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Identification and Mapping of QTLs Conferring Resistance to Ascochyta Blight in Chickpea

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ABSTRACT

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Lab., is a devastating disease of chickpea (*Cicer arietinum* L.) worldwide. Resistant germplasm has been identified and the genetics of resistance has been the subject of numerous studies. The objectives of the present study were to determine the genetics of resistance to ascochyta blight of chickpea and to map and tag the chromosomal regions involved using molecular markers. We used a set of 142 $F_{5:6}$ recombinant inbred lines (RILs) obtained from an interspecific cross of *C. arietinum* (FLIP84-92C, resistant parent) × *C. reticulatum* Lad. (PI 599072, susceptible parent). The RILs were scored for disease reactions in the field over 2 yr and were genotyped for polymorphic molecular markers [isozyme, random amplified polymorphic DNA (RAPD), and inter simple sequence repeat (ISSR)] in the laboratory. The disease was scored quantitatively and data were used for QTL analysis. A linkage map was established that comprised nine linkage groups containing 116 markers covering a map distance of 981.6 centimorgans (cM) with an average distance of 8.4 cM between markers. Two quantitative trait loci (QTLs), QTL-1 and QTL-2, conferring resistance to ascochyta blight, were identified which accounted for 50.3 and 45.0% of the estimated phenotypic variation in 1997 and 1998, respectively, and were mapped to linkage groups 6 and 1, respectively. Two RAPD markers flanked QTL-1 and were 10.9 cM apart while one ISSR marker and an isozyme marker flanked QTL-2 and were 5.9 cM apart. These markers can be used for marker-assisted selection for ascochyta blight resistance in chickpea breeding programs, and to develop durable resistant cultivars through gene pyramiding.

CHICKPEA ($2n = 16$), a self-pollinated diploid annual grain legume, is an important crop on the Indian subcontinent, West Asia, North Africa, southern Europe, and North and Central America. Among many diseases that affect chickpea, ascochyta blight is the most devastating worldwide, causing up to 100% yield losses in severely affected fields (Nene, 1984). Resistance breeding has relied on the use of screening nurseries where disease epidemics are created by inoculation with the pathogen and frequent sprinkler irrigation. With this approach, ascochyta blight resistance sources have been identified and many resistant cultivars have been developed (Reddy and Singh, 1983, 1984; Nene and Reddy, 1987; Singh and Reddy, 1996).

Previous reports indicate that resistance to ascochyta blight in chickpea is conferred by more than one gene (Singh and Reddy, 1983; Tewari and Pandey, 1986; Muehlbauer and Singh, 1987; Singh and Reddy, 1989; Kusmenoglu, 1990; Muehlbauer and Kaiser, 1994). Recently, Tekeoglu et al. (2000) showed that two complementary recessive genes conferred resistance. However, the locations of the genes conferring resistance are not known. Since multiple genes appear to condition resistance, knowledge of their genomic locations and linkage to molecular markers would facilitate gene transfer and pyramiding of the genes into acceptable genetic backgrounds through marker-assisted selection.

Molecular markers have been used to establish linkage maps for many crop species (O'Brien, 1993) and they have been utilized to determine gene number for particular traits and for gene tagging (Paterson et al., 1991; Lee, 1995). Many important disease resistance genes have been mapped and tagged in various crops (Staub et al., 1996; Mohan et al., 1997). RAPD markers (Williams et al., 1990; Welsh and McClelland, 1990) are simple and fast and have been employed widely for mapping genomes (Torres et al., 1993; Hunt and Page, 1995) and for tagging resistance genes (Staub et al., 1996; Mohan et al., 1997; Mayer et al., 1997).

Linkage analysis and mapping disease resistance genes using molecular markers has been limited in cultivated chickpea because of the minimal polymorphism available (Muehlbauer and Singh, 1987; Gaur and Slinkard, 1990; Kazan et al., 1993; Simon and Muehlbauer, 1997). Isozyme analysis has revealed insufficient polymorphism to be useful in finding tags for fusarium wilt and ascochyta blight resistance genes (Kusmenoglu et al., 1992). DNA marker systems such as RAPD, ISSR, sequence tagged microsatellite sites (STMS), and amplified fragment length polymorphism (AFLP) overcome the problem of minimal polymorphism and allow more detailed analysis of the genome. Recently, genes conferring resistance to fusarium wilt in chickpea have been tagged with RAPD and ISSR markers (Mayer et al., 1997; Ratnaparkhe et al., 1998).

