63.1	4	5.4	1.8	19	1113.0
115	57	59	15	521	38	13.7	13.7	4.8	2.9	114.5	5	7	2.0	38	3597.0
116	58	52	13	247	33	7.5	6.2	3	2.1	57.9	3	2	3.1	32	1658.0
117	60	56	10	274	59	4.6	12.2	3.6	3.4	72.1	3	7.2	1.7	54	3022.0
118	60	59	16	214	9	23.8	4.6	2.4	1.9	19.9	2	4.2	1.1	9	153.0
119	52	52	7	625	50	12.5	8.2	3.6	2.3	44.1	3	6.2	1.3	50	1490.0
120	59	61	18	291	24	12.1	10.3	4.2	2.5	65.7	3	3.2	3.2	19	1220.0
121	54	55	9	615	56	11.0	11.2	3.5	3.2	75.9	3	6.8	1.6	44	2577.0
122	55	60	19	416	23	18.1	9.8	3.4	2.9	48.2	4	8.2	1.2	20	664.0
123	64	67	12	473	13	36.4	7.4	2.8	2.6	62.1	3	4.9	1.5	8	397.0
124	56	59	18	443	24	18.5	10.4	3.6	2.9	47.7	4	8.5	1.2	21	683.0
125	61	67	24	368	21	17.5	9.2	2.7	3.4	63.6	4	5.1	1.8	18	1094.0

126	52	56	14	368	17	21.6	6.2	3.6	1.7	56.6	3	3.4	1.8	15	786.3
127	60	60	25	307	13	23.6	8.9	3.3	2.7	56.1	3	3.4	2.6	11	640.4
128	62	70	17	343	16	21.4	7.9	3.4	2.3	43.4	3	3.7	2.1	12	495.0
129	64	61	17	118	4	29.5	13	4	3.3	72.4	4	6.2	2.1	4	211.0
130	58	64	20	235	11	21.4	7.5	3.3	2.3	27.7	3	2.2	3.4	10	280.0
131	65	64	13	40	3	13.3	10.5	4	2.6	33.3	4	5.4	1.9	2	53.0
132	55	52	11	347	14	24.8	7.9	3.1	2.5	42.3	3	1.4	5.6	14	529.0
133	61	58	15	441	25	17.6	10.2	3.9	2.6	47.9	5	5.5	1.9	25	1019.0
134	71	64	14	668	30	22.3	9.9	3.5	2.8	53.9	3	7.5	1.3	23	1082.0
135	61	59	15	280	17	16.5	9.3	3.9	2.4	52.3	4	3.6	2.6	12	586.8
136	64	73	13	472	20	23.6	6.2	3.1	2.0	52.7	3	4.2	1.5	17	942.0
137	60	64	18	342	24	14.3	15.8	4.3	3.7	93.7	4	7.9	2.0	23	2069.0
138	70	63	12	170	5	34.0	11.7	3.5	3.3	71.7	2	9.2	1.3	5	308.0
139	60	61	12	768	64	12.0	5.3	2.5	2.1	7.1	2	4.4	1.2	61	476.0
140	70	58	11	468	18	26.0	7	3.3	2.1	31.8	3	2.4	2.9	15	512.0
141	57	60	9	219	12	18.3	11	3.9	2.8	39.5	3	4.9	2.2	11	391.0
142	60	55	18	287	37	7.8	14	4	3.5	65.9	5	4.4	3.2	36	2314.0
143	61	61	16	251	11	22.8	11.3	4.3	2.6	75.3	5	6.5	1.7	11	462.0
144	63	66	12	307	40	7.7	11.7	3.4	3.4	62.9	3	8.4	1.4	35	1941.0
145	69	69	15	170	6	28.3	11.2	4.8	2.3	79.5	5	9.4	1.2	6	440.0
146	60	59	16	214	9	23.8	4.6	2.4	1.9	19.9	2	4.2	1.1	9	153.0
147	60	55	18	287	37	7.8	14	4	3.5	65.9	5	4.4	3.2	36	2314.0
148	54	55	9	203	10	20.3	5.1	2.4	2.1	15.6	2	2	2.6	7	64.0
149	70	65	15	641	29	22.1	9.3	3.3	2.8	54.4	3	7.2	1.3	22	1063.0
150	60	56	11	404	46	8.8	14	3.6	3.9	61	4	7.2	1.9	41	2308.0
151	68	67	15	89	4	22.3	5.2	2.7	1.9	9.6	2	5.2	1.0	3	37.0
152	57	58	11	300	23	13.0	14.7	4.8	3.1	109.7	5	11.7	1.3	21	2139.0

