DEVELOPMENT OF MOLECULAR MAP AND IDENTIFICATION OF CLOSELY LINKED DNA MARKERS FOR FUSARIUM WILT RESISTANCE IN CHICKPEA (*Cicer arietinum* L.)

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PALB 1070

DEPARTMENT OF PLANT BIOTECHNOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

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in

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Dedicated to
Guide, Family &
Friends....

DEPARTMENT OF PLANT BIOTECHNOLOGY
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CERTIFICATE

This is to certify that the thesis entitled, **"DEVELOPMENT OF MOLECULAR MAP AND IDENTIFICATION OF CLOSELY LINKED DNA MARKERS FOR FUSARIUM WILT RESISTANCE IN CHICKPEA (*Cicer arietinum* L.)"** submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY in PLANT BIOTECHNOLOGY** of the University of Agricultural Sciences, Bengaluru, is a *bonafide* record of research work done by **Mr. PAVANKUMAR JINGADE, ID No. PALB 1070** 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 degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
August 2014

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Bengaluru

August, 2014 *(Pavankumar Jingade)*

"DEVELOPMENT OF MOLECULAR MAP AND IDENTIFICATION OF CLOSELY LINKED DNA MARKERS FOR FUSARIUM WILT RESISTANCE IN CHICKPEA (*Cicer arietinum* L.)"

PAVANKUMAR JINGADE

THESIS ABSTRACT

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the major disease, causing 10-90% yield loss annually. Eight races of pathogen have been reported and race 1A is more prevalent in India. The resistance genes to race 1A have been identified and attempts are being made to map the genes using molecular marker. The markers closely linked to H_1 locus have been identified. The Present study aims to map molecular markers linked to H_2 locus of wilt resistance using $F_{11:12}$ recombinant inbred lines (RILs) of an intraspecific cross between K 850 (Susceptible) and WR 315 (Resistant) segregating for H_2 loci alone.

A partial linkage map was developed using 31 polymorphic markers. The map had four linkage groups with coverage of 690.0 cM and marker density of 5.72 cM. A combined linkage map was developed using 72 polymorphic markers which included 41 markers identified earlier. The combined map had 64 markers mapped on six linkage groups with coverage of 1258.8 cM and marker density of 19.36 cM.

QTL mapping using phenotypic data for wilt resistance generated by screening RILs over two seasons 2007 and 2008 in wilt sick plot at ICRISAT along with linkage information from 31 markers detected five major QTLs. Two QTLs were detected using linkage information from combined linkage mapping. A stable QTL (GSSR 18 - TC14801) for wilt resistance was identified across both the seasons, and the QTL explained a variance of 69.80 per cent in first season and 60.80 in second season. Further, the RILs were phenotyped for seed yield and yield components over two seasons 2012 and 2013 *rabi* in replicated field trials. The QTL mapping using linkage information from combined map detected two stable QTLs (NCPGR 93 - GSSR 9) and (GSSR 9 - GSSR 77) for 100 seed weight.

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ಕಡಲೆಯ (*ಸೀಸರ್ ಆರಿಟಿನುಂ* ಎಲ್.) ಸಮಗ್ರ ಆಣ್ವಿಕ ನಕ್ಷೆ ಅಭಿರುದ್ದಿಕರ್ಣ ಮತ್ತು ಫ್ಯೂಸೇರಿಯಂ ವಿಲ್ಟ್ ರೋಗ ಪ್ರತಿರೋಧನೆಗೆ ನಿಕಟ ಸಂಪರ್ಕ ಹೊಂದಿರುವ ಡಿಎನ್ಎ ಗುರುತುಗಳ ಗುರುತಿಕರಣ

ಪವನಕುಮಾರ್ ಜಿಂಗಾಡೆ

ಪ್ರಬಂಧದ ಅಮೂರ್ತ

ಫ್ಯೂಸೇರಿಯಂ ಆಕ್ಸೀಸ್ಪೊರಿಯಂ ಸಿಸೇರಿನಿಂದ ಕಡಲೆಗೆ ಉಂಟಾಗುವ ವಿಲ್ಟ್ ರೋಗವು ಒಂದು ಪ್ರಮುಖ ರೋಗವಾಗಿದ್ದು ವಾರ್ಷಿಕ ೧೦-೯೦ ಪ್ರತಿಶತ ಬೆಳೆ ನಷ್ಟ ಉಂಟುಮಾಡಬಲ್ಲದ್ದಾಗಿದೆ. *ಫ್ಯೂಸೇರಿಯಂ ಆಕ್ಸೀಸ್ಪೊರಿಯಂ*ನಲ್ಲಿ ಎಂಟು ರೋಗಾಕರಗಳು ವರದಿಯಾಗಿದ್ದು ರೋಗಾಕರ ೧ ಎ ಭಾರತದಲ್ಲಿ ಹೆಚ್ಚು ಪ್ರಚಲಿತವಾಗಿದೆ. ರೋಗಾಕರ ೧ ಎ ಗೆ ರೋಗ ನಿರೋಧಕ ವಂಶವಾಹಿಗಳು ಗುರುತಿಸಲಾಗಿದ್ದು, ಡಿಎನ್ಎ ಗುರುತುಗಳನ್ನು ಬಳಸಿ ಈ ವಂಶವಾಹಿಗಳ ಆಣ್ವಿಕ ನಕ್ಷೆ ಅಭಿವೃದ್ದಿಸುವ ಪ್ರಯತ್ನಗಳು ಮಾಡಲಾಗುತ್ತಿದೆ. ಹೆಚ್ ೧ ಲೋಕಸ್ಗೆ ನಿಕಟ ಸಂಪರ್ಕದಲ್ಲಿರುವ ಡಿಎನ್ಎ ಗುರುತುಗಳನ್ನು ಗುರುತಿಸಲಾಗಿವೆ. ಈ ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಎರಡು ನಿರ್ದಿಷ್ಟ ಜಾತಿಯ ತಳಿಗಳ (ಕೇ ಲಖ೦ x ಡಬ್ಲೂಆರ್ ೩೧೫) ಮಿಲನದಿಂದ ರಚಿತವಾದ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳನ್ನು (ರಿಲ್ಸ್) ಬಳಸಿ ವಿಲ್ಟ್ ರೋಗ ಪ್ರತಿರೋಧಕ ಹೆಚ್ ೨ ಲೋಕಸ್ಗೆ ನಿಕಟ ಸಂಪರ್ಕದಲ್ಲಿರುವ ಡಿಎನ್ಎ ಗುರುತುಗಳ ಆಣ್ವಿಕ ನಕ್ಷೆ ಅಭಿವೃದ್ದಿಸುವ ಪ್ರಯತ್ನ ಮಾಡಲಾಗಿದೆ.

ಮೂವತ್ತೂಂದು ಬಹುರೂಪಿ ಗುರುತುಗಳನ್ನು ಬಳಸಿಕೊಂಡು ಭಾಗಶಃ ಸಂಪರ್ಕ ನಕ್ಷೆಯನ್ನು ಅಭಿವೃದ್ದಿಸಲಾಗಿದೆ. ಈ ನಕ್ಷೆಯು ನಾಲ್ಕು ಸಂಪರ್ಕ ಗುಂಪುಗಳನ್ನು ಹೂಂದಿದ್ದು ೬೯೦.೦cM ವ್ಯಾಪ್ತಿ ಮತ್ತು ೫.೭೨cM ಸಂದ್ರಾತೆಯುಳ್ಳದ್ದಾಗಿದೆ. ಈ ಹಿಂದೆ ಗುರುತಿಸಲಾದ ೪೧ ಡಿಎನ್ಎ ಗುರುತುಗಳು ಸೇರಿ ಒಟ್ಟು ೭೨ ಬಹುರೂಪಿ ಡಿಎನ್ಎ ಗುರುತುಗಳನ್ನು ಬಳಸಿಕೊಂಡು ಸಂಯೋಜಿತ ನಕ್ಷೆಯನ್ನು ಅಭಿವೃದ್ದಿಸಲಾಗಿದೆ. ಈ ಸಂಯೋಜಿತ ನಕ್ಷೆಯು ಆರು ಸಂಪರ್ಕ ಗುಂಪುಗಳನ್ನು ಹೂಂದಿದ್ದು ೧೨೫೮.೮cM ವ್ಯಾಪ್ತಿ ಮತ್ತು ೧೯.೩೬cM ಸಂದ್ರಾತೆಯುಳ್ಳದ್ದಾಗಿದೆ.

ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳನ್ನು (ರಿಲ್ಸ್) ೨೦೦೭ ಮತ್ತು ೨೦೦೮ ಹಿಂಗಾರಿನಲ್ಲಿ ಇಕ್ರಿಸ್ಯಾಟ್ನಲ್ಲಿರುವ *ಫ್ಯೂಸೇರಿಯಂ* ವಿಲ್ಟ್ ರೋಗಗ್ರಸ್ತ ತಾಕಿನಲ್ಲಿ ಬೆಳಿಸಿ ರೋಗ ನಿರೋಧಕತೆಯ ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗಿದೆ. ತದನಂತರ ೩೧ ಡಿಎನ್ಎ ಗುರುತುಗಳ ಸಂಪರ್ಕ ನಕ್ಷೆ ಮಾಹಿತಿಯನ್ನು ಆಧರಿಸಿ ಐದು ಪ್ರಮುಖ ಕ್ಯೂಟಿಎಲ್ ಗಳನ್ನು ಪತ್ತೆ ಮಾಡಲಾಯಿತು ಮತ್ತು ಸಂಯೋಜಿತ ಸಂಪರ್ಕ ನಕ್ಷೆ ಮಾಹಿತಿಯನ್ನು ಬಳಸಿ ಎರಡು *ಫ್ಯೂಸೇರಿಯಂ* ವಿಲ್ಟ್ ರೋಗ ನಿರೋಧಕ ಸಂಬಂಧಿತ ಕ್ಯೂಟಿಎಲ್ ಗಳನ್ನು ಪತ್ತೆ ಮಾಡಲಾಯಿತು. ಎರಡೂ ನಿರ್ದಿಷ್ಟ ಋತುಗಳಲ್ಲಿ ಒಂದು ಸ್ಥಿರ ಕ್ಯೂಟಿಎಲ್ಅನ್ನು (ಜಿಎಸ್ಎಸ್ಆರ್ ೧೮ - ಟಿಸಿ ೧೪೮೦೧) ಗುರುತಿಸಲಾಗಿದೆ. ಈ ಕ್ಯೂಟಿಎಲ್ ಮೊದಲ ಋತುವಿನಲ್ಲಿ ೬೯. ೮೦ ಪ್ರತಿಶತ ಮತ್ತು ಎರಡನೇ ಋತುವಿನಲ್ಲಿ ೬೦.೮೦ ಪ್ರತಿಶತ ಫೀನೋಟೈಪಿಕ್ ಭಿನ್ನತೆಯನ್ನು ವಿವರಿಸಿತು. ಆನಂತರ ಮರುಮಿಶ್ರಿತ ಸ್ವಾಭಾವಿಕ ಸಾಲುಗಳನ್ನು (ರಿಲ್ಸ್) ಎರಡು ಋತುಗಳಲ್ಲಿ ೨೦೧೨ ಮತ್ತು ೨೦೧೩ ಹಿಂಗಾರಿನಲ್ಲಿ ಇಳುವರಿ ಮತ್ತು ಇಳುವರಿ ಲಕ್ಷಣಗಳಿಗೆ ಸಂಬಂಧಪಟ್ಟ ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಯಿತು. ಸಂಯೋಜಿತ ಸಂಪರ್ಕ ನಕ್ಷೆಯ ಮಾಹಿತಿಯನ್ನು ಬಳಸಿ ೧೦೦ ಬೀಜ ತೂಕಕ್ಕೆ ಸಂಬಂಧಿತ ಎರಡೂ ಸ್ಥಿರ ಕ್ಯೂಟಿಎಲ್ಗಳನ್ನು (ಎನ್ಸಿಪಿಜಿಆರ್ ೯೩ - ಜಿಎಸ್ಎಸ್ಆರ್ ೯) ಮತ್ತು (ಜಿಎಸ್ಎಸ್ಆರ್ ೯ - ಜಿಎಸ್ಎಸ್ಆರ್ ೭೭) ಪತ್ತೆಮಾಡಲಾಯಿತು.

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ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES


INTRODUCTION

I. INTRODUCTION

Chickpea (*Cicer arietinum*, 2n=2x=16) a cool season legume with an genome size of ~738 mb (Varshney *et al.,* 2013; Jain *et al.,* 2013) belongs to genus *Cicer*, tribe *Cicereae,* family *Fabaceae*, and subfamily *Papilionaceae* which contains essentially all of the important legume crops, originated in south eastern Turkey and Syria. Based on seed size and colour cultivated chickpeas are of two types microsperma (*Desi* type) and macrosperma (*Kabuli* type). Consumption of *Desi* type is restricted primarily to the middle-east and south-east Asia, whereas *Kabuli* is a popular and valuable global commodity.

Among pulses, chickpea is one of the major legume crops of India. India ranks first in terms of production and consumption in the world. About 65 per cent of global area with 68 per cent of global production of chickpea is contributed by India (Reddy and Mishra 2010a). Though about 65 per cent of the world acreage and 68 per cent production are accounted in India, the production is still not adequate to meet the domestic demand. Further, there is a competition for area from other major crops like wheat, rice, *Bt*-cotton, sunflower *etc.* whose productivity and profitability is high. Consequently there is a drastic reduction in area and production in the traditional chickpea growing areas of north-west and north-east India. The area under chickpea has been decreased from 4.3 million ha. in 1970s to 1.1 m. ha and the production from 3.1 to 1.00 million tonnes in this region.

Chickpea's share of pulse production declined from 45% in 1970s to less than 30% in 2000. However, there has been expansion in the area of chickpea in central and peninsular India, particularly Andhra Pradesh, Karnataka, Maharashtra and Madhya Pradesh which together contribute to 91% of the production and 90% of the area under chickpea

(Anon. 2012). In the future, all the chickpea production will have to come from these areas, unfortunately, chickpea is always grown under harsh environments in the marginal and high risk prone areas in this regions. *Fusarium* wilt, caused by *Fusarium oxysporum ciceri* (*FOC*), is one of the major problems in this region. Annual yield losses from this disease have been estimated to range from 10 to 15%, but *Fusarium* wilt epidemics can be devastating to individual crops and cause 100% loss under favourable conditions.

Fusarium wilt of chickpea has been managed primarily by the use of resistant cultivars, but virulent races of the pathogen have undermined their importance in recent years (Haware *et. al.,* 1982). Till now, eight races of the pathogen have been reported of which six races (1A, 2, 3, 4, 5 and 6) cause wilting symptoms, whereas the race 0, the least virulent among all the races, induces progressive foliar yellowing compared with severe leaf chlorosis, flaccidity, and early wilting induced by other races (Jimenez-Diaz *et al.,* 1993; Kelly *et al.,* 1994). The genetic analysis studies have indicated that the resistance to *Fusarium oxysporum* f.sp. *ciceri* race 0 (*FOC0*) is governed by one or two independent genes (Tekeoglu *et al.,* 2000; Rubio *et al.,* 2003). Whereas, the resistance to *FOC2* is conferred by a single recessive gene (Sharma and Muehlbauer, 2007), resistance to *FOC3* is also monogenic (Sharma *et al.,* 2004) and resistance to race 4 (*FOC4*) was monogenic recessive (Tullu *et al.,* 1998; Sharma *et al.,* 2005) as well as digenic recessive (Tullu *et al.,* 1999) but resistance to race 5 is governed by single gene (Tekeoglu *et al.,* 2000; Sharma *et al.,* 2005).

The present study concentrates on race 1A, which is prevalent in peninsular India in epidemic form. Genetics of resistance to race1A (*FOC1*) suggested that two to three major independent loci H_1, H_2 and H_3 (Upadhyaya *et al.,* 1983a and b; Singh *et al.,* 1987, Brinda and

Ravikumar, 2005) govern wilt resistance in chickpea. Dominant alleles at both H_1 and H_2 loci cause early wilting and dominance at any one locus (either H_1 or H_2) lead to late wilting while homozygous recessive at H_1 and H_2 confer complete resistance.

High level of resistance to *FOC* race1 is available in the cultivated species and genotypes. However, the progress in the development of high yielding wilt resistant varieties is very slow and all the existing varieties, including national check Annegeri 1(A 1) are susceptible to wilt, which lead to spread of the inoculum in all chickpea growing areas of India. The selection for resistance in segregating population and maintenance of wilt sick plot is difficult task, hence molecular markers and marker based screening/selection for resistance are of great advantage and has been advocated as a highly efficient method to select targeted genes. Various types of genomic resources like microsatellite or simple sequence repeat (SSR)/sequence tagged microsatellite markers (STMS), expressed sequence tags (ESTs), single nucleotide polymorphism (SNP), cleaved amplified polymorphic sequences (CAPS), conserved intron spanning primers (CISP) and diversity arrays technology (DArT) markers have been developed in chickpea. The availability of molecular markers and genetic linkage maps has facilitated marker assisted selection, positional cloning and mapping of QTL for disease resistance and other traits of interest in many crops. A number of intraspecific and interspecific molecular maps for resistance to *Fusarium* wilt (Winter *et al.,* 2000; Benko-Iseppon *et al.,* 2003; Rubio *et al.,* 2003; Sharma *et al.,* 2004 Gowda *et al.,* 2009 and Millan *et al.,* 2010) as well as for other traits (Winter *et al.,* 1999; Santra *et al.,* 2000; Radhika *et al.,* 2007; Ali *et al.,* 2008; Cobos *et al.,* 2009 and Nayak *et al.,* 2010) have been developed in chickpea.

In case of chickpea, resistance genes associated with different races of *Fusarium oxysporum* were tagged. The H_1 locus has been tagged

and validated using different populations (Mayer *et al.,* 1997; Ravikumar *et al.,* 2003; Brinda and Ravikumar, 2005). The H_2 locus was tagged by using a mapping population specifically segregating for H_2 locus (Soregaon *et al.,* 2007). However, due to limitations of mapping population size, low precision of gene/QTL locations and non-validation of these markers limit their application in practical breeding, hence higher density of markers in the area of these genes is still necessary.

The present work focuses on development of molecular chickpea map using functional SSR and other markers in a mapping population (K 850 X WR 315) segregating for both H_1 and H_2 loci. The developed saturated maps would further be useful in development of resistance varieties by marker assisted selection. Hence, the study was under taken with the following specific objectives:

1. Development of molecular map using functional SSR and other markers in chickpea.
2. Characterization of RILs for *Fusarium* wilt resistance.
3. Identification and mapping of markers closely linked to *Fusarium* wilt resistance and other quantitative traits contributing to seed yield.
4. Validation of identified markers using selected genotypes.


REVIEW OF
LITERATURE

II. REVIEW OF LITERATURE

The major constraints in chickpea production are biotic and abiotic stresses, apart from narrow germplasm base, recalcitrant nature, requirement of specific agro-climatic factors etc. Among the biotic stresses a prime factor is *Fusarium* wilt disease caused by the fungal pathogen *Fusarium oxysporum* f sp. *ciceri* (*FOC*) which may cause up to 90 % yield loss. (Srivastava and Singh 1984; Gupta *et al.,* 1986; Jimenez-Diaz *et al.,* 1993; Cortes and Hav 2000). Breeding for disease resistance by identifying and incorporating gene(s) that confer inherent resistance is feasible approach to combat the problem. However the development and maintenance of wilt sick plots for screening of the material is a difficult task. Therefore, identification of molecular markers linked to *Fusarium* wilt resistance helps for quicker identification of resistant progeny in the segregating population (Mayer *et al.,* 1997).

The literature available pertaining to *Fusarium* wilt resistance in chickpea is furnished here under following headings.

2.1 Importance, origin and taxonomy of chickpea

2.2 Constraints in chickpea production

2.3 Phenotyping techniques for wilt resistance

2.4 Molecular markers in chickpea

2.5 Mapping in chickpea

2.6 Genetic variability, heritability and genetic advance studies in chickpea

2.1 Importance, origin and taxonomy of chickpea

2.1.1 Importance of chickpea

Chickpea (*Cicer arietinum* L.), the third most important cultivated grain legume in the world after soybean and beans (Anon 2012). It is the member of the *Fabaceae* family, which includes 18,000 species, grouped into 650 genera (http://www.ildis.org/Leguminosae). Chickpea is commonly called as gram, Bengal gram or garbanzo bean is mainly cultivated in the Indian subcontinent, west Asia, the Mediterranean, North Africa and the America (Croser *et al.,* 2003).

Chickpea has one of the highest nutritional compositions of any dry edible legume (Wood and Grusak 2007). Chickpea is an important source of protein in the diets of the poor and is particularly important in vegetarian diets, it is being used increasingly as a substitute for animal protein. Chickpea is a helpful source of zinc, folate and protein. Being low in glycemic index value and high in dietary fibre, chickpea is digested very slowly which helps maintain stable blood sugar levels and healthier glucose metabolism. This makes chickpea a great food especially for diabetics and insulin-resistant individuals (www. glycemic-index.org/ chickpeas-nutrition). Chickpea is low in fat and most of this is polyunsaturated.

According to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) chickpea seeds contain 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugar), 5% fat, 6% crude fibre and 3% ash. High mineral content has been reported for phosphorus (340mg/100g), calcium (190mg/100g), magnesium (140mg/100g), iron (7mg/100g) and zinc (3mg/100g).

Among the food legumes, chickpea is the most hypocholesterolemic agent, germinated chickpea seeds were reported to be effective in

controlling cholesterol level in rats (Geervani, 1991). Glandular secretion of the leaves, stems, and pods consists of malic and oxalic acids, giving a sour taste. Medicinal applications include use for aphrodisiac, bronchitis, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke and warts. Acids are supposed to lower the blood cholesterol levels (Duke, 1981). Recent studies have also shown that they can assist in lowering of cholesterol in the bloodstream (Pittaway *et al.,* 2008).

Chickpea also improves soil fertility by fixing atmospheric nitrogen, meeting upto 80% of its nitrogen (N) requirement from symbiotic nitrogen fixation (Saraf *et al.,* 1998). Chickpea returns a significant amount of residual nitrogen to the soil and adds organic matter, improving soil health and fertility (Gaur *et al.,* 2012).

2.1.2 Origin and taxonomy of chickpea

Chickpea (*C. arietinum* L.) is one of the pulse crops of ancient origin domesticated in the old world about 7000 years ago. Cytogenetic and seed protein analyses are consistent with *C. reticulatum* as the wild progenitor of domesticated *C. arietinum*, with south-eastern Turkey as the presumed centre of origin (Ladizinsky and Adler 1976). The proof of chickpea cultivation dates back as far as the early Bronze Age in Jericho (Hopf *et al.,* 1969). It is grown and consumed in large quantities from South East Asia to India and in the Middle East and Mediterranean countries.

Chickpea is probably originated from South eastern Turkey and spread with human movement toward the west and south west via the silk route (Singh *et al.,* 1997). Four centres of diversity were identified in the Mediterranean, Central Asia, the near east and India as well as a secondary centre of origin in Ethiopia (Vavilov and Freier 1951).

Taxonomically chickpea is classified to a separate tribe, *Cicereae* Alef in subfamily *papilionoideae* of l*eguminosae* family. The genus *Cicer* contains two subgenera viz., *Pseudononis* and *Viciastrum* which include 43 species (9 annuals, 33 perennial and 1 unspecified) (Van der Maesen, 1987). Now family (*Fabaceae*) and sub family (Faboideae) has been changed. *Leguminosae* is an older name still and considered valid (International Code of Botanical Nomenclature (ICBN) Art 18.5 Vienna code, 2006).

2.2 Constraints in chickpea production

Chickpea is a hardy, deep-rooted dry land crop and can grow to full maturity despite conditions that would prove fatal for most crops. It is grown on marginal land and rarely receives fertilizers or protection from diseases and insect pests (Singh and Reddy 1993). Despite of its economic importance chickpea productivity is low because of yield losses due to abiotic (drought, cold and salinity) and biotic stresses (*Helicoverpa*, *Ascochyta* blight, *Fusarium* wilt and Botrytis grey mold).

On a global basis, annual yield losses in chickpea were estimated to be 6.4 million tonnes due to abiotic stresses and 4.8 million tonnes due to biotic stresses (Ryan, 1997). The most common abiotic stresses affecting chickpea production, in the order of importance, are drought, heat and cold (Singh *et al.,* 1994; Croser *et al.,* 2003). Drought together with heat causes 3.3 million tonnes yield loss per annum (Ryan, 1997). Other abiotic stresses specific to some regions are salinity, waterlogging, soil alkalinity and acidity, and nutrient deficiencies and toxicities (Ryan, 1997; Siddique *et al.,* 2000).

Amongst the causal agents of biotic stresses, about 67 fungi, 3 bacteria, 22 viruses and 80 nematodes have been reported on chickpea (Nene *et al.,* 1978) but only few of these cause economically important

diseases (Haware *et al.,* 1992). The maximum number of pathogens has been reported from India alone with the number rising to 89 pathogens in 1995 from 35 in 1978 (Nene *et al.,* 1978). The insect *Helicoverpa armigera* which feeds on foliage, flowers and developing seeds, is the most important pest of chickpea, while stunt is the most important and prevalent viral disease in most chickpea growing regions of the world. Among economically important fungal diseases of chickpea are root diseases like *Fusarium* wilt and root rots caused by a complex of soil borne fungi, foliar diseases like *Ascochyta* blight and *botrytis* grey mould, of which wilt is most devastating disease at latitudes ranging from zero to 30 (Faris and Gowda, 1990; Haware *et al.,* 1990; Van Rheenen, 1991).

2.2.1 *Fusarium* wilt of chickpea

Fusarium wilt of chickpea was first mentioned in 1918 (Nene *et al.,* 1980). Prasad and Padwick (1939) reported that *Fusarium* species that they isolated was classified in the section, Elegans and subsection, Orthoceras. However, Prasad and Padwick (1939) did not name the fungal isolate as a separate species. Comparisons of the isolates with all known species of the sub-section under identical conditions was necessary. The problems were elaborately studied by Padwick (1940) who named the pathogen as *Fusarium orthoceras* var *ciceri*. Later based on several isolates collected in 1954, 1955 and 1956 from wilt affected plants the causative agent of this disease has been classified as *Fusarium oxysporum* f. sp. *ciceri* (*FOC*). *FOC* is internally seed borne and is found as chlamydospore-like structures in the hilum region of the seed (Haware *et al.,* 1978). At the time of infection *Fusarium oxysporum* commonly penetrates root hairs or epidermal cells just behind the root tip or within the zone of elongation and proceeds intercellularly and intracellularly to the primary meristem (MacHardy and Beckmann, 1981). After the endodermal and pericycle tissues have been penetrated, the xylem is colonized and the pathogen is distributed upward (Nelson, 1981). Among

other legumes, lentil, pea and pigeonpea are symptomless carriers of the chickpea wilt fungus (Haware and Nene, 1982).

Beckman (1987) reported that *Fusarium* wilt, caused by *Fusarium oxysporum* is a soil borne, facultative, vascular wilt fungus that provokes economically important losses in approximately 80 crops including chickpea. Annual yield losses due to wilt have been estimated at 10-90% (Jimenez-Diaz *et al.,* 1989). Many traditional Mediterranean Kabuli cultivars are susceptible to the wilt and sources of resistance (mainly *Desi* cultivars) are included in breeding programs (Singh *et al.,* 1987; Kaiser *et al.,* 1994). Infected seeds play an important role in long distance dispersal and in transmitting the disease to new areas. Once the inoculum is established in soil, it is difficult to eradicate as the chlamydospores survive in the soil for at least 6 years, and under favourable conditions germinate and infect the seedlings through tender roots (Haware *et al.,* 1996). The pathogen can also survive in infected crop residues buried in the soil and other *Cicer* species can also be affected under artificial inoculation conditions (Barve, 2003).

2.2.2 Pathogen growth and cultural characteristics

The fungus grows on potato sucrose agar at 25°C and appears as delicate, white and cottony growth becoming felted and wrinkled in older cultures (Nelson *et al.,* 1983). Fungal hyphae are septate and profusely branched. Microconidia are borne on simple short conidiophores, arising laterally on the hyphae. Microconidia and macroconidia are generally sparse on solid media, however, they are formed abundantly in potato sucrose broth. Microconidia are oval to cylindrical, straight to curved and measure 2.5-3.5 x 5-11µm. Macroconidia, which develop on the same conidiophores on which microconidia are formed (Nelson *et al.*, 1983), are thin walled, 3-5 septate, fusoid, pointed at both ends, fewer in number than microconidia, and measure 3.5-4.5 x 25-65µm.

Chlamydospores, formed in 15-day-old cultures are smooth or rough walled, terminal or intercalary, and may form singly, in pairs, or in chains.

2.2.3 *Fusarium* wilt disease symptoms in chickpea

Affected seedlings show a dull green colour of the foliage, sudden drooping of the petioles, rachis and leaves. The plants, when uprooted, show uneven shrinkage at the collar (Nene, 1978). There is no external rotting of roots and pith, however, when the roots are split vertically, internal discoloration may be seen in such wilted plants (Nene, 1978). Transverse sections of the infected roots examined under the microscope shows the presence of hyphae and spores of the fungus in the xylem, thereby confirming the diagnosis of vascular wilt (Nene, 1978).

Isolates of *FOC* may induce either fast wilting or a progressive yellowing syndrome, which develops 15-40 days after inoculation depending on the cultivar. Wilting can be observed in a susceptible cultivar within 25 days after sowing in *FOC* infested soil and this is known as 'early wilt' (Haware and Nene, 1982). Wilting may also occur during reproductive growth and is known as 'late wilt'. Early wilting is reported to cause 77-94% yield loss while late wilting causes 24-65% loss (Haware and Nene, 1982). Seed harvested from the late wilted plants is lighter and duller than that harvested from healthy plants. Plants grown from infected seeds wilt faster than the plants grown from healthy seeds. *Fusarium oxysporum* f.sp. *ciceri* can cause wilting of infected plant either by mechanical plugging of water dusts (xylem) by mycelium, wilt toxins or hydrolytic enzymes (Green, 1981).

Vascular discolouration or browning is a diagnostic symptom in vascular wilt diseases. Dark melanin pigment accumulation in host cells results from an increase in both phenolic and polyphenoloxidase and

other enzyme activity (Green, 1981). The browning originates in xylem parenchyma adjacent to colonized vessels and staining is transferred to the vessels (Pegg, 1981). Upon infection of host roots, the fungus crosses the cortex and enters the xylem tissues, produces mycelia and/ or spores in the vessels, and this result in the death of the plant (Beckman, 1987). The characteristic symptoms include sudden drooping of leaves and petioles, internal (xylem) discoloration, yellowing of leaves, stunting and finally death of the plant (Nene and Haware., 1980; Kraft *et al.,* 1994).

2.2.4 Classification of *Fusarium oxysporum* f.sp. *ciceri*

Fusarium is a large cosmopolitan genus of pleoanamorphic hyphomycetes whose members can cause a wide range of plant diseases (Farr *et al.,* 1989); mycotoxicoses and mycotic infections of other animals as well as humans (Nelson, 1981). *Fusarium* is noted for production of secondary metabolites such as plant growth hormones (gibberellin) as well as toxins (tricothecenes and fumosins). Many species of this fungus are recognized, out of which *F. oxysporum* is the commonest one. The species is well represented among the soil borne fungi and can be observed in diverse soil types all over the world (Burgess, 1981) and is considered to be a normal constituent of the rhizosphere of plants (Appel and Gordon, 1994). However, only some strains of *F. oxysporum* are pathogenic to plants where they penetrate into the roots and provoke root rots causing severe damage.

The vascular wilt causing form a species of *F. oxysporum* typically invade only living root tissues and are host specific, get suppressed by saprophytes (Hillocks, 2001). Based on the plant species and plant cultivars infected, *F. oxysporum* is classified into more than 120 specialis and races (Armstrong, 1981). The presently accepted classification for the *Fusarium* wilt pathogen *F. oxysporum* f. sp. *ciceri* belongs to Class:

Fungi Imperfecti, order: *Moniliales*, family: *Tuberculariaceae*, genus: *Fusarium*, species: *oxysporum*, specialis: *ciceri.*

2.2.5 Different races of *Fusarium oxysporum* f.sp. *ciceri*

Fusarium oxysporum f. sp. *ciceri* can also infect many crop plants which includes banana (*Musa spp.*) (*F. oxysporum* f. sp. *cubense*), cabbage (*Brassica spp.*) (*F. oxysporum* f. sp. *conglutinans*), cotton (*Gossypium spp.*) (*F. oxysporum* f. sp. *vasinfectum*), flax (*Linum spp.*) (*F. oxysporum* f. sp. *lini*), muskmelon (*Cucumis spp.*) (*F. oxysporum* f. sp. *melonis*), onion (*Allium spp.*) (*F. oxysporum* f. sp. *cepae*), pea (*Pisum spp.*) (*F. oxysporum* f. sp. *pisi*), tomato (*Lycopersicon spp.*) (*F. oxysporum* f. sp. *lycopersici*), watermelon (*Citrullus spp.*) (*F. oxysporum* f. sp. *niveum*), China aster (*Calistephus spp.*) (*F. oxysporum* f. sp. *callistephi*), carnation (*Dianthus spp.*) (*F. oxysporum* f. sp. *dianthi*), chrysanthemum (*Chrysanthemum spp.*) (*F. oxysporum* f. sp. *chrysanthemi*), gladioli (*Gladiolus spp.*) (*F. oxysporum* f. sp. *gladioli*), tulip (*Tulipa spp.*) and chickpea (*F. oxysporum* f. sp. *ciceri*) (Armstrong, 1981; MacHardy and Beckman, 1981).

Trapero-Casas and Jimenez-Diaz (1985) made attempts to distinguish the two pathotypes *F. oxysporum* f. sp. *ciceri* based on distinct yellowing or wilting symptoms and found that the yellowing pathotype induces progressive foliar yellowing with vascular discolouration, while the wilting pathotype induces rapid and severe chlorosis, flaccidity, and vascular discolouration. In addition to these two pathotypes, Haware and Nene (1982) reported existence of four physiological races (1, 2, 3, and 4) of *F. oxysporum* f.sp. *ciceri* in India using 10 chickpea lines as differentials. Two additional races (0 and 5) were later identified from Spain and later another race (race 6) was reported from California, USA (Phillips, 1988). Race 1 was subsequently divided into two races named as race 1A (from India) and race 1 B/C

(from Spain) based on variation in reaction on differential host lines (Jimenez Diaz *et al.,* 1993). Race 1 B/C was also found in USA (California), Syria, Turkey and Tunisia. Thus, a total of eight physiological races of the pathogen have been reported worldwide. Kelly *et al.,* (1994) found that unlike the other races, race 1A is more widespread and he also reported its presence in India, California, and the Mediterranean region.

Races 0 and 1B/C belong to the yellowing pathotype, whereas the remaining races form the wilting pathotype (Kelly *et al.,* 1994). The eight races also have a distinct geographic distribution. Races 2, 3, and 4 have been reported only in India, whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean region and California (Kelly *et al.,* 1994).

An intraspecific phylogeny of *FOC* races inferred from DNA fingerprinting with repetitive sequences indicated that each of the eight races forms a monophyletic lineage and that they have evolved in a simple stepwise pattern, with race 0 being hypothesized as ancestor of the wilting races (Jimenez-Gasco and Perez-Artes 2001).

Jimenez-Gasco, (2001) used 99 isolates of *FOC*, representative of the two pathotypes (yellowing and wilt) and the eight races described (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6), in the study and developed sequence characterized amplified regions (SCAR) markers for races 0 and 6, while a race 5 specific identification assay was developed using touchdown PCR.

Jimenez-Gasco and Milgroom (2004) studied the association between fingerprint lineages and geographic origin of isolates, neither UPGMA nor neighbour joining trees showed any association between fingerprint lineages and geographic origin of isolates, except for grouping of races 2, 3 and 4 which have been reported only from India.

Honnareddy and Dubey (2006) conducted a study to determine pathogenic and genetic variability of isolates of *FOC* collected from different parts of India. Pathogenic virulence study of 25 isolates of the pathogen on international set of differential cultivars was accomplished for characterization of new isolates in the known four races of the pathogen. Genetic variability within 24 isolates representing seven races of *FOC* was assessed by RAPD.

Singh *et al.* (1991) analysed thirty isolates of *FOC* obtained from rhizosphere soil of chickpea from different locations from northern India and evaluated amount of genetic variation by RAPD and IGS (Intergenic sequence) analysis further genetic similarity between each of the isolates was calculated and results indicated that there was little genetic variability among the isolates collected from the different locations.

Sharma *et al.* (2009) evaluated 48 isolates of *FOC* collected from different chickpea growing regions in India for genetic variations using amplified fragment length polymorphism (AFLP). Out of 48 isolates, 41 were found pathogenic and seven non-pathogenic. Pathogenic isolates differed in their virulence however, there was no apparent correlation between geographical origin and virulence of the isolates. UPGMA cluster analysis and principle coordinate analysis distinctly classified 48 isolates into two major groups, pathogenic and non-pathogenic. The pathogenic isolates could be further clustered into six major groups at 0.77 genetic similarities. Region specific grouping was observed with in few isolates.

