MOLECULAR MAPPING OF FLOWERING TIME GENES IN CHICKPEA (*Cicer arietinum* L.)

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In

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By

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CERTIFICATE

This is to certify that the thesis entitled **"MOLECULARMAPPING OF FLOWERING TIME GENES IN CHICKPEA (*Cicer arietinum* L.)"**submitted by **Mr.MALLIKARJUNA B.P.** for the degree of **DOCTOR OF PHILOSOPHY** in **GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Raichur is a record of bonafide research work done by him during the period of his study in this university under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or any other similar title.

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SEPTEMBER, 2015

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(AYYANAGAUDA PATIL)

DECLARATION

I, **MALLIKARJUNA B. P.**, hereby declare that the thesis entitled **"MOLECULAR MAPPING OF FLOWERING TIME GENES IN CHICKPEA (*Cicer arietinum* L.)"** submitted to the University Agricultural Sciences, Raichur for the degree of **Doctor of Philosophy in Agriculture** is the result of original research work done by me. I also declare that no material contained in this thesis has been published earlier in any manner.

Place: Raichur **(MALLIKARJUNA B. P.)**

Date: 30-09-2015 **I. D. No. PHD12AGR2015**

CERTIFICATE

Mr. MALLIKARJUNA B. P., has satisfactorily prosecuted the course of research and that research entitled **"MOLECULAR MAPPING OF FLOWERING TIME GENES IN CHICKPEA (*Cicer arietinum* L.)"** submitted is the result of original research and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither thesis nor its part thereof has been previously submitted by him for a degree of any University.

Place: Raichur
Date: 14-09-2015

(K. P. VISWANATHA)
Chairperson

AFFECTIONATELY DEDICATED

TO

MY BELOVED PARENTS
AND FRIENDS

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Date: 25-09-2015
Place: Raichur **(MALLIKARJUNA B.P.)**

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percentage
°C	:	degree Celsius
µl	:	microliter
AFLP	:	Amplified Fragment Length Polymorphism
BAC	:	Bacterial Artificial Chromosome
BES	:	BAC-end Sequences
BIBAC	:	Binary Bacterial Artificial Chromosome
BLAST	:	Basic Local Alignment Search Tool
bp	:	base pair
CaLG	:	*Cicer arietinum* Linkage Group
CaM	:	*Cicer arietinum* Microsatellites
CDC	:	Crop Development Center, University of Saskatchewan
CIM	:	Composite Interval Mapping
cM	:	centiMorgan
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DArT	:	Diversity Array Technology
DF	:	Days to first flower
DH	:	Doubled Haploids
DM	:	Days to Maturity
DNA	:	Deoxyribonucleic Acid
Efl	:	Early flowering gene
EST	:	Expressed Sequence Tag
FAO	:	Food and Agricultural Organization of United Nations
GMM	:	Genic molecular markers
ICCM	:	ICRISAT Chickpea Microsatellites
ICIM	:	Inclusive Composite Interval Mapping
ICRISAT	:	International Crops research Institute for the Semi-Arid Tropics

IM	:	Interval Mapping
ISSR	:	Inter Simple Sequence Repeats
kbp	:	kilo base pairs
LD	:	linkage disequilibrium
LG	:	Linkage Group
LOD	:	Logarithm of odds (base 10)
MAS	:	Marker-Assisted Selection
Mb	:	Million bases
mM	:	milliMolar
NIL	:	Near Isogenic Lines
NIPGR	:	National Institute for Plant Genome Research
PCR	:	Polymerase Chain Reaction
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restricted Fragment Length Polymorphism
RGA	:	Resistance Gene Analogues
RIL	:	Recombinant Inbred Line
SCAR	:	Sequence Characterized Amplified Region
SIM	:	Simple Interval Mapping
SNP	:	Single Nucleotide Polymorphism
SMA	:	Single Marker Analysis
SSR	:	Simple Sequence Repeats
STMS	:	Sequence Tagged Microsatellite Sites
Taq		*Thermus aquaticus*

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Molecular mapping of flowering time genes in chickpea (*Cicer arietinum* L.)

Mallikarjuna B.P. **2015** **K.P. VISWANATHA**
Major Advisor

ABSTRACT

Flowering time is an important component of adaptation and productivity of chickpea (*Cicer arietinum* L.) in semi-arid environments characterized by terminal drought stress. The present study was aimed at identifying molecular markers linked to flowering time genes in four F_2 populations of chickpea. Genetic studies revealed that flowering time was determined by a single major gene in the crosses ICCV 96029 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier. Whereas in the cross ICC 5810 × CDC Frontier, it was under digenic control with complementary gene action. The intra-specific genetic map developed consisted of 77 markers, spanning 262.25 cM in the cross ICCV 96029 × CDC Frontier and 76 markers with 335.74 cM map distance in the cross ICC 5810 × CDC Frontier. The genetic map of BGD 132 × CDC Frontier consisted of 68 markers covering 311.10 cM map distance and that of ICC 16641 × CDC Frontier had 67 SSRs with 385.13 cM genome coverage. Consensus map developed from four populations consisted 111 SSRs and covered the map distance of 364.44 cM. QTL analysis detected altogether seven major (*Qefl1*-2, *Qefl2*-1, *Qefl2*-2, *Qefl2*-3, *Qefl2*-4, *Qefl3*-3, *Qefl4*-1) and three minor QTLs (*Qefl1*-1, *Qefl3*-1, *Qefl3*-2) for flowering time that are distributed on linkage groups CaLG01, CaLG03, CaLG04, CaLG06 and CaLG08 of chickpea genetic map. Analysis of QTL regions provided important candidate genes like *SUVR5, SET6, HOS1, TEM1, EFL6, JMJ11* and homeotic genes like *AP2, ANT, SPT, AHL27* and *PTL*, that are known to be involved in various functions like regulation of flowering time and flower development. Flowering time was positively correlated with key phenological traits and showed no correlation with grain yield in all the crosses. Flowering time showed positive correlation with 100 seed weight in all the crosses except in the cross ICC 16641 × CDC Frontier, where the correlation was non-significant. Harvest index was negatively associated with flowering time. The identified genomic regions with linked markers can be deployed for introgressing early flowering trait into elite chickpea cultivars through marker-assisted selection (MAS) to develop early maturing cultivars better adapted to terminal stress conditions.

Introduction

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool season annual legume with a diploid set of chromosomes (2n=2x=16) and an estimated genome size of 738 Mb (Varshney *et al.*, 2013). Cultivated chickpea is highly self-pollinating legume crop that belongs to genus *Cicer*, tribe *Cicereae*, subfamily Papilionaceae and family Fabaceae. It is originated in the region of South-Eastern Turkey and adjoining areas of Syria (Singh, 1987). Globally, chickpea ranks second in area and production only after common bean. During 2013, it was grown on about 13.5 million ha area with production of 13.1 m tonnes and productivity of 966 kg ha^{-1} (FAOSTAT, 2015). Though chickpea is grown in over 50 countries globally, Southern and South-Eastern Asia accounts for 79.2 % of the global chickpea production. India is the largest chickpea producing country having cultivated in an area of 9.6 m ha with 8.8 m tonnes production and productivity of 920 kg ha^{-1}. The other major chickpea producing countries includes, Australia, Pakistan, Turkey, Myanmar, Iran, Ethiopia, Canada and USA.

Chickpea plays an important role in human nutrition for large population sectors in the developing world and is considered as a healthy food in many developed countries (Jodha and Subbarao, 1987). In addition to its high protein content (22-28 %) and carbohydrates (~60 %), chickpea is a good source of essential amino acids such as tryptophan and lysine, dietary fiber, minerals, unsaturated fatty acids and β-carotene (Awasthi *et al*., 1991 and Jukanti *et al*., 2012). As compared to other pulse crops, anti-nutritive components are very less in chickpea (Williams and Singh, 1987). Like other legumes, chickpea also fixes atmospheric nitrogen through symbiotic nitrogen fixation, thus plays an important role in improving and sustaining the overall productivity of the cropping systems.

Chickpea is traditionally a low-input crop and is grown extensively in the moisture stress environments. The major constraints limiting chickpea production globally include various abiotic and biotic stresses. Terminal drought (the soil moisture stress that occurs at the pod filling and seed development stage of the crop with increasing severity towards the end of season) is a major constraint to chickpea production in over 80 % of the global area. This is because the crop is largely grown under rainfed conditions in the post-rainy season (Gaur *et al*., 2008). In addition to

terminal drought, heat stress at the reproductive stage (in late sown conditions) has become a major constraint to chickpea production. Recently, there is a large shift in chickpea area from cooler long-season environments (northern and north-eastern India) to warmer, short-season environments (central and southern India) (Gaur *et al*., 2014). Therefore, phenology (time to flowering, podding and maturity) plays a critical role in adaptation of chickpea cultivars to different environments.

Flowering time is an important component of crop adaptation, particularly in semi-arid regions where the growth is restricted by water availability and by the seasonal temperature profile (Berger *et al*., 2006). Early flowering trait provides advantage of avoiding drought in such environments and thus avoids yield losses (Subbarao *et al*., 1995). Early maturity is also important in temperate environments for escaping end-of-season frost (Anbessa *et al*., 2006). Reduction of time to flowering and maturity has made a major contribution towards increasing and stabilizing chickpea productivity in the tropics. Super-early chickpea genotypes like ICCV 96029 have reduced crop duration from 160 to less than 130 days in the subtropics (Kumar and Rao, 2001) which further expanded opportunities for expansion of chickpea cultivation in newer niches and in specific situations where early podding is highly desired (Sandhu *et al*., 2007). The development and adoption of an extra-short-duration *kabuli* variety ICCV 2 and several early maturing, Fusarium wilt resistant varieties such as KAK 2, JG 11, Vihar *etc*., have shown a high impact on enhancement of the chickpea area under cultivation and productivity in short- season environments (Than *et al*., 2007 and Gaur *et al*., 2008).

Flowering time or days to flowering (recorded as number of days from sowing to the appearance of the first fully opened flower) can be recorded with high precision and provides a fairly good indication of succeeding phenological traits (time of podding and maturity) (Gaur *et al*., 2015). Time to flowering is a function of photoperiod and temperature in chickpea (Roberts *et al*., 1985) and it is a highly variable trait depends on season, date of sowing, altitude and latitude (Summerfield and Roberts, 1988). The wide variability for time to flowering in chickpea germplasm has been documented (Pundir *et al*., 1988 and Upadhyaya *et al*., 2007) which provides the opportunities to develop cultivars with desirable maturity duration. Also, selection for time to flowering is effective even in early segregating generations as it is reported to be controlled by only a

few major genes (Or *et al*., 1999; Kumar and van Rheenen, 2000 and Kumar and Rao, 1996).

A number of major loci controlling flowering time have been reported in other major legumes like pea (Weller *et al*., 1997) and soybean (Cober *et al*., 1996). In chickpea, oligogenic inheritance of flowering time has been reported by Gumber and Sarvjeet (1996), Anbessa *et al*. (2006) and Hegde (2010) who suggested that two genes control time to flowering. However, Kumar and van Rheenen (2000) in chickpea genotype ICCV 2 and Or *et al*. (1999) in ICC 5810 reported the presence of a major gene for flowering time *viz*., "*efl-1*" and "*ppd*" or "*efl-2*" respectively. Later, Hegde (2010) identified another flowering time gene "*efl-3*" in BGD 132 and very recently Gaur *et al*. (2015) also reported a new major flowering time gene "*efl-4*" in ICC 16641. Studies have shown that these four flowering time genes are non-allelic (Hegde, 2010 and Gaur *et al*., 2015).

Recent advances in the development of genomic resources have made it possible to locate genomic regions harbouring several agronomically important traits in chickpea (Gaur *et al*., 2014a). Quantitative trait loci (QTLs) controlling flowering time have also been identified on the LG1, LG2, LG3, LG4 and LG8 of the chickpea genetic map (Cho *et al*., 2002; Cobos *et al*., 2007 and 2009; Lichtenzveig *et al*., 2006; Aryamanesh *et al*., 2010; Hossain *et al*., 2010 and Jamalabadi *et al*., 2013). Detection of QTLs on different linkage groups showed that there may be several genes controlling flowering time in chickpea. Therefore, identification of specific genomic regions controlling different sources of earliness assumes great significance in chickpea improvement. The availability of chickpea genome sequence and annotation of genomic regions will further assist in identification of candidate genes and their regulatory sequences involved in expression of flowering time.

Hence, the present study was undertaken to identify genomic regions responsible for flowering time using four mapping populations derived from the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier. The objectives envisaged in the study were as follows:

1. Phenotyping of F_2 and F_3 segregating populations for early flowering, associated traits and yield parameters.
2. Identification of polymorphic SSR markers between parents.

3. Genotyping of F_2 segregating population with a number of SSR markers showing parental polymorphism.
4. Construction of genetic linkage map and identification of genes/QTLs for early flowering.

Review of Literature

II. REVIEW OF LITERATURE

The present investigation concerned primarily to study the genetics of flowering time, development of genetic linkage map, identification of genomic regions governing flowering time in chickpea and association studies. The available literature relevant to this study in chickpea and other legumes are reviewed and presented briefly under the following sections.

2.1 Chickpea: Botany, origin and evolution

2.2 Major constraints to chickpea production

2.3 Chickpea phenology

2.4 Genetics of flowering time genes in chickpea

2.5 Genetic mapping

2.6 Marker trait association

2.7 Association of earliness with other yield related traits

2.1 Chickpea: Botany, origin and evolution

Chickpea (*Cicer arietinum* L.) belongs to the genus *Cicer*, tribe *Cicereae*, sub-family Papilionaceae and family Leguminoseae. The genus *Cicer* encompasses 43 species, among them nine are annuals, 33 are perennial and one with unspecified life cycle, of the 9 annual species, *Cicer arietinum* L. is the only cultivated species. Based on the morphological characteristics, life cycle and geographical distribution, Van der Maesen (1987) classified the *Cicer* genus into four sections. Eight annual species, namely *C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. bijugum, C. judaicum, C. yamashitae* and *C. cuneatum* were placed in section Monocicer, *C. chorassanicum* and *C. incisum* (perennial species) in section Chamaecicer, 23 perennial species in section Polycicer and seven woody perennial species in section Acanthocicer. Two among the eight wild annual *Cicer* species native to eastern Turkey are closely related to the cultivated chickpea. The first, *Cicer echinospermum* P. H. Davis (echinate seed coat) grows in steppe plant formations on soils of basaltic origin. The second closely related species *Cicer reticulatum* Ladiz. (reticulate seed coat) is found in

oak shrub formations on hilly limestone bedrock (Ladizinsky, 1975). Based on meiotic chromosomes pairing data, *C. reticulatum* was suggested as the immediate wild progenitor of domesticated chickpea (Ladizinsky and Adler, 1976a and 1976b). This early identification is also supported by seed storage protein profiles (Ladizinsky and Adler, 1975) and by morphological comparisons (De Leonardis *et al.*, 1996) as well as by DNA marker analyses (Patil *et al.*, 1995).

The cultivated chickpea is a crop of ancient origin and is one of the first grain legumes domesticated in the old world (Van der Maesen, 1987). Chickpea originated in the region of South-eastern Turkey and adjoining areas of Syria (Singh, 1987). Vavilov (1926) identified Southwest Asia and the Mediterranean region as the two primary centres of origin, while Ethiopia as a secondary centre of origin. He further noted that large-seeded lines were abundant around the Mediterranean basin while small-seeded lines were dominant eastward. There are two distinct market types of chickpea: "*Kabuli*" (also known as macrosperma) and "*Desi*" (also known as microsperma) differing in their geographic distribution and seed characteristics. The *desi* types with predominantly pink-coloured flowers, having anthocyanin pigmentation on most of the plant parts are found in central Asia and in the Indian subcontinent. While, the *kabuli* types do not show anthocyanin pigmentation in any plant part and they have large beige or cream seed color and "ram's head" seeds with white flowers are mostly found in the Mediterranean region. There is linguistic evidence that large-seeded chickpea reached India via the Afghan capital, Kabul about two centuries ago and acquired the name in Hindi as *Kabuli chana* (chana = chickpea) (Van der Maesen, 1972). Local food preferences would have helped in the spread and adaptation of these types in different regions.

2.2 Major constraints to chickpea production

The major constraints limiting chickpea production globally include various abiotic and biotic stresses. Chickpea is often referred to as a cool-season subtropical legume, but much of the crop is grown in the tropics where at times during the growth cycle unfavourably high temperatures are encountered (Rheenen, 1991). Drought and heat stresses during the reproductive phase with increasing severity towards the end of the crop season are the major abiotic stresses of chickpea as the crop is generally grown on progressively receding soil moisture conditions. Soil salinity and chilling atmospheric temperatures are also important stresses in some growing environments (Gaur *et al.*, 2015).

Under biotic stress, more than 50 pathogens have been reported to affect chickpea, but only a few devastate the crop. Fusarium wilt (FW) [caused by *Fusarium oxysporum* f. sp. *ciceri*], dry root rot [caused by *Rhizoctonia bataticola*] and collar rot [caused by *Sclerotium rolfsi*] are the important root diseases of chickpea in areas where the chickpea growing season is dry and warm e.g., Southern and Eastern Asia (Central and Southern India) and Eastern Africa. While, Ascochyta blight (AB), [caused by *Ascochyta rabiei* (Pass.) Labr.] and botrytis grey mold (BGM) [caused by *Botrytis cineria* Pres.] are the important foliar diseases in the areas where the chickpea growing season is cool and humid. AB is important in west and central Asia, North Africa, North America, Australia, northern part of India and Pakistan, while BGM is important in Nepal, Bangladesh, northern India and Australia (Gaur *et al.,* 2010).

Pod borer (*Helicoverpa armigera* Hubner.) is the most important pest of chickpea worldwide. It is a highly polyphagous pest and can feed on various plant parts such as leaves, tender shoots, flower buds, and immature seeds. The extent of global losses to chickpea by this pest is estimated at over US$ 500 million (Ryan, 1997 and Gaur *et al.,* 2012). The viral diseases, rust (*Uromyces ciceris*-arietini), root nematodes (*Meloidogyne sp.*), Phytophthora root rot (*Phytophthora medicaginis*), powdery mildew (*Oidiopsis taurica* Lev.), cutworm (*Agrotis sp.*) and leaf miner (*Liriomyza cicerina*) are also important in some chickpea growing areas.

2.3 Chickpea phenology

Phenology is the most important adaptive criterion in annual crops that minimizes exposure to climatic stresses and maximizes productivity in target environments (Berger *et al.,* 2006). Crop plants have evolved various mechanisms to cope with the climatic stress patterns under which they naturally evolved or were domesticated. As proposed by Kumar and Abbo (2001), chickpea domestication in the Mediterranean was accompanied by increased day-length sensitivity to allow spring-sown crops to commence the reproductive phase prior to the onset of terminal drought regardless of biomass accumulation at this time. Further, they suggested that dissemination of the crop to Africa and the Indian subcontinent was only possible through the selection of day-length insensitive genotypes that would not delay flowering under the reducing photoperiod typically experienced in the chickpea growing seasons there.

Terminal drought is a major constraint to chickpea production in over 80 % of the global area. This is because the crop is largely grown under rainfed (residual moisture)

conditions in the post-rainy season (Gaur *et al.,* 2014). Drought escape under terminal drought enables selection of plants completing their life cycle in short period thus making judicious usage of available moisture condition and avoids yield loss (Subbarao *et al.,* 1995 and Kumar and Abbo, 2001). Singh *et al.,* (2008) suggested that development of early maturing cultivars coupled with early growth vigour may help the varieties utilize the available soil moisture efficiently and produce relatively higher yields. In the Mediterranean environments also the dryland chickpea crop is exposed to high evaporative demand and water stress during the reproductive stage. Thus, early flowering/podding chickpea cultivars may realize higher yield in winter-season chickpea by expanding duration of reproductive period (Kumar and Abbo, 2001). In temperate environments also early maturity minimizes risk of frost damage and enables producers to attain better harvest quality and a higher yield by escaping end-of-season frost (Anbessa *et al.,* 2006).

In chickpea, terminal drought escape through early phenology (short duration) has been the most successful breeding strategy (Gaur *et al.,* 2008). The number of days taken from sowing to flower initiation can be recorded easily which provides a good indication of the succeeding phenological traits (days to podding and maturity) since these traits are inter-correlated. The existence of wide genetic variation for flowering time in chickpea was documented by Pundir *et al.,* (1988) who reported a range from 33 to 107 days for time to 50% flowering in a collection of 12,018 accessions. Similarly, Robertson *et al.,* (1997) found variability for early flowering in *C. judaicum*. Later, several early flowering accessions of *desi* and *kabuli* types have been identified from germplasm collections and most of these originated from tropical India (Maharashtra and Karnataka), Ethiopia, Mexico, Iran and Pakistan (Pundir *et al.,* 1988 and Upadhyaya *et al.,* 2007). This wide variability for time to flowering in chickpea germplasm provides opportunities to develop cultivars with desirable maturity duration. Also, selection for time to flowering is effective even in early segregating generations as it is reported to be governed by few major genes (Or *et al.,* 1999; Kumar and van Rheenen, 2000; Hegde, 2010 and Gaur *et al.,* 2015). Therefore, it has been possible to develop breeding lines that mature earlier than both the parents by accumulating earliness genes from the two parents. For example, the super-early line ICCV 96029 which flowers in about 24 days at Patancheru was developed from a cross between two early lines ICCV 2 and ICCV 93929 which flower in 30 and 32 days, respectively (Kumar and Rao, 1996).

Subsequently, several early maturing high-yielding cultivars have been developed, for example, ICCV 2 (released in India, Sudan, and Myanmar), JGK 1 and KAK 2 (released in India) and ICCV 92318 (released in Ethiopia) in *kabuli* types and ICCC 37, JG 11 (released in India) and ICCV 88202 (released in Australia, Myanmar and India) in *desi* types. Adoption of early maturing, fusarium wilt resistant varieties such as KAK 2, JG 11,Vihar *etc*., has shown high impact on enhancement of the chickpea area under cultivation and productivity in short-season environments such as Myanmar (Than *et al.,* 2007) and Southern India (Gaur *et al.,* 2008). Development of super-early lines have further expanded opportunities for cultivation of chickpeas in areas and cropping systems where the cropping window available for chickpeas is narrow and in specific situations where early podding is highly desired, for example, when immature grains are used as vegetables (Sandhu *et al.,* 2007).

2.4 Genetics of flowering time genes in chickpea

Flowering time, recorded as the number of days from seeding to onset of flowering usually varies with local circumstances such as sowing date, altitude and latitude. In chickpea, flowering time is reported to be influenced by the seasonal temperature profile and the photoperiodic response of the plant with no interaction between the two factors (Summerfield and Roberts, 1988). Kumar and Abbo (2011) observed involvement of several genetic systems responding to day length and temperature in chickpea that causes a typical continuous frequency distribution of flowering time. Though chickpea is quantitatively long day in its response, but some relatively photoperiod insensitive genotypes are also available (Roberts *et al.,* 1985).

Genetic approaches have been used to study the flowering differences between varieties of the same species since the early decades of the twentieth century. Typically, this was conducted by making crosses between two varieties showing different flowering time and then following the segregation of flowering time among progeny of the cross. This classical approach was used to analyse flowering time in several crops including peas (Keeble and Pelly, 1910), cotton (Leake, 1911), wheat (Thompson, 1918) and tobacco (Allard, 1919).

A number of major loci controlling flowering time have been reported in other important legumes like pea (Weller *et al.,* 1997), soybean (Cober *et al.,* 1996) and common bean (Coyne, 1970 and Kornegay, *et al.,* 1993). However, information on the

genetic control of flowering time in chickpea is only beginning to accumulate. Kumar *et al.* (1985) reported that at least two different loci control flowering time in chickpea. Preliminary studies on genetics of flowering time was carried out by Gumber and Sarvjeet (1996), who reported two duplicate genes in homozygous recessive state cause early flowering in ICCV 2 and accounted for 35 days difference in flowering between ICCV 2 and late flowering parents GL 769, BG 276 and PGK 45 in a subtropical long-duration environment of northern India.

A major gene "*efl-1*" for early flowering was identified in a cross between the extra-early variety ICCV 2 and the medium-duration variety JG 62 (Kumar and van Rheenen, 2000). They studied recombinant inbred lines (RILs) of this cross and observed bimodal distribution with nearly equal peaks, one peak corresponding with flowering time of ICCV 2 × JG 62. They also observed segregation of other minor genes in this cross. This gene was responsible for about 3 weeks difference in flowering time between the two parents at ICRISAT, Patancheru.

Later, Kumar and Rao (1996) selected a super early chickpea segregant ICCV 96029 (flower in about 24 days at Patancheru) from the F_6 generation from a cross between two extra-early varieties ICCV 2 and ICCV 93929 (which flower in 30 to 32 days at Patancheru). ICCV 96029 inherited *efl-1* from ICCV 2 and at least one additional gene affecting early flowering from ICCV 93929 and flowered about a week earlier than either of the parents. This further indicated involvement of more than one gene controlling flowering time. Early flowering is beneficial to early maturity and also helps to prolong the reproductive period which is a major yield determinant in chickpea (Bonfil and Pinthus, 1995).

Two early flowering lines, 272-2 and 298T-9 derived from crosses that involved ICCV 96029 as one of the parents were studied for inheritance of flowering time in short-season temperate environment of Western Canada (Anbessa *et al.*, 2006). Crosses of these lines with late flowering Canadian cultivars 'CDC Anna' and 'CDC Frontier' segregated for two major genes with duplicate recessive epistasis and the interaction between these genes was mainly an additive x additive type. This study further supports presence of two major genes for flowering time in ICCV 96029.

ICC 5810 (Harigantras), a black-seeded landrace from central India has been another source of early flowering. This accession is relatively day length or photoperiod

insensitive (Roberts *et al.*, 1985) and flowered about two months earlier than the cultivar Hadas (days to flower 115 to 140) at Rehovot, Israel (Or *et al.*, 1999). Study of F_2 segregation suggested that differences in flowering time between ICC 5810 and Hadas are due to a major gene designated as '*ppd'* (photoperiod dependent). Later, Kumar and Abbo (2001) speculated that the recessive early flowering gene '*ppd*' of ICC 5810 and '*efl*-1' of ICCV 2 could be alleles of the same locus. However, Hegde (2010) studied allelic relationships between early flowering genes of ICCV 2 (*efl*-1) and ICC 5810 (*ppd*) by crossing two early flowering lines (ICCV 2 × ICC 5810). He observed that F_1 plants flowered about 13 and 20 days later than ICCV 2 and ICC 5810, respectively, indicated the complementation of dominant genes present in ICC 5810 and ICCV 2 lines. The F_2 plants segregated into 9 (late): 6 (early): 1 (super-early) indicated involvement of two duplicate dominant genes interacting with a cumulative but unequal effect on time of flowering. Therefore, he concluded that early flowering genes of ICCV 2 (*efl*-1) and ICC 5810 (*ppd*) were non-allelic. He renamed the "*ppd*" gene present in ICC 5810 as "*efl-2*". Recent allelic relationship studies at ICRISAT provided confirmatory evidence in support of these findings (Gaur *et al.*, 2015).

BGD 132 is another source of early flowering *kabuli* line derived as transgressive segregant of the cross ICCV 2 × ICCV 5. Hegde (2010) used BGD 132 in a series of different early × early (BGD 132 × ICC 5810, BGD 132 × BGD 9812, ICCV 2 × BGD 132 and BGD 132 × SBD 377) and late × early (BG 362 × BGD132) crosses. He observed late flowering in F_1s of all the crosses and indicated that late flowering was dominant over early flowering. He also indicated that genes for flowering time in these genotypes are non-allelic and provided a confirmatory evidence for the existence of several duplicate dominant genes for flowering time in chickpea. Further, he observed monogenic segregation for days to flowering in the cross BG 362 × BGD132 and concluded that BGD 132 has a dominant gene at one of the loci which is different from those in ICCV 2 (*efl-1*) and ICCV 5810 (*efl-2*). He designated this gene in BGD 132 as "*efl-3*".

Upadhyaya *et al.*, (2007) screened chickpea core collection consisting of 1,956 germplasm lines at ICRISAT and identified very early-maturing genotypes such as ICC 16641, ICC 16644, ICC 11040, ICC 11180, ICC 12424 and ICC 14648. Recent experiments at ICRISAT by Gaur *et al.* (2015) studied allelic relationships of the early flowering gene of ICC 16641/ICC 16444 with the previously identified early flowering

genes of ICCV 96029/ICCV 2 (*efl-1*), ICC 5810 (*efl-2*) and BGD 132 (*efl-3*) and concluded that the major early flowering gene of ICC 16641/ICC 16644 was not allelic to any of the previously identified early flowering genes and they designated this new early flowering gene as "*efl-4*".

Thus, so far four major genes for flowering time, *efl-1* (in ICCV 2 and ICCV 96029), *efl-2* (in ICC 5810), *efl-3* (in BGD 132) and *efl-4* (in ICC 16641 and ICC 16644) have been identified in chickpea (Kumar and van Rheenen, 2000; Kumar and Abbo, 2001; Hegde, 2010 and Gaur *et al.*, 2015).

2.5 Genetic mapping

Genetic mapping is a procedure of locating the molecular markers or gene loci/QTLs in order, indicating the relative distances among them and assigning to their linkage groups on the basis of their recombination values from all pairwise combinations. Genetic mapping is based on the principle that genes (markers or loci) segregates *via* chromosome recombination (called crossing-over) during meiosis (i.e., sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). In a segregating population, the frequency of recombinant genotypes can be used to calculate recombination fractions, which may be used to infer the genetic distance between markers (Collard *et al.*, 2005). By analysing the segregation of markers, the relative order and distances between markers can be determined. Markers that have a recombination frequency of 50% are described as 'unlinked' and assumed to be located far apart on the same chromosome or on different chromosomes (Hartl and Jones, 2001). The recombination fractions are converted into map distances (cM) by using mapping functions. Genetic linkage map construction requires following steps: (1) Development of appropriate mapping population, (2) Identification of polymorphic markers between parental genotypes and (3) Linkage analysis of markers. Linkage between markers is usually calculated using odds ratios (i.e., the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio and is called a logarithm of odds (LOD) value or LOD score (Risch, 1992). LOD >3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e., 1000:1) than no linkage (null hypothesis). LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values. Commonly used software programs include

Mapmaker/EXP (Lander *et al.*, 1987), MapManager QTX (Manly *et al.*, 2001) and GMendel (http://cropandsoil.oregonstate.edu/Gmendel). JoinMap is another commonly used program which is generally used for constructing and combining the genetic maps developed for different mapping populations (Stam, 1993).

The beginning of the linkage map development in chickpea was based on morphological and isozyme loci. However, their small numbers and the fact that expression of these markers is often influenced by the environment, makes them unsuitable for routine use. Further, limited polymorphism exhibited by cultivated chickpea for the molecular markers developed in the early phase forced researchers to use interspecific crosses in linkage mapping of chickpea. The first linkage map of chickpea was developed by Gaur and Slinkard (1990a and 1990b) using isozyme markers in the inter-specific crosses of *C. arietinum* with *C. reticulatum* and *C. echinospermum*. Later, DNA-based markers such as RFLP and RAPD (Simon and Muehlbauer, 1997), STMS and amplified fragment length polymorphism (AFLP) (Winter *et al.,* 1999), morphological isozyme, inter simple sequence repeat (ISSR) and RAPD loci (Santra *et al.,* 2000) and STMS markers (Tekeoglu *et al.,* 2002; Flandez-Galvez *et al.,* 2003; Udupa and Baum, 2003; Cho *et al.,* 2004 and Tar'an *et al.,* 2007) were used.

The interspecific RIL population of the cross *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777) has been considered as the reference mapping population and extensively used for genome mapping (Nayak *et al.,* 2010; Gujaria *et al.,* 2011; Thudi *et al.,* 2011; Choudhary *et al.,* 2012 and Hiremath *et al.,* 2012). The first integrated genetic map based on this population comprised 37 inter simple sequence repeats (ISSRs), 70 amplified fragment length polymorphisms (AFLPs), 118 sequence-tagged microsatellite sites (STMSs), 96 DNA amplification finger printings (DAFs), 17 RAPDs, 3 cDNAs, 8 isozymes and 2 sequence characterized amplified regions (SCARs) covering a total distance of 2077.9 cM (Winter *et al.,* 2000). An advanced genetic map with 521 markers including simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) with an inter marker distance of 4.99 cM spanning 2602.1 cM was developed from the above population (Nayak *et al.,* 2010). In this map, they integrated 71 SNP loci based on gene-specific primers developed by Choi *et al.* (2004). This effort demonstrated the power of a comparative genomics approach (Medicago and Chickpea) to identify molecular tools.

Later, this map was integrated with BES-SSRs, DArT and gene-based markers by Thudi *et al.* (2011), which comprised of 1291 loci on eight linkage groups (LGs). In this map, highest number of markers per LG was on LG 3 (218) and the lowest was on LG 8 (68) with an average inter-marker distance of 0.65 cM. Later, an advanced gene-rich map of chickpea comprising of 406 loci (including 177 gene-based markers) spanning 1,497.7 cM genetic distance has been developed for this reference population (Choudhary *et al.,* 2012). Recently, Hiremath *et al.* (2011 and 2012) developed large-scale KASPar assays for SNP genotyping and developed a genetic map comprising 1328 marker loci, including novel 625 CKAMs (Chickpea Kaspar Assay Markers), 314 TOG-SNPs and 389 published marker loci for this reference population. Gujaria *et al.* (2011) has developed a transcript map that comprised of 300 loci (including 126 genic molecular markers [GMMs]) and spans a genomic region of 766.56 cM.

Cho *et al.* (2002) developed the first intra-specific map of cultivated chickpea from ICCV 2 × JG 62 RILs. This map was developed using 3 ISSRs, 20 RAPDs, 55 STMSs along with two phenotypic markers comprised of 14 linkage groups and spanning 297.5 cM. This map was used to map genes for important morphological traits along with the double podded trait. Tekeoglu *et al.* (2002) developed a chickpea map from 65 STMS primer pairs and a population size of 142 RILs from an interspecific cross between FLIP84-92C (*Cicer arietinum*) and PI599072 (*Cicer reticulatum*).They also integrated this map with marker data from Santra *et al.* (2000) and reported a total genetic map with 167 markers on nine linkage groups covering 1,174.5 cM with an average distance of 7.0 cM between markers.

The genetic map reported by Flandez-Galvez *et al.* (2003) was based on 66 markers, including 51 SSRs and a population of 85 F_2 plants from an intraspecific cross between *desi* cultivars ICC12004 and Lasseter. Udupa and Baum (2003) generated a map from 52 SSRs and a population size of 97 RILs from an intraspecific cross *kabuli* type chickpea between ILC 1272 and ILC 3279. The genetic map reported by Cho *et al.* (2004) was generated from 53 STMS primer pairs based on the population of RILs from a cross between PI359075(1) and FLIP84-92C(2). Another map developed based on a "*kabuli* × *desi*" cross included a total of 134 molecular markers (3 ISSR, 13 STMSs and 118 RAPDs) mapped to 10 linkage groups (Cobos *et al.*, 2005). This map spanned a genomic region of 534.5 cM with an average marker interval of 8.1 cM. Later, Radhika *et al.* (2007) developed an integrated intraspecific map spanning a region of 739.6 cM,

including 230 markers at an average distance of 3.2 cM between markers. The genetic map published by Taran *et al.*, (2007) was generated from 135 primer pairs including 134 SSRs and was based on a population of 186 F_2 plants from an intraspecific cross of *desi* cultivar ICCV 96029 and *kabuli* cultivar CDC Frontier. Markers reported in this map were assigned to 8 linkage groups with a combined linkage distance of 1,285 cM with average linkage distance of 8.9 cM between primer pairs. Another map developed recently by Anuradha *et al.* (2011) included 144 markers assigned to 11 linkage groups, spanning 442.8 cM, with an average marker interval of 3.3 cM.

Consensus genetic maps using both interspecific and intraspecific populations were also developed. A consensus map based on five interspecific (*C. arietinum* × *C. reticulatum*) and five intraspecific (*Desi* × *Kabuli* types) populations was developed (Millan *et al.,* 2010). It integrated 555 marker loci including RAPDs (251), STMSs (149), AFLPs (47), 33 cross-genome markers, 28 gene-specific markers, 10 isozyme markers, 10 inter-simple sequence repeats (ISSRs) and 7 RGA loci. Recently, Varshney *et al.* (2014) developed an intraspecific genetic map of chickpea from ICC 4958 × ICC 1882 and ICC 283 × ICC 8261 RIL populations. The genetic maps comprised of 241 and 168 SSR loci with genome coverage of 621.51 cM and 533.06 cM, respectively. A consensus map developed from these two populations integrated 352 SSR loci covering map distance of 771.39 cM.

Common markers in these and future maps with SSR primer pairs could lead to the development of a high density genetic map of chickpea to identify tightly linked flanking markers for genes of interest, which ultimately helpful in marker-assisted selection (MAS) and positional cloning of agronomically important genes.

2.6 Marker trait association

The most crucial factor for marker-assisted breeding is the knowledge of the association between the markers and trait of interest and it is starting point to work for crop improvement. More closely the markers are associated with the traits, the higher the possibility of success and efficiency of use will be. This information can be determined in various ways, linkage-based approach or by linkage disequilibrium (LD) based association mapping, classical mutant analysis, linkage or recombination analysis, bulked segregant analysis, *etc*. In past, in several crops, genetic mapping based approaches were used to identify the QTLs/genes for a trait (Gupta and Varshney, 2004). For conducting

marker-trait association by using linkage maps, three widely used methods are: single marker analysis (SMA), simple interval mapping (SIM), and composite interval mapping (CIM) (Tanksley, 1993).

2.6.1 Mapping populations used for QTL mapping

Development of appropriate mapping population is necessary for constructing a genetic linkage map and dissecting complex traits. The first step in producing a mapping population is selecting two genetically diverse parents for one or more traits of interest. Further, the parents should be genetically divergent enough to exhibit sufficient polymorphism, and on the other hand, they should not be too distant that causes sterility of the progenies and expresses high level of segregation distortion during linkage analysis (Collard *et al.,* 2005). Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals (Mohan *et al.,* 1997), however larger populations are required for high-resolution mapping (Paterson, 1996).

