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ORIGINAL PAPER

Genetic analyses and conservation of QTL for ascochyta blight resistance in chickpea (*Cicer arietinum* L.)

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Abstract Ascochyta blight (AB) caused by *Ascochyta rabiei* (teleomorph, *Didymella rabiei*) Pass. Lab. is an important fungal disease of chickpea worldwide. Only moderate sources of resistance are available within the cultivated species and we hypothesized that the available sources may carry different genes for resistance, which could be pyramided to improve field resistance to AB. Four divergent moderately resistant cultivars CDC Frontier, CDC Luna, CDC Corinne, and Amit were each crossed to a highly susceptible germplasm ICCV 96029. Parents, F_1 and F_2 generations were evaluated under controlled conditions for their reactions to AB. A total of 144 simple sequence repeat (SSR) markers were first mapped to eight linkage groups (LG) for the CDC Frontier × ICCV 96029 population. Then based on the evidence from this population, 76, 61, and 42 SSR markers were systematically chosen and mapped in CDC Luna, CDC Corinne, and Amit populations, respectively. Frequency distributions of the AB rating in the F_2 generation varied among the four populations. Composite interval mapping revealed five QTLs (QTL1–5), one on each of LG 2, 3, 4, 6, and 8, respectively, distributed across different sources, controlling resistance to AB. CDC Frontier contained QTL2, 3, and 4 that simultaneously accounted for 56% of phenotypic variations. CDC Luna contained QTL 1 and 3. CDC Corinne contained QTL 3 and 5, while only QTL 2 was identified in Amit. Altogether these QTL explained 48, 38, and 14% of the estimated phenotypic variations in CDC Luna, CDC Corinne, and Amit populations, respectively. The results suggested that these QTLs could be combined into a single genotype to enhance field resistance to AB.

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Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop with a wide distribution across the tropics, subtropics, and temperate regions (Singh 1997). It accounts for about 15% (9.3 million tonnes) of the world's total pulse production (FAO 2007). In many areas of production including West and Central Asia, North Africa, southern and eastern Europe, North America, and Australia, the chickpea crop is affected by the foliar fungal disease ascochyta blight, which is the most destructive disease of chickpea, and in case of severe infection caused up to 100% yield loss (Nene and Reddy 1987; Singh and Reddy 1993; Chang et al. 2007). Successful chickpea cultivation in many growing areas nowadays depends on effective management of AB.

Ascochyta can infect all above ground parts of the chickpea plant and occurs throughout the growing season (Jayakumar et al. 2005). Application of fungicides may not be cost–effective due to the need for repeated applications under the prevalence of prolonged weather conditions favorable to disease development (Chongo et al. 2004) and due to insensitivity of the pathogen to some of the fungicides used (Cho et al. 2004; Chang et al. 2007). Use of resistant cultivars is considered the most viable option for long-term management of AB.

Chickpea cultivars with improved levels of resistance to AB have been developed and commercialized (Vandenberg et al. 2003; Warkentin et al. 2005). However, only partial resistance is available among the cultivated chickpea germplasm and the improved cultivars are only moderately resistant to AB. Singh and Reddy (1993) evaluated 19,343 germplasm collection which included 12,749 desi accessions from ICRISAT and 6,594 kabuli accessions from ICARDA and found at best accessions with a score of 4 in a 1–9 scale for resistance. The moderately resistant cultivars need to be supplemented with fungicide application for good protection of the crop in most years. Thus, further improvement in field resistance of chickpea to AB is required to increase and stabilize production levels.

One of the genetic strategies being considered to increase the level of resistance to AB in chickpea at the Crop Development Centre (CDC), University of Saskatchewan, Canada is to analyze different sources of resistance within the cultivated species that potentially carry different genes and pyramid these to improve the level of resistance. Combining key genes for resistance to AB may also increase the durability of resistance by giving protection against varying populations of ascochyta. Several studies have reported varying results related to the genetic control of resistance to AB including a single dominant or a single recessive gene depending on the parent (Singh and Reddy 1983; Tewari and Pandey 1986), two complementary dominant genes (Dey and Singh 1993), three major recessive genes with complementary effects and several modifiers (Tekeoglu et al. 2000). These clearly show the complexity of the genetic control of AB resistance, which may require marker-assisted selection to effectively manipulate these genes to build high and durable resistance (Rakshit et al. 2003).