153	59	59	9	390	65	6.0	9.4	3.5	2.7	51.4	5	6.9	1.4	65	2427.0
154	62	62	16	209	18	11.6	14.3	3.8	3.8	62	3	5.3	2.7	14	818.0
155	60	61	12	768	64	12.0	5.3	2.5	2.1	7.1	2	4.4	1.2	61	476.0
156	58	58	17	488	27	18.1	5.8	3.3	1.8	48.7	3	3.4	1.7	23	1119.5
157	60	64	18	342	24	14.3	15.8	4.3	3.7	93.7	4	7.9	2.0	23	2069.0
158	63	56	10	401	18	22.3	6.2	3.6	1.7	19.9	3	2.7	2.3	15	230.0
159	56	56	11	1005	47	21.4	9.3	3.8	2.4	53.1	4	11.7	0.8	45	2111.0
160	59	59	22	321	23	14.0	15.8	3.9	4.1	114.5	5	7.8	2.0	23	3597.0
161	56	54	19	478	49	9.8	9.6	3.6	2.7	48.8	3	7	1.4	41	1685.0
162	56	59	18	443	24	18.5	10.4	3.6	2.9	47.7	4	8.5	1.2	21	683.0
163	60	59	16	414	24	17.3	9.6	3.7	2.6	48.4	5	5.2	1.8	24	1000.0
164	66	58	16	159	21	7.6	5.5	3.4	1.6	16.9	3	1.7	3.2	20	316.0
165	70	68	22	217	12	18.1	15.2	4.8	3.2	114.5	5	6.2	2.5	9	1225.0
166	56	57	11	452	22	20.5	9.3	3.9	2.4	77.7	3	7.5	1.2	20	1383.0
167	62	66	23	395	22	18.0	9.8	2.9	3.4	63.1	4	5.4	1.8	19	1113.0
168	54	57	12	560	24	23.3	9.3	4.4	2.1	58	4	5.1	1.8	23	997.0
169	62	58	14	307	18	17.1	9.9	4.1	2.4	51.8	4	3.9	2.5	13	605.8
170	71	64	14	668	30	22.3	9.9	3.5	2.8	53.9	3	7.5	1.3	23	1082.0
171	53	57	12	495	31	16.0	9.8	2.8	3.5	35.8	3	6.4	1.5	23	677.0
172	54	56	11	522	32	16.3	10.4	3	3.5	35.3	3	6.7	1.6	24	696.0
173	57	56	16	395	64	6.2	10	3.5	2.9	31.9	4	6.2	1.6	62	1703.0
174	59	61	18	291	24	12.1	10.3	4.2	2.5	65.7	3	3.2	3.2	19	1220.0
175	65	64	13	40	3	13.3	10.5	4	2.6	33.3	4	5.4	1.9	2	53.0
176	60	56	11	404	46	8.8	14	3.6	3.9	61	4	7.2	1.9	41	2308.0
177	64	58	7	473	13	36.4	7	3	2.3	33.8	4	6.3	1.1	12	340.0
178	52	51	13	538	34	15.8	11.7	4.5	2.6	59.7	4	7.5	1.6	31	1656.0
179	62	62	16	209	18	11.6	14.3	3.8	3.8	62	3	5.3	2.7	14	818.0

180	56	54	19	478	49	9.8	9.6	3.6	2.7	48.8	3	7	1.4	41	1685.0
181	56	57	17	368	63	5.8	9.4	3.3	2.8	32.4	4	5.9	1.6	61	1684.0
182	60	58	8	417	66	6.3	10	3.7	2.7	50.9	5	7.2	1.4	66	2446.0
183	61	58	15	441	25	17.6	10.2	3.9	2.6	47.9	5	5.5	1.9	25	1019.0
184	55	56	11	587	25	23.5	9.9	4.6	2.2	57.5	4	5.4	1.8	24	1016.0
185	57	56	16	395	64	6.2	10	3.5	2.9	31.9	4	6.2	1.6	62	1703.0
186	66	55	3	398	16	24.9	11	3.9	2.8	72.7	3	5.5	2.0	15	1139.3
187	62	58	14	307	18	17.1	9.9	4.1	2.4	51.8	4	3.9	2.5	13	605.8
188	58	58	17	488	27	18.1	5.8	3.3	1.8	48.7	3	3.4	1.7	23	1119.5
189	58	62	19	264	23	11.5	9.7	4	2.4	66.2	3	2.9	3.3	18	1201.0
190	60	60	12	443	41	10.8	13	3.8	3.4	76.9	3	5.6	2.3	37	2377.0
191	64	65	14	13	2	6.5	9.9	3.8	2.6	33.8	4	5.1	1.9	1	34.0
192	58	53	16	335	58	5.8	8.9	3.3	2.7	36.7	3	5.7	1.6	50	1493.0
193	57	55	16	809	38	21.3	7.9	3.1	2.5	30.9	4	5.4	1.5	38	1182.0
194	60	54	13	64	3	21.3	8.9	3.3	2.7	26.5	3	5.8	1.5	2	46.0
195	54	53	6	625	85	7.4	6.8	3.5	1.9	28.1	4	6.1	1.1	71	2142.0
196	63	56	10	401	18	22.3	6.2	3.6	1.7	19.9	3	2.7	2.3	15	230.0
197	56	56	11	1005	47	21.4	9.3	3.8	2.4	53.1	4	11.7	0.8	45	2111.0
198	55	59	16	607	37	16.4	11.5	3.7	3.1	81.7	4	7.2	1.6	32	2012.0
199	60	60	14	418	19	22.0	7.8	3.6	2.2	31.6	3	5.5	1.4	12	440.3
200	60	58	8	417	66	6.3	10	3.7	2.7	50.9	5	7.2	1.4	66	2446.0

Annexure III

Morphological observations of mapping population for seed, leaf, fruit and vine related traits

