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FULL RESEARCH PAPER

Genetic relationships among Chickpea (*Cicer arietinum* L.) genotypes based on the SSRs at the quantitative trait Loci for resistance to Ascochyta Blight

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Abstract Breeding for resistance to ascochyta blight in chickpea has been challenged by several factors including the limited sources of good resistance. Characterization of a set of genotypes that may contain different genes for resistance may help breeders to develop better and more durable resistance compared to current cultivars. The objective of this study was to evaluate the genetic relationships of 37 chickpea germplasm accessions differing in reaction to ascochyta blight using Simple Sequence Repeat (SSR) markers linked to Quantitative Trait Loci (QTL) for resistance. The results demonstrated that ILC72 and ILC3279, landraces from the former Soviet Union, had SSR alleles that were common among the kabuli breeding lines and cultivars. A lower SSR allele diversity was found on LG4 than on other regions. No correlation was found between the dendrogram derived using SSRs at the QTL regions and the SSRs derived from other parts of the genome. The clustering based on 127 alleles of 17 SSRs associated with the QTL for ascochyta blight resistance enabled us to differentiate three major groups within the current germplasm accessions. The first group was the desi germplasm originating from India and cultivars derived from it. The second group was a mix of desi genotypes originating from India and Greece, and kabuli breeding lines from ICARDA and the University of Saskatchewan. The third and largest group consisted of landraces originating mostly from the former Soviet Union and breeding lines/cultivars of the kabuli type. Several moderately resistance genotypes that are distantly related were identified. Disease evaluation on three test populations suggested that it is possible to enhance the level of resistance by crossing moderately resistant parents with distinct genetic backgrounds at the QTL for resistance to ascochyta blight.

Keywords Chickpea · Ascochyta blight resistance · Quantitative Trait Loci (QTL) · Simple Sequence Repeat (SSR)

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Introduction

Ascochyta blight caused by the fungus *Ascochyta rabiei* is one of the most destructive diseases of chickpea worldwide resulting in reduced yield and quality. Yield losses of up to 100% have been reported in severely infected fields (Acikgoz et al. 1994). Seed treatment and foliar application of fungicides, as well as cultural practices are often unsuccessful and uneconomical for controlling this disease (Nene and Reddy 1987). The use of cultivars with high levels of resistance is considered the most

economical solution for long-term disease management. The use of resistant cultivars will also help to stabilize chickpea production. Breeding for resistance to ascochyta blight in chickpea has been limited by several factors, including the high pathogenic variability of the fungus and the limited sources of good resistance (Khan et al. 1999; Singh and Reddy 1993).

Early studies by Singh and Reddy (1993) demonstrated that only five genotypes (ICC4475, ICC6328, ICC12004, ILC200 and ILC6482) out of 19,343 accessions were resistant to ascochyta blight in repeated field and greenhouse evaluations. Chen et al. (2004) further reported that the germplasm lines ICC3996, ICC4475 and ICC12004 were resistant against a number of *A. rabiei* isolates originating from northwestern United States. Several other accessions of different origins with reported resistance to ascochyta blight included: ILC72, ILC195, ILC200, ILC482, ILC3279 and ILC6482 (Reddy and Singh 1992; Singh et al. 1992; 1993). Most breeding programmes worldwide have relied heavily on two kabuli genotypes, ILC72 and ILC3279, as sources for ascochyta blight resistance (Crino 1990; Muehlbauer et al. 1998, 2004; Muehlbauer and Kaiser 2002; Millan et al. 2003; Rubio et al. 2004). In many cases, the occurrence of new pathotypes or the increased aggressiveness of the current *A. rabiei* pathotypes have broken the resistance in several of these varieties. For example, cv. Sanford was initially considered as a resistant variety; however, after several years of production under Saskatchewan environments it became very susceptible. The use of additional resistance sources in breeding programmes is needed to diversify the genetic basis of resistance in elite chickpea germplasm and/or to increase the level of resistance through gene pyramiding. It is crucial, therefore, to characterize accessions from diverse origins that may contain different genes for resistance to ascochyta blight. This will allow breeders to select sources of resistance that may contain different genes and to accumulate those genes in one cultivar to enhance the levels of resistance.

Several approaches have been used to differentiate disease resistance genes. These include the use of differential isolates of the pathogen, the test of allelism, the localization of the resistance genes in the host genome and the use of molecular markers. Several classifications have been suggested for *A. rabiei* isolates based on the reactions of a set of differential host plants (Udupa et al. 1998; Chen et al. 2004; Chongo et al. 2004). However, the lack of universal differential host plants for isolate characterization and the use of different screening techniques or conditions, as well as the absence of a consensus as to whether the variability of *A. rabiei* is due to race or aggressiveness of a single race, make it difficult to distinguish different resistance genes using different pathogen isolates. Furthermore, reaction of different genotypes with potentially different genes for resistance to ascochyta blight often results in similar phenotypes. Therefore, resistance that may be contributed by different genes cannot be separated on the basis of disease evaluation alone. Molecular markers linked to the resistance genes offer an alternative tool for tracing genes for resistance to ascochyta blight. In addition, molecular markers can be used to assess the diversity at specific genomic regions that are associated with resistance to disease and to measure genetic relationships among genotypes. This approach has been used in wheat to separate germplasm with different resistance genes to fusarium head blight caused by *Fusarium graminearum* (McCartney et al. 2004).