Attempts have been made over the last decade to tag AB resistance genes in chickpea using DNA markers. Santra et al. (2000) identified two QTLs for resistance to AB, one tagged with random amplified polymorphic DNA (RAPD) and the other with inter simple sequence repeat (ISSR) markers in an interspecific recombinant inbred lines (RILs) derived from a cross of *C. arietinum* × *C. reticulatum*. Tekeoglu et al. (2002), using the same RIL population, integrated sequence-tagged microsatellite markers (STMS) also known as simple sequence repeats (SSR) into the map, where the two QTLs were located. One of the QTLs was linked to SSR marker GAA47 and the other linked to TA72s, TA2, and TS54, all of these markers were mapped to LG4 in the chickpea linkage map (Winter et al. 1999; Tar'an et al. 2007a). A separate QTL for resistance to AB linked to RAPD markers on LG2 was identified by Cobos et al. (2006) and later SSR markers were mapped in the vicinity by Iruela et al. (2007). This QTL was flanked by SSRs, TS58 and TS82 and tightly linked to TA194. Udupa and Baum (2003) and Cho et al. (2004) also reported a QTL in the same genomic region, but attributed the resistance to a specific pathotype of ascochyta. Additional QTLs for resistance to ascochyta on LG3 (Tar'an et al. 2007a), LG6 (Cho et al. 2004; Tar'an et al. 2007a), and LG8 (Flandez-Galvez et al. 2003; Lichtenzveig et al. 2006) have also been reported.

Despite many reports on QTLs for resistance to AB, the use of marker-assisted selection in breeding for resistance to AB in chickpea has been limited. Considering the genomic region of the QTLs alone, the reports from the different groups were inconsistent. This could be attributed to differences in resistance sources and pathogenic variability in the AB populations evaluated. In addition, the artifacts due to differences in environmental conditions during evaluation and the type of disease assessments used, for example, disease score versus area under the disease progress curve, could contribute to this inconsistency (Tekeoglu et al. 2000). Udupa and Baum (2003) argued that populations derived from interspecific crosses used by many of the authors for analysis of QTL for AB resistance, showed wider variability for morphophysiological traits that could interfere with disease scoring and is a potential source of bias. This warrants a more intensive examination of the QTLs reported for resistance to AB using populations derived from cultivated × cultivated crosses and using common SSR markers that display clear polymorphism. It is certainly important to elucidate the key QTLs for resistance to AB from different sources, such that these could be pyramided into a single genotype to build high and durable resistance. The objective of this study was to evaluate previously reported and new QTLs conferring resistance to AB in four chickpea populations derived from widely divergent cultivated resistance sources.

Materials and methods

Population and phenotyping

Four divergent moderately AB resistant cultivars CDC Frontier, CDC Luna, CDC Corinne, and Amit were each crossed to a highly susceptible germplasm ICCV 96029. The resistant parents were chosen to represent the most divergent sources of resistance to AB based on results of the SSR at the known QTLs for AB in chickpea (Tar'an et al. 2007b). CDC Frontier is an improved cultivar developed at the University of Saskatchewan from the cross FLIP 91-22C/ICC14912 (Warkentin et al. 2005). CDC Luna, formerly tested as FLIP97-133C (Taran et al. 2009a), is also an improved cultivar developed from a cross FLIP 91-123C/FLIP 84-79C/FLIP 90-127C in collaboration with the International Centre for Agricultural Research in the

Dry Areas (ICARDA). CDC Corinne is a recent release cultivar selected from a landrace (ICC12512-1) originated from India that was obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT; Taran et al. 2009b). Amit is an improved cultivar developed through selection from a Bulgarian landrace. CDC Frontier, CDC Luna, and Amit are of the kabuli market class, while CDC Corinne and ICCV 96029 are of the desi market class. In each cross the AB susceptible germplasm ICCV 96029 was used as the female parent. For simplicity, the four populations are hereafter referred to as CDC Frontier, CDC Luna, CDC Corinne, and Amit population, after their respective resistant parent.

A single F_1 seed from each cross was used to develop the F_2 population. To obtain a sufficient number of F_2 seeds, multiple clones derived from stem cuttings of the single F_1 plant were grown (Tar'an et al. 2007a). The F_2 populations, parents, and residual F_1s were evaluated for their reaction to AB under greenhouse conditions. A total of 186 individual F_2 genotypes for the CDC Frontier, 154 for the CDC Luna, 179 for the CDC Corinne, and 202 for the Amit populations were used. Three clones obtained from stem cuttings of individual F_2 genotypes were used as the replicates. Each population was treated as a separate set and laid out in a completely randomized design with three replications. Each plant was grown in a 10 cm^2 plot filled with Sunshine mix No. 4 medium (Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta, Canada). The greenhouse day/night temperature was 20/16°C and photoperiod was maintained at 16 h using fluorescent and incandescent lights.

