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QTL analysis for ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.)

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Abstract In both controlled environment and the field, six QTLs for ascochyta blight resistance were identified in three regions of the genome of an intraspecific population of chickpea using the IDS and AUDPC disease scoring systems. One QTL-region was detected from both environments, whereas the other two regions were detected from each environment. All the QTL-regions were significantly associated with ascochyta blight resistance using either of the disease scoring systems. The QTLs were verified by multiple interval mapping, and a two-QTL genetic model with considerable epistasis was established for both environments. The major QTLs generally showed additive gene action, as well as dominance inter-locus interaction in the multiple genetic model. All the QTLs were mapped near a RGA marker. The major QTLs were located on LG III, which was mapped with five different types of RGA markers. A CLRR-RGA marker and a STMS marker flanked QTL 6 for controlled environment resistance at 0.06 and 0.04 cM, respectively. Other STMS markers flanked QTL 1 for field resistance at a 5.6 cM interval. After validation, these flanking markers may be used in marker-assisted selection to breed for elite chickpea cultivars with durable resistance to ascochyta blight. The tight linkage of RGA markers to the major QTL on LG III will allow map-based cloning of the underlying resistance genes.

Keywords *Cicer arietinum* · Ascochyta blight · Disease resistance · QTLs · RGA

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Introduction

Chickpea (*Cicer arietinum* L.), a self-pollinating diploid (2n=16), is the third most important food legume worldwide (FAO 1996). Major producing countries include India, Turkey, Canada, Pakistan, Australia and Mexico; with Australia the largest chickpea exporter (FAOSTAT Database 2002). In spite of the availability of high yielding cultivars, the production of chickpea has been hampered by the widespread incidence of ascochyta blight—the most destructive foliar disease of chickpea. Ascochyta blight, caused by the fungal pathogen *Ascochyta rabiei* (Pass.) Lab., attacks all aerial parts of the plant at all growth stages, and can cause up to 100% yield loss (Nene and Reddy 1987; Jimenez-Diaz et al. 1993; Acikgoz et al. 1994).

In 1998 the disease affected all of the major chickpea growing regions of Australia, reducing the country's chickpea exports by 50% (FAOSTAT Database 2002). International breeding efforts have been directed towards the development of elite cultivars of chickpea with durable resistance to ascochyta blight. Genetic stocks for broad-based resistance were identified in accessions of several wild *Cicer* species (Singh et al. 1981; Haware et al. 1992; Singh and Reddy 1993; Stamigna et al. 1998; Collard et al. 2001). However, most of these novel resistance sources could not be readily incorporated into breeding programs because of reproductive barriers. This is particularly the case for the far-distant wild relatives

Cicer bijugum and *Cicer pinnatifidum*, within which sources for potential immunity were detected (Ahmad et al. 1988; Haware et al. 1992; Singh and Reddy 1993; Collard et al. 2001). Novel sources of resistance are therefore sought from within the cultivated species, for the immediate improvement of current chickpea cultivars. Fortunately, various sources of resistance were identified within the cultivated gene pool, and have been used in several genetic and breeding studies (Reddy and Singh 1992; Singh 1997a; b; Galvez et al. 2000; Meredith et al. 2000; Santra et al. 2000; Tekeoglu et al. 2000).

The current understanding of the genetics of ascochyta blight resistance (ABR) in chickpea strongly suggests polygenic inheritance of the trait. Tekeoglu et al. (2000) showed that in intra- and inter-specific recombinant inbred lines (RILs), ABR was conferred by at least three recessive and complementary genes with several modifiers. In an interspecific genetic background, Santra et al. (2000) mapped two quantitative trait loci (QTLs) which conditioned ABR over two years of field screening. Likewise, preliminary QTL mapping in a wide-cross between *C. arietinum* and *Cicer echinospermum* (resistance source) revealed two to three QTLs for seedling resistance in controlled glasshouse bioassays (Collard et al. 2003). Although the genetic mechanism of ABR has been studied in identified resistant accessions of *C. arietinum*, the number and genomic locations of the genes or QTLs conditioning resistance has yet to be verified.

The genetic complexity of both the resistance mechanism of chickpea and the pathogenicity of *A. rabiei*, has compelled resistance breeding towards pyramiding of broad genetic sources into elite genotypes via marker-assisted selection (MAS; Singh 1997b; Van Rheenen and Haware 1997). The increased efficiency of MAS compared to conventional breeding methods has been demonstrated in several important crops (e.g. Inukai et al. 1996; Young 1996; Eathington et al. 1997). In *Cicer*, molecular and isozyme markers linked to ABR-QTLs have been identified in *C. arietinum* × *Cicer reticulatum* (Santra et al. 2000; Rajesh et al. 2002; Tekeoglu et al. 2002) and *C. arietinum* × *C. echinospermum* (Collard et al. 2003) interspecific populations. Recently, in another intraspecific *C. arietinum* population, a major locus and two independent recessive loci were also identified by Udupa and Baum (2002) to confer resistance to the pathotype I and II of *A. rabiei*, respectively.

