

Identification of QTLs for resistance to *Fusarium* wilt and *Ascochyta* blight in a recombinant inbred population of chickpea (*Cicer arietinum* L.)

Tosh Garg · B. P. Mallikarjuna · Mahendar Thudi · Srinivasan Samineni · Sarvjeet Singh · J. S. Sandhu · Livinder Kaur · Inderjit Singh · Asmita Sirari · Ashwani K. Basandrai · Daisy Basandrai · Rajeev K. Varshney · Pooran M. Gaur

Received: 7 September 2017 / Accepted: 29 January 2018 / Published online: 5 February 2018
© Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract *Fusarium* wilt (FW; caused by *Fusarium oxysporum* f. sp. *ciceris*) and *Ascochyta* blight (AB; caused by *Ascochyta rabiei*) are two major biotic stresses that cause significant yield losses in chickpea (*Cicer arietinum* L.). In order to identify the genomic regions responsible for resistance to FW and AB, 188 recombinant inbred lines derived from a cross JG 62 × ICCV 05530 were phenotyped for reaction to FW and AB under both controlled environment and field conditions. Significant variation in response to FW and AB was detected at all the locations. A genetic map comprising of 111 markers including 84 simple sequence repeats and 27 single nucleotide polymorphism (SNP) loci spanning 261.60 cM was constructed. Five quantitative trait loci (QTLs) were detected for resistance to FW with phenotypic

variance explained from 6.63 to 31.55%. Of the five QTLs, three QTLs including a major QTL on CaLG02 and a minor QTL each on CaLG04 and CaLG06 were identified for resistance to race 1 of FW. For race 3, a major QTL each on CaLG02 and CaLG04 were identified. In the case of AB, one QTL for seedling resistance (SR) against ‘Hisar race’ and a minor QTL each for SR and adult plant resistance against isolate 8 of race 6 (3968) were identified. The QTLs and linked markers identified in this study can be utilized for enhancing the FW and AB resistance in elite cultivars using marker-assisted backcrossing.

Keywords *Ascochyta* blight · *Fusarium* wilt · Simple sequence repeat · Single nucleotide polymorphism

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10681-018-2125-3>) contains supplementary material, which is available to authorized users.

T. Garg · S. Singh · L. Kaur · I. Singh · A. Sirari
Department of Plant Breeding & Genetics, Punjab
Agricultural University, Ludhiana, India

T. Garg · B. P. Mallikarjuna · M. Thudi ·
S. Samineni · R. K. Varshney · P. M. Gaur
International Crops Research Institute for the Semi-Arid
Tropics (ICRISAT), Patancheru, India

J. S. Sandhu
Indian Council of Agricultural Research (ICAR), Krishi
Bhawan, New Delhi, India

A. K. Basandrai · D. Basandrai
Chaudhary Sarwan Kumar Himachal Pradesh Krishi
Vishvavidyalaya (CSKHPKV), Regional Station,
Dhaulakuan, India

P. M. Gaur (✉)
The UWA Institute of Agriculture, University of Western
Australia, Perth, Australia
e-mail: p.gaur@cgiar.org

Introduction

Chickpea (*Cicer arietinum* L. $2n = 2x = 16$) is the second most important food legume of the world which is grown in more than 54 countries. It is a self-pollinated cool season crop with an estimated genome size of 738 Mbp (Varshney et al. 2013). Globally, chickpea is cultivated on 13.9 million ha with a production of 13.6 million tonnes (FAO 2016). The major chickpea producing countries are India, Australia, Pakistan, Turkey, Myanmar, Iran, Ethiopia, Canada and USA. It serves as a major source of protein to predominantly the vegetarian population in the Indian Sub-continent. In addition, chickpea seeds contain important minerals (phosphorus, calcium, magnesium, iron and zinc), fibre and are free from anti-nutritive components (Jukanti et al. 2012).

The crop is grown under diverse ecological conditions and thus gets exposed to various biotic and abiotic stresses (Gaur et al. 2012a). Among the biotic stresses, chickpea production is largely constrained by Fusarium wilt (FW caused by *Fusarium oxysporum* f. sp. *ciceris*) and Ascochyta blight (AB caused by *Ascochyta rabiei*) in many chickpea growing areas. For instance, FW is the most destructive root disease in warm and drier areas and can cause yield loss ranging from 10 to 40% and sometimes up to 100% under specific conditions (Sharma et al. 2014; Li et al. 2015). FW has emerged as a major threat in recent times due to large shift in chickpea cultivation from cool long season environments (Northern India) to warm and short season environments (Central and Southern India) (Sharma and Pande 2013). In addition, the scenario of changing climatic conditions in the form of increased temperature and erratic rainfalls especially in semi-arid regions has enhanced the occurrence of FW (Sharma and Pande 2013). On the other hand, AB is recognized as a potentially important disease under cool, humid weather conditions and capable of causing complete yield losses under favourable conditions (Pande et al. 2005; Sharma and Ghosh 2016). Therefore, breeding for disease resistant cultivars is the most efficient method to reduce yield losses. For this, identification of quantitative trait loci (QTLs)/genes responsible for disease resistance is an important pre-requisite for undertaking marker assisted breeding (Sharma and Ghosh 2016).

In past, genomic resources have been deployed in chickpea to locate and tag the molecular markers linked to disease resistance (Gaur et al. 2012b, 2014). For FW, eight races (races 0, 1A, 1B/C, 2–6) have been identified and the genetics of resistance to six races has been reported as monogenic, digenic or quantitative (Singh et al. 1987; Kumar 1998; Tullu et al. 1999; Rubio et al. 2003; Tekeoglu et al. 2000; Sharma et al. 2004, 2005). Genetic control of resistance to races 1B/C and 6 is not reported so far. The genes/QTLs for resistance to six races (0, 1A, 2–5) of FW pathogen have been mapped on to the chickpea genetic map (Li et al. 2015). In case of AB, different models of inheritance from qualitative to quantitative resistance have been reported depending on the genotypes tested, screening method and fungal isolates (Kaur and Singh 2009; Bhardwaj et al. 2010) and also QTLs have been identified in different genomic regions (Sharma and Ghosh 2016). However, most of these studies used different mapping populations to identify QTLs for different diseases. In the present study, we constructed a genetic map using simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers in a recombinant inbred population (RIL) segregating for both FW and AB. This provides a unique opportunity to identify the genomic regions controlling resistance to FW and AB in a single population.

