GENETIC MAPPING OF FUNCTIONAL MARKERS LOCATED IN QTL REGION FOR *FUSARIUM* WILT RESISTANCE IN CHICKPEA (*Cicer arietinum* L.)

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DEPARTMENT OF PLANT BIOTECHNOLOGY
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in

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BENGALURU **FEBRUARY, 2017**


Affectionately Dedicated to
My Beloved Parents
My Sister
and
My Dear Friends

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU - 560065

CERTIFICATE

This is to certify that the thesis entitled **"Genetic Mapping of Functional Markers located in QTL Region for *Fusarium* Wilt Resistance in Chickpea (*Cicer arietinum* l.)"** submitted by **Mr. RAGHU, R.** I.D. No. **PALB 3067**, for the award of degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, GKVK, Bengaluru, is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

BENGALURU
February, 2017

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Bengaluru
February, 2017 ***(Raghu, R.)***

Genetic Mapping of Functional Markers located in QTL region for *Fusarium* Wilt Resistance in Chickpea (*Cicer Arietinum* L.)

RAGHU R

THESIS ABSTRACT

Fusarium wilt (FW) is a major disease of chickpea (*Cicer arietinum* L.) in all the chickpea growing areas. The present investigation utilized the FW linked QTLs/markers information to fine map the FW resistance regions. Forty six markers linked to FW resistance were reported which were physically mapped onto chickpea genome sequence. Among them, 23 markers were mapped on six chromosomes and six were mapped on unplaced genomic scaffold. After physical mapping, 16 genomic regions were selected for identification of genes and microsatellite repeats. Totally 36.85 Mb on chromosomes and 1.65 Mb on scaffolds were scanned for genes and SSR motifs. Altogether, 1,578 genes and 2,250 genic SSR motifs were identified and primers could be designed for only 941 genic SSR motifs. From among 941 markers 168 markers were selected for primer synthesis and 161 showed required amplification. Two intraspecific linkage maps of chickpea were constructed using recombinant inbred lines (RILs) of JG62×WR315 (JW) and K850×WR315 (KW) using a set of 23 and 22 polymorphic SSR markers respectively for genotyping after screening 168 markers. Three linkage groups spanning a distance of 144.51 and 100.46 cM with an average markers density of 6.53 and 5.23 cM were observed for population JW and population KW respectively. The RILs were phenotyped for wilt resistance over two seasons. Six QTLs (*qW60-07-1-1*, *q60pot-3-3*, *qW30-08-1-1*, *q30pot-3-1*, *qW30-07-3-1*, and *qW30-08-3-1*) in population JW with phenotypic variance ranging from 8.56-23.62 per cent and one QTL (*qWilt60-07-3*-1) in population KW explaining phenotypic variance of 9.45 per cent were identified. These seven redefined QTLs were physically mapped to three chromosomes (2, 4 and 6) suggesting that there are three different loci controlling FW resistance. The QTLs were redefined with a smaller interval harbouring 75 putative candidate genes for disease resistance.

February, 2017

Department of Plant Biotechnology
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Dr. R L Ravikumar
(Major advisor)

ಕಡಲೆಯ ಫುಸಾರಿಯಮ್ ವಿಲ್ಟ್ ಪ್ರತಿರೋಧಕ ಕ್ಯೂಟಿಎಲ್ ಪ್ರದೇಶದಲ್ಲಿರುವ ಕ್ರಿಯಾತ್ಮಕ ಗುರುತುಕಾರಕಗಳು ಅನುವಂಶಿಯ ಮ್ಯಾಪಿಂಗ್

ರಘು, ಆರ್.

ಪ್ರಬಂಧದ ಅಮೂರ್ತ

ಫುಸಾರಿಯಮ್ ವಿಲ್ಟ್ ಕಡಲೆಯ ಒಂದು ಗಂಭೀರ ರೋಗವಾಗಿದ್ದು, ಕಡಲೆ ಬೆಳೆಯುವ ಎಲ್ಲಾ ಪ್ರದೇಶದಲ್ಲಿ ಹರಡಿದೆ. ಈ ತನಿಖೆಯಲ್ಲಿ ಫುಸಾರಿಯಮ್ ವಿಲ್ಟ್ ಪ್ರತಿರೋಧ ಕೇಂದ್ರಗಳ ಸಂಪರ್ಕದಲ್ಲಿರುವ ಕ್ಯೂಟಿಎಲ್/ಗುರುತುಕಾರಕಗಳ ಆಧಾರದ ಮೇಲೆ ವಿಲ್ಟ್ ಜೀನೋಮಿಕ್ ಪ್ರತಿರೋಧಕ ಪ್ರದೇಶವನ್ನು ಫೈನ್ ಮ್ಯಾಪ್ ಮಾಡಲಾಗಿದೆ. ಮೊದಲೆ ಕಂಡುಹಿಡಿದಿರುವ ೪೬ ವಿಲ್ಟ್ ಪ್ರತಿರೋಧಕ್ಕೆ ಸಂಪರ್ಕದಲ್ಲಿರುವ ಗುರುತುಕಾರಕಗಳನ್ನು ಕಡಲೆಯ ಜೀನೋಮ್ ಅನುಕ್ರಮವನ್ನು ಬಳಸಿ ಫಿಸಿಕಲ್ ಮ್ಯಾಪಿಂಗ್ ಮಾಡಲಾಗಿದೆ. ಅದರಲ್ಲಿ ೨೩ ಗುರುತುಕಾರಕಗಳು ಕಡಲೆಯ ಆರು ವರ್ಣತಂತುವಿನಲ್ಲಿ ಮತ್ತು ಆರು ಗುರುತುಕಾರಕಗಳು ಕಡಲೆಯ ಸ್ಕ್ಯಾಫೋಲ್ಡ್ ಗಳಲ್ಲಿ ಮ್ಯಾಪ್ ಆಗಿವೆ. ಫಿಸಿಕಲ್ ಮ್ಯಾಪಿಂಗ್ನ ನಂತರ ವಂಶವಾಹಿಗಳು ಮತ್ತು ಮೈಕ್ರೋಸ್ಯಾಟಲೈಟ್ ಗಳನ್ನು ಕಂಡುಹಿಡಿಯಲು ೧೬ ಜೀನೋಮಿಕ್ ಪ್ರದೇಶಗಳನ್ನು ಆರಿಸಲಾಗಿದೆ. ಒಟ್ಟಾರೆ ೩೬.೮೫ ಮತ್ತು ೧.೬೫ ಎಮ್.ಬಿ ಜೀನೋಮಿಕ್ ಪ್ರದೇಶ ಕ್ರಮವಾಗಿ ವರ್ಣತಂತುವಿನಲ್ಲಿ ಮತ್ತು ಸ್ಕ್ಯಾಫೋಲ್ಡ್ ಗಳಲ್ಲಿ ವಂಶವಾಹಿ ಹಾಗೂ ಎಸ್ಎಸ್ಆರ್ ಗಳನ್ನು ಹುಡುಕಲಾಗಿದೆ. ಸರಾಸರಿ ೧,೫೭೮ ವಂಶವಾಹಿಗಳು ಹಾಗೂ ೨,೨೫೦ ಜೆನಿಕ್ ಎಸ್ಎಸ್ಆರ್ ಗಳನ್ನು ಗುರುತಿಸಲಾಗಿದೆ, ಅದರಲ್ಲಿ ೯೪೧ ಎಸ್ಎಸ್ಆರ್ ಗಳಿಗೆ ಪ್ರೈಮರ್ ಡಿಸೈನ್ ಮಾಡಲು ಸಾಧ್ಯವಾಗಿದೆ. ಇದರಿಂದ ೧೬೮ ಎಸ್ಎಸ್ಆರ್ ಪ್ರೈಮರ್ ಗಳನ್ನು ತಯಾರಿಸಲಾಗಿದ್ದು, ಅದರಲ್ಲಿ ೧೬೧ ಗುರುತುಕಾರಕಗಳು ಅಂಪ್ಲಿಫಿಕೇಶನ್ ತೋರಿಸಿರುತ್ತದೆ. ಕಡಲೆಯ ಎರಡು ನಿರ್ದಿಷ್ಟ ಜಾತಿಯ ಜೀವಿಗಳ ಸಂಪರ್ಕ ನಕ್ಷೆಗಳನ್ನು, ಜೆಜಿ೬೫ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ ಮತ್ತು ಕೆಎ೫೦ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ಗಳ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳ ಹಾಗೂ ೧೬೮ ಎಸ್ಎಸ್ಆರ್ ಗುರುತುಕಾರಕಗಳ ಪರೀಕ್ಷೆಯ ನಂತರ ಕ್ರಮವಾಗಿ ೨೩ ಮತ್ತು ೨೨ ಬಹುರೂಪಿ ಗುರುತುಕಾರಕಗಳನ್ನು ಬಳಸಿಕೊಂಡು ನಿರ್ಮಿಸಲಾಗಿದೆ. ಜೆಜಿ೬೫ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ ಮತ್ತು ಕೆಎ೫೦ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ ಗಳ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳ ಸಂಪರ್ಕ ನಕ್ಷೆ ಕ್ರಮವಾಗಿ ೧೪೪.೫ ಮತ್ತು ೧೦೦.೪೬ ಸೆಂಟಿಮೋರ್ಗನ್ ದೂರ ವಾಪಿಸಿದ್ದು, ಮೂರು ಸಂಪರ್ಕ ಗುಂಪುಗಳನ್ನು ಹೊಂದಿರುತ್ತದೆ, ಹಾಗೂ ಕ್ರಮವಾಗಿ ೬.೫೩ ಮತ್ತು ೫.೨೩ ಸೆಂಟಿಮೋರ್ಗನ್ ಗುರುತುಕಾರಕ ಸಾಂದ್ರತೆ ಕಂಡುಬಂದಿರುತ್ತದೆ. ಈ ಎರಡು ಗುಂಪುಗಳ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳನ್ನು ಎರಡು ಬೇರೆ ಬೇರೆ ಋತುವಿನಲ್ಲಿ ಬೆಳೆದು ಆ ಸಾಲುಗಳಲ್ಲಿ ಸಸ್ಯ ರೋಗ ಪ್ರತಿರೋಧವನ್ನು ಪರೀಕ್ಷಿಸಲಾಗಿದೆ. ಜೆಜಿ೬೫ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳಿಂದ ಆರು ಕ್ಯೂಟಿಎಲ್ಗಳನ್ನು (*ಕ್ಯೂ.ಡಬ್ಲ್ಯೂ 60-07-1-1, ಕ್ಯೂ.60ಪಾಟ್-3-3, ಕ್ಯೂ.ಡಬ್ಲ್ಯೂ 30-08-1-1, ಕ್ಯೂ.30ಪಾಟ್-3-1, ಕ್ಯೂ.ಡಬ್ಲ್ಯೂ 30-07-3-1, ಕ್ಯೂ.ಡಬ್ಲ್ಯೂ 30-08-3-1*) ಗುರುತಿಸಲಾಗಿದೆ. ಅವುಗಳ ತಳಿ ಪ್ರಕಟ ಲಕ್ಷಣ ವ್ಯತ್ಯಯನ ಶೇ. ೮.೫೬ ರಿಂದ ೨೩.೬೨ ರವರಗೆ ಕಂಡುಬಂದಿರುತ್ತದೆ. ಹಾಗೂ ಕೆಎ೫೦ x ಡಬ್ಲ್ಯೂಆರ್೩೧೫ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳಿಂದ ಒಂದು ಕ್ಯೂಟಿಎಲ್ (*ಕ್ಯೂ.ಡಬ್ಲ್ಯೂ 60-07-3-1*) ಗುರುತಿಸಲಾಗಿದ್ದು, ಅದರ ತಳಿ ಪ್ರಕಟ ಲಕ್ಷಣ ವ್ಯತ್ಯಯನ ಶೇ ೯.೪೫ ಹೊಂದಿರುತ್ತದೆ. ಈ ಏಳು ಹೊಸ ಕ್ಯೂಟಿಎಲ್ ಗಳು ಮೂರು ಕಡಲೆಯ ವರ್ಣತಂತುಗಳ (೨, ೪ ಮತ್ತು ೬) ಮೇಲೆ ಮ್ಯಾಪ್ ಆಗಿದ್ದು, ಮೂರು ವಿವಿಧ ಸ್ಥಾನಗಳಲ್ಲಿ ವಿಲ್ಟ್ ಪ್ರತಿರೋಧವನ್ನು ನಿಯಂತ್ರಿಸುತ್ತಿರುವುದು ಖಚಿತವಾಗಿದೆ. ಈ ಹೊಸ ಕ್ಯೂಟಿಎಲ್ ಗಳು ಕಡಲೆಯ ಸಣ್ಣ ಜೀನೋಮಿಕ್ ಪ್ರದೇಶದಲ್ಲಿ ಮ್ಯಾಪಿಂಗ್ ಆಗಿದ್ದು, ಅದರಲ್ಲಿ ೭೫ ರೋಗ ಪ್ರತಿರೋಧಕ ವಂಶವಾಹಿಗಳನ್ನು ಗುರುತಿಸಲಾಗಿದೆ.

ಫೆಬ್ರವರಿ ೨೦೧೭,

ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ,
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಂಗಳೂರು- ೫೬೦೦೬೫

(ಆರ್. ಎಲ್. ರವಿಕುಮಾರ್)
ಪ್ರಮುಖ ಸಲಹೆಗಾರರು

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I INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool season legume crop belongs to genus *Cicer*, tribe *Cicereae*, family *Fabaceae*, and subfamily *Papilionaceae*. The name *Cicer* derived from Greek word 'kikus' meaning force or strength. The genus *Cicer* originated in South-eastern Turkey (Ladizinsky, 1975). Chickpea has originated from its wild type *Cicer reticulatum* (Tanno and Willcox, 2006). It is adopted to relatively cooler climatic conditions and spread all over the world. The largest adaptation is in the Indian sub-continent and in recent years spread to Australia. The vernacular name of chickpea is bengal gram (English), chana (Hindi) and kadale (Kannada). It is grown in over 45 countries in all continents of the world.

Chickpea is an annual herbaceous plant with spreading type of branches. The cultivated chickpea were classified into two classes (Cubero, 1975) based on seed size and colour *i.e.,* Macrosperma (*Kabuli* type) and Microsperma (*desi* type). The seeds of *Kabuli* type are larger, round, cream-coloured and contribute about 20 per cent of total chickpea production whereas, *desi* type seeds are small, angular, brown in colour and contribute about 80 per cent of total chickpea production.

There is a great demand for chickpea due to its importance in nutritional value. Chickpea is an important component of diet contributing rich sources of protein (17-22 %) for vegetarian by choice (Hulse, 1991). Chickpea is also rich in carbohydrates followed by dietary fibres, unsaturated fatty acids (linoleic and oleic acids), vitamins (vitamin A precursor β-carotene, riboflavin, niacin, thiamine and folate) and also contains mineral nutrients (Ca, Mg, P and K). It is low in lipids and anti-nutritional factors. Consumption of chickpea has beneficial effect against important diseases *viz.*, cardiovascular disease (CVD), type 2 diabetes and digestive diseases (Jukanti *et al.*, 2012).

Chickpea is second most widely cultivated pulse crop after common bean (*Phaseolus vulgaris*) and ranks third in production after field pea (*Pisum sativum*) in the world. World-wide chickpea is cultivated in an area of 14.80 million ha, with production of about 14.23 million tonnes and productivity of 962 kg/ha. India is the largest producer of chickpea and contributes a major share in world's chickpea production (Anonymous, 2015). The area under cultivation in India is 10.74 million ha with a production of 9.88 million tonnes and productivity of 919.9 kg/ha (Anonymous, 2015). Despite its broad adaptation, production is restricted by several biotic and abiotic stresses. Among biotic stresses, *Fusarium* wilt is the major disease limiting the chickpea productivity.

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. c*iceri* (FOC). The disease is very severe causing annual yield losses of 10-15 per cent. But under favourable conditions for pathogen development, it may cause yield losses upto 90 per cent (Srivastava *et al.,* 1984; Gupta *et al.,* 1986; Jimenz-Diaz *et al.,* 1993 and Cortes *et al.,* 2000). FOC is a soil born pathogen and it also survives in seed in hilum region as chlamydospore-like structures (Haware *et al.,* 1978). Lentil, pigeonpea and pea were identified as alternate hosts without showing symptoms; the pathogen can also survive in

soil for more than five years without its host (Haware and Nene, 1982). Eight races of FOC (0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been identified in all chickpea growing areas of world. The race 1 is predominant in peninsular India (Haware and Nene, 1982; Phillips, 1988; Jimenez-Diaz *et al.,* 1993; Ghosh *et al.,* 2013). Being soil borne, the management of FW is difficult. Cultivation of FW resistant varieties can be an effective management strategy to reduce the yield loss. However, development of resistant varieties for wilt through conventional breeding methods and maintenance of wilt sick plot is tedious and time consuming. Hence, molecular breeding is now becoming an efficient strategy to speed up the development of wilt resistant genotypes.

DNA markers tightly linked to FW resistance genes can be used for screening resistant genotypes in early stage of plant growth without subjecting them to pathogens. Marker assisted selection (MAS) using molecular markers linked to FW resistance will hasten the development of resistant varieties by pyramiding resistance genes into agronomically superior genotypes. However, limited genetic polymorphism in the genome of cultivated chickpea species, limits the development of markers closely linked to wilt resistance (Mayer *et al.,* 1997). Nevertheless, many researchers initially tried to map the resistant genes. Mayer *et al.* (1997) was first to tag the resistant genes against race 1 using two molecular markers CS-27_{700} (allele specific) and UBC-170 (locus specific). Followed by that many researchers identified the markers closely linked to FW resistant genes for different races *viz., Foc0* (Cobos *et al.,* 2005; Halila *et al.,* 2010; Millan *et al.,* 2010), *Foc1* (Tekeoglu *et al.,* 2000; Sharma *et al.,* 2004; Millan *et al.,* 2006; Soregaon *et al.,* 2007; Gowda *et al.,* 2009; Millan *et al.,* 2010; Barman *et al.,* 2014), *Foc2* (Gowda *et al.,* 2009), *Foc3* (Sharma *et al.,* 2004; Millan *et al.,* 2006; Gowda *et al.,* 2009), *Foc4* (Winter *et al.,* 2000; Huettel *et al.,* 2002; Tekeoglu *et al.,* 2000; Sharma *et al.,* 2004; Millan *et al.,* 2006 and 2010) and *Foc5* (Winter *et al.,* 2000; Huettel *et al.,* 2002; Tekeoglu *et al.,* 2002; Iruela *et al.,* 2006; Millan *et al.,* 2006 and 2010). Subsequently, efforts were made to map quantitative trait loci (QTLs) for FW resistance. Sabbavarapu *et al.* (2013) identified two QTLs *FW-Q-APR-6-1* and *FW-Q-APR-6-2* for FW resistance for race 1 on linkage group (LG) 6. Two QTLs *Wilt 1* and *Wilt 2* on LG2 were identified for wilt resistance for race 1 at 30 and 60 days after sowing respectively by Patil *et al.* (2014). Recently, Jingade and Ravikumar (2015) detected five QTLs for *Foc1*, resistance among which one was stable QTL across seasons flanked by markers GSSR18-TC14801.

Comparison of different studies revealed that four FW resistance genes (*Foc1, Foc3, Foc4* and *Foc5*) clusters on the same linkage group (Winter *et al.,* 2000; Huettel *et al.,* 2002; Tekeoglu *et al.,* 2000; Sharma *et al.,* 2004; Millan *et al.,* 2006 and 2010) indicating that wilt resistance could be a super loci functioning against many races. It is important to fine map these loci to determine the candidate genes for resistance and to develop reliable robust markers for MAS. To drive fine mapping of QTLs linked to wilt resistance, development of microsatellite, single nucleotide polymorphism (SNP) and INDELs (insertion and deletions) markers in the genomic region controlling wilt resistance by physical mapping the QTLs is important.

High throughput next generation sequencing (NGS) technologies provides a rapid means of discovery of genes/markers based on gene/transcriptome sequences. Recently

chickpea genome has been sequenced and its draft genome sequence information is available in the database (Jain *et al.*, 2013; Varshney *et al.*, 2013). Based on available resources on genetic linkage map, markers linked to wilt resistance, QTLs associated with trait of interest, draft genome sequence and transcriptome resources of chickpea provide an opportunity to improve chickpea marker assisted breeding programs by developing robust markers linked to various traits including FW resistance.

In this context, development of *in silico* physical mapping of QTLs and its integration with genetic map are very much needed for development of tightly linked markers to fine map the FW resistance genes. Further, availability of chickpea genome sequence information and bioinformatics tools have helped in linking the physical map and already established genetic linkage map which provides better option for resistance breeding in chickpea.

In the present study, the molecular markers linked closely and flanking QTLs for FW resistance were utilized to align against chickpea genome sequence, based on sequence homology for *in silico* physical mapping. The information from genetic/QTL map is used to relate physical and genetic map to facilitate the better resolution of QTLs. The genomic regions of QTLs mapped *in silico* on chickpea genome sequence were used for identification of genes and microsatellite repeats in the regions. The genic simple sequence repeats (SSR) markers were developed from the genes identified in the QTL region for FW resistance. The genic SSR markers were validated in the laboratory for amplification and polymorphism between resistant and susceptible parental lines. The genic SSR markers which showed polymorphism for parents of recombinant inbred lines (RILs) were used for the construction of genetic map and physical map to fine map the wilt resistance loci in chickpea genome.

In this regard, the specific objectives of present investigation are formulated as follows,

1. Identification of genomic region of *Fusarium* wilt resistance by utilizing genome sequence information and previously reported QTL/linked markers.
2. Development of gene based markers for candidate genes located in *Fusarium* wilt resistance loci.
3. Genetic mapping of functional markers linked to *Fusarium* wilt resistance in chickpea.

II REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is a cool season legume crop belonging to the family *Fabaceae*. It is a self-pollinating diploid (2n=2x=16) crop with genome size of ≈738 Mb (Jain *et al.,* 2013; Varshney *et al.,* 2013). The origin of chickpea was traced long back 7500 years ago in Turkey and spread to central Asia (Zohary and Hopf, 2000). It is one of the nutritionally and economically important pulse crop.

But the production of chickpea is affected by many diseases. Among them, *Fusarium* wilt (FW) is one of the major disease of chickpea. It is a vascular disease caused by *Fusarium oxysporum* f. sp. *ciceri*. High level of genetic resistance is available in the cultivated taxa and the development of resistant genotypes is the best option to reduce the yield losses.

However, development and maintenance of uniform wilt sick plots for testing breeding lines to develop resistant varieties is very difficult. Furthermore, the severity of disease is affected by inoculum concentration, virulence and environmental conditions (Jimenez-Gasco *et al.,* 2004). The identification of reliable DNA markers closely linked to resistant genes increases the efficiency of selection of resistant genotypes at very early stage of growth in the absence of wilt sick plots. This helps in characterizing large numbers of chickpea genotypes/breeding lines. Moreover, wilt resistance could be a super locus with resistance genes function against many races. It is important to fine trap these loci to determine the candidate genes for resistance.

To drive fine mapping of QTLs linked to wilt resistance, development of microsatellite, SNP's and indel markers in the genomic region controlling wilt resistance is important. The development of markers in any genomic region has now become simpler and easy because of high throughput NGS provides a rapid means of discovery of genes/markers based on genome/transcriptome sequences. Recently chickpea genome has been sequenced and its genome sequence information is available in the public database (Varshney *et al.,* 2013).

In the present study, attempt has been made for targeted *in silico* physical mapping of resistance loci for development of the genic microsatellite markers in the region and fine mapping of resistance loci.

In this direction the available literature has been reviewed under the following headings,

2.1 Importance of chickpea
2.2 *Fusarium* wilt disease in chickpea
2.3 Genetics of *Fusarium* wilt resistance
2.4 Molecular markers linked to *Fusarium* wilt resistance and linkage map of chickpea
2.5 Genomic resources in chickpea
2.6 Physical mapping of chickpea
2.7 *In silico* physical mapping of chickpea
2.8 Fine mapping

2.1 Importance of chickpea

Nutritionally, chickpea is a rich source of protein for vegetarian population in the developing countries. The protein content of chickpea ranges from 17-22 per cent of total dry seed mass (Hulse, 1991). Kaur *et al*., (2005) reported that protein quality of the chickpea is better than other pulses *viz*., black gram (*Vigna mungo* L.), green gram (*Vigna radiata* L.) and red gram (*Cajanus cajan* L.). Further, amino acid profiling of chickpea showed that sulpur containing amino acids are limited (Wang and Daun, 2004). Chickpea is also rich in carbohydrate (60.7 g / 100 g) and total dietary fibres (17.4 g / 100 g). Chickpea also provides essential vitamins and minerals. Hundred grams of chickpea seeds contain about 5.0 mg of Fe, 4.1 mg of Zn, 138 mg of Mg and 160 mg of Ca. It is also a good source of vitamins such as riboflavin, niacin, thiamin, folate and the β-carotene. Even though, fats and lipids are present in very low amount (6 g / 100 g seeds), chickpea is nutritionally rich in unsaturated fatty acid *viz*., linoleic and oleic acids. Chickpea is one of the important crop having beneficial effect on important diseases *viz*., cardio vascular disease and type 2 diabetes (Jukanti *et al*., 2012).

Chickpea is one of the economically important pulse crop, because of its low cost of cultivation, requires less input and it is cultivated mostly in marginal land with residual moisture. Chickpea being a legume crop, form a very important component in diversifying cereal based cropping system through intercropping, because of its ability to fix atmospheric nitrogen and in breaking disease cycle through crop rotation (Arnon, 1972). Chickpea is cultivated as sole crop and mixed or intercrop. In India chickpea intercropped with rape-seed, mustard, linseed, barley and lentil. In crop rotation, it is often followed by wheat, barley, rice and also grown as catch crop in sugarcane fields (Pandey *et al.,* 2007). Despite, the benefits of intercropping and crop rotation, higher economic returns provide the potential for chickpea to replace fallow land to cultivation and to break continuous cereal crop cultivation in farming system (Zhang *et al.,* 2000). Even though, the chickpea production in the world is higher *i.e.,* 14.23 million tonnes (Anonymous, 2015), the productivity of chickpea is low due to biotic and abiotic stresses. Several diseases are known to limit the chickpea production worldwide. Among biotic stresses, *Fusarium* wilt (FW) is a serious disease cause potential yield losses in chickpea regularly. This disease is prevalent in all the chickpea growing areas of world. It is a serious problem in India, Iran, Pakistan, Nepal, Burma, Spain and Tunisia.

2.2 *Fusarium* wilt disease in chickpea

Fusarium wilt of chickpea is a soil borne vascular disease caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo and K. Sato. It was first reported from India (Butler, 1918). Padwick (1940) identified *Fusarium orthoceras* var. *ciceri* as the cause of chickpea wilt in India, later it was renamed as *F. oxysporum* f. sp. *ciceri* (FOC) which is now widely accepted. In India it is estimated that yield loss is 10-15 per cent annually under normal conditions (Jalali and Chand, 1992). But under favourable conditions for pathogen development, it may cause yield losses of about 90 per cent (Srivastava *et al.,* 1984; Gupta *et al.,* 1986; Jimenz-Diaz *et al.,* 1993 and Cortes *et al.,* 2000). Since, the fungus is soil-borne and can survive for more than 6 years in the soil, it is not possible to control disease through crop rotation (Haware *et al.,* 1978). Eight races of FOC have been reported so far among them 1A, 2, 3, 4, 5, and 6 cause wilting symptoms and the

races 0 and 1B/C cause yellowing symptoms (Haware and Nene, 1982; Jimenez-Diaz *et al.*, 1993; Kelly *et al.*, 1994).

The control of FW disease is difficult either through chemical control or by crop rotation, because of its soil borne nature. High level of genetic resistance is available in the cultivated taxa and the development of resistant genotypes with agronomically superior trait is the best option to reduce the yield losses. Identification of resistant cultivars/genotypes from the germplasm is traditionally done by screening the germplasms in wilt sick plots. Evaluation of a large number of germplasm and breeding lines for resistance to FW under field as well as greenhouse conditions is laborious, time consuming and costly. Further, the resistance is affected by inoculum concentration and environmental conditions (Jimenez-Gasco *et al.,* 2001; Landa-Blanca *et al.,* 2001).

2.2.1 Screening technique to identify wilt resistant chickpea genotypes/germplasm

Effective and efficient screening for resistance to soil borne pathogens such as *Fusarium spp*. requires suitable environmental conditions and uniform inoculum load across all the plants of test genotypes to discriminate between resistant and susceptible genotypes. In general, screening under field and controlled conditions (green house and laboratory conditions) has been suggested to identify resistant genotypes for FW resistance in chickpea.

2.2.1.1 Screening under field conditions

The most widely used method for screening of FW resistance and susceptibility was wilt sick plot (WSP) method. The main advantage of WSP method is that it allows screening of a large number of genetic materials under field conditions (Infantino *et al.,* 2006). Typical disease symptoms are the main criteria for evaluating germplasm or breeding lines, while the reisolation of the causal organism is a confirmatory test. In this method, inoculum load can vary with different races, environmental conditions, crop and ecotypes (e.g., *Desi* and *Kabuli*) of chickpea (Ali *et al.,* 1994). Haware *et al.* (1992) developed the field screening at ICRISAT, according to that protocol, the selection of a wilt sick-plot is done in slightly alkaline vertisols. The susceptible cultivar was grown for 2 to 3 season's leads to incorporation of the infected plant debris into the soil. Artificially and naturally infested soils were used successfully across the world to screen chickpea materials for resistance.

2.2.1.2 Screening under controlled conditions

In field screening there is no control over climatic conditions and the presence of other soil borne fungal pathogens and nematodes may affect the reaction of genotypes. Therefore, screening under controlled conditions in glasshouse is also suggested to confirm the results of WSP method. This is particularly important for studying inheritance pattern, QTL mapping and tagging studies (Choudhary *et al.,* 2013). Screening under controlled condition further classified into two methods *i.e.,* greenhouse condition and laboratory conditions.

A. Greenhouse screening

This technique is popularly known as wilt sick pot method. In this method the fungus is mass multiplied on sand: maize (9:1) meal medium for 15 days at 28-30 °C. After multiplying for 20 days, 200 g of this medium is mixed with 2 kg autoclaved soil and placed in 15 cm plastic pot and were incubated at 25-30 °C. After 2 days, 7-10 days old seedlings were transplanted in the pathogen infested pots. Wilt incidence is recorded at 30 and 60 days after transplanting (Nene and Haware, 1980; Brindha and Ravikumar, 2005).

B. Laboratory screening

It is also called as root dip method in which seedlings were inoculated by dipping their roots in the inoculum (1×10^6 spores/ml of culture) for 2-5 minutes and then they were transplanted in pot containing autoclaved sand, vertisol or alfisol soil (Bhatti, 1990). Apart from this Fusaric acid (FA), one of the toxins produced by the *Fusarium*, is used as the selective agent to screen chickpea genotypes *in vitro*. FA treatment involves two techniques *i.e.*, *in vitro* root feeding and *in vitro* screening based on pollens. The *in vitro* root feeding technique involves root feeding of FA (15 ppm) to 9 days old chickpea seeding which cause necrotic lesions and death of susceptible seedlings by 6^{th} day after transplanting, whereas resistant genotype survive for 13 days (Ravikumar and Ratnababu, 2007). In case of *in vitro* pollen based screening the concentration of FA to inhibit 50 per cent pollen tube growth differs for resistant, late wilting and susceptible types, which has also been validated by molecular markers (Ratnababu and Ravikumar, 2010). Further, screening for wilt incidence for different races, different mapping populations with different techniques were summarized in the Table 1.

2.3 Genetics of *Fusarium* wilt resistance

The genetic studies on *Fusarium* wilt resistance were started way back in mid 1930s. Resistance to wilt in chickpea has been shown to be race specific and governed by major resistance genes (Upadyayaya *et al.,* 1983a and b). Earlier studies on genetics of wilt resistance were restricted to race 1, where it was shown to be inherited by a recessive gene (Ayyar and Iyer, 1936; Kumar and Haware, 1982; Sindhu *et al.,* 1983). Later several studies on genetics of wilt resistance against race 1 indicated three independent loci designated as H_1, H_2 and H_3 govern resistance to wilt (Singh *et al.,* 1987). However, two major independent loci, H_1 and H_2 determine resistance to race 1 in chickpea was also suggested (Brindha and Ravikumar, 2005; Sabbavarapu *et al.,* 2013). The dominant alleles at both H_1 and H_2 loci result in early wilting and recessive at anyone loci ($h_1h_1H_2$_*or* H_1_h_2h_2) produce late wilting and recessive alleles at both the loci ($h_1h_1h_2h_2$) result in resistance (Upadhyaya *et al.,* 1983a, b; Brindha and Ravikumar, 2005; Soregaon and Ravikumar, 2010). Singh and Reddy, (1991) studied F_1 and F_2 generations of the two crosses H-208 x K850 and H-208 x C-104. They indicated that cultivar H-208 carried a dominant allele for late wilting to race 1 of *Fusarium oxysporum* f.sp. *ciceri* at a locus different from the earlier reported. Anupama (2001) reported two recessive genes under homozygous conditions for resistance to race 1 of *Fusarium* wilt in a cross between ICCV2 ($h_1h_1h_2h_2h_3h_3$) and JG62 ($H_1H_1H_2H_2h_3h_3$).

Table 1. Summary of different screening techniques used for phenotyping of chickpea genotypes for *Fusarium* wilt **resistance**

Sl. No.	Races	Genotypes/breeding lines	Generations	Techniques	References
1	Race 1	K850×C104 and K850×JG62	F_1, F_2 and F_3	Wilt sick pot method	Singh *et al.,* 1987
2	Race 1	C104×WR315	F_6	Wilt sick pot method	Mayer *et al.,* 1997
3	Race 4	JG62×Surutatto-77	F_2 and F_3	Root dip method	Tullu *et al.,* 1999
4	Race 4 and 5	ICC4958×PI489777	F_6	Root dip method	Ratanparke *et al.,* 1998a
5	Race 0 and 5	ICC4958×PI489777	F_8 and F_9	Root dip method	Tekeoglu *et al.,* 2000
6	Race 4 and 5	ICC4958×PI489777	F_7	Root dip method	Winter *et al.,* 2000
7	Race 4 and 5	ICC4958×PI489777	F_8	Root dip method	Benko-Iseppon *et al.,* 2003
8	Race 3	WR315×C104	F_7	Root dip method	Sharma *et al.,* 2004
9	Race 1	JG62×WR315	F_5	Corn meal sand method	Brindha and Ravikumar, 2005
10	Race 0	CA2156×JG62 and CA2139×JG62	F_7	Root dip method	Cobos *et al.,* 2005
11	All races	Varietal lines	-	Root dip method	Sharma *et al.,* 2005
12	Race 1	JG62×WR315	F_7	Wilt sick plot method	Soregaon *et al.,* 2007
13	Race1, 2 and 3	JG62×Vijaya	F_9	Corn meal sand method	Gowda *et al.,* 2009
14	Race 1	JG62×WR315 BG256×WR315	F_8 F_6	Wilt sick plot method	Ravikumar and Shindhe, 2010
15	Race 1	C214×WR315	F_3	Wilt sick plot method	Sabbavarapu *et al.,* 2013
16	Race 1	JG62×WR315	F_9	Wilt sick plot method	Patil *et al.,* 2014
17	Race 1	WR315×C104	F_8	Root dip method	Barman *et al.,* 2014

Similarly, resistance for FOC race 2 (*Foc2*) was governed by two (Gumber *et al.,* 1995) or three genes (Kumar, 1998), whereas resistance to FOC race 3 (*Foc3*), race 4 (*Foc4*) and race 5 (*Foc5*) is monogenic (Sharma *et al.,* 2005). Resistance to race 0 (*Foc0*) was governed either by single gene (Tekeoglu *et al.,* 2000) or digenic (Rubio *et al.,* 2003).

Inheritance of resistance to race 4 of *Fusarium* wilt was studied by Tullu *et al.* (1998) using 100 F_6 derived F_7 recombinant inbred lines (RILs), that had been developed from the cross of breeding lines C-104 (late wilter) x WR315 (resistant). They found that the gene for resistance to race 4 segregating in 1:1 ratio as expected for single gene. There are only a couple of studies on the inheritance of resistance to race 5 by a single gene (Tekeoglu *et al.,* 2000; Sharma *et al.,* 2005). Resistance for race 5 and race 0 were studied in F_6 RILs, which showed a 1 resistant : 1 susceptible segregation ratio for both races, indicating that resistance to each race is controlled by a single gene (Tekeoglu *et al.,* 2000). Further, a 3:1 resistant to susceptible ratio was observed in the RIL population of CA-2139 x JG62 cross indicating the involvement of two genes for resistance to race 0 of *Fusarium oxysporum* f.sp. *ciceri* of chickpea (Rubio *et al.,* 2003), presence of either gene is sufficient for resistance. Genetics of resistance to two races (race 1B/C and 6) is yet to be determined (Sabbavarapu *et al.,* 2013).

2.4. Molecular markers linked to *Fusarium* wilt resistance and linkage map of chickpea

DNA markers tightly linked to FW resistance genes can be used for screening resistant genotypes in early stage of plant growth without subjecting them to pathogens. Marker assisted selection (MAS) using molecular markers linked to FW resistance will hasten the development of resistant varieties by pyramiding resistance genes into agronomically superior genotypes. However, minimal genetic polymorphism in the genome of cultivated chickpea species, limits the development of markers linked to wilt resistance (Mayer *et al.,* 1997). Nevertheless, many researchers initially tried to map the resistant genes using restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) yield low success. Mayer *et al.,* (1997) was first to tag the resistant genes against race 1 using two RAPD markers CS-27_{700} and UBC-170_{550}. These two markers were tested using one hundred F_6 recombinant inbred lines (RILs) derived from intraspecific cross C-104 (late susceptible) × WR315 (resistant), which fits expected 1:1 segregation ratio. Six per cent recombination frequency was observed between two markers, whereas seven per cent recombination has been observed between these two markers and with the loci controlling resistance for wilt. After cloning and sequencing of these two fragments, they designed two pairs of allele-specific associated primer (ASAP), CS-27_{700}F/ CS-27_{700}R and UBC-170_{550}F/UBC-170_{550}R. CS-27_{700} amplifies a fragment of 700 base pair (bp) linked to the allele for susceptibility (H_1) to *Fusarium* wilt race-1, with an estimated distance of 6 cM. The other marker UBC-170_{550} produce single band for both resistance and susceptible genotypes indicated locus specificity. Later, Tullu *et al.,* (1998) demonstrated that the markers associated with race 1 (CS-27_{700} and UBC-170_{550}) were also associated with race 4 resistance, when tested with earlier intraspecific F_7 RILs mapping population. Both markers showed 1:1 segregation ratio expected for single gene.

Both RAPD loci were 9 map units apart from the resistance locus. Their results indicated that both the resistance loci for race 1 and race 4 were 5 map units apart.

Ratnaparkhe *et al.* (1998a) used 131 F_6 derived F_7 RIL mapping population of interspecific cross between *C. arietinum* (ICC-4958, resistant) and *C. reticulatum* (PI 489777, susceptible). They first time reported an ISSR marker $UBC855_{500}$ linked to the FW resistance gene for race 4 in repulsion at a distance of 5.2 cM units. The marker $UBC855_{500}$ is also located on the same side of $CS\text{-}27_{700}$ at 0.6 cM. Further, Ratnaparkhe *et al.* (1998b) identified one more ISSR marker $UBC825_{1200}$ and studied the inheritance of the marker using same RILs reported earlier, showing that marker linked to FW resistant gene for race 4 at 5 cM units.

A compressive reference linkage map of the chickpea for FW race 4 & 5 has been constructed using a set of 130 F_6-derived F_7 RILs of the interspecific cross of *C. arietinum* (resistant) × *C. reticulatum* (susceptible), in which a total of 354 markers were mapped on the RILs including 118 STMSs, 96 DAFs, 70 AFLPs, 37 ISSRs, 17 RAPDs, eight isozymes, three cDNAs, two SCARs and three loci that confer resistance against different races of *Fusarium* wilt. Out of these markers EAAMCTA12, ECAMCTA07 (AFLP), CS-27 and TA96 were associated to the FW resistance locus *Foc4* and other two markers TA27 and TA59 were linked to *Foc5* locus. They also reported that FW resistance loci *Foc4* and *Foc5* were mapped to the same linkage group (LG) 2 along with CS-27 previously shown to be linked to *Foc1* at a distance of 3.7 and 18 cM, respectively. This demonstrates that clustering of several FW resistance genes on LG 2 (Winter *et al.*, 2000). A study on inheritance of resistance to race 0 and 5 has been showed using a set of 131 F_6-derived RILs of cross ICC4958×PI489777. The resistance for each race was controlled by a single gene and linkage analysis showed that genes conferring resistance to race 0 and race 5 were found in different genomic regions (Tekeoglu *et al.,* 2000).

Huettel *et al.* (2002) mapped *Foc4* and *Foc5* on LG 2 flanked by markers CS-27-TA96 and ECAMCTA07-TA27 respectively using interspecific cross of ICC4958×PI489777 (F_8) RIL mapping population. Using same interspecific mapping population, Tekeoglu *et al.* (2002) mapped resistance genes for FW race 4 (*Foc4*) and race 5 (*Foc5*) on the same LG 2 and were separated by 11.2 cM units. Linkage analysis also shown ASAP marker CS-27 mapped between FW resistance loci *Foc4* and *Foc5* at a distance of 7.2 and 4 cM respectively.

Due to low genetic polymorphism within *C. arietinum,* earlier researchers tried to map the FW resistance gene using interspecific crosses with wild relative *C. reticulatum* (Ratnaparkhe *et al.,* 1998a and 1998b; Winter *et al.,* 2000 and Huettel *et al.,* 2002). However, the sources of resistance to FW are available within the cultivated *C. arietinum,* it is important to use intraspecific populations to map FW resistance. Marker assisted selection (MAS) require intraspecific linkage maps saturated with codominant markers *viz.,* SSRs, STMSs or SNPs (single nucleotide polymorphism). Such markers will also be useful for map based cloning as the differences in genetic and physical

distances among markers would be minimum (Winter *et al.,* 2000; Benko-Iseppon *et al.,* 2003) and also linkage drag would be minimum.

There were reports showing that linkage between DNA amplification fingerprinting (DAF) markers (closest one R-2609-1) and race 4 resistance locus (Benko-Iseppon *et al.,* 2003). An RAPD marker $OPJ20_{600}$, linked to resistance to race 0, was successfully used to study inheritance of resistance for race 0 in two $F_{6:7}$ RIL populations, indicating that two genes were controlling resistance to race 0 and either gene is sufficient to confer resistance (Rubio *et al.,* 2003). Sharma *et al.* (2004) developed linkage map using 100 F_7 RILs of intraspecific cross WR315×C-104, where they mapped *Foc3* at 0.6 cM from STMS (Sequence-tagged Microsatellite Sites) markers TA96, TA27 and CS-27A and on the other side TA194 flanked *Foc3* at 14.3 cM units.

Cobos *et al.* (2005) constructed a genetic map from two RIL mapping populations derived from an intraspecific crosses CA2156 × JG62 and CA2139 × JG62. Ten linkage groups (LGs) were obtained that includes morphological markers and 134 molecular markers. The wilt resistance genes for *Foc0* are flanked by RAPD marker $OPJ20_{600}$ and STMS marker TR59 which were mapped on LG3. Iruela *et al.* (2007) validated resistance to FW caused by race 5 was inherited as a single gene and mapped to LG2, flanked by the STMS markers TA110 (6.5 cM apart) and TA59 (8.9 cM apart).

An integrated intraspecific map has been developed using 186 $F_{8:9}$ RILs. A total of 273 different markers *viz.,* RAPD, SSR, ISSR, RGA (resistance gene analogue) and ASAP were used for analysis and generated eight linkage groups, genes for double pod and QTLs for seed weight were mapped (Radhika *et al.,* 2007). Further, Soregaon *et al*. (2007) identified an RAPD marker A07C amplifies 417 bp linked to H_2 locus of wilt susceptibility for race 1. The reliability of both A07C and $CS\text{-}27_{700}$ markers in identifying susceptible genotypes was confirmed by validating in different chickpea genotypes and crosses (Brindha and Ravikumar, 2005; Soregaon and Ravikumar, 2010).

One hundred F_9 RILs obtained from the cross between JG62 (susceptible) and Vijay (resistant) were used by Gowda *et al.* (2009) to map three FW resistance loci *i.e., Foc1*, *Foc2* and *Foc3.* They identified previously unreported markers tightly linked and flank the resistance loci. The resistant locus *Foc1* was flanked by SSR markers H_3A_{12} and TA110 at 3.9 and 2.1 cM, respectively. Similarly, *Foc2* was tagged with SSR markers TA96 and H_3A_{12} at a distance of 0.2 and 2.7 cM, respectively and *Foc3* locus was tagged with SSR markers H_1B06y and TA194 at 0.2 and 0.7 cM respectively.

A consensus genetic map of chickpea was constructed by merging linkage maps from 10 different intra and interspecific mapping populations, using STMS as bridging markers, for mapping different traits such as *Ascochyta* blight (AB), *Fusarium* wilt, rust resistance, seed weight, flowering time and days to flower. The integrated map developed from merging of interspecific crosses included 555 loci. Among them 135 STMSs and 33 cross-genome markers distributed on eight linkage groups and covers 652.67 cM. While the consensus map developed by merging intraspecific maps consisted of 99 STMSs, 3 SCARs, 1 ASAP, *Fusarium* wilt resistance gene, 5 morphological traits as well as RAPD

and ISSR markers distributed on eight linkage groups covering 426.99 cM. In that consensus map two STMS markers TA200 & TA110 flanks *Foc1*, *Foc4* & *Foc5* locus on LG 2. Similarly, TA96 and TA59 flanks *Foc3*, while $Foc0_2$ flanked by TS47 and TA27 on the same LG2 (Millan *et al.*, 2010).

Further, Soregaon (2011) reported that RAPD marker A07C linked to the FW resistance gene H_2 using RILs of the cross K850×WR315 and also validated using another mapping population JG62×WR315. Using K850×WR315 RIL mapping population, the genetic linkage map with 31 polymorphic markers was constructed, in that 28 markers mapped to five LG covering a total of 171.5 cM with an average marker density of 5.72 cM. Three QTLs for *Fusarium* wilt reaction at 30 DAS (days after sowing) with the phenotypic variance ranging from 4.30 to 13.50 per cent was observed. Two major QTLs (OPK9- $A07C_{417}$, H_4G_{11} and TA1-NCPGR33, SSR21) which explain 13.5 and 8.7 per cent phenotypic variance were located on LG 3. A co-dominant SSR marker H_4G_{11} linked to H_2 locus of *Fusarium* wilt resistance became a more convenient marker for identifying susceptibility for race 1 along with $CS\text{-}27_{700}$.

A linkage map of chickpea has been constructed by Bharadwaj *et al.* (2011) using F_2 population of *desi* × *kabuli* cross of BGD 112 and FLIP 90-166 utilizing STMS markers. They assessed 250 STMS markers for parental polymorphism and 49 of which were polymorphic. Linkage analysis revealed eight LG with 33 loci covering a distance of 471.1 cM. The molecular map developed from cross between *desi* × *kabuli* provide a better variability and diversity that can be directly utilized by breeder for MAS.

Subsequently, efforts were made to map quantitative trait loci (QTLs) for FW resistance and *Ascochyta* blight (AB) resistance by Sabbavarapu *et al.* (2013). Three hundred and seventy one SSR markers have been used for assessing parental polymorphism for parents of two $F_{(2:3)}$ populations *i.e.*, C-214 (FW susceptible) × WR315 (FW resistant) and C-214 (AB susceptible) × ILC 3279 (AB resistant). The polymorphic markers from 371 SSR markers have been used for genotyping of mapping populations. They did the linkage analysis and constructed two mapping populations with 57 (C 214 × WR 315) and 58 (C 214 × ILC 3279) genetic loci. By combining genotypic data and phenotypic data of mapping populations they identified two novel QTLs *FW-Q-APR-6-1* and *FW-Q-APR-6-2* for FW resistance for race 1 flanked by CaM1402–CaM1101 (36.3 cM) and CaM1125–TA22 (41.4 cM) respectively on LG 6, which explained 10.4-18.8 per cent of phenotypic variation. In case of AB resistance six QTLs were identified. One major QTL, flanked by STMS11-TA130 markers mapped on LG 4 explains phenotypic variation of 31.9 per cent for AB resistance, in both field and controlled conditions. The QTL was also reported from different resistant lines in many earlier studies (Sabbavarapu *et al.*, 2013).

Patil *et al.* (2014) constructed a molecular map of chickpea using ninety four F_9 derived F_{10} lines of cross early wilting genotype (JG62) × resistant genotype (WR315). One hundred and ten chickpea STMS and 48 AFLP markers have been utilized to screen parental polymorphism. Out of that 28 STMS and 5 AFLP were polymorphic for parents. Six out of 33 markers showed segregation distortion. They constructed linkage map with

20 STMS and 3 AFLP markers which covers five LG spanning a total of 300.2 cM units. They identified two novel QTLs using genotypic and phenotypic data for wilt reaction using wilt sick plot. One QTL *Wilt 1* flanked by TA27-TA59 (9 cM apart) for early wilting (at 30 DAS) with the 36 per cent phenotypic variation with the maximum LOD score of 9.19 and other QTL *Wilt 2* flanked by TA27-TA110 (22.2 cM apart) for late wilting (at 60 DAS) explained 16 per cent phenotypic variation with LOD score of 3.53. Both the QTLs were mapped on the same linkage group 2 and flanking markers of these QTLs have been reported previously in various studies.

Recently, Jingade and Ravikumar (2015) mapped the H_2 locus linked to FW resistance for race 1A using 300 genic and genomic SSR markers. They mapped 31 polymorphic markers on F_{10}–F_{11} RILs segregating for H_2 locus of wilt resistance from intraspecific cross between K850 (susceptible late wilter, $h_1h_1H_2H_2$) and WR315 (resistant, $h_1h_1h_2h_2$). The linkage map developed has four LG which covers 690 cM with marker density of 5.72 cM units. They used the phenotypic data of wilt sick plot tested over two seasons. They identified five QTLs by combining genotypic data and phenotypic data of two seasons for late wilting (at 60 DAS). Among five QTLs, a stable QTL flanked by GSSR18-TC14801, was identified for wilt resistance in both the seasons, and the QTL explained a variance of 69.80 and 60.80 per cent in 2007 and 2008 *Rabi* respectively.

From the earlier reports, forty four FW resistance linked markers covering 32 FW resistance loci and nine QTLs from various studies have been identified. It is clear that FW resistance is governed by many genes or QTLs. Comparison of different studies revealed that four FW resistance gene (*Foc1*, *Foc3*, *Foc4* and *Foc5*) clusters on the same LG 2 (Winter *et al.*, 2000; Huettel *et al.*, 2002; Tekegulu *et al.*, 2002; Sharma *et al.*, 2004; Millan *et al.*, 2006 and 2010). It clearly indicates that wilt resistance could be super loci function against many races. The markers linked to wilt resistance for different races or QTLs mapped were briefly summarized with their LG, genetic distance and resistant genes tagged in Table 2.

2.5. Genomic resources in chickpea

2.5.1. Chickpea genome sequence information

A good number of genomic approaches have been undertaken encompassing whole-genome sequencing to have a deep insight into the gene content and organization of the chickpea genome. The chickpea draft genome sequence, diversity analysis of chickpea cultivars and the marker resources generation are expected to help elucidate the molecular basis of agronomically important traits *viz.*, increase in yield, drought tolerance and disease resistance *etc.*, to facilitate the genetic improvement of a nutritionally and economically important crop.

The draft genome sequencing of chickpea was carried out simultaneously in the same year by Jain *et al.* (2013) and Varshney *et al.* (2013). Varshney *et al.* (2013) selected the genotype CDC Frontier, a Canadian *kabuli* chickpea variety cultivated worldwide and is resistant to several important fungal diseases, including *Ascochyta* blight, and insects like pod borer. They had done illumina sequencing which generated

Table 2. Summary of the markers and QTLs identified for *Fusarium* wilt resistance of different races

***Fusarium* races**	**QTLs/Resistant loci tagged**	**Markers linked**	**Genetic distance (cM)**	**Linkage group (LG)**	**References**
Race 1	*H_1*	UBC-170_{550} CS-27_{700}	-	-	Mayer *et al.,* 1997
Race 4	-	UBC-855_{500}	-	-	Ratnaparkhe *et al.,* 1998a
Race 4	*Foc4*	$UBC825_{1200}$	5.0	-	Ratnaparkhe *et al.,* 1998b
Race 4	*Foc4*	CS-27_{700} TA96	3.7 3.4	2	Winter *et al.,* 2000
Race 5	*Foc5*	ECAMCTA07 TA27	6.4 3.5	2	
Race4 Race 5	*Foc4* *Foc5*	CS-27-*Foc4*-TA96 ECAMCTA07-*Foc5*-TA27	7.1 9.9	2	Huettel *et al.,* 2002
Race 1, Race 4 Race 5	*H_1* *Foc4* *Foc5*	CS-27_{700}-*H_1* CS-27_{700}-*Foc4* CS-27_{700}-*Foc5*	14 7.2 4.0	2	Tekegulu *et al.,* 2002
Race 1 Race3 Race 4	*Foc1* *Foc3* *Foc4*	CS-27-*Foc1* *Foc1*-TA96, TA27, CS27A *Foc3*-TA96, TA27, CS27A *Foc3*-TA194 CS-27- *Foc4* *Foc4*-TA96, TA27, CS27A	7.1 9.2 8.1 14.3 8.2 8.1	2	Sharma *et al.,* 2004
Race 0	*Foc0*	TR59 $OPJ20_{600}$	2.0 3.0	3	Cobos *et al.,* 2005
Race 5	*Foc5*	TA110 TA59	6.5 8.9	2	Iruela *et al.,* 2006
Race 1, Race 4 Race 5	*Foc1* and *Foc4* *Foc3* and *Foc5*	GA16-TA96 TA96-TA27	-	2	Millan *et al.,* 2006
Race 1	*H_2*	*A07C (Full length sequence of RAPD marker)	21.7	-	Soregaon *et al.,* 2007

***Fusarium* races**	**QTLs/Resistant loci tagged**	**Markers linked**	**Genetic distance (cM)**	**Linkage group (LG)**	**References**
-	-	H4G11, H4E09, TR20	-	2	Radhika *et al.*, 2007
Race 1 Race2 Race3	*Foc1* *Foc2* *Foc3*	TA110-*Foc1*-H3A12 H3A12-*Foc2*-TA96 TA194-*Foc3*-H1BO6Y	6.0 2.9 0.9	2	Gowda *et al.*,2009
Race 0	*Foc0*$_2$	TS47-TA27	1.5	2	Millan *et al.*, 2010
Race 3	*Foc3*	TA59-TA96	2.0	2	
Race 1 Race 4 Race 5	*Foc1* *Foc4* *Foc5*	TA110-TA200	10.0	2	
Race 0	*Foc0*	TA59, TR59, TS47	-	2	Halila *et al.*, 2010
Race 1	*H*$_2$	OPK4, OPK9, A07C, H4G11, SSR14, TR59, NCPGR58, NCPGR74, TS72	-	3	Soregaon, 2011
Race 1	*FW-Q-APR-6-1* *FW-Q-APR-6-2*	CaM1402–CaM1101 CaM1125–TA22 TR44- CaM1402	36.3 41.4 2.1	6	Sabbavarapu *et al.*, 2013
Race 1	*Wilt 1* *Wilt 2*	TA27-TA59 TA27-TA110	9.0 22.2	2	Patil *et al.*, 2014
Race 1	*Foc1*	TA37 TA200 TR2	0.2 1.0 3.0	2	Barman *et al.*, 2014
Race 1 and Race 3	*Foc1* and *Foc3*	GA16, TA194, TS82, TAA60, TR19	-	2	Varshney *et al.*, 2014a
Race 1	*QTLs between*	TR 24 - EST SSR 21 ESTSSR21 - ESTSSR65 GSSR18 - TC14801 GSSR11 - ESTSSR 3	63.50 63.60 158.80 56.80	1	Jingade and Ravikumar, 2015

153.01 Gb of sequence data. Based on k-mer statistics, they estimated chickpea genome size to be 738.09 Mb, which includes 73.8 per cent of the genome is captured in scaffolds and remaining 36.3 per cent (non-assembled genome) is enriched for repetitive sequences. About 65.23 per cent of genome assembly was anchored to eight linkage group *i.e.,* Ca1 to Ca8. From the genome analysis they predicted 28,269 genes and also observed high levels of synteny between chickpea and *Medicago*. The chickpea genome assembly contains 81,845 SSRs and 4.4 million variants (SNPs and INDELs) (Table 3).

Jain *et al.* (2013) generated draft genome sequence of chickpea using next-generation sequencing platforms that is 454/Roche GS FLX Titanium platform, bacterial artificial chromosome end sequences and a genetic map. Chickpea cultivar ICC4958, a *desi* drought-tolerant and a popular breeding parent isolated from India has been used for this purpose. They estimated total genome size based on read alignment to be 740.52 Mb. A total of 520 Mb assembly covers 70 % of the predicted 740 Mb genome length. Genome analysis predicts the presence of 27,571 genes and 210 Mb as repetitive elements.

After availability of the draft genome sequence of chickpea, Misra *et al.* (2014) developed the Chickpea Genomic Web Resource (CGWR) is a web-based application dedicated to chickpea genome visualization and comparative analysis, based on next generation sequencing and assembly of *Cicer arietinum desi* genotype ICC4958. This web based program designed for mapping, scanning and browsing the significant chickpea genomic features *viz*., (a) sequence processing tools for nucleotide BLAST (Basic Local Alignment Search Tool) search, protein search, CDS (coding DNA sequence) search and repeat motifs (SSR) search, (b) genome browser for annotation of structural and functional genes, molecular markers, transcriptomes gene regulatory element and comparative genomics, (c) Maps for chromosomal map generation for carotenoids, flavonoids, resistance genes, chickpea specific genes and transposons. The CGWR is available freely under the open source license at http://www.nipgr.res.in/ CGWR/home.php.

Recently, Parween *et al.* (2015) reported an advanced version of the ICC4958 genome assembly named as version 2.0, generated using additional sequence data and an improved genetic map. In this advanced version 2.7 fold increases in the length of pseudomolecules and substantial reduction in sequence gaps. The new version genome assembly covered more than 94 per cent of the estimated gene space and predicted the presence of 30,257 protein-coding genes. This includes 2230 and 133 genes encoding transcription factors (TF) and resistance gene homologs, respectively. They also did pairwise comparison of pseudomolecules in the *desi* (ICC4958) and the earlier reported *Kabuli* (CDC Frontier) chickpea assemblies showed an extensive local collinearity with incongruity in the placement of large sequence blocks along the linkage groups, apparently due to use of different genetic maps. More than four thousand SNPs differentiating a *desi* group and a *kabuli* group of chickpea genotypes have been identified using SNP-based mining of intraspecific polymorphism.

Table 3. Chickpea reference genome (*Cicer arietinum* ASM33114v1) sequence information

Locus	Type	Name	Reference Sequence	INSDC	Size (Mb)	GC %	Protein	rRNA	tRNA	Other RNA	Gene	Pseudogene
		master WGS	-	ANPC00000000.1	863.58	31.0	-	-	-	-	-	-
Nucleus	Chromosome	Ca1	NC-021160.1	CM001764.1	48.36	32.1	3,687	-	81	141	3,190	248
Nucleus	Chromosome	Ca2	NC-021161.1	CM001765.1	36.63	32.2	2,584	-	48	80	2,276	182
Nucleus	Chromosome	Ca3	NC-021162.1	CM001766.1	39.99	31.7	3,303	-	65	145	2,868	227
Nucleus	Chromosome	Ca4	NC-021163.1	CM001767.1	49.19	31.2	4,360	-	82	146	3,600	211
Nucleus	Chromosome	Ca5	NC-021164.1	CM001768.1	48.17	32.3	3,739	-	80	167	3,220	241
Nucleus	Chromosome	Ca6	NC-021165.1	CM001769.1	59.46	31.8	4,505	-	101	180	3,945	305
Nucleus	Chromosome	Ca7	NC-021166.1	CM001770.1	48.96	31.7	3,690	-	79	139	3,225	245
Nucleus	Chromosome	Ca8	NC-021167.1	CM001771.1	16.48	32.2	1,866	-	28	75	1,554	107
Chloroplast	Chromosome	Pltd	NC-011163.1	EU835853.1	0.12531	33.9	75	4	29	-	108	-
	Uncharacterized	-	.	-	183.52	34.2	4,179	-	72	235	4,231	611

INSDC=International Nucleotide Sequence Database Collaboration, WGS=Whole Genome Sequence, Pltd=Plastid and Ca= *Cicer arietinum* L.
(Source: http://www.ncbi.nlm.nih.gov/genome/?term=chickpea)

2.5.2 Transcriptome data of chickpea

Garg *et al.* (2011a) did *de novo* assembly of chickpea transcriptome using short-read sequence data and for the first time they reported complete transcriptome of chickpea. A non-redundant set of 53,409 transcripts, representing about 28 Mb of unique transcriptome sequence have been generated and a total of 45,636 chickpea transcripts showed significant similarity with unigenes/predicted proteins from other legumes. They also analysed GC content, functional characterization of transcripts. They also identified transcriptome/EST based SSR, a total of 4,816 SSRs in 4,180 transcripts of chickpea with frequency of one SSR per 5.80 kb of the sequence.

Although several genes/EST involved in different stressed conditions like high salinity, cold, drought (Mantri *et al.*, 2007; Molina *et al.*, 2008), have been identified, the gene discovery and development of candidate genes were limited. In this regard, Garg *et al.* (2011b) have sequenced a set of 34,760 transcripts with an average length of 1,020 bp representing about 4.8 per cent (35.5 Mb) of the total chickpea genome. They assigned putative function and gene ontology terms for at least 73.2 per cent and 71.0 per cent of chickpea transcripts, respectively. Based on the comparison they identified two set of genes includes linage-specific (legume) and chickpea specific genes. The tissue-specific gene expression has also been analysed for mature leaves, flower buds and young pods. A total of 1,851 (5.33 %) transcripts encoding for transcription factors (TFs) have been identified. From *de novo* generated transcriptome a set of 4000 SSR have been identified using Perl script MISA, which can be developed into functional markers. For the first time they developed a public database, the Chickpea Transcriptome Database (CTDB), which provides a searchable interface to the chickpea transcriptome data (http://www.nipgr.res.in/ctdb.html). This database provides the transcriptome sequence of the chickpea genotype (ICC4958) which is used for the present study.

Hiremath *et al.* (2011) provided a large transcript dataset for chickpea and differential responses to drought using next generation sequencing technologies such as Roche/454 and Illumina/Solexa. A total of 1,03,215 tentative unique sequences (TUSs) have been produced and putative functions has been determined for 49,437 (47.8 %) of the TUSs, and gene ontology assignments have been determined for 20,634 (41.7 %) of the TUSs. They did comparison of the chickpea TUSs with the *Medicago truncatula* genome assembly resulted in 42,141 aligned TUSs. They analysed TUSs for drought stress resulted in the over expression of the genes coding for Zinc finger family protein, MYB domain containing family, WRKY, auxin response factor, pentatricopeptide-repeat containing protein, bZIP and early response to dehydration (ERD)-related protein of *Arabidopsis* were identified. The TUSs has been also used to identify a diverse set of markers, including 728 SSRs, 495 SNPs, 387 conserved orthologous sequence (COS) markers, and 2088 intron-spanning region (ISR) markers

Agarwal *et al.* (2012) sequenced the transcriptome of *kabuli* chickpea genotype (ICCV2), using GS-FLX Roche 454 and Illumina technologies. The hybrid assembly generated 43,389 transcripts with an average length of 1065 bp representing 46.2 Mb of *kabuli* chickpea transcriptome. They identified a total of 5409 SSRs in these transcript sequences.

Subsequently, Pradhan *et al.* (2014) studied the global transcriptome profile of chickpea seed development at four different stages *i.e.,* embryo formation, cell expansion, food and storage reserve formation. Using next generation sequence (NGS) platforms 51,099 unigenes were sequenced. Further, homology based comparison indicated 17.5 per cent of unigenes were seed specific and the expression studies revealed that genes involved in the biosynthesis of secondary metabolites. They also mined about 12,000 SSR markers from chickpea seed transcriptome and validated few markers.

Recently, Garg *et al.* (2016) performed whole transcriptome sequencing of chickpea genotypes to understand the molecular mechanism of drought and salinity stress response. A total of 4954 and 5545 genes exclusively regulated in drought-tolerant and salinity-tolerant genotypes respectively have been identified. The transcriptome profile and components of regulatory network associated with drought and salinity stress responses in chickpea in which the key enzymes involved in metabolic pathways, such as carbohydrate metabolism, photosynthesis, lipid metabolism, generation of precursor metabolites/energy, protein modification, redox homeostasis and cell wall component biogenesis have been showed differential expression during drought and salinity stresses.

Many research works have been carried out on chickpea transcriptome studies in various plant parts, whole plant, seed development and stressed conditions *viz.*, drought and salinity. But a limited number of studies have been conducted for transcriptome analysis of chickpea challenged for *Fusarium* wilt.

Gupta *et al.* (2009) first reported the transcriptome analysis of both *F. oxysporum* f. sp. *ciceris* race 1 induced and uninduced plants of JG62 (susceptible) and WR315 (resistant) cultivars by cDNA amplified fragment length polymorphism (cDNA-AFLP) analysis of the upregulated and downregulated genes at 48 and 96 hours of post-infection. They observed that transcript-derived fragments were homologous to genes for sucrose synthase, isoflavonoid biosynthesis, drought stress response, serine threonine kinases, cystatins, arginase and so on. They concluded that the pathogen invasion somehow triggers the signal transduction mediated by sugar sensing as supported by the elevated levels of sucrose synthase, invertase, β amylase, and so on. Apart from this, the susceptible plants showed upregulation of 14.3.3 protein suggesting sugar starvation in them, whereas in resistant cultivar overexpression of many sugar metabolizing genes provides better resistance for pathogen attack.

Ashraf *et al.* (2009) for the first time to demonstrate comparative analyses of genotype dependent EST and stress-responsive transcriptome of chickpea wilt. They challenge two chickpea genotypes JG62 (susceptible) and WR315 (resistant) with FW. A total of 6272 high quality ESTs have been identified, functionally annotated, computationally analysed and classified into different functional categories. The 2013 unigenes identified were classified into 209 gene families and also identified 262 genotype specific SNPs. The transcriptome analysis revealed that ESTs encoding proteins like dirigent and harpin-induced 1 in the resistant genotype indicated their role in pathostress response. Similarly, many other stress induced proteins, *viz.*, extensin, universal stress proteins, aquaporin, annexin, cold acclimation responsive protein

BudCAR4 and metallothionien have been identified as more abundant in the resistant genotype implicating their involvement in pathostress response apart from their role in abiotic stresses.

Later, Gurjar *et al.* (2012) demonstrated the gene expression analysis of chickpea genotypes JG62 (susceptible) and Digvijaya (resistant) infected and uninfected root tissues with *Fusarium oxysporum* f. sp. *ciceri* (FOC). They analysed chickpea gene expression profile using cDNA-RAPD, cDNA-AFLP and semi-quantitative real time techniques. Differentially expression studies revealed that 134 transcripts exhibiting >200 bp size. In that 65 per cent of plant related to defense, shock, structural and DNA/protein synthesis and 18 per cent of fungal origin genes has been expressed respectively.

Jain *et al.* (2015) sequenced the whole transcriptome of chickpea genotypes susceptible (JG62), tolerant (ICCV2 and K850) and resistant (WR315) challenged for wilt. They used Illumine platform for sequencing and assembled to identify microsatellite markers, SNPs and insertions/deletions (InDels). The transcriptome analysis, a total of 303 microsatellite markers, 14,462 SNPs and 1864 InDels among the chickpea cultivars has been identified.

Recently, Verma *et al.* (2015a) updated chickpea transcriptome database (CTDB) to latest version (v2.0) which is earlier developed by Garg *et al.,* (2011b) an integrated CTDB, which provides the comprehensive web interface for visualization and easy retrieval of transcriptome data in chickpea from *desi* (ICC4958), *kabuli* (ICCV2) and wild (PI489777) chickpea genotypes. The CTDB provides user-friendly utilities *viz*., homology search, functional annotation and gene expression data from different tissues/organs in chickpea and an option to conduct comparative genomics studies with/among different legumes. Furthermore, the CTDB provides an option to develop functional molecular markers (microsatellites and SNPs) in the transcriptomes of different chickpea genotypes.

2.5.3 Genic markers in chickpea

The availability of enormous amount of chickpea genome sequence data either complete or partial has made it possible to develop the molecular markers directly from the parts of genes. These markers are referred as "Genic Molecular Markers" (GMM). The development of GMMs is a targeted approach provides ample of nucleotide diversity in genes controlling agronomic traits in plant populations. These molecular markers developed directly from the parts of genes, BAC clones, ESTs, full length cDNA clones called genic molecular markers (GMM). Further genic molecular markers classified into two types that is gene targeted marker (GTM) and functional markers (FM).

Gene targeted marker (GTM) derived from polymorphism within the gene but not involved in phenotypic trait. Functional markers (FM) derived from polymorphic sites in gene and more likely involved in phenotypic trait variation. Expressed sequence tag (EST) is a short sub-sequence of a cDNA. ESTs provide a rapid and efficient approach for gene discovery and analysis of gene expression in eukaryotes (Varshney *et al.,* 2007).

In case of chickpea genome abundant SSRs have been available with high level of interspecific polymorphism suggesting SSR markers are well suited for chickpea genome mapping and gene tagging. But for intraspecific both genomic and transcript datasets can be utilized to develop polymorphic SSR markers to develop parental mapping population in chickpea.

A study on drought and salinity stress responsive ESTs in chickpea for gene discovery and markers development has identified 3,728 SSRs of which 177 new EST-SSR markers has been developed. A total set of 77 SSR markers has been validated on 24 genotypes revealed 230 alleles with an average of 4.6 alleles per marker and average polymorphism information content (PIC) value of 0.43. Beside EST-SSR markers, 21,405 high confidence SNPs in 742 contigs has been identified (Varshney *et al.,* 2009).

Gaur *et al.* (2011) constructed microsatellite enriched library of chickpea with 387 putative microsatellite containing clones. They designed 254 STMS primers of which 181 have been developed as functional markers. Along with previously reported STMS unmapped and 181 novel STMS markers have been utilized to genotype intraspecific mapping population of cross ICCV2 (single podded) × JG62 (double podded) and mapped with LOD score of 3.5. The 138 new markers positions have been obtained on eight linkage groups spanned 630.9 cM with an average marker density of 4.57 cM.

Choudhary *et al.* (2012) identified 487 novel EST-derived functional markers which included 125 EST-SSRs, 151 intron targeted primers (ITPs), 109 expressed sequence tag polymorphisms (ESTPs), and 102 SNPs by utilizing 2,496 ESTs generated from chickpea seeds. A total of 872 gene based markers utilized for analysis of parental polymorphism between *C. arietinum* ICC4958 and *C. reticulatum* PI489777, parents of the reference chickpea mapping population. A total of 487 gene based markers and along with 385 previously published markers, of which 318 (36.5 %) has found to be polymorphic. An advanced linkage map has been constructed with eight linkage groups containing 406 genetic loci spanned 1,497.7 cM.

Subsequently, a study on transferability test for 131 EST-SSR of *M. truncatula* in chickpea has been carried. In that thirty pairs of primers have been designed to amplify over 10 chickpea genotypes. The results showed that thirteen primer pairs (43 %) generated reproducible bands in at least one chickpea genotype, in that eight bands (61.5 %) showed polymorphic in the chickpea genotypes. A total of 24 alleles have been amplified with an average of 3 alleles per primer (Jafari *et al.,* 2013). In order to assist genomic studies and marker assisted breeding approaches, Doddamani *et al.* (2014) developed a user friendly database called Chickpea Microsatellite Database (CicArMiSatDB http://cicarmisatdb.icrisat.org). This database provides detailed information on SSRs along with their features in the genome.

A limited number of studies have been carried to develop gene based molecular markers linked to *Fusarium* wilt resistance in chickpea. In this regard Jain *et al.* (2015) did the transcriptome analysis of wilt challenged chickpea genotypes *i.e.,* susceptible JG62 and tolerant ICCV2, K850 and resistant WR315. The comparative analysis of

transcriptome revealed 303 polymorphic microsatellites, of which 64 per cent could be physically mapped on the chickpea genome and also identified a total of 14,462 SNPs and 1864 InDels among the chickpea cultivars analysed. They were able to design primers for at least 251 (82.8 %) polymorphic SSRs. Further, at least seven polymorphic SSRs, at least 243 SNPs and 44 InDels has been utilized to map the *Fusarium* wilt resistance related QTLs identified in previous studies (Gowda *et al.,* 2009; Sabbavarapu *et al.,* 2013; Patil *et al.,* 2014; Varshney *et al.,* 2014a), representing important candidate functional marker for FW resistance.

2.6 Physical mapping of chickpea

Several genetic linkage maps have been developed and markers linked to different traits have been identified in chickpea. Though these markers can be used in marker-assisted selection (MAS) for improving the trait, the molecular basis of traits remains unknown. Isolation and validation of genes underlying the QTL/genes for the traits of interest is an essential step to determine gene function. Development of a genome-wide physical map or local physical map around the QTL region and then sequencing those region(s) are the next steps in this direction (Gaur *et al.,* 2012). By comparing a genetic map and corresponding physical map, the actual physical position of any gene can be determined.

A physical map is an ordered set of DNA fragments, among which the distances are expressed in physical distance units, in terms of base pairs (bp). The resolution or accuracy with which this can be done ranges from mapping loci to a particular chromosome (low resolution) to the determination of the precise nucleotide sequence (high resolution) establishing the relationship between genetic and physical mapping there by increasing the efficiency of fine mapping (Boopathi, 2013).

Rajesh *et al.* (2004) developed BIBAC (Binary) library of chickpea consisting of 23,780 clones, with an average insert size of 100 kb and covering about 3.8X genomes of chickpea.

Lichtenzveig *et al.* (2005) developed BAC (Bacterial artificial Chromosomes) library and a BIBAC library from the nuclear DNA of chickpea, *Cicer arietinum* L., cv. *Hadas,* partially digested with Hind III and BamH I, respectively. The BAC library has 14,976 clones, with an average insert size of 121 kb, and the BIBAC library consists of 23,040 clones, with an average insert size of 145 kb. The combined libraries collectively cover 7.0X genomes of chickpea.

In terms of physical mapping, Zhang *et al.* (2010) developed a BAC/BIBAC-based physical map of chickpea. It consists of 1945 contigs and each contig contains an average of 28.3 clones and has an average physical length of 559 kb. In total, the contigs span about 1088 Mb. Using this map, they were able to identify BAC/BIBAC contigs containing or close to QTL, governing resistance to *Didymella rabiei* and QTL responsible for days to first flower.

Zatloukalova *et al.* (2011) integrated genetic and chromosome-based physical maps of chickpea by assigning linkage groups (LGs) in chickpea to different chromosomes using flow cytometry and PCR-based primers that amplify STMS markers. Using this approach, they were able to assign LGs: LG8 to chromosome H, LG5 to chromosome A, LG4 to chromosome E and LG3 to chromosome B. The two chromosomes (C & D) could not be sorted out; therefore, they were jointly assigned to LG6 and LG7. Similarly, LG1 and LG2 were assigned to chromosomes F and G.

Varshney *et al.* (2014b) integrated physical, genetic and genome map of chickpea. They developed physical map of chickpea using reference chickpea genotype (ICC-4958) from BAC libraries targeting 71,094 clones with ~12X genome coverage. Further High information content fingerprinting (HICF) of these clones produced an assembly of 1,174 contigs covering an average of 574 Mb genome with 0.49 Mb per contig and 3,256 singletons represent 407 Mb genome. The physical map was linked with two genetic maps with the help of 245 BAC-end sequence (BES)-derived SSR markers. This study allowed locating some of the BACs in the vicinity of some important quantitative trait loci (QTLs) for drought tolerance and resistance to *Fusarium* wilt and *Ascochyta* blight.

2.7 *In silico* physical mapping of chickpea

In recent years, advent of next generation sequencing technologies has made possible to generated massive amounts of genomic sequence informations. The utilization of this information in applied crop improvement programs has been well augmented by the availability of sophisticated bioinformatics tools. The availability of whole genome sequences information triggers the development of new gene based molecular markers and tools, such as molecular markers for precise genetic mapping and comprehensive molecular analysis of genome structure and function (Ramu *et al.,* 2010). The *in silico* physical mapping is a targeted approach which provides a great opportunities to understand the genetic variations in nucleotide sequences, differences in physical map, and genetic recombination in linkage maps, as well as benefits for markers enrichment in a specific genome region for fine mapping or QTL mapping (Hong *et al.,* 2012).

In rice, *in silico* comparison of genetic map marker sequences to a whole genome sequence has done using FLAST, a rapid sequence comparison program based on DDS by aligning marker sequence against BAC end sequencing database (Yuan *et al.,* 2000). Liu *et al.,* (2007) identified resistance gene content of the interval in *indica* rice variety Kasalath carries *Pi36,* a gene that determines resistance to Chinese isolates of rice blast by *in silico* approach with the genomic sequence of the reference *japonica* variety Nipponbare for the identification of candidate gene (s) for *Pi36* and results showed that candidate gene(s) for *Pi36* is located to a 17 kb interval on chromosome 8.

Ramu *et al.* (2010) aligned all publicly available sorghum SSR markers against sorghum genome sequence for development of sequence based physical map of sorghum. They correlated the physical map with already existing linkage maps. They also suggested that the novel markers can be first aligned on a sequence-based physical map and those located near the QTLs can be utilized for mapping, thereby reducing the number of markers to be tested in order to identify polymorphic flanking markers for the

QTL. Similarly, Hong *et al.* (2012) through *in silico* approaches aligned 142 newly developed and validated EST bases SSR loci as well as 223 SSR loci on different linkage map in grape genome and further, the physical location and orders of these SSR loci on the chromosome has been determined.

