Genetic Study of Ascochyta Blight Resistance in Chickpea and Lentil

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Abstract

Ascochyta blight is responsible for severe crop losses in most chickpea and lentil production areas around the world. The research was conducted to study the genetic basis for ascochyta blight resistance in chickpea and lentil by means of QTL analysis, and PCR-based approaches to identify resistance gene analogues (RGA) sequences in the lentil genome. An AFLP and three SSR markers were linked to the gene(s) for ascochyta resistance in a chickpea population derived from a cross between CDC Chico and CDC Marengo. Two QTL that explained 36 % and 29 % of the disease reaction variability were identified in a lentil RI population derived from a cross between ILL5588 and L692-16-1. These markers were converted into SCAR markers to simplify their use for marker-assisted selection.

Several RGA clones were generated from amplification of the lentil (line ILL5588) genome using degenerate primers targeting leucine rich repeats (LRR), nucleotide binding-sites (NBS), and kinase domain-containing sequences. Preliminary database search resulted in sequence similarities to known resistance genes such as *RPS2* (from Arabidopsis) and *L*6 (from flax).

Introduction

Lentil and chickpea production areas have increased dramatically in Saskatchewan in the past few years. Among many diseases that affect chickpea and lentil, ascochyta blights, caused by fungi *Ascochyta rabiei* and *Ascochyta lentis*, are the most devastating worldwide, causing severe yield losses in affected fields (Nene, 1984). These diseases pose a real threat to the sustainability of the crop production. The research was conducted to study the genetic basis for resistance to ascochyta in chickpea and lentil and to identify molecular markers linked to the resistance genes for ascochyta blights in a chickpea population developed from a cross between CDC Chico and CDC Marengo, and in a lentil recombinant inbred population derived from a cross between ILL5588 and L692-16-1. The results of the study will provide plant breeders with new tools to select for disease resistant plants and to pyramid disease resistance genes from different sources into elite breeding lines.

Results and Discussion

A chickpea segregating population was developed from a cross between CDC Chico (small kabuli; moderately resistant to ascochyta) and CDC Marengo (large desi; highly susceptible to

ascochyta). The reaction to ascochyta (on a scale of 0 to 9; where 0 = no symptom and 9 = plants are dead) was evaluated in both 120 lines of $F_{2:3}$ population and 74 recombinant inbred lines ($F_{6:7}$ generation) under greenhouse conditions. Based on phenotypic evaluation, the $F_{2:3}$ lines were classified into 29 resistant, 67 segregating and 24 susceptible, while the RIL's were grouped into 32 resistant, 7 segregating and 35 susceptible. $\div^2$ analyses indicated that these segregation ratios are not different ($P > 0.10$) from 1:2:1 and 1:1 expected ratios for the F_2 and F_6 generations, respectively. These results indicated that a major gene for resistance to ascochyta blight may be segregating in the population.

DNA was extracted from 110 F_2 plants and 74 RIL's. DNA samples from ten of each of the most resistant and susceptible lines were bulked. DNA's of the two parents and the resistant (R) and susceptible (S) bulks were screened for polymorphisms using 800 RAPD primers, 50 ISSR primers, 60 STMS's (**S**equence-**T**agged **M**icrosatellite **S**ites) from chickpea and cowpea, and 48 AFLP primer combinations.

No polymorphism between the R and S bulks was found from screening of all the RAPD and ISSR primers. Three STMS's (TA76s, TA110 and TA176) and one AFLP primer combination (EcoR1+aca and Mse1+cac) produced polymorphic bands between the parents and the two bulks. These markers were then tested across each lines of the $F_{2:3}$ and $F_{6:7}$ generations. The association of these markers with the reaction to ascochyta blight was determined by one-way ANOVA and the results are presented in Table 1. The aca-cac_{170} is linked to the major locus for the resistance and captured 32 % and 46 % of the variation in the F_2 and RI populations, respectively. The TA110 marker that was linked with the AFLP marker (aca-cac_{170}) at a distance of 16 cM on LG7 may be linked to the same gene for the resistance but at a farther distance than the ACA-CAC_{170} marker. Additional two loci on LG1 and LG4 that were tagged by TA76s and TA176 markers also contributed a smaller effect to the resistance to ascochyta in this population.