The limited number of QTL studies for ABR, particularly in a pure *C. arietinum* genetic background, has been primarily due to the limited number of intraspecific linkage maps available for the chickpea genome. Map-based approaches to identification and analysis of QTLs conferring various quantitative traits have been successfully utilized in other crops (e.g. Paterson et al. 1991; Bonierbale et al. 1994; McCouch and Doerge 1995; Michelmore 1995; Paterson 1996; Young 1996; Mohan et al. 1997, Cho et al. 2003). To-date, only two intraspecific linkage maps have been reported in chickpea that were based on populations segregating for ABR (Galvez et al. 2002; Udupa and Baum 2002; Flandez-Galvez et al. 2003).

Using an intraspecific population segregating for ascochyta blight resistance and a linkage map of chickpea by Flandez-Galvez et al. (2003), this study aimed to (1) analyze the QTLs conditioning ascochyta blight resistance in the field and controlled environments, and (2) understand the genetic mechanism of resistance based on QTL effects and inferred gene actions.

Materials and methods

Mapping population and the STMS-based linkage map

An intraspecific population of chickpea derived from a cross between the desi cultivars ICC1 2004 (ascochyta blight resistant) and Lasseter (highly susceptible), was used as a mapping population. The framework map was based on 85 F_2 progenies, and consisted of 51 sequence-tagged microsatellite sites (STMS), three inter-simple sequence repeats (ISSR) and 12 resistance gene analog (RGA) markers (Flandez-Galvez et al. 2003). Chickpea is a self-pollinating diploid (2n=16). The linkage map comprised eight linkage groups of the chickpea genome with a total coverage of 534.5 cM and an average marker density of 8.1 cM. F_3 progenies derived from open-pollination of each F_2 plant ($F_{2:3}$), were used to infer the disease reaction of the F_2 plants in the mapping population. The $F_{2:3}$ families were generated at the Victorian Institute for Dryland Agriculture (VIDA), Victoria, Australia (Meredith, pers communication).

Ascochyta blight infection trials

Ascochyta blight screening was conducted simultaneously in two environments: (1) in a controlled environment (20±2°C and a 12-h photoperiod) at the University of Melbourne, Victoria, and (2) in the field-screening nursery at VIDA, Horsham, Victoria from July to October 2001. Late winter/spring is the best time of the year to conduct ascochyta blight screening in the field in this location, as temperature and rainfall are optimal for infection. For the controlled environment trial, a virulent single-spore isolate of *A. rabiei* was used as the inoculum. Preparation of inoculum and the artificial inoculation procedure were as described by Collard et al. (2001), except that plants were sown in seedling trays, placed in plastic containers and covered with transparent polyethylene sheets during inoculation. For the field trial, natural infection was utilized but enhanced by spreader-rows (Porta-Puglia et al. 1994) of cultivar Howzat (moderately susceptible). Infected chickpea stubble was also scattered in the field one week after germination to increase infection.

Experimental design and resistance evaluation

Ten and 30 plants of each $F_{2:3}$ family were evaluated in the controlled environment and field trials, respectively. The parents, as well as the chickpea cultivar Macarena (susceptible) and an accession of *C. bijugum* (resistant), were included as control genotypes. In both trials, the experimental design was a randomized complete block. In the controlled environment, test plants were sown in a pair of seedling trays. Each pair of trays constituted one experimental block or replicate, and contained an individual plant of each of the 85 $F_{2:3}$ families and control genotypes. During inoculation, the ten replicates were boxed separately. In the field, test plants were sown in rows of 15 plants spaced at approximately 13 cm and replicated twice. Each block comprised eight plots of 250-plant rows, which were furrowed one meter apart. A spreader row was laid out between every two test rows.