Yasala *et al.* (2012) identified gall midge resistance genes (*Gm1* through *Gm11*) involved in rice-gall midge interactions by *in silico* analysis for gene content of 4.02 Mb genomic regions containing the gall midge R genes and the results revealed that five genes with one or more copy number were common among these regions such as the genes encoding NBS-LRR class proteins, no apical meristem protein, F-box family protein, pentatricopeptide repeat containing protein and SET domain containing protein.

Recently, Madrid *et al.* (2014) integrated the genetic map with sequence based physical map in chickpea for identification of QTL related to *Ascochyta* blight (AB) resistance located in LG2. They physically mapped 15 markers reported earlier in various genetic maps linked to AB resistance thorough *in silico* approaches. They constructed the physical map of chromosome 2 (Ca2) and identified the QTL position 32–33 Mb, comprises of 42 candidate genes including *ethylene insensitive 3-like gene* (Ein3), *Avr9*/*Cf9* and *Argonaute 4*, directly involved in disease resistance mechanisms.

2.8 Fine mapping

The use of large mapping population and great number of marker helps in identifying more tightly linked marker to trait is termed as fine mapping (high-resolution mapping). Therefore, the fine mapping of QTLs may be used to develop reliable markers (at least <5 cM but ideally <1 cM away from the gene) for marker assisted selection and also to discriminate between a single gene or several linked genes (Collard *et al.*, 2005). To carry out fine mapping three perquisites were necessary *i.e.*, a mapping population segregating for trait of interest, markers located in the vicinity of the locus involved in the trait and a robust phenotypic method to differentiate the trait in the mapping population.

A good number of studies have been carried in rice for fine mapping of agronomically important traits. Li *et al.* (2004) fine mapped a grain-weight QTL, *gw3*.1 using a set of near isogenic lines (NILs) on rice chromosome 3 by narrow down the location of the gene underlying this QTL to a 93.8 kb region. He *et al.* (2006) fine mapped the rice bacterial blight resistance gene, *Xa2*, confers resistance to T7147 of the bacterial blight pathogen *Xanthomonas oryzae* pv. *Oryzae* located near to SSR markers HZR950-5 and HZR970-4 which cover approximately 190-kb region on the long arm of chromosome 4. Xu *et al.* (2006) identified the gene *Sub1A-1* responsible for submergence tolerance in FR13A varieties form the *Sub1* locus, which is introgressed into a widely grown Asian rice cultivar through MAS. Thomson *et al.* (2010) located the position of QTL *Saltol* between RM23 and RM140 (10.7–12.2 Mb) on chromosome 1 using 140 IR29/Pokkali RILs, and identified additional QTLs associated with salinity tolerance in rice.

A major gray leaf spot resistance QTL, *qRgls2* fine mapped and narrow down from 110 Mb to 1 Mb, flanked by closest markers G346 and DD11 on maize

chromosome 4 (Xu *et al.*, 2014). Similarly, Zuo *et al.* (2015) fine mapped a major resistance QTL *qHSR1* for head smut in maize on chromosome 2 and identified the gene *ZmWAK* within *qHSR1* conferring quantitative resistance to maize head smut.

Maroof *et al.* (2010) fine mapped *Rvs 4* conferring resistance for all kind of soyabean mosaic virus strains on chromosome 2 limiting to physical distance of less than 100 Kb. Further, Asian soybean rust resistance gene *Rpp2* has been fine mapped into a 188.1 kb interval by closest SSR and SNP markers on chromosome 16 (Yu *et al.,* 2015).

A limited number of fine mapping studies were done in the chickpea. For the first time in chickpea fine mapping for *sfl* locus for double podding gene was carried out using novel STMS and SNP markers. The *sfl* locus was mapped between TR44 and the SNP scaffold1646p97220 flanking genetic distance 5.1 cM on LG6. The combined data of linkage analysis, markers physical positions and recombinant events fine mapped the *sfl* locus to 92.6 Kb on chromosome 6 consists of seven candidate genes. Among seven genes the regulator of axillary meristem-predicted gene could be a candidate gene for the simple/double podding gene (Ali *et al.,* 2016).

Kujur *et al.* (2015) fine mapped the QTL for seed weight *qSW5.1* through ultra-high density linkage map developed using SNPs. The QTL *qSW5.1* was dissected to identify the one regulatory SNP-carrying embryo defective gene responsible for seed weight.

Kale *et al.* (2015) followed two approaches *i.e.,* QTL and gene enrichment analysis for identified candidate genes in the "*QTL-hotspot*" region for drought tolerance present on the Ca4 pseudomolecule in chickpea. QTL analysis was done by combining a high-density recombinant bin map developed using 53,223 SNPs identified in the RIL population of ICC 4958 (drought tolerant) and ICC 1882 (drought sensitive) cross along with phenotypic data for drought. They split the "*QTL-hotspot*" region into two subregions namely "*QTL-hotspot_a*" and "*QTL-hotspot_b*" consists of 15 and 11 genes respectively. The second approach, gene enrichment analysis using significant marker trait associations based on SNPs from the Ca4 pseudomolecule with the phenotypic data, and identified candidate genes from the refined "*QTL-hotspot*" region. Totally 23 genes were enriched in this region. Twelve genes were found common in both approaches and four of them were validated for drought tolerance in chickpea.

III MATERIAL AND METHODS

The present investigation was undertaken to link the genetic map with physical map (*in silico*) for identification of QTL regions associated with *Fusarium* wilt (FW) resistance in chickpea genome sequence with the help of bioinformatics tools. Further, identification of genes and microsatellite repeats in FW resistance region in chickpea genome was also carried out. For those identified microsatellite repeats, primers pairs were designed. From these designed primers 168 primers were synthesised and tested for amplification and polymorphism. In that, polymorphic markers were utilized for segregation analysis of two RIL mapping populations for QTL mapping. DNA isolation and molecular marker analysis was carried out in the laboratory and phenotyping of RIL mapping populations was conducted in the green house during *Rabi* season 2016 in the Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru, Karnataka. The experiments conducted for the present study materials used are given below,

3.1 Physical mapping of molecular markers/QTLs linked to the *Fusarium* wilt resistance and identification of genes in the genomic region by utilizing chickpea genome sequence information.

3.2 Identification of microsatellites in the selected genes and development of SSR markers.

3.3 Genetic mapping of functional markers developed in this study and redefining QTLs linked to *Fusarium* wilt resistance.

3.1 Physical mapping of molecular markers/QTLs linked to the *Fusarium* wilt resistance and identification of genes in the genomic region by utilizing chickpea genome sequence information.

3.1.1 Selection of FW resistance linked markers for sequence based homology search against chickpea genome sequence

Based on available information, forty four FW resistance linked markers were selected for sequence based homology search against chickpea genome sequence (Table 4) for identification of FW resistance genomic regions. Along with these linked markers, two markers (H4E09 and TR20) on either side of a linked marker H_4G_{11} (Radhika *et al.,* 2007) were also selected for *in silico* physical mapping. Out of 46 markers selected, five were RAPD markers, two were SCAR markers, two were ISSR markers, one was AFLP marker and the remaining were SSR and STMS markers. The primer sequences (Table 4) of these linked markers were used as query sequence for sequence based homology search against chickpea genome sequence as a reference sequences available in public database.

3.1.2 Chickpea genome sequence

The annotated ≈738 Mb draft chickpea genome sequence of *kabuli* variety CDC Frontier (Varshney *et al.,* 2013) available in NCBI (national center for biotechnology information) was used as reference genome sequence (ASM33114v1 reference, Annotation release 101) for sequence based homology search. The chickpea

transcriptome database (CTDB) provides the annotated and expression profiling data along with putative function for each transcriptome of the ICC4958 (*desi*), ICCV2 (*kabuli*) and PI489777 (wild type) genotypes (Garg *et al.*, 2011b).

3.1.3 Anchoring sequence-based genetic markers to the chickpea sequence map

The selected FW resistance linked markers primer sequences were used as query sequences for BLAST (basic local alignment search tool) search (Program BLASTN 2.4.0) analysis against chickpea CDC Frontier genome assembly as a reference sequence in NCBI. The primer sequences of markers selected were collected in FASTA format (Table 4) and BLAST searched against chickpea genome sequence considering default parameters *viz*., the reference genome (ASM33114v1 reference, Annotation release 101), highly similar sequence (megablast) and algorithm parameters like maximum target sequences 100, expected threshold 10, word size 28, match/mismatch score 1-2 and species specific repeats for *Cicer arietinum.*

3.1.4. Analysis of BLAST search output

Output of BLAST search consists of three part *viz*., (a) graphical summary with color key for alignment score, (b) description of sequences producing significant alignments with maximum score, total score, query coverage, E-value (Expect value), identity and accession number, (c) corresponding alignment with range and also graphics. The BLAST hits of marker sequences (forward and reverse sequences) of each primer pairs were grouped together using following parameters (Ramu *et al.*, 2010).

(i) Sequence producing significant alignment with lowest E-value, maximum identity with no gaps.

(ii) Both primer pairs should hit on the same chromosome/scaffold but opposite to one another (positive or negative strand) on the chickpea genome assembly.

(iii) Forward and reverse primer sequence hit, in terms of start and end nucleotide number should be between 50 to 500 bp on genome assemble as expected amplicon size.

The marker sequences fulfilling these parameters were mapped on the chromosomes for physical mapping of QTLs.

3.2 Identification of microsatellites in the selected genes and development of SSR markers

3.2.1 Identification of genes present in the FW resistance region in chickpea genome

The QTL region flanked by linked markers in the chickpea genome was identified using NCBI map viewer (http://www.ncbi.nlm.nih.gov/projects/sviewer) displaying details of annotation on a region of the sequences. This map viewer represent entire chromosome along with genes in featured green colour and tRNA genes featured blue colour. With the help of marking tools the genomic region flanked by linked markers were mapped. The mapped region was scanned for number of genes, represented by genbank id. along with putative functions.

Table 4: Primer sequences of markers linked to *Fusarium* wilt resistance

Sl. No.	Markers	Forward primer sequences 5'-3'	Reverse primer sequences 5'-3'
1	$CS27_{700}$ (SCAR)	AGCTGGTCGCGGGTCAGAGGAAGA	AGTGGTCGCGATGGGGCCATGGTG
2	$UBC170_{550}$ (SCAR)	ATCTCTCCTGTGTGTGTG	ATCTCTCCTGCATCACAAG
3	H4G11	ATCTAAGTGAGCGGCTACTAAATCA	GTAGTCATGCAGCCTATAAAAACAA
4	TA186	ACAAAATTCTAAAAGTTCCTTCTACCA	GTTGTTAGTCGAATAATTGAGAAAAAGA
5	NCPGR58	TGAAGATCTCCAACGGTAAC	TTTCTTTTGATGTGTTCTTGG
6	NCPGR74	TCCGTCCACACATTTCTACT	CTTTTAGTTGGTCGAAGCC
7	SSR14	ACCTCCGTCCACACATTTCTAC	GTCGAAGCCATTGTTTTGTTG
8	TR20	ACCTGCTTGTTTAGCACAAT	CCGCATAGCAATTTATCTTC
9	H4E09	TGCTATTTGTACTAGGACTTAAGGAAA	TGTTTAAAGTACCCATTAAAAACGTAA
10	TA59	ATCTAAAGAGAAATCAAAATTGTCGAA	GCAAATGTGAAGCATGTATAGATAAA
11	TA110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA
12	TR59	AAAAGGAACCTCAAGTGACA	GAAAATGAGGGAGTGAGATG
13	TA96	TGTTTTGGAGAAGAGTGATTC	TGTGCATGCAAATTCTTACT
14	TA27	GATAAAATCATTATTGGGTGTCCTTT	TTCAAATAATCTTTCATCAGTCAAATG
15	H3A12	TCAATCTTTTGTTGTTACTATGAATCTG	AACCTTAGACTGTGTTCGCTGA
16	CaM1402	CACCCAAATCCCCAAA	TGCCTTTTGTATTTGAAAAATGTG
17	CaM1101	CGGGTAGAATGTAACACCCAG	TTAAATGGACGTGGGTAACG
18	CaM1125	CACCCATTTTGATGGTCTGA	CAACAATTCCACTGCCTCTG
19	TS82	TCAAGATTGATATTGATTAGATAAAAGC	CTTTATTTACCACTTGCACAACACTAA
20	TS47	GTTAATATTTTTCCGCTTCGT	TCAAATTGTGTTAAAAATCAAAGTGTT
21	TR19	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTTCTCCA
22	GA16	CACCTCGTACCATGGTTTCTG	TAAATTTCATCCTCTCCGGC
23	TAA60	TCATGCTTGTTGGTTAGCTAGAAC	GACATAATCGAGTTAAAGAAAA
24	TA194	TTTTTGGCTTATTAGACTGACTT	TTGCCATAAAATACAAAATCC
25	TA22	TCGTGTTTACTGAATGTGGA	TCTCCAACCCTTTAGATTGA
26	TS72	CAAACAATCACTAAAAGTATTTGCTCT	AAAAATTGATGGACAAGTGTTATTATG
27	TA37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA

Sl. No.	Markers	Forward primer sequences 5'-3'	Reverse primer sequences 5'-3'
28	TA200	TTTCTCCTCTACTATTATGATCACCAG	TTGAGAGGGTTAGAACTCATTATGTTT
29	TR2	GGCTTAGAGTTCAAAGAGAGAA	AACCAAGATTGGAAGTTGTG
30	TR44	TTAATATTCAAAAACTCTCTTGTGCAAT	TTTACAACAGCGCTTGTATTTAGTAAG
31	H1B06	GACTCACTCTCCAAATGGAACC	AAGCCCATGAAAACCATATATTC
32	GSSR18	CCCTCAAGCAACCCATAAAT	TTGACACCATATGTGTTCTCCC
33	TC14801	CAGATTCCAAACGTGCAGTG	ATTGCAATGTGAACCCACAA
34	GSSR11	CTGTTACGTGCAATGGATGC	TCGGTATGACACAAAAATGTGA
35	EST SSR3	ATGAAATTGCTCCGTTGAGG	GGGATTTGATTCGTGGAAGA
36	TR24	AACAACTTCCTCTTATTTTCCA	CAGTAAAAATCAGCCCAAAC
37	EST SSR21	GGTTTTGAGAGAGAGTGCGG	TCTTCCGCAAAACAAAAACC
38	EST SSR 65	CCTCAAGTGCAACAAAAACAA	TGCAAACATTTTCACACCAGA
39	A07C (RAPD)	GAAACGGGTGC	
40	CS27 (RAPD)	AGTGGTCGCG	
41	$OPJ20_{600}$ (RAPD)	AAGCGGCCTC	
42	OPK4 (RAPD)	CCGCCCAAAC	
43	OPK9 (RAPD)	CCCTACCGAC	
44	$UBC825_{1200}$ (ISSR)	ACACACACACACACACT	
45	$UBC855_{500}$ (ISSR)	ACACACACACACACACYT	
46	ECAMCTA07 (AFLP)	-	

DNA sequence of A07C polymorphic band 5'-3' (Soregaon *et al.*, 2007)

GAAACGGGTGCAGTGTGTAAGTTTGGAAACTGTTTTGGAGTTTACATCTCTCATATAAATAGGGAATCCAAGAAGCGTGTTACTAA
TGCATGTTTTCCTTTTTTTGCCAAATATAATTACAGCAAGGGCACCAACTATGGACAACCAGGAGGTTGATAACACATCCCAGACT
ATCAAACGGAGATTACCTAGGGAAATAAAGCTAAAACTTGCTAAAGTTGCTAGATTAGCGGTACTGGCTAACTCTCCATTCCTGAT
ATTGTGCTTGTAGAAATACATACAATTTCATATATTTTATTTTGATGTATATAAACATTGTCATATTTACTTGTGTTTTTTTGTTGCA
GCAGGCAAGCCAAGGGAAAGTATCAACGGAGTTGATTAATCGTCTTATGAGTAGTCTTGGGCACCCGTTTC

3.2.2 Identification and development of microsatellite markers for functional genes in the FW resistance genomic region in chickpea genome

The SSR motifs present in the genes on FW resistance regions were identified using online software called Webstat (http://purl.oclc.org/NET/websat/) developed by Martins *et al.* (2009). This software consists of two parts *i.e.*, TROLL (Tandem Repeat Occurrence Locator), it's an SSR finder program and primer3 software as a primer designing program. The sequence of each gene was downloaded in FASTA format as input for this software. The TROLL program identifies the all types of microsatellite repeats *viz*., mono, di, tri, tetra, penta and hexanucleotide repeats. The identified SSR motifs were highlight with yellow colour. Primer designing will be done by clicking on highlighted yellow coloured SSR. The designed primer sequences were highlighted in green colour where, SSR motifs in blue colour (Fig. 1). If, consensus sequences were not available for primer designing, this software shows failure of primer designing as an error. The primers designed were exported as output data by clicking on export data on the software. This output data was in the CVS format containing SSR motifs, forward primer sequence along with primer length and Tm˚C; reverse primer sequence along with primer length and Tm ˚C.

3.2.3 Primer synthesis

In the present study 168 primer pairs for genic SSRs were synthesised. The primers synthesised based on repetition of SSR motifs should be more than six for all types of repeat units (di, tri, tetra, penta and hexanucleotide repeats) excluding mononucleotide repeats (Choudary *et al.*, 2012).

3.3 Genetic mapping of functional markers developed in this study and redefining QTLs linked to *Fusarium* wilt resistance

3.3.1 Plant materials

Two set of previously developed F_{12} recombinant inbred lines (RILs) segregating for FW resistance and were used in the present investigation. First set of RILs were (referred as population JW) derived from the cross JG62 (susceptible) × WR315 (resistant) consist of 125 lines. Similarly second set 141 F_{12} RILs (referred as population KW) derived from cross K850 (late wilter) × WR315 (resistant) were also used. These mapping populations were used to map the genic SSR markers derived from FW resistance region in chickpea genome by *in silico* physical mapping approach.

3.3.2. Genotyping of RILs

3.3.2.1 DNA extraction

The genomic DNA was extracted from vegetative buds and young leaves of 125 RILs of population JW and 141 RILs of KW and their respective parental genotypes by following CTAB extraction method (mini preparation) with little modifications (Doyle and Doyle, 1987). The details are given below,

1. Approximately 0.5-1.0 g fresh tender growing bud of plants were harvested and crushed in 400 µl of extraction buffer (2 % w/v CTAB, 1.4 M NaCl, 0.1 M Tris HCl,

pH 8.0, 0.2 % β-Mercapto ethanol, 2 % PVP) pre-warmed to 65 ˚C using 1.5 ml centrifuge tube and micro pestle.

2. The crushed samples were centrifuged at 10000 rpm for 5 min at 4 ˚C.
3. The supernatant was transferred to fresh tube and equal volume of chloroform: isoamyl alcohol mixture (24:1 v/v) was added and inverted gently and centrifuged at 10000 rpm for 10 minutes at 4 ˚C.
4. After centrifuge over the upper aqueous layer was carefully transferred to fresh tube and equal volume (400 µl) of pre-chilled isopropanol was added and mixed gently.
5. Incubate for 2 hours or overnight at -20 ˚C to precipitate DNA.
6. The above solution was centrifuged at 10000 rpm for 10 minutes to pellet the DNA.
7. The supernatant was discarded and the pellet was washed with 70 per cent ethanol and then air dried for 15-20 minutes.
8. The DNA pellet was resuspended in 100 µl of TE buffer (10 mM Tris-Hcl, 1 mM EDTA maintained at pH 8) in sterile centrifuge tube and kept for 1 hr at room temperature.
9. After complete dissolving of DNA in TE buffer, the samples were stored at -20 ˚C

3.3.2.2 DNA quality and quantity estimation

The quality and concentration of DNA samples was assessed by running the genomic DNA on 0.8 per cent agarose gel in 1 X TBE (For 1000 ml 10X TBE-108 g of tris base, 55.2 g of boric acid and 40 ml of 0.5 M EDTA) and stained with 5 µl ethidium bromide (10 mg/ml stock) per 100 ml and checked for shearing of DNA, contamination of RNA and protein. The appropriate quantification of DNA was carried out by using uncut λ DNA as standard. The DNA stocks were diluted according to required working concentration of 25-50 ng/µl.

3.3.2.3 Polymerase Chain Reaction (PCR)

Newly developed 168 genic SSR markers were tested for amplification and polymorphism between parents of two RIL populations. The polymorphism between JG62 (susceptible) and K850 (resistant) was also tested. 15 µl PCR reaction mixture consists of 10.1 µl of PCR water, 2 µl taq buffer (10 X), 0.5 µl dNTPs (2 mM), 0.5 µl of forward and reverse primer each (10 pmole) from Eurofins genomic India pvt Ltd, 0.4 µl of 1 U/µl 3B DNA polymerase (3B BlackBio Biotech India Ltd.) and ~25-50 ng (1 µl) of plant genomic DNA as template. Polymerase chain reaction (PCR) was carried using Eppendorf mastercycler nexus gradient.

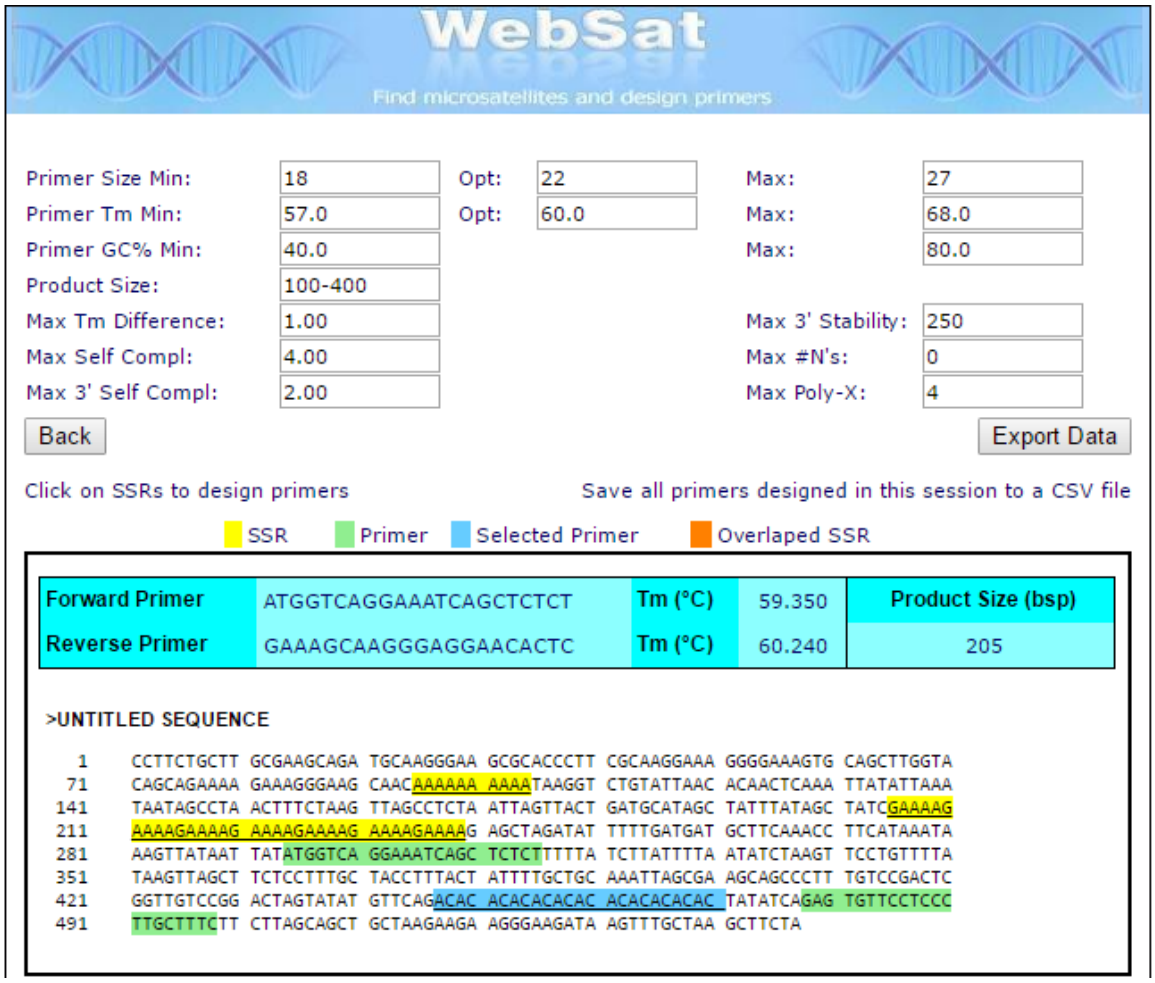


Fig. 1: Identification and primer designing for SSR motifs using online Webstat software. Colour code: Yellow colour highlight SSR identified; Green colour highlight designed primers with SSR in blue

3.3.2.4 PCR conditions

PCR conditions for amplification of novel genic SSR markers were as follows,

Sl. No.	Steps	Temperature (˚C)	Duration	Cycles
1	Initial denaturation	95	5 min	1
2	Denaturation	94	20 sec	40
3	Annealing	50-55*	45 sec	
4	Extension	72	1 min	
5	Final extension	72	8 min	1

Final hold at 4 ˚C

* Annealing temperature was determined to each primer using gradient PCR.

3.3.2.5 Electrophoresis

3.3.2.5.1 Agarose gel electrophoresis

The amplified product from each tube along with 3 µl of 6 X gel loading dye were separated on 3-4 per cent agarose in 1 X TBE buffer (pH 8) as electrolyte. 100 bp step-up ladder was used as molecular weight markers for PCR amplified SSR products. The gels were photographed using gel documentation system Alpha innotech, FluorChem® FC2 Imaging System. A few markers were resolved in PAGE (Polyacrylamide gel electrophoresis). PCR products were confirmed on 3 per cent gel before loading them to PAGE.

3.3.2.5.2. Polyacrylamide gel electrophoresis

After PCR amplification, 2.5 µl of PCR product and 2.5 µl 6 X gel loading dye were loaded to the 8 per cent non-denaturing PAGE. 80 ml gel was sufficient for BioBee® PAGE unit. The composition for 100 ml of 8 per cent non-denaturing gel is as follows, 27 ml of 29:1 w/w of acrylamide: bisacrylamide, 10 ml of TBE (10 X), 750 µl of 10 per cent Ammonium per sulphate (APS) prepared freshly, 62 ml of distilled water and at the last 100 µl of TEMED was added. The gel mixture was poured to end sealed glass plates and allowed for 10-15 minutes for solidification. After that 5 µl of samples + gel loading dye were loaded on PAGE unit. Electrophoresis was run at 200 V for three hours or until desired resolution reached. Amplified products were visualised by using silver staining protocol.

A. Protocol for gel preparation and electrophoresis

1. Glass plates were initially washed with distilled water. Then plates were washed with absolute alcohol or spirit. Leave it for 10-15 minutes for drying.
2. All accessories were washed with distil water and spirit.

3. **Solution A:** 500 µl of acetic acid + 9.5 ml of absolute alcohol for 10 ml (required for preparation of bindiscilin and repulscilin).
4. Bindiscilin solution (10 µl of 3-trimethoxy propyl in two ml of solution A) was applied to the mother plate.
5. Repulscilin solution (250 µl of Dimethyl dichloro silane + 750 µl of solution A) was applied to the notch plate.
6. Plates were set and comb was inserted in the reverse orientation.
7. Plates were placed on the table ensuring the comb portion to be at the elevated side.
8. 80 ml of acrylamide solution with 750 µl of 10 per cent APS solution and 100 µl of TEMED mixture was injected between the glass plates and allowed for solidification.
9. After solidification the comb and bottom seal of the plates were removed and placed on to the vertical electrophoresis unit. The upper tank and the bottom tank were filled with the 1 X TBE for electric conductivity. The wells were washed with the tank buffer using 1 ml pipette.
10. Cables and temperature sensors were fixed to the electrophoretic unit and power pack. The unit was run for 30-45 minutes at 80 V without sample to set the buffer temperature.
11. 5 µl of samples (2.5 µl of PCR product with 2.5 µl of gel SSR gel loading dye) were loaded to the each well. 200 V was applied for 2-3 hours or until desired resolution takes place.

B. Silver staining for visualization of SSR alleles

1. After electrophoresis, plates were separated carefully for staining.
2. Glass plate with gel was placed in the fixer/stop solution in shaking conditions for 15-20 minutes (200 ml of glacial acetic acid + 1800 ml of distilled water).
3. Later glass plate was washed twice with distilled water for 2 minutes each in shaking condition.
4. Glass plate was placed in staining solution (2 g of silver nitrate + 3 ml of formaldehyde and make up the volume to 2000 ml with distilled water) for 15-20 minutes in shaking conditions.
5. Plate was quickly washed with distilled water for 30 seconds to 1 minute.
6. Remove the water and add developing solution (45 g of sodium carbonate anhydrous + 300 µl sodium thiosulphate from 10 mg/ml stock, 3 ml of 37 per cent formaldehyde (at the time of staining) and finally make up the volume to 2000 ml with distilled water) to the plate and manually shaken till the band appears.
7. After removing developer, fixer/stop solution was added to terminate the reaction.
8. Glass plate was rinsed in distilled water for 5 minutes and dried and later observations were recorded.

3.3.3 Genotypic evaluation of RILs using polymorphic genic SSR markers

For mapping of genic SSR markers developed from FW resistance regions, all polymorphic markers (both on agarose gel electrophoresis and PAGE) were employed for genotyping all the RILs of two mapping populations (population JW and population KW). The RILs were scored for presence or absence of parental allele in homozygous or heterozygous state. Alleles similar to female parent (JG62 or K850) were scored as 2, allele like male parent (WR315) was scored as 0, for heterozygous alleles were scored as 1 and -1 for absent of allele.

3.3.4 Phenotyping of RILs for wilt reaction

The RILs of population JW were phenotyped for wilt resistance in wilt sick pot in the greenhouse in the present study. The available wilt reaction data of both the sets of RILs (JW and KW) in wilt sick field screening at ICRISAT over two years (*rabi* 2007 and *rabi* 2008) was used to mapping QTLs for FW resistance in this study (Shinde *et al.*, 2010; Soregaon, 2011; Jingade and Ravikumar, 2015).

3.3.4.1 Wilt sick pot method

The wilt reaction test was conducted for RILs of JW using wilt sick pots developed earlier in our lab in the Department of Plant Biotechnology, UAS, GKVK, Bengaluru. The wilt sick pots were developed by using, a pure culture of FOC 1A obtained from ICRISAT, Hyderabad. Further, fungal pathogen was inoculated with 6 mm disc of actively growing culture FOC 1A into autoclaved corn meal-sand (CMS) mixture in 9:1 ratio (450 g sand + 50g corn meal + 100ml distilled water in 1000 ml conical flask) for multiplication of pathogen. Infested CMS was thoroughly mixed with sterile soil sample in the ratio of 1:12 w/w to the pots *i.e.,* 250 g per pot (Brindha and Ravikumar, 2005; Nene and Haware, 1980). One pot per RILs was used. Six seeds from each RILs were sown in each pot at a depth of 2-3 cm along with susceptible check (JG62) at the centre in same pot. Typically, the susceptible check JG62 showed 100 per cent wilting symptoms and plant death within 25-30 days after sowing and was confirmed by discolouration of vascular tissue by uprooting and pathogen reisolation.

The evaluation of RILs was done by observing wilting symptoms (initial wilting *i.e.,* dropping of leaf tips) and death from days after sowing (DAS). The wilt reaction was measured in terms of number of wilted plants on 30^{th} and 60^{th} DAS to the total number of plants sown in each pot and expressed in terms of per cent wilt incidence. The per cent wilt incidence was calculated for 30 and 60 DAS (Haware and Nene, 1982; Barman *et al.,* 2014) separately.

3.3.5 Statistical analysis

3.3.5.1 Chi-square test for segregation of genic SSR markers

A statistical test commonly used for assessing goodness of fit between observed data with those of expected data. Here, chi-square test was performed on the genotypic data to test the goodness of fit for expected 1:1 monogenic segregation ratio in the RILs.

3.3.5.2 Single marker analysis (SMA)

Single markers analysis was performed to test the linkage of new SSR markers and wilt resistance in both the set of RILs separately. The phenotypic data on FW resistance obtained from wilt sick pot screening and wilt sick field screening along with the genotypic data of 23 and 24 polymorphic genic SSR markers for 125 and 141 RILs of JW and KW respectively were utilized for analysis. The SMA was performed using ICIM QTL IciMapping version 4.00 software (http://www.isbreeding.net) with a minimum threshold log likelihood ratio (LOD) score of 2.5.

3.3.5.3 Linkage map construction and mapping of genic SSR markers

The genotypic data of RILs of each population was utilized for construction of linkage map separately for each population using ICIM QTL IciMapping software (http://www.isbreeding.net). The genotypic data of twenty three polymorphic markers on 125 RILs of JW and twenty four polymorphic markers for 141 RILs of KW were subjected for linkage map construction. The minimum LOD of 2.5 and maximum recombination fraction of 0.25 were used for determination of linkage groups. After adding marker locus Ripple command was used to identify the marker order. The recombination frequencies obtained after linkage analysis were converted to genetic distance in centimorgans (cM) units using kosambi mapping function (Kosambi, 1943). The marker order and linkage distance obtained in linkage analysis was further used for QTL analysis to map the QTLs.

3.3.5.4 QTL mapping

The genotypic data combined with phenotypic data obtained from wilt sick pot and wilt sick field on RILs were analysed for identification of the QTLs using ICIM QTL Ici Mapping software version 4.00. The QTL analysis was performed by composite interval mapping (Jansen and Stam, 1994; Zeng, 1994) for each cross separately. A minimum LOD score of 2.5 was used for declaring the presence of a putative QTL.

3.3.5.5 QTL analysis

The coefficient of variance (R^2) explained by QTL was used as a measure of the magnitude of association and it is estimated as square of the partial correlation coefficient. Estimation of the additive genetic effect of each detected QTL, the total LOD score, the total proportion of phenotypic variance explained by all the detected QTLs were obtained by fitting a multiple regression model that simultaneously included all the detected QTLs for the trait.

The percentage of phenotypic variance (R^2) explained by QTL was estimated. This is based on the partial correlation of the putative QTL with observed variable, adjusted for cofactors (Kendall and Stuart, 1961). In the simultaneous fit, the cofactors are ignored and only the putative QTLs initially detected and their estimated position were used in multiple regressions to obtain the final estimates of the additive genetic effects and the percentage of phenotypic variation for the particular trait that could be explained by the QTLs. The additive effect was calculated as half the differences between genotypic values of two homozygotes (Falconer, 1989).

3.3.6 Physical mapping of QTLs identified

The redefined QTLs for FW resistance identified in the present study were *in silico* mapped on to the chickpea chromosomes based on the physical position of key markers flanking QTLs in the genetic map. The genetic and physical maps were compared to fine tune the FW resistance QTL locus on the chickpea genome. The physical distance of the QTLs identified were compared with the physical distance covered by the FW resistance markers in the beginning of the study to redefine the QTLs. Further, the putative candidate genes involved in the wilt resistance regions were identified in the redefined QTL regions.

IV RESULTS AND DISCUSSION

The results and discussion of experiments conducted in this study are presented in the following headings,

1. *In silico* physical mapping of markers linked to *Fusarium* wilt resistance on the chickpea chromosomes
2. *In silico* identification of genes, gene based microsatellites in the QTL region for *Fusarium* wilt resistance and development of genic SSR markers
3. Primer synthesis, identification of polymorphic markers and genotyping RILs of two mapping populations
4. Construction of linkage map and identification of QTLs and putative candidate genes for FW resistance

4.1 *In silico* physical mapping of markers linked to *Fusarium* wilt resistance on chickpea chromosomes

Physical map is defined as actual distance between genetic markers in term of base pairs. Physical mapping increases the efficiency of the fine mapping by establishing relationship between genetic and physical distances. However, there is no direct relationship between genetic distance in centimorgon (cM) and physical distance in base pairs (bp). In case of *Arabidopsis*, kilo base pair to centimorgan ratio varied from 30 to 550 kb per cM on chromosome 4 (Schmidt *et al.*, 1995), whereas in rice 1 cM on average equals to 258.5 kb (The Rice Genome Sequencing Project, 2005). In wheat, the variation is even more extreme, with 1 cM equivalent to 118 to 22,000 kb (Gill *et al.*, 1996a and b). The nonlinear relationship between genetic and physical map distances can hinder the ability to identify closely linked markers to the trait of interest. Therefore, genetically close markers may actually be far apart in terms of base pairs (or vice versa) due to differences in the frequency of recombination along the length of a chromosome. The identification of molecular markers closely linked to desirable trait through physical mapping facilitates, marker assisted selection (MAS), positional cloning and mapping of QTLs for disease resistance and other traits of interest in many crops (Paterson, 1996; Winter and Kahl, 1995).

The construction of complete physical map is very important for molecular breeding program in any crop. In chickpea bacterial artificial chromosome (BAC) library was first constructed from germplasm line, FLIP 84-92C, to facilitate positional cloning of disease resistance genes and physical mapping of the genome (Rajesh *et al.*, 2004). Lichtenzveig *et al.* (2005) constructed a BAC library and a binary BAC (BIBAC) library from the nuclear DNA of chickpea and identified positive BACs were sequenced for SSR marker development which could be employed for the generation of a physical map and as potential resources for whole genome sequencing. Similarly, Zhang *et al.* (2010) also constructed a genomic-wide, BAC/BIBAC-based physical map of chickpea by fingerprint analysis. Zatloukalova *et al.* (2011) developed integrated genetic and chromosome-based physical maps of chickpea by assigning linkage group (LG) in chickpea to different

chromosomes using flow cytometry and PCR based primers that amplify sequence tagged microsatellite site (STMS) markers.

Recently, chickpea whole genome sequencing has been reported (Jain *et al.*, 2013; Varshney *et al.*, 2013) which generated massive amount of genomic sequence information and utilization of this information in applied crop improvement programs has been augmented by the availability of sophisticated bioinformatics tools. The availability of whole genome sequences triggers the development of new molecular resources and tools, such as molecular markers for precise genetic mapping and comprehensive molecular analysis of genome structure and function (Ramu *et al.*, 2010). The chickpea genome sequencing projects provide the better resolution in anchoring molecular markers to respective chromosomes for better development of physical map to know the actual distance between the genetic markers linked to trait of interest using bioinformatics tools which aid in applied crop breeding programs.

In this regard, present investigation was conducted to develop physical map of FW resistance regions of chickpea genome by using molecular markers closely linked to FW resistance reported earlier through *in silico* approaches. A total of forty six markers linked to FW resistance were selected for *in silico* physical mapping against chickpea genome sequence in NCBI database. Among 46 markers, five were RAPD, one was AFLP and two were ISSR were not suitable for *in silico* physical mapping. However, the DNA sequence of polymorphic band of A07C an RAPD marker was available (Soregaon *et al.*, 2007) and used for physical mapping (Table 4). Among, thirty nine markers including DNA sequence of polymorphic band of A07C, 23 markers were mapped to different chickpea chromosomes (Table 5 and Fig. 2) and six were mapped to unplaced genomic scaffolds (Table 6). The remaining ten markers were not mapped to any of the chickpea chromosomes or unplaced genomic scaffolds. Recently, Madrid *et al.* (2014) performed *in silico* PCR against chickpea reference genome using similar criteria used in the present study to anchor the STMS markers tightly linked to the *Ascochyta* blight (AB) resistance on LG2 and identified the QTL_{AR3} region on chickpea chromosome 2 which flanked 32–33 Mb, comprising 42 genes.

Further, efforts were made in the present study to map the QTL regions in the chickpea genome by anchoring the flanking markers to physical positions on chickpea chromosomes. Similarly, NCBI sequence viewer has been used in the similar way by Niranjan *et al*. (2009) for *in silico* anchoring of the QTL genetic marker data on to the sequence based physical map of rice using 77 markers and they localized targeted QTL region on chromosome 1 using the flanking markers on the either side of QTL region. The physical mapping of all the resistance loci/QTL regions of chickpea specifying *Fusarium* wilt (FW) resistance (Mayer *et al.*, 1997; Winter *et al.*, 2000; Huettel *et al.*, 2002; Tekeoglu *et al.*, 2002; Sharma *et al.*, 2004; Cobos *et al.*, 2005; Iruela *et al.*, 2006; Millan *et al.*, 2006; Soregaon *et al.*, 2007; Gowda *et al.*, 2009; Millan *et al.*, 2010; Halila *et al.*, 2010; Soregaon, 2011; Sabbavarapu *et al.*, 2013; Patil *et al.*, 2014; Barman *et al.*, 2014; Varshney *et al.*, 2014; Jingade and Ravikumar, 2015) was done with molecular markers flanking QTLs and markers tightly linked to FW resistance.

Table 5. *In silico* mapping of molecular markers associated with *Fusarium* wilt resistance on chickpea chromosomes

Markers		E-value	Identity (%)	Gaps	+/- strands	Chromosomes (Ca)	Physical position (bp)	Gene id.	Putative functions
GSSR11	FP	0.002	100	0/20 (0 %)	+	Ca1	8377553-8377835	Intergenic	-
	RP	2e-04	100	0/22 (0 %)	-				
GSSR18	FP	0.002	100	0/20 (0 %)	+	Ca1	41069867-41070121	Intergenic	-
	RP	2e-04	100	0/22 (0 %)	-				
TA110	FP	3e-07	100	0/27 (0 %)	+	Ca2	9410378-9410585	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	-				
TA200	FP	3e-04	96	0/26 (0 %)	-	Ca2	15459886-15460087	LOC101492815	probable 6-phosphogluconolactonase 1
	RP	3e-07	100	0/27 (0 %)	+				
H3A12	FP	3e-07	100	0/27 (0 %)	-	Ca2	16257283-16257461	Intergenic	-
	RP	2e-04	100	0/22 (0 %)	+				
TA37	FP	3e-07	100	0/27 (0 %)	-	Ca2	17196292-17196573	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	+				
TA27	FP	1e-06	100	0/26 (0 %)	-	Ca2	17196296-17196535	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	+				
TA59	FP	3e-07	100	0/27 (0 %)	+	Ca2	23029043-23029285	LOC101503022	MADS-box protein SVP-like
	RP	3e-07	100	0/27 (0 %)	-				
TR19	FP	6e-04	100	0/21 (0 %)	+	Ca2	27187236-27187435	Intergenic	-
	RP	0.002	100	0/20 (0 %)	-				
GA16	FP	6e-04	100	0/21 (0 %)	-	Ca2	34747499-34747739	LOC101503585	BicD, Microtubule-associated protein Bicaudal-D
	RP	0.002	100	0/20 (0 %)	+				
TR24	FP	2e-04	100	0/22 (0 %)	-	Ca3	36209664-36209788	Intergenic	-
	RP	0.002	100	0/20 (0 %)	+				

Markers		E-value	Identity (%)	Gaps	+/- strands	Chromosomes (Ca)	Physical position (bp)	Gene id.	Putative functions
TC14801	FP	0.002	100	0/20 (0 %)	+	Ca3	37377922-37378174	LOC101497275	-
	RP	0.002	100	0/20 (0 %)	-				
TR20	FP	0.002	100	0/20 (0 %)	-	Ca4	23273185-23273356	Intergenic	-
	RP	0.002	100	0/20 (0 %)	+				
TS82	FP	9e-08	100	0/28 (0 %)	-	Ca4	25798007-25798169	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	+				
ESTSSR21	FP	0.002	100	0/20 (0 %)	+	Ca4	36209664-36209788	LOC101501064	C2H2-like zinc finger protein
	RP	0.002	100	0/20 (0 %)	-				
$A07C_{700}$		0.0	99	0/410 (0 %)	-	Ca4	38472483-38472892	LOC101491241	wound-responsive family protein
TS72	FP	3e-07	100	0/27 (0 %)	+	Ca4	40036747-40036944	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	-				
ESTSSR3	FP	0.002	100	0/20 (0 %)	-	Ca5	31182033-31182241	LOC101508239	epidermal growth factor receptor substrate 15
	RP	0.002	100	0/20 (0 %)	+				
TR44	FP	9e-08	100	0/28 (0 %)	-	Ca6	29073261-29073549	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	+				
CaM1125	FP	0.002	100	0/20 (0 %)	-	Ca6	29610051-29610331	Intergenic	-
	RP	0.002	100	0/20 (0 %)	+				
H4E09	FP	3e-07	100	0/27 (0 %)	+	Ca6	29648025-29648253	Intergenic	-
	RP	3e-07	100	0/27 (0 %)	-				
CaM1402	FP	0.21	100	0/16 (0 %)	-	Ca6	42884010-42883853	Intergenic	-
	RP	1e-05	100	0/24 (0 %)	+				
CaM1101	FP	6e-04	100	0/21 (0 %)	+	Ca6	56466205-56466494	Intergenic	-
	RP	0.002	100	0/20 (0 %)	-				

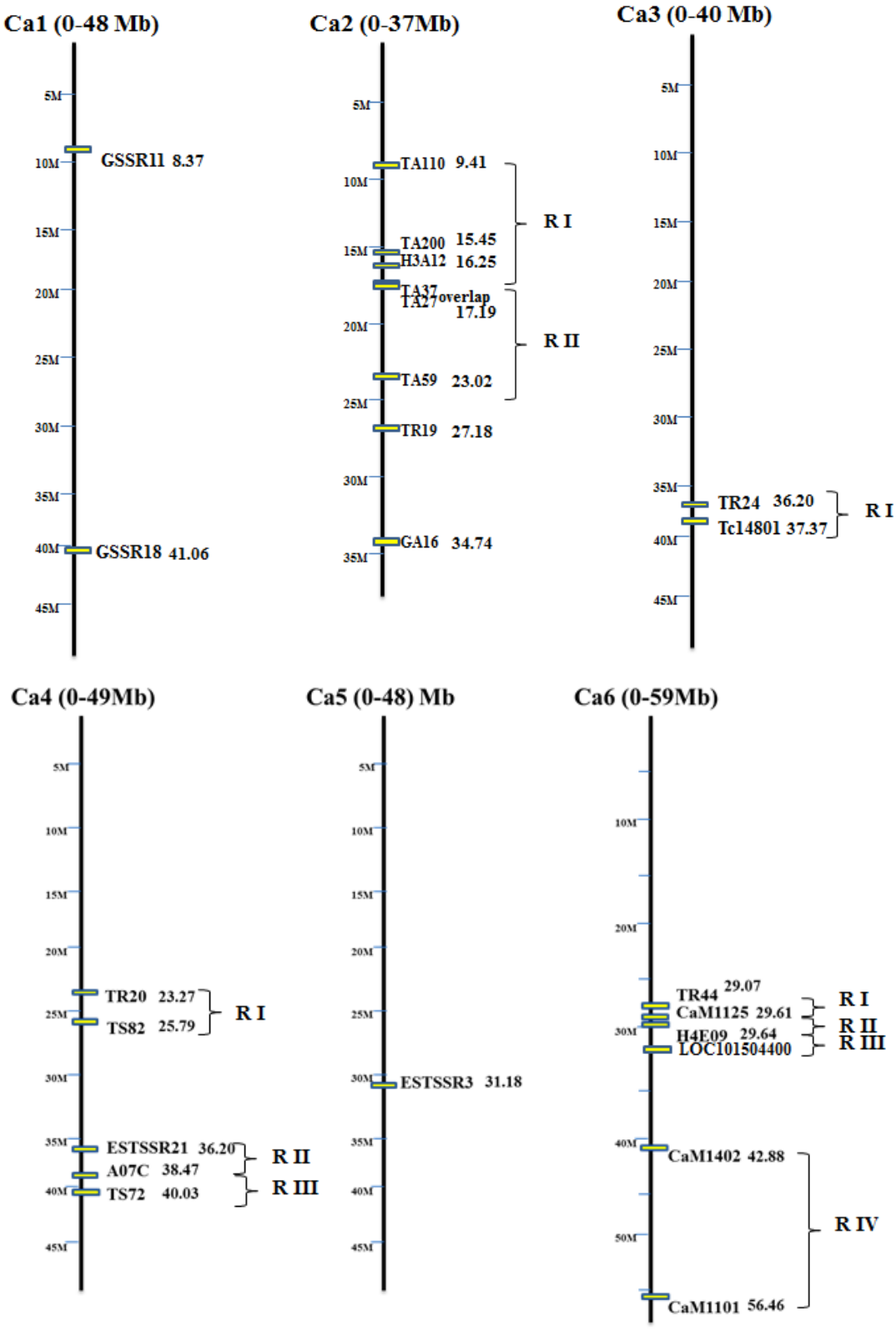


Fig. 2: Pictorial representation of *in silico* physically mapped *Fusarium* wilt linked markers on the *Cicer arietinum* chromosomes (Ca), R-represent genomic regions scanned

Table 6: Markers *in silico* physical mapped on chickpea unplaced genomic scaffolds

Markers		E-value	Identity (%)	Gaps	+/- strands	Scaffold	Physical positions (bp)	Gene id.	Putative functions
TA22	FP	0.002	100	0/20 (0 %)	+	96	156829- 157020	Intergenic	-
	RP	0.002	100	0/20 (0 %)	-				
H4G11	FP	4e-06	100	0/25 (0 %)	-	174	348420-348617	Intergenic	-
	RP	4e-06	100	0/25 (0 %)	+				
TA186	FP	3e-07	100	0/27 (0 %)	+	306	257945-259728	Intergenic	-
	RP	9e-08	100	0/28 (0 %)	-				
TA96	FP	6e-04	100	0/21 (0 %)	+	766	204309-204547	Intergenic	-
	RP	0.006	100	0/20 (0 %)	-				
ESTSSR65	FP	6e-04	100	0/21 (0 %)	-	7355	5101-5198	LOC101491980	CCT motif family protein
	RP	6e-04	100	0/21 (0 %)	+				
H1B06	FP	2e-04	100	0/22 (0 %)	+	415	12257-12435	Intergenic	-
	RP	5e-05	100	0/23 (0 %)	-				

Among 46 markers selected for physical mapping in our study 23 markers (50 %) were mapped on chickpea chromosomes, six markers (13.04 %) were mapped to chickpea unplaced genomic scaffolds and 10 markers could not be mapped as they did not follow preferred criteria for physical mapping (Ramu *et al.,* 2010). Of the 10 markers which did not follow the standard criteria for mapping, six markers (CS-27, TS47, TR2, H1A12, TR59 and TA194) had mis-match hits, *i.e.*, the forward and reverse primers of each primer pair were aligned to different chromosome/scaffolds or one or both primers of SSR markers did not align properly, thereby unsuitable for mapping. Although the remaining four markers (NCPGR74, SSR14, TAA60 and NCPGR58) aligned to same chromosome or scaffold, the fragment size exceeded 500 bp which is not preferred for mapping. In many physical mapping studies such results were common. For example in sorghum sequence based physical mapping of 7013 SSR markers, 1063 SSR (15.2 % of total SSR markers), and two CISP markers were dropped from physical mapping as they did not follow preferred criteria of physical map (Ramu *et al.,* 2010). Among 1063 SSRs, 599 (56.4 % of discarded SSR markers) had no hits, 144 (13.5 % of discarded SSR markers) had a fragment size >500 bp and were not assigned to a specific chromosome. The remaining markers either had mis-match hits (on different chromosome) or one or both primer pairs of the SSR markers did not align perfectly or the primer pairs were assigned to super clusters. Among, the perfectly assigned SSR markers on sorghum genome sequence, 118 were duplicated, 708 produced putatively overlapping fragments, and 132 annealed to regions which contained more than one SSR fragment (Ramu *et al.,* 2010). Similarly, Madrid *et al.* (2014) physically mapped 11 out of 15 marker selected for AB resistance on chickpea chromosome 2 to map QTL_{AR3}. Five out of the eight STMS primers and one genic marker were amplified by *in silico* PCR, three genic molecular markers were mapped by applying BLAST to the whole genome sequence and two out of the three were ESTs.

In the present study, the markers were distributed randomly in all the regions of chickpea chromosomes. Similar results were observed in the study conducted by Madrid *et al.* (2014) for AB resistance QTLs in chickpea. In sorghum sequence-based physical map SSR markers were saturated at telomeric regions of the chromosomes, no marker was located around the centromeric regions of chromosomes (Paterson *et al.,* 2009; Ramu *et al.,* 2010).

Earlier studies on resistance in chickpea for race 1 *Fusarium oxysporum* f. sp. *ciceri* evidence that crosses among late wilters, between early and late wilter and between early or late wilter and completely resistant lines indicated that resistance to race 1 is controlled by atleast three independent loci designated as H_1, H_2 and H_3 (Upadhyaya *et al.,* 1983b; Singh *et al.,* 1987). The homozygous recessive alleles both the loci H_1 and H_2 or partially recessive alleles in homozygous form at either the H_1 or H_2 locus and dominant allele at H_3 locus confers the complete resistance. Any one recessive allele at H_1 and H_2 or dominant allele at H_3 locus separately delays the wilting. These three resistance genes in chickpea were independent in contributing resistance for race 1. Hence, different region in genome controls resistance for FW which is indicated in the physical map.

Among the 23 markers mapped to chromosomes, two markers (GSSR11 and GSSR18) were mapped to chromosome (Ca) 1, eight markers (TA110, TA200, H3A12, TA37, TA27, TA59, TR19 and GA16) were mapped to Ca2, two markers (TR24 and TC14801) were mapped to Ca3, five markers (TR20, TS82, ESTSSR21, A07C and TS72) were mapped to Ca4, only one markers (ESTSSR3) was mapped to Ca5 and five markers (TR44, CaM1125, H4E09, CaM1402 and CaM1101) were mapped to Ca6 (Table 5 and Fig. 2).

The highest number of markers were mapped on Ca2 (eight), followed Ca4 (five) and Ca6 (five). The two markers (GSSR11 and GSSR18) mapped to Ca1 were physically very far *i.e.,* 8.37 and 41.06 Mb respectively. In Ca2 the marker density was more, the region between 15 Mb and 18 Mb is concentrated with four markers (TA200, H3A12, TA37 and TA27). The markers TA37 and TA27 overlaped at 17.19 Mb whereas, TA59 and TR19 were also close to each other with a distance of 4.16 Mb. Similarly, in Ca3 two markers TR24 and Tc14801 were mapped close to each other physically at 36.20 and 37.37 Mb respectively. In Ca4, out of five markers, two (TR20 and TS82) were mapped close to each other at 23.27 and 25.79 Mb respectively whereas, three markers (ESTSSR21, A07C and TS72) were concentrated between 36 Mb and 40 Mb. Only one marker located physically at 31.18 Mb on Ca5. In Ca6 out of five markers mapped, three markers were clustered between 29 Mb and 30 Mb whereas, two markers (CaM1402 and CaM1101) were mapped little far covering a distance of 13.58 Mb. The physical mapping of markers linked to FW resistance has led to the identification of two genomic regions in Ca2, one in Ca3, three in Ca4 and four in Ca6, which were saturated with more number of markers associated with FW resistance. In Ca2 one resistance loci *Foc1* (Gowda *et al.,* 2009; Barman *et al.,* 2014) and two QTLs (*Wilt 1* and *Wilt 2*) (Patil *et al.,* 2014) were physically mapped to the two regions. Similarly, in Ca6 one QTL (*FW-Q-APR-6-1*) (Sabbavarapu *et al.,* 2013) was anchored *in silico* between markers CaM1402 and CaM1101.

So far only one study has been carried out to exploit the whole chickpea genome sequence information together with previous QTL genetic information as similar to our study. Madrid *et al.* (2014) mapped QTL_{AR3} *Ascochyta* blight (AB) resistance on LG2 which correspond to Ca2 and identified candidate genes located in this genomic region to design diagnostic markers useful for MAS. They selected fifteen markers on LG2 for AB resistance for sequence based physical mapping in that, five STMS markers GA16, TA194, TS82, TR19 and TA110 were common for both AB and FW resistance. The markers TA110, TR19 and GA16 were mapped on Ca2 in their study. In our investigation the same markers TA110 (9410378 bp), TR19 (27187236 bp) and GA16 were also mapped on the Ca2 at same physical positions as mapped by Madrid *et al.* (2014). However, GA16 was mapped at 34747518 bp on Ca2 by Madrid *et al.* (2014) whereas, in our study the same marker was mapped Ca2 with little changes in physical position (*i.e.,* 34747499 bp). The little difference is because of update and improvement in the chickpea reference genome frequently. The markers TS82 and TA194 were not aligned to chromosomes in their study, but in present study TS82 was physically mapped at 25798007 bp on Ca4. The marker TA194 was not aligned to any of the chickpea chromosomes in this study also.

4.2 *In silico* identification of genes, gene based microsatellites in the QTL region for *Fusarium* wilt resistance and development of genic SSR markers

In silico physical mapping is a targeted approach to fine map the FW resistance genes in the chickpea genome. The SSR or microsatellite markers have become the marker of choice in plant breeding as they are abundant, uniformly distributed throughout the genome, multiallelic, co-dominant and show extensive polymorphisms (Gupta *et al.,* 2000). In case of chickpea genome abundant SSRs have been available with high level of interspecific polymorphism suggesting SSR markers are well suited for chickpea genome mapping and gene tagging, both genomic and transcript datasets can be utilized to develop SSR markers in chickpea.

In this regard based on physical mapping of molecular markers and QTLs linked to FW resistance and the physical nearness of linked markers, two regions in Ca2, one region in Ca3, three regions in Ca4 and four regions in Ca6 and six unplaced genomic scaffolds were chosen for identification of genes and the SSR in these regions.

4.2.1 Physical mapping of FW resistance loci/QTLs on chickpea

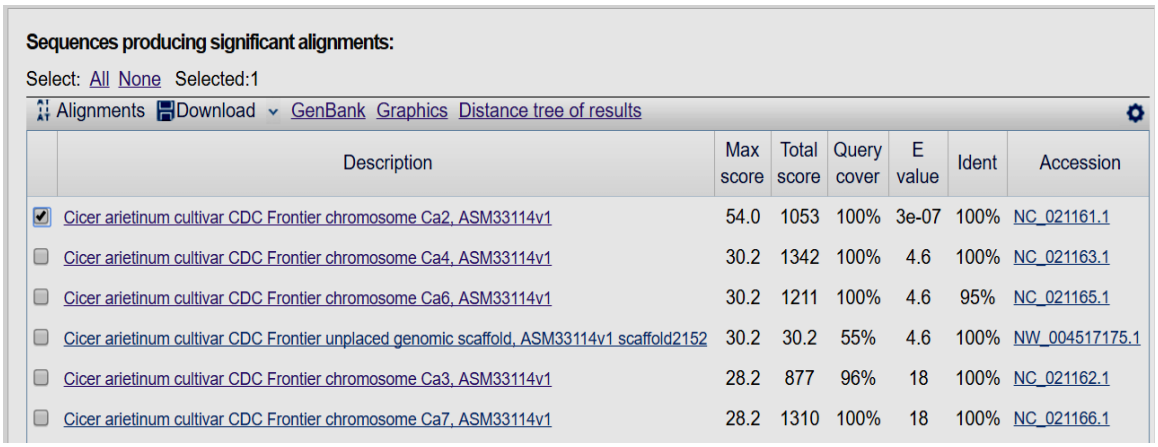
4.2.1.1 Chromosome 2 (Ca2)

The three marker intervals on chromosome 2 between markers TA110 and H3A12 (Gowda *et al.,* 2009), TA200 and TA37 (Barman *et al.,* 2014), TA27 and TA59 (Patil *et al.,* 2014) were selected after physical mapping of QTLs and associated markers linked to FW resistance. The genes present in these regions were identified.

The region I (Fig. 2) contained a QTL between TA110 and H3A12 markers at physical position 9.41 and 16.25 Mb (Fig. 3A and B) flanking 6.84 Mb. The genetic distance reported for the same loci was 6.0 cM on LG2 (Gowda *et al.,* 2009). The genomic region flanked by these markers was scanned for number of genes using map viewer (Fig. 4) and genic SSR using online webstat software. There were 310 genes and 314 genic SSR motifs (Appendix-I) present in the genomic region flanked by these markers. Among 314 genic SSR motifs identified, mononucleotide motifs (225; 71.65 %) were more abundant followed by di-(62; 19.74 %), tri-(18; 5.73 %), tetra-(7; 2.22 %), penta-(1; 0.3 %) and hexa-(1; 0.3 %) nucleotide motifs (Table 7 and Fig. 5A). Overall from 314 genic SSR, only for seventy three SSR motifs the primer sequences were available (Appendix II).

The region II contained QTL *Wilt 1* flanked by TA27 and TA59 markers covered a genomic region of 5.83 Mb, consisting of 135 genes and 204 genic SSRs (Appendix-III). Among 204 SSR motifs identified mononucleotide (135; 66.17 %) and dinucleotide (53; 25.98 %) were dominating followed by tri-(14; 6.86 %), tetra-(1; 0.49 %) and penta-(1; 0.49 %) (Table 7 and Fig 5C). Out of 204 genic SSR motifs in the region II, the primers could be designed for only 141 genic SSRs were able to design primers (Appendix-IV).

Barman *et al.* (2014) mapped *Foc1* on LG2 flanked by markers TA200 and TA37 in the same region which covers genetic distance of 1.2 cM. The flanking markers were



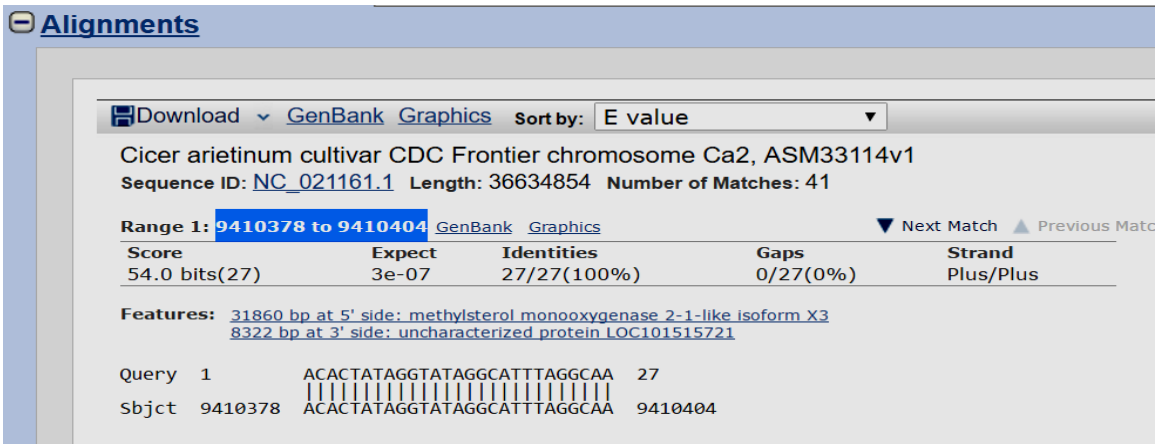
Sequences producing significant alignments:

Select: All None Selected:1

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	Cicer arietinum cultivar CDC Frontier chromosome Ca2, ASM33114v1	54.0	1053	100%	3e-07	100%	NC_021161.1
☐	Cicer arietinum cultivar CDC Frontier chromosome Ca4, ASM33114v1	30.2	1342	100%	4.6	100%	NC_021163.1
☐	Cicer arietinum cultivar CDC Frontier chromosome Ca6, ASM33114v1	30.2	1211	100%	4.6	95%	NC_021165.1
☐	Cicer arietinum cultivar CDC Frontier unplaced genomic scaffold, ASM33114v1 scaffold2152	30.2	30.2	55%	4.6	100%	NW_004517175.1
☐	Cicer arietinum cultivar CDC Frontier chromosome Ca3, ASM33114v1	28.2	877	96%	18	100%	NC_021162.1
☐	Cicer arietinum cultivar CDC Frontier chromosome Ca7, ASM33114v1	28.2	1310	100%	18	100%	NC_021166.1

(i) BLAST output of query sequence TA110 (forward primer)



Alignments

Download GenBank Graphics Sort by: E value

Cicer arietinum cultivar CDC Frontier chromosome Ca2, ASM33114v1

Sequence ID: NC_021161.1 **Length:** 36634854 **Number of Matches:** 41

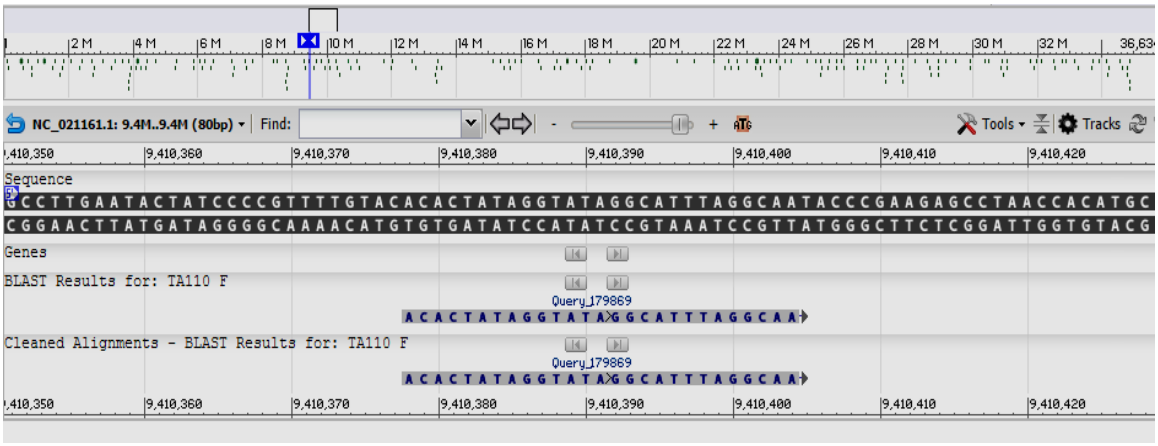
Range 1: 9410378 to 9410404 GenBank Graphics Next Match Previous Matc

Score	Expect	Identities	Gaps	Strand
54.0 bits(27)	3e-07	27/27(100%)	0/27(0%)	Plus/Plus

Features: 31860 bp at 5' side: methylsterol monooxygenase 2-1-like isoform X3
8322 bp at 3' side: uncharacterized protein LOC101515721

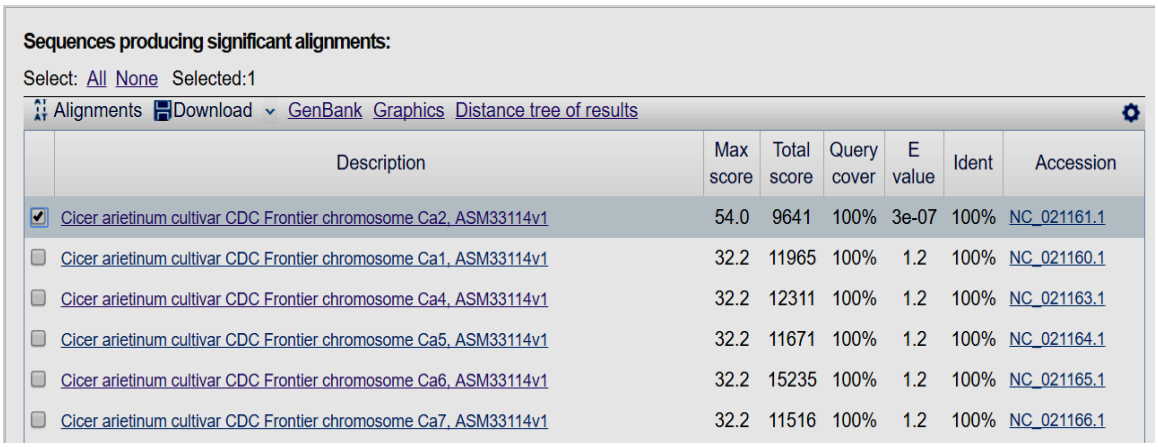
```
Query  1        ACACTATAGGTATAGGCATTTAGGCAA  27
               ||||||||||||||||||||||||||
Sbjct  9410378  ACACTATAGGTATAGGCATTTAGGCAA  9410404
```

(ii) Significant pairwise alignment of TA110 (forward primer)



(iii) Graphical display of BLAST output of query sequence TA110 (forward primer)

Fig. 3A: BLAST output of Query sequence TA110 (forward primer) producing significant alignment with chickpea genome on chromosome 2



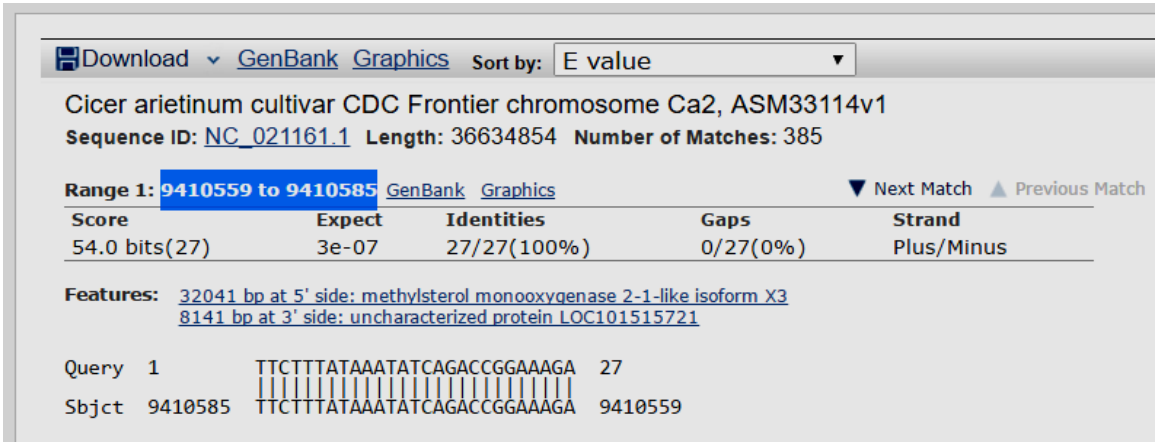
Sequences producing significant alignments:

Select: All None Selected:1

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Cicer arietinum cultivar CDC Frontier chromosome Ca2, ASM33114v1	54.0	9641	100%	3e-07	100%	NC_021161.1
Cicer arietinum cultivar CDC Frontier chromosome Ca1, ASM33114v1	32.2	11965	100%	1.2	100%	NC_021160.1
Cicer arietinum cultivar CDC Frontier chromosome Ca4, ASM33114v1	32.2	12311	100%	1.2	100%	NC_021163.1
Cicer arietinum cultivar CDC Frontier chromosome Ca5, ASM33114v1	32.2	11671	100%	1.2	100%	NC_021164.1
Cicer arietinum cultivar CDC Frontier chromosome Ca6, ASM33114v1	32.2	15235	100%	1.2	100%	NC_021165.1
Cicer arietinum cultivar CDC Frontier chromosome Ca7, ASM33114v1	32.2	11516	100%	1.2	100%	NC_021166.1

(i) BLAST output of query sequence TA110 (reverse primer)



Download GenBank Graphics Sort by: E value

Cicer arietinum cultivar CDC Frontier chromosome Ca2, ASM33114v1

Sequence ID: NC_021161.1 **Length:** 36634854 **Number of Matches:** 385

Range 1: 9410559 to 9410585 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
54.0 bits(27)	3e-07	27/27(100%)	0/27(0%)	Plus/Minus

Features: 32041 bp at 5' side: methylsterol monooxygenase 2-1-like isoform X3
8141 bp at 3' side: uncharacterized protein LOC101515721