Resistance to many diseases are reported to be inherited quantitatively (Young, 1996) and that such resistance is controlled by multiple loci, known as quantitative resistance loci (QRL) (Geiger and Heun, 1989). Three QTLs have been shown to confer resistance to ascochyta blight in pea (*Pisum sativum* L.) and accounted for 71% of the phenotypic variation (Dirlewanger et al., 1994). The objectives of the present study were to determine the genetics of resistance to ascochyta blight of chickpea and to map and tag the chromosomal regions involved using molecular markers.

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Abbreviations: AFLP, amplified fragment length polymorphism; cM, centimorgan; ISSR, inter simple sequence repeat; QTL, quantitative trait locus; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred lines; STMS, sequence tagged microsatellite sites.

MATERIAL AND METHODS

An interspecific cross between *C. arietinum* (FLIP84-92C, resistant parent) and *C. reticulatum* (PI 599072, susceptible parent) was made and the F_2 population was advanced by single seed descent to the F_5 in the greenhouse to develop RILs. Single rows of each of 142 $F_{5:6}$ RILs were planted in the ascochyta blight screening nursery at the Spillman Research Farm of Washington State University located at Pullman, WA, on 5 May 1997 and 30 April 1998. Because of the limited amount of seed of the RILs it was necessary to use a single replication of each line in an augmented experimental plot design in both years. Soil type at the farm was Palouse series fine-silty, mixed mesic Pachic Ultic Haploxerolls. Spreader rows of a highly susceptible line were planted every four rows and along the border of the plot to increase the distribution and uniformity of the disease within the nursery. Ascochyta blight infested crop debris was used as inoculum and was distributed within the plot area on 27 May 1997. The plants were about 10 cm tall at the time of inoculation. After inoculation with the infested debris, the field was sprinkle irrigated daily to maintain a moist environment favorable for disease development.

Each RIL was scored for disease symptoms using the internationally accepted (Reddy and Singh, 1984) and commonly used scale of 1 to 9 and was based on the degree of infection of leaves, stems, and pods. According to the scale, 1 = no visible lesions on the leaves (highly resistant); 2 = lesions visible on the leaves by close examination (highly resistant); 3 = A few lesions visible on the leaves which are easily seen (resistant); 4 = Many lesions visible, but lesions have not caused irreparable damage to the plants (resistant to moderately resistant); 5 = Large lesions on stems and leaves, some leaf and stem girdling, but the plant is still alive (intermediate); 6 = Many large lesions on stems and leaves, moderate stem and leaf girdling, but the plant probably will survive (moderately susceptible); 7 = Many large lesions on stems, leaves, and pods, stem and leaf girdling common, the plant may or may not die but will produce few seeds (susceptible); 8 = Large lesions on stems and leaves common, stem and leaf girdling common, the plant probably will die (susceptible); 9 = Infection severity such that the plant is dead or dying (highly susceptible). The first scoring was made the second week of June 1997 and 1998 when the susceptible check showed disease symptoms. Disease scores were recorded weekly thereafter for 3 wk. Final scores were made when all susceptible checks had scores of 9.

The RILs were placed into resistant and susceptible categories based on disease scores plus or minus the LSD estimates. Those RILs with mean disease scores equal to the resistant parent plus the LSD were classified as resistant, while those with mean disease scores equal to the susceptible parent minus the LSD were classified as susceptible. When the LSD estimates were considered, RILs with scores of 1 to 4 were classified as resistant, while RILs with scores of 5 to 9 were classified as susceptible. Our classification differed from that of Tewari and Pandey (1986) who subjectively classified lines with scores of 1 to 3 as resistant and those with scores of 4 to 9 as susceptible. Our classification also differed from that of Singh and Reddy (1983, 1989) who classified lines with scores of 1 to 5 as resistant, and those with scores of 6 to 9 as susceptible. Segregation ratios between resistant and susceptible classes were tested by χ^2 ($P < 0.05$) for goodness-of-fit to a 1 resistant:1 susceptible ratio expected for a single gene for resistance when using RILs, and also for goodness of fit to a 1 resistant: 3 susceptible ratio expected for RILs if resistance were conferred by two complementary recessive genes.

Table 1. Isozyme systems assayed to map and tag ascochyta resistance genes in chickpea.