Different types of populations, including progenies from F_2 generation, backcross (BC), recombinant inbred lines (RILs), double haploids (DH) and near isogenic lines (NILs) have been used for genetic mapping in chickpea. F_2 populations are developed by self-pollinating F_1 hybrids derived by crossing two parents, while BC population is produced by crossing F_1 to one of the parents). By repeated backcrossing for at least six generations (BC_6) with the recurrent parent, more than 99% of the genome can be recovered from the recurrent parent. Further selfing of selected individuals at BC_6F_1 or BC_7F_1 will produce lines that are homozygous for the target gene which are considered to be nearly isogenic with the recipient parent (NILs). NILs are mainly generated for fine mapping of a QTL/genomic region of interest. DH populations are generally developed by chromosome doubling of haploids developed though anther culture (pollen or microspore culture) of F_1 plants.

Recombinant inbred lines (RILs) are developed following single seed descent (SSD) advancement of F_2 plants by six or more generations and then developing single plant progenies. This process leads to lines that contain a different combination of linkage blocks from the original parents. Seed from RILs is predominantly homogeneous and abundant, so the seed can be shared across any lab interested in adding markers to an existing linkage map previously constructed with the RILs. Moreover, RILs can be grown

in replicated trials at several locations and/or over several years making them ideal for QTL mapping (Paterson, 1996 and Collard *et al.*, 2005).

2.6.2 Approaches for QTL mapping

The main principle of QTL detection is to obtain association between the trait value under consideration and the genotype of every marker. A significant correlation test means that at least one QTL may exist near the marker locus. To be able to detect QTL using the marker loci, linkage disequilibrium must exist between alleles at the marker loci and alleles of the linked QTL (Tanksley, 1993). Tests for QTL/trait association are often performed by the following approaches.

The single marker approach, also referred to as the single factor analysis of variance (SF-ANOVA) or single point analysis (Edwards *et al.*, 1987). In this method, SF-ANOVA is done for each marker locus independent of information from other loci. F-tests provide evidence whether differences between marker locus genotype classes are significant or not. Although this approach is computationally simple, it suffers from major limitations: (i) the likelihood of QTL detection significantly decreases as the distance between the marker and QTL increases (ii) the method cannot determine whether the markers are associated with one or more QTLs and (iii) the effects of QTLs are likely to be underestimated because they are confounded with recombination frequencies.

SIM (Simple Interval Mapping) was first proposed by Lander and Botstein (1989). This method takes full advantage of a linkage map and evaluates the target association between the trait values and the genotype of a hypothetical QTL (target QTL) at multiple analysis points between pair of adjacent marker loci (the target interval). Presence of a putative QTL is estimated if the log of odds ratio (LOD) exceeds a critical threshold. However, when multiple QTLs are segregating in a cross (which is usually the case), SIM fails to take into account genetic variance caused by other QTLs.

Composite Interval Maping (Zeng, 1994) and MQM (multiple-QTL model or marker-QTL-marker analysis) developed by Jansen and Stam (1994) combine interval mapping for a single QTL in a given interval with multiple regression analysis on marker associated with other QTL. The advantages of CIM are as follows; (i) mapping of multiple QTLs can be accomplished by the search in one dimension (ii) by using linked markers as cofactors, the test is not affected by QTL outside region, thereby increasing

the precision of QTL mapping and (iii) by eliminating much of the genetic variance by other QTL, the residual variance is reduced thereby increasing the power of detection of QTL. CIM is more precise and effective at mapping QTLs as compared to single-point analysis (SMA) and SIM, especially when linked QTLs are involved.

2.6.3 QTL mapping for flowering time genes in chickpea

Because of the paucity of markers and non-availability of genetic maps, QTL mapping in chickpea has been very slow. However, during the last few years, the development of large scale genomic resources has made some progress towards QTL mapping for several production constraints (Varshney *et al.,* 2012). As a result, genetic mapping in chickpea has focused on tagging agronomically relevant genes such as yield and its component traits, resistance to biotic and abiotic stresses (see Table 1). The QTLs controlling flowering time were also mapped on the chickpea genetic map by several studies are presented here.

Cho *et al.* (2002) reported one QTL for days to 50 % flowering on linkage group (LG) 3 with a LOD score of 3.03 (flanked by Ts57 and Ta127) in a RIL population derived from a cross between ICCV 2 × JG 62. An additional loosely linked QTL for days to first flower was also found on the same location with a LOD score of only 2.34. In fact, several weak QTLs for flowering were also detected on five other linkage groups with low LOD scores (1.14–1.96). These findings indicate that several unknown factors confer time to flowering in chickpea even though segregation for a major flowering gene was observed in this study.

In an intraspecific RIL population of the cross CA2156 × JG62, Cobos *et al.* (2007) reported a major QTL for days to 50 % flowering (QTL_{DF1}) on LG4 closely linked to marker GAA47 explaining around 20 % of phenotypic variation. Later, Cobos *et al.* (2009) used an interspecific RIL population *Cicer arietinum* (ICCL81001) × *Cicer reticulatum* (Cr5-9) and identified one QTL (designated as QTL_{DF3}) on LG3A with the closely associated STMS marker TA142 accounting 52 % (in greenhouse) and 26 % (in field) of the phenotypic variation. This QTL_{DF3} location was consistent with that obtained by Cho *et al.* (2002) from an intraspecific cross.

The major flowering time gene from ICC 5810 (*efl*-2 or *ppd*) was mapped on LG1 by Lichtenzveig *et al.* (2006) in a cross Hadas × ICC 5810. This QTL was flanked by the

markers H1F022 and GAA40 has a major effect explaining largest proportion of the response variance, 56 % and 53 % at two locations tested. The second QTL was found on LG2 between markers H4B09 and H1B06 with a minor effect and explaining a smaller proportion of the phenotypic variance of 22 % and 18 %. Also, a QTL on LG8 was identified with significant effect on days to flower and resistance to *Didymella rabiei*.

In an interspecific cross ICC 3996 × ILWC 184, Aryamanesh *et al.* (2010) reported two QTLs with epistatic interaction on LG3 controlling flowering time together explaining 90.2 % of the phenotypic variation and flanked by markers TA142 (3.2 cM) and TA64 (6.9 cM) and markers TS29 (7.7 cM) and TA76 (10.2 cM), respectively. The location of these QTLs on LG3 were different from the single QTL for days to flowering identified by Cho *et al.* (2002) on LG3 suggesting the presence of different genes for the control of flowering time.

Hossain *et al.*, (2010) used simple interval mapping approach in $F_{5:7}$ RILs derived from the cross ICC3996 × S95362 and identified flowering time QTL on LG3 between markers TS19-TR56 (explained 23 % phenotypic variance). Further, Rehman *et al.* (2011) studied the cross ILC 588 × ILC 3279 and mapped four QTLs controlling days to flowering each on LG1, LG3, LG4 and LG8 flanked by the marker intervals H5A08-TA8, TA6-NCPGR12, TA132-GA137 and TA159-GA6, respectively.

Recently, Jamalabadi *et al.* (2013) identified a closely linked marker (TA117) on linkage group LG3 for the days to flowering trait in a $F_{2:3}$ population derived from the cross ILC3279 × ICCV2. This QTL explained 33 % of the variation. Earlier, Cho *et al.* (2002) also reported a QTL for days-to-flowering on LG3 using an RIL from a cross between the same line employed in this study ICCV2 and JG 62. Therefore, the alleles of that QTL could be the same in both findings.

These results indicate that putative QTL for flowering time genes may be population-specific as indicated by different genomic regions (LG) that control the same trait(s) in different mapping populations. Presently the major flowering time gene from ICCV 2 (*efl*-1) has been mapped on LG3 (Chao *et al.*, 2002 and Jamalabadi *et al.*, 2013). The major flowering time gene from ICC 5810 (*efl*-2 or *ppd*) was mapped on LG1, LG2 and LG8 (Lichtenzveig *et al.*, 2006). The flowering time genes *efl-3* and *efl-4* are yet to be mapped.

2.7 Association of earliness with other yield related traits

Phenological traits (days to flowering, days to podding and maturity) are important traits of adaption in target environments. In chickpea, number of days taken from sowing to flowering initiation provides a good indication of the succeeding phenological traits (days to podding and to maturity), since these traits are inter-correlated. Several studies reported significant positive association among days to flowering and days to maturity in chickpea (Malik *et al.*, 1988; Atta *et al.*, 2008; Hasan *et al.*, 2008; Sidramappa *et al.*, 2010; Naveed *et al.*, 2012; Jivani *et al.*, 2013; Monpara and Dhameliya, 2013; Ramanappa *et al.*, 2013; Zeeshan *et al.*, 2013 and Gaur *et al.*, 2015).

The efficiency of selection for phenology and yield component characters mainly depends upon the direction and magnitude of association among these characters. This is particularly important for k*abuli* chickpea, where seed weight is an important yield component and a significant yield determinant. Sedgley *et al.* (1990) suggested that early pod set should be a prime strategy for avoiding drought stress in environments prone to end-of-season moisture stress thereby increasing productivity. However, penalties associated with earliness include short time available to accumulate biomass and development of a shallower root system. The first can limit the grain yield potential and the latter will render plants vulnerable to adverse effects of intermittent drought (Johansen *et al.*, 1997 and Kumar and Abbo, 2001). Significantly positive association between days to flowering and grain yield was reported by Naveed *et al.* (2012). However, Gul *et al.* (2013) reported significant negative association. While, Monpara and Dhameliya (2013), Arshad *et al.* (2003), Sidramappa *et al.* (2010), Atta *et al.* (2008) and Naveed *et al.* (2012) observed non-significant association for days to flowering with grain yield.

Or *et al.* (1999) observed a low but significant association among the days to flower and mean grain weight in F_2 population populations derived from cross between early flowering (*desi*) x late flowering (*kabuli*) cultivars. However, Hovav *et al.* (2003) observed positive and relatively high genetic correlations between time to flowering and seed weight and suggested that in certain genetic backgrounds it might be difficult to breed early-flowering cultivars without compromising seed weight. Similar findings are reported by Ali *et al.* (2011), Gul *et al.* (2013) and Zeeshan *et al.* (2013).

Despite the clear evidence for the gene action of flowering time genes in chickpea, flowering time is heavily affected by polygenes similar to grain yield. Under such circumstances, it becomes clear that numerous combinations between promoting and demoting alleles at any linked flowering and yield loci (major and/or minor) may exist (Kumar and Abbo, 2001). Therefore, such type of associations between the flowering loci and loci affecting agronomic performance suggests that selection to produce desired combinations in any direction should be possible.

Material & Methods

III. MATERIAL AND METHODS

The present study on "Molecular mapping of flowering time genes in chickpea" was carried out at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India (located at 18°N, 78°E, 545 m above sea level and 600 km away from the sea). The field experiments for phenotyping of the mapping populations were conducted during post rainy season (October to February) of 2013-14 and 2014-15 at Patancheru. The weather data (total rainfall during the crop season, minimum and maximum temperature, relative humidity, total evaporation, solar radiation and bright sunshine hours) for each cropping season at the experimental site is given in APPENDICES (A and B). The genotyping of the mapping populations were carried at Genotyping Service Laboratory (GSL), Centre of Excellence in Genomics (CEG), ICRISAT, Patancheru.

3.1 Experimental Material

3.1.1 Selection of parents and development of mapping populations

Four early flowering lines ICCV 96029, ICC 5810, BGD 132 and ICC 16641 were used as female parents to cross with common male parent "CDC Frontier" (a late flowering Canadian cultivar) to produce F_1s during post rainy season of 2011-12. All the female lines and male parental line differed for flowering time, maturity and various agronomic traits (see Table 2). The F_1s were planted during post rainy season of 2012-13 and selfed to obtain F_2 generation seeds.

3.1.2 Experimental design

The experiment for evaluation of flowering time and various agronomic traits was carried out in an un-replicated design in the black precision (BP-12) field at ICRISAT, Patancheru (Plate 1 and 2). Twenty seeds of each of the parents and F_1s and 190 F_2 seeds from each of the three crosses (ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier) and 146 F_2 seeds from the cross ICC 16641 × CDC Frontier were planted on 29th October, 2013. Seeds were treated with a mixture of 2 g of Thiram and 1 g of Carbendazim per kilogram of seeds and dibbled when sufficient moisture was available in the soil. The seeds were sown at a wider spacing of 60 cm x 20 cm with single seed per hill in the row length of 4m. Basal dose of 18 kg N and 46 kg

P_2O_5 ha^{-1} by using 100 kg/ha Diammonium phosphate fertilizer were applied to raise healthy crop. The seeds germinated during first week of November 2013. Plot was maintained weed free by regular weeding. One supplementary irrigation was given at 30 DAS to overcome moisture stress conditions. Control of pod borer (by spraying of 20 mL/ha Indoxacarb in 300 L water) was taken up when the pest incidence reached the economic threshold level. All other crop management practices were carried out to ensure good crop establishment.

3.2 Recording of observations:

The following observations were recorded on individual plant basis in all the parents, F_1s and F_2s in the field condition.

3.2.1 Pre-harvest observations

3.2.1.1 Days to flowering

The number of days was counted from sowing to the appearance of the first fully opened flower on the plant. The data was recorded on daily basis on all the parents and F_1s and all plants individually in F_2 populations.

3.2.1.2 Flower color

Color of the freshly opened flower i.e. pink or white was recorded on individual plant basis.

3.2.1.3 Single/double podding

Number of pods per peduncle was counted (as single or double) and recorded on individual plant basis.

3.2.1.4 Days to pod initiation

The number of days counted from the date of sowing to appearance of first pod on the plant.

3.2.1.5 Plant height at maturity (cm)

The plant height at maturity was measured in centimetres from ground level to the tip of the longest branch at maturity.

3.2.1.6 Days to maturity

Number of days after sowing when more than 90 % of pods of the plant have changed from green to yellow.

3.2.2 Post-harvest observations

3.2.2.1 Biological yield per plant (g)

The above ground plant weight of the fully dried plant was recorded as biological yield per plant.

3.2.2.2 Number of pods per plant

The number of pods per plant was recorded by counting the total number of filled pods on each plant.

3.2.2.3 Number of seeds per plant

The total number of seeds obtained after threshing of all the filled pods of a plant was counted manually.

3.2.2.4 Number of seeds per pod

The total number of seeds per pod was computed by dividing total number of seeds per plant by total number of filled pods per plant.

$$\text{No. of seeds per pod} = \frac{\text{Total number of seeds per plant}}{\text{Total number of pods per plant}}$$

3.2.2.5 Grain yield per plant (g)

Total seeds from each plant were weighed and recorded in gram (g), after threshing the dried pods.

3.2.2.6 100-seed weight (g)

Weight of 100 seeds was measured and expressed in gram. Those plants which had number of seeds less than 100, 100-seed weight was calculated by the following formula:

$$\text{100-seed weight} = \frac{\text{Weight of total seeds of the plant}}{\text{Total number of seeds of the plant}} \times 100$$

3.2.2.7 Harvest index (%)

It was calculated by the following formula:

$$\text{Harvest Index} = \frac{\text{Grain yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100$$

3.2.3 Evaluation of F_3 progenies

During post rainy season of 2014-15, F_3 progenies of four mapping populations along with parents were evaluated in BS-7C field at ICRISAT, Patancheru. Based on availability of sufficient seed (minimum 20 seed), a total of 164, 174, 182 and 102 progeny rows from the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier respectively were used. Each progeny row comprised of 20 plants in 2 meter row with a spacing of 60 cm × 10 cm. The sowing was done using tractor operated dibbler on 15th October, 2014. The crop was raised under rainfed conditions and all the crop management practices were taken to ensure good crop establishment. Each progeny was observed for time of flowering, flower color and single/double podding at regular intervals and classified them as non-segregating and segregating types and subjected to chi-square test. Other observations like plant height, biomass, grain yield and 100 seed weight were recorded (as described in above section) on one meter continuous plants in each progeny row.

3.3 Statistical analysis

The recorded data were subjected to following statistical analyses.

3.3.1 Parameters of descriptive statistics

i. Mean

Mean is the sum of all observations in a sample divided by the total number of observations (N).

$$\textbf{Mean } (\bar{X}) = \sum_{i=1}^{N} \frac{X_i}{N}$$

Where,

X_i = observation of i^{th} individual

N = total number of observations

ii. Range

It is the difference between largest and the smallest measurements of the individuals in the sample.

iii. Standard error

A measure of the mean difference between sample estimate of mean and the population parameter (μ) i.e. it is the measure of uncontrolled variation present in the sample which is estimated by dividing the standard deviation by the square root of number of observations in the sample.

$$\textbf{SE} = \frac{\textbf{SD}}{\sqrt{N}}$$

Where,

SD = standard deviation

N = total number of observations

iv. Chi-square test of goodness of fit

Chi-square test was used to test the goodness of fit of the observed ratio of segregation for flowering time based on data of F_2 population. To test the goodness of fit, suggested formula by Panse and Sukhatme (1989) was used.

Hypothesis tested:

H_o: Progeny segregate in the ratios tested.

H_1: Progeny does not segregate in the ratios tested.

The chi-square (χ^2) test statistic is defined by the following equation.

$$X^2 = \sum \frac{(O-E)^2}{E}$$

Where,

O = frequency of observed phenotypes

E = frequency of expected phenotypes

Chi-square probability distribution table was used to find p-value with the given chi-square test statistic at k-1 degrees of freedom (where k = Number of phenotypic classes). A p-value of 0.05 is used as cut-off between significant and not-significant results. If the p-value is less than or equal to 0.05 probability level, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

v. Correlation coefficients

Correlation refers to the degree and direction of association between two or more variables. Correlation coefficient (r) measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement of correlated traits. Its values vary between -1 (perfect negative correlation) and +1 (perfect positive correlation). Simple correlation coefficient between flowering time and yield contributing traits were calculated with SPSS software (SPSS Inc., 2015) by using the formula suggested by Panse and Sukhatme (1989).

$$r_{xy} = \frac{\sigma_{xy}}{\sqrt{\sigma_x^2 . \sigma_y^2}}$$

Where,

r_{xy} = Correlation coefficient between X and Y

σ_{xy} = Covariance of X and Y

σ_x^2 = Variance of X and σ_y^2 = Variance of Y

Significance of correlation coefficients was tested at (n-2) degrees of freedom using ‘t’ table from Fisher and Yates at 0.05 and 0.01 probability levels.

3.4 Construction of Genetic linkage map

3.4.1 Plant material

Four F_2 populations developed by crossing early flowering lines ICCV 96029, ICC 5810, BGD 132 and ICC 16641 with late flowering common male parent CDC Frontier were used for constructing genetic linkage map and to identify QTLs for flowering time. For this, 2.5 g of fresh leaf tissue was collected from 20 days old seedlings of parents, 190 F_2 plants in the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier and 146 F_2 plants in the cross ICC 16641 × CDC Frontier.

3.4.2 Genomic DNA isolation

DNA extraction was carried out by following high-throughput mini-DNA extraction protocol (Mace *et al.*, 2003) as given below.

i. Reagents required

1. 3 % CTAB (Cetyl Trimethyl Ammonium Bromide) buffer having 100 mM Tris, 1.4M NaCl, 20 mM EDTA and 3 % CTAB. The pH was adjusted to 8.0 using HCl. Just before use, mercaptoethanol (0.17 %) was added. It was used as extraction buffer.
2. Chloroform-isoamyl alcohol mixture (24:1) stored in the dark at room temperature.
3. Ice-cold isopropanol
4. RNase-A (10 mg/ml) dissolved in solution containing 10 mM Tris (pH 7.5) and 15 mM NaCl stored at –20 °C; working stocks were stored at 4 °C.
5. Phenol-chloroform-isoamyl alcohol mixture (25:24:1)
6. 3 M sodium acetate (pH 5.2)
7. Ethanol (absolute and 70 %)
8. $T_1E_{0.1}$ buffer (10 mM Tris and 1mM EDTA)
9. $T_{10}E_1$ buffer (0.5 M Tris and 0.05 M EDTA)

ii. Sample preparation

- Leaves were collected from 20 days old seedlings.
- Leaf tissues of 70-100 mg was placed in 12 × 8-well strip tube with strip cap (Marsh Biomarket, New York, USA) in a 96 deep-well plate together with two 4 mm stainless steel grinding balls (Spex CertiPrep, USA).

iii. CTAB extraction

- A volume of 450 µl of pre-heated (at 65 ºC for half an hour) extraction (CTAB) buffer was added to each extraction tube containing leaf sample and secured with eight strip caps.
- Samples were homogenized in a tissue lyser, EZ-LYZER (Genetix, BiotecAsia pvt. Ltd.) following the manufacturers' instructions at 500 strokes/min for 2 times at 2 min interval.
- Plate was fitted into locking device and incubated at 65 ºC for 10 min with shaking at periodical intervals.

iv. Solvent extraction

- A volume of 450µl of chloroform-isoamyl alcohol mixture (24:1) was added to each tube and mixed thoroughly by inverting.
- The samples were centrifuged at 5500 rpm for 10 minutes (Sigma centrifuge model 4K15C with Qiagen rotor model NR09100: 2 × 1120 g SW). The aqueous layer (approximately 300 µl) was transferred to fresh strip tubes.

v. Initial DNA precipitation

- To the tube containing aqueous layer, 0.7 volumes (approximately 210 µl) of cold isopropanol (kept at –20 ºC) was added. The solutions were carefully mixed and the tubes were kept at –20 ºC for 10 minutes.
- The samples were centrifuged at 5000 rpm for 15 minutes.
- Supernatant was decanted from each sample and pellet was air dried for 20 min.

vi. RNase treatment

- In order to remove co-isolated RNA, 200 µl of low salt TE buffer ($T_1E_{0.1}$) and 3 µl of RNase-A (stock 10mg/µl) were added to each tube containing dry pellet and mixed properly.
- The solution was incubated at 37 ºC for 30 minutes.

vii. Solvent extraction

- After incubation, 200 µl of phenol-chloroform-isoamyl alcohol mixture (25:24:1) was added to each tube, carefully mixed by inverting twice and centrifuged at 5000 rpm for 10 minutes.
- The aqueous layer was transferred to fresh tubes and chloroform-isoamyl alcohol (24:1) mixture was added to each tube, carefully mixed and centrifuged at 5000 rpm for 10 minutes. The aqueous layer was then transferred to fresh tubes.

viii. DNA precipitation

- To the tubes containing aqueous layer, 15 µl (approximately 1/10th volume) of 3M sodium acetate (pH 5.2) and 300 µl (2 volume) of absolute ethanol (kept at –20 ºC) were added and the tubes were subsequently incubated at –20 ºC for 5 minutes.
- Following incubation, the box containing tubes was centrifuged at 6200 rpm for 15 minutes.

ix. Ethanol wash

- After centrifugation, supernatant was carefully decanted from each tube having ensured that the pellets remained inside the tubes and 200 µl of 70% ethanol was added to the tubes followed by centrifugation at 5000 rpm for 5 minutes.

x. Final re-suspension

- Pellets were obtained by carefully decanting the supernatant from each tube and then allowed to air dry for one hour.
- Completely dried pellets were re-suspended in 100µl of $T_{10}E_1$ buffer and incubated overnight at room temperature to allow the pellets to dissolve completely.
- Dissolved DNA samples were stored at 4 ºC.

3.4.3 DNA quantification

The extracted DNA was quantified by loading the samples on 0.8 % agarose gel containing 0.5 µl/10 ml Ethidium bromide (10 mg/ml). After DNA quantification, the tubes containing DNA were labelled and stored at -20 ºC as stocks. The DNA was normalized to 5 ng/µl concentration with visual comparison by loading DNA samples with the standard λ DNA molecular weight markers (2.5 ng/µl, 5 ng/µl and 10 ng/µl) on 0.8 % agarose gel. Diluted DNA was used for PCR reactions at a concentration of 5 ng/µl.

3.4.4 Screening of SNP and SSR markers

A set of 625 SNPs (CKAMs) from Hiremath *et al.* (2012) and 89 SNPs from Jaganathan *et al.* (2013) was genotyped on a panel of 5 parental genotypes by KASPar genotyping assay for parental polymorphism study. The list of SNP markers is given in the Table3a and 3b. Also, a total of 472 SSRs including 146 SSR markers developed from BESs developed by Thudi *et al.* (2011) named as CaM-series, 124 novel SSR markers developed from SSRs-enrichment library (ICCM-series) by Nayak *et al.* (2010) and 135 SSR markers from Winter-series (TA-, TAA-, GA- and GAA-series) were used to screen the parents of mapping populations *viz.,* ICCV 96029, ICC 5810, BGD 132, ICC 16641 and CDC Frontier. In addition, a set of 57 SSR markers developed by Lichtenzveig *et al.* (2005) named as "H-series" and 10 SSR markers (NCPGR-series) developed by Sethy *et al.,* (2006) and Gaur *et al.* (2011) were used to screen for parental polymorphism (Table 4a - 4e).

3.4.5 KASPar SNP genotyping assay

2.5 µl of genomic DNA (5 ng/µl), 2.5 µl 2X KASP reaction mix and 0.055 µl assay mix of primers (12 µM each allele-specific forward primer and 30 µM reverse primer) were mixed in each well of a PCR plate (KBiosciences protocol). PCR with KASP thermal cycling program was carried out as: 94 ºC at 15 min (initial activation); 10 touchdown cycles of 94 ºC for 20 s and 65.57 ºC for 60 s (dropping 0.8 ºC per cycle); 26 cycles at 94 ºC for 20 s and 57 ºC at 60 s; and final holding at 10 ºC.

3.4.6 Analysis of genotypic data

The fluorescence endpoint reading of reactions was done using TECAN microplate reader (Infinite F200 Pro, Austria). Further details on principle,

procedure and chemistry of the KASPar assay are available at http://dna.uga.edu/wp-content/uploads/2013/12/KASPar-SNP-Genotyping-Manual-KBioscience.pdf. Genotyping data obtained based on the florescence detected from the KASPar assay was graphically viewed (Plate 3) and analysed through the KlusterCaller Version 3.4 software (http://results.lgcgenomics.com/software/klustercaller/).

3.4.7 Amplification of SSR markers

PCR reactions with final reaction mixture of 12 µl were conducted in 96-well and 384-well micro-titer plates in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) DNA thermocycler. The reaction mixture contained final concentration of 1 µl template DNA (5 ng/µl), 1.0 µl of 2 mM dNTPs, 0.5 µl of M-13 tailed forward and 1 µl of reverse primer, 1 µl of M-13 labelled primer (6FAM/NED/PET/VIC), 0.50 µl of $MgCl_2$, 0.05 µl of 500U Taq DNA polymerase (AmpliTaq Gold) and 1.0 µl of 1X PCR buffer (AmpliTaq Gold).

To amplify TA, TAA, GA, GAA and H-series markers, the two touch-down PCR profiles 60-55 ºC and 55-45 ºC were used. The PCR profile 60-55 ºC was with an initial denaturation for 15 min at 94 ºC, followed by 5 touch-down PCR cycles comprising of 94 ºC for 20 seconds (s), 60 ºC for 20 s and 72 ºC for 30 s were performed. These cycles were followed by 35 cycles of 94 ºC for 10 s with constant annealing temperature of 56 ºC for 20 s and 72 ºC for 30 s and a final extension was carried out at 72 ºC for 20 min.

PCR profile 55-45 ºC was with an initial denaturation for 15 min at 94 ºC, followed by 10 touch-down PCR cycles comprising of 94 ºC for 20 s, 55 ºC for 20 s and 72 ºC for 30 s were performed. These cycles were followed by 35 cycles of 94 ºC for 10 s with constant annealing temperature of 48 ºC for 20 s and 72 ºC for 30 s and a final extension was carried out at 72 ºC for 20 min.

For ICCM, CaM and NCPGR-series markers, a touch-down PCR profile (61-51 ºC) with an initial denaturation for 15 min at 94 ºC, followed by 10 touch-down PCR cycles comprising of 94 ºC for 20 s, 61 ºC for 20 s and 72 ºC for 30 s were performed. These cycles were followed by 35 cycles of 94 ºC for 10 s with constant annealing temperature of 54 ºC for 20 s and 72 ºC for 30 s and a final extension was carried out at 72 ºC for 20 min. The PCR products were stored at 4 ºC until further use.

3.4.8 Capillary electrophoresis

i. Sample preparation

After confirming the PCR amplification on 1.2 % agarose gel, the PCR products were size-separated by capillary electrophoresis using an ABI Prism 3730xl DNA analyzer (Applied Biosystems Inc.). For this, 1.5 µl PCR amplicon was mixed with 7 µl of Hi-Di formamide (Applied Biosystems, USA), 0.1 µl of the LIZ-500 size standard (Applied Biosystems, USA) and 8.0 µl of distilled water. The pooled PCR amplicons were denatured for 5 minutes at 95 °C and cooled immediately and resolved in automated 96 capillary ABI 3730xl DNA Analyser.

ii. SSR fragment analysis

The electrophoregram containing trace files produced from ABI Prism 3730 xl DNA analyzer were analysed using GeneMapper version 4.0 (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan 500™ LIZ®. GeneMapper version 4.0 software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped onto the curve and the sample data is converted from migration times to fragment size. The peaks were displayed with base pair size and height (amplitude) values in a chromatogram (Plate 4) and the allelic data were exported in to excel spread sheet for further analysis. The polymorphic markers were obtained based on the differences in base pair sizes between the parents of each cross.

3.4.9 Genotyping of mapping populations

The markers polymorphic between ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier were genotyped on 190 F_2s each and those polymorphic between ICC 16641 × CDC Frontier were genotyped on 146 F_2 plants. PCR amplification was carried out according to the conditions described in 3.4.7 using fluorescent-labelled primers (FAM/VIC/NED/PET). After checking the amplification on 1.2 % agarose, based on the amplicon sizes and florescent dyes used, the PCR products from each florescent dye (FAM/VIC/NED/PET) were pooled to construct PCR multi-pooling set in order to facilitate high-throughput multiplex capillary electrophoresis. Each

set consisted of four SSR markers with different labels and allele size. For pooling, 1.5 µl from each of PCR amplicon (with different dye or same dye with considerable difference in the amplicon sizes) were mixed with 0.1 µl of the LIZ-500 size standard (Applied Biosystems, USA), 3 µl of Hi-Di formamide and 7.0 µl of distilled water in a 384 well PCR plate and denatured at 95 °C for 5 minutes. Capillary electrophoresis of denatured pooled products was performed using ABI Prism 3730 DNA analyser (Applied Biosystems Inc.). Allele calling and exporting of the allelic data were carried out by using GeneMapper version 4.0 software (Applied Biosystems, California, USA).

3.4.10 Data scoring

The polymorphic markers were scored across segregating populations based on the amplicon sizes in the parents. Using GeneMapper software version 4.0, the allele of the female parent was always scored as 'A' irrespective of the size of the amplicon. Similarly, the allele of the male parent was always scored as 'B' and the genotypes having alleles from both the parents were designated as 'H' and missing data was scored as '-'. Therefore, the allele scoring was carried out as follows:

'A' – Allele of female parent (ICCV 96029, ICC 5810, BGD 132 and ICC 16641)

'B' – Allele of male parent (CDC Frontier)

'H '– Heterozygous (presence of both parental alleles)

'-' – Missing data (failed amplification)

3.4.11 Linkage map construction

Genotype data were assembled for all segregating makers on all F_2 individuals from each mapping population and linkage analysis was performed using JoinMap version 4.0 using ''Regression mapping algorithm'' (Van Ooijen, 2006). Before linkage analysis, marker segregations in all populations were subjected to Chi-square test ($P < 0.05$) to assess deviations from the expected Mendelian segregation ratio of 1:2:1 by using the ''Locus genotype frequency'' function. Markers showing segregation distortion were also integrated into the map.

Map calculations were performed with parameters like LOD value 3.0, recombination frequency 0.40 and a maximum threshold value of 5 for the jump. The best

marker order was determined using the 'Ripple' function (value of 1). Recombination frequencies were converted to map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi, 1944). Placement of markers into different linkage groups (LGs) was done with ''LOD groupings'' and ''Create group using the mapping tree'' commands. Mean χ^2 contributions or average contributions to the goodness of fit of each locus were also checked to determine the best fitting position for markers in genetic maps. The markers showing negative map distances and highly distorted in mean χ^2 values were discarded. Final maps were drawn with the help of MapChart version 2.2 (Voorrips, 2002). The linkage groups were named according to Varshney *et al.* (2014).

For the construction of consensus map, linkage groups previously obtained at LOD 3.0 were used to combine the data from the separate populations. Once we had established different ''group nodes'' in all populations, data were combined from four crosses. ''Combine Maps'' command was used to align genetic maps obtained in different populations for a visual inspection of the marker order. Groups with at least two markers in common could be combined using the ''Combine groups for Map Integration'' function. The heterogeneity test among different pairwise recombination frequencies provided information of discrepancies in different populations and allowed to decide if some could be discarded.

3.5 QTL mapping

The linkage map data and phenotyping data was used for QTL analysis using inclusive composite interval mapping (ICIM) through the QTL-ICiMapping software version 4.0 (Wang *et al*., 2014). ICIM-Add mapping performs first a stepwise regression to identify the most significant markers and marker-pair multiplications at 0.001 probability level and the scanning step of 1 cM. Then a one-dimensional scanning or interval mapping was conducted to identify additive QTL. The threshold levels to declare significant QTL were determined by performing 1000 permutations by maintaining the chromosome-wise type I error rate of 0.05 (Churchill and Doerge, 1994). The LOD score peaks were used to estimate the most likely position of QTL on the linkage map. The amount of variation explained by marker was determined using the coefficient of determination (R^2) value. In this study, a QTL that explains more than 10 %of total phenotypic variance is considered as major QTL.

3.6 Identification of candidate genes

Primer/gene sequences were blasted (BLASTN) against the CDC Frontier genome (Varshney *et al.,* 2013). For each primer pair, the best hit was selected based on the e-value and percent identity. The primer pairs not resulting in best hits on respective chromosomes were left out. Regions with >5 Mb on the chromosomes were filtered out and the selected regions were further analysed to find genes present in these regions. Gene ontology was carried out by the BLAST of these genes against the SWISS-PROT and TrEMBL database. The resulted genes are assigned with a Uniprot ID and the functions for these genes were retrieved from Uniprot KB database. These genes were further assigned into the classification of molecular function, cellular component and biological process.

Experimental Results

IV. EXPERIMENTAL RESULTS

4.1 Genetics of flowering time genes

4.1.1 Flowering time of parents, F_1 and F_2 populations

The flowering time of parental lines varied from 25-65 days (Table 5). All the female parents *i.e.,* ICCV 96029, ICC 5810, BGD 132 and ICC 16641 started flowering on 25, 28, 28 and 29 days respectively. The early-flowering parent ICCV 96029 flowered in 25 to 28 days and ICC 5810 in 28 to 30 days with a mean flowering time of 26.8 and 28.3 days, respectively. Whereas, the lines BGD 132 and ICC 16641 completed flowering within 29 days with mean of 28.5 and 29.0 days to first flower, respectively. Also, all the female lines were very early to mature with mean days to maturity of 75.5, 77.2, 78.9 and 79 days, respectively. The male parent, CDC Frontier was very late to flower having mean flowering time of 66.9 days with a range of 65-68 days and reached physiological maturity in 108 days. The F_1s of all the four crosses were late to flower with mean flowering time of 61.2, 54.1, 53.3 and 60.8 days in the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier respectively, indicating dominance of lateness in all four crosses studied (Table 6).

The F_2 populations of all the crosses showed a high range for flowering time (Table 6). The mean days to first flower in the F_2 population of the cross ICCV 96029 × CDC Frontier was 48.89 with a range of 25-77 days. F_2 individuals of the cross ICC 5810 × CDC Frontier started flowering from 27 to 76 days with mean flowering time of 49.47 days. While the crosses BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier had mean flowering time of 49.04 and 52.93 with range 26-75 and 26-82 days, respectively.

4.1.2 Frequency distribution for flowering time in F_2 populations

The F_2 populations showed high variation for flowering time (Table 6). The majority of the individuals in all the crosses fell between the two parents for time to flowering, but a few were one to two days earlier than the early parent and others were up to two weeks later than the late parent (Fig. 1-4). Inspection of flowering time data revealed that the distribution was skewed towards late flowering parent.

The quantitative data recorded on flowering time in each F_2 population were converted into qualitative data based on the distribution pattern in F_2. We classified the phenotypic classes (early and late) based on 'natural' break points in the distribution frequencies within each population. For example, natural break points of flowering time in cross ICCV 96029 × CDC Frontier is 40 days (Figure 1). Similarly, the break points were also observed in the crosses BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier for number of days to flowering (Figure 3&4) with no/few plants observed at 40^{th} day, thus separating the two phenotypic classes. Accordingly, all the F_2 plants that flowered up to 40 days from sowing were classified as early and those which flowered at 41 days and after as late in these crosses. Whereas for the cross ICC 5810 × CDC Frontier, the break point was observed at 45 days with a drop in frequency of individuals flowering at 45 days (Figure 2). Hence, all the F_2 plants that flowered up to 45 days of sowing were classified as early and those which flowered thereafter as late in this cross. The distribution of flowering time data in F_2 populations of these four crosses followed a bimodal pattern with clear break between two peaks, facilitating classification of phenotypic data into early and late flowering groups. The area under the two peaks was not equal in all the crosses with majority of individuals falling in late group. Thus, strongly suggesting involvement of major genes controlling flowering time trait in these crosses.

4.1.3 Segregation of flowering time genes in F_2 populations

The F_2 segregation analysis of flowering time trait was carried out in the four crosses. The expected values corresponding to the observed values for late: early flowering plants in the F_2s were calculated on the basis of the assumed Mendelian ratio. The deviations of these were subjected to the chi-square test in order to determine the goodness of fit.