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Query  1        TTCTTTATAAATATCAGACCGGAAAGA  27
               ||||||||||||||||||||||||||||
Sbjct  9410585  TTCTTTATAAATATCAGACCGGAAAGA  9410559
```

(ii) Significant pairwise alignment of TA110 (reverse primer)

(iii) Graphical display of BLAST output of query sequence TA110 (reverse primer)

Fig. 3B: BLAST output of Query sequence TA110 (reverse primer) producing significant alignment with chickpea genome on chromosome 2

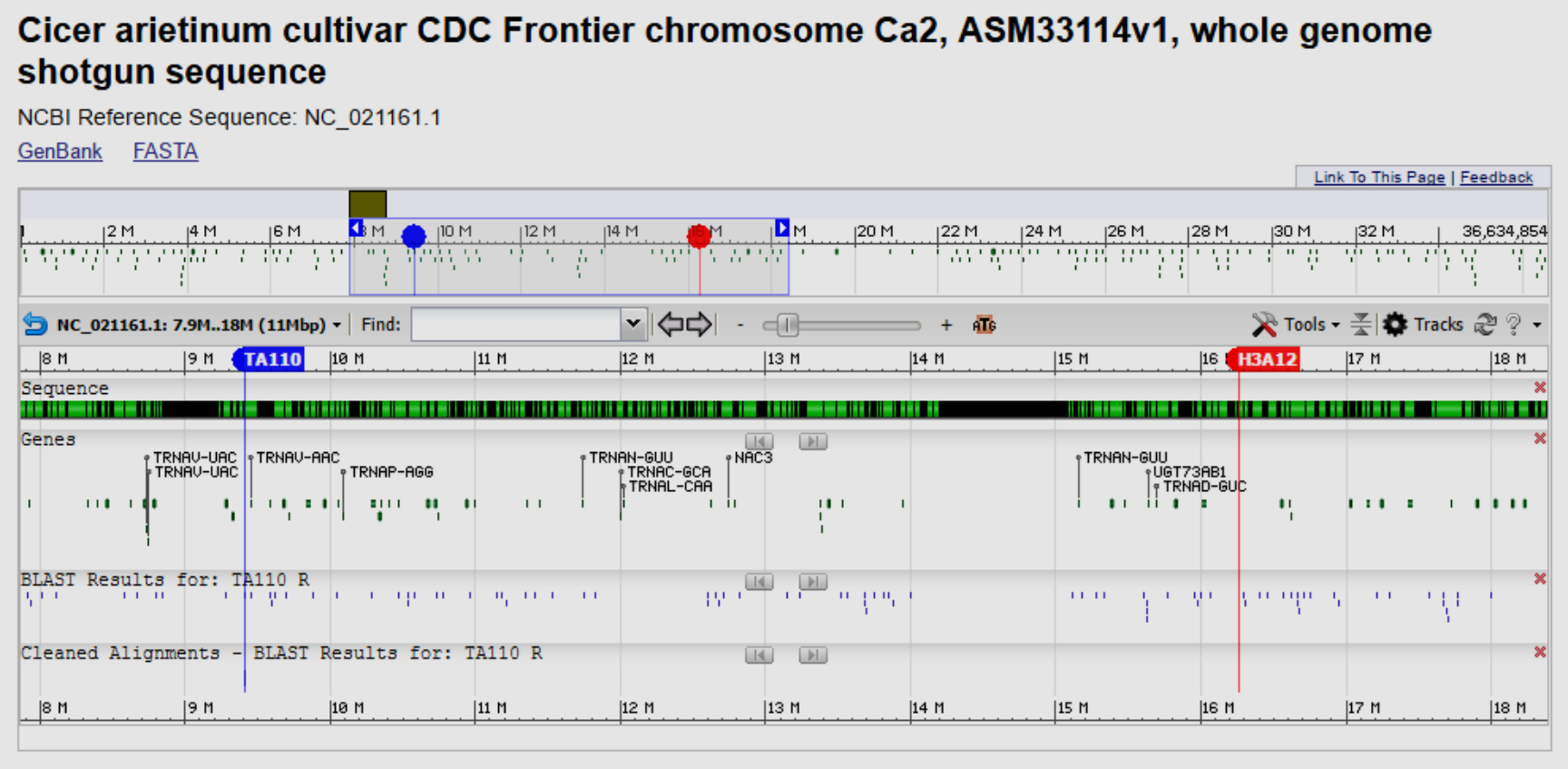


Fig. 4: Graphical display representing alignment of BLAST output result of both TA110 and H3A12 on to chromosome 2 of chickpea genome using NCBI sequence viewer

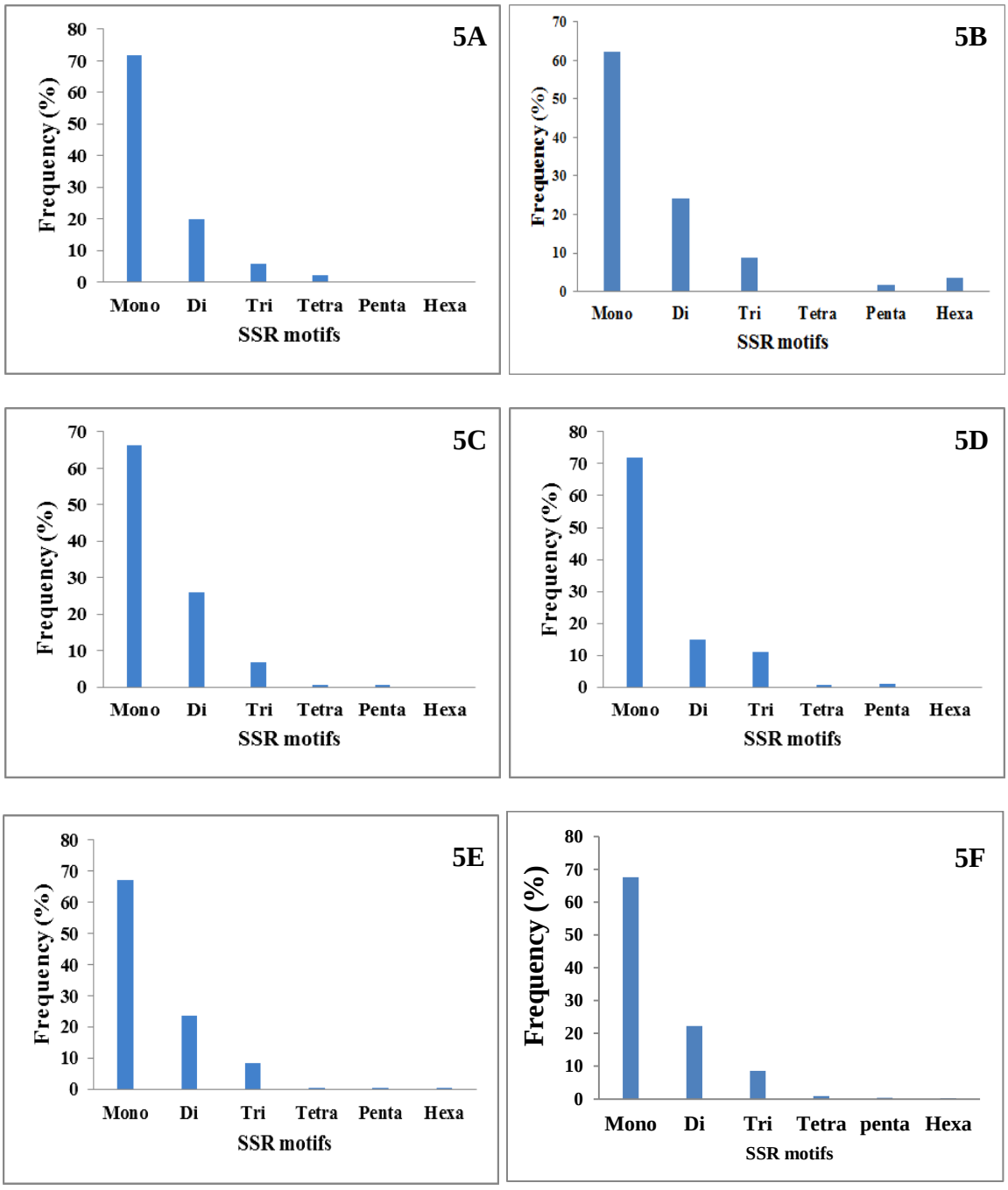


Fig. 5: Frequency of microsatellite repeats found in *Fusarium* wilt resistance regions of chickpea genome sequence, 5A-genomic region flanked by TA110-H3A12 on Ca2; 5B-genomic region flanked by TA200-TA37 on Ca2; 5C-genomic region flanked by TA27-TA59 on Ca2; 5D-genomic region flanked by A07C-TS72 on Ca4; 5E-genomic region flanked by CaM1402-CaM1101 on Ca6. 5F-Overall chickpea genome scanned

mapped to Ca2 at 15.49 and 17.19 Mb respectively. This region also overlaps with the QTL reported by Gowda *et al.,* (2009) between markers TA110 and H3A12. TA200 and TA37 flanks 1.73 Mb genomic region consists of 63 genes, in that 32 genes (LOC101492815 to LOC101514414) overlap with genes covered by TA110 and H3A12 (Appendix-I). In the remaining 31 genes, fifty eight genic SSR motifs were identified (Appendix V). Among identified genic SSRs mononucleotide motifs were most abundant (36; 62.06 %), followed by di-(14; 24.13 %), tri-(5; 8.62 %), hexa-(2; 3.44 %) and penta-(1; 1.72 %) nucleotide repeat units (Table 7 and Fig. 5B). Only for 46 SSRs out of 58 were able to design primers (Appendix VI). Similarly, Patil *et al.*, (2014) identified two FW resistance QTLs (*Wilt 1* and *Wilt 2)* on LG2. In that, QTL *Wilt 2* flanked by TA110 and TA27 markers, was found within the genomic region I from TA110 to TA37 which was already scanned.

4.2.1.2 Chromosome 3 (Ca3)

One region (R I) flanked by two FW resistance linked markers TR24 and TC14801 (Jingade and Ravikumar, 2015) were mapped at 36.20 and 37.37 Mb was selected on chromosome 3. These two markers did not flank any QTLs/ resistant loci, but linked to FW resistance and close to each other covering only 1.16 Mb genomic region. The interval consists of 153 genes and 179 genic SSR motifs (Appendix-VII). In this genomic region also mononucleotide (107; 59.77 %) repeats were highest in frequency followed by di-(43; 24.02 %), tri-(27; 15.08 %) and tetra-(2; 0.11 %) nucleotide motifs (Table 7). Only 102 microsatellites were able to design primers out of 179 microsatellite motifs (Appendix-VIII).

4.2.1.3 Chromosome 4 (Ca4)

Five markers *viz*., TR20, TS82, EST SSR21, A07C and TS72 linked to FW resistance in different studies (Table 2) were physically mapped on the Ca4. Since, these markers did not flank any QTLs, but linked to resistance loci and close to each other in physical map. Three genomic regions were selected for scanning for genes and microsatellites. The region I flanked by TR20 and TS82 covered a distance of 2.52 Mb consisting of eighty four genes and 117 genic SSR motifs (Appendix-IX). However, in this genomic region the large fraction of SSRs identified were mononucleotide (62.39 %) followed by di (20.51 %), tri (14.52 %) and tetra (2.56 %) nucleotide repeat units (Table 7). Out of 117 microsatellites, the primers could be designed only for ninety one SSR motifs (Appendix-X).

The region II and III were flanked by EST SSR21-A07C and A07C-TS72 respectively on Ca4. These three markers were mapped at 36.20, 38.47 and 40.03 Mb respectively. The total genomic region covered between EST SSR21-TS72 was 3.82 Mb. This genomic region covered 253 genes and 373 genic SSRs (Appendix-XI). The genomic region flanked by these marker enriched with mononucleotide motifs (71.04 %) followed by di (18.76 %), tri (8.57 %) tetra (1.07 %) and penta (0.53 %) (Table 7 and Fig. 5D). Of these 373 genic SSRs, it was possible to design primers for 255 genic SSR motifs (Appendix-XII).

Table 7: Occurrence of microsatellite (mono, di, tri, tetra, penta and hexanucleotide) repeats in the *Fusarium* wilt resistance region of chickpea genome sequence

Chromosomes (Ca)	Flanking markers	Mono	Di	Tri	Tetra	Penta	Hexa	Total
Ca2	Region I (TA110-H3A12 and *TA200-TA37)	225	62	18	7	1	1	314
		36	14	5	0	1	2	58
	Region II (TA27-TA59)	135	53	14	1	1	0	204
Ca3	TR24-TC14801	107	43	27	2	0	0	179
Ca4	Region I (TR20-TS82)	73	24	17	3	0	0	117
	Region II (EST SSR21-A07C)	149	46	14	3	0	0	212
	Region III (A07C-TS72)	116	24	18	1	2	0	161
Ca6	Region I (TR44-CaM1125)	57	25	8	0	1	2	93
	Region III (H4E09-LOC101504400)	39	14	3	0	0	0	56
	Region IV (CaM1402-CaM1101)	522	182	64	4	3	1	776
Scaffolds	-	61	14	5	0	0	0	80
	Total	**1520**	**501**	**193**	**21**	**9**	**6**	**2250**

*TA200-TA37-excluding genic SSRs present in 32 genes covered by TA110-H3A12

4.2.1.4 Chromosome 6 (Ca6)

In Ca6 five markers namely, TR44, CaM1125, H4E09, CaM1402 and CaM1101 linked to FW resistance were physically mapped (Table 5 and Fig. 2). The three markers TR44, CaM1125 and H4E09 were clustered in the same region at physical positions of 29.07, 29.61 and 29.64 Mb respectively.

The region I flanked by TR44 and CaM1125 covered a distance 0.53 Mb and consists of twenty one genes and ninety three genic SSRs (Appendix-XIII). The primers could be designed only for thirty two genic SSR motifs (Appendix-XIV). The mononucleotide repeat motifs were abundant (61.29 %) in this region followed by di (26.88 %), tri (8.60 %), hexa (2.15 %) and penta (1.07 %) nucleotide repeat motifs (Table 7).

The region II was flanked by markers CaM1125 and H4E09 covered genomic region of 0.038 Mb. However, there were no genes between them. An adjacent region (R III) from H4E09 (29.64 Mb) up to gene LOC101504400 (at 30.45 Mb) with putative function of nucleobase-ascorbate transporter 12 was screened for the functional genes. This genomic region covers twenty five genes and 56 genic SSRs motifs (Appendix-XV). This region is dominated by mono (69.64 %) followed by dinucleotide repeats (25 %) and trinucleotide repeats (5.35 %) (Table 7). Out of 56 genic SSRs, the primers could be designed only for 40 microsatellites (Appendix-XVI).

The region IV was flanked by markers CaM1402 and CaM1101 (Subbavarapu *et al.*, 2013) were physically mapped at 42.88 and 56.46 Mb respectively (Table 5 and Fig. 2). These two markers flanked 13.58 Mb, consisting 518 genes and 776 genic SSRs (Appendix-XVII). This genomic region covers a wide range of perfect SSR repeat units, among them mononucleotide (522; 67.26 %) repeats were most abundant followed by di- (182; 23.45 %), tri-(64; 8.24 %), tetra-(4; 0.51 %), penta-(3; 0.38 %) and hexa-(1; 0.12 %) nucleotide repeat motifs (Table 7 and Fig. 5E). Out of 776 genic SSRs, it was possible to design primers for only 138 genic SSRs (Appendix-XVIII).

4.2.1.5 Unplaced genomic scaffolds

Six markers were physically mapped to different chickpea unplaced genomic scaffolds (Table 6). The marker TA22 was mapped to unplaced scaffold 96 of size 347.24 Kb. The scaffold contains thirteen genes comprising of thirty genic SSRs. The primers could be designed for eight microsatellites. The marker H4G11 was mapped on scaffold 174 of size 498 Kb. Seventeen genes were identified in the scaffold and 34 genic SSRs were found in that region. Similarly, the other markers TA186, H1B06, TA96 and ESTSSR65 were mapped to scaffold 306, 415, 766 and 7355 respectively. Altogether, the six scaffolds covered 1.65 Mb genomic region, comprising of 48 genes (Appendix-XIX). From these scaffolds, 80 SSR motifs were found for twenty two motifs primers could be designed (Appendix-XX).

The physical mapping of all the linked markers represented ten genomic regions on four chromosomes and six unplaced genomic scaffolds covering 36.85 Mb and 1.65 Mb genomic regions respectively. The analysis of the region for genes and genic SSRs,

resulted in identification of 1,578 genes and 2,250 genic SSR motifs (Table 8). Among the microsatellite motifs, the mononucleotide repeats (1520; 67.55 %) were most abundant followed by di-(501; 22.26 %), tri-(193; 8.57 %), tetra-(21; 0.93 %), penta-(9; 0.4 %) and least hexa-(6; 0.2 %) nucleotide repeat units (Table 7 and Fig. 5F). Within the mononucleotide repeats, the fraction of (T) repeat motifs accounted for 54.44 per cent followed by (A) repeat motifs (32.43 %) whereas, in dinucleotide repeats AT repeats (30.73 %) were highest followed by TA (28.54 %) and TC (11.97 %) repeats.

In chickpea genome, the mononucleotide repeats were highest in frequency compared to others which was confirmed by earlier reports. The highest frequency of mononucleotide repeats (49.01 %) followed by tri- (28.75 %), di- (11.59 %), tetra- (6.96 %) and penta-nucleotide repeat (3.85 %) were observed by Gangadhar *et al.* (2010). Garg *et al.* (2011a) presented the occurrence of SSRs in chickpea transcript where, mono-nucleotide repeats represented the largest fraction (41.9 %) followed by trinucleotide (36.1 %) and di-nucleotide (19.3 %) repeats and only a small fraction of tetra-, penta- and hexa-nucleotide SSRs. However, when mononucleotide repeats were excluded the tri-nucleotide repeats were the most abundant type in cereals followed by di-, tetra-, penta- and hexa-nucleotide repeats (Varshney *et al.,* 2002; Kumari *et al.,* 2013; Jayashree *et al.,* 2006). In accordance with our results.

Agarwal *et al.* (2012) also reported the highest frequency of tri-nucleotide (54 %) SSRs followed by di-nucleotide (41 %) SSRs excluding mononucleotide repeats. However, contradict the present results. The SSRs found in the FW resistance genomic region, the occurrence of dinucleotide repeats were abundant followed by trinucleotide. Similarly, Choudhary *et al.* (2012) also reported the occurrence of highest frequency of trinucleotide repeat motifs (52.6 %) followed by di (28.2 %), penta (9.9 %) and tetra (9.3 %) nucleotide motifs in chickpea. Similarly, in global transcriptome analysis the largest fraction of SSRs identified were trinucleotides (44.46 %) followed by tetranucleotides (25.82 %) (Pradhan *et al.,* 2014).

The results observed by Jain *et al.,* (2015) contradict the present results. Among 303 polymorphic transcript based SSR markers for FW resistance, the largest number of di-nucleotides (170), followed by trinucleotide (124) repeats were observed. The number of tetra- (5), penta- (1) and hexa-nucleotide (3) repeats was very less.

Further, efforts were made to design primers for these identified genic SSR motifs using online webstat software. Out of 2,250 genic SSR identified in the FW resistance genomic region, it was possible to design primer pairs for only nine hundred and forty one genic SSR motifs. Among 941 microsatellites for which primers were designed 480 were mono, 326 were di, 111 were tri, 16 were tetra, six were penta and two were hexanucleotide repeat motifs. From among 941 markers 168 markers were selected for primer synthesis based on preferred criteria *i.e.,* a microsatellite should repeat more than seven times and excluding mononucleotide repeats (Choudary *et al.,* 2012).

Table 8: *In silico* physical mapping of *Fusarium* wilt resistance regions and the number of genes and SSR motifs in the regions

Flanking markers	References	Chromosomes	Physical positions	Genomic region covered (Mb)	No. of genes	No. genic SSR motifs	Primers designed
TA110-H3A12	Gowda *et al.*, 2009	Ca2	9410378-16257461	6.84	310	314	73
*TA200-TA37	Barman *et al.*, 2014	Ca2	15459860-17196573	1.73	31	58	46
TA27-TA59	Patil *et al.*, 2014	Ca2	17196296-23029285	5.83	135	204	141
TR24-TC14801	-	Ca3	36209664-37377922	1.16	153	179	102
TR20-TS82	-	Ca4	23273185-25798169	2.52	84	117	91
EST SSR21-A07C	-	Ca4	36209664-38472892	2.26	153	212	159
A07C-TS72	-	Ca4	38472892-40036944	1.56	100	161	96
TR44-CaM1125	Sabbavarapu *et al.*, 2013	Ca6	29073261-29610331	0.53	21	93	32
CaM1125-H4E09	-	Ca6	29610331-29648253	0.038	0	0	0
H4E09-LOC101504400	-	Ca6	29648253-30451787	0.81	25	56	40
CaM1402-CaM1101	Sabbavarapu *et al.*, 2013	Ca6	42884010-56466494	13.58	518	776	139
Scaffolds	-	-	-	1.65	48	80	22
			TOTAL	**38.508**	**1,578**	**2,250**	**941**

* TA200-TA37 flanking genomic region covers 63 genes out of that 32 genes were already covered in genomic region flanked by TA110-H3A12, hence rest of the genes and genic SSR markers were represented in this table.

These synthesised novel genic SSR markers were named as UASBC (University of Agricultural Sciences Bengaluru Chickpea) series primers. The primer names, forward and reverse primer sequences, SSR motifs, gene id., markers flanking genomic region from where primers were selected for synthesis and expected product size (bp) were summarised in the Table 9.

4.3 Primer synthesis, identification of polymorphic markers and genotyping RILs of two mapping populations

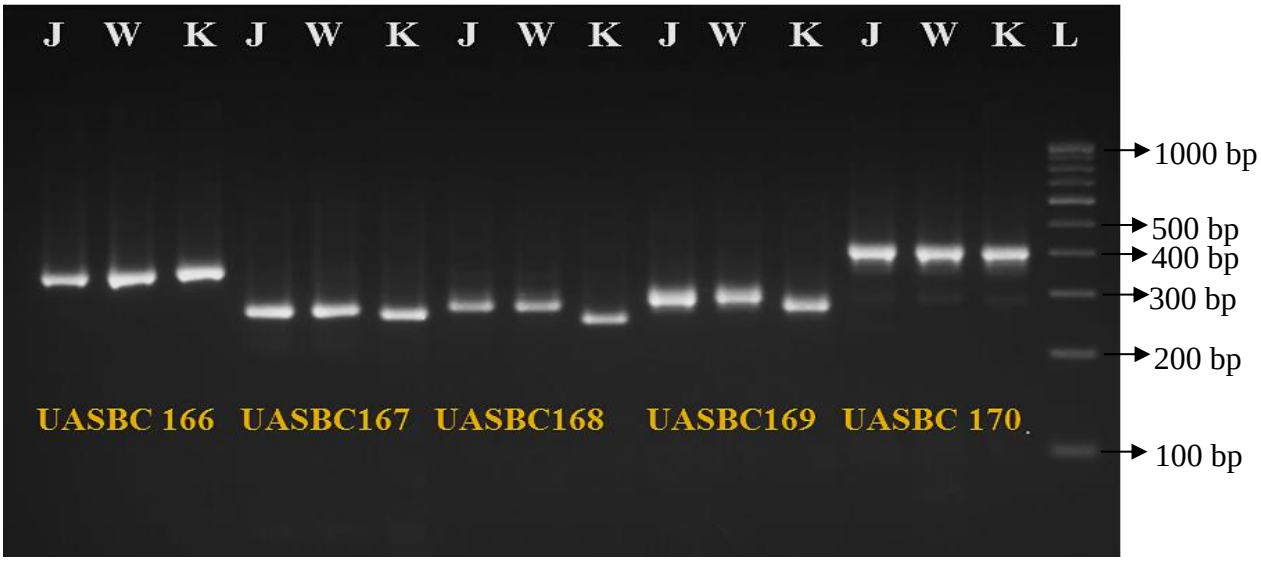
4.3.1 Experimental validation of newly synthesized genic SSRs and assessment of parental polymorphism

One sixty eight novel genic SSR markers were synthesised and tested for amplification of DNA template of three chickpea genotypes JG62, WR315 and K850. One hundred and sixty one out of 168 produced scorable profile in all the three genotypes (Plate 1). The amplified product produced was of expected product size in all the amplified primers. The parental polymorphism was studied using parental lines JG62, K850 and WR315 of the two sets of RILs used in this study. Twenty three markers (13.69 %) produced polymorphic bands between susceptible JG62 and resistant WR315, while twenty four markers (14.28 %) were polymorphic between late wilting (K850) and resistant (WR315) parental lines. Seventeen markers (10.11 %) recorded polymorphism between susceptible JG62 and late wilting K850 genotypes (Table 10). The RILs of two crosses *viz*., JG62 × WR315 and K850 × WR315 were used for mapping and ten primers were polymorphic for both the sets of RILs.

The frequency of polymorphic primers observed in the present study was in accordance with many of earlier studies *viz.,* Tullu *et al*. (1998) (14 %), Radhika *et al.* (2007) (9.5 % and 11.57 %), Gowda *et al.* (2009) (13.45 %), Nayak *et al.* (2010) (16.7 %), Patil *et al.* (2014) (21.30 %), Sabbavarapu *et al.* (2013) (20.8 %) and Jingadae and Ravikumar (2015) (10.3 %). However, Ratnaparkhe *et al*. (1998a) reported higher percentage of polymorphism (38 %) for the genic SSR in chickpea. The possible reason could be the use of intraspecific mapping population and genic markers (which are developed from conserved regions of the genome) employed in the present study, unlike interspecific mapping population and ISSR markers used by Ratnaparkhe *et al*. (1998a). Combined with knowledge of germplasm diversity and candidate gene regions, the analysis presented here should accelerate fine mapping of QTL region, identification and isolation of genes underlying QTL/gene responsible for wilt resistance.

In general, polymorphism observed in earlier studies in chickpea was low. The lack of detectable genetic polymorphism in cultivated germplasm is not uncommon, particularly for chickpea (Kazan and Muehlbauer, 1991), soybean (Keim *et al.*, 1990) and common bean (Gepts, 1991). Due to this low intra and inter-specific polymorphism (Udupa *et al.*, 1993; Labdi *et al.*, 1996 and Mayer *et al.*, 1997) progress in chickpea genomic research has been relatively slow, compared with legumes like soybean and *Medicago* (Radhika *et al.*, 2007). The lower level of polymorphism might be due to narrow genetic base of chickpea.

(a)



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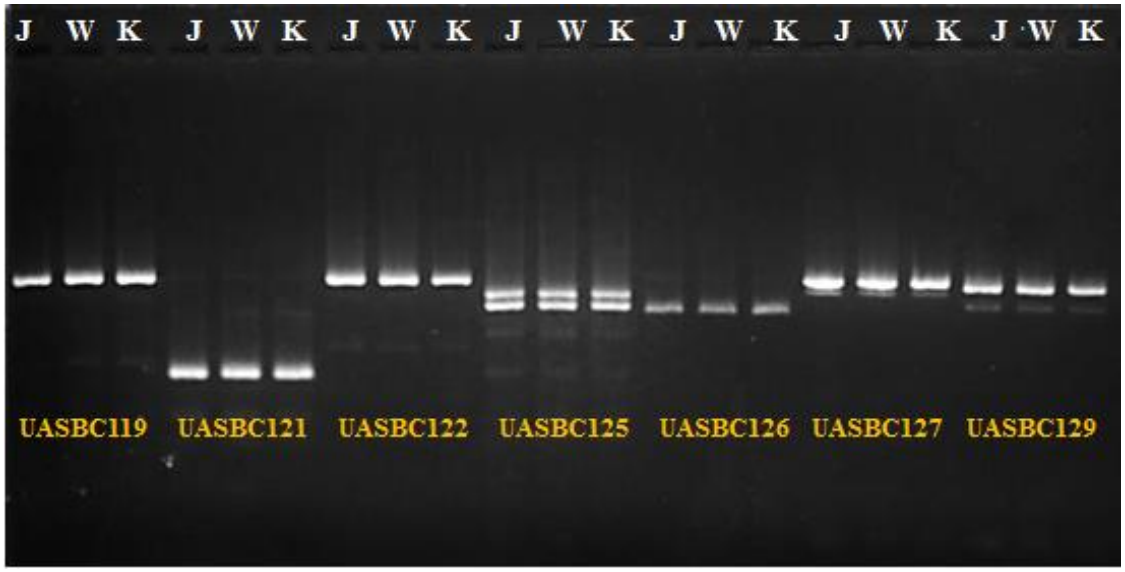


Plate 1: Amplification of genomic DNA by newly synthesised genic SSR primers (UASBC): J-JG62, W-WR315, K-K850 and L-100 bp step-up ladder

Table 9: Genic SSR markers developed through *in silico* physical mapping of *Fusarium* wilt resistance genomic regions

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
1	UASBC1	LOC101489302	Ca4	A07C-TS72	GTCTCACTTTTGTGCCTTCTAACA/ GTTGCTAAGAGTATCCAAACCCA	(TTTTG)6	378
2	UASBC2	LOC101491241	Ca4	A07C-TS72	GCTGCTGGGTTACATAAAATGA/ CGAAAGTAGCGGAGAGTGGTAT	(AT)6	393
3	UASBC3	LOC101493838	Ca4	A07C-TS72	GAACACATACATTTGACCGTGTG/ AAGGCAACCCTTACTTTTGTGA	(AAT)7	146
4	UASBC4	LOC101495550	Ca4	A07C-TS72	GAAACCTTAACGATCCTCATGC/ ACCTGATATGCTTCGCCTAAAA	(TTTA)10	195
5	UASBC5	LOC101507081	Ca4	A07C-TS72	TCAGGAAAGAGGTTGGAACTTG/ ATCAAACTGGGAATGCACTTG	(TA)9	260
6	UASBC6	LOC101508554	Ca4	A07C-TS72	GCTCTCTGTGTTGCTTAGGGAT/ GACTGACTCCTCAAGTGGAACC	(TAA)25	242
7	UASBC7	LOC101506964	Ca4	A07C-TS72	TAGCCTGTTCTCTATTGGGGTG/ TATCACATGGTTGTTTGGCAGT	(TG)6	230
8	UASBC8	LOC101509945	Ca4	A07C-TS72	AACCTTCCTTCCAACCACTAAAC/ GAAAACCACGCCAAAACTATGT	(AAT)20	168
9	UASBC9	LOC101509615	Ca4	A07C-TS72	GCCTAATGGAAGGATGGGTAA/ GCACTTTCGTTTCACTTTGCTT	(TAAAT)6	381
10	UASBC10	LOC101509301	Ca4	A07C-TS72	CGTGACATTGGGGAATAGTTGT/ GCACAATTTAGCTGAGGTTGCTA	(AAT)6	347
11	UASBC11	LOC101507600	Ca4	A07C-TS72	TGCTTTAGGGATTGTCTCATCA/ CTTGATAATGTAGTTGGCATACAG	(TC)8	396
12	UASBC12	LOC101506964	Ca4	A07C-TS72	TAGCCTGTTCTCTATTGGGGTG/ TATCACATGGTTGTTTGGCAGT	(TG)6	230
13	UASBC13	LOC101504059	Ca4	A07C-TS72	ACGGGAGAAGAGAAGAAGTTGA/ GTTAGGCCATGTTTGATGGTTA	(TA)16	389
14	UASBC14	LOC101489033	Ca2	TA110-H3A12	TTCTCTCCTTCCTCTCCTTCCT/ GAATATGGGCAAGTGTTGTTGA	(CA)12	334
15	UASBC15	LOC101498460	Ca2	TA110-H3A12	GTCCCTTCCTTCTCACAACAAC/ AGGGTGCTCTGTCTTTGTCTTC	(CT)8	181
16	UASBC16	LOC101491810	Ca2	TA110-H3A12	CTCTTTCTCCGCGCTAACC/ CGTATGGATCTTCCTGAGCTTC	(ACG)6	143

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
17	UASBC17	LOC101495484	Ca6	CaM1402-CaM1101	ACCCTCTAATGCAACATCATCA/ TATAAATCCCACTTGACCCACC	(TAA)7	348
18	UASBC18	LOC101515041	Ca6	CaM1402-CaM1101	CAACTGGTCATATCTCTGGTGC/ CCCCAAGAATACACGTAACAAA	(AT)13	374
19	UASBC19	LOC101498533	Ca6	CaM1402-CaM1101	CTATGACTTGGCAGCATTGAAG/ GATAGGTTCTCCATCTCTTTGAGC	(AT)7	253
20	UASBC20	LOC101501211	Ca6	CaM1402-CaM1101	CACTTTTACCCCTCTCCATTTG/ AGAGTAGCACTGGGGATTCAAG	(TC)20	356
21	UASBC21	LOC101511520	Scaffold174	-	CATCTATGTATGGTTGTGTGGTCA/ TTTGGCTTTAGCTTCCTACCAA	(AT)8	303
22	UASBC22	LOC101489480	Ca2	TA110-H3A12	CTAATTGTCCGAAGTTGACGTG/ GGAATGCAAGAAGGTGATAAGTG	(AT)15	386
23	UASBC23	LOC101512685	Ca2	TA110-H3A12	CACGGACAGTTTTACGATCTCA/ TCGAATCCAACATCACACAATC	(TATT)7	303
24	UASBC24	LOC101513982	Ca2	TA110-H3A12	TCAATGGGTGTATTAAGCACAG/ GTCAGTGTTTCTGGGTGTTTTC	(TAA)18	389
25	UASBC25	LOC101501439	Ca2	TA110-H3A12	TCGATTTGCTCTAATACTGCTGAC/ CCTCTGCCTTGAATACTCCTTG	(AT)11, (TA)9	344
26	UASBC26	LOC101502078	Ca2	TA110-H3A12	TACTCCTTCAACGTACCAACCC/ TGGCGAGACAAATACACAACTC	(TTTTA)6	393
27	UASBC27	LOC101490665	Ca2	TA110-H3A12	TGCCCCAACCTTAGAAGATAAA/ CTTTGCTCACAACACAACCATT	(AT)12, (A)11, (A)12	355
28	UASBC28	LOC101503797	Ca2	TA110-H3A12	TTGTTGCTCTTTATGGTCCTCA/ GTGCTTGCATTAGTCTTTCTTCC	(GATTAT)6	364
29	UASBC29	LOC101488825	Ca2	TA110-H3A12	ACCCTGCTCTTCTTCCTTTACC/ ACAACCCCAAATGCAATGTT	(GA)7	400
30	UASBC30	LOC101488825	Ca2	TA110-H3A12	GTGTGGTGTCTGTTGGGCTAT/ACCTC ATTAGAGAAGGTTTACATGC	(TTTA)6	378
31	UASBC31	LOC101510540	Ca2	TA110-H3A12	TGTGGGTTACAAAGCAAGAGAA/ TTCAAAACAGGAAGTGGGTCTT	(TA)12	399
32	UASBC32	LOC101505498	Ca2	TA110-H3A12	GGATGATGCTACCGTTGATTTC/ TTGGAGAAGGTCCGATAAGAGA	(AT)21	202
33	UASBC33	LOC101506689	Ca2	TA110-H3A12	CATCCAAATGCAGTCCTCAGT/ CCGTTTCATATAGTTGTGTCGG	(TAAT)6	377

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
34	UASBC34	LOC101494099	Ca6	CaM1402-CaM1101	AGGGAACAACACCCAGATAGTC/ TTAAGAAGGGTTGGGCTCAC	(AT)21	398
35	UASBC35	LOC101513415	Ca6	CaM1402-CaM1101	GGTCAAGCCCAATAAATCACTC/ CGTTGCACATCCACATTCTACT	(TTAT)11	195
36	UASBC36	LOC101498314	Ca6	CaM1402-CaM1101	GCTCCAAAAGATTCACAAAAGG/ AGCTGCACTTAATGGAAATGGT	(AAT)12	387
37	UASBC37	LOC101507117	Ca6	CaM1402-CaM1101	AACAATCAAGACGCTAATTGGG/ GGATCGGGGTAGCATAACAAC	(ATA)9, (AAC)9	380
38	UASBC38	LOC101508263	Ca6	CaM1402-CaM1101	CATACAAATATGGTGGGTGGC/ CAATGCAAGGACAGGAACATAA	(TA)21	395
39	UASBC39	LOC101499413	Ca6	CaM1402-CaM1101	CTTTGAGGACTTGGTCAGATTG/ CATGTTTCGTTATAGGAGGAGC	(ATA)17	395
40	UASBC40	LOC101497778	Ca6	CaM1402-CaM1101	GTTGGAATGTTGCGAAGATGTA/ GTTTTGTCCTTTGCGTCTCTCT	(ATT)13	365
41	UASBC41	LOC101514741	Ca2	TA200-TA37	GTCAAGAATGCCGATGTACG/ ATATTAGACCATTTCCTTGGGC	(AT)16	376
42	UASBC42	LOC101488826	Ca2	TA200-TA37	GGCAGGCAAACAAACTACATACT/ ATCACTCCACAAGCCGTTTAAT	(AAT)11	359
43	UASBC43	LOC101488826	Ca2	TA200-TA37	GCTTGTGGAGTGATGGTTTTAT/ AGAGTTGAATGAGAGAGAACGG	(TA)6, (A)10, (AT)7	335
44	UASBC44	LOC101492140	Ca2	TA200-TA37	TTCTGCTGGTAAGAAATGGGAT/ CAAAGATCAAGAGCAATGAGGA	(TGG)8	191
45	UASBC45	LOC101494422	Ca2	TA200-TA37	AACCCAGAGAAGTCAGATCCAA/ TACCATCCCAACGTGTCATCTA	(ATATT)14	338
46	UASBC46	LOC101494422	Ca2	TA200-TA37	CCAGCAATCAAAGAATGAACAC/ GCTAGTCTCACCAACGGGTC	(TA)13	230
47	UASBC47	LOC101512465	Ca2	TA200-TA37	GAAGTTGTAAGACCCGCATTTT/ TAGAAGTGGGGAACGTGGTATT	(TAA)18	306
48	UASBC48	LOC101507653	Ca2	TA110-H3A12	TCCTTTTCTCTTGAGCTTGACTG/ ACGATGGGATAACTGAGGAAGA	(AC)24	160
49	UASBC49	LOC101514850	Ca2	TA110-H3A12	GTACTCCCTCCCTGTCGC/ GCACCACACTTCATTCCTATGT	(AT)7, (TA)7	364
50	UASBC50	LOC101492815	Ca2	TA110-H3A12	ATCATGTGTTCCCCTATTTGTG/ AACTCTAATGCACACCAACCAA	(TAT)23	282

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
51	UASBC51	LOC101500751	Ca4	A07C-TS72	CAAGCTCTGGTAAGCATAAGCA/CTT TACTTCTACACTCAACGGATCA	(TA)10	369
52	UASBC52	LOC101508554	Ca4	A07C-TS72	ACGCTCTTGTCTTTTGTCACTT/ AGTAGTCATCCTCCGGTAATCA	(T)11	389
53	UASBC53	LOC101510924	Ca4	A07C-TS72	TATTTTGTACGGCATAGCCAGA/ GAAAACAATGAGACCCAACACA	(TA)22	313
54	UASBC54	LOC101494165	Ca4	A07C-TS72	ACTTCAGCAACAGAATCAACCC/ CCCATCATATACTCCAAGTCCAA	(A)17	399
55	UASBC55	LOC101512465	Ca2	TA27-TA59	GAAGTTGTAAGACCCGCATTTT/ TAGAAGTGGGGAACGTGGTATT	(TAA)18	306
56	UASBC56	LOC101490121	Ca2	TA27-TA59	CCAACAATTCAACTTCCTCCAT/ TGAGAAACAGATAAACGATACACCC	(AT)20	359
57	UASBC57	LOC105851616	Ca2	TA27-TA59	GACAAGGGAAAGAAGACATTGC/ AGTTTTGGGACTGCATGAGC	(ATA)21, (AT)10	252
58	UASBC58	LOC101498462	Ca2	TA27-TA59	TCCTGTGTGACCATTTCTATGC/ CCCTGTCTAGTAAGATCGTGGG	(AG)16	367
59	UASBC59	LOC101503022	Ca2	TA27-TA59	TCAGCCACACCATTCTAATCAC/ TAAAGTCACCAACCAAAACCCT	(TC)18	355
60	UASBC60	LOC101500167	Ca2	TA27-TA59	TTACTTTTACGAGGCGACTTCC/ ACAATTCGAGCACACAAATCAC	(GA)16	384
61	UASBC61	LOC101502609	Ca2	TA27-TA59	ATCAATATCGTGTGCCCATACA/ GTGTCAACAAACAAATCGGGTA	(AT)23	272
62	UASBC62	LOC101502926	Ca2	TA27-TA59	GGGAAATCGTTATTAGCCACAA/ CAAAGCAAAATCCACAACTCTG	(TTAAT)7	314
63	UASBC63	LOC101498007	Ca2	TA27-TA59	CGAGTAACAATAAGGAAAGGGC/ GTTTGGGTTCTCATGTAGCAAA	(TA)17	279
64	UASBC64	LOC101515069	Ca2	TA27-TA59	AGTATTCTTCACGCTGCAATCA/ CTCTTACAAACCACCAAAACACC	(TA)26	347
65	UASBC65	LOC101505338	Ca4	TR20-TS82	GCATTGGTTGTTTGCTCTGTAT/ TCACTTGGAGTCATTTCGATGT	(TTA)14	191
66	UASBC66	LOC101510592	Ca4	TR20-TS82	AGGCTGAAGGGATAATGACAAA/ TCTAGTGGAAAGGTCGAATGCT	(TA)23	397
67	UASBC67	LOC105851931	Ca4	TR20-TS82	CAAAGCAGAATGTCCATCAA/ CGTTTGTAGCGCAGAATGT	(AGA)18	382

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
68	UASBC68	LOC101499060	Ca4	TR20-TS82	TCCAGTGACAAATACAATTCCG/ ACAATAGCCCCTGAAACAGAGA	(TCAT)8	171
69	UASBC69	LOC101488417	Ca4	TR20-TS82	GAAAGAAGAAAGGAAGGGGAAG/ GTTAGTGATGTGATGAGTGTGATGA	(AT)19	340
70	UASBC70	LOC101507596	Ca4	TR20-TS82	CCAAACTCTCCTCCCTACCTCT/ AGACATTCCATCTCCCAAGAAA	(TCA)10	386
71	UASBC71	LOC101508452	Ca4	TR20-TS82	CCACACACAACACAAATCACAA/ AAGCACCAAAGGTAGTGAGGAA	(A)19	313
72	UASBC72	LOC101491183	Ca2	TA110-H3A12	CAAAGCGTTTCTGTGTAACCAA/ TTTGGATGAAGATGTGATGTCC	(ATT)8	285
73	UASBC73	LOC101515723	Ca2	TA110-H3A12	TCTTCCTCACTTCAGATCCCTC/ GAAAACTTCGCTCATCTTTGCT	(TTTC)8	192
74	UASBC74	LOC101493792	Ca2	TA110-H3A12	TGCTTTCGTTCTTGTTGTTG/ CCAGCACATCATAAATACATCC	(AAT)9	287
75	UASBC76	LOC101498314	Ca6	CaM1402-CaM1101	GCTCCAAAAGATTCACAAAAGG/ AGCTGCACTTAATGGAAATGGT	(AAT)12	387
76	UASBC77	LOC101497987	Ca6	CaM1402-CaM1101	AAGTCCTTAATGTATCTCGCGG/ CTTCCTTGTTTTGCAGCTTTCT	(GAT)10	278
77	UASBC78	LOC101499300	Ca6	CaM1402-CaM1101	AAACTCAGCATTTTAGGCCG/ GAAGGCTACCACTACGCAATCT	(TTAT)7	398
78	UASBC79	LOC101504726	Ca6	CaM1402-CaM1101	TTTTCTGAGGCAACATGCTCTT/ CCGAATGACAAGATTCCACACT	(TA)19	305
79	UASBC80	LOC101491914	Ca6	CaM1402-CaM1101	CTGGAATCTCCCATTCGGT/ GAGAACTTAGAGAAGGTCAATGCAA	(AT)6	396
80	UASBC81	LOC101514179	Ca6	CaM1402-CaM1101	CACAATTTCCCTTTCTCTGCTT/ TCTTACCCTGACATTTTCCTTGTAG	(T)53	238
81	UASBC82	LOC101510511	Ca6	CaM1402-CaM1101	TGCTGGTTACACCTTGCTTATG/ TCTAAAGCCAAAGGAATGGAAG	(ATT)26	271
82	UASBC83	LOC101492347	Ca6	CaM1402-CaM1101	ATGTGTGTTGTATCCATTGCGT/ GATTGATGCCTAAAATGCAGGT	(ATCT)22	355
83	UASBC84	LOC101510625	Ca6	CaM1402-CaM1101	TGCAACGTAGAGCAATTTTGAC/ TCATTTCCATGACTTTCTGAGC	(TAT)20	278
84	UASBC85	LOC101509650	Ca6	CaM1402-CaM1101	ATTAGAGGCAAACAAGAACCGA/ ATGTTGAAGTTGGGAAAACGAC	(TAT)22	391

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
85	UASBC86	LOC101509650	Ca6	CaM1402-CaM1101	TTTCAAGTTTTCAACCTAGCGG/ ATTCTCGTGCCAAACATTACCT	(TTA)14	239
86	UASBC87	LOC101507324	Ca6	CaM1402-CaM1101	AATGGAAAGGAAGAGAGCAACA/ TATTGTCCCCTAGCAAGCATTT	(TCA)21	314
87	UASBC88	LOC101502054	Ca6	CaM1402-CaM1101	CTTTCAGAGGAAAACGAACGAT/ GGAAGGGAGGCTATAAAATGCT	(TA)8	201
88	UASBC89	LOC101502054	Ca6	CaM1402-CaM1101	GAGCGCGAGGAGATTCAG/ CTGAGGTGGGAGAGTTAAGGAG	(CT)7	400
89	UASBC90	LOC101502054	Ca6	CaM1402-CaM1101	GTGTCATTGGCTTTGAGATTGT/ AACAACAGCAGGTTCGTCCTAT	(TC)7	264
90	UASBC91	LOC101489008	Ca6	CaM1402-CaM1101	ACTGGATGCTGGTTGGAGAT/ GTTGGCTTGTTCCCATCTTTT	(TA)18	395
91	UASBC92	LOC101500886	Ca6	CaM1402-CaM1101	GGACCTAGTTTTCCCGTCTTCT/ TGACAAGGACTATTTTGAGCGA	(AT)20	317
92	UASBC93	LOC101506664	Ca6	CaM1402-CaM1101	AGTGCAGGTGTAAATCATGTGG/ CCTTCGTACTCATCCTCTAAGGC	(AT)8	233
93	UASBC94	LOC101500576	Ca6	CaM1402-CaM1101	ACCAAGTGTATGGGACACCTTT/ ACATCTCCATTGCTTCCATCTT	(TA)8	174
94	UASBC95	LOC101489545	Ca6	CaM1402-CaM1101	GCGGTTCAAAAGAGTGAAGAAT/ CGTCATACTCATCAAGCGGTTA	(GAA)14	335
95	UASBC96	LOC101488897	Ca6	CaM1402-CaM1101	TGAAAGCACCACTATGGCTAAA/ TGTCAACTTTATGTCTCCGATAGC	(CT)23	391
96	UASBC97	LOC101506340	Ca6	CaM1402-CaM1101	TCTTCACTTCCCAGGTTCCTTA/ TGAGTTGCTTGTCTCACCAGTT	(T)44	368
97	UASBC98	LOC101499296	Ca6	CaM1402-CaM1101	GCGGCCACACACCTTATT/ AAAATAGGAGGGCTAATTCCGT	(AT)23	226
98	UASBC99	LOC101505364	Ca6	CaM1402-CaM1101	TGCAGAAGATATGGGGAGTTTT/ AAAGCTAGGAAACATAGGCACG	(TC)19	205
99	UASBC100	LOC101512664	Ca6	CaM1402-CaM1101	AGTGGAAGTAGAGGGATTAGACGA/ TGTAATGTGATTGGTAGACCGC	(CTATTA)33	368
100	UASBC101	LOC101514938	Ca6	CaM1125-H4E09	TTGAAAAGTCACGATGGATCAG/ ACCCTGCAACCAAAGAATGA	(TG)6	342
101	UASBC102	LOC101514938	Ca6	CaM1125-H4E09	AACAAACACGACCCTGTCACTA/ ATCAGTTGCTGCTTCCTCATCT	(T)16	350

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
102	UASBC103	LOC101507318	Ca6	CaM1125-H4E09	CATATCTCCAAACACGTCATGC/ AACCATATCACAACTGGGTCATC	(ATT)6	321
103	UASBC104	LOC101509330	Ca6	CaM1125-H4E09	TATCACACTCGATACACACCCC/ TAAATTCGCTCTTTCCTGCAAC	(T)65	241
104	UASBC105	LOC101508375	Ca6	CaM1125-H4E09	TCTATGGAAACCCAGTGAGCTA/ CTATGCACAAATTCATTCACCC	(TTA)18	332
105	UASBC106	LOC101494922	Ca6	CaM1125-H4E09	AGTCGAAATGAAGAACTCCACC/ TATATCTCATCCTGCACCCAAA	(AAT)17	365
106	UASBC107	LOC101494922	Ca6	CaM1125-H4E09	AGTCGAAATGAAGAACTCCACC/ TATATCTCATCCTGCACCCAAA	(TAT)20.333	365
107	UASBC108	LOC101490890	Scaffold 96	-	ATAGCACGGGGTCATATAATGG/ TCCCCATCAGAACCTTCGTA	(TA)18	333
108	UASBC109	LOC101490890	Scaffold 96	-	ATTTGTCATTTGAGTTGCGG/ ACACAAGGCTATGGAGGAAAAT	(TA)16	267
109	UASBC110	LOC101499861	Scaffold 766	-	TTGAGATGTGAGTGAAGTGATGTG/ AAAATGAAGACGACTGTTTCGG	(TA)13	396
110	UASBC111	LOC101500195	Scaffold 766	-	TCTTCGCACTCTTCAGGTATTG/ CCGCAGTCTATGTTGTTAGTTTG	(TA)14	303
111	UASBC112	LOC101488843	Scaffold 766	-	AGTTTCCTTTGTGAGAGGGTTG/ CCCATCATCTTTTACATGCTCA	(ATG)8	361
112	UASBC113	LOC101500363	Ca6	H4E09-LOC101504400	CACGCAGTTTTATAGTCGGTGT/ TTGACGAAGGATGACACTTGAT	(TA)17	374
113	UASBC114	LOC101500675	Ca6	H4E09-LOC101504400	ATTCGGATAGGTTGGTTTGAGT/ CTTTGTTTCATTTAGTCCTCGG	(TA)17	234
114	UASBC115	LOC101489737	Ca4	TR20-TS82	ATTGGCATCAATGTGTGAGAAG/ CTCAATGTCCCCTAAACCAAAA	(TGA)8	366
115	UASBC116	LOC101500679	Ca6	CaM1402-CaM1101	GATTCACCGCAAATTCAGACA/ AATGACCCCACACACCACTAAT	(TTATT)6	287
116	UASBC117	LOC101514293	Ca6	CaM1402-CaM1101	CTGTTGACACATGGCTTATTGC/ TCGTATCCAGGCCAACTAAAAT	(ATT)9	377
117	UASBC118	LOC101496017	Ca6	CaM1402-CaM1101	CAATGTGGGTTTGGTATGTTCA/ GGTGGAGTGTGCAAAGGTATTT	(TA)18	315
118	UASBC119	LOC101511722	Ca2	TA110-H3A12	CATGATTTTCGGTGTTCGACT/ TGGTGTGCGAGTTTTAACAAAG	(AAC)7	399

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
119	UASBC120	LOC101494641	Ca2	TA110-H3A12	TATGGGTGGTGATCTAGGGTTC/ ATTCATGTCTCAGTGTTGGTGC	(TTG)6	325
120	UASBC121	LOC101505729	Ca2	TA110-H3A12	AAGAATAAAAGAAGCGCGAGTG/ GGCGGTTGAGAGTAAGAGAGAG	(CAT)7	200
121	UASBC122	LOC101507653	Ca2	TA110-H3A12	TTGGATTCTCAGGGTTCATTCT/ CCATTTTCAAGCAAGTGCAA	(TTTA)6	390
122	UASBC123	LOC101507145	Ca2	TA110-H3A12	GTCATGTCAAATTAGCACGCTC/ AGAGACCAATCCTGAAAGCAAG	(TTG)7	363
123	UASBC124	LOC101490870	Ca2	TA110-H3A12	CTGGGTATATGCTATTCTGGGG/ TCGGGTACTTCTCCATCTTCAT	(CAT)7	262
124	UASBC125	LOC101498006	Ca2	TA27-TA59	GTGCTGAGGAGAAGTAGAAGCAA/ AATCAAACCACCATTCACACCT	(GGT)7	327
125	UASBC126	LOC101502926	Ca2	TA27-TA59	GGGAAATCGTTATTAGCCACAA/ CAAAGCAAAATCCACAACTCTG	(TTAAT)7	314
126	UASBC127	LOC101496486	Ca2	TA27-TA59	ATTTTGAATCTATGCAGGCACC/ TCCACCACCACAATCTTCATTA	(CAA)7	386
127	UASBC128	LOC105851616	Ca2	TA27-TA59	GGGTCATATAGGGTCACACAGC/ TCCCAGAGTAGAGGAGATGAGG	(TAAA)6	365
128	UASBC129	LOC101502968	Ca4	TR20-TS82	CAAGTTTTGCCTCCATCTATGA/ TCACCAAAGCAATCTTATCCG	(TTA)9	369
129	UASBC131	LOC101510183	Ca6	CaM1402-CaM1101	CTCTTTTGCATGAATTGGAGC/ GCCACACACAGAACTCAACTTT	(ATA)8	297
130	UASBC132	LOC101497650	Ca6	CaM1402-CaM1101	GGACCCTATTGCCTAAATCACA/ CTCCCTCCGTTTCTAAATACAAGA	(TAAT)6	283
131	UASBC133	LOC101493243	Ca6	CaM1402-CaM1101	TGTAAATGTGTGAACCTCGCTC/ GACGAAAACGAGAACGAAGAAG	(CTT)7	332
132	UASBC134	LOC101488343	Ca6	CaM1402-CaM1101	AGACGGAGGAGAATGACTGAAA/ AGGCAAGGTGAAGAAGCATAAC	(TTA)7	305
133	UASBC135	LOC101492117	Ca6	CaM1402-CaM1101	CACTTATTTCACACACACACTCTCTC/ TTGTCCCAAAAGGATCAACTCT	(ACA)7	162
134	UASBC136	LOC101492791	Ca6	CaM1402-CaM1101	TTCCTCTCTCTCTCCTCCTTTTC/ AAGGGTGTAATGGGTTGTTCTC	(CTT)8	114
135	UASBC137	LOC101503235	Ca6	CaM1402-CaM1101	ATTCAACTCGGGACCTTACTG/ AAAGGGTACATAACACAATGCC	(AT)20	397

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
136	UASBC138	LOC101498648	Ca6	CaM1402-CaM1101	CTTCTTCCCTCTTCTTCCAACA/ ACCTGATCCAAAACAACACACA	(TC)18	397
137	UASBC 139	LOC101495609	Ca2	TA110-H3A12	CCTTTTGTTGTTTGGGAAGCTA/ TATTTGAATGTGTAGGGCATGG	(AT)14	337
138	UASBC 140	LOC101500284	Ca2	TA110-H3A12	CCCACCACACAGAAGAAAGAA/ AGAAGGCAAGAGAGAAGAGGGT	(TC)13	113
139	UASBC 141	LOC101505729	Ca2	TA110-H3A12	AAGAATAAAAGAAGCGCGAGTG/ GGCGGTTGAGAGTAAGAGAGAG	(CAT)7	200
140	UASBC 142	LOC101506051	Ca2	TA110-H3A12	GTAATGAGCCCAAAACGAAGTC/ CTGATATGCGATTGTGTGTGTG	(TC)11	255
141	UASBC 143	LOC101506576	Ca2	TA110-H3A12	TGGTTATGAGTGGTTGACTGTG/ TATCTTGAATGACCCTGACTGC	(AT)12	398
142	UASBC 144	LOC101489481	Ca2	TA110-H3A12	CCTCTAGGGAAACACCTCAAAA/ CAATGCTTACTTCACTTGGTGC	(GAA)7	207
143	UASBC 145	LOC101508291	Ca2	TA110-H3A12	AAGGTAGGAAGGCTGTAGAGGG/ TCCATTTCTTCACTTTGGGACT	(AT)13	310
144	UASBC 146	LOC101497475	Ca2	TA110-H3A12	AGAGATAAGCAAAACTTGGCGA/ ATCAATGGGTCAAGAAACCTGT	(AT)10	182
145	UASBC 147	LOC105851616	Ca2	TA27-TA59	TCATTAAGAGATTCCCCGTTCT/ GGTGTATTTTGGATTTTGGCTC	(TA)16	345
146	UABCS 148	LOC101503575	Ca2	TA27-TA59	GGCTTGAATCTACTTCGACTCTTG/ TGCTCCCAAAACACGATTTTAC	(TA)11	316
147	UASBC 149	LOC101511725	Ca2	TA27-TA59	AGAGGCAATAATGGGTGTGTTT/ TGGCCTGACCTAGAAGCATATT	(AT)11	250
148	UASBC 150	LOC101511725	Ca2	TA27-TA59	GCCAAATACCGATGGAAAATG/ TCTCACCTGCAATAAGCATCAA	(TA)14	343
149	UASBC 151	LOC101495940	Ca2	TA27-TA59	AGTTTCTATGAATTTGGCGCTC/ TGTGTGTCTTCCCTTTATGACC	(CT)13	199
150	UASBC 152	LOC101496486	Ca2	TA27-TA59	CGTCCTCTTCTGCTGGTCTACT/ CTTATGATCGTGGTGCAAATGT	(T)34	330
151	UASBC 153	LOC101506851	Ca4	TR20-TS82	TACAGGTCAGAAGCAAAGGACA/ TGTAACCGAAAGTTCATCGCTA	(TA)11	368
152	UASBC 154	LOC101508663	Ca4	TR20-TS82	GCTCACAGTTGCCACAATTTAG/ ATTTATGGTCTCATGCAATCCC	(AT)13	398

Sl. No.	Marker name	Genbank Id.	Chromosome/ scaffold	Markers flanking genomic regions	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)
153	UASBC 155	LOC101507596	Ca4	TR20-TS82	TTCAGGGAGATAGACGACCATT/ TGGTAACTTGCATTCACCCAT	(TA)13	364
154	UASBC 156	LOC101511284	Ca2	TA200-TA37	GGTCTTTTGAGGGCTTATTTCC/ CGTGGGCATAGTTCATATCTCA	(TC)12	275
155	UASBC 157	LOC101498314	Ca6	CaM1402-CaM1101	ATGATGCGTGGAGAAGGAAA/ CACACCACCACTACCATAAATCA	(TAA)7	363
156	UASBC 158	LOC101501846	Ca6	CaM1402-CaM1101	GCGCTGTAAAAGACCTCCTTAT/ TGACCTGCCATAACTGATTTTC	(TA)11	348
157	UASBC 159	LOC101512758	Ca6	CaM1402-CaM1101	GTGTCATGTTCGGTGATGTCTT/ TCTCTCTCAAGCCAAACCTTTC	(GT)11	202
158	UASBC 160	LOC101498313	Ca6	CaM1402-CaM1101	GACAAACCTTGAAAGCACATGA/ AGGAGGGGTGTTGACATATAGC	(AT)13	377
159	UASBC 161	LOC101497651	Ca6	CaM1402-CaM1101	GAGCATCTTTTCAATCGACCTT/ AGGTGGTCTTCCAGAACTTTGA	(CAA)7	342
160	UASBC 162	LOC101489660	Ca6	CaM1402-CaM1101	TTCCTATTCTCCCAATGCAA/ GTACTCATCTTGCGGGTTTAAT	(AT)13	293
161	UASBC 163	LOC101502686	Ca6	CaM1402-CaM1101	ATTAACCATCGCGCTCTCTCT/ TGGATCAGAAATGACGAATCAC	(CT)14	209
162	UASBC 164	LOC101502383	Ca6	CaM1402-CaM1101	TTATCCCACGCGGTGTTATT/ TGTGGAGTGCTTTTGTCTCACT	(TA)15	242
163	UASBC 165	LOC101497985	Ca6	CaM1402-CaM1101	TATGGTTGAGTTTTGGGTAGGG/ ACGTGGGTTACGTGAAATCAG	(TA)12	226
164	UASBC166	LOC101500194	Ca3	TR24-TC14801	AACGCAAGTGAAGAAAGAAACC/ CGAACCAGTGTAATGCAATTCT	(CT)10	343
165	UASBC167	LOC101501469	Ca3	TR24-TC14801	AACCAAGTGTACCTGCTGTTTT/ GCATGACGACTACAAAATTACG	(TA)12	264
166	UASBC168	LOC101510893	Ca3	TR24-TC14801	GTTGATGGATTGGAATGTGTTG/ TATATGCAAAGCCGCCGT	(CT)73	380
167	UASBC169	LOC101503824	Ca3	TR24-TC14801	TTACTCTTTCTCTGCAACTGCG/ CAATTTAGCATCGGTTGGTTTC	(TTA)16	275
168	UASBC170	LOC101509709	Ca3	TR24-TC14801	ACCGTCCAATAAAGAATGTGCT/ GAAGTGAAAGCCGAATCAAAAG	(AT)16	397

Table 10: List of novel genic SSR markers polymorphic between chickpea genotypes

Sl. No.	Polymorphic between JG62 and WR315	Polymorphic between K850 and WR315	Polymorphic between JG62 and K850
1	UASBC4	UASBC6	UASBC4
2	UASBC6	*UASBC22	UASBC6
3	UASBC8	UASBC24	UASBC8
4	*UASBC22	UASBC34	*UASBC24
5	UASBC32	*UASBC45	UASBC32
6	UASBC39	UASBC50	UASBC34
7	UASBC48	UASBC51	UASBC50
8	UASBC50	UASBC53	UASBC53
9	UASBC53	*UASBC57	UASBC82
10	*UASBC54	UASBC79	UASBC84
11	UASBC57	UASBC84	*UASBC87
12	UASBC65	*UASBC86	UASBC92
13	*UASBC76	*UASBC87	UASBC95
14	UASBC79	UASBC98	UASBC104
15	UASBC82	UASBC100	UASBC114
16	UASBC92	*UASBC104	UASBC168
17	*UASBC95	UASBC109	UASBC169
18	UASBC98	UASBC114	-
19	UASBC100	*UASBC118	-
20	*UASBC116	*UASBC135	-
21	*UASBC144	UASBC164	-
22	*UASBC164	*UASBC165	-
23	*UASBC165	UASBC168	-
24	-	UASBC169	-

*Markers showed polymorphism in 8 % PAGE whereas, as remaining in 3.5 % agarose

However, in the present study, the diverse intraspecific parental lines were used which resulted in moderate level of polymorphism. The genotypes were also diverse at phenotypic level such as resistance to *Fusarium* wilt, double poddednesss, seed size and other morphological traits. Therefore the RILs used in the study were considered as an ideal population to map QTLs for wilt resistance.

4.3.2 Genotyping of RILs

The polymorphic SSR markers identified in the present study were used to genotype two mapping populations. The first mapping population consisted of 125 RILs (> F_{10}) of cross JG62 × WR315 JW and second mapping population consisted of 141 RILs (>F_{11}) of cross K850 × WR315 KW. All the polymorphic markers showed segregation in their respective RILs (Plate 2 and 3).

4.3.3 Chi square test (χ^2)

The χ^2 test was used for segregation analysis of individual markers for the expected monogenic 1:1 ratio in RILs. Twenty three markers were polymorphic in the RILs of JW. The χ^2 test showed that all the twenty three markers followed the expected Mendelian segregation ratio of 1:1 (Table 11). Twenty four markers were polymorphic and used for genotyping of 141 RILs of KW. The χ^2 test revealed that only two markers (UASBC168 and UASBC169) showed segregation distortion ($P<0.05$) from the expected Mendelian ratio of 1:1 (Table 12). Both these markers were skewed towards female parent (K850). Hence, only 22 markers were used for linkage map construction. In the present study only nine per cent of the markers showed segregation distortion in one of the crosses which is significantly low when compared to earlier reports of Tullu *et al.*, (1999) (30 %), Winter *et al.* (2000) (38.4 %) and Flandez-Galvez *et al.* (2003) (20.4 %). The observed segregation distortion in the present study is in favour of the maternal alleles (K850) as reported from Flandez-Galvez *et al.* (2003). 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. In the present study, the frequency of markers showed segregation distortion was nil in one set of RILs and only nine per cent in another crosses. The map estimated in the present study is based on only non-distorted markers hence not affected by segregation distortion and hence more reliable. All the 23 markers for JW and 22 markers for KW were used for the construction of linkage map in the present study.

4.4 Construction of linkage map and identification of QTLs for Wilt resistance

4.4.1 Genetic linkage map construction

In recent years, the use of molecular markers has accelerated plant breeding in a number of areas including disease resistance. The order and relative distance of genetic markers that are associated with trait of interest can be determined by genetic mapping (Xu, 2010). The recombination frequency between two linked genetic markers can be defined in terms of genetic distance known as centiMorgans (cM). The availability of molecular markers and genetic linkage maps is the foremost step for identification of QTL for traits of interest in any crops (Paterson, 1996; Winter and Kahl, 1995).

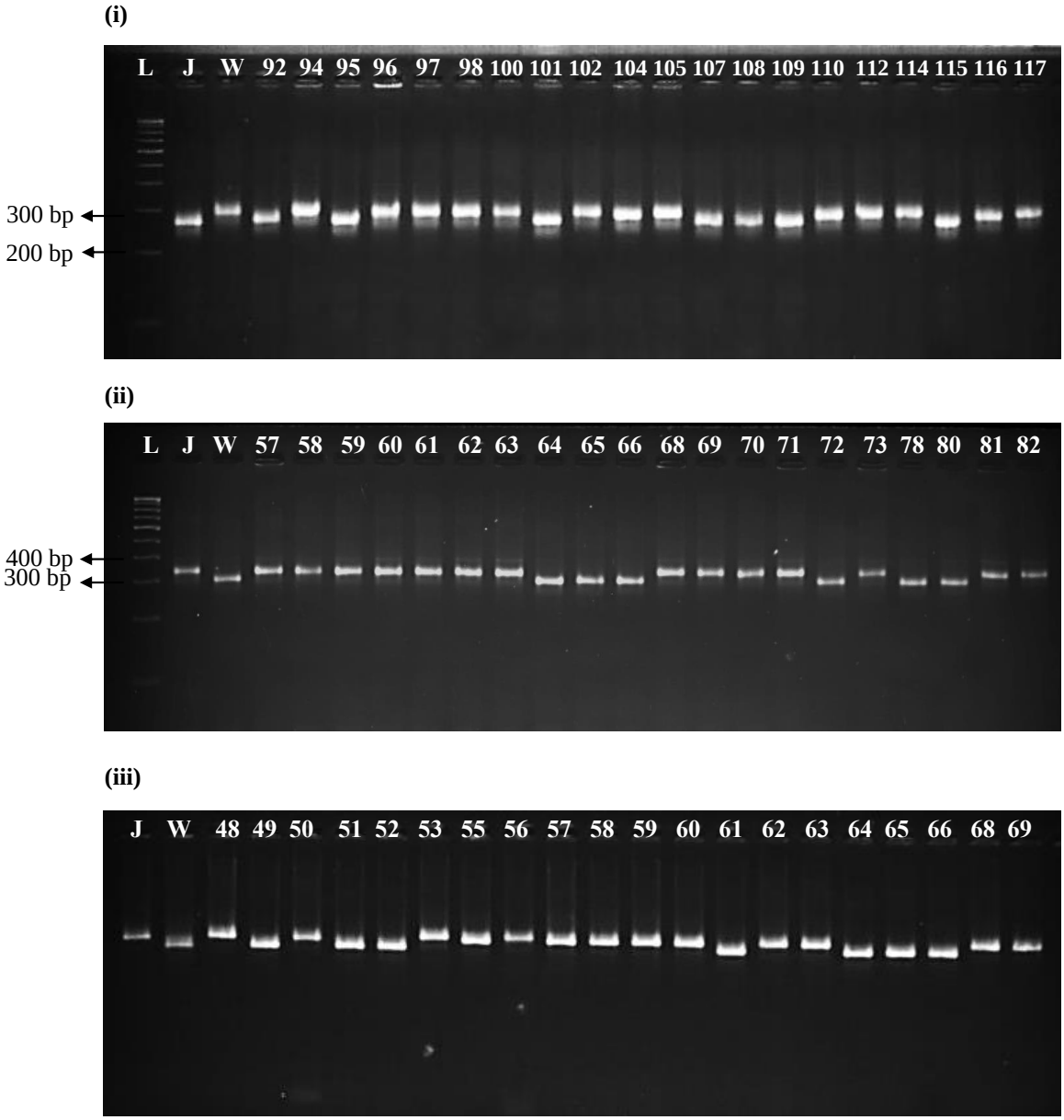


Plate 2a: RILs of JG62×WR315 showing segregation for Primers (i) UASBC57, (ii) UASBC82 and (iii) UASBC100 in 3.5 % agarose

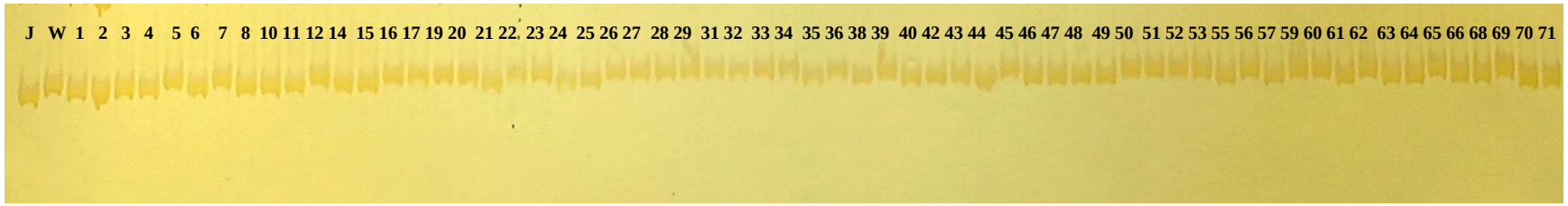


Plate 2b: RILs of JG62×WR315 showing segregation for Primers UASBC54 in 8 % PAGE

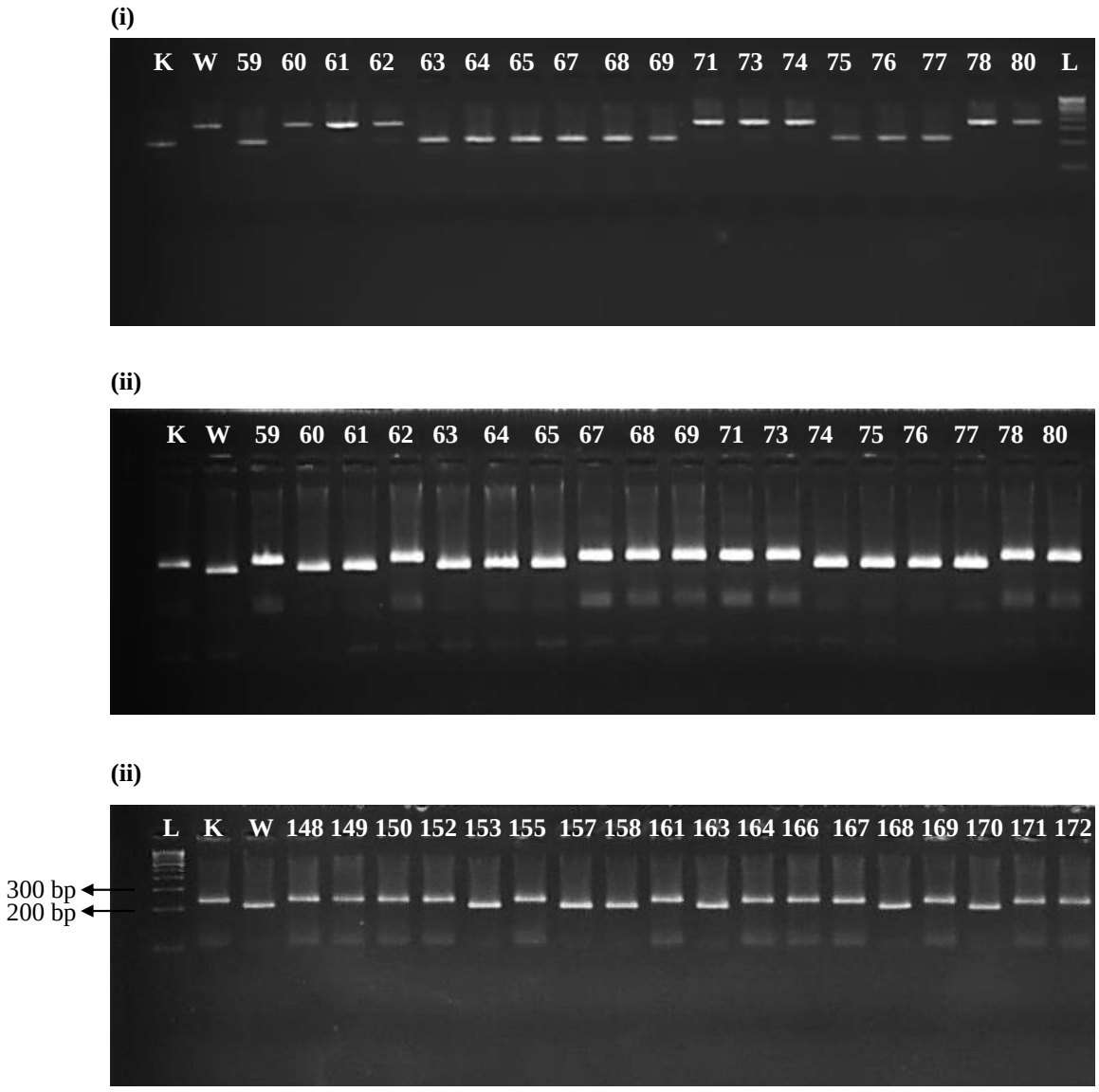
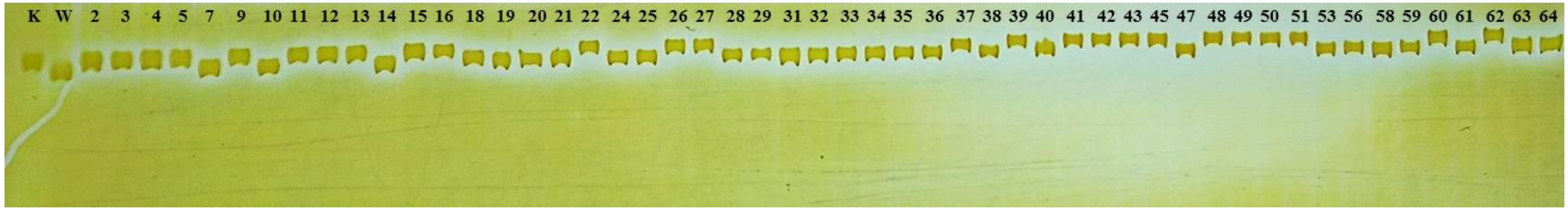


Plate 3a: RILs of K850×WR315 showing segregation for Primers (i) UASBC51, (ii) UASBC98 and (iii) UASBC114 in 3.5 % agarose

(i)



(ii)

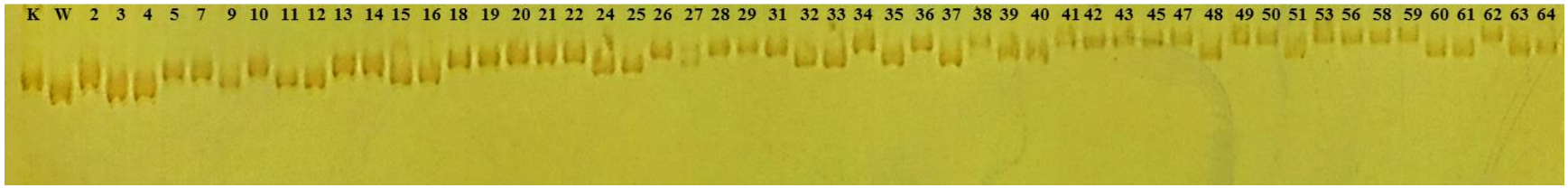


Plate 3b: RILs of K850×WR315 showing segregation for Primers (i) UASBC50, (ii) UASBC87 in 8 %

Table 11. Segregation of markers in RILs of JG62×WR315

Sl. No.	Markers	JG62	WR315	χ^2 values	χ^2 (1:1)
1	UASBC4	65	58	0.40	NS
2	UASBC6	61	64	0.07	NS
3	UASBC8	53	71	2.61	NS
4	UASBC22	71	53	2.61	NS
5	UASBC32	64	60	0.13	NS
6	UASBC39	60	58	0.03	NS
7	UASBC48	57	65	0.52	NS
8	UASBC50	70	53	2.35	NS
9	UASBC53	57	65	0.52	NS
10	UASBC54	70	55	1.80	NS
11	UASBC57	54	69	1.83	NS
12	UASBC65	60	60	0.00	NS
13	UASBC76	53	72	2.89	NS
14	UASBC79	61	64	0.07	NS
15	UASBC82	61	64	0.07	NS
16	UASBC92	57	66	0.66	NS
17	UASBC95	62	63	0.01	NS
18	UASBC98	66	59	0.11	NS
19	UASBC100	67	57	0.81	NS
20	UASBC116	65	59	1.61	NS
21	UASBC144	72	53	2.89	NS
22	UASBC164	59	66	0.39	NS
23	UASBC165	68	57	0.97	NS

NS-Non significant, S-Significant @ 5 %

Table 12. Segregation of markers RILs of K850×WR315

Sl. No.	Markers	K850	WR315	χ^2 values	χ^2 (1:1)
1	UASBC6	72	60	1.09	NS
2	UASBC22	67	73	0.26	NS
3	UASBC24	70	71	0.01	NS
4	UASBC34	81	60	3.13	NS
5	UASBC45	74	67	0.35	NS
6	UASBC50	82	59	3.76	NS
7	UASBC51	79	60	2.60	NS
8	UASBC53	57	70	1.33	NS
9	UASBC57	85	56	5.96	NS
10	UASBC79	67	74	0.35	NS
11	UASBC84	81	59	3.46	NS
12	UASBC86	83	58	4.43	NS
13	UASBC87	78	60	2.35	NS
14	UASBC98	82	59	3.75	NS
15	UASBC100	82	58	4.11	NS
16	UASBC104	82	59	3.75	NS
17	UASBC109	73	67	0.26	NS
18	UASBC114	83	58	4.43	NS
19	UASBC118	82	59	3.75	NS
20	UASBC135	71	70	0.00	NS
21	UASBC164	83	58	4.43	NS
22	UASBC165	77	64	1.20	NS
23	UASBC168	117	23	63.11	S
24	UASBC169	117	24	61.34	S

NS-Non significant, S-Significant @ 5 %

In chickpea the linkage map of both interspecific and intraspecific crosses are available for linkage analysis. Many researchers constructed the genetic linkage map for resistance to *Fusarium* wilt (Winter *et al.,* 2000; Tekeoglu *et al.,* 2000; Benko-Iseppon *et al.,* 2003; Rubio *et al.,* 2003; Sharma *et al.,* 2004; Cobos *et al.,* 2005, Iruela *et al.,* 2007; Gowda *et al.,* 2009; Halila *et al.,* 2009 and Millan *et al.,* 2010) as well as for other traits (Winter *et al.,* 1999; Santra *et al.,* 2000; Cho *et al.,* 2002; Flandez-Galvez *et al.,* 2003; Millan *et al.,* 2003; Pfaff and Kahl, 2003; Rakshit *et al.,* 2003; Rajesh *et al.,* 2004; Cobos *et al.,* 2006; Iruela *et al.,* 2006; Lichtenzveig *et al.,* 2006; Radhika *et al.,* 2007; Ali *et al.,* 2008; Cobos *et al.,* 2009 and Nayak *et al.,* 2010).

4.4.1.1 Mapping population JW

The genetic linkage map was constructed using 23 markers and 125 F_{12} RILs of cross JG62 × WR315. A minimum LOD score of 2.5 and recombination frequency of 0.25 was set as threshold value for linkage determination. The intermarker distances were used to construct the linkage map by ICIM QTL IciMapping version 4.00 software. Twenty two markers were mapped into three linkage groups (LG) (Fig. 6) spanning a total distance of 144.51 cM with an average marker distance of 6.53 cM (Table 13) and only one marker UASBC76 was unlinked. The length of the LG ranges from smallest 20.58 cM (LG2) to largest 73.85 cM (LG 1). The number of markers mapped per LG varied from six (LG2 and LG3) to 10 (LG1). The highest number of markers were observed in LG1 with an average marker density of 7.83 cM and the highest markers density of 3.43 was observed in the LG2 (Fig. 6 and Table 13). Only one marker (UASBC76) was unlinked in this study. Winter *et al.* (1999) constructed genetic linkage map using 120 STMS markers, of these 112 markers were linked to 11 LG covering a genetic distance of 613 cM. Winter *et al.* (2000) utilized 354 markers for construction of genetic map of chickpea with a LOD of 4. Of these 303 markers were mapped to 16 LG spanning a distance of 2077.9 cM and a total of 51(14.4 %) markers were unlinked. Cobos *et al.* (2005) reported a total of 14 markers unlinked out of 125 mapped to 11 LG spanning distance of 330.03cM. Radhika *et al.* (2007) developed integrated intraspecific map of chickpea with a total of 273 markers of which 230 were mapped to eight LG, rest of the markers were unlinked. Bharadwaj *et al.* (2011) did linkage analysis with 49 markers of which 33 markers were mapped on eight linkage groups whereas, 16 markers (32 %) were unlinked. Thudi *et al.* (2011) showed 323 (20 %) markers unlinked in genetic map out of 1,614 novel markers of different classes (ISSR, EST-SSR, CISR, CAPS, DArT and legacy markers) developed by BAC-End Sequences in chickpea. Patil *et al.* (2014) utilized 28 STMS and five AFLP markers for genetic map construction of which 20 STMS and three AFLP markers were mapped to five LG, rest of the markers were unlinked. Barman *et al.* (2014) constructed the genetic map using 57 markers. The 46 markers were assigned to nine LG spanning distance of 671.5 cM whereas, 11 markers (19.29 %) were unlinked. In the present study, the markers used for genetic map were developed through sequence based physical map of FW resistance region. For the first time they were used for construction of genetic linkage map. The markers physically represent a known genomic region of the chickpea chromosomes.

Table 13. Linkage groups, coverage and marker density in mapping population (JG62×WR315)

Linkage group (LG)	No. of SSRs	Length (cM)	Marker density/ Average distance (cM)
LG1	10	73.85	7.83
LG2	6	20.58	3.43
LG3	6	50.08	8.34
Unlinked	1	0	0
Total	**23**	**144.51**	**6.53**

4.4.1.2 Mapping population KW

Twenty two markers which showed normal Mendelian ratio of 1:1 in the mapping population of cross K850 × WR315 were used for construction of genetic map. Twenty two markers formed three linkage groups (LG) spanning a total length of 100.46 cM with an average markers density of 5.23 cM. Fourteen markers were mapped to LG1 with a highest marker density of 3.78 cM. Five and three markers were mapped to LG2 and LG3 with marker density of 5.42 and 6.78 cM respectively (Table 14 and Fig. 7). The genetic distance of LG1 (53 cM) was highest whereas, the genetic distance of LG3 (20.36 cM) was the lowest.

The 14 markers mapped on LG1 were developed from QTL region (*FW-Q-APR-6-1* and *FW-Q-APR-6-2*) (Subbavarapu *et al.,* 2013) and physically mapped on to Ca6. Similarly, the LG2 consisted of five markers developed from QTL-hotspot region for FW resistance on LG2 (Winter *et al.,* 2000, Cobos *et al.,* 2005; Gowda *et al.,* 2009; Patil *et al.,* 2014; Barman *et al.,* 2014), and physically mapped on to Ca2. Similarly, LG3 composed of three markers developed by FW resistance genomic region (Millan *et al.,* 2010; Radhika *et al.,* 2007; Soregaon, 2011; Jingadhe and Ravikumar, 2015) were mapped on Ca4 *in silico*. The markers developed from the same genomic region were clustered together in genetic linkage map. The results enhanced the reliability of the mapping populations used in this study for construction of genetic maps and identification of reliable QTLs for FW resistance and other traits.

Only three out of eight possible linkage groups were obtained in both populations in the present study. The number of markers used in this study was less, therefore the total distance covered in this study (144.51 in population JW and 100.46 cM in population KW) was also less compared to earlier workers (Winter *et al.,* 2000 (2077.9 cM, 16 LG); Cobos *et al.,* 2005 (330.03 cM, 11 LG); Radhika *et al.,* 2007 (509.3 cM, 7 LG and 623.9 cM 7 LG); Nayak *et al.,* 2010 (2602 cM, 8 LG); Sabbavarapu *et al.,* 2013 (347.9 cM, 8 LG) and Patil *et al.,* 2014 (300.2 cM, 5 LG)). The map developed by Winter *et al.* (2000) was considered as a reference map in chickpea till recently. However, the revised genetic map of Nayak *et al.* (2010) was larger in all aspects compared to any other map available till date in chickpea and yielded eight linkage groups which is in

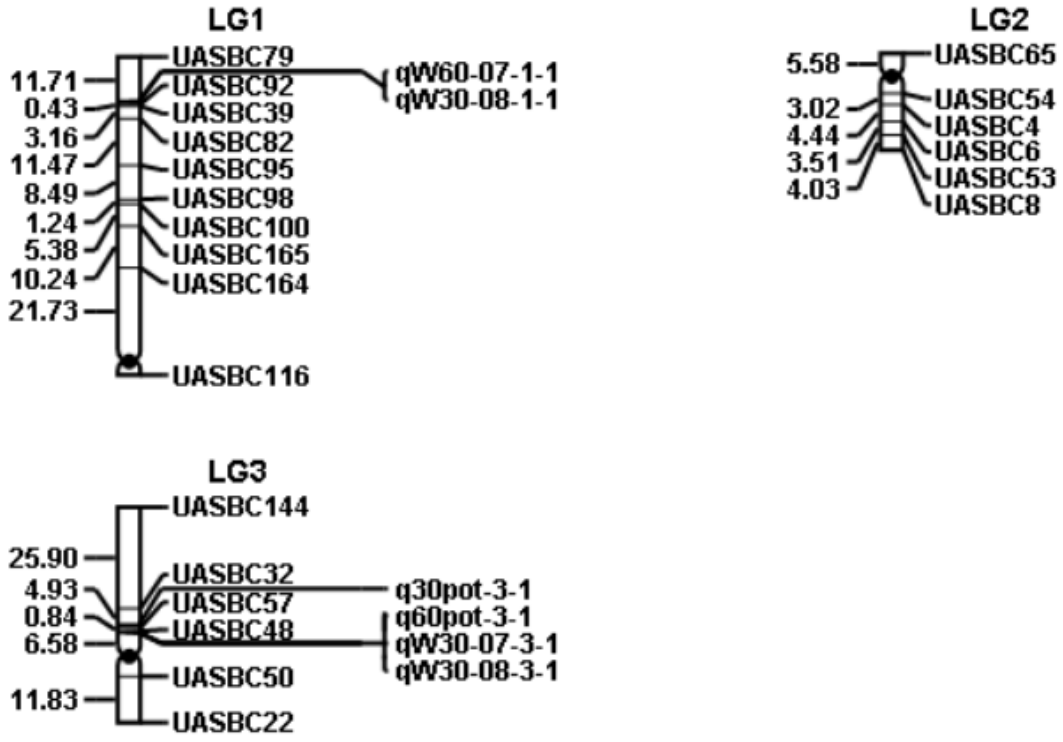


Fig. 6. Genetic linkage map showing marker positions and QTLs associated with *Fusarium* wilt resistance in mapping population (JG62×WR315)

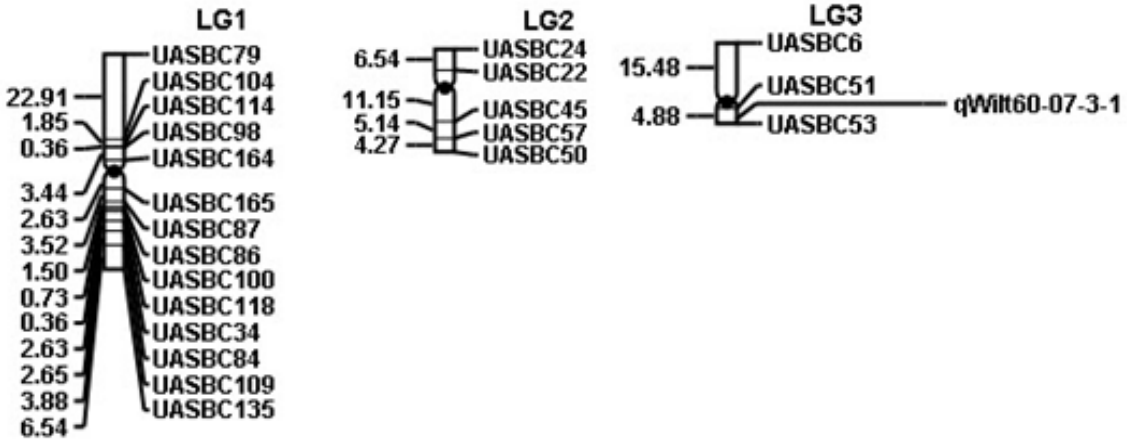


Fig. 7. Genetic linkage map showing marker positions and QTLs associated with *Fusarium* wilt resistance in mapping population (K850×WR315)

agreement with eight chickpea chromosomes, with an average intermarker distance of 4.99 cM. However, the marker used in the present study are all novel hence may be joined to the reference map of Nayak *et al.* (2010) to further improve the map.

The genetic linkage maps of both populations were compared in the present study. The markers which were polymorphic in both populations were mapped on same linkage group (LG) in both genetic maps. The LG1 in genetic map based on RILs of JW corresponds to LG1 based on RILs of KW in which five markers (UASBC79, UASBC98, UASBC100, UASBC164 and UASBC165) were common. The LG2 and LG3 of population JW corresponds to LG3 and LG2 of KW in which three (UASBC22, UASBC50 and UASBC57) and two (UASBC6 and UASBC53) markers were common. The positions and order of the common markers of two mapping populations were not concurrent. For example the order of five common markers of LG1 UASBC79, UASBC98, UASBC164, UASBC165 and UASBC100 were different in population JW and population KW. Millan *et al.* (2010) compared the genetic linkage map of wide and narrow crosses of chickpea. Out of five common markers between wide and narrow crosses in LG1, two were in differing positions. Cobos *et al.* (2005) compared the joint map obtained from two RIL populations with the reference map (Winter *et al.*, 2000) sharing common markers. The clustering and grouping of markers were same in both the populations supporting the reliability of genetic maps.

Table 14. Linkage groups, coverage and marker density in mapping population (K850×WR315)

Linkage group (LG)	No. of SSRs	Length (cM)	Marker density/Average distance (cM)
LG1	14	53	3.78
LG2	5	27.1	5.42
LG3	3	20.36	6.78
Total	**22**	**100.46**	**5.23**

4.4.2. Phenotyping of RIL's for wilt reaction

In the present investigation 125 RILs of population JW were tested for resistance to race 1 of *Fusarium oxysporum* f. sp. *ciceri* (FOC) in greenhouse condition using wilt sick pots. The wilt reaction was recorded as percentage of wilted plants in each RIL on 30^{th} and 60^{th} day after sowing (DAS). The highly susceptible RILs and susceptible parent (JG62) showed complete wilting (100 %) within 30 DAS, while the resistant RILs and parent (WR315) did not show any wilting symptoms (0 %) even after 60 DAS (Plate 4). The wilt reaction observed in wilt sick pots was reliable as the pathogen was uniformly spread in all the pots and disease scoring was unambiguous. The susceptible check JG62 wilted within 30 days in all the test pots. The mean wilting per cent ranged from 0.00-100.00 per cent for both 30 and 60 DAS (Table 15).

Table 15. Estimates of wilt score of RILs of JG62×WR315 in wilt sick pot testing

Trait	Mean wilt score (%)	Variance	Standard error	Range (%)	
				Minimum	Maximum
Wilt reaction (30 DAS)	50.35	1645.89	40.56	0.0	100
Wilt reaction (60 DAS)	70.30	1407.29	37.51	0.0	100

The mean wilt per cent at 30 DAS was significantly lower than mean wilt per cent at 60 DAS (Table 15). The frequency distribution of RILs for wilt reaction confirmed the involvement of many minor genes along with major genes governing the resistance (Fig. 8). The disease reaction of RILs showed continuous variation suggesting polygenic inheritance of wilt reaction along with major genes (Upadhyaya, 1983a and b; Singh *et al.*, 1987a and b).

Earlier reports indicated that resistance for FW was controlled by two to three loci. The resistance to race 1 is controlled by atleast three independent loci designated as H_1, H_2 and H_3 (Upadhyaya *et al.*, 1983b; Singh *et al.*, 1987). The homozygous recessive allele both the loci H_1 and H_2 or partially recessive alleles in homozygous form at either the H_1 or H_2 locus and dominant allele at H_3 locus confers the complete resistance. Later, Brindha and Ravikumar, (2005) showed that the early wilting at seedling stage was governed by dominant alleles at both the loci ($H_1H_1H_2H_2$) whereas, late wilting after flowering stage was controlled by recessive allele at either of these two loci ($H_1H_1h_2h_2$/ $h_1h_1H_2H_2$) and for resistance recessive alleles at both the loci ($h_1h_1h_2h_2$). Recently, number of studies using molecular markers have indicated presence of quantitative loci influencing wilt resistance in chickpea (Gowda *et al.*, 2009; Sabbavarapu *et al.*, 2013; Patil *et al.*, 2014; Jingadhe and Ravikumar, 2015) contradicting the earlier results that only major genes contribute to wilt resistance in chickpea. There could be genes with minor effects that modify the disease response.

The RILs of JW and KW were phenotyped earlier for wilt resistance under field conditions in wilt sick plot of ICRISAT over two years (2007 *rabi* and 2008 *rabi*) in early generations (Jingade and Ravikumar, 2015; Shinde *et al.*, 2010 and Soregaon, 2011). The same data was used for the present study also. The field reactions of RILs for wilt resistance over two years clearly showed that the RILs of population JW showed significantly higher mean wilt incidence on 30 DAS compared to RILs of population KW suggesting the influence of parental lines on the wilt reaction of RILs. JW is a cross between early wilting and resistance genotypes while KW is a cross between late wilting and resistance genotypes. Shinde *et al.*, (2010) observed similar results in sick pot experiment for wilt resistance in which the RILs derived from the cross BG256 x WR315 showed significantly lower mean wilt incidence compared to the RILs of JG62 x WR315 where, the female parent BG256 is a late wilting genotype when compared to JG62 suggesting the influence of parental wilt reaction on segregation of RILs.

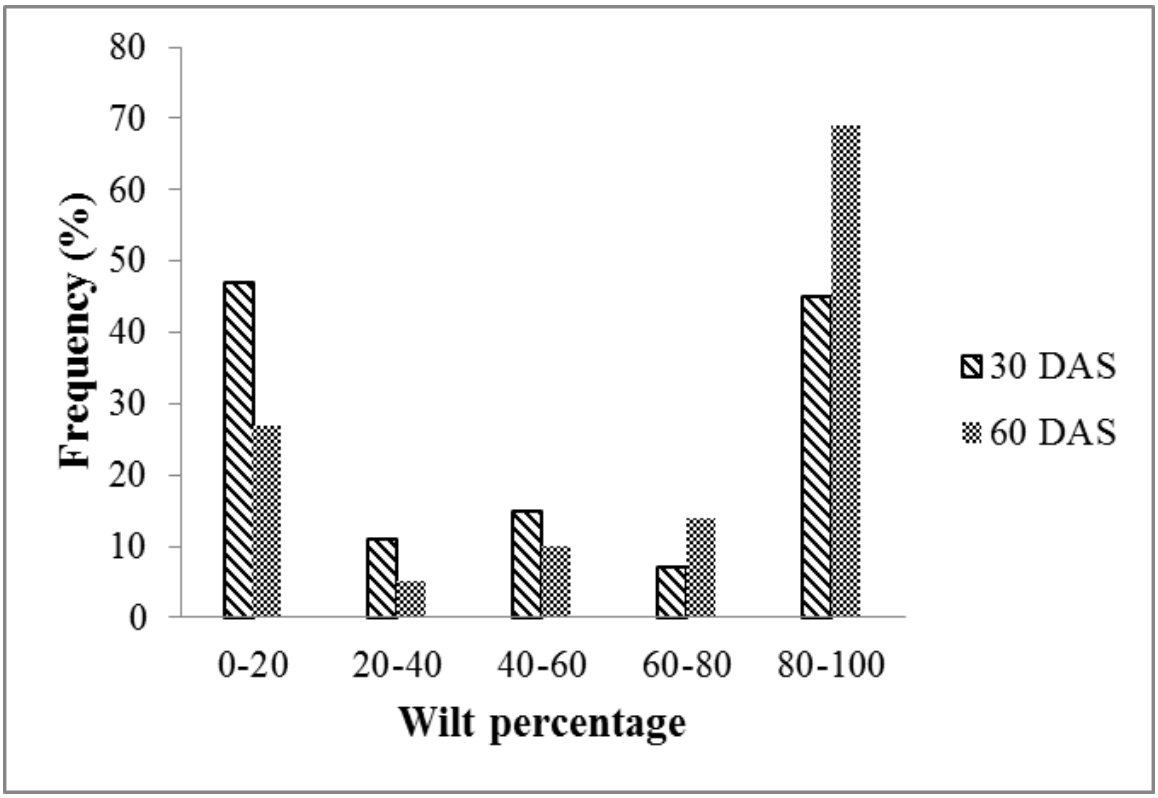
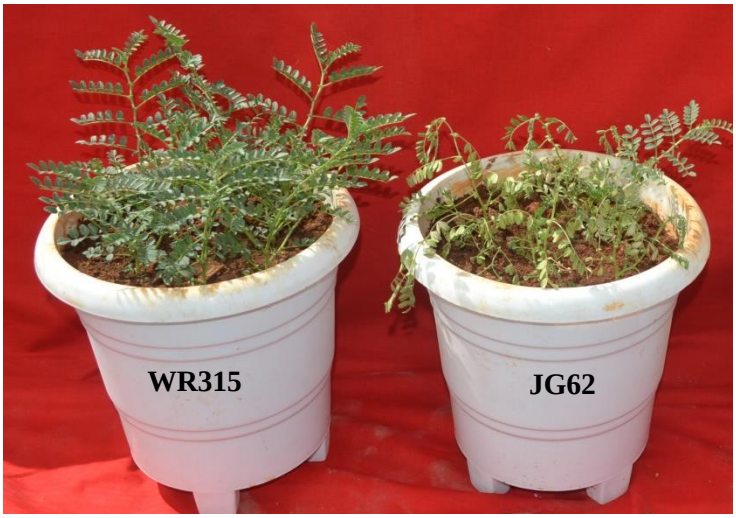
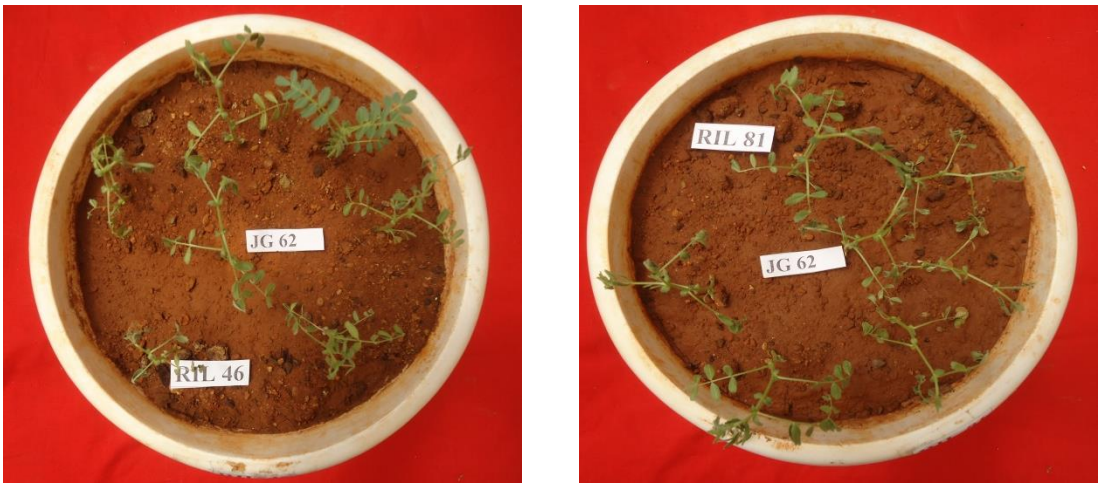


Fig. 8: Wilt reaction of RILs of population (JG62×WR315) at 30 and 60 DAS in wilt sick pot



A. Parental lines



B. Susceptible RILs (46 and 81) with JG62 in the centre for reference

C. Resistant RILs (4 and 40) with JG62 in the centre for reference

Plate 4: Wilt reaction of parental lines and selected RILs of JG62×WR315 in wilt sick pot

4.4.3 Single marker analysis

The assumption that if there is association between trait of interest and marker type, it is likely that the marker type is close to the trait locus (Xu, 2010). Single marker analysis (SMA) is also called as single point analysis. It is the simplest method for detecting QTLs associated with single markers. The statistical methods used for single-marker analysis include t-tests, analysis of variance and linear regression (Collard *et al.,* 2005). Most commonly used statistical method for SMA is linear regression, because the coefficient of determination (R^2) from the markers explains the phenotypic variations arising from the QTL linked to the marker. The main advantages of SMA are the, analysis can be performed using basic statistical software and does not requires complete linkage map (Collard *et al.,* 2005).

4.4.3.1 Mapping population JW