Table 1. Genomic location, percentage of the phenotypic variation and donor parent for marker loci associated with reaction to ascochyta blight in $F_{2:3}$ and RI populations of chickpea derived from a cross between CDC Chico and CDC Marengo.

Marker locus	Linkage Group*	% variation ($F_{2:3}$)	% variation (RIL's)	Source of resistance
aca-cac_{170}	7	32 % ($P < 0.000$)	46 % ($P < 0.000$)	CDC Chico
TA76s	1	14 % ($P < 0.000$)	19 % ($P < 0.000$)	CDC Chico
TA110	7	10 % ($P < 0.004$)	13 % ($P < 0.000$)	CDC Chico
TA176	4	7 % ($P < 0.01$)	9 % ($P < 0.01$)	CDC Chico

*) The STMS markers were previously assigned into linkage groups based on the analysis of a RIL population developed from an interspecific cross between *C. arietinum* (ICC4958) and *C. reticulatum* (P.I. 489777; Winter et al. 1999)

The AFLP marker (aca-cac_{170}) was cloned and sequenced in order to develop a SCAR (**S**equence **C**haracterized **A**mplified **R**egion) marker to simplify its use for marker-assisted selection. The sequence of this marker contains a tandem GA repeat (16 *n*). This SCAR marker was designated SCAR $AFAG_{140}$. The forward and reverse primer sequences of this SCAR are

5’-ttc aca aag taa tat acc tg-3’ and 5’-atg agt cct gag taa cac gga-3’, respectively. At 54 ºC of annealing temperature these primers generated a band of 140 bp.
Advanced generations of breeding lines and check cultivars that were assessed for ascochyta reaction under field conditions (natural infection) in Assiniboia in the year of 2001 were used to test the utility of these markers. DNA was extracted from individual lines and scored for the presence of the SCAR and TA76s markers. The result of the analysis is presented in Fig. 1. Mean disease score of the lines that had both markers was 3.8 ± 0.8 which is significantly lower than the lines that had either or none of the markers present. This result demonstrated the potential of the markers for marker-assisted selection.

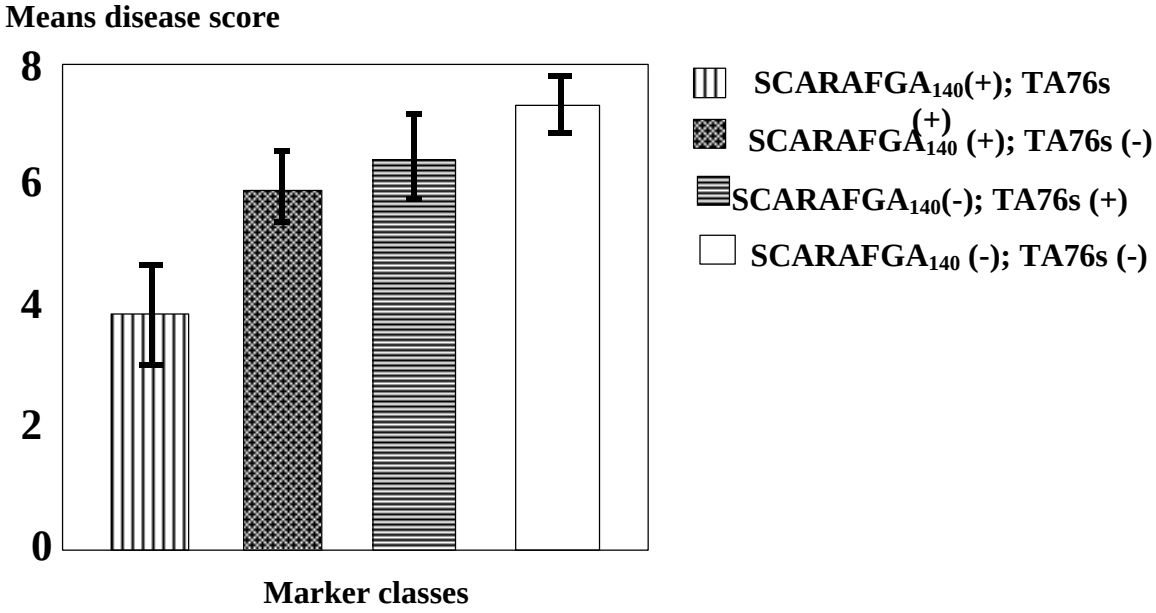

Figure1. Mean ascochyta blight score of advanced chickpea breeding lines for each combination of $SCARAFGA_{140}$ and TA76s markers. Vertical bars indicate the SE of the means. + = marker is present; - = marker is absent.