Classification of the flowering time data into two classes - early and late in all the crosses did not deviate significantly from the assumed Mendelian ratio (Table 7). The 190 F_2 plants of the cross ICCV 96029 × CDC Frontier segregated into 138 late: 52 early. These numbers are in good fit with the expected ratio of 3 late: 1 early (χ^2 = 0.57, P = 0.50-0.30). The cross BGD 132 × CDC Frontier consisted of 190 F_2 individuals which fell into two phenotypic classes *i.e.* late and early flowering plants in 3:1 ratio with 143 late: 47 early types (χ^2 = 0.01, P = 0.95-0.90). Similarly, in the cross ICC 16641 ×

CDC Frontier also the 146 F_2 plants segregated into 110 late and 36 early flowering plants. This ratio is in good fit with expected 3:1 ratio (χ^2 = 0.01, P = 0.95-0.90). Thus, time to flowering in the crosses ICCV 96029 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier is controlled by a single major gene indicating simple inheritance of this trait.

Whereas in the cross ICC 5810 × CDC Frontier, 190 F_2 individuals segregated into 108 late (later than 45 days) and 82 early (45 days or earlier) flowering types which did not fit into 3:1 ratio for monogenic control. A more detailed genetic analysis revealed that the classification of the time to flowering data into late and early flowering did not deviate significantly from a 9 late: 7 early flowering segregation ratio (χ^2 = 0.03, |P = 0.90-0.80).This shows that time to flowering in this cross is governed primarily by two genes with duplicate recessive epistasis between them.

4.1.4 Segregation of flowering time genes in F_3 progenies

The F_2 segregation pattern was also confirmed by studying 164, 174, 182 and 102 F_3 progeny rows of the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier, respectively. Each progeny row comprising 20 plants was observed for flowering time on individual row basis at regular intervals and classified them as non-segregating and segregating types (Plates 5-8). This data was subjected to the chi-square test (Table 8).

In the cross ICCV 96029 × CDC Frontier, all the 37 early flowering F_2 plants did not segregate in F_3 progenies for flowering time (χ^2 = 0, P=1.0). Of the 127 late flowering plants, 87 segregated into late and early flowering plants, 40 progenies showed no segregation and produced only late flowering plants (χ^2 = 0.19, P = 0.70-0.50) in F_3.

Whereas in the cross BGD 132 × CDC Frontier, all the 44 early flowering F_2 plants did not segregate for flowering time in F_3 progenies (χ^2 = 0, P=1.0). 94 out of 138 late flowering F_2 plants segregated into late and early flowering plants and remaining 44 progenies produced only late flowering plants (χ^2 = 0.13, P = 0.80-0.70).

Similarly in the cross ICC 16641 × CDC Frontier, all the 25 early flowering F_2 plants flowered early in F_3 progenies (χ^2 = 0, P=1.0). Of the 77 late flowering F_2 plants, 54 segregated and 23 progeny lines produced only late flowering plants (χ^2 = 0.41,

P = 0.70-0.50). These results in F_3 gave conclusive evidence of monogenic inheritance of flowering time genes in the crosses ICCV 96029 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier.

In case of ICC 5810 × CDC Frontier, 27 progenies out of 71 early flowering F_2 plants produced only early plants and remaining 44 progenies segregated for early and late plants (χ^2 = 0.67, P = 0.50-0.30). Whereas, 103 late flowering F_2 plants produced 16 F_3 progenies which are late without segregating, while 87 segregated to produce late and early plants (χ^2 = 2.04, P = 0.20-0.10) in F_3. Thus, the segregation pattern in F_3 progenies of this cross confirmed the digenic control of flowering time.

These results confirm that time to flowering in the crosses ICCV 96029 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier is controlled by a single major gene, indicating the simple inheritance of this trait. While in the cross ICC 5810 × CDC Frontier, it is under digenic control with complementary effect.

4.1.5 Inheritance of flower color

Inheritance of flower color (pink vs white flower) was studied in two crosses that involved pink flower (ICCV 96029 and ICC 5810) and white flower (CDC Frontier) as one of their parents.

4.1.5.1 Inheritance of flower color in the cross ICCV 96029 × CDC Frontier

The cross between ICCV 96029 (pink flower) and CDC Frontier (white flower) produced pink flowered F_1s indicating pink flower color is dominant over white flower color. The observed ratio of 153 pink: 37 white flowered plants in F_2 that corresponded to the expected 3:1 ratio (χ^2=3.09, P = 0.10-0.05) (Table 9). These results suggested that a single gene control difference in the flower color between the parents of this cross. The F_2 segregation was confirmed by studying the breeding behaviour of 183 F_3 families. All the 34 white flowered F_2 plants did not segregate in F_3 progenies (χ^2=0, P = 1.0). Out of 149 pink flowered F_2 plants, 102 segregated into pink and white flowered plants in F_3 and 47 progenies showed no segregation which produced only pink flowered plants (χ^2=1.24, P = 0.30-0.20) (Table 10). These results further confirmed monogenic control of flower color trait. The segregation pattern of each F_3 family (pink or white or segregating) was used to determine the genotype of respective F_2 individuals (as A or B or H) and this data were used for linkage analysis.

4.1.5.2 Inheritance of flower color in the cross ICC 5810 × CDC Frontier

The cross between ICC 5810 (pink flower) and CDC Frontier (white flower) produced pink flowered F_1s. The F_2 data (N=190 plants) fitted well to the ratio of 3: 1 with 146 pink and 44 white flowered plants (χ^2=0.34, P = 0.70-0.50) (Table 9). In this case also 185 F_3 families were studied for segregation pattern of flower color. Out of 143 pink flowered F_2 plants, 101 segregated to produce pink and white flowered progenies in F_3 and remaining 42 did not show any segregation which produced only pink flowered plants (χ^2=1.17, P = 0.30-0.20). Whereas, 42 white flowered F_2 plants bred true to produce only white flowered plants in F_3 (χ^2=0, P = 1.0) (Table 10). These results clearly establish that flower color is controlled by single locus in this cross. Here also the F_3 phenotypic data was used to determine the genotype of F_2 and used for linkage analysis.

4.1.6 Inheritance of double podding trait

Inheritance of double podding trait was studied in the cross ICCV 96029 × CDC Frontier involving double podding (ICCV 96029) and single podding (CDC Frontier) parents. All the F_1s of this cross were single podded, indicating single podding is dominant over double podding trait. There were 146 single podded and 44 double podded plants in F_2. This gave a good fit to the monogenic 3:1 ratio (χ^2=0.34, P = 0.70-0.50) (Table 11). F_3 segregation pattern indicated that out of 138 single podded F_2 plants, 93 F_3 families segregated into single and double podded plants and 45 F_3 progenies produced only single podded plants without segregation (χ^2=0.03, P = 0.90-0.80). While all the 45 double podded F_2 plants did not show any segregation and produced only double podded plants in F_3 (χ^2=0, P = 1.0) (Table 12). These results confirmed monogenic control of single/double podding trait. The segregation pattern of each F_3 family (double podded or single podded or segregating) was used to determine the genotype of respective F_2 individual (A or B or H) and used in linkage analysis.

4.2 Genetic linkage mapping

4.2.1 Parental polymorphism and markers for mapping

A set of 625 SNPs (CKAMs) from Hiremath *et al.* (2012) and 89 SNPs (KBD) from Jaganathan *et al.* (2013) and a total of 472 SSR markers (from Thudi *et al.,* 2011; Nayak *et al.,* 2010; Huttel *et al.,* 1999; Winter *et al.,* 1999; Lichtenzveig *et al.,* 2005; Sethy *et al.,* 2006 and Gaur *et al.,* 2011) were screened for polymorphism between the

parents of four mapping populations. The polymorphism status of SNP and SSRs is presented here (Table 13 and 13a).

4.2.1.1 SNP markers

Out of the 714 SNPs screened, 49 (6.86%), 44 (6.16%), 36 (5.04%) and 29 SNPs (4.06%) showed polymorphism between parents of the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier, respectively (Table 14).

4.2.1.2 SSR markers

a) CaM-series

Initially, 146 CaM markers were screened on five chickpea genotypes *i.e.* ICCV 96029, ICC 5810, BGD 132, ICC 16641 and CDC Frontier. A total of 112 (76.71%) SSR markers produced scorable amplicons and 18 (12.33%) markers did not yield any scorable amplicon. About 16 (10.95%) markers produced multiple amplicons which were difficult to score. A total of 18 (12.33%) SSR primer pairs showed polymorphism between the parents of cross ICCV 96029 × CDC Frontier. A set of 18 (12.33%) markers showed polymorphism between the parents of the cross ICC 5810 × CDC Frontier. Similarly, 16 (10.96%) and 14 (9.59%) primer pairs revealed parental polymorphism between the parents of the crosses, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier, respectively.

b) ICCM-series

Out of 124 ICCM markers used for parental polymorphism survey, 105 (84.68%) markers produced amplification, while 19 (15.32%) markers did not amply. A set of 8 (6.45%) markers exhibited polymorphism between the parents of the cross ICCV 96029 × CDC Frontier. Similarly for the parents of the crosses ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and BGD 132 × CDC Frontier, total number of polymorphic primer pairs observed was 5 (4.03%), 5 (4.03%) and 7 (5.65%) respectively.

c) Winter-series

A set of 135 published markers from Dr. Peter Winter's group (Huttel *et al.,* 1999 and Winter *et al.,* 1999) were screened on all the parental genotypes that gave 95 (70.37%) scorable amplicons and 53 (39.30%) markers showed polymorphism for cross

ICCV 96029 × CDC Frontier and 56 (41.48%) markers found polymorphic for the cross ICC 5810 × CDC Frontier. Likewise, 48 (35.56%) and 51 (37.78%) markers exhibited polymorphism for the crosses BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier respectively.

d) H-series

Another set of 57 H-series markers were used that are developed from screening BAC and BIBAC libraries of chickpea by Lichtenzveig *et al.* (2005). Out of these, 45 (80.36%) markers gave scorable markers and a total of 16 (28.07%), 13 (22.81%), 17 (29.82%) and 16 (28.07%) markers were found polymorphic between the parents of the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier respectively.

e) NCPGR-series

Furthermore, 10 published markers from NIPGR, New Delhi (Sethy *et al.*, 2006 and Gaur *et al.*, 2011) were screened for parental polymorphism. As a result, 9 (90%) markers gave scorable amplicons. Out of which, 5 (50%) revealed polymorphism for the cross ICCV 96029 × CDC Frontier, 3 (30%) markers for cross ICC 5810 × CDC Frontier. Similarly, 4 (40%) and 5 (50%) markers were found polymorphic for the crosses BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier respectively.

The list of polymorphic SSR markers with their respective amplicon size used for the linkage analysis are given in Table 15a to 15d. In this study, a very low number of polymorphic SNPs were obtained and most of these were located within a narrow (i.e. 10-16 Mb) region on chromosome Ca4 of chickpea genome. Therefore, we considered these as less informative and excluded from further genotyping and linkage analysis. Hence, the F_2 mapping population (N=190) of the cross ICCV 96029 × CDC Frontier was genotyped with 76 polymorphic SSR markers. These 76 SSR markers along with two morphological markers i.e. flower color (*B/b*) and double podding ('*Sfl/sfl*') trait were used for linkage map construction. A total of 77 polymorphic SSR markers were screened on the F_2 individuals (N=190) of the cross ICC 5810 × CDC Frontier and used for linkage analysis along with one morphological trait i.e. flower color (*B/b*). A total of 68 polymorphic SSR markers was screened on each F_2 population of the crosses BGD 132 × CDC Frontier (N=190) and ICC 16641 × CDC Frontier (N=146) respectively and the genetic linkage map was developed using JoinMap v 4.0.

4.2.2 Segregation distortion analysis

The segregation of individual SSR markers was tested for goodness of fit to the expected 1:2:1 ratio by χ^2 test ($P < 0.05$). Out of 77 markers, 66 (85.71%) markers gave a good fit to the expected segregation ratio in the cross ICCV 96029 × CDC Frontier and only 11 (14.29%) markers showed χ^2 values significant at 5% level showing segregation distortion (Table 16). In the cross ICC 5810 × CDC Frontier, 63 (82.89%) markers segregated according to the expected Mendelian ratio, while 13 (17.11%) markers significantly deviated from the expected ratio (Table 17). Similarly in the cross BGD 132 × CDC Frontier, 66 markers (97.06%) out of 68 showed a good fit to the expected 1:2:1 segregation ratio, while only 2 (2.94%) markers distorted from the expected ratio (Table 18). In the cross ICC 16641 × CDC Frontier, only 4 (5.97%) out of 67 markers significantly deviated from the expected segregation ratio and remaining 63 (94.03%) markers segregated perfectly into the expected ratio 1:2:1 (Table 19). All the polymorphic markers screened on the mapping populations including those exhibited segregation distortion were used for linkage map construction for each cross separately.

4.2.3 General features of the linkage map

The genotyping data of the polymorphic markers were compiled and these data were used to construct the intraspecific genetic map for each population using JoinMap v 4.0 programme. The features of genetic maps are presented crosswise in the following sections.

4.2.3.1 Genetic linkage map of the cross ICCV 96029 × CDC Frontier

A total of 78 (16-CaM series, 5-ICCM series, 11-H series, 4-NCPGR series, 40-Winter series and 2 morphological) markers were used to construct the intra-specific genetic map. The developed intra-specific genetic map consisted of 77 marker loci spanning a genetic distance of 262.25 cM (Figure 5) with an average inter marker distance of 3.41 cM. At LOD-score of 3.0, one marker (TA76s) remained unlinked to any of the linkage groups but this marker did not deviate from Mendelian ratio of 1:2:1. Number of marker loci mapped ranged from 5 (on CaLG01 and CaLG08) to 14 (on CaLG06). The group CaLG06 of this intra-specific map covered the highest genetic map distance of 70.44 cM followed by the CaLG04 (50.68 cM). While the group CaLG05 with 5.46 cM distance covered the least map distance and it is the densest group with

average marker density of 0.55 cM. On the other hand, CaLG08 had only five widely spaced markers with inter marker distance of 9.04 cM. The '*B/b*' locus for flower color mapped between the markers ICCM0192a and GAA47 on CaLG04, while double podding locus '*Sfl/sfl*' was mapped between the markers TR44 and GA34 on CaLG06. Linkage groups CaLG01, CaLG02, CaLG03, CaLG04, CaLG05, CaLG06 and CaLG07 showed non-random distribution of markers with dense sub-clusters either at the central region or at distal ends. The number of marker loci mapped along with the respective map distances and inter-marker distances of this intra-specific genetic map is given in Table 16.

4.2.3.2 Genetic linkage map of the cross ICC 5810 × CDC Frontier

The genetic map of this cross consists of 76 segregating markers (14-CaM series, 4-ICCM series, 11-H-series, 3-NCPGR series, 43-Winter series and one morphological marker) distributed across the 8 linkage groups (CaLGs) and covered 335.74 cM with an average marker density of one marker per 4.42 cM (Table 17). At LOD-score of 3.0, two markers (TA76 and TA93) remained unassigned to any of the LGs even though they segregated according to expected Mendelian ratio 1:2:1. The CaLG05 is the smallest group in terms of map distance (6.74 cM) with 6 closely spaced markers, while the linkage group CaLG04 is the largest group with 14 markers mapped covering 88.88 cM of the map distance. The other large groups include CaLG06 (76.28 cM), CaLG08 (56.62 cM) and CaLG03 (40.88 cM). The linkage group CaLG08 is the sparsest group having only 5 widely spaced markers at 11.32 cM. On the other hand, CaLG07 is the densest group with average inter marker distance of 0.88 cM. In this population also the '*B/b*' locus for flower color was mapped on CaLG04 between the markers GAA47 and TR33 (Figure 6). Dense sub-clusters of markers were observed either in the central region or at distal ends of the groups CaLG01, CaLG02, CaLG03, CaLG04, CaLG05, CaLG06 and CaLG07.

4.2.3.3 Genetic linkage map of the cross BGD 132 × CDC Frontier

The general features of the intraspecific genetic map of the cross BGD 132 × CDC Frontier is given in Table 18. All the 68 markers used (12-CaM series, 3-ICCM series, 15-H-series, 3- NCPGR series and 35-Winter series) were mapped onto 8 linkage groups (CaLGs) that spanned a total of 311.10 cM at a LOD-score of 3.0. The smallest LG (CaLG01) was made of 4 markers. Whereas, the largest (CaLG03) comprises 15 markers

spanning 80.12 cM map length. The linkage group CaLG05 is having smallest map distance of 7.88 cM. The average marker density in CaLGs is 4.57 cM. CaLG05 is the densest group with average marker density of 0.99 cM followed by CaLG02 (1.30 cM). The linkage group CaLG01 is sparsest group having only 4 widely spaced (8.19 cM) markers (Figure 7). In this map also the markers were distributed unevenly in all the groups.

4.2.3.4 Genetic linkage map of the cross ICC 16641 × CDC Frontier

The intraspecific linkage map of this cross consisted of 67 SSR markers (14-CaM series, 5-ICCM series, 9-H series, 5- NCPGR series and 34-Winter series) that are mapped onto eight linkage groups spanning 385.13 cM of the chickpea genome at an average marker density of 5.75 cM (Table 19). CaLG03 represented the largest linkage group in terms of the number of markers mapped (14). While, CaLG04 was covered largest map distance (74.75 cM) with 13 markers mapped on it. On the other hand, CaLG05 was the shortest among the eight linkage groups having only 10.74 cM containing 9 markers and it is the densest group with average marker density of 1.19 cM. CaLG03 is the second most-dense linkage group which had a marker density of 4.19 cM. While, CaLG08 was made up of five widely spaced markers and was the sparsest (9.25 cM) linkage group. Only one marker i.e., TA93 was unassigned to any of the linkage groups, but it showed Mendelian inheritance in the population. Uneven marker distribution was observed in CaLG05 and CaLG06 having dense sub-cluster at the central region (Figure 8). Whereas, in CaLG01, CaLG02, CaLG03, CaLG04 and CaLG07 distal sub-cluster was observed.

4.2.3.5 Consensus map developed from four crosses

The developed consensus map comprised of 111 loci, including 109 SSRs and two morphological traits (flower color and single/double podding). All these loci were distributed on eight linkage groups, covering 387.63 cM with an average density of 3.28 cM/marker (Table 20). The length of CaLGs ranged from 6.94 cM (CaLG05) to 82.23 cM (CaLG04). The density of markers on the map ranged from 0.53 cM/marker on CaLG05 to 4.57 cM/marker on CaLG04. The flower color locus (*B/b*) was mapped on CaLG04 of the consensus map between the SSR markers ICCM0192a and TR33. While, the gene for double podding (‘*Sfl/sfl*’) was assigned to CaLG06 between the markers TR01 and TR44 (Figure 9).

A detailed comparison between consensus map and population specific/ component genetic maps was made. This revealed a very high congruency in terms of grouping of markers on the respective LGs. The groups CaLG01 and CaLG02 each had one marker common for the four crosses. The CaLG03 showed five markers in common for all the crosses. The linkage group CaLG04 also had five markers common. In addition, the gene for flower color was found common in two populations i.e. ICCV 96029 × CDC Frontier and ICC 5810 × CDC Frontier. The linkage groups CaLG05 and CaLG06 each had four markers that are common for all four crosses. Similarly, the group CaLG07 had six markers in common and two markers in CaLG08 were common for all four maps. In general, a total of 28 markers were common between four populations, 33 markers were common for three crosses and 26 markers was found common in two crosses (Table 20).

4.3QTL mapping for flowering time genes

Phenotypic data of flowering time and the genotypic data were analysed for identification of the putative QTLs using QTL-ICiMapping software version 4.0 (Wang *et al.*, 2014) following inclusive composite interval mapping (ICIM-ADD) approach. The details of QTLs identified for flowering time are presented in Table 21 and also explained population wise in the following sections.

4.3.1 QTLs for flowering time in the cross ICCV 96029 × CDC Frontier

All the linkage groups were scanned at LOD threshold of 2.5 with 1000 permutations (P<0.05%) using QTL-ICiM software. ICIM-ADD method detected presence of major QTL for flowering time "*Qefl1*-2" on CaLG04 between GAA47 and ICCM0192a (LOD = 5.95) explaining 12.34% of the phenotypic variation (Figure 5 and 10). Single marker regression analysis revealed that GAA47 within this QTL was significantly associated with flowering time and accounted for 14.27% of total phenotypic variation. Another minor QTL "*Qefl1*-1" was also detected on CaLG03 (LOD = 3.55) between CaM1122 and TR13. This QTL showed a small phenotypic effect explaining only 7.07% of phenotypic variance. The estimated additive effect was -4.44 and -5.42, respectively. Therefore, the QTL "*Qefl1*-2" is considered as major (showing >10% phenotypic variation) QTL for flowering time and the associated marker i.e. GAA47 can be used further for marker-assisted selection (MAS).

4.3.2 QTLs for flowering time in the cross ICC 5810 × CDC Frontier

A total of four QTLs (*Qefl2*-1, *Qefl2*-2, *Qefl2*-3 and *Qefl2*-4) was identified for flowering time in this population (Figure 6 and 11). The QTL "*Qefl2*-1" flanked by markers TA122 and TA30 was identified on CaLG01 with LOD value of 12.77 explaining 20.13% of total phenotypic variation. Another QTL on CaLG03, "*Qefl2*-2" explained the highest phenotypic variation (25.19%) with a LOD value of 16.82.This QTL was flanked by markers CaM1358 and TA142. Third QTL, "*Qefl2*-3" was detected on CaLG04 (LOD = 9.10) between the markers NCPGR21 and GAA47 with 10.44% phenotypic variation. Similarly, the QTL "*Qefl2*-4" was identified between the markers GA6 and TA118 on CaLG08 (LOD = 17.68) which accounted for 25.57% of total phenotypic variation. The additive effect detected for all these QTLs is -3.26, -6.65, -4.4 and -7.03, respectively. These results indicate that all the four QTLs (*Qefl2-1*, *Qefl2-2*, *Qefl2-3* and *Qefl2-4*) was found to be major (showing >10% phenotypic variation).

4.3.3 QTLs for flowering time in the cross BGD 132 × CDC Frontier

One major QTL (*Qefl3*-3) and two minor QTLs (*Qefl3*-1, *Qefl3*-2) were detected for the flowering time in this cross (Figure 7 and 12). The major QTL "*Qefl3*-3" was identified on CaLG08 flanked by the markers TA127 and H1D24 with LOD value of 44.83. Within the QTL region, the marker H1D24 was found closely associated. This QTL had a large contribution to the phenotypic variance explaining 65.35% of the total variation. The additive effect estimated for this QTL was -13.0. The remaining two minor QTLs (*Qefl3*-1 and *Qefl3*-2) were detected on CaLG03 defined by marker intervals CaM1515-TR13 and TA142-TA64, respectively. These QTLs explained phenotypic variation of 4.28% (at LOD = 5.17) and 3.99% (at LOD = 4.21) with estimated additive effect of -1.23 and -3.36, respectively. Based on these results, the QTL "*Qefl3*-3" is considered as the major QTL for flowering time in this cross.

4.3.4 QTLs for flowering time in the cross ICC 16641 × CDC Frontier

A single major QTL (*Qefl4*-1) for flowering time was observed on CaLG06 flanked by markers TA14 and TR44 in this cross (Figure 8 and 13). The marker, TR44 within this QTL was closely associated. The phenotypic variation contributed by this QTL was 88.19 % with a LOD value of 55.60. Also, a higher additive effect (-16.75) was

detected for this QTL. Therefore, this major QTL appears to be promising for introgressing into desired genetic backgrounds.

In general, seven major QTLs (which contributed more than 10% phenotypic variation) and three minor QTLs (with less than 10% phenotypic variation) were detected for flowering time in this study (Figure 14). A major QTL of the cross ICC 5810 × CDC Frontier (*Qefl2*-2) and a minor QTL of the cross BGD 132 × CDC Frontier (*Qefl3*-2) detected on CaLG03 had a common marker TA142 as one of two flanking markers explaining phenotypic variation ranging from 3.99-25.19%. Another two minor QTLs i.e. "*Qefl1*-1" and "*Qefl3*-1" of the crosses ICCV 96029 × CDC Frontier and BGD 132 × CDC Frontier respectively, were also identified on CaLG03 that shared TR13 as one of two flanking markers explaining 4.28-7.07% of phenotypic variation. Similarly, two major QTLs detected on CaLG04 in the crosses ICCV 96029 × CDC Frontier (*Qefl1*-2) and ICC 5810 × CDC Frontier (*Qefl2*-2) had GAA47 as one of the markers flanking the QTL region contributing about 10.44-12.34% of total phenotypic variation. On CaLG08, two major QTLs were detected i.e. "*Qefl2*-4" (in the cross ICC 5810 × CDC Frontier) and "*Qefl3*-3" (in the cross BGD 132 × CDC Frontier) but at different genomic regions. While, the major QTL "*Qefl2*-1" identified on CaLG01 is unique for the cross ICC 5810 × CDC Frontier and the major QTL "*Qefl4*-1" detected on CaLG06 is unique for the cross ICC 16641 × CDC Frontier (Figure 9). In case if one of two flanking markers is common in more than one QTL of different cross, we have considered that region as only one genomic region that contains more than one QTL (followed from Varshney *et al.*, 2014). Therefore, these results suggest that the genomic regions on CaLG01, CaLG03, CaLG04, CaLG06 and CaLG08 may contain one or more key genes responsible for flowering time in chickpea.

4.3.5 Identification of candidate genes in QTL regions

After identification of QTLs for flowering time, identification of candidate genes in the QTL regions were done by aligning flanking SSR markers on CDC Frontier genome sequence assembly (Varshney *et al.*, 2013). The results of BLAST search indicated that out of 17 primer pairs, four primer sequences i.e. CaM1122, CaM1515, TA127, and H1D24 exhibited multiple hits on different chromosomes or scaffold regions. Hence, these markers were not analysed further. Whereas, remaining fourteen markers (GAA47, ICCM192a, TA122, TR13, TA30, CaM1358, TA142, NCPGR21, GA6,

TA118, TA64, TA14, and TR44) showed best hits on their respective chromosomes with high percent identity and low E-value (Table 22).

Analysis of BLAST hits revealed that SSR markers GAA47-ICCM192a on CaLG04 with the genome sequence identified a region of 0.7 Mb (starting at 8.2 Mb and ending at 8.9 Mb) on Ca4 chromosome. Another region of 18.3 Mb was identified between the markers TA122-TA30 with starting at 5.4 Mb and ending at 23.7 Mb on the chromosomeCa1. Similarly, the markers CaM1358-TA142 identified 8.1 Mb region with starting at 18.1 Mb and ending at 26.2 Mb on Ca3 chromosome. Likewise, the markers GAA47-NCPGR21 identified 1.8 Mb (starting at 8.2 Mb and ending at 10 Mb) on Ca4, GA6-TA118 identified 3.5 Mb (starting at 1.4 Mb and ending at 4.9 Mb) on Ca8 and TA142-TA64 identified 3.3 Mb (starting at 26.2 Mb and ending at 29.5 Mb) on Ca3. On Ca6, a region of 29.1 Mb (starting at 29 Mb and ending at 58.1 Mb) was identified between the markers TR44-TA14. The regions having >5 Mb were filtered out which resulted in two regions with relatively narrow region on Ca4 (0.7 Mb region between the markers GAA47-ICCM192a) and Ca8 (region of about 3.5 Mb on Ca8 within the markers GA6-TA118). The identified regions were further analysed which resulted in a total of 561 genes present on Ca4 and Ca8. Gene ontology analysis by the blast of these genes against the SWISS-PROT and TrEMBL database resulted in 554 genes (162 genes on Ca4 and 362 genes on Ca8 region) assigned with a Uniprot ID.

Based on Gene Ontology (GO) descriptions (UniProt database, UniProt-GO), all 554 genes (162 in the Ca4 and 392 in Ca8 region) were further assigned to three functional categories i.e. molecular function, cellular component and biological processes (Table 23). Out of 554 genes, 360 genes were assigned to “molecular function” category, 364 genes to “cellular component” category and 397 genes to “biological process” category. However, the sum of genes assigned to different functional categories (1121) is higher than the total number of genes (554), as a given gene may fall in more than one category. The highest number of genes under molecular function category fell into binding (253) followed by catalytic activity (214). Under cellular component category, majority of genes fell into cell and cell part (327), followed by organelle (264). Similarly, in the biological process category, a maximum number of genes fell into metabolic process (309) followed by cellular process (303).

Among 162 genes present in the "*Qefl1*-2" on Ca4, the GO annotation provided genes related to various functions of flower development and regulation (Table 24). Important ones include*EFL6* and *JMJ11*-Jumonji class proteins that are known to be involved in regulation of FT (flowering locus T) and*TEM1*- transcriptional repressor of flowering time in long day plants. In addition, *PTL*- a chromatin binding protein involved in flower development was found. Similarly, the important flowering related genes identified in the "*Qefl2*-4" (on Ca8) region includes, *SUVR5, CZS, SDG6, SET6, HOS1* and *INRPK1* which are reported to be involved in regulation of flower timing and floral induction. A cold responsive gene *VRN2* known to be involved in vernalization was also identified. In addition homeotic genes like *AP2, ANT, CKC1, DRG, OVM, SPT, BHLH24, EN99* and *AHL27* which are involved in flower initiation and floral organogenesis were identified. Another important stress responsive gene *UPF1* was identified on Ca8 which is reported to be involved in photoperiod dependent phenotypes and stress response.

4.4 Association between flowering time and other yield related traits

The data collected on individual F_2 plants were used to estimate correlation coefficients between flowering time and other phenological, yield related traits in all the crosses (Table 25).Flowering time showed significant positive correlations with days to pod initiation in all the four crosses i.e., ICCV 96029 × CDC Frontier (0.988), ICC 5810 × CDC Frontier (0.987), BGD 132 × CDC Frontier (0.992) and ICC 16641 × CDC Frontier (0.997). Flowering time and days to maturity showed highly significant positive correlation (0.882, 0.888, 0.934 and 0.950) in all the crosses. Flowering time showed positive and significant correlation with plant height (0.507, 0.189, 0.453 and 0.471) and biomass (0.264, 0.181, 0.331 and 0.280) in all the crosses. In all the crosses, no significant correlation was found for flowering time with pods per plant, seeds per plant and grain yield. Significant positive correlation was observed between flowering time and 100 seed weight in the crosses ICCV 96029 × CDC Frontier (0.281), ICC 5810 × CDC Frontier (0.309) and BGD 132 × CDC Frontier (0.237). Whereas in the cross ICC 16641 × CDC Frontier, the values of correlation coefficients were not significant. Flowering time showed significant negative correlation with harvest index in all the crosses i.e. ICCV 96029 × CDC Frontier (-0.431), ICC 5810 × CDC Frontier (-0.442), BGD 132 × CDC Frontier (-0.486) and ICC 16641 × CDC Frontier (-0.616).

Discussion

V. DISCUSSION

Terminal (end-of-season) drought is the most important constraint to chickpea production accounting for 40–50% yield reduction globally (Ryan, 1977). This is because the crop is largely grown under rainfed conditions in the post-rainy season where the growing season is short (90-120 days) because of the risk of extreme drought or high temperatures at the end of the season (the pod filling stage of the crop) (Gaur *et al.,* 2014). Development of early-maturing varieties has been the most effective strategy to escape from terminal drought. However, it is necessary to match the crop maturity with the crop season length to obtain high yield. Therefore, flowering time is the key phenological trait for adaptation of chickpea to short-season environments as it helps crop to escape from end-of-season stresses (drought, high/low temperature extremities).

The present study deals with the study of genetics of flowering time genes, construction of intraspecific genetic linkage maps, QTL analysis for identification of genomic regions controlling flowering time and association of flowering time with other yield relate traits in chickpea. These results have been discussed in the context of available studies.

5.1 Genetics of flowering time genes

Analysis of the genetic basis of time to flowering in chickpea contributes to our understanding of its inheritance mechanism and is of practical importance because the choice of effective selection/breeding methods depends in part upon the genetic basis of the trait. The F_2 populations used in this study was derived from crosses between four different early flowering lines (ICCV 96029, ICC 5810, BGD 132 and ICC 16641) and late flowering (CDC Frontier) cultivar. Therefore, different alleles of genes responsible for flowering must be present in these crosses and segregation can be clearly observed in the data distribution of the F_2 plants growing in a short season environment (Fig. 1-4). In the present study, the F_2s grown during the post rainy season flowered when days were long and temperatures were not very high. The range of expression for flowering time revealed considerable variation in F_2 of all the crosses (Table 6). Both temperature and photoperiod have been reported to affect flowering time in chickpea (Roberts *et al.,* 1985 and Kumar and Abbo, 2001). Transgressive segregation in these assays may be the result of new genetic combinations related to photo-thermal response. In fact, major genes

(*efl-1, ppd/efl-2, efl-3* and *efl-4*) controlling flowering time have been reported in chickpea (Kumar and van Rheenen 2000; Or *et al.,* 1999; Hegde 2010 and Gaur *et al.,* 2015) and complementary gene actions seem to be evident in crosses between chickpea genotypes (Kumar *et al.,* 1985 and Anbessa *et al.,* 2006). In other legumes also, the number of flowering time genes identified varies considerably. For example, six major genes have been identified in pea (Murfet, 1985), eight in soybean (Bernard, 1971; Buzzell and Voldeng, 1980; McBlain and Bernard, 1987; Cober and Voldeng, 2001 and Ray *et al.,* 1995), two in pigeonpea (Koebner *et al.,* 1991 and Craufurd *et al.,* 2001), one in lentil (Sarker *et al.,* 1999) and one in common bean (Coyne and Mattson, 1964).

In the present study, F_1 hybrids of all the four crosses were late to flower indicating dominance of lateness as late flowering is known to be dominant over early flowering in chickpea (Gumber and Sarvjeet, 1996; Or *et al.,* 1999; Kumar and van Rheenen, 2000; Anbessa *et al.,* 2006; Hegde, 2010 and Gaur *et al.,* 2015) and soybean (McBlain and Bernard, 1987; Ray *et al.,* 1995 and Cober and Voldeng, 2001). Whereas in pigeonpea (Saxena and Sharma, 1990) and common bean (Coyne and Mattson, 1964), early flowering was found to be dominant to late flowering.

Segregation for flowering time among the F_2 segregants of the cross ICCV 96029 × CDC Frontier showed that this trait is under monogenic control (Table 7). The confirmatory evidence came from the segregation pattern of F_3 progenies (Table 8). Earlier in a preliminary report based on crosses among the early (ICCV 2) and two late (GL769 and BG276) parents, Gumber and Sarvjeet (1996) reported that time to flowering was controlled by duplicate genes. However, using the same early flowering parent ICCV 2 and another late flowering parent JG 62, Kumar and van Rheenen (2000) observed bimodal distribution for flowering time and reported a single major gene plus polygenic mode of inheritance for time to flowering. They proposed this single recessive gene for time of flowering (*efl-1*) in the line ICCV 2. ICCV 96029, a super early line used as a source of early flowering gene for one of the crosses in our study was developed from a cross of two early flowering genotypes, ICCV 2 and ICCV 93929 (Kumar and Rao, 2001). It was about one week earlier than either of the parents at ICRISAT, Patancheru, India (Kumar and Rao, 2001). In addition to major gene (*efl-1*), this genotype likely has additional alleles for early flowering, which is also supported by the findings of Anbessa *et al.* (2006).

The distribution pattern of flowering time in the F_2 populations of the cross ICC 5810 × CDC Frontier (Figure 2) suggests the involvement of major genes affecting the flowering time trait. Classification of the flowering time data into two classes - late (after 45 d) and early (up to 45 d) resulted into 9 late: 7 early classes indicate involvement of two genes with duplicate recessive epistasis governing time to flowering in this cross (Table 7). Based on the assumed F_2 genotypic ratio, one of the nine late F_2 plants is expected to show no-segregation in F_3, while eight out of nine plants expected to segregate into late and early plants. Similarly, 3/7 of early F_2 plants are expected to breed true in F_3, whereas 4/7 plants expected to segregate into early and late plants (Table 8). Thus, the segregation pattern in F_3 progenies for time of flowering confirmed the segregation observed in F_2 supporting involvement of two genes with complementary effect. This result is similar to the observations on inheritance of duration from seeding to heading in rice (Chang *et al.,* 1969). In chickpea, Anbessa *et al*. (2006) and Gaur *et al.,* (2015) reported two major genes with duplicate recessive epistasis that controlled time to flowering. By contrast, Hegde (2010) in a cross involving ICC 5810 reported involvement of two dominant genes with cumulative but unequal effect on time of flowering. Earlier, Gumber and Sarvjeet (1996) also observed two duplicate dominant genes for flowering time in chickpea. In other legumes such as common bean (*Phaseolus vulgaris*) and pigeonpea (*Cajanus cajan*) also the duration from sowing to flowering is under the control of two genes (Craufurd *et al.,* 2001 and Kornegay *et al.,* 1993).

The cross between BGD 132 and CDC Frontier showed late flowering was dominant over early flowering. Similar results were reported by Or *et al.* (1999), Kumar and Rheenen (2000) and Anbessa *et al.* (2006). F_2 plants of this cross segregated as 3 late: 1 early following monogenic segregation for flowering time (Table 7). The inheritance pattern observed for time of flowering in F_2 was confirmed in F_3 (Table 8). Similar results were reported by Or *et al.* (1999) and Kumar and van Rheenen (2000). The results of this study further support the findings of Hegde (2010), who proposed a single recessive gene (*efl-3*) conferring earliness in the line BGD 132.

In the cross ICC 16641 × CDC Frontier, late flowering was found dominant over earliness. The distribution pattern of F_2 plants for flowering time suggests major gene control of the trait. The F_2 segregants of this cross were clearly grouped into 3 late: 1 early (Table 7). This was confirmed by studying the breeding behaviour of F_3 families (Table 8). Therefore, we report single gene (*efl-4*) control difference in flowering time

between the parents of this cross. Similar results were reported by Or *et al.,* (1999) and Kumar and van Rheenen (2000) in their respective studies.

The present study revealed that time to flowering in chickpea under short season environment typical of semi-arid tropics followed major gene inheritance. These results are consistent with previous reports on the inheritance of time to flowering in chickpea (Gumber and Sarvjeet, 1996; Or *et al.,* 1999; Kumar and van Rheenen, 2000; Anbessa *et al.,* 2006; Hegde, 2010 and Gaur *et al.,* 2015). The simple genetic basis of the flowering time genes observed in this study reveals that early-flowering trait can be easily incorporated into modern high-yielding cultivars either by backcross breeding or by simple selection in F_2 and subsequent generations.