In the present investigation simple linear regression method was used to perform single marker analysis (SMA). The single marker analysis indicated 10 markers (UASBC8, UASBC32, UASBC39, UASBC48, UASBC50, UASBC53, UASBC57, UASBC82, UASBC92 and UASBC144) were associated with FW resistance across both the stages, field and pot experiments (Table 16). The marker having strong relationship was measured using phenotypic variance (PVE) values which indicate the overall percentage of variability of FW resistance at particular scoring time. The phenotypic variance explained by markers ranged from 9.22 to 25.54 per cent for FW resistance. Six markers (UASBC53, UASBC8, UASBC32, UASBC57, UASBC48 and UASBC50) were associated with FW resistance at 30 DAS in pot culture experiment with a phenotypic variance ranged from 10.40 (UASBC53) to 21.69 (UASBC57) per cent. Similarly, in the field experiment five markers in 2007 and seven markers in 2008 were associated with FW resistance at 30 DAS. Among them five markers (UASBC92, UASBC8, UASBC57, UASBC48 and UASBC50) were common for both the seasons (2007 and 2008). The phenotypic variance ranged from 9.35 to 16.75 per cent in 2007 and 9.22 to 10.65 per cent in 2008 (Table 16). Three markers (UASBC8, UASBC48, and UASBC57), showed consistent association with FW resistance across pot and field experiments at 30 DAS.

Five markers (UASBC8, UASBC32, UASBC57, UASBC48 and UASBC50) were associated with wilt score at 60 DAS in pot culture experiment and the phenotypic variance ranged from 9.50 (UASBC50) to 25.54 per cent (UASBC48). For field reaction four markers (UASBC92, UASBC39, UASBC82 and UASBC48) with phenotypic variance of 9.93 (UASBC82) and 12.51 (UASBC92) per cent were identified in 2007 at 60 DAS. None of the markers were associated with WR at 60 DAS in 2008. Only one marker UASBC48 was common for wilt reaction at 60 DAS in both pot and field experiment in 2007 (Table 16). Five markers (UASBC8, UASBC32, UASBC48, UASBC50 and UASBC57) were common at 30 and 60 DAS in pot experiment. Only one marker (UASBC48) was consistently associated with FW resistance at 30 and 60 DAS in both pot as well as field experiments.

Table 16. Single marker analysis for *Fusarium* wilt resistance in mapping population (JG62×WR315)

Trait	LG	Markers	Max LOD	PVE (%)	Additive effect
W30pot	2	UASBC53	2.98	10.40	13.06
W30pot	2	UASBC8	3.58	12.38	14.35
W30pot	3	UASBC32	5.21	17.47	16.90
W30pot	3	UASBC57	6.63	21.69	18.92
W30pot	3	UASBC48	6.48	21.23	18.66
W30pot	3	UASBC50	3.52	12.16	14.22
W60pot	2	UASBC8	3.83	13.18	13.69
W60pot	3	UASBC32	4.62	15.66	14.79
W60pot	3	UASBC57	7.65	24.56	18.62
W60pot	3	UASBC48	8.00	25.54	18.93
W60pot	3	UASBC50	2.71	9.50	11.62
W30-07	1	UASBC92	2.64	9.35	7.96
W30-07	2	UASBC8	2.91	10.25	8.40
W30-07	3	UASBC57	4.44	15.21	10.19
W30-07	3	UASBC48	4.93	16.75	10.67
W30-07	3	UASBC50	2.95	10.39	8.45
W60-07	1	UASBC92	3.59	12.51	7.78
W60-07	1	UASBC39	3.08	10.81	7.23
W60-07	1	UASBC82	2.81	9.93	6.92
W60-07	3	UASBC48	2.82	9.97	6.95
W30-08	1	UASBC92	2.95	10.65	8.63
W30-08	1	UASBC39	2.54	9.22	8.02
W30-08	2	UASBC53	2.62	9.51	8.19
W30-08	2	UASBC8	2.82	10.17	8.51
W30-08	3	UASBC144	2.63	9.52	8.23
W30-08	3	UASBC57	2.62	9.52	8.19
W30-08	3	UASBC48	2.95	10.65	8.64

Where,

W30-pot= Wilt reaction of RILs in pot culture experiment (30th DAS)
W60pot= Wilt reaction of RILs in pot culture experiment (60th DAS)
W30-07= Wilt reaction of RILs in field experiment (30th DAS 2007)
W60-07= Wilt reaction of RILs in field experiment (60th DAS 2007)
W30-08= Wilt reaction of RILs in field experiment (30th DAS 2008)
W60-08= Wilt reaction of RILs in field experiment (60th DAS 2008)

4.4.3.2 Mapping population KW

The marker data and phenotypic data of RILs were used to identify the marker associated with FW resistance at 30 and 60 DAS for wilt reaction in 2007 and 2008 field experiments. The variation in wilt reaction co-segregate with one marker UASBC53 for wilt score at 60^{th} DAS in 2007. The marker UASBC53 explained the phenotypic variance of 8.38 per cent with a LOD score of 2.60. None of the markers were associated with FW resistance at 30 DAS in this population.

Three markers (UASBC8, UASBC48, and UASBC57) consistently associated with FW resistance in both pot as well as field experiment in mapping population JW. Among these three markers UASBC48 was common at both stages as well as pot and field experiments. The marker UASBC53 was associated with wilt resistance in both the mapping populations. Four markers (UASBC8, UASBC48, UASBC53 and UASBC57) showed consistent linkage with FW resistance and hence useful for screening genotypes for wilt resistance and markers assisted chickpea breeding for wilt resistance.

The major disadvantages of SMA is, a QTL far from a marker, the less likely it will be detected. The recombination between the marker and QTL could lead to the underestimation of a QTL effect. This limitation can be overcome by utilizing the large number of markers spread throughout the genome (Tanksley, 1993). In the present study the number of polymorphic markers used for QTL detection were limited. To overcome this limitation composite interval mapping (CIM) was used for the detection of QTLs in the present study. This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Jansen, 1993; Jansen and Stam, 1994; Zeng, 1994). The main advantage of CIM is that it is more precise and effective at mapping QTLs compared to single-point analysis.

4.4.4 QTL mapping and analysis

Quantitative trait loci **(**QTLs**)** are loci controlling quantitative traits. The chromosomal region associated with a quantitative trait flanked by markers is defined as QTL (Geldermann, 1975). QTL analysis is a statistical method that links phenotypic data with genotypic data (molecular marker data) to explain the genetic basis of variations in complex traits (Falconer and Mackay, 1996; Kearsey, 1998). The important step towards QTL mapping is to have a linkage map with good coverage of markers. In the present study, instead of concentrating on the whole genome only already known QTLs regions were saturated with newly developed markers.

Many researchers identified FW resistance loci and QTLs in chickpea. In previous studies several markers were used to map the resistance genes *Foc1* (Mayer *et al.,* 1997), *Foc*-0 (Rubio *et al.,* 2003), *Foc2* (Sharma and Muehlbauer, 2007; Gowda *et al.,* 2009), *Foc3* (Sharma *et al.,* 2004), *Foc4* and *Foc5* (Ratnaparkhe *et al.,* 1998a; Tullu *et al.,* 1998; Tekeoglu *et al.,* 2000). The disease reaction of RILs showed continuous variation suggesting polygenic inheritance of wilt reaction along with major genes (Upadhyaya, 1983a and b). Further studies using molecular markers illustrated the presence of QTLs

influencing FW reaction in chickpea rather than only major genes contributing for FW resistance (Gowda *et al.*, 2009; Patil *et al.*, 2014).

The resistance loci *Foc1*, *Foc2* and *Foc3* flanked by markers TA110-H3A12, H3A12-TA96 and TA194-H1B06Y respectively were mapped on linkage group 2 (LG2) (Gowda *et al.*, 2009). Recently, Barman *et al.* (2014) mapped *Foc1* resistance loci flanked by markers TA37 and TA200. Few studies were conducted to discover QTLs involved in wilt resistance. Subbavarapu *et al.* (2013) identified the two novel QTLs (*FW-Q-APR-6-1* and *FW-Q-APR-6-2*) flanked by markers CaM1402-CaM1101 and CaM1125-TA22 respectively on LG6 for FW race. Patil *et al.*, (2014) identified two major QTLs (*Wilt 1* and *Wilt* 2) on LG2 flanked by markers TA27-TA59 and TA27-TA110 respectively for race 1. Recently, Jingadhe and Ravikumar (2015) identified five QTLs for Wilt resistance against race 1, among them one stable QTL flanked by GSSR18-Tc14801 was identified on LG1, explained phenotypic variance of 69.80 and 60.80 per cent in 2007 and 2008 *rabi* respectively. After physical mapping ten QTLs/genomic regions (Fig. 2) were selected for markers development and used in fine mapping the regions. The QTL mapping in this study was conducted using ICIM QTL IciMapping version 4.00 software with minimum LOD score of 2.5.

4.4.4.1 Mapping population JW

The QTL analysis was conducted with wilt reaction of RILs on 30^{th} and 60^{th} days under wilt sick pot and wilt sick field (2007 and 2008) and a partial linkage map developed using 23 markers. A total of six QTLs were identified for FW resistance with phenotypic variance ranging from 8.56 to 25.97 per cent. Out of six QTLs identified, two QTLs were located on LG1 and four QTLs were located on LG3 (Table 17 and Fig. 6).

Two QTLs (*qW60-07-1-1* and *qW30-08-1-1*) were flanked by common markers UASBC79 and UASBC92 at position 11 cM with LOD score of 3.55 and 2.50 respectively on LG1. One QTL (*q30pot-3-1*) was flanked by UASBC32 and UASBC57 with a LOD score of 6.78 at 30 cM on LG3. Three QTLs (*q60pot-3-1, qW30-07-3-1* and *qW30-08-3-1*) were flanked by common markers UASBC48 and UASBC50 with LOD score of 7.93, 4.94 and 2.59 respectively at 32 cM on LG3 (Table 17).

For wilt reaction at 30 DAS, four QTLs (*qW30-08-1-1, q30pot-3-1, qW30-07-3-1,* and *qW30-08-3-1*) were identified. Out of four, one QTL (*qW30-08-1-1*) was present on LG1 and three QTLs (*q30pot-3-1, qW30-07-3-1,* and *qW30-08-3-1*) were present on LG3. Further, one QTL (*q30pot-3-1*) on 30 DAS was identified based on pot screening while, the remaining three were from field data. Among four QTLs for 30 DAS, the QTL *q30pot-3-1* has highest phenotypic variation of 23.62, followed by QTLs *qW30-07-3-1, qW30-08-1-1,* and *qW30-08-3-1* with phenotypic variation of 17.31, 8.56 and 8.64 per cent (Table 17). All the QTLs had positive additive effect with contribution of favourable alleles from male parent. Two QTLs (*qW30-07-3-1* and *qW30-08-3-1*) for 30 DAS were identified at same position (32 cM).

Table 17. QTLs detected for *Fusarium* wilt resistance based on population (JG62×WR315) in chickpea

Trait	QTL detected	LG	Flanking markers	QTL positions (cM)	Max LOD	R^2 (%)	Additive effect	Donor parent
W30pot	*q30pot-3-1*	3	UASBC32-UASBC57	30.00	6.78	23.62	19.71	Male
W60pot	*q60pot-3-1*	3	UASBC48-UASBC50	32.00	7.93	25.97	19.08	Male
W30-07	*qW30-07-3-1*	3	UASBC48-UASBC50	32.00	4.94	17.31	10.84	Male
W60-07	*qW60-07-1-1*	1	UASBC79-UASBC92	11.00	3.55	12.82	7.87	Male
W30-08	*qW30-08-1-1*	1	UASBC79-UASBC92	11.00	2.50	8.56	7.74	Male
W30-08	*qW30-08-3-1*	3	UASBC48-UASBC50	32.00	2.59	8.64	7.78	Male

Where,

30pot= Wilt reaction of RILs in pot culture experiment (30^{th} DAS)
60pot= Wilt reaction of RILs in pot culture experiment (60^{th} DAS)
W30-07= Wilt reaction of RILs in field experiment (30^{th} DAS 2007)
W60-07= Wilt reaction of RILs in field experiment (60^{th} DAS 2007)
W30-08= Wilt reaction of RILs in field experiment (30^{th} DAS 2008)

Two QTLs (*qW60-07-1-1* and *q60pot-3-3*) were identified for 60 DAS in LG1 and LG3 respectively. One QTL (*q60pot-3-3*) was identified based on pot screening and explained the highest phenotypic variation (25.97 %). The other QTL *qW60-07-1-1* was identified based on field data (2007 *rabi*) and explained a phenotypic variation of 12.82 per cent. Both the QTLs had positive additive effect with contribution of favourable alleles from male parent.

The novel genic SSR marker developed from FW resistance region in the chickpea genome were utilized for the first time for redefining QTL positions. On LG1 two QTLs were identified in which one was major QTL (*qW60-07-1-1*) and another one was minor QTL (*qW30-08-1-1*). The LG1 was composed of 10 markers which were developed from the QTL region *FW-Q-APR-6-1* (mapped on Ca6 *in silico*) after physical mapping (Subbavarapu *et al.,* 2013). The QTL identified in the Subbavarapu *et al.* (2013) was narrowed down and split into one major (*qW60-07-1-1*) and one minor (*qW30-08-1-1*) QTL on LG1 which corresponds to LG6 in Subbavarapu *et al.* (2013) study and Ca6 in sequence based physical map in the present study.

Among the four QTLs identified in LG3, three were major (*q30pot-3-1*, *q60pot-3-1* and *qW30-07-3-1*) and only one QTL was minor (*qW30-08-3-1*). The markers on LG3 were developed from the QTL region on LG2 proposed by Gowda *et al.,* 2009, QTLs (*Wilt 1* and *wilt 2*) on LG2 from Patil *et al.,* (2013) and *Foc1* locus on LG2 (Barman *et al.,* 2014) which were *in silico* mapped to Ca2. In previous studies, resistance genes to races (0, 1, 2, 3, 4, and 5; *Foc*-0, *Foc*-1, *Foc*-2, *Foc*-3, *Foc*-4 and *Foc*-5) have been found to form a cluster located on the LG2 of chickpea reference map (Winter *et al.,* 2000; Ratnaparkhe *et al.,* 1998; Tekeoglu *et al.,* 2000; Sharma and Muehlbauer, 2007; Cobos *et al.,* 2009; Gowda *et al.,* 2009). The above mentioned LG2 was represented by LG3 of Radhika *et al.,* (2007) composite map, which is an 'hot spot' for *Fusarium* wilt resistance genes. Recently, Patil *et al.,* (2014) identified two major QTLs (*Wilt 1* and *Wilt 2*) which explained the phenotypic variance of 36 and 16 per cent with LOD of 9.19 and 3.53 respectively in this region. In physical mapping these resistance loci and QTLs for FW races were mapped on chickpea chromosome (Ca2) confirming that chromosome 2 contains a hot spot for FW resistance genes.

The hot spot region for FW resistance genes/QTLs were redefined by discovering four stable QTLs in the same region in this study confirming that the loci is an hotspot for FW resistance genes (Winter *et al.,* 2000; Ratnaparkhe *et al.,* 1998; Tekeoglu *et al.,* 2000; Sharma and Muehlbauer, 2007; Cobos *et al.,* 2009; Gowda *et al.,* 2009) on chromosome 2.

4.4.4.2 Mapping population KW

In this investigation only one QTL (*qWilt60-07-3-1*) was identified for FW resistance at 60 DAS in the field experiment 2007 and was located on LG3 (Fig. 7). The QTL (*qWilt60-07-3-1*) was flanked by markers UASBC51 and UASBC53 covered an interval of 4.88 cM with a LOD score of 2.69. The phenotypic variance explained by this QTL was 9.45 per cent. The favourable allele in case of this QTL (*qWilt60-07-3*-1) was contributed by resistant parent with positive additive effect of 8.16. The markers

(UASBC51 and UASBC53) flanking QTL (*qWilt60-07-3*-1) were developed from the FW resistance region flanked by A07C and TS72 mapped on chromosome 4 (Ca4) (Soregaon *et al.,* 2007; Soregaon, 2011). In the present investigation the FW resistance genomic region on Ca4 was narrowed down by identifying a QTL (*qWilt60-07-3*-1). The LG3 from KW population corresponds to LG3 in genetic map constructed by Soregaon (2011) using same RILs.

Earlier studies on genetics of resistance to FW showed that resistance to race 1A was governed by two to three genes ($h_1h_1h_2h_2h_3h_3$) (Upadhyaya *et al.,* 1983b, Singh *et al.,* 1987, Sharma and Muehlbaur, 2007). The combination of any two genes in recessive form confers resistance, whereas when only one of these genes in recessive, late wilting occurs (Gumber *et al.,* 1995; Kumar, 1998). Since, the parent K850 (late wilter) segregating for only one resistance locus ($h_1h_1H_2H_2$) (Jingadhe and Ravikumar, 2015). In the present study the genetic mapping of FW resistance in population KW has resulted in one QTL on LG3 unlike, in population JW which segregate for early wilting and resistance and resulted in six QTLs. The result indicated that FW resistance is controlled by two-three genes and many QTLs with major and minor effects.

Overall, the QTLs identified on LG1 in JW were redefined QTLs from earlier QTLs (*FW-Q-APR-6-1*) identified by subbavarapu *et al.* (2013) which was physically located on chromosome 6. The four QTLs on LG3 in genetic map of population JW were redefined from *Foc1* resistance locus and QTL (*Wilt 1*) identified by Gowda *et al.* (2009) and Patil *et al.* (2014) respectively, which were *in silico* mapped to chromosome 2. Hence, LG3 considered as QTL-hotspot region for FW resistance. In KW the QTL on LG3 was redefined from H_2 locus identified by Soregaon *et al.* (2007) which was physically mapped on chromosome 4. These results conclude that there are three independent resistant loci against FOC race 1A and are located on chromosome 2, 4 and 6.

The linkage maps developed by using SSR markers in an intra-specific mapping populations in the cultivated gene pool is more important to breeding programme. The uneven recombinations and segregation distortion in inter-specific mapping populations (Cho *et al.,* 2002; Flandez-Galvez *et al.,* 2003) may underestimate the genetic distances. Overall the SSR markers flanking resistant QTLs (UASBC32-UASBC57, UASBC48-UASBC50, UASBC79-UASBC92 and UASBC51-UASBC53) represent a small genomic area and hence more reliable for marker assisted breeding for resistance to FW in chickpea.

4.4.5 Comparison of genetic and physical map and identification of closely linked markers to FW resistance

For any breeding program genetic map is the first step to illustrate the arrangement of DNA markers on a chromosomes with relative distances between marker positions on a genetic map calculated using recombination frequencies. The main advantage of constructing genetic map is to identify chromosomal locations containing genes and QTLs associated with traits of interest (Paterson, 1996). By analysing the segregation of markers, the relative order and distances between markers can be

determined. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome (Collard *et al.,* 2005). Genetic map entirely depends on recombination frequency which may depend on population size, the parents and trait selected. The distance between markers and genes are expressed in term of map units. The marker close to a gene in genetic map may be far in terms of physical distance. The information on recombination frequency is of limited value for gene isolation through map-based cloning. While, this limitations can be overcome by physical mapping (Wu *et al.,* 2003).

A physical map is based on the actual number of nucleotide pairs between loci. These maps are a key resource for understanding genome organisation. The actual distance between markers and genes can be identified, which is a limitation in genetic map. Physical map is the basis for map-based cloning, fine mapping, robust markers development and marker-assisted selection, serving as a bridge between breeding and sequencing research. A complete genome sequence is a physical map at its highest resolution (O'Rourke, 2002). The integrated physical/genetic map will facilitate cross referencing for positional cloning and fine mapping of genes or QTLs of interest (Reddy *et al.,* 2009).

4.4.5.1 Mapping population JW

Three linkage groups were constructed using 23 polymorphic SSR markers and 125 RILs in population JW. All the markers of LG1 were physically mapped on the chromosome 6 (Ca6) and all markers of LG2 and LG3 were mapped on Chromosome 4 (Ca4) and 2 (Ca2) respectively (Fig. 9).

In linkage group 1, ten markers were present and all the ten markers were developed from the genes identified in the QTL region on chromosome 6. It is interesting to observe that the markers developed from the same physical region were linked in genetic map also, although limited number of markers were used in this study. Such association of linkage between genetic and physical map were reported earlier in different crops *viz*., rice, soyabean, peanut *etc*., (Zhao *et al.,* 2002: ott *et al.,* 2011; Khera *et al.,* 2015). The markers UASBC39 and UASBC92 which were very close physically were also together in genetic map. Similarly, UASBC165 and UASBC164 were also together in both physical and genetic maps. However, the order of physical and genetic maps were not same for all the markers in LG1. There was no co-linearity between genetic and physical map in these regions. This kind of non-collinearity is due to inaccuracies in mapping populations with less number of individuals, leading to inversions in the order of the markers (Ferreira *et al.,* 2006).

The LG2 composed of six markers and were developed from the genes identified in the QTL region of chromosome 4. The order of markers in genetic map was same as that of physical map on the chromosome 4. The markers UASBC54 and UASBC4 were close to each other in both physical as well as genetic map. Similarly, UASBC6, UASBC53 and UASBC8 were close together in physical and genetic maps also. The probable reason may be due co-dominant mapping population used in the present study. These results were supported by earlier results of Ferreira *et al.,* (2006) that the more

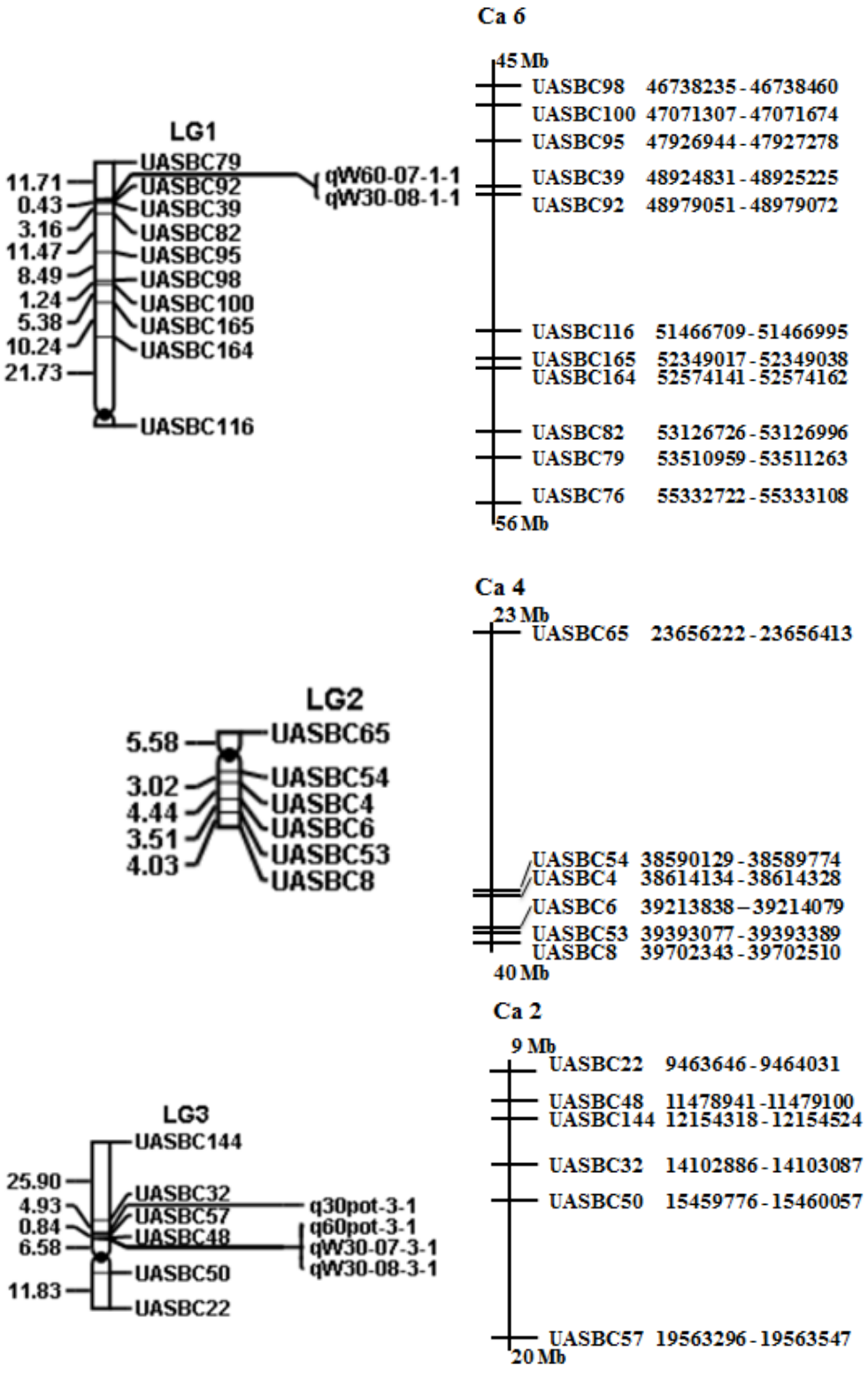


Fig. 9. Comparison of genetic and physical map derived from mapping population (JG62×WR315) in chickpea

accurate maps were obtained for the co-dominant F_2 and RILs whereas the maps for the dominant F_2 population were less accurate

In LG3 six markers were present and all the six markers were developed from the genes identified in the QTL region of chromosome 2. The alignment of physical and genetic map indicated that the marker order was different in genetic and physical map. The markers UASBC32, UASBC57 and UASBC48 were close together in genetic map but not in physical map. Such discrepancies in genetic and physical maps were reported earlier in different crops. Linkage maps are inherently subjected to scoring errors, statistical ambiguities and differences in distances and order depending on the mapping population and markers used (Gale *et al.,* 2005).

The two QTLs (*qW60-07-1-1* and *qW30-08-1-1*) were identified in the LG1 for wilt score at 60 and 30 DAS in field experiment 2007 and 2008 respectively. These two QTLs were flanked by common markers UASBC79 and UASBC92 with an interval of 11.71 cM. In physical map, these two markers flanked a genomic region of 4.53 Mb on chromosome 6. The new polymorphic SSR markers flanking QTLs identified in this study were designed from the genomic region covered by QTL (*FW-Q-APR-6-1*) flanked by markers CaM1402 and CaM1101 on Ca6 (Sabbavarapu *et al.,* 2013). The physical map of flanking markers CaM1402 and CaM1101 represented a region of 13.58 Mb consisting of 520 genes. In the present study the QTL region was narrowed down to 4.53 Mb (Table 18 and Fig. 9). Further two QTLs (*qW60-07-1-1* and *qW30-08-1-1*) were physically mapped to this narrowed region on Ca6 which consists of only 241 genes. The overall marker density in the QTL region is enhanced which will be helpful to fine map the QTLs. The integrated map will enable structural and functional genomic studies including marker assisted chickpea breeding for wilt resistance, map based gene cloning, QTL analysis and comparative synteny mapping. In linkage group 2 no QTLs were detected for FW resistance. The markers used for genetic mapping were designed from genomic region flanked by FW resistance linked markers A07C-TS72-ESTSSR21 and TR20-TS82 (Radhika *et al.,* 2007; Soregaon, 2011; Jingadhe and Ravikumar, 2015) on Ca4 represented 6.34 Mb and 337 genes. The failure to detect major QTLs from this region from our study could be because of the small mapping population and markers used in the study. Multiple mapping populations serving with higher population size could increase the possibility to detect QTLs in this region.

Four QTLs were identified in LG3. One QTL (*q30pot-3-1*) flanked by UASBC32 and UASBC57 with an interval of 4.93 cM, covered 5.46 Mb genomic region on Ca2. The marker UASBC32 was designed and synthesised from genomic region for FW resistance locus (*Foc1*) flanked by TA110 and H3A12 (Gowda *et al.,* 2009) on Ca2 which covers genomic region of 6.48 Mb whereas, UASBC57 was designed and synthesis from QTL (*Wilt 1*) region for FW resistance flanked by markers TA27 and TA59 (Patil *et al.,* 2014) covers genomic region of 5.83 Mb in *in silico* physical map. A total of 13.61 Mb region was covered in that total region narrow down to 5.46 Mb in the present investigation. The genomic region flanked by both QTL (*Wilt 1*) and *Foc1* locus was fine-tuned by identifying redefined QTL (*q30pot-3-1*) on Ca2 narrowing the genomic region to 5.46 Mb, consisting of 150 genes (Table 18).

Table 18. Comparison of genetic and physical map derived from mapping population (JG62×WR315) in chickpea

Genetic map					*In silico* physical map				
LG	**Genetic distance (cM)**	**No. of markers**	**QTL identified**	**Flanking markers**	**Corresponding chromosome**	**Physical distance (Mb)**	**No. of markers**	**Genomic region covered by QTL flanking markers (Mb)**	**No. of genes in QTL region**
LG1	73.85	10	*qW60-07-1-1* *qW30-08-1-1*	UASBC79-UASBC92	Ca6	8.59	11*	4.53	241
LG2	20.58	6	-	-	Ca4	16.04	6	-	
LG3	50.08	6	*q30pot-3-1* *q60pot-3-1* *qW30-07-3-1* *qW30-08-3-1*	UASBC32-UASBC57 UASBC48-UASBC50 UASBC48-UASBC50 UASBC48-UASBC50	Ca2	10.09	6	5.46 3.98	150 168

*****Unlinked marker UASBC76 was physically mapped onto the Ca6

On LG3 three QTLs (*q60pot-3-1, qW30-07-3-1 and qW30-08-3-1*) for FW resistance were discovered with common flanking markers UASBC48 and UASBC50. All the three QTLs covered a genetic distance of 6.58 cM (Fig 9). The physical map with flanking markers were mapped on Ca2 and covered a distance of 3.98 Mb. Both markers UASBC48 and UASBC50 were designed and synthesised from genomic region covered by TA110 and H3A12 which flanks *Foc1* locus (Gowda *et al.,* 2009). The QTL flanking markers TA110 and H3A12 in physical map covered a genomic region of 6.48 Mb consisting of 310 genes. From the QTL analysis of the present study using novel markers the redefined QTLs (*q60pot-3-1, qW30-07-3-1 and qW30-08-3-1*) for FW resistance and the new markers fine mapped the region with an interval of 3.98 Mb genomic region consisting of only 168 genes (Table 18 and Fig. 9).

The new closely linked markers to FW resistance identified in this study will be robust and reliable in chickpea molecular breeding for FW resistance. Such fine mapping of QTL regions for disease/stress resistance through physical mapping of QTLs has been reported earlier. For examples, in rice *in silico* analysis of a QTL region flanked by the genetic markers RM212 and RM319 on chromosome 1 identified 16 candidate genes for drought resistance tolerance (Wang *et al.,* 2005). He *et al.* (2006) fine mapped the rice bacterial blight resistance gene, *Xa2,* which confers resistance to bacterial blight pathogen *Xanthomonas oryzae* pv. *Oryzae*. The flanking SSR markers HZR950-5 and HZR970-4 covered approximately 190-kb region on the long arm of chromosome 4. Maroof *et al.* (2010) fine mapped *Rvs 4* conferring resistance for all kind of soyabean mosaic virus strains on chromosome 2 limiting to physical distance of less than 100 Kb. Thomson *et al.* (2010) identified the position of QTL *Saltol* between RM23 and RM140 on chromosome 1 through physical mapping of the QTL region for salt tolerance in rice. Xu *et al.* (2014) physically mapped a major gray leaf spot resistance QTL, *qRgls2* and fine mapped by closest markers G346 and DD11 on maize chromosome 4.

4.4.5.2 Mapping population KW

Twenty two markers and 141 RILs were used to generate QTL mapping for Fusarium wilt resistance. Out of twenty two SSR markers, 21 were *in silico* anchored to three chromosomes (Ca2, Ca4 and Ca6) and one marker UASBC109 was mapped on unplaced genomic scaffold 96 in chickpea genome (Fig. 10).

The careful analysis of all the three LGs and the physical map of these markers suggest that there is physical co-localization of genetic loci of majority of markers on the chromosomes. There is a conservation of loci. For example 11 out of 14 markers in LG1 were co-localised to Chromosome 6 (Ca6). The remaining two (UASBC118 and UASBC114) were mapped on chromosome (Ca2) and one (UASBC109) on scaffold 96 at 14.23 Mb. The LG1 in this study corresponds to Ca6. The marker UASBC109 from unplaced genomic scaffold 96 was mapped on LG1 in genetic map indicates that this scaffold could be a part of the Ca6. The genetic distance covered by LG1 was 53 cM and 27.29 Mb distance on Ca6 in physical map. However, the marker order is not conserved between physical and genetic map in LG1. The markers UASBC165 and UASBC164 were close to each other both in genetic and physical map and also follow same order. But the order in genetic map was UASBC79, UASBC92 UASBC39 and UASBC82

whereas, in physical map these markers were mapped in different order. Many earlier researchers showed the collinearity of marker order in genetic and physical map. For example Zhu *et al.* (1999) constructed the physical map of chromosome 7 in the rice blast fungus *Magnaporthe grisea* using BAC library and 20 RFLP markers through hybridization technique. The anchored marker in physical map of chromosome 7 collinear with the genetic map. The order of markers in a high density genetic map remains the same as that of the physical map. For example Kunzel *et al.* (2000) compared the high density genetic map with physical map of barley chromosome 2H and observed the same marker order in both genetic map. However, the order of markers in genetic and physical map are not without errors. The inversions of marker order in genetic maps can results from genotyping errors, use of limited number of informative meiosis to generate map, which lead to underestimation of recombination frequencies. On the other hand the error in the order of markers in physical map can be due to incorrect identification of marker positions (Clerget-Darpoux *et al.,* 1986; Goddard *et al.,* 2000; Collins *et al.,* 2001; Reich *et al.,* 2001).

Five markers on LG2 were co-localised to chromosome 2 (Ca2) along with one more markers (UASBC118) from LG1. The LG2 in the present study corresponds to Ca2. The genetic distance covered by LG2 was 27.10 cM whereas, 35.88 Mb distance was covered in Ca2. Similarly, from LG3 three markers were co-localised to chromosome 4 (Ca4) along with one more marker (UASBC114) from LG1. The LG3 in this study corresponds to Ca4. The genetic distance covered by LG3 was 20.36 cM whereas, 14.17 Mb distance was covered in Ca4 in physical map. The markers order in both the LG2 and LG3 showed non-collinearity with Ca2 and Ca4 respectively in physical map. Such discrepancies were also observed in many studies. For example in fungus *Gibberella zeae* differences in gene order in genetic map was observed in comparison with physical map (Lee *et al.,* 2008). Cloutier *et al.* (2012) compared three genetic maps with physical map and observed a large collinearity in the marker order except few marker inversions in flux.

Only one QTL (*qWilt60-07-3-1*) was identified for FW resistance at 60 DAS in field experiment 2007 on LG3 flanked by markers UASBC51 and UASBC53 with an interval of 4.88 cM. In physical map this two markers flanked a region 0.53 Mb on Ca4. The new polymorphic SSR flanking QTL identified in this study were designed from the genomic region covered by two FW linked markers A07C and TS72 (Soregaon *et al.,* 2007; Soregaon, 2011). The physical mapping of flanking markers A07C and TS72 represented 1.56 Mb consisting 100 genes. In the present study this FW resistance loci was narrow down to 0.53 Mb consists of only 37 genes.

In the present study a few markers UASBC109, UASBC114 and UASBC118 were although genetically linked to LG1, they showed non-conservation in physical map. Such discrepancies are due to limited number of markers used. The high density genetic map with more number of markers can solve this kind of errors (Cloutier *et al.,* 2012).

Several studies have been reported on integration of genetic and physical map in different crops such as rice, wheat, chickpea *etc*. to identify closely linked markers to

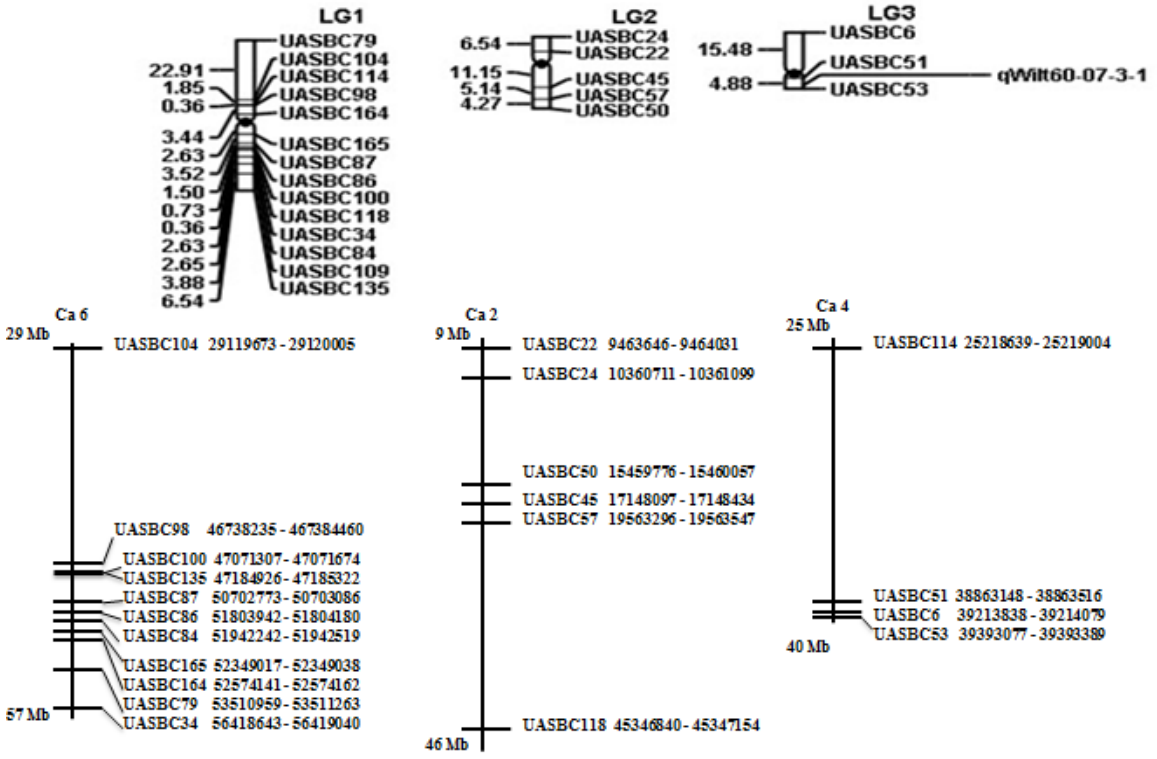


Fig. 10. Comparison of genetic and physical map derived from mapping population (K850×WR315) in chickpea

many quantitative traits. In case of chickpea, Madrid *et al.* (2014) constructed the physical map by linking genetic map. A QTL related to *Ascochyta* blight resistance located in LG2 (QTL_{AR3}) where single-copy markers based on candidate genes located in the Ca2 pseudomolecule were used for refining the QTL position. They predicted 32–33 Mb genomic region, comprising 42 genes in which candidate genes located in this region include *ethylene insensitive 3-like* (*Ein3*), *Avr9*/*Cf9* and *Argonaute* 4, directly involved in disease resistance mechanisms. Similarly, Varshney *et al.* (2014b) identified the *QTL-hotspot* region flanked by tightly linked markers for drought on CaLG04. The physical mapping of the markers identified 7 Mb region with a total of 654 genes. They also represented the QTL region/genes *ara1* and *ara2a* for *Ascochyta* blight resistance in physical map and identified a 3 Mb region on Ca2 that consist a total of 306 genes.

The comparison of physical and genetic map revealed three important regions for FW resistance, one each on chromosome 2, 4 and 6.

Through genetic analysis a QTL hot spot was also identified for FW resistance. *In silico* physical mapping in the present study revealed that the QTL-hotspot region/FW resistance region on LG2 in genetic map for different races identified in many studies such as, resistance loci *$Foc0_2$, Foc1, Foc3, Foc4, Foc5* (Winter *et al.,* 2000; Huttel *et al.,* 2002; Sharma *et al.,* 2004; Iruela *et al.,* 2006; Millan *et al.,* 2006 and 2010; Barman *et al.,* 2014) and QTLs (*Wilt 1* and *Wilt 2*) (Patil *et al.,* 2014) was physically mapped on chickpea Ca2. This indicates that chromosome 2 carries the potential QTL-hotspot region for FW resistance. In this study, the QTL hot spot has been redefined to 3.98 Mb with closely linked markers UASBC48 and UASBC50 through genetic mapping. Dissecting the present QTL in this study provided better insight into potential candidate genes for FW resistance. In the present study, a stable QTL for wilt score at 30 DAS in two seasons and QTL for wilt score at 60 DAS in pot experiment with common flanking markers UASBC48-UASBC50 were identified in JW cross. The region narrowed down to 3.98 Mb consisting of 168 genes Ca2. Further, the putative candidate genes were identified based on gene function involved in disease resistance. To narrow down and fine map the genes involved in FW resistance in the QTL region, requires the development of single nucleotide polymorphism (SNPs) in this region.

4.5 Identification of putative candidate genes in redefined QTL regions of FW resistance

QTL mapping has successfully been employed to identify FW resistance loci and has been shown to be a powerful strategy to identify candidate genes. In this study QTL resolution has been enhanced by mapping QTLs by developing novel markers in the QTL region which has increased marker density in the region. A refined physical region of QTLs of FW resistance identified by novel markers was used to identify annotated candidate genes.

One redefined QTL (*q30pot-3-1*) flanked by UASBC32-UASBC57 and three redefined QTLs (*q60pot-3-1, qW30-07-3-1 and qW30-08-3-1*) with common flanking markers (UASBC48-UASBC50) were physically mapped on Ca2. The flanking markers of the QTLs identified in the study were overlapping on chromosome 2 covering 8.08 Mb

which consists of 291 genes. Of these, 61 genes function were not yet annotated. Thirty putative candidate genes for disease resistance were identified and classified according to their functions (Table 19). Two more QTLs (*qW60-07-1-1* and *qW30-08-1-1*) flanked by common markers (UASBC79 and UASBC92) were identified in this study and were physically mapped to Ca6 covering 4.53 Mb genomic region. The 4.53 Mb contained 241 genes of which 28 genes were uncharacterized. Among them, 25 putative candidate genes involved in disease resistance were identified (Table 19). Similarly, another QTL identified in the study was *in silico* mapped to Ca4, covering a physical distance of 0.53 Mb consisting 37 genes. Of these, 20 putative candidate genes were identified in this region (Table 19). Further, the putative candidate genes for disease resistance identified from all the three genomic regions of FW resistance were classified based on their function.

Fine mapping and *in silico* candidate genes identification is a common approach and in many crop plants putative candidate genes for different traits have been identified. In rice Wang *et al.* (2005) physically mapped the QTL flanked by markers RM212 to RM319 for drought stress tolerance on Chromosome 1 and identified 16 putative candidate genes. Similarly, QTL responsible for resistance specificity to *Xanthomonas oryzae* pv.*oryzae iso* 6 in rice was *in silico* anchored to chromosome 10 consists of 201 genes. In that, 28 putative positional candidate genes were identified and were classified based on their functions (Reddy *et al.*, 2008).

In soyabean two of the Conrad QTL on chromosome 19 contributing partial resistance to multiple *Phytophthora sojae* isolates were dissected through sequence and expression profiling. Out of 153 genes, 11 candidate genes encode proteins potentially involved in signal transduction, hormone-mediated pathways, plant cell structural modification, ubiquitination, and basal resistance were identified (Wang *et al.*, 2012). The map position of the maturity date locus was refined from 3.56 Mb to 220 kb in peach. Among 25 annotated genes identified within this interval two genes ppa007577m and ppa008301m were identified as the most likely candidates, encoding transcription factors of the NAC (NAM/ATAF1, 2/CUC2) (Pirona *et al.*, 2013) family.

A few studies were conducted in chickpea to identify the candidate genes for various traits through *in silico* physical mapping approach. Madrid *et al.* (2014) *in silico* anchored QTL_{AR3} for AB resistance on chickpea Ca2 and identified an *ethylene insensitive 3-like gene (Ein3)* which explained the highest percentage (44.3 %) of the total phenotypic variation for resistance to blight. Bajaj *et al.* (2015) identified one major genomic region of 126.8 Kb harbouring a strong seed weight (SW)-associated robust QTL. High-resolution QTL mapping with comprehensive marker-based comparative genome mapping lead to identification of one potential regulatory SNP (G/A) in the *cis*-acting element of candidate *ERF* (ethylene responsive factor) transcription factor (TF) gene governing seed weight in chickpea. Similarly, Verma *et al.* (2015b) analysed the genomic sequence corresponding to five robust QTLs related seed traits and led to the identification of 684 putative candidate genes whose expression profiling revealed that 101 genes exhibited seed specific expression in chickpea. Recently, Ali *et al.* (2016) performed sequence based analysis and identified a candidate gene for the simple/double

podding gene *regulator of axillary meristem* between TR44 (STMS marker) and the SNP scaffold1646p97220.

However, this is the first report on candidate genes identification for *Fusarium* wilt resistance through *in silico* approach by redefining the QTL positions through fine mapping the loci. The candidate genes identified in the redefined QTL regions for FW resistance in chickpea genome encodes protein which are potentially involved in plant defense responses *viz.*, cell wall modification, biosynthetic pathway, metabolism, signaling transduction, RNA silencing complex, transcription factors (TF), regulatory pathway, protein-protein interactions, stress response, defense response, cellular redox and energy metabolism (Table 19).

Among several genes located in the QTL regions, two genes encoding proteins annotated as cell wall remodulation, that is extensins and expansins. Extensins are essential for cell-wall assembly and growth by cell extension and expansion. Ashraf *et al.* (2009) identified the extensin protein involved in basal defense response, accumulated excess in resistance genotype when compared with susceptible chickpea genotypes which were challenged with *Fusarium oxysporum* f.sp. *ciceri.* Expansins involved in cell wall remodulation by cell wall loosening and are known for their endogenous function in cell wall extensibility. Recently, the *Arabidopsis expansin-like A2* (*EXLA2*) gene has been reported to link plant development and defense (Abuqamar *et al.,* 2013).

Three candidate genes encoding in the biosynthetic pathway encoding for cytosolic sulfotransferase like, glycerol kinase like and spermine synthase like. The cytosolic sulfotransferases perform the sulfation of smaller molecules. Glucosinolates (GS) are secondary metabolites play an important role in plant defense against herbivores, insects, fungi, and bacteria. The cytosolic sulfotransferases play an important role in biosynthesis of GS by transferring a sulfo group onto a desulfo backbone of GS, which is important as desulfo-GS do not possess biological activities (Gigolashvili *et al.,* 2012). Glycerol-3-phosphate (G3P) contribute to basal resistance against the hemibiotrophic pathogen, *Colletotrichum higginsianum.* Glycerol kinase (GK) mediated phosphorylation of glycerol, or the G3P dehydrogenase mediated reduction of dihydroxyacetone phosphate to produce G3P (Mandal *et al.,* 2011). Spermine was proposed to act as a salicylate-independent inducer of acidic pathogenesis-related protein expression in tobacco mosaic virus-infected tobacco plants (Yamakawa *et al.,* 1998). In biosynthetic pathway of spermine synthesis putrescine is then successively aminopropylated to produce spermidine and spermine by the spermidine and spermine synthase enzymes, respectively (Cohen, 1998).

A gene encoding for Glutathione S-transferases (GSTs) protein was also identified. GSTs are induced by diverse environmental stimuli, with increased GST levels used to maintain cell redox homeostasis and protect organisms against oxidative stress. GSTs are also stimulated by various stresses including pathogen infection (Chen *et al.,* 2012). Plant GSTs play direct roles in reducing oxidative damage and enhancing tolerance to stresses (Chen *et al.,* 2012). Since, FW is a vascular disease affects the water uptake and causes plant to wilt due to lack of water supply to shoot portion (Nene, 1978).

Table 19: Putative candidate genes for disease resistance in redefined QTL for *Fusarium* wilt resistance in chickpea

Sl. No.	Putative candidate genes	Gene id.	Number of genes
Chromosome 2			
1	Cytosolic sulfotransferase 15-like and 14-like	LOC101491627, LOC101514415	2
2	FAR-RED IMPAIRED RESPONSE 1-like	LOC101492268	1
3	Glutathione S-transferase U10-like	LOC101509885	1
4	Expansin-A15-like	LOC101504973	1
5	NAC transcription factor 29-like, 25-like and 8-like	LOC101506808, LOC101507022, LOC101490669	3
6	Early-responsive to dehydration stress protein (ERD4)	LOC101488482	1
7	Protein argonaute 7-like	LOC101488825	1
8	Amino acid Putative protein kinase family protein	LOC101491500	1
9	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	LOC101491812,	1
10	Receptor-like protein kinase 2-like	LOC101495505	1
11	F-box/LRR-repeat protein	LOC101509241, LOC101499837, LOC101500167, LOC101500487, LOC101504222	5
12	WRKY transcription factor 57-like	LOC101498460	1
13	14-3-3-like protein GF14 iota-like	LOC101502079	1
14	Subtilisin-like protease-like	LOC101512261	1
15	Selenoprotein K-like	LOC101492140	1
16	Metal-nicotianamine transporter YSL3-like	LOC101512465, LOC101512465, LOC101502295	3
17	Peroxidase 11	LOC101504752	1
18	Protein srg1-like	LOC101510002, LOC101510541, LOC101510861	3
19	Aminoacylase-1-like	LOC101495721	1
Chromosome 4			
1	Cysteine-rich receptor-like protein; Putative protein kinase	LOC101491877, LOC101509077	2
2	Leucine-rich repeat protein kinase family protein	LOC101501377	1
3	Ethylene-responsive transcription factor CRF3-like	LOC101502348	1
4	DNAJ heat shock N-terminal domain-containing protein	LOC101505779	1

5	Chaperone DnaJ-domain superfamily	LOC101505444	1
6	Protein spermine synthase like	LOC101492874	1
7	RCAR3 a regulatory component of ABA receptor	LOC101505121	1
8	Putative protein kinase	LOC101493206, LOC101493523, LOC101508554	3
9	Probable receptor Putative protein kinase	LOC101507916, LOC101509729, LOC101509405, LOC101510048	4
10	Cell wall-associated Putative protein kinase	LOC101494796	1
11	Receptor serine/threonine Putative protein kinase	LOC101510924, LOC101510377, LOC101495452, LOC101510804	4
Chromosome 6			
1	Extensin-2-like	LOC101492912, LOC101492583, LOC101492248	3
2	WRKY transcription factor 33-like and 40-like	LOC101512877, LOC101509113	2
3	Protein phosphatase 2C 60-like	LOC101502686	1
4	Calcium-transporting ATPase 9, plasma membrane-type-like	LOC101492347	1
5	Glucan endo-1,3-beta-glucosidase-like	LOC101509650, LOC101494403	2
6	CCR4-associated factor 1 homolog 11-like	LOC101508067	1
7	Glycerol kinase-like	LOC101499194	1
8	Leucine-rich repeat receptor-like protein kinase At1g35710-like	LOC101497648	1
9	Cysteine-rich receptor-like protein	LOC101505159, LOC101501213	2
10	Phospholipase D alpha 1-like	LOC101501626	1
11	Late embryogenesis abundant protein 1-like and 2-like	LOC101503652, LOC101504724	2
12	Heat shock 70 kDa protein 15-like	LOC101491162, LOC101491477	2
13	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	LOC101490196	1
14	Aspartic proteinase CDR1-like	LOC101512979	1
15	Peroxidase 48-like	LOC101511060	1
16	F-box/LRR-repeat protein 3-like	LOC101509439	1
17	Disease resistance response protein 206-like	LOC101504947, LOC101504628	2

Therefore GSTs play an important role in FW resistance. The genes encoding proteins like FAR-RED IMPAIRED RESPONSE 1-like, Ca^{2+} transport ATPase like, 14-3-3 proteins, subtilisin-like, phospholipases like and Cell wall-associated Putative protein kinases were identified in redefined QTL regions. Protein FAR-RED IMPAIRED RESPONSE 1-like plays an important role in regulating plant immunity, through integrating chlorophyll biosynthesis and the salicylic acid (SA) signaling pathway (Wang *et al.,* 2016). Protein phosphatase 2C involved in the defense response by activating the downstream signaling cascades like activation of NB-LRR disease resistance protein (Widjaja *et al.,* 2010). Ca^{2+} transport ATPase play an important role in Ca^{2+} efflux processes as well as Ca^{2+} influx events in shaping defined Ca^{2+} signals which involved in the activation of SA signaling pathway and glucoinolate in response to pathogen infection (Cheval *et al.,* 2013). The 14-3-3 proteins act as a phosphosensors, play role in pathogen perception by effector molecules and interaction between downstream defense related proteins (Lozano-Duran and Silke Robatzek, 2015). 14-3-3 proteins were accumulated at high amount in chickpea genotype (resistant) when compared susceptible genotype when challenged with *Fusarium oxysporum* f.sp. *ciceri* (Ashraf *et al.,* 2009). Subtilisin-like (subtilases) are serine proteases that specifically induced following pathogen infection and very recently an *Arabidopsis* subtilase (SBT3.3) was hypothesized to function as a receptor located in the plasma membrane activating downstream immune signaling processes (Figueiredo *et al.,* 2014). Phospholipases involved in production of important defense signaling molecules, which has been shown to modulate the activity of a variety of proteins involved in defense signaling (Canonne *et al.,* 2011). Cell wall-associated putative protein kinases serve a vital role in cell elongation and are required for plant development. Recently, rice wall-associated protein kinases (*Oryza sativa* WAK) functional evidence that induction of *Os*WAK1 as a novel wall associated protein kinase plays important roles in plant disease resistance (Li *et al.,* 2009).

Two candidate genes encoding for peroxidase like and cysteine-rich receptor like proteins involved in cellular redox and energy metabolism were identified. Peroxidase induced during the host plant defence participated in physiological processes, such as lignin and suberin formation, cross-linking of cell wall components, and synthesis of phytoalexins, or participate in the metabolism of ROS (reactive oxygen species) and RNS (reactive nitrogen species), both switching on the hypersensitive response, a form of programmed host cell death at the infection site associated with limited pathogen development (Almagro *et al.,* 2009). Cysteine-rich receptor like protein could be involved in direct ROS sensing. This protein has been linked to many different physiological processes, such as plant development, pathogen defense and abiotic stress response (Molendijk *et al.,* 2008).

Candidate genes involved in cell signaling, transcription and RNA processing are known to regulate many cellular responses in an organism. In the present study, gene encoding for serine/threonine protein kinase or protein kinase and aspartic acid proteinase were identified. Ashraf *et al.* (2009) showed that serine/threonine protein kinases were highly expressed in susceptible chickpea genotype than resistance challenged with FW. Identification of constitutive disease resistance gene (CDR1) encoding an apoplastic

aspartic protease reveals that it might function by generating an endogenous peptide elicitor to induce local and systemic defense responses (Xia *et al.,* 2004).

Protein F-box/LRR domain like and argonaute 7-like involved in protein-protein interaction were identified. The F-box proteins potentially involved in Hypersensitive reaction (HR) (Van-Den-Burg *et al.,* 2008) whereas, LRR (leucine rich repeats) were evolutionarily conserved in many proteins associated with innate immunity in plants (Padmanabhan *et al.,* 2009). Argonaute4 (AGO4) in *Arabidopsis* works independently of other components of the RNA-directed DNA methylation (RdDM) pathway in mediating resistance to *Pseudomonas syringae* pv *tomato* DC3000 (Agorio and Vera, 2007).

The putative candidate genes for transcription factors (TF) (NAC TF like and WRKY 33-like TF) were identified in the present study. NAC TFs are central components of many aspects of the plant innate immune system, basal defense, and systemic acquired resistance (Nuruzzaman *et al.,* 2013). WRKY TF involved in repression as well as activation of defense related genes/pathways during biotic and abiotic stress (Chi *et al.,* 2013). To support the present investigation both NAC transcription factor and WRKY like TF were upregulated in FW challenged chickpea resistant genotype (WR315) than susceptible genotype (JG62) (Ashraf *et al.,* 2009).

Pathogen attack is often accompanied by the accumulation of elevated levels of transcripts of disease related proteins. In the present investigation *in silico* approach helps in identifying the putative candidate genes involved in the disease resistance (Table 19). Late embryogenesis abundant (LEA) proteins accumulate in response to water limitation in vegetative tissues. Hanin *et al.* (2011) showed that over-expression of group 2 LEA proteins from *Arabidopsis* affects the expression of genes related to plant defense responses. The gene encoding for Selenoprotein K like identified in QTL region is a membrane protein involved in antioxidant defense, calcium regulation and the ER-associated protein degradation pathway (Liu *et al.,* 2014). Two genes encoding for DNAJ and heat shock protein 70 (Hsp70) were identified in the QTL region. Kanzaki *et al.* (2003) reported that Hsp70 chaperone plays an important role in plant disease resistance and they showed in tobacco that Hsp70 is required for resistance to *Pseudomona* chicorii. DnaJ also called as heat shock proteins 40 (Hsp40) is a co-chaperone component of the Hsp70 system (Kampinga and Craig, 2010).

The gene encoding for protein SRG1-like, aminoaclyase 1-like, glucan endo-1, 3-beta-glucosidase, CCR-4 associated factor 1 homolog 11-like, and disease response protein 206 involved in disease resistance were identified in the present investigation (Table 19). Truesdell, 1998 identified a novel alfalfa gene, *SRG1*, which was induced in response to plant stress. Nakano *et al.* (2014) identified DS2 gene encoding for aminoaclyase like protein in *Nicotiana benthamiana* which was involved in negative regulation of plant defense responses against *Phytophthora infestans* via NbMEK2 and SA-dependent signaling pathway. Hrmova and Fincher (1997) reported that glucan endo-1, 3-beta-glucosidase involved in protection against microbial invasion of germinated barley grain through its ability to degrade fungal cell wall polysaccharides.

Sarowar *et al.,* (2006) reported the over-expression of the pepper CCR-4 associated factor 1 (*CAF1*) gene in tomato plants resulted in enhanced resistance against the *oomycete* pathogen, *Phytophthora infestans*. In addition, multiple defence related genes, including *PR1* and *PR6*, are constitutively up-regulated in these transgenic plants. The disease resistance response protein (DRR206) gene is induced upon infection with pathogens involved in non-host resistance and treatment with abiotic agents, and moderately induced by wounding (Choi *et al.,* 2004). Of few genes in the redefined QTL regions were not yet annotated, if the functions of these unknown genes are deduced will provide much better resolution about resistance genes.

In conclusion, we detected three redefined QTL regions which explained more phenotypic variation for FW resistance and had higher LOD scores. We delimited the small region harbouring 75 putative genes for disease resistance. Our results provide useful robust markers for marker assisted breeding and the localization of candidate genes for understanding molecular basis for FW resistance in chickpea through saturation of the region with additional SNP and SSR markers developed in the region. The loci and candidate genes identified in this study will be useful for chickpea improvement. The candidate genes nominated in this study need to be verified and functionally characterised.

V SUMMARY