Isozyme name	Symbol	EC number
Aspartate amino transferase	AAT	EC2.6.1.1
L-alanyl β-napthylamide aminopeptidase	AAP	–
Aconitase	ACO	EC4.2.1.3
Acid phosphatase	ACP	EC3.1.3.2
Adenylate kinase	ADK	EC2.7.4.3
Aldolase	ALD	EC4.1.2.13
α-amylase	AMY	EC3.2.1.1
Fluorescent esterase	EST	EC3.1.1.2
Endopeptidase	ENP	EC3.4.21
Peptidase	PEPT	–
Fructose kinase	FK	EC2.7.1.4
β-D-galactosidase	GAL	EC3.2.1.23
Glucose phosphate isomerase	GPI	EC5.3.1.9
Glutamic pyruvic transaminase	GPT	EC2.6.1.2
Glucose-1-phosphate transferase	G1PT	–
Phosphoglucomutase	PGM	EC5.4.2.2
6-phosphogluconate dehydrogenase	PGD	EC1.1.1.44
Peroxidase	PRX	EC1.11.1.7
Malate dehydrogenase	MDH	EC1.1.1.37
Shikimate dehydrogenase	SKDH	EC1.1.1.25
Triose phosphate isomerase	TPI	EC5.3.1.1
Leucine aminopeptidase	LAP	EC2.6.1.6
Diaphorase	DIAP	EC1.8.1.4
Fructose 1,6-bis phosphatase	FBP	EC3.1.3.11

Isozyme analysis and isozyme loci nomenclature followed the methods of Kazan et al. (1993). Twenty-four isozymes were assayed (Table 1). Young leaf tissue from single plants of each RIL was harvested, lyophilized under liquid nitrogen, and stored at −70°C. DNA was extracted by the miniprep method of Doyle and Doyle (1987) with modifications as described by Simon and Muehlbauer (1997). One to two gram samples of young leaf tissue of each RIL were submerged in liquid nitrogen, ground to a fine powder, and quickly transferred to a tube containing 7.5 mL of ice-cold extraction buffer (0.35 *M* sorbitol, 0.1 *M* Tris, 5 m*M* EDTA, pH 7.5). The tube was briefly shaken and 7.5 mL nuclei lysis buffer [(2 *M* NaCl, 0.2 *M* Tris, 50 m*M* EDTA, 2% (v/v) hexadecyltrimethylammonium bromide (CTAB), pH 7.5] was then quickly added followed by 3 mL of 5% (w/v) sarkosyl solution. Sample sets were incubated in a 65°C water bath for 20 min, allowed to cool for a few min, and 18 mL of chloroform/isoamyl alcohol (24:1) added to each tube. The tubes were centrifuged at 5000 × g for 15 min, the aqueous layer was removed and extracted with a 15 mL chloroform/isoamyl alcohol mixture. The aqueous layer was transferred to a new tube and DNA was precipitated with a double volume of chilled ethanol. A dry DNA pellet was suspended in 500 μL TE buffer (10 m*M* Tris-HCl and 1 m*M* EDTA, pH 8.0) and quantified according to the mini-gel method (Sambrook et al., 1989) by comparing ethidium bromide stain intensity with that of standard lambda/*Hin*dIII DNA marker (Gibco BRL, Bethesda, MD). DNA was then diluted to 5ng/μL concentration and used in polymerase chain reactions (PCR).

Eight hundred 10-mer RAPD primers (UBC 1-800) and 100 ISSR primers (15–23-mer based on microsatellite sequences, UBC 801-900) were obtained from Biotechnology Laboratory, University of British Columbia, Vancouver, BC, Canada. Seventy additional 10-mer RAPD primers (CS 1-70) were obtained from Genosys Biotechnologies (The Woodlands, TX). RAPD and ISSR analyses were performed according to established procedures (Simon and Muehlbauer, 1997; Ratnaparkhe et al., 1998). For RAPD analysis, PCR amplification was performed in 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 0.1% (v/v) Triton X-100, 2.5 m*M* $MgCl_2$, 100 μ*M* dNTPs, 0.24 μ*M* of primer, 20 to 25 ng of DNA and 1 unit of *Taq* polymerase per 25-μL reaction volume with the following cycle repeated