Plant name	Number of seeds	Seed length (mm)	Seed breadth (mm)	Leaf size (cm^2)	Internodal length (cm)	Vine length (m)	Stem girth (cm)	Number of side branches	Fruit color	Fruit ends	Fruit shape	Leaf color
Priyanka	21.2	15.4	8.2	137.23	8.3	3.1	1.9	17	Light green	Both ends pointed	Spindle	Light green
IC634896	18.3	9.5	5.1	35.32	6.2	2.7	0.6	21	Green	Blossom end pointed	Rhomboid	Dark green
1	15.1	11.7	6.9	102.6	7.3	3.5	1.4	17	White	Both ends pointed	Spindle	Light green
2	18.2	12.2	7.0	50.6	7.6	3.1	1.2	11	White	Both ends pointed	Spindle	Light green
3	16.1	11.5	6.1	105.6	7.4	3.9	1.5	15	White	Both ends pointed	Spindle	Light green
4	7.0	11.6	7.0	30.8	6.9	4.0	1.1	18	Light green	Blossom end pointed	-	Dark green
5	8.0	11.7	6.7	66.6	5.9	3.1	0.7	24	Light green	Blossom end pointed	Oblong	Light green
6	19.2	12.0	6.2	53.6	7.7	3.5	1.3	9	White	Both ends pointed	Spindle	Light green
7	10.0	11.9	7.1	64.2	6.4	1.9	0.9	14	White	Both ends pointed	-	Dark green
8	11.0	11.0	6.0	116.5	7.5	4.7	1.9	15	White	Both ends pointed	Cylindrical	Light green
9	15.0	11.0	6.6	72.2	6.9	3.6	1.5	25	Light green	Both ends pointed	Spindle	Light green
10	8.0	10.9	6.9	33.6	6.1	2.4	0.7	19	Light green	Both ends pointed	-	Light green
11	8.0	11.4	6.2	33.8	7.0	4.4	1.2	16	Light green	Blossom end pointed	-	Dark green
12	13.0	12.5	7.5	49.7	6.3	4.8	0.8	16	White	Both ends pointed	Spindle	Light green
13	20.0	12.2	7.1	89.2	6.9	3.6	1.4	13	White	Blossom end pointed	Oblong	Light green
14	10.0	11.9	7.1	70.6	7.8	4.1	1.1	11	White	Blossom end pointed	Elliptical	Light green
15	9.0	11.5	5.9	69.6	6.0	3.5	0.8	22	Light green	Blossom end pointed	Oblong	Light green

16	11.0	11.7	6.3	67.2	6.5	2.3	1.0	12	White	Both ends pointed	-	Dark green
17	13.0	11.3	7.7	33.9	6.1	4.7	1.1	18	White	Both ends pointed	Spindle	Dark green
18	16.0	11.7	7.4	64.2	6.4	4.9	0.8	13	Light green	Both ends pointed	Spindle	Dark green
19	12.0	10.7	6.5	64.7	6.1	5.1	1.6	23	White	Both ends pointed	Spindle	Light green
20	12.0	10.8	5.2	119.5	7.6	5.1	2.0	13	White	Both ends pointed	Cylindrical	Light green
21	22.0	10.1	5.8	71.8	5.7	5.8	1.6	16	Green	Blossom end pointed	Elliptical	Light green
22	18.0	12.1	7.2	81.1	6.3	2.9	1.2	-	Light green	Both ends pointed	-	Light green
23	14.0	10.9	6.5	87.4	6.8	3.0	1.8	18	Light green	Both ends pointed	-	Light green
24	10.0	9.9	5.7	43.1	4.8	3.8	0.9	-	Green	Blossom end pointed	-	Dark green
25	14.0	11.0	5.8	91.8	7.2	5.6	1.2	13	Light green	Both ends pointed	Spindle	Light green
26	14.0	10.0	6.3	91.1	7.6	4.0	1.8	14	Green+white	Blossom end pointed	Cylindrical	Dark green
27	9.0	10.4	6.3	59.8	6.6	5.6	2.3	23	Green+white	Blossom end pointed	Elliptical	Dark green
28	21.0	9.5	5.3	89.1	6.1	1.6	0.8	-	White	Both ends pointed	-	Dark green
29	19.0	9.5	5.6	71.5	5.9	4.2	1.6	23	Green	Blossom end pointed	Elliptical	Dark green
30	8.0	9.9	5.7	21.1	4.8	1.9	1.2	17	Green	Blossom end pointed	Globular	Dark green
31	17.0	11.4	7.0	77.3	6.1	5.2	1.9	30	Green	Blossom end pointed	Elliptical	Dark green
32	16.0	12.5	8.0	87.4	6.8	4.8	2.1	20	Light green	Blossom end pointed	Elliptical	Dark green
33	10.0	10.1	6.1	19.5	7.1	1.6	0.5	-	Green	Blossom end pointed	-	Light green
34	18.0	10.9	6.5	87.3	6.3	3.0	0.9	15	Light green	Blossom end pointed	Spindle	Dark green
35	24.0	14.2	8.6	-	8.7	4.1	1.8	13	Light green	Both ends round	Cylindrical	Light green
36	14.0	9.9	6.4	16.5	5.6	1.4	0.7	12	Light green	Blossom end pointed	Elliptical	Dark green
37	24.0	13.2	7.7	164.5	9.4	3.5	1.2	14	Light green	Both ends pointed	Oblong	Dark green