Fusarium wilt (FW) is a serious problem for chickpea production in India. It is caused by soil borne pathogen *Fusarium oxysporum* f. sp. *ciceri*. It can cause major yield loss upto 90 per cent when the conditions are favourable for disease development. Due to soil borne nature of pathogen, it is very difficult to manage the disease. High level of resistance is available in cultivated varieties. However, the major problem encountered in resistance breeding is the development and maintenance of uniform wilt sick plots for genotype selection. To overcome all these problems, attempts were made to identify molecular markers linked to FW resistance. In recent years several researchers identified markers linked to wilt resistance genes/QTLs. However, the QTLs/markers represent a wider genomic region making them unreliable in chickpea FW resistance breeding programme. Therefore, the present investigation was undertaken to redefine the QTLs with closely linked markers.

The present investigation utilized the previous QTLs information to fine map the FW resistance regions in the chickpea genome by exploring available genomic resources, such as the annotated draft genome sequence of chickpea from NCBI and transcriptome data from CTDB to identify the relative position of markers tightly linked and/or flanking QTLs for FW resistance through sequence based physical map of chickpea using BLAST search tool against whole genome sequence of chickpea.

Overall, 46 markers linked to FW resistance were utilized for physical mapping in chickpea genome. Among 46 markers, five RAPD, one AFLP and two ISSR markers were not suitable for physical map hence, not included in this study. However, the DNA sequence of polymorphic band of A07C an RAPD marker was available and utilized for mapping. Out of thirty nine markers, 23 were mapped on chickpea chromosomes and six were mapped on unplaced genomic scaffold. The remaining ten markers could not be mapped to any of the chromosomes or unplaced genomic scaffolds as they did not meet the standard marker mapping criteria.

Of these 23 markers mapped, two markers (GSSR11 and GSSR18) were mapped to chromosome (Ca) 1, eight markers (TA110, TA200, H3A12, TA37, TA27, TA59, TR19 and GA16) were mapped to Ca2, two markers (TR24 and TC14801) were mapped to Ca3, five markers (TR20, TS82, ESTSSR21, A07C and TS72) were mapped to Ca4, only one markers (ESTSSR3) was mapped to Ca5 and five markers (TR44, CaM1125, H4E09, CaM1402 and CaM1101) were mapped to Ca6.

After physical mapping, ten genomic regions were selected for identification of genes and microsatellite repeats on chromosome 2, 3, 4 and 6. On chromosome 2, two genomic regions were selected. The region I contained a QTL between TA110 and H3A12 flanking 6.84 Mb, 310 genes and 314 genic SSR motifs. The region II contained QTL *Wilt 1* flanked by TA27 and TA59 markers covered a genomic region of 5.83 Mb, consisting of 135 genes and 204 genic SSRs. On chromosome 3 only one region flanked by two FW resistance linked markers TR24 and TC14801 was mapped. The region covered 1.16 Mb genomic region consisting of 153 genes and 179 genic SSRs. On

chromosome 4 three genomic regions were mapped. The region I flanked by TR20 and TS82 covered a distance of 2.52 Mb consisting of eighty four genes and 117 genic SSR motifs. The region II and III were flanked by EST SSR21-A07C and A07C-TS72 respectively, the total genomic region covered between EST SSR21-TS72 was 3.82 Mb consisting of 253 genes and 373 genic SSRs. On chromosome 4 four genomic regions were scanned for genes and SSR motifs. The region I flanked by TR44 and CaM1125 covered a distance 0.53 Mb and consists of twenty one genes and ninety three genic SSRs. The region II was flanked by markers CaM1125 and H4E09 covered genomic region of 0.038 Mb. The genomic region III covering twenty five genes and 56 genic SSRs motifs. The region IV was flanked by markers CaM1402 and CaM1101 covered 13.58 Mb, consisting 518 genes and 776 genic SSRs.

On unplaced genomic scaffolds six markers (TA22, H4G11, TA186, TA96, ESTSSR65 and H1B06) were physically mapped. From six scaffolds overall genomic region covered was 1.65 Mb, comprising of 48 genes and 80 SSR motifs.

After physical mapping, totally 36.85 Mb and 1.65 Mb genomic regions in chromosomes and scaffolds respectively, were scanned for genes and SSR motifs. Altogether, 1,578 genes and 2,250 genic SSR motifs were identified. Among the microsatellite motifs, the mononucleotide repeats were most abundant followed by di, tri, tetra, penta and least hexa nucleotide repeat units. Out of 2,250 genic SSR identified in the FW resistance genomic region, it was possible to design primer pairs for only 941 genic SSR motifs. From among 941 markers 168 markers were selected for primer synthesis based on preferred criteria *i.e.,* a microsatellite should repeat more than seven times and excluding mononucleotide repeats. These synthesised novel genic SSR markers were named as UASBC (University of Agricultural Sciences, Bengaluru Chickpea) series primers.

Of these 168 genic SSR markers were tested for amplification of DNA template of three chickpea genotypes JG62, WR315 and K850. One hundred and sixty one out of 168 produced scorable and predicted profile in all the three genotypes. Twenty three markers (13.69 %) produced polymorphic bands between susceptible JG62 and resistant WR315, while twenty four markers (14.28 %) were polymorphic between late wilting (K850) and resistant (WR315) parental lines. Seventeen markers (10.11 %) recorded polymorphism between susceptible JG62 and late wilting K850 genotypes.

The polymorphic SSR markers identified in the present study were used to genotype two RIL populations –population (JW) and population (KW). The χ^2 test showed that all the twenty three markers followed the expected Mendelian segregation ratio of 1:1 for population (JW). For population (KW) out of 24 polymorphic markers, the χ^2 test revealed that twenty two markers showed a normal Mendelian segregation (1:1), the remaining two markers (UASBC168 and UASBC169) showed segregation distortion ($P<0.05$).

The genetic linkage map was constructed separately for both populations using ICIM QTL IciMapping version 4.00 software. For population JW the genetic linkage map

was constructed using 23 markers and 125 F_{12} RILs. Twenty two markers were mapped into three linkage groups (LG) spanning a total distance of 144.51 cM with an average marker distance of 6.53 cM. Only one marker (UASBC76) was unlinked. For population KW, 22 markers which showed normal Mendelian ratio of 1:1 and 141 RILs were used for linkage map construction. Twenty two markers formed three linkage groups spanning a total length of 100.46 cM with an average markers density of 5.23 cM.

The RILs of population JW was evaluated for wilt resistance at 30 and 60 DAS by growing them in wilt sick pot under greenhouse conditions in the Department of Plant Biotechnology, UAS, Bengaluru. The RIL populations of JW and KW were also phenotyped in the earlier generations (F_8 and F_9) for wilt reaction in wilt sick field over two years 2007 and 2008 at ICRISAT, Hyderabad, India. The data was also used for present study. Under both field and pot conditions, the RILs were scored for wilt reaction based on the actual number of wilted plants on 30^{th} and 60^{th} DAS and expressed as percentage values.

Single marker analysis based on linear regression, identified a total of 10 markers explaining 9.22 to 25.54 per cent variance for *Fusarium* wilt resistance in population JW. The wilt reaction in both the stages, field and pot experiments were considered. Three markers (UASBC8, UASBC48, and UASBC57), showed consistent association with FW resistance across pot and field experiments at 30 DAS. For population KW only one marker UASBC53 explained the phenotypic variance of 8.38 per cent with a LOD score of 2.60.

For the mapping population (JW), the QTL mapping resulted in the identification of four (*qW30-08-1-1, q30pot-3-1, qW30-07-3-1,* and *qW30-08-3-1*) for wilt score at 30^{th} day and two (*qW60-07-1-1* and *q60pot-3-3*) for wilt score at 60^{th} day were obtained for wilt reaction with a LOD score ranged from 2.50 to 7.93 in population JW. For the mapping population KW only one QTL (*qWilt60-07-3-1*) was identified for wilt score at 60^{th} day with a LOD score of 2.69.

Overall, seven QTLs were identified in the present study. Four major QTLs (*q30pot-3-1, q60pot-3-1, qW30-07-3-1* and *qW60-07-1-1*) explained a phenotypic variation of 23.62, 25.97, 17.31 and 12.82 per cent respectively. Three minor QTLs (*qW30-08-1-1, qW30-08-3-1* and *qWilt60-07-3-1*) were also identified with a phenotypic variation of 8.56, 8.64 and 9.45 per cent respectively from both the populations.

The present genetic map indicated that there were many regions in the linkage map which contained more than one QTL for FW resistance. For example in population JW the interval between UASBC79 and UASBC92 had two QTLs (*qW60-07-1-1* and *qW30-08-1-1*), one each for 30^{th} day wilt score in field screening (2007) and 60^{th} day wilt score in field screening (2008). Similarly, three QTLs (*q60pot-3-1, qW30-07-3-1* and *qW30-08-3-1*) were identified in the interval between the markers UASBC48 and UASBC50 for 60^{th} day wilt score in pot experiment and 30^{th} day wilt score in field screening (2007 and 2008) respectively.

The comparison of genetic map of JW with physical map revealed that, all the markers of LG1 were physically mapped on the chromosome 6 (Ca6) and all markers of LG2 and LG3 were mapped on Chromosome 4 (Ca4) and 2 (Ca2) respectively. The two QTLs (*qW60-07-1-1* and *qW30-08-1-1*) flanked by common markers UASBC79 and UASBC92 on LG1 were mapped to chromosome 6. These two markers were developed in the QTL region flanked by CaM1402 and CaM1101 which represented a region of 13.58 Mb consisting of 520 genes. The identification of novel markers linked to FW resistance in the same genomic region has resulted in the narrowing down of QTL to 4.53 Mb on chromosome 6. Only 241 genes were found in this new region compared to 520 found in the same QTL region earlier. One QTL (*q30pot-3-1*) flanked by UASBC32 and UASBC57 and three QTLs (*q60pot-3-1, qW30-07-3-1 and qW30-08-3-1*) flanked by common markers UASBC48 and UASBC50 on LG3 were mapped on to chromosome 2. These flanking markers were developed from QTL region *Foc1* and *Wilt 1* covering 13.61 and 6.48 Mb respectively on chromosome 2. The flanking markers UASBC32-UASBC57 and UASBC48-UASBC50 has narrowed down the QTL region to 5.46 and 3.98 Mb, consisting of 150 and 168 genes.

Out of twenty two mapped SSR markers, 21 were *in silico* anchored to three chromosomes (Ca2, Ca4 and Ca6) and one marker UASBC109 was mapped on unplaced genomic scaffold 96 in chickpea genome in population (KW). The QTL (*qWilt60-07-3-1*) which was flanked by markers UASBC51 and UASBC53 on LG3 was mapped to chromosome 4. These flanking markers were developed from the genomic region covered by two FW linked markers A07C and TS72, representing 1.56 Mb consisting 100 genes. The physical mapping of redefined QTL narrowed down the QTL region to 0.53 Mb consisting of only 37 genes.

The present study is the first report on candidate genes identification for *Fusarium* wilt resistance through *in silico* approach by redefining the QTL positions through fine mapping the loci. The identification of redefined QTLs on three chromosomes (2, 4 and 6) showed that there are three different loci controlling FW resistance. The present study delineated small regions harbouring 75 putative candidate genes for disease resistance. The candidate genes identified in the redefined QTL regions for FW resistance in chickpea genome encodes protein which are potentially involved in plant defense responses *viz.,* cell wall modification, biosynthetic pathway, metabolism, signaling transduction, RNA silencing complex, transcription factors (TF), regulatory pathway, protein-protein interactions, stress response, defense response, cellular redox and energy metabolism.

Future line of work

1. Development of additional markers like SNPs in the redefined QTL regions to saturate the genomic region to identify robust markers tightly linked to FW resistance genes.
2. Validation closely linked SSR markers identified in this study in different genetic backgrounds and utilization of these markers in MAS for FW resistance breeding in chickpea.

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APPENDIX-I: List genes and genic SSRs present in chickpea genomic region flanked by markers TA110-H3A12 on chromosomes 2 (Ca2)

TC- Transcript ID from Chickpea transcriptome database

Sl.No.	Gene ID	Putative function	SSR motifs identified
1	LOC101515721	Uncharacterised	(TC)7, (T)10
2	LOC101488480	Cyclin-D1-1-like	(AAT)7
3	LOC101488823	TC12529: RNA-binding (RRM/RBD/RNP motifs) family protein	(T)10, (T)13
4	LOC101489149	TC06405: RNase H family protein	No SSR
5	LOC101489480	Putative disease resistance protein RGA3-like	(AT)15
6	LOC101489794	Putative disease resistance protein RGA3-like	No SSR
7	LOC101491932	DNA polymerase beta-like	No SSR
8	LOC101490118	TC28713: Chromosome-associated kinesin KIF4A	No SSR
9	LOC101495285	Agamous-like MADS-box protein AGL16-like	No SSR
10	LOC101492479	Formin-like protein 4-like	No SSR
11	LOC101493141	Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial-like	(TTC)6
12	LOC101495609	Uncharacterised	(AT)14
13	LOC101493694	Paired amphipathic helix protein Sin3-like 4-like	(A)10, (T)12
14	LOC101494012	Paired amphipathic helix protein Sin3-like 3-like	(T)11
15	LOC101494958	Ribosomal RNA small subunit methyltransferase NEP1-like	NO SSR
16	LOC101508922	ruBisCO-associated protein-like	(A)11, (A)12, (TA)6, (TA)27, (TA)6 (TA)12
17	LOC101496598	PXMP2/4 family protein 4-like	(A)10
18	LOC101509239	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
19	LOC101496920	TC14775: Oxidoreductase family protein	No SSR
20	LOC101497247	TC00644: Cysteine-rich receptor-like protein; Putative protein kinase.	(T)11, (A)10
21	LOC101497808	Programmed cell death protein 4-like	(A)10
22	LOC101498334	TC18639: F14O23.16 protein	(T)10
23	LOC101498677	Ras-related protein Rab7-like	(T)14
24	LOC101499001	Quinone oxidoreductase-like protein At1g23740, chloroplastic-like	(T)10
25	LOC101509563	DEAD-box ATP-dependent RNA helicase 39-like	No SSR
26	LOC101499321	UPF0544 protein C5orf45 homolog	(CT)6, (A)10
27	LOC101500164	DEAD-box ATP-dependent RNA helicase 39-like	(T)10
28	LOC101499835	TC32192: Expressed protein	No SSR
29	LOC101501439	Choline/ethanolamine kinase-like	No SSR
30	LOC101501754	Dehydrogenase/reductase SDR family member 12-like	(T)10
31	LOC101502078	Type I inositol 1,4,5-trisphosphate 5-phosphatase 1-like	(TC)7, (CT)6, (TC)6, (T)11, (A)15, (A)11, (TTTTA)6
32	LOC101502811	30S ribosomal protein S1 homolog A-like	(GAA)6
33	LOC101509883	Sensor protein RcsC-like	(T)10
34	LOC101503134	Ran-binding protein 1 homolog a-like	(T)16, (T)12
35	LOC101503678	Cytochrome P450 82A1-like	(A)10
36	LOC101504005	Probable pectinesterase 29-like	(A)16
37	LOC101504324	TC22206: Munc13 homology 1	(T)10
38	LOC101504654	40S ribosomal protein S18-like	(T)10
39	LOC101504971	Uncharacterised conserved protein UCP009193	(TC)7, (T)13
40	LOC101505283	TC08202: Protein with a putative role in mRNA splicing	(T)10
41	LOC101506478	Transcription factor GTE6-like	(T)17

42	LOC101506806	Expressed protein	(A)12
43	LOC101507143	Nucleolar complex protein 2 homolog	(A)10
44	LOC101510200	Uncharacterised	No SSR
45	LOC101507461	Gibberellin-regulated protein 12-like	NO SSR
46	LOC101510536	Gibberellin-regulated protein 12-like	(A)11
47	LOC101507770	Microtubule-associated protein TORTIFOLIA1-like	No SSR
48	LOC101508092	UPF0392 protein RCOM_0530710-like	NO SSR
49	LOC101508407	TC07359: Cofactor assembly of complex C (CCB4)	NO SSR
50	LOC101511393	Transcription factor MYB1R1-like	(A)10, (A)11, (T)10
51	LOC101511722	Transcription factor MYB1R1-like	(AAC)7, (A)10
52	LOC101512039	Expressed protien	No SSR
53	LOC101512364	CLAVATA3/ESR (CLE)-related protein 25-like	(AT)6, (T)14
54	LOC101512685	Serine/threonine-protein phosphatase PP2A catalytic subunit-like	(A)12, (TATT)7
55	LOC101513003	Phospholipid-transporting ATPase 3-like	(T)10, (T)10, (TA)7, (AT)6
56	LOC101495832	Uncharacterised	NO SSR
57	LOC101513328	TC18539: Nuclear localized serine-arginine-aspartate-rich protein	(TA)6
58	LOC101513654	Transcription factor bHLH79-like	(CT)9
59	LOC101496155	TC00501: DNA GYRASE B3 (GYRB3)	(T)11
60	LOC101513982	Myosin-10-like	(TAA)18
61	LOC101514526	TC09325: CW14	(A)10, (T)10
62	LOC101514849	Probable beta-1,3-galactosyltransferase 19-like	(A)13, (T)11
63	LOC101496483	Basic 7S globulin 2-like	No SSR
64	LOC101515172	TC05867: Putative membrane lipoprotein	NO SSR
65	LOC101515492	Glutathione S-transferase U17-like	No SSR
66	LOC101488245	Glutathione S-transferase U17-like	NO SSR
67	LOC101488574	Tetratricopeptide repeat protein 38-like	(T)10, (T)10
68	LOC101489033	Probable serine/threonine-protein kinase At1g18390-like	(CA)12
69	LOC101489362	TC12371: Actin cross-linking protein	(A)14, (T)10, (T)10, (A)11, (T)10
70	LOC101489688	TC12272: Uncharacterised	(A)11, (TG)7
71	LOC101490008	TMV resistance protein N-like	NO SSR
72	LOC101496819	TC18789: Resistance to leptosphaeria maculans 3 (rlm3)	(ATA)27
73	LOC101490542	dnaJ homolog subfamily B member 13-like	NO SSR
74	LOC101490867	Thioredoxin H2-like	(T)10
75	LOC101491183	Thioredoxin H2-like	(A)11, (T)10, (ATT)8, (A)11
76	LOC101491626	Thioredoxin H2-like	NO SSR
77	LOC101491934	Keratin, type I cytoskeletal 10-like	(T)10, (T)12
78	LOC101492266	Nitrate transporter 1.7-like	(T)10, (T)14, (A)10, (A)10, (A)10
79	LOC101497473	Nitrate transporter 1.7-like	(A)10, (T)10, (A)12, (A)13, (T)15
80	LOC101497809	Expressed protien	(A)12, (T)10, (T)12
81	LOC101498127	Nitrate transporter 1.7-like	(TA)12, (A)12
82	LOC101492605	TC07647: 5'-AMP-activated protein Putative protein kinase-related	No SSR
83	LOC101492936	Ultraviolet-B receptor UVR8-like	(T)13, (A)10, (A)10, (A)11, (C)11
84	LOC101498459	F-box protein SKIP23-like	No SSR
85	LOC101498803	TC21805: Cysteine-rich receptor-like protein kinase	(A)11, (A)12
86	LOC101493268	TC14521: Spc7, Spc7 kinetochore protein	NO SSR
87	LOC101493589	5-Formyltetrahydrofolate cyclo-ligase-like	(T)10, (TA)12, (A)10
88	LOC101494328	Uncharacterised	(T)10
89	LOC101494641	CAM calmodulin	(TTG)6, (T)11

90	LOC101494959	TC10087: RINT-1 / TIP-1 family	(T)10
91	LOC101495504	Cyclin-dependent kinase C-2-like	(T)10
92	LOC101499441	Probable polygalacturonase At3g15720-like	(A)11, (TA)9
93	LOC101499747	Probable polygalacturonase At3g15720-like	(TA)9, (TTA)8
94	LOC101502506	F-box protein SKIP23-like	No SSR
95	LOC101500284	Homeobox-leucine zipper protein HAT5-like	(TC)13, (CA)6
96	LOC101500597	Nitrate transporter 1.2-like	(T)13, (A)11, (A)11, (T)10, (AT)7
97	LOC101500908	TC26323: Glyoxal oxidase-related protein	No SSR
98	LOC101500908	Protein TWIN LOV 1-like	No SSR
99	LOC101502813	Uncharacterised	No SSR
100	LOC101501755	Expressed protein	No SSR
101	LOC101503136	Probable receptor-like protein kinase At5g15080-like	(TAA)6
102	LOC101503679	Uncharacterised, Expressed protein	No SSR
103	LOC101504221	Alpha-amylase 3, chloroplastic-like	(T)14, (T)11, (T)10
104	LOC101490665	NV18643	No SSR
105	LOC101505176	Probable pectate lyase 3-like	(T)10, (A)10
106	LOC101505729	Preprotein translocase subunit SECE1-like	(CAT)7
107	LOC101506051	Uncharacterised	(TC)11, (CA)6, (T)13, (G)15
108	LOC101506576	Single-stranded DNA-binding protein WHY1, chloroplastic-like	(TA)12, (CT)11
109	LOC101490989	Pentatricopeptide repeat-containing protein At2g02750-like	No SSR
110	LOC101491301	Probable LRR receptor-like serine/threonine-protein kinase At1g14390-like	(T)11
111	LOC101507343	SNF1-related protein kinase regulatory subunit gamma-1-like	(T)15
112	LOC101507653	Protein IQ-DOMAIN 31-like	(TTTA)6, (T)11, (AC)24
113	LOC101491627	Cytosolic sulfotransferase 15-like	No SSR
114	LOC101491936	Uncharacterised	NO SSR
115	LOC101492268	protein FAR-RED IMPAIRED RESPONSE 1-like	(A)10
116	LOC101507978	protein kinase 2B, chloroplastic-like	No SSR
117	LOC101492606	uncharacterised	No SSR
118	LOC101508290	UDP-galactose/UDP-glucose transporter 3-like	No SSR
119	LOC101508608	transcriptional activator Myb-like	(A)10, (T)11, (T)10
120	LOC101508923	TC12459: NAD(P)-linked oxidoreductase superfamily protein	(T)11
121	LOC101509676	Homeobox-leucine zipper protein ATHB-13-like	(CT)6, (T)10, (T)10
122	LOC101509999	TC18887: RNA-binding (RRM/RBD/RNP motifs) family protein	No SSR
123	LOC101510537	TC24818: Dual-targeted threonyl-tRNA synthetase	NO SSR
124	LOC101510317	TC21713: Dual-targeted threonyl-tRNA synthetase	NO SSR
125	LOC101510761	Transcription factor bHLH135-like	(T)13
126	LOC101492937	Uncharacterised	No SSR
127	LOC101511086	Probable peptidyl-prolyl cis-trans isomerase-like	No SSR
128	LOC101511602	F-box/kelch-repeat protein SKIP11-like	No SSR
129	LOC101512365	Uncharacterised	No SSR
130	LOC101512686	TC10570: Mucin-related	NO SSR
131	LOC101513004	HVA22-like protein c-like	(T)10, (T)11
132	LOC101513549	Transcription factor TCP15-like	(TTCT)8, (T)12
133	LOC101514086	Ran guanine nucleotide release factor-like	(T)10
134	LOC101514850	Peroxisomal adenine nucleotide carrier 1-like	(T)10, (AT)7, (TA)7, (T)10
135	LOC101493269	Probable membrane-associated kinase regulator 6-like	(A)12, (T)10
136	LOC101515173	Cullin-3A-like	No SSR

137	LOC101515493	Uncharacterised	No SSR
138	LOC101494230	Uncharacterised	No SSR
139	LOC101488481	Haloacid dehalogenase-like hydrolase domain-containing protein 3-like	(T)15
140	LOC101494536	Pollen-specific leucine-rich repeat extensin-like protein 2-like	No SSR
141	LOC101488824	Origin recognition complex subunit 6-like	(A)19, (T)10
142	LOC101489150	Sphinganine C(4)-monooxygenase 1-like	(T)10
143	LOC101489481	Protein PHYTOCHROME KINASE SUBSTRATE 1-like	(GAA)7
144	LOC101489795	Probable methyltransferase PMT2-like	No SSR
145	LOC101490349	Protein FEZ-like	No SSR
146	LOC101509885	Glutathione S-transferase U10-like	(A)10
147	LOC101495834	60S ribosomal protein L34-like	No SSR
148	LOC101496157	Enolase-phosphatase E1-like	(T)10, (A)10
149	LOC101496485	Uncharacterised	No SSR
150	LOC101496707	TC15394: RING/FYVE/PHD zinc finger superfamily protein	(T)10
151	LOC101497016	PHD finger protein MALE STERILITY 1-like	No SSR
152	LOC101497346	Probable apyrase 6-like	(GA)10
153	LOC101497674	Acetolactate synthase small subunit 2, chloroplastic-like	(T)13
154	LOC101510202	Sugar transport protein 14-like	No SSR
155	LOC101510539	Uncharacterised	No SSR
156	LOC101498225	Pentatricopeptide repeat-containing protein At2g02980-like	No SSR
157	LOC101498556	Ribonuclease 1-like	(T)11, (T)11, (T)12
158	LOC101498891	Ribonuclease 1-like	No SSR
159	LOC101510860	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
160	LOC101499215	Ribonuclease 1-like	(AT)10, (A)18, (A)12
161	LOC101499535	Ribonuclease 3-like	No SSR
162	LOC101499836	Beta-1,3-galactosyltransferase 15-like	No SSR
163	LOC101500165	50S ribosomal protein L18, chloroplastic-like	(T)10
164	LOC101500485	Uncharacterised	No SSR
165	LOC101500806	(R)-mandelonitrile lyase-like	No SSR
166	LOC101511181	Uncharacterised	No SSR
167	LOC101501127	Putative two-component response regulator like APRR6-like	(A)11, (A)10
168	LOC101501865	Mediator of RNA polymerase II transcription subunit 8-like	(T)10
169	LOC101503458	Cyclic dof factor 3-like	No SSR
170	LOC101503797	Uncharacterised	(GATTAT)6
171	LOC101504118	TC03056: A locus involved in embryogenesis	(A)11
172	LOC101504656	Agamous-like MADS-box protein AGL11-like	(A)10
173	LOC101504432	Expressed protein	(A)10
174	LOC101511498	Uncharacterised	No SSR
175	LOC101504973	expansin-A15-like	(T)10
176	LOC101512464	TC07751: RING/U-box superfamily protein	No SSR
177	LOC101505496	Methyltransferase-like protein 7A-like	No SSR
178	LOC101505833	TC11557: cAMP-regulated phosphoprotein 19-related protein	No SSR
179	LOC101506148	CASP-like protein MTR_5g041900-like	(T)11, (A)17
180	LOC101513108	psbQ-like protein 1, chloroplastic-like	No SSR
181	LOC101506479	Uncharacterised	(T)10, (AT)6
182	LOC101506808	NAC3 NAC transcription factor 29-like	No SSR
183	LOC101513436	Uncharacterised	No SSR
184	LOC101507145	TC08591: Embryo defective 1579 (emb1579)	(A)14, (TTG)7

185	LOC101513747	Transcription factor MYB29-like	(T)14, (T)10, (TAT)18, (T)12
186	LOC101507654	TC07243: Arabinanase/levansucrase/invertase	No SSR
189	LOC101514087	Putative protein TPRXL-like	No SSR
190	LOC101514411	TC14491: Integrase	No SSR
191	LOC101507979	Phosphate transporter PHO1 homolog 10-like	No SSR
192	LOC101508291	Multiple myeloma tumor-associated protein 2 homolog	(T)10, (T)14, (CA)12, (AT)13
193	LOC101508609	TC05107: O-fucosyltransferase family protein	(T)10
194	LOC101508924	Transmembrane emp24 domain-containing protein p24delta9-like	No SSR
195	LOC101509240	Transmembrane emp24 domain-containing protein p24delta7-like	No SSR
196	LOC101515065	trichohyalin-like	No SSR
197	LOC101509564	Transmembrane emp24 domain-containing protein p24delta7-like	No SSR
198	LOC101507022	NAC transcription factor 25-like	(AT)9, (A)11
199	LOC101515387	uncharacterised	No SSR
200	LOC101515723	Vesicle transport v-SNARE 12-like	(TTTC)8, (T)13, (GT)6, (T)10
201	LOC101488482	TC05744: Early-responsive to dehydration stress protein (ERD4)	(T)10
202	LOC101488825	Protein argonaute 7-like	(TC)8, (GA)7, (T)10, (AATA)6, (TTTA)6
203	LOC101489151	Protein UXT homolog	(T)13, (T)10
204	LOC101489690	Probable S-acyltransferase At1g69420-like	(TC)9, (T)12
205	LOC101490667	Uncharacterised	(T)10
206	LOC101491186	Phosphoribosylamine--glycine ligase, chloroplastic-like	(T)15
207	LOC101507655	Uncharacterised	No SSR
208	LOC101507980	Uncharacterised	No SSR
209	LOC101508292	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
210	LOC101491500	TC15116: Amino acid Putative protein kinase family protein	(T)10
211	LOC101508610	Mitotic checkpoint protein bub3-like	No SSR
212	LOC101491809	Cell division topological specificity factor homolog, chloroplastic-like	No SSR
213	LOC101508925	TC19067: Transposase-like gene with conserved domains from the family of hAT transposases	(TA)10, (GA)6, (T)10
214	LOC101492137	Uncharacterised	(T)14, (T)10
215	LOC101492480	Chorismate mutase, chloroplastic-like	No SSR
216	LOC101492814	Glutamyl-tRNA(Gln) amidotransferase subunit B, chloroplastic/mitochondrial-like [Cicer arietinum	(A)11, (A)11
217	LOC101493143	Uncharacterised	(T)14
218	LOC101509241	F-box/LRR-repeat protein 3-like	No SSR
219	LOC101493470	EGF domain-specific O-linked N-acetylglucosamine transferase-like	(T)10, (A)10
220	LOC101509565	Serine/arginine repetitive matrix protein 1-like	No SSR
221	LOC101493792	Uncharacterised	(A)11, (T)11, (AAT)9, (A)10
222	LOC101509886	Agamous-like MADS-box protein AGL16-like	No SSR
223	LOC101494114	Putative pentatricopeptide repeat-containing protein At1g69350, mitochondrial-like	No SSR
224	LOC101494419	TC14547: uvrB/uvrC motif-containing protein	(T)7
225	LOC101495610	Acidic endochitinase-like	No SSR
226	LOC101496600	Protein GDAP2 homolog	(T)13, (T)11, (T)12
227	LOC101496271	Uncharacterised	No SSR

228	LOC101495937	26S proteasome non-ATPase regulatory subunit 10-like	No SSR
229	LOC101497141	COBW domain-containing protein 1-like	No SSR
230	LOC101510203	ATP-dependent DNA helicase PIF1-like	No SSR
231	LOC101510540	Floral homeotic protein APETALA 1-like	(A)10, (TA)12, (AT)7
232	LOC101497475	Beta-glucosidase 40-like	(AT)10
233	LOC101497812	Probable UDP-glucose 6-dehydrogenase 1-like	No SSR
234	LOC101498129	UPF0396 protein-like	No SSR
235	LOC101498460	Probable WRKY transcription factor 57-like	(CT)8, (A)17, (A)11, (AT)11
236	LOC101498805	Pentatricopeptide repeat-containing protein At2g44880-like	No SSR
237	LOC101499121	Putative SNAP25 homologous protein SNAP30-like	(T)11, (A)10, (AT)6
238	LOC101499639	Probable leucine-rich repeat receptor-like protein kinase At1g35710-like	No SSR
239	LOC101499943	Pentatricopeptide repeat-containing protein At2g20540-like	No SSR
240	LOC101500598	TC06110: RING/U-box superfamily protein	No SSR
241	LOC101500909	TATA-binding protein-associated factor 2N-like	No SSR
242	LOC101501237	Beta-glucosidase 12-like	No SSR
243	LOC101511499	Putative pentatricopeptide repeat-containing protein At1g26500-like	No SSR
244	LOC101511826	Uncharacterised	(T)11
245	LOC101502079	14-3-3-like protein GF14 iota-like	No SSR
246	LOC101501756	Putative fasciclin-like arabinogalactan protein 20-like	No SSR
247	LOC101502406	Uncharacterised	(TC)6
248	LOC101512138	TC05096: *Arabidopsis thaliana* VILLIN4	No SSR
249	LOC101502709	Interaptin-like	No SSR
250	LOC101504869	Triose phosphate/phosphate translocator, chloroplastic-like	No SSR
251	LOC101503902	Pentatricopeptide repeat-containing protein At1g26460, mitochondrial-like	No SSR
252	LOC101505178	Glucan endo-1,3-beta-glucosidase-like protein At1g69295-like	(T)10
253	LOC101505498	TC06282: Alpha/beta-Hydrolases superfamily protein	(TC)6, (T)10, (T)10, (AT)21
254	LOC101505834	Anthranilate synthase component I-1, chloroplastic-like	No SSR
255	LOC101506149	Formin-like protein 3-like	No SSR
256	LOC101506689	Latent-transforming growth factor beta-binding protein 4-like	(A)11, (TAAT)6
257	LOC101513109	Proline-rich receptor-like protein kinase PERK9-like	No SSR
258	LOC101513655	Ureide permease 2-like	No SSR
259	LOC101514412	Ureide permease 1-like	(T)14, (A)12, (A)10, (T)10
260	LOC101514739	Pentatricopeptide repeat-containing protein At5g50280, chloroplastic-like	No SSR
261	LOC101515066	UPF0420 protein C16orf58 homolog	(A)10, (T)11, (T)11
262	LOC101488576	Pentatricopeptide repeat-containing protein At1g69290-like	No SSR
263	LOC101488920	Probable inactive purple acid phosphatase 1-like	No SSR
264	LOC101489567	Probable inactive purple acid phosphatase 1-like	No SSR
265	LOC101489243	Magnesium transporter MRS2-5-like	(A)11
266	LOC101498892	Uncharacterised	No SSR
267	LOC101490544	glu S.griseus protease inhibitor-like	(A)15
268	LOC101490870	Ninja-family protein AFP1-like	(CAT)7
269	LOC101491188	Protein SPIRAL1-like 2-like	(AT)9
270	LOC101499216	Serine/threonine-protein phosphatase 7 long form homolog	No SSR

271	LOC101491502	Cytochrome P450 78A3-like	(TC)12
272	LOC101500166	Uncharacterised	No SSR
273	LOC101491810	Serine/threonine-protein kinase dst1-like	(ACG)6
274	LOC101500486	Pentatricopeptide repeat-containing protein At1g20230-like	No SSR
275	LOC101492138	Uncharacterised	No SSR
276	LOC101492481	Uncharacterised	(T)13
277	LOC101500807	Uncharacterised	No SSR
278	LOC101492815	probable 6-phosphogluconolactonase 1-like	(TAT)23
279	LOC101493906	TC09857: Fructoputative protein kinase-like protein	(CT)10, (T)11
280	LOC101493373	Folic acid synthesis protein fol1-like	No SSR
281	LOC101494231	Peptidyl-prolyl cis-trans isomerase E-like	No SSR
282	LOC101494538	Pentatricopeptide repeat-containing protein At1g69290-like	No SSR
283	LOC101501128	Uncharacterised	(T)11, (A)11
284	LOC101501440	Uncharacterised	(AGA)9
285	LOC101501757	Protein FAR1-RELATED SEQUENCE 5-like	No SSR
286	LOC101494858	Cytochrome b5-like	(CT)6
287	LOC101495182	PI-PLC X domain-containing protein At5g67130-like	(A)10, (T)11, (GT)6, (T)10, (T)10, (T)10
288	LOC101502080	TC01124: Cysteine-rich receptor-like protein; Putative protein kinase	No SSR
289	LOC101495505	Receptor-like protein kinase 2-like	No SSR
290	LOC101495835	Pentatricopeptide repeat-containing protein At1g12300, mitochondrial-like	No SSR
291	LOC101496377	UGT73AB1 UDP-glycosyltransferase 73D1-like	No SSR
292	LOC101496708	Alpha-1,4-glucan-protein synthase	No SSR
293	LOC101502407	Uncharacterised	No SSR
294	LOC101497017	Salicylate O-methyltransferase-like	(T)11, (T)10, (T)19
295	LOC101497348	Protein CRABS CLAW-like	(T)10, (AT)6
296	LOC101497906	Uncharacterised	(T)17
297	LOC101505936	Putative tnp2 transposase	No SSR
298	LOC101506250	T-complex protein 1 subunit alpha-like	No SSR
299	LOC101503680	Floral homeotic protein APETALA 1-like	(AT)6, (TA)8, (A)10, (T)10, (AT)6, (AT)10
300	LOC101504006	MADS-box protein CMB1-like	(TA)6, (T)13, (T)13, (T)17, (TA)6, (A)8
301	LOC101504974	Uncharacterised	No SSR
302	LOC101506577	Putative transposase protein	No SSR
303	LOC101506913	Plant transposase (Ptta/En/Spm family)	(T)13
304	LOC101505285	Squamosa promoter-binding-like protein 6-like	No SSR
305	LOC101507240	Uncharacterised	No SSR
306	LOC101507868	Adenine nucleotide alpha hydrolases-like superfamily protein	No SSR
307	LOC101513438	Uncharacterised	No SSR
308	LOC101514089	Uncharacterised	No SSR
309	LOC101508180	Dentin sialophosphoprotein-like	(T)10
310	LOC101514414	Uncharacterised	No SSR

APPENDIX II: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by TA110-H3A12 on chromosome 2

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101515721	(TC)7	217	TTTCCTTCCCTCTCTTCATTCA	22	AGTGACCAGGGTTTGTTTTCAT	22
LOC101488480	(AAT)7	249	GCTTGCTGATGTTTTGAGTACG	22	TGAAGGTCCAATAAAGAAGGGA	22
LOC101489480	(AT)15	386	CTAATTGTCCGAAGTTGACGTG	22	GGAATGCAAGAAGGTGATAAGTG	23
LOC101493141	(TTC)6	252	TTACCCTCACTCCTCTGGTGAT	22	AGAAGGTTGGTAGATCGGAATG	22
LOC101495609	(AT)14	337	CCTTTTGTTGTTTGGGAAGCTA	22	TATTTGAATGTGTAGGGCATGG	22
LOC101511722	(AAC)7	399	CATGATTTTCGGTGTTCGACT	21	TGGTGTGCGAGTTTTAACAAAG	22
LOC101512685	(TATT)7	303	CACGGACAGTTTTACGATCTCA	22	TCGAATCCAACATCACACAATC	22
LOC101513003	(TA)7	302	CGGGAAAAGATGTTAGATGAGG	22	AACTTGAGGGGAGTGTTGACAT	22
LOC101513003	(AT)6	397	AGGGTGCAGCTAGGTTAAAACA	22	GTGCAGGTAATGGGTACAAACA	22
LOC101513328	(TA)6	263	CACACGTCTCTCTCATTCAACAG	23	ATAGGCATTTTCATATCGGTGG	22
LOC101513654	(CT)9	198	CTACTCTCCACTCTCCCAACCA	22	GTAGGTGATAGGATGCCGTGTT	22
LOC101513982	(TAA)18	389	TCAATGGGTGTATTAAGCACAG	22	GTCAGTGTTTCTGGGTGTTTTC	22
LOC101489033	(CA)12	334	TTCTCTCCTTCCTCTCCTTCCT	22	GAATATGGGCAAGTGTTGTTGA	22
LOC101489688	(TG)7	288	TTGGGTACACTTGTTTGAGGTG	22	GCAATATAGAGCCGCGATTAAC	22
LOC101501439	(AT)11	344	TCGATTTGCTCTAATACTGCTGAC	24	CCTCTGCCTTGAATACTCCTTG	22
LOC101501439	(TA)9	344	TCGATTTGCTCTAATACTGCTGAC	24	CCTCTGCCTTGAATACTCCTTG	22
LOC101502078	(TTTTA)6	393	TACTCCTTCAACGTACCAACCC	22	TGGCGAGACAAATACACAACTC	22
LOC101502078	(A)15	288	GATCCTCCTTGAACTGTCTTGC	22	ACGGATACGACGGAGAAACTTA	22
LOC101502078	(CT)6	259	TCCACAGTCAAAACCTCTCTCA	22	CAACAAACCCCACACAACATAG	22
LOC101502811	(GAA)6	392	CCTACCCATGTTTCATTTTGCT	22	TCATTTCCTCCAACAACAACAC	22
LOC101504971	(TC)7	326	TCACCAGAAACTCTCCTTCCAT	22	ATCAGGTCGGTGTATAAGAGGG	22
LOC101499321	(CT)6	235	TGTCAACACTCTTCATCGCTCT	22	GAATGTCCTTGGCTTGATAACC	22
LOC101491183	(ATT)8	285	CAAAGCGTTTCTGTGTAACCAA	22	TTTGGATGAAGATGTGATGTCC	22
LOC101494641	(TTG)6	325	TATGGGTGGTGATCTAGGGTTC	22	ATTCATGTCTCAGTGTTGGTGC	22
LOC101500284	(TC)13	113	CCCACCACACAGAAGAAAGAA	21	AGAAGGCAAGAGAGAAGAGGGT	22
LOC101500284	(CA)6	385	GTCTGAGCTTTGGCTTTTGAAT	22	TGGTATTTGTATCGGTGCTAAGTG	24
LOC101503136	(TAA)6	394	TCAAAGGCTGCTAGTGTATGAGTT	24	TACTGGTCGTTCAGCTTCTTCA	22
LOC101490665	(AT)12	355	TGCCCCAACCTTAGAAGATAAA	22	CTTTGCTCACAACACAACCATT	22
LOC101490665	(A)11	355	TGCCCCAACCTTAGAAGATAAA	22	CTTTGCTCACAACACAACCATT	22
LOC101490665	(A)12	355	TGCCCCAACCTTAGAAGATAAA	22	CTTTGCTCACAACACAACCATT	22
LOC101505729	(CAT)7	200	AAGAATAAAAGAAGCGCGAGTG	22	GGCGGTTGAGAGTAAGAGAGAG	22
LOC101506051	(TC)11	255	GTAATGAGCCCAAAACGAAGTC	22	CTGATATGCGATTGTGTGTGTG	22
LOC101506576	(AT)12	398	TGGTTATGAGTGGTTGACTGTG	22	TATCTTGAATGACCCTGACTGC	22
LOC101506576	(CT)11	353	GAGCTTGCAGTTTTCAATTCAC	22	CCACTTCTTTCCTAATTTCGCT	22
LOC101507653	(TTTA)6	390	TTGGATTCTCAGGGTTCATTCT	22	CCATTTTCAAGCAAGTGCAA	20

LOC101507653	(AC)24	160	TCCTTTTCTCTTGAGCTTGACTG	23	ACGATGGGATAACTGAGGAAGA	22
LOC101514850	(AT)7	364	GTACTCCCTCCCTGTCGC	18	GCACCACACTTCATTCCTATGT	22
LOC101514850	(TA)7	364	GTACTCCCTCCCTGTCGC	18	GCACCACACTTCATTCCTATGT	22
LOC101488824	(A)19	227	AATCCACATCTCAACCCTCATT	22	TTAGGGAATGCAACTTACGACA	22
LOC101489481	(GAA)7	207	CCTCTAGGGAAACACCTCAAAA	22	CAATGCTTACTTCACTTGGTGC	22
LOC101497346	(GA)10	317	ATTCTTTCTCGTCCACTCCGTA	22	GTTGCACTTTGAAGCGTTGTAA	22
LOC101503797	(GATTAT)6	364	TTGTTGCTCTTTATGGTCCTCA	22	GTGCTTGCATTAGTCTTTCTTCC	23
LOC101507145	(TTG)7	363	GTCATGTCAAATTAGCACGCTC	22	AGAGACCAATCCTGAAAGCAAG	22
LOC101508291	(AT)13	310	AAGGTAGGAAGGCTGTAGAGGG	22	TCCATTTCTTCACTTTGGGACT	22
LOC101507022	(AT)9	347	AGATGGAAGCCGGTCCTT	18	CTGTTGTGTCCACCATTTGATT	22
LOC101515723	(TTTC)8	192	TCTTCCTCACTTCAGATCCCTC	22	GAAAACTTCGCTCATCTTTGCT	22
LOC101488825	(TC)8	290	TCGTCTCTCTCACTTGTTCTCAA	23	GTGATGATGGTGGTGATGATG	21
LOC101488825	(GA)7	400	ACCCTGCTCTTCTTCCTTTACC	22	ACAACCCCAAATGCAATGTT	20
LOC101488825	(AATA)6	308	TGATCCCTTATGTTCCAATGCT	22	TATAGCCCAACAGACACCACAC	22
LOC101488825	(TTTA)6	378	GTGTGGTGTCTGTTGGGCTAT	21	ACCTCATTAGAGAAGGTTTACATGC	25
LOC101489690	(TC)9	382	CCAACAACAGAAACAACACACA	22	AAATGCAAACACCAAAGGGTAG	22
LOC101493792	(AAT)9	287	TGCTTTCGTTCTTGTTGTTG	20	CCAGCACATCATAAATACATCC	22
LOC101510540	(TA)12	399	TGTGGGTTACAAAGCAAGAGAA	22	TTCAAAACAGGAAGTGGGTCTT	22
LOC101510540	(AT)7	372	GAGAAGGAGAAGGCTGTAGCAC	22	GTGGTTCAAGTGTCAGGTCAAG	22
LOC101497475	(AT)10	182	AGAGATAAGCAAAACTTGGCGA	22	ATCAATGGGTCAAGAAACCTGT	22
LOC101498460	(CT)8	181	GTCCCTTCCTTCTCACAACAAC	22	AGGGTGCTCTGTCTTTGTCTTC	22
LOC101499121	(AT)6	113	TGCACGCAAATTAGTAGCAAAG	22	AACAAATCACAACCGATGAGTG	22
LOC101502406	(TC)6	210	ACCCAAAGAGGAGGAACAAGAT	22	TCAAGAGCTTCCAACAGTCTCA	22
LOC101505498	(AT)21	202	GGATGATGCTACCGTTGATTTC	22	TTGGAGAAGGTCCGATAAGAGA	22
LOC101505498	(TC)6	329	ATGCGGATTCAAAGATTACCTG	22	CACCAAAGATACCCTCACCATT	22
LOC101506689	(TAAT)6	377	CATCCAAATGCAGTCCTCAGT	21	CCGTTTCATATAGTTGTGTCGG	22
LOC101490870	(CAT)7	262	CTGGGTATATGCTATTCTGGGG	22	TCGGGTACTTCTCCATCTTCAT	22
LOC101491188	(AT)9	247	TGCTTGCTGCTTCTTGTAATGT	22	GGGGTAAAGGAAATAGTTGGGT	22
LOC101491810	(ACG)6	143	CTCTTTCTCCGCGCTAACC	19	CGTATGGATCTTCCTGAGCTTC	22
LOC101492815	(TAT)23	282	ATCATGTGTTCCCCTATTTGTG	22	AACTCTAATGCACACCAACCAA	22
LOC101494858	(CT)6	304	TCAATATGCCTTGGGTTAGGTC	22	AGAGACCAGAGAAAAGTGTCGC	22
LOC101495182	(GT)6	361	TGAAGGCATAGCTTATCAGTGG	22	ATTCCATCCTTACCATCTGCAC	22
LOC101497348	(AT)6	290	TCACCAACAAGTTCAATTCCAG	22	CATAACATGAAATCCTCCACCA	22
LOC101503680	(AT)10	378	GAGAAGGAGAAGGCTGTAGCAC	22	GTGGTTCAAGTGTCAGGTCAAG	22
LOC101503680	(TA)8	379	CTATGTGTACGTTGTGCAGGAAA	23	GGGTACGGATGCTCTTGAGAC	21
LOC101503680	(AT)6	386	CTGGAACGCCATGAAAGGTAT	21	GTCATTTGTTAGCATCACCCGT	22
LOC101504006	(TA)6	296	ACCATACCTACTAAAACTCAAGGACAC	27	CAGCATCACAAAGGACAGAGAG	22
LOC101504006	(TA)6	379	TTTGTATCTGGTGGTGAGCTTG	22	CTTATGGCTTGGAAAAGCATGT	22

APPENDIX-III: List of genes and genic SSRs present in chickpea genomic region flanked by markers TA27-TA59 on chickpea chromosomes 2

Sl.No.	Gene ID	Putative functions	SSR motifs identified
1	LOC101495506	Proline-rich receptor-like protein kinase perk9	(A)10, (AT)6, (T)16
2	LOC101512465	Metal-nicotianamine transporter ysl3-like	(TAA)18
3	LOC101512783	Uncharacterised	No SSR
4	LOC101496043	Hd domain-containing protein c4g3.17	(A)15, (T)10
5	LOC101496486	Transcription factor fama	(T)34, (T)10, (CAA)7, (T)15
6	LOC105851610	Uncharacterized	No SSR
7	LOC101497018	Importin-9	(T)10, (T)10, (T)10
8	LOC101497349	Uncharacterized	No SSR
9	LOC101497676	Cytochrome P450 71d10-Like	No SSR
10	LOC101498006	Uncharacterised	(GGT)7
11	LOC101498337	Uncharacterised	No SSR
12	LOC101514090	Uncharacterized	No SSR
13	LOC101514415	Cytosolic sulfotransferase 14-like	(T)13, (A)10, (A)12
14	LOC101515068	Protein far1-related sequence 5-like	(T)13, (T)10
15	LOC101515388	Uncharacterized	No SSR
16	LOC101499004	S-adenosylmethionine decarboxylase proenzyme	No SSR
17	LOC101410582	Trnah-gug transfer rna histidine	No SSR
18	LOC101488484	Uncharacterized	No SSR
19	LOC101499837	F-box/fbd/lrr-repeat protein at1g13570-like	No SSR
20	LOC101499536	Protein nsp-interacting kinase 2	(T)17, (A)11, (T)11
21	LOC101500167	F-box/fbd/lrr-repeat protein at1g13570-like	(GA)16
22	LOC101500487	F-box/fbd/lrr-repeat protein at1g13570-like	No SSR
23	LOC101500808	60s Acidic Ribosomal Protein P2-1-Like	No SSR
24	LOC101501129	Uncharacterized	(A)12, (A)12, (TA)8
25	LOC101490121	Fatty acyl-coa reductase 8-like	(AT)20
26	LOC105851611	Uncharacterized	No SSR
27	LOC101501963	Fatty acyl-coa reductase 8-like	(A)10, (A)16, (AT)8, (A)12, (AT)8
28	LOC101502295	Metal-nicotianamine transporter ysl3-like	No SSR
29	LOC101502609	Fatty acyl-coa reductase 8-like	(A)10, (A)16, (AT)8, (A)10, (AT)23, (AT)11
30	LOC101502926	Uridine-cytidine kinase c-like	(TTAAT)7, (T)10, (T)11, (T)13
31	LOC101504222	F-box/fbd/lrr-repeat protein at1g13570-like	(T)10
32	LOC101504752	Peroxidase 11	No SSR
33	LOC101505075	Uncharacterized	No SSR
34	LOC101491725	Uncharacterized	No SSR, no hit found
35	LOC101505395	Asc1-like protein	(T)10
36	LOC105851613	Uncharacterized	No SSR, no hit found
37	LOC101505938	N-acetyltransferase ycf52	No SSR
38	LOC101506252	Chaperonin 60 subunit beta 4, chloroplastic	No SSR
39	LOC101506691	Basic leucine zipper 43-like	No SSR
40	LOC101492385	Protein sis2-like	No SSR
41	LOC101507024	Fe(2+) transport protein 1-like	(TTA)6, (T)12
42	LOC105851614	Uncharacterized	No SSR
43	LOC101507657	Probable indole-3-acetic acid-amido synthetase gh3.1	No SSR
44	LOC101493049	Uncharacterized	No SSR
45	LOC101493374	Uncharacterized	(A)10
46	LOC101507982	Wpp domain-interacting tail-anchored protein 2	(A)12, (AT)6
47	LOC101509357	Uncharacterized	No SSR
48	LOC101509680	Transcription factor bhlh49	(T)13, (AT)6
49	LOC101493696	Uncharacterized	No SSR
50	LOC101510002	Protein srg1-like	No SSR

51	LOC101510541	Protein srg1-like	(AT)10, (A)10
52	LOC101510861	Protein srg1-like	(T)11, (TA)18, (TA)6, (ATA)10
53	LOC101511182	Probable terpene synthase 2	(T)11
54	LOC101494014	Probable terpene synthase 2	No SSR
55	LOC101494739	Alpha-xylosidase 1-like	(T)10, (T)10
56	LOC101495067	Transmembrane Emp24 Domain-Containing Protein p24delta3-Like	No SSR
57	LOC101495395	Transmembrane Emp24 Domain-Containing Protein p24delta4-Like	(T)12, (A)10
58	LOC101495721	Aminoacylase-1-like	(A)12, (T)10
59	LOC105851615	Uncharacterised	No SSR
60	LOC105851616	Uncharacterised	(A)10,(T)10,(A)16, (A)11, (T)10,(A)12, (TA)16, (AT)10, (ATA)21, (A)10, (A)11, (A)11, (A)13, (A)14, (TA)16, (TA)7, (AT)8, (AT)8, (AT)12, (T)12, (A)11, (TAAA)6, (A)11C(A)11
61	LOC105851617	Uncharacterized	No SSR
62	LOC101500059	Protein fantastic four 1	(AGA)6
63	LOC101497019	Agamous-like mads-box protein agl5	(A)11, (A)10, (T)12, (ATA)6
64	LOC101497350	Zinc finger ccch domain-containing protein 15 homolog	(T)10
65	LOC101497677	Low affinity sulfate transporter 3-like	No SSR
66	LOC101498007	Sulfate transporter 1.3	(TA)17
67	LOC101501653	Ring-h2 finger protein atl47-like	No SSR
68	LOC101501965	Ring-h2 finger protein atl47-like	No SSR
69	LOC105851618	Uncharacterized	No SSR
70	LOC101502610	Uncharacterized	No SSR
71	LOC101502927	Synaptonemal complex protein 1-like	(T)10, (AT)10, (A)15, (T)11, (T)11, (T)10
72	LOC101503257	Cryptochrome-1	(TC)6, (A)10, (T)12, (T)10
73	LOC101504223	Nodulin-26-like	(T)10
74	LOC101503575	Uncharacterized	(TA)11, (A)10, (T)10
75	LOC101504870	Bidirectional sugar transporter sweet2-like	No SSR
76	LOC101505179	RNA-binding protein 12-like	No SSR
77	LOC101505499	Serine/threonine-protein kinase sapk2-like	(T)12
78	1433	14-3-3-like protein a-like	No SSR
79	LOC101506367	Cytochrome c oxidase subunit 6b-1-like	(GA)6
80	LOC101507346	Uncharacterized protein at1g04910	(GA)7
81	LOC101507869	Uncharacterized	No SSR
82	LOC101506692	Thylakoid lumenal 29 kda protein, chloroplastic	(TC)7, (A)11
83	LOC101508181	Probable polyamine transporter at3g13620	No SSR
84	LOC101511089	Uncharacterized	No SSR
85	LOC101511396	Uncharacterized	No SSR
86	LOC101508506	Uncharacterized	(T)11
87	LOC101508820	Ring-h2 finger protein atl54	No SSR
88	LOC101511725	Upf0483 protein agap003155	(T)10, (T)11, (A)10, (AT)11, (A)12, (A)10, (TTA)22, (TA)14
89	LOC101512041	Uncharacterized	No SSR
90	LOC101512369	Chaperone protein dnaj 1, mitochondrial	(TAT)7, (A)11
91	LOC105851619	Uncharacterized	No SSR
92	LOC101513552	Purine permease 1	No SSR
93	LOC101513857	Probable 2-oxoglutarate/fe(ii)-dependent dioxygenase	(AT)11, (T)10, (ATA)15, (TAT)16, (A)11, (T)12, (TA)10, (TTA)19, (TA)7
94	LOC101488248	Uncharacterized	(TC)10, (T)15
95	LOC101488578	UDP-glucose flavonoid 3-o-glucosyltransferase 6-like	No SSR

96	LOC101491408	Uncharacterized	No SSR
97	LOC105851620	Zinc finger bed domain-containing protein ricesleeper 2-like	No SSR
98	LOC105851621	Uncharacterized	No SSR
99	LOC101488922	Zinc-finger homeodomain protein 2-like	(A)24, (A)10
100	LOC101492042	Probable terpene synthase 2	No SSR
101	LOC101489245	Ribosomal RNA processing protein 36 homolog	(T)11
102	LOC101489568	Transport inhibitor response 1-like protein	(T)11
103	LOC101493050	Uncharacterized	No SSR
104	LOC101493375	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
105	LOC101489884	Mitochondrial succinate-fumarate transporter 1	(T)16
106	LOC101493697	Proline-rich receptor-like protein kinase perk10	No SSR
107	LOC101490446	Upf0483 protein agap003155-like	(T)10, (A)10, (A)10
108	LOC101494329	Uncharacterized	(A)10, (A)10, (A)10, (A)12, (A)10, (AT)18
109	LOC101494962	Uncharacterized protein k02a2.6-like	No SSR
110	LOC101513749	Xyloglucan galactosyltransferase katamari1 homolog	No SSR
111	LOC101495613	Transcription factor bhlh143-like	(CT)8, (T)10
112	TRNAA-UGC	Transfer RNA alanine (anticodon UGC)	
113	LOC101495940	Cyclin-d2-1-like	(CT)13, (GA)10, (A)8
114	LOC101515069	Uncharacterized	(A)11, (TA)10, (TA)26, (TA)15, (A)11, (T)12
115	LOC101515389	Proline-rich receptor-like protein kinase perk9	(A)12, (T)10, (T)10, (T)15, (A)10, (AT)6
116	LOC101496274	Histone H3.3	(T)10
117	LOC101496602	Dnaj homolog subfamily b member 14	(T)13, (TC)7
118	LOC101497574	Glucose-6-phosphate isomerase 1, chloroplastic	(TG)8, (T)10, (AG)6
119	LOC101498131	Uncharacterized	(TC)7, (T)11
120	LOC101498462	Uncharacterized	(T)12, (T)12, (AG)16
121	LOC101488485	Uncharacterized	No SSR
122	LOC101498807	Rho GTPase-activating protein ren1	(T)10, (AC)7, (TC)6, (AT)7, (A)12, (A)11, (T)10
123	LOC105851624	Uncharacterized	(T)10
124	TRNAA-AGC	Transfer RNA alanine (anticodon AGC)	No SSR
125	LOC101499123	Uncharacterized	(A)11
126	LOC101489800	Uncharacterized	No SSR
127	LOC101490123	Glutathione s-transferase t3-like	No SSR
128	LOC101499444	Uncharacterized	(A)12, (TC)10
129	LOC101500600	Ubiquitin carboxyl-terminal hydrolase 16-like	No SSR
130	LOC101500910	At-hook motif nuclear-localized protein 17-like	(TAA)6
131	LOC101501240	AP-4 complex subunit mu-like	(T)10
132	LOC101501758	Beta carbonic anhydrase 5, chloroplastic	(A)17, (A)11, (A)16, (A)10, (T)10
133	LOC101502081	Transcription factor bhlh30-like	No SSR
134	LOC101502408	Uncharacterized	No SSR
135	LOC101503022	MADS-box protein SYP-like	(TC)18, (TAT)7, (TA)22, (A)10, (T)11, (ATA)25, (T)11, (A)15, (A)13, (T)11

APPENDIX-IV: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by TA27-TA59 on chromosome 2

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101495506	(A)10	371	ATCAAACAACCAAAGTCACGG	21	AACCCAACCAATGTCTCAAAGT	22
LOC101495506	(AT)6	389	GGGAAAACCTTTGAGGAGAGAT	22	ACTTACAAGAGGAGCAGAGGCA	22
LOC101495506	(T)16	249	TGAAAAGGTATGTTGCTCCTGA	22	TATCCCTATCCGTCGTTCGAT	21
LOC101512465	(TAA)18	306	GAAGTTGTAAGACCCGCATTTT	22	TAGAAGTGGGGAACGTGGTATT	22
LOC101497018	(T)10	309	AGGTGCTACATAGTTTTGGCCT	22	GCAACGACTTTTGATAATGCAC	22
LOC101497018	(T)10	323	AAGGTTGAGGTGACAAATGGAG	22	TAAATAGGCTTCCAGACAAGGC	22
LOC101498006	(GGT)7	327	GTGCTGAGGAGAAGTAGAAGCAA	23	AATCAAACCACCATTCACACCT	22
LOC101514415	(T)13	353	AGAAGCGGCCAAAACTAATTC	21	TTCACAACACCCTAATTTGACG	22
LOC101514415	(A)10	342	CGTAGAGTCGGTCCAAGGTAAG	22	TAAGTAGGGAGTGTTTGTGGGG	22
LOC101514415	(A)12	377	GGTGTGGTAGATTGATGTGACG	22	GAGTGTCCGAGTGTTAAGGGTC	22
LOC101515068	(T)13	258	CATTGAGGGGTTCATTTGATAG	22	TCACACGTTTAGGTTTTCCTTG	22
LOC101515068	(T)10	392	CCTCAAGGAAAACCTAAACGTG	22	AAATACCCGTAATCCAACACCA	22
LOC101501129	(A)12	367	CAGTTTTCTTGCTGGGTTTTAG	22	CTGTGACCATAGCAGACTCAAC	22
LOC101490121	(AT)20	359	CCAACAATTCAACTTCCTCCAT	22	TGAGAAACAGATAAACGATACACCC	25
LOC101501963	(A)16	386	AGACACTCAAAGGCTCGTCAA	21	GAATTGTTTTATCCTCGGAACG	22
LOC101501963	(AT)8	245	CAAAACTGCATACTCATTGGGG	22	AAATTCATGTGCGAATGGGA	20
LOC101501963	(A)12	324	TGTGCAACTTTGGCATGTAGTT	22	ATGGTGGTGTGGTATGTGTGAT	22
LOC101501963	(AT)8	242	ATCAATATCGTGTGCCCATACA	22	GTGTCAACAAACAAATCGGGTA	22
LOC101502609	(A)13	387	AGACACTCAAAGGCTCGTCAA	21	GAATTGTTTTATCCTCGGAACG	22
LOC101502609	(AT)8	246	ACCTCATCACAAAACTGCACAC	22	GTGCGAATGGGACGATCT	18
LOC101502609	(A)10	388	TGTGCAACTTTGGCATGTAGTT	22	CTTTTGGGTCCGATGTTTACTC	22
LOC101502609	(AT)23	272	ATCAATATCGTGTGCCCATACA	22	GTGTCAACAAACAAATCGGGTA	22
LOC101502926	(TTAAT)7	314	GGGAAATCGTTATTAGCCACAA	22	CAAAGCAAAATCCACAACTCTG	22
LOC101502926	(T)10	372	TGGTTTCTGTTTCTGGTGTGTT	22	ACTTCTATCCCTAATTTCGCCC	22
LOC101502926	(T)11	258	CCTACTTTGCTGATTTATGCCAG	23	CCAAGACGTTTATGATGGGAAT	22
LOC101502926	(T)13	136	GATGCTAGTCGAATTGTTGACG	22	TTCACGTCCTCAGAAAAGAACA	22
LOC101504222	(T)10	370	CCAATCACAATAACTAACCGCA	22	CAAGAGACAATACGAAAGAACGG	23
LOC101505395	(T)10	266	ATATTCTGGGAAACAAGACGCT	22	CACTTTCTCGTCACAAACATGA	22
LOC101507024	(TTA)6	397	CCTTTGCTTCCATCAACTCTTT	22	CCACGTAACACCATAGCTGAAG	22
LOC101507982	(A)12	293	GAGTGAGTAGAATGACAAGCCCA	23	ATATGAGGGAGGGACCAAAACT	22
LOC101507982	(AT)6	287	GTTTTGGTCCCTCCCTCATATT	22	ACAGTTTTGGTCCCTCATTCTG	22
LOC101509680	(T)13	157	GGGTATCTTTCTCCTCCCACTT	22	AAGCCCTAGTCAGAGTTGCAGA	22

LOC101509680	(AT)6	279	ACACCCTACAAAAGCTGGATGT	22	CAAGCACCACAAATCAAAACC	21
LOC101510861	(T)11	282	TGGGTAGGTGTATAGCTTTTCTGT	24	TGCAAGTCAATGTCAAGATGAG	22
LOC101510861	(TA)6	319	CAATGTTGCTGGTTCTGGTATG	22	GGTCTTAAATTGTGGTTATGGCAG	24
LOC101511182	(T)11	309	GATAACCTTCATTCTCTTGCCG	22	GTTTCCTTGCTCGTCTTTGAAT	22
LOC101494739	(T)10	262	CTTCTTTTCTGGACCTACCCCT	22	ACCATTCGCATCTGGTAAGC	20
LOC101494739	(T)10	148	TAACATGCCAAGACAAACAAGG	22	CATGATTGGAGGATGTAAGTGC	22
LOC101495395	(T)12	282	TTGAAGGAATAGTGGAAGCCAT	22	TCAAACACGAGACCTCACAGTT	22
LOC101495395	(A)10	397	AACTGTGAGGTCTCGTGTTTGA	22	CTTCCCTGTAGCAAAGTCCAAG	22
LOC101495721	(T)10	253	TCCTTTTCCATCCCTCTGAATA	22	TGTTCTCTCCTTTTCCTCCATT	22
LOC105851616	(T)10	344	GCATTAGGAGATATGTAGGAGCA	23	AATGGAGTCACCGTCATAGTTT	22
LOC105851616	(A)11	354	GGTTTGTATGATTGGGGTTGTT	22	CTCCGTCTTCCACATTTTCAG	21
LOC105851616	(T)10	320	AGTCACCTAAACCGACACATGA	22	ACATCTTGTTTGAATGGGTTCC	22
LOC105851616	(A)12	349	CATCTCCATTCCCATCCGTA	20	TCGATTGGCTTAGTGAAAATCC	22
LOC105851616	(TA)16	345	TCATTAAGAGATTCCCCGTTCT	22	GGTGTATTTTGGATTTTGGCTC	22
LOC105851616	(AT)10	252	GACAAGGGAAAGAAGACATTGC	22	AGTTTTGGGACTGCATGAGC	20
LOC105851616	(ATA)21	252	GACAAGGGAAAGAAGACATTGC	22	AGTTTTGGGACTGCATGAGC	20
LOC105851616	(A)10	381	GCTCATGCAGTCCCAAAACT	20	GCATAATTGCTCATGCGGTA	20
LOC105851616	(A)11	373	ATGGTCACGAAGGTGTGAGTAG	22	AGAGGAGAAAAGTAGAGAAGATGGA	25
LOC105851616	(A)11	392	GAGAACGACAAGGTAGTCCCAC	22	CAGTTTTGGCCTTAATGGAAAG	22
LOC105851616	(A)13	316	CGTTGAGCAAGAATGAAGAGTG	22	CATTTGGGAGTAGGCTTTCCTT	22
LOC105851616	(A)11	229	CTAGTTGGAAGTCGCCATTTG	21	TAGTTGTGGACGCTCTCTCAAG	22
LOC105851616	(TAAA)6	365	GGGTCATATAGGGTCACACAGC	22	TCCCAGAGTAGAGGAGATGAGG	22
LOC105851616	(A)11	340	GCTGCTTGTGTTGTTTTGTTG	21	TTTGTCACTCGTGTTTCACTCA	22
LOC105851616	(A)10	340	GCTGCTTGTGTTGTTTTGTTG	21	TTTGTCACTCGTGTTTCACTCA	22
LOC101500059	(AGA)6	302	GCTCAAACTCAAAACCTTGCTT	22	ACTTCATGTGGACTTGTTGGTG	22
LOC101497019	(A)11	293	CCATTACCTTCCCTTTCTCTCC	22	GATGAGGCCATTTCTACGTTTC	22
LOC101497019	(A)10	378	TGGTCAGATGTATTGTCCTTGC	22	GAAGAGCGAGATTTGTGAAGGT	22
LOC101497350	(T)10	391	CTCCTTTTCTTGCTTTCCCTTT	22	CAGCATCCTACCGAAGTCCTTA	22
LOC101498007	(TA)17	279	CGAGTAACAATAAGGAAAGGGC	22	GTTTGGGTTCTCATGTAGCAAA	22
LOC101502927	(T)10	342	CTGGAGCATATTCGTGCATTAG	22	AGAGCATCTGAACTCGCTGAC	21
LOC101502927	(A)15	384	GGAGCAAGAGTTTTACAATGAGATG	25	ATTGCATTTCCCGTAGTGAAGA	22
LOC101502927	(T)11	165	TTGCATGGAGAGGTAATAGCAA	22	ATTCTGACATCAATCACCCAGA	22
LOC101502927	(T)11	189	CCAAGAAAAGGAAAACGAGAAG	22	TGGGTCCTGAAAGAGAGAAGAA	22
LOC101503257	(TC)6	267	CCCCACTCATTTAACCAATCC	21	TTGGGAGAGATTTTAGGCCAT	21
LOC101503257	(A)10	282	CACCTCTTCTCCCACCTAAAAG	22	GTGTCAAGTGTGCGACTTCAAT	22
LOC101503257	(T)12	369	GGTACTCTCCCTGATGGTCGT	21	TTGTAAATGCCAAAACGCTG	20
LOC101503257	(T)10	301	TCGAACATGCCCTAGATAAATG	22	AACTGTCCCGATGAACTGAAAT	22

LOC101504223	(T)10	307	GCTTCGCTTTAACTGTCGCTAT	22	GCACCCATTAACTGACACAACA	22
LOC101503575	(TA)11	316	GGCTTGAATCTACTTCGACTCTTG	24	TGCTCCCAAAACACGATTTTAC	22
LOC101503575	(A)10	377	TGATTGAGGGACTGCTTGTTA	21	CAGCCGAGTCTGAAACATATCTA	23
LOC101503575	(T)10	377	TGATTGAGGGACTGCTTGTTA	21	CAGCCGAGTCTGAAACATATCTA	23
LOC101505499	(T)12	270	ATTTTGTGTGCCCTCCTCTTTA	22	TGTGTTGATGTCGTGTTGATGT	22
LOC101506367	(GA)6	259	GCTTAACCACTTGGCTTTTGTT	22	ATAATGACCACTCAACATCCCC	22
LOC101507346	(GA)7	301	CTTCGAGAAAACCCAAAACAAC	22	ATCAATTTCCGTCTCTGCATTC	22
LOC101506692	(TC)7	215	GATTGGGGCAGAAATAAGAGAA	22	TTGGATTATGAGCAACAAGTGG	22
LOC101506692	(A)11	364	CCACTTGTTGCTCATAATCCAA	22	ATGGCTCACTTACCAAACCAAT	22
LOC101508506	(T)11	230	GGGTTTGTTTAGGAGAGGGTTT	22	CATTGCGGTGAGAGAGATAGAA	22
LOC101511725	(T)10	211	AGTGGTTCCAATTCAACAAGGT	22	AATCCAAGAAGACCATCAAACG	22
LOC101511725	(T)11	211	AGTGGTTCCAATTCAACAAGGT	22	AATCCAAGAAGACCATCAAACG	22
LOC101511725	(A)10	250	AGAGGCAATAATGGGTGTGTTT	22	TGGCCTGACCTAGAAGCATATT	22
LOC101511725	(AT)11	250	AGAGGCAATAATGGGTGTGTTT	22	TGGCCTGACCTAGAAGCATATT	22
LOC101511725	(A)12	173	GTGGACAGCATGTGGTATGAGT	22	GCCTTAGCCTGACCTACGATTA	22
LOC101511725	(A)10	295	GTGTTGGATTGACCCTCTCAAA	22	CTTTAGGAACCTTCGTCAGTGC	22
LOC101511725	(TA)14	343	GCCAAATACCGATGGAAAATG	21	TCTCACCTGCAATAAGCATCAA	22
LOC101513857	(T)10	380	AGCCCCTTGGTTTTAGGTATTC	22	AAAGGTTGGGTCCATGAAGTC	21
LOC101488248	(TC)10	155	TTAGTCTTTTGTGGGCCTCTTC	22	CTTTGCTTGTCATGTTTTGGTG	22
LOC101488922	(A)24	396	TAGCATTTGCAGAACAACACG	21	CCACCCACTAAAATCCCATAAA	22
LOC101488922	(A)10	357	CTGCTAAGGGATATTTGGGACA	22	TGATGTTGTGGTGGTTGAAAAG	22
LOC101489568	(TC)8	162	ACTCACTCACCTCATCTCCCAT	22	GATGGAATCTTTGCTTCTTTGG	22
LOC101489884	(T)16	296	GATTCTTCATCAATGCCCCA	20	ATGAGCCAGAGATTGCTTTCAT	22
LOC101490446	(T)13	353	AAGGCAAATCTCTTGTTGAAGG	22	TGCAAACACTCATCGAAGTTG	21
LOC101494329	(A)10	400	GGTTGACACAGAGGGAACAAA	21	TTTCCGATTTGAGAGGGTAAAG	22