Gujaria *et al.* (2011) used gene specific markers, ITS-RFLP, ISSR and AFLP for distinguishing Indian *F. oxysporum* f. sp. *cicer* races and report for the first time that *F. oxysporum* f. sp. *ciceri* race 3, a wilt pathogen of chickpea in India, is actually *F. proliferatum* based on phylogenetic analysis with EF-1α sequence data and easily distinguished *F. oxysporum* f. sp. *ciceri* races 1, 2 and 4 from "race 3" (*F. proliferatum*)

by PCR amplification with oligonucleotides Hop78 transposon (Hop 78), cutinase (Cut), desaturase (Dst). *F. oxysporum* f. sp. *ciceri* race 4 was distinguished with the xylanase 3 (xyl3) gene based marker.

Dubey *et al.* (2012) analysed seventy isolates of *Fusarium oxysporum f.sp. ciceri* (*FOC*) causing chickpea wilt representing 13 states and four crop cultivation zones of India for their virulence and genetic diversity and based on differential responses, the isolates were characterized into eight races of the pathogen. Further molecular characterization with four different molecular markers (RAPD, SSR, ITS and universal rice primers) grouped the isolates into eight categories at genetic similarities ranging from 37 to 40%, and the molecular groups partially corresponded to the states of origin/chickpea-growing region of the isolates as well as races of the pathogen characterized in the study. The majority of southern, northern and central Indian populations representing specific races of the pathogen were grouped separately into distinct clusters.

2.2.6 Genetic basis of *Fusarium* wilt resistance

A diverse range of organisms constantly challenge plants, but not all of them are able to cause disease hence the understanding of resistance mechanism is a pre-requisite for developing appropriate breeding as well as molecular strategies. Particularly breeding for disease resistance requires a thorough understanding of race pattern and genes governing resistance to each race. With the proper documentation of race pattern and genetics of resistance to each race, tremendous success has been achieved for resistance to rust in wheat. Although field, laboratory and greenhouse techniques to screen for resistance to soil borne pathogens like *Fusarium oxysporum* have been developed; there are contradicting reports on genetics of resistance. The reports on genetics of

resistance to race 1 (which is more prevalent in India) and other races reviewed below separately.

2.2.6.1 Genetics of resistance to race 1

Several studies reported that resistance of chickpea to *Fusarium* wilt is due to a single recessive gene (Pathak and Singh 1975; Tewari *et al.*, 1981; Kumar and Haware, 1982; Sindhu *et al.,* 1983; Phillips, 1983). However, Kumar and Haware (1982) also reported that segregation for JG 62 x WR 315 cross did not fit 3:1 (Susceptable: Resistance) ratio and that probably more genes were involved.

Sindhu *et al.* (1983) assigned the gene symbol *rfo* to this recessive gene. However, the studies of Upadhyaya *et al.* (1983a) suggested that two pairs of recessive genes are needed for resistance. Resistance in chickpeas to race 1 of *FOC* is known to be controlled by at least three independent loci two incompletely recessive and one dominant gene, individually delaying wilting and any two in combination confer complete resistance (Smithson *et al.,* 1983).

Upadhyaya *et al.* (1983a) confirmed that the cultivar C 104 appears to differ from WR 315 and CPS 1 by a single locus, which results in delayed wilting when in homozygous recessive form. The same group also suggested that data are consistent with the hypothesis that JG 62 carried the two genes in a homozygous dominant condition ($H_1H_1H_2H_2$), C 104 is homozygous recessive at the second locus ($H_1H_1h_2h_2$) and the resistant parents (WR 315, CPS 1, BG 212 and P 436-2) are homozygous recessive at both loci ($h_1h_1h_2h_2$). They also identified another late wilting genotype K 850.

Upadhyaya *et al.* (1983b) suggested that two independent loci govern resistance to race 1 of the *Fusarium* wilt causing pathogen. Dominant alleles at both loci ($H_1\_H_2\_$) result in early wilting reaction,

homozygous recessive at either of the loci (H_1_h_1h_2 or $h_1h_1H_2$_) result in late wilting reaction while homozygous recessive at both loci ($h_1h_1h_2h_2$) result in complete resistance. Further similar inheritance pattern was observed (Brinda and Ravikumar 2005).

Singh *et al.* (1987) studied the reactions of parents, F_1 and F_2 generations of crosses of chickpea cultivars K 850 with C 104 and JG 62 and F_3 progenies of K 850 x C 104 to race 1 of *FOC* and confirmed that K 850 carries a recessive allele for resistance at a locus different from and independent of that carried by C 104 and that the recessive alleles at both loci together confer complete resistance. The genetics of wilt resistance against race 1A indicated three independent loci Designated as H_1, H_2 and H_3 govern resistance to wilt. However few other studies indicated two major independent loci, H_1 and H_2 determine resistance to race 1 in chickpea. Mandal and Bahl (1980) reported simple nature of genetics of inheritance to wilt in chickpea in a cross involving two resistant and one susceptible parents in F_1 and F_2 generations. It was not clear whether there was one strong gene or the other gene(s) were not segregating between the parents used in that particular study.

Singh *et al.* (1991) studied F_1 and F_2 generations of the two crosses H 208 x K 850 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 two reported earlier.

Anupama (2001) reported two recessive genes under homozygous condition for resistance to race 1 of *Fusarium* wilt in a cross between ICCV 2 ($h_1h_1h_2h_2h_3h_3$) and JG 62 ($H_1H_1H_2H_2h_3h_3$). The digenic ratio of 9:6:1 obtained in F_2 generation was confirmed by the F_3 progeny and RIL population. Recently Girase and Deshmukh (2002) also obtained trigenic ratio in two crosses viz., JG 62 x ICC 4958 and JG 62 x Vijay for *Fusarium* wilt resistance.

2.2.6.2 Genetics of resistance to other races

A good number of reports on the inheritance of resistance to *Fusarium* wilt race 1 (ICRISAT isolate) in chickpea are available. Very little information is available regarding race 2 (Kanpur isolate) and other races of *Fusarium* wilt. Similar to race 1A, resistance to race 2 was initially found to be conferred by a single recessive gene (Pathak *and* Singh, 1975), however, later studies revealed involvement of two or three genes.

The parents F_1, F_2 and F_3 of three crosses (JG 62 x JG 74, JG 62 x PG 114 and JG 74 x PG 114) were screened, utilizing the Pantnagar isolate (race 3) of *F. oxysporum* f.sp. *ciceri* by Dikshit and Singh (1994).

Gumber *et al.* (1995) found that resistance to race 2 of *Fusarium* wilt is controlled by two genes one of which (A) must be present in the homozygous recessive form, and the other (B) in the dominant form, whether homozygous heterozygous, for complete resistance. Early wilting results if the plant is homozygous recessive for bb. Late wilting occurs if both loci are dominant.

Kumar (1998) developed F_2 and RILs with a set of three crosses *viz.,* WR 315 x C 104 (resistant x susceptible), WR 315 x K 850 (resistant x late wilter) and K 850 x GW 517 (late wilter x later wilter) and studied the genetics for wilt resistance to race 2, the study indicated the involvement of the three loci (two recessive alleles and one dominant allele), homozygous recessive forms at the first two loci confer resistance and a dominant allele at the third locus complements the other two loci to late wilting whereas susceptibility occurred when first two loci were in dominant form. Later Sharma *et al.* (2005) demonstrated that resistance to race 2 was governed by a single recessive gene. The cause of the

discrepancies among the three studies could be due to different strains of race 2 being used or due to environmental conditions and evaluation techniques used.

Inheritance of resistance to race 4 of *Fusarium* wilt was studied by Tullu *et al.* (1998) using 100 F_5 derived F_7 recombinant inbred lines (RILs), that had been developed from the cross of breeding lines C 104 (late wilter) x WR 315 (resistant). They found that the gene for resistance to race 4 segregating in 1:1 ratio as expected for single gene.

Tullu *et al.* (1999) performed genetic analysis on the F_1, F_2 and F3 families from the cross of JG 62 x Surutato 77. Results indicated that two independent genes controlling resistance to race 4 of *Fusarium* wilt of chickpea. Similar to races 1 and 2, the phenomenon of late wilting was also detected for race 4.

Tekeoglu *et al.* (2000) used race 5 and race 0 to study *Fusarium* wilt resistance in F6 derived recombinant inbred lines. 1:1 (resistant: susceptible) segregation ratio was observed for both races, indicating that resistance to each race is controlled by a single gene. More recently a 3:1 resistant to susceptible ratio was observed in the RIL population of CA 2139 x JG 62 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 (1 B/C and 6) is yet to be determined.

The cross JG 74 (resistant) x JG 62 (susceptible) showed monogenic recessive inheritance of resistance. However, PG 114 (resistant) x JG 62 (susceptible) showed resistance to wilt under influence of two recessive genes. Sharma *et al.* (2004 and 2005). Resistance to race-3 of the pathogen was found to be monogenic but its

dominant or recessive nature is not yet ascertained (Sharma and Muehlbauer, 2007).

Monogenic recessive nature of resistance to race 4 was also reported by Sharma *et al.* (2005). Further, Two genes controlling resistance to *Fusarium* wilt race 0 (*FOC-1* and *FOC-2*) have been respectively located in LG5 (Cobos *et al.* 2005) and LG2 (Halila *et al.* 2009).

2.3 Phenotyping techniques for wilt resistance

Screening techniques play an important role in precise screening of the genotypes for wilt reaction. During the last decade of 20th century different screening strategies have been adopted by the researchers for screening of chickpea wilt.

Nene *et al.* (1980) standardized greenhouse based screening technique using the pot culture method. The large earthen pots measuring 35 cm in diameter were filled with non-autoclaved vertisol followed by the application of the *Fusarium* wilt fungus and autoclaved chickpea stem bits (200 g) in the top 15 cm of vertisol. The susceptible cultivar, JG 62 was planted to each pot and after wilting the plants were chopped and incorporated in the soil in the pot.

Nene, (1980b) standardized water culture screening technique. The screening procedure involved a single spore culture of *F. oxysporum* f.sp. *ciceri*, multiplication of the inoculum in 100 ml liquid media in 250 ml flasks on a shaker for 10 days at 25-30°C, adjusting the inoculum concentration to 6.5 x 10^5 or 1 x 10^6, inoculation of 14 days old seedlings grown in autoclaved sand and transferring the seedlings to glass tubes containing 20 ml of inoculum. A susceptible cultivar is used as a control and is inoculated along with each set of lines being evaluated. Also, for each test line an uninoculated seedling was kept as a check.

Haware *et al.* (1992) developed wilt sick-plot in slightly alkaline vertisols and grown a susceptible cultivar for 2 to 3 seasons and the infected plant debris were incorporated into the soil. Iqbal, (2005) screened 51 chickpea germplasm accessions under greenhouse against *F. oxysporum.* Sixteen lines exhibited highly resistant reaction. The remaining 35 lines were found to be resistant, moderately resistant, susceptible and highly susceptible. While, in field screening none of the 51 test lines were found to be highly resistant or resistant, only 6 lines showed moderate resistance, all other were susceptible to highly susceptible.

Ratnaparkhe *et al.* (1998) used plastic trays filled with coarse perlite for screening of RILs in greenhouse (21-26°C). At 3-4 nodal stage seedlings were removed from the perlite, pruned and were submerged in the spore suspension and after about 5 min in the spore suspension were replanted into the perlite. Plants were then scored as susceptible or resistant.

Sharma *et al.* (2004) cultured the *FOC 3* race in V8 liquid medium at 25° C for 20 days and later the culture was filtered through four layers of cheese cloth to separate mycelium from the spores the microconidial load was adjusted to 1x 10^6 spores per millilitre. The pre-germinated seeds of RILs grown in 20x10x2.25 tray filled with perlite were uprooted at 4-5 leaf stage and the roots were trimmed and dipped in inoculum for 5 minutes and the inoculated plants were again transferred in trays containing perlite and potting soil 1:1 v/v after planting nutrient solution was applied twice a week. The inoculated and control plants (dipped in water) were incubated at temperature regime of 26°C/22°C for 12/12 hours and 16h illumination was provided by florescent bulb. The number of wilted and live plants were noted at weekly intervals and it was found that 44 RILs were resistant and 51 were susceptible.

Ravikumar *et al.* (2007) developed a simple screening technique for *Fusarium* wilt resistance through root feeding of toxin. Further the possibility of screening the diverse genotypes as well as RILs of chickpea for wilt resistance through *in-vitro* pollen tube growth inhibition in the presence of pathotoxin was studied by Ratnababu and Ravikumar (2009 and 2010). They investigated that genes conferring wilt resistance in chickpea determine the resistance at both sporophyte and gametophyte phases. Their study demonstrated that pollen bioassay can be used to select resistance in pollen grains and consequently the genotype of the sporophyte producing pollen grains.

Chaudhry *et al.* (2007) screened 119 genotypes in wilt sick plot developed by repeated incorporation of various cultures of *Fusarium oxysporum* f. sp. *ciceri* and sowing of susceptible cultivars AUG 424, each test line was sown in a single row subplot of 3 meter and they found that none of the test line was immune or highly resistant to wilt.

Shah *et al.* (2009) screened 249 mutants in M4 generation developed through gamma irradiation and Ethyl methane sulphonate (EMS) along with four parents for *Fusarium* wilt resistance in natural wilt sick plot and found that out of 249 mutants 75 mutants exhibited highly resistant reaction (less than 10%) followed by 31 mutants resistant (11 to 20%), 34 mutants moderately resistant/tolerant (21 to 30%), 35 mutants susceptible (31 to 50%) and 75 mutants were highly susceptible (50 to 100%).

Gujaria *et al.* (2011) studied gene expression analysis of chickpea roots during *FOC* infection was performed the seeds were infected with *FOC* races 1, 2 and 4 culture initially the seeds were wrapped in wet sterile muslin cloth and stored at room temperature (24°C to 26°C) till sprouting. The sprouted seeds were transferred to trays containing sterile water with macro and micro nutrients (half strength Hoagland's nutrient

medium) and kept at 22°C and 60% relative humidity under normal day conditions (14 h light/10 h dark). Freshly prepared spore suspension (1×10^6 spores/ml) of *FOC* races 1, 2 and 4 was added individually to the sterile hydroponic trays containing seven days old chickpea plants. Seedlings grown in similar trays with no pathogen (un-inoculated plants) were used as control, the study unveiled the genes, involved in chickpea defence against *Fusarium* wilt which were not reported previously.

Varshney *et al.* (2014a) screened BC_3F_4 families, along with their parents in both sick plot sick pots specific to race 1 isolates at ICRISAT Patancheru during the off-season, and in sick pot method. The experiment was laid out in two replications using randomized complete block Design (RCBD) and three lines that showed resistance reaction, similar to donor parent.

2.4 Molecular Markers and linkage mapping in chickpea

The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. As compared to morphological and biochemical markers, molecular markers have several advantages as they are phenotypically neutral and are not influenced by pleiotropic and epistatic interactions, and their expression is not dependent on age/part of the plant (Jones *et al.,* 1997).

Various types of genomic resources like microsatellite or simple sequence repeat (SSR)/sequence tagged microsatellite markers (STMS), expressed sequence tags (ESTs), single nucleotide polymorphism (SNP), cleaved amplified polymorphic sequences (CAPS), conserved intron spanning primers (CISP) and diversity arrays technology (DArT) markers have been developed for chickpea. Among several types of markers available SSR have become popular for molecular genetic studies in crop

plants including chickpea. Similarly thousands of molecular markers including simple sequence repeats have been developed in chickpea Huttel *et al.* (1999) developed 28 SSR markers, Winter *et al.* (1999) developed 174, Sethy *et al.* (2003), developed 280 SSRs Lichtenzveig *et al.* (2005) developed 233 SSRs Qadir *et al.* (2007) developed 63 SSRs and Garg *et al.* (2011a) developed 4816 SSRs with frequency of one SSR per 5.80 kb of the chickpea sequence. Single feature polymorphism markers were developed by Saxena *et al.* (2011), single nucleotide polymorphism (SNP) by Hiremath *et al.* (2012); Saxena *et al.* (2011), and expressed sequence tags (ESTs) or transcript reads (Varshney *et al.*, 2009; Dubey *et al.*, 2012; Hiremath *et al.*, 2011; Kudapa *et al.*, 2012) were developed. Very recently, draft genome sequences have also become available for chickpea (Varshney *et al.*, 2013; Jain *et al.*, 2013).

2.4.1 Molecular mapping in chickpea

Linkage map (Genetic map or meiotic map) is defined as a linear arrangement of genes or molecular markers on the chromosomes, it is a necessary tool for genome analysis, marker-assisted breeding and map-based cloning. A linkage map may be thought of as a "Road Map" of the chromosomes derived from two different parents. Genetic maps indicate the position and relative genetic distances between markers along chromosomes, which is analogous to signs or land marks along a highway where the genes are houses (Paterson, 1996). Genetic mapping is based on the principle that genes and markers segregate via chromosome recombination (called crossing over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). It is therefore most desired in chickpea which is a valuable and important agricultural crop, where yield potential is dramatically affected by several biotic and abiotic stresses.

Therefore chickpea breeders throughout the world have been focusing on increasing the yield by pyramiding the Desirable genes for resistance/tolerance into agronomically superior varieties through integration of advanced technologies like marker assisted breeding along with conventional approaches (Millan *et al.,* 2006).

2.4.1.2 Molecular mapping in chickpea using intraspecific crosses

Mayer *et al.* (1997) for the first time tagged the wilt resistance gene H_1 (*foc-1*) of race 1 in chickpea. In bulk segregant analysis of selected F_3 plants of intraspecific cross (C 104 x WR 315), they identified two primers UBC-170_{550} and CS-27_{700} that amplified a DNA fragment linked to *Fusarium* wilt resistance and susceptibility respectively. Analysis of the RILs with these primers yielded an estimate of 7 per cent recombination between the two markers and the locus for wilt resistance and 6 per cent recombination between the loci corresponding to the two RAPD markers (UBC-170 and CS-27). After cloning and sequencing they designed two allele specific associated primers (ASAPs), CS-27_{700} and UBC-170_{550}. CS-27 amplified a fragment linked to the allele for susceptibility to race 1 (H_1 locus) of *Fusarium* wilt, whereas UBC-170 produced a single fragment for both resistant and susceptible genotypes, thus demonstrating locus specificity rather than allele specificity. Further analysis with additional markers placed the two loci near one end of linkage group VI on the linkage map of Simon and Muehlbauer (1997).

Tullu *et al.* (1998) developed an intraspecific cross (C 104 x WR 315), to determine the inheritance of resistance to race 4 and identified RAPD markers linked to the gene for resistance to race 4 and observed that resistance is controlled by single recessive gene. The RAPD markers (CS-27_{700} and UBC-170_{550}) previously shown to amplify fragments linked to race 1 resistance also amplified fragments associated with race 4

resistance and were located nine map units from the race 4 resistance. Resistance to Race 1 and 4 are five map units apart.

Tullu *et al.* (1999) developed F_1s, F_2s and F_3 population by cross JG 62 x Surutato 77 and carried genetic analysis which indicated involvement of two independent genes for resistance to race 4 further linkage analysis of candidate RAPD marker, CS-27_{700} and the phenotypic data from F_2 showed that the marker locus was linked to one of the resistance genes.

Cho *et al.* (2002) constructed an intraspecific genetic linkage map and determined map positions of genes that confer double podding(s) and seed traits using a population of 76 F_{10} derived RILs from the cross ICCV 2 (large seeds and single pods) x JG 62 (small seeds and double podded). They used 55 STMS, 20 RAPD, 3 ISSR and 2 phenotypic markers to develop a genetic map that comprised 14 linkage groups covering 297.5 cM. The gene for double podding was mapped to linkage group 6 and linked to Tr44 and Tr35 at a distance of 7.8 cM and 11.5 cM, respectively. The major gene for pigmentation was mapped to linkage group 8 and was loosely linked to Tr 33 at a distance of 13.5 cM. Four QTLs for 100- seed weight (located on LG4 and LG9), seed number per plant (LG4), days to 50 per cent flowering (LG3) were identified.

Sharma *et al.* (2004) mapped Sequence-tagged sites (STS) markers for *Fusarium* wilt (race 3) resistance using 100 F_7 RILs derived from an intra-specific cross between WR 315 and C 104. The mapping population was phenotyped in plastic plates filled with perlite under controlled condition. The linkage between markers and resistance genes was established using the map constructed with a LOD score of 4.0 and the Kosambi function. The results showed that *foc-3* gene was mapped 0.6 cM from STMS markers TA96 and TA27 and STS marker CS27A. Another STMS marker, TA194, at 14.3 cM, flanked the gene on the other side.

Linkage between *foc-3* and two other chickpea wilt resistance genes, *Foc-1* (syn. h1) and *Foc-4*, was established. *Foc-3* was mapped 9.8 cM from *foc-1* and 8.7 cM from *foc-4*, whereas *foc-1* and *foc-4* are closely linked at 1.1 cM.

Soregaon *et al.* (2007) developed linkage map for *Fusarium* wilt resistance used 164 RILs (F_8 generation) derived from crossing late wilting genotype, K 850 ($h_1h_1H_2H_2$) with resistant WR 315 (h_1h_1 h_2h) segregating for H2 locus and a second population segregate for both H_1 and H_2 loci derived from crossing of early wilting susceptible JG 62 (H_1H_1 H_2H_2) with resistant WR 315 (h_1h_1 h_2h_2)· the mapping population was phenotyped for their wilt reaction by growing in the wilt sick plot at ICRISAT Patancheru, and genotyping was done using four polymorphic RAPD obtained by screening 79 RAPD primers for parental polymorphism. The linkage map was developed by MAPMAKER programme using a LOD score of 3 and the results indicated that only one marker (A07C417) showed linkage to H_2 locus (across different genetic backgrounds) and susceptibility with a distance of 21.7 cM.

Halila *et al.* (2009) mapped the *foc 2/foc 2* gene using RIL populations derived from two intraspecific crosses between (CA2 156 × JG 62 and CA 2139 × JG 62) with 12 STMS markers. Linkage analysis was performed using JOINMAP 3.0 with a LOD score threshold of 3 and a maximum recombination fraction of 0.25 along with the Kosambi mapping function. The results suggest that all the 12 markers were linked, to LG2 tested in this study and covered a genetic distance of 38.44 cM, with an average distance of 3.49 cM between markers. The longest distance between markers was 12.38 cM and the shortest was 0.4 cM. Further QTL analysis revealed the presence of two QTLs one in LG2 and other in LG5 for resistance against *FOC* race 0. The QTL in LG5 was same as previously reported by Cobos *et al.* (2005) and TR59 was the

closest marker. The QTL on LG2, was closely flanked by two markers (TS47 and TA59), and located in a genomic region with a high density of STMS markers which were also linked to other wilt race-specific resistance genes. The marker TA59 was the closest to the new QTL and explained between 21.8% and 26.2% of the total phenotypic variation of resistance with maximum LOD scores of 4.37 and 5.41, respectively. Further the multiple QTL models (MQM) mapping analysis, employing the markers TA59 (LG2) and TR59 (LG5) as cofactors, detected two strong QTL in both years, with maximum LOD score values for LG2 and LG5 of 5.0 and 9.15 in the first year, and 6.14 and 8.52 in the second one, respectively.

Gowda *et al.* (2009) in order to map the *Fusarium* wilt resistance genes evaluated one hundred F_9 recombinant inbred lines (RILs) derived from a cross between two *Cicer arietinum* cultivars, JG 62 and Vijay, Eight hundred 10-mer RAPD primers, 500 chickpea SSR primers and an allele specific associated marker (CS27A) were used in the study and after parental screening, 175 primers with reproducible profiles were selected as polymorphic to screen the mapping population. The RILs were screened for the disease reaction for all the three *FOC* races (1, 2 and 3) in wilt sick pots at Pulses Research Station, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri. The linkage analysis was carried out using JoinMap ver. 3.0. Map was constructed at LOD 4.0 with Kosambi mapping function. Upon linkage analysis 19 markers showed association with one or more wilt resistance genes. The simple sequence repeat markers H_3A_{12} and TA110 flanked the *foc-1* locus at 3.9 and 2.1 cM, respectively, while *foc-2* was mapped 0.2 cM from TA96 and 2.7 cM from H_3A_{12}. The H_1B_{06} and TA194 markers flanked the *foc-3* locus at 0.2 and 0.7 cM, respectively. Further they validated the linked markers using 16 diverse chickpea genotypes.

Hossain *et al.* (2010) used two RIL populations derived from intraspecific crosses of a *Kabuli* type (S95362) crossed to two *Desi* types (Howzat and ICC3996) both the RILs were assessed across two environments. A total of 80 previously characterized polymorphic SSRs were used for genotyping and linkage analysis was performed with a LOD score of 3.0 on the non-distorted markers using Map Manager QTX further QTL analysis using MapManager QTX ver. 0.23 revelled, two major QTLs were one on LG 4 (QTL1) and another on LG 1 (QTL2) that together accounted for 20% of the seed size trait.

Gowda *et al.* (2011) used two 197 and 108 $F_{8:9}$ recombinant inbred line of chickpea derived from the crosses JG 62 x Vijay (JV population) and Vijay x ICC 4958 (VI population) respectively, the mapping population was phenotyped for seven agronomic) and yield traits (plant height, plant spread, number of branches per plant, number of pods per plant,) yield per plant, 100-seed weight and days to maturity) in RCBD Design at four (VI population) and three (JV population) different environment condition. The linkage maps developed using JV population had 123 markers on eight linkage groups and covered 623.2 cM with marker density of 6.1 cM, whereas the VI population had 176 markers spanning 688.4 cM with an average of 5.9 cM interval between two markers. The QTL analysis through Composite Interval Mapping using Windows QTL Cartographer Ver. 2.5 with a threshold LOD scores of 3.0 scanning intervals of 2.0 cM between the markers was used for declaring presence of the QTLs, 41 significant QTLs (LOD≥3.0) and 47 suggestive QTLs (2.0>LOD< 3.0) were identified for the seven traits in JV population and majority of the QTLs (14) were found to be associated with LG2. Sixty-five significant QTLs and 77 suggestive QTLs were detected for the seven traits in VI population. Further it was found that most of the QTLs were environment specific, however 24 QTLs expressed in more than one environment

Sabbavarapu *et al.* (2013) developed a linkage map for both *Fusarium* wilt (FW) and Ascochyta blight resistance using 188 F_2 plants of each mapping population ($F_{2:3}$) derived from C 214 (FW susceptible) x WR 315 (FW resistant) and C 214 (AB susceptible) x ILC 3279 (AB resistant). The mapping population was screened for *Fusarium* wilt in wilt-sick plot at Patancheru, India during crop season 2011–2012 in two replications using randomized complete block and for Ascochyta blight the screening was done in plastic trays (40 x 30 x 5 cm) filled with sand and vermiculate mixture in 10:1 ratio under controlled environment facility (CEF) at ICRISAT-Patancheru. The genotyping was done using 298 SSR markers and linkage analysis was performed by JoinMap 3.0 program with a LOD value of 3.0. In case of FW resistance mapping 57 markers were mapped onto 8 linkage groups spanning a total map length of 347.9 cM and in case of AB resistance mapping 58 markers were mapped onto 10 linkage groups with the total map length of 386.3 cM.

2.4.1.1 Molecular mapping in chickpea using inter-specific crosses

The beginning of the linkage map development in chickpea was based on morphological and isozyme loci. However their small number and the fact that expression of these markers is often influenced by environment make them unsuitable for routine use. Further there was extreme low level of polymorphism among cultivated genotypes of chickpea therefore inter-specific crosses were exploited for development of genetic linkage map (Gaur and Slinkard 1990).

The first inter-specific linkage map of chickpea was developed by Gaur and Slinkard (1990) using F_2 population derived from *C. arietinum* x *C. reticulatum* and *C. arietinum* x *C. echinospermum* with 3 morphological and 26 isozyme markers and identified 7 linkage groups with a genome coverage of 200 cM.

Kazan *et al.* (1993) studied inheritance and linkage relationships of five morphological and 23 isozyme markers using F_2 as mapping population derived from two different inter-specific crosses by crossing (*C. arietinum* x *C. reticulatum*) and (*C. arietinum* x *C. echinospermum*), obtained 8 linkage groups with an genome coverage of 257 cM.

Ratnaparkhe *et al.* (1998) also used $F_{6:7}$ RILs (131) developed from the inter-specific cross between *C. arietinum* (ICC-4958, resistant) and *C. reticulatum* (PI 489777, susceptible) through single seed decent procedure. They reported that 38 ISSR primers out of 100 were found to be polymorphic and segregated in a 1:1 Mendelian ratio. They first time reported an ISSR marker UBC-855550 linked to the gene for resistance to *Fusarium* wilt race 4 at a distance of 5.2 cM. It co segregated with CS-27_{700}, resistance to *Fusarium* wilt race 1 and was mapped to linkage group 6 of the cicer genome. The marker UBC-855_{550} is located 0.6 cM from CS-27_{700} and is present on the same side of the wilt resistance gene.

Winter *et al.* (1999) mapped a total of 120 sequence-tagged microsatellite site (STMS) markers in 90 recombinant inbred lines developed from an inter-species cross between *C. arietinum* and *C. reticulatum*. The results showed that at a LOD score of 4 the markers were arranged in 11 linkage groups and with a genome covering of 613 cM.

Santra *et al.* (2000) used a set of 142 $F_{5:6}$ RILs obtained from an interspecific cross of *C. arietinum* (FLIP84 92C Ascochyta blight resistance resistant parent) with *C. reticulatum* Lad. (PI 599072, susceptible parent) and linkage map was established that comprised nine linkage groups containing 116 markers covering a map distance of 981.6 cM with an average distance of 8.4 cM between markers. Two QTLs, QTL-1 and QTL-2, conferring resistance to Ascochyta blight, were

identified and mapped to linkage groups 6 and 1 respectively. Two RAPD markers flanked QTL-1 and were 10.9 cM apart while one ISSR marker and an isozyme marker flanked QTL-2 and were 5.9 cM apart. Further the map was extended by Tekeoglu *et al.* (2002) by employing 50 SSRs and one Resistance gene analogue (RGA) marker.

Benko-Iseppon *et al.* (2003) developed 131 RIL population by inter-specific cross between *Cicer arietinum* (Resistant parent) with *Cicer reticulatum* (Susceptible parent) segregating resistant genes against *Fusarium oxysporum* f.sp. *ciceri* races 4 and 5, and developed DNA amplification finger printing (DAF) markers linked to both resistance loci. The results obtained by performing BSA revealed 19 new markers on linkage group 2 of the genetic map on which the resistance genes are located and the closest linkage of 2.0 cM distance was observed between marker R-2609-1 and the race 4 resistance locus. Further they found that seven other markers flanking the wilt resistant locus at a range of 4.1 to 9.0 cM distance. The sequencing of the linked markers revealed that was highly similar to genes encoding proteins involved in plant pathogen response, such as PR-5.

Rajesh *et al.* (2004) performed BSA on a resistant bulk and a susceptible bulk along with parents for Ascochyta blight resistance using RGA primers Designed based on the conserved motifs present in characterized R-genes of all available RGAs and their 48 different combinations. Only one RGA showed polymorphism during BSA. This marker was evaluated in an $F_{7:8}$ population of 142 RILs from an interspecific cross of *C. arietinum* (FLIP 84-92C) x *C. reticulatum* (PI599072) and was mapped to *cicer* linkage map. They compared genomic locations of chickpea RGA with the locations of mapped chickpea R-genes. That is the first RGA marker mapped to chickpea linkage map.

Abbo *et al.* (2005) developed a F_2 mapping population from a cross between an Israeli cultivar and wild *Cicer reticulatum* the mapping population was phenotyped for carotenoid concentration and seed weight, the population was genotyped using 91 sequence tagged microsatellite site markers and two CytP450 markers to generate a genetic map consisting of nine linkage groups and a total length of 344.6 cM. Further QTL mapping with a LOD score of 2 revealed four QTLs for beta-carotene concentration, a single QTL for lutein concentration and three QTLs for seed weight, the most significant QTL for seed weight was located on LG 4B flanked by markers GA24 and STMS11 on one side and GA2 on the other and two other, less significant seed weight QTLs were detected on LG 1B (QTL2) and 2B (QTL3).

Cobos *et al.* (2006) obtained a linkage map in a RIL population derived from an interspecific cross between *Cicer arietinum* (ILC72) x *Cicer reticulatum* (Cr5-10) resistant and susceptible to blight, caused by *Ascochyta rabiei*, respectively, using RAPD, ISSR, STMS, isozyme (pdf6) and flower colour (pink/white) markers. The map comprised 10 linkage groups and covered a distance of 601.2 cM. A QTL explaining 28 per cent of the variation for resistance and was located in linkage group 2 (LG2). Five RAPD markers on this linkage group showed significant association with resistance (OPA04372, UPC881621, OPAI09352 and OPAC12700) and the major QTL peak lay midway between OPAI09746 and UBC881621 which are 14.1 cM apart.

Cobos *et al.* (2009) constructed a genetic map from a RIL population from the cross *Cicer arietinum* (ICCL81001) x *Cicer reticulatum* (Cr5-9), which is composed of 10 linkage groups covering 848.1 cM. The lines were grouped with RAPD, ISSR and STMS markers and phenotyped with respect to flowering time, flower colour, growth habit, seed size, seed coat reticulation, seed coat thickness. Segregation distortion was

observed for 35 per cent of markers, mainly clustered on LG4, LG7 and two unassigned linkage groups. Rs/rs (seed coat reticulation) together with a QTL accounted for >50 per cent of the variation for seed coat thickness. Both Hg/hg (growth habit) and a flowering time QTL mapped to LG3A. The STMS marker TA142 was closely linked to this QTL. Two QTL for seed size were detected one on LG4 linked to STMS markers GAA47 and STMS11, and the other on LG2 linked to STMS TA110.

Choudhary *et al.* (2012) reported development of an advanced transcript map of chickpea. Chickpea seeds were used to generate 2,496 ESTs, which led to development of 487 novel EST-derived functional markers including EST-SSRs (125), intron targeted primers (ITPs) (151), expressed sequence tag polymorphisms (ESTPs) (109), and single nucleotide polymorphisms (SNPs) (102). These 487 markers along with 385 previously published markers were used for genotyping the reference population derived from a cross between *C. arietinum* (ICC4958) and *C. reticulatum* (PI489777) and linkage map was constructed with 406 loci distributed on eight linkage groups spanning 1,497.7 cM with average marker density was 3.68 cM and the average number of markers per LG was 50.8.

2.4.1.1 Integrated mapping – through consensus map approach

Knowledge of the inheritance of agronomic characters is a basic requirement to identify and integrate interesting genes in linkage maps and to utilise these maps for marker-assisted selection (MAS) of these characters to accelerate the development of new cultivars hence most of the chickpea geneticists agree that the generation of an integrated genetic map of the crop, comprising loci of both economic and scientific importance, presently is a central goal of chickpea genetics (Millan *et al.,* 2006).

Simon and Muehlbauer (1997) developed first integrated genetic linkage map of chickpea. The map consists of 9 morphological, 27 isozyme, 10 RFLP, and 45 RAPD markers covering 550 cM. The map was made from segregation data from populations of three interspecific crosses of cultivated chickpea (*C. arietinum*) and a closely related wild species (*C. retlculatum*). The linkage map had 10 linkage groups representing the eight chromosomes of chickpea. They also observed that several regions of the genome were found to be slightly skewed from the expected Mendelian ratios of alleles and by comparing the map with published maps of pea and lentil they found five regions of the chickpea map have gene orders that were similar to those found in the pea genome.

Winter *et al.* (2000) constructed a comprehensive reference map using 130 F6:7. RILs from the interspecific cross between cultivated chickpea line ICC 4958 x *C. reticulatum* (PI 489777). The same population was used previously (Winter *et al.,* 1999) to generate a core STMS marker map. A total of 354 markers were mapped on the RILs including 118 STMs, 96 DAFs, 70 AFLPs, 37 ISSRs, 17 RAPDs, 8 isozymes, 3 CDNAs, 2 SCARs and 3 loci that confer resistance against different races of *Fusarium* wilt. At a LOD score of 4.0, 303 markers covered 2077.9 cM in eight large and eight small linkage groups at an average distance of 6.8 cM between markers. Eight large groups probably represent the eight chickpea chromosomes. However in view of so many unlinked (51) markers, they reported that large regions of the genome are still not covered by markers.

