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Mapping and validation of QTLs for resistance to an Indian isolate of Ascochyta blight pathogen in chickpea

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Abstract Ascochyta blight (AB) caused by *Ascochyta rabiei*, is globally the most important foliar disease that limits the productivity of chickpea (*Cicer arietinum* L.). An intraspecific linkage map of cultivated chickpea was constructed using an F_2 population derived from a cross between an AB susceptible parent ICC 4991 (Pb 7) and an AB resistant parent ICCV 04516. The resultant map consisted of 82 simple sequence repeat (SSR) markers and 2 expressed sequence tag (EST) markers covering 10 linkage groups, spanning a distance of 724.4 cM with an average marker density of 1 marker per 8.6 cM. Three quantitative trait loci (QTLs) were identified that contributed to resistance to an Indian isolate of AB, based on the seedling and adult plant reaction. QTL1 was mapped to LG3 linked to marker TR58 and explained 18.6% of the phenotypic variance (R^2) for AB resistance at the adult plant stage. QTL2 and QTL3 were both mapped to LG4 close to four SSR markers and accounted for 7.7% and 9.3%, respectively, of the total phenotypic variance for AB resistance at seedling stage. The SSR markers which flanked the AB QTLs were validated in a half-sib population derived from the same resistant parent ICCV 04516. Markers TA146 and TR20, linked to QTL2 were shown to be significantly associated with AB resistance at the seedling stage in this half-sib population. The markers linked to these QTLs can be utilized in marker-assisted breeding for AB resistance in chickpea.

Keywords Ascochyta blight · Chickpea · *Cicer arietinum* · Genetic linkage map · QTL mapping · SSR markers

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Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated edible food legume, highly valued as a good source of protein (20–23%). It is grown on over 10 M ha across more than 50 countries worldwide. India is a major producer of chickpea and represents 65% of the world's total chickpea production (FAOSTAT data 2006). The average yield of chickpea is low (780 kg ha^{-1}) and has only marginally improved over the last three decades. Among the several abiotic and biotic constraints that limit productivity, Ascochyta blight (AB) caused by the pathogen *Ascochyta rabiei* (Pass.) Labr., is a major disease causing significant losses in grain yield and quality (Gaur and Singh 1996; Pande et al. 2005). Disease development is favored by cool and wet weather conditions, often resulting in

100% yield losses (Reddy et al. 1990; Singh et al. 1992; Singh and Reddy 1993). The pathogen spreads via airborne spores and although the disease can be controlled by fungicide treatment, this is not economical for most developing country farmers. Consequently, breeding efforts have focused on the development of resistant germplasm, based on host plant resistance. Developing chickpea varieties with high levels of resistance to AB has been challenging because of the following factors: (i) paucity of high levels of resistance in the primary genepool, (ii) complex genetic basis of resistance conferred by several quantitative trait loci (QTLs), (iii) high pathogen variability, and (iv) the emergence of new pathotypes due to natural recombination through a sexual reproduction phase in the AB life cycle (Pande et al. 2005).

There have been many reports of low levels of genetic polymorphism in cultivated chickpea (Ahmad et al. 1992; Udupa et al. 1993; Labdi et al. 1996). As a result of limited polymorphism, interspecific mapping populations of *C. arietinum* and *C. reticulatum* have been developed and used to create skeletal linkage maps based on isozymes (Gaur and Slinkard 1990a, b; Kazan et al. 1993), restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers (Simon and Muehlbauer 1997). A comprehensive interspecific linkage map was first generated using a diverse range of markers including simple sequence repeats (SSR), DNA amplification fingerprints (DAF), amplified fragment length polymorphisms (AFLP), inter-simple sequence repeats (ISSR), RAPD, isozymes, sequence characterized amplification regions (SCAR) and disease resistance analogues (Winter et al. 2000). Forty-seven gene specific markers were subsequently added to this map which has become the basic reference map in chickpea (Pfaff and Kahl 2003). SSR markers from this reference map have been extensively used for genetic linkage analysis of chickpea including the mapping of genes for disease resistance and agronomically important traits (Tekeoglu et al. 2002; Benko-Iseppon et al. 2003; Rakshit et al. 2003; Pfaff and Kahl 2003; Abbo et al. 2005). Another interspecific linkage map (*C. arietinum* × *C. echinospermum*) was developed using RAPD, SSR, ISSR and RGA markers (Collard et al. 2003).