To measure the disease reactions of test plants, two disease scoring systems were evaluated in both trials using the 1–9 visual scale (Reddy and Singh 1984) modified by Collard et al. (2001): (a)

initial disease score (IDS) for the initial disease reaction, and (b) area under the disease progress curve (AUDPC) for the progress of disease over time. The IDS was taken when the disease reaction of the mapping population was best differentiated by reference with the parental and control genotypes. In the controlled environment, IDS was scored one week after inoculation or 15 days after sowing, whereas in the field IDS was scored 50 days after sowing. For the AUDPC, disease reactions were scored weekly after the evaluation of the IDS. The AUDPC was calculated using the formula

$$Y = \sum [(X_i + X_{i+1})/2](t_{i+1} - t_i),$$

where Y is the AUDPC, X_i is the blight score of the ith evaluation, X_{i+1} is the blight score of the i+1th evaluation and $(t_{i+1}-t_i)$ is the number of days between two evaluations (Campbell and Madden 1990). Disease evaluation was stopped when the susceptible parent and control were dead. Three and four consecutive weekly scores were obtained to calculate the AUDPC in the controlled environment and field, respectively.

Data analysis

All data were analysed by ANOVA using standard procedures and the residuals were examined for normality and heteroscedasticity. Data from the controlled environment and field trials were analyzed separately, as environmental components of variance were assumed to be larger in the field. Simple product moment correlations were calculated between individual AUDPC and IDS within and between environments, and genetic correlations between environments were estimated by the method of Burdon (1977) to test the importance of possible genotype by environment interaction.

Using the linkage map (F_2) genotype data and family-mean IDS and AUDPC of the $F_{2:3}$ families, putative QTLs for resistance to ascochyta blight were identified by single-point analysis or one-way ANOVA at $P \leq 0.05$ using the GLM procedure of SAS (SAS Institute Inc. 1996), and verified by composite interval mapping (CIM—Windows QTL Cartographer version 1.30; Basten et al. 2001; Wang et al. 2002). For the CIM, five markers were used as background controls and were searched outside the 10 cM window by stepwise regression at $P \leq 0.1$ (Basten et al. 2001; Wang et al. 2002). Putative QTLs were declared when the likelihood ratio (LR) value exceeded 10.8 (equivalent to LOD $\geq$2.4). This critical LR gave an approximate 95% confidence on the location of QTLs on the marker-intervals, and was determined after 1,000 repetitions of a data permutation test (Churchill and Doerge 1994). To further reduce the influence of possible background genetic effects, adjacent QTLs detected within a 10 cM region were collectively marked as one QTL.

Finally, a QTL was declared either when the region was detected by CIM and the flanking markers were significant by one-way ANOVA (single-point analysis), or when the region was mapped with both the IDS and AUDPC disease scores regardless of the association of the flanking markers. This was to protect against type I and type II errors in declaring QTLs. In cases where multiple QTLs were detected, inter-locus interaction or epistasis was determined by simultaneous analyses of the QTLs in a multiple regression model using the multiple interval mapping (MIM) method of QTL Cartographer.

Results

Frequency distribution

The frequency distribution of the disease reaction of the $F_{2:3}$ mapping population to ascochyta blight was approximately normal (Fig. 1), consistent with the polygenic control of resistance. However, due to the optimum infection condition, distributions of IDS (6.9 to 9.0) and AUDPC (191.2 to 206.2) scores in the controlled environment were very high (Fig. 1, unfilled graphs). Mean blight scores of the resistant parent by both disease scores (IDS=7.9±1.0 and AUDPC=200.5±6.2) were not significantly different from that of the susceptible parent (IDS=8.3±0.7 and AUDPC=202.8±4.3).

