RAPD and AFLP Markers Linked to Anthracnose Resistance Gene in PI 320937 Lentil (*Lens culinaris* Medik.)

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Key words: Lentil, anthracnose, resistance gene, RAPD and AFLP markers

Abstract

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore is the causal fungus for anthracnose disease in lentils. A germplasm accession, 'PI 320937', is among the lines used as a resistance source to develop cultivars in the breeding program. A cross of Eston (susceptible) and PI 320937 (resistant) was used to develop 147 recombinant inbred lines (RILs) to study the genetics of resistance and identify markers associated to the resistance gene. The $F_{5:6}$ RILs were inoculated with *C. truncatum* isolate 95B36 at 10^5 conidia ml^{-1} and scored for anthracnose reactions over 2 replications in the greenhouse. About 600 RAPD and 10 AFLP primers were screened. We used bulk segregant analysis to construct contrasting DNA bulks, one containing only resistant and the other only susceptible plants based on the greenhouse tests. These polymorphic markers between parental lines were used to genotype RILs and make linkage analysis. Segregation data indicated that a single major gene (*LCt-2*) confers resistance. Minor genes also modified the level of resistance. Two RAPD markers; namely, OPE $O6_{1250}$ and UBC 704_{700} were linked in repulsion and coupling at 6.4 and 10.8 cM, respectively, to the resistance gene. Also, 3 AFLP markers were identified within 30 cM distance from the resistance locus. These markers will be useful in lentil breeding via marker-assisted selection towards developing cultivars with anthracnose resistance.

Introduction

Lentil (*Lens culinaris* Medikus) is one the most important annual seed crops in the Prairies. Canada is the biggest lentil producer and exporter in the world. Lentils are becoming increasingly popular in developed countries as they are perceived as a healthy component of the diet. However, lentil production is threatened by two major diseases, ascochyta (caused by *Ascochyta fabae* f. sp. *lentis*) and anthracnose (caused by *Colletotrichum truncatum* (Schwein.) Andrus &W.D. Moore). Currently, the disease is widespread in western Canada. The fungus overwinters in infested plant debris as microsclerotia that serve as primary inoculum in the field. Periodic disease epidemics result in drastic yield losses (20-100%) in isolated fields (Gibson et al. 1991; Buchwaldt et al. 1992). Genetic resistance is a viable option for reducing losses from anthracnose infection of lentil. Only few lines have been used as sources of resistance in the Crop Development Centre (CDC), University of Saskatchewan pulse breeding program. However, genetic information on the mode of inheritance of resistance to anthracnose is limited. The objective of our study was to analyze pattern of inheritance and find markers linked to the resistance gene.

Materials and methods

F_5 derived F_6 recombinant inbred lines (RILs) were developed using single descent from a cross of Eston x xPI 320937. The RILs were infected with anthracnose isolate to determine the resistance pattern and screened with RAPD and AFLP primers to find markers linked to the resistance gene. Leaf samples for DNA was collected from F_5 plants while the disease data was obtained from the F_5-derived-F_6 RILs.

Results and discussion

The data for the F_5 derived F_6 RILs were used to infer the genotype of the individual F_5 plants from which DNA samples were collected. The F_5 population consisted of 70 resistant plants and 77 susceptible plants giving a good fit to a 1 resistant : 1 susceptible ratio that suggested a single gene controlling resistance. The resistance gene is called as *LCt-2*. However, while resistance for anthracnose was associated with a major gene effect, resistance was modified by minor gene effects. Two RAPD and 3 AFLP markers were identified linked to the resistance locus. The 2 RAPD markers OPE $O6_{1250}$ and UBC 704_{700} were linked in repulsion and coupling, and 6.5 and 10.6 cM away from the resistance locus where as, three AFLP markers were within 30.6 cM distance on one side of the gene for resistance. Sequence information and chi-square test for segregation ratios of markers is given in Table 1. These markers would be useful in marker-assisted selection.

Acknowledgements

The authors acknowledge the financial support from Agriculture Development Fund (ADF) and Agri-Food Innovative Fund (AFIF). Technical assistance from Brent Barlow and Pulse Field Lab Crew, and Carmen Breitkreutz are highly appreciated.

References

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Table 1. Sequence information and chi-square test for segregation ratios of AFLP and RAPD markers in $F_{5:6}$ RILs from a cross of Eston and PI 320937 lentil

Primers	Sequences	Segregation ratio (1:1)		χ^2 (1:1)	P
UBC 704	GGAAGGAGGG	48(+)	45(–)	0.10	0.75-0.90
OPEO6b	CCACGGGAAC	46(–)	49(+)	0.10	0.75-0.90
OPEF4	GGTGATCAGG	48(+)	47(–)	0.01	0.9-0.95
UBC 229B	CCACCCAGAG	48(–)	47(+)	0.01	0.9-0.95
OPER4	CCCGTAGCAC	38(–)	56(+)	3.45	0.05-0.10
UBC 18b	GGGCCGTTTA	45(+)	48(–)	0.10	0.75-0.90
EMCTTAAG	CTT/AAG*	47(+)	45(-)	0.04	0.75-0.90
EMCTTACA	CTT/ACA*	50(+)	42(–)	0.70	0.25-0.50
EMCTAAAG	CTA/AAG*	45(-)	48(+)	0.10	0.75-0.90

+ = presence of the band, – = absence of the band
EM = corresponds to EcoR1 and Mse1 primers while * = indicates the 3 selective sequences for the primers, respectively.