40 times: denaturing at 94°C for 20 s, annealing at 36°C for 1 min, 3 min ramp to 72°C and elongation at 72°C for 1 min. Final elongation segment was held for 8 min. In ISSR analysis, the amplification reaction mixture was identical to that used with RAPD except that the dNTPs concentration was 200 μM instead of 100 μM, and the following cycle was repeated 35 times: denaturing at 94°C for 1 min, annealing at 50°C for 1 min, 2 min at 72°C for elongation. The final elongation segment was held for 8 min. Polymerase chain reactions were carried out in a Perkin Elmer Cetus (The Perkin-Elmer Corporation, Norwalk, CT) Gene Amp PCR system 9600. The PCR products were separated electrophoretically in 2% (w/v) agarose gel using 1× TBE buffer (Sambrook et al., 1989). Banding patterns were visualized on a UV-transilluminator after staining the gels with ethidium bromide at a concentration of 0.5 μg/mL. To score the loci, RAPD and ISSR gels were photographed on Thermal photographic paper (#K65H, Mitsubishi Electric Corporation, Tokyo, Japan) using a Benchtop Digital Documentation System (FOTO/Analyst Mini Visionary, #FCR-10, Fotodyne, Inc., Hartland, WI).

Since RAPDs and ISSR markers are dominant, a marker locus was considered to be polymorphic if the band was present in one parent and not in the other. Segregation of polymorphic bands in the progeny was confirmed by analyzing the parents and 10 RILs chosen at random. Only clear and reproducible DNA bands were scored. The RAPD and ISSR marker loci were designated according to the primer name. For primers which amplified more than one polymorphic band, a subscript of a, b, and c (starting from highest to lowest molecular weight band) was given after the primer name.

All marker loci were scored at least twice from photographs of the gels to minimize interpretation errors. Segregation of marker loci was tested for goodness-of-fit to the expected Mendelian segregation ratio of 1:1 using the χ^2 test ($P < 0.05$). Linkage groups were established using MAPMAKER/EXP 3.0 (Lander et al., 1987). A two-point linkage analysis was conducted to determine maximum likelihood recombination frequency and the LOD score for each possible pair of loci. Linkage groups were established using the "group" command on the two-point data with a recombination value (θ) of 0.25 and constant LOD score of 4.0. Three-point linkage analyses were then conducted to determine the most likely order of loci within groups using "compare" command for smaller groups. For large linkage groups, a framework was established based on two-point linkage analysis and additional markers were mapped using the "try" command and linkage order was verified using the "ripple" command. Data quality were checked using both Mapmaker "error detection" command as suggested by Lincoln and Lander (1992) and Map Manager QTb (version2.8) "double crossover" command (Manly, 1998). Unlikely double crossovers due to possible genotyping errors were corrected by rechecking the data. Mapmaker linkage order results were compared to the results obtained from Map Manager using the "rearrange" option which rearranges the loci for specified groups based on simulated annealing by minimizing double recombinants. The Kosambi mapping recombination function was used to determine distance in centimorgans between markers (Kosambi, 1944). The linkage map was compared with previously reported maps (Gaur and Slinkard, 1990; Kazan et al., 1993) for assigning linkage group designations. Common linkage group designations were assigned based on presence of common isozyme markers in both maps.

The SAS statistical program (SAS, Institute, 1996) was used for two-tailed *t*-tests to determine single marker associations with blight resistance. Single point and interval analyses were performed for QTL analysis of the phenotypic and marker data. The program Qgene, version 2.30 (Nelson, 1997) was used to perform single marker and interval mapping analyses based on a linear regression model. Interval mapping was performed to identify and map putative QTLs involved in resistance. Markers with $P < 0.001$ were considered to be significantly associated with a putative QTL. Qgene was used to analyze two-way interactions between QTLs, and between QTLs and other markers. Qgene was also used to conduct multivariate analyses. The R^2 value of the best marker associated with each QTL was used to calculate the proportion of phenotypic variation explained by the QTL.

Table 2. χ^2 test for segregation ratios (1:1 and 1:3) of ascochyta blight resistant and susceptible recombinant inbred lines in chickpea.

Year	Resistant RILs	Susceptible RILs	χ^2 (1:1)	Significant ($P < 0.05$)	χ^2 (1:3)	Significant
1997	35	107	36.5	yes	0.5	no
1998	40	102	27.0	yes	0.5	no

RESULTS AND DISCUSSION

When the RILs were classified as resistant or susceptible, the segregation ratio between resistant and susceptible lines fit a 1 resistant: 3 susceptible ratio ($P < 0.05$) (Table 2). If two complementary recessive genes control a qualitative trait, a 1:3 segregation ratio would be expected for two discrete classes in a recombinant inbred line population. In our case, the segregation into resistant and susceptible classes closely fit a 1 resistant: 3 susceptible ratio in both years and was consistent with the genetic model that two complementary genes conferred resistance to ascochyta blight in the resistant parent (FLIP 84-92C) we used. The frequency distribution (Fig. 1) of disease scores of the RILs was bimodal with two phenotypic classes in an approximate ratio of 1:3 and was consistent with the two gene model for resistance. Differences among RILs were highly significant when data from the 2 yr of evaluation were combined.