A RIL population of lentil that was developed at ICARDA from a cross between ILL5588 and L692-16-1 was used in the current study. ILL 5588 is one of the most important donor lines for ascochyta resistance in both Australian and Canadian lentil breeding programs. The lines were tested for their reaction to two isolates (RM 389-1 and PC-1) of *A. lentis* in a growth chamber. The isolates were chosen based on the differential reaction of the two parents to the disease. The scoring was done at 14 days after inoculation on a scale of 0 (no symptoms) to 7 (plants are dead).

DNA’s from the two parents and the RI lines were extracted following the CTAB procedure. A total of 818 RAPD and ISSR primers were screened for polymorphisms between the two parents and the resistance and susceptible bulks. Primers that produce polymorphic bands between parents and the two bulks were tested on the individual RI lines. The marker data generated from this analysis were integrated into the existing linkage map developed from the same

population (Eujayl et al. 1998). QTL analysis for the association between marker loci and the reaction to two isolates was done using QTL Cartographer program Version 1.13.

Of the 818 RAPD and ISSR primers that were screened, only 5 primers resulted polymorphic bands between the two parents and the resistance and susceptible bulks. QTL analysis indicated that only two marker loci generated using RAPD primers OPB18 and OPV1 were associated (LOD > 3.0) with the reaction to ascochyta blight. These two QTL ($OPB18_{680}$ and $OPV1_{800}$) were located on LG 2 and LG 4 (Table 2) of the lentil map and explained 36 % and 29 %, respectively, of the disease reaction variability. The $OPB18_{680}$ marker is identical to RB18 marker that was previously identified from the cultivar Northfield by the Australian group (Ford et al. 1999). The $OPB18_{680}$ marker was sequenced and converted into a SCAR marker to simplify the analysis. The forward and reverse primer sequences for this SCAR marker are following: 5'-ccacagcagtcacaaaccctatatga-3 ' and 5 ' -ccacagcagtattattatcaatattttg-3 ' , respectively. At a 60ºC annealing temperature, these primers will amplify a single band at a size of 680 bp (Fig. 2).

Linkage group	Closest marker to the LOD peak	LOD value	% variation	Additive effect	Source of resistance
2	SCAR $B18_{680}$	8.0	0.36 (P <0.000)	-0.84	ILL5588
4	$OPV1_{800}$	8.3	0.29 (P <0.000)	-1.06	ILL5588

Table 2. Summary of QTL for reaction to ascochyta blight in a RI population of lentil derived from a cross between ILL5588 and L692-16-1.

Figure 2. Co-segregation of the SCAR $B18_{680}$ marker and the ascochyta disease reaction in the RIL's derived from a cross between ILL5588 x L692-16-1

To examine whether the cultivar ILL5588 contains DNA fragments similar to known resistance genes, a PCR-based approach to isolate resistance gene analogues (RGA) sequences from the lentil genome was employed. This approach relies on the use of degenerate primers, which were designed based on the conserved peptide motifs of cloned resistance genes from arabidopsis, flax and tobacco to amplify leucine rich repeats (LRR), nucleotide binding-sites (NBS) and kinase domain-containing sequences from lentil cultivar ILL5588. More than 48 RGA fragments (sizes range from 400 to 700 bp) that were generated from the amplification of lentil ILL5588 were cloned and sequenced. Sequence analysis indicated several of these genomic fragments were homologous to each other (64 to 92 % identity). These sequences will be deposited into the Genebank database at the National Center for Biotechnology Information at Bethesda, Maryland. Database search resulted some significant sequence similarities to known resistance genes, such as *RPS2* (from Arabidopsis; Bent et al. 1994) and *L*6 (from flax;Lawrence et al. 1995).

Conclusion

1. Three QTL were associated with resistance to *A. rabiei* in a chickpea population derived from a cross between CDC Chico and CDC Marengo
2. Two QTL were identified to control the resistance to ascochyta blight in a RI population of lentil derived from a cross between ILL5588 and L692-16-1.
3. The use of degenerate primers targeting NBS-LRR and kinase domain-containing sequences has a potential to isolate disease resistance-related sequences from lentil genome.

References

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