5.1.1 Inheritance of flower color

Segregation of flower color was observed in two crosses ICCV 96029 × CDC Frontier and ICC 5810 × CDC Frontier involving pink and white colored parents. The F_1s of both the crosses produced pink flower indicating dominance of pink over white. A similar observation was made by Kumar *et al.* (2000). The segregation in F_2 revealed the monogenic control of flower color (*B/b*) and this was confirmed by segregation analysis of F_3 progenies (Table 9-10). This result is in agreement with the report of Hasan and Deb (2013) and Cho *et al.* (2002). Earlier, Khan and Akhtar (1934) and Kumar *et al.* (1999) reported complementary gene action for flower color. Later, Kumar *et al.* (2000) reported two independent loci with supplementary type of gene action controlling flower color in chickpea. However, Gaur and Gour (2001) reported presence of three factors in controlling petal color in chickpea. Flower color is a valuable phenotypic marker and also useful in molecular marker studies in chickpea.

5.1.2 Inheritance of double podding trait

Chickpea typically produces one pod per peduncle, but a limited number of accessions in the chickpea germplasm produce two pods per peduncle at some reproductive nodes (Pundir *et al.,* 1988 and Srinivasan *et al.,* 2005). A breeding line ICCV 96029 was used as the donor parent with double podding trait in this study. The F_2 population of the cross ICCV 96029 × CDC Frontier segregated into 3 single podding: 1 double podding plants (Table 11-12), confirming the single recessive gene inheritance hypothesis for double podding (Kumar *et al.,* 2000; Cho *et al.,* 2002 and Anbessa *et al.,*

2007). This implies that the double podding trait can be easily incorporated into the desired genetic backgrounds. Under well expressed environmental conditions, double podding trait is an important contributing yield trait in chickpea (Sheldrake *et al.*, 1978).

5.2 Genetic linkage mapping

SSR and SNP markers are considered as preferred marker systems in plant breeding due to their unique advantages. Though sufficient number of SNP (714) and SSR (472) markers that represent most of chickpea genome were attempted in this study, a very low polymorphism between parents was the main bottleneck for their utilization in linkage analysis. The proportion of polymorphic markers obtained for SNP markers was 6.86%, 6.16%, 5.04% and 4.06% between the parents of the intraspecific crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier, respectively. While for SSRs, the level of polymorphism found was 21.18%, 20.13%, 19.07%, and 19.70%, respectively which is similar to that found by Thudi *et al.,* (2011) who reported 10-19% between the parents of intra-specific crosses. While Udupa and Baum (2003), Cho *et al.* (2004), Tar'an *et al.* (2007) and Kottapalli *et al.* (2009) demonstrated that the frequencies of SSR polymorphism between two *C. arietinum* parents were in the range of 30% to 50%.A higher polymorphism frequency (77%) was found by Tekeoglu *et al.* (2002) between parents of a cross between *C. arietinum* and *C. reticulatum*. This issue of low polymorphism might be resolved by combining SSRs with new types of markers such as those based on single nucleotide polymorphisms (Tar'an *et al.,* 2007).

When segregating markers were tested for goodness of fit to the expected 1: 2: 1 ratio using χ^2 test ($P < 0.05$), most of the markers gave a good fit to the expected segregation ratio in all the populations. While, only 11 (14.29%), 13 (17.11%), 2 (2.98%) and 4 (5.97%) markers showed segregation distortion in the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier, respectively which is relatively less distortion compared to Tullu *et al.,* (1999) [30%], Winter *et al.* (2000) [38.4%] and Flandez-Galvez *et al.,* (2003) [20.4%] where they used RIL population in their studies. Winter *et al.* (1999) reported a general trend of distorted segregation in a RIL population from a wide cross and noted that distorted frequencies are more pronounced in RILs than in F_2 population (for this comparison, see Tanksley *et al.*, 1992). Segregation distortion affects the estimation of map distances and

the order of markers when many distorted markers are used for linkage map construction and hence affects the QTL analysis. However, we used all the markers for the linkage analysis in spite of the distorted segregations observed for only few markers.

In the present study, we constructed four intraspecific genetic maps with the 77, 76, 68 and 67 segregating markers distributed across all eight linkage groups (CaLGs) in each map (Figure 5, 6, 7 and 8). Since F_2 populations were used, the intraspecific maps in this study represent the coarse genetic maps spanning 262.25 cM with an average inter-marker distance of 3.41 cM in the cross ICCV 96029 × CDC Frontier. The genetic map of the cross ICC 5810 × CDC Frontier spanned 335.74 cM with average marker distance of 4.42 cM. Similarly, the genetic map of the cross BGD 132 × CDC Frontier spanned 311.10 cM with an average distance between adjacent markers of 4.57 cM and genetic map of the cross ICC 16641 × CDC Frontier covered 385.13 cM of the genome with an average of 5.75 cM between adjacent markers. These results further indicate that, the intraspecific maps obtained are less dense compared to earlier workers (Winter *et al.,* 2000 [2077.9 cM; 16 LG]; Radhika *et al.,* 2007 [509.3 cM; 7 LG and 623.9 cM; 7 LG]; Nayak *et al.,* 2010 [2602 cM; 8 LG]; Thudi *et al.,* 2011 [845.56 cM; 8 LG] and Varshney *et al.,* 2014 [621.51 cM; 8 LG and 533.06 cM; 8 LG]). Considering the 740 Mbp physical size of the chickpea genome (Arumuganathan and Earle, 1999) and ignoring the fact that recombination rates can vary widely within the genome, 1 cM distance in present maps roughly equates to 2.82 Mbp, 2.20 Mbp, 2.38 Mbp and 1.98 Mbp in all the four crosses which is high compared to Winter *et al.,* (2000) [1 cM = 0.36 Mbp], integrated map of Radhika *et al.,* (2007) [1 cM = 1 Mbp] and Nayak *et al.,* (2010) [1 cM = 0.28 Mbp]. Also, some of the linkage groups have short span, which could be due to the unavailability of polymorphic markers. Hence, attention should be given in the future to identify more polymorphic markers to extend the length of these linkage groups.

In general, dense sub-clusters of marker loci were observed either in the central region or at distal ends of most of the linkage groups in all the genetic maps of this study (Fig. 5, 6, 7 and 8).However, such apparent clustering of markers at distinct and often central positions of linkage groups is not unusual and was observed for all types of markers in very advanced maps (e.g. Tanksley *et al.,* 1992; Winter *et al.,* 1999; Nayak *et al.,* 2010; Radhika *et al.,* 2007 and Milan *et al.,* 2010). It may reflect the low level of recombination in centromeric and subtelomeric genomic regions (Tanksley *et al.,* 1992).

When the genetic maps were compared with earlier maps, a reasonable genomic synteny was found among them, also between the current intraspecific chickpea maps and earlier maps of Millan *et al.* (2010), Thudi *et al.* (2011), Jaganathan *et al.* (2014) and Varshney *et al.* (2014) which encourages the use of SSR markers and the possibility of integration of different maps through common markers. The distribution of markers to linkage groups is comparable with earlier published maps of Thudi *et al.,* (2011), Jaganathan *et al.* (2014) and Varshney *et al.* (2014). However, the order of marker loci on intra-specific maps differed in several instances. Possible reason for this is that earlier maps were developed from RIL populations, whereas the present maps were developed from F_2 mapping populations. So, the reversal of markers order may occur in few cases due to slight variation in recovery of recombinants.

Through consensus map approach, one can integrate different genetic maps through anchored markers and can locate markers/QTLs/genes for important traits which might not segregate in one mapping population but in the other. This is particularly important for crops like chickpea where very low level of polymorphism have initially been reported (Udupa *et al.,* 1993). In the present study, the data sets from four populations were joined to develop the consensus map. While comparing four intra-specific genetic maps, 28 marker loci were found common between four maps (Table 20). These markers were considered as anchor markers and used for merging the genetic maps for construction of the consensus genetic map. The consensus map developed contained 111 markers that covered 387.63 cM, which is less dense compared to the consensus maps of Millan *et al.* (2010) and Varshney *et al.* (2014). A detailed comparison between maps from individual component maps and consensus map reflects a general coincidence. Although differences in marker order exists, linkage groups are generally conserved. The sub clusters and gaps were also observed in most of the LGs either at central or in distal regions. The flower color locus (*B/b*) was mapped on CaLG04 between the markers TR33 and ICCM0192a. This result is in agreement with the earlier reports of Santra *et al.* (2000) and Millan *et al.* (2010) who reported GAA47 as closely linked marker. In our study we mapped a new marker (ICCM0192a) between *B/b* locus and GAA47. The gene for double podding (‘*Sfl/sfl*’) was mapped on CaLG06 of the chickpea consensus map flanked by the markers TR01 and TR44. Similar result was reported earlier by Cho *et al.* (2002).In general, the consensus map is still low in marker density and these regions need to be filled with more markers for any map based cloning of agronomically important genes.

5.3 QTL mapping for flowering time genes

Flowering time is considered to be an important adaptive trait as the crop is grown in different thermal and photoperiod regimes (Khanna-Chopra and Sinha, 1987). So far, several studies have reported QTL for flowering time on linkage groups LG1, LG2, LG3, LG4 and LG8 of the chickpea genetic map (Cho *et al.,* 2002; Cobos *et al.,* 2007 and 2009; Lichtenzveig *et al.,* 2006; Aryamanesh *et al.,* 2010; Hossain *et al.,* 2010 and Jamalabadi *et al.,* 2013) indicating, genomic regions controlling flowering time are distributed throughout the genome. In the present study, flowering time data of four F_2 populations was used along with the genetic mapping data QTL analysis using QTL-ICiMapping version 4.0 software.

A major QTL "*Qefl1*-2" for flowering time was detected in the cross ICCV 96029 × CDC Frontier on CaLG04 flanked by the markers GAA47 - ICCM192a explaining 12.34% of phenotypic variation. In addition, a minor QTL "*Qefl1*-1" (PVE=7.07) was identified on CaLG03. Mendelian inheritance revealed flowering time was governed by a single major gene. However, the low phenotypic contribution explained by the QTLs may be an indication of the possible involvement of additional unidentified loci which have not been detected either because of incomplete genome coverage or the small population size (Dholakia *et al.,* 2003). Earlier, Cobos *et al.,* (2007) reported a major QTL for days to 50% flowering (QTL_{DF1}; 20% PV) on LG4, this QTL had a common marker (*i.e.* GAA47) with the QTL reported in this study. Therefore, they may refer to the same QTLs. Later, Cho *et al.* (2002) and Jamalabadi *et al.* (2013) also reported a QTL for days to flowering in LG3 using a RIL population from a cross involving the same line ICCV 2 as one of the parents. The line ICCV 2 is an indirect source of earliness (*efl-1*) in our cross. However, lack of common markers does not allow a definitive conclusion that these two QTL represents the same locus of this study. Based on these findings, it is apparent that several unknown factors confer time to flowering in chickpea even though segregation for a major flowering gene was observed in this study. Similar findings was reported by Cho *et al.,* (2002).

Four major QTLs each on CaLG01 (*Qefl2*-1, PVE=20.13%), CaLG03 (*Qefl2*-2, PVE=25.19%), CaLG04 (*Qefl2*-3, PVE=10.44%) and CaLG08 (*Qefl2*-4, PVE=25.57%) were identified in the cross ICC 5810 × CDC Frontier. Genetic studies revealed that two major genes with complementary gene action controlling flowering time in this cross

(Table 7). Earlier, Cho *et al.,* (2002) detected single QTL for flowering time on LG3 between the markers TS57 and TA127. Recently, Jamalabadi *et al.* (2013) also identified a QTL on LG3 closely linked to the marker TA117. However, these markers were not mapped in this present map and hence the exact chromosomal location could not be compared. Whereas, Cobos *et al.* (2009) and Aryamanesh *et al.* (2010) mapped a QTL for flowering time on LG 3 closely linked to marker TA142 which is also detected in our study. Therefore, the alleles of these QTLs could belong to same set of genes. Another QTL for flowering time was identified by Cobos *et al.,* (2007) on LG4 (explaining 20% PV) closely linked to STMS GAA47. In our study also a QTL on CaLG4 (*Qefl2*-3, 10.44% PV) was detected having GAA47 as flanking marker. Therefore, the alleles of that QTL could be the same in both findings. In all these studies, the parents used were different. However, Lichtenzveig *et al.* (2006) in the cross involving Hadas and ICC 5810 reported three QTLs on LG1, LG2 and LG8 for flowering time and recently Rehman *et al.* (2011) reported four QTLs for flowering time on LG1, LG3, LG4 and LG8. None of these QTLs were found similar to the QTLs detected in the present study. In our study however, LG2 was not associated with any effect on time to flowering. One possible explanation for this is that absence of common markers in our map due to less number of polymorphic markers available for linkage analysis. Therefore, further studies are needed to confirm two major complementary genes out of four major QTLs detected in this study responsible for flowering time in ICC 5810.

In the cross BGD 132 × CDC Frontier, a major QTL (*Qefl3*-3) for flowering time on CaLG08 is reported for the first time in this study. This QTL is flanked by markers TA127 and H1D24 contributing phenotypic variance of 65.35%. Linkage analysis of flowering time based on F_3 segregating data resulted into mapping of flowering time locus "*Efl-3/efl-3*" on CaLG08 of the chickpea genetic map of this cross (Fig. 12a). Previously, Cho *et al.* (2002) reported a QTL for flowering time on LG3 flanked by markers TS57 and TA127. However, LG3 of Cho *et al.* (2002) is equivalent to CaLG8 in this study based on the common markers of the current map and genetic maps of Tar'an *et al.* (2007) and Varshney *et al.* (2014). Therefore the alleles of both the QTLs could be same. Recently, Lichtenzveig *et al.* (2006) and Rehman *et al.* (2011) also reported a QTL for flowering time on LG8. Two additional QTLs *i.e.* "*Qefl3*-1" and "*Qefl3*-2" with minor effect (4.28% and 3.99%) were also detected on CaLG03 in this study. Hence, CaLG08 appears to be strong candidate linkage group having major QTL controlling time to flowering in this cross.

In the cross ICC 16641 × CDC Frontier, only one putative QTL (*Qefl4*-1) for flowering time was detected on CaLG06 between markers TA14 and TR44 with a LOD value of 55.60 that explained 88.19% of the phenotypic variation. This novel QTL is unique for this cross and is reported for the first time in this study. Mendelian inheritance also revealed monogenic inheritance of flowering time in this cross. This is further confirmed by linkage analysis and mapping of major flowering time gene "*Efl-4/efl-4*" on CaLG06 (Fig. 13a) of the chickpea genetic map between the markers TA14 and TR44.

In general, additive effect in negative direction was found important for flowering time in all QTLs. This suggests that favourable alleles for flowering time were contributed by the female parent in all the crosses. Altogether, a total of 10 genomic regions distributed across the linkage groups CaLG01, CaLG03, CaLG04, CaLG06 and CaLG08 were identified to be associated with flowering time trait in this study (Fig. 14). The genomic regions on CaLG03 showed significant effect on flowering time in the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier. While the QTLs on CaLG04 shared a common associated marker GAA47 in the crosses ICCV 96029 × CDC Frontier and ICC 5810 × CDC Frontier. This candidate marker was reported to be associated with a number of important traits including days to first flower, yield and seed weight (Cobos *et al.,* 2007), resistance to Ascochyta blight (Aryamanesh *et al.,* 2010). Very recently, Varshney *et al.* (2014) reported a *"QTL-hotspot"* region on CaLG04, this region contains a number of QTLs for several drought component traits including days to first flowering (DFF). Therefore, CaLG04 seems to be an interesting genomic region for targeting several agronomically important traits through molecular breeding.

Similarly, the genomic regions on CaLG08 conferred major effect on flowering time in the crosses ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier. While the putative QTL for flowering time detected on CaLG01 and CaLG06 are unique for crosses ICC 5810 × CDC Frontier and ICC 16641 × CDC Frontier respectively. These results indicate that genes governing flowering time are distributed throughout the genome. Therefore, it is necessary to saturate specific genomic regions with additional markers in order to refine these regions containing QTLs for flowering time. Candidate genes could then be identified by homology with regulatory genes involved in the control of flowering time. Also, these genomic regions/QTLs can be deployed for introgressing early flowering trait into elite chickpea cultivars through marker-assisted selection (MAS).

5.3.1 Identification of candidate genes in flowering time QTL regions

Availability of genome sequence of chickpea (Varshney *et al.,* 2013) facilitated identification of candidate stress responsive genes present in the QTL regions of important traits such as Drought tolerance, Ascochyta blight resistance and Fusarium wilt resistance in chickpea (Varshney *et al.,* 2014a). Similarly, in the present study also the effort was made to identify the genes involved in regulation of flowering time. The filtering of BLAST primers pairs resulted in two narrow regions on "*Qefl1*-2" (on Ca4) and "*Qefl2*-4" (Ca8) which contained a total of 162 and 362 genes, respectively.

Among 554 genes identified in these regions, Gene Ontology (GO)-annotation indicated presence of important genes like Histone methyltransferase (*SUVR5*) and Zinc ion binding (*SET6*) activity genes which are reported to be involved in downregulation of flower timing in Arabidopsis (Krichevsky *et al*., 2007). Ubiquitin-protein ligase (*HOS1*) that controls flowering time in response to ambient temperature and intermittent cold in Arabidopsis (Lee *et al*., 2012) was also identified. Genes like Early flowering 6 (*EFL6*)- a lysine-specific demethylase and *JMJ11*- Jumonji/zinc finger class proteins that regulates the expression of Flowering locus T (FT) mRNA levels (Jeong *et al*., 2009) and flowering time in Arabidopsis (Noh *et al*., 2004 and Yu *et al.,* 2008) were also found on Ca4. In addition, genes like *CLF* (Polycomd group protein *CURLY* LEAF) and *SET1* (produces SET domain group1 proteins) that are reported to be involved in photoperiod insensitive flowering (Goodrich *et al*., 1997) were identified. Further, these regions also harbours genes like *TEM1* (TEMPRANILLO gene family) which down regulates the timing of flowering (Castillejo and Pelaz, 2008). The cold responsive gene *VRN2* which is reported to play central role in vernalization (Gendall *et al.,* 2001) was also found in Ca8 region (Table 24).

One important stress responsive gene *UPF1* was identified on Ca8 which is shown to be involved in long day light signalling and Non-sense mediated mRNA decay (NMD) mediated pathways influencing plant development, defence response and adaptation to environmental stresses (Shi *et al.,* 2012). Several other homeotic family genes like *AP2* (producing floral homeotic protein family), *ANT* (AP2 like ethylene-responsive transcription factor), *SPT* (Transcription factor SPATULA), *AHL27* (AT-hook motif nuclear-localized protein 27), *PTL* (Trihelix DNA binding protein PETAL LOSS) were also identified. These genes are reported to play key role in homeotic functions such as

specification of floral organ identity, perianth architecture, floral organogenesis, regulation of flowering and flower development (Drews *et al*., 1991; Klucher *et al*., 1996; Alvarez and Smyth, 1999; Xiao *et al*., 2009 and Brewer *et al*., 2004).

These results indicate that genomic regions on Ca4 and Ca8 contains several important candidate genes responsible for flower development and regulation of flowering time. Similarly, other major flowering time QTLs/genomic regions identified in this study are also expected to contain several important candidate genes related to flower development and regulation of flowering time. Further, the major flowering genes identified in this study forms the appropriate genetic material for validation of these candidate genes by using functional genomics approaches which ultimately helps in breeding for early maturity in chickpea.

5.4 Association between flowering time and other yield related traits

The study of associations among various traits is useful to breeders in selecting genotypes possessing desired characteristics. Selection for one component may bring about a simultaneous change in the other. Thus, correlation is the powerful tool to study the character association and is therefore very useful to facilitate selection for improvement of characters without scarifying the gain in the other.

In the present study, the data collected on individual F_2 plants were used to estimate correlation coefficients between flowering time and other phenological, morphological and yield traits (Table 25). In F_2 generations with no replicated progeny, it is impossible to subtract the environmental variance component. Still the correlation values may indicate if a strong genetic association exists between the tested traits (Or *et al.,* 1999). In all the F_2 populations, flowering time showed significant and highly positive correlations with days to pod initiation in all the crosses suggesting that early flowering leads to early podding. Flowering time and days to maturity was positively correlated in all the crosses. These results suggest that in general, the early flowering lines also mature early. Also, observations on flowering time can be recorded with more precision than on days to maturity, particularly in long growing season environments thus flowering time can be used to select for early maturity. Similar results were found by Malik *et al.* (1988), Atta *et al.* (2008), Sidramappa *et al.* (2010), Naveed *et al.* (2012), Jivani *et al.* (2013), Monpara and Dhameliya (2013), Ramanappa *et al.* (2013), Zeeshan *et al.* (2013) and Gaur *et al.* (2015).

In all the crosses, flowering time showed positive and significant correlation with plant height and biomass. These results indicate that extra-early and early plants of these F_2 populations matured very early and could not accumulate enough biomass (had less plant height and biomass) than the late maturing plants. Singh *et al.,* (1990) reported that days to flowering and days to maturity contribute to seed yield in chickpea mainly via biological yield and harvest index. Thus, reducing the growth period after a threshold level may have a penalty on grain yield.

In all the F_2 populations, no significant correlation was detected for flowering time with pods per plant, seeds per plant and grain yield indicating that there are no constraints in combining yield with earliness in chickpea thereby allowing simultaneous selection for both traits. Non-significant association for flowering time with grain yield has been reported by Monpara and Dhameliya (2013), Arshad *et al.* (2003), Sidramappa *et al.* (2010), Atta *et al.* (2008) and Naveed *et al.* (2012).

Flowering time showed either significant positive correlation (in the crosses ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier) or no correlation (in the cross ICC 16641 × CDC Frontier) with 100-seed weight. Highly positively significant genetic correlations between time to flowering and seed weight were also reported by Hovav *et al.* (2003) suggesting that in certain genetic backgrounds it might be difficult to breed early-flowering cultivars without compromising seed weight. While Gaur *et al.* (2015) observed non-significant correlation between flowering time and mean seed weight in early-flowering segregants in the crosses where ICCV 96029 (*efl-1*), ICC 5810 (*efl-2*), BGD 132 (*efl-3*) and ICC 16641 (*efl-4*) was one of the parents. These findings suggest that in certain crosses there is scope for combining large seed size with earliness in chickpea. This is also supported by the fact that there are many large-seeded *kabuli* varieties with early maturity (Gaur *et al.,* 2007).

Flowering time showed significant negative association with harvest index in all the crosses. These results indicate that extra-early and early genotypes are more efficient in their yield partitioning and accumulated biomass necessary to ensure optimum seed yield within shorter duration possibly through a higher crop growth rate. These results encourage to combine earliness with high harvest index in these crosses. High harvest index and drought escape through early flowering and early maturity are considered as important attributes of adaption in chickpea under drought stressed environments (Berger *et al.,* 2004).

Future line of research

Future research work arising from this thesis should be directed towards the following areas:

- Increasing the genome coverage and marker density of the linkage maps developed in this study would improve its usefulness by increasing the likelihood that markers will be tightly linked to flowering time genes. This will also facilitate the alignment of different chickpea genome maps and ultimately will help in the development of a dense chickpea consensus map.

- F_2 populations used in this study were only tested in one only environment (at Patancheru). Hence, these F_2s can be advanced to develop Recombinant inbred lines which can be tested in multiple environments to identify stable QTLs.

- SSR markers linked to flowering time needs to be validated on different genotypes in order to assess their efficiency in Marker assisted selection.

- Major QTL regions identified in this study can be validated through functional genomics approach to identify more candidate genes in all the flowering time QTLs.

Summary & Conclusions

VI. SUMMARY AND CONCLUSIONS

Recent years have witnessed significant progress in the development of genomic resources in chickpea that has led to identification of molecular markers for genes/quantitative trait loci (QTLs) controlling several agronomic traits in chickpea. The present research entitled "Molecular mapping of flowering time genes in chickpea (*Cicer arietinum* L.)" was carried out to identify molecular markers linked to flowering time genes in chickpea. This study provides insight into the genetic control of flowering time in chickpea which will facilitate breeding of early maturing chickpea cultivars better adapted to terminal stress environments. The specific objectives of the research were: to study the genetics of flowering time genes, construction of genetic linkage maps, identification of QTLs governing flowering time genes and association of flowering time with maturity and other agronomic traits. The findings from the research are briefly summarized below:

High polymorphism was observed for flowering time between the parents of all the four crosses. The F_1s of all the crosses were late to (61, 54, 53 and 60 days) flower suggesting the dominance of late flowering over early flowering in all the crosses studied. A wide range of variation for flowering time was observed in F_2 progenies of all the crosses i.e. ICCV 96029 × CDC Frontier (25-77), ICC 5810 × CDC Frontier (27-76), BGD 132 × CDC Frontier (26-75) and ICC 16641 × CDC Frontier (26-82). Bimodal distribution of flowering time data with unequal peaks in F_2 facilitated classification of phenotypic data into early and late flowering groups in all the crosses. This strongly indicated that the difference in flowering time between the parents of all the crosses is under major genes.

The F_2 segregation analysis for flowering time indicated that total F_2 plants of the crosses ICCV 96029 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier segregated as 138 late: 52 early, (χ^2 = 0.57, P = 0.50-0.30), 143 late: 47 early (χ^2 = 0.01, P = 0.95-0.90) and 110 late: 36 early (χ^2 = 0.01, P = 0.95-0.90), respectively (Table 9). The 3:1 segregation of late: early individuals among the F_2 progeny was interpreted as an evidence to a major gene action affecting flowering in these crosses. While the F_2 plants of the cross ICC 5810 × CDC Frontier segregated into 108 late: 82 early (χ^2 = 0.03, P = 0.90-0.80). This suggests that two duplicate recessive genes were

interacting with a complementary effect on time of flowering in ICC 5810. The segregation pattern observed for time of flowering in F_2 was confirmed in F_3 (Table 8). This simple genetic basis of the flowering time genes observed in the present study reveals that it can be easily incorporated the early-flowering habit into modern high-yielding cultivars either by backcross breeding or by simple selection in F_2 and subsequent generations.

The parental genotypes of intra-specific mapping populations (ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier, BGD 132 × CDC Frontier and ICC 16641 × CDC Frontier) were screened with 714 SNPs and 472 SSR markers (146-CaM, 124-ICCM, 57-H-series, 10-NCPGR, 135-Winter-series). As a result, a very low number of SNP markers (49, 44, 36 and 29) and a total of 100, 95, 90 and 93 SSR markers respectively were found polymorphic. The intraspecific maps developed in this study contained 77, 76, 68 and 67 SSR markers that spanned 262.25 cM, 335.74 cM, 311.10 cM and 385.13 cM of map length at an average density of 3.41 cM, 4.42 cM, 4.57 cM and 5.75 cM respectively. Most of the markers mapped in the F_2s showed Mendelian segregation (1:2:1). However, there were a few markers (14.29 %, 17.11 %, 2.94 % and 5.97 % of markers) that showed a significant deviation from the expected ratio. Use of common markers will enable alignment and integration of different maps (for example, Winter *et al.,* 2000 and Tar'an *et al.,* 2007) and allows for the possibility of developing a consensus map of chickpea. In this study, 28 markers were found common between four genetic maps and the developed consensus map consisting of 111 markers which covered 364.44 cM of the chickpea genome having average inter-marker distance of 3.28 cM. Comparatively larger maps have been reported in earlier studies (see Discussion, chapter V) where they combined different marker types including AFLP, SSR, SNP, DArT etc. This emphasizes the need to incorporate more number of different types of markers in these maps.

In this study, 10 genomic regions were found to be associated with flowering time trait. In the cross ICCV 96029 × CDC Frontier, a major QTL (*Qefl1*-2) on CaLG04 was detected at the position 41.0 cM between the markers GAA47 and ICCM0192a with 12.34 % PVE. In addition, a minor QTL (*Qefl1*-1) was detected on CaLG03 flanked by the markers CaM1122 and TR13 explaining 7.07 % of phenotypic variation. Four major QTLs (*Qefl2*-1, *Qefl2*-2, *Qefl2*-3 and *Qefl2*-4) were detected for flowering time in the cross ICC 5810 × CDC Frontier all together explained a total of 81.33 % of phenotypic

variation. Inheritance studies showed that time to flowering is determined by two major genes in this cross. A major QTL (*Qefl3*-3) for flowering time with significant effect (65.35 % of phenotypic variation) was identified between the markers TA127 and H1D24 on CaLG08 of the genetic map of BGD 132 × CDC Frontier. In addition, two minor QTLs were also detected on CaLG03 (*Qefl3*-1 and *Qefl3*-2) together explaining 8.27 % phenotypic variation. In the cross ICC 16641 × CDC Frontier, a single major QTL (*Qefl4*-1) with very high phenotypic variation of 88.19 % was identified on CaLG06 flanked by the markers TA14 and TR44. This is the novel genomic region for flowering time identified for the first time in this study.

In summary, we mapped the major flowering time gene "*efl-1*" from ICCV 96029 on CaLG04 (*Qefl1*-2, PVE = 12.34 %), "*efl-3*" from BGD 132 on CaLG08 (*Qefl3*-3, PVE = 65.35 %) and "*efl-4*" from ICC 16641 on CaLG06 (*Qefl4*-1, PVE = 88.19 %). Whereas for the flowering time gene "*efl-2*", four major QTLs were detected on CaLG01 (*Qefl2*-1, PVE = 20.13 %), CaLG03 (*Qefl2*-2, PVE = 25.19 %), CaLG04 (*Qefl2*-3, PVE = 10.44 %) and CaLG08 (*Qefl2*-4, PVE = 25.57 %). Genetic studies revealed that it is under digenic control with complementary gene action. Therefore, further studies are needed to confirm two major complementary genes out of four major QTLs detected for flowering time in ICC 5810. The major genes and their genomic regions (QTLs) governing flowering time can be deployed for introgressing early maturity into elite chickpea lines through marker-assisted selection (MAS).

Identification of candidate genes in the flowering time QTL regions of "*Qefl1*-2" and "*Qefl2*-4" indicated presence of several important genes like *SUVR5* and *SET6* that are reported to be involved in downregulation of flower timing in Arabidopsis. Also, genes like *HOS1* and *TEM1* which regulates flowering time, *EFL6* and *JMJ11* genes that are involved in regulation of Flowering locus T (FT) and flowering time in Arabidopsis were found. In addition, a gene (*VER2*) that has shown their central role in vernalization was also found. Further, genes like *CLF* and *SET1* involved in photoperiod insensitive flowering were also identified in this region. A stress responsive gene *UPF1* was also found that is known to be involved in photoperiod-dependent phenotypes and adaptation to environmental stresses. Several other important genes of homeotic gene family (*AP2, ANT, SPT, AHL27* and *PTL*) were also identified which has their role in various functions from floral meristem identity to flower development. These candidate genes can be further validated by using functional genomics approach in order to clone the genes for

utilizing in breeding programmes for developing early maturing chickpea better adapted to different environments.

Association study revealed that the flowering time was positively correlated with days to first podding (r = 0.988, r = 0.987, r = 0.992 and r = 0.997) and days to maturity (r = 0.882, r = 0.888, r = 0.934 and r = 0.950). Hence, flowering time can be used as primary selection criteria for developing early maturing chickpea genotypes. However, in breeding programmes aimed at early flowering, other important agronomic traits must be considered. Non-significant association was observed between flowering time with pods per plant, seeds per plant and seed yield. Which allows simultaneous selection of these traits for the development of early maturing and high yielding cultivars. Seed weight is an important yield component and a significant price determinant especially in *kabuli* chickpeas. In the present study, correlation between flowering time and seed weight was positive in some crosses i.e. ICCV 96029 × CDC Frontier, ICC 5810 × CDC Frontier and BGD 132 × CDC Frontier suggesting that in these genetic backgrounds it might be difficult to breed early-flowering cultivars without compromising seed weight. However, a non-significant association in the cross ICC 16641 × CDC Frontier suggests that earliness in chickpea can be combined with large seed size without any difficulties in such crosses. Harvest index showed significant negative association with flowering time indicating possibility of developing early maturing cultivars with high harvest index under terminal drought stress environments.

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VII. REFERENCES

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The format of “Literature cited” presented above is in accordance with the guidelines for the Thesis presentation for University of Agricultural Sciences, Raichur.

Table 1. Summary of trait mapping for important traits in chickpea

Trait studied	Cross	QTL/genes	Reference
Biotic stress			
Ascochyta blight	ICC 4958 × PI489777	QTLs	Tekeoglu *et al*. (2002)
	Lasseter × PI527930	QTLs	Collard *et al*. (2003)
	ICC1 2004 x Lasseter	QTLs	Flandez-Galvez *et al*. (2003)
	ICC 4958 × PI489777	QTL	Rakshit *et al*. (2003)
	ILC 1272 × ILC3279	*ar1, ar2a, ar2b*	Udupa and Baum (2003)
	PI359075 × FLIP84-92C	QTLs	Cho *et al*. (2004)
	ILC3279 × WR315	QTLAR1, QTLAR2	Iruela *et al*. (2006)
	Hadas × ICC5810	$QTL_{4.1}$, $QTL_{4.2}$, QTL_{8}	Lichtenveig *et al*. (2006)
	ILC3279 × WR315	QTLAR3	Iruela *et al*. (2006)
	ICCV96029 × CDC Frontier	QTLs	Taran *et al*. (2007)
	ICCV 96029/CDC Frontier	QTL2, QTL3, QTL4	Anbessa *et al*. (2009)
	ICCV 96029×CDC Luna	QTL1, QTL3	Anbessa *et al*. (2009)
	ICCV 96029×CDC Corinne	QTL3, QTL5	Anbessa *et al*. (2009)
	ICCV 96029×Amit	QTL2	Anbessa *et al*. (2009)
	(ICC 4991 × ICCV 04516)	QTLs	Kottapalli *et al*. (2009)
	ICC 3996 × ILWC 184	QTL3, QTL4	Aryamanesh *et al*. (2010)
	C 214 × ILC 3279	AB-Q-SR-4-1 and 2	Sabbavarapu *et al*. (2013)
Fusarium wilt	C104 × WR315	*Foc1, Foc4*	Tullu *et al*. (1999)
	WR315 × C104	*Foc3*	Sharma *et al*. (2004)
	WR315 × C104	*Foc4*	Sharma *et al*. (2005)
	CA2156 × JG62	*Foc0*	Cobos *et al*. (2005)
	ILC3279 × WR315	*foc5*	Iruela *et al*. (2006)
	ICCL81001 × Cr5-9	*Foc0, foc5*	Cobos *et al*. (2009)
	JG62 × Vijay	*Foc1, Foc2, Foc3*	Gowda *et al*. (2009)
	C 214 × WR 315	FW-Q-APR-6-1 and 2	Sabbavarapu *et al*. (2013)
Botrytis gray mold	ICCV 2 × JG62	QTL1, QTL2, QTL3	Anuradha *et al*. (2011)
Rust	ILC72 × Cr5-10	Uca1/uca1	Madrid *et al*. (2008)

Contd….