The RILs were genotyped for one morphological trait locus *P* (for anthocyanin pigmentation), 11 isozyme, 111 RAPD, and 21 ISSR marker loci. Segregation for all loci was tested for goodness of fit to the expected 1:1 ratio by χ^2 test ($P < 0.05$). Most of the markers fit the

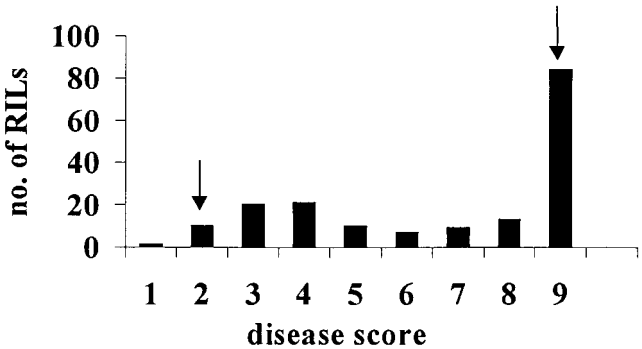

Fig. 1. Frequency distribution of disease scores for the RILs. Disease was scored on a 1 to 9 scale, where 1 indicated no infection and 9 indicated dead plants. Disease scores of *C. arietinum* (FLIP84-92C, resistant parent) and *C. reticulatum* (PI 599072, susceptible parent) were 2 and 9 (indicated by arrows), respectively.