LOC101494329	(A)10	373	ACTTTACCCTCTCAAATCGGAA	22	CTCTCACACTCGTTCTTAGTTTTGT	25
LOC101494329	(A)10	373	ACTTTACCCTCTCAAATCGGAA	22	CTCTCACACTCGTTCTTAGTTTTGT	25
LOC101495613	(CT)8	157	TCAACTCCCCTACACTCCATTT	22	CAGGATGAAGCAAGTGAAACAG	22
LOC101495613	(T)10	249	ACCAGATTTAGGGCATTGAAAC	22	ACGTCACAGGTTTAAGCAACTG	22
LOC101495940	(CT)13	199	AGTTTCTATGAATTTGGCGCTC	22	TGTGTGTCTTCCCTTTATGACC	22
LOC101495940	(GA)10	326	GGTCATAAAGGGAAGACACACA	22	CAAAACTTGGTGCCATTTCA	20
LOC101495940	(A)8	326	GGTCATAAAGGGAAGACACACA	22	CAAAACTTGGTGCCATTTCA	20
LOC101515069	(TA)10	347	AGTATTCTTCACGCTGCAATCA	22	CTCTTACAAACCACCAAAACACC	23
LOC101515069	(TA)26	347	AGTATTCTTCACGCTGCAATCA	22	CTCTTACAAACCACCAAAACACC	23
LOC101515069	(A)11	389	GGTGTTTTGGTGGTTTGTAAGAG	23	GGCGTAGAGTGTGTGTGAGAGA	22
LOC101515069	(T)12	204	CTATATTGCATTTGGCTGCTGA	22	ATCTTCGTTCTTTTGGATGAGG	22
LOC101515389	(A)12	385	ATATAGCGTGTTGGTCAGCGAT	22	GGAAGATGAAGAGGGTGGTGTA	22

LOC101515389	(T)10	327	TAGAAGAAGAACGAAGAAGGGG	22	TTTTGTGAACCTTAGACAGCG	21
LOC101515389	(AT)6	356	TAGGGCATATTTGTGCGAACC	21	TCTGTAAGCATGTATTTGTCGTGC	24
LOC101496274	(T)10	320	TTTCGCAGCACAAGAAAGC	19	GGATGGATGTAAGAAACCACAAC	23
LOC101496602	(T)13	376	TTTCCTCTCCGCTCTCTCTAAA	22	CCTCGTTTATCTCTTGAAACCG	22
LOC101496602	(TC)7	395	ATGAGTAGACCAAAAGAGGCCA	22	TCCACAACAAGCAAGTAGGAGA	22
LOC101497574	(TG)6	297	AAAATTAAGGGGTCTCAATCGG	22	CCTCCAATCCCAACAGAAAGTA	22
LOC101497574	(T)10	218	GCTAGGTTTCCCATGTTTGACT	22	TACTCCTATTGGCCTCATCCAT	22
LOC101497574	(AG)6	148	ATCAGGTGCGTTTTAGATTCGT	22	GACCTCTTTCACTCCCTCTGAA	22
LOC101498131	(TC)7	320	TCTCTCTTCACTCTCTCATTCACA	24	CTCTCTAACCCATCGAAGTGC	21
LOC101498131	(T)11	287	AATGCACTTCGATGGGTTAGA	21	CTTCCTTCCAACAATCAACAAC	22
LOC101498462	(T)12	334	CTTGAACTCATCAACGCTTCTG	22	TGAGCTACACAACAACACACACA	23
LOC101498462	(T)12	334	CTTGAACTCATCAACGCTTCTG	22	TGAGCTACACAACAACACACACA	23
LOC101498462	(AG)16	367	TCCTGTGTGACCATTTCTATGC	22	CCCTGTCTAGTAAGATCGTGGG	22
LOC101498807	(T)10	397	TGCTTCATTTCTGACCAAGGTA	22	TGAGGCTTAGGAGAGGGAATAG	22
LOC101498807	(AC)7	347	TAATGAGAGGGGCAGAGATAGG	22	ATCACACCAAAACACTCAACCA	22
LOC101498807	(AT)7	299	TACGCTCTTGCTACTCTTGTGC	22	AAATTCTTGGAAGGGAGAAACC	22
LOC101498807	(A)12	391	TTACCCTTTCTTTGTCGCTTGT	22	GAGATTGTGTTTGGGAACCATT	22
LOC101498807	(A)11	179	ACAAGCGACAAAGAAAGGGTAA	22	AGACACACGGTTTCACCACAT	21
LOC101498807	(T)10	387	ACACTTTCCACAGACCAAGACA	22	TCCTGTCAAATAGATCACCCG	21
LOC105851624	(T)10	270	CAGGAAACAACGCATACACAAT	22	CACGTAATCTGCATTCATGGAC	22
LOC101499444	(A)12	370	TGCTGAGACCCAATTCATCTAA	22	CTTATCAACACCATCCTTTCCC	22
LOC101499444	(TC)10	348	GTCATAGACAAGTAGACAACACCTTTG	27	GAACCCCGCAACTTTGTAAAT	21
LOC101500910	(TAA)6	358	CGCCGTAGATGTAGCAAAAGTA	22	GCTCACAGATATGACAGACGGA	22
LOC101501240	(T)10	300	TAAAGCTCTGGATGGGATTGTT	22	GGGCTGTCCCTTGAACTCTTAT	22
LOC101501758	(T)10	357	TGATGTAGGGAATATGGCTGAA	22	TGAGTCTGCACAAGCAATGAC	21
LOC101503022	(TC)18	355	TCAGCCACACCATTCTAATCAC	22	TAAAGTCACCAACCAAAACCCT	22
LOC101503022	(TAT)7	226	AGGAGCTTTCAGTGTTGTGTGA	22	CGTGGTTAATGTTTTGACTGTACC	24
LOC101503022	(A)15	173	GGAAGTTGCTGATAAAACCCAA	22	ATTCCTTCAAAATCCTCACCCT	22
LOC101503022	(A)13	282	ATTTCTCTTGAGTTTTGGTCCC	22	ATTGCAGTTTTGATCCCCTT	20
LOC101503022	(T)11	363	CCATGTGTTTGTTCATTTGCC	21	GAGTCAATTCGATGGTAAGGTCA	23
LOC101496486	(T)34	330	CGTCCTCTTCTGCTGGTCTACT	22	CTTATGATCGTGGTGCAAATGT	22
LOC101496486	(T)10	400	CTATTTCGGTCAACCTATTCTAGC	24	CCAATGTTTGTAGGACCCC	19
LOC101496486	(CAA)7	386	ATTTTGAATCTATGCAGGCACC	22	TCCACCACCACAATCTTCATTA	22
LOC101500167	(GA)16	384	TTACTTTTACGAGGCGACTTCC	22	ACAATTCGAGCACACAAATCAC	22
LOC101501129	(A)12	367	CAGTTTTCTTGCTGGGTTTTAG	22	CTGTGACCATAGCAGACTCAAC	22

APPENDIX-V: List of genes and genic SSRs present in chickpea genomic region flanked by markers TA200-TA37 on chickpea chromosomes 2

The genomic region covered by these markers 1.73 Mb, but 0.79 Mb (797601) of genomic region was overlapping with TA110-H3A12 which covers 32 genes (LOC101492815 to LOC101514414) (Appendix-I)

TC- Transcript ID from Chickpea transcriptome database

Sl. No.	Gene Id	Putative function	SSR motifs identified
1	LOC101508719	Uncharacterized	(T)10, (T)14, (T)11, (TG)6
2	LOC101514741	Mediator-associated protein 1-like	(T)11, (TTATAT)8, (TATTTA)89, (AT)16, (AT)6, (T)11
3	LOC101510111	Clavaminate synthase-like protein At3g21360-like	(ATT)23, (A)15, (T)10
4	LOC101510436	Uncharacterised	(T)10, (A)15, (T)12, (T)13, (T)10
5	LOC101510975	Uncharacterized	No SSR
6	LOC101511284	TC00386: RVT_1, Reverse transcriptase	(TC)12, (c) 17, (T)11
7	LOC101515724	Uncharacterised	No SSR
8	LOC101488483	TC11413: Aminotransferase-like, plant mobile domain family protein	No SSR
9	LOC101511604	Transcription factor BIM2-like	(T)11
10	LOC101511928	Protease 2-like	(T)11, (T)10
11	LOC101512261	Subtilisin-like protease-like	No SSR
12	LOC101512782	Probable phosphatidylinositol 4-kinase type 2-beta At1g26270-like	(T)11,
13	LOC101488826	Uncharacterised	(AT)6, (T)14, (AAT)11, (TA)6, (A)10,(AT)7, (T)10, (A)11, (AT)9, (T)11, (T)13
14	LOC101513111	Probable GMP synthase [glutamine-hydrolyzing]-like	(TA)8, (T)10
15	LOC101489152	TC06714: MuDR family transposase	No SSR
16	LOC101489482	(-)-germacrene D synthase-like	(T)16, (TTA)24
17	LOC101489798	BTB/POZ domain-containing protein NPY5-like	No SSR
18	LOC101490350	Transcription factor bHLH30-like	(A)10, (A)19, (CA)7, (T)13,(T)11
19	LOC101490669	NAC domain-containing protein 8-like	(T)11
20	LOC101492816	Uncharacterized	No SSR
21	LOC101490991	Uncharacterized	No SSR
22	LOC101491303	Serine/threonine-protein kinase STN7, chloroplastic-like	(A)11
23	LOC101491812	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase At2g14440-like	(T)12
24	LOC101493793	52 kDa repressor of the inhibitor of the protein kinase-like	No SSR
25	LOC101492140	selenoprotein K-like	(TGG)8
26	LOC101511500	52 kDa repressor of the inhibitor of the protein kinase-like	No SSR
27	LOC101494116	Protein decapping 5-like	No SSR
28	LOC101494422	phosphoenolpyruvate carboxylase 4-like	(ATATT)14, (AT)9, (TA)13, (GT)6, (TA)8
29	LOC101494960	Putative phospholipid-transporting ATPase 9-like	(T)10
30	LOC101495506	Proline-rich receptor-like protein kinase PERK9-like	(A)10, (AT)6, (T)16
31	LOC101512465	Metal-nicotianamine transporter YSL3-like	(TAA)18

APPENDIX-VI: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by TA200-TA37 on chromosome 2

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101508719	(T)10	392	CAATCCTCAAGAACTCTAATGGG	23	CCACAAGGCACATAGAAAGAGA	22
LOC101508719	(T)14	256	TTGGGTGTGTTGAGTTAGCAAT	22	TATAAGGGTTGGGGACAAAGTG	22
LOC101508719	(T)11	309	TTGTTGACTTGTCTTTTCAGGG	22	CATGAACCATCCACCATCAG	20
LOC101508719	(TG)6	337	AAGGGCAGAAGTAACAACCAAA	22	ACCATAACCATTTTGCATCTCC	22
LOC101514741	(T)11	247	GACATCACTTCAGATCAGTGGC	22	CGAATACAACAACAAGTCAGTGG	23
LOC101514741	(AT)16	376	GTCAAGAATGCCGATGTACG	20	ATATTAGACCATTTCCTTGGGC	22
LOC101514741	(AT)6	342	CTTGGCTCTAAGTCATTTGCG	21	AAACAACAAGAACGCGAGGTAT	22
LOC101510111	(T)10	275	GCGAATTGGTATATGGTGTCCT	22	GCTTGGAACAAAAGGTGTCTCT	22
LOC101510436	(T)10	200	TGGGGTCTCATCTATCTCATCC	22	CGAAACCTTTGACTTGAACCTT	22
LOC101510436	(A)15	375	GTAGTGTTTGGTTGGTCGGAA	21	AATTTGGCCCTTTCTTTCTCTC	22
LOC101510436	(T)12	269	TAATTGCCAGAAAACAGAGGGT	22	GGTTAGAGAAGAGAAAGCAAGAGTG	25
LOC101510436	(T)13	223	TCAAGTTTCCTTGCCTGTAAGC	22	TTGAAAGTGAAGCACCAACATC	22
LOC101511284	(TC)12	275	GGTCTTTTGAGGGCTTATTTCC	22	CGTGGGCATAGTTCATATCTCA	22
LOC101511284	(C)17	373	CAAATGCAGAATCACAACACCT	22	GCTCCATGAGATAACCTTCACC	22
LOC101511284	(T)11	327	AAGAGAGGCAGAAAATTCCGTA	22	TTGAGACTGCTACAACCATTCG	22
LOC101511604	(T)11	289	GCCCCTAACACCAACAATAAAG	22	TCTTCTTCGCTGCTCAGTTACA	22
LOC101511928	(T)11	386	AGGACCCATATCACTGGATGTC	22	CTCTCATTGCATACCCATCAAA	22
LOC101511928	(T)10	325	TAACGTGATCCAGGTGTTGTC	21	AAGTACGGGTAGTGAAATATGACC	24
LOC101488826	(AT)6	377	GCATGGTATCGTTTGTCTTGTG	22	GGAACATGAGATAAGGTCTCCAA	23
LOC101488826	(T)14	396	GTATGTTTAATTCGGCAAGCAC	22	TTCCCTTTGAATCTAGTATGCG	22
LOC101488826	(AAT)11	359	GGCAGGCAAACAAACTACATACT	23	ATCACTCCACAAGCCGTTTAAT	22
LOC101488826	(TA)6	335	GCTTGTGGAGTGATGGTTTTAT	22	AGAGTTGAATGAGAGAGAACGG	22
LOC101488826	(A)10	335	GCTTGTGGAGTGATGGTTTTAT	22	AGAGTTGAATGAGAGAGAACGG	22
LOC101488826	(AT)7	335	GCTTGTGGAGTGATGGTTTTAT	22	AGAGTTGAATGAGAGAGAACGG	22
LOC101488826	(T)10	229	CCGTTCTCTCTCATTCAACTCT	22	ATGCCTCATAACGTGTAGACAA	22
LOC101488826	(A)11	370	TTGGTTGTCTACACGTTATGAGG	23	TCCCTTCGACTTTAGTATGTGTTTC	25
LOC101488826	(AT)9	370	TTGGTTGTCTACACGTTATGAGG	23	TCCCTTCGACTTTAGTATGTGTTTC	25
LOC101488826	(T)11	388	CACATCAAGAAGCGATCAATAC	22	CACCATACATATTACTTTGCCG	22
LOC101513111	(TA)8	160	GTGTTGGGGAGTTCCATCTTAC	22	ACCGTTCTGACTTACTATCGCA	22
LOC101513111	(T)10	136	CCTAGCAGAAAGACCTTGGAAA	22	GAAAGGCAAACAAACAAGCAC	21
LOC101489482	(T)16	305	ACCTCCCTAGAAATGTTGCAGA	22	CAAAATGATAGCTCACACCCAA	22
LOC101490350	(A)19	240	AAGTTTAGAGGGAGTACAACTGGA	24	TTCGGATTACAAGAAGGAGAGA	22

LOC101490350	(CA)7	100	TAAAAGCCAGACAGACAAACCA	22	GCATGTTGTTATAGATGAGAGGAGA	25
LOC101490669	(T)11	384	AATAGCTCTGCAATGTGTTGGA	22	CGGGTAAGTTTTGAGGATGAGT	22
LOC101491303	(A)11	353	AGGCTAATGCACAGAGAAATGC	22	GTGCTGTCAAAAGATTCCAGGT	22
LOC101491812	(T)12	320	GTTTACACTGATGCTGGGAAAT	22	CAGAAAACCAAAAGGCTAGACA	22
LOC101492140	(TGG)8	191	TTCTGCTGGTAAGAAATGGGAT	22	CAAAGATCAAGAGCAATGAGGA	22
LOC101494422	(ATATT)14	338	AACCCAGAGAAGTCAGATCCAA	22	TACCATCCCAACGTGTCATCTA	22
LOC101494422	(AT)9	376	CACACACCTTTGCCATTTTATC	22	TCTACAACCCATTTGTCACGAG	22
LOC101494422	(TA)13	230	CCAGCAATCAAAGAATGAACAC	22	GCTAGTCTCACCAACGGGTC	20
LOC101494422	(GT)6	224	TTTACTGCGAAATGCAAGGTAG	22	GTGTTCATTCTTTGATTGCTGG	22
LOC101494960	(T)10	396	TCTTCTTCAATAGCCACCTTTCTC	24	CCTCCTTCTTTTACCCCTCATT	22
LOC101495506	(A)10	371	ATCAAACAACCAAAGTCACGG	21	AACCCAACCAATGTCTCAAAGT	22
LOC101495506	(AT)6	389	GGGAAAACCTTTGAGGAGAGAT	22	ACTTACAAGAGGAGCAGAGGCA	22
LOC101495506	(T)16	249	TGAAAAGGTATGTTGCTCCTGA	22	TATCCCTATCCGTCGTTCGAT	21
LOC101512465	(TAA)18	306	GAAGTTGTAAGACCCGCATTTT	22	TAGAAGTGGGGAACGTGGTATT	22

APPENDIX-VII: List of genes and genic SSRs present in chickpea genomic region flanked by markers TR24-TC14801 on chickpea chromosomes 3

Sl. No.	Gene Id	Putative function	SSR motifs identified
1	LOC101498827	CASP-like protein 1E1	(T)10, (T)15
2	LOC101499146	Zinc finger protein CONSTANS-LIKE 13	(TC)9, (A)10
3	LOC101500194	membrane-anchored ubiquitin-fold protein 3-like	(A)10, (A)12, (CT)6, (T)10, (T)12
4	LOC101499860	40S ribosomal protein S15-4	No SSR
5	LOC101500507	Monothiol glutaredoxin-S11	No SSR
6	LOC101500829	Glutaredoxin-C11	No SSR
7	LOC101501158	OTU domain-containing protein 6B	No SSR
8	LOC101501469	Monothiol glutaredoxin-S6	(TA)12
9	LOC101501787	Monothiol glutaredoxin-S6-like	No SSR
10	LOC101502110	Uncharacterized	(A)10
11	LOC101502431	BTB/POZ domain-containing protein At1g03010-like	(TA)8
12	LOC101503168	Peptide chain release factor APG3, chloroplastic	(T)10
13	LOC101503487	Spermatogenesis-associated protein 20	(T)17, (AT)6
14	LOC101503823	Uncharacterized	(A)11, (AG)6, (T)11, (ATT)6
15	LOC101504783	40S ribosomal protein S26-1-like	(T)12
16	LOC101505100	cullin-1	(T)12, (T)10, (TA)8, (C) 13, (C)15
17	LOC101505423	Homeobox protein LUMINIDEPENDENS	(CT)9
18	LOC101505978	Protein indeterminate-domain 2-like	No SSR
19	LOC101506286	Uncharacterized	(T)10
20	LOC101506607	Potassium transporter 5-like	No SSR
21	LOC101506941	Wee1-like protein kinase	No SSR
22	LOC101507267	uncharacterized	(A)10, (T)15, (A)10, (T)11
23	LOC101490901	Autophagy-related protein 18a-like	No SSR
24	LOC101507580	Pentatricopeptide repeat-containing protein At5g56310-like	No SSR
25	LOC101507896	Dephospho-CoA kinase	No SSR
26	LOC101508205	Cyclin-dependent kinase 12-like	(TA)6, (ACC)6
27	LOC101508530	Bifunctional protein FolD 2	No SSR
28	LOC101508843	protein LOW PSII ACCUMULATION 1, chloroplastic	(T)10
29	LOC101509166	Rapid alkalinization factor	No SSR
30	LOC101509496	Heat shock factor-binding protein 1-like	No SSR
31	LOC101509815	dol-P-Man:Man(5)GlcNAc(2)-PP-Dol alpha-1,3-mannosyltransferase	(T)12
32	LOC101491220	Uncharacterized	(AT)7
33	LOC101510570	Translocator protein homolog	(AT)26
34	LOC101510893	Xyloglucan 6-xylosyltransferase 2	(CT)73
35	LOC101511207	Thiamine pyrophosphokinase 1	(CTT)6, (TC)7
36	LOC101511529	Probable U6 snRNA-associated Sm-like protein LSm4	No SSR
37	LOC101511855	WD repeat-containing protein 89 homolog	(T)10
38	LOC101512168	Uncharacterized	(T)10
39	LOC101512497	Probable E3 ubiquitin-protein ligase BAH1-like 1	No SSR
40	LOC101512811	Universal stress protein A-like protein	No SSR
41	LOC101513141	Phytochrome A-associated F-box protein	No SSR
42	LOC101491540	Serine/arginine-rich-splicing factor SR34	(T)10
43	LOC105851843	NADH dehydrogenase [ubiquinone] iron-sulfur protein 5-B	(TTA)6, (T)11
44	LOC101513776	21 kDa protein-like	(AAC)6, (T)11
45	LOC101514116	Caffeoylshikimate esterase-like	No SSR
46	LOC101491846	Uncharacterized	(AC)11, (T)11
47	LOC101514663	U1 small nuclear ribonucleoprotein A	(T)10

48	LOC101514990	Indole-3-acetic acid-induced protein ARG2-like	No SSR
49	LOC101515317	Uncharacterized protein At5g01610-like	No SSR
50	LOC101515642	Exocyst complex component SEC15B	No SSR
51	LOC101488396	60S ribosomal protein L18	No SSR
52	LOC101488724	Bifunctional epoxide hydrolase 2	No SSR
53	LOC101489059	RING-H2 finger protein ATL5	No SSR
54	LOC101489391	Uncharacterized	No SSR
55	LOC101489717	Probable pectinesterase/pectinesterase inhibitor 7	No SSR
56	LOC101490034	Probable pectinesterase/pectinesterase inhibitor 41	No SSR
57	LOC101490578	Pectinesterase	(T)11, (TA)16, (AAT)11, (AAT)11, (TA)16, (AAT)7, (A)10, (T)11
58	LOC101490898	Sucrose synthase 2	(T)27
59	LOC101491961	Putative GTP diphosphokinase RSH1, chloroplastic	(T)16, (T)17, (T)12
60	LOC101491431	Uncharacterized	(ATTA)6
61	LOC101492517	60S ribosomal protein L19-1-like	No SSR
62	LOC101492847	Ethylene response sensor 1	(AG)8
63	LOC101492520	Nicotinamide adenine dinucleotide transporter 1, chloroplastic-like	(T)17, (AC)7
64	LOC101493396	Syntaxin-43	(CT)7,, (T)10
65	LOC101493720	Cellulose synthase-like protein D5	No SSR
66	LOC101494260	Probable galacturonosyltransferase-like 7	No SSR
67	LOC101492850	Uncharacterized	No SSR
68	LOC101494982	Uncharacterized	No SSR
69	LOC101493182	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific-like	(AAT)7
70	LOC101495308	Zinc finger CCCH domain-containing protein 1	No SSR
71	LOC101495856	Protein NDR1-like	No SSR
72	LOC101496184	Uncharacterized	No SSR
73	LOC101496512	Uncharacterized	No SSR
74	LOC101496845	Transcription factor MYB12-like	(T)10, (T)11, (T)12, (A)10
75	LOC101497170	dnaJ protein ERDJ3B	(AAG)7, (TA)7
76	LOC101493817	Uncharacterized	(AT)10
77	LOC101497500	Uncharacterized	(T)19, (T)13, (ATT)7
78	LOC101497836	Probable beta-D-xylosidase 2	(T)10, (T)11, (A)10, (A)10
79	LOC101498153	Uncharacterized	No SSR
80	LOC101499148	Uncharacterized	No SSR
81	LOC101498829	E3 ubiquitin-protein ligase MARCH1-like	No SSR
82	LOC101498481	DNA mismatch repair protein MSH6	(T)11
83	LOC101499469	DNA replication licensing factor MCM7	(CT)8, (T)14, (T)11
84	LOC101499969	Vacuolar protein sorting-associated protein 51 homolog	(A)10, (AT)20, (AT)21
85	LOC101500317	OCS element-binding factor 1	(TC)18
86	LOC101500634	Calvin cycle protein CP12-2, chloroplastic-like	No SSR
87	LOC101500939	Probable glutamyl endopeptidase, chloroplastic	(T)11
88	LOC101501470	Protein trichome birefringence-like 6	(T)10
89	LOC101494145	histone-lysine N-methyltransferase EZA1-like	(A)10
90	LOC101494454	DELLA protein RGL2-like	No SSR
91	LOC101494771	Uncharacterized	No SSR
92	LOC101501992	Probable WRKY transcription factor 69	(AT)6, (T)10
93	LOC101502320	ATP-dependent Clp protease proteolytic subunit 5, chloroplastic-like	(CTT)6
94	LOC101502631	Protein IWS1 homolog A	No SSR
95	LOC101502950	Proline-rich receptor-like protein kinase PERK3	(A)12, (T)10
96	LOC101503277	DEAD-box ATP-dependent RNA helicase 24	No SSR
97	LOC101503824	Nodal modulator 1	(TTA)16, (AT)12, (T)11, (TG)8

98	LOC101504141	Glycine-rich cell wall structural protein-like	No SSR
99	LOC101504893	Stachyose synthase	(A)15
100	LOC101495104	Probable WRKY transcription factor 23	No SSR
101	LOC101495434	Flowering time control protein FCA	(T)10, (T)11
102	LOC101505198	Uncharacterized	(CTT)7
103	LOC101505979	Long chain acyl-CoA synthetase 1	(T)16, (ATT)20, (TAT)8, (TAT)6, (TTA)9, (T)16, (ATT)20, (TAT)10, (TTA)7, (ATT)13
104	LOC101506287	nifU-like protein 1, chloroplastic	(A)10, (A)10
105	LOC101506608	Pentatricopeptide repeat-containing protein At4g02750-like	(T)11, (AT)9
106	LOC101506942	SWR1-complex protein 4	(T)10, (T)10, (T)10
107	LOC105851830	Uncharacterized	(TA)13, (A)13, (T)10
108	LOC101508206	ADP-ribosylation factor	No SSR
109	LOC101508531	Nitrogen regulatory protein P-II homolog	(TA)9, (TA)9
110	LOC101509062	Uncharacterized	No SSR
111	LOC101509386	Probable boron transporter 2	No SSR
112	LOC101509709	Polygalacturonase At1g48100	(AT)16
113	LOC101510238	Glycogen synthase kinase-3 homolog MsK-3	(TC)10, (T)11, (T)11
114	LOC101510572	Short-chain dehydrogenase reductase 3b-like	No SSR
115	LOC101511112	Zinc finger protein 598	(TCT)7
116	LOC101511417	Uncharacterized	(T)17
117	LOC101511749	S-Adenosylmethionine synthase	(T)11, (T)10
118	LOC101512064	Squamosa promoter-binding-like protein 1	No SSR
119	LOC101512393	pto-interacting protein 1-like	(T)22, (TA)19, (TA)11
120	LOC101512928	Probable sugar phosphate/phosphate translocator At3g17430	(CT)8, (TG)8
121	LOC101513253	Uncharacterized	No SSR
122	LOC101513583	Uncharacterized	(T)11, (T)17
123	LOC101513899	Pectinesterase-like	No SSR
124	LOC101514239	Peptide chain release factor 1-like, mitochondrial	(T)11
125	LOC101514554	Two-pore potassium channel 5	No SSR
126	LOC101514880	3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase	No SSR
127	LOC101515207	Uncharacterized	No SSR
128	LOC101515528	Protein transport protein Sec23A	(A)11
129	LOC101488280	Putative proline--tRNA ligase C19C7.06	(T)10
130	LOC101488610	Probable polygalacturonase	(GA)9, (TCT)6, (TG)7, (A)10, (T)10,
131	LOC101489172	Probable WRKY transcription factor 47	(TA)8, (T)10, (TA)7, (AT)6
132	LOC101489718	Chitinase 10	(A)10
133	LOC101490035	Auxin-responsive protein IAA30	No SSR
134	LOC101490374	Transcription factor PIF3	(A)11
135	LOC101490696	Uncharacterized	(T)10
136	LOC101491013	Transcription repressor MYB6-like	(ATTA)6
137	LOC101492848	Uncharacterized	NO SSR
138	LOC101492067	Pectin acetylesterase 3-like	(TC)13, (T)11, (A)10
139	LOC101491328	Peptidyl-prolyl cis-trans isomerase, chloroplastic	(T)15
140	LOC101493180	Putative expansin-A17	NO SSR
141	LOC101493501	Glucose-1-phosphate adenylyltransferase large subunit 2, chloroplastic-like	(T)11, (AT)7
142	LOC101493816	Paladin-like	(AGA)7, (ATT)6, (T)10
143	LOC101494142	Cathepsin B-like	(T)13
144	LOC101495766	Cathepsin B-like	NO SSR
145	LOC101494449	Uncharacterized	(T)13, (TAT)17, (T)10, (T)62, (A)11, (A)10, (T)15
146	LOC101494767	Uncharacterized	(T)10

147	LOC101495098	Pentatricopeptide repeat-containing protein AT2G20540-like	(T)10
148	LOC101495761	DEAD-box ATP-dependent RNA helicase 3, chloroplastic-like	NO SSR
149	LOC101495427	Transmembrane protein 184A-like	NO SSR
150	LOC101496082	Phytochrome A-like	NO SSR
151	LOC101496626	Rhicadhesin receptor-like	(ACC)6, (T)15
152	LOC101496945	Protein NRT1/ PTR FAMILY 4.5-like	(A)9, (TA)9
153	LOC101497275	Pectin acetylesterase 12-like	(TC)6, (AGA)7

Appendix-VIII: List of primers designed for genic SSRs in the chickpea genomic region flanked by TR24-TC14801 on chromosome 3

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101498827	(T)10	327	TGGAGTTGAGAATGTGGAGATG	22	CGAACGAGGATACTTCTTTGAGAT	24
LOC101499146	(TC)9	374	TTTGAGTTGTGAGATTGATGGG	22	GAGCCAAAATTGCTTATTGAGG	22
LOC101499146	(A)10	208	GCGGCCTATATTCTTTGGATTT	22	ATCGCCCTCTAACTCTTGTCCT	22
LOC101500194	(CT)6	136	TGGGTCTCCTCTCGTGACTTAT	22	GGTTTCTTTCTTCACTTGCGTT	22
LOC101500194	(CT)10	343	AACGCAAGTGAAGAAAGAAACC	22	CGAACCAGTGTAATGCAATTCT	22
LOC101500194	(T)10	343	AACGCAAGTGAAGAAAGAAACC	22	CGAACCAGTGTAATGCAATTCT	22
LOC101500194	(T)12	255	TTGGAGAAGCAAGTGGATCATA	22	ATCAGAGAAATGAGCATCAGCA	22
LOC101501469	(TA)12	264	AACCAAGTGTACCTGCTGTTTT	22	GCATGACGACTACAAAATTACG	22
LOC101502110	(A)10	386	ATGGGTCTATTTGGTTTGATGG	22	TCCTACCCGACCATACATTCAT	22
LOC101502431	(TA)8	262	TGATTTGTGGTGTAGAATGCTC	22	GAAGTGCAAAGGTTGATGCTA	21
LOC101503168	(T)10	232	TTGTGATGTTAGGTTGTGTCCA	22	ATATCATTTCCGCCATATCCTC	22
LOC101503487	(T)17	386	TTCTCTCAATGGCAACTCTTCA	22	CACGAACACCACATTACCACAT	22
LOC101503487	(AT)6	236	AAGGGTTTCTGTAAATTGGGTC	22	GAAGAACTCCAACAAATGCAGA	22
LOC101503823	(AG)6	385	AACGCGGCTGCTGTATTC	18	CCCTCCAACTTAAACCTTCACA	22
LOC101503823	(T)11	244	GAAAGAGAAACGACAGAGACAACA	24	TCCACCAAATAAATCAACCTCC	22
LOC101503823	(ATT)6	244	GAAAGAGAAACGACAGAGACAACA	24	TCCACCAAATAAATCAACCTCC	22
LOC101504783	(T)12	237	GGACAAGGCAATCAAGAGATTT	22	ATGGATAGCACATGATACGCAG	22
LOC101505423	(CT)9	383	GATTTATCGGAGCTGGAGATTG	22	GCACCAAGAGGTTCTAGCAGTT	22
LOC101506286	(T)10	336	ATCACTTTCACTTTGCTGCTGA	22	CGACTGCGATTTGTTGACAG	20
LOC101507267	(A)10	398	TCGTTACCTATTGATTGGGAGG	22	ACATGAAAACGCCTAACACTTG	22
LOC101507267	(T)15	398	GGTGGAGACTATAAATGGTAAGTGTG	26	TTGGGGTATAAAATGAGCTTCC	22
LOC101507267	(A)10	398	GGTGGAGACTATAAATGGTAAGTGTG	26	TTGGGGTATAAAATGAGCTTCC	22
LOC101507267	(T)11	396	AGCTCTGGACGTTTCAACTCTC	22	CGTATGATGGGTGCATTGATTA	22
LOC101508205	(TA)6	376	AAGATAAGCAAGCTACCAACCG	22	GACACACATGGAAAATTGGAGA	22
LOC101508205	(ACC)6	246	TGTTTACCCATATCCACCACAA	22	ATGGAGCAATATCCACCTTCAT	22
LOC101508843	(T)10	183	TTCAGCTTTTCTCCAAAGGAAG	22	GGATTGGGATTCAAAGTAATCG	22
LOC101509815	(T)12	367	GAGGTTTTCTGCCATTGACCTA	22	CCACTCAACTCTTCTCACCACA	22
LOC101491220	(AT)7	195	GTAGGGCCATTTTAGCATCAAC	22	TCCCTTTCATCACCTGAGTTTT	22
LOC101510893	(CT)73	380	GTTGATGGATTGGAATGTGTTG	22	TATATGCAAAGCCGCCGT	18
LOC101511207	(CTT)6	140	AAGTTTGGGAGAAAGAGAACCA	22	GCACAACGAGAGCGTATGTTAG	22
LOC101511207	(TC)7	258	AATCAAAGGTGACATGGACTCA	22	ATTACTTTCGTTCCCTGCAAGA	22
LOC101511855	(T)10	400	ATTGTATTCTTGGTCCCCTCAA	22	CCTTCCATTAGCAACTACGATG	22

LOC101512168	(T)10	358	CAAAATCAATGTAGGAGTGCGA	22	ATGACACACCCCACCAAATTAC	22
LOC105851843	(TTTA)6	392	CCCTATCTGCTTCCATTGTCTC	22	TCCAGCTAACAGAAAACGAGTG	22
LOC105851843	(T)11	372	TCTAGTGCATCCAAACATCTGC	22	AGGGGAAGGGAAAACTAAAAGA	22
LOC101513776	(AAC)6	241	GACCATTACATTTCCCCTTATGTC	24	CTCCCCTATTGCTACTGACACC	22
LOC101491846	(AC)8	207	CATAAAACGAGATCGCCAGAG	21	GCAGCAAGAGTTCCTTCATCTT	22
LOC101514663	(T)10	362	GTCAATGGTCATGTTAGAGGCA	22	GATTTTGCACCACCTGGATAAG	22
LOC101490578	(T)11	200	CTCCAAACTCAAGCACAACAAG	22	TCCAATCCCATCTCCAACTAAC	22
LOC101490578	(T)11	307	GTTGCGTGTAAAGTGTGGTTTC	22	AGGCAGAACAAAGAGTGTGTGA	22
LOC101490898	(T)27	149	TAACTTCATGGGGTCAGGTTCT	22	ACTCTTTCTAGTCGCTGGTTGC	22
LOC101491961	(T)16	343	CCATGTCAGGTACGTCCAACTA	22	TCATATCTTCCATTTCCATCCC	22
LOC101491961	(T)17	344	CCATGTCAGGTACGTCCAACTA	22	TCATATCTTCCATTTCCATCCC	22
LOC101491961	(T)12	371	TTCAAGATGTGAAAGCAGAGGA	22	GCGAGGCCACTTTTGTATTATC	22
LOC101491431	(ATTA)6	314	TTGGATGACGATCAAGACATTC	22	CGTTCCTCACTGTAATTCCTCC	22
LOC101493396	(CT)7	286	GGATGCTATTGTCCCACCTAAA	22	ATCCTCCAAACCAAAGGTAACA	22
LOC101493396	(T)10	379	TGTTACCTTTGGTTTGGAGGAT	22	CAATTTCTCTTTCTCTTTCCGC	22
LOC101493182	(AAT)7	116	ACCACAATCCTATTATCCACCG	22	CTTGAAACGGCATCATCGT	19
LOC101496845	(T)10	337	GGGCTCCTTGTTGTGAGAAA	20	TATTTCCCCTTTTGAGATCAGC	22
LOC101496845	(T)11	337	GGGCTCCTTGTTGTGAGAAA	20	TATTTCCCCTTTTGAGATCAGC	22
LOC101496845	(T)12	337	GGGCTCCTTGTTGTGAGAAA	20	TATTTCCCCTTTTGAGATCAGC	22
LOC101497170	(AAG)7	375	ACGTGGAGAACAGAGATTGGTT	22	CGCCATTGAAGATTACAAGACA	22
LOC101497170	(TA)7	211	AAGGGAGGGGTATTTCATCACT	22	TCAGGCACCAACATGATTAGAC	22
LOC101493817	(AT)10	394	AGGTTGGATCTTTGATGGCTAA	22	AAAATGTCTCTGAACCCTGCAT	22
LOC101497500	(ATT)7	381	TTCTTGTAATGTGAATCCTGCG	22	TGAATTTAGGGATCGGTTCAAG	22
LOC101497836	(T)10	390	ATTGGACGGGTCAGATTTCTTT	22	CAACTATTATTACGGGACAAAGCG	24
LOC101497836	(T)11	386	TTATGTTAAGGGGCTACAGGGA	22	CAGAAGATTCCCATTTTCGCTA	22
LOC101497836	(A)10	360	CACTAAAGCAACCGCTCTAAGTC	23	GGCCTCACAACATGATAAATCC	22
LOC101497836	(A)10	396	GCCAAATTATAGCCCTAGTTCA	22	TTCGAGCATATCTTCCTATTCC	22
LOC101498481	(T)11	384	GCATTTGGGAAGAGACTGCTAA	22	CAAGATGAACGGTGATTGACAT	22
LOC101499469	(CT)8	336	ACATCACTTCACCCTCAACCC	21	CGTTGTTGTACTTTGGTGTTCG	22
LOC101499469	(T)14	317	CCCTCTCTCTCTCTCTCCCT	22	CGTTGTTGTACTTTGGTGTTCG	22
LOC101499469	(T)11	302	TTCATCTCTCACATCGAACACC	22	TCAGCGAAAATCCCAATATACC	22
LOC101500939	(T)11	377	GCGAAGACGAAGAGTAAAATCC	22	GACAGATGACAAACATCGTGGT	22
LOC101501470	(T)10	114	CATCTCATTTCAGGTTTGCTTC	22	TGTGTGGGACTAGATTTACAGTCTT	25
LOC101494145	(A)10	353	GCGAGAATGTGATGCAGATGTA	22	CCGACGTTACACCCAAAGATAA	22
LOC101501992	(AT)6	333	TGAAAAGAGGAAGCTATGGACA	22	GATATGGAGAGCCTTTGATTGG	22
LOC101501992	(T)10	212	TTTGGGTCTTGGAGGAAATATG	22	TATGAGTTGCGGAATGTAATGC	22
LOC101502320	(CTT)6	392	TTGGCTCTCTTCTTCTTTCCAC	22	CCTCCCAAATTCCTGTAAATCA	22

LOC101502950	(A)12	384	CACTACCACAACACTAGCAGCA	22	TACCGATTAACATGACTGACGC	22
LOC101502950	(T)10	330	CTCCAAAATCCTCCATTCAAAC	22	CTTCTCAAGCATAATCGGTGTC	22
LOC101503824	(TTA)16	275	TTACTCTTTCTCTGCAACTGCG	22	CAATTTAGCATCGGTTGGTTTC	22
LOC101503824	(AT)12	336	ATGGCTATAAATGCAGGATGTG	22	ATCTTGGGAATGTTTGGTAAGC	22
LOC101503824	(T)11	137	CCTAAGTTGTCTTCCCAACCAT	22	CCCCTCAAGCTAAACAGTAAAAC	23
LOC101504893	(A)15	305	AAACATTGGTGGCAGCTTTT	20	TGGACAAGAACAGAGTAAAACAGAG	25
LOC101495434	(T)10	307	CTGGAGGAGATTCACAAAGACC	22	GGAACTGGTGCAACGTAGACTT	22
LOC101495434	(T)11	316	TGAGGAGCATTATTGGAGAGTT	22	ATTTGTAGGCAGATCAGGTGAG	22
LOC101505198	(CTT)7	271	CACACCATTTCAATCTCTTTCG	22	TTCTTCGTCAGAACCTGCATTA	22
LOC101506287	(A)10	299	CAACTACAACGATGAAAATGGG	22	CTATGCGCCAACACAAAATAAC	22
LOC101506942	(T)10	110	CACCGAGACTGAATGAGAGAGA	22	ACCACTCAAAAGATGCCCTAAA	22
LOC101506942	(T)10	386	TTGTTTTAGCCTTGTGATGTGG	22	CTTTTATGAGCCGAGGTAGGTG	22
LOC101508531	(TA)9	377	CTTCCAAAGGTTTCACACACAA	22	ACATACCTCCCTGCCTCTCTTT	22
LOC101508531	(TA)9	285	AAGAGAGGCAGGGAGGTATGTA	22	TTCCCATTTTCAACAAAGCC	20
LOC101509709	(AT)16	397	ACCGTCCAATAAAGAATGTGCT	22	GAAGTGAAAGCCGAATCAAAAG	22
LOC101510238	(TC)10	255	CTTTCTTACCCATTCCTAATCCC	23	AGCAGTGAGTGTGATCTGAAGG	22
LOC101510238	(T)11	381	CATTTTGGTGTTGGATGTTCAC	22	GTCCAGTTTCAGTTCCATTTCC	22
LOC101510238	(T)11	389	AGCTTATATTCGGTGCGACTG	21	ACTTGATTTCTTCCCTTGTTGG	22
LOC101511112	(TCT)7	271	ATTGGGTCTTGTTTGGTGTAGC	22	TGCAATAAGGGGAAGAAAGAGA	22
LOC101511417	(T)17	368	CAAAATTCAGGTTTAGGTTCGG	22	CCAGGTGACAAATCAAATCAAC	22
LOC101511749	(T)11	343	CCAAGTATTTTGGCTCAAGTGT	22	TCTCTGCGTAACAATGGAAGAT	22
LOC101512393	(T)10	314	ACAAGGTTTTGGGTATGTTTGG	22	TTTTACGGCCTCCATCTCAC	20
LOC101512928	(CT)8	235	TCTCGGATTTATCGTTTCCAGT	22	ATCAGTGGGATTCAGAGTAGGG	22
LOC101512928	(TG)8	304	TTCACATTGCATCAAGCCTAGT	22	AGAATCCCACAGCATAATCCAT	22
LOC101513583	(T)11	369	CAGCTTCACAATCTCTGACACC	22	GATTCCCACACCTTCTGTTTGT	22
LOC101513583	(T)17	321	TGCTGTTTGTGGCTATTCTGAT	22	CTCCATATCAAGTCCCCAAGAG	22
LOC101514239	(T)11	400	TGAAACGTGAAAAGCTGAGAAG	22	TACGTGTAAGAGATCCGGTGAA	22
LOC101489718	(A)10	303	GAGTCAGATCCGCCAAATTAGT	22	GGACATGCTGTGTCATCTTTGT	22
LOC101490374	(A)11	114	CATCTGAAAGGGTGAGGAAAAT	22	TCTTCTCTTCTGCAAACATCCA	22
LOC101490696	(T)10	321	GTGAGGGATTTCATAGCAAAGG	22	GTACATGGTCGAGACACAAAGG	22
LOC101491013	(ATTA)6	265	GCTTAGGAAGGGTCTATGGTCA	22	GCCTACAACTCTTACCACATCTTTG	25
LOC101497275	(TC)6	388	GATTCCAAACGTGCAGTGATAA	22	ACAGAGGATTTCCCTCAACAAA	22
LOC101497275	(AGA)7	388	GATTCCAAACGTGCAGTGATAA	22	ACAGAGGATTTCCCTCAACAAA	22

APPENDIX-IX: List of genes and genic SSRs present in chickpea genomic region flanked by markers TR20-TS82 on chickpea chromosomes 4

Sl. No.	Gene Id	Name	SSR motifs identified
1	LOC101502344	UPF0505 protein C16orf62 homolog	(AT)9, (A)11, (T)13, (AT)6, (T)10, (TA)6
2	LOC101502654	Glucan endo-1,3-beta-glucosidase 12-like	(T)14
3	LOC101502968	Embryo defective 2016 (EMB2016)/ TC00989	(T)10, (TTA)9, (TC)6
4	LOC101491979	dof zinc finger protein DOF3.5-like	No SSR
5	LOC101503516	E3 ubiquitin-protein ligase PUB23-like	No SSR
6	LOC101503852	E3 ubiquitin-protein ligase PUB24-like	No SSR
7	LOC101504165	BEL1-like homeodomain protein 1	(ATT)6, (TTC)6
8	LOC101504489	digalactosyldiacylglycerol synthase 1, chloroplastic-like	(A)13
9	LOC101505338	Replication factor C subunit 3-like	(TTC)6
10	LOC101506192	Ribosome biogenesis protein NSA2 homolog	(T)12
11	LOC101505670	Uncharacterized	(A)11
12	LOC101506520	Uncharacterized	(T)12, (T)10
13	LOC101506851	Exocyst complex component EXO70A1	(T)11, (TA)11
14	LOC101507186	Protein YLS9-like	No SSR
15	LOC101493311	Protein FAR1-RELATED SEQUENCE 5-like	(A)10
16	LOC101493633	Uncharacterized	(A)10, (T)13
17	LOC101507504	Protein YLS9-like	No SSR
18	LOC101493948	Uncharacterized	No SSR
19	LOC101508350	Uncharacterized	(AT)6, (T)11
20	LOC101508663	Uncharacterized	(TA)10, (AT)13
21	LOC101509609	Cytokinin riboside 5'-monophosphate phosphoribohydrolase LOG7	(T)10, (T)10, (T)12, (TTA)6
22	LOC101509940	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
23	LOC101510264	2-Cys peroxiredoxin BAS1, chloroplastic-like	(T)10, (T)10, (GT)6
24	LOC101510592	Transcription factor E2FA	(TA)6, (TA)23, (A)14, (T)12
25	LOC101510920	Histidine--tRNA ligase	No SSR
26	LOC101511232	Putative HVA22-like protein g	(A)11, (T)13
27	LOC101495227	Transcription repressor OFP6	(AAT)6
28	LOC101495546	Transcription repressor OFP15	No SSR
29	LOC101511554	Probable CCR4-associated factor 1 homolog 9	No SSR
30	LOC105851930	RING finger protein 141-like	(T)10
31	LOC101512077	Ubiquitin-conjugating enzyme E2 variant 1D	(T)10, (A)10
32	LOC101512833	Uncharacterized	(TC)10, (T)10
33	LOC101513160	Pentatricopeptide repeat-containing protein At2g44880-like	No SSR
34	LOC101496209	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
35	LOC101513483	40S ribosomal protein S17-like	No SSR
36	LOC101514026	Uncharacterized	No SSR
37	LOC105851931	Uncharacterized	(AGA)18
38	LOC101497195	Pentatricopeptide repeat-containing protein At2g44880-like	No SSR
39	LOC105851932	Uncharacterized	(T)13, (T)10, (AATA)6
40	LOC101497526	Uncharacterized	(T)14, (T)10, (A)10, (T)10
41	LOC101497738	Pentatricopeptide repeat-containing protein At2g44880-like	No SSR
42	LOC101498065	Uncharacterized CRM domain-containing protein At3g25440, chloroplastic	(CT)9, (A)11
43	LOC101498401	Regulatory protein NPR3-like	No SSR
44	LOC101498740	Uncharacterized	No SSR

45	LOC101499581	Xylulose 5-phosphate/phosphate translocator, chloroplastic-like	No SSR
46	LOC101499060	BRCA1-A complex subunit BRE-like	(TCAT)8, (T)10
47	LOC101499883	Tubulin-folding cofactor E-like	(TA)6
48	LOC101500222	Uncharacterized	No SSR
49	LOC101504369	Serine/threonine-protein kinase CTR1-like	(A)10, (T)10, (A)14, (A)10, (A)10, (T)12
50	LOC101500533	Uncharacterized	(T)12
51	LOC101505020	Uncharacterized	No SSR
52	LOC101505340	Uncharacterized	(T)12
53	LOC105851933	Exocyst complex component 5-like	No SSR
54	LOC101501060	Protein kinase and PP2C-like domain-containing protein	(TC)6, (T)12
55	LOC101501372	Arginine/serine-rich coiled-coil protein 2	No SSR
56	LOC101502753	Pentatricopeptide repeat-containing protein At1g80270, mitochondrial-like	(T)10, (T)21, (TC)6
57	LOC101503066	Pentatricopeptide repeat-containing protein At1g80270, mitochondrial-like	No SSR
58	LOC101503394	GDSL esterase/lipase At1g29670-like	(T)19, (A)10, (A)11, (TTA)22, (TAT)23, (TTG)17.3, (TA)11
59	LOC101488417	Uncharacterized	(A)11, (TA)29, (T)10, (T)13, (CCA)11, (T)12, (AT)9, (AT)8
60	LOC101507290	Probable indole-3-pyruvate monooxygenase YUCCA3	(TGA)6, (T)12
61	LOC101507596	7-hydroxymethyl chlorophyll a reductase, chloroplastic	(TCA)10, (TA)13
62	LOC101489737	B3 domain-containing protein REM20-like	(TGA)8, (ATG)7
63	LOC101490052	Uncharacterized	(TC)6, (T)10, (T)11, (AT)7
64	LOC101508132	ATP-dependent zinc metalloprotease FTSH 4, mitochondrial-like	No SSR
65	LOC101508452	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13-B	(A)19
66	LOC101508756	PHD finger protein ING1	No SSR
67	LOC101490389	Putative protein TPRXL	No Ssr
68	LOC101490714	40S ribosomal protein S8-like	(AAT)34, (T)10, (A)11, (A)10, (T)19, (T)20, (TAA)8, (A)10, (T)13, (A)13, (A)11, (AT)6, (T)14
69	TRNAL-CAA	Transfer RNA leucine (anticodon CAA)	No SSR
70	LOC101491349	Probable ubiquitin-like-specific protease 2B	No SSR
71	LOC101492314	Ethylene-responsive transcription factor ERF118-like	No SSR
72	LOC101492978	Probable serine incorporator	(T)10, (T)10
73	LOC101509402	Dirigent protein 18-like	No SSR
74	LOC101509726	Dirigent protein 18-like	No SSR
75	LOC101493312	Putative B3 domain-containing protein At1g78640	No SSR
76	LOC101510045	Hydroxyethylthiazole kinase	No SSR
77	LOC101493635	F-box protein At2g26160-like	No SSR
78	LOC101493950	LOB domain-containing protein 12-like	(A)13
79	LOC101510372	Xylosyltransferase 2	(T)10
80	LOC101510701	GDT1-like protein 2, chloroplastic	(TTTA)6
81	LOC101511023	GATA transcription factor 1	No SSR
82	LOC101511329	Lachrymatory-factor synthase-like	No SSR
83	LOC105851935	Pentatricopeptide repeat-containing protein At2g44880-like	No SSR
84	LOC101511654	Uncharacterized	(T)10

APPENDIX-X: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by TR20-TS82 on chromosome 4

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101502344	(AT)9	347	GGTTAAAACAAGAGAGGGAAGGA	23	CACGTCAACGGCATAATTTTC	21
LOC101502344	(A)11	148	TTCCTCGCAATCTGTATTTTCC	22	TGTGACTATGGATGCCACTTTC	22
LOC101502344	(T)13	243	GAAAGTGGCATCCATAGTCACA	22	TCAAGAAAGCCTAGATTCACCC	22
LOC101502344	(AT)6	279	CATAAGGCTGAGGGTTTAGTCTCT	24	GAGTGGAAGGTAGATTGATGGC	22
LOC101502344	(T)10	243	GTTGCCTTCTTCCTTGTTCTTC	22	CAGCAGCAATAGCAAACTGAAC	22
LOC101502344	(TA)6	343	AGGAGGGTGTTGACAGATAGCA	22	GCCATGCTCACGACAAATCTA	21
LOC101502654	(T)14	381	CTCATCGCTTCATCATCAGGTA	22	TCGATTTGAGTTGAGGGGTAGT	22
LOC101502968	(TTA)9	369	CAAGTTTTGCCTCCATCTATGA	22	TCACCAAAGCAATCTTATCCG	21
LOC101502968	(TC)6	250	TGTCTCCACTCTCTACCAACCA	22	TCCAAGGATGAAATACAAAGGG	22
LOC101504165	(ATT)6	272	GTTGAAGTGGAAATTGAAGAGC	22	TCTTTCTTTTGGGACAGAGACA	22
LOC101504165	(TTC)6	317	TCCTTGTGTGTCTCATCATTCC	22	CTTGTGAATCTGGTTGGTGTGT	22
LOC101505338	(TTA)14	191	GCATTGGTTGTTTGCTCTGTAT	22	TCACTTGGAGTCATTTCGATGT	22
LOC101506192	(T)12	159	TGGCTACTTTGATTGCCCTAAT	22	TGAGGCTAAAAGGACACAAACA	22
LOC101505670	(A)11	278	ATATGGCCGTGTTGGAAGTAAT	22	ACTGTGGGGTGTCTAAGTCTCAT	23
LOC101506520	(T)12	349	AGGCATTTCTGAGGAGTTTGAG	22	GTACCACACCAGATCATTTCCC	22
LOC101506520	(T)10	349	AGGCATTTCTGAGGAGTTTGAG	22	GTACCACACCAGATCATTTCCC	22
LOC101506851	(T)11	372	GCTTTAATATGGCCTGGAAGC	21	AGAGTCCGGTAAGCAGTCAAAA	22
LOC101506851	(TA)11	368	TACAGGTCAGAAGCAAAGGACA	22	TGTAACCGAAAGTTCATCGCTA	22
LOC101493633	(A)10	235	TCATCTAAAGTGGGGAGAGGAA	22	AGATATGGAATGTCAAACCGGA	22
LOC101493633	(T)13	274	CGCACAAGATGGAGTGTGTATT	22	ACTATTGTCCAGCCTACCAACC	22
LOC101508350	(AT)6	369	TGTGCATCGAAACTTGAGACAT	22	CTGCTCCACTGATACCAAAACA	22
LOC101508663	(TA)10	131	AGGTATGTGGTGGGTAATTGCT	22	AATGTCTCCATCTCAGCCTCTC	22
LOC101508663	(AT)13	398	GCTCACAGTTGCCACAATTTAG	22	ATTTATGGTCTCATGCAATCCC	22
LOC101509609	(T)10	280	GCAAACAAACACTCACTCAACA	22	TGGCTTAATGACAATGATACGG	22
LOC101509609	(T)10	307	GGTCTTTTACATTTTCGGCCAT	22	CTTGACAGGCAGATTCCAGCTA	22
LOC101509609	(T)12	307	GGTCTTTTACATTTTCGGCCAT	22	CTTGACAGGCAGATTCCAGCTA	22
LOC101509609	(TTA)6	229	GTGTCTGATATGCACCAAAGGA	22	ACCCCGTCCAATAGTAAACCTC	22
LOC101510592	(TA)6	368	TACCATGTTGAAGTTGTGGGA	21	GATCTAAGGGTTGAGAGTAGTCATTC	26
LOC101510592	(TA)23	397	AGGCTGAAGGGATAATGACAAA	22	TCTAGTGGAAAGGTCGAATGCT	22
LOC101510592	(T)12	365	GTATCCTTCTTGTTTTCGTGCC	22	TTGACACTCCACAGCTTCTGAC	22
LOC101511232	(A)11	385	AGAAAGAGAGAATCAGAGGCCA	22	CCCTAACATTTTCAACCGCTAT	22
LOC101495227	(AAT)6	382	ACACACACCTTCCTCTCACTCC	22	ACCTTCATTTCCAACTCTTCCA	22

LOC105851930	(T)10	356	TTGAGCCAACTACCACTTCTTG	22	CCTTCTCATCCATTGCCTACAT	22
LOC101512077	(T)10	128	AGTTGTTGCCGTTGACCTAAAT	22	TCAGAAACCGATCCATAATTCC	22
LOC101512077	(A)10	232	CGTGCTGTTGATGGATATTGAC	22	GCAGATGCTGATGACAGAAAAC	22
LOC101512833	(T)10	289	CAAGTTCTGCATTCTCTGCTTC	22	TAGACTTCCCTAGCATTCCCAA	22
LOC105851931	(AGA)18	382	CAAAGCAGAATGTCCATCAA	20	CGTTTGTAGCGCAGAATGT	19
LOC105851932	(T)13	390	CCTTCAAACACCAATCTCAACA	22	ATTAACAAACACGGGAAACTCG	22
LOC105851932	(T)10	285	TCTGCCCAAGGCTATAAGGATA	22	AAGCTCACAATCGTCTTCACAA	22
LOC105851932	(T)13	324	GGGTGGATGTTATTGGACTCAT	22	GACGCAGACGAGTTGTAAGTTG	22
LOC101497526	(T)14	322	GGTCTGAGCCTGACTTACGATT	22	GTTTGAGAGGTAATAATGGGTGC	23
LOC101497526	(T)10	322	GGTCTGAGCCTGACTTACGATT	22	GTTTGAGAGGTAATAATGGGTGC	23
LOC101497526	(T)10	261	GCAAAACGTACCATTGACGATA	22	ATGCCCTGATTGATGACCATA	21
LOC101498065	(CT)9	220	ATCTCATCCCCTTCTTCTCCTC	22	TTTATCCAAACATGGCCTTAGC	22
LOC101498065	(A)11	356	TACCTTGCAGCTTGAACAACTC	22	CGGCAGAGGAAGACAACTAACT	22
LOC101499060	(TCAT)8	171	TCCAGTGACAAATACAATTCCG	22	ACAATAGCCCCTGAAACAGAGA	22
LOC101499060	(T)10	214	TCCCTGTTTCTAGGTCTTTTGC	22	CCATAAGTCAGAATGTGGGTCA	22
LOC101499883	(TA)6	211	GATCCGTCAATGGTGTGAAGTA	22	GTGAGTGTCTTGTGTCCATGCT	22
LOC101504369	(A)10	289	TTATCAGGGATGGCAAGCTAAT	22	GGTGAAGGGATCAAAACTGAAG	22
LOC101504369	(T)10	155	TAGATTTCATCACCTGGGCTTT	22	GTTCATATCCTCTCAGGCACAA	22
LOC101504369	(A)14	330	ATGAACAACTTACTGCTGTGGC	22	TGGTCCCTTCTCTTCTCAAACT	22
LOC101504369	(A)10	173	TTGGATGCACACTAGAAAATGG	22	AACGCTACAGCTTCCTCAAAAG	22
LOC101504369	(A)10	173	TTGGATGCACACTAGAAAATGG	22	AACGCTACAGCTTCCTCAAAAG	22
LOC101504369	(T)12	399	AAAGTACAACCTTCGCTGTGTG	22	CAGCAGATACAATACGGAACGA	22
LOC101500533	(T)12	178	AATTACCTAAAACCAGCAGCGA	22	CCCAATTCCAGTTTACCCTATG	22
LOC101505340	(T)12	329	GCAAGCTGAAATTGATAGGCTT	22	GCTGGACTCAGATGAACGTCTAC	23
LOC101501060	(TC)6	294	TTTATGAAGGCACACTCAATGG	22	CCAAACAGATCCTTATCATCCC	22
LOC101501060	(T)12	293	TATGAGACCAGAGGGAGCATTT	22	ATACATAAAGCAAACCGCACCT	22
LOC101502753	(T)10	349	CACACAATCAACCTGCACTTTA	22	AGGTCTTGGATTTCTGTTTTGC	22
LOC101502753	(TC)6	312	TCTTAATTCGTAGATGGCACCC	22	CAGAGTCACATGGAATGACCAA	22
LOC101503394	(T)19	265	GGTTAAATTCAGGGTCAGCAAT	22	GGGAAGAGGAGTTGAGAGTTGA	22
LOC101503394	(A)10	241	GTTTTCCGCAATCACACAGTTA	22	GTAATTTGCAGCCACAACCAT	21
LOC101503394	(A)11	308	TAGTAGGGACGTAAAAGACCCG	22	GGATGCAGAGAGGAGAAGAAAA	22
LOC101488417	(A)11	228	CTAAAACAAGCAAGTGTGGCAA	22	CTTACAACGTGACAACCCCAAT	22
LOC101488417	(T)10	334	TCACTACCACAATCAAACACAGC	23	ATTAACGATGGGGAGAACTCTG	22
LOC101488417	(T)12	136	GCAACTTTCCTTCCTGACACTT	22	CCCCTTCCTTTCTTCTTTCTTC	22
LOC101488417	(AT)19	340	GAAAGAAGAAAGGAAGGGGAAG	22	GTTAGTGATGTGATGAGTGTGATGA	25
LOC101488417	(AT)8	400	CACACTCATCACATCACTAACTTCTC	26	GTTTCCTCATCTACTGCTTCCG	22
LOC101507290	(TGA)6	245	TTTGGTCTTTGGAGGGTTAAGA	22	ACAAGCACTTTCTTTCCACCAT	22

LOC101507290	(T)12	392	GAAGCTCGGTGAGTATCGAAAT	22	AAAACTGGTGTCTTCCCTGAAA	22
LOC101507596	(TCA)10	386	CCAAACTCTCCTCCCTACCTCT	22	AGACATTCCATCTCCCAAGAAA	22
LOC101507596	(TA)13	364	TTCAGGGAGATAGACGACCATT	22	TGGTAACTTGCATTCACCCAT	21
LOC101489737	(TGA)8	366	ATTGGCATCAATGTGTGAGAAG	22	CTCAATGTCCCCTAAACCAAAA	22
LOC101489737	(ATG)7	366	ATTGGCATCAATGTGTGAGAAG	22	CTCAATGTCCCCTAAACCAAAA	22
LOC101490052	(TC)6	204	CAACACCAGAATCCATCTCAAA	22	ACGGAGAAATCGAAAGTGTAGG	22
LOC101490052	(T)10	115	GGCACCTCTGTCTGAAATCCTA	22	CGATGCTGAACCAAAGTTGATA	22
LOC101490052	(T)11	331	GCCGGGTATGTATTTGATTTTC	22	AACTAATCTCTCCACCCCAACC	22
LOC101490052	(AT)7	351	GTTACAGCACGAGATCAAAGCA	22	ATTACCTCGGCATGGAGTTTTA	22
LOC101508452	(A)19	313	CCACACACAACACAAATCACAA	22	AAGCACCAAAGGTAGTGAGGAA	22
LOC101490714	(A)10	167	AGCCTTCACATATCCTTCATCG	22	ACTGCTATAAGCCGCACAAAA	21
LOC101490714	(T)19	189	CTACTTTGTATTTGTCCTGTCCTTG	25	TATTCCCAGTTTGCGATAAGC	21
LOC101490714	(T)20	312	CAAACTGGGAATACGACGATCT	22	GCGATAAGCAACTGGACCTAAA	22
LOC101490714	(A)13	378	TCCAATGTAATAAGCAAGACGG	22	ATTCGTGATCGGGTTTGTATTC	22
LOC101490714	(A)11	378	TCCAATGTAATAAGCAAGACGG	22	ATTCGTGATCGGGTTTGTATTC	22
LOC101490714	(AT)6	123	GAATACAAACCCGATCACGAAT	22	GGATGAACGATGAAGAATGGAT	22
LOC101490714	(T)14	225	AAAACTCCATCTACCAAGGGTT	22	CTAAGCAACCAAATCAGGTCAG	22
LOC101492978	(T)10	215	TCTTCCTGTTCGAGTTTCACAA	22	CTAGATCCGCACCAATGAGTTA	22
LOC101492978	(T)10	323	ATGCTGATTTCCCCAGTTGT	20	GACTCTCATCCAAAGCAAGAAA	22
LOC101493950	(A)13	388	GATGCAACCTCCTATTTCACAG	22	ACACTGCAAATTAAAGCCACC	21
LOC101510372	(T)10	297	TCTGATGTTTGTGATGTTCGTG	22	TTGGTTTCAACTTCCTCAACCT	22
LOC101511654	(T)10	327	GCAAGCTGAAATTGATAGGCTT	22	GCTGGACTCAGATGAACGTCTAC	23

APPENDIX-XI: List of genes and genic SSRs present in chickpea genomic region flanked by markers ESTSSR21-TS72 on chickpea chromosomes 4

Sl. No.	Gene Id.	Putative function	SSR motifs identified
1	LOC101508668	ABC transporter G family member 26-like	(CA)6, (CA)6, (T)11
2	LOC101508986	Uncharacterized	No SSR
3	LOC101509518	E3 ubiquitin-protein ligase At4g11680	(T)13, (T)10
4	LOC101501910	Chaperone protein ClpC1, chloroplastic-like	(A)11
5	LOC101502240	Uncharacterized	No SSR
6	LOC101509841	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase	No SSR
7	LOC101502559	Uncharacterized	No SSR
8	LOC101511978	Formin-binding protein 4	No SSR
9	LOC101503199	Tobamovirus multiplication protein 3-like	No SSR
10	LOC101512527	Pentatricopeptide repeat-containing protein At5g13770, chloroplastic-like	No SSR
11	LOC101503518	CASP-like protein 1C1	(T)14, (T)10
12	LOC101503855	Putative clathrin assembly protein At1g25240	No SSR
13	LOC101504168	Uncharacterized	No SSR
14	LOC101512838	Pentatricopeptide repeat-containing protein At5g39710	No SSR
15	LOC101504493	Agamous-like MADS-box protein AGL80	No SSR
16	LOC101513382	Armadillo repeat-containing protein 6	No SSR
17	LOC101513696	Serine/threonine-protein kinase SRK2E	(AG)10, (AG)6, (A)10, (T)16, (A)11
18	LOC101514031	Uncharacterized	(T)10, (T)10, (GT)8
19	LOC101514584	Probable N-acetyl-gamma-glutamyl-phosphate reductase, chloroplastic	(T)10, (T)10
20	LOC101504807	Gibberellin 20 oxidase 2-like	(T)10
21	LOC101514908	Flavonol synthase/flavaNone 3-hydroxylase-like	No SSR
22	LOC101515231	Uncharacterized	(T)11
23	LOC101515557	Leucine-rich repeat extensin-like protein 3	No SSR
24	LOC101488311	Uncharacterized	(TC)6, (T)18
25	LOC101488634	B3 domain-containing transcription factor VRN1-like	(T)10, (T)17, (T)10
26	LOC101488973	Uncharacterized	No SSR
27	LOC101505120	Extensin-like	No SSR
28	LOC101489301	Uncharacterized	No SSR
29	LOC101489625	Extra-large guanine nucleotide-binding protein 1-like	(T)11
30	LOC101490167	Allene oxide synthase, chloroplastic-like	(A)10, (TA)7
31	LOC101490490	Long chain acyl-CoA synthetase 2-like	(T)10
32	LOC101491038	Uncharacterized	No SSR
33	LOC101492086	UPF0496 protein At4g34320-like	(T)13
34	LOC101492430	Cell division control protein 48 homolog C-like	No SSR
35	LOC101492763	Desi-like protein At4g17486	(T)10
36	LOC101493314	G-box-binding factor 1-like	(T)10, (T)10
37	LOC101493637	Pentatricopeptide repeat-containing protein At3g48810	No SSR
38	LOC101505443	Uncharacterized	No SSR
39	LOC101493953	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha	(T)10, (T)10
40	LOC101494474	Splicing factor U2af small subunit B	No SSR
41	LOC101495005	Mitotic spindle checkpoint protein BUBR1	(AAG)7
42	LOC101506418	Titin	No SSR
43	LOC105851070	Squamosa promoter-binding-like protein 14	(CAC)7, (T)12, (T)10
44	LOC101495335	Uncharacterized	(TAT)8
45	LOC101495662	Uncharacterized	(T)11, (T)11, (A)10
46	LOC101495986	Uncharacterized	(A)12, (TA)8, (AT)12

47	LOC101496321	Protein ROOT INITIATION DEFECTIVE 3	(A)12, (TTTA)7, (A)10
48	LOC101496648	Protein indeterminate-domain 5, chloroplastic-like	(T)10, (A)15, (A)10
49	LOC101497407	Protein CHROMATIN REMODELING 19	No SSR
50	LOC105851982	Uncharacterized	No SSR
51	LOC105851983	Mucin-5AC-like	No SSR
52	LOC105851984	Zinc finger BED domain-containing protein RICESLEEPER 2-like	(A)10
53	LOC101497743	Peptidyl-prolyl cis-trans isomerase CYP21-4-like	(T)71, (T)30
54	LOC101498068	Histone-lysine N-methyltransferase family member SUVH9-like	(T)10, (GT)6, (GT)6
55	LOC101507403	B3 domain-containing transcription factor VRN1-like	(A)10, (A)10, (T)17, (T)13, (T)11
56	LOC101498848	B3 domain-containing protein LOC_Os12g40080-like	(T)13, (T)10
57	LOC101507713	B3 domain-containing transcription factor VRN1-like	No SSR
58	LOC101508042	B3 domain-containing transcription factor VRN1-like	(T)10, (T)10, (TAT)19, (T)12
59	LOC101508553	Alpha,alpha-trehalose-phosphate synthase [UDP-forming] 6-like	(GA)17, (A)12
60	LOC101509076	Probable RNA-dependent RNA polymerase 1	(T)17
61	LOC101509613	Transcription factor bHLH121-like	No SSR
62	LOC101504917	Probable RNA-dependent RNA polymerase 1	(A)11
63	LOC101509943	CRAL-TRIO domain-containing protein YKL091C	(T)11, (T)10
64	LOC101510474	Two-component response regulator ARR2	(A)13
65	LOC101511657	Uncharacterized	No SSR
66	LOC101511125	Dynamin-related protein 1C-like	(TC)6, (T)10
67	LOC101511979	Microtubule-associated protein 70-2-like	(TC)8, (T)15, (T)10
68	LOC101512307	Cleavage and polyadenylation specificity factor subunit 3-II	(T)17, (A)10, (T)10, (TA)20
69	LOC101512637	40S ribosomal protein S2-4-like	No SSR
70	LOC101505220	E3 ubiquitin protein ligase RIE1-like	(A)10
71	LOC101512947	E3 ubiquitin protein ligase RIE1	(A)12, (A)10
72	LOC101513273	Probable UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase 2	(A)13
73	LOC101513602	tRNA:m(4)X modification enzyme TRM13 homolog	(TC)14
74	LOC101514138	Protein VAC14 homolog	(TC)6, (GT)11, (A)10
75	LOC101514465	Ankyrin repeat-containing protein At2g01680-like	(T)10
76	LOC101514789	Probable calcium-binding protein CML25	No SSR
77	LOC101515113	Uncharacterized	No SSR
78	LOC101515434	Uncharacterized	No SSR
79	LOC101515766	Nudix hydrolase 17, mitochondrial-like	(A)13
80	LOC101488524	Zinc finger protein VAR3, chloroplastic	(A)10, (T)11, (T)13, (T)11
81	LOC101488870	1-aminocyclopropane-1-carboxylate oxidase homolog 4-like	No SSR
82	LOC101489193	zinc finger HIT domain-containing protein 2	(T)12, (T)15, (T)14
83	LOC101489951	Probable peroxygenase 4	(T)12, (AT)12, (A)10, (A)10, (T)10, (TAA)7, (T)10, (A)10, (A)12, (T)10, (A)10
84	LOC101490284	Probable peroxygenase 5	(T)11
85	LOC101490602	Protein TIFY 6A	(T)13
86	LOC101490924	Lysine-rich arabiNogalactan protein 19	No SSR
87	LOC101491240	Mediator of RNA polymerase II transcription subunit 23	(TA)7, (TG)9, (A)12, (TC)10, (AT)8, (T)13, (T)10, (T)10
88	LOC101491566	Glucan endo-1,3-beta-glucosidase 3-like	(CT)7
89	LOC101491876	Dynein light chain 2, cytoplasmic	(T)11
90	LOC101506854	Uncharacterized	(T)10
91	LOC101492547	Exosome complex component RRP42-like	(T)10, (A)10
92	LOC101492210	Uncharacterized	No SSR
93	LOC101492873	Uncharacterized	(A)16, (T)11

94	LOC101493205	Uncharacterized	No SSR
95	LOC101493746	Cysteine-rich receptor-like protein kinase 10	(AT)9
96	LOC101494066	Uncharacterized	(T)11
97	LOC101495006	Uncharacterized	(TTC)6, (T)10
98	LOC101495336	Phosphoribosylamine--glycine ligase-like	(T)10
99	LOC101496649	Cytochrome c oxidase subunit 2	No SSR
100	LOC101495880	DNA-directed RNA polymerase III subunit RPC7	(AT)14
101	LOC101496964	Pentatricopeptide repeat-containing protein At1g22960, mitochondrial	(T)10
102	LOC101497295	Uncharacterized	(AG)6
103	LOC101497615	Probable ADP-ribosylation factor GTPase-activating protein AGD13	No SSR
104	LOC101497947	MLP-like protein 328	(TAT)16, (AT)6, (T)16
105	LOC101498273	Uncharacterized	(CT)8, (AT)10, (T)10
106	LOC101507188	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
107	LOC101498849	Glycerophosphodiester phosphodiesterase protein kinase domain-containing GDPDL2-like	(GTT)6
108	LOC101499887	Probable receptor-like protein kinase At5g39030	(TA)14
109	LOC101500438	Carbonyl reductase [NADPH] 1-like	(T)10
110	LOC101500961	Nudix hydrolase 25	(CTC)7, (T)18
111	LOC101501281	Uncharacterized	(TA)8, (A)11, (AT)11, (T)11, (A)20
112	LOC101502241	Pentatricopeptide repeat-containing protein At1g62350	(A)10
113	LOC101502756	Auxin efflux carrier component 4-like	(AT)7, (T)10, (T)10
114	LOC101503069	Uncharacterized	(ATT)10
115	LOC101503624	Leucine-rich repeat extensin-like protein 6	No SSR
116	LOC101507816	F-box/kelch-repeat protein At3g23880-like	No SSR
117	LOC101504266	MLP-like protein 43	(AT)13
118	LOC101503951	MLP-like protein 43	(AT)6
119	LOC101508135	Uncharacterized	No SSR
120	LOC105851988	Uncharacterized	No SSR
121	LOC101508454	Nicotinate-nucleotide pyrophosphorylase [carboxylating], chloroplastic	(ATT)10, (A)10, (T)10
122	LOC101508987	Uncharacterized	(T)10
123	LOC101512309	Cationic amiNo acid transporter 1-like	No SSR
124	LOC101509300	T-complex protein 1 subunit theta-like	No SSR
125	LOC101509614	Uncharacterized	(AT)8, (AT)8
126	LOC101509944	Heavy metal-associated isoprenylated plant protein 26-like	No SSR
127	LOC101512949	WAT1-related protein At4g08290-like	(T)11, (TA)6
128	LOC101510267	WAT1-related protein At1g44800	(T)10, (A)17, (AATT)6
129	LOC101510803	Bifunctional protein FolD 1, mitochondrial-like	
130	LOC101511126	Putative glutathione-specific gamma-glutamylcyclotransferase 2	(T)10
131	LOC101511435	Uncharacterized	(CT)7
132	LOC101511766	Uncharacterized	(A)11
133	LOC101512308	UDP-galactose transporter 1	(TTA)6
134	LOC101512638	Transcription factor IIIA	No SSR
135	LOC101512948	Long chain acyl-CoA synthetase 9, chloroplastic-like	(T)12, (AT)6, (T)10
136	LOC101514585	Uncharacterized	(T)11, (TA)12, (ATA)17, (TAAA)8, (A)10, (T)16, (T)12, (A)10, (T)10, (T)11
137	LOC101513697	Uncharacterized	No SSR
138	LOC101514032	Filament-like plant protein 3	(T)12, (T)25, (A)10
139	LOC101515232	Chlorophyllide a oxygenase, chloroplastic	No SSR

140	LOC101514909	Uncharacterized	No SSR
141	LOC101514360	BTB/POZ domain-containing protein At1g21780-like	No SSR
142	LOC101515558	Aldehyde dehydrogenase family 3 member H1	(A)11, (T)15, (T)10, (T)18, (T)13
143	LOC101515233	Uncharacterized	(T)13, (A)17
144	LOC101488525	Aspartic proteinase Asp1-like	No SSR
145	LOC101488871	Uncharacterized	No SSR
146	LOC101489194	Amino acid permease 3-like	(TA)9, (A)10, (A)11, (TA)7
147	LOC101515559	Probable polygalacturonase At3g15720	(T)11, (AAT)6, (ATT)33, (A)10, (T)10
148	LOC101489741	Probable methyltransferase PMT10	(T)16, (T)11
149	LOC101489741	Tetratricopeptide repeat protein 33	(T)16, (TA)6
150	LOC101488312	Uncharacterized	No SSR
151	LOC101490391	Probable receptor-like serine/threonine-protein kinase At5g57670	No SSR
152	LOC101490717	Peptidyl-prolyl cis-trans isomerase CYP59	No SSR
153	LOC101491241	Uncharacterized	(AT)6
154	LOC101490717	Cyclophilin, consisting of a peptidyl-prolyl cis-trans isomerase (PPIase) domain	No SSR
155	LOC101489302	DNA topoisomerase 2-binding protein 1-A-like	(TTTTG)6, (AT)6, (A)10
156	LOC101491241	Wound responsive family protein	(AT)6
157	LOC101491763	Floral homeotic gene encoding a MADS domain transcription factor	(A)11
158	LOC101492087	Putative TATA binding protein associated factor 21 kDa	No SSR
159	LOC101492431	Protein with NAD Putative protein kinase activity	(GAA)6, (T)10, (T)10
160	LOC101492982	Tetratricopeptide repeat (TPR)-like superfamily protein	(ATA)32, (ATA)7
161	LOC101493522	Putative protein kinase	No SSR
162	LOC101494165	ACT domain repeat 3 (ACR3)	(T)10, (A)17, (A)12
163	LOC101489952	Uncharacterized	No SSR
164	LOC101493838	Embryo defective	(A)10, (T)10, (AAT)7
165	LOC101496214	ras-related protein RABC1-like	(T)11, (A)12, (A)10, (A)10
166	LOC101495881	DNA repair protein XRCC3 homolog	(T)11, (A)12
167	LOC101495550	DNAJ heat shock N-terminal domain-containing protein	(TTTA)10
168	LOC101490285	Uncharacterized loci	No SSR
169	LOC101496539	SUR7SUR7/PalI family	No SSR
170	LOC101495232	AtSKP2 (F-box protein SKP2B-like)	(T)10
171	LOC101496871	Scarecrow-like protein (SCL1)	(T)11, (A)10
172	LOC101497744	Transcriptional co-regulator of AGAMOUS	(TA)8, (AT)11, (AT)6
173	LOC101497200	Polynucleotidyl transferase ribonuclease 21H-like superfamily protein	(T)13, (A)10
174	LOC101498610	Exostosin family protein	(T)14, (A)14, (T)10
175	LOC101498274	Nodulin MtN3 family protein/bidirectional sugar transporter SWEET1-like	(T)10
176	LOC101490603	F-box/LRR-repeat protein At3g48880-like	(A)14
177	LOC101490925	F-box/LRR-repeat protein At3g4880-like	No SSR
178	LOC101491242	Tracheary element differentiation related 7 (TED7)/ proline-rich receptor-like protein kinase PERK10-like	No SSR
179	LOC101498940	Probable auxin efflux carrier component 6-like	No SSR
180	LOC101499585	Plant glycogenin like starch initiation protein 2 (PGSIP2)	No SSR
181	LOC101499266	Plant stearoyl-acyl-carrier-protein desaturasr family protein	(T)15, (T)17

182	LOC101499888	Nucleolar essential protein-related /ribosomal RNA small subunit methyltransferase NEP1-like	No SSR
183	LOC101491567	LEC1-Like (L1L)/nuclear transcription factor Y subunit B-3-like	(ATT)9, (T)11
184	LOC101500439	Uncharacterized	(T)12
185	LOC101500751	LEC1-Like (L1L)	(TA)10, (AT)11
186	LOC101491877	Cysteine-rich receptor-like protein; Putative protein kinase.	(T)11, (T)10, (T)10
187	LOC101501377	Leucine-rich repeat protein kinasefamily protein	No SSR
188	LOC101501063	Glycolipid transfer protein2 (GLTP2)/ pleckstrin homology domain-containing family A member 8-like	(A)11, (AT)13, (A)11, (T)15
189	LOC101492211	*Pht1* (inorganic phosphate transporter 1-11-like)	No SSR
190	LOC101502348	Ethylene-responsive transcription factor CRF3-like	(T)10
191	LOC101502018	Integral outer envelope membraneprotein (homolog of PDV2)	(A)14
192	LOC101501693	Arabinogalactan peptide 16-like	(GA)10
193	LOC101503731	Dihydrofolate synthetase	No SSR
194	LOC101502656	Ubiquitin-conjugating enzyme E2 10-like	(T)13, (TAT)6
195	LOC101503200	SUMO-conjugating enzyme UBC9-like	(T)10, (T)10
196	LOC101492548	Transcription factor SPEECHLESS-like	(A)10
197	LOC101504808	Arabidopsis homolog of polypyrimidine tract-binding (PTB) protein	No SSR
198	LOC101504169	Nodulin MtN3 family protein	(A)10, (T)11
199	LOC101505779	DNAJ heat shock N-terminal domain-containing protein	(T)10
200	LOC101492874	Protein spermine synthase like	(T)16, (T)12, (T)10
201	LOC101505121	RCAR3 a regulatory component of ABA receptor	No SSR
202	LOC101506091	Cyclic nucleotide gated channel family	No SSR
203	LOC101505444	Chaperone DnaJ-domain superfamily protein	No SSR
204	LOC101504494	FtsH proteas e/ATP-dependent zinc metalloprotease FTSH 11 chloroplastic/mitochondrial-like	No SSR
205	LOC101506419	Putative protein with 6-phosphoglucunolactonase activity	No SSR
206	LOC101507081	Polycomb group protein - fertilization independent endosperm-like	No SSR
207	LOC101506745	Putative protein with 6-phosphoglucunolactonase activity	No SSR
208	LOC101507599	Highly ABA-induced *PP2C* gene 2 (HAI2)	No SSR
209	LOC101493206	Protein kinase superfamily protein	(A)11
210	LOC101493523	Putative protein kinase	(T)13
211	LOC101493839	Uncharacterized	No SSR