To date, a number of Quantitative Trait Loci (QTL) for resistance to ascochyta blight were identified by different groups (Santra et al. 2000; Tekeoglu et al. 2002; Flandez-Galvez et al. 2003; Millan et al. 2003; Udupa and Baum 2003; Cho et al. 2004; Iruela et al. 2006). The use of common Simple Sequence Repeat (SSR) markers in most of these studies provided general conclusions that a major QTL on LG 2 close to the GA16 marker controlled the resistance to pathotype I of *A. rabiei*. Another region on LG2 at the proximity of TA37 locus also contributed to the resistance to pathotype I. Most reports demonstrated that the resistance to pathotype II is located on LG4. A number of SSR loci (GAA47, TA130, TR20, TA72, TS72 and TA2) were mapped within this region (Winter et al. 2000; Udupa and Baum 2003; Cho et al. 2004). By single-point analysis Cho et al. (2004) identified an additional SSR marker (TA46) that was strongly associated with the resistance derived from FLIP84-92C. This marker explained between 59% and 69% of the variation for resistance using different isolates under controlled environments; however, this marker did not show

linkage to other markers on the map. Using ICC12004 as the source of resistance, Flandez-Galvez et al. (2003) identified additional QTL for resistance to ascochyta blight under field conditions on a 5.6 cM interval between TS12b and STMS28 on LG1. Furthermore, a region flanked by TS45 and TA3b on LG2 was significantly associated with the disease reaction under controlled environments (Flandez-Galvez et al. 2003). The SSR marker loci on LG2 of the map reported by Flandez-Galvez et al. (2003) correspond to LG8 of the map constructed by Winter et al. (2000).

The current study used the available SSRs from previous mapping and QTL studies to evaluate the genetic relationships among 37 chickpea germplasm accessions differing in reaction to ascochyta blight. The chickpea germplasm accessions used for the analysis were derived from diverse geographical origins. The study provided information for the effective use of diverse genetic resources to improve ascochyta blight resistance in chickpea.

Materials and methods

Plant materials and field disease screening

One hundred and eighty-two chickpea germplasm accessions with putative resistance to ascochyta blight derived from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), International Centre for Agricultural Research in the Dry Areas (ICARDA), Washington State University, Regional Plant Introduction Station (WRPIS), United States Department of Agriculture (USDA) and Crop Development Centre, University of Saskatchewan collections were tested in the field in Saskatoon, Canada in the summers of 2003 and 2004. In this screening the germplasm accessions were exposed to naturally occurring mixed populations of *A. rabiei*. To increase the disease pressure, dried infected plant debris collected from chickpea production areas in Saskatchewan, Canada in the previous year was spread throughout the plot area and high humidity was maintained with misting irrigation. Thirty-five genotypes with the lowest disease scores (6 or lower using the same scale as for indoor disease screening) were selected and planted in the greenhouse for further disease evaluation under controlled environments. The summary of indoor disease screening is presented in Table 1.

Indoor disease screening

A single-spore derived culture of *A. rabiei* isolate *ar68–2001* was used for indoor disease screening. The *ar68–2001* isolate was collected from cv. Sanford from a commercial production field in Saskatchewan in 2001. The isolate was selected for a high level of aggressiveness from a collection of more than 250 isolates obtained from different chickpea cultivars and production areas across Saskatchewan between 1998 and 2002. The isolate was grown at room temperature under continuous fluorescent light. Primary inoculum was produced by diluting 7 day-old colonies with sterile distilled water followed by agitating the cultures with a sterile glass rod. The suspensions were filtered through a Miracloth layer and adjusted to the final concentration of 2×10^5 conidia ml^{-1} using a hemacytometer. Tween 20 surfactant (polyoxyethylene sorbitan monolaurate) was added at a rate of one drop 100 ml^{-1} suspension.

Ten seeds of each chickpea genotype were grown in 10 cm square pots (1 seed per pot) in a greenhouse for four weeks. The plants were inoculated by spraying 2 ml of conidial suspension per plant or until run-off using an atomizer. Immediately after inoculation, the plants were transferred into a misting chamber covered with a translucent plastic sheet to provide 100% RH during the infection period. After 48 h incubation, the plants were transferred to a greenhouse bench. The temperature was maintained at 20/16°C (day/night) and 16 h photoperiod with fluorescent and incandescent lights. Plant reactions were scored visually two weeks after inoculation. Scoring was made on an individual plant basis on a scale of 0–9 (Singh and Reddy 1993; Chongo et al. 2004); where 0 = immune, no symptoms of disease; 1 = few, very small lesions (<2 mm^2) on leaves and stems (1–2% plant area infected); 2 = many, very small lesions and few small lesions (2–5 mm^2) on leaves and stems (3–5% plant area infected); 3 = many small lesions (6–10% plant area infected); 4 = few small and few large lesions (>5 mm^2), 11–25% plant area infected; 5 = many small and large lesions (26–50% plant area infected); 6 = many small and large lesions, lesions coalescing (51–75% plant area

Table 1 Seed type, status and origin of 37 chickpea germplasm accessions differing in reaction to ascochyta blight (AB)