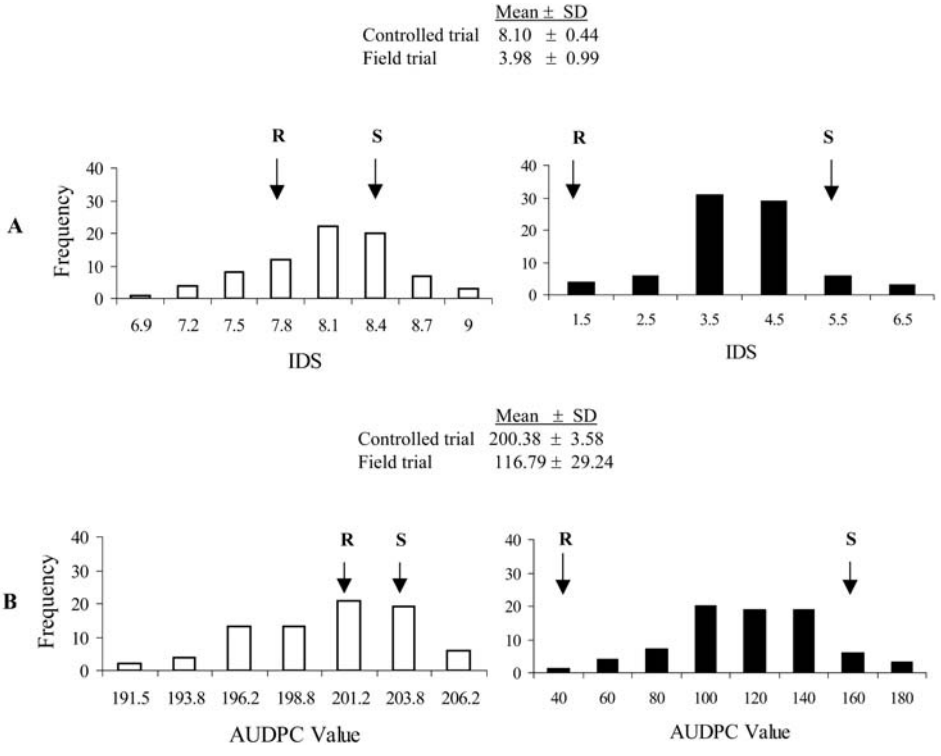

Fig. 1A, B Frequency distribution of the initial disease score (IDS; **A**) and area under disease progress curve (AUDPC; **B**) of the $F_{2:3}$ families in controlled environment (*unfilled bar*) and field (*filled bar*) trials. Mean disease scores of the parents in each trial are indicated by *arrows*