Cobos *et al.* (2005) developed an integrated map using two $F_{6:7}$ RIL populations from intra-specific crosses of CA 2156 x JG 62 and CA 2139 x JG 62. CA 2156 and CA 2139 are white flowered, single podded, Spanish Kabuli landrace with thin seed coat, whereas JG 62 is

an Indian *Desi* cultivar with purple flowers and double podding with thick seed coat. CA 2156 is susceptible to *Fusarium* wilt *FOC-0* whereas CA 2139 and JG 62 are resistant to *FOC-0*. A QTL for wilt (*FOC-0*) resistance in RIL population CA 2139 x JG 62, was identified and the QTL explained a variation of 34.8 per cent to 37.8 per cent. Three morphological traits and 48 common molecular markers enabled joining of maps obtained independently. Flower colour (B/b) and seed coat thickness (Tt/tt) appeared to be linked to STMS marker GAA47 on LG1; this confirmed the earlier results of Tekeoglue *et al.* (2002). The single/double podding locus (Sfl/sfl) was located on LG9 jointly with two RAPD markers and STMS TA80. The association between TA80 and Sfl/sfl was reported by Rajesh *et al.* (2004) and Cho *et al.* (2002) confirmed it in RILs population.

Radhika *et al.* (2007) developed an integrated intra-specific map spanning a region of 739.6 cM, including 230 markers at an average distance of 3.2 cM between markers. Fifty STMS markers and a RGA locus were integrated by Tekeoglu *et al.* (2002) into a chickpea genetic map that was previously constructed using 142 F_6 derived recombinant inbred lines (RILs) from a cross of *C. arietinum* x *C. reticulatum* Lad. The map covered 1,174.5 cM with an average distance of 7.0 cM between markers in nine linkage groups and nine markers including the RGA showed distorted segregation. Six codominant STMS markers were integrated into two previously reported major QTL conferring resistance to Ascochyta blight caused by *Ascochyta rabiei*. Using common STMS markers as anchors, three maps developed from different mapping populations were joined and Three yield related traits, double podding (Sfl), seeds per pod (Spp) and seed weight (Sw) were mapped genes for double podding (Sfl) were tagged by the markers NCPGR33 and UBC249z at 2.0 and 1.1 cM, respectively on LG-2, whereas, seeds per pod (Spp), was tagged by the markers Ta2x and UBC_{465} at 0.1 and 1.8 cM,

respectively further genes for resistance to *Ascochyta* blight and *Fusarium* wilt, were located on the combined linkage map.

Millan *et al.* (2010) constructed a consensus genetic map by merging linkage maps from 10 different populations, using STMS as bridging markers. Those populations derived from five wide crosses (*C. arietinum* x *C. reticulatum*) and five narrow crosses (Desi x Kabuli types) were previously used for mapping genes for several agronomic traits such as Ascochyta blight, *Fusarium* wilt, rust resistance, seed weight, flowering time and days to flower. The integrated map obtained from wide crosses consists of 555 loci including, among other markers, 135 STMS and 33 cross genome markers distributed on eight linkage groups and covered 652.67 cM. The map obtained from narrow crosses comprises 99 STMS, 3 SCARs, 1 ASAP, *Fusarium* resistance gene, 5 morphological traits as well as RAPD and ISSR markers distributed on eight linkage groups covering 426.99 cM. Comparison between maps from wide and narrow crosses reflects a general coincidence, although some discrepancies are discussed.

Thudi *et al.* (2011) developed a comprehensive genetic map for Chickpea with SSR and DArT markers. A BAC-library comprising 55,680 clones was constructed and 46,270 BESs were generated and primer pairs were designed for 1,344 SSRs. In parallel, DArT array with 15,000 clones was developed. Parental screening of these markers found 253 BES-SSR markers and 675 DArT markers polymorphic. These polymorphic markers resulted in comprehensive genetic map comprising 1,291 markers on eight linkage groups (LGs) spanning a total of 845.56 cM distance based on recombinant inbred line (RIL) population ICC 4958 × PI 489777. The number of markers per linkage group ranged from 68 (LG 8) to 218 (LG 3) with an average inter-marker distance of 0.65 cM.

Varshney *et al.* (2014b) constructed two individual genetic maps comprising 241 loci and 168 loci for ICCRIL03 (ICC 4958 × ICC 1882) and ICCRIL04 (ICC 283 × ICC 8261) mapping population respectively, and a consensus genetic map comprising 352 loci, the mapping population was phenotyped for a total of 20 drought component traits in 7 seasons at 5 locations in India. Further QTL mapping revelled 45 robust main-effect QTLs explaining up to 58.20 % phenotypic variation and 973 epistatic QTLs explaining up to 92.19 % phenotypic variation for several target traits. Five robust main QTLs (4 for Plant height and 1 for Shoot Dry Weight) with up to 30.20 % PV were identified and among five robust QTLs, a QTL named 'QR3sdw01' flanked by 'TAA170–NCPGR21' on CalG04 appeared consistently for two seasons.

2.5.4 Physical Mapping

A physical map is fundamental to any progress towards a more complete understanding of the structure, composition and function of the genome. This cannot be achieved by mere recombination mapping, the construction of a complete physical map of a genome, is challenge for chickpea genomics (Millan *et al.* 2006).

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. In the case of chickpea, a BIBAC library consisting of 23,780 clones, with an average insert size of 100 kb and covering about 3.8X genomes of chickpea was developed (Rajesh *et al.,* 2004). However, multi-enzyme

BAC libraries with higher genome coverage are required for comprehensive genome research (Ren *et al.,* 2005; Wu *et al.,* 2004a; Wu *et al.,* 2004b). Subsequently, Lichtenzveig *et al.* (2005) developed a BAC and BIBAC library in chickpea from the Hadas genotype; digested with HindIII and BamHI enzymes, respectively. These BAC and BIBAC libraries consist of 14,796 and 23,040 clones, respectively, with an average insert size of 121 kb (BAC) and 145 kb (BIBAC). These libraries jointly represent about 7.0X genome coverage of chickpea. Furthermore, Zhang *et al.* (2010) constructed BAC and BIBAC libraries consisting of 22,272 and 38,400 clones, respectively. Analysis of random clones showed combined genome coverage of 11.5X. Very recently, a BAC-library was developed from ICC 4958 that comprise 55,680 clones digested with Hind III (Thudi *et al.,* 2011). Sequencing of 25,000 clones has provided 46,270 BAC-end sequences (BESs) that were used to develop SSR markers. SSR markers (157), derived from BESs, have been integrated into the genetic map based on the ICC 4958 × PI 489777 population.

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. A pioneer work towards integration of genetic and chromosome-based physical maps has been done recently by Zatloukalova *et al.* (2011). They were able to assign linkage groups (LG) in chickpea to different chromosomes using flow cytometry and PCR-based primers that amplify sequence tagged microsatellite site 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. This ability to isolate individual chromosomes will be useful in high-throughput physical mapping. This could also be used to discover genes and determine the order of low-copy genic regions on a chromosome as recently ~738 mb draft whole genome sequence of Kabuli chickpea variety has been released, and these sequence resources assist genomics-based breeding approaches such as genotyping by sequencing, genome-wide association studies and genomic selection (Varshney *et al.,* 2013; Jain *et al.,* 2013).

Varshney *et al.* (2014a) developed Physical map of chickpea for the reference chickpea genotype (ICC 4958) using bacterial artificial chromosome (BAC) libraries targeting 71,094 clones (~12X coverage). The physical map was linked with two genetic maps with the help of 245 BAC-end sequence (BES)-derived simple sequence repeat (SSR) markers, and located some of BACs in the vicinity of important quantitative trait loci (QTLs) for drought tolerance and resistance to *Fusarium* wilt and *Ascochyta* blight, further comprehensive analysis of markers in abiotic and biotic stress tolerance QTL regions led to identification of 654, 306 and 23 genes in drought tolerance "QTLhotspot" region, Ascochyta blight resistance QTL region and *Fusarium* wilt resistance QTL region, respectively.

2.6 Genetic variability, heritability and genetic advance studies in chickpea

2.6.1 Genetic variability studies

In chickpea substantial variability does exists, but much of it cannot be used directly as it is mostly associated either with low yield or poor seed features, making it unacceptable. Hence, the information on

the magnitude of variability for different quantitative and qualitative trait along with their heritability.

2.6.2 Variability, heritability and genetic advance

Variability is the most distinctive feature of living beings and forms the foundation of plant improvement. In a highly self-pollinated crop like chickpea, the crop improvement strategy for varietal development by breeding programme, involves selection of potential genotypes from the existing germplasm, utilizing them in the hybridization programme and isolation of superior genotypes in the segregating population. This necessitates a thorough knowledge of genetic variability, heritability and the genetic advance that can be achieved through selection. The available information on variability, heritability and genetic advance is summarized in tables 1 and 2.

Table 1: Summary of review on variability for various traits in chickpea

Sl. No.	Character	Variability	Material studied	Reference
1	Plant height	High	28 genotypes	Dasgupta and Islam (1992)
			96 cultivars	Bhatia *et al.* (1993)
			10 exotic genotypes	Wahid and Ahmed (1999)
			16 cultivars	Sial (2003)
			31 genotypes	Sharaan *et al.* (2008)
			28 genotypes	Sharma *et al.* (2005)
			20 genotypes	Ali *et al.* (2011)
			20 genotypes	Akthar *et al.* (2011)
		Low	40 diverse genotypes	Arora(1991)
2	Number of branches per plant	High	28 genotypes	Dasgupta and Islam (1992)
			96 cultivars	Bhatia *et al.*(1993)
			70 genotypes	Chavan *et al.* (1994)
			2 crosses CP-9623xT39-1 and RS-11xT39-1	Vijayalaxmi *et al.* (2000)
			20 genotypes	Sharma *et al.* (2005)
			31 genotypes	Sharaan *et al.* (2008)
			20 genotypes	Ali *et al.* (2008)
			20 genotypes	Malik *et al.* (2010)
			28 genotypes	Sharma *et al.* (2010)
			20 genotypes	Ali *et al.* (2011)
3	100 Seed weight	High	40 diverse genotypes	Arora(1991)
			96 cultivars	Bhatia *et al.*(1993)
			44 varieties	Rao *et al.* (1994)
			50 genotypes	Kumar *et al.* (2001)
			40 cultivar	Nimbalkar (2000)
			2 crosses CP 9623xT39-1 and RS-11xT39-1	Vijayalaxmi *et al.* (2000)
			40 genotypes	Arora and Jeena(2001)
			36 genotypes	Jeena and Arora(2001)
			33 genotypes	Yadav *et al.*, (2003)
			14 genotypes	Burli *et al.,*(2004)
			20 genotypes	Sharma *et al.* (2005a)

			15 Kabuli genotypes	Yucel *et al.* (2006)
			18 elite genotypes & 2 varieties	Saleem *et al.* (2005)
			25 genotypes	Dwevedi and Gabriel (2009)
			31 genotypes	Sharaan *et al.* (2008)
			32 genotypes	Atta *et al.* (2008)
			4 varieties	Bicer *et al.* (2009)
			28 genotypes	Sharma *et al.* (2010)
			20 genotypes	Ali *et al.* (2011)
4	Number of pods per plant	High	40 diverse genotypes	Arora(1991)
			96 cultivars	Bhatia *et al.*(1993)
			70 genotypes	Chavan *et al.* (1994)
			44 varieties	Rao *et al.* (1994)
			40 lines derived via multiple inter varietal crosses	Rao(1996)
			100 genotypes	Tripathi (1998)
			20 genotypes	Sharma *et al.* (2005a)
			31 genotypes	Sharaan *et al.* (2008)
			362 accessions	Kumar *et al.* (2001)
			24 kabuli genotypes	Naghavi and Jahansouzs (2005)
			18 elite genotypes and 2 varieties	Saleem *et al.* (2005)
			17 genotypes	Saleem *et al.* (2008)
			360 landraces and lines	Fashadfar *et al.* (2008)
			32 genotypes	Atta *et al.* (2008)
			25 gnotypes	Dwevedi and Gabriel (2009)
			28 genotypes	Sharma *et al.*(2010a)
			20 genotypes	Ali *et al.* (2011)
		Moderate	28 genotypes	Dasgupta *et al.* (1992)
		Low	140 Varieties	Islam *et al.* (1984)
			17 genotypes	Saleem *et al.* (2008)

5	Seed yield per plan	High	40 genotypes	Arora (1991)
			44 Varieties	Rao *et al.* (1994)
			40 lines derived from multiple inter varietal cross	Rao, (1996)
			30 F4 progenies	Wanjarin and Patil (1996)
			100 genotypes	Tripathi *et al.* (1998)
			10 exotic genotypes	Wahid and Ahmed (1999)
			40 genotypes obtained by single, double and multiple crosses	Rao and Kumar (2000)
			40 genotypes obtained by single, double and multiple crosses	Jeena and Arora (2001)
			136 genotypes	Hegde *et al.* (2002)
			30 genotypes	Sable *et al.* (2000)
			33 diverse genotype	Yadav *et al.* (2003)
			14 genotype	Burli *et al.* (2004)
			F3 progenies and biparental population	Singh *et al.*,(2004)
			362 accessions	Naghavi and Johansour (2005)
			20 genotypes	Sharma *et al.* (2005)
			32 genotypes	Atta *et al.* (2008)
			4 varieties	Bicer (2009)
			20 genotypes	Malik *et al.* (2009)
			28 genotypes	Sharma *et al.* (2010a)
			20 genotypes	Ali *et al.* (2011)
			20 genotypes	Akthar *et al.* (2011)
		Moderate	2 crosses CP-9623xT39-1 and RS-11xT39-1	Vijayalaxmi *et al.* (2000)

Table 2: Summary of review on heritability and Genetic advance for various traits in chickpea

Sl. No.	Character	Heritability	Reference	Genetic advance	Reference
1	Plant height at maturity	High	Misra (1991), Rao *et al.* (1994), Arshad *et al.* (2002), Singh *and* Sing (2002), Burli *et al.* (2004), Saleem *et al.* (2005), Sharma *et al.* (2005a), Saleem *et al.* (2008), Atta *et al.* (2008), Sharma *et al.* (2010), Ali *et al.* (2011) and Akthar *et al.* (2011)	High	Dumbre *et al.* (1984), Rao *et al.* (1994), Arshad *et al.* (2002), Sharma *et al.* (2005a), Saleem *et al.* (2008), Atta *et al.* (2008), Sharma *et al.* (2010) and Ali *et al.* (2011)
		Moderate	Salimath and Patil *et al.* (1990) and Sharaan *et al.* (2008)	Moderate	Saleem *et al.* (2005)
				Low	Singh and Sing (2002)
2	No. of branches	High	Wahid and Ahmed (1999), Khargade *et al.*(1985), Kampli *et al.* (2002) and Sial *et al.* (2003) Vijayalakshmi *et al.* (2000), Singh and Sing (2002), Saleem *et al.* (2008), Atta *et al.* (2008), Sharma *et al.* (2005a) and Sharma *et al.* (2010)	High	Khargade *et al.* (1985), Misra (1991 Arora and Jeena (2001), Kampli *et al.* (2002), Sharma *et al.* (2005a), Saleem *et al.* (2008) and Sharma *et al.* (2010)
				Moderate	Vijayalakshmi *et al.* (2000)
		Low	Arshad *et al.* (2002), Sharaan *et al.* (2008) and Ali *et al.* (2011)	Low	Arshad *et al.* (2002), Singh and Sing (2002) and Ali *et al.* (2011)

3	Days to 50% flowering	High	Govil and Kumar (1989), Misra (1991), Panchabhai *et al.* (1992), Jahagirdar *et al.* (1994), Jahagirdar *et al.* (1996), Kumar and Krishna (1998), Singh and Sing. (2002), Sial *et al.* (2003), Arshad *et al.* (2004), Burli *et al.* (2004), Bicer *et al.* (2005), Misra (1991), Sharma *et al.* (2005a), Sharaan *et al.* (2008), Saleem *et al.* (2008) and Sharma *et al.* (2010)	High	Khorgade *et al.* (1985), Govil and Kumar (1989), Jahagirdar *et al.* (1994), Jahagirdar *et al.* (1996), Burli *et al.* (2004) and Saleem *et al.* (2008)
				Low	Singh and Sing (2002), Arshad *et al.* (2004), Sharma *et al.* (2005a) and Sharma *et al.* (2010)
4	100 seed weight	High	Misra (1991), Jahagirdar *et al.* (1994), Rao *et al.* (1994), Jahagirdar *et al.* (1996), Mathur and Mathur (1996), Wanjari and Patil (1996), Kumar and Krishna (1998), Kumar *et al.* (1999), Nimbalkar (2000), Jeena and Arora (2001), Arora and Jeena (2001), Singh and Singh (2002), Sail *et al.* (2003), Arshad *et al.* (2004), Burli *et al.* (2004), Jeena *et al.* (2005), Saleem *et al.* (2005), Sharma *et al.* (2005a), Bicer *et al.* (2005), Saleem *et al.* (2008), Atta *et al.* (2008) and Sharma *et al.* (2010),	High	Jahagirdar *et al.* (1996), Mathur and Mathur (1996), Kumar *et al.* (1999) Nimbalkar (2000), Arora and Jeena (2001), Jeena and Arora (2001), Singh *et al.* (2002), Sial *et al.* (2003), Burli *et al.* (2004), Jeena *et al.* (2005), Sharma *et al.* (2005a), Saleem *et al.* (2008), Atta *et al.* (2008), Sharma *et al.* (2010a) and Ali *et al.* (2011)
				Moderate	Misra (1991)
				Low	Arshad *et al.* (2004) and Saleem *et al.* (2005)
		Moderate	Vijayalakshmi *et al.* (2000), Sharaan *et al.(*2008) and Ali *et al.* (2011)		

5	Number of pods per plant	High	Misra (1991), Chavan *et al.* (1994), Jahagirdar *et al.* (1994), Jahagirdar *et al.* (1996), Kumar *et al.* (1999), Singh *et al.* (2000), Kumar *et al.* (2001), Singh and Singh (2002), Jeena *et al.* (2005), Bicer *et al.* (2005), Saleem *et al.* (2005), Atta *et al.* (2008), Sharma *et al.* (2005a), Sharma *et al.* (2010) and Ali *et al.* (2011)	High	Jahagirdar *et al.* (1996), Tripathi (1998), Kumar *et al.* (1999), Singh *et al.* (2000), Kumar *et al.* (2001), Arshad *et al.* (2002), Sial *et al.* (2003), Jeena *et al.* (2005), Sharma *et al.* (2005a), Atta *et al.* (2008), Dwevedi and Gabriell (2009), Sharma *et al.* (2010a) and Ali *et al.* (2011)
				Moderate	Singh and Singh (2002) and Saleem *et al.* (2008)
		Moderate	Salimath *et al.* (1990), Wahid and Ahmed (1999), Sial *et al.* (2003), Sharaan *et al.* (2008) and Saleem *et al.* (2008)	Low	Saleem *et al.* (2005)
		Low	Arshad *et al.* (2002)		
6	Seed yield per plant	High	Jahagirdar *et al.* (1996), Mathur and Mathur (1996), Kumar *et al.* (1998), Singh and Singh (2002), Deshmukh *et al.* (2004), Jeena *et al.* (2005), Saleem *et al.* (2005), Bicer *et al.* (2005), Sharma *et al.* (2005a), Ali *et al.* (2011), Sharaan *et al.* (2008), Atta *et al.* (2008), Sharma *et al.* (2010a) and Akthar *et al.* (2011)	High	Jhagirdar *et al.* (1996), Mathur and Mathur (1996), Tripathi (1998), Kumar *et al.* (1998), Sial *et al.* (2003), Jeena *et al.* (2005), Sharma *et al.* (2005a), Atta *et al.* (2008) and Sharma *et al.* (2010a)
		Moderate	Wanjari and Patil (1996), Kumar and Krishna (1998) and Wahid and Ahmed (1999)	Moderate	Misra (1991) and Vijayalakshmi *et al.* (2000)
		Low	Arshad *et al.* (2002), Sial *et al.* (2003)	Low	Arshad *et al.* (2002) and Saleem *et al.* (2005)

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present study was carried out with the objectives to map the genic and genomic SSR markers and to identify the QTLs influencing wilt resistance and other yield and yield components. The details of the experiments conducted and the materials used are presented below.

3.1 Evaluation of RILs for seed yield and yield components

3.1.1 Details of experimental site

All the field experiments were conducted at the experimental field of University of Agricultural Sciences GKVK Bengaluru. The experimental field belongs to the AINP (All India Network Project on Acarology) at 'K' Block of UAS GKVK Bengaluru, Karnataka. The experimental area was situated at 12.96° N latitude and 77.56° E longitude at an altitude of 920 meter above the sea level with an average rainfall of about 900 mm. The soil type of the experimental block was alfisols with pH range of 5.5 to 6.5.

3.1.2 Plant material

A set of 141 intraspecific F_{10} derived F_{11} recombinant inbred lines (RILs) derived by single seed descent method by crossing K 850 (susceptible late wilter, $h_1h_1H_2H_2$) and WR 315 (resistant, $h_1h_1h_2h_2$) segregating for wilt resistance were used in the present study.

The RILs were grown in two replications in Randomized Complete Block Design (RCBD). Each line was grown in a single row of 5 m per replication accommodating 32 plants. The susceptible early wilting genotypes JG 62 and resistant WR 315 were sown after every 20 rows as checks and wilting of JG 62 in all the rows was conformed for uniform distribution of inoculum of *Fusarium oxysporum* throughout the experimental block. The number of plants showing wilt symptoms in

each line was recorded in percentage on 30th day for early wilting and 60th and 90th day for late wilting. The death due to wilting was confirmed by observing the discolouration of xylem by uprooting the plants.

3.1.3 Experimental methods

The above mentioned 141 RILs were evaluated for six morphological traits under field conditions over two seasons 2012 *rabi* and 2013 *rabi* in two different experimental Designs at UAS, GKVK, Bengaluru. The details of the Designs and observations recorded are given below.

3.1.3.1 First season evaluation

The 141 (F_{10} derived F_{11}) recombinant inbred lines along with their parents (K 850 and WR 315) and checks (JG 62 {Double podded, early flowering, small seeded, *Fusarium* wilt susceptible}, A 1{National check high yielding} and JG 11 {Late wilting Susceptible}) were evaluated for agronomic and productive traits in AINP (All India Network Project on Acarology) field at UAS GKVK Bengaluru during *rabi* 2012. The trial was laid out in augmented design, each genotype was grown in a row of each 2.5 m length with 30 cm spacing between rows and 10 cm with in the row. Recommended agronomic practices with regular irrigations were followed for proper crop growth.

3.1.3.2 Second season evaluation

The same 141 F_{11} derived F_{12} recombinant inbred lines harvested from the previous season (2012 *rabi*) along with their parents were planted in RCBD (Randomized Complete Block Design) with two replications in AINP (All India Network Project on Acarology) field at UAS GKVK Bengaluru during *rabi* 2013. Each entry was represented by a single row of two meter length per replication with a spacing of 30 cm between rows and 10 cm between plants within the row, and

recommended agronomic practise including regular irrigation to raise a good crop growth.

For both seasons the observations on six quantitative characters were recorded on five competitive plants selected at random per RIL per replication. The procedure followed for recording observations for each trait is presented below.

a) **Days to 50 per cent flowering:** The number of days taken from sowing to the opening of flowers in 50 per cent of plants in each genotype was recorded.

b) **Plant height at maturity:** The plant height was measured in centimetre from ground level to the tip of main stem at harvest.

c) **Number of branches per plant:** The total number of branches arising directly from the main stem was counted at the time of harvest.

d) **Number of pods per plant**: Total number of pods per plant was counted at the time of harvest.

e) **100 seed weight:** The weight in grams (g) of a counted sample of 100 seeds from each plant was recorded. In case where the seed number was less than 100, it was calculated from the weight of available number of seeds and converted to 100 seed weight.

f) **Seed yield per plant:** The weight of seeds in grams (g) from each plant was recorded.

3.1.3.3 Phenotyping RILs for wilt reaction

The RILs were grown in two replications in Randomized Complete Block Design (RCBD). Each line was grown in a single row of 5 m per

replication accommodating 32 plants. The susceptible early wilting genotypes JG 62 and resistant WR 315 were sown after every 20 rows as checks and wilting of JG 62 in all the rows was confirmed for uniform distribution of inoculum of *Fusarium oxysporum* throughout the experimental block. The number of plants showing wilt symptoms in each line was recorded in percentage on 30th day for early wilting and 60th and 90th day for late wilting. The death due to wilting was confirmed by observing the discolouration of xylem by uprooting the plants.

3.1.3.4 Statistical analysis

The mean of data collected for six agronomic traits of 141 RILs and two parents (K 850 and WR 315) were subjected to following statistical analysis.

3.1.3.4.1 Analysis of variance (ANOVA)

The analysis of variance for both first (2012 *rabi* in augmented Design) and second (2013 *rabi* in RCBD with two replications) season data for different characters was carried out using their mean data in order to partition variability due to different sources by following Panse and Sukhatme (1961).

In order to assess and quantify the genetic variability among the RIL population for the characters under study, estimation of the genetic parameters such as genotypic coefficient of variability (GCV), phenotypic coefficiency of variability (PCV), heritability (h2), and genetic advance mean (GAM) were carried out using Windowstat 8.0 (developed by Indostat Services 18, Ameerpet, Hyderabad, India).

Structure of ANOVA for augmented Design

Source of variation	Df	MSS	'F' ratio
Block (eliminating checks + RILs)	(b-1)	MSS(b)	MSS(b)/ EMSS
Entries (checks + RILs progenies) (ignoring blocks)	(e-1)	MSS (e)	MSS(e)/EMSS
Checks	(c-1)	MSS (c)	MSS(c)/EMSS
RILs	(v-1)	MSS (v)	MSS(v)/EMSS
Checks vs. (RILs)	(c-1) (v-1)	MSS (vc)	MSS (vc)/EMSS
Non genetic (error)	(c-1) (b-1)	EMSS	

Structure of ANOVA for RCBD

Sources of variation	d.f.	MSS	F value
Replication	(r-1)	MSS r=M1	-
Genotypes	(g-1)	MSS g=M2	M2/M3
Error	(r-1) (g-1)	EMSS=M3	-
Total	(rg-1)	M1+M2+M3	-

Where,
g = number of genotypes
r = number of replications

3.1.3.4.2 Estimation of genetic parameters

In order to assess and quantify the genetic variability among the RILs for the characters under study, the following parameters were estimated.

a. Estimation of variance components

Phenotypic and genotypic variances were estimated using the following formula (Singh and Chaudhary, 1979).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MSS (genotype)} - \text{MSS (error)}}{\text{Number of replications}} = \frac{M2 - M3}{r}$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \text{MSS (error)} = \frac{M2 - M3}{r} + M3$$

b. Coefficient of variability

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

i) Genotypic coefficient of variability (GCV)

$$GCV = \frac{\sqrt{\sigma^2 g}}{\bar{X}} X\ 100$$

(ii) Phenotypic coefficient of variability (PCV)

$$PCV = \frac{\sqrt{\sigma^2 p}}{\bar{X}} X\ 100$$

Where,

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

$\bar{X}$ = General mean of the characters

The GCV and PCV values were classified as described by Sivasubramanian and Menon (1973).

GCV and PCV values	Classification
0 to 10%	Low
10 to 20%	Medium
20% and above	High

c. Heritability (h^2)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \text{ X } 100$$

Where,

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

Heritability estimates were classified into low, moderate and high by following Hanson *et al.,* (1956).

Heritability (h^2)	**Classification**
0 to 30%	Low
30 to 60%	Medium
60% and above	High

d. Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson *et al.* (1955).

$$GA = h^2 K \sigma p$$

Where,

h^2 = Heritability in broad sense

K = Selection differential which is equal to 2.06 at 5 per cent intensity of selection (Lush, 1949).

σp = Phenotypic standard deviation

e. Genetic advance as per cent of mean (GAM)

$$GAM = \frac{GA}{\bar{X}} X\ 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean of the character

GAM was categorized as follows by Johnson *et al.* (1955).

GAM	Classification
0 to 10%	Low
10 to 20%	Medium
20% and above	High

3.2 Genetic mapping of molecular markers

3.2.1 Genomic DNA extraction, PCR amplification and electrophoresis

3.2.1.1 DNA extraction from both parents and RIL population

The genomic DNA was extracted from vegetative buds and young leaves of RILs along with their parental genotypes by following CTAB method of extraction with slight modifications (Doyle and Doyle, 1990).

1. Approximately two grams of fresh young leaves from shoot apex was harvested and crushed in liquid nitrogen in a pre-cooled pestle and mortar to make fine powder.
2. The ground powder was transferred to a 2 ml sterile polypropylene tube containing 1 ml of extraction buffer (2% w/v CTAB, 1.4 M NaCl, 0.1 M Tris HCl, pH 8.0, 0.03% β Mercapto ethanol, 0.1% PVP) pre-warmed to 65°C.

3. The samples were incubated at 65°C for 30 minutes with intermittent mixing of tubes every 15 minutes.
4. After bringing back tubes to room temperature, equal volume (1 ml) of Chloroform:Isoamyl alcohol (CIA) (24:1 v/v) was added to each tube containing sample and extraction buffer and mixed by inverting.
5. Tubes were then centrifuged at 8500 rpm for 30 minutes.
6. Upper aqueous phase was carefully transferred to new sterile tube. Then equal volume of prechilled isopropenol was added and mixed gently.
7. Tubes were kept overnight at -20°C and centrifuged at 8500 rpm for 15 minutes to pellet the DNA.
8. The supernatant was decanted and the pellet was washed with 1 ml of 70 per cent ethanol and then air dried completely for 1-2 hour until no traces of alcohol was found.
9. The DNA pellet was suspended in 200-400 µl of Tris EDTA (10 mM Tris-HCl; 1 mM EDTA maintained at pH8) in sterile centrifuge tube.
10. When DNA was fully dissolved, 1 µl of RNase (stock 10 mg/ml) per 100 µl volume of sample was added (Final RNase concentration 100 µg/ml) and incubated at 37°C for one hour or at room temperature for overnight.
11. DNA samples were stored in -20°C for future use.

3.2.1.2 Assessment of DNA quality and quantity

The extracted DNA samples were loaded on 0.8% agarose gel prepared in 1x TAE buffer (For 250 ml 50x TAE - 60.5 gm of Trisbase, 14.25 ml glacial acetic acid and 25 ml of 0.5 M EDTA) containing 0.5 µl/10 ml ethidium bromide (10mg/ml) followed by visualization and documentation using Alpha digidoc 1000 system (Alpha Innotech

Corporation, USA). Further the quality of DNA was assessed by checking for the shearing of DNA and contamination with RNA (which usually runs ahead in gel).

DNA quantification was done by nanodrop and also by gel electrophoresis using 1 per cent agarose gel with known concentrations of uncut lambda DNA of 50 ng/µl, 100 ng/µl and 200 ng/µl. The DNA stocks of the samples were diluted accordingly to make it to required 20-30 ng/µl. Then the diluted DNA was confirmed with uncut lambda DNA on 1 per cent agarose gel. The DNA quantification was also done based on intensity of bands by loading 5 µl of sample in 1 per cent agarose gel.

3.2.1.3 PCR (polymerase chain reaction)

The polymerase chain reaction was carried out in 15 µl reaction volume containing 20-30 ng/ µl genomic DNA, 1X PCR buffer, 2 mM MgCl2, 200 µM dNTPs (3B BlackBio Biotech India Ltd), 10 pM of each forward and reverse primer (Eurofins Pvt. Ltd.) and 0.5U Taq DNA polymerase enzyme (3B BlackBio Biotech India Ltd). The reaction components were prepared as follows.

PCR reaction mixture

Deionised nuclease free water	10.0 µl
10X PCR buffer	1.5 µl
2 mM dNTPs	0.5 µl
Forward primer (10pmol/µl)	0.5 µl
Reverse primer (10pmol/µl)	0.5 µl
Taq DNA polymerase (1U/µl)	0.5 µl
DNA sample template (20-30 ng)	1.5 µl
Total	**15.0 µl**

The PCR amplification was carried out in Thermal Cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany). The amplification cycle consisted of initial denaturation step at 95°C for 5 min, cyclic denaturation step at 94°C for 40 seconds, primer annealing step ranged from 50-58°C (depending on the primer) for 40 s and the primer extension step at 72°C for 1 min. The cycle was repeated 35 times and ended with the final extension at 72°C for 10 min.

3.2.1.4 Agarose gel electrophoresis

The PCR products from each tube along with 5 µl of loading dye were separated electrophoretically using 3.0% agarose gels containing 0.05 µg/mL ethidium bromide. The amplification products were examined under UV light and photographed using Alpha digidoc 1000 system (AlphaInnotech Corporation, USA) gel documentation system.

3.2.1.5 Types of markers used

In this present study both genic and genomic SSR's developed at National Institute of Plant Genome Research (NIPGR), New Delhi along with other available SSR markers were used for mapping. The genic SSRs were developed from transcriptome sequence of various tissues of chickpea variety ICC4958, using next generation sequencing platforms whereas the genomic SSR's were developed based on the draft genome sequence of *Desi* chickpea (Garg *et al.* 2011a).