Genetic maps constructed from an interspecific cross may not represent the true recombination distance (cM) and map order found in the cultivated genome. Polymorphic loci identified in interspecific maps may be monomorphic in cultivated genotypes and thus have little direct application in breeding programs. Thus, intra-specific maps have been generated utilizing SSR, DAF, AFLP. STMS, ISSR and RGA markers and are being increasingly applied in chickpea mapping (Cho et al. 2002; Flandez-Galvez et al. 2003a; Udupa and Baum 2003; Cho et al. 2004). More recently, new SSR markers have been developed and utilized for mapping QTLs conferring resistance to AB (Sethy et al. 2003; Lichtenzveig et al. 2005; Tar'an et al. 2007).

Recent studies have confirmed the quantitative inheritance of AB resistance in chickpea. Two to seven QTLs associated with resistance to AB at seedling/adult plant stage have been reported in both interspecific (Santra et al. 2000; Tekeoglu et al. 2000, 2002; Collard et al. 2003) and intraspecific populations (Flandez-Galvez 2003b; Udupa and Baum 2003; Cho et al. 2004). As there are variations in the pathotypes of AB pathogen, there is a need to identify and validate QTLs against AB pathotypes prevalent in the target region. Thus, this study was conducted to identify and validate QTLs for resistance to a highly virulent Indian isolate of AB. Intraspecific populations were used in this study so that the markers identified would have a high probability of being polymorphic in breeding programs.

Materials and methods

Plant material

The AB resistant cultivar used in the study was ICCV 04516 which was developed at ICRISAT-Patancheru from a double cross [(C 235 × NEC 138-2) × (FLIP 87-4C × ILC 4421)]. It showed a consistent disease score of 3–4 (on a scale of 1–9, where 1 = most resistant and 9 = most susceptible) in AB resistance when screened under controlled environment conditions. It was crossed with ICC 4991 (Pb 7), a cultivar highly susceptible to AB with a disease score 9. SSR markers were used to identify the F_1 hybrids and only confirmed hybrids were advanced to develop the F_2 population. A total of 179 F_2 plants were developed to construct an intraspecific linkage map and identification of AB resistance QTLs conferring AB resistance at the adult plant stage. While $F_{2:3}$ progenies were

used for QTL identification at the seedling stage. Identified markers associated with QTLs were subsequently validated in another F_2 population ($n = 94$) generated from the cross ICCV 10 × ICCV 04516.

DNA extraction and genotyping

Total genomic DNA was isolated from 20 to 30 mg leaf tissue harvested from 14 day old seedlings using a CTAB based, high throughput DNA extraction protocol described by Mace et al. (2004). The parents of the mapping population ICC 4991 and ICCV 04516 were pre-screened with 232 SSR markers (Winter et al. 1999; Hüttel et al. 1999), 108 EST markers (Buhariwalla et al. 2005) and 15 chickpea RGA markers (Hüttel et al. 2002) to identify polymorphic markers. PCR amplification was achieved in a 5 µl reaction volume containing between: 10 and 15 ng of genomic DNA; 0.2–0.6 pico moles of forward and reverse primer; 0.1–0.25 mM of each dNTP; 1.0–3.5 mM $MgCl_2$; 0.1–0.5 U of Taq DNA Polymerase (Bioline) and 1× Buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) using the specific concentrations optimized for each primer. The optimized concentrations of the different PCR reagents were determined for each primer using an adapted Cobb and Clarkson 5 grid optimization protocol (Buhariwalla et al. 2005). PCR amplifications were carried out using one of the three PCR touchdown amplification programmes viz Cp65-60, Cp60-55 and Cp55-45 optimized for each primer depending upon the Tm value of the primer as described by Buhariwalla et al. (2005). Amplifications were carried out in 384-well plates using Perkin Elmer Gene-Amp PCR Sys 9700 (Norwalk Conn.) thermal cycler. Amplified products were electrophoresed on 6% PAGE at 650 V in 0.5× TBE buffer for 3.5 h. PAGE gels were stained using a modified silver staining protocol (Buhariwalla et al. 2005). Alternatively, capillary electrophoresis was used where PCR amplification was carried out using fluorescent-labeled primers (FAM, PET, NED and VIC). PCR amplification products (1.0 µl each) were multiplexed, in a mixture of high Hi-Di™ Formamide containing an internal size standard LIZ (500), denatured for 5 min at 94°C and separated by capillary electrophoresis using an ABI PRISM 3700 (ABI, Foster City, CA, USA). Fragment sizes generated by the different SSR markers were calculated by comparison with internal standard GeneScan-500 LIZ using Genotyper Version 3.7 software (Applied Biosystems). Polymorphic markers were scored across segregating populations. Data was recorded as A for the allele (band) of susceptible parent, B for the allele (band) of resistant parent and H for the heterozygotes. Similarily, in case of CE, alleles corresponding to the susceptible and resistant parents corresponded to peaks were analyzed.