38	26.0	14.2	8.6	35.7	6.9	3.1	0.9	10	Light green	Both ends round	Cylindrical	Light green
39	12.0	10.3	6.8	36.0	7.4	4.0	1.1	28	Light green	Blossom end pointed	Elliptical	Dark green
40	13.0	-	-	16.2	5.8	1.5	0.5	25	Light green	Blossom end pointed	Rhomboid	Dark green
41	20.0	12.4	7.9	148.4	8.8	4.2	1.2	26	Light green	Blossom end pointed	Spindle	Dark green
42	11.0	11.1	7.1	115.6	8.6	4.3	2.4	16	White	Both ends pointed	Spindle	Dark green
43	13.0	13.8	8.3	92.1	6.1	4.8	1.5	13	Green	Both ends round	Elliptical	Dark green
44	9.0	11.2	6.4	43.1	4.8	1.7	0.8	22	Light green	Both ends pointed	Spindle	Dark green
45	12.0	10.8	8.0	45.7	6.7	6.3	2.1	13	Green	Blossom end pointed	Cylindrical	Dark green
46	12.0	12.6	7.0	13.7	4.6	1.3	0.5	17	Light green	Blossom end pointed	Spindle	Dark green
47	17.0	11.9	7.1	131.5	8.2	5.0	2.3	22	White	Both ends pointed	Spindle	Light green
48	13.0	12.0	6.7	80.9	7.6	4.9	1.6	12	White	Blossom end pointed	Elliptical	Dark green
49	10.0	12.1	7.2	-	7.1	2.3	1.6	21	Light green	Blossom end pointed	Spindle	Dark green
50	11.0	11.0	6.0	-	5.2	-	-	-	Green	Both ends pointed	-	Dark green
51	14.0	12.2	5.8	85.7	8.8	5.1	1.6	18	Light green	Blossom end pointed	Spindle	Light green
52	13.0	10.0	6.3	-	-	-	-	-	Light green	Both ends pointed	-	Light green
53	16.0	10.8	5.8	75.2	7.0	4.0	1.6	23	Light green	Both ends pointed	Spindle	Light green
54	16.0	9.5	5.3	22.0	5.3	5.5	1.4	19	Green	Both ends pointed	Rhomboid	Dark green
55	9.0	10.7	6.1	36.6	6.2	2.8	0.8	17	Light green	Both ends pointed	-	Light green
56	13.0	-	-	19.4	5.9	1.2	0.9	20	Light green	Blossom end pointed	Spindle	Dark green
57	19.0	-	-	91.1	6.7	4.6	1.2	28	Green	Both ends pointed	Cylindrical	Dark green
58	15.0	-	-	23.2	4.2	1.7	0.7	24	Light green	Both ends pointed	-	Dark green
59	18.0	9.1	4.9	-	5.3	1.7	0.7	24	Light green	Blossom end pointed	-	Dark green

60	19.0	-	-	114.6	5.6	1.9	0.6	16	Light green	Both ends pointed	-	Dark green
61	24.0	11.8	7.1	109.8	6.8	5.2	1.6	15	Light green	Both ends pointed	Cylindrical	Light green
62	23.0	11.2	7.2	121.2	6.1	1.2	0.7	16	Light green	Both ends pointed	-	Dark green
63	15.0	-	-	113.9	8.3	4.2	0.9	11	White	Both ends pointed	Spindle	Dark green
64	13.0	-	-	-	5.1	1.3	-	-	Green	Both ends pointed	-	Dark green
65	7.0	-	-	36.5	6.1	4.1	0.9	12	Light green	Both ends pointed	Spindle	Light green
66	11.0	-	-	21.8	5.9	2.2	0.7	16	White	Both ends pointed	Spindle	Dark green
67	23.0	11.9	7.5	63.1	6.9	4.7	1.3	13	Light green	Both ends pointed	Spindle	Light green
68	21.0	10.9	5.6	51.8	5.6	3.4	1.2	14	Light green	Both ends pointed	Elliptical	Light green
69	19.0	-	-	117.6	7.8	5.7	1.9	10	White	Both ends pointed	Spindle	Light green
70	14.0	12.3	6.7	52.7	6.4	5.2	0.9	14	White	Both ends pointed	Spindle	Light green
71	25.0	-	-	81.2	8.4	3.2	1.2	13	Light green	Both ends pointed	Oblong	Dark green
72	25.0	14.0	7.8	-	8.8	4.5	1.9	11	Light green	Both ends round	Cylindrical	Light green
73	21.0	12.0	6.3	92.2	7.0	4.0	1.5	11	White	Blossom end pointed	Oblong	Light green
74	11.0	11.7	6.3	73.6	7.9	4.5	1.2	9	White	Blossom end pointed	Elliptical	Light green
75	12.0	12.6	7.0	13.7	4.6	1.3	0.5	17	Light green	Blossom end pointed	Spindle	Dark green
76	23.0	11.9	7.5	63.1	6.9	4.7	1.3	13	Light green	Both ends pointed	Spindle	Light green
77	14.0	11.1	6.9	36.9	6.2	5.1	1.2	16	White	Both ends pointed	Spindle	Dark green
78	17.0	11.5	6.6	67.2	6.5	5.3	0.9	11	Light green	Both ends pointed	Spindle	Dark green
79	7.0	11.6	7.0	30.8	6.9	4.0	1.1	18	Light green	Blossom end pointed	-	Dark green
80	20.0	12.2	7.1	89.2	6.9	3.6	1.4	13	White	Blossom end pointed	Oblong	Light green
81	8.0	10.9	6.9	33.6	6.1	2.4	0.7	19	Light green	Both ends pointed	-	Light green