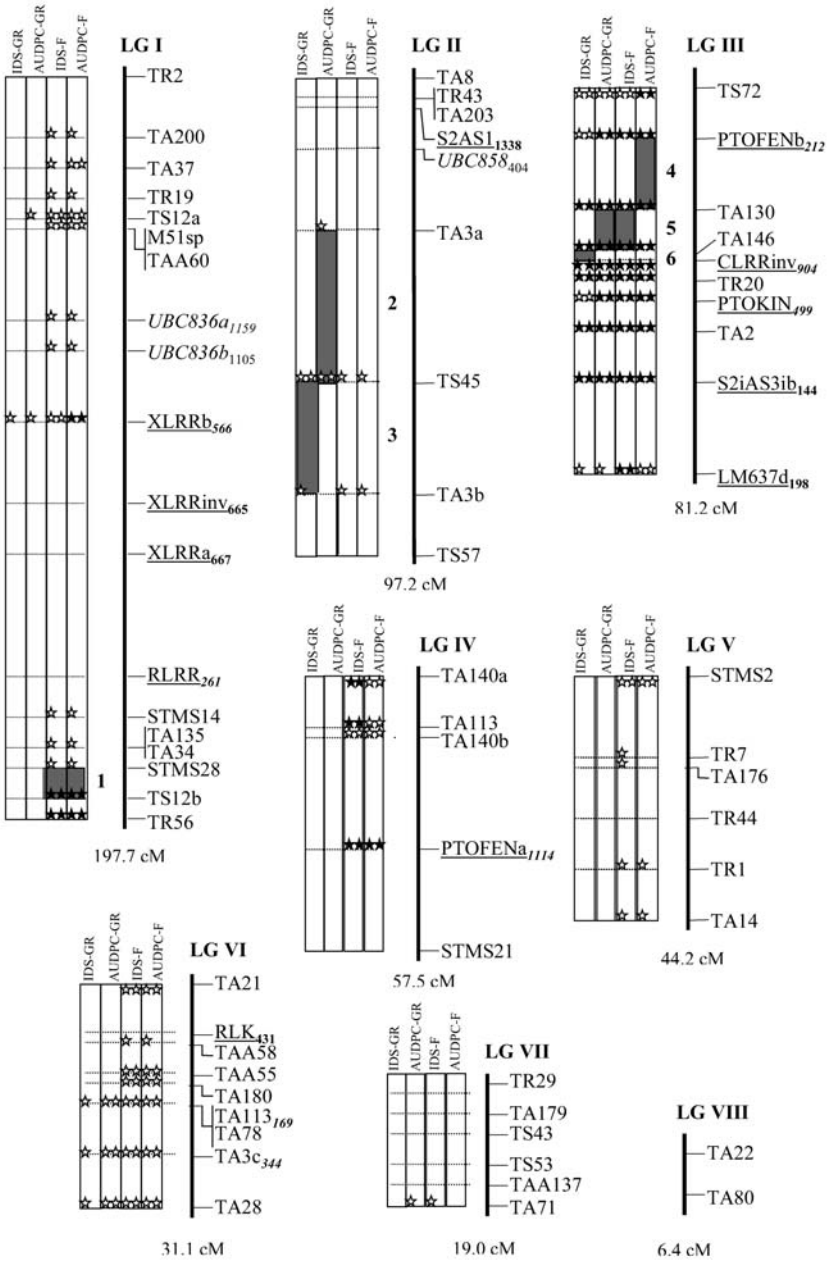

Fig. 2 Locations of putative QTLs (QTLs 1–6) for ABR in the intraspecific chickpea map (Flandez-Galvez et al. 2003) based from controlled environment (GR) and field (F) trials, and using either the initial disease score (IDS) or the area under disease progress curve (AUDPC) disease parameter. *Shaded bars* represent intervals mapped by composite interval mapping. Whereas, *stars* mark loci that were significantly associated with ABR by 1-way ANOVA: *single unfilled stars* at $P \leq 0.05$, *double unfilled stars* at $P < 0.01$ and *double filled stars* at $P < 0.001$. The markers on the linkage map are predominantly STMS markers. RGA markers are *underlined* and ISSR markers *italicized*

Verification of phenotype data

The IDS and AUDPC scores were strongly correlated in the controlled environment (r=0.96) and field (r=0.98), but only moderately correlated between the two screening environments (r=0.55 to 0.60). Separate and combined ANOVAs for the two trials revealed highly significant variation between the $F_{2:3}$ families for both IDS and AUDPC ($P<0.01$) and the repeatabilities of the family means were 0.74 and 0.76 for IDS, and 0.71 and 0.84 for AUDPC, in the controlled environment and field, respectively. They also indicated there was a significant genotype × environment interaction for both disease scores, but between environment genetic correlations for IDS (0.63) and AUDPC (0.70) they were moderately high. This indicated that the interaction was largely due to differences in the scale of genetic effects between environments rather than changes in the ranking of the families (Falconer 1952).

Single-marker QTLs

Using single-point analysis, putative QTLs were detected in seven of the eight marker-linkage groups; with all the markers on LG III significantly associated to both IDS and AUDPC disease scores (Fig. 2). In the controlled environment, 16 markers were associated with IDS and 18 with AUDPC. Whereas, 42 and 39 markers where

Table 1 Putative QTLs for ABR identified by composite interval mapping (CIM) at the critical LR threshold of 10.8 (α=0.05)

Test parameter	Linkage group	Interval length (cM)[a]	Flanking markers	QTL position (cM)[b]	LR[c]	Genetic effects[d]		Gene action[e]	R^{2f}(%)
						Add.	Dom.		
Controlled trial									
IDS	II (3)	22.3	TS45-TA3b	14.0	15.61	0.23	0.05	PD	14.2
	III (6)	0.1	TA146-ClRRinν_{904}	0.04	35.18	0.40	0.06	A	36.3
AUDCP	II (2)	35.5	TA3a-TS45	16.0	11.70	0.47	−4.94	OD	48.9
	III (5)	10.4	TA130-TA146	6.0	39.46	3.91	1.14	PD	47.8
Field trial									
IDS	I (1)	5.6	STMS28-TS12b	4.0	13.31	0.36	−0.35	D	8.0
	III (5)	10.4	TA130-TA146	2.0	51.85	1.0	0.31	PD	37.3
AUDCP	I (1)	5.6	STMS28-TS12b	4.0	12.11	10.29	−7.33	PD	6.5
	III (4)	15.4	PTOFENb$_{212}$-TA130	10.0	67.09	32.78	11.73	PD	50.2

[a] Interval between the two flanking markers (cM)
[b] QTL position from the left flanking marker (cM)
[c] Peak value of the maximurn-likelihood-ratio (LR) test statistic observed for the QTL in question
[d] Additive gene and dominance gene effects
[e] A = additive gene action (|d/a|<0.2), PD = partial dominance (0.2<|d/a|<0.8), D = dominance (0.8<|d/a|<1.2) and OD overdominance (|d/a|>1.2)
[f] Proportion of phenotypic variance explained by the QTL

associated with IDS and AUDPC in the field trial, respectively.

Flanking-marker QTLs

Using CIM, six QTLs were mapped on three linkage groups (Fig. 2 and Table 1): LG I (QTL 1), LG II (QTLs 2 and 3) and LG III (QTLs 4, 5 and 6). All their flanking markers were also significantly associated with ABR by one-way ANOVA ($P \leq 0.05$). The multiple QTLs on LG III were located more than 10 cM apart based on the position of QTL peaks or maximum LOD scores, and thus were declared as different QTLs. This QTL-region was associated with ABR in both the controlled environment and field, however the specific QTL which affected resistance differed depending on whether the disease reaction was measured by IDS or AUDPC (Fig. 2). Furthermore, QTLs on LG II and LG I were only detected in the controlled environment and field, respectively. All six QTLs mapped by CIM were incorporated into multiple regression, and analyzed for inter-locus interactions or epistasis.