Disease resistance evaluation

Inoculum preparation

A single spore isolate of a virulent culture of *A. rabiei* collected from infected chickpea plants in Hissar (northern India) was multiplied on sterile seeds of chickpea genotype ICCV 88901. Chickpea seeds were soaked overnight in water, autoclaved at 121°C for 25 min, and inoculated with a 1 cm disc of an actively growing culture of *A. rabiei* on Czapek Dox Agar. Inoculated seeds were incubated at 20°C for 8 days and 12 h photoperiod. Profusely sporulated seeds were stirred in sterile distilled water (SDW) to facilitate the release of pycnidiospores into the water. The suspension was filtered through a muslin cloth and diluted to 50,000 spores/ml and used as an inoculum.

Adult plant resistance

A cut-twig method was used to evaluate AB resistance in 80 day old F_2 plants. Three twigs each with a minimum of five pinnules were collected from individual F_2 plants, wrapped with a cotton plug and placed in test tubes (15 × 100 mm) filled with SDW. The twigs were transferred to a controlled environment facility, maintained at a temperature of 20 ± 1°C under a light intensity of ~1,500 lux with a photoperiod of 12 h a day. Inoculum was sprayed on to the foliage using a hand-operated atomizer. Thereafter, 100% relative humidity (RH) was provided for the initial 4 days after inoculation (DAI) and later 100% RH was maintained for 6–8 h a day until 10 DAI. The disease severity was scored 10 DAI on a 1–9 disease scale. The mean data over the three repetitions was used for QTL mapping.

Seedling resistance

Seedlings of the $F_{2:3}$ families (24 plants each) along with the susceptible check ICC 4991 were raised in

plastic trays (40 × 30 × 5 cm) filled with a sand and vermiculite mixture (10:1), maintained in a greenhouse at a temperature of 25 ± 3°C with a 12–13 h photoperiod. Pathogen inoculum was sprayed on to the foliage of 10 day old seedlings which were incubated under conditions as described above in the cut twig method. Disease severity was scored at 10 DAI. Eight seedlings for each $F_{2:3}$ progeny were raised in three replications in a randomized design. The mean data over the three replicates was used to compute the best linear unbiased predictors (BLUPs) of the random effect in restricted maximum likelihood (REML) variance components analysis using Genstat version 8.0 with replicates as fixed models and genotypes as random effects. BLUPs were used for QTL mapping.

Disease scoring

Ascochyta blight disease severity on inoculated seedlings and cut-twigs was scored on a scale of 1–9, where 1 = no symptoms, 2 = minute lesions prominent on the apical stem, 3 = lesions up to 5 mm in size with slight drooping of the apical stem, 4 = obvious lesions on all plant parts with clear drooping of the apical stem, 5 = obvious lesions on all plants parts, defoliation initiated with slight to moderate breaking and drying of branches, 6 = lesions as in 5, defoliation, broken, dry branches common, some plants killed, 7 = lesions as in 5, defoliation, broken, dry branches very common, up to 25% of the plants/twigs killed, 8 = symptoms as in 7 but up to 50% of the plants/twigs killed, 9 = symptoms as in 7 but up to 100% of the plants/twigs killed. Based on the disease score, the plants were categorized for their reaction to AB infection as follows: 1 = immune (I); 1.1–3 = resistant (R); 3.1–5 = moderately resistant (MR), 5.1–7 = susceptible (S) and 7.1–9 = highly susceptible (HS).

Data analysis

Linkage map construction

A genetic linkage map with segregating markers was constructed using Join Map 3.0 Software (Van Ooijen and Voorrip 2001) based on principles described by Stam (1993). A Logarithm of odds (LOD) score >3.0 was used to create linkage groups. Recombination values were converted to genetic distances using the Kosambi (1944) mapping function.