Plants were inoculated with a single spore-derived suspension of *Ascochyta rabiei* isolate ar68-2001 at the seedling stage. This is an aggressive isolate collected from commercial chickpea fields in Saskatchewan and is being used as a standard for pathological and genetic studies in the CDC chickpea breeding program. The inoculum preparation and inoculation process were as described by Tar'an et al. (2007a). The inoculated plants were kept in a misting chamber at 100% relative humidity for a 48-h period to facilitate infection and were thereafter transferred to the greenhouse. Disease reactions were scored 2 weeks after inoculation on a 0–9 scale (Chongo et al. 2004); where 0 = no symptoms, 1 = <2% plant area affected (PAA) with very small (<2 mm^2) lesions on leaves or stems, 2 = 2–5% PAA with very small lesions, 3 = 5–10% PAA with very small and small (≤2 to 5 mm^2) lesions, 4 = 10–25% PAA with small and few large (>5 mm^2) lesions, 5 = 25–50% PAA with many large lesions, 6 = 50–75% PAA and lesions coalescing, 7 = 75–90% PAA and lesions coalescing with stem girdling, 8 = >90% PAA and stem girdling and breakage, and 9 = plants dead.

Genotypic analysis

Young leaves were harvested from the F_2 plants and parental genotypes, and freeze-dried for 24 h. These were later stored in a −80°C freezer until the date of DNA extraction. Genomic DNA was extracted following the cetyl-trimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1990). The first phase of this study focused on the CDC Frontier population and mapping of 144 SSR markers to this population (Tar'an et al. 2007a). Then these SSRs were screened on the parents of the remaining three populations and those that showed polymorphism between the two parents were used for genotyping their respective F_2 populations. The polymerase chain reaction (PCR) for these analyses contained 5 µl of 10 ng/µl DNA, 16.3 µl dH_2O, 2.5 µl 10× buffer, 1 µl dNTP's, and 1U Taq DNA polymerase. The PCR products were separated on a 6% polyacrylamide gel and visualized with silver staining. The bands were scored as resistant parent, susceptible parent, or the heterozygote type.

Statistical analysis

The significance of the differences in the mean AB ratings of the F_1s and their respective crossing parents was assessed using unpaired two-sample *t* test (Steel and Torrie 1980). Analysis of variance was conducted using the PROC GLM of SAS for the disease rating data of the F_2s. The mean disease rating of each F_2 was computed using the LSMEANS option of the PROC GLM. The frequency distribution of the AB ratings based on these mean values was illustrated graphically. Further these mean values were used for QTL analysis. Genetic linkage mapping was carried out using MAPMAKER/EXP program version 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA). A threshold logarithm of odds ratio (LOD) value of 3.0 was used to declare linkage between any two adjacent markers. The QTL Cartographer program (North Carolina State University, Raleigh, NC) was employed to identify loci associated with AB resistance. A single point analysis was used initially to identify markers significantly associated with AB resistance. Then QTL location and effects were determined by composite interval mapping.

Results

The mean AB rating for the resistant parents CDC Frontier, CDC Luna, CDC Corinne, and Amit ranged from 4.8 to 5.3, whereas the susceptible parent ICCV 96029 was on average rated 8.2 on a 0–9 rating scale (Table 1). The F_1s were intermediate with a mean rating of 5.0 to 7.8 depending on the cross. *T* test revealed that the mean AB ratings of the F_1s

Table 1 Comparison of the parents, the F_1s and F_2 populations for mean ascochyta blight rating in four chickpea populations

Population	AB rating			*t* test	
	Parent 1 (±SE)	Parent 2 (±SE)	F_1 (±SE)	F_1 vs. P_1	F_1 vs. P_2
1	8.2 ± 0.22	4.9 ± 0.26	5.2 ± 0.26	$t = 10.3$; $P = 0.00$	$t = 1.0$; $P = 0.36$
2	8.2 ± 0.22	5.3 ± 0.29	7.8 ± 0.23	$t = 1.0$; $P = 0.38$	$t = 8.4$; $P = 0.00$
3	8.2 ± 0.22	4.8 ± 0.31	5.0 ± 0.32	$t = 9.6$; $P = 0.00$	$t = 1.6$; $P = 0.18$
4	8.2 ± 0.22	4.8 ± 0.33	5.1 ± 0.41	$t = 5.2$; $P = 0.01$	$t = 0.1$; $P = 0.96$

Population: 1—ICCV 96029/CDC Frontier; 2—ICCV 96029/CDC Luna; 3—ICCV96029/CDC Corinne; 4—ICCV 96029/Amit

Number of individual F_1s evaluated: population 1 = 9, population 2 = 8, population 3 = 11, and population 4 = 9

from the CDC Frontier, CDC Corinne, and Amit crosses were not significantly different from the rating of their respective resistant parents. However, the differences between the AB ratings of the F_1s and the susceptible parent in these crosses were significant. Conversely, for the CDC Luna population the mean AB rating of the F_1 plants was comparable to that of the susceptible parent ICCV 96029 and was significantly higher than that of the resistant parent.