Genetic effects and epistatic interactions of QTLs

Table 2 summarizes the results of the multiple regression analysis of the putative ABR-QTLs. All six QTLs mapped by CIM were validated by multiple interval mapping (MIM). However, only two QTLs with significant epistatic interaction were resolved to have a simultaneous effect on ABR in each trial and for both IDS and AUDPC (Table 2). QTL 5 and the adjacent QTLs 4 and 6 were effective in all cases, had the highest LR value and explained the largest proportion of phenotypic variance. This was consistent when QTLs were analyzed singly by CIM (Table 1). Except for QTL 2, all the QTLs were positioned <10 cM from the nearest flanking marker, with QTL 6 (controlled environment-IDS) only 0.04 cM away from TA146 (Table 2). QTL 5 from both controlled environment and field trials, and QTL1 from the field trial, were 4.4 to 2.0 cM and 1.6 cM away from the nearest flanking markers, respectively. By simultaneous analysis, the QTL effects including additive, dominance and epistasis gene actions, accounted for 48 to 76% of the variance in disease reactions either in the controlled environment or field. As shown in Table 2, variances explained by interacting QTLs were similar regardless of location or infection condition, when the AUDPC was measured rather than the IDS. In the controlled environment, epistatic interaction of QTLs was not detected using the IDS disease scoring system.

The positive values of the additive genetic effects indicated that all six QTLs contained resistance-enhancing alleles from the susceptible parent. Without epistasis, the major QTLs (QTLs 4 and 5) measured by either IDS or AUDPC, conferred pure additive gene action in both the controlled environment and field. Whereas, a considerable dominance was affected by the interacting minor QTL in the two-QTL genetic model, for each disease score and environment. Furthermore, the negative values of the dominance genetic effects of the minor QTL indicated that the resistance conferred by this locus was either completely dominant or over-dominant. The positive partial-dominance gene action of the major QTL 6 for IDS in the controlled environment also indicated the deviation of the disease score from mid-parent value towards susceptibility. In the field, epistatic interactions of QTLs were detected between heterozygotes (dominance × dominance), while between heterozygotes and

Table 2 Estimates of QTL effects and interactions obtained by multiple interval mapping (MIM). QTLs identified by CIM were used to establish the multiple genetic model

Test parameter	σ^2_P[a]	QTL (pair)	Marker[b]	QTL position (cM)[c]	LR[d]	Genetic effects[e]						Gene action	R^{2g} (%)
						Additive		Dominance		Epistasis			
						σ^2_A	Value	σ^2_D	Value	σ^2_I	Value		
Controlled trial:													
IDS	0.19	3	TA3b	8.3	6.11	0.02	0.20	0.00	0.04			A	10.9
		6	TA146	0.04	19.86	0.07	0.41	0.00	0.09			PD	36.7
Total					25.97	0.09		0.00					47.6
AUDCP	12.62	2	TA3a	16.0	8.48	0.17	0.50	3.68	−3.70			OD	30.5
		5	TA146	4.4	16.50	3.63	2.43	0.05	−0.31			A	29.1
		2×5			6.47					1.50	4.27	DA	11.9
		2×5			1.39					0.55	−1.93	AD	4.4
Total					32.84	3.80		3.73		2.05			75.9
Field trial:													
IDS	0.96	1	TS12b	1.6	7.70	0.06	0.26	0.04	−0.38			OD	9.8
		5	TA130	2.0	33.87	0.51	1.08	−0.01	0.13			A	52.3
		1×5			7.18					0.07	1.04	DD	7.1
Total					48.75	0.57	8.32	0.03		0.07			69.2
AUDCP	844.0	1	TS12b	1.6	7.30	52.84	33.95	16.64	−8.18			D	8.2
		4	TA130	5.4	38.47	509.50		−6.49	3.53			A	59.6
		1×4			8.37					63.27	31.80	DD	7.5
Total					54.14	562.34		10.15		63.27			75.3

[a] σ^2_P = phenotypic variance
[b] Nearest flanking marker
[c] QTL position from the nearest flanking marker (cM)
[d] Peak value of the (partial) maximum LR test statistic observed for the QTL in question
[e] σ^2_A = additive, σ^2_D = dominance and σ^2_I = epistatic genetic variances
[f] A=additive gene action (|d/a|<0.2). PD = partial dominance (0.2|d/a|<0.8), D=dominance (0.8|d/a|<1.2)and OD=overdominance (|d/a|>1.2); AA=additive × additive, DD = dominance × dominance and AD = additive × dominance QTL epistatic interactions
[g] (Partial) phenotypic variance explained by the QTL

homozygotes (dominance × additive and additive × dominance) in the controlled environment (Table 2).