3.2.1.6 Assessment of parental polymorphism and genotyping of RILs

Initially the parents K 850 and WR 315 were screened for polymorphism in agarose gel (3%) using a total of 300 primers which included 253 genic (EST-SSRs) and 47 genomic SSR's (GSSR's) (Table 3). Out of 300 primers screened for parental polymorphism, 31 (10.3%) primers were found to be polymorphic. Subsequently the 31

Table 3: List of SSR markers used to screen for parental polymorphism in the present study

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
1	EST SSR 1	GAAAAGCGGAGAAAGTGTGC	AAACTCTCTCTCCCACGCCT
2	EST SSR 2	AAGCATCAGAAGAATCAGACAGG	CTTCCTCCTCGAGATCCTCC
3	EST SSR 3	ATGAAATTGCTCCGTTGAGG	GGGATTTGATTCGTGGAAGA
4	EST SSR 4	CGAGGATCTCTGGGAAATGA	AAGCGTGCTGCTTCAGAAAT
5	EST SSR 5	TGGTTACAAATGTACAATGCCA	GGCAGATTCCTCACCCTACA
6	EST SSR 6	GGGTGTGGATAGCAATGGTT	AGCTCAATTGCCAGGAAGAA
7	EST SSR 7	ATCACTTCTTGCCATGTCCC	GGTGGTGGAATGAGGAGAAA
8	EST SSR 8	AATTAGGGTTGGATGGAGGG	CTTCCGAACCACTACGCTTC
9	EST SSR 9	AGTGGTGGAAGTATCCGTGG	AAGGATGAAAAACAGAGGGTG
10	EST SSR 10	GGTTCATTTCAAAAGCGGAA	CTTCCTTCAAACCAAGCCAA
11	EST SSR 11	CCTCTTCAAACCATCCTCACA	TCTCGAGCGATCCATCTTTT
12	EST SSR 12	CAAAGCCTGGTTCTTCATCA	GGAAGTAGCCTGAGGTGCAA
13	EST SSR 13	TCTTTCGCTTTCACTCCCTC	GGTGGAAGCGGTTATTGAGA
14	EST SSR 14	AATACGCATCCAATCCATCC	GTGTGGTGCTGCACAGAGTT
15	EST SSR 15	ACCATTTCCTCCTGTGTTGC	CATAGCCACAGCCATTGAAA
16	EST SSR 16	GCTTGTGCATGGAAGACAAA	GCGAGTGATAAATACTGCCCA
17	EST SSR 17	TTTTTCATCCATCACATCATCA	TTGATGCTTTACAACGTCGC
18	EST SSR 18	CAAAGTAAACCCCACCGCTA	ACCGCAATATGGAACACGAT
19	EST SSR 19	CGTTTCTCGCTCTGGAGGTA	TTTCGTTGGTTACACGGTCA
20	EST SSR 20	CCTAATTCACCACCACCACC	ATGCAACCCATTTTGGAGAA
21	EST SSR 21	GGTTTTGAGAGAGAGTGCGG	TCTTCCGCAAAACAAAAACC
22	EST SSR 22	GCCTCATGCATCACAAGAAA	GCATTTTGCATGTTTGAACC
23	EST SSR 23	CCAACCAATGAAAGCTAGGC	TCCTATACCAATCCCCCACA
24	EST SSR 24	TCACTCCCTCGATCCTCAAC	AGAAACTTGGCAAAAGCAGC
25	EST SSR 25	GAACTGAGCGAGGAAGTTGG	ACATCTCCGAACTCGACCAC
26	EST SSR 26	CTGCATCAACCACACCAATC	CAACGGATAATGCACTCCCT
27	EST SSR 27	TAGTTGCTGCCTTCGGAACT	GGCGGATACTACTTTGTCGC
28	EST SSR 28	TCGCATAGAAGATGTCGTCG	ACGCTACTCGATGACGAGGT
29	EST SSR 29	CCTCGCCAAATAATCTCAGG	CCGAAGAGCAGAGGAAGATG
30	EST SSR 30	ACATTGTTGGTCGTGTTTGC	GGGTGATGTTGGAAGGATGA
31	EST SSR 31	ACAGAGAGGGAGGTTCGTCA	CCCTTCTTCCATGTACCACC
32	EST SSR 32	TCCCGTGAAACTGTCACAAA	CACCTGGAGAAGCTGAAATTG
33	EST SSR 33	GTCCCCCGCAGTTACTGTTA	GTAATTGTGAAGCCGGTCGT
34	EST SSR 34	CGGTAAGAGAAGAAGCCACG	TGCATTCAATTCAATTCCACA
35	EST SSR 35	CGCAACTTCTCCCTTTCATC	TCATGGATTTCCTTTGCCTC
36	EST SSR 36	TCAACCCTGTTTTGGTTCCT	TTGTTCCTTATTGTCACAACACC
37	EST SSR 37	GTGTTGGAGTTGGAGGAGGA	CTTGTTGCTGTTGTGCACCT
38	EST SSR 38	AATGGTGGTGGTTTGAAAGG	CCATTCGCACTTTTTGTCCT
39	EST SSR 39	GTGGCCAGGTGAGAGAAGAG	ATGGTAGTTTGGCGGTGAAG
40	EST SSR 40	TTGATGAGGAAGATGGAGGG	TTGATGAGGAAGATGGAGGG
41	EST SSR 41	GACTAAGGCCTCAAAACCCC	ATCACCACTTCTTGCATCCC
42	EST SSR 42	TCCCAACCTCTTTTCCCTTT	AAACGGTGTTGACCATAGGAA
43	EST SSR 43	TTCTCCAATTTCTCCCTTTTGA	AATTGAGCCTTTTGCCATTG
44	EST SSR 44	GACTAAGGCCTCAAAACCCC	ATCACCACTTCTTGCATCCC
45	EST SSR 45	GCAGTGGAGGTGAATTCGAT	GGGTGCACCTGTTTCAGAAT
46	EST SSR 46	GCGATCTCTCGAAAACCCTA	GAACGCAAACCACATGATTG
47	EST SSR 47	TGAACCCAACAAGGAACCAT	CCCCATGTGCTAAAAGCAAC
48	EST SSR 48	CCGATACCGGAGGATGTAGA	CCCAAACTCGACCTTGTTGT

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
49	EST SSR 49	TATGCTGCTGCAACTCCAAC	ACACAACCTGCTCATTGCTG
50	EST SSR 50	CGAAGCATACCAGGTTGATG	GGGATCTTGTTTCACGCTGT
51	EST SSR 51	TCCATTCCAACCTTAACCACA	GGAATGGAAGAAGAGAAGGGA
52	EST SSR 52	CGCATCTTCAATTCCATCCT	GGGCCCAACTCAAAAGTTTC
53	EST SSR 53	TCAAAGGGAAAGGATTTTGG	AGGGTCCCTCAGTATTGCCT
54	EST SSR 54	AACCCATTTTGCAATCTGCT	CGCTGAGGAGAGAGTTCCAC
55	EST SSR55	TTTTTCCCTTTATCGCATGG	TAGGGGAAGGCAAATGTACG
56	EST SSR 56	CTCTTGCAACTTCCCCACTC	AAAGCAAAGGAGGGTTTGGT
57	EST SSR 57	ATCACAACGTGGTGGTAGCC	TCTTCTTCTCCTGGGGATCA
58	EST SSR 58	CAGGCCTTGTTTGTTGAGGT	TTCTCCTCGATTTCAATGGG
59	EST SSR 59	TAATGAAAATTGTGGGAGAAG	TGACCCTCTTGTCATACTCAT
60	EST SSR 60	CGCCACAACAACACCATATC	CGAAAGAAATTGATGCGAGA
61	EST SSR 61	TGCTGGTCTTGTAGCTGGTG	GCAGCTTGTAAAGGGTTTGG
62	EST SSR 62	AGATCCACCTCCACCTTGTG	TTGGAGGTTGTGTTGTTGGA
63	EST SSR 63	ACACCACCCACCAGTAGGAA	GGGACAAGTCAGTCCGGTAA
64	EST SSR 64	CGGCCATTGAAATTGAAAA	GTTGGAAACAAACACGCCTT
65	EST SSR 65	CCTCAAGTGCAACAAAAACAA	TGCAAACATTTTCACACCAGA
66	EST SSR 66	TTTCAACAATGCCAACCAAA	TCGAAGAAGGGGAAGAAACA
67	EST SSR 67	ACCATTTGTTTGGGCATTTC	CGAATTGAGGGTTCTTCCAA
68	EST SSR 68	ACGGTCAGTGAGCTGCTTCT	TCCCAATCCTAGCTACCACG
69	EST SSR 69	CCAGTTGTTGCCTCGGTATT	CAGGTTGATGTCCGAATGTG
70	EST SSR 70	GCATCACAAGCTTCAACAGC	TTTTGGGTTGATGGGTGATT
71	EST SSR 71	CGTCGTTCACATACCAAACG	CCAGAAGGAGATCCTGAACG
72	EST SSR 72	CGGTGATGAACCTGTTGTTG	AAGCCACTCAAGACGCTGTT
73	EST SSR 73	TTCCAGATCTCCGGTAGGTG	ACTCTCCACTCTCCCAACCA
74	EST SSR 74	CATTCCCATATTTTCCTCCG	AAGACAATCGAATCCAACGG
75	EST SSR 75	AAACTTGAGGGCAAGCCTTT	CAAAACACAGAGTTGAAGGAACA
76	EST SSR 76	GAGCATCATAAGCGACGTGA	CCGCCAATCATATCCATTCT
77	EST SSR 77	CAGGAACCAAGATTGCAAGA	GGGAAAGAGTTCAAAACCCA
78	EST SSR 78	GGGACAAGTCAGTCCGGTAA	ACACCACCCACCAGTAGGAA
79	EST SSR 79	GGCAGCGACAACTACAACAA	TAATTGAGAATCGGGTTCGG
80	EST SSR 80	TCCTCCAACAACAACACCAA	AGGAGGAACCTTTGAAACCC
81	EST SSR 81	CGGTGTGAATTTGGTGATGA	CAACAACAACCGAACGAGTG
82	EST SSR 82	TCACCATCGTGTGATGGACT	TTGTTGGGTCCTCTTTTGTTTT
83	EST SSR 83	AAACACAGATGTCGTCGCAA	CACTGATATTCGGCAACACG
84	EST SSR 84	CCGGCTTAATGAAAGAGGAA	GACCATGGGATGGATGTTTC
85	EST SSR 85	GGTTTTGAGCGTGGTTTGAT	TGCAAAATGTGAAGCACAGA
86	EST SSR 86	CGTTACGATATTCGGGTGCT	GCTTCCCTTACGCTTAACCC
87	EST SSR 87	TGATGATTTTGTGATGGTTTTGA	GCTGCATCACAGAAAGTTGG
88	EST SSR 88	CTTGCATTGCACTTCTTCCA	TGCAGAAGGAATTGTGCTTG
89	EST SSR 89	GACGCTTTCCAGAAGATTGC	CCACTGGAATGGAGGAAAAA
90	EST SSR 90	CTGGTCCAAAGATCACAGCA	CTTTCACCCCCACAAACACT
91	EST SSR 91	ACGGTGAATTCATCCCAGAC	ATCATTGCTGTGAAAGCACG
92	EST SSR 92	ATGATGCTACTGGAGGTGGC	CCCAAATCCTCCTCTTCCTC
93	EST SSR 93	CCATCACCACCAACCCTAAC	AGAAGAGAGGGCGGAGTAGG
94	EST SSR 94	TCACCATGCCATTAACTCCA	GTGCCAGGAGAAGGTCTCAG
95	EST SSR 95	GCTGGTACCACCCGAACTAA	GTGAAGTAACGGGGATCCAA
96	EST SSR 96	TGAGCGATGTTACGTGTTGAG	TGGTGGACATGAGAGGATGA
97	ca4958_TC16800	TGGTTTGTGCCTGAGAGAGA	TCCCAAACAAGACCTGATGA
98	ca4958_TC16812	TCGCTTCTGTAAACGACTGG	GCAAACAAACAAACAAACAAA
99	ca4958_TC16903	AAGTGGCTATCAGGGGTGTC	GGGGGAGAGTGACACTGAAT

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
100	ca4958_TC16909	CCTGACGGGGAAAATCATAG	TGACTCAGAATCGGACAAGG
101	ca4958_TC17100	TTGGAACATCCCATCAGGTA	TCCTCCTCCGTGTGTAACAG
102	ca4958_TC17173	TCACTTCTTCCACCAAACCA	ATCCTCAAATGGATCCAACC
103	ca4958_TC17568	TTGGGCTCTTCTCCTTCTTC	CAACAATGTCACCCAGCTTT
104	ca4958_TC17608	GGAAAGGGATGTTGGATGTT	ACCATGATACCTCACCACCA
105	ca4958_TC17719	GCGAGAATTTGCCATTTGTT	TAATTTGGTGGCTGTTTCCA
106	ca4958_TC17933	TCTAGGGGTTCGTGTGAGTG	GCCATGAATCTTCGTCCTCT
107	ca4958_TC17959	TTCGCTTCATTGAATGTTGG	CGATCACAAGTCAGAACAAGG
108	ca4958_TC18003	CCACCCTCGTTACCTCTCTT	TTGGGTCTCTCCTCTCTCGT
109	ca4958_TC18114	CACTCCAATGGCTAATCTGC	ACAATCTCCGGTGGTGTTTT
110	ca4958_TC18156	TGTTTTTCTTTGCTGCTCCA	GCTGCCACTTGTTGCATATC
111	ca4958_TC18156	TGTTTTTCTTTGCTGCTCCA	GCAAAATTGAGAGGTGGAATG
112	ca4958_TC18452	TGCAACTCCACTTTCCTTCA	CCAACACTGCAAACACACAC
113	ca4958_TC18471	ATGGGACCCACACAGGTAGT	TGGTTTGATCAACTCCATGA
114	ca4958_TC18703	CACCCTCCACTTTTCCACTT	TGGAATTTGCTGTGAAGATCA
115	ca4958_TC18714	TGGAACAGATCAATTTGTGAA	TGCTATGGCAATGGTAAAGG
116	ca4958_TC18714	CCGCTGTTCTGATTTTTGAT	TCCAGTTCTCCCAATCTCAA
117	ca4958_TC18917	ACCTTCAGACTCCGGTTATT	AATGCAGATGAATGCGAGAG
118	ca4958_TC18930	CAAATGGCAGCTATTCATTCA	TTGCTGACCAGGAAACAGAG
119	ca4958_TC23243	GAAGATGAACGGTGGTTGTG	GCACTCCACATGAACATGAA
120	ca4958_TC23820	CACTTCGCCACTAAGTCACC	TGGACTGAAAAAGGAGACTCG
121	ca4958_TC24185	TCTCGAAGAATGTGGTGCTC	AAAGTTAGGCGTTCCACCTG
122	ca4958_TC24913	CAATTCATCATGAGGGTGGA	TCTATCTTTTCCCCCAAAGG
123	ca4958_TC25280	TCTTCACAAAACCGCCATAA	GGTGGGTTTGTTTGGGTTTA
124	ca4958_TC28113	AAGGGTTGGTGGAGTCAAAA	TGGCATAAAAGTTTGGTTCAG
125	ca4958_TC28408	GCTCAGTGGAGAGAGAGTGG	AAGGCTTCTCCTTGATCACAA
126	ca4958_TC29834	AAACGCATTGATACGGATTG	CAGATCCTGAGGAGGAGGAG
127	ca4958_TC31257	AGAAACCGAGGTTGATGGAG	TGACCACCTAAAGCTTGTCCT
128	ca4958_TC31939	ATGCTGATACTGTGCGAAGC	TGTTGTTGTTCCTCCTTTCG
129	ca4958_TC32962	CGTCGTCATCTTCATTTTCAA	AACGACGACGACGATAACAA
130	ca4958_TC33024	GTAGAGGTGGAGGTGGTGGT	CCATACCCTGAACCATACCC
131	ca4958_TC33024	AAGGGTATGGTGGAGGATCA	ACCACCTTCACCTTTTCCAC
132	ca4958_TC09239	TCTGCATTATTATCATACTACTCATC	TCATGATTATGATTACTTTTCATTT
133	ca4958_TC06880	TCATTTTGTTGGGATTTTCAA	TCCCTAATCATCACTCACTCA
134	ca4958_TC14433	ATGCTCCATGGTGGTCATAA	ACGAGCTCCAATTCACATAA
135	EST_icc1882_con0917	CATCACAATCCCTTCTTCAA	GAAGAGAGGAGGGAGAGAGAG
136	ca4958_TC05038	TTCCGTTTTCAAACCCAAAC	AACAGAGAGCTCCGAATCCA
137	ca4958_TC05167	CCAGTTGTTGCCTCGGTATT	CAGGTTGATGTCCGAATGTG
138	ca4958_TC14801	CAGATTCCAAACGTGCAGTG	ATTGCAATGTGAACCCACAA
139	ca4958_TC16913	TCCATTCCAACCTTAACCACA	GGAATGGAAGAAGAGAAGGGA
140	ca4958_TC16245	GGCCACGAAAAACATCAAGT	TTGGAAATGGGTTCTCAACA
141	ca4958_TC10205	TAGTTGCTGCCTTCGGAACT	GGCGGATACTACTTTGTCGC
142	ca4958_TC08452	AGCTTCTTTTGGCTTGTGGA	GAAGCATGTTTGCTGCAACT
143	ca4958_TC07232	AATGGAGCTTGAATCTGGGA	TGGGAAGCTTGTTGAAGTGA
144	ca4958_TC10126	TTTCGTCCCCAAATCCAATA	ATTGGTCCCCAATGTCTTGA
145	ca4958_TC15151	TTTTTCATCCATCACATCATCA	TTGATGCTTTACAACGTCGC
146	ca4958_TC03453	TCACAACCACCTGTAGCAGC	TGACCCTTTTGGGGTCAGTA
147	ca4958_TC05036	TCGAGGAAGATTGCAGGAGT	ATTGCTATTTTGGTCGGACG
148	ca4958_TC06179	GGTTCATTTCAAAAGCGGAA	CTTCCTTCAAACCAAGCCAA
149	ca4958_TC07757	TGAACAAAGGACCACACACC	TGTTCCAAGAAAAGCAGAGGA
150	ca4958_TC07870	TTTTTCCCTTTATCGCATGG	TAGGGGAAGGCAAATGTACG

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
151	ca4958_TC10124	CCCAGATACAATGCATACGCT	CCGATTTTCCTTCCTCAACA
152	ca4958_TC10363	TGCAGCAGAGCATCAAATCT	GTTGTCTGAAGGTCCCCAAA
153	ca4958_TC11468	CGGTGATGAACCTGTTGTTG	AAGCCACTCAAGACGCTGTT
154	ca4958_TC12179	ATTGCCATGAGAAATGGAGG	CCAGAAGATCCAAAGCCTGA
155	ca4958_TC15401	GTCTTGGGAACCTTGCGTTA	GATGCGTAACTACAACCGCA
156	ca4958_TC15544	AAACTTGAGGGCAAGCCTTT	CAAAACACAGAGTTGAAGGAACA
157	ca4958_TC15615	AACCCATTTTGCAATCTGCT	CGCTGAGGAGAGAGTTCCAC
158	ca4958_TC16715	CCAAAACCATTTTCCTTCAATC	TATGGATCGGAAGACGAACC
159	ca4958_TC18432	TACGGAGGCTATGGATGGTC	CACTCTTCCACTCCTTTGTGC
160	ca4958_TC03722	GATTCACCAAACCAAGCCAT	AAAGAGTGCAGGGGATGTTG
161	ca4958_TC05739	TCATCTGGGAAAAGGGAGTG	AAGGACCTCATCAAAACCAGAA
162	ca4958_TC06046	CAGGCCTTGTTTGTTGAGGT	TTCTCCTCGATTTCAATGGG
163	ca4958_TC08447	TCCATTTCCTTCCTTTTCCTC	GGTAGAAGGTGCTTGACCGA
164	ca4958_TC09085	GGCTGGGTTGATTTATGGTG	AGACAGCAAGCAAGCAGTGA
165	ca4958_TC1130	TGCTGGTCTTGTAGCTGGTG	GCAGCTTGTAAAGGGTTTGG
166	ca4958_TC11140	AGTCTTGGTGGTGTTTTCGG	TCTACCCCCAGTGCATTCTT
167	ca4958_TC11147	TGGGCATCTCAGAAAATGCT	ATTTGGGCCTGAGAAGGTTT
168	ca4958_TC12117	TTTCAACAATGCCAACCAAA	TCGAAGAAGGGGAAGAAACA
169	ca4958_TC12490	TGCAAGTTAACACGAGCACC	TTCCAGCAAATTTTGGAACC
170	ca4958_TC14627	TTATGAGGCGGCATAAAAGG	TTGAATGTTCCATCAGCACG
171	ca4958_TC17527	GCGTTTTGGTTTTGGAAAGA	AACTTGCAGCCAACACTTCC
172	ca4958_TC11653	TCACCATCGTGTGATGGACT	TTGTTGGGTCCTCTTTTGTTTT
173	ca4958_TC11038	CCACCTCTTCCCATCACTCA	TCATGGAGAATCCCAGATCC
174	ca4958_TC00696	TGGGGTGTGTTTTCTTTTCTC	ACAGATCAGTAGCCCGTGAA
175	ca4958_TC01951	CACATGGAAGACCACCACTT	TGTTTCACATTTCCCTGCAT
176	ca4958_TC02355	GGGCATAAAGAGGTGGTGTT	AACCCGAGATCATGAGGATT
177	ca4958_TC02355	TGTTGTTGTTCCTCCTTTCG	CCACTCGTGACGGTAGTGTT
178	ca4958_TC02624	AAGTGGCCATTGGTATCCTC	ACGGTCACCAGAACTTGTTG
179	ca4958_TC02706	CATTGCTAATTTGCTGAGGA	GAATGCGCAGAGATCCATAA
180	ca4958_TC02857	GATCGGAGGAAGAATCGAAA	TTGGATGTGGATGCTTATGG
181	ca4958_TC03008	CCCTTTGGTGATTTTGAACC	CATTGATTGCCCACAACACT
182	ca4958_TC03244	TCGGATCTGTTTGTTTCTGC	GCCATGAAATGAAACCCCTA
183	ca4958_TC03560	TCCCAAAACAATACCAACCA	GGAAAAGGTTCCATTTTTGG
184	ca4958_TC03875	GGGGAAAAGTCGAAATTGAA	CACCCCTTCCTTATTTGCAT
185	ca4958_TC03897	GGAACCAGGAAGAGCCATTA	CGCCTCGTAGAAGCAGATAC
186	ca4958_TC03990	TGGCTTCTCCATCAAAACAA	TTCCGACCTTATCAATGGAA
187	ca4958_TC04232	TTACATTGTGTTGATCCTTCACC	TTCCCTTTATTCATTTATTTGAACC
188	ca4958_TC04313	TTTGGGTCGTTTGTTTTGAA	CCGCCATAACCCTTTTCTT
189	ca4958_TC04502	CAATGCAAGTTCAAATGGAGA	TTGTCTCCCACCAAACTCAA
190	ca4958_TC04586	TTGAACTCCCTTGTTGAGGA	CCTCACATGATATTCTCCAAA
191	ca4958_TC04757	GTGGAGGAGGTGAAGGTGAT	CCTCCCCCACCATATGTTTA
192	ca4958_TC05016	TTGCTGCATCATTTCTGTCA	TCAGCATCATCAGAGGGTTC
193	ca4958_TC05274	CGGGTTTAGGTTGGACTGAT	CCGTTACCCTTATGGCATCT
194	ca4958_TC05331	TTCCAGTTTGTCGAGGTTGA	AGGAGGTTGAGCTGATTTGAA
195	ca4958_TC05934	GGTTTTTGTTTTGCTCGGTA	TCAACCTTTGTTCAGCAACAC
196	ca4958_TC06040	CCAAGGTGTTCCTTTCTTCC	CGGGTTGAGGTACCGAGTTA
197	ca4958_TC06482	CTCTACTTTGGCATGAAACCA	TTGAATGGAGCAATGAATCC
198	ca4958_TC07057	AGCTAGGTGGGGAATGTGAC	GGGCTTAATTTCACGGACAG
199	ca4958_TC07193	AGGGTGAAGGATCCAAAGTG	TGATATTCGGGTTCAAATGC
200	ca4958_TC07193	AGGGTGAAGGATCCAAAGTG	TGATATTCGGGTTCAAATGC
201	ca4958_TC07410	GCCTGAATTTTGGGAATCTG	CGAGCGTTTTCTCTTCCTTG

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
202	ca4958_TC07421	AAATCTCCACCACCTCGAAT	ATATCACCGGTGGTTTTGGA
203	ca4958_TC07645	GCAGCAATTCCAAAACATTG	CCTTGAGCAGCCCAATAAAG
204	ca4958_TC07722	GATGATGGTCTTTGGCATCC	GGCAAAGTTCGCAGACATAA
205	ca4958_TC07975	TATCCGCAATGTCAGTGTCC	TGGAGCGAAGAATACCACAA
206	ca4958_TC07975	CCATTTTTCTTGCATGTTCG	TAGAACCAGAGGCAGAGCAA
207	ca4958_TC08322	TCCTTCAAGCCCTAGTTTGG	GCTTCTGTTTCTGTCCCATTT
208	ca4958_TC08531	TGAAGGCGAGATGCTTGTAG	GCTTCCTGTGTGCTGGTAGA
209	ca4958_TC08568	GATCGGAAGTTTGGTGGATT	CCCCCTCTTCATACTTCCAA
210	ca4958_TC08885	GCACCAATTTTTCTTTTTGAA	TGAAATTTGGTTTTGGGTTG
211	ca4958_TC08918	CCCAACCCCTTCACATTTTA	GCTGACAGTTTTCCGTTTGA
212	ca4958_TC08953	TTGCCCTCTTCTTCTTCCTC	GCGAGCAAGACAGTGAAGAT
213	ca4958_TC08953	TTGCCCTCTTCTTCTTCCTC	GCGAGCAAGACAGTGAAGAT
214	ca4958_TC09117	TGGAGCATGGAACCCTAAAT	GGTGCTCTTCTCCTTTCACC
215	ca4958_TC09222	CAGAACTCTCTTGCCATTGCT	ATCAAGTGGTGGCAAATCAA
216	ca4958_TC09378	GGGATTATTCTTCGGTGACG	AGACGAAGGTTCATCCCAAT
217	ca4958_TC09444	CCCAGTTCTCTTAGCTCACCA	TCACCATTTTGTGGAGGATT
218	ca4958_TC09513	GCTCCGGGCTCTATTAACCT	TACGAAGATGCAGACGAAGC
219	ca4958_TC09806	GAGTCCTCCCAATTCCTTCA	AAGGAGGAACACAAGGGGTA
220	ca4958_TC09904	CAACAACAGCATCAACAACAA	TGATGCAAATGGTGAGGATT
221	ca4958_TC09904	CCCTCCTTTTTCTTCATCCA	TTCTCTGCCATGGTGAAATC
222	ca4958_TC10046	TTGGTGAATGAGGATGAACTTTA	GCTATGTATCCACTTGCTATGTTTT
223	ca4958_TC10273	ATGCCTGGGAATTCATTGTT	GCCACGTTTGTTGTGTTTTT
224	ca4958_TC10422	TGTGGCGATGAAAGTTTGTT	AACCAAATGCACCACTCTCA
225	ca4958_TC10535	GTCCATGTCAACCTTTGCAG	GCAAAATGCATTAATTTGCAC
226	ca4958_TC10802	TTTTTCTTGTTGCAGCTTGG	TTTGTTGATTCCTGTGTTGGA
227	ca4958_TC10910	CAGGCAAGACAGGCTACTGA	GCATTCTCCGGTTATGATCC
228	ca4958_TC11191	AGATAGTTGCACGCCTTTGA	AAGGGTGCTGCCTAATTCTC
229	ca4958_TC11203	ACAGCAGGTGCACTCAAAAG	TGGGCACTTTACAATCTTGC
230	ca4958_TC11243	TTGCTGACTTCTATTTCGATG	AATCGTCGCCATTGGTAGTT
231	ca4958_TC11253	CCCACTGATGTTTGAGAAGG	AGCTTTTTCGCCGTTTAATG
232	ca4958_TC11327	GCCGATCTTCCACAGTGAG	TGTCGAAGATGGACACTGCT
233	ca4958_TC11367	TGCAACAAATGAATGGAACA	TTGGTATCTTCTGGACGGTTA
234	ca4958_TC11369	GCGTTGTTTGAGGATGAGAG	TCAGATCTGTTTTTGCCAGA
235	ca4958_TC11613	GGATGCGGTGTTGAACATAC	TTTGGTTCTTGAGCTTGGAA
236	ca4958_TC11838	TTTGTGTTGATCATCATTGGA	GTGGTAGAGGACCCACCATT
237	ca4958_TC11910	TTTTGAACCAAGGCTTTGAA	GGAAATTGTGGTACACCTTGG
238	ca4958_TC11988	AATCAAAACCAACACGACGA	CTGCTTCGCCTTTCTCCTAA
239	ca4958_TC12039	TCCCTTGTCTCTTTGGTGAA	TCGATTCATCTCCTCATTGG
240	ca4958_TC12240	AGGTTTTTATGAGAGGCGTCA	GCCAAGGTGGAACCAATTAC
241	ca4958_TC12253	CCTCATCTCCTTTGCCTGAT	TAGACCCGGAACTTTGATCC
242	ca4958_TC12253	TCTGTCGAAAACAAGCCAAC	TGCTTTGGTGCGTCAATTAG
243	ca4958_TC12255	GGATGTCACCGTACACGTTAG	TCTGATCAATCACCTTCTCG
244	ca4958_TC12255	GGATGTCACCGTACACGTTAG	TCTGATCAATCACCTTCTCG
245	ca4958_TC12644	TGGTGGAGGGAAGATAAAGG	GAATGTGGCTGAGTTCAACG
246	ca4958_TC12848	AATGCTTGCTAGGGGACAAT	TGGTGATGATGATGATGGTG
247	ca4958_TC13118	TGACAAAGTCCAAGACTCCAA	AGAAAAAGTCCCACATCATGG
248	ca4958_TC13754	ATGAGACTGTGGGACCCAAT	ACTGCCTGAATCGCTACCAT
249	ca4958_TC13761	TTGGTTAGATCGGATTAGTGGTT	TTCATTTCTCTTCTTTTTCTTTTT
250	ca4958_TC13876	CAAAGCTTGGGTTACTCCAT	TTGGTTCTATCGATGGTCTCC
251	ca4958_TC14483	GCAAGCATTTCTTCCACAGA	CCCTCCTTTCCCTCTCTTCT
252	ca4958_TC14528	GAAGAGCCATGGTGACAGAA	CAACTTCTCCACACCCTTCA

Sl. No.	SSR ID	Forward primers (5'-3')	Reverse primers (5'-3')
253	ca4958_TC14781	TGGTGAGTGGAGCTGAAGAG	GCGGTTCTCCTCTAAGCAAT
254	GSSR4	AACCACATTTGTTGTCGCTG	CAAAATAAATGAAGGAGAGAATAAAGC
255	GSSR6	TGAGTGTGTACTGGGGTGAAA	AACACGCCCCTTCTTATCCT
256	GSSR7	GCGAAAAAGACAACGAAAGC	TCATCGGATTGGTCCTCTTC
257	GSSR9	GACAAGCCTCCGTGTGATTT	ATCGACGTCGCTAAATGCTT
258	GSSR11	CTGTTACGTGCAATGGATGC	TCGGTATGACACAAAAATGTGA
259	GSSR12	GACCTAGTCCGCGACTCAAC	AGACCCAAACATGTCGGTAAA
260	GSSR13	TCATGCAACAGACGAATTGA	AACATGTTATAAAACGTAAGGCG
261	GSSR14	CAATCAAGCTCGTATGGAACA	CATCAATTATTCAACTACCAATTTTC
262	GSSR15	TGTGACAAAACAAATAACGTGTCA	CGTCAAAGTTAACGCAGTTGAG
263	GSSR18	CCCTCAAGCAACCCATAAAT	TTGACACCATATGTGTTCTCCC
264	GSSR19	TGTCACAAACATGTAAGACAGTTGTT	AAGATTTGGCTGGCTTCATC
265	GSSR21	CCTTGCGTGAGTTTGACGTA	CATCGCGGTCATGTGAGTTA
266	GSSR24	TCATTTTTGAATGTCCATGCTT	TCAATTTCTTATACTTTTCGCAACA
267	GSSR27	GCAAAGTGGAATTTGTGGGT	AAAACATCAATTTACCATCATTCA
268	GSSR37	CGAACACTGATGCCAAACAA	AAAATGTATTTTATGAGTTTGGCCTAT
269	GSSR39	GTGCACACGGGTTACACAGT	GTGCGGTGGTTGAAGGTAGT
270	GSSR40	TCACTTTTACATTACACACAAGGATT	CATGTCTTTTGATATACTACCTCCACA
271	GSSR41	CCAAGCTATAGTGCAATCATCGT	AGGAGGCCTTTGAAAAATCA
272	GSSR42	AAAACATCATGTGCTGGTGC	CAGCTCATCCTCCTACTGTGG
273	GSSR47	CCACCAAAATCCGTCAACTT	TGTTCCATCATATTTTCAACCG
274	GSSR50	CCAAAAGGTTACGTTCAAAAAGA	GCTTTTTGCATTTGTTTCCAA
275	GSSR57	AACGCTTTCCTACCAATTTTT	GGAGGAAGTCGTTAACAAAAGA
276	GSSR58	TTATATAAAATTCGATATGCAGGATCT	TCTCAATTCAAACTTCCACTCAA
277	GSSR60	TTTTTCTTTTCCATAATGTGCG	CACCGGTTCAATTTTCGATT
278	GSSR62	TTTTTGTTACAACCATTTTTGGAT	GAAGCTATTTTGAATTCTTTTGTTAAA
279	GSSR68	GAGCTCAATAATTGGATTAGATTTATG	TGGTACAAAATACCAGGATCAA
280	GSSR69	GCCCAGTCTCTCCCTCTTTC	ACGTCACTCCCTTCCACAAA
281	GSSR77	TGTCAACAAAAGACTAGGCTAGGA	AATTTTGTAACGACCAACAACA
282	GSSR81	GAAATTGAACCCACAATTCCA	AATTGGCAACGGTGAGAAAA
283	GSSR82	TCAATTTTGAACGAGAAGTGAATC	CCAAAGAGTCTACTAGCGGAACA
284	GSSR85	TGTGAGTCGTGTCACAATAAAAA	TGTTTATCAGGTTCAAGCCG
285	GSSR87	GAAGGAAATATATCCCTTACAAGTGTG	ACGGTAAACTATAAGACGTGTCG
286	GSSR94	TAAAACCTTATTTTGCCGGA	GGGATAACGCCAAGGACATA
287	GSSR106	TGAAATTCAGCAACGAAATGA	CCCAGTAACCGCTCTCTCTG
288	GSSR107	GCAGGGGCTATATGGATTTG	GCATGAATTCAGCAGAAGAAAA
289	GSSR114	TTATCCCGTAAGCCTCGCTA	CCACGTCGGTTCTCTTTGAT
290	TR 2	GGCTTAGAGTTCAAAGAGAGAA	AACCAAGATTGGAAGTTGTG
291	TR 56	TTGATTCTCTCACGTGTAATTC	ATTTTGATTACCGTTGTGGT
292	TR 20	ACCTGCTTGTTTAGCACAAT	CCGCATAGCAATTTATCTTC
293	TR 24	AACAACTTCCTCTTATTTTCCA	CAGTAAAAATCAGCCCAAAC
294	TR 29	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG
295	TR 59	AAAAGGAACCTCAAGTGACA	GAAAATGAGGGAGTGAGATG
296	TA 42	ATATCGAAATAAATAACAACAGGATGG	TAGTTGATACTTGGATGATAACCAAAA
297	TA 53	GGAGAAAATGGTAGTTTAAAGAGTACTAA	AAAAATATGAAGACTAACTTTGCATTTA
298	TA 186	ACAAAATTCTAAAAGTTCCTTCTACCA	GTTGTTAGTCGAATAATTGAGAAAAAGA
299	TS 45	TGACACAAAATTGTCTCTTGT	TGTTCTTAACGTAACTAACCTAA
300	TA 135	TGGTTGGAAATTGATGTTTT	GTGGTGTGAGCATAATTCAA

markers which were consistently polymorphic between the parents (K 850 and WR 315) were screened on 141 RILs using 3.0 per cent agarose gel electrophoresis and the gels were scored for the presence or absence of the parental alleles and the data generated by 31 different markers was recorded and subsequently used for mapping studies.

3.2.1.7 Statistical analysis

3.2.1.7.1 Chi-square test to determine segregation of markers

The x^2 test was used to assess goodness-of-fit to the expected 1:1 segregation ratio for each of the 31 marker tested.

3.2.1.7.2 Construction of linkage map and mapping of molecular markers for wilt resistance

The data generated by screening 31 polymorphic markers on 141 RILs was used for construction of linkage map. The linkage analysis was performed using G-Mendel programme of iMAS (Integrated Marker Assisted Selection) developed by ICRISAT, Hyderabad. A minimum LOD of 2.5 and maximum recombination fraction of 0.4 were set as threshold value for linkage group determination. The Haldane mapping function was used to convert recombination fraction into map distances in centi Morgans (cM).

GMendel 2.0 is unique and performs multipoint linkage analysis on populations with complex genetic structures. GMendel 2.0 generates two point maximum likelihood estimates for all pair wise markers. Linkage phases are correctly assigned based on probability rules and gene order is estimated using an advance multipoint mapping algorithm. Multipoint gene order is determined by GMendel 2.0 using a powerful method called the simulated annealing algorithm (SAA). The marker ordering is validated by Monte Carlo and bootstrap methods. Recombination fraction was converted into map distances in

centi Morgans (cM) using Haldane mapping function. The intermarker distances calculated from GMendel were used to construct the Linkage map by using linkage map building module. Further the marker order and the linkage distances obtained were used for QTL analysis.

3.3 QTL analysis and mapping

3.3.1 Phenotypic data

Two season phenotypic data of 141 RILs obtained in the field experiments as described in 3.1.3.1 and 3.1.3.2 along with earlier wilt data available in the lab was used to carryout marker trait association studies. The wilt reaction of the RILs to *Fusarium* wilt was determined by growing the F_8 generation RILs in wilt sick plot of International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Field screening of all the RILs and parental genotypes along with early wilting check JG 62 and resistant WR 315 for wilt reaction was carried out in two seasons 2007 and 2008 at wilt sick plots at ICRISAT, Patancheru, near Hyderabad, situated at an altitude of 545 m above MSL and at a latitude of 17°32' North and longitude of 78°16' East. Using the field data of both the seasons two individual QTL maps were developed and later another QTL map using mean data from both seasons was developed.

3.3.2 QTL analysis

Genotypic and phenotypic data obtained on 141 RILs, as presented above, were analysed for identification of the QTLs using QTL Cartographer version 2.5 (Basten *et al.* 1994) following composite interval mapping (CIM) approach, with a walk speed of 2 cM. QTL analysis was conducted using data of individual seasons collected on yield and yield related traits during *rabi* 2012 and *rabi* 2013 and *Fusarium* wilt resistance data of two seasons (2007 *rabi* and 2008 *rabi*) available in the laboratory (Soregaon, 2011). Besides this, the analysis was also

conducted using the data pooled over the means of the two seasons for both traits. A minimum LOD score of 3.0 was used for declaring the presence of a putative QTL.

The presence of putative QTL in an interval was tested by using a critical value for LOD threshold of 3.0 as determined by WQTL Cartographer using the Bonferroni chisquare approximation (Zeng, 1994) corresponding to genome wise type-I error. As the mapping population comprised of RILs, the additive model "AA" was used for analysis in which additive x additive epistatic effects were included. The point at which the LOD score had the maximum value in the interval was taken as the estimated QTL position. The coefficient of determination also known as coefficient of variance (R^2) explained by the QTL was used as a measure of the magnitude of association and it is estimated as the square of the partial correlation coefficient. Estimates 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 linear regression model that simultaneously included all the detected QTLs for the trait in question. The LOD score was calculated from the F value for the multiple regressions (Haley and Knott, 1992).

$$LOD=n/n\ (1+p8F/Df)*0.2171$$

Where,

p = number of parameters fitted (Haley and Knott, 1992)

F ratio = SSR (full)-SSR (red)/pMSE (full)

SSR (full) = Sum of square for regression with full model i.e with QTL and cofactors

SS (red) = Sum of square for regression with reduced model i.e without the QTL

MSE (full) = SSE/DEF=Residual mean square (full model)

pMSE = Number of estimated QTL effects

Df = Number of degrees of freedom for residual sum of square in multiple regression

The percentage of phenotypic variance (R2) explained by a QTL was estimated. This is based on the partial correlation of 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).

Additive effect= (Parent P2-Parent P1)/2

3.3.3 Development of combined genetic map and QTL mapping

In the present study screening of 300 genic and genomic SSRs on parental lines (K 850 and WR 315) resulted in identification 31 polymorphic markers. However, the earlier work in the same laboratory has identified 41 additional markers which were polymorphic among the same parental lines. The genotypic data on all the RILs was also available (Soregaon, 2011). Altogether the genotypic data on 72 markers are available so an attempt has been made to develop combined linkage map using G-Mendel programme of iMAS (Integrated Marker Assisted Selection). A minimum LOD of 2.5 and maximum recombination fraction of 0.4 were set as threshold value for linkage group determination and the Haldane mapping function was used to convert recombination fraction into map distances in centi Morgans (cM), the linkage map was developed by following steps as mentioned in section 3.2.1.7.2. Further

the linkage map and the linkage distances obtained were used for QTL analysis.