Highly significant differences ($P < 0.01$) were observed in AB ratings among the F_2 genotypes within each population (Table 2). In agreement with the F_1 data, the mean AB rating was higher (6.7) for the CDC Luna population as compared to 4.4 to 5.4 for the other three populations (Fig. 1). The frequency distribution of the AB rating was continuous, but differences were apparent among the four populations in the mean, mode, and range of the AB ratings (Fig. 1). This distribution was skewed toward the susceptible parent particularly for the CDC Luna population.

A total of 144 SSR markers were mapped for the CDC Frontier population during the first phase of our study (Fig. 2). These markers were mapped to eight LGs, which corresponds with the chromosome number of chickpea. These SSRs that were selected from 322 SSR showed polymorphism between the two parents, produced clear bands for scoring and had an observed ratio that fit to the expected 1:2:1 ratio (Tar'an et al. 2007a). The map covered a combined linkage distance of 1, 285 cM with an average distance of 8.9 cM between pairs of markers. The LGs were numbered from one to eight in accordance with the map of Winter et al. (1999, 2000) by matching the common SSR markers, and throughout this paper the LG number of associated markers refers to this notation.

Based on the evidence from the CDC Frontier population, a total of 76, 61 and 42 SSR markers were systematically chosen and mapped to the CDC Luna, CDC Corinne, and Amit populations, respectively (Fig. 3). These covered a distance of 497, 391, and 276 cM in the CDC Luna, CDC Corinne, and Amit populations, respectively. All the markers were mapped to the same linkage groups as in the map of the CDC Frontier population. However, some differences in the marker orientations and distances within a linkage group were detected among the four populations.

Table 2 Mean squares of ascochyta blight ratings in four F_2 chickpea populations

Population	*df*		Mean squares	
	F_2 genotypes	Error	F_2 genotypes	Error
ICCV 96029/CDC Frontier	186	357	4.05*	0.79
ICCV 96029/CDC Luna	153	272	6.07*	0.73
ICCV 96029/CDC Corinne	179	321	2.92*	0.39
ICCV 96029/Amit	202	383	3.14*	0.90

* Indicates highly significant differences at $P \leq 0.01$

Single point analysis using QTL Cartographer revealed that some markers were significantly associated with AB resistance. Subsequent composite interval mapping demonstrated that a total of five different QTLs associated with resistance to AB existed in the populations being studied. Some of these QTLs were common across different populations while others were unique to a given population (Table 3). One QTL, each on LG2, LG3, LG4, LG6, and LG8 hereafter referred to as QTL1, QTL2, QTL3, QTL4, and QTL5, respectively, were identified. CDC Frontier contained QTL2, 3, and 4 that simultaneously accounted for 56% of the total estimated phenotypic variation. CDC Luna contained QTL 1 and 3 that altogether explained 48% of the total estimated phenotypic variation. CDC Corinne contained QTL 3 and 5 that explained 38% of the total estimated phenotypic variation, while only QTL 2 was identified in Amit, which accounted for 14% of phenotypic variations (Table 3; Figs. 2 and 3). The marker nearest to the maximum LOD peak for the QTL2 and QTL3, which was common across CDC Frontier, CDC Luna, and Amit populations, were the same except for the CDC Corinne population in QTL3. Simple linear regression analysis confirmed the dependence of mean AB rating on the alleles of the SSRs (TR19 for QTL1, TA64 for QTL2, TS54 for QTL3 in CDC Frontier and Amit; and TA132 for QTL3 in CDC Corinne population; TA176 for QTL4 and TS45 for