82	13.0	10.5	5.7	67.7	6.2	5.5	1.7	21	White	Both ends pointed	Spindle	Light green
83	23.0	9.9	5.0	74.8	5.8	6.2	1.7	14	Green	Blossom end pointed	Elliptical	Light green
84	16.0	11.7	7.4	64.2	6.4	4.9	0.8	13	Light green	Both ends pointed	Spindle	Dark green
85	19.0	11.9	6.4	84.1	6.4	3.3	1.3		Light green	Both ends pointed	-	Light green
86	15.0	10.7	5.7	90.4	6.9	3.4	1.9	16	Light green	Both ends pointed	-	Light green
87	16.0	9.5	5.3	22.0	5.3	5.5	1.4	19	Green	Both ends pointed	Rhomboid	Dark green
88	11.0	9.7	4.9	46.1	4.9	4.2	1.0		Green	Blossom end pointed	-	Dark green
89	15.0	10.8	5.0	94.8	7.3	6.0	1.3	11	Light green	Both ends pointed	Spindle	Light green
90	24.0	14.2	8.6	-	8.7	4.1	1.8	13	Light green	Both ends round	Cylindrical	Light green
91	15.0	9.8	5.5	94.1	7.7	4.4	1.9	12	Green+white	Blossom end pointed	Cylindrical	Dark green
92	10.0	10.2	5.5	62.8	6.7	6.0	2.4	21	Green+white	Blossom end pointed	Elliptical	Dark green
93	14.0	9.9	6.4	16.5	5.6	1.4	0.7	12	Light green	Blossom end pointed	Elliptical	Dark green
94	22.0	9.3	4.5	92.1	6.2	2.0	0.9		White	Both ends pointed	-	Dark green
95	26.0	14.2	8.6	35.7	6.9	3.1	0.9	10	Light green	Both ends round	Cylindrical	Light green
96	20.0	9.3	4.8	74.5	6.0	4.6	1.7	21	Green	Blossom end pointed	Elliptical	Dark green
97	15.1	11.7	6.9	102.6	7.3	3.5	1.4	17	White	Both ends pointed	Spindle	Light green
98	9.0	9.7	4.9	24.1	4.9	2.3	1.3	15	Green	Blossom end pointed	Globular	Dark green
99	10.0	9.9	5.7	43.1	4.8	3.8	0.9	-	Green	Blossom end pointed	-	Dark green
100	18.0	11.2	6.2	80.3	6.2	5.6	2.0	28	Green	Blossom end pointed	Elliptical	Dark green
101	24.0	11.8	7.1	109.8	6.8	5.2	1.6	15	Light green	Both ends pointed	Cylindrical	Light green
102	17.0	12.3	7.2	90.4	6.9	5.2	2.2	18	Light green	Blossom end pointed	Elliptical	Dark green
103	8.0	9.9	5.7	21.1	4.8	1.9	1.2	17	Green	Blossom end pointed	Globular	Dark green

104	11.0	9.9	5.3	22.5	7.2	2.0	0.6		Green	Blossom end pointed	-	Light green
105	17.0	11.4	7.0	77.3	6.1	5.2	1.9	30	Green	Blossom end pointed	Elliptical	Dark green
106	19.0	10.7	5.7	90.3	6.4	3.4	1.0	13	Light green	Blossom end pointed	Spindle	Dark green
107	25.0	14.0	7.8		8.8	4.5	1.9	11	Light green	Both ends round	Cylindrical	Light green
108	15.0	9.7	5.6	19.53	5.7	1.8	0.8	10	Light green	Blossom end pointed	Elliptical	Dark green
109	24.0	13.2	7.7	164.495	9.4	3.5	1.2	14	Light green	Both ends pointed	Oblong	Dark green
110	25.0	13.0	6.9	167.495	9.5	3.9	1.3	12	Light green	Both ends pointed	Oblong	Dark green
111	27.0	14.0	7.8	38.664	7.0	3.5	1.0	8	Light green	Both ends round	Cylindrical	Light green
112	13.0	10.1	6.0	38.952	7.5	4.4	1.2	26	Light green	Blossom end pointed	Elliptical	Dark green
113	12.0	10.7	6.5	64.672	6.1	5.1	1.6	23	White	Both ends pointed	Spindle	Light green
114	12.0	10.9	6.3	118.632	8.7	4.7	2.5	14	White	Both ends pointed	Spindle	Dark green
115	14.0	13.6	7.5	95.106	6.2	5.2	1.6	11	Green	Both ends round	Elliptical	Dark green
116	10.0	11.0	5.6	46.123	4.9	2.1	0.9	20	Light green	Both ends pointed	Spindle	Dark green
117	13.0	10.6	7.2	48.74	6.8	6.7	2.2	11	Green	Blossom end pointed	Cylindrical	Dark green
118	13.0	12.4	6.2	16.703	4.7	1.7	0.6	15	Light green	Blossom end pointed	Spindle	Dark green
119	18.0	11.7	6.3	134.535	8.3	5.4	2.4	20	White	Both ends pointed	Spindle	Light green
120	14.0	11.8	5.9	83.949	7.7	5.3	1.7	10	White	Blossom end pointed	Elliptical	Dark green
121	11.0	11.9	6.4		7.2	2.7	1.7	19	Light green	Blossom end pointed	Spindle	Dark green
122	20.0	12.4	7.9	148.371	8.8	4.2	1.2	26	Light green	Blossom end pointed	Spindle	Dark green
123	14.0	-	-	19.156	5.9	1.9	0.6	23	Light green	Blossom end pointed	Rhomboid	Dark green
124	21.0	12.2	7.1	151.371	8.9	4.6	1.3	24	Light green	Blossom end pointed	Spindle	Dark green
125	11.0	11.1	7.1	115.632	8.6	4.3	2.4	16	White	Both ends pointed	Spindle	Dark green