QTL mapping

QTL mapping was done using the software QTL Cartographer Version 2.0 (Wang et al. 2003) using the AB disease data of the F_2 population and $F_{2:3}$ progenies. Phenotypic data of both F_2 and $F_{2:3}$ generations was used separately to identify adult plant and seedling resistance QTLs, respectively. The CIM (composite interval mapping) method (Jansen and Stam 1994; Zeng 1994) was used to position the QTLs. Forward and reverse regression analysis was employed for QTL detection. Cofactors were selected by the program using Model 6 with genetic background controlled by five markers and window size set at 10 cM. All the linkage groups were scanned at a minimum default threshold of LOD 2.4 with 300 permutations ($P < 0.05\%$). The phenotypic variance explained by a single QTL was estimated by the square of the partial correlation coefficient (R^2). Estimates of R^2 value and additive effects for each QTL at its peak LOD position were obtained from the QTL analysis using Zmapqtl program of QTL Cartographer. For validation studies the data from the genotyping was subjected to regression analysis against the F_2 AB disease scores using Genstat version 8.0 to compute the phenotypic variance explained by the markers.

Results

Intraspecific linkage map of ICC 4991 × ICCV 04516

Ninety-six SSR markers (41.4%) from 232 markers used to screen the parental genotypes were consistently polymorphic. Only three out of 108 EST markers, AGLC11, AGLC29 and AGLC66 were polymorphic. All fifteen RGA markers screened were monomorphic. Each segregating marker was tested for goodness-of-fit to the expected 1:2:1 ratio using a χ^2 test ($P < 0.05$). Inspite of segregation distortion with a few polymorphic markers, all marker data were used for linkage analysis. A total of 84 markers (82 SSR and two EST), were mapped onto eight major and two minor linkage groups. Fifteen markers (15.5%) GA6, TAA169, TA11, CaSTMS10, TS29, AGLC66, TA118, TR5, TA136, CaSTMS25, GA26, TS46, GAA60, TA196 and CaSTMS21 remained unlinked.