Trait studied	Cross	QTL/genes	Reference
Abiotic stress			
Salinity tolerance	ICCV 2 × JG-62	QTL	Vadez *et al*. (2012)
Drought tolerance score	ILC 588 × ILC 3279	Q1-1, Q3-1, Q7-1	Rehman *et al*. (2011)
Stomatal conductance	ILC 588 × ILC 3279	Q8-1	Rehman *et al*. (2011)
Canopy temperature differential	ILC 588 × ILC 3279	Q1-1	Rehman *et al*. (2011)
Drought tolerance traits	ICC 4958 × ICC 1882 ICC 283 × ICC 8261	*"QTL hotspot"*	Varshney *et al*. (2014)
Agronomic traits			
Plant growth habit	ICCL81001 × Cr5-9	*Hg/hg*	Cobos *et al*. (2009)
Plant growth habit	ICC 3996 × ILWC 184	*prostrate*	Aryamanesh *et al*. (2010)
Anthocyanin pigmentation	ICC 3996 × ILWC 184	P	Santra *et al*. (2000)
Pigmentation	ICCV 2 × JG-62	C	Cho *et al*. (2002)
Flower color	CA2156 × JG62 and CA2139 × JG62	B/b	Cobos *et al*. (2005)
Stem colour	ICC3996 X S95362	QTL_1 St. Cl	Hossain *et al*. (2010)
Days to 50% flower	ICCV 2 × JG-62	QTL	Cho *et al*. (2002)
Flowering time	Hadas × ICC5810	QTLs	Lichtenveig *et al*. (2006)
Days to flowering	CA2156 × JG62	QTLDF1	Cobos *et al*. (2007)
Flowering time	ICCL81001 × Cr5-9	QTLDF3	Cobos *et al*. (2009)
Flowering time	ICC 3996 × ILWC 184	QTL 3	Aryamanesh *et al*. (2010)
Flowering time	ICC3996 × S95362 and S95362 × Howzat	QTL_1 F.T	Hossain *et al*. (2010)
Days to flowering	ILC 588 × ILC 3279	Q1-1, Q3-1, Q4-2, Q8-2	Rehman *et al*. (2011)
Flowering time (under salinity)	ICCV 2 × JG-62	QTL	Vadez *et al*. (2012)
Days to flowering	ILC3279 × ICCV2	QTL_{DF}	Jamalabadi *et al*. (2013)
Days to maturity	ILC 588 × ILC 3279	Q1-1, Q3-1, Q7-1	Rehman *et al*. (2011)

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Trait studied	Cross	QTL/genes	Reference
Reproductive period	ILC 588 × ILC 3279	Q1-1, Q3-1, Q7-1	Rehman *et al*. (2011)
Single-/double-podding locus	CA-2156 × JG-62	*Sfl/sfl*	Rajesh *et al*. (2002)
Double podding	ICCV 2 × JG-62	*s*	Cho *et al*. (2002)
Single/double-podding locus	CA2156 × JG62 and CA2139 × JG62	*Sfl/sfl*	Cobos *et al*. (2005)
Double Podding	JG62 ×Vijay	*Sfl*	Radhika *et al*. (2007)
Plant height	ILC 588 × ILC 3279	Q1-1, Q4-3	Rehman *et al*. (2011)
Seeds per pod	ICCV 2 × JG-62	QTL	Cho *et al*. (2002)
Seeds per pod	JG62 ×Vijay	*Spp*	Radhika *et al*. (2007)
100 seed weight	ICCV 2 × JG-62	QTL	Cho *et al*. (2002)
Seed weight	Hadas x Cr205	QTL1, QTL2, QTL3	Abbo *et al*. (2005)
Seed size	CA2156 × JG62	QTLSW1, QTLSW2	Cobos *et al*. (2007)
Seed weight	JG62 ×Vijay	*Qncl.Sw1 to Qncl.Sw8*	Radhika *et al*. (2007)
Seed size	ICCL81001 × Cr5-9	QTLSW1, QTLSW3	Cobos *et al*. (2009)
Seed coat thickness	ICCL81001 × Cr5	QTLTt	Cobos *et al*. (2009)
Seed shape	ICC3996 × S95362 and S95362 × Howzat	QTL1 RI W	Hossain *et al*. (2010)
Seed coat thickness	ICCL81001 × Cr5-9	QTLTt	Cobos *et al*. (2009)
Seed shape	ICC3996 × S95362 and S95362 × Howzat	QTL_1 RI	Hossain *et al*. (2010)
Yield	CA2156 × JG62	QTLYD	Cobos *et al*. (2007)
Grain yield	ILC 588 × ILC 3279	Q1-1, Q4-1	Rehman *et al*. (2011)
Harvest index	ILC 588 × ILC 3279	Q1-1, Q3-1	Rehman *et al*. (2011)
Beta-carotene	Hadas x Cr205	QTL1 to QTL4	Abbo *et al*. (2005)
Lutein concentration	Hadas x Cr205	QTL	Abbo *et al*. (2005)

Table 2. Characteristics of the parental lines used in this study

Sl. No.	Genotype	Origin/pedigree/alternative name(s)	Days to flowering	Key traits	References
1.	ICCV 96029	A breeding line developed at ICRISAT, India from a cross between two extra-early lines ICCV 2 (*kabuli*) and ICCV 93929 (*desi*)	28	*Desi* type, pink flower, double-podded, semi-erect growth habit, and brown seed. It flowered about a week earlier than both extra-early parents and thus called 'super-early'. It was reported to be the world's earliest flowering chickpea germplasm.	Kumar and Rao (1996), Kumar *et al.* (2001) and Gaur *et al*. (2015)
2.	ICC 5810	A landrace from Maharashtra Province of India. Also known as 'Harigantras'	30	*Desi* type, pink flower, semi-spreading growth habit, black seed, and small seed size. It was described as the earliest flowering and the least photoperiod sensitive genotype	Roberts *et al.* (1985) and Gaur *et al*. (2015)
3.	BGD 132	A breeding line developed by Indian Agricultural Research Institute (IARI), at Dharwad center	27	*Kabuli* type, white flower, semi-spreading growth habit extra-early, white seed, and medium seed size (having 100 seed weight of 27.55 g).	IARI, (2006), Hegde, (2010) and Gaur *et al*. (2015)
4.	ICC 16641	A landrace from Punjab province of Pakistan	28	*Kabuli* type, white flower, semi-spreading growth habit, super-early, white seed, and medium seed size (having 100 seed weight of 21.94 g).	Gaur *et al*. (2015)
5.	CDC Frontier	A cultivar developed by Crop Development Centre, University of Saskatchewan, Canada.	53	*Kabuli* type, white flower, fair Ascochyta blight resistant, medium maturity, medium-large seed size (having 100 seed weight of 31.62 g).	Warkentin *et al*. (2005)

Table 3a. List of CKAMSNP markers used for parental polymorphism study

Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name
1	CKaM0003	41	CKaM0272	81	CKaM0474	121	CKaM0662
2	CKaM0005	42	CKaM0278	82	CKaM0476	122	CKaM0668
3	CKaM0010	43	CKaM0285	83	CKaM0488	123	CKaM0695
4	CKaM0014	44	CKaM0288	84	CKaM0493	124	CKaM0701
5	CKaM0016	45	CKaM0291	85	CKaM0499	125	CKaM0706
6	CKaM0017	46	CKaM0294	86	CKaM0511	126	CKaM0709
7	CKaM0018	47	CKaM0295	87	CKaM0512	127	CKaM0711
8	CKaM0019	48	CKaM0298	88	CKaM0515	128	CKaM0715
9	CKaM0021	49	CKaM0307	89	CKaM0520	129	CKaM0722
10	CKaM0027	50	CKaM0310	90	CKaM0525	130	CKaM0723
11	CKaM0029	51	CKaM0314	91	CKaM0539	131	CKaM0727
12	CKaM0032	52	CKaM0320	92	CKaM0541	132	CKaM0730
13	CKaM0034	53	CKaM0325	93	CKaM0542	133	CKaM0732
14	CKaM0037	54	CKaM0332	94	CKaM0544	134	CKaM0735
15	CKaM0039	55	CKaM0338	95	CKaM0553	135	CKaM0737
16	CKaM0042	56	CKaM0340	96	CKaM0554	136	CKaM0742
17	CKaM0044	57	CKaM0342	97	CKaM0557	137	CKaM0745
18	CKaM0163	58	CKaM0343	98	CKaM0558	138	CKaM0747
19	CKaM0166	59	CKaM0351	99	CKaM0560	139	CKaM0749
20	CKaM0168	60	CKaM0356	100	CKaM0561	140	CKaM0750
21	CKaM0170	61	CKaM0358	101	CKaM0568	141	CKaM0752
22	CKaM0173	62	CKaM0359	102	CKaM0571	142	CKaM0755
23	CKaM0174	63	CKaM0360	103	CKaM0573	143	CKaM0759
24	CKaM0185	64	CKaM0366	104	CKaM0586	144	CKaM0761
25	CKaM0190	65	CKaM0375	105	CKaM0588	145	CKaM0769
26	CKaM0191	66	CKaM0387	106	CKaM0592	146	CKaM0770
27	CKaM0196	67	CKaM0391	107	CKaM0596	147	CKaM0775
28	CKaM0206	68	CKaM0400	108	CKaM0605	148	CKaM0779
29	CKaM0207	69	CKaM0411	109	CKaM0609	149	CKaM0784
30	CKaM0212	70	CKaM0414	110	CKaM0610	150	CKaM0787
31	CKaM0213	71	CKaM0422	111	CKaM0612	151	CKaM0788
32	CKaM0229	72	CKaM0430	112	CKaM0622	152	CKaM0791
33	CKaM0232	73	CKaM0434	113	CKaM0626	153	CKaM0792
34	CKaM0244	74	CKaM0438	114	CKaM0628	154	CKaM0793
35	CKaM0246	75	CKaM0441	115	CKaM0634	155	CKaM0795
36	CKaM0256	76	CKaM0443	116	CKaM0636	156	CKaM0796
37	CKaM0257	77	CKaM0455	117	CKaM0638	157	CKaM0798
38	CKaM0261	78	CKaM0460	118	CKaM0646	158	CKaM0801
39	CKaM0265	79	CKaM0463	119	CKaM0649	159	CKaM0802
40	CKaM0270	80	CKaM0468	120	CKaM0651	160	CKaM0811

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Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name
161	CKaM0812	201	CKaM0907	241	CKaM1012	281	CKaM1113
162	CKaM0814	202	CKaM0910	242	CKaM1013	282	CKaM1119
163	CKaM0819	203	CKaM0911	243	CKaM1014	283	CKaM1120
164	CKaM0820	204	CKaM0914	244	CKaM1017	284	CKaM1121
165	CKaM0822	205	CKaM0918	245	CKaM1018	285	CKaM1123
166	CKaM0826	206	CKaM0920	246	CKaM1020	286	CKaM1124
167	CKaM0827	207	CKaM0921	247	CKaM1024	287	CKaM1125
168	CKaM0830	208	CKaM0923	248	CKaM1030	288	CKaM1126
169	CKaM0835	209	CKaM0925	249	CKaM1032	289	CKaM1129
170	CKaM0837	210	CKaM0927	250	CKaM1033	290	CKaM1136
171	CKaM0839	211	CKaM0928	251	CKaM1035	291	CKaM1140
172	CKaM0842	212	CKaM0931	252	CKaM1039	292	CKaM1144
173	CKaM0843	213	CKaM0932	253	CKaM1041	293	CKaM1146
174	CKaM0844	214	CKaM0934	254	CKaM1042	294	CKaM1147
175	CKaM0845	215	CKaM0935	255	CKaM1043	295	CKaM1155
176	CKaM0846	216	CKaM0937	256	CKaM1045	296	CKaM1158
177	CKaM0847	217	CKaM0941	257	CKaM1049	297	CKaM1159
178	CKaM0848	218	CKaM0943	258	CKaM1052	298	CKaM1162
179	CKaM0854	219	CKaM0944	259	CKaM1053	299	CKaM1166
180	CKaM0856	220	CKaM0947	260	CKaM1054	300	CKaM1171
181	CKaM0860	221	CKaM0948	261	CKaM1056	301	CKaM1177
182	CKaM0867	222	CKaM0953	262	CKaM1058	302	CKaM1178
183	CKaM0869	223	CKaM0960	263	CKaM1062	303	CKaM1179
184	CKaM0870	224	CKaM0966	264	CKaM1063	304	CKaM1181
185	CKaM0871	225	CKaM0967	265	CKaM1064	305	CKaM1185
186	CKaM0874	226	CKaM0971	266	CKaM1068	306	CKaM1186
187	CKaM0875	227	CKaM0978	267	CKaM1073	307	CKaM1190
188	CKaM0876	228	CKaM0979	268	CKaM1074	308	CKaM1191
189	CKaM0880	229	CKaM0982	269	CKaM1075	309	CKaM1193
190	CKaM0885	230	CKaM0984	270	CKaM1076	310	CKaM1194
191	CKaM0888	231	CKaM0990	271	CKaM1080	311	CKaM1195
192	CKaM0891	232	CKaM0992	272	CKaM1082	312	CKaM1196
193	CKaM0892	233	CKaM0996	273	CKaM1088	313	CKaM1198
194	CKaM0894	234	CKaM0999	274	CKaM1089	314	CKaM1200
195	CKaM0895	235	CKaM1000	275	CKaM1096	315	CKaM1201
196	CKaM0897	236	CKaM1002	276	CKaM1097	316	CKaM1202
197	CKaM0900	237	CKaM1005	277	CKaM1099	317	CKaM1205
198	CKaM0901	238	CKaM1006	278	CKaM1100	318	CKaM1209
199	CKaM0905	239	CKaM1007	279	CKaM1101	319	CKaM1210
200	CKaM0906	240	CKaM1010	280	CKaM1108	320	CKaM1212

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Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name
321	CKaM1217	361	CKaM1328	401	CKaM1424	441	CKaM1506
322	CKaM1220	362	CKaM1330	402	CKaM1425	442	CKaM1508
323	CKaM1224	363	CKaM1333	403	CKaM1428	443	CKaM1512
324	CKaM1232	364	CKaM1334	404	CKaM1430	444	CKaM1515
325	CKaM1233	365	CKaM1338	405	CKaM1431	445	CKaM1517
326	CKaM1237	366	CKaM1339	406	CKaM1432	446	CKaM1518
327	CKaM1242	367	CKaM1340	407	CKaM1434	447	CKaM1519
328	CKaM1245	368	CKaM1344	408	CKaM1436	448	CKaM1522
329	CKaM1247	369	CKaM1345	409	CKaM1437	449	CKaM1523
330	CKaM1248	370	CKaM1347	410	CKaM1442	450	CKaM1526
331	CKaM1250	371	CKaM1348	411	CKaM1444	451	CKaM1529
332	CKaM1252	372	CKaM1349	412	CKaM1447	452	CKaM1535
333	CKaM1254	373	CKaM1351	413	CKaM1448	453	CKaM1537
334	CKaM1257	374	CKaM1353	414	CKaM1449	454	CKaM1538
335	CKaM1261	375	CKaM1354	415	CKaM1450	455	CKaM1539
336	CKaM1264	376	CKaM1356	416	CKaM1455	456	CKaM1541
337	CKaM1265	377	CKaM1359	417	CKaM1456	457	CKaM1543
338	CKaM1268	378	CKaM1362	418	CKaM1458	458	CKaM1544
339	CKaM1270	379	CKaM1364	419	CKaM1460	459	CKaM1546
340	CKaM1273	380	CKaM1374	420	CKaM1461	460	CKaM1547
341	CKaM1275	381	CKaM1378	421	CKaM1463	461	CKaM1550
342	CKaM1277	382	CKaM1382	422	CKaM1464	462	CKaM1553
343	CKaM1280	383	CKaM1384	423	CKaM1468	463	CKaM1555
344	CKaM1282	384	CKaM1386	424	CKaM1469	464	CKaM1562
345	CKaM1285	385	CKaM1393	425	CKaM1471	465	CKaM1563
346	CKaM1286	386	CKaM1394	426	CKaM1473	466	CKaM1564
347	CKaM1287	387	CKaM1397	427	CKaM1474	467	CKaM1574
348	CKaM1288	388	CKaM1399	428	CKaM1477	468	CKaM1575
349	CKaM1289	389	CKaM1402	429	CKaM1481	469	CKaM1576
350	CKaM1290	390	CKaM1403	430	CKaM1483	470	CKaM1581
351	CKaM1292	391	CKaM1404	431	CKaM1484	471	CKaM1583
352	CKaM1301	392	CKaM1405	432	CKaM1485	472	CKaM1586
353	CKaM1302	393	CKaM1407	433	CKaM1488	473	CKaM1587
354	CKaM1303	394	CKaM1408	434	CKaM1493	474	CKaM1589
355	CKaM1304	395	CKaM1410	435	CKaM1494	475	CKaM1590
356	CKaM1307	396	CKaM1413	436	CKaM1497	476	CKaM1592
357	CKaM1309	397	CKaM1414	437	CKaM1498	477	CKaM1593
358	CKaM1311	398	CKaM1417	438	CKaM1499	478	CKaM1598
359	CKaM1312	399	CKaM1420	439	CKaM1501	479	CKaM1600
360	CKaM1316	400	CKaM1422	440	CKaM1502	480	CKaM1605

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Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name	Sl. No.	Marker (KASPar assay) name
481	CKaM1606	518	CKaM1685	555	CKaM1763	592	CKaM1845
482	CKaM1607	519	CKaM1689	556	CKaM1765	593	CKaM1854
483	CKaM1608	520	CKaM1690	557	CKaM1766	594	CKaM1855
484	CKaM1611	521	CKaM1691	558	CKaM1767	595	CKaM1859
485	CKaM1612	522	CKaM1693	559	CKaM1768	596	CKaM1860
486	CKaM1614	523	CKaM1695	560	CKaM1769	597	CKaM1863
487	CKaM1618	524	CKaM1701	561	CKaM1770	598	CKaM1871
488	CKaM1619	525	CKaM1703	562	CKaM1772	599	CKaM1872
489	CKaM1621	526	CKaM1704	563	CKaM1773	600	CKaM1878
490	CKaM1623	527	CKaM1706	564	CKaM1774	601	CKaM1883
491	CKaM1624	528	CKaM1707	565	CKaM1775	602	CKaM1889
492	CKaM1629	529	CKaM1710	566	CKaM1776	603	CKaM1890
493	CKaM1630	530	CKaM1712	567	CKaM1782	604	CKaM1893
494	CKaM1631	531	CKaM1715	568	CKaM1783	605	CKaM1903
495	CKaM1632	532	CKaM1716	569	CKaM1786	606	CKaM1904
496	CKaM1634	533	CKaM1717	570	CKaM1788	607	CKaM1905
497	CKaM1636	534	CKaM1718	571	CKaM1790	608	CKaM1908
498	CKaM1638	535	CKaM1719	572	CKaM1796	609	CKaM1914
499	CKaM1640	536	CKaM1720	573	CKaM1805	610	CKaM1920
500	CKaM1643	537	CKaM1721	574	CKaM1808	611	CKaM1921
501	CKaM1645	538	CKaM1722	575	CKaM1810	612	CKaM1928
502	CKaM1646	539	CKaM1726	576	CKaM1812	613	CKaM1933
503	CKaM1647	540	CKaM1727	577	CKaM1818	614	CKaM1946
504	CKaM1649	541	CKaM1732	578	CKaM1819	615	CKaM1960
505	CKaM1651	542	CKaM1733	579	CKaM1820	616	CKaM1968
506	CKaM1653	543	CKaM1734	580	CKaM1824	617	CKaM1975
507	CKaM1658	544	CKaM1738	581	CKaM1825	618	CKaM1977
508	CKaM1662	545	CKaM1740	582	CKaM1826	619	CKaM1978
509	CKaM1663	546	CKaM1742	583	CKaM1828	620	CKaM1984
510	CKaM1665	547	CKaM1743	584	CKaM1830	621	CKaM1990
511	CKaM1667	548	CKaM1747	585	CKaM1832	622	CKaM1999
512	CKaM1669	549	CKaM1748	586	CKaM1834	623	CKaM2003
513	CKaM1670	550	CKaM1750	587	CKaM1836	624	CKaM2004
514	CKaM1676	551	CKaM1754	588	CKaM1840	625	CKaM2005
515	CKaM1677	552	CKaM1758	589	CKaM1842		
516	CKaM1678	553	CKaM1760	590	CKaM1843		
517	CKaM1681	554	CKaM1761	591	CKaM1844		

Table 3b. List of KBD SNP markers used for parental polymorphism study

Sl. No.	SNP marker	Sl. No.	SNP marker	Sl. No.	SNP marker
1	KBD Ca4_10938784	31	KBD Ca4_12483233	61	KBD Ca4_14180364
2	KBD Ca4_11091561	32	KBD Ca4_12525394	62	KBD Ca4_14180446
3	KBD Ca4_11151645	33	KBD Ca4_12558541	63	KBD Ca4_14191063
4	KBD Ca4_11212154	34	KBD Ca4_12591453	64	KBD Ca4_14207137
5	KBD Ca4_11274281	35	KBD Ca4_12766702	65	KBD Ca4_14454011
6	KBD Ca4_11275171	36	KBD Ca4_12982420	66	KBD Ca4_14782609
7	KBD Ca4_11276225	37	KBD Ca4_13260961	67	KBD Ca4_14953388
8	KBD Ca4_11276413	38	KBD Ca4_13320618	68	KBD Ca4_14960491
9	KBD Ca4_11276484	39	KBD Ca4_13391772	69	KBD Ca4_15446980
10	KBD Ca4_11277138	40	KBD Ca4_13641318	70	KBD Ca4_15596429
11	KBD Ca4_11277574	41	KBD Ca4_13687249	71	KBD Ca4_15608950
12	KBD Ca4_11304561	42	KBD Ca4_13702641	72	KBD Ca4_15651804
13	KBD Ca4_11319018	43	KBD Ca4_13704532	73	KBD Ca4_15703929
14	KBD Ca4_11332734	44	KBD Ca4_13704532	74	KBD Ca4_15735041
15	KBD Ca4_11396052	45	KBD Ca4_13718704	75	KBD Ca4_15744761
16	KBD Ca4_11441735	46	KBD Ca4_13724666	76	KBD Ca4_15905044
17	KBD Ca4_11459336	47	KBD Ca4_13726718	77	KBD Ca4_15925936
18	KBD Ca4_11490100	48	KBD Ca4_13742690	78	KBD Ca4_15926040
19	KBD Ca4_11490496	49	KBD Ca4_13769052	79	KBD Ca4_15930674
20	KBD Ca4_11514870	50	KBD Ca4_1387649	80	KBD Ca4_15934901
21	KBD Ca4_11528896	51	KBD Ca4_13796035	81	KBD Ca4_15942209
22	KBD Ca4_11599868	52	KBD Ca4_13838796	82	KBD Ca4_15942388
23	KBD Ca4_11847494	53	KBD Ca4_13839771	83	KBD Ca4_16149795
24	KBD Ca4_11989829	54	KBD Ca4_13841279	84	KBD Ca4_16149998
25	KBD Ca4_11991929	55	KBD Ca4_13844704	85	KBD Ca4_16278600
26	KBD Ca4_12004209	56	KBD Ca4_13858972	86	KBD Ca4_16278671
27	KBD Ca4_12108718	57	KBD Ca4_13872964	87	KBD Ca4_16284657
28	KBD Ca4_12205230	58	KBD Ca4_13974649	88	KBD Ca4_16343743
29	KBD Ca4_12269922	59	KBD Ca4_14019005	89	KBD Ca4_16507090
30	KBD Ca4_12289417	60	KBD Ca4_14180094	-	-

Table 4a. List of CaM markers used for parental polymorphism study

Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker
1	CaM0034	38	CaM0539	75	CaM0909	112	CaM1497
2	CaM0038	39	CaM0574	76	CaM0919	113	CaM1502
3	CaM0063	40	CaM0598	77	CaM0955	114	CaM1506
4	CaM0111	41	CaM0599	78	CaM0958	115	CaM1515
5	CaM0113	42	CaM0600	79	CaM1007	116	CaM1529
6	CaM0123	43	CaM0610	80	CaM1016	117	CaM1536
7	CaM0173	44	CaM0620	81	CaM1020	118	CaM1542
8	CaM0232	45	CaM0624	82	CaM1036	119	CaM1581
9	CaM0233	46	CaM0629	83	CaM1042	120	CaM1590
10	CaM0244	47	CaM0639	84	CaM1068	121	CaM1591
11	CaM0251	48	CaM0645	85	CaM1072	122	CaM1607
12	CaM0260	49	CaM0656	86	CaM1079	123	CaM1620
13	CaM0277	50	CaM0658	87	CaM1084	124	CaM1637
14	CaM0284	51	CaM0661	88	CaM1098	125	CaM1648
15	CaM0286	52	CaM0677	89	CaM1101	126	CaM1666
16	CaM0317	53	CaM0691	90	CaM1122	127	CaM1668
17	CaM0336	54	CaM0698	91	CaM1125	128	CaM1714
18	CaM0340	55	CaM0705	92	CaM1129	129	CaM1722
19	CaM0345	56	CaM0717	93	CaM1132	130	CaM1750
20	CaM0358	57	CaM0720	94	CaM1135	131	CaM1763
21	CaM0368	58	CaM0726	95	CaM1149	132	CaM1782
22	CaM0403	59	CaM0740	96	CaM1158	133	CaM1809
23	CaM0416	60	CaM0743	97	CaM1159	134	CaM1827
24	CaM0421	61	CaM0751	98	CaM1218	135	CaM1853
25	CaM0423	62	CaM0753	99	CaM1228	136	CaM1868
26	CaM0435	63	CaM0790	100	CaM1238	137	CaM1903
27	CaM0443	64	CaM0795	101	CaM1239	138	CaM1975
28	CaM0446	65	CaM0799	102	CaM1337	139	CaM2036
29	CaM0463	66	CaM0805	103	CaM1354	140	CaM2045
30	CaM0464	67	CaM0806	104	CaM1358	141	CaM2049
31	CaM0475	68	CaM0812	105	CaM1360	142	CaM2064
32	CaM0480	69	CaM0821	106	CaM1377	143	CaM2085
33	CaM0486	70	CaM0862	107	CaM1402	144	CaM2094
34	CaM0491	71	CaM0864	108	CaM1451	145	CaM2168
35	CaM0492	72	CaM0880	109	CaM1469	146	CaM2186
36	CaM0507	73	CaM0881	110	CaM1477	-	-
37	CaM0519	74	CaM0886	111	CaM1496	-	-

Table 4b. List of ICCM markers used for parental polymorphism study

Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker
1	ICCM0001a	32	ICCM0074a	63	ICCM0159	94	ICCM0243a
2	ICCM0001b	33	ICCM0075	64	ICCM0160	95	ICCM0243c
3	ICCM0002	34	ICCM0076	65	ICCM0161a	96	ICCM0244a
4	ICCM0003	35	ICCM0077b	66	ICCM0161b	97	ICCM0245
5	ICCM0004	36	ICCM0078a	67	ICCM0162c	98	ICCM0246a
6	ICCM0008a	37	ICCM0078b	68	ICCM0166a	99	ICCM0247
7	ICCM0008b	38	ICCM0079	69	ICCM0166c	100	ICCM0249
8	ICCM0008c	39	ICCM0080	70	ICCM0178	101	ICCM0250
9	ICCM0009a	40	ICCM0081	71	ICCM0185	102	ICCM0251c
10	ICCM0010b	41	ICCM0082	72	ICCM0189	103	ICCM0256c
11	ICCM0014b	42	ICCM0088	73	ICCM0190a	104	ICCM0257
12	ICCM0019b	43	ICCM0089a	74	ICCM0191	105	ICCM0259
13	ICCM0022	44	ICCM0090b	75	ICCM0192a	106	ICCM0263a
14	ICCM0024	45	ICCM0093c	76	ICCM0194	107	ICCM0265c
15	ICCM0026	46	ICCM0094	77	ICCM0196	108	ICCM0269a
16	ICCM0030b	47	ICCM0097a	78	ICCM0197a	109	ICCM0272a
17	ICCM0034	48	ICCM0101b	79	ICCM0200	110	ICCM0273
18	ICCM0042	49	ICCM0103	80	ICCM0202b	111	ICCM0277
19	ICCM0043	50	ICCM0104	81	ICCM0205	112	ICCM0278b
20	ICCM0045	51	ICCM0107b	82	ICCM0207	113	ICCM0281b
21	ICCM0052	52	ICCM0120a	83	ICCM0212a	114	ICCM0282a
22	ICCM0059b	53	ICCM0120b	84	ICCM0215a	115	ICCM0282c
23	ICCM0060	54	ICCM0121a	85	ICCM0216a	116	ICCM0284a
24	ICCM0061	55	ICCM0123a	86	ICCM0216b	117	ICCM0284b
25	ICCM0062	56	ICCM0124	87	ICCM0224	118	ICCM0286a
26	ICCM0063	57	ICCM0125b	88	ICCM0236b	119	ICCM0288
27	ICCM0065b	58	ICCM0130a	89	ICCM0236c	120	ICCM0289
28	ICCM0068	59	ICCM0134	90	ICCM0237b	121	ICCM0290
29	ICCM0069	60	ICCM0155	91	ICCM0240b	122	ICCM0293
30	ICCM0072	61	ICCM0156a	92	ICCM0242a	123	ICCM0295
31	ICCM0073a	62	ICCM0156b	93	ICCM0242b	124	ICCM0297

Table 4c. List of NCPGR markers used for parental polymorphism study

Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker
1	NCPGR4	3	NCPGR10	5	NCPGR19	7	NCPGR27	9	NCPGR93
2	NCPGR7	4	NCPGR12	6	NCPGR21	8	NCPGR89	10	NCPGR142

Table 4d. List of Winter-series markers used for parental polymorphism study

Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker
1	TA05	28	TA186	55	TA71	82	TR26	109	TS38
2	TA103	29	TA189	56	TA72	83	TR28	110	TS39
3	TA106	30	TA191	57	TA76s	84	TR29	111	TS45
4	TA108	31	TA194	58	TA78	85	TR31	112	TS46
5	TA11	32	TA196	59	TA8	86	TR32	113	TS5
6	TA110	33	TA199	60	TA80	87	TR33	114	TS54
7	TA113	34	TA2	61	TA87	88	TR35	115	TS57
8	TA114	35	TA200	62	TA9	89	TR40	116	TS58
9	TA116	36	TA203	63	TA93	90	TR42	117	TS62
10	TA117	37	TA206	64	TA96	91	TR43	118	TS71
11	TA118	38	TA21	65	TAA104	92	TR44	119	TS72
12	TA12	39	TA22	66	TAA169	93	TR56	120	TS74
13	TA122	40	TA23	67	TAA170	94	TR57	121	TS81
14	TA125	41	TA28	68	TAA55	95	TR58	122	TS83
15	TA127	42	TA29	69	TAA58	96	TR59	123	TS84
16	TA13	43	TA30	70	TAA60	97	TR60	124	GA6
17	TA130	44	TA34	71	TR01	98	TR7	125	GA16
18	TA132	45	TA36	72	TR03	99	TS104	126	GA20
19	TA135	46	TA39	73	TR08	100	TS105	127	GA21
20	TA14	47	TA4	74	TR13	101	TS11	128	GA22
21	TA140	48	TA46	75	TR14	102	TS12	129	GA26
22	TA142	49	TA47	76	TR17	103	TS17	130	GA34
23	TA144	50	TA5	77	TR18	104	TS24	131	GA117
24	TA176	51	TA53	78	TR19	105	TS27	132	GA137
25	TA179	52	TA59	79	TR2	106	TS29	133	GAA40
26	TA18	53	TA64	80	TR20	107	TS34	134	GAA46
27	TA180	54	TA65	81	TR24	108	TS36	135	GAA47

Table 4e. List of H-series markers used for parental polymorphism study

Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker	Sl. No.	Marker
1	H1A10	13	H1G22	25	H1O12	37	H3C041	49	H4D08
2	H1A12	14	H1H07	26	H2A08	38	H3D05	50	H4D11
3	H1A18	15	H1H11	27	H2B18	39	H3F08	51	H4G11
4	H1B04	16	H1H15	28	H2B202	40	H3F09	52	H4H11
5	H1B06	17	H1H22	29	H2E13	41	H3H021	53	H4H12
6	H1B11	18	H1H24	30	H2L102	42	H3H07	54	H5A08
7	H1B17	19	H1I01	31	H3A03	43	H3H121	55	H5B04
8	H1C092	20	H1I18	32	H3A10	44	H4F07	56	H5E02
9	H1C22	21	H1J07	33	H3B01	45	H4F09	57	H6D11
10	H1D24	22	H1L161	34	H3C06	46	H4A03	-	-
11	H1E22	23	H1N12	35	H3C10	47	H4A04	-	-
12	H1F14	24	H1O01	36	H3C11	48	H4B06	-	-

Table 5. Mean with standard error and range of flowering time and maturity of parental lines

Sl. No.	Parents	N	Days to flower		Days to Maturity	
			Mean ± SE	Range	Mean ± SE	Range
1	ICCV 96029	20	26.85 ± 0.21	25-28	75.55 ± 0.30	75-81
2	ICC 5810	20	28.35 ± 0.15	28-30	77.25 ± 0.16	77-80
3	BGD 132	20	28.55 ± 0.11	28-29	78.95 ± 0.23	78-83
4	ICC 16641	20	29.00 ± 0.00	29-29	79.00 ± 0.00	79-79
5	CDC Frontier	20	66.90 ± 0.25	65-68	108.15 ± 0.59	104-112

Table 6. Mean with standard error and range of flowering time and maturity of F_1s and F_2 populations

Sl. No.	Cross	F_1					F_2				
		N	Days to flower		Days to maturity		N	Days to flower		Days to maturity	
			Mean ± SE	Range	Mean ± SE	Range		Mean ± SE	Range	Mean ± SE	Range
1	ICCV 96029 × CDC Frontier	20	61.20 ± 0.33	59-63	92.95 ± 0.46	90-95	190	48.89 ± 0.80	25-77	89.63 ± 0.56	73-116
2	ICC 5810 × CDC Frontier	20	54.15 ± 0.19	53-55	89.75 ± 0.76	84-95	190	49.47 ± 0.69	27-76	89.92 ± 0.49	76-113
3	BGD 132 × CDC Frontier	20	53.30 ± 0.31	51-55	94.50 ± 0.53	91-98	190	49.04 ± 0.92	26-75	92.19 ± 0.66	73-120
4	ICC 16641 × CDC Frontier	20	60.80 ± 0.39	59-64	94.65 ± 0.60	90-99	146	52.93 ± 1.26	26-82	91.83 ± 0.92	72-120

Table 7. Segregation of flowering time in F_2 of four chickpea crosses

Sl. No	Cross	N	Observed		Expected		Ratio tested	χ^2	P-value*
			Late	Early	Late	Early			
1	ICCV 96029 × CDC Frontier	190	138	52	142.5	47.5	3:1	0.57	0.5-0.3
2	ICC 5810 × CDC Frontier	190	108	82	106.88	83.13	9:7	0.03	0.9-0.8
3	BGD 132 × CDC Frontier	190	143	47	142.5	47.5	3:1	0.01	0.95-0.9
4	ICC 16641 × CDC Frontier	146	110	36	109.5	36.5	3:1	0.01	0.95-0.9

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 8. Segregation of flowering time in F_3 progenies of four chickpea crosses

Sl. No.	Cross	Phenotypic class	No. of progeny tested	Observed		Expected		Ratio tested	χ^2	P-value*
				Segregating	Non-segregating	Segregating	Non-segregating			
1	ICCV 96029 × CDC Frontier	Late	127	87	40	84.67	42.33	2:1	0.19	0.7-0.5
		Early	37	0	37	0	37	0:1	0	1.0
2	ICC 5810 × CDC Frontier	Late	103	87	16	91.56	11.44	8:1	2.04	0.2-0.1
		Early	71	44	27	40.57	30.43	4:3	0.67	0.5-0.3
3	BGD 132 × CDC Frontier	Late	138	94	44	92	46	2:1	0.13	0.8-0.7
		Early	44	0	44	0	44	0:1	0	1.0
4	ICC 16641 × CDC Frontier	Late	77	54	23	51.33	25.67	2:1	0.416	0.7-0.5
		Early	25	0	25	0	25	0:1	0	1.0

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 9. Segregation of flower color in F_2 of two chickpea crosses

Cross	N	Observed		Expected		Ratio tested	χ^2	P-value*
		Pink	White	Pink	White			
ICCV 96029 × CDC Frontier	190	153	37	142.5	47.5	3:1	3.09	0.1-0.05
ICC 5810 × CDC Frontier	190	146	44	142.5	47.5	3:1	0.34	0.7-0.5

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 10. Segregation of flower color in F_3 progenies of two chickpea crosses

Cross	Phenotypic class	No. of progeny tested	Observed		Expected		Ratio tested	χ^2	P-value*
			Segregating	Non-segregating	Segregating	Non-segregating			
ICCV 96029 × CDC Frontier	Pink	149	102	47	91.5	45.75	2:1	1.24	0.3-0.2
	White	34	0	34	0	34	0:1	0	1.0
ICC 5810 × CDC Frontier	Pink	143	101	42	92.5	46.25	2:1	1.17	0.3-0.2
	White	42	0	42	0	42	0:1	0	1.0

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 11. Segregation of single/double podding trait in F_2 of the cross ICCV 96029 × CDC Frontier

Cross	N	Observed		Expected		Ratio tested	χ^2	P-value*
		Single podding	Double podding	Single podding	Double podding			
ICCV 96029 × CDC Frontier	190	146	44	142.5	47.5	3:1	0.34	0.7-0.5

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 12. Segregation of single/double podding trait in F_3 progenies of the cross ICCV 96029 × CDC Frontier

Cross	Phenotypic class	No. of progeny tested	Observed		Expected		Ratio tested	χ^2	P-value*
			Segregating	Non-segregating	Segregating	Non-segregating			
ICCV 96029 × CDC Frontier	Single podding	138	93	45	91.5	45.75	2:1	0.03	0.9-0.8
	Double podding	45	0	45	0	45	0:1	0	1.0

*Null hypothesis of the test is that progeny segregate in the ratios tested. If the p-value (probability) is less than or equal to 0.05, then reject the null hypothesis. Otherwise, one fails to reject the null hypothesis.