Materials and methods

Mapping population

The mapping population used in this study comprised of 188 RILs (F_8) developed from a cross between JG 62 and ICCV 05530 at ICRISAT, Patancheru. The variety JG 62 is highly susceptible to FW (all races except for race 0, Jiménez-Díaz et al. 2011) and AB (Tewari and Pandey 1986), while ICCV 05530 is an advanced breeding line highly resistant to FW and moderately resistant to AB (Pande et al. 2007).

Phenotyping of RILs for FW resistance

The RIL population was evaluated during 2011–2012 in a wilt-sick plot with race 1 (at ICRISAT, Patancheru) and race 3 [at Punjab Agricultural University (PAU), Ludhiana] of FW in a randomized

complete block design with two replications. The plot size consisted of a single row of 2 m length at ICRISAT, Patancheru and 4 m at PAU, Ludhiana. JG 62 was used as susceptible check for FW at both the locations. Observations for FW severity were recorded at 45 days after sowing and then at monthly intervals till maturity. The wilt incidence was measured using the formula (% wilt incidence = (number of wilted plants/total number of plants) × 100). Lines showing up to 50% wilt incidence were considered as resistant, whereas those with more than 50% wilt incidence as susceptible.

Phenotyping of RILs for AB resistance

Seedling resistance (SR)

For testing of SR, cloth chamber screening technique as described by Gurha et al. (2003) was used at PAU, Ludhiana during 2011–2012. Seeds of both parental lines and RIL population were sown in two replications along with highly susceptible varieties C 214 and L 550 as indicator-cum-infector plants. Ten days old seedlings were inoculated by spraying spore suspension of isolate 8 of race 6 (3968) of *A. rabiei* at 4×10^4 spores ml⁻¹ (Singh 1990). The disease symptoms started appearing 7 days after inoculation. Observations were recorded 13 days after inoculation on 1–9 scale as described by Singh and Sharma (1998).

The RIL population was also screened for SR at ICRISAT, Patancheru during 2011–2012. Seedlings of the parental lines and RIL population along with the susceptible check ICC 4991 were raised under controlled environmental conditions of 25 ± 3 °C temperature with 12–13 h photoperiods. Pathogen inoculum of ‘Hisar race’ of *A. rabiei* (Sharma et al. 2010) was sprayed on to the foliage of 10 days old seedlings. Disease severity was scored 10 days after inoculation on a 1–9 disease scale (Singh and Sharma 1998). The lines with disease score ≤ 5 were considered as resistant and above 5 as susceptible.

Adult plant resistance (APR)

For APR, field screening technique as described by Gurha et al. (2003) was used to develop the disease and evaluation of population for disease reaction at PAU, Ludhiana. During 2011–2012, the RIL population along with susceptible checks L 550 and C 214

were planted in two replications keeping 2 m row length and 40 cm row to row space. The population was artificially inoculated by spraying the isolate 8 of race 3968 of pathogen at 4×10^4 spores ml⁻¹ (Singh 1990) during the flowering stage of the crop. The epiphytotic conditions were created with the help of perfo-sprayer system to maintain the relative humidity beyond 85% and temperature around 25 °C. The disease symptoms started appearing after 10–15 days of inoculation.

At Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Hill Agricultural Research and Extension Centre, Dhaulakuan, India (a hot spot for AB) the RILs were planted with two replications in 1.5 m row length having 30×10 cm² spacing during 2011–2012. The plots were inoculated at the flowering stage of the crop by frequently spraying of conidial inoculum of pathotype I isolate AB 4 (10^6 conidia ml⁻¹). AB infected debris were also broadcasted in each plot along with the first spray for uniform development of the disease and to prevent disease escape. Observations were recorded on 1–9 rating scale, where 1 is highly resistant and 9 is highly susceptible (Singh and Sharma 1998). The lines with disease score ≤ 5 were considered as resistant and above 5 as susceptible.

The mean data over the replicates for each disease were used to compute the best linear unbiased predictors (BLUPs) of the random effect in restricted maximum likelihood (REML) variance components analysis using Genestat version 14.0, with genotypes as random effects and replicates as fixed models. The variance components were estimated using WIN-DOWSTAT software. BLUP values were used for QTL mapping.

DNA isolation and genotyping

Total genomic DNA from young leaves of parental lines and 188 RILs was isolated during the crop season 2011–2012 by following a high-throughput mini DNA extraction protocol (Mace et al. 2003). The parental polymorphism was studied using a total of 450 previously published SSR markers evenly distributed in the genome (Winter et al. 1999; Lichtenzveig et al. 2005; Huttel et al. 1999; Nayak et al. 2010; Thudi et al. 2011). SSR marker genotyping including PCR amplification, separation, visualization and analysis of amplified products were carried out by following the

method as described by Nayak et al. (2010). In addition, 32 SNP markers (CKAM-series) were genotyped as described in Hiremath et al. (2012). The polymorphic markers were used to genotype on the RIL population.

Genetic map construction and QTL analysis

The genotyping data generated in this study was used for linkage analysis using JoinMap v 4.1 (Van Ooijen 2006). Segregation ratios for both alleles (1:1) of SSR markers were tested for goodness of fit to assess significant deviations from the expected Mendelian segregation. Only the markers showing goodness of fit were used for map construction. The markers were grouped into linkage groups (LGs) using the independence LOD parameter with $\text{LOD} \geq 2.0$. Markers were placed into different LGs with “LOD groupings” and “Create group using the mapping tree” parameters. Regression mapping algorithm was used to order the loci in each LG using recombination frequency ≤ 0.40 , LOD threshold > 1.0 , value of 5 for the jump and ripple value of 1. Recombination frequencies were converted into map distances using Kosambi mapping function (1994).