212	LOC101507916	Putative PR5-like receptor Putative protein kinase	No SSR
213	LOC101508231	One of the closely related 12-oxophytodienoic acid reductases	No SSR
214	LOC101508554	Putative protein kinase	(A)16, (T)11, (A)11, (T)16
215	LOC101509729	Putative PR5-like receptor Putative protein kinase	No SSR
216	LOC101509077	Cysteine-rich receptor-like protein; Putative protein kinase.	(T)69
217	LOC101509405	Probable receptor-like protein kinase At1g67000-like (SNC4-suppressor of npr1-1 constitutive 4)	No SSR
218	LOC101494475	RNA polymerase-associated protein LEO1-like	No SSR
219	LOC101494796	Cell wall-associated Putative protein kinase	(A)12, (T)10, (A)10
220	LOC101510048	Probable receptor-like protein kinase At1g67000-like	(AT)11, (T)10, (AT)27, (A)12
221	LOC101510924	Receptor serine/threonine Putative protein kinase	(T)10, (T)10, (T)11, (CA)6, (TAA)9, (TA)22

222	LOC101511436	One of the closely related 12-oxophytodienoic acid reductases	(T)12
223	LOC101510703	Uncharacterised	(T)11, (T)12, (T)12, (A)11
224	LOC101495125	Probable 2-oxoglutarate/Fe (II)-dependent dioxygenase-like	(AT)9, (T)11, (A)13
225	LOC101510377	Receptor serine/threonine putative protein kinase	No SSR
226	LOC101496103	Aminotransferase-like plant mobile domain family protein	No SSR
227	LOC101495452	Receptor serine/threonine Putative protein kinase	No SSR
228	LOC101495781	Putative RING-H2 finger protein ATL21B-like	No SSR
229	LOC101509945	One of the closely related 12-oxophytodienoic acid reductases (G-type lectin S-receptor-like serine/threonine-protein kinase B120-like)	(T)10, (T)15, (A)11, (T)10, (A)10, (AAT)20
230	LOC101511127	One of the closely related 12-oxophytodienoic acid reductases	No SSR
231	LOC101493426	Uncharacterised	(T)10, (T)12
232	LOC101510804	Receptor serine/threonine Putative protein kinase	No SSR
233	LOC101492764	Uncharacterized	No SSR
234	LOC101510475	One of the closely related 12-oxophytodienoic acid reductases	No SSR
235	LOC101493098	Pentatricopeptide repeat (PPR) superfamily protein	No SSR
236	LOC101509615	NADH-dependent glutamate synthase	(T)10, (TAAAT)6
237	LOC101508758	Sulfite exporter TauE/ SafE family protein	(T)13
238	LOC101509301	Got1/Sft2-like vescicle transport protein family	(AAT)6
239	LOC101508455	La1 a highly abundant phosphoprotein La proteins	(T)11
240	LOC101507917	putative c-myb-like transcription factor	(TA)8
241	LOC101507600	Probable sodium/metabolite co-transporter BASS4 chloroplastic-like	(TC)8, (T)10, (AT)6, (T)12
242	LOC101506964	ENTH/VHS/GAT family protein	(T)12, (TG)6
243	LOC101505780	D-arabinono-14-lactone oxidase family protein	No SSR
244	LOC101507293	D-aminoacid aminotransferase-like PLP-dependent enzymes superfamily protein	(TTA)6, (A)12
245	LOC101506310	Dentin sialophosphoprotein-like	No SSR
246	LOC101506632	Subunit K of photosystem I reaction center.	No SSR
247	LOC101504059	Exostosin family protein (probable 94glycosyltransferase At5g03795-like).	(TA)16, (A)10, (TA)7, (T)15
248	LOC101503732	Expressed protein	(A)10, (T)11
249	LOC101505122	Tonoplast intrinsic protein4 (aquaporin TIP4-1-like).	No SSR
250	LOC101505445	Casein kinase1 like3 (ckl3)	No SSR
251	LOC101503398	The DREB subfamily A-4of ERF/AP2 transcription factor family	No SSR
252	LOC101494067	Uncharacterised	No SSR
253	LOC101504809	Tonoplast intrinsic protein 4	(T)15, (T)10, (A)10, (A)10

APPENDIX-XII: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by ESTSSR21-TS72 on chromosome 4

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101508668	(CA)6	381	CTTTTCATCCTTGAGGTTCAGC	22	TGATTGTTCCAGACAAGATTCG	22
LOC101508668	(CA)6	378	CTATGACCCTTTTCACCGTAGG	22	CCACCAAAGTAGCCCTAACAAA	22
LOC101508668	(T)11	353	TTCCTCCTAACTGGTGGCTACT	22	GAGGTCGTCTCTATTGTATGAAACC	25
LOC101509518	(T)13	371	CCAATCTTCACCTTCTTCATCC	22	GTGTGCTATTTCCCTCAAGACC	22
LOC101509518	(T)10	284	GGTCTTGAGGGAAATAGCACAC	22	GGAGGCATGTTTTGGACAATAG	22
LOC101501910	(A)11	357	CCAATCAAGGCATAAAGCATAC	22	CATGAAGCAATCCCAAAAGTAG	22
LOC101513696	(AG)10	103	TTGGAGTGAGTGATGAGAAAGG	22	GCTCGTGAATGTGAATGTGAAT	22
LOC101513696	(AG)6	103	TTGGAGTGAGTGATGAGAAAGG	22	GCTCGTGAATGTGAATGTGAAT	22
LOC101513696	(A)10	400	ATTCACATTCACATTCACGAGC	22	AGACCCAATATCACGAACAAGC	22
LOC101513696	(T)16	247	ATGAAACACGGATACTGACACG	22	CAACTTCAACATCGACCAACAT	22
LOC101513696	(A)11	311	TCTCCTCGAATATCAAGCGAAT	22	ACAAGACCACACATCTGCAATC	22
LOC101514031	(T)10	251	GAGAGCTTGATTTGTTCGCTTT	22	GTGTTTGGGTCAGTGCTTCATA	22
LOC101514031	(T)10	398	CTCCACGACAAAGGGTAAAATC	22	GCAAACCATAGAGTCCTCAACA	22
LOC101514031	(GT)8	299	AACCCTAACCCTGATCCTCATT	22	GGCTCTGTCTTACTCCTGCTGT	22
LOC101514584	(T)10	361	TTGGGAAGATGGAAAATAGTGC	22	AATACAGATGAAATTGCCTGCC	22
LOC101514584	(T)10	374	GTCTTGCCTCGGTTTCATGT	20	TAAGTTGTTTCCAAAAGCTCCC	22
LOC101504807	(T)10	394	CTGATGGTCAATGGATTTTGG	21	TCTCCTTCATCGTGTTTCTTGA	22
LOC101515231	(T)11	348	GTTTTCTCCCCTCTCCATTCTC	22	CGACTATCGGCTAAATCAGTAACA	24
LOC101488311	(TC)6	274	CTCTGGTCTCGATTACGAAAGAA	23	TATGCTCCCATCACCTGTAGTG	22
LOC101488311	(T)18	267	GTCTGTGCAGTCATTTGGGTT	21	ACCTTAGCCAGCCTCTTTTCTT	22
LOC101490167	(A)10	354	ATGAAAAGCCAACCGTCATC	20	TGTTGCTGAACTAGAAAATGCC	22
LOC101490167	(TA)7	354	ATGAAAAGCCAACCGTCATC	20	TGTTGCTGAACTAGAAAATGCC	22
LOC101490490	(T)10	375	TGACCGTATTTGTGCTGGTAAA	22	GCGAAAAGAACTCGCAAAGAT	21
LOC101492763	(T)10	351	ACTCCCTGAAGCCCTTAAAACT	22	GGTTCCTATGGAGAAACATGGA	22
LOC101493953	(T)10	289	ATACCAACCTGAACTCCCTCCT	22	GGTGTCCATAAGTATTGGGGAA	22
LOC101493953	(T)10	287	GGGCTACTGCTAAAGTTCCTGA	22	CCAAGAAATCCAAGTAAAACGC	22
LOC105851070	(CAC)7	394	CGTTCACATACCAAACGACAAT	22	TTTCCCTTCATCAAACTCCAAC	22
LOC105851070	(T)12	395	ATCAGGTTGCTGTTGCGG	18	AGCTGGACGAGAATTACATAACTAGC	26
LOC105851070	(T)10	364	GCTAGTTATGTAATTCTCGTCCAGC	25	GGAACTTGTGACCTTTCCTCAA	22
LOC101495335	(TAT)8	344	CTGCTCTTATGGCTCATCCTTT	22	TTTGTGAACACCCCTACTTCTG	22
LOC101495662	(T)11	368	CTCTCCTGCCTACTCCTTTTCA	22	CAACAACGCATAATTTCAGCTC	22
LOC101495662	(T)11	368	CTCTCCTGCCTACTCCTTTTCA	22	CAACAACGCATAATTTCAGCTC	22

LOC101495662	(A)10	371	GTTGTTGAGTGTGAGGCGTTT	21	CAAAGAGGGGAGAAGGACATAA	22
LOC101495986	(A)12	204	CTCGATATTTTCCCCTTTCACA	22	CTCACTACACCTTCCCTTCCAC	22
LOC101496321	(TTTA)7	370	GGTTCTCACTTCATCGCTTCTT	22	TGAAAATTGGAAACTCACCTCC	22
LOC101496321	(A)10	400	GATGAATTGTTTTGGAGGTCAG	22	GAACCCTCAATCTCCTTATCACTC	24
LOC101496648	(T)10	376	CTTCTTGGTGTTTTCTGGGAAT	22	GCTGTGGAAGATTGATTTTGTG	22
LOC101496648	(A)15	376	CTTCTTGGTGTTTTCTGGGAAT	22	GCTGTGGAAGATTGATTTTGTG	22
LOC105851984	(A)10	364	GTGGAGCATATTGGTTTTAGGAG	23	ATGAGGGGCAGGAACATAAATA	22
LOC101497743	(T)71	260	TGAGTGATTTGGTTGGCATT	20	AGGTTTGATCCTCCCCATTTAT	22
LOC101497743	(T)30	260	TGAGTGATTTGGTTGGCATT	20	AGGTTTGATCCTCCCCATTTAT	22
LOC101507403	(T)11	228	GCAACCAGCAAACTAAGCATTC	22	AAAGTGGAGAAGCCAAAACTCA	22
LOC101498848	(T)13	372	ATCATTGTTTTGGAACCGTG	20	TGTGTGCCATTTTAGTGTAACC	22
LOC101508042	(T)10	399	TTGCATAACGGAGAGCTTGTAA	22	AACCTTGAAATAGGGTCCTCCT	22
LOC101508553	(GA)17	217	TTTGCTCAGTGGAGAGAGAGTG	22	CCAGTTTTGGTTGAGACTTGAG	22
LOC101508553	(A)12	240	TGGAACTCAAGTCTCAACCAAA	22	CTATGATGAACAACAAGGGCAA	22
LOC101509076	(T)17	339	CGCTTTCTCAAGGTCTGTCACT	22	TCAGCAAAAGAAGCACATGACT	22
LOC101504917	(A)11	372	CGAAAACTGGAGGGTTAAGAGA	22	CAGCAGAAACATTTGATGGG	20
LOC101509943	(T)11	398	GATGAGGTGGTATTTATCCCCA	22	ATGAACCCATTAGGCACCATAG	22
LOC101509943	(T)10	331	GGATATGGTTATCGGCAGTGAG	22	TACATAAGATTCAAAAGCCCCG	22
LOC101510474	(A)13	384	GTGTTTTGGGAGTTTTCGTTGT	22	GTACAATTCTGTGGTGTAGGATCAA	25
LOC101511125	(TC)6	309	TGATTGAGTCCTCTGATTGTGC	22	ACGGCATTAGTTCCTTTATCCA	22
LOC101511125	(T)10	349	CGGGCAGTTAGAGAGAGAGAGA	22	CCACGGCCTTTGGTATAGAAT	21
LOC101511979	(TC)8	220	AGCAAAACCTCTTCACAACACA	22	GCACAGAGAGAACAAAGCAGAG	22
LOC101511979	(T)15	352	TGAAGGTGGAGCTTAATCGTCT	22	TTACTCCCAAACCATGACCAAT	22
LOC101511979	(T)10	400	GTTACACCCTCGTTTTGCTTTT	22	TGGCTCAAAGTAGACCCTTGAT	22
LOC101512307	(T)17	395	TGGTGAACATCAATGGAAAGAG	22	AAGTAAACCAACGCTCCAACAT	22
LOC101512307	(A)10	353	GGCACCTAAGTTTTGTCCATGT	22	AAGGGTGTGTGCATCTATTTCA	22
LOC101512307	(T)10	350	CAATACTGTGTGCATGAGGATG	22	AATCTGTGTGTCGGGATCTGTA	22
LOC101512307	(TA)20	248	TCTATTCTGGGCCTTACTCTGAA	23	ATAACATGCTTTGGGGAGAGAA	22
LOC101512947	(A)12	197	CTACATTGCATAAGTCCATTGC	22	CCGCAACATAAAACTAGAGTCA	22
LOC101512947	(A)10	340	GGTGATATGGGTGGAATGAAGT	22	ACGGTCCAGGTGTAAGAGTTGT	22
LOC101513273	(A)13	321	GCTGATGATGTTGAGGTCAGAA	22	AACCAAACACAAAGTAGCAGCA	22
LOC101513602	(TC)14	259	ATCATATCTCAAGGTCCGAAGG	22	TAGGTAGCACCAACACTTCACG	22
LOC101514138	(TC)6	373	ATCTTCGATTCTCCCAACTCAA	22	CCCTCAACCTATTTCCACAAAA	22
LOC101514138	(GT)11	240	GCTGCTACTGTTGGGTTGACTA	22	TATCTTTCCTTTGGGTGATGCT	22
LOC101514138	(A)10	272	GAAGCATTTGATGTAGGAGCAA	22	GATTCGAGTAGCCTCCCATTC	21
LOC101514465	(T)10	372	CTCATTTTGTGGGGTTGGTTAT	22	TTGCATTCATGGCAGAGTTATC	22
LOC101488524	(A)10	367	GCAACAAGTAATGGCTGTTCAT	22	TTGAAATTAAGGCCCGTTTG	20

LOC101488524	(T)11	367	GCAACAAGTAATGGCTGTTCAT	22	TTGAAATTAAGGCCCGTTTG	20
LOC101488524	(T)13	367	GCAACAAGTAATGGCTGTTCAT	22	TTGAAATTAAGGCCCGTTTG	20
LOC101488524	(T)11	367	GCAACAAGTAATGGCTGTTCAT	22	TTGAAATTAAGGCCCGTTTG	20
LOC101489193	(T)12	390	GGTTTCTGTTTCAGCCTCAGC	21	CATGTTTGGGTGATGTCTATTCG	23
LOC101489193	(T)15	244	ACTCCATCGCTGAATGTGTATG	22	TCATAGGCCACCTCACAAAGTA	22
LOC101489193	(T)14	383	GCATTCTTAGCTTTATCGCTGG	22	CCCAAATCAAACATAACCACCT	22
LOC101489951	(AT)12	145	CACTACCAAACCCTTAAACCAA	22	GAACTCGGAAGCTCACAATTA	21
LOC101489951	(T)10	393	CTTTTCTGTTGCCGTCACAAT	21	CAAAACGAATGTATGGCCG	19
LOC101489951	(TAA)7	393	CTTTTCTGTTGCCGTCACAAT	21	CAAAACGAATGTATGGCCG	19
LOC101489951	(T)10	399	TGAGAGAGTTTAAGCCACCACA	22	GACACTACCCTGCGTCAAATTA	22
LOC101489951	(A)10	281	TCGCAATCAACTGAATAACACC	22	ATATACCTCGCAAATGCCCTC	21
LOC101490602	(T)13	400	ATGAAGGGTGCAAAGATTCAG	21	CTATGTCTCTGTGTGGATTGGG	22
LOC101491240	(TA)7	209	AAGTTACCCTTTTCGTCTTTGG	22	TCCGCTCATATTAAAACACCTC	22
LOC101491240	(TG)9	329	TACAGGCTTTTATCTCAGGGGA	22	GAGAGGAGCAGAGAGCCATTAG	22
LOC101491240	(A)12	271	ATTTTGTCGTACCAGTTTTGGG	22	CAACGATGCAAAGTAGCTGTTC	22
LOC101491240	(TC)10	292	AGTAGGGATGTTTTGGGTTGTG	22	ATCATGGTGGTAAGTGAGCCTT	22
LOC101491240	(AT)8	264	GAATCCCTTTTATGTTTTCCCC	22	CCAAACCCACAATTTCCTACAT	22
LOC101491240	(T)13	235	ACACAGGAGTATTCAAACGGGT	22	TATGGTTTCAGCCACAAGAAGA	22
LOC101491240	(T)10	297	TCTATATTTGGCTCAGTGGTCG	22	TTAATGGGATGTCTTGTCCCTT	22
LOC101491240	(T)10	283	TAGGAACGAGAGAAATGAAGCC	22	AAACGGAGAATATAGGGCCAGT	22
LOC101491566	(CT)7	198	GCTTCTCCTCTTTCATTTCACA	22	CATCACAGCCATTGAAGTTTCT	22
LOC101491876	(T)11	267	TAAACACTTGGCACTTTCCCTT	22	CACATGGATTTGGGTTATCCTT	22
LOC101506854	(T)10	209	TACTTCTTGGACGGCTGAAAAT	22	TTCTGTCATTGGCTCATCAAAC	22
LOC101492547	(A)10	231	TCGGCCAGAGTTAGAATAGGTG	22	AGACTTTCCCTTTGTCAGGTTG	22
LOC101492547	(T)10	334	GCTTGTTGTCAGTTCAGATGGA	22	GGTAACTTCAAACCATTTCCCA	22
LOC101492873	(A)16	379	ACCTAGTTGATTTGAGCCAACA	22	AGGAGTAGGGGTGTAAATAGCG	22
LOC101492873	(T)11	214	GTGCATATCTCAACAAGAGTATTCC	25	GCAGGTTTGTAGTCCTGAATGT	22
LOC101493746	(AT)9	283	GATATGATGGTTTGATTGACGC	22	TCGCATGACCAGTTACAAAGTC	22
LOC101494066	(T)11	378	TCTCCCGTCAGTGTCAGTTTTA	22	ATCTATCACCATCACACCATCG	22
LOC101495006	(TTC)6	302	TTATGTGAAGGTGGAAGACACG	22	AAAGAAGAGGAAGCAGAGCGTA	22
LOC101495006	(T)10	339	TGTCAAACCTCATCTCATCCAA	22	ATTCAAGGCTGGGTATTTTCCT	22
LOC101495336	(T)10	347	TGAGGAGTGGTGTGTATTGCTT	22	TCCGACAAAATGAAACTACTGC	22
LOC101495880	(AT)14	390	GTTGGAGGAGCCTAAACTCAAT	22	AAGAACTATCCGTGTTTGACGA	22
LOC101496964	(T)10	296	GTGCTCACGACACGAGTAAGAC	22	GGACAACTACAGGTAACAGCCC	22
LOC101497295	(AG)6	367	CACGAACAACAACAAGAGAAGG	22	GCCCTTTAACCACTAGACCTCA	22
LOC101498273	(CT)8	256	CGTGGAGTGAAAGAGGAAGATT	22	CAGTTACAGCACCTTGCATCAC	22
LOC101498273	(AT)10	183	CAAATACCGAAGCAAATCAGC	21	CCACACCCGTCTACACAACATA	22

LOC101498273	(T)10	327	TTGCTCCGGTAAAGCATACATA	22	ATTAGTGGTTGTGGGGACAAAT	22
LOC101498849	(GTT)6	225	ACAGAGTCAAAGACAGAGTACCAAA	25	ATCAAAATCAAAGGCTGGGTAG	22
LOC101500438	(T)10	365	ACTCCCTGTAAGTAAGTGGGCA	22	CTCCGTTCTTTAGCCTTCTCCT	22
LOC101500961	(CTC)7	399	TTCACAATACAGCGTACCATCC	22	CCAGGTTTAGTAGAGCCGAAAA	22
LOC101500961	(T)18	399	TTCACAATACAGCGTACCATCC	22	CCAGGTTTAGTAGAGCCGAAAA	22
LOC101501281	(TA)8	390	AAAGTTGGACACGATTTTACCC	22	TCTATCTCAATACGTTGCTGCC	22
LOC101501281	(A)11	219	CCTTATCTTGGAAACTGGATGG	22	GCAGGAGCAGATGACAGTAAAG	22
LOC101501281	(AT)11	329	ACCTTCATGGGGTATTATGTGC	22	AGAGCCTATTTCGATCCTCCTT	22
LOC101501281	(T)11	302	TTACCCTTTCTTTGTCGCTTGT	22	AGAAGGTGAGATGCAAACAGGT	22
LOC101501281	(A)20	302	TTACCCTTTCTTTGTCGCTTGT	22	AGAAGGTGAGATGCAAACAGGT	22
LOC101502756	(AT)7	399	TTGGAATGGCTATGTTCAGCTT	22	CATGGGATTGCATTTTACTCCT	22
LOC101502756	(T)10	185	AGCCGTCCATTCTAGTGTTGTT	22	GTTGTCCATTGTGTCTGGGAG	21
LOC101503069	(ATT)10	318	CTGAAAACAGTGACAAAGTCCG	22	TCCCAAACTGGAATGCTGTAA	21
LOC101504266	(AT)13	327	ATTAAGTGGGAAAGTGGAGGGT	22	GCAAGGACACACCAACAAATTA	22
LOC101508454	(ATT)10	352	AAGGGCAATCAAAACTCAGGTA	22	ACTCTTCTCGTTGTTACGCCTC	22
LOC101508454	(A)10	352	AAGGGCAATCAAAACTCAGGTA	22	ACTCTTCTCGTTGTTACGCCTC	22
LOC101508454	(T)10	134	CCTTTTCTCATTAGGCGATGTC	22	CCAGATGGAGGGTAGTCCAATA	22
LOC101508987	(T)10	370	TTGACCATAGAGAGGGAGAGGA	22	TATATTTCTGTTGGACCTGCCG	22
LOC101509614	(AT)8	362	CATATTCGCAAGTGATGTGGAT	22	ATTGCTTACCGCTTCAAAACC	21
LOC101509614	(AT)8	362	CATATTCGCAAGTGATGTGGAT	22	ATTGCTTACCGCTTCAAAACC	21
LOC101512949	(T)11	352	GTTTCTCACTTCCCTCAACTCA	22	ATGTAGGTCGATCATGCACA	20
LOC101510267	(AATT)6	330	TCACATTGAAGATGTACCCAGC	22	GAAGTAACAAAGACAGGGCCAC	22
LOC101511126	(T)10	335	TATCTGGAAGTGAGGGAGAAGC	22	ACTTTCTTATCTGGGGATGCAA	22
LOC101511435	(CT)7	261	ATCTACTTGGGGACTTGCTTTG	22	AAACCCGTCCATACAAAACCTA	22
LOC101511766	(A)11	386	CTCCTAAAGTGCGGAAAGTGTT	22	CATTGAGCTGTTGAGACATGAA	22
LOC101512308	(TTA)6	376	ATGGTGGGCTTTCAATGTTACT	22	ATCATTCGCCAGACTTTCCTAA	22
LOC101512948	(T)12	301	AACGAGCACTGACAAAGCATAA	22	CTGAAACAACCCTGAAGGAAAA	22
LOC101512948	(AT)6	318	TGTTTAGAAAGGTCCGAGCAAT	22	ATCAGTGTTTATGGTTGGAGAGG	23
LOC101512948	(T)10	345	GTGGGACATTCTCTGATTTTGA	22	GCATTCCTTACATTTCACTGCT	22
LOC101514585	(T)11	148	TTGGATCGTTATAGGCCAAATC	22	CCTGTGAACCCAATACATTTCA	22
LOC101514585	(TA)12	389	GGCAACGACAAATCGACA	18	TGTCATAAACTAGAATGAGTCCCTG	25
LOC101514585	(ATA)17	254	TACCGTTTGAGTTGCACTTTTG	22	TTTTGGTTTGACTGGGTATCG	21
LOC101514585	(TAAA)8	243	AGGTCAACAAAGCAACAACAAC	22	GAAGGAAGCTCAAAGGAACCTA	22
LOC101514585	(T)12	395	CAACAGCAGGATCAACAATCA	21	TGAAAGTGTTTACCATATCGGG	22
LOC101514585	(A)10	351	GATCCTCCCATTCCCCTAAATA	22	GTGATCCGCATGATTTGTTGT	21
LOC101514032	(T)12	147	GCTGGTCAAAGGTATTTCATCC	22	GGTTACATCCAATTTCAGCCAT	22
LOC101514032	(T)25	385	TCCTCAAGCCATTCTTCATAGC	22	TATTGTTTTCTGACACTCGGCA	22

LOC101514360	(A)10	112	ATTCGTTCGTAACAGGGATGAG	22	GGATTAGTGCCATACGGTTTTC	22
LOC101515558	(T)15	291	TGTCATCCACAGATGTTGTACG	22	CTTTTCTTTGCCTCTTGTTTGG	22
LOC101515558	(T)18	397	CTAACTTTTGGAGTTGCCCAAT	22	TCAAGTGTGTCCGAATGATGA	21
LOC101515558	(T)13	376	TGGTCGTAGGTGGCTTATCTTT	22	ACATCTCAAGTGTGTCCGAATG	22
LOC101515233	(T)13	279	GGGAGGCAAACCGGAAAT	18	TATTTGATTGTGTAGGAGGCCG	22
LOC101515233	(A)17	321	TCTCTTGCCCATGATATGACAG	22	TAAACCAGAGGATGCGGGT	19
LOC101489194	(TA)9	183	CAAGAAAGGCACAAAAGCTACA	22	TGGCTTGGCTGTTTTCAGTA	20
LOC101489194	(A)10	287	GCTTACACACTTCATAACAACCCA	24	AGGATAAGGTTCGGCTTTTCTT	22
LOC101489194	(A)11	317	TACCAAAGTGGAGCACAAAATG	22	TGGAGATTGAGAATACAGAGCAAG	24
LOC101515559	(A)10	395	CGATGGCTTTGACATTTCTGAT	22	GACGGCAATACAATCATCACCT	22
LOC101515559	(T)10	331	TAAGACAGTTCCGGTAAGCACA	22	TGTGTACCTCATTATCAACACCG	23
LOC101489741	(T)16	272	GCAGCCGAAGAAAAGAACAAT	21	GGTGAAGAGAGGAATGGATTTG	22
LOC101489741	(T)11	275	GATAGTTATGTGCGGGCTTACC	22	CGATCATATAGAACAGGCAACG	22
LOC101489741	(T)18	357	ATAGAAGAGAAATTGCGGTGGA	22	TATAGGGATTTAAGGGCCAACC	22
LOC101489741	(TA)6	346	TGTGTGGTGAAGAACGGTAATC	22	CCCTCCTACAAGAAAGACGATG	22
LOC101491241	(AT)6	393	GCTGCTGGGTTACATAAAATGA	22	CGAAAGTAGCGGAGAGTGGTAT	22
LOC101489302	(TTTTG)6	378	GTCTCACTTTTGTGCCTTCTAACA	24	GTTGCTAAGAGTATCCAAACCCA	23
LOC101489302	(AT)6	328	AGCTGGCAAATAGAAGTTGGAA	22	TCTGGAGCGTAAGAACAGTCAA	22
LOC101489302	(A)10	270	CGCTGTCATTAGTTTGGTTTGA	22	AATCAACTTTAGCACACCCGTT	22
LOC101491241	(AT)6	393	GCTGCTGGGTTACATAAAATGA	22	CGAAAGTAGCGGAGAGTGGTAT	22
LOC101491763	(A)11	372	TGCGTTTGTCTGTTGTTGTAGA	22	GGAGGTCTCACATTTGTACTTGG	23
LOC101492431	(GAA)6	328	CCCCAACTCTTTTCTTTTCCTT	22	TGTGCGGCGATAACTAACTTC	21
LOC101492431	(T)10	299	TGAAGTTAGTTATCGCCGCAC	21	CTTTTCTATCCACACAAACCAGC	23
LOC101492431	(T)10	317	TGGTCAAATGGATGGTAGTTCA	22	ATCCGAAAGTGTAGCGAATGTT	22
LOC101494165	(A)17	399	ACTTCAGCAACAGAATCAACCC	22	CCCATCATATACTCCAAGTCCAA	23
LOC101493838	(T)10	230	GGTGTCGAGAGTAATAGTGGGG	22	CTCCTAATCACCATGCCAAAAT	22
LOC101493838	(AAT)7	146	GAACACATACATTTGACCGTGTG	23	AAGGCAACCCTTACTTTTGTGA	22
LOC101496214	(T)11	296	GTGCGTGCGTTTATTGTAATTC	22	ACGCTACACTTCTCGGTTCTTC	22
LOC101496214	(A)10	338	GAGGGAAATACTTTGAAGGGCT	22	GGATAGGGGTAGCAAAACCAA	21
LOC101496214	(A)10	338	GAGGGAAATACTTTGAAGGGCT	22	GGATAGGGGTAGCAAAACCAA	21
LOC101495881	(T)11	296	GTGCGTGCGTTTATTGTAATTC	22	ACGCTACACTTCTCGGTTCTTC	22
LOC101495550	(TTTA)10	195	GAAACCTTAACGATCCTCATGC	22	ACCTGATATGCTTCGCCTAAAA	22
LOC101495232	(T)10	269	GACTTTGAGGGTGTAGTGATCCA	23	CGTCTATGTGTCGGTGTCGTAT	22
LOC101497744	(AT)11	157	CGATCCCAAATAGCAGTGTGTA	22	TTCTTTATCCCACATTCCCAAC	22

LOC101497744	(AT)6	283	GGATGTAGACTGGTTCTTTGGC	22	TGCATGTGCTTAATGGATAAGG	22
LOC101497200	(A)10	373	TGCAAGCGTTGTCTGAAAGT	20	CAGAGTGGGACAGAGGGAGTAT	22
LOC101498610	(T)14	137	TGTTTTCCCGAGGTAATAATGC	22	CCAAGCCAACCAATGTCTAAAT	22
LOC101498610	(A)14	261	CAGAACAGAAGCAACAATCCAG	22	TTGGTATTCGACATCAGCATTC	22
LOC101498610	(T)10	312	AAGGAAAAGCTGGTCGTCTTC	21	ACCGTTTTGGTTTGACTGAACT	22
LOC101498274	(T)10	372	CAACTGTCTTCTTTCTGCTTGG	22	TGGTGTAGTTTCAAGATTTGGC	22
LOC101490603	(A)14	251	ACTCTGTCACCCTCGAATGTTT	22	TCCAGAAAATGGCAGGACTAAT	22
LOC101500439	(T)12	253	AGTTATTGGGCTGTTGCTGATT	22	GCCTTAATTTCTTCCTTAGTGGC	23
LOC101499266	(T)15	375	TTTGTATCTTTCTGGTCGGGTT	22	TCCAGTACAGTCAACACTAATTCACTC	27
LOC101499266	(T)17	260	TGGAGATTAGAGAAGCTGGAGG	22	CAAAGTTCCTTGGTGTGTCAAG	22
LOC101500751	(TA)10	369	CAAGCTCTGGTAAGCATAAGCA	22	CTTTACTTCTACACTCAACGGATCA	25
LOC101491877	(T)10	388	CTTAGGCAACGAGGACGAGT	20	GTTGTTCAAATGAGAAGGAGAGTG	24
LOC101501063	(AT)13	224	GGGTAACTCAAACAAAAGATACGAG	25	AATGATGAGGTGAAATCCAAGG	22
LOC101501063	(A)11	263	CCTTAGTTTTGGTCCTCTAAACAGA	25	GAAGGAGATGGAGATGGAGATG	22
LOC101501693	(GA)10	176	AGCTCCCTGGTATTCTCTCTCC	22	GCCACTGACATAAGAGTTGCAT	22
LOC101502018	(A)14	289	TTGATGAAAAGGACAGTGAGGA	22	TATTTGGCAGAGTGAGAGACCA	22
LOC101502656	(T)13	365	CTTGATTAGGTTTACGCGATGG	22	TCAAACAACAACCACTGCAACT	22
LOC101502656	(TAT)6	365	CTTGATTAGGTTTACGCGATGG	22	TCAAACAACAACCACTGCAACT	22
LOC101507081	(TA)9	260	TCAGGAAAGAGGTTGGAACTTG	22	ATCAAACTGGGAATGCACTTG	21
LOC101505779	(T)10	396	TTCACTCCTTCATTACTTCGCA	22	ACAGCAAGACCACGGTAAAAGT	22
LOC101504169	(A)10	324	CTTCGTATGGCTGTTGCTGTT	21	ACTGCCCAAGTAAAATCAAGGA	22
LOC101492874	(T)16	344	TCTCTCTGGTCATCATCTTGCT	22	AGTTCACCATCCTTAGCCAAAC	22
LOC101492874	(T)12	344	TCTCTCTGGTCATCATCTTGCT	22	AGTTCACCATCCTTAGCCAAAC	22
LOC101492874	(T)10	361	AATTGTTTGGCTAAGGATGGTG	22	CCTCATAACTCCAGGTTTGTTCA	23
LOC101493206	(A)16	162	GACATTGCTCCTTCACTAACTTCA	24	GACAAAAGACAAGAGCGTTACATC	24
LOC101493523	(T)10	301	GGAATGATGGATTATGTCTCCC	22	TCTCTTCTCTTTCTTCTCCCACA	23
LOC101507081	(TA)9	260	TCAGGAAAGAGGTTGGAACTTG	22	ATCAAACTGGGAATGCACTTG	21
LOC101505779	(T)10	396	TTCACTCCTTCATTACTTCGCA	22	ACAGCAAGACCACGGTAAAAGT	22

LOC101504169	(A)10	324	CTTCGTATGGCTGTTGCTGTT	21	ACTGCCCAAGTAAAATCAAGGA	22
LOC101492874	(T)16	344	TCTCTCTGGTCATCATCTTGCT	22	AGTTCACCATCCTTAGCCAAAC	22
LOC101492874	(T)12	344	TCTCTCTGGTCATCATCTTGCT	22	AGTTCACCATCCTTAGCCAAAC	22
LOC101492874	(T)10	361	AATTGTTTGGCTAAGGATGGTG	22	CCTCATAACTCCAGGTTTGTTCA	23
LOC101508554	(A)16	162	GACATTGCTCCTTCACTAACTTCA	24	GACAAAAGACAAGAGCGTTACATC	24
LOC101508554	(T)11	389	ACGCTCTTGTCTTTTGTCACTT	22	AGTAGTCATCCTCCGGTAATCA	22
LOC101508554	(A)11	338	TAAATAGGGAGACGCCACACTC	22	GCCTTTGTGAGAAGACAAGGAT	22
LOC101508554	(T)16	259	ATCACCCCTCATAGCATTCAAG	22	AGTCTGCAAGGAAAAGACATGG	22
LOC101508554	(A)12	394	TGCTCTCCTTTCTCCTCTTTTG	22	AATTTCTGGTGGTGGGAATATG	22
LOC101508554	(TAA)25	242	GCTCTCTGTGTTGCTTAGGGAT	22	GACTGACTCCTCAAGTGGAACC	22
LOC101508554	(T)10	271	GGGGCTTAGGTGCAACATC	19	TTACCGTCATTTGAGTGTTTGG	22
LOC101508554	(T)10	300	CCACTGTTGCTTAGGAGTGTTC	22	AGTTTACCCTGACATTGCCCTA	22
LOC101508554	(T)10	177	TTGTTGCAGATTATATCGGTGG	22	GGTCCCCATGTTTATGGTTAGA	22
LOC101509077	(T)10	301	GGAATGATGGATTATGTCTCCC	22	TCTCTTCTCTTTCTTCTCCCACA	23
LOC101493206	(A)16	162	GACATTGCTCCTTCACTAACTTCA	24	GACAAAAGACAAGAGCGTTACATC	24
LOC101493523	(T)10	301	GGAATGATGGATTATGTCTCCC	22	TCTCTTCTCTTTCTTCTCCCACA	23
LOC101494796	(T)10	301	GGAATGATGGATTATGTCTCCC	22	TCTCTTCTCTTTCTTCTCCCACA	23
LOC101494796	(A)10	204	ACACACCAAAGAAGGAAACTCA	22	TTTGAAGACTGTACCGAAACCA	22
LOC101510924	(T)10	370	ATTTGAGGTTTCGTGGATGAGT	22	GCAGCTTTGATGGAGTTCCTAA	22
LOC101510924	(T)10	370	ATTTGAGGTTTCGTGGATGAGT	22	GCAGCTTTGATGGAGTTCCTAA	22
LOC101510924	(TA)22	313	TATTTTGTACGGCATAGCCAGA	22	GAAAACAATGAGACCCAACACA	22
LOC101511436	(T)12	382	AGGGGAAGAAGAATGGTTCAAT	22	GGTAGGGGCATATAATGGATGA	22
LOC101495125	(T)11	363	TGTTGATTAGGAAAGGGAGGAA	22	AGGGATACATGGTTTTAGTGTGG	23
LOC101495125	(A)13	249	TTACCCTTCATGGACCTTCTTT	22	AACGAGTATTGAGTGTGATGTTACC	25
LOC101509945	(T)15	366	GATTTGAGGTTTCGTGGATGAG	22	GCAGCTTTGATGGAGTTCCTAA	22
LOC101509945	(A)12	366	GATTTGAGGTTTCGTGGATGAG	22	GCAGCTTTGATGGAGTTCCTAA	22
LOC101509945	(T)11	332	GGTTGAAAGTTCGTGACCATTA	22	CACAGAGCAGGAGAAGGATTTT	22
LOC101509945	(T)10	298	CATTCCCAATCGAAGGTATTCT	22	CGGTTATCAATTATCCGCAA	20
LOC101509945	(A)11	294	GTGTGTCAACTACTTCGCCTTA	22	ATTAAAGTGAGGGGCCAAAT	20
LOC101509945	(AAT)20	168	AACCTTCCTTCCAACCACTAAAC	23	GAAAACCACGCCAAAACTATGT	22
LOC101493426	(T)10	337	ATGGTGTTGCTCTTGCTTTCTT	22	TGCGGAATAAATAGACATGCAC	22
LOC101493426	(T)12	337	ATGGTGTTGCTCTTGCTTTCTT	22	TGCGGAATAAATAGACATGCAC	22
LOC101509615	(T)10	284	TCTGCCTCATGCTTTCTATCAA	22	ACTACCTGACTGGGGAACAAAA	22
LOC101509615	(TAAAT)6	381	GCCTAATGGAAGGATGGGTAA	21	GCACTTTCGTTTCACTTTGCTT	22
LOC101509301	(AAT)6	347	CGTGACATTGGGGAATAGTTGT	22	GCACAATTTAGCTGAGGTTGCTA	23
LOC101508455	(T)11	362	CTGAGACGGTGGTGTTCTTTT	21	CAATTACCTCTTCTGGTTTGGG	22

LOC101507600	(TC)8	396	TGCTTTAGGGATTGTCTCATCA	22	CTTGATAATGTAGTTGGCATACAGC	25
LOC101507600	(T)10	386	GCAGTTGATTTTGATCGGAGTT	22	TTCTAAGTGTTGCGTGAGTTGC	22
LOC101507600	(AT)6	255	TTAGTGGGATAAGGGTTGGTTG	22	TTTGTCTCTTACACCGGGAAGT	22
LOC101507600	(T)12	340	CACAATATCGCTTTCCTACTGC	22	ACAAAGTCAGCCACACCTGTAA	22
LOC101506964	(T)12	317	ATTCGGGGATGAAACATAAGC	21	ATGACCATTTTACGACCATTCC	22
LOC101506964	(TG)6	230	TAGCCTGTTCTCTATTGGGGTG	22	TATCACATGGTTGTTTGGCAGT	22
LOC101507293	(A)12	302	AATGGACGGGTTAGTGATTTTC	22	TGCACAAAGGAGAGTGAATGTT	22
LOC101504059	(T)10	321	AATCTCATGGTCCCCAATACAC	22	CACATTTGAACCCACCAAAA	20
LOC101504059	(TA)16	389	ACGGGAGAAGAGAAGAAGTTGA	22	GTTAGGCCATGTTTGATGGTTA	22
LOC101504059	(T)15	254	TTTTCTTCTCGTGTGTATCGCA	22	GTGACTTGCCATTAACCAAACA	22
LOC101503732	(A)10	356	AGGTAATCATAAACCCACACCG	22	TACGAAGCGGTATTGACGAAAT	22
LOC101503732	(T)11	356	AGGTAATCATAAACCCACACCG	22	TACGAAGCGGTATTGACGAAAT	22
LOC101505780	(T)10	374	AAAAGCCTCCTTAATCCGAGG	21	ATTTTGGTTTGGTCCCCTAGC	21
LOC101504809	(T)10	321	AATCTCATGGTCCCCAATACAC	22	CACATTTGAACCCACCAAAA	20

APPENDIX-XIII: List of genes and genic SSRs present in chickpea genomic region flanked by markers TR44-CaM1125 on chickpea chromosomes 6

Sl. No.	Gene Id	Putative function	SSR motifs identified
1	LOC101507318	Serine carboxypeptidase-like	(ATT)6
2	LOC101507844	Vacuolar protein sorting-associated protein 24 homolog 1	(CT)6, (A)14, (T)10, (T)12
3	LOC101508375	Probable xyloglucan endotransglucosylase/hydrolase protein 32	(TTA)8
4	LOC101508689	Probable glucuronoxylan glucuronosyltransferase F8H	(T)12, (T)11
5	LOC101509010	Uncharacterized	(A)11
6	LOC101509330	Shugoshin-1	(T)65, (T)29, (T)10
7	LOC101509644	Uncharacterized	(TC)7, (A)11, (CAA)6, (CTA)6, (GAA)6
8	LOC101509971	DNA ligase 1	No SSR
9	LOC101510503	Protein UPSTREAM OF FLC	(A)13, (T)10
10	LOC101511054	RING finger and CHY zinc finger domain-containing protein 1	(T)11, (A)11, (T)33, (T)42, (TTC)6
11	LOC101512011	Transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR	(AAT)6
12	LOC101512334	Superoxide dismutase [Cu-Zn], chloroplastic	(A)12
13	LOC101512661	Zinc finger protein ZAT5	(A)12, (CT)8
14	LOC101512971	50S ribosomal protein L9, chloroplastic	(T)10
15	LOC101513298	Pheophorbide a oxygenase, chloroplastic	No SSR
16	LOC101513622	Protein argonaute 5	(CT)6
17	LOC101492342	Vacuolar protein sorting-associated protein 41 homolog	(T)13, (TG)6
18	LOC101514171	Putative pentatricopeptide repeat-containing protein At1g26500	No SSR
19	LOC101514493	coiled-coil domain-containing protein 132	(T)12, (TA)6, (AT)8
20	LOC101514938	Serine carboxypeptidase-like 51	(T)12, (TG)6, (T)16
21	LOC101515457	Uncharacterized	No SSR

APPENDIX-XIV: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by TR44-CaM1125 on chromosome 6

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101514938	(T)12	342	TTGAAAAGTCACGATGGATCAG	22	ACCCTGCAACCAAAGAATGA	20
LOC101514938	(TG)6	342	TTGAAAAGTCACGATGGATCAG	22	ACCCTGCAACCAAAGAATGA	20
LOC101514938	(T)16	350	AACAAACACGACCCTGTCACTA	22	ATCAGTTGCTGCTTCCTCATCT	22
LOC101514493	(T)12	311	GTTGGACATGCAATCAACACTT	22	CACACCACGACTCATCTCTTGT	22
LOC101514493	(TA)6	148	TTTGTAGGATGAGGAGGGACAT	22	TGTAAGTGAGCCTGGAAATGAA	22
LOC101514493	(AT)8	264	GCAGAGGGCAATCTAACTCTTG	22	TCTGGTACAGCATCCACCTAAA	22
LOC101492342	(T)13	387	AGGCATTAGCGGAGTTGTATGT	22	GAAACCTTGCAGTTGTGTGAGT	22
LOC101492342	(TG)6	384	CAGTCGTTGTTTTCTTTTGCTG	22	ATAGGAACCGGCATTTCAAGTA	22
LOC101513622	(CT)6	168	CTCGCTCTTCTCATTTTCCATT	22	ATGGTTGGTTATGGCGTATCTC	22
LOC101512661	(A)12	118	ACGCAAGGAAGATTTTGTGTG	21	TTGGGAGAGAGAGAAAGAGGAA	22
LOC101512661	(CT)8	369	GTTGGAAACGCAAGGAAGATT	21	AGCAACACCACAAGATGACAAA	22
LOC101512334	(A)12	347	TGACGGTGACTCCTCTTCTCTT	22	GAGATTTCAGCAACAGCATCG	21
LOC101512011	(AAT)6	264	CATTCCTTTCATACCCTGCAAT	22	CTTTCATTCGTACTCTTCGCCT	22
LOC101511054	(T)11	378	AGAAGGAGATGCAAGATCGTAG	22	TGCTCTATTGTTGGTTGGAGA	21
LOC101511054	(A)11	378	AGAAGGAGATGCAAGATCGTAG	22	TGCTCTATTGTTGGTTGGAGA	21
LOC101511054	(T)33	378	AGAAGGAGATGCAAGATCGTAG	22	TGCTCTATTGTTGGTTGGAGA	21
LOC101511054	(T)42	378	AGAAGGAGATGCAAGATCGTAG	22	TGCTCTATTGTTGGTTGGAGA	21
LOC101510503	(T)10	400	GGGAATTTGATTGCTTGACAG	21	GTCCTGCATATTTGTGAATTGG	22
LOC101509644	(TC)7	198	TACCTTCATTTCAACCATTCCC	22	CCTTTCCTTGGCAGTAGTAAGC	22
LOC101509644	(A)11	141	CCTTCCTACCTCCCATTTCTCT	22	GAAGAGGGGTACGTTGATTGTC	22
LOC101509644	(CAA)6	324	CAATGGAGCTTGAACAAACAAG	22	TCAAAGGGTCAATAACTGGTGA	22
LOC101509644	(CTA)6	126	CTTCACCAACCTTACAACACCA	22	CAGAACCTTCACAGAAAACCCT	22
LOC101509644	(GAA)6	398	TCTACCAATTTTCCTGCTATGG	22	ACTCCCTCACACACACTCAAAT	22
LOC101509330	(T)65	241	TATCACACTCGATACACACCCC	22	TAAATTCGCTCTTTCCTGCAAC	22
LOC101509330	(T)29	241	TATCACACTCGATACACACCCC	22	TAAATTCGCTCTTTCCTGCAAC	22
LOC101509330	(T)10	311	GTTGCAGGAAAGAGCGAATTTA	22	AACTACTAACCGCTAACATGAGGC	24
LOC101508689	(T)12	237	ATCTCACGATTTCGGTTCTTGT	22	CGGTATCACAACATGCTCAACT	22
LOC101508375	(TTA)18	332	TCTATGGAAACCCAGTGAGCTA	22	CTATGCACAAATTCATTCACCC	22
LOC101507844	(A)14	382	TTTAGTCAAGCTCCGCCACT	20	GAAAAGGATGAAGGTTTGGTCA	22
LOC101507844	(T)10	205	ATCCAGGGTTTTATGATTGTGG	22	AACTTCTTTTGCTTGTTCTGGC	22
LOC101507844	(T)12	359	TCGATCTTCTTTGGATAGCGTT	22	GTCATTCCTCTTTGCAGCTTCT	22
LOC101507318	(ATT)6	321	CATATCTCCAAACACGTCATGC	22	AACCATATCACAACTGGGTCATC	23

APPENDIX-XV: List of genes and genic SSRs present in chickpea genomic region flanked by markers H4E09-LOC101504400 on chickpea chromosomes 6

Sl. No.	Gene Id.	Putative function	SSRs motifs Identified
1	LOC101494298	Axial regulator YABBY 1-like	(CT)6, (CA)8, (CT)8, (TA)26, (T)10, (AT)12
2	LOC105852328	Uncharacterized	(A)10, (A)13, (A)10, (T)10
3	LOC101494922	Uncharacterized	(CT)7, (AAT)17, (AAT)8, (TAT)20.3, (T)10, (T)10, (TC)7, (A)13, (A)12, (A)10
4	LOC101496128	Uncharacterized	(C)33, (T)15, (T)10
5	LOC101497106	Cyclin-dependent kinases regulatory subunit 1	No SSR
6	LOC101497438	Uncharacterized	No SSR
7	LOC101497976	Aspartic proteinase-like protein 2	(T)10
8	LOC101498523	SRSF protein kinase 2-like	(T)12
9	LOC101501620	GDSL esterase/lipase At5g22810-like	(T)12, (A)10, (TA)7, (A)10, (A)11, (A)10
10	LOC101499092	recQ-mediated genome instability protein 2	No SSR
11	LOC101501933	GDSL esterase/lipase At5g22810-like	(T)10
12	LOC101499408	Putative phospholipid:diacylglycerol acyltransferase 2	(A)10
13	LOC101500026	UDP-D-apiose/UDP-D-xylose synthase 2	(A)12, (T)14, (T)10
14	LOC101502264	Uncharacterized	(T)10, (T)10, (A)11, (A)10, (A)12, (T)18
15	LOC101500363	AT-hook motif nuclear-localized protein 9-like	(T)16, (A)10, (TA)17
16	LOC101500989	GABA transporter 1-like	(AT)22, (T)10, (T)10
17	LOC101500675	GABA transporter 1-like	(TA)17, (AT)8, (T)10, (A)12, (AT)7
18	LOC101502778	Glucomannan 4-beta-mannosyltransferase 2	(T)12, (AT)7
19	LOC105852329	Uncharacterized	No SSR
20	LOC101503096	Histone deacetylase HDT1-like	No SSR
21	LOC101503425	Uncharacterized	No SSR
22	LOC101494924	Uncharacterized	No SSR
23	LOC101503764	Uncharacterized	No SSR
24	LOC101504092	Arogenate dehydratase/prephenate dehydratase 6, chloroplastic-like	No SSR
25	LOC101504400	Nucleobase-ascorbate transporter 12	(T)10, (GT)6

APPENDIX-XVI: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by H4E09-LOC101504400 on chromosome 6

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101494298	(CT)6	308	TCGATCCTCTATACATAAGTGGC	23	AGGGAGATAGTTGTTGTTGCTG	22
LOC101494298	(CA)8	283	TGGCATTGATCTCTCTCTCTCTT	23	ATAGTTGTTGTTGCTGGTCCG	21
LOC101494298	(CT)8	192	GCAACAACAACTATCTCCCTCC	22	TGCAAGGAACACTCACCTAAAA	22
LOC105852328	(A)10	345	CAGCTAGTGTACTGAATCGTTGC	23	AAGGAATTAGGGGTTACATTGG	22
LOC105852328	(A)10	358	TATTGAAGTGCGGTATCATTGG	22	GTTCTTCCTCCGTTGTCTGTTT	22
LOC105852328	(T)10	300	TCAGTACCCGCATGAATAAACA	22	GGGTGAAGGCATAGTTGAGAAA	22
LOC101494922	(CT)7	298	TTCTAAAATCGCCGCTCAGT	20	AAACGAGAGAAAGATGCGAAAC	22
LOC101494922	(AAT)17	365	AGTCGAAATGAAGAACTCCACC	22	TATATCTCATCCTGCACCCAAA	22
LOC101494922	(AAT)8	365	AGTCGAAATGAAGAACTCCACC	22	TATATCTCATCCTGCACCCAAA	22
LOC101494922	(TAT)20.33	365	AGTCGAAATGAAGAACTCCACC	22	TATATCTCATCCTGCACCCAAA	22
LOC101494922	(T)10	353	GCGATTGTGGAATCTGAGG	19	TCACGAGCTTTAGAGTTGTCCA	22
LOC101494922	(T)10	396	TGGACAACTCTAAAGCTCGTGA	22	TCATCCTCAATCTCTCTCCACA	22
LOC101494922	(TC)7	330	TGAACTCTTTTAGACCTCCCTCC	23	TTTGATACCATGAAGTTGTGGG	22
LOC101494922	(A)13	181	GAAGGGAAGTAGAAAAGGACAGG	23	GCTTCATACACTCCTAGTCGCA	22
LOC101494922	(A)12	125	GAAGAAGAAGGCAACGTGAACT	22	GCGTATTCCCAAAGCCTAGAA	21
LOC101494922	(A)10	342	GTGTGCATGAGTTCTCTTCAGG	22	GTTTGGTGTGATGATTGTGGAC	22
LOC101496128	(T)15	350	ATTAGCGAAGCAGTTTGGTGTC	22	ACAGATAGCGGAGAGTAGCAGG	22
LOC101496128	(T)10	160	ATCACATAGGGTGTTTGAAGGA	22	CAATCTGAGCAGCAGTACCAAT	22
LOC101498523	(T)12	308	AAAGGATTTGCCCAGGATAGTT	22	CCCAGTTGGTTGACTTTACACTT	23
LOC101501620	(A)10	292	GGTCCCACATGAATACACTTTT	22	ATTTTCAGGATTTCCCTCCA	20
LOC101501620	(TA)7	171	ATATCTTGTTGGTGCTGGGACT	22	ATTGAAAATCGGTCTGGTTGAG	22
LOC101501620	(A)10	376	GCTATGCCAATTTCATTCAGGT	22	GGTCTTCGGATTTTGATTTGAG	22
LOC101501620	(A)11	379	ACTTGAGAAAAGTGCTTCCTCG	22	ATCGCAAACTCGGGCTCT	18
LOC101501620	(A)10	240	TAAAGAGCCCGAGTTTGCG	19	TTAGTTAGGCATCGCCATTTGT	22
LOC101501933	(T)10	385	TACCATGATTCTACGGCCAAAC	22	GCCCTGCTATTTTCATCAACTC	22
LOC101500026	(T)14	220	CTCCTGCTGATTACAACACGC	21	CGTTTTCCCATACACTTCACAA	22
LOC101500026	(T)10	191	AAGTGTATGGGAAAACGATTGG	22	TTGAACTACCACCGACACATTC	22
LOC101502264	(T)10	302	TTACGTTCGTGGTCCCTTAACT	22	CTTCTCGATTTAGTCCTTTATTCCC	25
LOC101502264	(A)11	302	TTACGTTCGTGGTCCCTTAACT	22	CTTCTCGATTTAGTCCTTTATTCCC	25
LOC101502264	(A)10	315	GGGAATAAAGGACTAAATCGAGAAG	25	GGAGGAGGAGTAAAGTGATCCA	22
LOC101502264	(A)12	315	GGGAATAAAGGACTAAATCGAGAAG	25	GGAGGAGGAGTAAAGTGATCCA	22
LOC101500363	(T)16	385	GAGAGAGAAAACAAAGAACAGAGAG	25	TTAACACCTTGTAATCCCGAAC	22

LOC101500363	(A)10	301	TTTTGGATGGATTGAGGATACC	22	CTTCCCCTTGACTTTTATGGC	21
LOC101500363	(TA)17	374	CACGCAGTTTTATAGTCGGTGT	22	TTGACGAAGGATGACACTTGAT	22
LOC101500989	(T)10	372	TGGGGCTGTAGTGGCTAGTAAT	22	GACCCATTTGGATTTGATAGGA	22
LOC101500675	(TA)17	234	ATTCGGATAGGTTGGTTTGAGT	22	CTTTGTTTCATTTAGTCCTCGG	22
LOC101500675	(AT)8	234	ATTCGGATAGGTTGGTTTGAGT	22	CTTTGTTTCATTTAGTCCTCGG	22
LOC101500675	(A)12	356	GTGGCTTGTAATCTTTTGGGAG	22	GACCCATTTGGATTTGATAGGA	22
LOC101504400	(T)10	320	GAGGTTTGAGGAAGTGCTTGAT	22	GGAACAATGACAAGTGGAATGA	22
LOC101504400	(GT)6	177	TCATTCCACTTGTCATTGTTCC	22	CGATACCACCAAAGAAGTTTCC	22

APPENDIX-XVII: List of genes and genic SSRs present in chickpea genomic region flanked by markers CaM1402-CaM1101 on chickpea chromosomes 6

Sl. No.	Gene ID	Name	SSR identified
1	LOC101494099	MLO-like protein 1-like	(A)10, (T)15, (T)11, (AT)21, (AT)18, (T)11, (T)11, (A)10, (A)12, (T)10, (AT)6, (TA)6, (T)10, (TA)7, (TA)6
2	LOC101492349	Expressed protein	No SSR
3	LOC101492586	Expressed protein	No SSR
4	LOC101492013	Uncharacterised	No SSR
5	LOC101512112	Transcription factor MYB12-like	(TA)6
6	LOC101491698	MYB-related protein 315-like	(T)10
7	LOC101491384	TC19210: Integrase	(A)10
8	LOC101511797	Equilibrative nucleotide transporter 3-like	NO SSR
9	LOC101511474	Equilibrative nucleotide transporter 3-like	(CT)10, (T)11, (T)13
10	LOC101491070	Uncharacterised	NO SSR
11	LOC101511163	Expressed protein	NO SSR
12	LOC101510951	Expressed protein	NO SSR
13	LOC101510627	Diacylglycerol kinase iota-like	(AT)6, (T)10, (A)10
14	LOC101510298	Protein THYLAKOID FORMATION1, chloroplastic-like	NO SSR
15	LOC101509770	TC18684: Equine herpesvirus glycoprotein gp2	(A)10, (A)11
16	LOC101490422	exocyst complex component 3-like	NO SSR
17	LOC101490098	Ethylene-responsive transcription factor ERF119-like	NO SSR
18	LOC101509443	TC17487: Wound-responsive family protein	NO SSR
19	LOC101509217	Expressed protein	(T)13, (AT)20
20	LOC101488798	E3 ubiquitin ligase BIG BROTHER-like	NO SSR
21	LOC101508901	TC17487: Wound-responsive family protein	NO SSR
22	LOC101508589	NAD(P)H dehydrogenase B3, mitochondrial-like	(A)11
23	LOC101508266	NAD(P)H dehydrogenase B1, mitochondrial-like	(T)10
24	LOC101507951	TC14502: Embryo defective 1923 (emb1923)	NO SSR
25	LOC101507118	TC16881: Expressed protein	(T)10
26	LOC101505259	Oral cancer-overexpressed protein 1-like	No SSR
27	LOC101504408	Protein ULTRAPETALA 1-like	(CT)6, (T)10, (T)10, (T)24, (T)10
28	LOC101514718	Uncharacterized	No SSR
29	LOC101504097	H/ACA ribonucleoprotein complex Non-core subunit NAF1-like	(T)11
30	LOC101503773	Secretory carrier-associated membrane protein 1-like	(AC)6
31	LOC101503434	Protein STRUBBELIG-RECEPTOR FAMILY 3-like	(CAC)6
32	LOC101514389	Expressed protein	No SSR
33	LOC101514064	Aspartic proteinase-like protein 2-like	(T)11
34	LOC101513728	Aspartic proteinase-like protein 2-like	(T)12, (T)12, (A)13
35	LOC101513415	Eukaryotic aspartyl protease family protein	TC20338, (T)13, (T)10, (AT)7, (TTAT)11, (T)11, (T)10, (A)10, (A)10, (T)10
36	LOC101503109	Xyloglucan endotransglucosylase/ hydrolase protein 9-like	(AT)17, (A)12, (T)20, (A)16
37	LOC101513088	Aspartic proteinase-like protein 2-like	(T)10, (T)10, (T)11
38	LOC101512759	Unknown function	(A)11
39	LOC101512439	Aspartic proteinase-like protein 2-like	No SSR
40	LOC101498314	BEL1-like homeodomain protein 9-like	(CT)9, (TAA)7, (ACA)6, (AAT)12
41	LOC101497987	Expressed protein	(GAT)10, (A)13

42	LOC101497652	Galactose oxidase-like	No SSR
43	LOC101502169	Probable peptide/nitrate transporter At3g53960-like	(T)10
44	LOC101501846	Probable peptide/nitrate transporter At3g53960-like	(TA)9, (A)10, (TA)11, (T)11
45	LOC101501529	Putative peptide/nitrate transporter At2g37900-like	No SSR
46	LOC101501216	Uncharacterised	No SSR
47	LOC101497328	Putative peptide/nitrate transporter At2g37900-like	(A)10, (T)10, (T)12, (T)10, (T)12, (T)11, (A)12
48	LOC101500263	Uncharacterised	NO SSR
49	LOC101496460	Elongation factor 1-delta 2-like	NO SSR
50	LOC101499923	UPF0481 protein At3g47200-like	NO SSR
51	LOC101496136	Expressed protein	No SSR
52	LOC101499615	TC20678: Gag-pro	(T)11, (T)17
53	LOC101499300	Expressed protein	(TTAT)7, (T)13
54	LOC101495586	Expressed protein	(T)10, (A)11
55	LOC101495041	Expressed protein	(TA)6, (A)10, (T)11
56	LOC101498652	Anaphase-promoting complex subunit cdc20-like	No SSR
57	LOC101494717	ROP guanine nucleotide exchange factor 1-like	(AT)19, (T)10
58	LOC101494404	Probable glutathione S-transferase-like	No SSR
59	LOC101494097	Probable glutathione S-transferase-like	No SSR
60	LOC101493776	Universal stress protein A-like protein-like	(T)10, (T)10, (T)10, (A)10
61	LOC101491915	Scarecrow-like protein 33-like	(T)10
62	LOC101491604	Scarecrow-like protein 33-like	No SSR
63	LOC101491282	Uncharacterised	No SSR
64	LOC101493246	Ubiquitin fusion degradation protein 1 homolog	No SSR
65	LOC101490970	TC15333: Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	No SSR
66	LOC101490522	ATP-dependent zinc metalloprotease FTSH 10, mitochondrial-like	(T)10
67	LOC101490200	Uncharacterised	(CT)6, (AT)8
68	LOC101489865	Laccase-2-like	(T)11
69	LOC101489335	E3 ubiquitin-protein ligase SDIR1-like	(T)11
70	LOC101489011	E3 ubiquitin-protein ligase RING1-like	(TC)8, (A)12
71	LOC101488797	TC31352: Acyl-acyl carrier protein thioesterase	(T)13, (T)10, (TA)7
72	LOC101515591	Pumilio homolog 2-like	(T)10
73	LOC101515043	Two-component response regulator ARR2-like	(AT)6
74	LOC101514716	Pentatricopeptide repeat-containing protein At5g59600-like	No SSR
75	LOC101514388	Protein TOC75, chloroplastic-like	No SSR
76	LOC101514063	Low-temperature-induced 65 kDa protein-like	No SSR
77	LOC101513727	D-xylose-proton symporter-like 3, chloroplastic-like	(T)12
78	LOC101513414	Serine/arginine repetitive matrix protein 1-like	(T)11
79	LOC101513087	Protein TIC 62, chloroplastic-like	No SSR
80	LOC101492250	Transcriptional regulator TAC1-like	No SSR
81	LOC101512758	Tropinone reductase homolog	(GT)11
82	LOC101512438	Metal tolerance protein B-like	(T)11
83	LOC101512110	RAS-related protein Rab11C-like	No SSR
84	LOC101510626	Transcription elongation factor B polypeptide 1-like	No SSR
85	LOC101511161	Pentatricopeptide repeat-containing protein At3g46870-like	No SSR
86	LOC101511472	TC30929: Maternal effect embryo arrest 60 (MEE60)	No SSR
87	LOC101511795	TC17976: Adenine nucleotide alpha hydrolases-like superfamily protein	(T)14
88	LOC101510297	TC05834: IMP dehydrogenase/GMP reductase	(T)10, (T)11
89	LOC101509977	Copper transporter 2-like	No SSR
90	LOC101509333	UDP-glycosyltransferase 76E12-like	(A)10
91	LOC101509651	18.5 kDa class I heat shock protein-like	No SSR

92	LOC101508587	Uncharacterised	No SSR
93	LOC101507849	Probable serine/threonine-protein kinase At5g41260-like	(T)11
94	LOC101507326	Uncharacterised	No SSR
95	LOC101507000	Uncharacterised	No SSR
96	LOC101503882	Non-cyaNogenic beta-glucosidase-like	(A)10, (AT)10, (ATA)35, (T)10, (TTA)8, (TAA)8, (A)13
97	LOC101502484	Unconventional myosin heavy chain 6-like	No SSR
98	LOC101501940	Non-cyaNogenic beta-glucosidase-like	(T)10, (AT)11, (ATA)6, (ATA)6, (T)10, (A)11, (A)10, (T)13, (A)11
99	loc101500680	Beta-glucosidase 12-like	(T)10
100	LOC101501310	Beta-glucosidase 12-like	(A)10
101	LOC101506664	Beta-glucosidase 12-like	(AT)8
102	LOC101500576	Beta-glucosidase 12-like	(TA)8
103	LOC101506343	Non-cyaNogenic beta-glucosidase-like	(TA)7, (A)12
104	LOC101500261	Beta-glucosidase 13-like	(T)14
105	LOC101506028	TC08267: Beta glucosidase 16 (BGLU16)	(T)10, (T)11
106	LOC101505703	Linoleate 13S-lipoxygenase 2-1, chloroplastic-like	No SSR
107	LOC101505370	Linoleate 13S-lipoxygenase 2-1, chloroplastic-like	(A)10, (T)10, (T)10, (AT)6, (T)19
108	LOC101499921	Linoleate 13S-lipoxygenase 2-1, chloroplastic-like	(A)10, (A)15, (A)11
109	LOC101499614	Putative Non-ribosomal peptide synthetase	(TA)7
110	LOC101499299	Lysine-specific demethylase 8-like	(AT)6
111	LOC101498775	TC06806: Dor1, Dor1-like family	(TA)6
112	LOC101498313	TC21053: Cysteine-rich receptor-like protein kinase	(T)19, (T)10, (AT)13, (CTT)6, (TA)9
113	LOC101497986	Uncharacterised	No SSR
114	LOC101497651	Transcription factor bHLH122-like	(T)10, (T)14, (CAA)7, (ATTTT)8
115	LOC101497327	DNA-damage-repair/toleration protein DRT100-like	No SSR
116	LOC101505050	DNA-damage-repair/toleration protein DRT100-like	No SSR
117	LOC101496135	Catalytic activity	(T)13, (T)10
118	LOC101495812	Serine/threonine-protein kinase OXI1-like	No SSR
119	LOC101495484	AP2-like ethylene-responsive transcription factor PLT2-like	(TA)7, (TAA)6
120	LOC101495160	Zinc finger A20 and AN1 domain-containing stress-associated protein 8-like	No ssr
121	LOC101494831	Cytochrome c-type biogenesis protein CcmE-like	No SSR
122	LOC101494307	Zinc transporter ZTP29-like	No SSR
123	LOC101493985	Copper transporter 6-like	No SSR
124	LOC101504726	Putative copper-transporting ATPase HMA5-like	(A)14, (T)12, (A)14, (TA)19, (TTA)8, (A)10, (A)10
125	LOC101493244	E3 ubiquitin ligase BIG BROTHER-like	(AG)11, (TA)7, (T)14
126	LOC101492912	Extensin-2-like	No SSR
127	LOC101492583	Extensin-2-like	(C)13, (C)10
128	LOC101492248	Extensin-2-like	NO SSR
129	LOC101491914	Aldehyde dehydrogenase family 3 member F1-like	(CTT)6, (TA)9, (T)11, (A)12, (A)11, (AT)7, (A)10, (CT)7, (AT)6, (T)12, (T)11
130	LOC101491603	GTPase Der-like	(T)14
131	LOC101491281	tRNA-specific 2-thiouridylase MnmA-like	No SSR
132	LOC101490969	Transcription factor MYB46-like	(A)10, (TA)6
133	LOC101490640	Dicarboxylate transporter 1, chloroplastic-like	No SSR
134	LOC101515590	Aquaporin NIP6-1-like	(T)12
135	LOC101490321	Uncharacterised	No SSR

136	LOC101515041	Aquaporin NIP6-1-like	(TA)6, (AT)13, (T)11
137	LOC101514715	Probable CCR4-associated factor 1 homolog 7-like	(A)12, (A)14
138	LOC101514179	40S ribosomal protein S12-like	(T)53
139	LOC101513833	Pentatricopeptide repeat-containing protein At1g71460, chloroplastic-like	No SSR
140	LOC101489660	Cinnamoyl-CoA reductase 2-like	(A)24, (AT)13, (A)11, (T)12
141	LOC101513526	CBL-interacting serine/threonine-protein kinase 25-like	(A)10, (A)10, (T)11
142	LOC101513204	V-type proton ATPase subunit D-like	(TC)7
143	LOC101512877	Probable WRKY transcription factor 40-like	No SSR
144	LOC101512572	Probable GMP synthase [glutamine-hydrolyzing]-like	(CT)8
145	LOC101512016	TC08923: Novel subunit of the chloroplast NAD(P)H dehydrogenase complex	(T)10, (A011
146	LOC101511698	TC18158: Single-copy phospholipid N-methyltransferase	No SSR
147	LOC101511369	Uncharacterised	(T)12, (TA)6
148	LOC101510838	Uridylyltransferase-related	NO SSR
149	LOC101510511	3-oxoacyl-[acyl-carrier-protein] synthase 3 A, chloroplastic-like	(T)10, (A)10, (ATT)26
150	LOC101510183	DNA/RNA-binding protein KIN17-like	(ATA)8
151	LOC101509864	TC13095: T26F17.17	(CTT)6
152	LOC101509116	Putative cyclic nucleotide-gated ion channel 8-like	(T)11
153	LOC101489334	Uncharacterised	(T)10
154	LOC101489010	Galactoside 2-alpha-L-fucosyltransferase-like	No SSR
155	LOC101488668	Uncharacterised	No SSR
156	LOC101488344	Galactoside 2-alpha-L-fucosyltransferase-like	(T)11, (T)10, (T)10, (AT)10, (A)10, (T)11
157	LOC101508585	Pectinacetylesterase family protein	NO SSR
158	LOC101508068	Uncharacterised	(A)14, (GAT)6
159	LOC101505160	Transcriptional activator DEMETER-like	(A)10, (A)14
160	LOC101507743	Probable methyltransferase WBSCR22 homolog	(TTA)19
161	LOC101504846	Galactoside 2-alpha-L-fucosyltransferase-like	(A)10, (T)10
162	LOC101504532	Uncharacterised	(T)11
163	LOC101504200	F-box protein At5g07610-like	No SSR
164	LOC101503881	lamin-like protein-like	No SSR
165	LOC101503555	TC17826: Os12g0556400 protein (Fragment)	No SSR
166	LOC101503238	Chaperone protein dnaJ 8, chloroplastic-like	(A)10
167	LOC101502686	Probable protein phosphatase 2C 60-like	(CT)14
168	LOC101502383	Shikimate O-hydroxycinnamoyltransferase-like	(TA)15, (T)10
169	LOC101501844	Shikimate O-hydroxycinnamoyltransferase-like	(T)13, (T)10, (T)10
170	LOC101500680	Glycine-rich RNA-binding protein GRP1A-like	(A)13, (T)12
171	LOC101500369	rRNA 2'-O-methyltransferase fibrillarin-like	(T)11, (AT)7, (GAG)6
172	LOC101507439	Uncharacterised	(T)11
173	LOC101499518	Uncharacterised	(A)10, (T)12, (AG)6, (AG)10
174	LOC101500032	Uncharacterised	(TC)6, (T)12
175	LOC101499195	Protein ASPARTIC PROTEASE IN GUARD CELL 2-like	(T)10
176	LOC101498874	Cyclin-dependent kinase B1-2-like	(TA)10
177	LOC101498533	AP2-like ethylene-responsive transcription factor At1g16060-like	(AT)7, (T)10
178	LOC101497985	Uncharacterised	(TA)12
179	LOC101497650	Uncharacterised	
180	LOC101496894	TC08393: Ubiquitin-specific protease family C19-related protein	(T)10, (T)14
181	LOC101496243	Kelch repeat-containing protein At3g27220-like	(T)11
182	LOC101495912	F-box/kelch-repeat protein At1g51550-like	(A)10, (T)10

183	LOC101495584	TC10917: Pyridoxamine 5'-phosphate oxidase family protein	(TGA)6, (T)10
184	LOC101495264	Uncharacterised	(T)10, (A)10
185	LOC101494931	TC06635: Protein with a B-box domain predicted to act as a transcription factor	No SSR
186	LOC101494617	KH domain-containing protein At4g18375-like	(A)10
189	LOC101494096	GATA transcription factor 28-like	No SSR
190	LOC101493560	Mannosyl-oligosaccharide 1,2-alpha-manNosidase MNS1-like	(T)17, (T)12, (T)10
191	LOC101493243	Metal tolerance protein C4-like	(CTT)7, (T)11, (A)11
192	LOC101492681	60S ribosomal protein L7-4-like	NO SSR
193	LOC101492347	Calcium-transporting ATPase 9, plasma membrane-type-like	(A)10, (TA)15, (TA)6, (AT)6, (TA)7, (T)10, (T)10, (A)10, (AT)14, (T)13, (ATAT)22, (T)13, (AT)9, (T)10, (A)10
194	LOC101492011	O-fucosyltransferase family protein	TC11565, (CT)7, (TG)9, (AC)6
195	LOC101507117	Uncharacterised	(T)11, (TTA)11, (T)11, (AT)6, (AAC)9, (ATA)9
196	LOC101506780	F-box/kelch-repeat protein At3g23880-like	No SSR
197	LOC101506455	Putative F-box protein At3g16210-like	No SSR
198	LOC101491067	Ribonuclease S-7-like	(A)11, (A)11
199	LOC101490198	Protein AF-9 homolog	(A)14
200	LOC101506125	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
201	LOC101488899	Indole-3-acetic acid-induced protein ARG7-like	No SSR
202	LOC101489549	TC00172: Putative integrase core domain protein	No SSR
203	LOC101489864	Auxin-induced protein 6B-like	No SSR
204	LOC101515468	Auxin-induced protein X10A-like	No SSR
205	LOC101488222	Auxin-induced protein X10A-like	No SSR
206	LOC101514823	Indole-3-acetic acid-induced protein ARG7-like	(T)10, (TA)9
207	LOC101514502	TC22449: SAUR-like auxin-responsive protein family	(TA)9
208	LOC101515149	Auxin-induced protein X10A-like	(T)14, (AT)6
209	LOC101513525	Auxin-induced protein 6B-like	NO SSR
210	LOC101505810	TC10595: SAUR-like auxin-responsive protein family	(A)10
211	LOC101514178	Auxin-induced protein 6B-like	(A)10
212	LOC101513832	Indole-3-acetic acid-induced protein ARG7-like	No SSR
213	LOC101511582	Auxin-induced protein X10A-like	No SSR
214	LOC101512243	Auxin-induced protein 10A5-like	(T)10
215	LOC101511911	Auxin-induced protein X10A-like	No SSR
216	LOC101512876	Auxin-induced protein X10A-like	(A)15
217	LOC101512571	Auxin-induced protein X10A-like	No SSR
218	LOC101513203	Auxin-induced protein 10A5-like	No SSR
219	LOC101511262	Auxin-induced protein 15A-like	No SSR
220	LOC101505472	Auxin-induced protein X10A-like	No SSR
221	LOC101510950	Probable pectinesterase/pectinesterase inhibitor 36-like	(T)13, (TA)31, (T)10, (TA)19
222	LOC101510625	Uncharacterised	(AT)9, (TAT)20
223	LOC101509650	Glucan endo-1,3-beta-glucosidase-like	(T)19, (TAA)6, (A)10, (T)10, (T)10, (TAT)22, (T)11, (T)10, (A)11, (A)16, (T)11, (TTA)14, (T)11, (AT)13, (A)15, (AT)6, (TAT)6, (TA)8, (TA)14, (T)11

224	LOC101508067	Probable CCR4-associated factor 1 homolog 11-like	No SSR
225	LOC101507438	TC24292: TNP1	No SSR
226	LOC101507116	TC01644: Plant transposase (Ptta/En/Spm family)	No SSR
227	LOC101506779	50S ribosomal protein L1, chloroplastic-like	(TTAT)6
228	LOC101506454	Vacuolar amiNo acid transporter 1-like	(CT)10, (T)10, (A)10, (T)10, (A)13, (A)10, (A)10, (A)13, (A)12, (T)10, (A)10, (A)15, (A)11, (T)10, (T)11
229	LOC101506124	Mediator of RNA polymerase II transcription subunit 15a-like	(TA)11, (TA)7, (ATA)9, (AAT)6, (AT)15, (AT)6, (A)10, (AT)12, (T)10, (T)10, (TA)10, (ATA)19, (ATA)11, (T)12, (T)10, (TA)7
230	LOC101500679	TC06643: Sterile alpha motif (SAM) domain-containing protein	(TTATT)6
231	LOC101500368	Protein TOPLESS-like	(TC)7, (T)11, (T)10, (T)16, (T)13, (T)11, (T)10
232	LOC101499815	Internalin-A-like	(T)12, (TC)9, (GT)8
233	LOC101499517	2-methoxy-6-polyprenyl-1,4-benzoquiNol methylase, mitochondrial-like	(TA)14, (T)10
234	LOC101499194	Glycerol kinase-like	(T)11, (T)10
235	LOC101498873	TC24796: VQ motif-containing protein	(TC)13, (TC)9, (ATG)6
236	LOC101498532	Clustered mitochondria protein-like	(T)11, (ATT)13, (AT)7, (T)12, (A)11, (T)18, (T)19
237	LOC101497983	Haloalkane dehalogenase-like	(TA)6
238	LOC101505809	Putative pentatricopeptide repeat-containing protein At3g15930-like	No SSR
239	LOC101497648	Probable leucine-rich repeat receptor-like protein kinase At1g35710-like	No SSR
240	LOC101497001	Signal recognition particle 54 kDa protein 2-like	(AAT)6, (T)12
241	LOC101496689	Uncharacterised	No SSR
242	LOC101496358	TC17255: diacylglycerol cholinephosphotransferase	(T)10, (T)10
243	LOC101495693	Uncharacterised	No SSR
244	LOC101496020	TC15305: Chloroplast-targeted protein	No SSR
245	LOC101495366	Palmitoyl-moNogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like	(T)10
246	LOC101505159	TC00957: Cysteine-rich receptor-like protein; Putative protein kinase	NO SSR
247	LOC101504845	TC00061: eIF4E protein	(A)10, (CA)6, (A)10, (AT)11, (A)10
248	LOC101504531	TC11413: Aminotransferase-like, plant mobile domain family protein	No SSR
249	LOC101495038	Histidine-containing phosphotransfer protein 6-like	(A)11, (T)10, (T)11, (T)12,
250	LOC101494715	Protein LURP-one-related 12-like	(A)19, (T)11, (T)12, (A)13
251	LOC101504199	High mobility group B protein 13-like	(T)12
252	LOC101494403	Glucan endo-1,3-beta-glucosidase 14-like	(A)11
253	LOC101494095	Methyl-CpG-binding domain-containing protein 10-like	(A)10
254	LOC101493775	TC01644: Plant transposase	No SSR
255	LOC101503880	Uncharacterised	No SSR
256	LOC101503554	TC01644: Plant transposase	No SSR
257	LOC101503237	Uncharacterised	(T)10
258	LOC101502904	Uncharacterised	No SSR
259	LOC101502587	TC21680: Aminotransferase-like, plant mobile domain family protein	No SSR
260	LOC101493454	Uncharacterised	(A)12, (A)11

261	LOC101502272	EPIDERMAL PATTERNING FACTOR-like protein 8-like	No SSR
262	LOC101492460	Uncharacterised	(T)12, (T)10, (T)15
263	LOC101492119	Uncharacterised	(TC)10
264	LOC101501626	Phospholipase D alpha 1-like	(T)11
265	LOC101501309	TC00722: RVT_1, Reverse transcriptase (RNA-dependent DNA polymerase)	(T)10, (AT)9
266	LOC101500995	Uncharacterised	No SSR
267	LOC101509115	Two-component response regulator ARR10-like	(A)10
268	LOC101508797	Ethylene-responsive transcription factor WIN1-like	(A)10
269	LOC101508263	DEAD-box ATP-dependent RNA helicase 56-like	(T)12, (GT)7, (TA)21
270	LOC101507949	TC06583: O-fucosyltransferase family protein	NO SSR
271	LOC101507628	TC17069: Alba DNA/RNA-binding protein	(CA)6, (T)12
272	LOC101506997	TC17112: Lactoylglutathione lyase / glyoxalase I family protein	(T)11, (A)10, (T)10
273	LOC101507324	Uncharacterised	(TCA)21
274	LOC101506662	Peptide deformylase 1A, chloroplastic-like	(GT)6
275	LOC101506342	Uncharacterised	(GA)6, (GA)6, (A)10
276	LOC101506025	Lipid transfer-like protein VAS-like	(ATT)26, (A)11
277	LOC101505367	3-isopropylmalate dehydratase small subunit 2-like	(TTC)6
278	LOC101505048	TC18449: Ran BP2/NZF zinc finger-like superfamily protein	(A)10, (T)12
279	LOC101504724	Late embryogenesis abundant protein 2-like	(T)12
280	LOC101503652	Late embryogenesis abundant protein 1-like	No SSR
281	LOC101503980	Probable peptide transporter At1g52190-like	(T)10,
282	LOC101502685	WD repeat-containing protein 43-like	No SSR
283	LOC101502382	Peroxyureidoacrylate/ureidoacrylate amidohydrolase RutB-like	(T)14
284	LOC101502054	Cytochrome c oxidase subunit 5b-2, mitochondrial-like	(CT)7, (CT)7, (T)10, (T)13, (TA)8, (TC)7
285	LOC101501725	(6-4)DNA photolyase-like	(A)10
286	LOC101490639	Putative pectinesterase 10-like	(A)10, (A)10
287	LOC101501411	TC01826: Alpha/beta-Hydrolases superfamily protein	(GAAGA)6, (TC)7, (T)10
288	LOC101501096	Plastidic ATP/ADP-transporter-like	(CT)8
289	LOC101500573	Root phototropism protein 3-like	No SSR
290	LOC101500031	Intracellular protein transport protein USO1-like	(T)10
291	LOC101490320	TC14230: PEA3 subfamily ETS-domain transcription factor N terminal domain	No SSR
292	LOC101499720	Pentatricopeptide repeat-containing protein At1g15510, chloroplastic-like	(A)10
293	LOC101499415	Molybdate transporter 2-like	(ACC)6
294	LOC101499099	TC07708: Uncharacterised	No SSR
295	LOC101498872	TC22865: Uncharacterised	(AT)7, (A)12, (AT)8
296	LOC101498531	Pleiotropic drug resistance protein 1-like	No SSR
297	LOC101498203	Pleiotropic drug resistance protein 1-like	No SSR
298	LOC101497885	S-Norcoclaurine synthase 1-like	(T)12, (T)10
299	LOC101497551	Feruloyl CoA ortho-hydroxylase 1-like	(A)11
300	LOC101489658	Putative pentatricopeptide repeat-containing protein At1g17630-like	(T)12
301	LOC101497000	Malonate--CoA ligase-like	(T)17, (T)12, (A)10
302	LOC101496688	Katanin p60 ATPase-containing subunit A1-like	(AG)18, (A)10, (T)11
303	LOC101496134	bifunctional aspartate amiNotransferase and glutamate/aspartate-prephenate amiNotransferase-like	(T)18, (T)11, (AT)6
304	LOC101495811	50S ribosomal protein L14-like	(AAG)6
305	LOC101495483	TC21371: hAT dimerisation domain-containing protein / transposase-related	No SSR

306	LOC101495159	DEAD-box ATP-dependent RNA helicase 52C-like	No SSR
307	LOC101489332	Uncharacterised	(T)17, (A)10, (T)10, (T)10, (T011, (T)15, (A)14
308	LOC101489008	Auxin-induced protein IAA6-like	(TA)18, (TA)18, (TA), (A)12, (A)11
309	LOC101494830	N-alpha-acetyltransferase 15, NatA auxiliary subunit-like	(TC)9, 9CT)6, (T)10, (TG)7, (T)11, (AC06, (CA)6, (TA)7, (AT)8
310	LOC101488667	Uncharacterised	No SSR
311	LOC101494515	F-box/kelch-repeat protein At1g80440-like	No SSR
312	LOC101493983	TC19696: UDP-Glycosyltransferase superfamily protein	(T)11, (AT)11
313	LOC101493452	Stress response protein nst1-like	(T)11, (T)10
314	LOC101493122	E3 SUMO-protein ligase RanBP2-like	No SSR
315	LOC101488343	Tetratricopeptide repeat (TPR)-like superfamily protein	TC03944, (TTA)7, (T)10, (A)11, (T)10, (T011, (A)12, (T)10, (A)10, (A)10
316	LOC101492792	Coatomer subunit beta'-2-like	(CT)12, (T)10
317	LOC101492458	Protein TIC 20, chloroplastic-like	(T)11, (AG)7
318	LOC101492117	Probable sulfate transporter 3.4-like	(ACA)7, (T)13, (T)10, (T)10, (A)12, (T)17