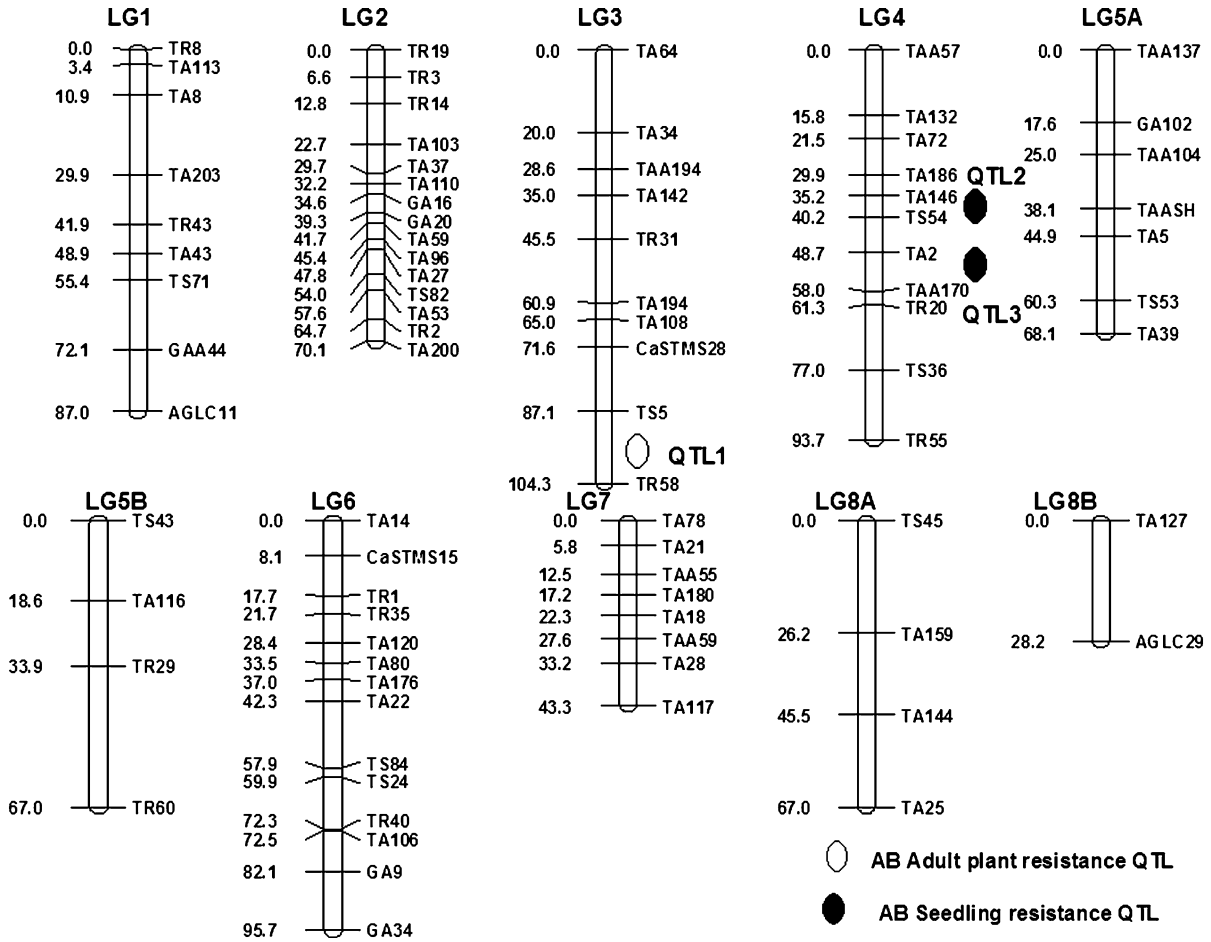

Fig. 1 Intraspecific genetic linkage map of ICC 4991 × ICCV 04516 depicting positions of Ascochyta blight resistance QTLs

An intraspecific genetic linkage map was constructed using the Kosambi mapping function applied to SSR and EST marker data (Fig. 1). Markers were included on the map if they had a LOD value >3.0. The total map length spanned a distance of 724.4 cM with an average marker density of 8.6 cM. Eight major linkage groups (LG1 to LG8) were assigned corresponding to the basic chromosome number of chickpea. LG5 and LG8 consisted of two minor subgroups LG5A, LG5B and LG8A, LG8B, respectively. These subgroups remained unlinked because of insufficient markers mapping to that region of the linkage group. The linkage groups were numbered in line with previously published intraspecific maps. AGLC11 a new EST marker was placed at the distal end of the LG1 and AGLC29 was placed at the distal end of LG8B. LG2 contained the highest number of evenly distributed markers. Forty-two markers on our map were placed in the same linkage groups as those reported by Winter et al. (2000).

Phenotypic variation

The mean disease severity scores of the resistant parent ICCV 04516 and susceptible parent ICC 4991 were 3.7 and 9.0, respectively. The frequency distribution of the average disease score of three cut twigs, across 179 adult F_2 plants and F_3 seedlings is presented in Fig. 2a, b. Segregation of the AB disease reaction was found to be continuous in both the generations reflecting a polygenic basis. However, a large number of plants were classified into major categories of moderate resistant class (3.1–5.0) and susceptible class (5.1–7.0). As expected only a few plants were either highly resistant or susceptible to AB. The mean AB disease score of eight seedlings for each $F_{2:3}$

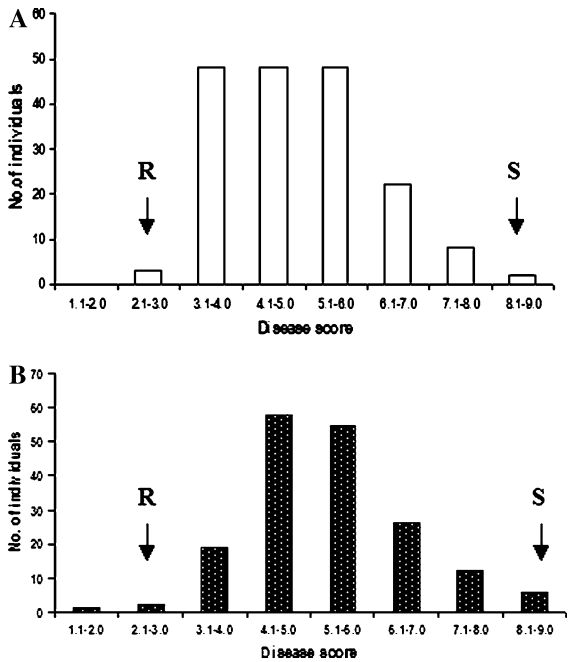

Fig. 2 Frequency distribution of disease scores for Ascochyta blight resistance. (**a**) F_2 population (cut-twig method) and (**b**) $F_{2:3}$ populations (seedling inoculation method)

progeny in the three replications were subjected to analysis of variance (ANOVA). The calculated *F* value was significant, at 1% level of significance, suggesting that the genotypes under consideration showed considerable variation in disease reactions to AB. The standard error of the mean (0.30) and standard error of difference (0.42) were low. The estimated variance and standard error were 1.24 and 0.16 respectively indicating there was a good amount of variation found for the character studied.