Germplasm accessions	Seed type	Status	Origin/Breeding Institution	AB score ± Se
CDC Ebony	D	Cultivar	Univ. of Sask., Canada	4.8 ± 0.42
CDC Vanguard	D	Cultivar	Univ. of Sask., Canada	5.0 ± 0.45
304–31	D	Breeding line	Univ. of Sask., Canada	5.0 ± 0.66
304–40	D	Breeding line	Univ. of Sask., Canada	5.0 ± 0.52
95NN12	K	Breeding line	Univ. of Sask., Canada	4.6 ± 0.34
Amit	K	Cultivar	Bulgaria	4.5 ± 0.58
CDC Chico	K	Cultivar	Univ. of Sask., Canada	8.2 ± 0.52
CDC Frontier	K	Cultivar	Univ. of Sask., Canada	4.6 ± 0.52
FLIP82-150C	K	Breeding line	ICARDA, Syria	5.2 ± 0.62
FLIP83-48	K	Breeding line	ICARDA, Syria	5.5 ± 0.67
FLIP84-92C	K	Breeding line	ICARDA, Syria	4.8 ± 0.28
FLIP91-2	K	Breeding line	ICARDA, Syria	5.5 ± 0.64
FLIP91-46	K	Breeding line	ICARDA, Syria	5.6 ± 0.47
FLIP97-133C	K	Breeding line	ICARDA, Syria	5.2 ± 0.42
FLIP98-133C	K	Breeding line	ICARDA, Syria	5.3 ± 0.54
ICC76	D	Germplasm	India	4.9 ± 0.44
ICC1400	D	Germplasm	Unknown	5.1 ± 0.32
ICC1468	D	Germplasm	India	4.6 ± 0.54
ICC1532	D	Germplasm	Unknown	5.2 ± 0.64
ICC3996	D	Germplasm	India	4.0 ± 0.68
ICC4475	D	Germplasm	Unknown	4.5 ± 0.64
ICC4936	D	Germplasm	Greece	4.6 ± 0.48
ICC5124	K	Germplasm	India	5.3 ± 0.44
ICC12004	D	Germplasm	Unknown	4.0 ± 0.52
ICC12512-1	D	Germplasm	India	4.4 ± 0.37
ICC12952	D	Germplasm	India	5.0 ± 0.67
ICC12961	K	Germplasm	Former USSR	5.2 ± 0.52
ICC14911	K	Germplasm	Unknown	5.6 ± 0.44
ILC72	K	Germplasm	Former USSR	5.2 ± 0.34
ILC202	K	Germplasm	Former USSR	4.8 ± 0.72
ILC2506	K	Germplasm	Russia	5.2 ± 0.44
ILC2956	K	Germplasm	Former USSR	5.2 ± 0.47
ILC3279	K	Germplasm	Former USSR	4.8 ± 0.56
ILC3856	K	Germplasm	Morocco	4.8 ± 0.68
ILC5913	K	Germplasm	Unknown	5.6 ± 0.54
ILC5928	K	Germplasm	Morocco	5.0 ± 0.47
Sanford	K	Cultivar	USDA/ARS, USA	8.5 ± 0.50

Disease score was average of 10 plants under greenhouse conditions (Se = standard error)

infected); 7 = many small and large lesions, lesions coalescing, stem girdled (76–90% plant area infected); 8 = many small and large lesions, lesions coalescing, girdling stem breakage (>90% plant area infected), and 9 = plants dead. The disease score for each genotype was averaged from ten plants.

SSR analysis

Ten seeds of each genotype were grown in 10 cm square pots, one seed per pot, in a greenhouse. Two weeks after sowing, equal quantities of fresh leaf tissue from an average of eight plants of each

genotype were harvested and bulked for DNA extraction. Genomic DNA was prepared according to the protocol described by Doyle and Doyle (1990). The DNA was initially analyzed using 17 SSRs that were associated with the QTL for ascochyta blight resistance (Flandez-Galvez et al. 2003; Udupa and Baum 2003; Cho et al. 2004). Subsequently the DNA was analyzed using 24 SSRs from eight linkage groups of the chickpea SSR map (Tar'an et al. 2007) as a whole genome diversity analysis. Three SSRs that were distantly located from each other in each linkage group were selected. SSR loci that were linked to the QTL for ascochyta blight resistance were excluded from selection.

The SSR analysis was done following the protocol described by Winter et al. (1999). Both 10 bp and 50 bp DNA ladders were used as molecular weight markers for each gel. The SSR bands were visualized using silver staining protocol. The glass plates were scanned to create electronic files for band sizing and documentation. SSR allele sizing was done using AlphaEase software (Alpha Innotech Corporation, California, USA).

Polymorphic information content (PIC), genetic similarity and cluster analyses

PIC values were calculated with the following formula (Botstein et al. 1980):

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} p^2{}_{ij}$$

Where n is the number of marker alleles for marker i, and p_{ij} is the frequency of the jth allele for marker i.

Band profiles were compiled onto a data matrix on the basis of the presence (1) or absence (0) of the allele bands. Genetic similarity (GS) between a pair of lines was calculated using the Dice index of similarity (Nei and Li 1979). Cluster analysis was conducted on the GS matrix using the UPGMA procedure of the NTSYS-pc programme version 2.02 g (Rohlf 1998). The resulting clusters were expressed as a dendrogram. The dendrogram presents a pictorial representation of the clustering process by indicating the order of individuals and groups joined together because of their similarity. The goodness of fit of the dendrogram was examined using Mantel's test for matrix correlation between the dendrogram and the GS (Mantel 1967). Two dendrograms, one based on the SSRs at the QTL regions and the other based on the SSRs from other regions of the chickpea genome, were constructed. The correspondence between the two dendrograms was tested with the Mantel Z statistic (Mantel 1967). This procedure examines the matrix-correspondence by taking the two matrices together and plots one against the other, element by element, except for the diagonal elements. This test gives the product-moment correlation, r, and a statistic test, Z, to measure the degree of relationship between two matrices. Significance of Z was determined by comparing the observed Z values with a critical Z value obtained by calculating Z for one matrix with 1,000 permuted variants of the second matrix. All computations were done with the NTSYS-pc programme version 2.02 g (Rohlf 1998).