Discussion

QTLs which conditioned ascochyta blight resistance (ABR) in a pure *C. arietinum* genetic background were identified using a segregating population that was either infected with a single-spore *A. rabiei* isolate in a controlled environment, or naturally infected in the field. The genetic effects of single-locus QTLs and QTLs in combination were determined relative to their map positions in the chickpea genome.

By single-locus CIM analysis, six QTLs for ABR were located on the chickpea map. However, multiple interval mapping (MIM) resolved only two QTLs that had simultaneous as well as epistatic interaction effects for resistance, for each disease-scoring system and blight condition. Although different QTLs on LG III were detected with IDS and AUDPC, LG III was a major QTL region for ABR having been consistently mapped for adjacent QTLs from both controlled environment and field trials. The shift of QTL peaks could be due to the specificity of the two disease scoring systems, or to the limitation of the linkage map used as a framework in the analysis of QTLs. IDS measured the initial disease reaction of the chickpea plant to ascochyta blight, while AUDPC measured the total disease severity over time. However, the F_2-based framework map unto which the QTLs were positioned, consisted of only 66 markers (Flandez-Galvez et al. 2003). Even if LG III seemed to be well covered with markers, the whole linkage map is still far from marker-saturation to precisely locate the QTLs. LG III can be conservatively declared as a major QTL region for ABR, whereas LG II and LG I were QTL regions more specific for either a controlled environment or field-detected resistance. QTLs on LG II (QTLs 2 and 3) were detected only from the controlled environment trial while QTL 1 on LG I was detected only from the field trial.

The QTLs verified by MIM were in similar regions to those mapped by Santra et al. (2000) in an interspecific *C. arietinum* × *C. reticulatum* population. QTL 1 was mapped on the linkage group containing the UBC836—the QTL 2-ISSR marker detected in the interspecific population. QTLs 2 and 3 were also mapped adjacent to the UBC858, the QTL3-ISSR marker of Santra et al. (2000). Although no common marker was available between the two regions, the major QTL region in this study (QTLs 4, 5 and 6) can also be associated with the previous QTL 2 based on the alignment of a few STMS-

anchor markers between the *C. reticulatum* interspecific linkage map and a rudimentary *C. arietinum* intraspecific linkage map (Tekeoglu et al. 2002). The resolution of the previous QTL region (QTL 2; Santra et al. 2000) into three interacting QTLs could be due to the different types of mapping populations, pathotypes of *A. rabiei* and/or disease scoring systems used in the two mapping studies. The map-positions of associated markers may have also been refined in the intraspecific linkage map and, with good marker density of the regions, have enhanced the mapping resolution around these resistance loci. High-resolution mapping is the strategy resorted by geneticists to determine whether a QTL is comprised of a single gene or several linked genes affecting the trait (Tanksley 1993). In another interspecific cross involving *C. echinospermum* as the resistance donor (Collard et al. 2003) and in an intraspecific population (Udupa and Baum 2002), the same major QTL region was also strongly associated with ABR in a glasshouse inoculation trial. In this region, Udupa and Baum (2002) also mapped two recessive major loci which conferred resistance to the pathotype II of *A. rabiei*.

The MIM results suggested that QTL epistasis, in the form of dominance x dominance (field) and dominance × additive interactions (controlled environment), was an important genetic component for ascochyta blight resistance in chickpea. The major QTLs (QTLs 5 and 6) explaining the largest proportion of the disease variation in the population, largely conferred additive genetic effects in both environments as measured by either IDS or AUDPC. In all cases, the major QTLs were also involved in dominance epistatic interactions. Since the resistance-enhancing alleles involved in all the QTLs detected came from the susceptible parent, perhaps only when an epistasis-gene action occurred was the resistance conferred. The dominant resistance conferred by the minor QTLs in the two-QTL models, supported this possible resistance mechanism. By classical genetic analysis of disease segregation, epistatic interactions between two (Dey and Singh 1993) or three (Tekeoglu et al. 2000) ABR genes were also suggested in inter- and intra-specific populations of chickpea. In rice, a similar conclusion was drawn for yield and yield-component traits whereby, a strong additive gene epistasis was found to be important in controlling the expression of the traits (Li et al. 1997; Xing et al. 2002).