QTL mapping

Adult plant resistance

The phenotyping for resistance to Ascochyta blight disease was carried out in F_2 as well as $F_{2:3}$ progenies of ICC 4991 × ICCV 04516. F_2 plants (n = 179) were genotyped and screened against AB pathogen using the cut twig method, 80 day old plants were used. The destructive seedling screening method was avoided in F_2 population in order to be able to harvest seeds from all plants and thus develop an unbiased population of the next generation. Composite interval mapping (CIM) detected a peak on LG3 (Figs. 1, 3) and presence of a QTL (QTL1), 9.2 cM away from marker TR58 at a LOD of 2.03 (Table 1). This QTL explained 18.6% of phenotypic variance (R^2).

Seedling resistance

Composite interval mapping confirmed the presence of two QTLs (QTL2 and QTL3) both on LG4 flanked by SSR markers TA146, TS54, TA2 and TAA170 (Figs. 1, 4; Table 1). Both QTL2 and QTL3 accounted for 7.75% and 9.28% of the total phenotypic variance, respectively. The combined QTLs on LG4 explained over 17% of the total phenotypic variation for seedling resistance to AB in the $F_{2:3}$ population.

Validation of QTL markers in the mapping population derived from ICCV 10 × ICCV 04516

Eleven reported SSR markers associated with AB resistance, TA2, TS54, TA146, TS45, TA28, GA16,

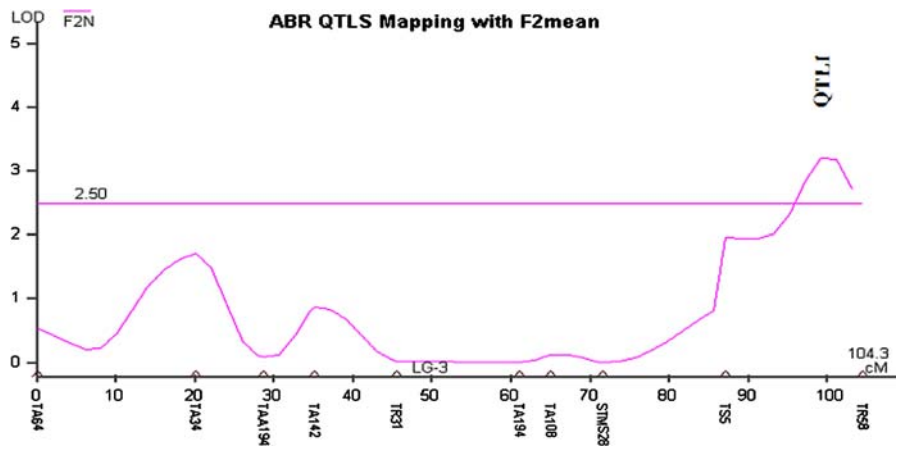

Fig. 3 Graphical representation of Ascochyta blight resistance QTL1 identified at adult plant stage on linkage group 3 of ICC 4991 × ICCV 04516, an intraspecific map with F_2 means

Table 1 Map location and estimated effects of quantitative trait loci governing adult plant and seedling resistance to Ascochyta blight in F_2 and $F_{2:3}$ populations based on composite interval mapping

Mapping population	Linkage group	Marker	Position	LOD	R^2	Additive effect
F_2 resistance at adult plant stage	LG3	TR58	95.11	2.03	0.18	0.67
$F_{2:3}$ resistance at seedling stage	LG4	TS54	40.21	2.68	0.08	0.53
$F_{2:3}$ resistance at seedling stage	LG4	TA2	50.71	2.15	0.09	0.57