Test populations

Three populations were developed by crossing moderately resistance lines with distinct SSR alleles at the QTL for the resistance to ascochyta blight. These crosses included ICC12004 × FLIP84-92C, ICC4475 × CDC Frontier and ICC3996 × Amit. A single F_1 plant from each cross was vegetatively propagated by stem cutting to maximize production of F_2 seeds for population development. Stimroot no.1 (Evergro Canada Inc., Delta, British Columbia, Canada) containing the active ingredient indole-3-butyric acid (IBA) was used to induce root development. Each F_2 plant was also vegetatively propagated. Three to four cuttings were made from each F_2 plant. Initially, the cuttings were grown in a peat pellet and incubated in a high humidity chamber with fluorescent light for about 10 days. The cutting-derived plants were then transferred into individual 10 cm square pots filled with Sunshine mix no. 4 medium (Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta, Canada). Three cutting-derived plants from each F_2 plant that were relatively uniform in size from each population were selected. These cutting-derived plants served as replication in a completely randomized design in a greenhouse for disease evaluation using the same conditions and procedure as for indoor disease screening.

Analysis of variance (ANOVA) was done using the SAS package (SAS Institute Inc., 1999). Genotypic variance (σ^2 g) and phenotypic variance

($\sigma^2 p = \sigma^2 g + \sigma^2 e$) were determined based on expected mean squares of the ANOVA. Genetic coefficient of variation (GCV%) was calculated as % of the square root of genetic variance to population mean. The ratio of genetic variance to the total phenotypic variance served as the heritability estimate. Genetic advance (GA) was calculated based on the formula of $GA = k \times h^2 \times \sigma p$ (Falconer 1989), where k is a selection differential for which a standardized value (2.06) for 5% selection intensity was used in this analysis and σp is the standard deviation of the phenotypic variance. The GA is expressed as % of the mean population.

Results

There were only 35 germplasm lines and cultivars out of 182 accessions that consistently showed moderate to good resistance to ascochyta blight from the 2003 and 2004 field and greenhouse disease evaluations. These lines were selected for further disease evaluation and molecular characterization. Table 1 presents the mean reaction of the 35 chickpea germplasm accessions plus two susceptible cultivars (CDC Chico and Sanford) to *A. rabiei* infection under controlled conditions. Twenty lines had disease scores of 5.0 or lower. These lines had many small and few large lesions with less than 50% plant area infected. Six of these lines were selections made at the University of Saskatchewan, whereas the remaining genotypes in this category were germplasm lines originating from India, Greece, Russia, Morocco and ICARDA. Fifteen lines had disease scores ranging from 5.1 to 5.6. The disease scores for CDC Chico and Sanford (susceptible checks) were 8.2 and 8.5, respectively.

The 17 SSRs associated with the QTL for resistance to ascochyta blight used in the analysis detected 2 to 13 alleles (mean = 7.6) across Chico and Sandford and the 35 accessions and PIC values ranged from 0.47 to 0.87 with mean value of 0.71 (Table 2). On average the SSR loci on LG4 contained fewer alleles compared to the loci on LG2B (Table 2). The average PIC value of the SSR markers on LG4 (0.64) was much smaller than the average PIC value (0.82) of the markers in LG2A and LG2B combined. FLIP 84-92C and ILC3279 had identical SSR alleles for the QTL located on LG4 and LG8, except for the TA72 locus; however, their alleles were distinctly different for the QTL on LG1 and LG2A + B (Table 3). ILC72, ICC12961 and ILC2956 had the same alleles for 16 SSR loci at all QTL regions. Available passport data and current analysis revealed that ILC72 and ICC12961 were derived from the same landrace. ILC 72 is maintained by ICARDA, while ICC12961 is maintained by ICRISAT. ICC3996 and ICC12004 also had identical SSR alleles on 15 loci (Table 3).

In LG4 the ILC72 alleles were identical with eight other genotypes (Table 3). In LG4, ILC3279 and ILC3856 had common alleles to that of ILC72, except for TA72 locus. Similarly for LG4, CDC Chico and Sanford had the same alleles as ILC72, except for the TA2 locus. Different alleles than that of ILC72 at all six loci on LG4 were found in a number of germplasms such as CDC Ebony, FLIP82-150C, ICC12952 and ICC3996.

Four genotypes, ILC72, ICC12961, ILC2956 and CDC Chico, had identical alleles at all four loci on

Table 2 Summary of 17 SSR loci associated with QTL for resistance to AB in different linkage groups of the chickpea genetic map

Linkage group (Interval length)	SSR Locus	Number of alleles	PIC	Amplicon size range (bp)
LG1[a]	STMS28	6	0.54	230–252
	TS12	13	0.64	245–300
LG2A[b]	GA20	7	0.83	130–205
	GA16	8	0.84	230–275
LG2B[b]	TA37	5	0.69	258–300
	TR19	11	0.87	206–274
	TA22s	11	0.86	192–280
	TA176s	12	0.82	210–280
LG4[c]	GAA47	2	0.47	154–170
	TA130	7	0.60	180–230
	TR20	7	0.62	148–178
	TA72	8	0.68	220–256
	TA2	6	0.79	130–182
	TS72	7	0.69	230–295
LG8[c]	TS45	7	0.68	224–250
	TA3	4	0.69	260–294
Unassigned	TA46	6	0.51	150–178