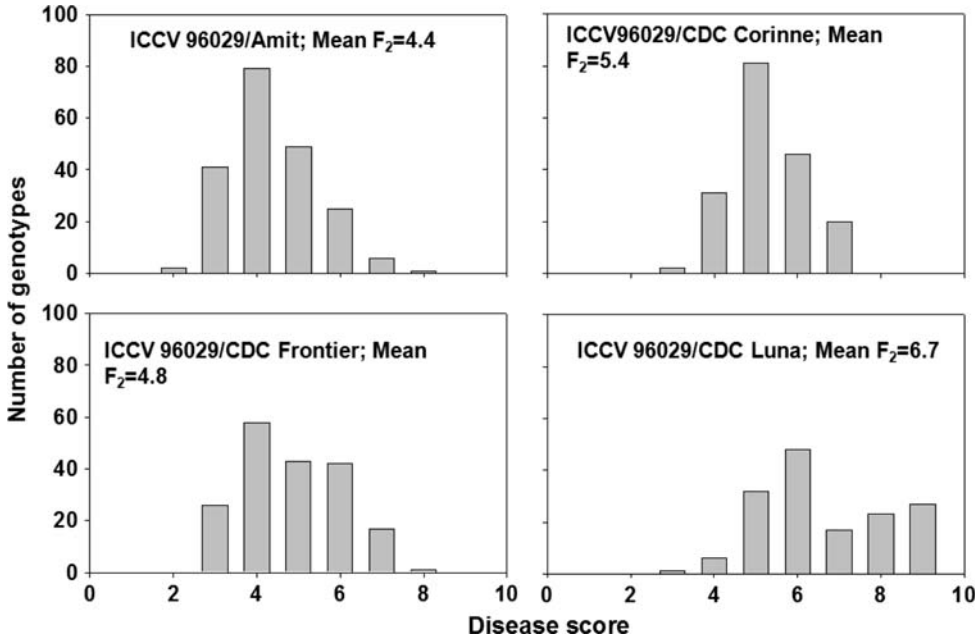

Fig. 1 Frequency distribution and mean of ascochyta blight disease ratings (1–9 scale) in four F_2 chickpea populations

QTL5 on LG8) nearest to the maximum LOD peak of each QTL.

Discussion

Efforts to improve AB resistance in chickpea have been hampered by the lack of sources of high levels of resistance within the primary gene pool of the crop. Virulent strains of *Ascochyta rabiei* always cause disease under favorable conditions even in the resistant germplasm (Cho et al. 2004). In this study we assessed whether four resistance sources may carry different genetic factors for resistance which could potentially be combined to improve field resistance to AB. The gene pyramiding approach is also desired to build durable resistance such that the crop will be safeguarded against existing or more aggressive strains that could emerge due to mutation or recombination in the AB pathogen which is known to have a sexual stage (Singh and Reddy 1993; Armstrong et al. 2001; Vail and Banniza 2008).

The AB rating for the resistant parents CDC Frontier, CDC Luna, CDC Corinne, and Amit under indoor conditions in the current study ranged from 4.8 to 5.3. These scores correlated well with those of the same cultivars under field conditions (Warkentin et al. 2005; Taran et al. 2009a, b), though the field rating tended to be slightly lower due to unfavorable environmental conditions for the disease. The results of this research revealed disparity in the frequency distribution of the AB ratings among the four F_2 populations (Fig. 1). The mean AB ratings for the F_1 and F_2 generations fell in between the resistant parent and the mid-parent in CDC Frontier, CDC Corinne, and Amit populations, whereas it was between the mid-parent and the susceptible parent for the CDC Luna population. This provided evidence that separate genes for resistance were carried by the different parental sources evaluated in this study. Unique resistance sources have also previously been noted by Singh and Reddy (1983), Tewari and Pandey (1986), and Tekeoglu et al. (2000). However, it was not possible to mark out precisely from the frequency distribution alone the genetic basis of AB resistance in each population or the common and unique genetic factors across the four populations.

Molecular analysis revealed five QTLs conferring resistance to AB distributed across different parental sources. Although previous authors using different resistant parents reported single recessive, single dominant, two dominant, or three recessive genes responsible for AB resistance (Singh and Reddy 1983; Tewari and Pandey 1986; Tekeoglu et al. 2000), no allelism test was carried out to determine the number of unique genes responsible for AB resistance. On the other hand, different QTLs for resistance to AB have previously been reported by different authors (Santra et al. 2000; Tekeoglu et al. 2002; Udupa and Baum 2003; Flandez-Galvez et al. 2003; Cho et al. 2004; Lichtenzveig et al. 2006; Iruela et al. 2007). However, because of the use of different methodology by different authors, interspecific versus intraspecific population, field versus greenhouse evaluation, disease score versus area under disease progress curve for disease assessment, and different strains of the pathogen, these results were difficult to compare. This marks the significance of this study in clearly elucidating the number of unique genetic factors underlying resistance to AB in pure *C. arietinum* genetic backgrounds