The QTL analysis was carried out using the phenotypic data pooled over the means of the two seasons (2012 and 2013 *rabi*) and the pooled genotypic data of 72 markers screened on the same set of mapping population obtained from present and the earlier study was used and QTL map was developed by following the procedure as mentioned in section 3.3.2.

3.4 Validation of molecular marker linked to *Fusarium* wilt resistance

To test the reliability of ten (TR 24, GSSR 18, GSSR 11, GSSR 21 GSSR 41, NCPGR 33, TC 14801, EST SSR 3, EST SSR 21, EST SSR 65) identified markers linked to *Fusarium* wilt resistance, a set of ten RILs derived from another intraspecific cross between JG 62 ($H_1H_1H_2H_2$, susceptible early wilter) x WR 315 ($h_1h_1h_2h_2$ resistant), whose wilt reaction for *Fusarium* wilt race 1 was well characterized in earlier experiments conducted in wilt sick plot at ICRISAT were selected for validation of markers. Five among ten RILs (RIL 63, 72, 73, 104, and 129) were resistant with variable degree of wilting percentage and remaining five were phenotypically susceptible for the wilt reaction. The DNA isolation and PCR reactions as mentioned in section 3.2.1.1 and 3.2.1.3 respectively, was carried out. Based on comparison between known phenotypic characters of the genotypes for wilt reaction and the genotypic characters as explained by the linked markers, the markers classified as validated markers and non-validated markers.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The present investigation was aimed to develop genetic linkage map and identification of QTLs for *Fusarium* wilt resistance, and an attempt was also made to develop combined genetic linkage map by clubbing genotypic data generated from the present study with earlier data (Soregaon 2011) generated for the same set of mapping population. The mapping population was evaluated for yield and yield related traits. The estimation of genotypic coefficient of variation (GCV) phenotypic coefficient of variation (PCV), broad sense heritability (h^2) and genetic advance mean (GAM) for six different characters over two seasons was carried out and results are presented below under following headings.

4.1 Genetic variability studies for seed yield and yield components

4.2 Phenotyping of RILs for their wilt reaction

4.3 Genetic mapping of molecular markers

4.4 Identification of QTLs for *Fusarium* wilt resistance, yield and yield components

4.6 Validation of the markers linked to *Fusarium* wilt resistance

4.1 Genetic variability studies for seed yield and yield components

4.1.1 Analysis of variance for quantitative traits

The results pertaining to analysis of variance are presented in tables 4 and 5. The analysis of variance (ANOVA) for *rabi* 2012 seasons indicated that, the variance due to genotypes were significant for days for 50 per cent flowering, plant height, seed yield per plant and 100 seed weight during 2012 *rabi*. Similarly, for *rabi* 2013 all the characters viz., days for 50 per cent flowering, plant height, number of branches, number of pods per plant, seed yield per plant and 100 seed weight were significant.

Table 4: Analysis of variance (ANOVA) for morphological traits in chickpea RIL population during *rabi* 2012

Source of variation	Degrees of Freedom (df)	Mean Squares					
		Days for 50% Flowering	Plant height	Number of branches	Number of pods per plant	Seed yield per plant	100 seed weight
Block (eliminating check+Var.)	2	02.07	35.45*	1.47	1001.99	16.23	4.11
Entries (ignoring Blocks)	145	12.25***	51.96***	0.81	540.44	50.77*	29.75***
Checks	4	05.07**	51.27**	0.17	322.57	117.08**	97.46***
Varieties	140	11.82***	52.05***	0.83	539.76	49.19*	27.92***
Checks vs. Varieties	1	101.82***	42.42*	0.71	1506.75	5.75	15.10*
Error	8	0.56	4.78	0.56	438.99	12.35	2.56

(* significant at P<0.5, ** significant P<.01 & *** significant P<0.001)

Table 5: Analysis of variance (ANOVA) for morphological traits in chickpea RIL population during *rabi* 2013

Source of variation	Degrees of Freedom (df)	Mean Squares					
		Days for 50% Flowering	Plant height	Number of branches	Number of pods per plant	Seed yield per plant	100 seed weight
Treatments	140	7.77***	52.10***	0.73***	452.62***	31.49***	29.315***
Replication	1	0.60	9.72	0.39	23.25	0.98	0.003
Error	140	0.38	6.37	0.13	42.37	8.01	0.070

(* significant at P<0.5, ** significant P<.01 & *** significant P<0.001)

4.1.2 Genetic variability parameters

The mean, range, genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), broad sense heritability (h^2) and genetic advance mean (GAM) values for *rabi* 2012 and *rabi* 2013 are presented in tables 6 and 7, respectively. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters in both the seasons.

The mean performance of 141 RILs for six yield and yield attributing traits recorded during r*abi* 2012 and *rabi* 2013 was almost similar across both season except for pod number, seed yield per plant and 100 seed weight which were slightly higher in *rabi* 2012 when compared to *rabi* 2013 (Fig 1).

4.1.3 Days for 50 per cent flowering

The number of days taken for 50 per cent flowering in first season ranged from 44.27 to 60.47 days with a mean of 49.19 days. The lowest value of 44.27 days was observed in RIL-170 and RIL-179 whereas the highest value of 60.47 was observed in RIL-35. For second season, days for 50 per cent flowering value ranged from 43.50 to 55.50 days with a mean value of 48.87, the lowest value was observed in RIL-61 and highest value in RIL-73. The trait showed low genotypic coefficient of variation (6.50 % and 3.90 %) and phenotypic coefficient of variation (6.72 % and 4.13 %) for both the seasons. The GAM was moderate (13.10) and low (7.70 %) for first and second season respectively with high broad sense heritability value in both (90.00 % and 94.84 %) seasons.

4.1.4 Plant height at maturity

The plant height during *rabi* 2012, showed a wide range from 19.52 to 74.65 cm, the least value being recorded in RIL-73 while the

Table 6: Estimates of genetic variability parameters for yield and yield components in chickpea RIL population during *rabi* 2012

Traits	Mean	Range		GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Max				
Days to 50 per cent flowering	49.19	44.27	60.47	6.50	6.72	94.40	13.1
Plant height at maturity (cm)	40.61	19.52	74.65	15.90	16.70	90.00	31.0
Number of branches per plant	3.92	1.95	6.95	13.00	23.30	31.30	15.0
Number of pods per plant	62.64	19.51	153.0	16.10	38.60	17.30	13.8
100 seed weight (g)	24.89	10.59	41.32	19.10	20.10	90.10	37.4
Seed yield/ plant (g)	17.83	4.22	44.34	33.40	39.00	17.30	13.8

Table 7: Estimates of genetic variability parameters for yield and yield components in chickpea RIL population during *rabi* 2013

Traits	Mean	Range		GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Max				
Days to 50 per cent flowering	48.87	43.50	55.50	3.90	4.13	0.90	7.71
Plant height at maturity (cm)	41.25	28.30	56.30	11.50	13.10	0.78	21.10
Number of branches per plant	03.36	2.16	5.50	16.30	19.50	0.70	28.10
Number of pods per plant	55.10	20.16	106.60	25.90	28.50	0.83	48.70
100 seed weight (g)	20.76	13.50	30.00	18.40	18.40	0.99	37.80
Seed yield/ plant (g)	13.37	4.50	27.40	25.60	33.20	0.55	40.60

GCV= genotypic coefficient of variation
PCV= phenotypic coefficient of variation
h^2=broad sense heritability
GAM= genetic advance mean

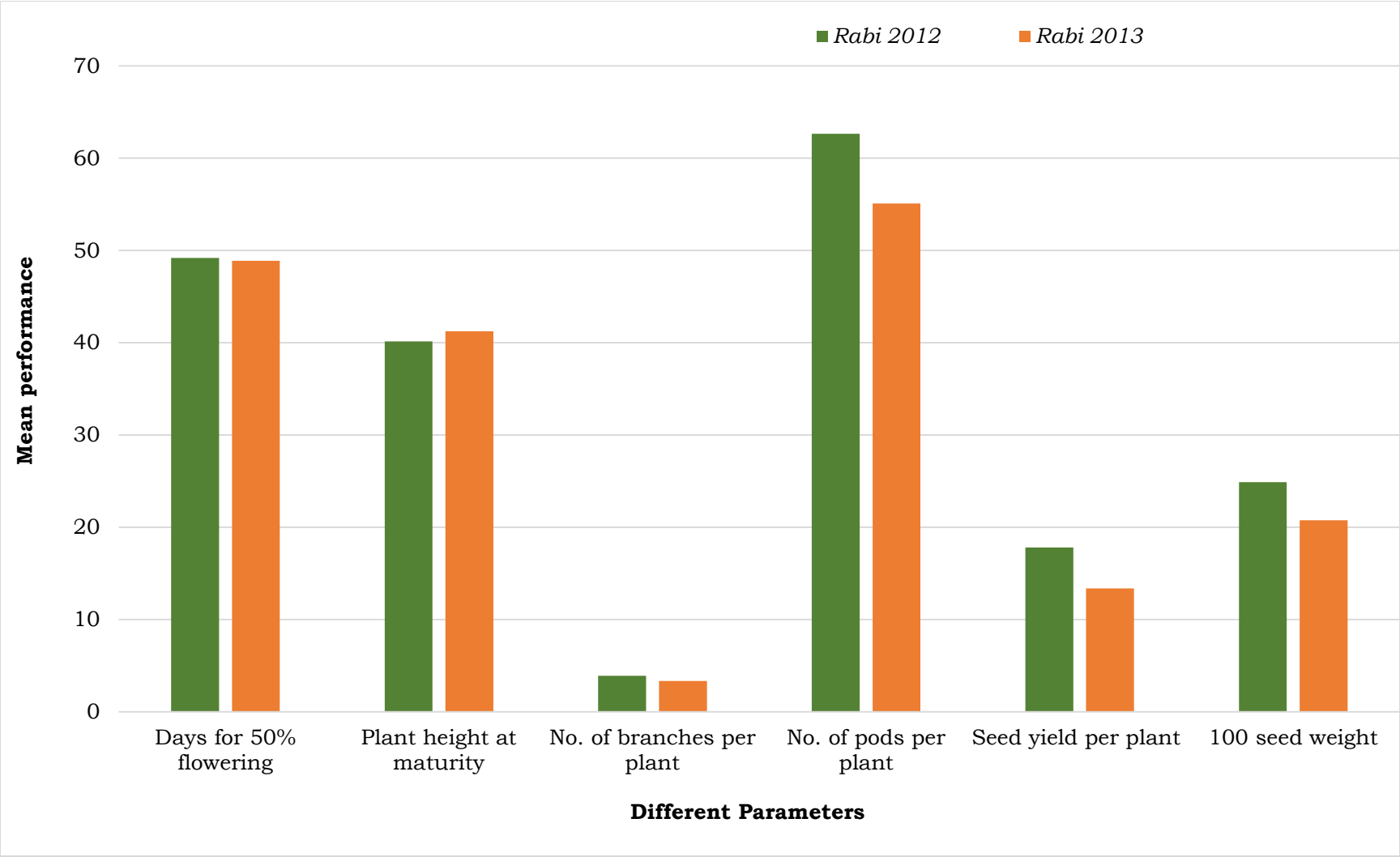


Fig. 1: Mean performance of RILs over both seasons for yield and yield attributing trait

highest value was observed in RIL-170 with a mean value of 40.61 cm. In *rabi* 2013 the value ranged from 28.3 cm (RIL-33) to 56.3 cm (RIL-11) with a mean value of 41.25 cm. The phenotypic coefficient of variation (16.70 and 13.10%) and genotypic coefficient of variation (15.90 and 11.50%) were moderate for both seasons. The trait recorded high broad sense heritability of 90 and 78 per cent with a higher GAM of 31.0 and 21.1per cent for both the first and second seasons.

4.1.5 Number of branches per plant

The branches per plant showed a range from 1.95 (RIL-129) to 6.95 (RIL-11) with a mean value of 3.92 during *rabi* 2013, whereas in *rabi* 2014 the value ranged from 2.17 (RIL-117) to 5.50 (RIL-145) with a mean of 3.36. The GCV (13.0 and 16.3%) and PCV (23.30 and 19.50%) levels were moderate for both the seasons. Moderate broad sense heritability 31.3 per cent was observed in first season whereas the broad sense heritability (70.0 %) was high for the second season. The genetic advance mean was low for *rabi* 2013 (15.0%), while it was high for the year 2014 *rabi* (28.1%).

4.1.6 Number of pods per plant

Wide range of variability was observed for number of pods per plant among the RILs in both the seasons. The value in the first season ranged from 19.51 to 153.0 with a mean value of 62.64 pods per plant. The minimum number of pods per plant (19.51) was recorded in RIL-126 whereas RIL-38 recorded maximum number of pods per plant (153.0). During second season the value ranged from 20.16 (RIL-121) to 106.0 (RIL-5) with a mean value of 55.10. Moderate GCV of (16.1%) and higher PCV value (38.60 and 28.50%) were recorded in both the season. The broad sense heritability value was low (17.3%) in first season and high (83.0%) in second season. The trait recorded a moderate GAM value of 13.8% in first season and higher value of 48.7% in second season.

4.1.7 100 seed weight

The trait showed a range of 10.59 g (RIL-88) to 41.32 g (RIL-63) with a mean of 24.89 g during first season and in second season it ranged from 13.5 g (RIL-87) to 30.0 g (RIL-153) with a mean value of 20.76 g. The trait showed moderate GCV (19.1 and 18.4) and PCV (20.1 and 18.4%) values in both the seasons. Higher broad sense heritability values of 90.1 and 99.0% were observed in *rabi* 2012 and *rabi* 2013, respectively. Moderate levels of GAM (37.4 and 37.8%) were observed for both first and second seasons.

4.1.8 Seed yield per plant

A wide range of variability for the seed yield per plant was observed in both the season. During first season the RILs-12 and 5 recorded lowest value of 4.22 g and highest value of 44.34 g, respectively with a mean value of 17.83 g. In second season the RIL-59 and RIL-153 recorded the lowest and highest value of 4.50 g and 27.4 g, respectively. The mean value was 13.37 g in the second season. By comparing the means of seed yield per plant for *rabi* 2012 and *rabi* 2013 with the JG 11(national check). Twenty six RILs out of 141 in *rabi* 2012 and two RILs in *rabi* 2013 were found superior over the national check (JG11) (Table 8).

The trait recorded a higher value for GCV and PCV in both the seasons. The genotypic coefficient of variation values observed was 33.4 and 25.6% and the phenotypic coefficient of variation values observed were 39.0 and 33.2% in first and second season respectively. However the h^2 value was low in first season (17.3%) and moderate in (13.8%) second season. High GAM values (13.8 and 40.6%) were observed in both the seasons.

Table 8: Comparison of mean seed yield per plant of RILs with national check JG 11

Sl No.	2012 *rabi*		Wilt score at 60DAS	2013 *rabi*	
	RIL numbers	Seed yield per plant (g)		Seed yield per plant (g)	Wilt score at 60DAS
1	5	44.34	69.53	-	
2	11	34.87	87.91	-	
3	15	26.19	11.87	-	-
4	19	25.81	52.56	-	-
5	20	31.27	11.59	-	-
6	36	28.33	77.21	-	-
7	67	31.51	52.37	-	-
8	75	26.01	77.17	-	-
9	76	25.69	35.96	-	-
10	91	28.54	59.58	-	-
11	94	26.79	92.10	25.58	92.10
12	95	31.28	23.66	-	-
13	102	30.83	67.10	-	-
14	103	27.77	73.70	-	-
15	106	25.59	38.43	-	-
16	107	22.53	73.66	-	-
17	110	27.21	66.21	-	-
18	115	27.11	54.76	-	-
19	137	31.08	37.64	-	-
20	140	27.29	48.65	-	-
21	144	33.48	66.61	-	-
22	153	29.37	85.71	27.38	29.37
23	155	26.24	42.49	-	-
24	169	37.63	46.31	-	-
25	174	26.96	45.50	-	-
26	178	26.08	62.20	-	-
27	JG 11 (National check)	25.3	-	24.7	-

DAS =Days after sowing

4.2 Phenotyping of RILs for their wilt reaction

The 141 RILs derived from an intraspecific late wilting cross were phenotyped for their wilt reaction in wilt sick plot of ICRISAT, Patancheru, near Hyderabad. The RILs at F_7 and F_8 generations were evaluated over two seasons 2007 *rabi* and 2008 *rabi.* The mean value of disease incidence for first season was 52.46% and 58.62% for second season suggesting that the disease incidence pattern was uniform for both seasons. The range observed for 2007 was 0.0 to100 per cent while it was 6.67 to 100 per cent for *rabi* 2008.

4.3 Genetic mapping of molecular markers and linkage map development

4.3.1 Assessment of parental polymorphism

In order to develop linkage map 141 RILs of F_{11} generation derived from an intraspecific cross between K 850 and WR 315 were used. A set of 300 genic and genomic SSRs markers were screened for polymorphism on the parents of RILs (K 850 and WR 315). Among 300 SSR markers tested on parents a total of 280 (93.3%) SSR markers produced scorable amplicons and 31 (11.1%) of the 280 amplified primers were found to be consistently polymorphic between the parents (K 850 and WR 315) in agarose gel at 3%. The 31 polymorphic primers consist of 23 genomic SSRs and eight genic SSRs, among the 23 polymorphic genomic SSRs 15 were novel and remaining eight were mapped in earlier studies using different mapping populations, whereas all the eight genic SSRs used in the present study were novel and were not mapped in any of the earlier studies.

4.3.2 Genotyping of mapping population

In order to map the molecular markers, all polymorphic primers were used and the entire mapping population consisting of 141 RILs was screened Plate1).

4.3.3 Chi square test to test the segregation pattern of markers

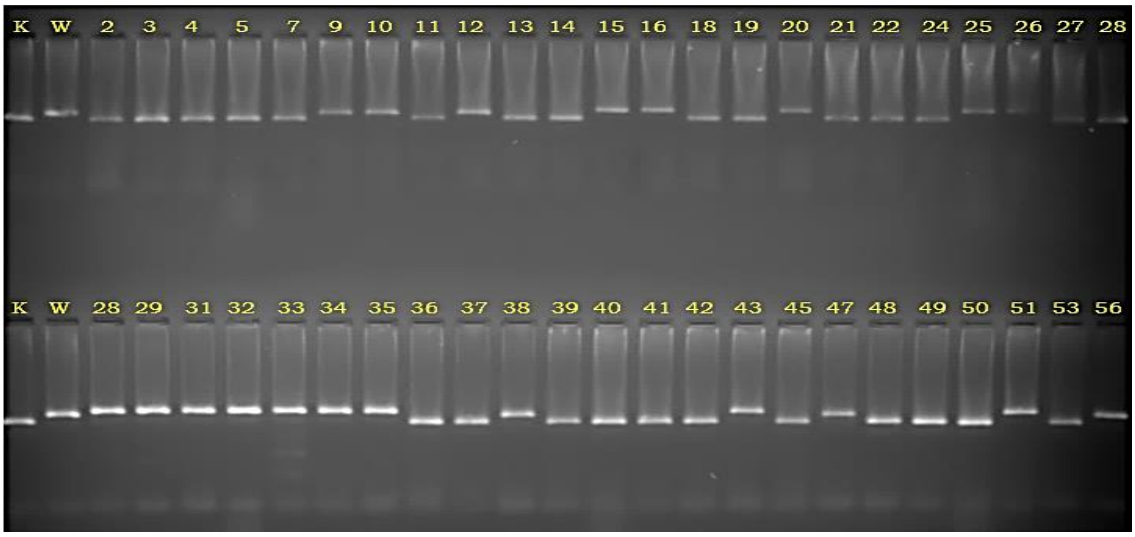
The segregation of individual markers for the expected monogenic 1:1 ratio in the RILs was tested using Chi square test (Table 9). Out of 31 SSR markers screened 4 (12.9%) markers showed segregation distortion from the expected Mendelian ratio (1:1) and were skewed towards female parent. However, all the 31 markers were used in construction of linkage map.

4.3.4 Linkage map construction

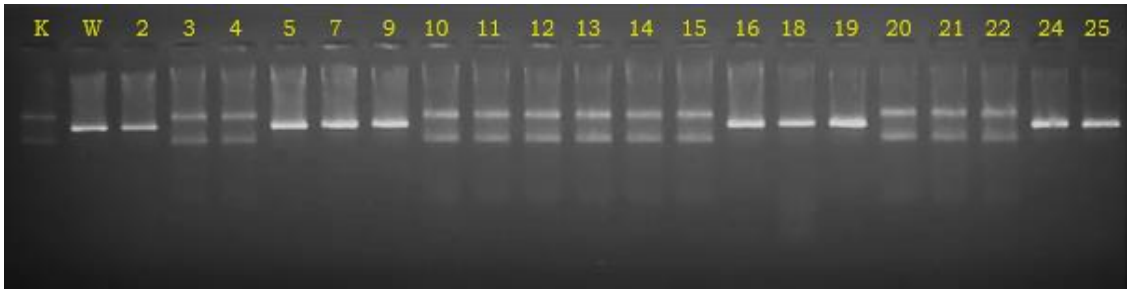
The linkage analysis was performed using G-Mendel programme of iMAS (Integrated Marker Assisted Selection) developed by ICRISAT, Hyderabad. A minimum LOD of 2.5 and maximum recombination fraction of 0.4 were set as threshold value, of the 31 markers used for mapping, 23 were mapped in four linkage groups (LGs) spanning a total length of 690.0 cM with an average marker density of 30 cM. The length of the linkage group ranged from smallest 12.8 cM (LG 2) to largest 588.60 cM (LG 1). The number of markers mapped per linkage group varied from 2 (LG 2) to 14 markers (LG 1). The highest marker density was observed in LG 2 with an average marker density of 6.4 cM and the least marker density of 42.04 cM was observed in LG 1 (Fig.2).

4.3.5 Genetic mapping of molecular markers and linkage map development using combined mapping approach

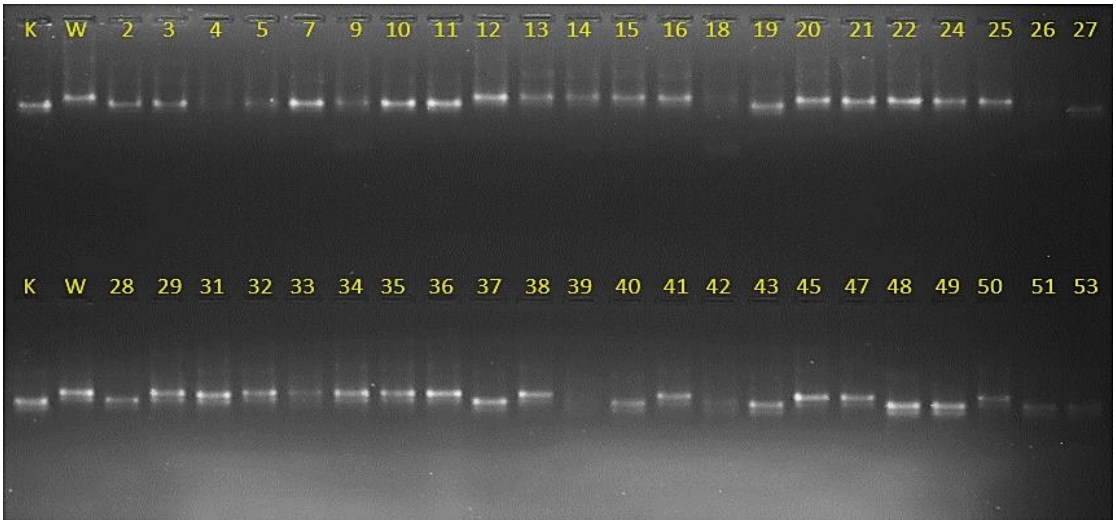
The combined linkage map was developed using 72 polymorphic markers which included 31 markers from the present study and 41



A: Segregation pattern of genic marker (EST-SSR 3) across chickpea RILs and parents K 850 and WR 315



B: Segregation pattern of genomic marker (GSSR 50) across chickpea RILs and parents K 850 and WR 315



C: Segregation pattern of genomic SSR marker (TA 42) across chickpea RILs and parents K 850 and WR 315

Plate 1: Segregation pattern of SSRs in 3% agarose gel across chickpea RILs and their parents (K 850 and WR 315)

Table 9: χ^2 test for segregation of markers across RIL population

Sl. No	SSR ID	K 850	WR 315	χ^2 value	χ^2 (1:1)
1	EST SSR 2	76	65	0.86	NS
2	EST SSR 3	84	57	5.17	S
3	EST SSR 8	97	44	19.92	S
4	EST SSR 21	73	68	0.18	NS
5	EST SSR 34	68	73	0.18	NS
6	EST SSR 65	70	71	0.01	NS
7	EST SSR 91	67	74	0.35	NS
8	TC14801	73	68	0.18	NS
9	TR 2	69	72	0.06	NS
10	TR 56	68	73	0.18	NS
11	TR 24	74	67	0.35	NS
12	TR 29	73	68	0.18	NS
13	TA 42	70	71	0.01	NS
14	TR 59	68	73	0.18	NS
15	TA 53	75	66	0.57	NS
16	TA 186	77	64	1.20	NS
17	GSSR 4	71	70	0.01	NS
18	GSSR 9	74	67	0.35	NS
19	GSSR 11	75	66	0.57	NS
20	GSSR 12	71	70	0.01	NS
21	GSSR 15	87	54	7.72	S
22	GSSR 18	74	67	0.35	NS
23	GSSR 21	76	65	0.86	NS
24	GSSR 37	74	67	0.35	NS
25	GSSR 41	75	66	0.57	NS
26	GSSR 50	74	67	0.35	NS
27	GSSR 57	76	65	0.86	NS
28	GSSR 77	76	65	0.86	NS
29	GSSR 87	72	69	0.06	NS
30	GSSR 94	73	68	0.18	NS
31	GSSR 106	114	27	53.68	S

NS–Non-significant, S-Significant @ 5%

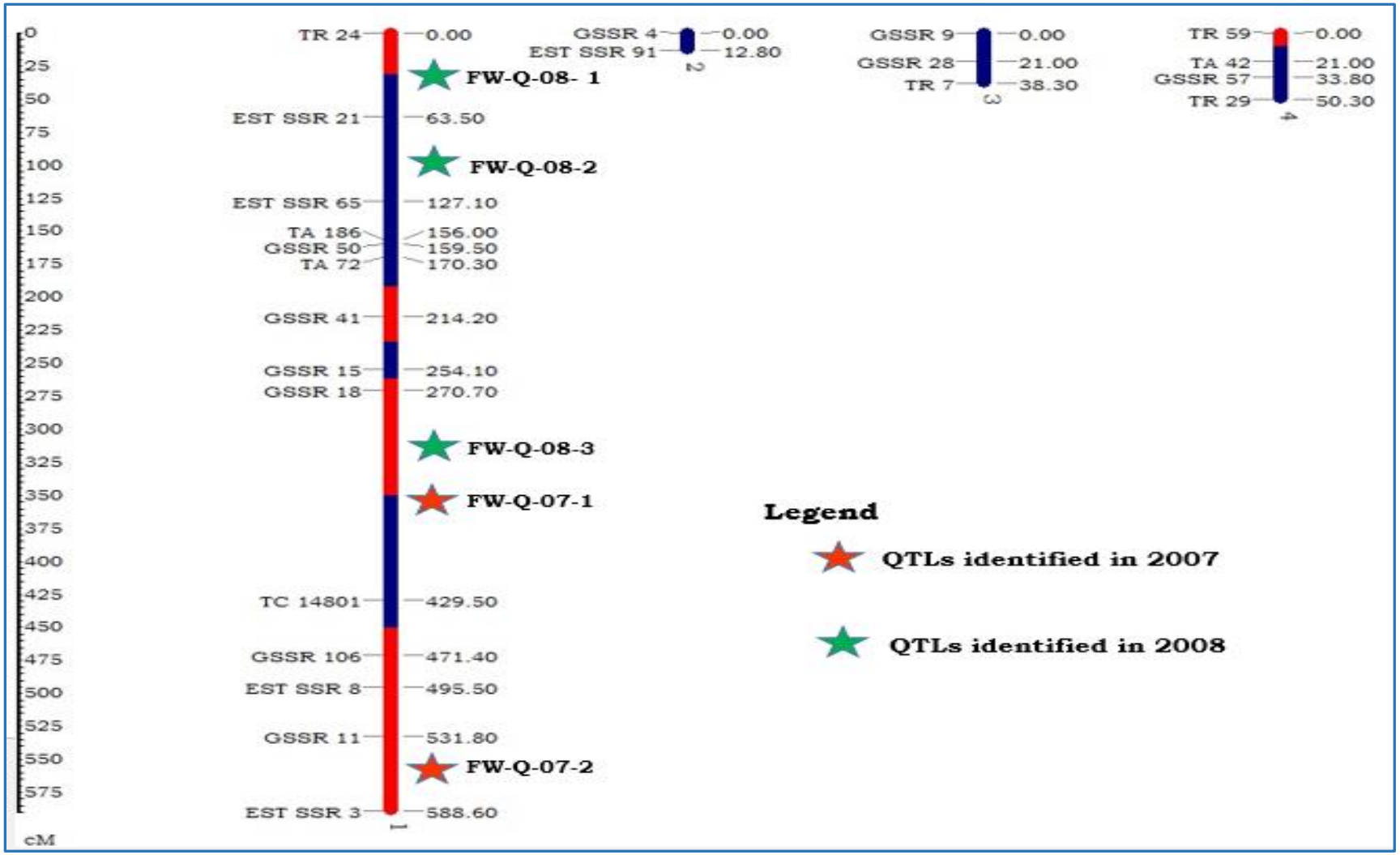


Fig. 2: Genetic linkage map depicting marker position and QTLs associated with *Fusarium* wilt resistance in chickpea

markers screened on the same set of 141 RILs (Soregaon, 2011). The mapping with combined markers was done using G-Mendel programme of iMAS developed by ICRISAT, Hyderabad. A minimum LOD of 3.0 and maximum recombination fraction of 0.35 were set as threshold value for construction of the combined linkage map, of the 72 markers used for mapping, 64 markers were mapped to six linkage groups (LGs) spanning a total length of 1258.8 cM with an average marker density of 19.66 cM. The length of the linkage group ranged from 13.80 cM (LG 3) to 1708.60 cM (LG 1). The number of markers mapped per linkage group varied from 2 (LG 3) to 44 markers (LG 1). The highest marker density was observed in LG 3 with an average marker density of 6.6 cM and the least marker density of 24.5 cM was observed in LG 1 (Fig.3).

4.4 Identification of QTLs for *Fusarium* wilt resistance, yield and yield attributing traits

4.4.1 QTL analysis and identification of QTLs for *Fusarium* wilt resistance

4.4.1.1 Phenotyping of mapping population

The phenotypic data for wilt reaction obtained from wilt sick plot experiments for two seasons (2007 *rabi* and 2008 *rabi*) as described in section 3.1.3.3 along with the mean data over both seasons were used for QTL analysis.

4.4.1.2 Detection of QTLs using two season data along with mean data over both the seasons

QTL analysis was conducted with data set on F_7 and F_8 lines obtained from wilt sick plot experiment conducted in *rabi* 2007, *rabi* 2008 and mean data from both the seasons, which detected a total of five QTLs (FW-Q-07-1, FW-Q-07-2, FW-Q-08-1 FW-Q-08-2 and FW-Q-08-3) for *Fusarium* wilt resistance. Among five QTLS, two QTLs were for *rabi*

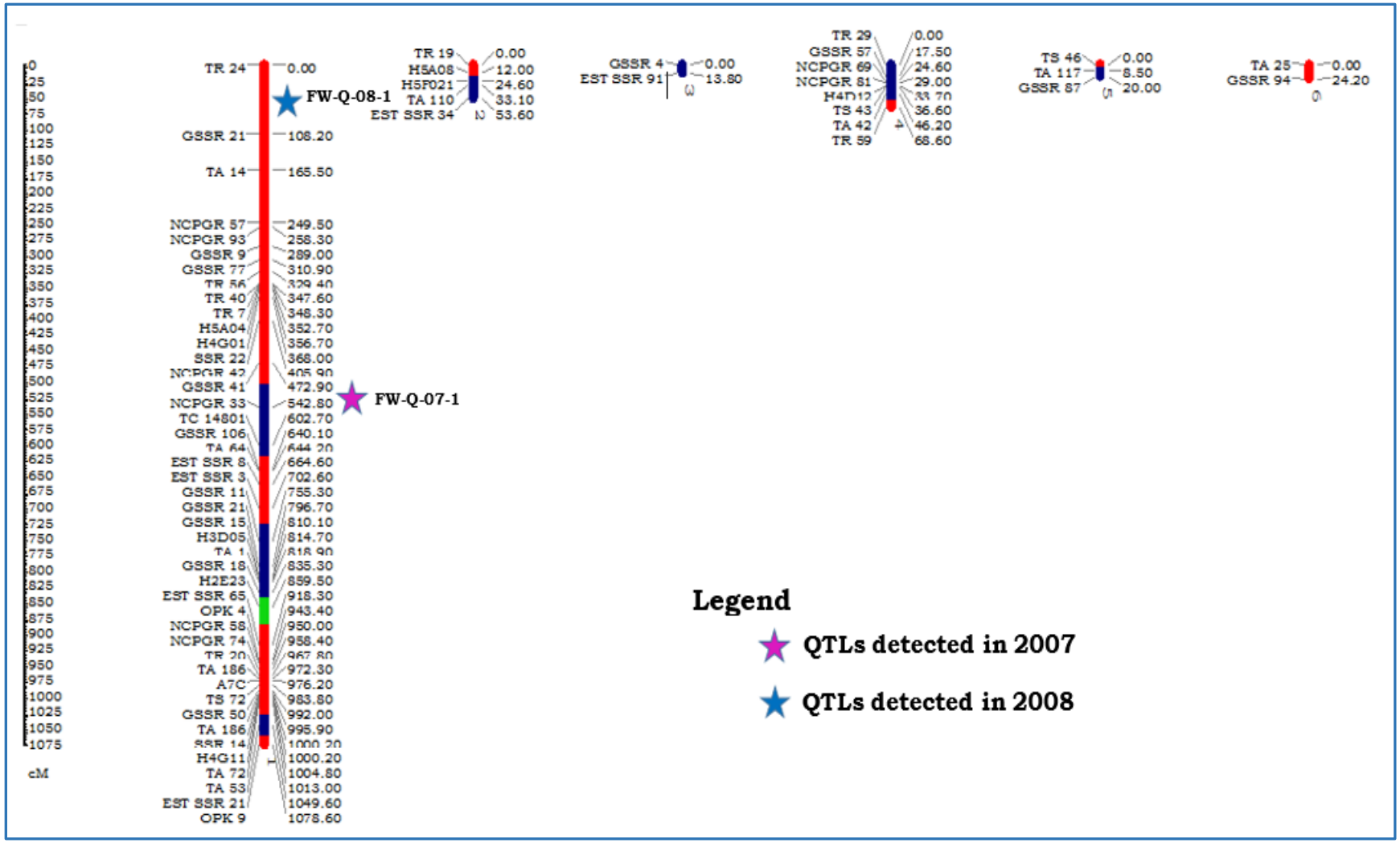


Fig. 3: Genetic linkage map depicting marker position and QTLs associated with *Fusarium* wilt resistance in chickpea using combined map

2007 (FW-Q-07-1 and FW-Q-07-2) and three QTLs were for *rabi* 2008 (FW-Q-08-1 FW-Q-08-2 and FW-Q-08-3). However, no QTLs were detected when mean data pooled over both the seasons were used for analysis.

4.4.1.2.1 QTLs detected in *rabi* 2007

Two QTLs (FW-Q-07-1 and FW-Q-07-2) with a LOD score of 8.90 and 3.05 for *Fusarium* wilt resistance were identified in 2007 *rabi* (Fig 4a), both QTLs were located on first linkage group (LG 1). The first QTL (FW-Q-07-1) was located at 340.71 cM flanked by markers (GSSR 18 and TC 14801). The QTL explained phenotypic variance of 69.80 per cent for *Fusarium* wilt reaction and it had an additive gene effects of -25.0 with the contribution of favorable alleles from female parent K 850. The second QTL (FW-Q-07-2) explained phenotypic variation of 68.80 per cent for the trait and was located at 559.81 cM flanked by markers GSSR 11 and EST SSR 3. The QTL had additive gene effect of 24.43 with a contribution of favorable alleles from resistant parent WR 315 (Table 10).

4.4.1.2.2 QTLs detected in *rabi* 2008

A total of three QTLs were detected in 2008 *rabi*, (Fig 4b) all the QTLs were located on LG 1, among the three QTLs the first QTL (FW-Q-08-1) was detected with a LOD score of 4.43 and it explained the phenotypic variation of 56.4 per cent for *Fusarium* wilt reaction. The QTL was located at 32.01 cM flanked by markers TR 24 (32.01 cM) and EST SSR 21 (31.49 cM). The QTL exhibited additive effect of 21.98 with the contribution of favorable alleles from male parent WR 315.