Linkage assignment is based on [a] Flandez-Galvez et al. (2003); [b] Cho et al. (2004);

[c] Winter et al. (2000)

Table 3 SSR allele distribution across 37 chickpea germplasm accessions differing in reaction to ascochyta blight

Germplasm accessionss	LG1		LG2A		LG2B				LG4						LG8		Un
	STMS28	TS12	GA20	GA16	TA37	TR19	TA22s	TA176s	GAA47	TA130	TA72	TR20	TS72	TA2	TS45	TA3	TA46
CDCEbony	b	cl	a	a	b	e	f	i	b	f	d	f	c	d	e	ad	d
CDCVanguard	a	hj	b	b	b	e	h	be	b	c	a	c	e	f	g	bc	d
304–31	a	hj	b	b	b	e	h	be	b	c	a	c	e	f	f	b	d
304–40	a	hj	b	be	b	e	h	be	ab	c	a	c	e	f	f	b	d
95NN12	c	ci	d	h	e	h	e	i	b	e	g	b	a	e	e	a	c
Amit	c	hj	c	a	a	h	b	j	a	c	a	c	f	b	c	a	d
CDCChico	ab	eg	e	f	a	d	e	h	a	c	a	c	e	a	ab	a	d
CDCFrontier	c	hj	f	g	a	g	g	i	a	c	a	c	e	b	c	b	d
FLIP82-150C	c	c	d	e	e	f	d	i	b	e	g	b	a	e	d	b	c
FLIP83-48	c	e	f	g	a	d	e	g	a	c	a	c	e	b	c	c	d
FLIP84-92C	c	hj	f	g	b	g	g	i	a	c	a	c	e	b	c	b	d
FLIP91-2	c	e	f	g	d	g	g	f	a	c	a	c	e	f	c	c	d
FLIP91-46	a	c	f	g	b	g	k	j	a	c	d	c	e	b	c	c	d
FLIP97-133C	c	hj	f	g	b	g	g	h	a	c	a	c	e	b	c	b	c
FLIP98-133C	b	eh	c	d	a	f	i	h	a	b	b	f	c	e	bd	c	d
ICC12004	e	bd	g	h	d	a	b	d	b	f	d	f	b	d	e	d	d
ICC12512-1	f	fl	c	a	a	e	f	i	b	c	a	d	d	c	f	a	a
ICC12952	d	g	e	f	b	f	f	i	b	b	b	d	d	c	d	a	b
ICC12961	c	hj	e	f	a	d	e	h	a	c	a	c	e	b	c	a	d
ICC1400	b	fj	d	e	c	e	h	k	b	b	a	d	b	c	c	c	a
ICC1468	0	cd	g	h	a	c	f	a	b	f	h	f	b	d	e	d	d
ICC14911	b	c	d	f	a	e	i	j	b	e	c	e	g	a	c	c	b
ICC1532	b	lm	e	e	b	j	c	f	b	a	e	g	f	a	e	b	e
ICC3996	e	bc	g	h	d	a	a	d	b	f	f	f	b	c	e	d	d
ICC4475	e	ad	g	h	d	b	a	c	b	f	f	f	b	c	d	d	d
ICC4936	d	g	d	f	a	f	f	h	b	b	b	d	d	c	d	a	b
ICC5124	c	hj	b	c	a	f	j	l	a	d	b	a	e	d	c	b	f
ICC76	e	bd	g	h	d	f	b	b	b	f	f	f	d	c	e	d	d
ILC202	c	c	e	f	c	i	e	h	a	c	a	c	f	b	c	b	d
ILC2506	c	hj	c	a	b	h	b	i	a	c	a	c	e	b	c	a	d
ILC2956	c	hj	e	f	a	d	e	h	a	c	a	c	e	b	c	a	d

Table 3 continued

Germplasm accessionss	LG1		LG2A		LG2B				LG4				LG8			Un
	STMS28	TS12	GA20	GA16	TA37	TR19	TA22s	TA176s	GAA47	TA130	TA72	TR20	TS72	TA2	TS45	TA3	TA46
ILC3279	c	c	e	f	b	i	e	h	a	c	b	c	e	b	c	b	d
ILC3856	c	hj	c	a	b	h	b	i	a	c	b	c	e	b	c	a	d
ILC5913	a	f	b	b	b	c	d	l	b	c	a	c	e	a	c	b	c
ILC5928	c	hk	d	a	b	i	e	i	a	c	b	c	F	a	c	a	c
ILC72	c	hj	e	f	a	d	e	h	a	c	a	c	E	b	c	b	d
Sanford	b	eg	e	f	a	d	e	i	a	c	a	c	E	a	b	b	d

Accessions with the same letter at a given SSR locus share the same allele

LG2B. Two additional genotypes (Sanford and FLIP83-48) also had alleles that were common with ILC72, except the allele for the TA176s locus (Table 3). Three desi genotypes (CDC Vanguard, 304–31 and 304–40) which are sister lines, had common alleles on LG2B region. There were ten and seven genotypes that had identical SSR alleles with ILC72 on LG1 and LG2A, respectively. At LG8, seven genotypes had alleles in common with ILC72. These included two resistance sources ILC3279 and FLIP84-92C.