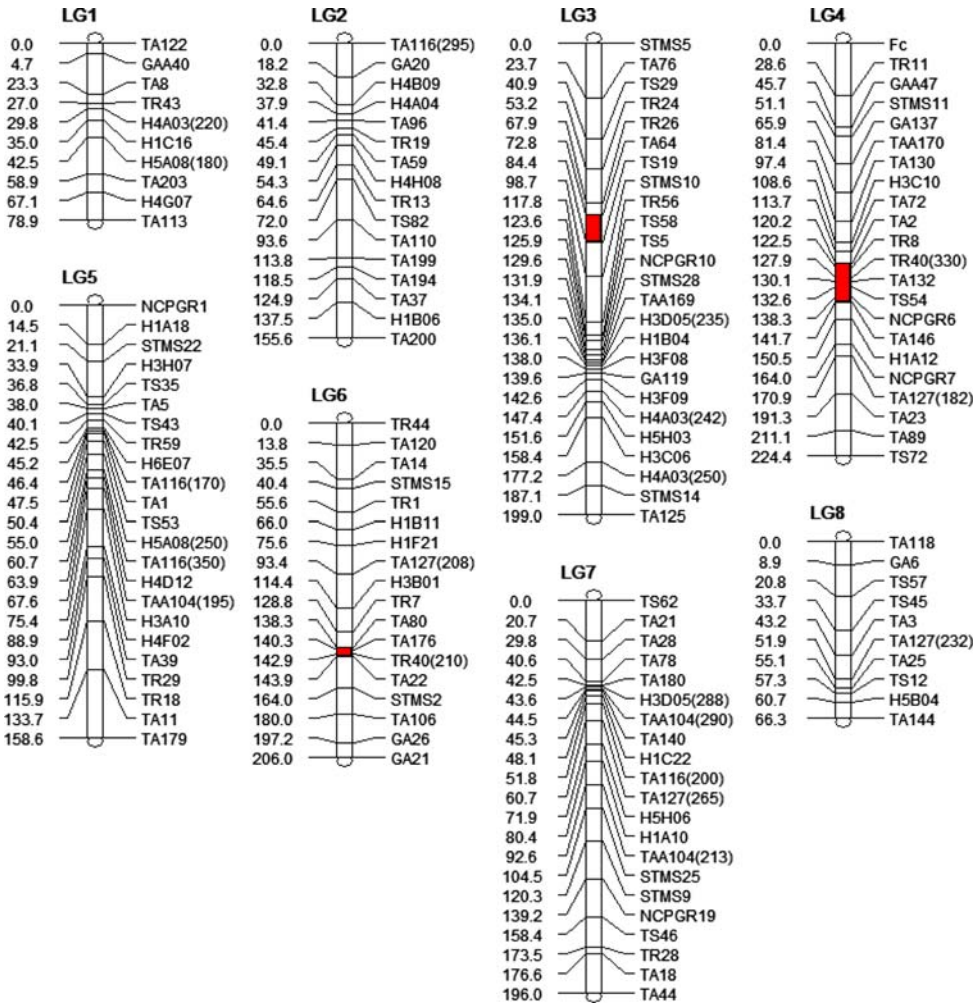

Fig. 2 SSR linkage map of chickpea derived from ICCV 96029/CDC Frontier population (adapted from Tar'an et al. 2007a). The shadowed areas are the locations of the QTL for ascochyta blight resistance

using widely divergent resistant parents from across desi and kabuli types and from varied countries of origin.

QTL1, which is located on LG2, corresponds with the QTL that was previously reported by Udupa and Baum (2003), Cho et al. (2004), Cobos et al. (2006), and Iruela et al. (2007). This QTL had relatively large effects (38%) on the total estimated phenotypic variation in reaction to AB in CDC Luna population. Tar'an et al. (2007b) reported that the resistant parents used in the previous studies, as was CDC Luna, were closely related and had the resistance derived from landraces originating from the former Soviet Union. Nevertheless, the confirmation of QTL1 by research groups in Mediterranean environments of Syria and Spain and temperate climatic conditions of Washington State, USA and Saskatchewan, Canada using different AB isolates and evaluation techniques suggested the importance of this locus. Udupa and Baum (2003) reported another QTL co-segregating with QTL1 on LG2; however, this QTL was not significant against the isolate used in the current study.

QTL2 was detected on LG3 for both CDC Frontier and Amit populations and accounted for 13% and 14%, respectively, of the phenotypic variations in AB ratings. A QTL for AB resistance on LG3 derived from ICCV 04516 that accounted for 19% of the phenotypic variation was previously reported by Kottapalli et al. (2009). The proximity of the locations of QTL2 on LG 3 suggested that this QTL may be common among CDC Frontier, Amit, and ICCV 04516. Even though the QTL on LG3 is only moderate contribution to AB variation, incorporation of this QTL in gene pyramiding using linked markers is undoubtedly important.