The second QTL (FW-Q-08-2) was detected with a LOD score of 3.13 and R^2 value of 56.8 per cent. The QTL was located at 95.51 cM flanked by markers EST SSR 21 (32.01 cM) and EST SSR 65 (31.59 cM) with male parent contributing the favourable alleles. The third QTL

(FW-Q-08-3) explaining a variance of 60.8 per cent was detected with a LOD score of 4.43. The QTL was located at 316.71 cM with GSSR 18 (46.01 cM) and TC14801 (112.79 cM) as flanking markers (Table 10).

Among all the five QTLs detected for both 2007 and 2008 *rabi*, a QTL (FW-Q-07-1) detected in 2007 *rabi* had highest LOD score of 8.9 and explained a variance of 69.8 per cent for the trait. Among the three QTLs detected in 2008 *rabi* third QTL (FW-Q-08-3) had the highest LOD score of 4.43 and explained a variance of 60.8 per cent for wilt resistance.

A common QTL over both the seasons flanked by same markers (GSSR 18 and TC 14801) was identified. Both the QTL (FW-Q-07-1) identified in 2007 *rabi* and QTL (FW-Q-08-3) identified in 2008 *rabi* were located at a distance of 340.71cM and 316.71 cM on LG 1. However the QTL (FW-Q-07-1) identified in 2007 *rabi* had a LOD score of 8.90 and explained a variance of 69.80 per cent, unlike a LOD score of 4.43 and variance of 60.8 per cent as explained by the QTL (FW-Q-08-3) identified in 2008 *rabi*. Both the QTLs had additive effect and contribution of favourable alleles from female parent K 850.

4.4.1.3 QTL analysis and identification of QTLs for *Fusarium* wilt resistance using combined linkage map

The linkage information and marker order generated by combined linkage map developed using 72 polymorphic markers along with the two season phenotypic data for wilt reaction of mapping population along with mean data over both the season was used in QTL analysis and two QTLs (FW-QC-07 and FW-QC-08) were identified one in 2007 *rabi* and another for 2008 *rabi*, However, no QTLs were identified in mean data pooled over both the seasons.

4.4.1.3.1 QTLs detected in 2007 *rabi*

A single QTL (FW-QC-07) with a LOD score of 8.73 and R^2 value of 71.4 per cent was detected (Fig 4c) using 2007 *rabi* data. The QTL was located on LG 1 at 508.91 cM flanked by markers GSSR 41 (36.01 cM apart) and NCPGR 33 (33.89 cM apart). The favorable allele for these QTL with additive effect was contributed by female parent K 850 (Table 11).

4.4.1.3.2 QTLs detected in 2008 *rabi*

A single QTL (FW-QC-08) with a LOD score of 3.99 explaining a phenotypic variation of 53.34 per cent with additive effect (21.77) for wilt reaction was detected in 2008 *rabi* data and linkage information from combined mapping (Fig 4d), the QTL was located on linkage group 1 at 52.01 cM flanked by markers TR 24 (52.01 cM apart) and GSSR 21 (56.19 cM apart) (Table 11).

4.4.2 QTL analysis and identification of QTLs for yield and yield components

4.4.2.1 Evaluation of mapping population for yield and yield components

The phenotypic data obtained from evaluating the mapping population consisting of 141 RILs for six morphological traits under field conditions over two seasons *rabi* 2012 and *rabi* 2013 as described in section 3.1.3.1 and 3.1.3.2, along with genotypic data generated by screening 31 polymorphic markers on the 141 RILs were used for QTL analysis. The QTL analysis was performed separately for *rabi* 2012, *rabi* 2013 and mean data pooled over both the seasons. A total of nine QTLs, two for r*abi* 2012, three QTLs for *rabi* 2013 and four QTLs for pooled data over both the seasons were identified.

Table 10: QTLs detected for *Fusarium* wilt resistance in chickpea for 141 RILs derived from the cross K 850 x WR 315

Seasons	Trait	Linkage group	Flanking marker	QTL Position (cM)	Max LOD	R2 (%)	Genetic effect		Donor parent
							Additive	Dominant	
Rabi 2007	% wilt @ 60 DAS	1	GSSR 18 - TC14801	340.71	8.90	69.80	-25.00	0.0	Female
	% wilt @ 60 DAS	1	GSSR 11 - EST SSR 3	559.81	3.05	68.80	24.43	0.0	Male
Rabi 2008	% wilt @ 60 DAS	1	TR 24 - EST SSR 21	32.01	4.43	56.80	21.98	0.0	Male
	% wilt @ 60 DAS	1	EST SSR 21 - EST SSR 65	95.51	3.13	56.20	21.94	0.0	Male
	% wilt @ 60 DAS	1	GSSR 18 - TC14801	316.71	4.43	60.80	-22.33	0.0	Female

DAS= Days after sowing

Table 11: QTLs detected for *Fusarium* wilt resistance in chickpea for 141 RILs derived from the cross K 850 x WR 315 using combined map

Seasons	Trait	Linkage group	Flanking marker	QTL Position (cM)	Max LOD	R2 (%)	Genetic effect		Donor parent
							Additive	Dominant	
Rabi 2007	% wilt @ 60 DAS	1	GSSR 41 – NCPGR 33	508.91	8.73	71.41	-25.06	0.0	Female
Rabi 2008	% wilt @ 60 DAS	1	TR 24 – GSSR 21	52.01	3.99	53.34	21.77	0.0	Male

DAS= Days after sowing

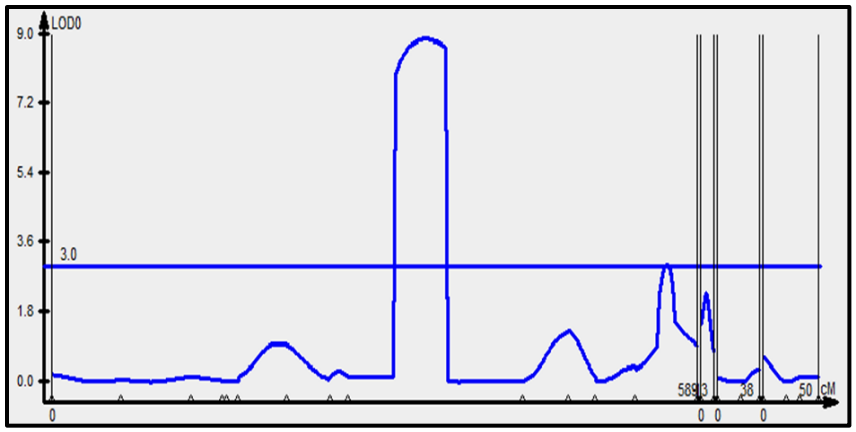


Fig. a: Two QTL detected on LG1 in *rabi* 2007

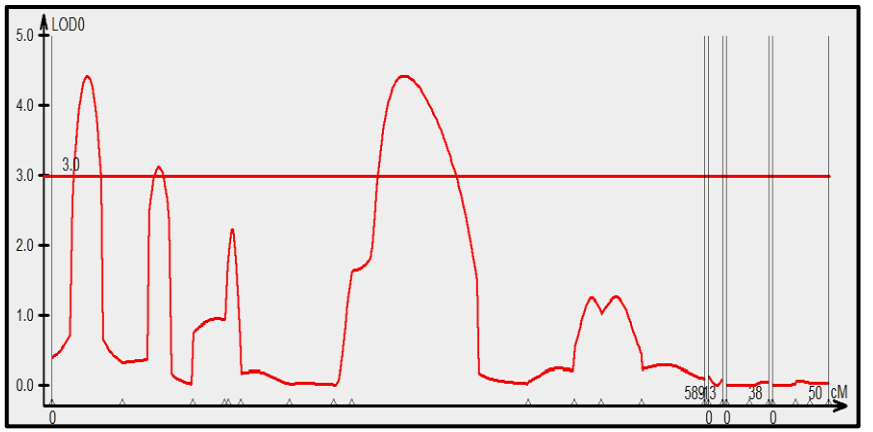


Fig. b: Three QTL detected on LG1 in *rabi* 2008

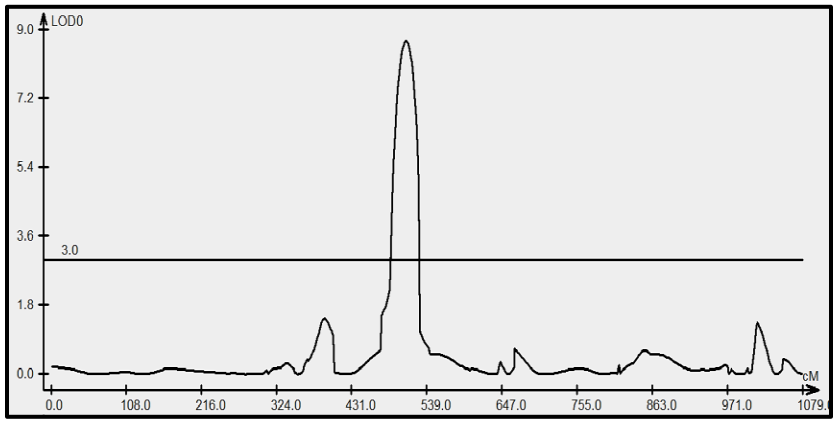


Fig. c: QTL detected in *rabi* 2007 by combine mapping

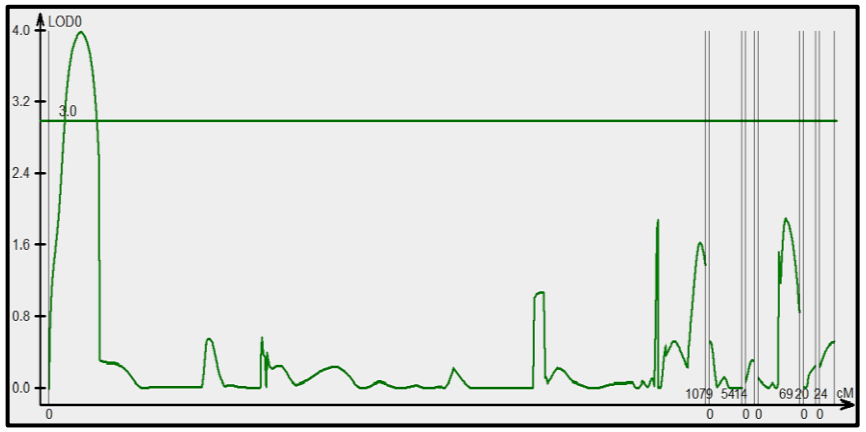


Fig. d: QTL detected in *rabi* 2008 by combine mapping

Fig. 4: Graph depicting LOD peak for *Fusarium* wilt resistance QTLs detected in chickpea

4.4.2.2 QTLs detected in *rabi* 2012 for yield and yield components

Two QTLs for seed yield per plant (TA 186 - GSSR 50 and GSSR 50 - TA 72) were identified in 2012 *rabi*. The first QTL (TA 186 - GSSR 50) was identified with a LOD score of 3.13 and explained phenotypic variation of 8.12 per cent for the trait. The QTL was located at 156.01 cM between markers TA186 and GSSR 50 and had additive effect with male parent contributing for favourable allele. The second QTL (GSSR 50- TA 72) explaining a variance of 26.18 per cent was identified with a LOD score of 5.58 (Fig 5a). The QTL was located at 165.51 cM flanked by GSSR 50 and TA 72 markers with male parent contributing for favourable alleles (Table 12).

4.4.2.3 QTLs detected in *rabi* 2013 for yield and yield components

A total of three QTLs were identified in 2013 *rabi*, one QTL (TA 72 - GSSR 41) for plant height at maturity (Fig 5b) and two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) for 100 seed weight (Fig 5c). The QTL (TA 72 - GSSR 41) for plant height at maturity was identified with the LOD score of 3.45. The QTL was located at 178.31 cM and flanked by markers (TA 72- GSSR-41) and explained a variance of 15.35 per cent for the trait. Two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) for 100 seed weight were located at 165.51 cM and 184.31 cM on LG 1, both the QTLs were detected with a LOD score of 5 and 4.23 respectively. The first (GSSR 50 - TA 72) and second (TA 72 - GSSR 41) QTLs explained a variance of 22.24 and 26.73 per cent for the trait (Table 12).

4.4.2.4 QTLs detected in pooled data for yield and yield components

Four QTLs with additive gene action ranging from 0.92 to 1.49 were detected in pooled data. Among the four QTLs detected, one QTL each for plant height at maturity (TA 72 - GSSR 41) (Fig 6a), seed yield per plant (GSSR 50 - TA 72) (Fig 6b) and two QTLs (GSSR 50 -TA 72 and

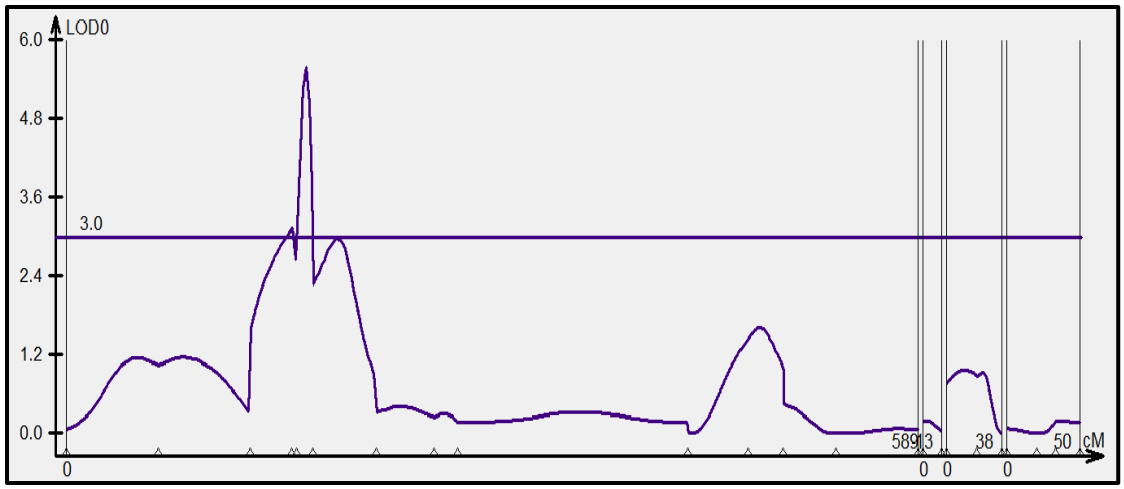


Fig. a: Two QTLs identified for seed yield per plant in *rabi* 2012

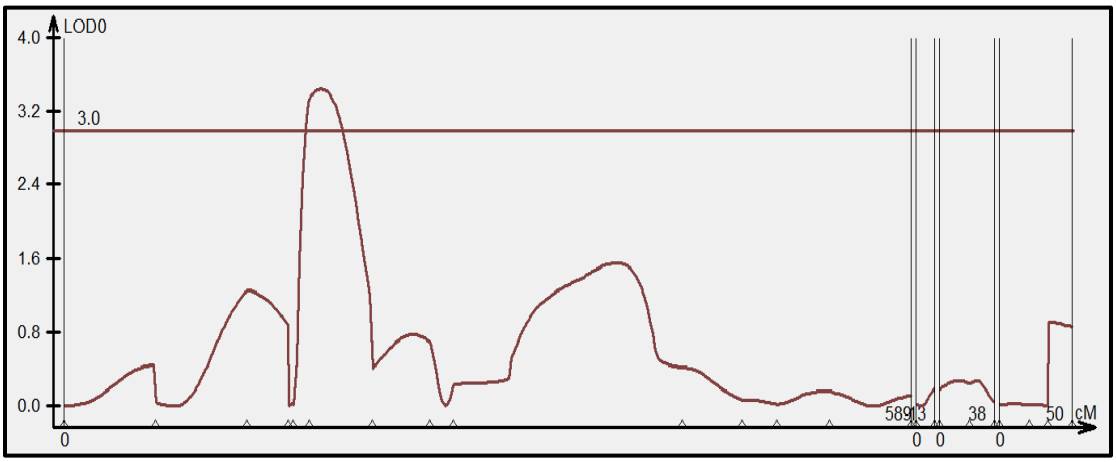


Fig. b: QTL identified for plant height at maturity in *rabi* 2013

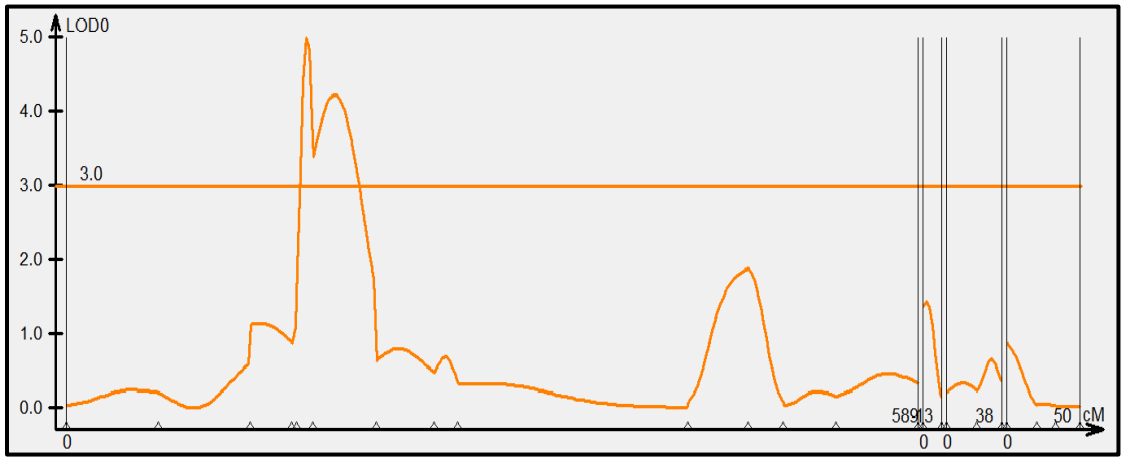


Fig. c: Two QTLs identified for 100 seed weight in *rabi* 2013

Fig. 5: LOD peaks of QTL identified for yield and yield components using *rabi* 2012 and 2013 data

TA 72 - GSSR 41) for 100 seed weight (Fig 6c). The QTL detected for plant height at maturity was similar to that of QTL (TA 72 - GSSR 41) detected in 2013 *rabi* for the same trait but the position was 8 cM apart from the earlier and the LOD score was slight higher 3.80 against 3.45 in 2013 *rabi*. The QTL (TA 72 - GSSR 41) based on pooled data explained 10.47 per cent variance for plant height unlike 15.35 per cent as explained by earlier QTL identified in *rabi* 2013 (Table 12).

The QTL (GSSR 50 - TA 72) detected for seed yield per plant in pooled data set was same as that detected in 2012 *rabi* and was located at the same position (165.51 cM) with slight reduced LOD score of 3.75 against 5.0 as observed in 2012 *rabi*. The QTL explained a variance of 16.53 per cent for the trait with additive gene effect and favourable allele for both QTLs came from male parent WR 315.

Two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) with additive effect of 1.22 and 1.38 with a contribution of favourable alleles from male parent for 100 seed weight were identified in pooled data set. The QTL (GSSR 50 - TA 72) was same as that identified in 2013 *rabi*. Both the QTLs were located at same position of 167.51cM. However the QTL based on pooled data explained less variance for the trait (18.78%) with a LOD score of 4.37 unlike 22.24 per cent as explained by single season QTL with a LOD score of 5.0. Another QTL (TA 72 - GSSR 41) identified for 100 seed weight with a LOD score of 4.33 matched with the QTL identified for the same trait in 2013 *rabi* with a LOD score of 4.23. Both the QTLs were located on LG 1 with QTL identified during *rabi* 2013 was at a position of 184.31 cM as compared to location of QTL identified based on pool data at 180.31 cM, flanked by the same pair of markers (TA 72 - GSSR 41).

Table 12: QTLs detected for yield traits in 141 RILs (K 850 x WR 315) of chickpea using two individual season and pooled data combined over seasons

Seasons	Trait	Linkage group	Flanking marker	QTL Position (cM)	Max LOD Score	R2 (%)	Genetic effect		Donor parent
							Additive	Dominant	
Rabi 2012	Seed Yield per plant	1	TA186 - GSSR 50	156.01	3.13	8.12	1.71	0.0	Male
		1	GSSR 50 - TA 72	165.51	5.58	26.18	2.78	0.0	Male
Rabi 2013	Plant height at maturity	1	TA 72 - GSSR 41	178.31	3.45	15.35	2.05	0.0	Male
	100 seed weight	1	GSSR 50 - TA 72	165.51	5.00	22.24	1.84	0.0	Male
		1	TA 72 - GSSR 41	184.31	4.23	26.73	1.99	0.0	Male
Pooled	Plant height at maturity	1	TA 72 - GSSR 41	170.31	3.80	10.47	0.92	0.0	Male
	Seed yield per plant	1	GSSR 50 - TA 72	165.51	3.75	16.53	1.49	0.0	Male
	100 seed weight	1	GSSR 50 - TA 72	167.51	4.37	18.78	1.22	0.0	Male
		1	TA 72 - GSSR 41	180.31	4.33	24.38	1.38	0.0	Male

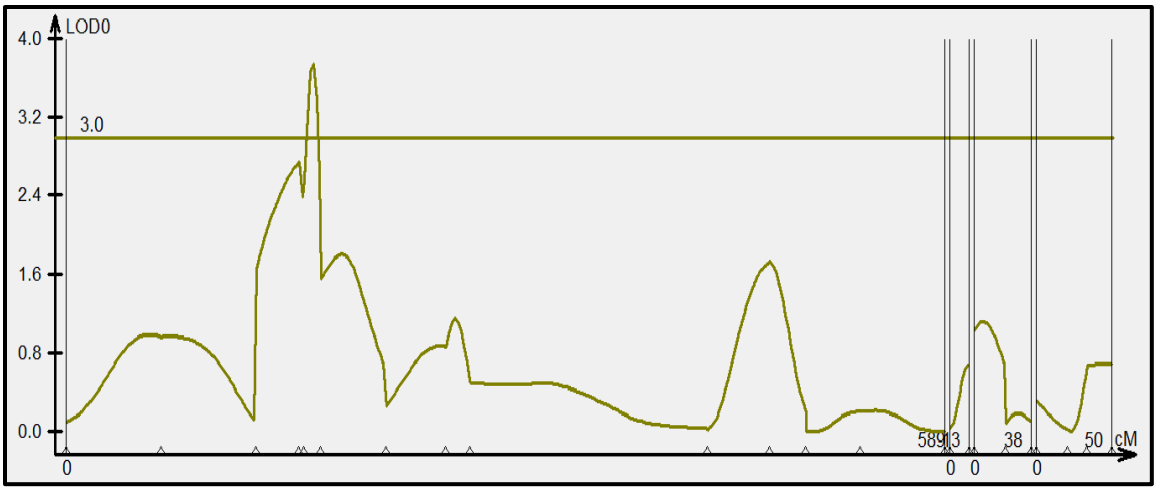


Fig. a: QTLs identified for seed yield per plant in pooled data set

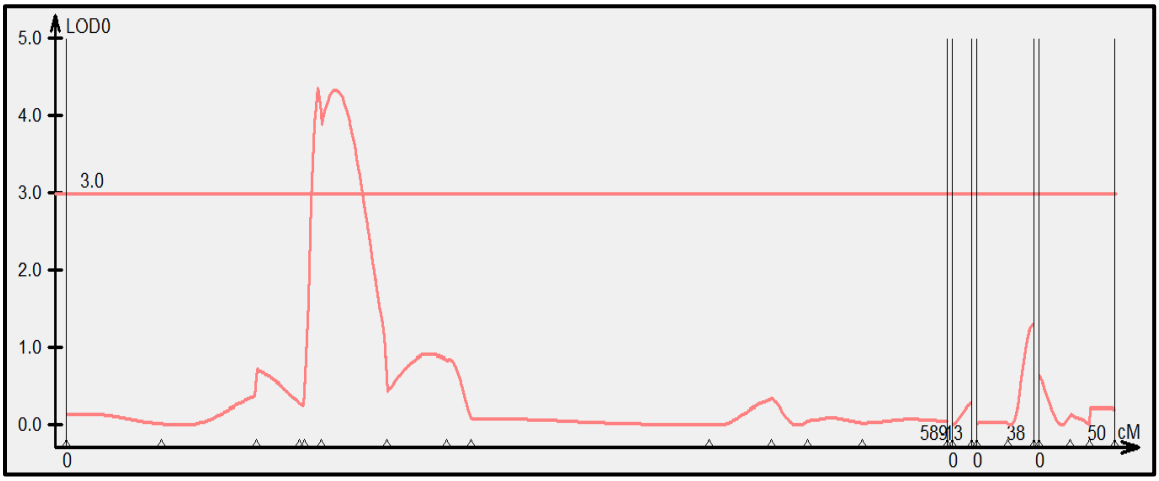


Fig. b: QTLs identified for 100 seed weight in pooled data set

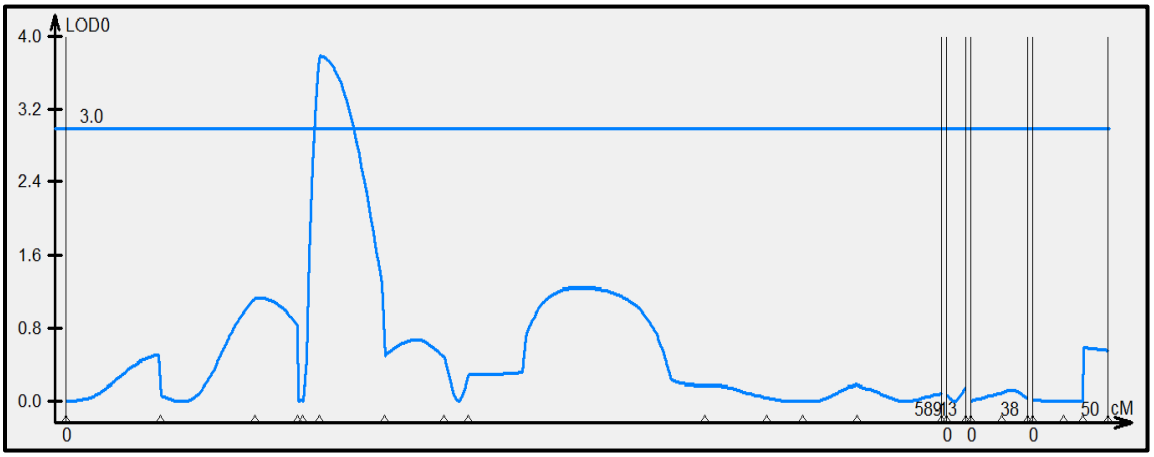


Fig. c: QTL identified for plant height at maturity in pooled data

Fig. 6: LOD peaks of QTL identified for yield and yield components in pooled data set

4.4.2.5 QTL analysis and identification of QTLs for yield and yield components using combined linkage map

The phenotypic data as described in section 3.1.3.1 and 3.1.3.2, along with genotypic data generated by screening 72 polymorphic markers on the 141 RILs were used for QTL analysis. The QTL analysis revealed a total of 28 QTLs, 19 QTLs in *rabi* 2012, five in 2013 *rabi* and four QTLs in pooled data set over both seasons.

4.4.2.5.1 QTLs detected in *rabi* 2012

A total of 19 QTLs were identified in 2012 *rabi* for yield and yield component traits. Among the 19 QTLs identified five QTLs (NCPGR 93 - GSSR 9, GSSR 9 - GSSR 77, GSSR 41 - NCPGR 33, A7C - TS 72, TS 72 - GSSR 50) on LG 1 were for days to 50 per cent flowering (Fig 7a). The QTLs were minor QTLs with the R^2 value ranging from 1.05 to 8.20 per cent and LOD score ranging from 3.25 to 4.01, respectively. The highest phenotypic variation of 8.20 per cent was explained by QTL (GSSR 41 - NCPGR 33) with a LOD score of 3.25, followed by QTL (NCPGR 93 - GSSR 9) with a variance of 6.17 per cent and LOD score of 3.77, the lowest variance of 1.05 per cent was explained by QTL (A7C - TS 72) with a LOD score of 3.27. All the five QTLs had additive gene action and male parent contributed the favourable alleles for all except for QTLs (GSSR 41 - NCPGR33) which had contribution of favourable alleles form female parent.

Among the 14 QTLs identified for 100 seed weight 11 were located on LG 1 and one each was located on LG 2, LG 3 and LG 6. All the QTLs except one were minor QTLs. The R^2 value ranged from 0.20 to 36.21 per cent and LOD score ranging from 3.30 to 9.28. The major QTL (TR 24 - GSSR 21) explained 36.21 % of variance and the LOD score (9.28) was also highest. This was followed by another QTL flanked by same markers (TR 24 - GSSR 21) with a LOD score of 6.42 and R^2 value

of 5.96 per cent. The least R^2 value of 0.20 was observed in QTL (TA 110 - EST SSR 34) located in LG 2 (Table 13). Among 14 QTLs, the favourable alleles for six QTLs were contributed by male parent with additive gene effect, the favourable alleles for other eight QTLs were contributed by female parent.

Among 14 QTLs identified for 100 seed weight (Fig 7b) during *rabi* 2012 two QTLs with a position of 54.01 cM and 104.01 cM on LG 1 were flanked by common markers (TR 24 - GSSR 21), the markers (NCPGR 33 - TC 14801) flanked two QTLs located on 558.81 cM and 578.81 cM on LG 1 and the marker interval (H_2E_{23} - EST SSR 65) had two QTLs located at 877.51 cM and 893.51 cM on LG 1.

4.4.2.5.2 QTLs detected in 2013 *rabi*

As compared to 2012 *rabi* very less number of QTLs were detected in 2013 *rabi*. Out of five QTLs detected two were for plant height at maturity (Fig 7c and 8a) and other three QTLs were for 100 seed weight (Fig 8b). The QTL (TS 72 - GSSR 50) among the two QTLs detected for plant height explained a phenotypic variation of 14.52 per cent with a LOD score of 4.19 per cent, the QTL had additive gene action with male parent WR 315 contributing for the favourable alleles. Another QTL (H_5F_{021} – TA 110) identified with a LOD score of 3.15 was located on LG 2, the QTL explained a variation of 8.47 per cent for plant height with favourable alleles contributing from male parent. The QTL was 0.01 cM away from the flanking marker H_5F_{021}.

A total of three QTLs were detected for 100 seed weight in 2013 *rabi*. All the three QTLs (NCPGR 93 - GSSR 9, GSSR 9 - GSSR 77 and GSSR 50 - TA 186) were located on LG 1 and were major QTLs explaining variance ranging from 16.57 (GSSR 9 - GSSR 77) to 26.11 (NCPGR 93 - GSSR 9) per cent. The LOD score for the QTLs ranged from

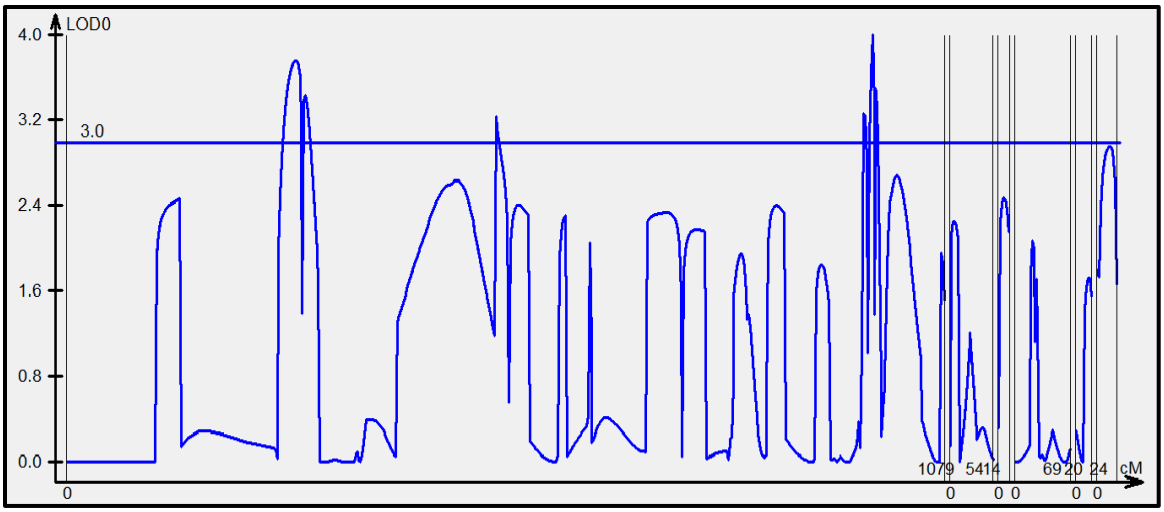


Fig. a: Five QTLs identified for 50% flowering using *rabi* 2012 data

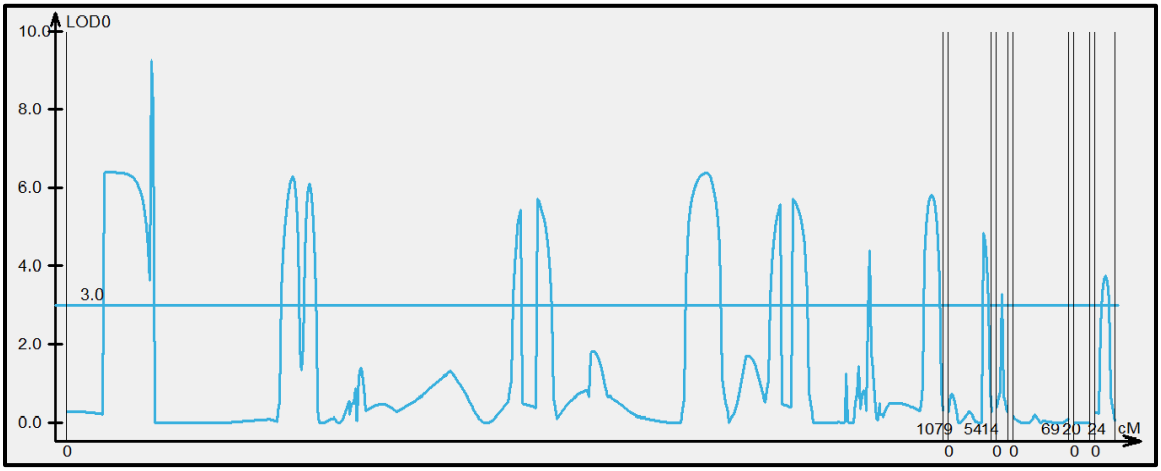


Fig. b: Fourteen QTLs identified for 100 seed weight using *rabi* 2012 data

Fig. c: QTL identified for plant height at maturity in LG1 using *rabi* 2013 data

Fig. 7: LOD peaks of QTLs identified for yield and yield components in *rabi* 2012 and 2013 data using combined map

3.50 (GSSR 50 - TA 186) to 4.29 (NCPGR 93 - GSSR 9) (Table 13). The QTL with the highest R^2 value of 26.11 was flanked by marker (GSSR 9) at 10.69 cM apart. All the three QTLs (NCPGR 93 - GSSR 9, GSSR 9 - GSSR 77 and GSSR 50 - TA 186) had additive gene effect with favourable alleles coming from male parent.

4.4.2.5.3 QTLs detected in pooled data

One QTL each for four different traits, (plant height at maturity (Fig 8c), number of pods per plant (Fig 9a), seed yield per plant (Fig 9b) and 100 seed weight (Fig 9c) were identified in the pooled data. The remaining three QTLs were located on LG 1. The QTL (TS 43 – TA 42) for seed yield was located on LG 4 at 40.61 cM with a LOD score of 3.30 and explained a variance of 10.44 per cent.

The QTL (GSSR 9 - GSSR 77) for plant height at maturity was located on LG 1 at 295.01 cM, the QTL was detected with a LOD score of 4.66 and explained a phenotypic variation of 17.68 per cent with additive gene action. In the present study only one QTL (TR 56 - TR 40) with additive gene effect for number of pods per plant was detected and was located at 335.41 cM on LG 1. The QTL had an R^2 value of 4.87 and a LOD score of 3.12 per cent. A QTL (TS 72 - GSSR 50) was identified for 100 seed weight with a LOD score of 4.61 and R^2 value of 15.47 per cent (Table 13).