For QTL analysis, datasets from two sick plot experiments were used for FW and four datasets (two each for SR and APR) were used for AB. QTL analysis was done using composite interval mapping in QTL-IciMapping software version 4.0 (Wang et al. 2014). A stepwise regression was performed to identify most significant markers and marker-pair multiplications at 0.001 probability level at the scanning step of 1 cM. The genome-wide threshold LOD scores for detection of QTL were calculated based on 1000 permutations at $P \leq 0.05$. QTL with LOD scores above the threshold LOD were considered as the significant QTL. The additive effect and proportion of phenotypic variance

(PVE%) explained by each QTL were estimated at the peak LOD score. The final image of linkage map and QTLs were generated with map option in ICIM software.

Results

Phenotypic variation of parental lines and RILs for reaction to FW and AB

Under field conditions at Patancheru, JG 62 was found completely susceptible to FW (i.e., 100% FW incidence) while at Ludhiana 89% FW incidence was recorded. Nevertheless, ICCV 05530 exhibited resistance against FW with mean disease incidence of almost 0 at both the locations (Table 1; Fig. 1). In the case of AB, an average disease score of 9 and 6.3 was recorded for JG 62 at Patancheru and Ludhiana locations, respectively for SR. For APR, an average disease score of 7.5 and 6.8 was recorded at Dhaulakuan and Ludhiana, respectively. Whereas, ICCV 05530 exhibited an average score of 4 and 3.5 for SR (at Patancheru and Ludhiana) and disease score of 2.5 and 3 for APR (at Dhaulakuan and Ludhiana) indicating resistant reaction. Analysis of variance indicated a considerable variation among the RILs for reaction to FW and AB as indicated by significant calculated F value at 0.1% level of significance (Table 2). A high broad sense heritability of 0.96 was estimated for APR of FW at Patancheru and Ludhiana. For AB also, a high broad sense heritability of 0.85 and 0.82 were recorded for SR at Patancheru and Ludhiana, respectively. For APR, a high broad sense heritability was recorded at Dhaulakuan (0.94) and Ludhiana (0.83). The frequency distribution of the RILs for FW incidence and AB disease scores based on mean BLUP values is illustrated in Figs. 1 and 2.

Table 1 Reaction of parents and RIL population for Fusarium wilt and Ascochyta blight disease

	Fusarium wilt (%)		Ascochyta blight (score)			
	Adult plant resistance		Seedling resistance		Adult plant resistance	
	Patancheru	Ludhiana	Patancheru	Ludhiana	Dhaulakuan	Ludhiana
JG 62	100	89	9	6.3	7.5	6.8
ICCV 05530	0	0	4	3.5	2.5	3
RIL mean	71	35	8	5	5	5
Range	0–100	0–96	5–9	0–8.5	0–8.5	2–9
SE	5.1	3.6	0.09	0.57	0.36	0.57

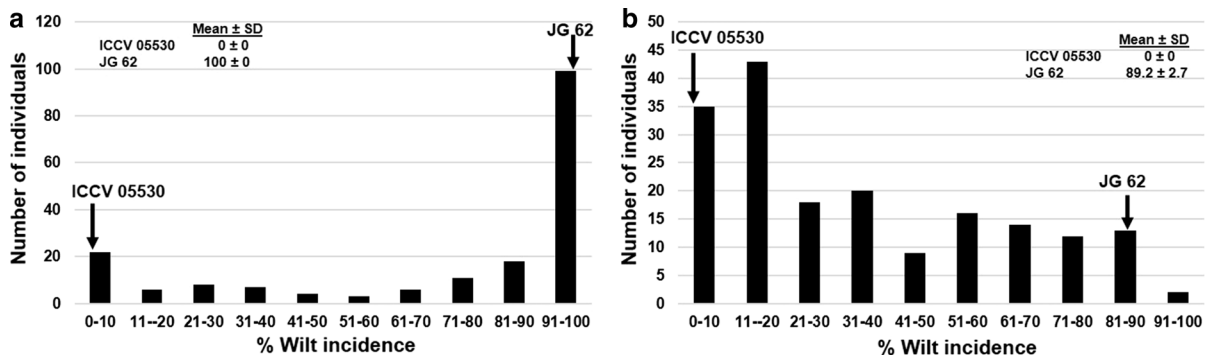


Fig. 1 Frequency distribution of RILs in response to Fusarium wilt incidence against **a** race 1 at Patancheru and **b** race 3 at Ludhiana. The susceptible parent JG 62 showed complete wilting, while ICCV 05530 exhibited resistant reaction at both the locations

Table 2 Analysis of variance of RIL population for reaction against Fusarium wilt and Ascochyta blight diseases

Source of variation	Fusarium wilt		Ascochyta blight			
	Adult plant resistance		Seedling resistance		Adult plant resistance	
	Patancheru	Ludhiana	Patancheru	Ludhiana	Dhaulakuan	Ludhiana
Genotype	2704.88*** ⁽¹⁸⁷⁾	1504.35*** ⁽¹⁸⁷⁾	5.48*** ⁽¹⁸⁷⁾	6.63*** ⁽¹⁸⁷⁾	8.63** ⁽¹⁸⁷⁾	7.3*** ⁽¹⁸⁷⁾
Error	52.62	25.97	0.29	0.64	0.26	0.65
Heritability (BS)	0.96	0.96	0.85	0.82	0.94	0.83
CV	10.26	14.63	7.0	16.46	10.30	14.96

Numbers in parenthesis denote the degrees of freedom

Significant at $P = 0.01$, *significant at $P = 0.001$

Majority of the RILs ($n = 137$) exhibited susceptible reaction ($> 50\%$ disease incidence) against race 1 of FW at Patancheru. However at Ludhiana, RIL population was continuously distributed for FW disease incidence with majority of RILs ($n = 125$) exhibited less disease incidence ($< 50\%$) against race 3 of FW. For SR of AB at Patancheru, majority of RILs exhibited susceptible reaction (5.1–9.0), but only few RILs showed resistance reaction (< 5.0). While at Ludhiana, RIL population exhibited continuous distribution for reaction to AB in the range of 1–9. For APR at Dhaulakuan, majority of RILs were classified within the class 3.1–7.0. While at Ludhiana, continuous distribution was observed among the RILs (2.1–9.0) for APR of AB.