The result of UPGMA cluster analysis based on the 17 SSRs associated with the QTL for resistance to ascochyta blight is presented in Fig. 1. The cluster analysis grouped the chickpea genotypes with identical SSR alleles and tended to group the genotypes based on seed types and country of origin. The Mantel Z test statistics showed a non-significant correlation ($r = 0.16$; $P = 0.99$) between the matrix of genetic relationships based on the QTL regions (Fig. 1) and the matrix of genetic relationships based on the whole genome (Fig. 2).

For the dendrogram based on the QTL regions, three groups were distinguished by truncating the dendrogram at the GS value of 0.164 (Fig. 1). The first group consisted of five germplasm accessions and cv. CDC Ebony, which was derived from a cross between ICC7524 and ICC1468. The germplasm in this group included ICC76, ICC1468, ICC3996, ICC4475 and ICC12004, which all are desi type. Each genotype in this group had a disease score of 5.0 or lower. Two were from India, while the origin of the others is unknown.

Seven genotypes, 4 desi and 3 kabuli, were clustered in group II. Two of the kabuli types in this group, 95NN12 and FLIP82-150C, are breeding lines developed at the University of Saskatchewan and ICARDA, respectively, whereas the origin of ICC14911 is unknown. Of the four desi types, two were collected from India, one from Greece and one of unknown origin.

Group III formed the major cluster, which consisted of twenty-three genotypes. The majority of genotypes in group III are of the kabuli seed type, except for CDC Vanguard, 304–31 and 304–40. Four subclusters were visible within group III at the cut-off value of 0.52. These subclusters tended to group the genotypes based on their country of origin, breeding institution or pedigree. CDC Vanguard and its sister lines (304–31 and 304–40) derived from a complex

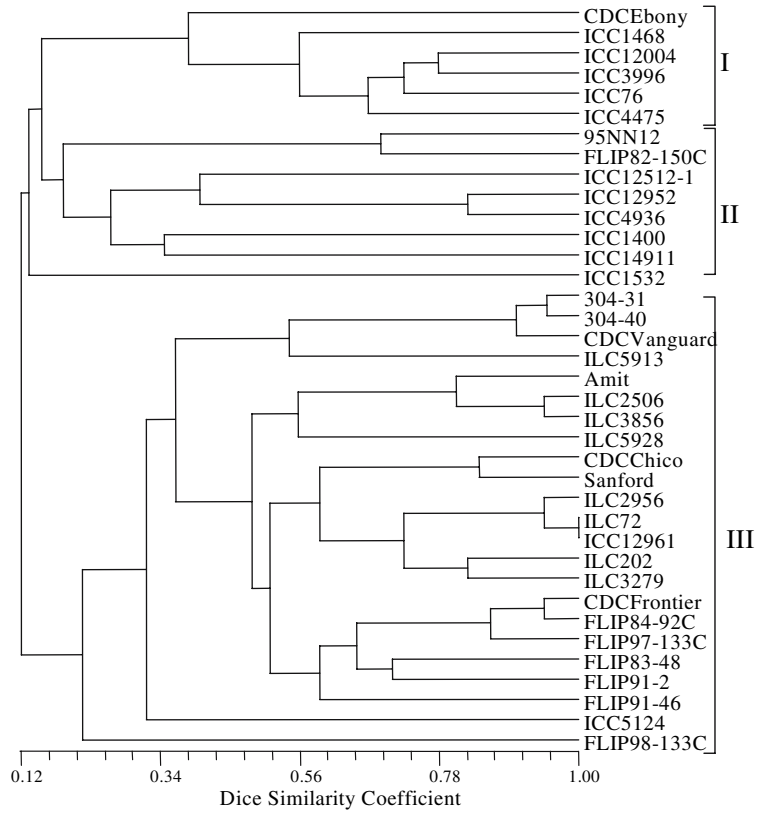

Fig. 1 UPGMA cluster analysis of 37 chickpea germplasm accessions differing in reaction to ascochyta blight based on 17 SSR loci at the QTL regions. Groups of accessions based on the cut-off value of 0.164 are indicated on the right

cross involving ICRISAT selections were grouped together with ILC5913. ILC2506, ILC3856 and ILC5928 were placed together with cv. Amit. Two genotypes (ILC3856 and ILC5928) in this subcluster originated from Morocco, whereas Amit was a selection from a landrace originating in Bulgaria. Five genotypes (ILC2956, ILC72, ICC12961, ILC202, ILC3279) which are landraces collected in the former Soviet Union formed a separate subcluster. Two susceptible cultivars (CDC Chico and Sanford) were placed within this group. The fourth subcluster consisted of five breeding lines developed at ICARDA and a cultivar (CDC Frontier) released by the University of Saskatchewan. Several genotypes that are distantly related based on the SSRs at the QTL regions were identified. These included ICC3996, ICC12004 and ILC2956.

Figure 3 shows the distribution of the disease scores in three segregating populations that were developed by crossing parental lines with distinct SSR alleles at the QTL for ascochyta blight resistance. For population A, the disease score of the F_2 plants ranged from 3.6 to 6.8 (mean = 4.35) while the disease score of ICC12004 and FLIP84-92C was 4.4 and 4.9, respectively. In population B, the scores of ICC4475 and CDC Frontier were 4.4 and 4.7, respectively. Disease scores varing from 3.5 to 7.0 were observed among the F_2 plants derived from the ICC4475 and CDC Frontier cross. The same trend was also observed on population C derived from the ICC3996 × Amit cross from which disease scores ranging from 3.4 to 7.0 were observed. F_2 plants both with enhanced levels of resistance and higher disease scores than the parents were found in each of the F_2 populations. Moderate amounts of genetic coefficient of variations (19–25%) exist within these populations (Table 4). Broad sense heritability estimates ranged from 0.38 to 0.43. At 5% selection intensity, the estimated genetic gain as % of the mean population ranged from 12% for ICC12004 × FLIP82-94C cross to 16% for ICC3996 × Amit cross suggesting that there were some different alleles for resistance to asscochyta blight, in the two parental lines. These results suggest that it is possible to develop chickpea cultivars with a higher level of resistance by accumulating resistance alleles from genetically distant sources.