The mapping of QTLs adjacent to RGA markers has provided a preliminary understanding on the most-likely biological significance of these statistical estimates and evidence of the location of candidate ABR genes in the region. Although nucleotide-sequence confirmation is required, the mapping of QTL 6 at approximately 0.06 cM from CLRRinv suggests that the major candidate gene at this locus may have conferred resistance to ascochyta blight in a similar mechanism as the *Cf9* gene in tomato. RGA marker CLRRinv was designed from the leucine-rich repeat regions of the *Cf9* gene in tomato against *Cladosporium fulvum* (Chen et al. 1998). The *Cf9* gene was involved both in first-defense pathogen recognition and interaction with a *Pto* gene-product to activate a protein kinase cascade of defense responses (Hammond-Kosack and Jones 1997). Indeed, QTL 4 was adjacent to QTLs 5 and 6, and was linked to the RGA marker PTOFEN which was designed from the protein kinase domain of the *Pto* gene in tomato which conferred resistance to *Pseudomonas syringae* pv *tomato* (Chen et al. 1998). The *Pto* gene has both signal transduction and binding/deactivating capacities to the *AvrPto*-proteins (Scofield et al. 1996; Tang et al. 1996). Furthermore, the strong association of other different RGA markers which flanked these major QTLs by one-way ANOVA, might explain the consistent association of this region for ABR across different genetic backgrounds, disease scoring systems and possibly different *A. rabiei* pathotypes. Complementary to this theory, QTLs 1, 2 and 3 may have conferred resistance through similar mechanisms as the *RPS2* gene of *Arabidopsis thaliana* and the *N* gene of tobacco because they were mapped adjacent to the RGA markers of these resistance genes.

A direct implication of the two-QTL genetic model established for each disease scoring system and blight condition, is that selection on the associated markers is likely to be effective in the breeding program. Although other minor QTLs may have yet to be resolved, the cumulative phenotypic effect (individual and epistatic) of the QTLs in the genetic model (about 75%) is already large for effective MAS breeding. Due to the cost and complexity in handling several genes or QTLs, the best combinations of resistance loci should be identified and used to prioritize breeding efforts for MAS, especially for multigenic traits (Tabien et al. 2002). Although some encouraging results have been reported with MAS for multigenic traits, the use of DNA markers in gene manipulation is still most effective with a single gene or a gene responsible for a high percentage of the trait's phenotypic variance (Lande and Thompson 1990; Ribaut and Hoisington 1998). Thus, breeding efforts using DNA markers have concentrated on manipulation of the smallest number of genes/QTLs with the greatest effect; e.g. grain yield and component traits, and insect resistance in maize (Stuber and Sisco 1991; Ajmonemarsan et al. 1995; Ribaut and Hoisington 1998) and rice blast resistance in rice (Inukai et al. 1996; Tabien et al. 2000, 2002). Furthermore, the considerable epistasis and possible genotype × environment interaction have to be taken into account in selecting the best QTL/marker combinations and the most effective MAS breeding scheme. With epistasis, the major QTLs in the genetic models were shown to be dependent, and affected by the inter-acting minor loci. Therefore, the minor loci and epistatic effects have to be considered when designing a breeding strategy, even if their phenotypic variance effects seemed insignificant in comparison with that of the major QTLs.

Tightly linked markers were identified for the major QTLs (controlled environment and field resistance) and QTL 1 (field resistance). However, further fine-mapping may be necessary to detect markers closely flanking the other QTLs detected within a controlled environment

(QTLs 2 and 3). Due to non-marker saturation and incomplete genome coverage of the framework map (Flandez-Galvez et al. 2003), the recombination distances may also have been under-estimated. Moreover, since the major QTL-region on LG III has also been consistently associated with ABR in another intraspecific population (Udupa and Baum 2002) and two interspecific populations of chickpea (Santra et al. 2000; Collard et al. 2003), high-resolution mapping using a larger mapping population should be directed at this region to dissect and tag likely clusters of ABR genes for *Cicer*. Once identified and tagged with informative DNA markers, the broad genetic sources for ABR can then be pyramided into one genotype and incorporated in a chickpea improvement program via marker-assisted selection. The tight linkage of the RGA markers to the major QTLs could also lead to map-based cloning of the underlying cluster of ABR genes. As shown in other crops, the candidate-gene approach has been successfully utilized to identify genes for resistance to various pathogens (Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996; Feuillet et al. 1997). Finally, CLRRinv and PTOFEN RGAs (and possibly other potential RGAs in the region) are most suitable for conversion into the sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers for selecting ABR in chickpea. In an interspecific population, CAPS coupled with RFLP analysis has proven effective in isolating and mapping resistance gene analogues of chickpea associated with a fusarium resistance gene (Hüttel et al. 2002).

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