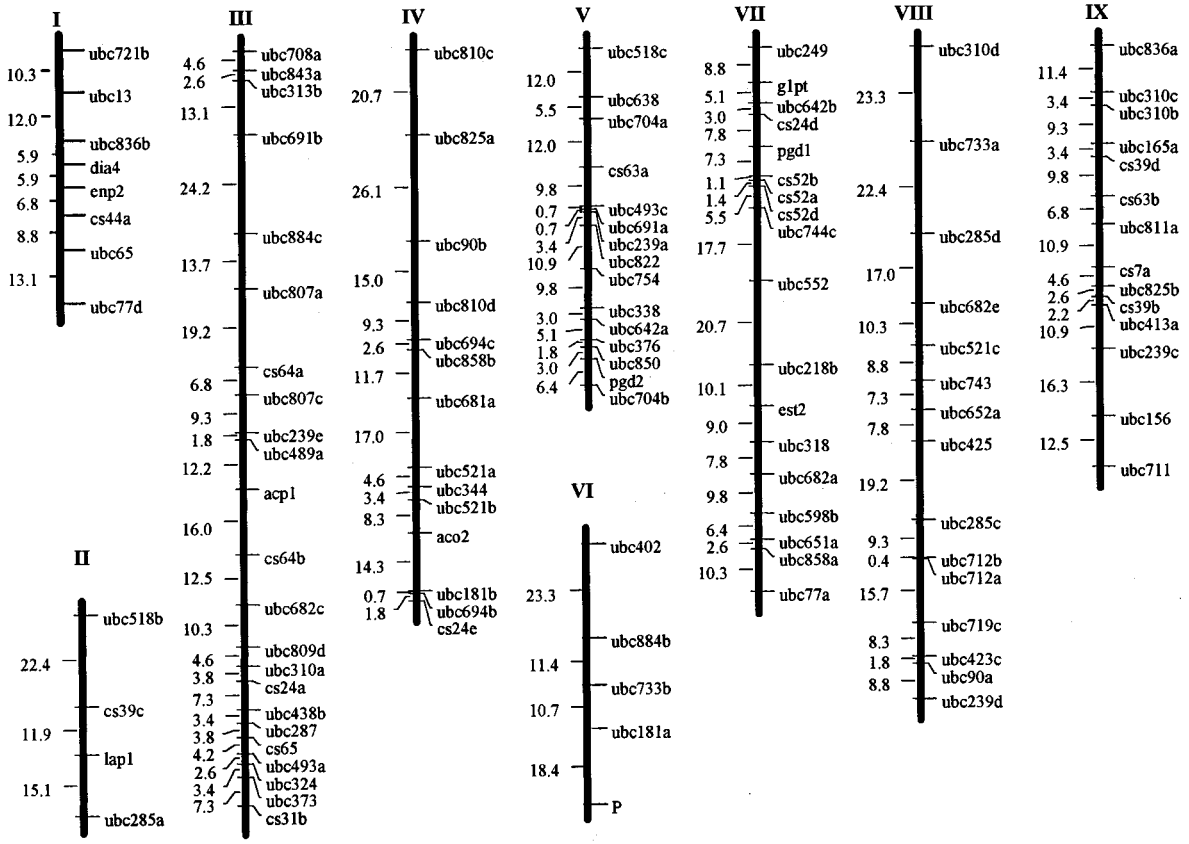

Fig. 2. Linkage map of the *Cicer* genome based on a morphological gene, isozyme, RAPD, and ISSR marker loci with LOD 4.0 and maximum distance between markers of 30 cM.

expected 1:1 ratio. However, two isozyme loci, three ISSR loci, and 10 RAPD loci had distorted segregation ratios and were omitted from linkage analysis. Out of 129 markers used for linkage map construction, 116 formed nine linkage groups, while 13 markers remained unassigned. The linkage map (Fig. 2) comprised nine linkage groups and included one morphological trait locus, nine isozyme loci, 17 ISSR, and 89 RAPD loci covering 981.6 cM with an average distance of 8.4 cM between two consecutive markers.

Disease scores were treated as quantitative data and 15 markers were significantly associated ($P < 0.001$) with blight resistance by the two-tailed t-test and by linear regression analysis when data from the 2 yr were used. When interval mapping was performed, three QTLs, namely QTL-1, QTL-2, and QTL-3, with LOD scores of 17.23, 7.31, and 3.04, respectively, were detected using 1997 data. However, only QTL-1 and QTL-2 with LOD scores of 16.19 and 6.52, respectively, were detected using 1998 data. QTL-1, QTL-2, and QTL-3, were mapped to linkage groups 6, 1, and 4, respectively.

The flanking markers, linkage group location, LOD scores and proportion of phenotypic variation (R^2) explained by each QTL are shown in Table 3. QTL-1 accounted for an estimated 42.5 and 41.4% of the variation in blight reaction in 1997 and 1998, respectively,

Table 3. Markers associated with three QTLs conferring resistance to ascochyta blight in chickpea.

QTL	Marker	Molecular weight	Sequence	Linkage group	[R^2]†	*P*	LOD value†	Distance (cM)‡
1	UBC733b	850bp	GGGAAGGGAG	6	0.413 (0.403)	<0.00001	17.18 (16.19)	10.7
	UBC181a	1850bp	ATGACGACGG		0.315 (0.264)	<0.00001		
2	UBC836b	700bp	$(AG)_8$YA§	1	0.199 (0.172)	<0.00001	7.27 (6.52)	5.9
	Dia4		Diaphorase		0.183 (0.135)	<0.00001		
3	UBC681a	1750bp	CCCCCGACT	4	0.097 (–)	0.0002	3.03 (1.03)	11.7
	UBC858b	1400bp	$(TG)_8$RT¶		0.056 (–)	0.001		

† R^2 and LOD values in parentheses are based on second year disease scoring data.
‡ Genetic distance between flanking markers.
§ Y = Cytosine [C] or Thymine [T].
¶ R = Adenine [A] or Guanine [G].

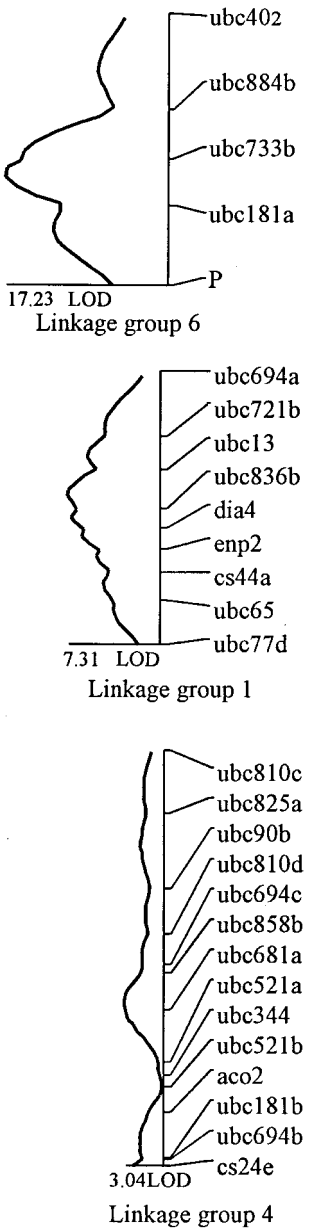

Fig. 3. Linkage groups 1, 4 and 6 of the *Cicer* genome where QTLs for ascochyta blight resistance are mapped.