126	12.0	10.8	5.2		5.3				Green	Both ends pointed	-	Dark green
127	14.0	9.8	5.5						Light green	Both ends pointed	-	Light green
128	16.0	-	-	26.218	4.3	2.1	0.8	22	Light green	Both ends pointed	-	Dark green
129	24.0	11.0	6.4	124.18	6.2	1.6	0.8	14	Light green	Both ends pointed	-	Dark green
130	12.0	-	-	24.796	6	2.55	0.8	14	White	Both ends pointed	Spindle	Dark green
131	20.0	-	-	117.611	5.7	2.3	0.7	14	Light green	Both ends pointed	-	Dark green
132	14.0	-	-	22.409	6	1.6	1	18	Light green	Blossom end pointed	Spindle	Dark green
133	15.0	12.0	5.0	88.729	8.9	5.45	1.7	16	Light green	Blossom end pointed	Spindle	Light green
134	20.0	-	-	94.138	6.8	5	1.3	26	Green	Both ends pointed	Cylindrical	Dark green
135	14.0	10.9	6.5	87.36	6.8	3	1.8	18	Light green	Both ends pointed	-	Light green
136	19.0	8.9	4.1	-	5.4	2.05	0.8	22	Light green	Blossom end pointed	-	Dark green
137	25.0	11.6	6.3	112.845	6.9	5.55	1.7	13	Light green	Both ends pointed	Cylindrical	Light green
138	16.0	-	-	116.936	8.4	4.6	1	9	White	Both ends pointed	Spindle	Dark green
139	17.0	9.3	4.5	25.001	5.4	5.9	1.5	17	Green	Both ends pointed	Rhomboid	Dark green
140	14.0	-	-	-	5.2	1.7	-	-	Green	Both ends pointed	-	Dark green
141	8.0	-	-	39.5	6.2	4.5	1	10	Light green	Both ends pointed	Spindle	Light green
142	24.0	11.7	6.7	66.122	7	5.1	1.4	11	Light green	Both ends pointed	Spindle	Light green
143	22.0	10.7	4.8	54.835	5.7	3.8	1.3	12	Light green	Both ends pointed	Elliptical	Light green
144	20.0	-	-	120.581	7.9	6.1	2	8	White	Both ends pointed	Spindle	Light green
145	26.0	-	-	84.168	8.5	3.6	1.3	11	Light green	Both ends pointed	Oblong	Dark green
146	13.0	12.4	6.2	16.703	4.7	1.65	0.6	15	Light green	Blossom end pointed	Spindle	Dark green
147	24.0	11.7	6.7	66.122	7	5.1	1.4	11	Light green	Both ends pointed	Spindle	Light green

148	8.0	11.4	6.2	33.811	7	4.35	1.2	16	Light green	Blossom end pointed	-	Dark green
149	19.0	-	-	91.138	6.7	4.6	1.2	28	Green	Both ends pointed	Cylindrical	Dark green
150	21.0	12.0	6.3	92.247	7	4	1.5	11	White	Blossom end pointed	Oblong	Light green
151	9.0	10.7	6.1	36.621	6.2	2.8	0.8	17	Light green	Both ends pointed	-	Light green
152	25.0	14.0	7.8	-	8.8	4.53	1.9	11	Light green	Both ends round	Cylindrical	Light green
153	18.0	10.9	6.5	87.332	6.3	2.96	0.9	15	Light green	Blossom end pointed	Spindle	Dark green
154	17.0	11.5	6.6	67.217	6.5	5.3	0.9	11	Light green	Both ends pointed	Spindle	Dark green
155	17.0	9.3	4.5	25.001	5.4	5.9	1.5	17	Green	Both ends pointed	Rhomboid	Dark green
156	11.0	9.7	4.9	46.123	4.9	4.2	1	-	Green	Blossom end pointed	-	Dark green
157	25.0	11.6	6.3	112.845	6.9	5.55	1.7	13	Light green	Both ends pointed	Cylindrical	Light green
158	9.0	9.7	4.9	24.054	4.9	2.34	1.3	15	Green	Blossom end pointed	Globular	Dark green
159	18.0	11.2	6.2	80.324	6.2	5.56	2	28	Green	Blossom end pointed	Elliptical	Dark green
160	25.0	13.0	6.9	167.495	9.5	3.9	1.3	12	Light green	Both ends pointed	Oblong	Dark green
161	13.0	10.5	5.7	67.672	6.2	5.5	1.7	21	White	Both ends pointed	Spindle	Light green
162	21.0	12.2	7.1	151.371	8.9	4.62	1.3	24	Light green	Blossom end pointed	Spindle	Dark green
163	14.0	12.2	5.8	85.729	8.8	5.05	1.6	18	Light green	Blossom end pointed	Spindle	Light green
164	15.0	9.7	5.6	19.53	5.7	1.8	0.8	10	Light green	Blossom end pointed	Elliptical	Dark green
165	27.0	14.0	7.8	38.664	7	3.5	1	8	Light green	Both ends round	Cylindrical	Light green
166	16.1	11.5	6.1	105.598	7.4	3.93	1.5	15	White	Both ends pointed	Spindle	Light green
167	12.0	10.9	6.3	118.632	8.7	4.74	2.5	14	White	Both ends pointed	Spindle	Dark green
168	8.0	11.7	6.7	66.643	5.9	3.1	0.7	24	Light green	Blossom end pointed	Oblong	Light green
169	15.0	10.7	5.7	90.36	6.9	3.4	1.9	16	Light green	Both ends pointed	-	Light green