Table 13. Polymorphism survey of SNP and SSR markers between parental lines of four intraspecific mapping populations

Markers	No. of markers screened	ICCV 96029 × CDC Frontier		ICC 5810 × CDC Frontier		BGD 132 × CDC Frontier		ICC 16641 × CDC Frontier	
		No. of polymorphic markers	Polymor-phism (%)	No. of polymorphic markers	Polymor-phism (%)	No. of polymorphic markers	Polymor-phism (%)	No. of polymorphic markers	Polymor-phism (%)
SNP	714	49	6.86	44	6.16	36	5.04	29	4.06
SSR markers									
CaM-series	146	18	12.33	18	12.33	16	10.96	14	9.59
H-series	57	16	28.07	13	22.81	17	29.82	16	28.07
ICCM-series	124	8	6.45	5	4.03	5	4.03	7	5.65
NCPGR-series	10	5	50.00	3	30.00	4	40.00	5	50.00
Winter-series	135	53	40.00	56	41.48	48	35.56	51	37.78
Total	**472**	**100**	**21.40**	**95**	**20.13**	**90**	**19.07**	**93**	**19.70**

Table 13a. Primers sequence of polymorphic SSR markers used for genetic linkage mapping

Marker	SSR repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	GenBankID
CaM0038	(TAA)43	CATGCTCGAATCTTATTTTGAGG	TCGATATAGCAAGGGAGAGGA	EI847480
CaM0111	(AAT)13	CCTCTCCTAGAACACCCCAA	GGTGTCAACAACCTAACTGTTTATTTT	EI849398
CaM0113	(TTA)52	AATGGAGAATGATGGGTTGC	GCCCCGTGTCCCTTATAAAT	EI849479
CaM0244	(TCTCT)6	TTTCCCCTTCTTTCTCAACA	TTCAGAGATTGGATGAGAAGGTT	EI852407
CaM0317	(AAT)13	TGGCCTAAATGTCTCAGCAA	AGAGGCAAACAAGAACCGAA	EI854030
CaM0443	(TAA)37	TCGTTTGCATAAGATGGAACA	GTACAACCGCCGCAAATATC	EI856442
CaM0475	(TA)12	TGTGTCGTATATTGATTTGTGTATCG	TGGTTTGACAAGGGGAAGAC	EI857186
CaM0507	(ATT)21a(ATT)9N(A)15	GAAGGAGAGAAGAAGGGGGA	AATTAGGTTTTGACACGTCCG	EI857918
CaM0661	(TAA)9tag(TAA)43	TCGTTTGCATAAGATGGAACA	TGCTATTAAGTGTGACCAGCAA	EI861555
CaM0753	(TTA)23	AATTGCGGCGAGAGAAGATA	TCAGTTTCTCTTTTCGATTCTTTC	EI863632
CaM0805	(TAA)24N(T)11	TGGTTAAAACATGCTCAAATCCT	TGGCGTTAATTTTAGGGACG	EI864627
CaM0886	(AT)17	TGAGAATTTTTCTTGAACTTGAACTG	AAGTCTTTTCCAGCACTTTTGC	EI866534
CaM1020	(AT)14	CCGATTTACACAATGCTATCCA	TCAGTATGGGTAGAGCATGTAGG	EI869214
CaM1122	(AG)21	CCAAAGGGGTGAGTTTTTGA	CCCCCTTAATTTCTTTCTCCA	EI871582
CaM1228	(ATA)23	TTTTCTTTTTACGATCAAGATCAAAC	CAATTGAATGTGTGGTTAATGGA	EI873918
CaM1358	(TA)5N(TA)15N(AT)5	GATGTGAGTGAAGTGACGTGG	AGAAAAGGAAGACGTTCGCA	EI876349
CaM1402	(ATA)35	CACCCAAATCCCCAAAATAA	TGCCTTTTGTATTTGAAAAATGTG	EI877412
CaM1515	(AG)5N(TA)18	GCAATGAGAAGGGAAGGAAA	GCGGAAAACCAATTTACCAA	EI879617
CAM2049	(AGAT)15	CCCTTTGGAAAGAGAGGAGG	AAGCCGATTCTTGGGACTTT	EI889687
GA16	(GA)22	CACCTCGTACCATGGTTTCTG	TAAATTTCATCCTCTCCGGC	-
GA34	(CT)11	CCTTTGCATGTATGTGGCAT	CCGTTTATAAAGGATGTAZGAGAC	-
GA6	(GA)23	ATTTTTCTCCGGTGTTGCAC	AAACGACAGAGAGTGGCGAT	-
GAA47	(GAA)11	CACTCCTCATGCCAACTCCT	AAAATGGAATAGTCGTATGGGG	-
H1B04	(TTA)35	TAGTTGAAACACACGGGTTA	AAAGTGAAATATGTCATCCTTATTA	-
H1C092	(GAA)5 119 bp (TAA)29	CAATAAAACACTTTGTTCCTTTTT	TGTAGAAAGAAAGCTAGCATGG	-
H1C22	(CAA)5 (TAA)47 AA (TAA)2 TAAAA (TAA)22	ATTTATACAAAGTTTTTGAAGTCG	CTTGTAAGTAGATAGTTTCACCAAA	-
H1D24	(TTA)14 TTG (TTA)6	TTTCGGTGAACAAAAACTAACTA	ACGGTTAAATAGATGAGTCAAAA	-

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Marker	SSR repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	GenBankID
H1F14	(TTTA)4 (TTA)11	GAGAGAGAGGAAGGGAAACG	TCCTAACTTGCTCCTTAACCTTG	-
H1H15	(GA)9 23 bp (GA)11	GGCGAGAAGGAAATTAAACG	TTGGCACGTGTTGGATACTT	-
H1N12	(CA)5 46 bp (CA)5	AAAAATTGGTTCTCAAGAGTAAA	ATGAGGATTGGACGTAATCA	-
H3A10	(TTA)24	TTTAAGGCTTCAGGTATTGATTTCT	TCACACATGCCAACTTAAAATAAAA	-
H3C06	(TAA)23	AATTTCGTGAATCATTAAAAATAGAGG	CACATGACTATCTAGACATTTTATTTATC	-
H3D05	(TAA)41	AGACGTGTTCCCTTTCTTTTAACTA	GCCGACACAAAGTTTATGATTTT	-
H3F08	(TTA)34	AAACACCCGTGATTCTCTAAAGTT	TGACACCTAATTTTATTCGGTTTTT	-
H3F09	(TTA)44	AGCATGTAGTAGGAGGCAAGTATG	GTAGGTTCCCGCTACATTACTTTTA	-
H4A04	(TTA)32	GCAAATTCTCACCATTTCTTTTT	TGTTTTGACGAATGAGAAGTAAAGA	-
H4D08	(TAA)4 9 bp (TAA)25	TGTCCTTTATTTCTTAAGCACACAT	GAGATGGATGTTATTGGACTCATC	-
H4G11	(TAA)18	ATCTAAGTGAGCGGCTACTAAATCA	GTAGTCATGCAGCCTATAAAAACAA	-
H5A08	(TTA)18 CCA (TTA)8 TT(TTA)3	AGGAGAGAAAATGTAACATCCTAAATC	CAAATTGGTTATTGATTACAATTAGGT	-
H6D11	(TAA)11	AAAGATGGGAACTTGAGATGTTG	AATAGCTACTCAAGGCTGAAGAAA	-
ICCM0130a	(AAT)22	GGATTTCGACTTTTATCCCTTTTT	CGGACTGGAATCAAAAGCTC	FI856734
ICCM0160	(AAC)4N(TAA)25	TTGCTTGAAACAACCTTTCG	CGGGTACAACCGTAGCAAAT	FI856816
ICCM0192a	(TAT)15c(ATT)15	GCTGCCCAAATTTTGACATTA	CCGGGGATCAAATTCTTCTT	FI856879
ICCM0243c	(GA)41tagat(AG)10	ACGACGATTCTGGATTTTGG	AGTTTTGGTAGGGGGTCGAG	FI856930
ICCM0250	(TAT)40	TTTCAAACACAATCTGAACGAGA	CCACCTTCGGGTAGGATACA	FI856940
ICCM0263a	(TATT)7	CGGGGATAAATCAACACACC	GGGCAAGGTCTTACCCTTGT	FI856635
ICCM0297	(TAA)18	CATGATTTGATTTGATTTGATTTC	GGAGTGGGAAACCTTAAGCC	FI856987
NCPGR10	(GA)42	AATCATTCAACTTACGGT	GACTTGTATGCTACTGTTGA	AY255884
NCPGR12	(CT)35	CCTTGTTAGTGTGTATAGGT	GTAATGACCAAGTGAACA	AY255886
NCPGR19	(GA)19	TCCATTGTAGCTTAGCTTAG	TCTTACTCTTAGCTTACCTCTT	AY255889
NCPGR21	(CT)15	TCTACCTCGTTTTTCGTGCC	TTGCTCCTTCAACAAAACCC	AY446327
NCPGR7	(CA)14	GACCAAGATTAGTAGAACCT	CTTGATAAGGATGAGTCATG	AY255883
NCPGR89	(GA)28	AAAGGGCCTTCAAGTTGTAT	ACTTTTGGAGTGAGAGGCT	AY446390
NCPGR93	(CA)2 (CT)24 (CA)13	CAAAGTTTGTTGCTAGGATTC	GAAGATCTCCGACGATGATA	AY446394
TA05	(TTA)29	ATCATTTCAATTTCCTCAACTATGAAT	TCGTTAACACGTAATTTCAAGTAAAGAT	-

Contd….

Marker	SSR repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	GenBankID
TA106	(TAA)26	CGGATGGACTCAACTTTATC	TGTCTGCATGTTGATCTGTT	-
TA11	(TTA)17	CATGCCATAAACTCAATACAATACAAC	TTCATTGAGGACAATGTGTAATTTAAG	-
TA110	(TTA)22	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA	-
TA116	(TAA)5TT(A)3(TAA)20	AATTCAATGACGAATTTTTATAAGGG	AAAAAGAAAAGGGAAAAGTAGGTTTTA	-
TA118	$(TAA)_{45}$	ACAAGTCACATGTGTTCTCAATA	GGA AAGGTTAAGA AATTTTACAATAC	-
TA122	$(TAA)_{28}$	AATGCTACAAATTATTAAAAACAGTC	AACTTTTAGTGTGCTGA CGA GT	-
TA127	(GTT)5(ATT)23	AAATTGTAAGACTCTCATTTTTCTTTATT	TCAAATTAACTACATCATGTCACACAC	-
TA132	$(TAA)_{28}$	CGA ATAACTGA GA AAAAGA AATTAG	TTCTAAAACTTCCTTCTACCATTAG	-
TA14	(TAA)22ATGA(TAA)4T(A)3TGAT(AAT)5ATT(A)3TGATAATAAAT(GAT)4(TAA)5	TGACTTGCTATTTAGGGAACA	TGGCTAAAGACAATTAAAGTT	-
TA142	(TTA)15	TGTTAACATTCCCTAATATCAATAACTT	TTCCACAATGTTGTATGTTTTGTAAG	-
TA144	(TAA)27	TATTTTAATCCGGTGAATATTACCTTT	GTGGAGTCACTATCAACAATCATACAT	-
TA176	(TAA)40(GAA)9	ATTTGGCTTAAACCCTCTTC	TTTATGCTTCCTCTTCTTCG	-
TA179	(TAA)40(TAAA)8	CAGAAGACGCAGTTTGAATAACTT	CGAGAGAGAGAAAGGAAGAAGAG	-
TA180	(TAA)30	CATCGTGAATATTGAAGGGT	CGGTAAATAAGTTTCCCTCC	-
TA196	(TAA)19	TCTTTTTAAATTTCATTATGAAAATACAAATTATA	CCTCGGGAGAGGTAAATGTAATTTC	-
TA200	(TTA)37	TTTCTCCTCTACTATTATGATCACCAG	TTGAGAGGGTTAGAACTCATTATGTTT	-
TA21	(TAA)51	GTACCTCGAAGATGTAGCCGATA	TTTTCCATTTAGAGTAGGATCTTCTTG	-
TA22	(ATT)40	TCTCCAACCCTTTAGATTGA	TCGTGTTTACTGAATGTGGA	-
TA28	(TAA)37CAA(TAA)30	TAATTGATCATACTCTCACTATCTGCC	TGGGAATGAATATATTTTTGAAGTAAA	-
TA30	(TAA)18TA(TAA)19	TCATTAAAATTCTATTGTCCTGTCCTT	ATCGTTTTTCTAAACTAAATTGTGCAT	-
TA34	(AAT)34	AAGAGTTGTTCCCTTTCTTTT	CCATTATCATTCTTGTTTTCAA	-
TA59	(TAA)29	ATCTAAAGAGAAATCAAAATTGTCGAA	GCAAATGTGAAGCATGTATAGATAAAG	-
TA64	(TAA)39	ATATATCGTAACTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA	-
TA71	(AAT)32	CGATTTAACACAAAACACAAA	CCTATCCATTGTCATCTCGT	-
TA78	(TTA)30	CGGTAAATAAGTTTCCCTCC	CATCGTGAATATTGAAGGGT	-
TA8	(TAA)44	AAAATTTGCACCCACAAAATATG	CTGAAAATTATGGCAGGGAAAC	-

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Marker	SSR repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	GenBankID
TA80	(TTA)23	CGAATTTTTACATCCGTAATG	AATCAATCCATTTTGCATTC	-
TA96	(AT)3(TTA)30(AT)3	TGTTTTGGAGAAGAGTGATTC	TGTGCATGCAAATTCTTACT	-
TAA170	(TTA)33	TATAGAGTGAGAAGAAGCAAAGAGGAG	TATTTGCATCAATGTTCTGTAGTGTTT	-
TAA58	(AAT)41	CATTGCTTAAGA ACCAAAATGG	CAATTTTACATCGA CGTGTGC	-
TR01	(TAA)31	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGAAAGT	-
TR13	$(TTA)_{47}TCA(TTA)_{6}$	CTTATTATTCAACTTCCATTTTGTT	TTTGTAATTTATGACACATTAATAGTTAG	-
TR14	$(TAA)_{36}$	TAAAGGGA CCAAAATCTCACAATTA	GA AATTAAGTTAAAAGA CCTCATGA	-
TR18	$(TAA)_{8}TAGTAATAG(TAA)_{32}$	CCCACTGA AAAATAAAAAGA TATAA	GTTTAACATTTTCGGTAGTTATCAA	-
TR19	(TAA)27	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTTCTCCA	-
TR29	(TAA)8TAGTAATAG(TAA)32	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG	-
TR32	(TTA)30	TTATTTTAACAWCTTCCTCTTATTGTCC	AAAACGGGTTTGATGTTTGATG	-
TR33	(TTA)22	TCTGATTTAATTTCCTATCATTAGTTGC	ATTTTTGTCGGGGAGTACATAATA	-
TR40	(TAA)44	AAGTGAAATATGTCATCCTTATTACTAACT	AGGAAACTGTGTTTCGTCTTTTTATT	-
TR42	$(TAA)_{57}$	TCTGTCATTCATAATGA TGTATTCT	CAACTCAACATGCTTTAATTGA T	-
TR43	(TAA)24	AGGACGAAACTATTCAAGGTAAGTAGA	AATTGAGATGGTATTAAATGGATAACG	-
TR44	(TAT)16	TTAATATTCAAAAACTCTCTTGTGCAAT	TTTACAACAGCGCTTGTATTTAGTAAG	-
TR56	(TAA)21	TTGATTCTCTCACGTGTAATTC	ATTTTGATTACCGTTGTGGT	-
TR59	(TA)3(TAA)17T(TAA)4	AAAAGGAACCTCAAGTGACA	GAAAATGAGGGAGTGAGATG	-
TR7	(TTA)25	GCATTATTCACCATTTGGAT	TGTGATAATTTTCTAAGTGTTTT	-
TS104	(ATT)40	TCAAGATTGATATTGATTAGATAAAAGC	CTTTATTTACCACTTGCACAACACTAA	-
TS36	$(TTA)_{3}CTA(TTA)_{22}$	ACCCTTTCAAATTTTCAACCTA	ATTACATCTAAATAAAGA CCCGTTA	-
TS45	(TAA)8(A)3(TAA)18	TGACACAAAATTGTCTCTTGT	TGTTCTTAACGTAACTAACCTAA	-
TS46	(TAA)46(CAA)2(TAA)3	GTTGATATTTTTGTGTGTGCGTAG	TAATTACTTGCAAAAATAAATGGACAC	-
TS5	(TTA)35	GTTGAATAGTACTTTCCCACTTGAGTC	TGAGACTAAAAATCATATATTCCCCC	-
TS72	(ATT)39	CAAACAATCACTAAAAGTATTTGCTCT	AAAAATTGATGGACAAGTGTTATTATG	-

Table 14. List of polymorphic SNP markers for the four crosses

Sl. No.	ICCV 96029 × CDC Frontier	ICC 5810 × CDC Frontier	BGD 132 × CDC Frontier	ICC 16641 × CDC Frontier
1	CKAM1101	CKAM1064	CKAM1772	CKAM1101
2	CKAM0709	CKAM0709	CKAM0709	CKAM1772
3	CKAM1312	CKAM1312	CKAM1312	CKAM1808
4	CKAM1691	CKAM1461	CKAM1691	CKAM0709
5	CKAM1461	CKAM0246	CKAM1461	CKAM0722
6	CKAM0291	CKAM0291	CKAM0291	CKAM1312
7	CKAM0493	CKAM0493	CKAM0493	CKAM1691
8	CKAM0612	CKAM0612	CKAM0612	CKAM1461
9	CKAM0722	CKAM0722	CKAM0722	CKAM1904
10	CKAM1790	CKAM1790	CKAM1790	KBDCa4_12004209
11	KBDCa4_10450575	KBDCa4_11212154	KBDCa4_11212154	KBDCa4_12483233
12	KBDCa4_11212154	KBD Ca4_11274281	KBDCa4_11274281	KBDCa4_12525394
13	KBDCa4_11274281	KBD Ca4_11275171	KBDCa4_11275171	KBDCa4_12558541
14	KBDCa4_11275171	KBD Ca4_11276413	KBDCa4_11276413	KBDCa4_13391772
15	KBDCa4_11276413	KBD Ca4_11277138	KBDCa4_11277138	KBDCa4_13641318
16	KBDCa4_11277138	KBD Ca4_11277574	KBDCa4_11277574	KBD Ca4_13687249
17	KBDCa4_11277574	KBD Ca4_11319018	KBDCa4_11319018	KBD Ca4_13702641
18	KBDCa4_11319018	KBD Ca4_11332734	KBD Ca4_11332734	KBD Ca4_13704532
19	KBDCa4_11332734	KBD Ca4_11396052	KBD Ca4_11396052	KBD Ca4_13718704
20	KBDCa4_11396052	KBD Ca4_11441735	KBD Ca4_11441735	KBD Ca4_13724666
21	KBDCa4_11441735	KBD Ca4_11459336	KBD Ca4_11459336	KBD Ca4_13726718
22	KBDCa4_11459336	KBD Ca4_11490100	KBD Ca4_11490100	KBD Ca4_13769052
23	KBDCa4_11490100	KBD Ca4_11490496	KBD Ca4_11490496	KBD Ca4_1387649
24	KBD Ca4_11490496	KBD Ca4_11514870	KBD Ca4_11514870	KBD Ca4_13838796
25	KBD Ca4_11514870	KBD Ca4_12004209	KBD Ca4_12004209	KBD Ca4_13839771
26	KBD Ca4_12004209	KBD Ca4_12483233	KBD Ca4_12483233	KBD Ca4_13841279
27	KBD Ca4_12483233	KBD Ca4_12525394	KBD Ca4_12525394	KBD Ca4_13844704
28	KBD Ca4_12525394	KBD Ca4_12558541	KBD Ca4_14180446	KBD Ca4_15651804
29	KBD Ca4_12558541	KBD Ca4_13391772	KBD Ca4_15925936	KBD Ca4_16149795
30	KBD Ca4_13391772	KBD Ca4_13641318	KBD Ca4_15926040	
31	KBD Ca4_13641318	KBD Ca4_13687249	KBD Ca4_15930674	
32	KBD Ca4_13687249	KBD Ca4_13702641	KBD Ca4_15934901	
33	KBD Ca4_13702641	KBD Ca4_13704532	KBD Ca4_15942388	
34	KBD Ca4_13704532	KBD Ca4_13718704	KBD Ca4_16149998	
35	KBD Ca4_13718704	KBD Ca4_13724666	KBD Ca4_16278600	
36	KBD Ca4_13724666	KBD Ca4_13726718	KBD Ca4_16284657	
37	KBD Ca4_13726718	KBD Ca4_13769052		
38	KBD Ca4_13769052	KBD Ca4_1387649		
39	KBD Ca4_1387649	KBD Ca4_13838796		
40	KBD Ca4_13838796	KBD Ca4_13839771		
41	KBD Ca4_13839771	KBD Ca4_13841279		
42	KBD Ca4_13841279	KBD Ca4_13844704		
43	KBD Ca4_13844704	KBD Ca4_15651804		
44	KBD Ca4_15925936	KBD Ca4_16149795		
45	KBD Ca4_15926040			
46	KBD Ca4_15930674			
47	KBD Ca4_15934901			
48	KBD Ca4_15942388			
49	KBD Ca4_16149795			

Table 15a. List of polymorphic SSRs for the cross ICCV 96029 × CDC Frontier

Sl. No.	Marker	ICCV 96029	CDC Frontier	Sl. No.	Marker	ICCV 96029	CDC Frontier
		Allele size	Allele size			Allele size	Allele size
1	CaM0038	289.72	341.64	51	TA05	191.58	200
2	CaM0111	300.66	294.79	52	TA106	258.39	234.92
3	CaM0244	221.18	216.36	53	TA11	208.76	208.77
4	CaM0443	236.71	290.32	54	TA110	241.86	221.43
5	CaM0507	222.39	254.21	55	TA116	178.75	181.63
6	CaM0661	383.23	311.22	56	TA118	216.22	230.83
7	CaM0753	201.03	207.3	57	TA122	157.24	172.8
8	CaM0799	208.18	202.96	58	TA127	251.38	242.77
9	CaM0805	293.13	311.43	59	TA132	178.04	197.08
10	CaM0886	258.82	268.98	60	TA14	269.2	242.74
11	CaM1122	266.4	256.46	61	TA144	265.82	260.14
12	CaM1158	225.94	230.51	62	TA176	281.37	213.22
13	CaM1228	239.73	227.57	63	TA180	208.14	202.14
14	CaM1358	241.07	229.38	64	TA196	194.37	191.26
15	CaM1402	238.3	211.64	65	TA200	294.82	276.89
16	CaM1451	252.85	264.97	66	TA203	222.36	231.45
17	CaM1515	244.49	240.08	67	TA21	352.21	346.24
18	CaM2049	199.4	187.39	68	TA22	250.89	191.2
19	GA34	173.99	178.06	69	TA28	395.8	338.5
20	GA6	208.18	192.23	70	TA59	250.09	235.42
21	GAA47	151.4	167.39	71	TA64	202.82	236.26
22	H1B04	287.34	281.28	72	TA71	199.24	214.22
23	H1C092	202.45	232.01	73	TA76s	228.24	234.32
24	H1C22	274.21	250.57	74	TA78	209.37	203.68
25	H1D24	181.23	187.7	75	TA8	192.7	208.88
26	H1G22	207.94	181.59	76	TA80	204.88	201.77
27	H1H15	223.28	234.17	77	TA93	152.15	142.42
28	H3A10	307.82	273.64	78	TA96	273.27	254.97
29	H3C06	190.23	205.22	79	TAA104	212.88	219.03
30	H3C11	199.24	250.86	80	TAA170	229.66	257.03
31	H3F08	214.8	218.23	81	TAA58	303.64	273.97
32	H3F09	226.28	205.25	82	TR01	227.62	213.32
33	H4A04	276.6	258.48	83	TR13	240.52	258.76
34	H4D08	225.39	210.73	84	TR18	239.65	245.66
35	H4G11	211.44	232.62	85	TR19	201.75	216.62
36	H5A08	240.4	225.33	86	TR24	154.54	157.88
37	H6D11	271.5	288.74	87	TR29	208.43	215.04
38	ICCM0080	175.67	163.13	88	TR33	253.31	247.29
39	ICCM0120a	223.02	216.94	89	TR40	264.36	255.69
40	ICCM0160	272.64	327.74	90	TR42	247.25	349.00
41	ICCM0192a	313.04	322.56	91	TR43	375.75	298.34
42	ICCM0250	478.92	494.68	92	TR44	282.17	288.67
43	ICCM0263a	275.27	282.54	93	TR56	234.08	242.69
44	ICCM0269a	313.26	322.48	94	TR59	273.49	285.77
45	ICCM0297	375.31	300.42	95	TS104	162.42	211.69
46	NCPGR10	289.33	261.71	96	TS36	130.55	186.63
47	NCPGR19	308.51	298.13	97	TS45	243.61	239.84
48	NCPGR21	154.85	165.48	98	TS46	224.5	242.24
49	NCPGR7	221.11	217.5	99	TS5	379.25	322.41
50	NCPGR93	313.91	297.67	100	TS83	257.8	248.45

Table 15b. List of polymorphic SSRs for the cross ICC 5810 × CDC Frontier

Sl. No.	Marker	ICC 5810	CDC Frontier	Sl. No.	Marker	ICC 5810	CDC Frontier
		Allele size	Allele size			Allele size	Allele size
1	CaM0038	274.74	341.64	49	TA127	236.36	242.77
2	CaM0111	275.37	294.79	50	TA132	176.17	197.08
3	CaM0113	173.96	177.23	51	TA135	210.05	193.26
4	CaM0244	221.27	216.36	52	TA14	260.36	242.74
5	CaM0317	272.94	285.47	53	TA142	127.39	134.18
6	CaM0443	305.19	290.32	54	TA144	277.83	260.14
7	CaM0507	241.26	254.21	55	TA176	272.09	213.22
8	CaM0661	266.28	311.22	56	TA179	200.81	207.73
9	CaM0691	212.72	248.13	57	TA180	208.97	202.14
10	CaM0799	208.23	202.96	58	TA200	267.73	276.89
11	CaM0805	308.19	311.43	59	TA203	196.29	231.45
12	CaM0886	250.51	268.98	60	TA21	337.9	346.24
13	CaM1020	267.62	264.21	61	TA22	275.62	191.2
14	CaM1358	235.93	229.38	62	TA28	369.64	338.5
15	CaM1402	244.64	211.64	63	TA30	220.06	208.37
16	CaM1515	244.58	240.08	64	TA34	246.95	223.5
17	CaM1853	176.07	171.4	65	TA39	357.39	337.72
18	CaM2049	219.11	187.39	66	TA59	258.86	235.42
19	GA16	266.22	258.29	67	TA64	245.79	236.26
20	GA34	157.44	178.06	68	TA71	198.86	214.22
21	GA6	233.89	192.23	69	TA76	230.85	234.32
22	GAA47	151.6	167.39	70	TA78	209.55	203.68
23	H1C22	289.2	250.57	71	TA80	234.42	201.77
24	H1F14	203.86	197.75	72	TA93	149.16	142.42
25	H3A10	317.13	273.64	73	TA96	282.38	254.97
26	H3C06	219.53	205.22	74	TAA170	253.94	257.03
27	H3C11	202.26	250.86	75	TAA58	267.75	273.97
28	H3D05	384.83	362.14	76	TR01	227.74	213.32
29	H3F08	244.98	218.23	77	TR13	252.43	258.76
30	H3F09	311.83	205.25	78	TR14	203.24	194.37
31	H4A04	285.79	258.48	79	TR19	228.39	216.62
32	H4D08	228.47	210.73	80	TR24	160.41	157.88
33	H4G11	238.27	232.62	81	TR31	202.73	214.28
34	H5A08	237.35	225.33	82	TR33	243.99	247.29
35	H6D11	297.34	288.74	83	TR40	234.48	255.69
36	ICCM0080	174.46	163.13	84	TR42	262.33	349
37	ICCM0160	343.12	327.74	85	TR43	323.42	298.34
38	ICCM0243c	264.49	244.98	86	TR44	279.15	288.67
39	ICCM0250	475.8	494.68	87	TR56	236.64	242.69
40	ICCM0297	316.14	300.42	88	TR7	204.3	210.5
41	NCPGR21	173.3	165.48	89	TS104	190.78	211.69
42	NCPGR89	279.18	226.37	90	TS27	263.52	254.71
43	NCPGR93	320.43	297.67	91	TS36	130.81	186.63
44	TA106	252.63	234.92	92	TS45	228.68	239.84
45	TA11	208.69	208.77	93	TS46	218.42	242.24
46	TA116	175.68	181.63	94	TS5	343.63	322.41
47	TA118	221.93	230.83	95	TS72	274.33	261.67
48	TA122	178.91	172.8	-	-	-	-

Table 15c. List of polymorphic SSRs for the cross BGD 132 × CDC Frontier

Sl. No.	Marker Name	BGD132	CDC Frontier	Sl. No.	Marker Name	BGD132	CDC Frontier
		Allele size	Allele size			Allele size	Allele size
1	CaM0038	289.87	341.64	46	TA106	216.37	234.92
2	CaM0111	275.52	294.79	47	TA11	208.61	208.77
3	CaM0113	174.09	177.23	48	TA110	241.82	221.43
4	CaM0443	284.16	290.32	49	TA116	178.65	181.63
5	CaM0507	223.46	254.21	50	TA118	251.46	230.83
6	CaM0661	284.22	311.22	51	TA122	166.82	172.80
7	CaM0691	212.66	248.13	52	TA125	214.54	232.86
8	CaM0799	208.19	202.96	53	TA127	251.43	242.77
9	CaM0805	293.23	311.43	54	TA14	269.49	242.74
10	CaM0886	254.55	268.98	55	TA142	124.53	134.18
11	CaM1122	266.28	256.46	56	TA144	265.95	260.14
12	CaM1228	239.39	227.57	57	TA180	226.51	202.14
13	CaM1402	205.68	211.64	58	TA196	194.41	191.26
14	CaM1515	244.46	240.08	59	TA200	294.95	276.89
15	CaM1853	190.83	171.40	60	TA21	357.73	346.24
16	CAM2049	188.55	212.50	61	TA22	220.94	191.20
17	GA6	230.03	192.23	62	TA59	250.04	235.42
18	GAA47	151.40	167.39	63	TA64	231.02	236.26
19	H1B04	290.66	281.28	64	TA71	199.03	214.22
20	H1C22	295.09	250.57	65	TA76	228.14	234.32
21	H1D24	181.24	187.70	66	TA78	227.60	203.68
22	H1G22	196.44	181.59	67	TA93	145.66	142.42
23	H1H15	237.73	234.17	68	TA96	273.31	254.97
24	H1N12	182.04	176.95	69	TAA104	213.10	219.03
25	H3A10	307.55	273.64	70	TAA170	236.03	257.03
26	H3C06	181.24	205.22	71	TAA58	297.36	273.97
27	H3C11	202.30	250.86	72	TR01	230.90	213.32
28	H3D05	376.51	362.14	73	TR13	228.38	258.76
29	H3F08	211.69	218.23	74	TR18	239.64	245.66
30	H3F09	235.15	205.25	75	TR19	204.59	216.62
31	H4A04	276.68	258.48	76	TR24	160.61	157.88
32	H4D08	225.50	210.73	77	TR29	176.55	215.04
33	H4G11	214.46	232.62	78	TR33	234.80	247.29
34	H5A08	240.53	225.33	79	TR40	249.37	255.69
35	H6D11	328.25	288.74	80	TR42	283.85	349.00
36	ICCM0192a	312.89	322.56	81	TR44	282.51	288.67
37	ICCM0250	251.47	494.68	82	TR56	233.16	242.69
38	ICCM0263a	275.49	282.54	83	TR59	273.34	285.77
39	ICCM0269a	313.02	322.48	84	TR7	219.45	210.50
40	ICCM0297	313.47	300.42	85	TS104	205.66	211.69
41	NCPGR10	255.71	261.71	86	TS36	130.85	186.63
42	NCPGR12	214.72	256.42	87	TS46	250.87	242.24
43	NCPGR19	308.49	298.13	88	TS5	370.46	322.41
44	NCPGR21	171.32	165.48	89	TS5	370.46	322.41
45	TA05	191.58	200.00	90	TS72	249.53	261.67

Table 15d. List of polymorphic markers for cross ICC 16641 × CDC Frontier

Sl. No.	Marker	ICC 16641	CDC Frontier	Sl. No.	Marker	ICC 16641	CDC Frontier
		Allele size	Allele size			Allele size	Allele size
1	CaM0038	263.3	341.6	48	TA11	208.7	208.8
2	CaM0244	221.5	216.4	49	TA110	227.2	221.4
3	CaM0443	248.6	290.3	50	TA116	187.3	181.6
4	CaM0475	207.9	231.4	51	TA118	207.3	230.8
5	CaM0507	227.0	254.2	52	TA122	148.7	172.8
6	CaM0661	272.2	311.2	53	TA125	235.7	232.9
7	CaM0805	296.1	311.4	54	TA127	248.5	242.8
8	CaM0886	247.6	269.0	55	TA135	198.3	193.3
9	CaM1122	266.4	256.5	56	TA14	260.5	242.7
10	CaM1228	236.7	227.6	57	TA142	127.8	134.2
11	CaM1358	239.1	229.4	58	TA176	219.0	213.2
12	CaM1402	200.2	211.6	59	TA179	200.0	207.7
13	CaM1515	244.1	240.1	60	TA180	206.3	202.1
14	CaM2049	201.8	187.4	61	TA196	185.3	191.3
15	GA16	264.7	258.3	62	TA200	270.8	276.9
16	GA6	218.1	192.2	63	TA203	210.6	231.5
17	GAA47	152.4	167.4	64	TA21	332.0	346.2
18	H1C22	274.2	250.6	65	TA22	215.2	191.2
19	H1D24	181.3	187.7	66	TA64	260.8	236.3
20	H1F14	204.0	197.8	67	TA71	202.2	214.2
21	H1G22	202.4	181.6	68	TA76s	222.5	234.3
22	H1H15	223.1	234.2	69	TA78	206.8	203.7
23	H3A10	243.0	273.6	70	TA8	201.8	208.9
24	H3C06	181.5	205.2	71	TA93	139.0	142.4
25	H3C11	220.2	250.9	72	TAA104	242.9	219.0
26	H3D05	345.4	362.1	73	TAA170	272.3	257.0
27	H3F08	260.3	218.2	74	TAA58	303.9	274.0
28	H3F09	256.4	205.3	75	TR01	210.2	213.3
29	H4A04	261.5	258.5	76	TR13	267.7	258.8
30	H4D08	216.4	210.7	77	TR18	233.7	245.7
31	H4G11	211.7	232.6	78	TR19	237.2	216.6
32	H5A08	237.4	225.3	79	TR24	182.2	157.9
33	H6D11	291.4	288.7	80	TR31	196.4	214.3
34	ICCM0080	173.0	163.1	81	TR32	212.6	188.5
35	ICCM0130a	286.3	265.4	82	TR33	259.1	247.3
36	ICCM0160	309.2	327.7	83	TR40	249.4	255.7
37	ICCM0192a	288.0	322.6	84	TR42	259.7	349.0
38	ICCM0250	466.9	494.7	85	TR43	311.0	298.3
39	ICCM0269a	288.0	322.5	86	TR44	276.5	288.7
40	ICCM0297	313.6	300.4	87	TR56	215.6	242.7
41	NCPGR10	285.4	261.7	88	TS104	171.1	211.7
42	NCPGR19	308.1	298.1	89	TS36	130.9	186.6
43	NCPGR21	169.4	165.5	90	TS45	246.2	239.8
44	NCPGR7	221.8	217.5	91	TS46	221.4	242.2
45	NCPGR93	318.3	297.7	92	TS5	299.8	322.4
46	TA05	185.9	200.0	93	TS72	274.3	261.7
47	TA106	202.5	234.9				

Table 16. Features of Genetic linkage map of the cross ICCV 96029 × CDC Frontier

Linkage group	Number of mapped markers			Map distance (cM)	Inter-marker distance (cM)
	Mendelian segregation	Distorted markers	Total		
CaLG01	5	0	5	5.85	1.17
CaLG02	6	1	7	13.9	1.99
CaLG03	3	9	12	43.69	3.64
CaLG04	11	1	12	50.68	4.22
CaLG05	10	0	10	5.46	0.55
CaLG06	14	0	14	70.44	5.03
CaLG07	12	0	12	27.02	2.25
CaLG08	5	0	5	45.22	9.04
Total	**66**	**11**	**77**	**262.25**	**3.41**

Table 17. Features of Genetic linkage map of the cross ICC 5810 × CDC Frontier

Linkage group	Number of mapped markers			Map distance (cM)	Inter-marker distance (cM)
	Mendelian segregation	Distorted markers	Total		
CaLG01	6	0	6	28.33	4.72
CaLG02	9	0	9	29.18	3.24
CaLG03	13	0	13	40.88	3.14
CaLG04	6	8	14	88.88	6.35
CaLG05	6	0	6	6.74	1.12
CaLG06	9	3	12	76.28	6.36
CaLG07	11	0	11	8.84	0.80
CaLG08	3	2	5	56.62	11.32
Total	**63**	**13**	**76**	**335.74**	**4.42**

Table 18. Features of Genetic linkage map of the cross BGD 132 × CDC Frontier

Linkage group	Number of mapped markers			Map distance (cM)	Inter-marker distance (cM)
	Mendelian segregation	Distorted markers	Total		
CaLG01	4	0	4	32.76	8.19
CaLG02	8	0	8	10.39	1.30
CaLG03	15	0	15	80.12	5.34
CaLG04	7	2	9	45.17	5.02
CaLG05	8	0	8	7.88	0.99
CaLG06	9	0	9	65.36	7.26
CaLG07	10	0	10	37.59	3.76
CaLG08	5	0	5	31.83	6.37
Total	**66**	**2**	**68**	**311.10**	**4.57**

Table 19. Features of Genetic linkage map of the cross ICC 16641 × CDC Frontier

Linkage group	Number of mapped markers			Map distance (cM)	Inter-marker distance (cM)
	Mendelian segregation	Distorted markers	Total		
CaLG01	1	4	5	41.05	8.21
CaLG02	5	0	5	41.77	8.35
CaLG03	14	0	14	58.65	4.19
CaLG04	13	0	13	74.75	5.75
CaLG05	9	0	9	10.74	1.19
CaLG06	8	0	8	65.58	8.2
CaLG07	8	0	8	46.32	5.79
CaLG08	5	0	5	46.26	9.25
Total	**63**	**4**	**67**	**385.13**	**5.75**

Table 20. Features of consensus map developed from four populations

Linkage group	Number of common markers between			Total no. of markers mapped	Map distance (cM)	Inter-marker distance (cM)
	Four Populations	Three Populations	Two Populations			
CaLG01	1	4	1	8	32.04	4.01
CaLG02	1	7	1	11	36.91	3.36
CaLG03	5	6	5	22	61.65	2.80
CaLG04	5	4	7	18	82.23	4.57
CaLG05	4	2	4	13	6.94	0.53
CaLG06	4	5	4	17	73.41	4.32
CaLG07	6	4	2	13	33.87	2.61
CaLG08	2	2	1	9	37.39	4.15
Total	**28**	**34**	**25**	**111**	**364.44**	**3.28**

Table 21. QTLs identified for flowering time in four chickpea crosses

Sl. No.	Cross	QTL	CaLG	Position (cM)	LOD	Additive effect	PVE (%)	Flanking markers		Closest marker
								Left marker	Right marker	
1	ICCV 96029 × CDC Frontier	*Qefl1*-1	3	0.00	3.55	-4.44	7.07	CaM1122	TR13	CaM1122
		***Qefl1*-2**	**4**	**36.00**	**5.95**	**-5.42**	**12.34**	**GAA47**	**ICCM0192a**	**GAA47**
2	ICC 5810 × CDC Frontier	***Qefl2*-1**	**1**	**15.00**	**12.77**	**-3.26**	**20.13**	**TA122**	**TA30**	**TA30**
		***Qefl2*-2**	**3**	**21.00**	**16.82**	**-6.65**	**25.19**	**CaM1358**	**TA142**	**TA142**
		***Qefl2*-3**	**4**	**55.00**	**9.10**	**-4.40**	**10.44**	**NCPGR21**	**GAA47**	**GAA47**
		***Qefl2*-4**	**8**	**15.00**	**17.68**	**-7.03**	**25.57**	**GA6**	**TA118**	**TA118**
3	BGD 132 × CDC Frontier	*Qefl3*-1	3	5.00	5.17	-1.23	4.28	CaM1515	TR13	TR13
		Qefl3-2	3	31.00	4.21	-3.36	3.99	TA142	TA64	TA142
		***Qefl3*-3**	**8**	**2.00**	**44.83**	**-13.0**	**65.35**	**TA127**	**H1D24**	**H1D24**
4	ICC 16641 × CDC Frontier	***Qefl4*-1**	**6**	**9.00**	**55.60**	**-16.75**	**88.19**	**TA14**	**TR44**	**TR44**