4.5 Validation of the markers linked to *Fusarium* wilt resistance

The DNA isolated from the ten F_{10} generation RILs derived from cross between JG 62 (early wilting) x WR 315 (resistant) were amplified with all the ten markers identified to be linked to *foc* 1 locus in the present study. Five out of ten RILs used for validation were resistant to wilt in sick plot and the other five were susceptible. One among ten primers (GSSR 21) screened, amplified the resistant allele in all the RILs

Table 13: QTLs detected yield traits in 141 RILs (K 850 x WR 315) of chickpea using two individual season and pooled data combined over seasons by combined mapping approach

Seasons	Trait	Linkage group	Flanking marker	QTL Position (cM)	Max LOD	R2 (%)	Genetic effect		Donor parent
							Additive	Dominant	
Rabi 2012	Days for 50% flowering	1	NCPGR 93 - GSSR 9	280.31	3.77	6.17	2.45	31.00	Male
		1	GSSR 9 - GSSR 77	293.01	3.45	3.44	1.86	32.13	Male
		1	GSSR 41 - NCPGR 33	526.91	3.25	8.20	-2.36	33.96	Female
		1	A7C - TS 72	978.21	3.27	1.05	1.47	26.97	Male
		1	TS 72 - GSSR 50	989.81	4.01	6.05	1.86	29.32	Male
	100 seed weight	1	TR 24 – GSSR 21	54.01	6.42	5.96	0.03	10.14	Male
		1	TR 24 – GSSR 21	104.01	9.28	36.21	-5.13	15.11	Female
		1	NCPGR 93 - GSSR 9	278.31	6.30	4.72	0.63	10.16	Male
		1	GSSR 9 - GSSR 77	299.01	6.12	5.07	0.71	10.24	Male
		1	NCPGR 33 - TC 14801	558.81	5.47	4.66	-0.34	10.15	Female
		1	NCPGR 33 -TC 14801	578.81	5.73	4.89	-0.34	10.15	Female
		1	GSSR 11 - GSSR21	787.31	6.41	4.27	-0.27	10.12	Female
		1	H2E23 - EST SSR 65	877.51	5.59	4.90	-0.36	10.15	Female
		1	H2E23 - EST SSR 65	893.51	5.74	4.99	-0.35	10.15	Female
		1	TS 72 - GSSR 50	987.81	4.43	3.67	0.76	10.11	Male
		1	EST SSR 21 - OPK9	1063.61	5.85	2.90	0.04	10.15	Male
		2	TA 110 – EST SSR 34	43.11	4.88	0.20	-0.14	10.14	Female
		3	GSSR 4- EST SSR 91	6.01	3.30	0.22	0.10	10.15	Male
		6	TA 25- GSSR 94	12.01	3.77	0.62	-0.10	10.15	Female
Rabi 2013	Plant height at maturity	1	TS 72 - GSSR 50	989.81	4.19	14.52	1.88	16.53	Male
		2	H5F021 – TA 110	24.61	3.15	8.47	1.91	-5.67	Male
	100 seed weight	1	NCPGR 93 - GSSR 9	278.31	4.29	26.11	2.05	-2.4393	Male
		1	GSSR 9 - GSSR 77	295.01	3.74	16.79	1.62	-1.0845	Male
		1	GSSR 50 - TA 186	994.01	3.50	16.57	1.58	-1.1075	Male
Pooled	Plant height at maturity	1	GSSR 9 - GSSR 77	295.01	4.66	17.68	1.17	0.35	Male
	No of pods per plant	1	TR 56 - TR 40	335.41	3.12	4.87	1.71	41.38	Male
	Seed yield per plant	4	TS 43 – TA 42	40.61	3.30	10.44	1.21	-3.07	Male
	100 seed weight	1	TS 72 - GSSR 50	989.81	4.61	15.47	1.05	9.40	Male

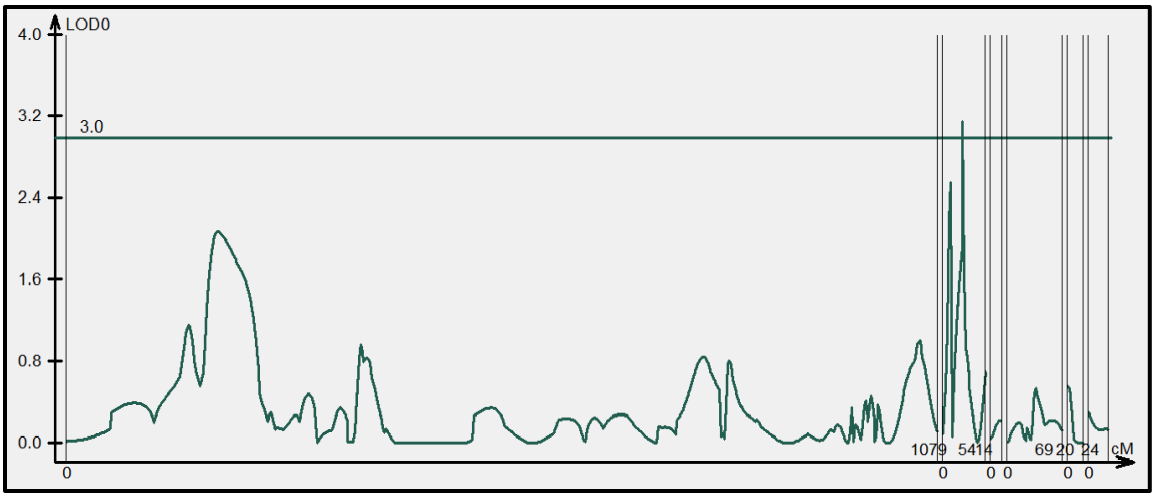


Fig. a: QTL identified for plant height at maturity in LG2 using *rabi* 2013 data

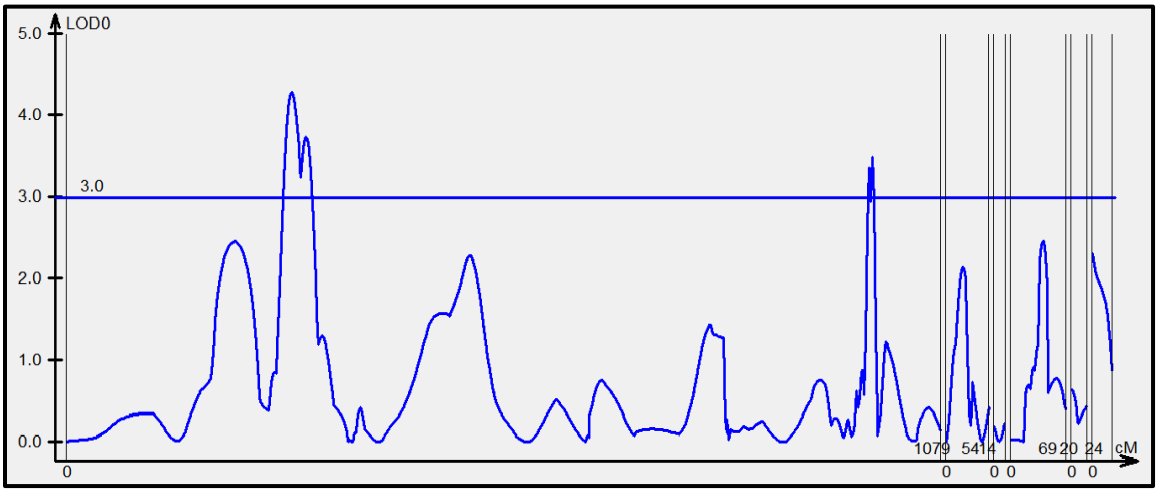


Fig. b: Three QTLs identified for 100 seed weight in LG1 using *rabi* 2013 data

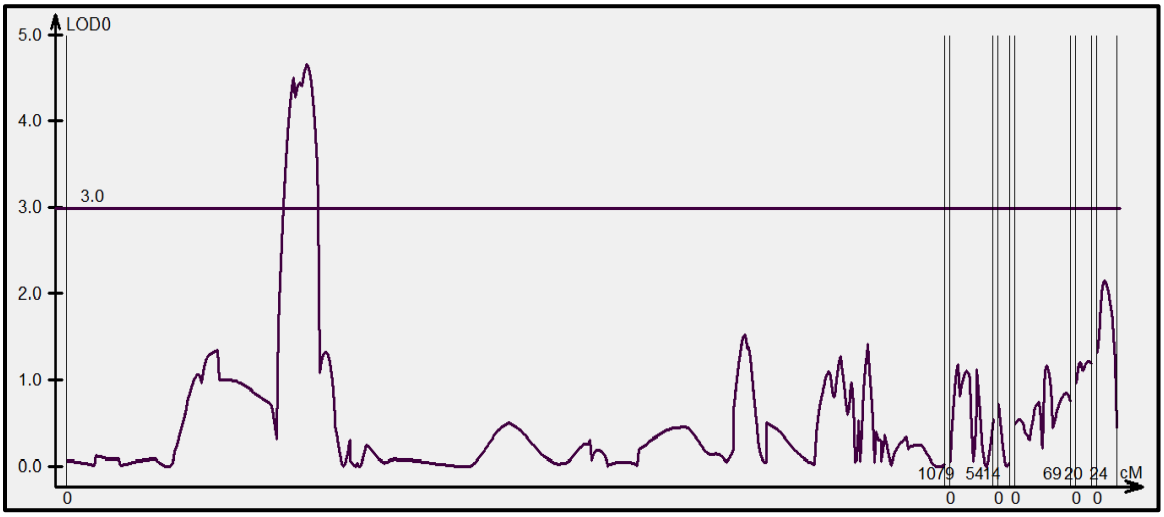


Fig. c: QTL identified for plant height at maturity using pooled data set

Fig. 8: LOD peaks of QTLs identified for yield and yield components in 2013 *rabi* and pooled data using combined map

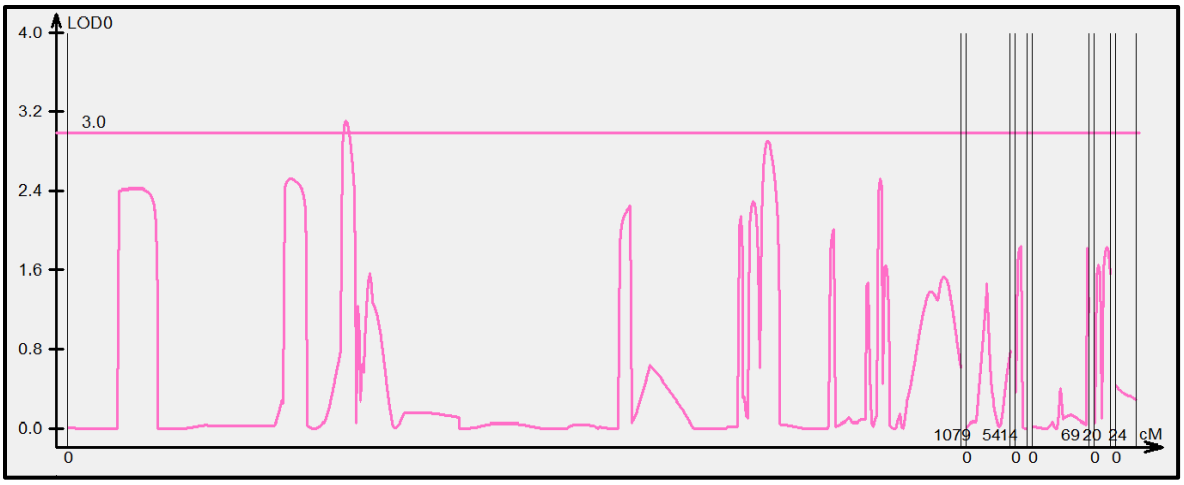


Fig. a: QTL identified for number of pods per plant using pooled data set

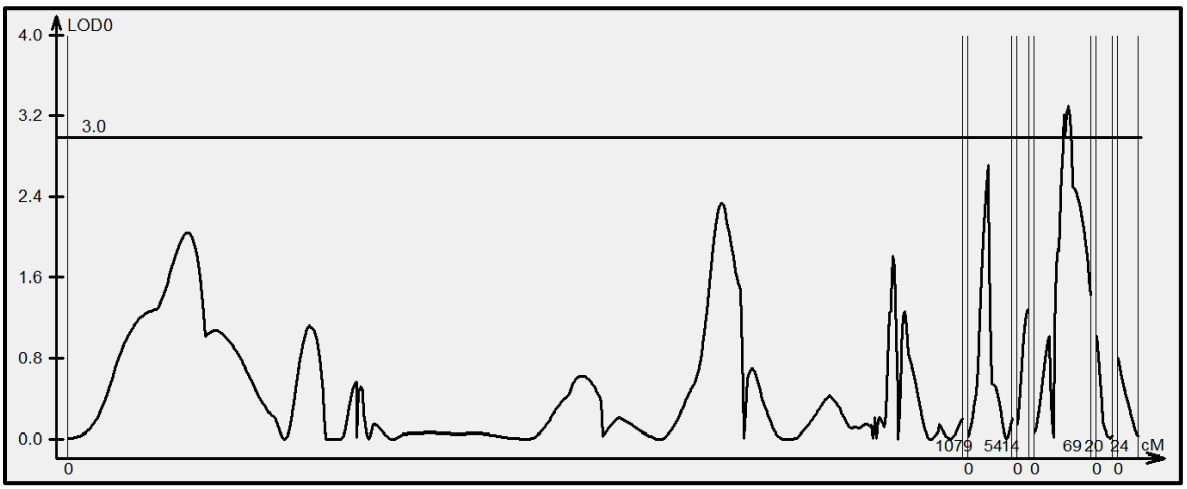


Fig. b: QTL identified for seed yield per plant in LG 4 using pooled data set

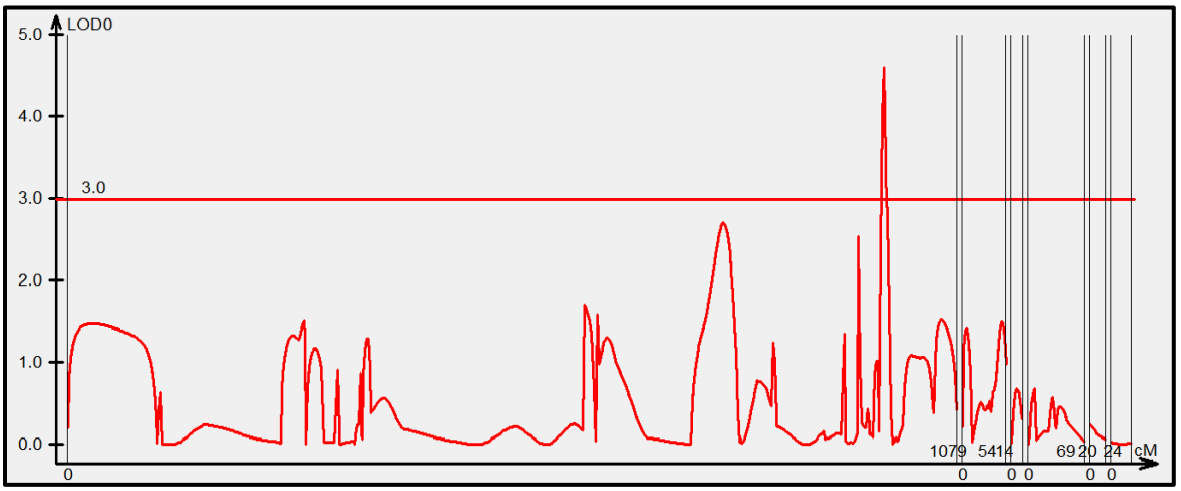
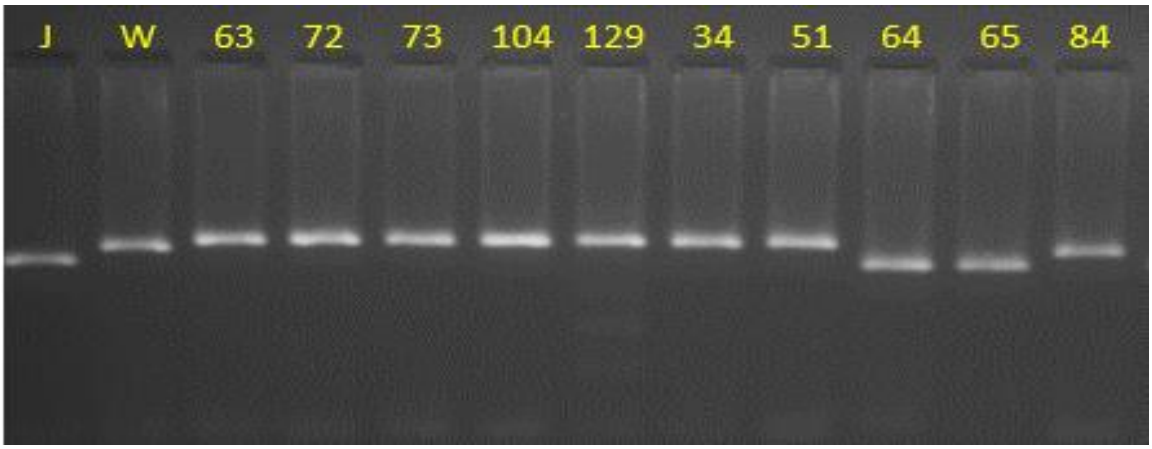
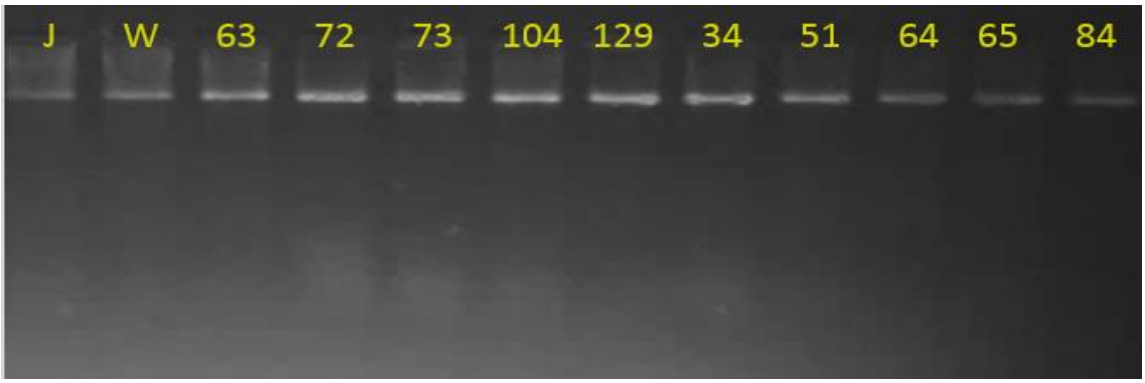


Fig. c: QTL identified for 100 seed weight using pooled data set

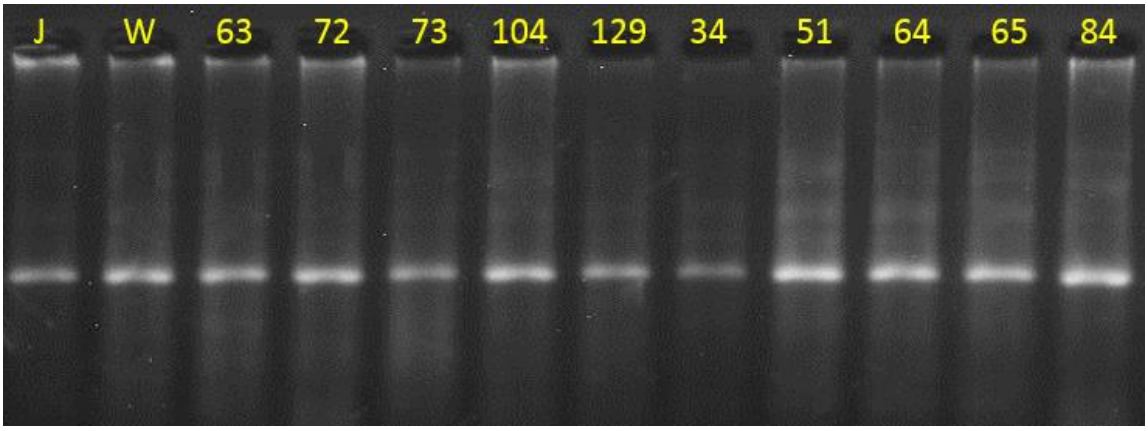
Fig. 9: LOD peaks of QTLs identified for yield and yield components in pooled data using combined map



A: Segregation pattern of SSR marker GSSR 21 linked to wilt resistance across (JG 62 X WR 315) chickpea RILs



B: Segregation pattern of SSR marker GSSR 41 linked to wilt resistance across (JG 62 X WR 315) chickpea RILs



C: Segregation pattern of SSR marker GSSR 11 linked to wilt resistance across (JG 62 X WR 315) chickpea RILs

Plate 2: Gel picture depicting validation of SSR marker linked to wilt resistance across RILs (JG 62 X WR 315) chickpea

which were phenotypically resistant for wilt reaction (Plate 2). However the amplified allele size for three out of five phenotypically susceptible RILs was that of resistant parent (WR 315) hence the marker was declared as non-validated , on contradictory the other nine markers (TR 24, GSSR 18, GSSR 11, GSSR 41, NCPGR 33, TC 14801, EST SSR 3, EST SSR 21, and EST SSR 65) liked with the *Foc* 1 locus failed to differentiate between the parent JG 62 and WR 315 and had the similar banding pattern for both the parents JG 62 and WR 315 hence all these markers were classified under non validated category.


DISCUSSION

V. DISCUSSION

Chickpea (*Cicer arietinum* L.) is one among the major legume crops grown across the world. India ranks first in terms of production and consumption in the world. About 65 per cent of global area with 68 per cent of global production of chickpea is contributed by India (Reddy and Mishra 2010b). Though about 65 per cent of the world acreage and 68 per cent production are accounted in India, the production is still not adequate to meet the domestic demand. One of the major constraints in realization of full yield potential of chickpea is occurrence of *Fusarium* wilt.

Fusarium wilt of chickpea is a vascular disease caused by *Fusarium oxysporum* sp. *ciceri*. The disease is widespread and causes substantial crop losses in many parts of the world. Majority of available cultivars are susceptible, the disease has been managed primarily by the use of resistant cultivars, but virulent races of the pathogen have undermined their importance in recent years (Haware and Nene, 1982).

Till now, eight races of the pathogen have been reported (Jimenez-Diaz *et al.,* 1993; Kelly *et al.,* 1994). Resistance for many of the diseases of crop plants is genetically simple and have been analysed extensively by traditional methods of plant pathology, breeding, and genetics (Flor, 1955; Nelson and Ullstrup, 1964). Genetically complex forms of disease resistance, by contrast, are more poorly understood (Geiger and Heun, 1989). The genetic analysis studies conducted in the past has indicated that the resistance to *Fusarium oxysporum* sp. *ciceri* race 0 (*FOC0*) is governed by one or two independent genes (Tekeoglu *et al.,* 2000; Rubio *et al.,* 2003). Resistance to Race 1A, which is prevalent in peninsular India in epidemic form is governed by two to three major independent loci H_1, H_2 and H_3 (Upadhyaya *et al.,* 1983a and b; Singh *et al.,* 1987,

Brinda and Ravikumar, 2005). Whereas, the resistance to *FOC2* is conferred by a single recessive gene (Sharma and Muehlbauer, 2007), resistance to *FOC3* is also monogenic (Sharma *et al.*, 2004), and resistance to race 4 (*FOC4*) is monogenic recessive (Tullu *et al.,* 1998; Sharma *et al.,* 2005) as well as digenic recessive (Tullu *et al.,* 1999) and resistance to race 5 is governed by a single gene (Tekeoglu *et al.,* 2000; Sharma *et al.,* 2005).

Molecular markers and linkage maps are pre-requisite for marker assisted selection (MAS) in crop improvement programme. However, the progress towards development of markers and linkage map is slow in chickpea, this could be because of presence of low level of genetic diversity in cultivated gene pools of chickpea. However, with the advent of genomic tools and availability of robust genotyping platforms progress has been made in development of various types of genomic resources like microsatellite or simple sequence repeat (SSR)/sequence tagged microsatellite markers (STMS), expressed sequence tags (ESTs), single nucleotide polymorphism (SNP), cleaved amplified polymorphic sequences (CAPS), conserved intron spanning primers (CISP) and diversity arrays technology (DArT) markers in chickpea which facilitated in mapping and tagging of *Fusarium* wilt resistance and many other agriculturally important genes.

Resistance genes to different races of *Fusarium oxysporum* were tagged and mapped by different groups using different mapping populations. The first *Fusarium* wilt resistance gene to be tagged in chickpea was H_1 (Mayer *et al.*, 1997) for race 1A using ASAP primer. Subsequently, markers linked closely to *foc*-1 (Sharma *et al.*, 2004, Sharma and Muehlbauer, 2005), *foc*-01 (Rubio *et al.*, 2003; Cobos *et al.*, 2005), *foc*-2 (Sharma and Muehbauer, 2005), *foc*-3 (Sharma *et al.*, 2004; Sharma and Muehlbauer, 2005), *foc*-4 (Ratnaparkhe *et al.*, 1998,, Tullu

et al., 1998; Tullu *et al.*, 1999; Tekeoglu *et al.*, 2000; Benko-Iseppon *et al.*, 2003; Sharma *et al.*, 2004; Sharma and Muehlbauer, 2005), the second resistance gene for race 4 (Tullu *et al.*, 1999) and *foc*-5 (Ratnaparkhe *et al.*, 1998; Tekeoglu *et al.*, 2000; Winter *et al.*, 2000; Benko-Iseppon *et al.*, 2003; Sharma and Muehlbauer, 2005) were identified. However, resistance to H_2 locus of race 1A, race 1 B/C and race 6 are limited (Soregaon *et al.*, 2007).

A number of intraspecific and interspecific molecular maps to map resistance to *Fusarium* wilt (Winter *et al.,* 2000; Benko-Iseppon *et al.,* 2003; Rubio *et al.,* 2003; Sharma *et al.,* 2004 Gowda *et al.,* 2009 and Millan *et al.,* 2010) as well as for other traits (Winter *et al.,* 1999; Santra *et al.,* 2000; Radhika *et al.,* 2007; Ali *et al.,* 2008; Cobos *et al.,* 2009 and Nayak *et al.,* 2010) have been developed in chickpea.

Most of the studies have considered *Fusarium* wilt resistance as qualitative and mapped the genes and markers. However, a few studies have considered *Fusarium* wilt as quantitative trait and mapped QTL since the timing and duration of wilting vary in each genotype. A few examples where *Fusarium* wilt was considered as QTL are Cobos *et al.* (2005), Radhika *et al.* (2007), Halila *et al.* (2009) and Millan *et al.* (2010).

The present study was directed towards development of linkage map in chickpea and identification of QTLs for *Fusarium* wilt resistance against race 1 which is prevalent in India. Further an attempt was also made for development of combined linkage map by making use of genotypic data generated in present study and data generated on same mapping population in earlier study (Soregaon *et al.,* 2011). The RILs were also evaluated for different quantitative traits *viz.,* days for 50 per cent flowering, plant height at maturity, number of branches per plant, number of pods per plant, seed yield per plant and 100 seed weight and QTLs were also identified for these traits.

The results of the experiments conducted in the present investigations are discussed below under the following headings.

5.1 Genetic mapping of molecular markers and linkage map development

5.2 Identification of QTLs for wilt resistance and other quantitative traits

5.3 Validation of markers linked to *Fusarium* wilt resistance

5.4 Genetic variability studies for seed yield and yield components

5.1 Genetic mapping of molecular markers and linkage map development

Considerable developments in the field of biotechnology have led plant breeders to think for more efficient selection system for crop improvement in contrast to conventional breeding methods. Molecular markers have been integral part of breeding programs due to their advantages over traditional methods. One of the main uses of markers in agricultural research is in the construction of linkage maps. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling both simple and complex traits (Mohan *et al.,* 1997). The identification of molecular markers closely linked to desirable traits facilitates marker assisted selection, positional cloning and mapping of QTLs for disease resistance and other traits of interest in many crops (Patterson and Garwick 1988; Winter and Kahl, 1995).

5.1.1 Linkage mapping and map development

Several linkage maps have been developed in the past to map *Fusarium* wilt resistance genes in chickpea using both intraspecific and interspecific mapping population, (Tekeoglu *et al.,* 2000; Winter *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; Millan *et al.,* 2010; Gowda *et al.,* 2011; Thudi *et al.,* 2011; Choudhary *et al.,* 2012; Sabbavarapu *et al.,* 2013; Varshney *et al.* 2014)

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.,* 2006; Pfaff and Kahl, 2003; Rakshit *et al.,* 2003; Rajesh *et al.,* 2004; Cobos *et al.,* 2006; Lichtenzveig *et al.,* 2005; Radhika *et al.,* 2007; Ali *et al.,* 2008; Cobos *et al.,* 2009 and Nayak *et al.,* 2010; Hossain *et al.,* 2010; Gowda *et al.,* 2011) are available in chickpea.

In the present study, linkage map was developed by screening 31 polymorphic SSRs (genic and genomic) on recombinant inbred lines (F_{11}) developed from intraspecific cross between K 850 and WR 315 genotypes of chickpea. The 31 polymorphic SSRs resulted from screening of 300 SSRs for parental polymorphism. The per cent polymorphism obtained in the present study (10.3%) is in accordance with majority 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%) and Sabbavarapu *et al.* (2013) (20.8%). However, when compared with reports by Ratnaparkhe *et al.* (1998) (38%) the per cent polymorphism obtained in the present study was less (10.3 %). The possible reason could be the use of intraspecific mapping population and more number (84.3%) of 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.* (1998).

The segregation of individual markers for the expected monogenic 1:1 ratio in the RILs was tested using Chi square test, four (12.9%) among 31 markers tested recorded a significant deviation ($P<0.05$) from the expected Mendelian segregation ratio (1:1), which is relatively less compared to Tullu *et al.* (1999) (30%), Winter *et al.* (2000) (38.4%) and Flandez-Galvez *et al.* (2003) (20.4%). The observed segregation distortion in present study is in favour of the maternal alleles (K 850) 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. Though Winter *et al.* (2000) observed different amounts of segregation distortion for different classes of markers, he reported that segregation distortion is less related to the class of affected markers than to the genomic region where they resided.

Out of 31 SSR markers 23 SSRs (8 genic and 15 genomic) were mapped. Sixteen out of 23 were novel markers and these markers were mapped for the first time in chickpea. Hence it is an addition to already existing integrated map (Nayak *et al.,* 2010).

The novel microsatellite markers were developed at the National Institute of Plant Genome Research, New Delhi were used. (Jhanwar *et al.,* 2012; Kujur *et al.,* 2013) The genic SSRs were developed from transcriptome sequence of various tissues of chickpea variety ICC4958, using next generation sequencing platforms (Garg *et al.,* 2011b). The genomic markers are based on the draft genome sequence of *Desi* chickpea (Jain *et al.,* 2013).

The linkage map was constructed using 31 polymorphic markers with the software GMendel programme of iMAS. The genetic linkage map represents 23 markers loci with four linkage groups spanning a total distance of 690.0 cM with the average marker distance of 30 cM. Only four out of eight possible linkage groups were obtained in the present study and map obtained is also less dense 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) and Nayak *et al.* (2010) (2602 cM, 8 LG). It may be due to use of less number of markers for mapping. Earlier, Winter *et al.* (2000) map was considered as reference map, but recently revised genetic map of Nayak *et al.* (2010) is still larger in all aspects compared to any other maps available till date and yielded eight linkage groups which is in agreement with eight

chickpea chromosomes, with an average intermarker distance of 4.99 cM.

Considering the 740 Mbp physical size of the chickpea genome (Arumuganathan *et al.,* 1999) and ignoring the fact that recombination rates can vary widely within the genome, 1 cM distance in present map equates to roughly 4.31 Mbp which is high compared to Winter *et al.* (2000) (1 cM = 0.36 Mbp), integrated map of Radhika *et al.* (2007) (1 cM = 1 Mbp) and Nayak *et al.* (2010) (1 cM = 0.28 Mbp (285 kb)). Though less number of markers used for construction of linkage map, per cent polymorphism is in accordance with earlier reports. The limited polymorphism in chickpea remained the biggest constraint in the construction of a complete saturated linkage map. STMSs, though highly polymorphic tend to cluster on chickpea linkage maps and their distribution is not uniform (Lichtenzveig *et al.*, 2005). AFLP and SNP can be the other category of markers which can be exploited to map those genomic regions where microsatellite density is low. Recently whole genome sequencing of chickpea by (Varshney *et al.,* 2013; Jain *et al.,* 2013) revealed the size of genome was ~738 mb. With the advent of chickpea whole genome sequence and availability of SNPs high density saturated maps can be developed in chickpea.

5.1.2 Development of combined linkage map

Knowledge of the inheritance of agronomic characters is a basic requirement to identify and integrate the genes of economically importance. A substantial support to chickpea improvement is expected to come from genetic mapping and comparative genome analysis using linkage maps (Zatloukalova *et al.,* 2011). Hence most of the chickpea geneticists agree that the generation of an integrated genetic map of the crop, comprising loci of both economic and scientific importance, presently is a central goal of chickpea genetics (Millan *et al.,* 2006).

The first integrated genetic linkage map in chickpea was developed in 1977 by Simon and Muehlbauer using three interspecific crosses, later different scientific groups have developed various genetic maps (Winter *et al.,* 2000; Flandez-Galvez *et al.,* 2003) and finally mapping data from ten different populations were merged into a consensus map (Millan *et al.,* 2010). Additionally, an integrated genetic map including gene-based markers as anchor points for comparing the genomes of *M. truncatula* and chickpea was published (Nayak *et al.,* 2010) as well as a transcript map based on genic molecular markers (Gujaria *et al.,* 2011). These maps provide the basis for marker-assisted selection (MAS) and facilitate cloning of agronomically relevant genes. Nevertheless, saturation of genome regions harbouring useful genes and quantitative trait loci is necessary to increase the efficacy of MAS.

In the present study two maps one with 31 markers (solo map) and another with 72 markers (combined map) involving 31 markers from the present study and 41 markers from earlier study carried out in the same laboratory were used (Soregaon 2011). A comparative study between both the maps revealed the presence of four linkage groups with 23 marker mapped in map developed using 31 markers against six linkage groups with 64 markers mapped in combined map.

The map constructed using 72 polymorphic markers with the software GMendel programme of iMAS produced genetic linkage map with 64 markers loci. Only six out of eight possible linkage groups were obtained in the present study and map obtained is also less dense compare with obtained by 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) and Nayak *et al.* (2010) (2602 cM, 8 LG).

The maps (combined map and map developed using 31 markers) shared a total of 17 markers with nearly same order and position on LG 1 and LG 4. All the 14 markers (TR 24, EST SSR 21, EST SSR 65, TA 186, GSSR 50, TA 53, GSSR 41, GSSR 15, TC 14801, GSSR 18, GSSR 106, EST SSR 8, GSSR 11 and EST SSR 3) of LG 1 and 3 markers (TR 59, TA 42, GSSR 57 and TR 29) from LG 4 of map developed using 31 markers (solo map) were retained on their respective linkage groups in combined map with 72 markers although some differences in their order are obvious. However, the three markers (GSSR 9, GSSR 77 and TR 56) from LG 3 of solo map were mapped on first linkage group of combined map with the same order and position as in solo map. The linkage group 2 of the solo map resembled the LG 3 of the combined map for the marker order and number. The two markers GSSR 4 and EST SSR 91 mapped on LG 2 of solo map were mapped on LG 3 of the combined map with the order and position.

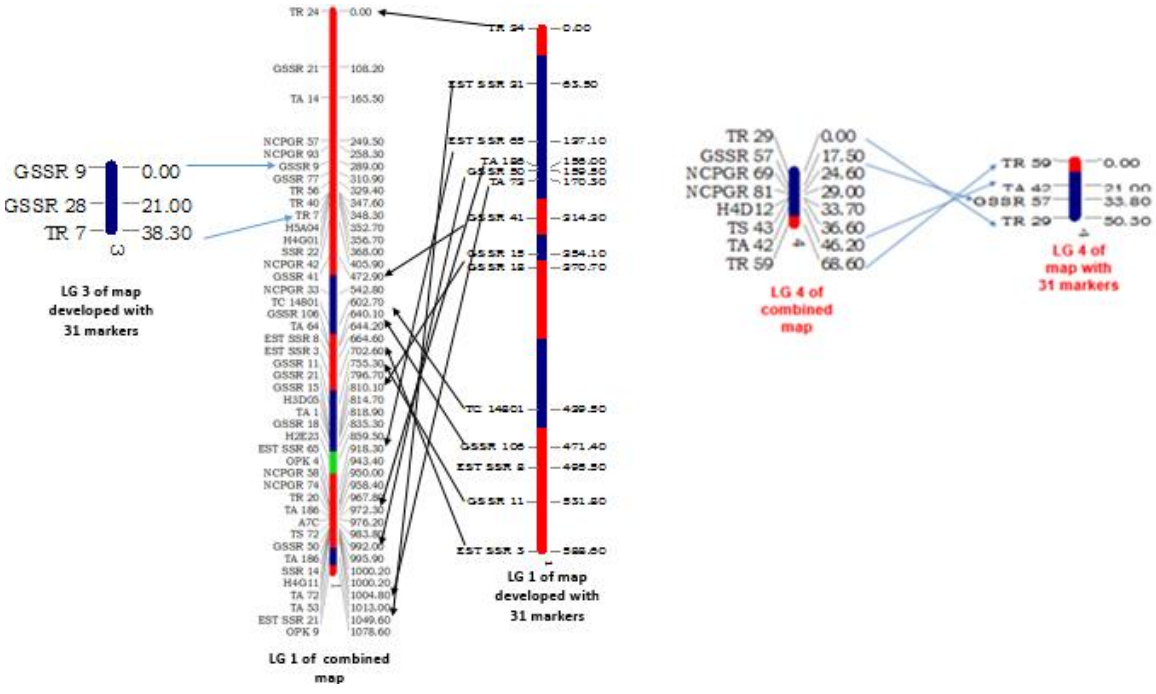


Figure depicting common markers between map developed using 31 markers and combined map

Some of the markers mapped in this study were also mapped in earlier studies in different genetic backgrounds, the marker TA 1 mapped on LG 1 and TA 110 mapped on LG 2 in the present study was in agreement with earlier study (Nayak *et al.* 2010). The marker TR 19 mapped on LG 2 was in agreement with earlier study (Winter *et al.,* 2000). However, two markers TR 20 and TS 72 in the present study were mapped on LG 1 unlike on LG 4 in earlier study (Nayak *et al.* 2010; Winter *et al.,* 2000), similarly markers TR 56 and TA 64 mapped on linkage group 1 in the present study were not in agreement with earlier study wherein they were mapped on LG 3 (Nayak *et al.* 2010), the marker TR 59 located on LG 4 in the present study was mapped on LG 5 of the integrated map developed in earlier study (Winter *et al.,* 2000) and on LG 1 of composite map (Radhika *et al.,* 2007), The marker TA 14 mapped on LG 6 (Winter *et al.,* 2000) and LG 4 (Radhika *et al.,* 2007) was mapped on LG 1 in the present study.