170	20.0	-	-	94.138	6.8	5	1.3	26	Green	Both ends pointed	Cylindrical	Dark green
171	11.0	11.0	6.0	116.458	7.5	4.65	1.9	15	White	Both ends pointed	Cylindrical	Light green
172	12.0	10.8	5.2	119.458	7.6	5.05	2	13	White	Both ends pointed	Cylindrical	Light green
173	23.0	9.9	5.0	74.76	5.8	6.2	1.7	14	Green	Blossom end pointed	Elliptical	Light green
174	14.0	11.8	5.9	83.949	7.7	5.3	1.7	10	White	Blossom end pointed	Elliptical	Dark green
175	20.0	-	-	117.611	5.7	2.3	0.7	14	Light green	Both ends pointed	-	Dark green
176	21.0	12.0	6.3	92.247	7	4	1.5	11	White	Blossom end pointed	Oblong	Light green
177	11.0	11.7	6.3	73.562	7.9	4.5	1.2	9	White	Blossom end pointed	Elliptical	Light green
178	14.0	11.1	6.9	36.889	6.2	5.05	1.2	16	White	Both ends pointed	Spindle	Dark green
179	17.0	11.5	6.6	67.217	6.5	5.3	0.9	11	Light green	Both ends pointed	Spindle	Dark green
180	13.0	10.5	5.7	67.672	6.2	5.5	1.7	21	White	Both ends pointed	Spindle	Light green
181	22.0	10.1	5.8	71.76	5.7	5.8	1.6	16	Green	Blossom end pointed	Elliptical	Light green
182	19.0	10.7	5.7	90.332	6.4	3.36	1	13	Light green	Blossom end pointed	Spindle	Dark green
183	15.0	12.0	5.0	88.729	8.9	5.45	1.7	16	Light green	Blossom end pointed	Spindle	Light green
184	9.0	11.5	5.9	69.643	6	3.5	0.8	22	Light green	Blossom end pointed	Oblong	Light green
185	23.0	9.9	5.0	74.76	5.8	6.2	1.7	14	Green	Blossom end pointed	Elliptical	Light green
186	19.0	11.9	6.4	84.14	6.4	3.3	1.3	-	Light green	Both ends pointed	-	Light green
187	15.0	10.7	5.7	90.36	6.9	3.4	1.9	16	Light green	Both ends pointed	-	Light green
188	11.0	9.7	4.9	46.123	4.9	4.2	1	-	Green	Blossom end pointed	-	Dark green
189	13.0	12.0	6.7	80.949	7.6	4.9	1.6	12	White	Blossom end pointed	Elliptical	Dark green
190	15.0	10.8	5.0	94.769	7.3	5.95	1.3	11	Light green	Both ends pointed	Spindle	Light green
191	19.0	-	-	114.611	5.6	1.9	0.6	16	Light green	Both ends pointed	-	Dark green

192	15.0	9.8	5.5	94.148	7.7	4.35	1.9	12	Green+white	Blossom end pointed	Cylindrical	Dark green
193	10.0	10.2	5.5	62.773	6.7	6	2.4	21	Green+white	Blossom end pointed	Elliptical	Dark green
194	22.0	9.3	4.5	92.106	6.2	2	0.9	-	White	Both ends pointed	-	Dark green
195	20.0	9.3	4.8	74.483	6	4.62	1.7	21	Green	Blossom end pointed	Elliptical	Dark green
196	9.0	9.7	4.9	24.054	4.9	2.34	1.3	15	Green	Blossom end pointed	Globular	Dark green
197	18.0	11.2	6.2	80.324	6.2	5.56	2	28	Green	Blossom end pointed	Elliptical	Dark green
198	17.0	12.3	7.2	90.36	6.9	5.15	2.2	18	Light green	Blossom end pointed	Elliptical	Dark green
199	11.0	9.9	5.3	22.476	7.2	2.02	0.6	-	Green	Blossom end pointed	-	Light green
200	19.0	10.7	5.7	90.332	6.4	3.36	1	13	Light green	Blossom end pointed	Spindle	Dark green

‘-’ Missing data

MAPPING THE QTL FOR YIELD TRAITS IN BITTER GOURD

(*Momordica charantia* L.)