Table 22. BLAST results of SSR markers flanking the QTL regions

QTL	qseqid	sseqid	pident	length	Mismatch	gapopen	qstart	qend	sstart	send	e-value	bitscore
Qefl1-1	CaM1122_F	Ca2	100	20	0	0	1	20	981568	981549	0.006	38.1
	CaM1122_R	Ca2	100	21	0	0	1	21	981330	981350	0.002	39.9
	TR13_F	Ca3	100	25	0	0	1	25	12673925	12673901	1.00E-05	47.3
	TR13_R	Ca3	100	29	0	0	1	29	12673735	12673763	1.00E-07	54.7
Qefl1-2	GAA47_F	Ca4	100	20	0	0	1	20	8284223	8284242	0.006	38.1
	GAA47_R	Ca4	100	22	0	0	1	22	8284391	8284370	4.00E-04	41.7
	ICCM0192a	Ca4	99.06	427	0	2	22	447	8913622	8913199	0	763
Qefl2-1	TA122_F	Ca1	100	26	0	0	1	26	5461632	5461607	3.00E-06	49.1
	TA122_R	Ca1	100	22	0	0	1	22	5461480	5461501	4.00E-04	41.7
	TA30_F	Ca1	100	27	0	0	1	27	23772489	23772515	1.00E-06	51
	TA30_R	Ca1	100	27	0	0	1	27	23772699	23772673	1.00E-06	51
Qefl2-2	CaM1358_F	Ca3	100	21	0	0	1	21	18161335	18161315	0.002	39.9
	CaM1358_R	Ca3	100	20	0	0	1	20	18161106	18161125	0.006	38.1
	TA142_F	Ca3	100	28	0	0	1	28	26262125	26262098	4.00E-07	52.8
	TA142_R	Ca3	100	26	0	0	1	26	26261991	26262016	3.00E-06	49.1
Qefl2-3	NCPGR21_F	Ca4	100	20	0	0	1	20	10077165	10077184	0.006	38.1
	NCPGR21_R	Ca4	100	20	0	0	1	20	10077311	10077292	0.006	38.1
	GAA47_F	Ca4	100	20	0	0	1	20	8284223	8284242	0.006	38.1
	GAA47_R	Ca4	100	22	0	0	1	22	8284391	8284370	4.00E-04	41.7
Qefl2-4	GA6_F	Ca8	100	20	0	0	1	20	1492393	1492374	0.006	38.1
	GA6_R	Ca8	100	20	0	0	1	20	1492222	1492241	0.006	38.1
	TA118_F	Ca8	100	23	0	0	1	23	4935826	4935848	1.00E-04	43.6
	TA118_R	Ca8	100	26	0	0	1	26	4936003	4935978	3.00E-06	49.1
Qefl3-1	CaM1515_F	scaffold1475	100	20	0	0	1	20	67445	67426	0.006	38.1
	CaM1515_R	Ca8	100	16	0	0	5	20	10772532	10772547	0.94	30.7
	TR13_F	Ca3	100	25	0	0	1	25	12673925	12673901	1.00E-05	47.3
	TR13_R	Ca3	100	29	0	0	1	29	12673735	12673763	1.00E-07	54.7
Qefl3-2	TA142_F	Ca3	100	28	0	0	1	28	26262125	26262098	4.00E-07	52.8
	TA142_R	Ca3	100	26	0	0	1	26	26261991	26262016	3.00E-06	49.1
	TA64_F	Ca3	100	27	0	0	1	27	29598023	29597997	1.00E-06	51
	TA64_R	Ca3	100	26	0	0	1	26	29597809	29597834	3.00E-06	49.1
Qefl3-3	TA127_F	scaffold198	100	29	0	0	1	29	64108	64080	1.00E-07	54.7
	TA127_R	C11117724	100	27	0	0	1	27	2810	2836	1.00E-06	51
	H1D24_F	scaffold983	100	23	0	0	1	23	306373	306351	1.00E-04	43.6
	H1D24_R	scaffold983	100	23	0	0	1	23	306185	306207	1.00E-04	43.6
Qefl4-1	TA14_F	Ca6	100	21	0	0	1	21	58199744	58199724	0.002	39.9
	TA14_R	Ca6	100	21	0	0	1	21	58199351	58199371	0.002	39.9
	TR44_F	Ca6	100	28	0	0	1	28	29073549	29073522	4.00E-07	52.8
	TR44_R	Ca6	100	27	0	0	1	27	29073261	29073287	0.000001	51

Table 23. Functional categorization of genes present in the flowering time QTL regions

Functional category	Number of genes in QTL regions
Molecular_function	360
Nucleic acid binding transcription factor activity	45
Transcription cofactor activity	5
Catalytic activity	214
Peroxidase activity	5
Structural molecule activity	14
Transporter activity	29
Binding	253
Electron carrier activity	5
Enzyme regulator activity	4
Nutrient reservoir activity	2
Molecular transducer activity	25
Cellular_component	364
Extracellular region	23
Cell	327
Plasmodesma	24
Membrane	152
Extracellular matrix	2
Macromolecular complex	65
Organelle	264
Extracellular region part	3
Organelle part	119
Membrane part	109
Cell part	327
Postsynaptic membrane	1
Intracellular organelle lumen	30
Biological_process	397
Reproduction	12
Immune system process	11
Behavior	3
Metabolic process	309
Cellular process	303
Reproductive process	34
Signaling	63
Killing of cells of other organism	2
Multicellular organismal process	59
Developmental process	63
Growth	11
Locomotion	2
Single-organism process	252
Rhythmic process	3
Response to stimulus	136
Localization	70
Multi-organism process	30
Biological regulation	137
Cellular component organization or biogenesis	70

Table 24. Candidates genes identified in the flowering time QTL regions on Ca4 and Ca8

Ca#	Gene id	Uniprotid	Gene names	Organism	GO_IDs and Molecular Function	Protein Families Name	Biological Function	Reference
Ca4	Ca_08477	Q6BDA0	***ELF6 JMJ11 PKDM9B***	Arabidopsis thaliana	Dioxygenase activity; histone H3-K9 demethylation; metal ion binding; negative regulation of long-day photoperiodism, flowering; negative regulation of short-day photoperiodism, flowering;	JHDM3 histone demethylase family – Probable lysine-specific demethylase ELF6 (EC 1.14.11.-) (Early flowering 6) (Jumonji domain-containing protein 11) (Probable lysine-specific histone demethylase ELF6)	Acts as a repressor of the photoperiodic flowering pathway and of FT. Binds around the transcription start site of the FT locus downregulates FT locus	Noh *et al*. (2004); Jeong *et al* (2009) Yu *et al*. (2008)
Ca4	Ca_08351	P93194	***INRPK1***	Ipomoea nil (Japanese morning glory)	ATP binding; extracellular region; protein phosphorylation; protein serine/threonine kinase activity; transmembrane receptor protein kinase activity	Protein kinase superfamily, Ser/Thr protein kinase family	Possible role in short-day photoperiod floral induction	Bassett *et al*. (2000)
Ca4	Ca_08436	Q9C6M5	***TEM1***	Arabidopsis thaliana	DNA binding; ethylene-activated ignalling pathway; nucleus; photoperiodism, flowering;	AP2/ERF transcription factor family, RAV subfamily	Transcritptional repressor of flowering time on long day plants. Acts directly on FT expression by binding 5'-CAACA-3' and 5'-CACCTG-3 sequences.	Castillejo and Pelaz (2008)
Ca4	Ca_08415	Q9LZS0	***PTL***	Arabidopsis thaliana	Regulation of flower development –chromatin binding	-	Regulates perianth architecture in flower. Required for the establishment of auxin flux.	Brewer *et al*. (2004)
Ca8	Ca_02096	O64827	***SUVR5 CZS SDG6 SET6***	Arabidopsis thaliana	Chromosome; histone-lysine N-methyltransferase activity; nucleus; zinc ion binding	Class V-like SAM-binding methyltransferase superfamily	Histone methyltransferase that functions together with its binding partner LDL1/SWP1 as one of the regulators of flower timing in Arabidopsis.	Krichevsky, *et al*. (2007)
Ca8	Ca_02330	Q84JU6	***HOS1***	Arabidopsis thaliana	Cytoplasm; ligase activity; metal ion binding nucleus; protein ubiquitination; regulation of signal transduction; response to cold; ubiquitin-protein transferase activity; vegetative to reproductive phase transition of meristem	E3 ubiquitin-protein ligase HOS1 (EC 6.3.2.-) (Protein high expression of osmotically responsive gene 1) (RING finger protein HOS1)	Controls flowering time in response to ambient temperatures (16 and 23 degrees Celsius) and intermittent cold, probably via the regulation of FT and TSF levels	Lee *et al*. (2012)

Ca#	Gene id	Uniprotid	*Gene names*	Organism	GO_IDs and Molecular Function	Protein Families Name	Biological Function	Reference
Ca8	Ca_02166	P93831	***CLF ICU1 PIF1 PIF2 SDG1 SET1***	Arabidopsis thaliana	Cell differentiation; DNA mediated transformation; flower development; histone-lysine N-methyltransferase activity; vegetative to reproductive phase transition of meristem	Class V-like SAM-binding methyltransferase superfamily, Histone-lysine methyltransferase family, EZ subfamily	Required to regulate floral development by repressing the AGAMOUS homeotic gene in leaves, influorescence stems and flowers.	Goodrich*et al*. (1997)
Ca8	Ca_02321	Q8W5B1	***VRN2***	Arabidopsis thaliana	Chromatin silencing complex; metal ion binding; nucleus; regulation of gene expression by genetic imprinting; response to cold; sequence-specific DNA binding transcription factor activity; vernalization response	Polycomb group (PcG) protein.VEFS (VRN2-EMF2-FIS2-SU(Z)12) family	Plays a central role in vernalization by maintaining repressed the homeotic gene FLC, a floral repressor, after a cold treatment. Associates constitutively along the whole FLC locus	Gendall *et al*. (2001)
Ca8	Ca_02168	P47927	***AP2***	Arabidopsis thaliana	Cell differentiation; DNA binding; flower development; meristem maintenance; ovule development; seed development ; specification of floral organ identity	Floral homeotic protein family - AP2/ERF transcription factor family, AP2 subfamily	Probable transcriptional activator that promotes early floral meristem identity and subsequently transition of an inflorescence meristem into a floral meristem.	Drews*et al*. (1991)
Ca8	Ca_02245	Q38914	***ANT CKC1 DRG OVM***	Arabidopsis thaliana	Flower development; maintenance of shoot apical meristem identity - sequence-specific DNA binding transcription factor activity	AP2/ERF transcription factor family, AP2 subfamily	functions as a class A homeotic gene	Klucher *et al*. (1996)
Ca8	Ca_02162	Q9FUA4	***SPT BHLH24 EN99***	Arabidopsis thaliana	Carpel development; circadian rhythm; response to cold and red light - DNA binding	-	Transcription factor that plays a role in floral organogenesis	Alvarez and Smyth (1999)
Ca8	Ca_02384	Q9S7C9	***AHL27 ESC ORE7***	Arabidopsis thaliana	Vegetative to reproductive phase transition of meristem - AT DNA binding, histone binding	-	Acts redundantly with AHL18, AHL22 and AHL29 in the regulation of flowering and regulation of the hypocotyl elongation	Xiao *et al*. (2009)
Ca8	Ca_02294	Q9FJR0	***UPF1***	Arabidopsis thaliana	Defense response to bacterium; long-day photoperiodism- zinc ion, ATP, DNA, RNA binding and helicase activity	DNA2/NAM7 helicase family	Light signaling and Non-sense mediated mRNA decay (NMD) pathways influencing plant development, stress response and adaptation to environmental stresses	Shi *et al.* (2012)

Table 25. Correlation coefficients between flowering time and other important traits

Cross	Days to pod initiation	Days to maturity	Plant height	Pods per plant	Seeds per plant	Biomass	Seed yield per plant	100 Seed weight	Harvest index
ICCV 96029 × CDC Frontier	0.988**	0.882**	0.507**	-0.032	-0.047	0.264**	0.055	0.281**	-0.431**
ICC 5810 × CDC Frontier	0.987**	0.888**	0.189**	-0.088	-0.126	0.181*	-0.01	0.309**	-0.442**
BGD 132 × CDC Frontier	0.992**	0.934**	0.453**	0.133	0.093	0.331**	0.135	0.237**	-0.486**
ICC 16641 × CDC Frontier	0.997**	0.950**	0.471**	-0.052	-0.088	0.280**	-0.087	-0.059	-0.616**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Plate 1. General view of the experimental plot of evaluation of F_2 populations for flowering time conducted during 2013-14

Plate 2. Segregation for flowering time and maturity in one of the F_2 population i.e. ICC 5810 x CDC Frontier

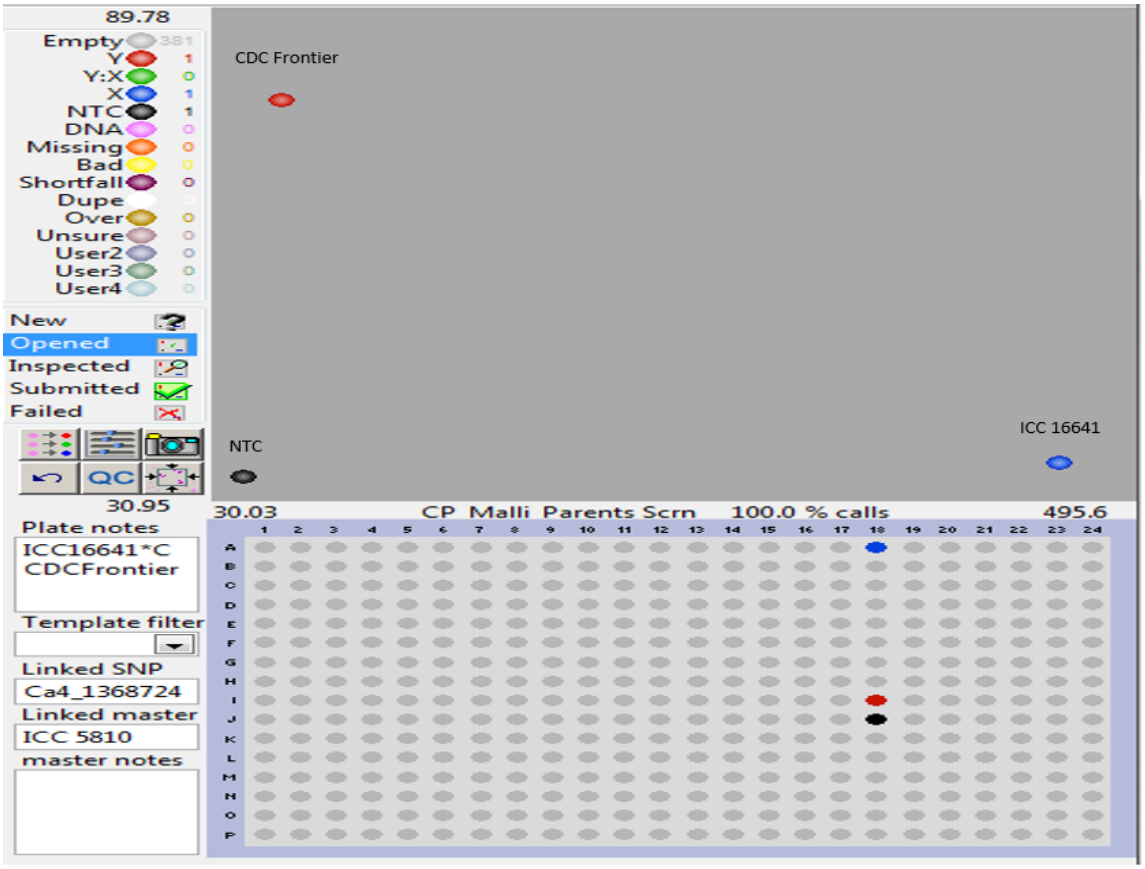

Plate 3. SNP genotypic data plotted using Kbiosciences KlusterCaller software showing polymorphism between the parents ICC 16641 and CDC Frontier

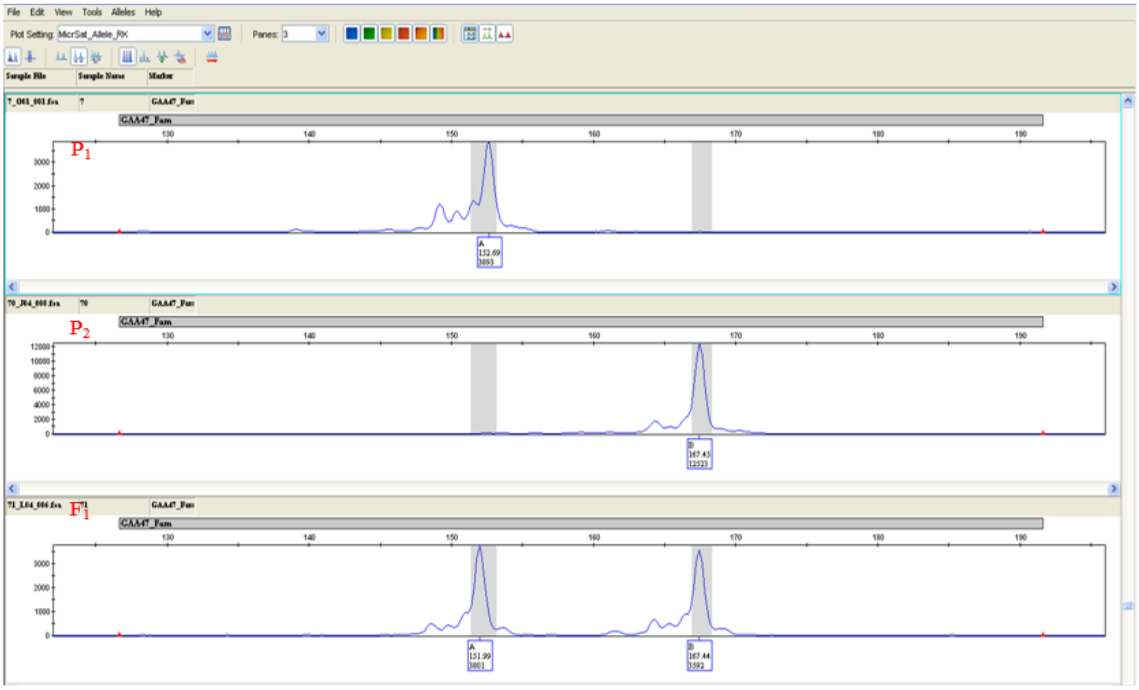

Plate 4. Electropherogram displaying allele calling of SSR genotyping results showing polymorphism between the parental genotypes

Plate 5. F_3 Progeny rows from the cross between ICCV 96029 x CDC Frontier, exhibiting uniform early flowering, segregation into early and late flowering and uniform late flowering phenotypes indicating monogenic inheritance of flowering time gene "*efl-1*"

Plate 6. F_3 Progeny rows from the cross between ICC 5810 x CDC Frontier, exhibiting uniform late flowering, segregation into early and late flowering and uniform early flowering phenotypes showing major gene ("*efl-2*") inheritance of flowering time

Plate 7. F_3 Progeny rows from the cross between BGD 132 x CDC Frontier, exhibiting uniform late flowering, segregation into early and late flowering and uniform early flowering phenotypes indicating monogenic inheritance of flowering time "*efl-3*"

Plate 8. F_3 Progeny rows from the cross between ICC 16641 x CDC Frontier, exhibiting uniform early flowering, segregation into early and late flowering and uniform late flowering phenotypes indicating monogenic inheritance of flowering time gene "*efl-4*"

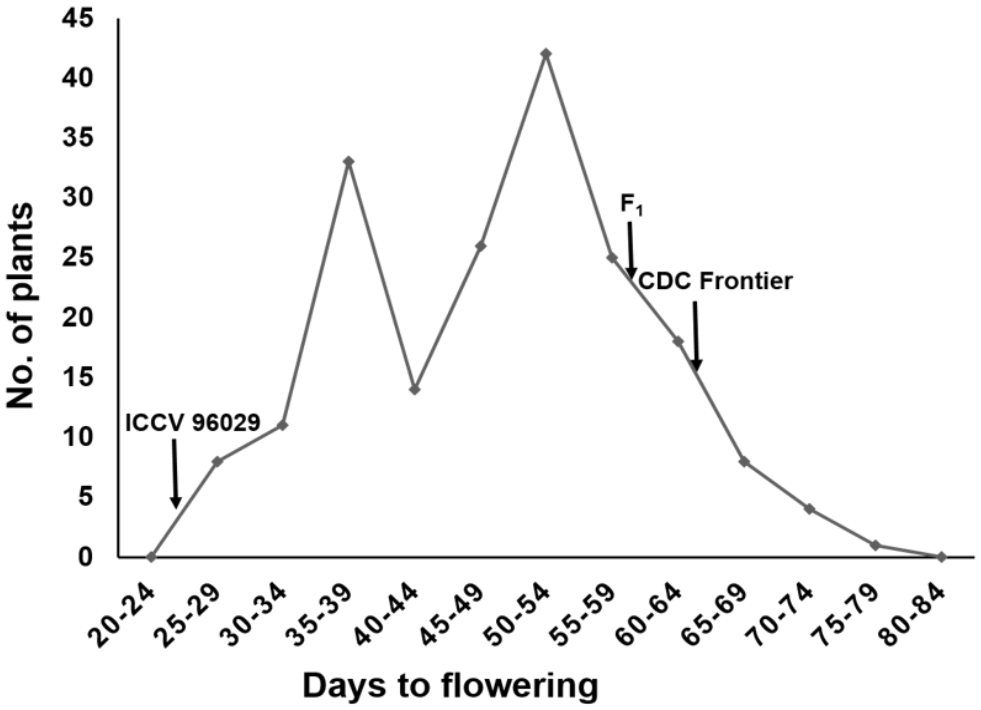

Figure 1. Distribution of flowering time in F_2 population of the cross ICCV 96029 x CDC Frontier

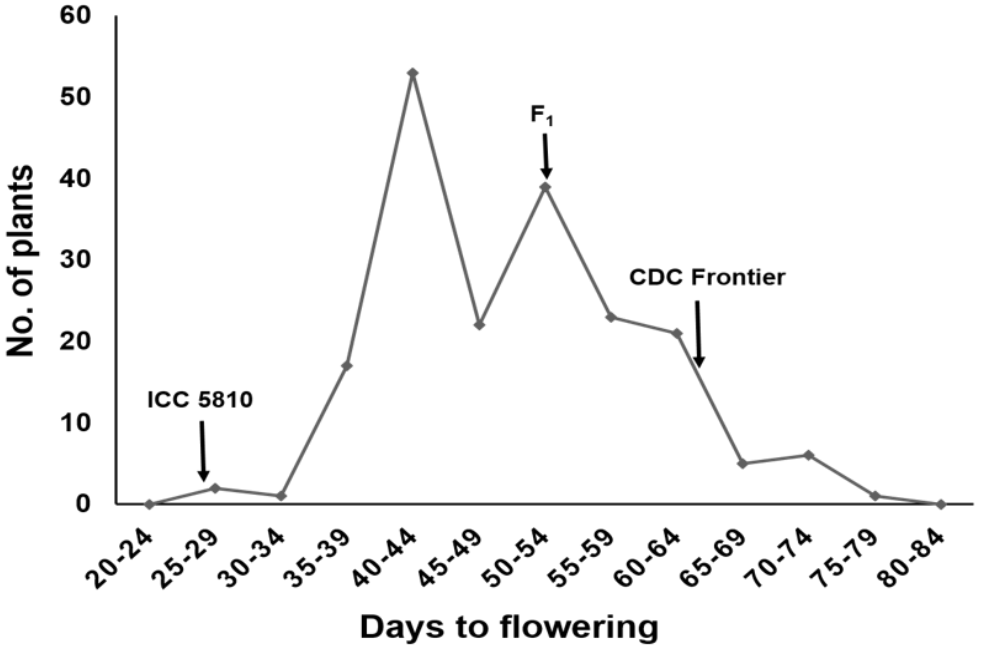

Figure 2. Distribution of flowering time in F_2 population of the cross ICC 5810 x CDC Frontier

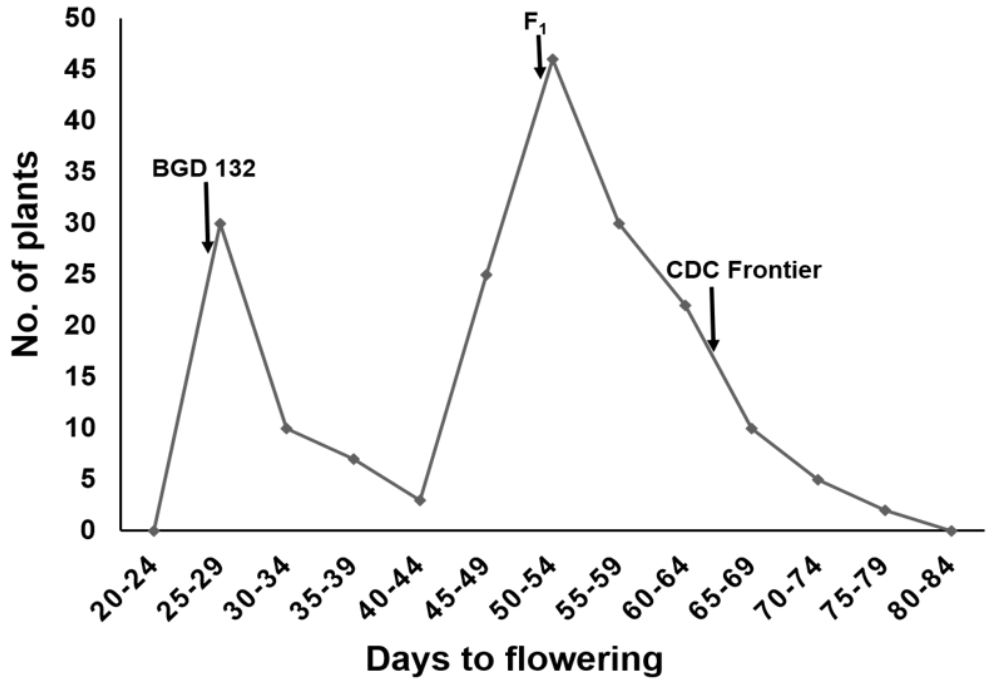

Figure 3. Distribution of flowering time in F_2 population of the cross BGD 132 x CDC Frontier

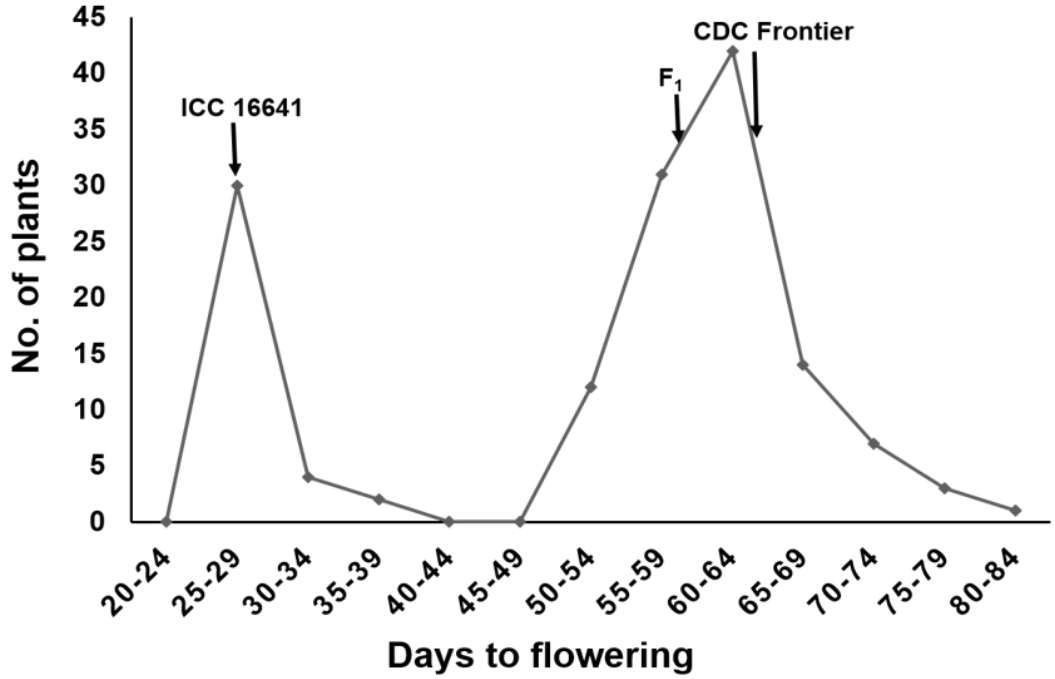

Figure 4. Distribution of flowering time in F_2 population of the cross ICC 16641 x CDC Frontier