while QTL-2 accounted for an estimated 19.9 and 17.2% of the variation in blight reaction in 1997 and 1998, respectively. QTL-1 and QTL-2 together accounted for an estimated 50.3% of the variation in reaction to ascochyta blight in 1997, whereas QTL-1 and QTL-2 together explained 45.0% of the variation in 1998.

The markers flanking QTL-1, UBC733b, and UBC 181a were 10.7 cM apart on linkage group 6, whereas QTL-2 is flanked by ISSR marker UBC836b and an isozyme marker *Dia4* that were spaced 5.9 cM apart on linkage group 1 (Table 3, Fig. 3). Two ISSR markers, UBC681a and UBC858b, flank QTL-3 and were spaced 11.7 cM apart on linkage group 4. Markers UBC836b and UBC858b have primer sequences based on di-nucleotide $(AG)_n$ and $(TG)_n$ repeats, respectively.

The linkage map in chickpea based on molecular markers is less well developed when compared to maps of other crops. Difficulties of mapping the *Cicer* genome are due to the minimal amount of polymorphism available. The first genetic linkage map of *Cicer* genome consisted of four linkage groups based on isozyme markers (Gaur and Slinkard, 1990). Later, a map of 10 linkage groups was reported using three separate F_2 populations and included 28 isozyme, 44 RAPD, 9 RFLP, and 6 other markers (Simon and Muehlbauer, 1997). Recently, a linkage map of *Cicer* based on a RIL population and using STMS was reported (Winter et al., 1999). However, none of the mapping populations used to date have segregated for ascochyta blight resistance and therefore the resistance loci have not been mapped. To determine map positions of the loci that confer resistance to ascochyta blight we developed a skeletal linkage map using a population of RILs that segregated for ascochyta blight resistance.

The total phenotypic variation explained by the detected QTLs in both years was less than the sum of the individual QTL effects which might be indicative of residual noise due to the genetic model (Nelson, 1999, personal communication) or epistatic interactions or presence of other minor genes. However, epistatic interactions between detected QTLs and between the QTLs and all other markers were not significant.

In most cases, relatively few QTLs with major effects confer quantitative disease resistance (Young, 1996) and our data concurs with this finding. Therefore, it seems that resistance to ascochyta blight in FLIP84-92C is controlled by at least two QTLs and possibly a few minor QTLs, one of which was detected with 1997 data. However, larger populations may be needed to detect other minor QTLs. Further investigations are also required to determine whether QTL-1 and QTL-2 are present in other chickpea germplasm lines reported to be resistant to ascochyta blight and if the QTLs are specific to the *A. rabiei* pathotypes. Our results showing the quantitative nature of resistance to ascochyta blight in chickpea are similar to that of quantitative resistance to ascochyta blight of pea (Dirlewanger et al., 1994). This may indicate synteny between the genomes of pea and chickpea as previously suggested by Weeden et al. (1992).

Studies of the inheritance of ascochyta blight indicated that either a single dominant or a single recessive gene conferred resistance in chickpea (Singh and Reddy, 1983; Tewari and Pandey, 1986; Singh and Reddy, 1989). Dey and Singh (1993) reported two dominant complementary genes in chickpea genotypes GLG84038 and GL84099, whereas one dominant and one recessive independent gene were found in ICC1468. Kusmenoglu (1990) used $F_{2:3}$ families to determine the genetics of ascochyta blight resistance in chickpea which indicated that resistance to ascochyta blight was controlled by at least two recessive genes. Susceptible parents used, methods used for disease evaluation, and classification of lines into resistant and susceptible groups are based on the subjective scoring scale. The source of resistance used in the development of resistant parents in our study and in Kusmenoglu (1990) was the same. Recently, Tek-

eoglu et al. (2000) using the same RIL populations as in the present study showed that two complementary recessive genes conferred resistance to ascochyta blight in chickpea. Therefore we consider that the two QTLs (QTL-1 and QTL-2) identified in the present study likely coincide with the two recessive genes reported by Kusmenoglu (1990) and Tekeoglu et al. (2000).