Comparison of the present combined map with earlier maps developed by different groups in chickpea revealed a moderate linkage conservation in at least two (LG 1 and LG 2) of the linkage groups with fair density of markers. However the map distance and marker orders of common markers differed possible due to intraspecific nature of our mapping population along with use of 23 (8 genic and 15 genomic) novel SSRs which were not used in any of the earlier mapping studies and comparatively less number of markers used in the present study.

5.3 Identification of QTLs for wilt resistance and other quantitative traits

5.3.1 Identification of QTLs for wilt resistance

Some forms of plant disease resistance are genetically simple and have been analysed extensively by traditional methods of plant pathology, breeding, and genetics (Flor 1955). Genetically complex forms

of disease resistance, by contrast, are more poorly understood (Geiger and Heun, 1989). The classical quantitative genetics provided the tools for studying complex disease resistance (Falconer, 1989). However, quantitative genetics is unsuited for dissecting polygenic resistance characters into discrete genetic loci or defining the roles of individual genes in disease resistance. An effective approach for studying complex and polygenic forms of disease resistance is known as "Quantitative Trait Locus" (QTL) mapping, which is based on the use of DNA markers (Tanksley, 1993).

A main requirement to perform proper QTL analyses is to have parents with contrasting character for disease infection. The lack of detectable genetic diversity in cultivated germplasm is common in most of the legumes, particularly in chickpea (Kazan and Muehlbauer, 1993), soybean (Keim *et al.,* 1990) and common bean (Gupt and Sharma 1991). In the present study, the RIL population derived from diverse genetic background of the intraspecific parental lines (K 850 and WR 315) provided moderate level of polymorphism both at genotypic and phenotypic level. Therefore this population is generally considered as an ideal population to map QTLs for wilt resistance and other yield and yield attributing traits.

Apart from vertical form of resistance, slow wilting resistance in chickpea after inoculation with *FOC* has been observed (Sharma *et al.,* 2005). The phenomenon of slow wilting in chickpea is similar to that of slow mildewing and slow rusting in crops such as pea and wheat. The slow rusting and slow mildewing resistance in other crops are usually governed by polygenes and quantitative trait loci (QTL) which have additive effect (Singh *et al.,* 2000). The first *Fusarium* wilt resistance gene in chickpea to be tagged in chickpea was H_1 (Mayer *et al.,* 1997). The gene was 7.0 cM from RAPD markers CS-27_{700} and UBC-170_{550} and an

Allele Specific Associated Primer (ASAP) marker. Subsequently, marker linked closely to *foc-1* were tagged (Sharma and Muehlbauer 2005). The present study mainly focus on identification of QTLs for *foc-1* using RILs derived from late wilting cross. The parents used in the development of RILs were K 850 (late wilting) x WR 315 (resistant). The conventional Mendelian genetic analysis indicated that these two parents have the same alleles at H_1 locus (h_1h_1) and different alleles at H_2 and H_3 locus (Upadhyaya *et al.* 1983b: Brinda and Ravikumar (2005). Hence, K 850 is phenotyped as late wilting and WR 315 as resistant. The RILs of the cross K 850 x WR 315 segregate for late wilting.

In the present study the wilt reaction of F_7 and F_8 generation RILs were tested under field conditions in wilt sick plot for *rabi* 2007 and *rabi* 2008. The QTL analysis was conducted with data set obtained from wilt sick plot experiment conducted in 2007 *rabi*, 2008 *rabi* and mean data pooled over both the seasons. As expected majority of the RILs did not show early wilting (wilting at 30 DAS), wilting started after 30-40 days hence wilting data recorded at 60DAS was used for QTL mapping.

The QTL mapping with 31 markers in present study identified five QTLs for *Fusarium* wilt resistance. Two QTLs (FW-Q-07-1 and FW-Q-07-2) base on 2007 *rabi* wilt data and three QTLs (FW-Q-08-1, FW-Q-08-2 and FW-Q-08-3) based on 2008 *rabi* wilt data. However, no QTLs were identified when pooled data set over both the seasons was used for QTL analysis. All the QTLs identified in this study were major QTLs with R^2 value more than 50 per cent. The LOD score was also high for all the QTLs. It is interesting to note that one QTL based on 2007 wilt data and another QTL based on 2008 wilt data were in the same locus and the flanking markers for those QTL were GGSR 18 and TC 14801. In addition the favourable alleles were donated by female and additive effect was (-25.00 and -22.33), respectively. Hence, it can be considered as

relatively stable QTL. Although the flanking markers were same, the QTL position were not same. The QTL of 2007 and 2008 were 24 cM apart the remaining three QTLs; one in 2007 (GSSR 11 - EST SSR 3) and two in 2008 (TR 24 - EST SSR 21 and EST SSR 21 - EST SSR 65) were also major QTLs with high additive effect and the contributing allele for all the three were from male parent WR 315. The QTLs found during 2008 were very close to each other while the second QTL of 2007 was at the other end of linkage group 1. All the five markers identified in this study were present only in LG 1 at different position.

In earlier studies efforts were also made to map quantitative trait loci (QTLs)/genes and markers flanking QTLs were reported for different *Fusarium* wilt races. For instance, markers flanking "Foc0" locus ($OPJ20_{600}$ and TR59), *foc1* locus (TA110 and H_3A_{12}), *foc2* locus (H_3A_{12} and TA96), *foc3* locus (TA96 and TA194), *foc4* locus (TA96 and CS27) and *foc5* locus (TA59 and TA96) determining resistance to race 0 (Cobos *et al.*, 2005), race 1 (Gowda *et al.,* 2009), race 2 (Gowda *et al.,* 2009); race 3 (Sharma *et al.,* 2004; Gowda *et al.,* 2009); race 4 (Winter *et al.,* 2000; Sharma *et al.,* 2004, 2005) and race 5 (Cobos *et al.,* 2009) respectively were reported.

In earlier study (Sharma *et al.,* 2004) considering wilt resistance as qualitative trait three markers (CS27, TA96 and TA27) linked to *foc-1* locus were identified. The marker CS27 was linked to *foc-1* locus mapped on the LG2 at a distance of 7.1 cM, CS27 was 16.9 cM away from a two STMS marker flanking *foc*-1 locus at the other end. The study also showed that *foc*-1 and *foc*-4 locus were closely linked at 1.1 cM. In the present study the major QTL (FW-Q-07-1) was identified in LG 1 which was in contradictory with the earlier study (Cobos *et al.,* 2005) wherein the QTL was identified on LG3. The QTL identified in the present study was flanked by markers (GSSR 18-TC1480). However, the QTL identified

by Cobos *et al.*, (2005) was located between markers (OPJ 20 $_{600}$ - TR 59). Recently in a study by Sabbavarapu *et al.* (2013) using new ($F_{2:3}$) mapping 'population derived by crossing C 214 (FW susceptible) and WR 315 (FW resistant) two novel QTL (FW-Q-APR-6-1 and FW-Q-APR-6-2) for FW race 1 have been identified. The identified QTLs were mapped on LG 6 unlike in the present study the QTL was located on LG1. The major QTL identified in the present study was placed between two novel SSR markers (GSSR 18-TC14801) which were not used in any of the earlier studies. However, the QTL identified by Sabbavarapu *et al.* (2013) was flanked between bacterial artificial chromosome ends (BAC-end) derived SSR markers (CaM1402–CaM1101). The QTL identified in the present study had explained a phenotypic variation of 69.80 per cent against 10.4 to 18.8 per cent as explained in earlier study. Both the QTL (FW-Q-APR-6-1 and FW-Q-APR-6-2) identified on LG2 by Sabbavarapu *et al.* (2013) were declared as novel QTLs since both the QTLs were not detected in any of the earlier studies which in agreement with the present study. The possible reason could be use of novel markers in both the studies. Resistant parent used for development of mapping population in both the studies was same (WR 315). However, in the present study F_{11} RILs were used but in earlier study F ($_{2:3}$) was used.

Winter *et al.* (2000) in a study to localization resistance genes for *Fusarium* wilt races 4 and 5 using interspecific cross identified the linkage between CS27 marker and genes for resistance to races 4 and 5 at distances of 3.7 and 18 cM, respectively. The marker was mapped on LG2, whereas the major QTL for race 1 identified in the present study was mapped on LG 1.

QTL analysis using combined linkage map (72 markers) detected two QTLs one each in 2007 *rabi* and 2008 *rabi*. The QTL identified in 2007 *rabi* was major QTL flanked between markers (GSSR 41-NCPGR 33)

on LG 1 had a LOD score of 8.73 with contribution of major alleles form susceptible late wilting parent (K 850). The QTL explained phenotypic variation of 71.41 per cent for wilt reaction. The second QTL (FW-Q-08-1) identified in combined mapping approach in 2008 *rabi* and the QTL identified in 2008 *rabi* were 20 cM away flanked by same markers (TR 24 - GSSR 21). It is also present on LG 1. In an earlier study (Sabbavarapu *et al.,* 2013) two QTLs (FW-Q-APR-6-1 and FW-Q-APR-6-2) for *FOC* 1 were identified with a LOD score of 8.0 and 7.6 which was in accordance to major QTL identified in the present study. However, the QTLs identified in earlier study were located on LG 6 and explained a variance of 10.4 and 18.8 per cent but the QTL identified in the present study was located on LG 1 and comparatively explained a higher variance of 71.41 per cent. The QTL (GSSR 41-NCPGR 33) identified in the present study was found in both the map developed using 31 markers and combined map developed using 72 markers.

All the QTLs identified in the present study for wilt resistance against race 1 were located on LG 1 and explained a higher phenotypic variation ranging from 53.34 to 71.41 per cent and had a higher LOD score of 8.90 per cent. The QTLs detected were not reported so far in any of the earlier studies hence the QTLs may be considered as novel. This is in agreement with earlier study by Sabbavarapu *et al.* (2013) reported two novel QTL (FW-Q-APR-6-1 and FW-Q-APR-6-2) identified for *Fusarium* wilt resistance for race 1 explaining 10.4 to 18.8% of phenotypic variation.

The probable reason for identification of these novel QTLs for race 1 of *Fusarium* as compared with earlier studies (Gowda *et al.*, 2009; Sharma *et al.*, 2004) involving a common resistant parent WR 315 may be explained due to variation in screening technique for the disease reaction in our study and earlier studies. In the present study the

mapping population was screened disease in wilt sick plot at ICRISAT for two seasons (2012 *rabi* and 2013 *rabi*), where as in earlier studies the phenotyping was carried out in lab condition using wilt sick pots.

The identification of closely linked markers or stable QTLs for the *FOC* resistance genes, will facilitate introgression of resistant genes from *FOC* resistant cultivars carrying individual genes into commercially competitive chickpea varieties, as witnessed in the recent study by Varshney *et al.*, (2014a) wherein introgress of resistance genes for *Fusarium* Wilt in to C 214, an elite cultivar of chickpea was carried out using MAS. For introgressing of *foc1* locus conferring resistance to race 1 of *FOC*, three SSR markers (TR19, TA194, and TAA60) present in the genomic region on linkage group CaLG02 and a few adjoining markers (GA16, TS82, and TA110) in the same region (Sharma *et al.,* 2004; Sharma and Muehlbauer, 2005; Millan *et al.,* 2006; Gowda *et al.,* 2009) were targeted for deployment. The foreground selection conducted with six markers (TR19, TA194, TAA60, GA16, TA110 and TS82) linked to *foc1*. Background selection employed with 40 evenly distributed markers. Further phenotyping of lines derived from backcrossing identified three resistant lines for race 1. In future the development of high density maps enables pyramiding of all the five race specific resistance genes into a single chickpea cultivar, thus greatly enhancing the spread and durability of wilt resistance.

5.3.2 Identification of QTLs for yield and yield component traits

Most of the mapping studies with molecular markers have used either F_2 populations or backcrosses. But recombinant inbred lines (RILs) developed by single seed descent method from F_2 population have several advantages over other populations for genetic mapping. RlLs are homozygous and, hence, they can be evaluated in different environments, which is useful in the analysis of quantitative traits as it

helps in the accurate assessment of the genetic component of variance. For the RlLs developed by selfing, there is a 2 fold to 4 fold increase in the recombination frequency between two linked markers.

In the present study RILs were also evaluated for six yield and yield attributing traits *viz.,* days to 50 per cent flowering, plant height at maturity, number of branches per plant, number of pods per plant, 100 seed weight and seed yield per plant. The ANOVA indicated significant variability for all the traits suggesting the suitability of the RILs for QTL analysis. The QTL analysis using linkage information generated 31 markers revealed total nine QTLs, two QTLs (TA186 - GSSR 50 and GSSR 50 - TA 72) in *rabi* 2012, three QTLs (TA 72 - GSSR 41, GSSR 50 - TA 72 and TA 72 - GSSR 41) in *rabi* 2013 and four QTLs (TA 72 - GSSR 41, GSSR 50 - TA 72, GSSR 50 - TA 72 and TA 72 - GSSR 41) in pooled data.

Both the QTLs (TA186 - GSSR 50 and GSSR 50 - TA 72) identified in *rabi* 2012 were for seed yield per plant. One QTL (TA 72 - GSSR 41) among three QTLs identified in *rabi* 2013 were for plant height at maturity and other two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) were for 100 seed weight. Among four QTLs identified in pooled data two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) for 100 seed weight and one each for plant height at maturity (TA 72 - GSSR 41) and a QTL (GSSR 50 - TA 72) for seed yield per plant were identified.

A total of 28 QTLs were identified using linkage information generated from combined linkage map. Among 28 QTLs 19 QTLs in *rabi* 2012, five QTLs in *rabi* 2013 and four QTLs in pooled data were identified. Out of 19 QTLs identified in *rabi* 2012 five QTLs were for days to 50 per cent flowering and 14 were for 100 seed weight. Three out of five QTLs identified in *rabi* 2013 were for 100 seed weight and two were for plant height at maturity. In pooled data set one each QTL for plant

height at maturity, number of pods per plant, seed yield per plant and 100 seed weight were identified.

Two QTL (TA186 - GSSR 50 and GSSR 50 - TA 72) for seed yield per plant in *rabi* 2012 and one QTL (GSSR 50 - TA 72) in pooled data was identified using linkage information generated from 31 markers. A common QTL (GSSR 50 - TA 72) for seed yield per plant identified across *rabi* 2012 and pooled data. The QTL explained a variance of 26.18 per cent in *rabi* 2012 and 16.53 per cent in pooled data. Both the QTLs had additive gene action and contribution of favourable alleles from male parent. Another QTL (TS 43 – TA 42) for the same trait was identified in pooled data set of combined linkage map. The QTL unlike those identified using 31 markers was located in LG 4 and explained a comparatively lesser variance of 10.44 per cent.

Three QTLs (TA186 - GSSR 50, GSSR 50 - TA 72 and GSSR 50 - TA 72) for seed yield per plant were identified on linkage group 1 in the present study using linkage information from 31 markers. A single QTL for the same trait was identified in combined map on LG 4. However, in earlier study by Gowda *et al.* (2011) five QTLs were identified for the same trait. Three out of five identified QTLs were on LG-2 and other two were mapped on LG-5 and LG-7 each. The QTLs identified in earlier study were flanked by five different STMS markers *viz.,* TA 47, TS 53, TR 29, TA 127 and UBC $_{299.}$ Among the five flanked markers, the marker TR 29 was common in both studies, but it was mapped to LG 2 in earlier study. In the present study TR 29 was mapped to LG 4 of map developed using 31 markers but unlike in earlier study the marker did not flank an of the QTL for seed yield per plant as in the present study.

One (GSSR 50 - TA 72) among four QTLs identified for seed yield per plant in the present study was major QTL, explaining a phenotypic variation of 26.13 per cent for seed yield per plant, whereas all the five

QTLs identified in earlier study (Gowda *et al.,* 2011) were minor QTLs with R^2 value ranging from 9.7 to 13.0 per cent. In the present study the QTL with highest R^2 value had a LOD score of 5.58 against 3.0 as in earlier study. Two QTLs for seed yield per plant explaining a variance ranging from 13.98 to 15.71 were identified in earlier study (Varshney *et al.,* 2014a). QTLs for seed weight per plant were also mapped by Radhika *et al.* (2007) in concensus mapping approach. The QTL was mapped to LG 2 flanked by markers TA2x and UBC_{465} at 0.1 and 1.8 cM, respectively. Through concensus map approach one can integrate earlier reported map with present map through anchored markers and can locate markers/QTLs or genes for important traits which might not segregate in one mapping population but in the other (Radhika *et al.,* 2007). This is particularly important for crops like chickpea where very low level of polymorphism have initially been reported (Udupa *et al.,* 1993; Labdi *et al.,* 1996). The major QTL (GSSR 50 - TA 72) for seed yield per plant identified in the present study explained the highest phenotypic variation for the trait. Hence upon validation in other genetic background the QTL can be made use in improvement of chickpea for yield traits in marker assisted selection programme.

Two QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) for 100 seed weight identified using map developed by 31 markers were shared between *rabi* 2013 and pooled data. Both the QTLs were flanked by same markers and were located on LG 1 at similar positions. The QTLs identified in *rabi* 2013 explained a variance ranging from 22.24 to 26.73 per cent against a variance range of 18.73 to 24.38 as explained by QTL detected in pooled data. All the four QTLs had the contribution of favourable alleles from male parent. Two stable QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) for 100 seed weight in rabi 2013 and pooled data identified in the present study was in accordance with the earlier study (Varshney *et al.,* 2014a) wherein two QTLs for 100 seed weight were

identified. Both the QTLs were consistent across three seasons and were stable across all the five different locations tested. The QTLs (GSSR 50 - TA 72 and TA 72 - GSSR 41) identified in the present study explained variance ranging from 22.24 to 26.73 per cent which was moderate when compared to earlier QTLs explaining a variance ranging from 10.31 to 58.20 per cent.

QTL analysis using combined map revealed 14 QTLs in *rabi* 2012, three QTLs in *rabi* 2013 and a one QTL in pooled data for 100 seed weight. Among 14 QTLs detected in *rabi* 2012 for 100 seed weight a single major QTL explaining a variance of 36.21 per cent was detected. The QTL had additive gene action and contribution of favourable alleles from female parent. Among all 17 QTLs detected in combined map, the QTL (TR 24-GSSR 21) was shared between *rabi* 2012 and pooled data with same flanking markers. However, the QTL detected in *rabi* 2012 had contribution of favourable alleles from female parent unlike the one detected in pooled data which had contribution of favourable alleles from male parent. Among the 17 QTLs identified in combined map, except three QTLs all other QTLs were located on LG 1. One each out of three QTLs was located on LG 2, LG3 and LG 6. In earlier study (Gowda *et al.* 2011) two QTLs each one on LG 2 and LG 4 were identified. A common QTL on LG 2 was found in both studies. However, both QTLs were located on different positions and flanked by different markers. The QTL identified in the present study explained a variance of 0.20 per cent unlike a higher variance of 11.4 per cent in the earlier study.

In present study a single QTL (TR 56 - TR 40) for number of pods per plant was detected in pooled data in combined map which was in contradictory to earlier study (Gowda *et al.,* 2011) wherein six QTLs for number of pods per plant were identified. Four out of six QTLs identified were mapped to LG 2 and one each on LG 3 and LG 4. None of the QTLs

were identified in the previous. The QTL had a R^2 value of 4.87 per cent with additive gene action and were mapped to LG 1 unlike the single QTL identified in the present study for number of pods per plant was mapped. All the QTLs identified in earlier study for number of pods per plant were minor QTLs with variance ranging from 9.1 to 12.7 per cent, which was in agreement with the present study (R^2 value 4.87). The favourable alleles for the QTL identified in the present study came from male parent.

A common QTL (TA 72 - GSSR 41) for plant height at maturity was identified using linkage information generated by mapping 31 markers in *rabi* 2013 and pooled data. Both the QTLs were located on LG 1. The QTL detected in *rabi* 2013 had a LOD score of 3.45 and explained a variation of 15.35 per cent. The QTL detected in pooled data had LOD score of 3.80 with a R^2 value of 10.47 per cent.

Two QTLs (TS 72 - GSSR 50 and H5F021 - TA 110) for plant height at maturity were identified in *rabi* 2013 using linkage data generated from combined map. Both the QTLs were minor QTLs with R^2 value ranging from 8.47 to 14.52 per cent. Another QTL (GSSR 9 - GSSR 77) for the same trait was identified in pooled data of combined map. The QTL had additive gene action and favourable alleles came from male parent. Gowda *et al.* (2011) identified five QTLs for plant height. Two out of five identified QTLs were located on LG 1 other two on LG 2 and a single QTL on LG 4. The R^2 value ranged from 9.6 to 12.8 per cent which was almost same as that of QTLs identified in the present study. However, one (H_5F_{021} – TA 110) out of five QTLs identified for plant height in the present study was mapped on LG 2 rest all were mapped on LG 1. A single QTL for plant height was identified on LG 4 by Varsheny *et al.* (2014a). The QTL explained a variance of 10.0 per cent which was on par with the QTL identified in the present study by using linkage information

combined map. However, the QTL in the previous study was mapped to LG 4 against LG 2 in the present.

Five QTLs for days to 50 per cent flowering mapped on LG 1 were identified in *rabi* 2012 using linkage information generated from combined map. However, no QTLs for the trait were identified in map developed using 31 markers. In the present study in both maps no QTLs were identified for number of branches per plant, these results were contradictory with those obtained by Varsheny *et al.* (2014a) in their study identified two QTLs on linkage group 8 the QTLs explained a variance ranging from 10.51 to 26.87 per cent. In the present study five QTLs for plant height were detected. Three out of five QTLs detected were located on LG 2 and one QTL was mapped to LG 3. In present study Composite Interval Mapping using Windows QTL Cartographer Ver. 2.5 with a threshold LOD score of 3.0 scanning intervals of 2.0 cM between the markers was used for declaring presence of the QTLs this was in accordance with the earlier study (Gowda *et al.,* 2011).

5.4 Validation of molecular marker linked to of wilt resistance

DNA markers have enormous potential to improve the efficiency and the precision of conventional plant breeding via marker assisted selection (MAS) (Collard and Mackill 2008). Marker validation is referred to determining the target phenotype in independent populations and different genetic backgrounds (Cakir *et al.,* 2003). Although, MAS is very useful in crop improvement programmes, the large scale application has not been realized. The major problem is that most of the markers are not functional in different genetic backgrounds. Hence Validation of markers linked to trait of interest in diverse genetic background is essential for their effective utilization in marker assisted selection.

In the present study an attempt has been made to validate the ten identified markers linked to wilt resistance in a validation population consisting of ten RILs derived from a cross between JG 62 (early wilter) x WR 315 (resistant).

Out of ten markers only one marker (GSSR 21) was found polymorphic among parents (JG 62 and WR 315) of validating population. The polymorphic marker (GSSR 21) was screened on selected on extreme genotypes of validating population. All the five resistant RILs showed susceptible alleles for GSSR 21 that is the allele associated with susceptible parent JG 62. However, among the five resistant RILs only three showed allele associated with resistant parent the remaining two were as that of susceptible parent. Hence the marker was not validated. Kottapalli *et al.* (2009) in their study on mapping of Ascochyta blight resistance also observed that only one among 11 identified marker was validated in different genetic background.

In earlier study (Soregaon, 2011) an attempt was made to validate SSR markers (TA110, TA96, $H_1B_{06}y$ and TA194) identified by Gowda *et al.,* (2009) during genetic mapping of the *foc1*, *foc2* and *foc3* genes with previously unreported SSRs. However, these markers were not validated. In agreement with the earlier results the identified linked SSR markers for *FOC* 1 in the present study using combined mapping approach with 72 markers including 23 novel markers were not validated in different genetic background. In contradictory to present study the ASAP marker CS-27_{700} linked to H_1 locus has been validated in different genetic backgrounds (Ravikumar *et al.,* 2003; Brinda and Ravikumar, 2005).

The possible reason for non-validation of the identified marker linked to *Fusarium* wilt in the present study could be the absence of the close linkage between the marker and the QTL. The marker GSSR 21 was 56.91cM apart from the QTL (FW-Q-08-1). In chickpea, numbers of

polymorphic markers are limited (Ratnaparkhe *et al.,* 1998). Hence only low density molecular maps are available to identify and map the markers linked to resistance loci. Hence there is an urgent need to explore additional markers closely linked to the *Fusarium* wilt resistance genes/QTLs in different populations and their validation must be checked in genotypes of diverse genetic background to ensure the proper utilization in breeding programs.

5.5 Genetic variability studies for seed yield and yield components

The main purpose of the variability study in the present investigation is not to record the variability present in mapping population, instead to show that the mapping population has requisite variability for many quantitative traits under observation and is suitable for mapping. It may not have major implication in selection of plant improvement for yield parameters.

The coefficient of variation indicates only the extent of variability existing for various traits, but does not give any information about the heritable portion of it, as the estimates of heritability. As such the heritability in a broader perspective is the proportion of genotypic variability to the total variability. Heritability estimates in the broad sense alone is not a true indicator of effectiveness of selection for the trait since it is restricted by their interactions with the environment. Hence, heritability values considered along with predicted genetic gain increases the reliability of the parameter as a tool in selection programme.

Days to 50 per cent showed the moderate genetic advance mean (GAM) in 2012 *rabi* and low GAM in 2013 *rabi* with a high level of heritability (h^2) in both seasons similar kind of results were reports by

Burli *et al.* (2004), Bicer *et al.* (2009), Misra (1991), and Sharma *et al.* (2010).

Plant height showed high heritability coupled with high GAM in both seasons. Similar kind of results was reported by Ali *et al.* (2011). Moderate level of heritability coupled with low GAM was noticed for number of branches in 2012 *rabi* this was in accordance with findings of Arshad *et al.* (2002), Singh *et al.* (2002) and Ali *et al.* (2011). In 2013 *rabi* high heritability coupled with high GAM was noticed for the trait and this was in accordance with findings of Kampli *et al.* (2002).

For the character number of pods per plant, in 2012 it was observed that the heritability was moderate coupled with low level of GAM was observed, this was in agreement with earlier study (Saleem *et al.*, 2005). A high level of heritability coupled with high GAM was observed in 2013 *rabi*, similar kind of findings were reported by Dwevedi and Gabriell (2009), Sharma *et al.,* (2010). The trait 100 seed weight recorded high estimates of heritability along with moderate levels of GAM in both the seasons. This was in agreement with earlier studies by Misra (1991).

The trait seed yield per plant recorded a lower heritability with a moderate GAM levels in 2012 *rabi* which was in accordance with earlier studies (Arshad *et al.,* 2002; Sial*,* 2003; Misra 1991 and Vijayalakshmi *et al.* 2000). In second season moderate heritability coupled with higher genetic advance mean in 2013 *rabi* which was in accordance with (Wahid and Ahmed 1999; Sharma *et al.,* 2005 and Sharma *et al.* 2010).

The mean values of 141 RILs for seed yield per plant over two seasons (*rabi* 2012 and *rabi* 2013) was compared with the mean value of national check (JG 11) for respective seasons. Among 141 RILs 26 RILs in *rabi* 2012 and two in *rabi* 2013 were found superior over national

check (JG 11). However, two lines (RIL 93 and RIL 153) were found superior over national check for seed yield per plant across both seasons.

One out of two RILs (93 and 153) found superior for seed yield the RIL 93 was susceptible to wilt (92.10%) and the other line (RIL 153) was moderately resistant (29.37%) for wilt reaction. The RIL 153 proposed to be high yielding wilt resistant genotype. The superiority of the line has to be tested in large scale yield trials and wilt resistance in wilt sick plot.


SUMMARY

VI. SUMMARY

Chickpea (*Cicer arietinum* L.) is an economically important crop of India. Several biotic and abiotic factors limit its production in the country. Wilt caused by *Fusarium oxysporum* f.sp. *cicer* is one of the major limiting factors for chickpea production. It is difficult to control the disease as it is soil borne. Development of high yielding resistant chickpea varieties appears to be the most viable strategy to overcome this problem. Several features of patho system, as well as the development and maintenance of uniform wilt sick plots for selection of resistance hinder the development of resistant cultivars.

Till today, eight (0, 1A, 1 B/C, 2, 3, 4, 5 and 6) races of *Fusarium oxysporum* f.sp. *ciceri* have been reported. Among eight races, race 1A is the most prevailing in India, causing significant yield losses. Genetic inheritance of resistance to different races has been well established. Resistance to race 1 A of *Fusarium* wilt is governed by two major independent H_1 and H_2 loci. Molecular markers linked to H_1 locus (CS27700) and H_2 locus (A07C417) have been identified. The resistance genes to all identified races of *Fusarium* wilt have been identified and mapped except H_2 locus of race 1 A, race 1 B/C and race 6. The present study was formulated to map SSR markers with an attempt to develop combined linkage map as well as to identify QTLs influencing wilt resistance and productivity traits. The salient features of present work are as follows.

A set of 300 SSR markers were screened on two parental lines K 850 and WR 315 from which 141 RILs (segregating for late wilting alone) were derived. Among 300 SSRs, 31 markers were found consistently polymorphic. The 31 polymorphic markers were screened on the mapping population to develop partial linkage map. The map had

four linkage groups with coverage of 690.0 cM. Out of 31 markers 23 markers were mapped with a marker density of 30 cM. Among the 23 markers mapped 16 were novel and were not mapped in any of the earlier studies.

An attempt was made to develop a combined linkage map using 72 polymorphic markers which included 31 markers identified in the present study along with 41 markers identified in earlier study using same mapping population. The map had 64 markers mapped on six linkage groups with coverage of 1258.8 cM and marker density of 19.36 cM.

The RILs were evaluated for seed yield and yield component traits by growing them in AINP (All India Network Project on Acarology) field at UAS GKVK Bengaluru during 2012 and 2013 *rabi*. The observation on six trait *viz.,* days to 50 per cent flowering, plant height at maturity, number of branches per plant, number of pods per plant, 100 seed weight and seed yield per plant. The phenotypic data generated for wilt reaction over two seasons (2007*rabi* and 2008 *rabi*) was used for QTL mapping.

QTL mapping using composite interval mapping (CIM) identified a major QTL (FW-Q-07-1) for *Fusarium* wilt resistance in 2007 *rabi*. The QTL was common across both the map developed using 31 markers from the present study and combined map (using 72 markers) with a LOD score ranging from 8.73 to 8.90. The phenotypic variance contributed by the QTL ranged from 69.80 per cent to 71.41 per cent and the contribution of favourable allele came from susceptible (late wilting) parent, K 850. The QTL was located at position of 340.71 and 508.91 cM, was flanked between markers (GSSR 18 - TC 14801 and GSSR 41 - NCPGR 33) on LG 1. Hence this is considered as s stable QTL for wilt resistance. The major QTL (FW-Q-07-1) identified in the present

study for *FOC* 1 resistance was stable QTL and explained a higher phenotypic variation for *Fusarium* wilt resistance when compared with earlier studies wherein a maximum of 18..8 per cent of variance for the trait was observed.

A total of five QTL's were identified using linkage information from map developed with 31 markers. Two out of five QTL's identified were in *rabi* 2007 and three were for *rabi* 2008. Two QTL's were detected by using linkage information from combined map developed using 72 markers. Among the two QTL's detected, a QTL (GSSR-41-NCPGR 33) was identified in *rabi* 2007 and the other QTL (TR 24-GSSR-21) was identified in *rabi* 2008.

QTL mapping for yield and yield component traits using linkage information from 31 markers along with two season morphological data revealed a total of nine QTLs, two QTLs (TA 186 - GSSR 50 and GSSR 50 - TA 72) in 2012 *rabi* and three QTLs in 2013 *rabi*, two out of three QTLs were flanked by markers (TA 72 - GSSR 41), the other was flanked between markers (GSSR 50 - TA 72). Two out of four QTLs, one each for 100 seed weight (GSSR 50 - TA 72) and plant height (TA 72 - GSSR 41) detected in pooled season were also found in 2013 *rabi*.

A total of nine QTL's for three different traits *viz.*, seed yield per plant, plant height at maturity and 100 seed weight were identified using linkage information from map developed by 31 markers. Two QTL's (TA 186- GSSR 50 and GSSR 50- TA 72) for seed yield were identified in *rabi* 2012. The QTL's explained a variance of 8.12 to 26.18 per cent. In *rabi* 2013 one QTL (TA 72-GSSR 41) for plant height at maturity and two QTL's (GSSR 50- TA 72 and TA 72- GSSR-41) for seed yield were identified. The QTL for plant height at maturity explained a variance of 15.3 per cent, with contribution of favourable alleles from male parent.

Among four QTL's identified in pooled data over seasons, one QTL each for plant height at maturity (TA 72- GSSR 41), for seed yield per plant (GSSR 50-TA 72) and two QTL's for 100 seed weight were identified. The QTL's for 100 seed weight explained a variance ranging from 18.78 to 24.38 per cent.

QTL mapping for yield and yield component traits using linkage information obtained from combined map and two season data on yield and yield attributing traits identified a total of 28 QTLs (19 QTLs in 2012 *rabi*, five in 2013 *rabi* and four QTLs in pooled data set over both seasons).

Among 19 QTLs detected in 2012 *rabi* a major QTL (TR 24 - GSSR 108) explaining a variance of 36.21 per cent for 100 seed weight was detected. The QTL (TR 24 - GSSR 108) was located on LG 1 and had a LOD score of 9.28 with additive gene effect and favourable alleles came from female parent (K 850). One QTL (NCPGR 93 - GSSR 9) for 100 seed weight out of five QTLs detected in 2013 *rabi* by combined mapping approach was a major QTL. The QTL explained a phenotypic variation of 26.11 per cent for the trait and it was located on linkage group 1 at a position of 278.31 cM.

A total of two QTL's (TS 72-GSSR 50 and GSSR 9-GSSR-77) for plant height at maturity were detected using linkage information from combined map. The QTL (TS 72-GSSR 50) was identified in *rabi* 2013 and explained a variance of 14.52 per cent with LOD score of 4.19.The favourable alleles for the QTL were contributed from the male parent (WR 315). However, no QTL's for plant height at maturity were identified in *rabi* 2012. A major QTL (GSSR 9 - GSSR 77) for plant height explaining a variance of 17.68 per cent for plant height at maturity was detected in pooled data set combined over seasons.

Five QTL's for days to 50 per cent flowering were identified in combined linkage map. The QTL's explained a variance ranging from 1.05 – 8.20 per cent with a contribution of favourable alleles from male parent for all except for QTL (GSSR 41-NCPGR 33), which had the contribution of favourable alleles from female parent. Only one QTL (TR 56-TR-40) was identified for number of pods per plant in pooled data using combined map. The QTL explained a variance of 4.87 per cent for the trait.

Analysis of variance for productive traits over two seasons indicated significant variation among the RILs for all six traits. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters in both the seasons indicating the presence of environmental effect. The frequency distribution of RILs for wilt and all quantitative traits except for number of pods per plant was normal. The frequency distribution for pod number skewed towards male parent WR 315.

Ten markers linked to *Fusarium* wilt resistance QTLs were identified. All the ten linked markers were validated using extreme genotypes of another cross derived from a cross between JG 62 (susceptible early wilter) x WR 315 (resistant). Nine out of ten markers identified were monomorphic for JG 62 and WR 315 parental lines. A marker GSSR 21 linked with a QTL (FW-Q-08-1) at a distance of 56.91cM was polymorphic among parental lines but failed to validate indicating the need for requirement of high density maps developed using different genetic backgrounds in chickpea.

Future line of work

1. Development of new molecular markers like SNPs using sequence information generated from chickpea whole genome sequence.
2. Development of high density maps using novel markers.
3. Integration of current map with already published maps through consensus approach.
4. The QTLs identified for yield and yield attributing traits upon validation can be made use in future crop improvement programme.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