Genetic map construction

On screening 450 SSR markers, we identified 101 polymorphic markers and genotyped on the RIL population. In addition, 32 SNP markers were also

genotyped and used for genetic map construction. Out of 133 polymorphic markers used, a total of 111 markers were mapped on eight LGs (CaLGs) covering a total genetic length of 261.60 cM (Supplementary Table 1; Supplementary Fig. 1). Twenty three markers remained unlinked. The map length of eight LGs ranged from 6.94 cM (CaLG03) to 66.46 cM (CaLG06) with an average of 32.7 cM per LG. The inter-marker distance ranged from 0.69 cM (CaLG03) to 5.01 cM (CaLG07) with an average marker density of 2.36 cM per LG. Based on the common markers, eight LGs in the present map were assigned to corresponding LGs of chickpea as per Varshney et al. (2014).

QTLs for Fusarium wilt resistance

A major QTL *FW-Q-APR-2-1* was identified on CaLG02 for FW resistance against race 1 at Patancheru (Table 3; Fig. 3). This QTL explained 24.56% phenotypic variance with LOD score of 18.54 and

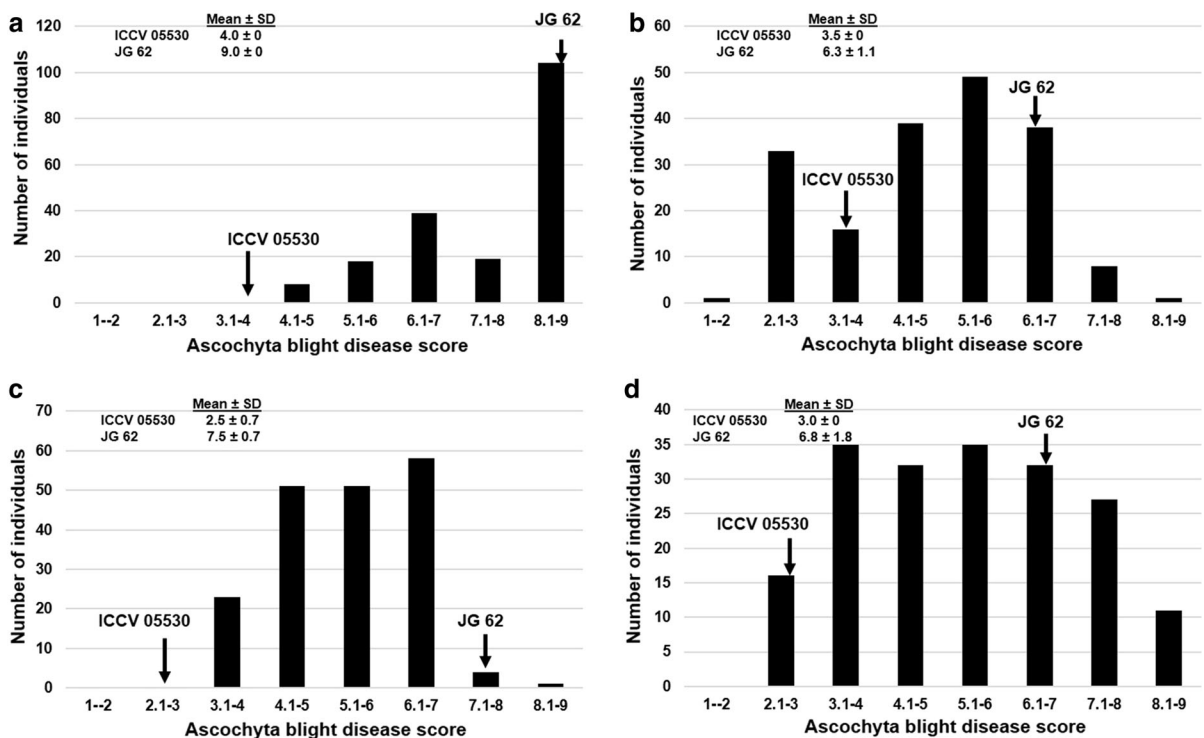


Fig. 2 Frequency distribution of RILs for seedling resistance (SR) against Ascochyta blight at **a** Patancheru and **b** Ludhiana and for adult plant resistance (APR) at **c** Dhaulakuan and

d Ludhiana. The parent JG 62 showed susceptible reaction to both SR and APR, while ICCV 05530 exhibited resistant reaction in all the locations

flanked by the markers TR19 and H2B061. In addition, two minor QTLs, i.e., *FW-Q-APR-4-1* (LOD = 5.6; PVE = 7.94%) and *FW-Q-APR-6-1* (LOD = 4.38; PVE = 6.63%) were identified on CaLG04 and CaLG06, respectively. The QTL *FW-Q-APR-4-1* was flanked by the markers TA132 and TA46 (Fig. 4). Whereas the QTL *FW-Q-APR-6-1* was flanked by TA80 and CaM0594 (Supplementary Fig. 2). In case of screening RILs in wilt-sick plot for FW resistance against race 3 at Ludhiana, two major QTLs viz., *FW-Q-APR-2-1* and *FW-Q-APR-4-1* were identified on CaLG02 and CaLG04, respectively (Figs. 3, 4). The QTL *FW-Q-APR-2-1* was flanked by the markers TR19 and H2B061 explaining 17.53% phenotypic variance with LOD value of 12.31. Another QTL (*FW-Q-APR-4-1*) explaining 31.55% phenotypic variance with LOD score of 21.02 was flanked by the markers CKAM1256 and TS72.