Our phenotypic data indicated that the AB reaction of CDC Frontier and Amit populations were similar (Table 1; Fig. 1). However, only one QTL was detected for the Amit population as opposed to three in the CDC Frontier population. The markers used for Amit population were a subset and chosen systematically based on the results from the CDC Frontier population in the first phase of this study. The smaller map coverage of only 276 cM for the Amit population as compared to about 1,200 cM for the CDC Frontier population (Figs. 2 and 3) may have prevented the identification of other loci contributing to AB resistance in Amit population.

QTL3 on LG4 was detected in three (CDC Frontier, CDC Luna, and CDC Corinne) of the four populations used

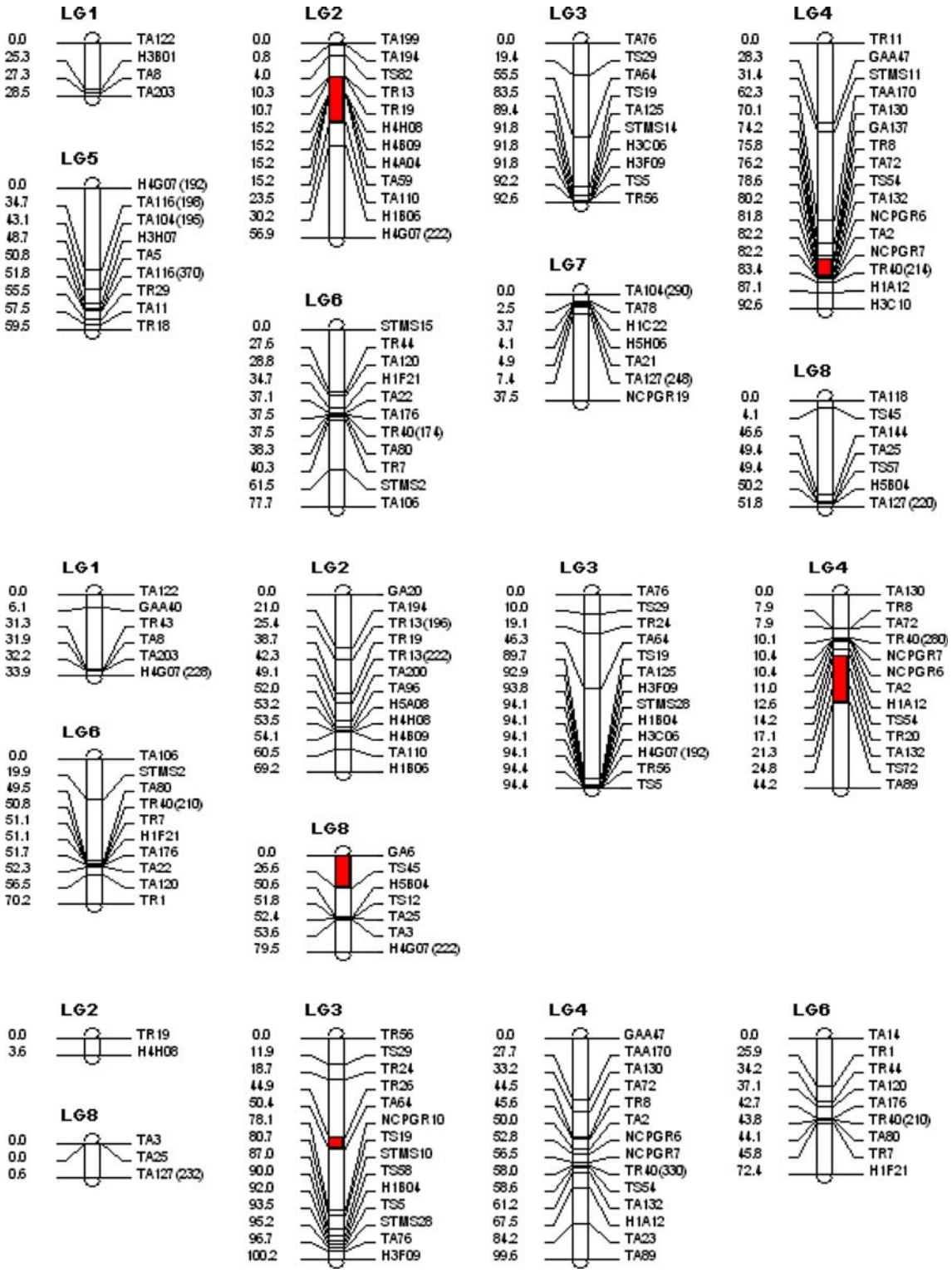

Fig. 3 SSR linkage map of chickpea derived from ICCV96029/CDC Luna (top), ICCV96029/CDC Corinne (middle), and ICCV96029/Amit (bottom) populations. The shadowed areas are the locations of the QTL for ascochyta blight resistance

in the current study and accounted for 29, 21, and 24% of the phenotypic variation of AB reaction, respectively. QTL3 was also the most frequently reported QTL by previous authors (Tekeoglu et al. 2002; Flandez-Galvez et al. 2003; Millán et al. 2003; Udupa and Baum 2003; Cho et al. 2004; Iruela et al. 2006; Lichtenzveig et al. 2006). Thus, repeatability of QTL3 in several previously reported populations and in 3 out of the 4 current populations indicated