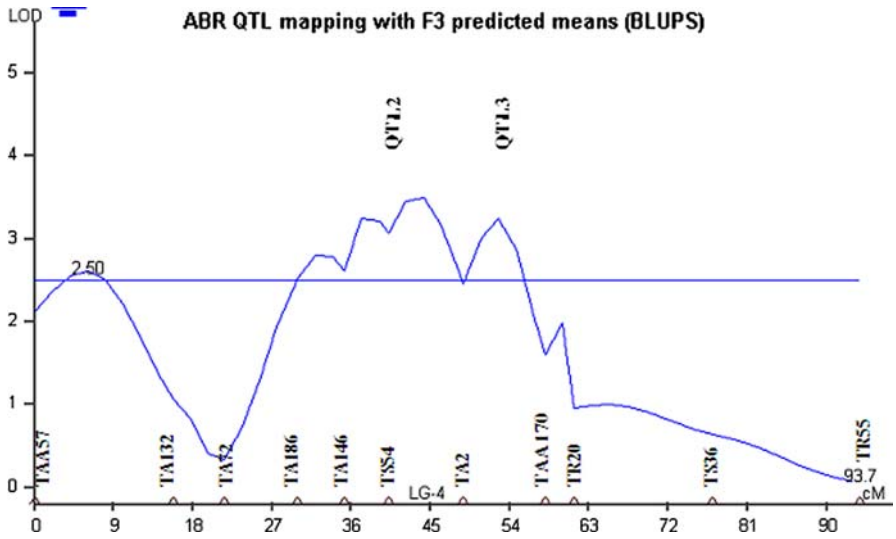

Fig. 4 Graphical representation of AB resistance QTLs (2 and 3) for seedling stage resistance on linkage group 4 of ICC 4991 × ICCV 04516, an intraspecific map with $F_{2:3}$ BLUPs

TA72, TR20, TA37, TA200 and GA20, were used to screen the parental genotypes ICCV 10 and ICCV 04516 which had been used to generate a half-sib validation population. Of these markers GA20, TA37, TA146, TS54, TR20 and TA2 were polymorphic and were used to genotype the entire validation population. Regression analysis of the genotype data against the F_2 AB disease scores was carried out to obtain the phenotypic variance explained by each marker. Marker TA146 was found to be significantly associated with the seedling resistance, and explaining 18.9% of the phenotypic variation followed by TR20 which explained 2.5% of the phenotypic variation (Table 2).

Table 2 Association of marker loci with Ascochyta blight disease reaction scores based on simple linear regression analysis of F_2 data

Population	Size	Markers	% Phenotypic variance	SE_m
F_2 (ICCV 10 × ICCV 04516)	94	TA146	18.89	1.19
		GA16	0.00	1.25
		TR20	2.50	1.23

Discussion

In this study we report the identification of QTLs from ICCV 04516 that are associated with resistance to a highly virulent Indian isolate of Ascochyta blight. Among the various DNA-based markers chosen for mapping, 41% of SSR markers screened were polymorphic, which is comparable with previous findings (Flandez-Galvez et al. 2003a; Hüttel et al. 1999; Udupa and Baum 2003). In contrast, the gene-based markers (108 EST markers) detected a low level of polymorphism between the parental genotypes. Only three markers, AGLC11 (arm repeat containing protein) AGLC29 (hypothetical protein) and AGLC66 (probable cystein proteinase), were polymorphic between the parental genotypes ICC 4991 and ICCV 04516. Two of these markers AGLC11 and AGLC29 were mapped to LG 1 and LG 8B, respectively. Gene-based markers offer a more precise tool for marker-assisted selection compared to linked markers but are clearly much more difficult to identify.

The estimated physical size of the chickpea genome is 750 mega base pairs (Arumuganathan and Earle 1991). Thus, the genetic distance of 1 cM is equivalent to approximately 1.4 Mbp (1,400 Kbp). On this basis at least 107 evenly distributed markers are required to provide an average marker density of 5 cM, which is the upper limit required for marker-assisted pyramiding of genes (Winter 1997). Despite the availability of interspecific linkage maps in chickpea (Winter et al. 1999, 2000; Santra et al. 2000; Collard et al. 2003), there has been little direct breeding application of markers from such maps as breeding populations are predominantly based on intraspecific crosses. This has led to an increasing focus on the development of genetic linkage maps based on intraspecific mapping populations. The first reported intraspecific maps included: 68 SSR markers distributed across 14 LGs (Cho et al. 2002); 52 SSR markers distributed across 8 LGs (Udupa and Baum 2003) and 53 SSR markers distributed across 8 LGs (Cho et al. 2004). In the present study, we have mapped 82 SSR and 2 EST markers across 8 major and 2 minor LGs. More recently a new set of SSR markers has been developed by Sethy et al. (2003) and Lichtenzveig et al. (2005) and these have been integrated into an existing linkage map by Tar'an et al. (2007) comprising 144 SSR markers. Distribution of markers across linkage groups was comparable to earlier published maps of Winter et al. (2000) and Millán et al. (2003), except that GAA44 was assigned to LG 1 in our map compared to LG 7 in the interspecific map of Winter et al. (2000) and Millán et al. (2003). Only four markers TS43, TA116, TR29 and TR60 were distributed across the second sub-group LG5B at an average density of 16.75. Udupa and Baum (2003) and Millán et al. (2003) assigned TS43 to LG5. TS43 and TR29 were designated on LG 5 by Winter et al. (2000). Based on these previous reports, LG5B was designated as a sub-group of LG 5. Though these two sub-groups were in the same grouping node when analyzed by Joinmap linkage analysis, they could not be joined due to insufficient linkage. Similarly two sub-groups LG8A (67 cM) and LG8B (28.2 cM) were considered to be part of linkage group 8 as marker TA127 has been mapped to LG 8 in a skeletal map based on F_2 mapping population derived from the cross ILC 272 × ILC 3279 (H.K. Buhariwalla, unpublished results).