QTLs for Ascochyta blight resistance

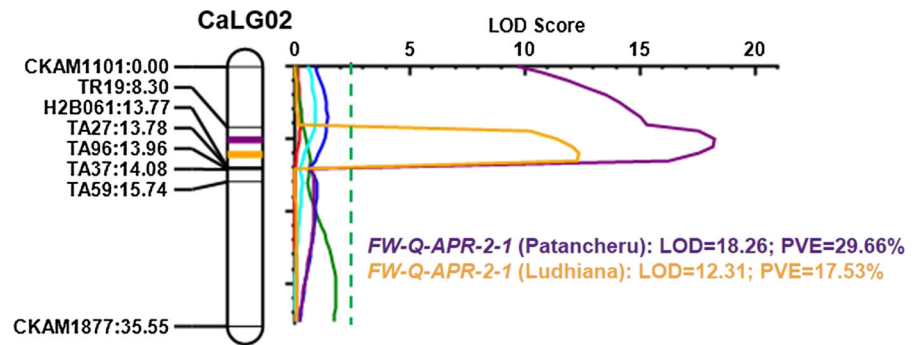
QTL analysis was conducted for data obtained at seedling and adult plant stage at different locations. For SR, a single minor QTL (*AB-Q-SR-4-1*) on CaLG04 flanked by markers ICCM0068 and CaM1158 was identified for Hisar race of AB at Patancheru (Table 3; Fig. 4). This QTL explained 6.44% phenotypic variance with LOD score of 2.73. Similarly, a minor QTL (*AB-Q-SR-4-1*; LOD = 2.76; PVE = 6.76%) flanked by the markers CKAM0847 and CKAM0964 was identified for SR of AB at Ludhiana. For APR against isolate 8 of race 6 (3968) of AB at Ludhiana, single minor QTL, i.e., *AB-Q-APR-4-1* was detected on CaLG04 with LOD value of 2.88 and explained phenotypic variation of 6.98% (Fig. 4). The QTL *AB-Q-APR-4-1* was flanked by the markers CKAM0847 and CKAM0964. No QTLs were identified for APR of AB at Dhaulakuan.

Table 3 List of QTLs identified for Fusarium wilt and Ascochyta blight resistance in the cross JG 62 × ICCV 05530

Diseases	Location	QTL	CaLG	Position (cM)	LOD	Additive effect	PVE (%)	Flanking markers	
								Left marker	Right marker
Fusarium wilt	APR at Patancheru	<i>FW-Q-APR-2-1</i>	02	10	18.54	27.4	24.56	TR19	H2B061
		<i>FW-Q-APR-4-1</i>	04	16	5.6	15.56	7.94	TA132	TA46
		<i>FW-Q-APR-6-1</i>	06	37	4.38	9.79	6.63	TA80	CaM0594
	APR at Ludhiana	<i>FW-Q-APR-2-1</i>	02	12	12.31	12.02	17.53	TR19	H2B061
		<i>FW-Q-APR-4-1</i>	04	9	21.02	16.19	31.55	CKAM1256	TS72
Ascochyta blight	SR at Patancheru	<i>AB-Q-SR-4-1</i>	04	4	2.73	0.34	6.44	ICCM0068	CaM1158
	SR at Ludhiana	<i>AB-Q-SR-4-1</i>	04	8	2.73	0.41	6.76	CKAM0847	CKAM0964
	APR at Ludhiana	<i>AB-Q-APR-4-1</i>	04	8	2.88	0.48	6.98	CKAM0847	CKAM0964

APR adult plant resistance, SR seedling resistance

Fig. 3 QTLs identified on CaLG02 of the cross JG 62 × ICCV 05530. Two major QTLs were identified for resistance to Fusarium wilt between common markers TR19 and H2B061



Discussion

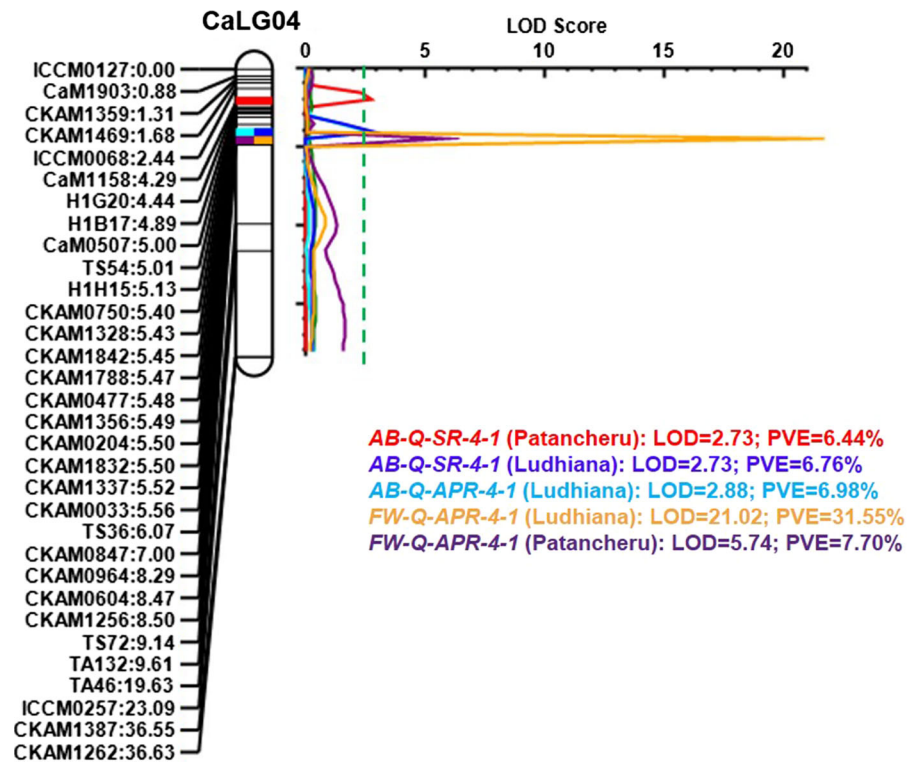
FW and AB are two major constraints for chickpea production causing significant yield losses globally. Developing resistant varieties is the most promising strategy to manage these diseases, especially for smallholding farmers across semi-arid tropics. However, effective use of this strategy requires identification of QTLs and molecular markers linked to disease resistance. In this context, the present study was conducted with an objective to identify the genomic regions controlling FW and AB resistance in a multiple disease resistant donor of chickpea, which could then be used for developing improved chickpea cultivars with enhanced resistance to both the diseases.