Use of recombinant inbred lines instead of an F_2 population is advantageous for mapping ascochyta blight resistance genes because nearly homozygous lines are scored rather than individual heterozygous plants. There was little segregation within RILs and this simplified scoring disease reactions. Seed sterility was not a problem in the RILs although the lines were developed from an interspecific cross. Interspecific crosses (*C. arietinum* × *C. reticulatum*) were also used for mapping isozyme and DNA markers in chickpea (Gaur and Slinkard, 1990; Kazan et al., 1993; Simon and Muehlbauer, 1997). To the best of our knowledge, this is the first report indicating (i) that ascochyta blight resistance of chickpea is quantitative in nature with two major genes and (ii) that specific molecular markers linked to ascochyta blight resistance genes have been identified.

Of the six markers that flank the three QTLs, three (UBC733b, UBC181a, and UBC681a) are RAPD markers, and two (UBC836b and UBC858b) are ISSR markers. This indicates that microsatellite based ISSR markers are useful for tagging disease resistance genes in chickpea. Our previous study indicated similar results in tagging of fusarium wilt resistance genes in chickpea (Ratnaparkhe et al., 1998). All markers identified in this study were linked to the QTLs for resistance in coupling phase. These associations of markers with resistance loci need to be verified in other segregating populations and shown to be effective in marker assisted selection. In addition, the conversion of linked markers to more easily applied markers such as sequence characterized amplified regions (SCARs) would simplify their use in breeding programs.

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Comparison of Bioclimatic Indices for Prediction of Maize Yields

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ABSTRACT

Yield prediction across a target production zone with varying strategies of agronomic practices has been a challenging problem to plant breeders when testing new genotypes for release. This study focused on comparing the importance of a new bioclimatic index called biological windows and six other traditional environmental indices as predictor variables of maize yields across sites (farmers' fields) and years, using a simple linear regression model. The yield data were collected for six hybrids evaluated in strip tests at 57 to 186 sites throughout Iowa during 1987–1994. The biological windows index was based on the Newhall Simulation Model and estimated the number of days the soil was moist and above 5°C. The environmental indices were July precipitation, temperature, the product of July precipitation and temperature, and the equivalent values for August. Because the actual values for the indices were not recorded at each site, all the indices were estimated for each site as the weighted averages of the data from 112 Iowa weather stations. Across years and within the Iowa sites, the mean percentiles of R-square distributions showed that biological windows had less predictive value for maize yields than the more traditional indicators such as August precipitation and temperature. For all indices, across years and within sites had much greater mean R-squares than across sites and within years, which had very low predictive values. For predicting yield across years within sites, there appeared to be an advantage in using August precipitation or the product of August precipitation and temperature over the five other indices. The R-square values for these two indices were at least 0.60 in 80% of the test sites for five hybrids.

Genotype × environment interaction (GEI) is a common problem that plant breeders face when releasing advanced plant materials for use in any relatively large target zone. Lin et al. (1986) reviewed alternative methods addressing this problem via stability analysis of genotypes and/or prediction over environments. A major limitation of such approaches in regression type analyses is that the use of the mean of the genotype in each environment as the independent environmental index variable often doesn't adequately characterize environments. Performance of a genotype in an environment relates not only to the type of environments, but also the interaction, intensity and timing of processes or events (Cooper and Blyth, 1996). Crop response depends on a suite of bioclimatic variables that relate to particular characteristics of the genotypes being evaluated (Corbett, 1998). Consequently, there is considerable need for the identification and use of bioclimatic variables for the evaluation and comparison of advanced genotypes across large growing regions. The identification of such key bioclimatic variables coupled with a simple model with good yield prediction capability over large production zones in the presence of GEI would reduce the efforts invested at the early stage of genotypic evaluation. A good predictive model could help with minimizing the number of test sites needed within years and would aid with seed production decisions by predicting genotypic performance in future years.

Crop models that use direct or derived weather, agroclimatic, soil, and agricultural practice variables with varying degree of complexity have been developed for various crop prediction purposes. Bondavalli et al. (1970) and Nielson et al. (1996) found that the most important weather variables that affect grain yield prediction of maize (*Zea mays* L.) in the U.S. Corn Belt region were precipitation and temperature occurring during the months of May and August. However, other studies stressed the importance of these two weather variables on maize yields during the critical period of July (tasseling and silking) and August (grain filling)

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