Accurate phenotyping is a pre-requisite for QTL mapping. Here we used two highly reproducible techniques for resistance screening under controlled environment conditions; the cut twig and seedling inoculation methods, based on screening techniques reported previously (Pande et al. 2005). The controlled environment screening methods used in this study have specific advantages over field screening techniques, including: minimizing environmental variability especially periods of high humidity and also enabling the evaluation of resistance against a single pathogen isolate. Cho et al. (2004) used a controlled environment screening method to identify AB isolate specific resistance QTLs. We have found the cut-twig method used in this study to be non-destructive and valuable in phenotyping F_2 populations. Additionally, the results from this screening method correlate well to that of the seedling inoculation method.

The frequency distribution of AB disease reaction phenotypes at adult plant and seedling stages resembled a quantitative trait and are in concordance with previous reports (Santra et al. 2000; Cho et al. 2004). Among the three QTLs identified, QTL1 contributed to resistance at the adult plant stage, whereas QTL2 and QTL3 contributed to resistance at the seedling stage. QTLs conditioning AB resistance during the different plant development stages have been reported previously (Collard et al. 2003). In the present study we identified a new QTL (QTL1) that contributed to resistance during the adult plant stage and was associated with the marker TR58. In addition, QTL2 and QTL3 detected by composite interval mapping (CIM) in the $F_{2:3}$ progenies contributed to resistance during the seedling stage. Interestingly, markers TA146 and TS54, associated with these QTLs were previously reported to be linked to AB resistance QTL3 (Tekeoglu et al. 2004) and QTL5 (Flandez-Galvez et al. 2003b). Similarly, the markers TA2, TS54 and TA146 linked to QTL2 and 3 were also found to be associated with AB resistance QTL2 reported by Tekeoglu et al. (2004).

All published reports suggest a polygenic basis of AB resistance, controlled by two QTLs (Tekeoglu et al. 2002), three QTLs (Santra et al. 2000), four QTLs (two for seedling resistance and two for adult plant resistance; Collard et al. (2003)) were identified using intraspecific mapping populations. Similarly, three QTLs (Udupa and Baum 2003), five QTLs (Cho et al. (2004) and seven QTLs (Flandez-Galvez et al. 2003a) were identified from studies that used interspecific populations.

The successful use of MAS for AB resistance requires validation of linked markers to QTLs across populations and environments. In this study, we were able to validate the marker TA146 linked to QTL2 associated with AB resistance at the seedling stage in a half-sib population derived from ICCV 10 × ICCV 04516. In addition, the QTL marker TR20 reported by Flandez-Galvez et al. (2003b) was also validated using the same half-sib population challenged with the Indian isolate of AB. These results suggest that QTLs linked to markers TA146 and TR20 are effective for AB resistance across environments, diverse mapping populations (both intra and interspecific) and against a different isolates of the pathogen.

In conclusion, we developed a new intraspecific genetic linkage map from the cross between Pb 7 (ICC 4991) and ICCV 04516 spanning a distance of 724.4 cM with an average unit marker density of 8.6 cM. Eighty-two SSR markers along with two EST markers were mapped to ten linkage groups (8 major and 2 minor groups). One QTL associated with adult plant resistance was identified on LG3 and two QTLs for AB resistance at the seedling stage were identified on LG4. Together, the three QTLs explained over 36% of the total phenotypic variation for AB resistance. The SSR markers, TA146 and TR20 linked to the QTLs were further validated across environments, diverse mapping populations (both intra and interspecific) and against a highly virulent Indian isolate of AB. These markers are now ready for routine use in marker-assisted breeding of AB resistance in chickpea.

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