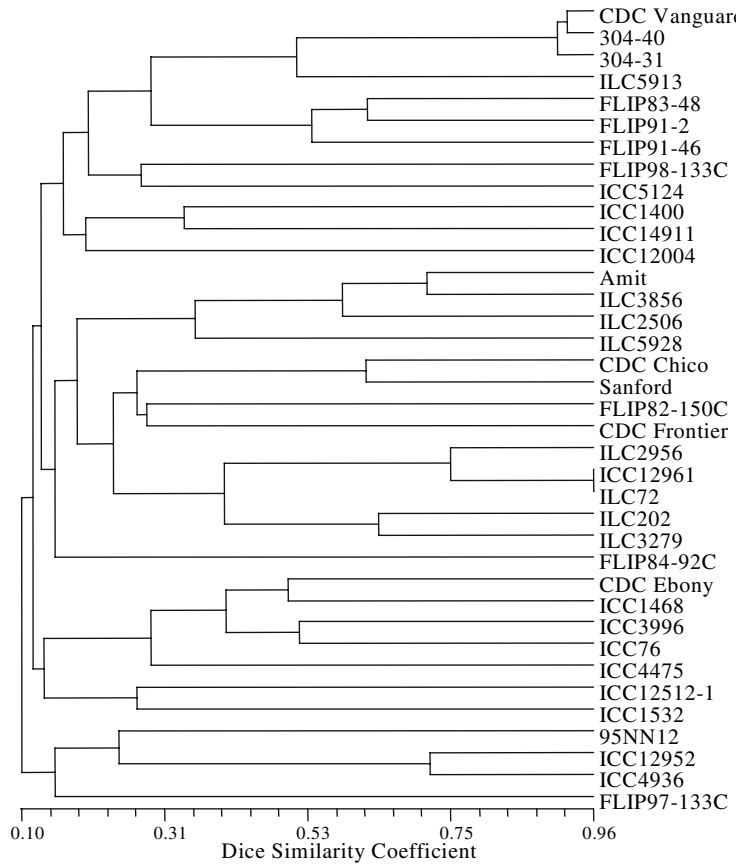

Fig. 2 UPGMA cluster analysis of 37 chickpea germplasm accessions differing in reaction to ascochyta blight based on 24 SSR loci distributed over eight linkage groups of the chickpea linkage map

Discussion

The current study provides an illustration of allele diversity at SSR loci associated with QTL for ascochyta blight resistance across a diverse collection of chickpea germplasm accessions. The hierarchical clustering based on these SSR alleles enabled us to differentiate three major groups of these chickpea germplasm accessions differing in reaction to ascochyta blight (Fig 1). The largest group (group III) was dominated by accessions of kabuli seed type. Within this group, the SSR alleles of the landraces collected from the former Soviet Union such as ILC72, ILC2506, ILC2956 and ILC3279 were the most common among the kabuli genotypes. This was expected since ILC72 and ILC3279 have been widely used as sources of ascochyta blight resistance around the world (Crino 1990; Muehlbauer et al. 1998, 2004; Muehlbauer and Kaiser 2002; Millan et al. 2003; Rubio et al. 2004). Pedigree information also demonstrated that ILC72 was used as the donor for the resistance in FLIP84-92C (Tekeoglu et al. 2000). FLIP84-92C is a moderately resistant germplasm accession that has been frequently used for studying the genetics of resistance to ascochyta blight (Santra et al. 2000; Tekeoglu et al. 2002; Cho et al. 2004). Our disease screening revealed that FLIP84-92C had slightly better resistance to ascochyta blight compared to ILC72 suggesting that FLIP84-92C may also have inherited the resistance alleles from the other parent (ILC215). Two cultivars (CDC Chico and Sanford), which were initially released as moderately resistant to ascochyta blight, also had ILC72 in their background. Under Saskatchewan conditions, the occurrence of new pathotypes or the increased of aggressiveness of the current of *A. rabiei* pathotypes has overcome the resistance in these cultivars. Our analysis demonstrated that CDC Chico and Sanford shared common SSR alleles with ILC72 on LG2A, LG2B and LG 4 except for TA176s and TA2 loci on LG2B and LG4, respectively. Their SSR profiles were distinctly different for the QTL regions at LG1 and