By

LAVALE SHIVAJI AJINATH

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ABSTRACT

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CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

COLLEGE OF AGRICULTURE, VELLANIKKARA

THRISSUR – 680656

KERALA, INDIA

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Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)

ABSTRACT

Bitter gourd (*Momordica charantia*), being a rich source of phytonutrients such as carbohydrates, minerals, vitamins, and other medicinal compounds, has a great importance in healthy dietary habits. Breeders always seek to breed bitter gourd varieties for the traits such as early maturity and high yield. However, limited investigations have been made to identify the genetic loci governing yield related traits. Marker assisted selection (MAS) assures the presence of favourable alleles and fast recovery of recurrent parent genome in the cultivar under improvement. The success of MAS mainly depends on the availability of a marker-dense genetic linkage map locating quantitative trait loci (QTL) for the target traits. The present study "Mapping the QTL for yield traits in bitter gourd (*Momordica charantia* L.)" was carried out during October, 2018 to December, 2021 with the objective to map the quantitative trait loci and to develop chromosome-wise maps for the yield traits in bitter gourd.

To develop the mapping population, high yielding bitter gourd cultivar Priyanka (*Momordica charantia* var. *charantia*) and a wild bitter gourd accession IC634896 (*M. charantia* var. *muricata*), were used as parents. A set of 450 microsatellites were screened for polymorphism using genomic DNA of parents and 47 were found polymorphic. Bitter gourd genome (GenBank acc. no. GCA_013281855.1) was scanned and new hyper-variable microsatellites were identified using Genome wide Microsatellite Analysing Tool (GMATo) and named as KAUBG_n where n is a serial number. From the 75 microsatellites identified, 69 were validated through successful PCR amplification and 38 among them were polymorphic between the parents. This led to the development of a set of 85 markers polymorphic between the parents.

Crosses were made between the parental lines and hybrids from the cross Priyanka × IC634896 yielded more number of fruits and total fruit produce compared to the reciprocal hybrid. An $F_{2:3}$ population was developed through single seed descent method from the cross Priyanka × IC634896. A panel of 200 $F_{2:3}$ plants were evaluated for twenty seven traits, including fruit-, flower-, seed-, vine-, and leaf-related traits, contributing directly or indirectly to the total yield. Wide variation was observed among the $F_{2:3}$ plants for the traits studied. A group of ninety plants was selected from 200 $F_{2:3}$ plants such that they represent the variation of the base population. Genomic DNA of these plants were genotyped using 85 polymorphic markers.

Genotypic data from the screening of 85 markers in the mapping population were used to generate a linkage map spanning 1287.99 cM distance across eleven linkage groups (LGs) corresponding to eleven chromosomes, using IciMapping software. LG 7 (28 markers) consisted of maximum number of markers followed by LG 2 and LG 9, each having 11 markers. LG 1 had 10 markers whereas LG 3, 4 and 8 had seven markers each. LG 5, 6, 10 and 11 had only one marker each. LG 7 covered maximum map distance of 384.19 cM where LG 8 covered least map distance of 68.58 cM.

The genetic map and phenotypic data were used to generate the QTL maps, using Inclusive Composite Interval Mapping (ICIM) method to locate twenty seven traits on *Momordica* genome. Sixty QTL, including 37 major QTL with LOD values ranging from 3.1 to 15.2, explaining 1.8 to 35.9 per cent of the phenotypic variation were identified for 24 traits, on seven chromosomes. Twenty three QTL were identified for fruit-traits with LOD values ranging from 3.1 to 7.6, explaining 5.5 to 35.9 per cent of phenotypic variation. Thirteen QTL were identified for flower-related traits with LOD value ranging from 3.1 to 15.2, explaining 7.0 to 26.0 per cent of phenotypic variation. Seven QTL each were identified for seed and leaf-related traits with LOD values ranging from 3.2 to 10.8 and 3.5 to 6.5, explaining 5.6 to 26.3 and 3.2 to 15.8 per cent of phenotypic variation, respectively. Ten QTL were identified for vine-related traits with 3.2 to 8.7 LOD values and explaining 1.8 to 17.6 per cent of phenotypic variation. Single marker analysis was performed to identify markers co-segregating with the yield contributing traits. There were 129 hits for the marker-trait association with LOD values more than 3.0, explaining 11.62 to 29.34 per cent of the phenotypic variation. Using the least and best performing $F_{2:3}$ plants, markers S13, KAUBG_5 and KAUBG_11 were validated for co-segregation with fruit breadth, first pistillate flower node, and number of pistillate flowers and fruits per plant, respectively.

This study gives insights into the relative locations of microsatellites and major effect QTL for yield traits in *Momordica* genome. QTL with shorter marker interval (qFrtL-8-1, qDPF-3-1, qDSF-3-1, qDSF-7-1, qFrtShp-8-1) can be directly used in MAS for improving yield characters. Linkage observed between microsatellites identified in this study with yield traits signifies their importance in further fine mapping as well as marker assisted selection. The linkage map constructed in this study, being the first with microsatellites from *Momordica* genome, paves the path for comparative and consensus map generation with other marker types. Further, fine mapping using markers within the identified QTL hotspots can lead to possible identification and cloning of genes underlying the yield traits.