Phenotyping of RILs under controlled and field conditions at different locations indicated a wide range of variation among the lines for their reaction to FW and AB. The parent JG 62 showed 89–100% wilt incidence against races 1 and 3 of FW. While, ICCV 05530 maintained its resistance against both the FW

racers. The disease score of > 6.0 (SR and APR) of susceptible parent JG 62 and a score of < 4.0 (SR and APR) of resistant parent ICCV 05530 indicated that JG 62 was susceptible, while ICCV 05530 was found resistant to AB at seedling and adult plant stages. A high range of variation among the RILs grown under controlled and field conditions was observed and similar kind of variation was earlier reported for reaction to FW (Sabbavarapu et al. 2013) and AB (Kottapalli et al. 2009; Sabbavarapu et al. 2013). In this study, a high heritability estimates for FW (0.96) and AB (0.82–0.94) resistance were detected. Presence of high heritability indicates higher influence of genetic variability than environmental variability. This suggests that selection could be effective to improve these traits under respective environments.

For FW, a major QTL *FW-Q-APR-2-1* was identified on CaLG02 for race 1 at Patancheru. In addition, minor QTLs were identified on CaLG04 (*FW-Q-APR-4-1*) and CaLG06 (*FW-Q-APR-6-1*). For race 3 of FW at Ludhiana, a major QTL was identified each on

Fig. 4 QTLs identified on CaLG04 of the cross JG 62 × ICCV 05530. A major and minor QTL was identified for resistance to Fusarium wilt. For Ascochyta blight, two minor QTLs for seedling resistance and a minor QTL for adult plant resistance were identified



CaLG02 (*FW-Q-APR-2-1*) and CaLG04 (*FW-Q-APR-4-1*) (Table 3; Figs. 3, 4). The major QTLs identified on CaLG02 for races 1 and 3 had common flanking markers (TR19 and H2B061) indicating that same genomic regions could be controlling resistance for these two races. Previously, Gowda et al. (2009) also reported resistance genes *foc1* and *foc3* on LG02 supporting the idea that a cluster of FW resistance genes may exist in this genomic region. The common flanking marker TR19 identified on CaLG02 was previously reported to be located in the same genomic region having a group of markers (TA200, TS47, TA59, TA27, TA96 and TA37) linked to other wilt resistance genes *foc1A*, *foc2*, *foc3*, *foc4* and *foc5* (Winter et al. 2000; Sharma et al. 2004; Sharma and Muehlbauer 2007; Cobos et al. 2009; Halila et al. 2009; Barman et al. 2014). These cluster of markers were physically located within 15.45–27.18 Mb region on the pseudomolecule 2 of the chickpea reference genome (Supplementary Table 2). Recently, Sabbavarapu et al. (2013) reported two major QTLs on CaLG06 for race 1 of FW involving WR 315 as resistant parent. In this study also a minor QTL for race 1 was identified on CaLG06 but linked with different markers. Further, a major QTL identified on

CaLG04 for race 3 and a minor QTL for race 1 could be considered novel as the QTLs for FW resistance on LG04 were not reported so far (Jendoubi et al. 2017). This could be due to a new resistant parent ICCV 05330 used in this study as compared to some earlier studies on QTL mapping where WR 315 was predominantly used as resistant parent (Sharma et al. 2004; Gowda et al. 2009; Sabbavarapu et al. 2013). The physical position of the linked markers was located on Chromosome 4 of chickpea genome (Supplementary Table 2). This helps in identification of candidate genes responsible for FW resistance.

For seedling resistance of AB, a minor QTL was identified on CaLG04 for ‘Hisar race’ at Patancheru. Also, a minor QTL was detected on CaLG04 for SR at Ludhiana. Previously, two minor QTLs on LG04 for resistance to ‘Hisar race’ at seedling stage were identified by Kottapalli et al. (2009) but were linked to different set of markers. However, markers linked to QTLs in these studies were physically located on Chromosome 4 of the reference genome. For APR at Ludhiana, a minor QTL was detected on CaLG04. The physical position of indicative markers was located on Ca4. Earlier, Tar’an et al. (2007) reported one major QTL on LG4 and a minor QTL on LG6 for APR of

AB. Also, Sabbavarapu et al. (2013) reported a minor QTL on LG6 but linked to different markers. Some of the indicative markers from these studies were physically located in the chickpea genome and some were not located because of no hits in the reference genome due to short primer sequences (Supplementary Table 2). Several other studies have identified QTLs for resistance to AB on LG01 (Santra et al. 2000), LG02 (Cobos et al. 2006), LG03 (Tar'an et al. 2007), LG04 (Tekeoglu et al. 2002; Flandez-Galvez et al. 2003; Udupa and Baum 2003) and LG06 (Santra et al. 2000; Cho et al. 2004) with higher phenotypic variation compared to the present study. The genetic background, experimental conditions and the methodology of QTL analysis employed in previous studies may have resulted in identifying QTLs with major effect. However, we could not detect any QTLs in our population linked to APR of AB at Dhaulakuan. The reason may be due to differences in experimental conditions, virulence properties of the pathogen that may not have permitted detection of QTL. Also, a less number of markers available for genetic mapping, which clearly demands inclusion of more number of polymorphic markers like SSRs and SNPs to map these resistant genes.