319	LOC101491791	Protein SYS1 homolog	(TC)13
320	LOC101491162	Heat shock 70 kDa protein 15-like	No SSR
321	LOC101491477	Heat shock 70 kDa protein 15-like	(T)10
322	LOC101513306	30S ribosomal protein S17-like	(T)11, (A)10, (AT)14
323	LOC101490842	TC13964: Transposon protein, CACTA, En/Spm sub-class	No SSR
324	LOC101490196	TC31355: Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	No SSR
325	LOC101512979	Aspartic proteinase CDR1-like	No SSR
326	LOC101489862	TC13964: Transposon protein, CACTA, En/Spm sub-class	No SSR
327	LOC101489547	TC13872: Possible role in N-terminal protein myristoylation	(T)10
328	LOC101489331	TC06238: TMA7, Translation machinery associated TMA7	No SSR
329	LOC101488666	Mitochondrial arginine transporter BAC2-like	(TTA)6, (T)10
330	LOC101488342	Probable pyridoxal biosynthesis protein PDX1.2-like	No SSR
331	LOC101511060	Putative Peroxidase 48-like	(T)13, (A)10
332	LOC101515365	Probable ATP-dependent RNA helicase DDX11-like	(TAT)6, (T)12, (T)10, (T)11
333	LOC101514821	60S ribosomal protein L37-3-like	No SSR
334	LOC101514293	Phox-associated domain	TC04677, (T)10, (T)14, (ATT)9, (A)11
335	LOC101513957	ras-related protein Rab7-like	(T)10
336	LOC101510738	ras-related protein Rab7-like	No SSR
337	LOC101513304	Glyoxylate/hydroxypyruvate reductase A HPR2-like	(T)10
338	LOC101512436	Thylakoid membrane phosphoprotein 14 kDa, chloroplastic-like	No SSR
339	LOC101512977	Rho guanine nucleotide exchange factor 8-like	(A)11
340	LOC101511469	Probable plastidic glucose transporter 3-like	(T)11, (A)10, (T)10
341	LOC101510623	Malonate--CoA ligase-like	No SSR
342	LOC101510085	Protein SCAR2-like	(CT)6, (AT)11
343	LOC101509766	Solute carrier family 40 member 2-like	No SSR
344	LOC101509439	F-box/LRR-repeat protein 3-like	(CT)8
345	LOC101509113	Probable WRKY transcription factor 33-like	(T)10, (T)10
346	LOC101509768	CASP-like protein Os05g0344400-like	(A)10, (T)16, (TA)6

347	LOC101509441	TC11413: AmiNotransferase-like, plant mobile domain family protein	No SSR
348	LOC101508795	TC16236: Methyltransferases	(A)10
349	LOC101504947	Disease resistance response protein 206-like	No SSR
350	LOC101504628	Disease resistance response protein 206-like	No SSR
351	LOC101504406	TC00189: Embryo defective 2753	(T)12
352	LOC101504096	Macrophage migration inhibitory factor homolog	(T)13, (A)11
353	LOC101503770	TC13964: Transposon protein, CACTA, En/Spm sub-class	No SSR
354	LOC101503432	Macrophage migration inhibitory factor homolog	(CT)9, (A)10
355	LOC101503106	PRA1 family protein B4-like	(T)10
356	LOC101502785	Probably inactive leucine-rich repeat receptor-like protein kinase IMK2-like	(ATG)6
357	LOC101502166	TC11812: Herpes virus major outer envelope glycoprotein (BLLF1)	(CT)6, (T)10, (T)12, (A)12, (T)11
358	LOC101501410	30S ribosomal protein S17-like	No SSR
359	LOC101507847	TC00583: Integrase	(A)11
360	LOC101501213	TC03608: Cysteine-rich receptor-like protein; Putative protein kinase	No SSR
361	LOC101500886	TC13170: TBL (TRICHOME BIREFRINGENCE-LIKE) gene family protein	(AT)20
362	LOC101500030	AP-1 complex subunit sigma-1-like	No SSR
363	LOC101499718	Proteasome subunit alpha type-4-like	(A)11
364	LOC101499413	TC08573: SGNH hydrolase-type esterase superfamily protein	(A)11, (ATA)17, (A)10
365	LOC101499097	Abscisic acid receptor PYL4-like	No SSR
366	LOC101498435	Ferritin-3, chloroplastic-like	(T)10, (T)10, (T)12
367	LOC101506555	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
368	LOC101506227	TC06714: MuDR family transposase	(AT)9
389	LOC101497778	Gamma-interferon-inducible lysosomal thiol reductase-like	(CTT)6, (ATT)13, (T)10
390	LOC101505915	Gamma-interferon-inducible lysosomal thiol reductase-like	No SSR
391	LOC101497444	Gamma-interferon-inducible lysosomal thiol reductase-like	(A)11, (A)11
392	LOC101505587	TC09926: TTF-type zinc finger protein with possible role in HAT dimerisation domain	(A)12, (T)10, (T)12
393	LOC101497110	Ethylene-overproduction protein 1-like	(AG)6, (A)18, (A)11
394	LOC101495691	TMV resistance protein N-like	(T)11, (A)12
395	LOC101494930	Peptide transporter PTR1-like	No SSR
396	LOC101494616	Laccase-4-like	(T)11, (T)16, (A)10, (T)10, (T)11
397	LOC101496791	Nuclear-encoded mitochondrial ribosomal protein S14	TC19055, NO SSR
398	LOC101494305	Transcription factor DIVARICATA-like	No SSR
399	LOC101493773	Queuine tRNA-ribosyltransferase-like	(T)10, (A)10, (A)13
400	LOC101493451	Queuine tRNA-ribosyltransferase-like	(TG)7, (TC)8, (TA)7, (T)11, (TTA)19, (A)10, (TA)7, (T)11
401	LOC101493121	BAHD acyltransferase DCR-like	(A)10
402	LOC101492791	GDP-manNose-dependent alpha-manNosyltransferase-like	(CTT)8
403	LOC101492245	Hepatoma-derived growth factor-related protein 2-like	(T)13, (T)10, (A)10, (A010
404	LOC101491912	UPF0481 protein At3g47200-like	(AAT)18
405	LOC101490420	Protein FAR1-RELATED SEQUENCE 6-like	No SSR

406	LOC101490096	30S ribosomal protein S31, chloroplastic-like	(TA)11, (A)12
407	LOC101489545	Protein ycf2-like	(T)10, (T)11, (T)11, (GAA)14
408	LOC101489223	Chlorophyll a-b binding protein CP29.1, chloroplastic-like	(A)14, (T)10, (TA)7
409	LOC101488897	Pyridoxal biosynthesis protein PDX2-like	(CA)6, (CT)23
410	LOC101488341	E3 ubiquitin-protein ligase RNF8-like	(T)12
411	LOC101515588	selT-like protein-like	(TA)6
412	LOC101515266	Omega-3 fatty acid desaturase, chloroplastic-like	No SSR
413	LOC101508162	Unique electron-transfer flavoprotein	TC02250, (TCT)8, (T)15
414	LOC101507846	Trihelix transcription factor GT-3b-like	No SSR
415	**LOC101507532**	Leucoanthocyanidin dioxygenase-like	(A)12, (A)10, (T)10, (T010, (T012, (T)11
416	LOC101507216	PHD finger protein ALFIN-LIKE 1-like	(CT)6
417	LOC101506887	dnaJ homolog subfamily B member 1-like	No SSR
418	LOC101514612	Zinc knuckle (CCHC-type) family protein	TC07304, (T)11, (T)10, (A)11
419	LOC101506340	Symplekin-like	(CT)9, (AC)6, (TC)9
420	LOC101506024	Rhodanese-like domain-containing protein 10-like	(A)11, (T)12, (T)11, (AT)6
421	LOC101513956	Proline-rich receptor-like protein kinase PERK12-like	No SSR
422	LOC101505700	GDSL esterase/lipase CPRD49-like	(T)10
425	LOC101505366	LIMR family protein At5g01460-like	(A)17
426	LOC101504843	Protein-lysine methyltransferase METTL21B-like	(TC)15, (T)10
427	LOC101504298	LIMR family protein At3g08930-like	No SSR
428	LOC101512976	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
429	LOC101503235	Vacuolar cation/proton exchanger 3-like	(AT)18, (TA)9, (TA)10, (T)12, (TA)9, (A)10, (TA)6, (T)11, 9A)12, (T)11, (A)12, (T)10, (AT)20, (ATG)6, (TA)6, (T)12, (T)14, (A)11, (T)11, (AT)6
430	LOC101503552	Thylakoid ADP,ATP carrier protein, chloroplastic-like	(A)23, (A)11
431	LOC101512664	Probable leucine-rich repeat receptor-like protein kinase At1g35710-like	(T)15, (A)14, (T)12, (A)12, (AT)8, (CTATTA)33, (T)10, (A)11, (A)14, (CT)13,
432	LOC101511695	Beta-glucosidase 11-like	(T)10, (T)11, (T)14
433	LOC101502902	Monothiol glutaredoxin-S16, chloroplastic-like	(T)12
434	LOC101500993	Pentatricopeptide repeat-containing protein At2g13600-like	No SSR
435	LOC101511367	Putative pentatricopeptide repeat-containing protein At1g68930-like	No SSR
436	LOC101500678	EMB-1 protein-like	(T)12
437	LOC101500140	AMP deaminase-like	(T)14, (A)16
438	LOC101510737	Pentatricopeptide repeat-containing protein At4g28010-like	No SSR
439	LOC101499918	TC00125: Peroxisomal targeting signal type 2 receptor	No SSR
440	LOC101499611	Pyruvate decarboxylase isozyme 2-like	(A)12, (T)11
441	LOC101510408	Cytochrome P450 76C3-like	No SSR
442	LOC101499296	Geraniol 8-hydroxylase-like	(AT)23
443	LOC101498977	Receptor-like protein 12-like	No SSR
445	LOC101498650	Cell division control protein 6 homolog	No SSR
446	LOC101498311	Zinc finger protein ZPR1-like	(A)10, (CT)7, (AT)18, (T)11
447	LOC101497777	Receptor-like protein 12-like	No SSR

448	LOC101497443	Tubulin beta-1 chain-like	(TC)6
449	LOC101497109	Leucine-rich repeat receptor protein kinase EXS-like	No SSR
450	LOC101496790	UPF0098 protein MTH_273-like	No SSR
451	LOC101509438	UPF0098 protein MTH_273-like	(A)13
452	LOC101509112	Eukaryotic initiation factor 4A-8-like	No SSR
453	LOC101507741	Putative pentatricopeptide repeat-containing protein At1g12700, mitochondrial-like	No SSR
454	LOC101507436	50S ribosomal protein L19, chloroplastic-like	No SSR
455	LOC101495363	Ankyrin repeat-containing protein At3g12360-like	No SSR
456	LOC101507114	Ankyrin repeat domain-containing protein 27-like	(A)10
457	LOC101505585	serine acetyltransferase 3, mitochondrial-like	(A)12
458	LOC101495035	aspartic proteinase CDR1-like	No SSR
459	LOC101494400	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
460	LOC101493772	Extensin-1-like	(A)10, (A)11, (A)10
461	LOC101492457	Calponin homology domain-containing protein DDB_G0272472-like	(T)11, (T)13, (A)10
462	LOC101503978	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
463	LOC101492116	ABC transporter C family member 3-like	No SSR
464	LOC101491790	ABC transporter C family member 3-like	No SSR
465	LOC101491161	DUF21 domain-containing protein At1g55930, chloroplastic-like	No SSR
466	LOC101502901	Vacuolar protein 8-like	(A)11
467	LOC101502584	Disease resistance protein RPM1-like	No SSR
468	LOC101502270	Zinc finger CCCH domain-containing protein 18-like	No SSR
469	LOC101490840	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
470	LOC101501723	Scarecrow-like protein 5-like	No SSR
471	LOC101501211	Probable WRKY transcription factor 20-like	No SSR
472	LOC101490194	Serine/threonine-protein phosphatase 7 long form homolog	No SSR
473	LOC101500884	Probable serine/threonine-protein kinase WNK11-like	(TA)6
474	LOC101500570	Heterogeneous nuclear ribonucleoprotein 1-like	(T)14, (A)11
475	LOC101500255	Protein TORNADO 1-like	No SSR
476	LOC101499515	TC16729: Calcium-dependent lipid-binding (CaLB domain) family protein	No SSR
477	LOC101499192	3-ketoacyl-CoA thiolase 2, peroxisomal-like	(T)10, (T)10, (A)10
478	LOC101498648	Kinesin-like protein KIN12B-like	(TC)18, (T)14
479	LOC101498309	Probable cytokinin riboside 5'-moNophosphate phosphoribohydrolase LOGL3-like	No SSR
480	LOC101489222	TC08532: Putative lysine decarboxylase family protein	(T)13
481	LOC101497776	TC17691: Ankyrin repeat family protein	(T)11, (T)12
482	LOC101497442	Disease resistance protein RPM1-like	No SSR
483	LOC101497108	Disease resistance protein RPM1-like	(A)11
484	LOC101496356	Leucine-rich repeat receptor protein kinase EXS-like	No SSR
485	LOC101496017	BAG family molecular chaperone regulator 4-like	(CTT)9, (T)10, (TA)18, (A)10,
486	LOC101515464	Ammonium transporter 2-like	No SSR
487	LOC101495033	Pentatricopeptide repeat-containing protein At3g16610-like	(A)14, (T)10, (A)10, (A)10, (T)10, (A)13
488	LOC101493449	Probable receptor-like protein kinase At5g15080-like	(T)13
489	LOC101492910	Protein MAK16 homolog B-like	No SSR
490	LOC101491278	L-type lectin-domain containing receptor kinase I.7-like	No SSR

491	LOC101490638	Pentatricopeptide repeat-containing protein At1g80150, mitochondrial-like	(T)11
492	LOC101514176	Pentatricopeptide repeat-containing protein At1g80150, mitochondrial-like	No SSR
493	LOC101490966	Pentatricopeptide repeat-containing protein At1g52620-like	No SSR
494	LOC101513830	Pentatricopeptide repeat-containing protein At1g52620-like	No SSR
495	LOC101513523	Probable transcription factor PosF21-like	(A)11, (A)14, (TA)6
496	LOC101490319	Tubulin beta-2 chain-like	(TC)8, (T)11
497	LOC101512875	18.5 kDa class I heat shock protein-like	No SSR
498	LOC101512570	60S ribosomal protein L36-2-like	No SSR
490	LOC101489984	Heme-binding-like protein At3g10130, chloroplastic-like	No SSR
491	LOC101489454	Immediate early response 3-interacting protein 1-like	(A)10
492	LOC101511260	TC20678: Gag-pro	No SSR
493	LOC101489122	Fructokinase-2-like	(T)17
494	LOC101488794	TC19072: O-fucosyltransferase family protein	(A)16, (T)10, (T)10, (T)11
495	LOC101515697	Laccase-7-like	(A)10, (A)10, (A)11
496	LOC101508381	TC06117: Cysteine-rich receptor-like protein; Putative protein kinase	No SSR
497	LOC101513082	Uracil phosphoribosyltransferase-like	(TAT)8, (TTG)6
498	LOC101512568	delta-1-pyrroline-5-carboxylate synthase-like	(T)12, (AT)14, (AT)9, (T)10, (AT)14, (AT)9, (T)10, (A)11, (ATT)6, (A)10, (TA)8, (A)10, (T)12
499	LOC101512240	Glucose-induced degradation protein 4 homolog	(T)12, (A)10
500	LOC101511579	UPF0160 protein MYG1, mitochondrial-like	(T)10, (A)10
501	LOC101511059	F-box/LRR-repeat protein 13-like	(A)10
502	LOC101510508	Aldo-keto reductase family 4 member C9-like	(T)11, (T)10
503	LOC101510180	CASP-like protein At3g53850-like	(T)11
504	LOC101506338	Chaperone protein DnaJ-like	(T)11, (T)12
505	LOC101506022	Putative gamma-glutamylcyclotransferase At3g02910-like	(T)11
506	LOC101505698	Nuclear anchorage protein 1-like	(GT)7
507	LOC101505364	Transcription factor SPT20 homolog	(T)11, (A)13, (TC)19
508	LOC101505046	CASP-like protein RCOM_1174750-like	(TA)11, (A)13
509	LOC101504721	Riboflavin synthase-like	No SSR
510	LOC101504404	TC15374: EXORDIUM like 3 (EXL3)	No SSR
511	LOC101507947	Protein FAR-RED IMPAIRED RESPONSE 1-like	No SSR
512	LOC101503551	Signal recognition particle 14 kDa protein-like	(TC)7, (T)11
513	LOC101503103	TC00644: Cysteine-rich receptor-like protein; Putative protein kinase	(T)12, (A)24, (T)14
514	LOC101502164	Adenylate kinase-like	(T)17
515	LOC101501841	Putative pectinesterase/pectinesterase inhibitor 26-like	No SSR
516	LOC101501210	Transcription factor GTE8-like	(TTC)8, (TTA)13, (ATT)14, (TTG)5, (T)11
517	LOC101496789	Putative E3 ubiquitin-protein ligase XBAT31-like	(A)11
518	LOC101498975	Zinc finger MYM-type protein 1-like	(A)12

APPENDIX-XVIII: List of primers designed for genic SSR motifs in the chickpea genomic region flanked by CaM1402-CaM1101 on chromosome 6

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
LOC101494099	(AT)21	398	AGGGAACAACACCCAGATAGTC	22	TTAAGAAGGGTTGGGCTCAC	20
LOC101494099	(AT)6	345	GCCATCTACGTTGTCTCAGGTA	22	TCAACAAACTCCTTCACTCCAA	22
LOC101511474	(CT)10	201	ACAGAGTCACGGTTGTTGATGT	22	ACCCTTTGCAGGTGATATTTTG	22
LOC101510627	(AT)6	391	GTAATTCCGTATCAGCATTTCG	22	ATCATTGGGAGATGGAGAGAG	21
LOC101504408	(CT)6	304	TGTTTTGCTATACGCCAGCTAA	22	TCAACAACCTTCACACAACTCA	22
LOC101503773	(AC)6	133	CAACGCACTCTTTCTTTCTGTG	22	TGAGAGCTAAGGGATCTTTGGA	22
LOC101503434	(CAC)6	372	AGAAGGATGTGCAAAGAATGGT	22	CATCAGGAAGCTCTGCTCTGTA	22
LOC101513415	(TTAT)11	195	GGTCAAGCCCAATAAATCACTC	22	CGTTGCACATCCACATTCTACT	22
LOC101498314	(CT)9	365	TCTCACCACCAACAATAGCAAC	22	AACCCTCATCAGCCATAGAAAA	22
LOC101498314	(TAA)7	363	ATGATGCGTGGAGAAGGAAA	20	CACACCACCACTACCATAAATCA	23
LOC101498314	(AAT)12	387	GCTCCAAAAGATTCACAAAAGG	22	AGCTGCACTTAATGGAAATGGT	22
LOC101497987	(GAT)10	278	AAGTCCTTAATGTATCTCGCGG	22	CTTCCTTGTTTTGCAGCTTTCT	22
LOC101501846	(TA)11	348	GCGCTGTAAAAGACCTCCTTAT	22	TGACCTGCCATAACTGATTTTC	22
LOC101499300	(TTAT)7	398	AAACTCAGCATTTTAGGCCG	20	GAAGGCTACCACTACGCAATCT	22
LOC101490200	(AT)8	181	TTTGCCTTTCTCTACTCTCGCT	22	GATGACTTGGTTGATGAGCTTG	22
LOC101515043	(AT)6	318	AGGGTTGTCTTTGCTGTCTGAT	22	TCAAGAATGTGAATGTGGGAAG	22
LOC101512758	(GT)11	202	GTGTCATGTTCGGTGATGTCTT	22	TCTCTCTCAAGCCAAACCTTTC	22
LOC101506664	(AT)8	233	AGTGCAGGTGTAAATCATGTGG	22	CCTTCGTACTCATCCTCTAAGGC	23
LOC101500576	(TA)8	174	ACCAAGTGTATGGGACACCTTT	22	ACATCTCCATTGCTTCCATCTT	22
LOC101506343	(TA)7	174	ACCAAGTGTATGGGACACCTTT	22	ACATCTCCATTGCTTCCATCTT	22
LOC101505370	(AT)6	400	AGCAAGGTCTTCAGGGTATTTT	22	AAAGGCACGAAGTTTCATCA	20
LOC101498775	(TA)6	395	TTCACGCTCTGTTTCTCAGTTC	22	ATAAGGTTGGTGTTGTTTGGGA	22
LOC101498313	(AT)13	377	GACAAACCTTGAAAGCACATGA	22	AGGAGGGGTGTTGACATATAGC	22
LOC101498313	(CTT)6	264	TGGAACTCAAAAGTGCAGAAGA	22	CATGGCACACTAAGGAAACTTG	22
LOC101498313	(TA)9	354	TGTTTTAGGGTCCAGCTAGGTT	22	GTTTGACACCTCCCTCTCTGTC	22
LOC101497651	(CAA)7	342	GAGCATCTTTTCAATCGACCTT	22	AGGTGGTCTTCCAGAACTTTGA	22
LOC101495484	(TAA)7	348	ACCCTCTAATGCAACATCATCA	22	TATAAATCCCACTTGACCCACC	22
LOC101504726	(TA)19	305	TTTTCTGAGGCAACATGCTCTT	22	CCGAATGACAAGATTCCACACT	22
LOC101493244	(AG)11	374	AACCAGCTTCACCAAAGTGTCT	22	ATTCGTCCTCGTAAGGAATCAA	22
LOC101491914	(AT)6	396	CTGGAATCTCCCATTCGGT	19	GAGAACTTAGAGAAGGTCAATGCAA	25
LOC101515041	(AT)13	374	CAACTGGTCATATCTCTGGTGC	22	CCCCAAGAATACACGTAACAAA	22
LOC101514179	(T)53	238	CACAATTTCCCTTTCTCTGCTT	22	TCTTACCCTGACATTTTCCTTGTAG	25

LOC101489660	(AT)13	293	TTCCTATTCTCCCAATGCAA	20	GTACTCATCTTGCGGGTTTAAT	22
LOC101489660	(A)24	380	GTTGGGAGAGTTGAAACATCA	21	TTCTGCATTCATTTCGAGTG	20
LOC101510511	(ATT)26	271	TGCTGGTTACACCTTGCTTATG	22	TCTAAAGCCAAAGGAATGGAAG	22
LOC101510183	(ATA)8	297	CTCTTTTGCATGAATTGGAGC	21	GCCACACACAGAACTCAACTTT	22
LOC101509864	(CTT)6	157	GGCTATTTCATCTCCACCTCAC	22	CGAAGGTTCTCTTTCGGTTTTA	22
LOC101508068	(GAT)6	170	CAAACTGAACCAGATCAACCAG	22	TCAGAAATCCAGTCCCAATCTT	22
LOC101502686	(CT)14	209	ATTAACCATCGCGCTCTCTCT	21	TGGATCAGAAATGACGAATCAC	22
LOC101502383	(TA)15	242	TTATCCCACGCGGTGTTATT	20	TGTGGAGTGCTTTTGTCTCACT	22
LOC101500369	(GAG)6	378	GACAAAGTTGAGGTTGTGGAAA	22	ACGAGGTGATTAAAGCCAAAAG	22
LOC101499518	(AG)10	106	AGAGAGAGAGAGGGAGATGGGA	22	CCAATCATAACAACTAAGCAGCG	23
LOC101500032	(TC)6	183	TTTGTTCTCTTTACCTCAACGC	22	CATCATCTTGTTGTGTCTCCAA	22
LOC101498874	(TA)10	342	TTTCATATAGCAGGGTGTTGGT	22	ACACGAAACATCACAGGACAA	21
LOC101498533	(AT)7	253	CTATGACTTGGCAGCATTGAAG	22	GATAGGTTCTCCATCTCTTTGAGC	24
LOC101497985	(TA)12	226	TATGGTTGAGTTTTGGGTAGGG	22	ACGTGGGTTACGTGAAATCAG	21
LOC101497650	(TAAT)6	283	GGACCCTATTGCCTAAATCACA	22	CTCCCTCCGTTTCTAAATACAAGA	24
LOC101495584	(TGA)6	231	TCGTTGATCGGTCTGTTGTTTA	22	GAAAAGTCCAGCCCTTGTACC	21
LOC101493560	(T)17	327	CAGAGAACACCAAATCCAGCTT	22	AATTCACCAATCAGTGTCAACG	22
LOC101493243	(CTT)7	332	TGTAAATGTGTGAACCTCGCTC	22	GACGAAAACGAGAACGAAGAAG	22
LOC101492347	(ATCT)22	355	ATGTGTGTTGTATCCATTGCGT	22	GATTGATGCCTAAAATGCAGGT	22
LOC101492347	(AT)14	298	GTTAAAGAGTGGGGCTGGTTG	21	GCAACAAACTTAATGAATGGGG	22
LOC101492347	(AT)9	249	GTCATATTCCTTGGATGCACAA	22	GTCTGCACGTCGAACAATTTTA	22
LOC101492011	(TG)9	198	CACAATATGCCACCTGATAGTTATG	25	CGGTGACGAAAGATGATTTAGA	22
LOC101507117	(TTA)11	317	TTGTCTTTCTATCAGTGGGCAA	22	ATTAGCGTCAATTTCGAGGATG	22
LOC101507117	(ATA)9	380	AACAATCAAGACGCTAATTGGG	22	GGATCGGGGTAGCATAACAAC	21
LOC101507117	(AAC)9	380	AACAATCAAGACGCTAATTGGG	22	GGATCGGGGTAGCATAACAAC	21
LOC101514823	(TA)9	397	ATCAAAGGCAAACGAATAGCC	21	CCAAAATTCCACCACATCTAGC	22
LOC101514502	(TA)9	397	CCAAAATTCCACCACATCTAGC	22	ATCAAAGGCAAACGAATAGCC	21
LOC101515149	(T)14	310	GAAGCGGTTTGTTATTCCCATA	22	TGACCATGACTATCCCTCTCAA	22
LOC101510625	(TAT)20	278	TGCAACGTAGAGCAATTTTGAC	22	TCATTTCCATGACTTTCTGAGC	22
LOC101509650	(TAT)22	391	ATTAGAGGCAAACAAGAACCGA	22	ATGTTGAAGTTGGGAAAACGAC	22
LOC101509650	(TTA)14	239	TTTCAAGTTTTCAACCTAGCGG	22	ATTCTCGTGCCAAACATTACCT	22
LOC101509650	(AT)13	342	CAAGGAGAAGCAAGAAGGAAGA	22	TGAAGAATTGGACACCAGAGAG	22
LOC101509650	(TA)8	354	TTTCTCCCTAACTCTCACGCTT	22	CGTGCTCGTACTCCATTGTAAA	22
LOC101509650	(TAA)6	240	CCAGGTGCAATAGGAAAATCA	21	CCATCTGTCTCGTTCACATACC	22
LOC101506779	(TTAT)6	263	ATAAATCGCTCTCTTGGCACAT	22	GCACAGCAGACAGGACATTAAG	22
LOC101506454	(CT)10	246	CGATTCTATGGTTCCTTCTTTG	22	TCTTCGGTTCCTAAATCACTCA	22
LOC101506124	(TA)11	387	TATGTGTGTGTGCCAACTTTGA	22	TTAAACACGGGACTTACAACCC	22

LOC101506124	(AT)15	400	CGTCTTATTGCGGTGTACTTTG	22	GTCTTGTGCGAATTGTCAGGTA	22
LOC101506124	(AT)12	335	GGGGAGGAATTGGTTAAGGG	20	GGAGGGAGGACAACATCAACTTA	23
LOC101500679	(TTATT)6	287	GATTCACCGCAAATTCAGACA	21	AATGACCCCACACACCACTAAT	22
LOC101500368	(TC)7	370	CAAGTTCAAATGTCCCCTTCTC	22	TGTACCTAAAGTGTCAGCCCAA	22
LOC101499815	(TC)9	287	GTCAATTTTCCTATGGCGTCTC	22	ATCGCAAAGATAGTCGCAGAAT	22
LOC101499815	(GT)8	114	TTTATGGAGGGTATGAATTGCC	22	AGCATCGTTCCTTTACATCACA	22
LOC101499517	(TA)14	354	TGATCTAATGAGTGCTGGGTTG	22	CATTCCAAGAAAAGGATTCAGC	22
LOC101498873	(TC)13	353	ACACTTCCCCATTCCTCAAAC	21	GCCCATTATTGTTTCTGTTGGT	22
LOC101498873	(ATG)6	305	ACCAACAGAAACAATAATGGGC	22	TAGGAGAGGGGTGCAAATAGAA	22
LOC101504845	(AT)11	378	AAAATCTAGCCAGCCCATAAGC	22	AATATAGCGAAGGAAAATCGGC	22
LOC101501309	(AT)9	328	TGAACAGAGGAAGAAGATGGAA	22	TGATGCCGTTTAGAATTACTCG	22
LOC101508263	(TA)21	395	CATACAAATATGGTGGGTGGC	21	CAATGCAAGGACAGGAACATAA	22
LOC101508263	(GT)7	239	AGATGCTGGAATCACTTGGTCT	22	TATCTTTGCTGAGTGTAGCCGA	22
LOC101507324	(TCA)21	314	AATGGAAAGGAAGAGAGCAACA	22	TATTGTCCCCTAGCAAGCATTT	22
LOC101506662	(GT)6	281	AAAGCTCCTGGTGTTGGTTTAG	22	CTACTATGACAGCGCCTCACAC	22
LOC101506342	(GA)6	214	ACCAAGAGACCTAGAAACTTCCAC	24	ATCTGTCATCCCAAACAAGACC	22
LOC101505367	(TTC)6	398	AGGGAACCAGAAAGAGTGAGTG	22	CAATTAAAGCAAAGGAACCGAG	22
LOC101502054	(TA)8	201	CTTTCAGAGGAAAACGAACGAT	22	GGAAGGGAGGCTATAAAATGCT	22
LOC101502054	(CT)7	400	GAGCGCGAGGAGATTCAG	18	CTGAGGTGGGAGAGTTAAGGAG	22
LOC101502054	(CT)7	176	TGTTTCTTTACACTCCCTCCGT	22	ATTGGCACTACATCCTCAACCT	22
LOC101502054	(TC)7	264	GTGTCATTGGCTTTGAGATTGT	22	AACAACAGCAGGTTCGTCCTAT	22
LOC101501096	(CT)8	356	GCCGTCTCTCTAAAAGGAATGA	22	AACAATGACAAGAACAAAGGGG	22
LOC101499415	(ACC)6	259	ACATCATCTCCAACAACGAACA	22	ATAGGAAGCCCAAATAAAAGGC	22
LOC101498872	(AT)7	372	AACAATGCTGAAGGATAGGCAT	22	TGGGAATGTTGAAGTTACAGAGG	23
LOC101498872	(AT)8	361	GTGGAGAGATGGTGATGATGAA	22	TGGGAATGTTGAAGTTACAGAGG	23
LOC101495811	(AAG)6	365	CAGAGGAACAAAACAGGGAAAC	22	ATAGCACCCTAAACACGCTGAT	22
LOC101489008	(TA)18	395	ACTGGATGCTGGTTGGAGAT	20	GTTGGCTTGTTCCCATCTTTT	21
LOC101494830	(CT)6	178	TTCTCTCTCTCTCTCTCTCGCA	22	CAATAAGCAAACAGGAATAGCG	22
LOC101494830	(TG)7	244	CAGCCATGACAACCATTGATAA	22	AAAGAAACCACTGCAACAAAGC	22
LOC101494830	(CA)6	354	CAAGTTTCAACACACACACACG	22	TGTCAACCTGGGAGAATCCTAT	22
LOC101493983	(AT)11	155	ATGCAAAAGCTCTCCAAGTAGT	22	AGGTTTCTCCCAATCCCTATAC	22
LOC101488343	(TTA)7	305	AGACGGAGGAGAATGACTGAAA	22	AGGCAAGGTGAAGAAGCATAAC	22
LOC101492792	(CT)12	217	ATCTGCAAAACACCACTCTCCT	22	TCGAAGAGGAAACTCACCATTT	22
LOC101492458	(AG)7	344	GACCCTGTTTGGATTAGCAACT	22	TTTTCACACATCCTTGTTGAGC	22
LOC101492117	(ACA)7	162	CACTTATTTCACACACACACTCTCTC	26	TTGTCCCAAAAGGATCAACTCT	22
LOC101491791	(TC)13	258	CCCCAATTCTCTTCCCAATAA	21	ACAATGGTTCACGTTCTTTCAG	22
LOC101513306	(AT)14	265	GGAGATGCGACCATATACACAG	22	TCTGCAAAATAGGATAGCGTTG	22

LOC101488666	(TTA)6	349	TAGCAAACAAAATAGCCACCG	21	TATGGGCACTTTACAATCTTGC	22
LOC101515365	(TAT)6	243	TAACCCTAACCCTAACCCCAAT	22	ACAGCATCTTCTTCTTCTTGCC	22
LOC101514293	(ATT)9	377	CTGTTGACACATGGCTTATTGC	22	TCGTATCCAGGCCAACTAAAAT	22
LOC101510085	(AT)11	239	CCTTCTTGATAAGTATGTTGGCCT	24	CTGAGGACTTGTACTGTGGCTG	22
LOC101509768	(TA)6	348	TTTTGATTAGGTGCAAGTGTGG	22	AGACATAGCAACCGATTTAGGC	22
LOC101503432	(CT)9	259	TTCCATCCTCTCTGAAGCTACC	22	TTACAAAGCTGAAAACCCCTTC	22
LOC101500886	(AT)20	317	GGACCTAGTTTTCCCGTCTTCT	22	TGACAAGGACTATTTTGAGCGA	22
LOC101499413	(ATA)17	395	CTTTGAGGACTTGGTCAGATTG	22	CATGTTTCGTTATAGGAGGAGC	22
LOC101497778	(ATT)13	365	GTTGGAATGTTGCGAAGATGTA	22	GTTTTGTCCTTTGCGTCTCTCT	22
LOC101497778	(CTT)6	260	CCTCAACGCTCATTCTTCTTCT	22	GTACCATTAGGTGCAATCCCAG	22
LOC101497110	(A)18	337	TCCTTCTTATTCCCTTTCACCA	22	CTACGGTGTCATTGGAAGATCA	22
LOC101493451	(TG)7	277	AGTCACCAGTAGATGGAAAGCC	22	AAAATAACAGGGAGATGATGGG	22
LOC101492791	(CTT)8	114	TTCCTCTCTCTCTCCTCCTTTTC	23	AAGGGTGTAATGGGTTGTTCTC	22
LOC101490096	(TA)11	388	GTTCTCTTTTCCCATTTTACCC	22	CAATTCACCACTACGCCACT	20
LOC101489545	(GAA)14	335	GCGGTTCAAAAGAGTGAAGAAT	22	CGTCATACTCATCAAGCGGTTA	22
LOC101488897	(CT)23	391	TGAAAGCACCACTATGGCTAAA	22	TGTCAACTTTATGTCTCCGATAGC	24
LOC101508162	(TCT)6	309	TTCCAATGAAGAAAAGTGACCC	22	TTGAACCAACATCACCATATCC	22
LOC101506340	(CT)9	236	GAAGCCAGTCATAGTTTGGGTC	22	ACTTTGGGCAGAAAATGAACAG	22
LOC101506340	(TC)9	389	AAATTGCACCAAACCAGTAACC	22	TCCTACCATTATTCACGCGG	20
LOC101506340	(T)44	368	TCTTCACTTCCCAGGTTCCTTA	22	TGAGTTGCTTGTCTCACCAGTT	22
LOC101504843	(TC)15	283	TACATTCTCTGCATCATCGCTC	22	CCAAAACAAAACACACATGAGG	22
LOC101503235	(AT)20	397	ATTCAACTCGGGACCTTACTG	21	AAAGGGTACATAACACAATGCC	22
LOC101512664	(CTATTA)33	368	AGTGGAAGTAGAGGGATTAGACGA	24	TGTAATGTGATTGGTAGACCGC	22
LOC101499296	(AT)23	226	GCGGCCACACACCTTATT	18	AAAATAGGAGGGCTAATTCCGT	22
LOC101498311	(AT)18	325	TGCAGCTTCTTACCTTGGAAAA	22	TTCGGTGGTAGTAATTCAACGAG	23
LOC101501211	(TC)20	356	CACTTTTACCCCTCTCCATTTG	22	AGAGTAGCACTGGGGATTCAAG	22
LOC101498648	(TC)18	397	CTTCTTCCCTCTTCTTCCAACA	22	ACCTGATCCAAAACAACACACA	22
LOC101496017	(CTT)9	396	AAGTTGCAGAGAAAAGCTGAGG	22	TGTGAACATACCAAACCCACAT	22
LOC101496017	(TA)18	315	CAATGTGGGTTTGGTATGTTCA	22	GGTGGAGTGTGCAAAGGTATTT	22
LOC101490319	(TC)8	176	CAAAACCCTTTCGAGTTTCA	20	CAGAGTCACCAACGTATCTTCC	22
LOC101505364	(TC)19	205	TGCAGAAGATATGGGGAGTTTT	22	AAAGCTAGGAAACATAGGCACG	22
LOC101505046	(TA)11	325	GTGGATATGGTAATGTCCTGAAAG	24	TGAGTCCCACAATTTAGAACGA	22

APPENDIX-XIX: List of genes and genic SSRs present in chickpea unplaced genomic scaffold

Sl. No.	Gene Id.		SSR motifs identified
TA22 on Scaffold 96			
1	LOC101499861	Flocculation protein FLO11-like	(A)10, (TA)13, (T)12
2	LOC101500195	Zinc finger MYM-type protein 1-like	(T)10, (TA)14, (T)11, (AT)22, (T)11
3	LOC101500833	Uncharacterized	No SSR
4	LOC101501789	Uncharacterized	(T)11
5	LOC101499564	Uncharacterized	NO SSR
6	LOC101502113	Caffeoylshikimate esterase-like	NO SSR
H4G11 on Scaffold 174			
1	LOC101510996	Zinc finger A20 and AN1 domain-containing stress-associated protein 8-like	(T)10, (T)12
2	LOC101511520	Chaperone protein dnaJ 6-like	(AT)8
3	LOC101512384	Serine/threonine-protein kinase Nek5-like	NO SSR
4	LOC101512056	Pentatricopeptide repeat-containing protein At3g49740-like	NO SSR
5	LOC101513245	Carboxyl-terminal-processing peptidase 3, chloroplastic	(TC)6, (A)10
6	LOC101512918	Arabinogalactan peptide 23-like	NO SSR
7	LOC101513574	High mobility group B protein 6-like	(T)10, (T)10
8	LOC101515743	Uncharacterized	NO SSR
9	LOC101488506	Uncharacterized	(TA)7, (A)14, (A)12, (A)10
10	LOC101513884	Zinc finger protein WIP2-like	(TA)6, (A)11
11	LOC101514228	Uncharacterized	NO SSR
12	LOC101488843	DNA-directed RNA polymerase I subunit rpa1-like	(ATG)8
13	LOC101489165	Uncharacterized	No SSR
14	LOC101514434	DNA-directed RNA polymerase I subunit rpa1-like	(A)16, (T)10, (T)11, (CA)6, (ATG)6
15	LOC101514758	1-acyl-sn-glycerol-3-phosphate acyltransferase 2-like	(TAT)6, (T)10, (A)13, (A)10, (T)15
16	LOC101489814	Uncharacterized	(T)10, (A)12, (T)12, (TA)9
17	LOC101515084	Probable helicase MAGATAMA 3	(CT)7, (T)10, (T)10, (T)11, (T)12, (T)10
TA186 on scaffold 306			
1	LOC101510780	GTP-binding protein ERG	(AT)8
2	LOC101510449	Transcriptional regulator ATRX homolog	(T)12
3	LOC101511411	Zinc finger protein 4-like	(CT)10, (T)36, (T)44, (TA)6
4	LOC101511106	Imidazoleglycerol-phosphate dehydratase	(T)21
5	LOC101510127	CST complex subunit STN1	NO SSR
6	LOC101513887	Methanol O-anthraniloyltransferase-like	(AT)9
7	LOC101514232	13-hydroxylupanine O-tigloyltransferase-like	(T)12, (A)20, (A)11, (A)10, (A)11, (AT)9
8	LOC101511955	13-hydroxylupanine O-tigloyltransferase-like	(T)18, (T)12, (AT)14
9	LOC101514546	Uncharacterized	NO SSR
10	LOC101514873	13-hydroxylupanine O-tigloyltransferase-like	NO SSR
11	LOC101512283	13-hydroxylupanine O-tigloyltransferase-like	(AT)12, (T)15, (A)11, (T)10, (A)15, (TTA)16, (TTA)18, (A)14, (A)11, (A)12, (T)8, (T)12, (A)13, (A)13
12	LOC101513249	13-hydroxylupanine O-tigloyltransferase-like	(A)12
13	LOC101515200	Uncharacterized	NO SSR
14	LOC101515520	Plastid division protein PDV2-like	NO SSR
15	LOC101513577	13-hydroxylupanine O-tigloyltransferase-like	(AT)9

16	LOC101488270	Putative uncharacterized protein DDB_G0290521-like	NO SSR
17	LOC101488601	Uncharacterized	NO SSR
18	LOC101488951	Probable ascorbate-specific transmembrane electron transporter 1	(A)14, (T)10
TA96 on scaffold 766			
1	LOC101499861	Flocculation protein FLO11-like	(A)10, (TA)13, (T)12
2	LOC101500195	Zinc finger MYM-type protein 1-like	(T)10, (TA)14, (T)11, (AT)22, (T)11
3	LOC101500833	Uncharacterized	NO SSR
4	LOC101501789	Uncharacterized	(T)11
5	LOC101499564	Uncharacterized	NO SSR
6	LOC101502113	Caffeoylshikimate esterase-like	NO SSR
EST SSR65 on scaffold 7355			
1	LOC101491980	Uncharacterized	(CT)7

APPENDIX-XX: List of primers designed for genic SSR motifs in the chickpea unplaced genomic regions

Gene Id.	SSR motifs	Product size (bp)	FW-Primer 5'-3'	FP length (bp)	RV-Primer 5'-3'	RP length (bp)
TA22 on unplaced genomic scaffold 96						
LOC101515513	(ATT)6	255	TCCCGAGAAATTAGAACGAAAC	22	CCAGTTTTCCCTTTCCATATCA	22
LOC101490890	(TA)8	397	TATTATCTGCGCTATGCACGG	21	TAAAATGAGGGGACCAAAACTG	22
LOC101490890	(AT)9	202	CTTTTCGTTTCCTTTTCCTTCC	22	TAAGTGCGGTGAATCCTTTACG	22
LOC101490890	(TA)18	333	ATAGCACGGGGTCATATAATGG	22	TCCCCATCAGAACCTTCGTA	20
LOC101490890	(AT)7	312	TTTTCACACTCTTTGGCCTCTC	22	GCCGCAACTCAAATGACAA	19
LOC101490890	(TA)16	267	ATTTGTCATTTGAGTTGCGG	20	ACACAAGGCTATGGAGGAAAAT	22
LOC101490890	(AT)11	281	TCAGACACTACCCGACATCAAG	22	ATATTCGACCGTTTTCTGCACT	22
LOC101489703	(CT)6	295	AACAGGGAACCAATACAAAGGA	22	GAAACCTGAATAATGGGCTTGA	22
H4G11 on unplaced genomic scaffold 174						
LOC101511520	(AT)8	303	CATCTATGTATGGTTGTGTGGTCA	24	TTTGGCTTTAGCTTCCTACCAA	22
LOC101488506	(TA)7	368	GTGCTGTTAGGCGTGTAAACAA	22	AGGTTAGTTGTCATGTGTCCCC	22
LOC101488843	(ATG)8	361	AGTTTCCTTTGTGAGAGGGTTG	22	CCCATCATCTTTTACATGCTCA	22
LOC101514434	(ATG)6	192	CAGATGCACAACCAAATTCTTC	22	GTGTTTCCTCTTCAGGACCATC	22
LOC101514758	(TAT)6	182	AGGAAGAGAAGAGGATTGGCAT	22	GCCAGAAGCGAAGAAGAGAATA	22
LOC101515084	(CT)7	399	CCTCGTCATTTTCTCCATTCTC	22	CCACCTTCTTCTTCTTTCCCTT	22
LOC101489814	(TA)9	378	GTCTCCATTCTTCCGATTTCAT	22	CTCTCAATTCTAAAACCCAGCA	22
TA186 on unplaced genomic scaffold 306						
LOC101510780	(AT)8	356	CCTCAGACACAATACCACGAGA	22	AGACACCGCTAATGTCCAAAAT	22
LOC101511411	(CT)10	106	GACTCCTCTGACTTTGCTCCAT	22	ATTATGCTACTTGGGACGTTGG	22
TA96 on unplaced genomic scaffold 766						
LOC101499861	(TA)13	396	TTGAGATGTGAGTGAAGTGATGTG	24	AAAATGAAGACGACTGTTTCGG	22
LOC101500195	(TA)14	303	TCTTCGCACTCTTCAGGTATTG	22	CCGCAGTCTATGTTGTTAGTTTG	23
EST SSR 65 on unplaced genomic scaffold 7355						
LOC101491980	(CT)7	353	AAACACCACCCTCACAAATCTT	22	GTTCAAAGGGGCATAGAAAGTG	22

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Development of Novel Microsatellite Markers Using Genome Sequence Information in Chickpea (*Cicer arietinum* L.)

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Abstract

Fusarium wilt (FW) is a major disease of chickpea (*Cicer arietinum* L.) limiting the productivity in all the chickpea growing areas. Several DNA markers linked to FW resistance were reported earlier. However, these markers were identified based on limited size of mapping population and limited number of markers representing wide genomic region and not reliable for Marker Assisted Selection (MAS). In the present study, QTLs and/or markers linked to wilt resistance in genetic msap were *in-silico* physically mapped on chickpea genome sequence. Majority of FW linked markers mapped to chromosome (Ca) 2, 4 and 6 in sequence based physical map. The highest number of markers and QTLs were located on Ca2 indicating hotspot for wilt resistance. Scanning of genomic regions of FW resistance flanked by TA110-H3A12, TA200-TA37 and TA27-TA59 on Ca2, and CaM1402-CaM1101 on Ca6, covers a region of 14.41 Mb and 13.58 Mb, respectively covering 1027 genes and 1385 genic SSR repeats. Four hundred and nine genic SSR primer pairs were designed from identified genes and out of which 165 primers were synthesised and checked for amplification in chickpea. All newly designed primers except seven gave expected amplicon size and hence will be used for fine mapping the *Fusarium* wilt resistance loci.

Chickpea is the second most cool season legume crop after common bean (*Phaseolus vulgaris*) with a genome size of H"738Mb (Varshney *et al.,* 2013). Even though chickpea production has increased, the productivity (962 kg/ha) has remained low (Anonymous, 2016). The low productivity is due to several biotic and abiotic stresses that affect the crop at all growth stages.

Fusarium wilt caused by soil borne fungal pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) is a serious pathogen of chickpea. Among eight race of FOC (0, 1A, 1B/C, 2, 3, 4, 5 and 6) (Jimenez-Diaz *et al.,* 1993), race 1 is predominant in peninsular India. On an average the annual yield loss from this disease is estimated to be 10 per cent which can go up to 90 per cent under favourable conditions (Singh and Reddy, 1991). Being soil borne, the management of FW is difficult. Cultivation of FW resistant varieties can be an effective management strategy to reduce the yield loss. However, development of resistant varieties for wilt through traditional breeding methods and maintenance of wilt sick plot is difficult and time consuming. Hence, molecular breeding could become an efficient strategy to speed up the development of wilt resistant genotypes.

Mayer *et al.,* (1997) was first to report molecular markers CS-27_{700} (allele specific) and UBC-170 (locus specific) linked to resistance genes for FW against race 1 (*Foc1*). Sharm *et al.,* (2004) mapped the resistance loci *Foc1*, *Foc3* and *Foc4* on linkage group (LG) 2. Gowda *et al.,* (2009) also mapped *Foc1*, *Foc2* and *Foc3* loci on LG2 and Barman *et al.,* (2014) mapped *Foc1* on LG2. Sabbavarapu *et al.,* (2013) identified the two new QTL for FW resistance for race 1 *i.e., FW-Q-APR-6-1* and *FW-Q-APR-6-2* on LG6 with phenotypic variation 10.4 and 18.8 per cent, respectively. Patil *et al.,* (2014) mapped two QTLs associated with early and late wilting on LG2, i.e., *Wilt 1* and *Wilt 2,* respectively.

The two wilt resistance loci located on LG2 and LG6 could be super loci function against many races. It is important to fine map these loci to determine the candidate genes for resistance and to develop reliable robust markers for MAS. To drive fine mapping of QTLs linked to wilt resistance, development of microsatellite, SNP and indel markers in the genomic region controlling wilt resistance is important. The development of markers in any genomic region has now become simpler and easy. High throughput NGS provides a rapid means of discovery of genes/markers

based on genome/transcriptome sequences. Recently chickpea genome has been sequenced and its genome sequence information is available in the database (Varshney *et al.*, 2013). The physical mapping of QTLs associated with FW resistance and development of polymorphic markers in QTL regions would facilitate fine mapping of QTLs and development of robust markers of MAS.

Based on the information available 29 markers closely linked to wilt resistance to race 1 and/or markers flanking QTLs for the FW resistance were selected for BLAST (Basic Local Alignment Search Tool) analysis against chickpea genome in the NCBI (National Center for Biotechnology Information) database. Out of 29 markers, CS-27 is an Allele Specific Associated Primer marker, A07C is a RAPD marker and the remaining 27 were SSR markers. The forward and reverse primer sequences of selected markers were used as query sequences for homology search in chickpea genome sequence in NCBI. The following parameters *viz*., (a) sequence producing significant alignment with lowest E-value (Expect value), (b) maximum identity, (c) no gaps, and (d) both primer pair sequences should fall on same chromosome on +/- strands opposite to one another with in 500bp were used for placing query sequence on the sequence based physical map of chickpea.

Four *Foc1* loci flanked by CS27-TA96 (Sharma et al., 2004), TA110-H3A12 (Gowda *et al.*, 2009), TA110-TA200 (Millan *et al.*, 2010), and TA200-TA37 (Barman *et al.*, 2014) on LG2; and four QTLs *FW-Q-APR-6-1* and *FW-Q-APR-6-2* (Sabbavarapu *et al.*, 2013) on LG6, *Wilt 1* and *Wilt 2* (Patil *et al.*, 2014) on LG2 flanked by CaM1402-CaM1101, CaM1125-TA22, TA27-TA59 and TA27-TA110, respectively were selected from genetic map for *in silico* physical map studies. In sequence based physical mapping, genes were identified from the genomic region flanked by these markers covering QTLs/resistant loci using NCBI map viewer (http://www.ncbi.nlm.nih.gov/projects/sviewer). All the genes identified were scanned for SSRs (mono, di, tri, tetra, penta and hexanucleotide repeats) using online Webstat software (http://purl.oclc.org/NET/websat/). Primer designing was tried using webstat software for all the genic SSR repeats identified (Martins *et al.*, 2009). A few genic SSR primers were selected for synthesis based on following two criteria, (a) SSR should repeat more than seven times and (b) avoid mono-nucleotide repeats These synthesised primers were used for amplification of genomic DNA of three chickpea genotypes.

Among 29 markers selected for *in silico* physical mapping, five markers (CS-27, TS47, TR2, H1A12, and NCPGR58) did not follow the criteria mentioned above; hence these markers were no longer considered for the study. Eight SSR markers were mapped on the unplaced genomic scaffold and in this region we identified genes and genic microsatellites for which primers were designed for future use. The remaining 16 markers were mapped to chromosomes. In chickpea till now four QTLs (Sabbavarapu *et al.*, 2013; Patil *et al.*, 2014) and four genomic regions (Sharma *et al.*, 2004; Gowda *et al.*, 2009; Millan *et al.*, 2010; Barman *et al.*, 2014) for *Fusarium* wilt resistance has been reported independently.

The physical mapping of four QTLs using flanking genetic markers resulted in the identification of only two chromosomal regions (Table I). Similarly out of four *Foc1* loci only two could be physically mapped using flanking markers.

Totally by physical mapping four chromosomal regions were identified for FW resistance. The chromosomal region one is a combined genomic region of two *Foc1* loci (flanked by TA110-TA200 and TA110-H3A12) and one QTL (flanked by TA27-TA110). This region one was mapped to *C. arietinum* chromosome 2 (Ca 2) covering 6.84 Mb genomic region. This genomic region covers 310 genes out of which 49 were uncharacterised in NCBI database (Table 1). Totally from all the genes in this region, 288 genic SSR repeats comprises of 203 mono, 57 di, 19 tri, seven tetra, one penta and one hexanucleotide repeats were observed. The second chromosomal region flanked by markers TA37and TA200 was mapped to Ca2 flanking 1.73 Mb genomic region which covers about 62 genes out of which 19 were uncharacterised in NCBI database (Table 1). Ninety genic SSR repeats were identified, out of which 57

TABLE I

In silico physical mapping of DNA markers linked to FW resistance in chickpea genome sequence.

Genetic map				*In silico* physical map					
QTLs\resistant loci	Flanking markers	LG	References	Chromo-some	Position of flanking marker (bp)	Distance covered (Mbp)	No. of genes	No. genic SSR markers	Primers designed
Foc 1 locus	TA110-H3A12	2	Gowda *et al.*, 2009	Ca2	9410378-16257461	6.84	310	288	74
Foc1 locus	TA200-TA37	2	Barman *et al 2014*	Ca2	15459860-17196573	1.73	62	90	55
Wilt-1 (QTL)	TA27-TA59	2	Patil *et al.*, 2014	Ca2	17196296-23029285	5.83	135	204	141
FW-Q-APR-6-1	CaM1402-CaM1101	6	Sabbavarapu *et al.*, 2013	Ca6	42884010-56466494	13.58	520	803	139

mono, 25 di, seven tri, one penta, two hexa and zero tetranucleotide repeat. Both the *Foc1* loci were present on LG2 in genetic map reported.

The third region flanked by TA27-TA59 covering 5.83 Mb of genomic region comprises 135 genes out of which 46 were uncharacterised in NCBI database (Table 1). Two hundred and four genic SSR repeats have been identified comprises 135 mono, 53 di, 14 tri, one tetra, one penta and zero hexanucleotide repeats.

The fourth region flanked by markers CaM1402-CaM1101 was physically mapped on Ca6. These markers cover 13.58 Mb genomic regions. This genomic region covers 520 genes out of which 40 were uncharacterised in NCBI data base (Table I). This genomic region contains about 803 genic SSR repeats out of which 522 were mono, 182 di, 64 tri, four tetra, 30 penta and one hexanucleotide repeat.

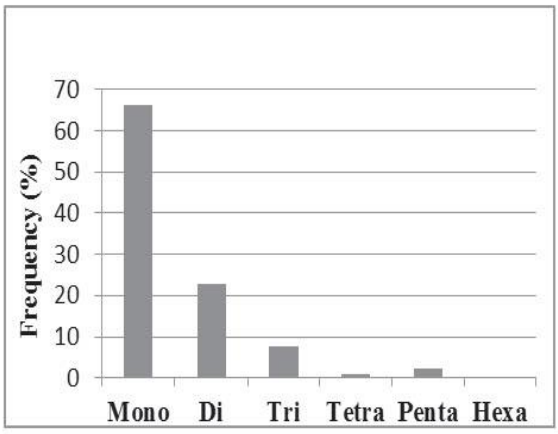

Fig. 1: Frequency of microsatellite repeats found in FW resistance regions of chickpea genome sequence.

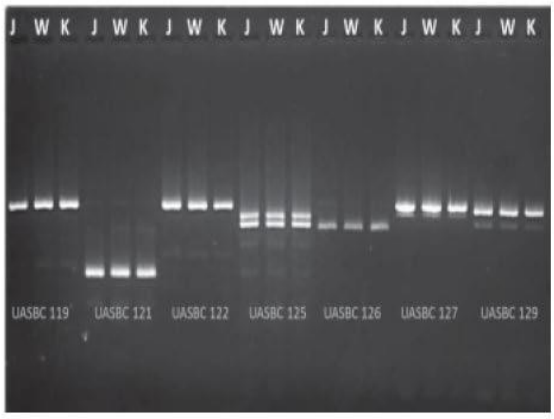

Fig. 2: Amplification of genomic DNA by newly synthesised genic SSR primers

The four physically mapped regions contain 1027 genes and 1385 SSR repeats. Among SSR repeats mononucleotide repeats were highest in frequency followed by di, tri and penta nucleotide repeats (Fig 1). Totally 409 primer pairs were designed for genic SSRs of which 270 were in Ca2 and 139 were in Ca6. From 409 SSR primers, 165 were selected for synthesis. These synthesised SSRs were tested for amplification of chickpea genomic DNA of three genotypes JG62, WR315 and K850. Out of 165 primer pairs synthesised 158 showed perfect amplification and produced the expected product size in all the three genotypes (Fig. 2). Hence, the new primers developed based on *in silico* physical mapping of FW resistance loci will be useful for fine mapping the genomic region linked to wilt resistance. The development of closely linked markers support marker assisted selection in chickpea breeding.

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Distribution of Genic Microsatellite Repeats in Chickpea (*Cicer arietinum* L.) Genome: An *In silico* Approach

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ABSTRACT

Chickpea (*Cicer arietinum*L.) is a cool season legume crop belongs to family *Fabaceae*. The microsatellites distributed in the complete genome sequence would help to understand the genome organization, population studies and in paternity test. The objective of the present study is to identify the occurrence and distribution pattern of microsatellites in selected region of chickpea genome. The gene sequences in the selected regions of chickpea genome were retrieved from NCBI database and webstat online software was used for the identification of simple sequence repeats in the genic region. Totally 2,170 microsatellites were identified from 897genes in the 36.04 Mb chickpea genome. The frequency of occurrence of mononucleotide repeats were highest (67.24 %), followed by di- (22.44 %) and trinucleotide repeats (8.66 %). The tetra-, penta- and hexa-nucleotide repeats were comparatively very less (< 2%). Among mononucleotide poly-A motifs were highest (64 %), followed by poly-T (36 %). In case of dinucleotide repeats, AT and TA repeats were abundant. The genic region was enriched with trinucleotide repeats TTA and AAT. Apart, an essential amino acid isoleucine was found abundant in the genic region of chickpea genome encoded by two (ATA and ATT) trinucleotide repeat motifs. The identified microsatellites will be used for development of novel markers in chickpea.

Key words *Microsatellites, Chickpea, SSR, and Genic region*

Chickpea is an important cool season legume, cultivated extensively in Indian sub-continent, West Asia, North and East Africa, Southern Europe, Ethiopia, America and Australia. It is rich in protein (17-22 %), contributes protein source for vegetarian diet (Hulse, 1991). It is second most widely cultivated pulse crop after common bean (*Phaseolus vulgaris*) and ranks third in production after field pea (*Pisum sativum*) in the world. World-wide chickpea is cultivated in an area of 14.80 million ha, with production of about 14.23 million tonnes and productivity of 962 kg/ha. India is the largest producer of chickpea and contributes a major share in world's chickpea production. The area under cultivation in India is 10.74 million ha with a production of 9.88million tonnes and productivity of 919.9kg/ha (Anonymous, 2015).

Microsatellites are also called as simple sequence repeats (SSRs) or short tandem repeats (STR), composed of 1 to 6 nucleotides repeats in tandem (Field and Wills, 1998). Microsatellites occur due to DNA polymerase slippage during replication and/or repair, leads to rapid mutation (Streisinger and Owen, 1985). These repeats have important advantages in molecular breeding as genetic markers because of their multiallelic nature, high reproducibility and codominant inheritance properties (Gupta *et al.*,2000). The development of SSR-based markers provide great opportunities in the field of genetic studies *viz*., genetic variation, linkage mapping, gene tagging, and evolution (Varshney *et al.*,2002). In chickpea many researchers identified the transcript based SSRs (Garg *et al.*, 2011; Agarwal *et al.*,2012; Choudhary *et al.*,2012; Jain *et al.*, 2015). Recently, chickpea genome has been sequenced (Jain *et al.*, 2013; Varshney *et al.*, 2013) which provide the genomic facilities for the identification of microsatellites followed by development of SSR markers. The present objective of the study is to assess the frequency of different types microsatellites (mono, di, tri, tetra, penta and hexanucleotide repeats) in the chickpea genome and their potentiality in the development of microsatellite markers.

MATERIALS AND METHODS

Nucleotide sequence retrieval

Totally six genomic regions were randomly selected for the identification of genic SSRs on four chickpea chromosomes based on physical map (data unpublished). The genomic region between markers TA110 (9.41 Mb) - TA59 (23.0 Mb) and TR24 (36.2 Mb) – Tc140841 (37.37 Mb) on chromosome 2 and Chromosome 3 respectively were selected. On chromosome 4 two genomic region were selected between TS72 (23.27 Mb) – EST SSR21 (25.79 Mb) and TR20 (36.2 Mb) – TS82 (40.0 Mb). Similarly in chromosome 6 two regions between H4E09 (29.0 Mb) – CaM1125 (30.45 Mb) and CAM1101 (42.88 Mb) – CaM1402 (56.46 Mb) were selected. Altogether, 36.04 Mb genomic region was sampled for identification of genes and SSRs. A total of 1,530 gene sequences were taken from the National Center for Biotechnology Information (NCBI) for identification of microsatellites (https://www.ncbi.nlm.nih.gov/genome/?term=chickpea [Last seen July 2016]). The genes were identified through NCBI map viewer (http://www.ncbi.nlm.nih.gov/projects/sviewer).

Identification of genic microsatellite repeat motifs

In order to identify the microsatellite repeats in genic region of chickpea genome, the webstat online software (Martins *et al.*,2009) was used. The sequences were searched for presence of mono-, di-, tri-, tetra-, penta- and hexa-nucleotide repeat motifs.

RESULTS AND DISCUSSION

Totally six genomic regions were selected to study the occurrence of microsatellites in genic region of chickpea genome. One region from chromosome 2, one from chromosome 3, two each from chromosome 4 and

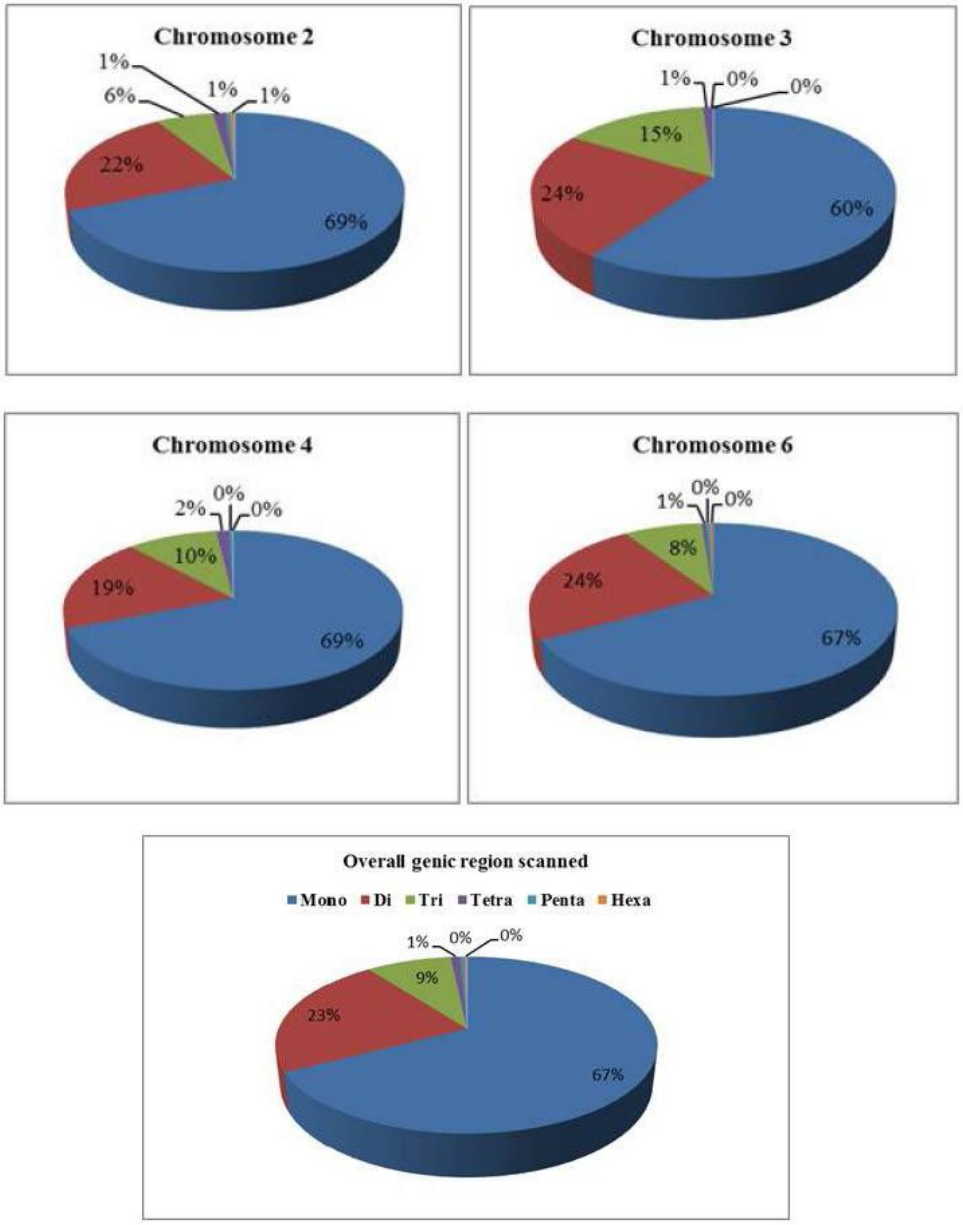

Fig. 1. Frequency of simple sequence repeat motifs in the genic region of chickpea genomes

chromosome 6. In chromosome 2 a total of 13.59 Mb genomic region was scanned and identified 476 genes. Of these 223 genes did not contain any SSR in them. Totally 576 SSR motifs were identified in chromosome 2. In chromosome 3, 1.16 Mb genomic region was scanned, consists of 153 genes and 179 SSRs. Similarly in chromosome 4 and 6, 6.34 and 14.95 Mb genomic region consists of 337 and 564 genes were scanned and identified 490 and 927 SSRs respectively.The detailed summary of genomic region scanned for SSR was given in Table 1.

Overall, 1,530 genes in the selected genomic regions of chickpea were scanned for SSR motifs using webstat online software. A total of 2,170 genic SSRs were identified from 897 genes, rest of the 633 genes did not contain any microsatellite repeats. The microsatellites such as mononucleotide (67.24 %) repeats were abundant in chickpea genome followed by dinucleotide (22.44 %) and trinucleotide (8.66 %). The occurrence of tetra-, penta- and hexa-nucleotide repeats were comparatively very less (< 2 %) (Figure1). The occurrence of mononucleotide repeats were comparatively higher followed by di-, tri, tetra-, penta- and hexa-nucleotide repeats in genic region of chickpea

Table 1. Occurrence of microsatellite (mono, di, tri, tetra, penta and hexanucleotide) repeats in the genic region of chickpea genome sequence

Chromosomes (Ch)	Genomic region scanned (Mb)	No. of genes	Mono	Di	Tri	Tetra	Penta	Hexa	Total
Ch2	13.59	476	396	129	37	8	3	3	576
Ch3	1.16	153	107	43	27	2	0	0	179
Ch4	2.52 (R-I)	84	338	94	49	7	2	0	490*
	3.82 (R-II)	253							
Ch6	1.37 (R-I)	46	618	221	75	4	4	3	925*
	13.58 (R-II)	518							
Total	**36.04**	**1,530**	**1459**	**487**	**188**	**21**	**9**	**6**	**2,170**

* SSR motifs from both R-I and R-II, R- genomic region

and same results were observed in other studies also. For example, the highest frequency of mononucleotide repeats (49.01 %) followed by tri- (28.75 %), di- (11.59 %), tetra- (6.96 %) and penta-nucleotide repeat (3.85 %) were observed in chickpea by Gangadhar *et al.* (2010). Garg *et al.* (2011) presented the occurrence of SSRs in chickpea transcript where, mono-nucleotide repeats represented the largest fraction (41.9 %) followed by trinucleotide (36.1 %) and di-nucleotide (19.3 %) repeats and only a small fraction of tetra-, penta- and hexa-nucleotide SSRs. The results observed by Jain *et al.* (2015) contradict the present results. Among 303 polymorphic transcript based SSR markers for FW resistance in chickpea, the largest fraction of di-nucleotides (170), followed by trinucleotide (124) repeats were observed and the fraction of tetra- (5), penta- (1) and hexa-nucleotide (3) repeats was very less.

The mononucleotide repeats A and T were abundant in chickpea genome whereas, occurrence of G and C type repeats were less than 1 %. The T-type repeat was highest contributing 64 % (885) of mononucleotide repeats followed by A-type repeats (36 %; 502) (Figure 2). A/T rich repeats were abundant compared to C/T rich repeats or G/C rich repeats of all SSRs in this study. Similar results were observed in chickpea by Thudi *et al.* (2011), where poly-A motifs were abundant, accounts for 49.84 % of all microsatellites observed. In *Acanthaceae* family plants among mononucleotide repeats, A-type repeats were highest and enriched with A/T repeats than C/T (Kaliswamy *et al.*, 2015).

We tried to identify the twelve different dinucleotide repeats (AT, AG, AC, TA, TG, TC, GA, GT, GC, CA, CT and CG) in genic region of chickpea genome, which we found in about 22.44 %. The dinucleotide repeat AT (32 %) showed highest frequency followed by TA (30 %), TC (13 %), CT (10 %) and 3 % each AG, TG, GA, GT and CA were observed in the genic region of chickpea. The GC and CG repeat types were not present in this genomic region (Figure3). The earlier research findings showed that the occurrence AT-type repeats were highest in plants like soyabean and *Arabidopsis* (Akkaya *et al.*, 1992; Bell and Ecker, 1994; Morgante and Olivieri, 1993). Similar to the earlier findings in the present study also AT repeats were highest in the genic region of chickpea.

The trinucleotide repeats accounts for about 8.66 % where, around 33 types of repeats were found in genic region. In that trinucleotide repeats TTA type was highest in frequency (21) followed by AAT, TAT, ATT, ATA and TAA with frequencies of 20, 18, 17, 15 and 11 respectively.

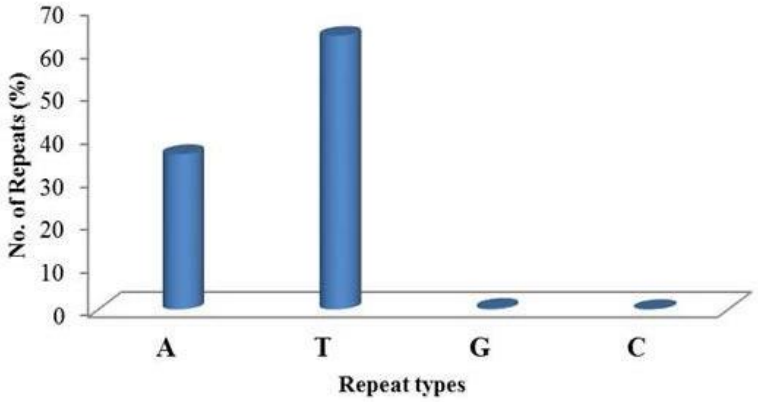

Fig. 2. Frequency distribution of mononucleotide repeats in the genic region of chickpea

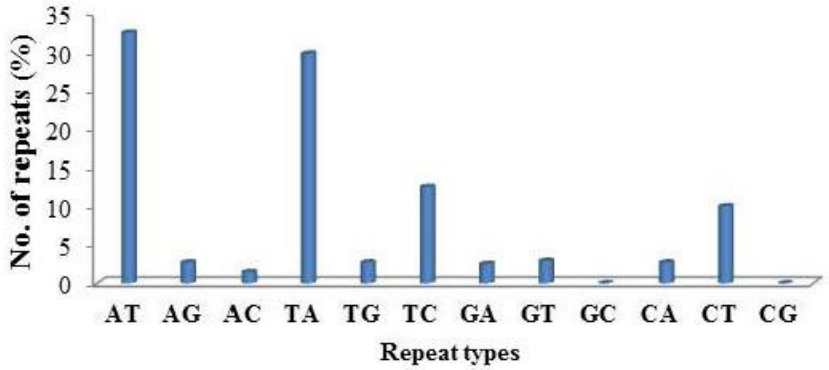

Fig. 3. Frequency distribution of dinucleotide repeats in the genic region of chickpea

Trinucleotide repeat TTA contributes 32 % followed by AAT (30 %) in genic region. Many researchers found that the trinucleotide repeats AAG and AAT were found in high frequency in the plant genome (Akkaya *et al.*, 1992; Lagercrantz *et al.*, 1993; Morgante and Olivieri, 1993; Wang *et al.*, 1994). Similarly in our study AAT repeats were also occurs at high frequency in the genic region of chickpea.

The tetra-nucleotide repeat TTTA was highest in frequencies. Nine penta and four hexanucleotide repeats were observed in the genic region of chickpea genome sequence. The tetra-, penta- and hexa-nucleotide repeats were occurred less in number when compared to the other SSRs, and it was similar to the results observed by other researches in chickpea (Garg *et al.*, 2011, Gangadhar *et al.*, 2010; Jain *et al.*, 2015).

The triplet codons codes for specific amino acids. The genic region was enriched with TTA and AAT trinucleotide repeats which codes for predominant essential amino acid leucine (Leu) and non-essential amino acid asparagine (Asn) respectively. Apart, the repeat types ATA and ATT were also abundant encodes for isoleucine (Ile). The SSR motif TAT encodes for tyrosine (Tyr). The SSR motif TAA one of the stop codon was enriched in the genic region than other two stop codons. Interestingly, both ATA, and ATT type repeats encodes for amino acid isoleucine which was highest in frequency than others. Isoleucine is one of the essential amino acid, required for hemoglobin formation and also maintaining blood glucose level (Doi *et al.*, 2005). Therefore, consumption of chickpea has beneficial effect against type 2 diabetes (Jukanti *et al.*, 2012) as they contain enriched isoleucine in their genome. It was also reported that in medicinal plant *Acanthaceae* species, same amino acid distribution was seen and also *Acanthaceae* family plants have been used to treat diabetes (Kaliswamy *et al.*, 2015).

Due to variation occurs in microsatellite loci that is heterozygous microsatellites where, length polymorphism was exploited for the analysis of plant population genetic structure of both wild (Zucchi *et al.*, 2002) and crop species (Pinto *et al.*, 2003) because of their co-dominance nature. Since development of microsatellite markers involves high cost however, in many studies the primers designed form one species can be used for another species of same genus or different genus of same family generally called as transferability of SSR markers (Oliveira *et al.*, 2006). The genic microsatellites identified in the study using chickpea genome sequence from NCBI database. Designing primers for identified microsatellites and polymorphism check in chickpea genotypes will leads to development of new genic SSR marker, which will be used for further studies in breeding programme in chickpea.

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