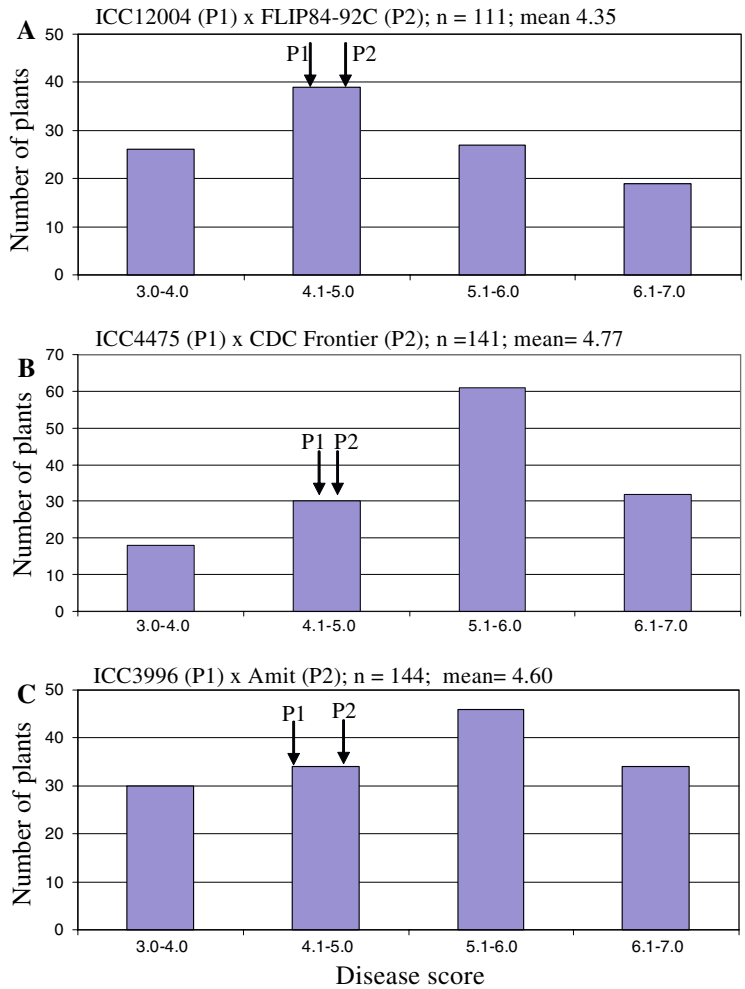

Fig. 3 Frequency distributions of three F_2 populations of chickpea derived from crosses of moderately resistant parents (A = ICC12004 × FLIP84-92C; B = ICC4475 × CDC Frontier; C = ICC3996 × Amit). The number of F_2 plants (n) for each population and mean disease score for each populations are presented. Arrows show disease score for each parental line in each population. The disease was rated using a 0 to 9 scale, where 0 = no symptoms and 9 = plants dead

LG8. Further analyses are needed to examine if these differences may contribute to the maintenance of resistance in ILC72 under Saskatchewan conditions.

The SSR allele diversity analysis demonstrated a highly conserved allele combination for the SSR across the QTL regions on LG4 compared to the QTL on other linkage groups. These results suggested that this region might have been targeted for selection for ascochyta blight resistance reducing the overall variation compared to other genomic regions.

Table 4 Mean ascochyta blight (AB), genetic coefficient of variation (GCV), heritability (H^2) and predicted genetic advance (GA) of three F_2 populations derived from crosses of moderately resistant genotypes

Cross	Population size	Mean AB	GCV (%)	H^2	GA (%)
ICC12004 × FLIP84-92C	111	4.35	19	0.38	12
ICC4475 × CDC Frontier	141	4.77	23	0.41	14
ICC3996 × Amit	144	4.60	25	0.43	16

The relationships among the chickpea germplasm accessions as revealed by the SSR alleles at the QTL regions were not correlated with those based on the SSR loci derived from other regions of different linkage groups, suggesting that the diversity at the QTL regions may not reflect the overall diversity at the whole genome. However, to some extent, sub-clusters containing few genotypes that had common parents in their pedigree were consistent on both dendrograms. For example, the sub-cluster of CDC Vanguard, 304–431 and 304–40, which are sister lines, were clustered together on both dendrograms.

Several genotypes such as ICC3996, ICC12004 and ILC2956 were distantly related based on the SSRs at the QTL regions. These lines might be used as sources of resistance to broaden the genetic base for the newer cultivars. For example, the SSR alleles on LG2A + B and LG4 in ICC3996 were relatively rare in this germplasm collection and were completely different from those in ILC72 and ILC3279. Moderate amounts of genetic variability exist within the population derived from a cross between ICC3996 and Amit. Disease evaluation of this population demonstrated some transgressive segregants with enhanced resistance and some with increased susceptibility compared to the parents. These results suggested that there were different minor resistance genes with additive gene actions in each of the two parental lines. The same results were also found from the other test populations from crosses between ICC12004 × FLIP84-92C and ICC4475 × CDC Frontier. The estimated genetic gain from selection in these three test populations averaged 14%. These results suggest that it is possible to develop chickpea cultivars with a higher level of resistance by accumulating resistance genes from different sources.

The current analysis of the genetic diversity using SSRs at the QTL regions associated with resistance to ascochyta blight suggest that the SSR alleles of the germplasm originating from the former Soviet Union were relatively common among the collection of chickpea germplasm accessions used in the study. Available pedigree information also showed that only a few sources were widely used in breeding programmes to develop resistant cultivars. Several potential sources of resistance from germplasm or breeding lines from different geographical origins may be used in breeding programmes in combination with adapted varieties to develop better and possibly more durable resistance to ascochyta blight. The current analyses provided information on genotypes with distinct genetic backgrounds at genomic regions associated with the QTL for ascochyta blight resistance. Our evaluation using three F_2 populations derived from crosses of moderately resistance parents with diverse genetic backgrounds at regions associated with resistance suggest that it is possible to recover progeny with better resistance to ascochyta blight than either parent.

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