In summary, FW and AB had moderate to high heritability and phenotypic selection should be effective for these traits in conventional breeding programme. However, given the difficulty of disease screening methods and environmental factors affecting resistance reactions, modern breeding strategy involving marker-assisted selection for resistance could be a desirable approach. In this study, three major QTLs and one minor QTL (including novel QTLs on CaLG04) for resistance to FW and three minor QTLs (on CaLG04) for resistance to AB were identified. The physical location of indicative markers linked to QTLs will facilitate discovery of candidate resistance genes and development of molecular markers for improving resistance to FW and AB.

References

- Barman P, Handique AK, Tanti B (2014) Tagging STMS markers to *Fusarium wilt* race-1 resistance in chickpea (*Cicer arietinum* L.). Indian J. Biotechnol 13:370–375
- Bhardwaj R, Sandhu JS, Livinder Kaur, Gupta SK, Gaur PM, Varshney RK (2010) Genetics of Ascochyta blight resistance in chickpea. Euphytica 171:337–343
- Cho S, Chen W, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to Ascochyta blight. Theor Appl Genet 109:733–739
- Cobos MJ, Rubio J, Strange RN, Moreno MT, Gil J, Millán T (2006) A new QTL for Ascochyta blight resistance in an RIL population derived from an interspecific cross in chickpea. Euphytica 149:105–111
- Cobos MJ, Winter P, Kharrat M, Cubero JI, Gil J, Millán T, Rubio J (2009) Genetic analysis of agronomic traits in a wide cross of chickpea. Field Crop Res 111:130–136
- FAO (2016) FAOSTAT data. <http://faostat.fao.org/>
- Flandez-Galvez H, Ades PK, Ford R, Pang ECK, Taylor PWJ (2003) QTL analysis for Ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.). Theor Appl Genet 107:1257–1265
- Gaur PM, Jukanti AK, Srinivasan S, Gowda CLL (2012a) Chickpea (*Cicer arietinum* L.). In: Bharadwaj DN (ed) Breeding of field crops. Agrobios (India), Jodhpur, pp 165–194
- Gaur PM, Jukanti AK, Varshney RK (2012b) Impact of genomic technologies on chickpea breeding strategies. Agronomy 2:199–221
- Gaur PM, Thudi M, Srinivasan S, Varshney RK (2014) Advances in chickpea genomics. In: Gupta S, Nadarajan N, Gupta DS (eds) Legumes in the omic era. Springer, New York, pp 73–94
- Gowda SJM, Radhika P, Kadoo NY, Mhase LB, Gupta VS (2009) Molecular mapping of wilt resistance genes in chickpea. Mol Breed 24:177–183
- Gurha SN, Singh G, Sharma YR (2003) Diseases of chickpea and their management. In: Ali M, Kumar S, Singh NB (eds) Chickpea research in India. Indian Institute of Pulses Research, Kanpur, pp 195–227
- Halila I, Cobos MJ, Rubio J, Millán T, Kharrat M, Marrakchi M, Gil J (2009) Tagging and mapping a second resistance gene for Fusarium wilt race 0 in chickpea. Eur J Plant Pathol 124:87–92
- Hiremath PJ, Kumar A, Penmetsa RV, Farmer A, Schlueter JA, Chamarthi SK, Whaley AM, Carrasquilla-Garcia N, Gaur PM, Upadhyaya HD, Kavi Kishor PB, Shah TM, Cook DR, Varshney RK (2012) Large-scale development of cost-effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes. Plant Biotechnol J 10:716–732
- Huttel B, Winter P, Weisind K, Chromosome W, Weigand F, Kahl G (1999) Sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217
- Jendoubi W, Bouhadida M, Boukteb A, Béji M, Kharrat M (2017) Fusarium wilt affecting chickpea crop. Agriculture. <https://doi.org/10.3390/agriculture7030023>
- Jiménez-Díaz RM, Jiménez-Gasco MM, Landa BB, Castillo P, Navas-Cortés JA (2011) Fusarium wilt of chickpea. In: Chen W, Sharma HC, Muehlbauer FJ (eds) Compendium of chickpea and lentil diseases. APS Press, St. Paul, pp 16–20. ISBN 978-0-89054-384-9

- Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN (2012) Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *Br J Nutr* 108:S11–S26
- Kaur L, Singh V (2009) Inheritance of resistance in four elite chickpea genotypes to *Ascochyta* blight. *J Food Legumes* 22(4):239–240
- Kosambi D (1994) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175
- Kottapalli P, Gaur PM, Katiyar SK, Crouch JH, Buhariwalla HK, Pande S, Gali KK (2009) Mapping and validation of QTLs for resistance to an Indian isolate of *Ascochyta* blight pathogen in chickpea. *Euphytica* 165:79–88
- Kumar S (1998) Inheritance of resistance to *Fusarium* wilt (race 2) in chickpea. *Plant Breed* 117:139–142
- Li H, Rodda M, Gnanasambandam A, Aftab M, Redden R, Hobson K, Rosewarne G, Materne M, Kaur S, Slater AT (2015) Breeding for biotic stress resistance in chickpea: progress and prospects. *Euphytica* 204:257–288
- Lichtenzweig J, Scheuring C, Dodge J, Abbo S, Zhang HB (2005) Construction of BAC and BIRAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, *Cicer arietinum* L. *Theor Appl Genet* 110:492–510
- Mace ES, Buhariwalla HK, Crouch JH (2003) A high throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Mol Biol Rep* 21:459a–459h
- Nayak SN, Zhu H, Varghese N, Datta S, Choi HK, Horres R, Jüngling R, Singh J, Kishor PB, Sivaramkrishnan S, Hoisington DA, Kahl G, Winter P, Cook DR, Varshney RK (2010) Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theor Appl Genet* 120(7):1415–1441
- Pande S, Siddique KHM, Kishore GK, Bayaa B, Gaur PM, Gowda CLL, Bretag TW, Crouch JH (2005) *Ascochyta* blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity and disease management. *Aust J Agric Res* 56:317–332
- Pande S, Gaur PM, Sharma M, Rao JN, Rao BV, Krishna Kishore G (2007) Identification of single and multiple disease resistance in desi chickpea genotypes to *Ascochyta* blight, *Botrytis* gray mold and *Fusarium* wilt. *SAT eJournal* 3(1)
- Rubio J, Hajj-Moussa E, Kharrat M, Moreno MT, Millan T, Gill J (2003) Two genes and linked RAPD markers involved in resistance to *Fusarium oxysporum* f. sp. *ciceris* race 0 in chickpea. *Plant Breed* 122:188–191
- Sabbavarapu MM, Sharma M, Chamarthi SK, Swapna N, Rathore A, Thudi M, Gaur PM, Pande S, Singh S, Kaur L, Varshney RK (2013) Molecular mapping of QTLs for resistance to *Fusarium* wilt (race 1) and *Ascochyta* blight in chickpea (*Cicer arietinum* L.). *Euphytica* 193:121–133
- Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000) Identification and mapping of QTL conferring resistance to *Ascochyta* blight in chickpea. *Crop Sci* 40:1606–1612
- Sharma M, Ghosh R (2016) An update on genetic resistance of chickpea to *Ascochyta* blight. *Agronomy* 6:18
- Sharma KD, Muehlbauer FJ (2007) *Fusarium* wilt of chickpea: physiological specialization, genetics of resistance and resistance gene tagging. *Euphytica* 157:1–14
- Sharma M, Pande S (2013) Unravelling effects of temperature and soil moisture stress response on development of dry root rot [*Rhizoctonia bataticola* (Taub.) Butler] in chickpea. *Am J Plant Sci* 4:584–589
- Sharma KD, Winter P, Kahl G, Muehlbauer FJ (2004) Molecular mapping of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea. *Theor Appl Genet* 108:1243–1248
- Sharma KD, Chen W, Muehlbauer FJ (2005) Genetics of chickpea resistance to five races of *Fusarium* wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. *Plant Dis* 89:385–390
- Sharma M, Pande S, Rathore A (2010) Effect of growth stages of chickpea on the genetic resistance of *Ascochyta* blight. *Eur J Plant Pathol* 128:325–331
- Sharma M, Nagavardhini A, Thudi M, Ghosh R, Pande S, Varshney RK (2014) Development of DArT markers and assessment of diversity in *Fusarium oxysporum* f. sp. *ciceris*, wilt pathogen of chickpea (*Cicer arietinum* L.). *BMC Genomics* 15:454
- Singh G (1990) Identification and designation of physiological races of *Ascochyta rabiei* in India. *Indian Phytopathol* 43(1):48–52
- Singh G, Sharma YR (1998) *Ascochyta* blight of chickpea. In: Mukherji KG, Rajar RL, Upadhyay RK (eds) *IPM system in agriculture*. Aditya Book Pvt. Ltd., New Delhi, pp 163–196
- Singh H, Kumar J, Haware MP (1987) Genetics of resistance to *Fusarium* wilt in chickpeas. In: Day PR, Jellis GJ et al (eds) *Genetics and plant pathogenesis*. Blackwell Scientific Publications, Oxford, pp 339–342
- Tar'an B, Warkentin TD, Tullu A, Vandenberg A (2007) Genetic mapping of *Ascochyta* blight resistance in chickpea (*Cicer arietinum* L.) using a simple sequence repeat linkage map. *Genome* 50:26–34
- Tekeoglu M, Tullu A, Kaiser WJ, Muehlbauer FJ (2000) Inheritance and linkage of two genes that confer resistance to *Fusarium* wilt in chickpea. *Crop Sci* 40:1247–1251
- Tekeoglu M, Rajesh PN, Muehlbauer FJ (2002) Integration of sequence tagged microsatellites to the chickpea genetic map. *Theor Appl Genet* 105:847–854
- Tewari SK, Pandey MP (1986) Genetics of resistance to *Ascochyta* blight in chickpea (*Cicer arietinum* L.). *Euphytica* 35(1):211–215
- Thudi M, Bohra A, Nayak SN, Varghese N, Shah TM, Varma RP, Nepolean T, Gudipati S, Gaur PM, Kulwal PL, Upadhyaya HD, Kavikishor PB, Winter P, Kahl G, Town CD, Kilian A, Cook DR, Varshney RK (2011) Novel SSR markers from BAC-End sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS ONE* 6(11):e27275
- Tullu A, Kaiser WJ, Kraft JM, Muehlbauer FJ (1999) A second gene for resistance to race 4 of *Fusarium* wilt in chickpea and linkage with a RAPD marker. *Euphytica* 109:43–50
- Udupa SM, Baum M (2003) Genetic dissection of pathotype-specific resistance to *Ascochyta* blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. *Theor Appl Genet* 106:1196–1202
- Van Ooijen J (2006) JoinMap version 4.0: software for the calculation of genetic linkage maps in experimental population. Kyazma BV, Wageningen

- Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Taran B, Millans T, Zhang X, Ramsay LD, Iwata A, Wang Y, Nelson W, Farmer AD, Gaur PM, Sodarlund C, Penmetsa RV, Xu C, Bharti AK, He W, Winter P, Zhao S, Hane JK, Garcia NC, Condie JA, Upadhyaya HD, Luo MC, Thudi M, Gowda CLL, Singh NP, Lichtenzweig J, Gali KK, Rubio J, Nadarajan N, Dolezel J, Bansal KC, Xu X, Edwards D, Zhang G, Khal G, Gil J, Singh KB, Datta SK, Jackson SA, Wang J, Cook DR (2013) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat Biotechnol* 31:240–246
- Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, Jaganathan D, Koppolu J, Bohra A, Tripathi S, Rathore A, Jukanti AK, Jayalakshmi V, Vemula A, Singh SJ, Yasin M, Sheshshayee MS, Viswanatha KP (2014) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). *Theor Appl Genet* 127:445–462
- Wang J, Li H, Zhang L, Meng L (2014) Users' manual of QTL IciMapping. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico
- Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC, Sahim S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ, Kahl G (1999) Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mol Genet Genomics* 262:90–91
- Winter P, Benko-Iseppon HB, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for *Fusarium* wilt races 4 and 5. *Theor Appl Genet* 101:1155–1163