Table 3 Linkage group, associated markers and percent phenotypic variation (R^2) accounted for by the QTLs for resistance to ascochyta blight detected in four F_2 populations of chickpea

Population	Linkage group (QTL)	Interval	Nearest locus[a]	Max. LOD score	R^2 (% variation)
ICCV 96029/CDC Frontier	LG3 (QTL2)	TA64–TS19	TA64	4.27	13
	LG4 (QTL3)	TA2–TA146	TS54	9.58	29
	LG6 (QTL4)	TA80–TA22	TA176	3.88	12
			Total variation		56
ICCV 96029/CDC Luna	LG2 (QTL1)	TR13–TA110	TR19	14.32	38
	LG4 (QTL3)	TR8–NCPGR6	TS54	4.09	21
			Total variation		48
ICCV 96029/CDC Corinne	LG4 (QTL3)	TS54–TS72	TA132	5.53	24
	LG8 (QTL5)	GA6–TS45	TS45	2.92	16
			Total variation		38
ICCV 96029/Amit	LG3 (QTL2)	TR26–TA64	TA64	3.16	14

[a] Marker nearest to the maximum LOD peak

that QTL3 like the QTL1 on LG2 could be a major genetic factor for AB resistance in chickpea. However, the nearest markers to the peak of LOD scan differed across the three populations, namely; TS 54 in CDC Frontier and CDC Luna populations and a neighboring TA132 in CDC Corinne. This could be due to the fact that fewer markers were mapped to LG4 in CDC Corinne population that may have biased the positions of the associated markers to precisely locate the QTLs. It is also possible that the QTL3 is comprised of more than one linked genes controlling resistance to AB and the relative effect of these genes may vary depending on the genetic background (i.e., other interacting genes on LG4 or elsewhere in the genome). Similarly, Flandez-Galvez et al. (2003) and Lichtenzveig et al. (2006) reported two or three interacting QTLs on LG4, supporting the idea that a cluster of AB resistance genes may exist in this genomic region.

QTL4 was found for the CDC Frontier population and located on LG6 flanked between SSR markers TA80 and TA22 (Fig. 1). Cho et al. (2004) also reported a QTL for AB resistance in the proximity of this genomic region. When considering the proportion of phenotypic variations accounted for, this QTL along with the QTL2 on LG3 appeared to have a relatively minor role for AB resistance. These regions may play the role of modifier genes as reported by Singh and Reddy (1983) and Tekeoglu et al. (2000), in which the absence of QTL2 and QTL4 may not lead to susceptibility, but their presence enhances the degree of resistance.

QTL5 on LG8 was detected in the CDC Corinne population flanked by SSRs GA6 and TS54. Flandez-Galvez et al. (2003) and Lichtenzveig et al. (2006) also reported a QTL for AB resistance on LG 8. The position of the current QTL on LG 8 matched well with the QTL reported by Flandez-Galvez et al. (2003). Flandez-Galvez et al. (2003) used the desi cultivar ICC 12004 as the resistant parent as is CDC Corinne, suggesting that the two resistance sources may share this QTL for AB resistance.

In summary, this study reported five key QTLs for resistance to AB in chickpea, distributed across four resistance sources. The resistant parents carried one, two, or three of the five QTLs, each determining 12 to 38% of the phenotypic variation in AB ratings. Altogether these QTL explained 56, 48, 38, and 14% of the estimated phenotypic variations in CDC Frontier, CDC Luna, CDC Corinne, and Amit populations, respectively. Combining these QTLs into a single genotype should increase field resistance to AB. A systematic crossing scheme is in progress designed to incorporate QTL1 from CDC Luna and QTL5 from CDC Corinne into CDC Frontier background. Subsequent selection in the segregating generations with the aid of tightly linked molecular markers developed in this study should allow for substantial progress in the improvement of field resistance to AB in chickpea. The gene pyramiding approach is also desired to build durable resistance such that the crop will be safeguarded against existing or potential new strains.

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