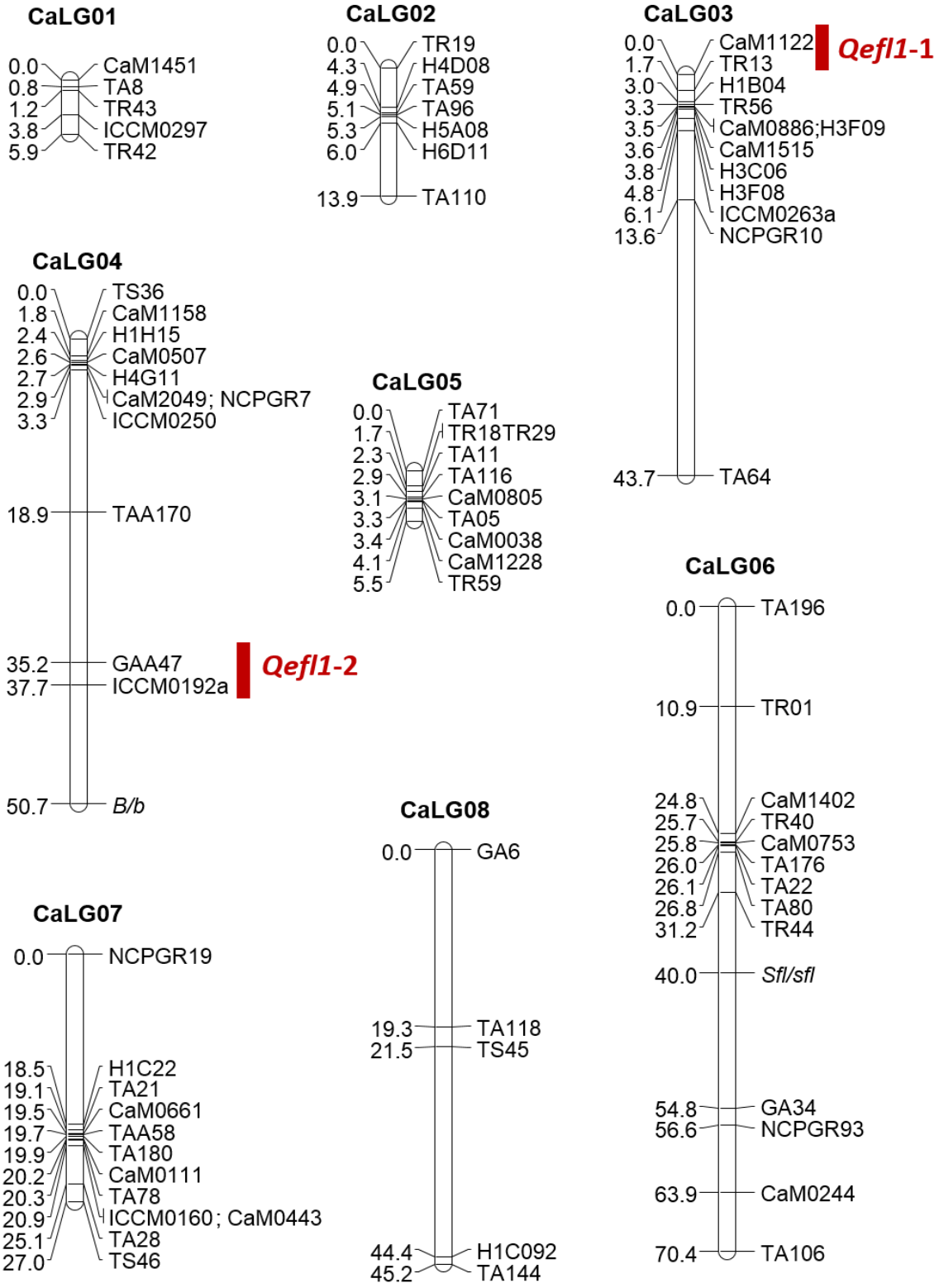

Figure 5. Genetic linkage map of the cross ICCV 96029 × CDC Frontier

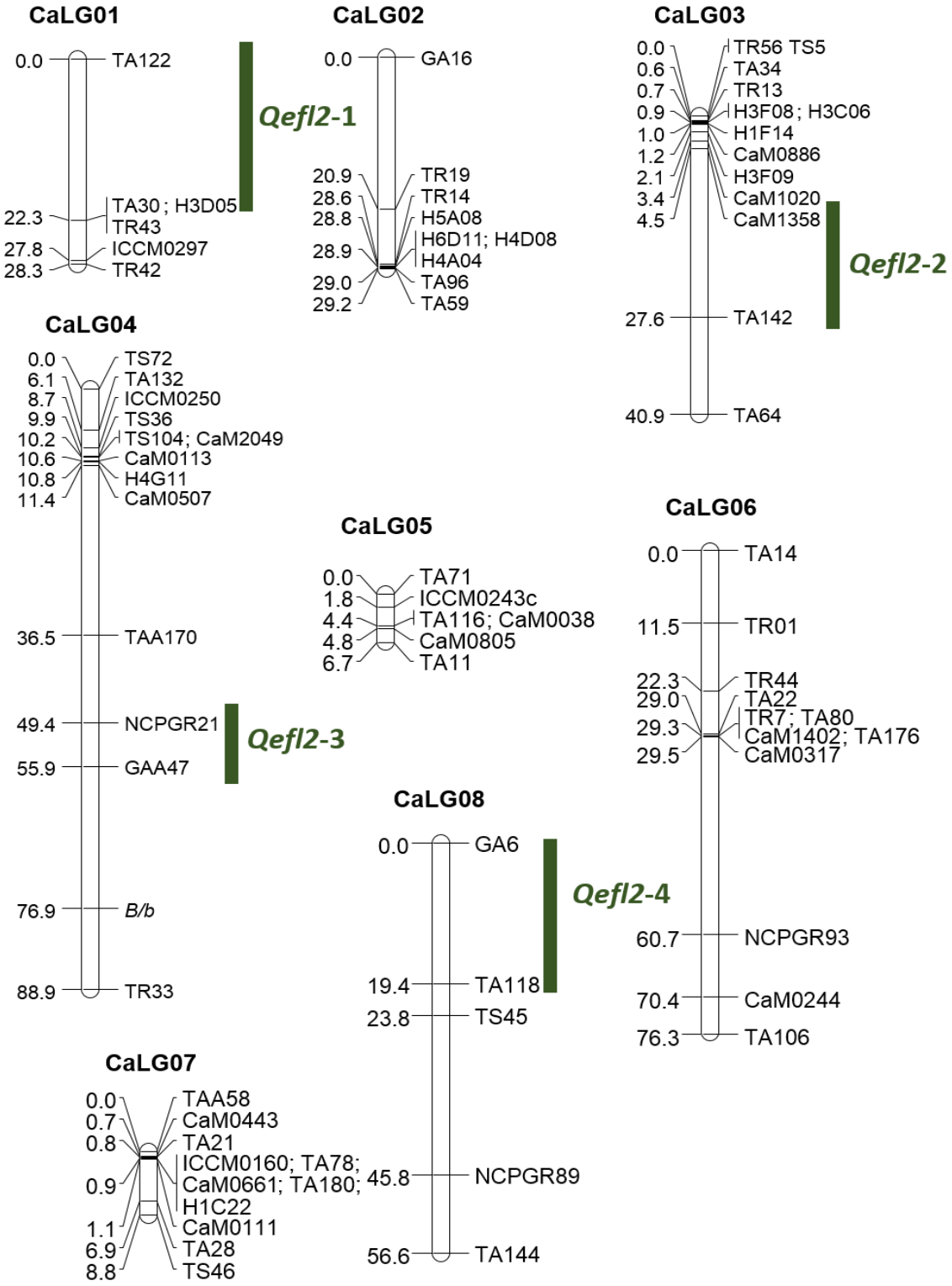

Figure 6. Genetic linkage map of the cross ICC 5810 × CDC Frontier

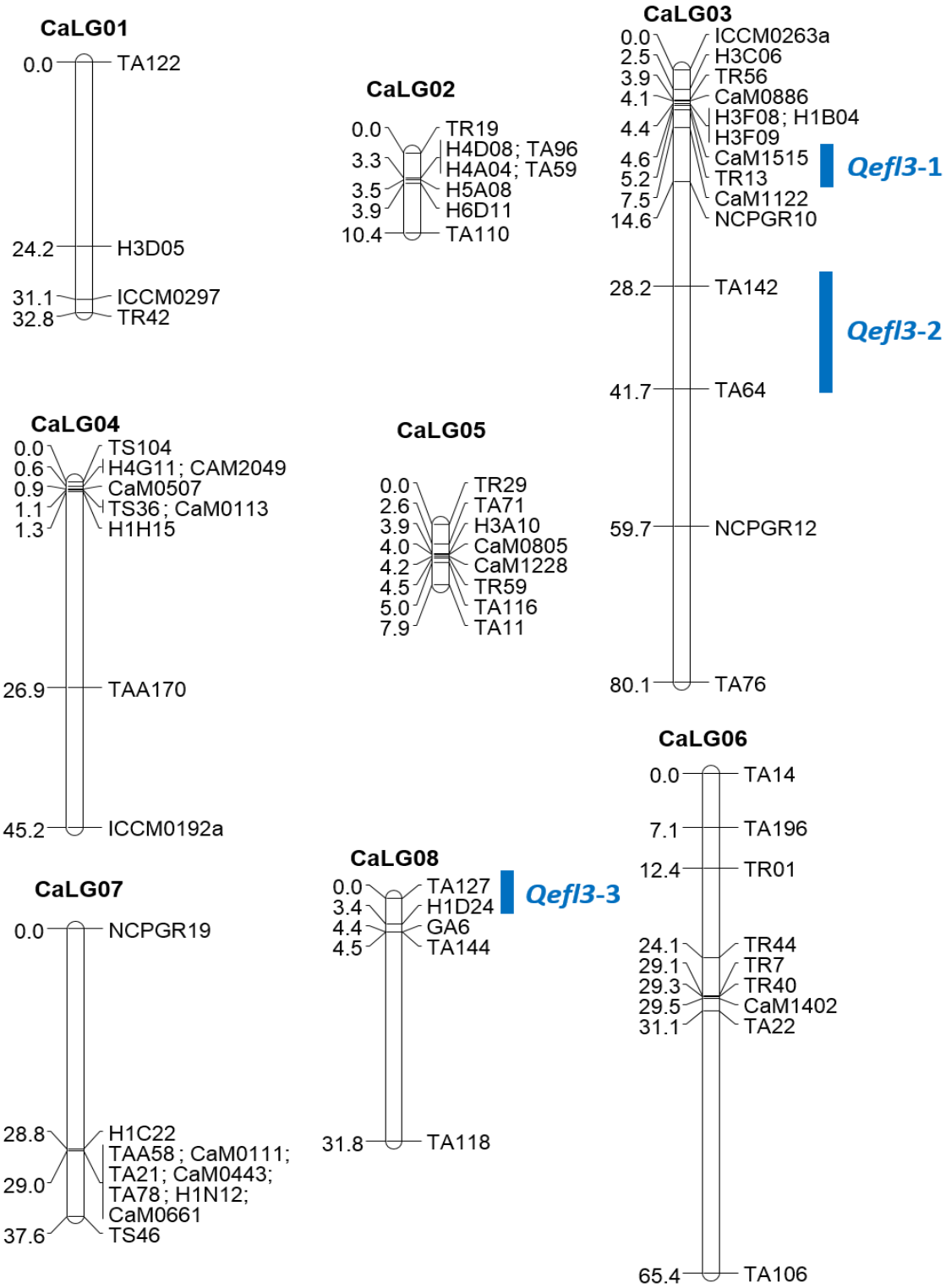

Figure 7. Genetic linkage map of the cross BGD 132 × CDC Frontier

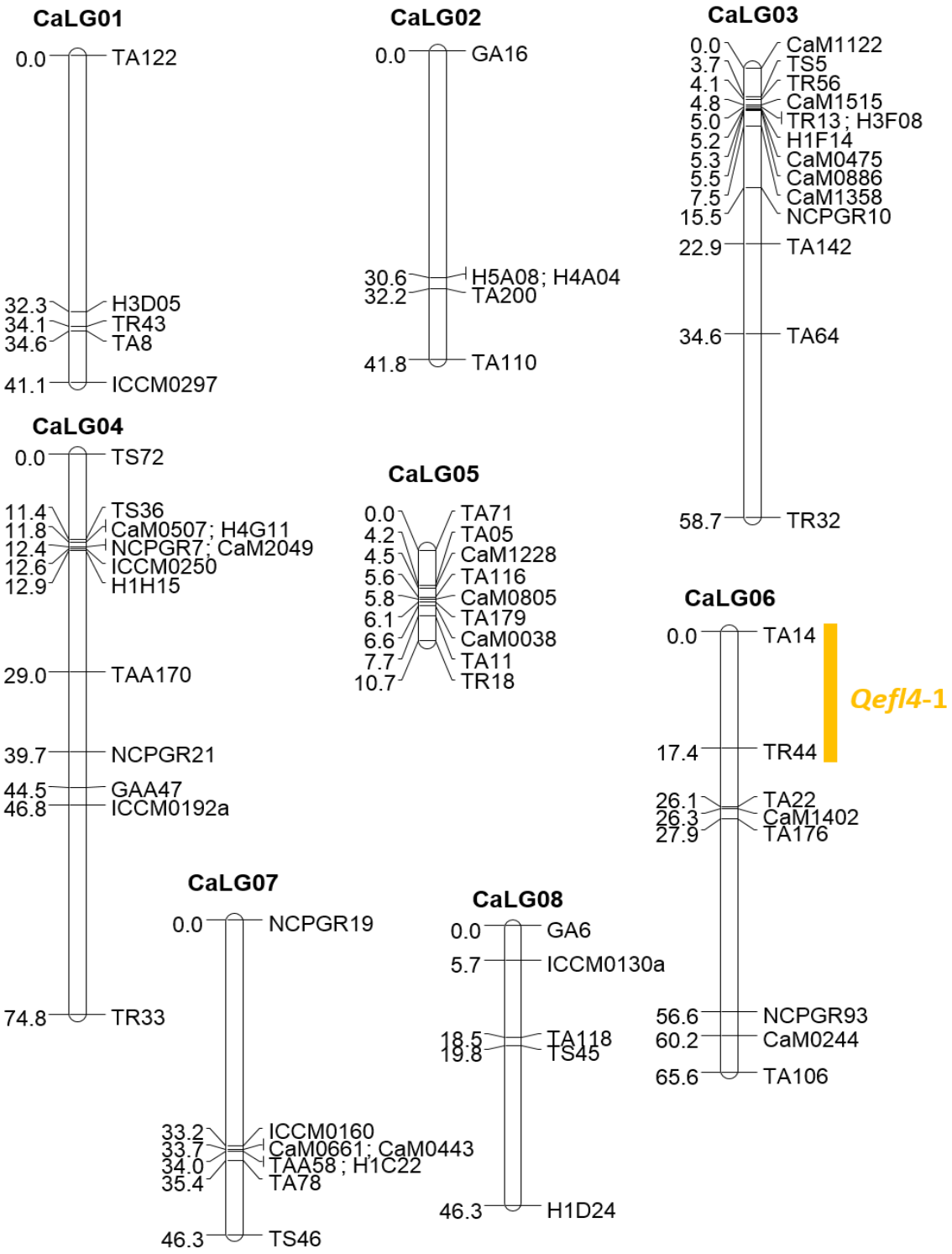

Figure 8. Genetic linkage map of the cross ICC 16641 × CDC Frontier

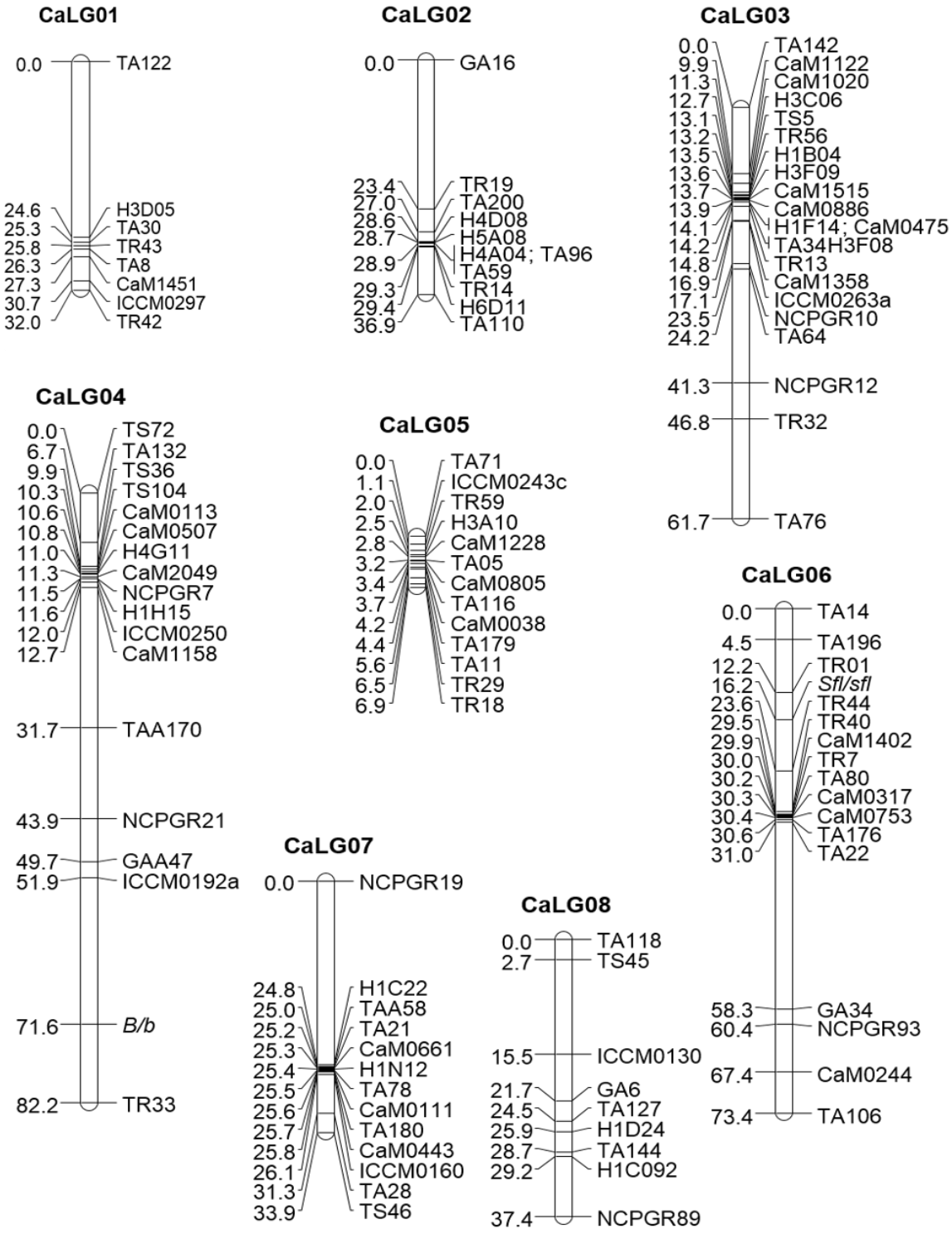

Figure 9. Consensus map of chickpea developed from four populations

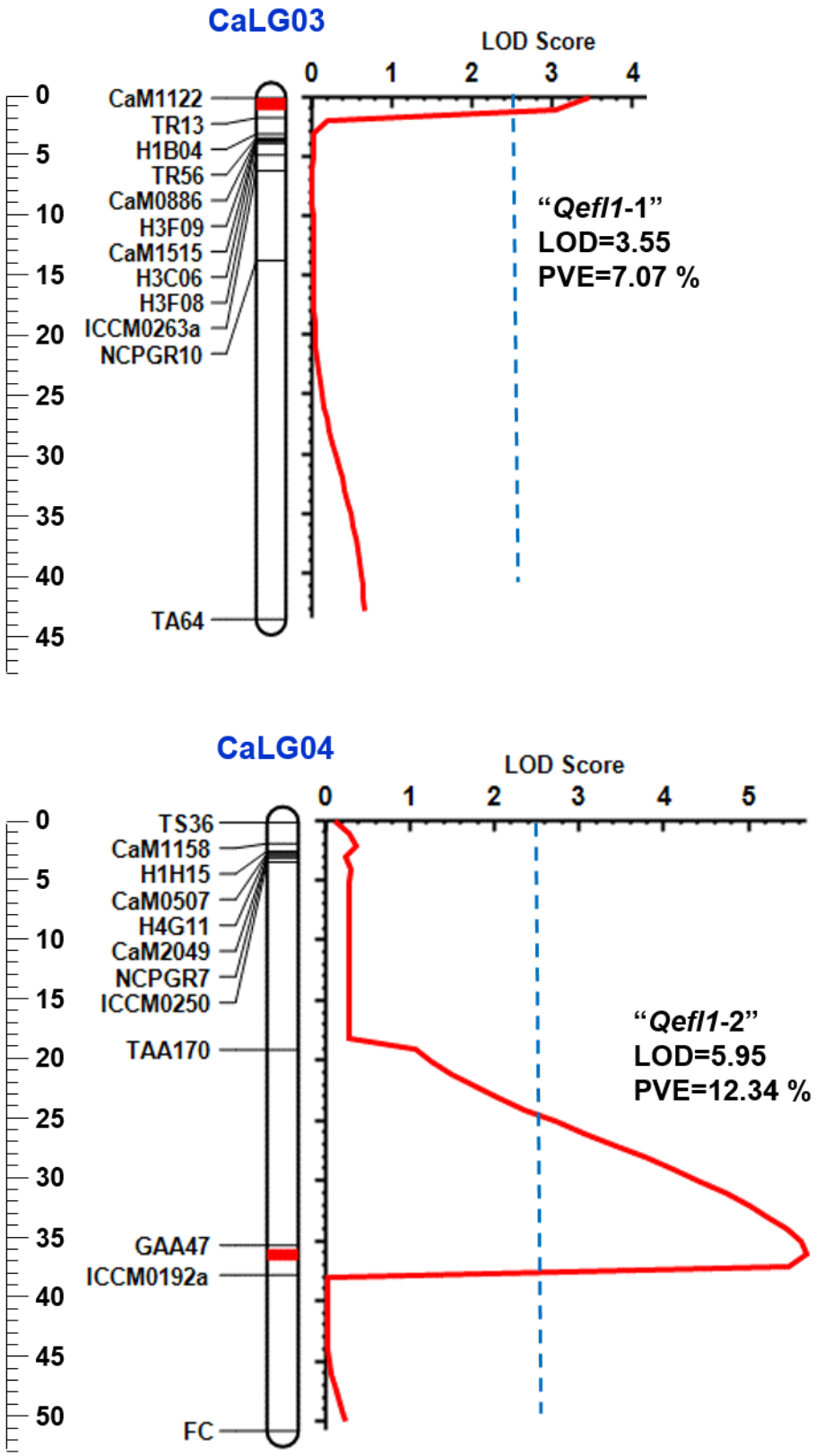

Figure 10. QTLs for flowering time in the cross ICCV 96029 × CDC Frontier

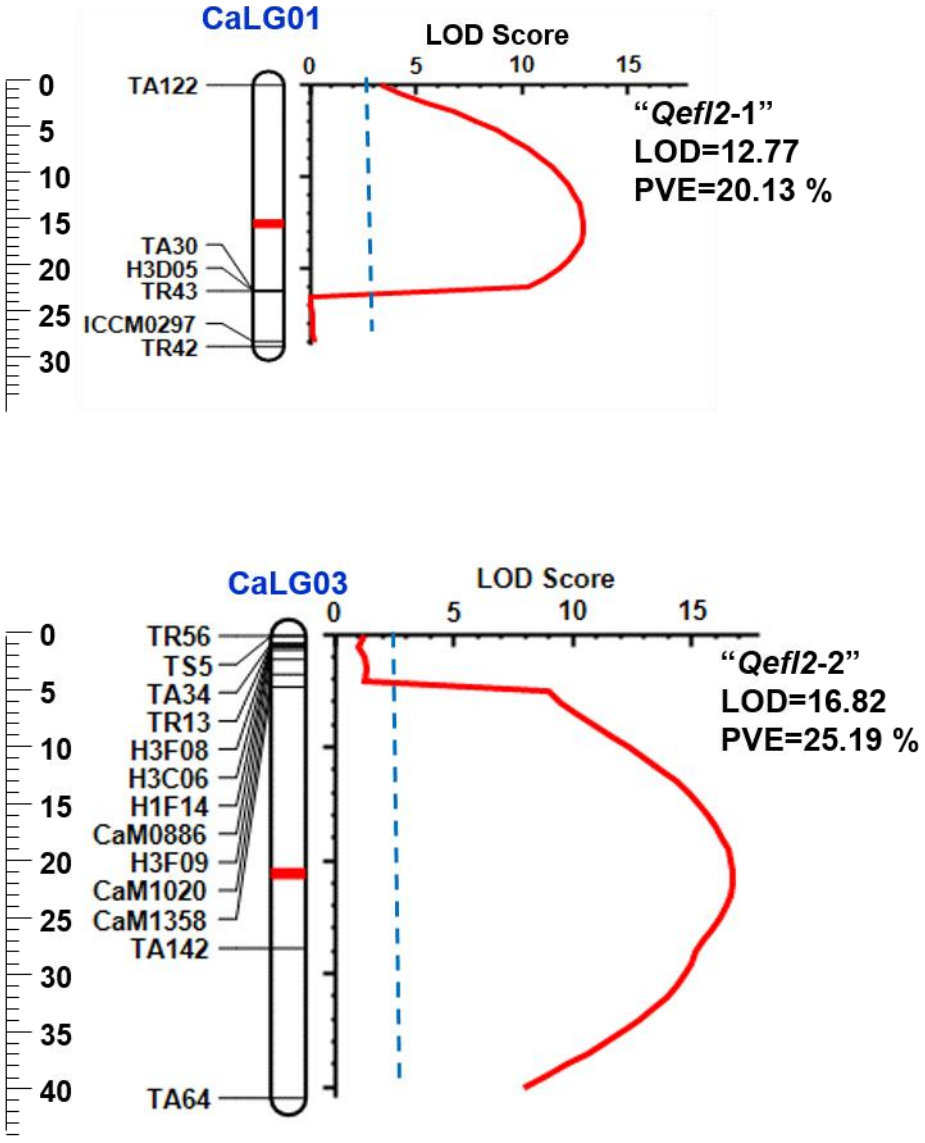

Figure 11. QTLs for flowering time in the cross ICC 5810 × CDC Frontier

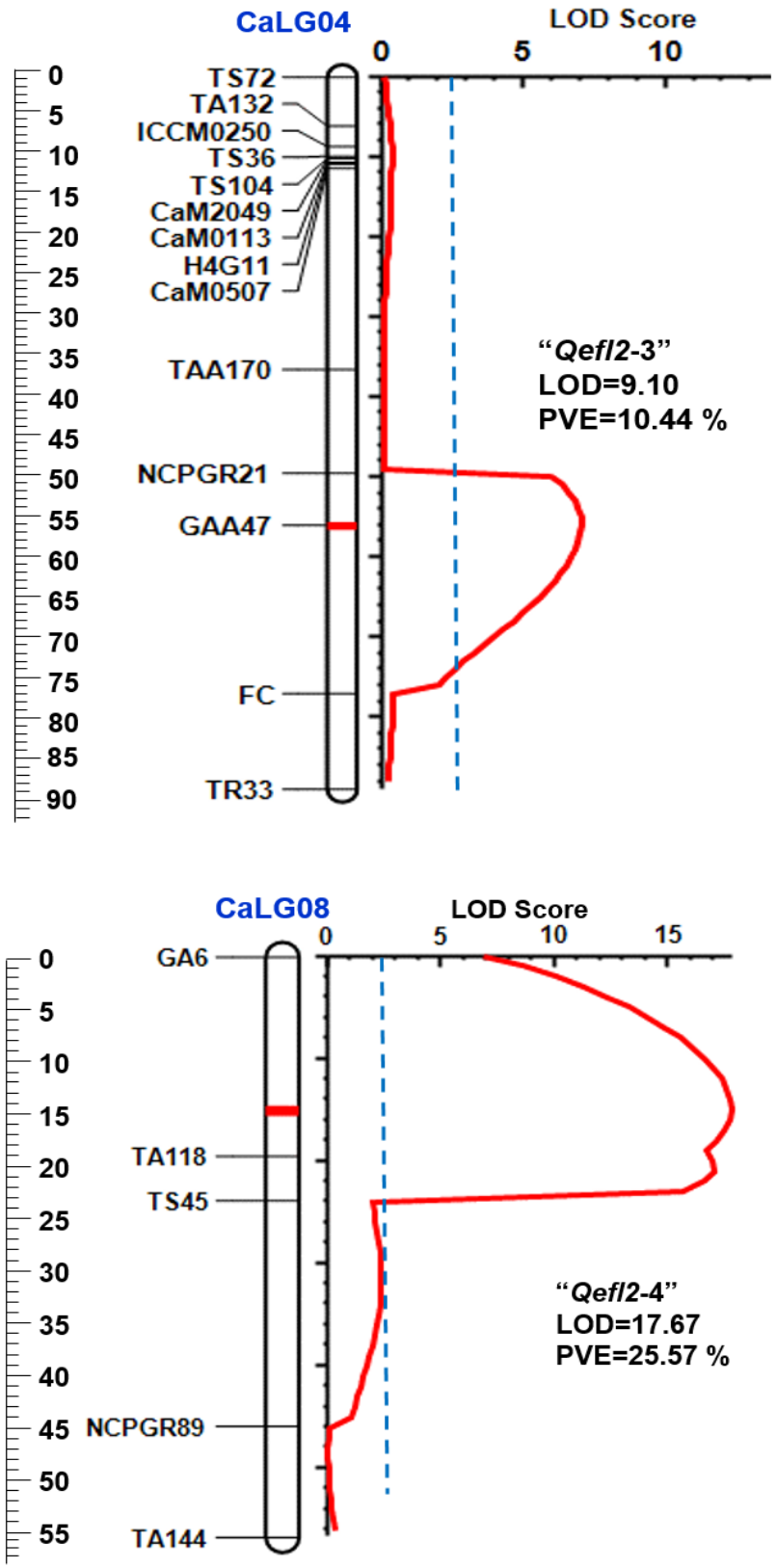

Figure 11. QTLs for flowering time in the cross ICC 5810 × CDC Frontier

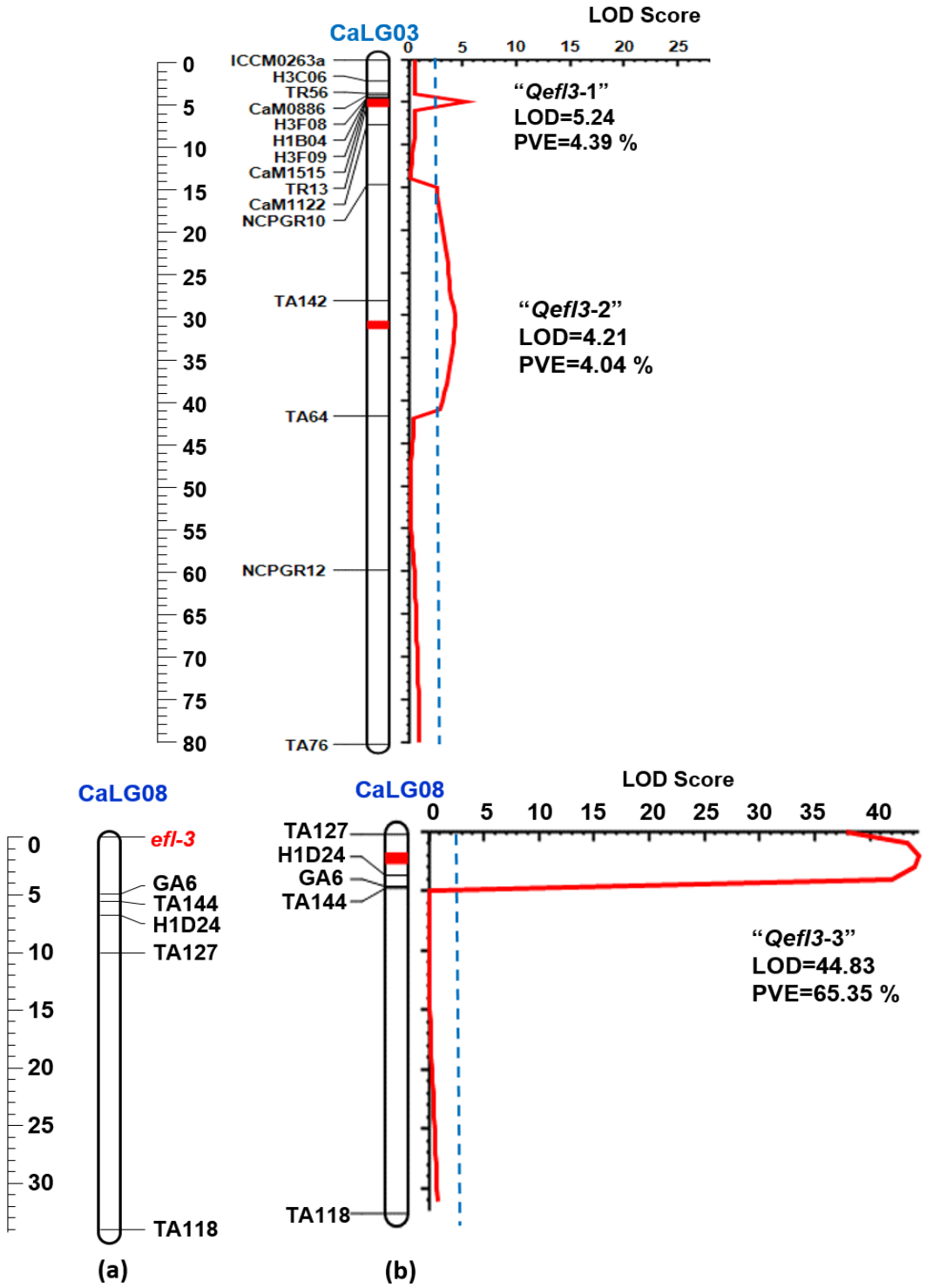

Figure 12 (a) Mapping of major flowering time gene "*efl-3*" on CaLG08 based on F_3 segregating data of the cross BGD 132 x CDC Frontier (b) Identification of major QTL for flowering time "*efl3*-3" on CaLG08

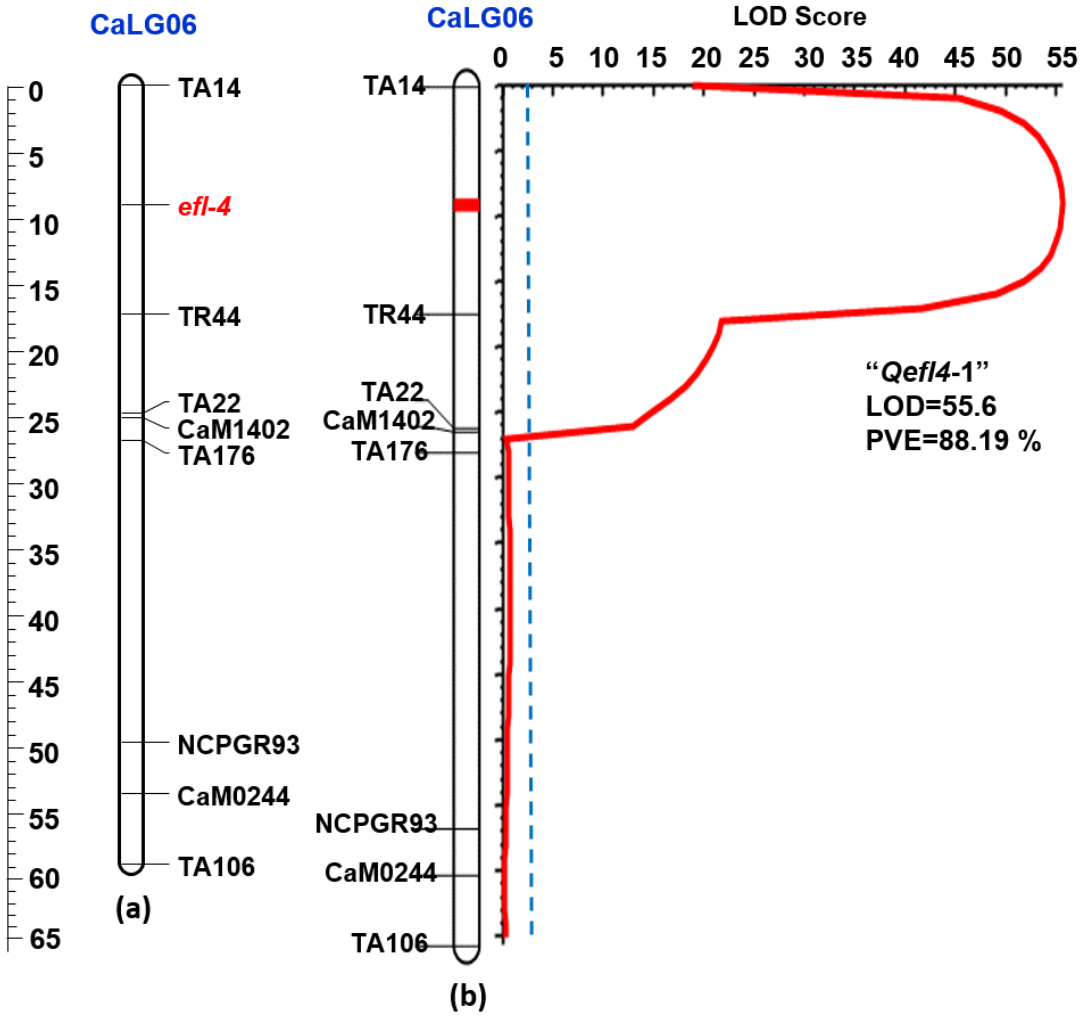

Figure 13 (a) Mapping of major flowering time gene "*efl-4*" on CaLG06 based on F_3 segregating data of the cross ICC 16641 x CDC Frontier (b) Identification of major QTL for flowering time "*Qefl4*-1" on CaLG06

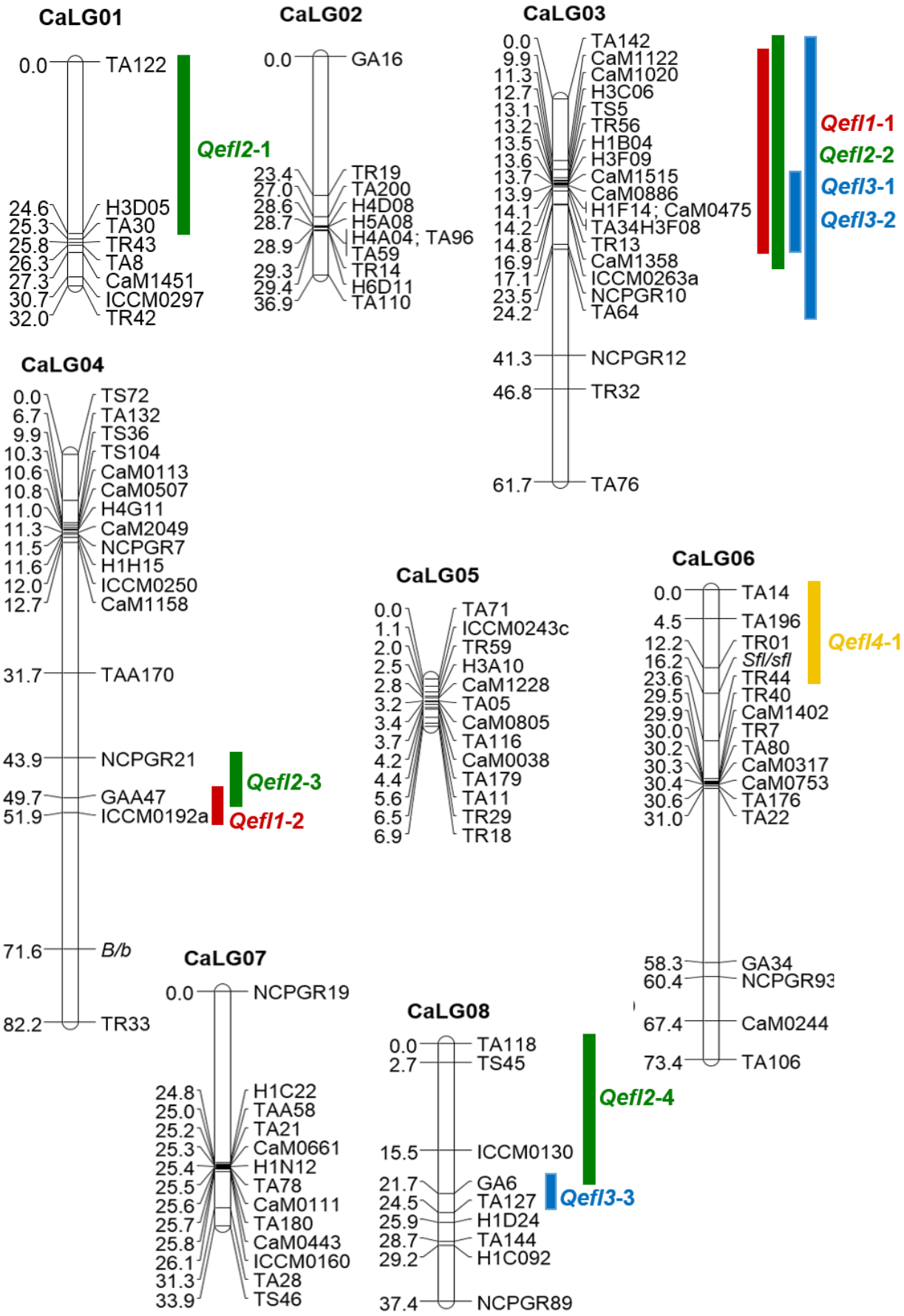

Figure 14. Summary of QTLs identified for flowering time in four crosses

Appendices

APPENDIX – A

Weekly average weather data from October 2013 to March 2014 at ICRISAT, Patancheru

Year	Std. Week	Date	Rain (mm)	Evap (mm)	Max Temp. (ºC)	Min. Temp. (ºC)	Relative Humidity-1 at 07:17 (%)	Relative Humidity-2 at 14:17 (%)	Wind Velocity (kmph)	Solar Radiation (mj/ m^2)	Bright Sunshine (Hrs)
2013	44	29/Oct-4/Nov	0	24.50	29.87	18.46	94.14	54.42	3.43	17.71	7.68
2013	45	5/Nov-11/Nov	0	24.10	28.53	15.16	92.70	50.14	3.56	16.53	7.50
2013	46	12/Nov-18/Nov	0	30.60	27.96	13.02	90.29	37.71	3.54	18.12	8.57
2013	47	19/Nov-25/Nov	18.69	22.00	28.03	16.19	93.00	55.42	4.24	13.99	6.05
2013	48	26/Nov-2/Dec	2	20.60	28.44	16.05	93.85	53.28	4.08	14.39	6.41
2013	49	3/Dec-9/Dec	0	25.50	27.62	12.18	95.29	45.28	3.81	16.53	8.07
2013	50	10/Dec-16/Dec	0	24.30	28.60	8.31	94.29	30.00	2.67	18.42	9.85
2013	51	17/Dec-23/Dec	0	25.00	28.10	10.83	92.56	36.00	4.33	16.78	9.50
2013	52	24/Dec-31/Dec	0	27.10	26.55	12.42	93.62	46.38	5.62	15.27	8.00
2014	1	1/Jan-7/Jan	0	23.89	28.03	13.40	95.85	44.42	5.09	15.16	8.19
2014	2	8/Jan-14/Jan	0	28.00	28.96	14.91	91.70	41.00	6.50	15.63	8.18
2014	3	15/Jan-21/Jan	0	32.39	28.83	15.36	91.00	42.57	8.18	15.46	7.79
2014	4	22/Jan-28/Jan	0	29.60	27.82	15.59	89.00	46.85	6.61	14.67	6.25
2014	5	29/Jan-4/Feb	0	29.19	28.26	13.52	90.00	39.28	4.58	16.00	7.79
2014	6	5/Feb-11/Feb	0	37.50	32.22	13.71	84.14	26.00	4.41	17.89	9.69
2014	7	12/Feb-18/Feb	0	40.29	29.69	15.52	77.00	34.00	7.50	15.86	7.30
2014	8	19/Feb-25/Feb	0	42.89	30.39	18.07	80.00	39.28	9.28	17.94	7.71
2014	9	26/Feb-4/Mar	3	39.79	30.16	18.33	86.14	49.00	9.35	16.33	7.00
2014	10	5/Mar-11/Mar	9.8	28.89	28.05	18.94	86.29	55.00	8.99	14.50	4.71
2014	11	12/Mar-18/Mar	0	52.20	33.50	18.32	75.70	30.42	6.68	20.60	7.91

APPENDIX – B

Weekly average weather data from October 2014 to March 2015 at ICRISAT, Patancheru

Year	Std. Week	Date	Rain (mm)	Evap (mm)	Max Temp. (ºC)	Min. Temp. (ºC)	Relative Humidity-1 at 07:17 (%)	Relative Humidity-2 at 14:17 (%)	Wind Velocity (kmph)	Solar Radiation (mj/ m^2)	Bright Sunshine (Hrs)
2014	42	15/Oct-21/Oct	23.6	39.1	32.68	20.94	91.56	46.14	6.5	18.03	8.68
2014	43	22/Oct-28/Oct	23.19	27.19	28.39	18.58	91.7	55.57	4.4	12.4	4.86
2014	44	29/Oct-4/Nov	0	35.1	30.85	14.03	91.56	34	2.81	19.12	8.5
2014	45	5/Nov-11/Nov	2.6	33.39	30.96	15.74	87.14	36	3.79	16.44	7.23
2014	46	12/Nov-18/Nov	19.39	22.89	29.85	18.85	93.14	55.14	4.81	13.26	5.49
2014	47	19/Nov-25/Nov	33.79	27.6	30.05	14.66	94.7	45.28	2.89	16.89	8
2014	48	26/Nov-2/Dec	0	31.39	29.6	11.53	89.85	33.28	3.43	17.76	8.66
2014	49	3/Dec-9/Dec	0	29.19	29.89	10.08	89.85	32.57	3.16	17.32	9.4
2014	50	10/Dec-16/Dec	0	20.69	28.3	18.17	90.85	53.71	5.93	11.58	4
2014	51	17/Dec-23/Dec	0	26.39	27.44	8.9	87.85	38.57	3.14	16.42	7.93
2014	52	24/Dec-31/Dec	0	31.89	28.3	11.47	88.75	39.25	4.04	16.71	8.5
2015	1	1/Jan-7/Jan	4.59	21.8	28.39	16.19	92	55	4.7	12.9	5.26
2015	2	8/Jan-14/Jan	0	31.89	27.69	6.87	85.14	28.14	3.16	18.78	10
2015	3	15/Jan-21/Jan	0	33.79	28.26	11.39	82.29	32.85	5.4	17.53	9.21
2015	4	22/Jan-28/Jan	0	40.7	29.01	14.74	87.43	35.85	9.1	17.8	8.67
2015	5	29/Jan-4/Feb	0	36.7	29.05	13.14	90.29	38.14	6.33	18.42	8.68
2015	6	5/Feb-11/Feb	0	50.7	30.53	14.76	81.14	28.85	9.26	19.69	9.68
2015	7	12/Feb-18/Feb	0	44.5	32.5	13.1	81	29.85	4.44	20.35	10
2015	8	19/Feb-25/Feb	0	52.79	33.46	15.39	74.14	28.42	6.44	19.96	9.18
2015	9	26/Feb-4/Mar	26	45.2	32.13	17.35	80.56	37.28	7.63	17.6	7.4
2015	10	5/Mar-11/Mar	46.2	25.5	30.3	18.78	90.56	54.85	4.71	15.56	5.08