Linkage of RAPD Markers and Ascochyta Resistance in Lentil

C.P. Andrahennadi, A.E. Slinkard and A. Vandenberg
Crop Development Centre
University of Saskatchewan, Saskatoon, Canada.

Genetic linkage of RAPD markers a ith genes for resistance to ascochyta blight caused by Ascochyta fabae f sp lentis Gossen et al was studied in lentil (Len culinaris Medikus) Template DNA was extracted from leaf tissue of' 70 F_2 plants in two hybrid populations Ascochyta inoculation was performed on F_3-derived F_3 families in a disease nursery with a susceptible check. Spanish Brown lentil Bulked segregant analysis was used to identify polymorphic RAPD bands in four bulks for 300 random primers. One polymorphic band was detected in Indianhead x Eston population indicating linkage RAPD assay s on individual DNA samples confirmed that this RAPD marker (UBC primer # 227) is linked to the recessive gene (ral_2) in Indianhead lentil with a map distance of 14.1±4.4 cM

Key words: Ascochyta, Genetic linkage, Lentil, RAPD markers

Introduction

Ascochyta blight, caused by *Ascochyta fabae* f. sp. *lentis,* is the most serious disease of lentil *(Lens culinaris* Medikus) in western Canada. Cool, wet and humid conditions are very conducive to this disease and it progresses quickly and develop up to epidemic levels under these conditions.. It is seed-borne as well as stubble-borne and attacks all above ground parts of the lentil plant. First visible symptoms are whitish to greyish necrotic lesions which appear on leaflets, stems and petioles. Disease development is accelerated by rainy weather during pod filling and seed maturation which results in high levels of seed mfection. Income losses of 70% or more have been reported due to the combination of yield and seed quality losses by ascochyta (Gossen and Morrall **1983).**

The management of ascochyta blight is successful only through an integrated approach. Crop rotation, use of ascochyta-free seeds, seed treatment and foliar fungicides have proven useful in managing this disease (Russell et al. 1987). However, the use of resistant lentil cultivars is by far the least expensive, the most environmentally sound and the most effective means of controlling this disease. An effective and efficient method of selecting resistant genotypes from segregating populations is essential to the lentil breeding program for ascochyta resistance. Recently researchers have utilized molecular markers as tools for indirect selection for disease resistance. This approach takes advantage of the close linkage between the molecular marker and the disease resistance gene. Accordingly, resistant genotypes can be selected by screening the segregating plants for the linked molecular marker.

Random amplified polymorphic DNA (RAPD) is mamly a method of amplifymg a specific sequence of DNA of a particular genotype. RAPD polymorphisms are the result of either a nucleotide base change that alters the primer binding site, or an insertion or deletion within the amplified region (Williams et al. 1990, Parks et al. 1991). Therefore, the resulting amplified products appear as absence or presence of a band for a single locus. Hence. RAPD markers are dominant markers. Individuals with two copies of an

allele are not distinguished from those with one copy of the allele The process that results in RAPD bands is known as polymerase chain reaction (PCR). Thus, the RAPD is, in fact, one type of PCR-based molecular marker.

RAPD markers possess several advantages over conventional markers, such as morphological, isozyme and restriction fragment length polymorphism (RFLP) markers One of the most Important of them is the availability of abundant polymorphic loci. In addition. RAPD assay is quick, easy to perform and relatively Inexpensive DNA-based molecular markers have been developed for disease resistance genes using near-isogenic lines and either RFLP (Young et al. 1988, Sarfatti et al. 1989, Schuller et al. 1992) or RAPD analysis (Martin et al. 1991, Paran et al. 1991, Penner et al. 1993).

Bulked segregant analysis

The application of bulked segregant analysis (BSA) in molecular marker screening is an efficient method of detecting linked molecular markers. This has been successfully employed to develop RAPD markers for a downy mildew resistance gene in lettuce (Michelmore et al 1991). This method uses two bulked DNA samples collected from individuals segregating in a single population. Each bulk consists of individuals that differ for a specific phenotype or genotype or individuals at either extreme of a segregating population. RAPD markers linked to this locus should appear polymorphic between the pools for alternative parental alleles. Within each pool or bulk the individuals are identical for the trait or gene of interest, but are arbitrary for all other genes. Two or few pools contrasting for a trait (e.g., resistant and susceptible to a disease) can be analyzed to identify markers that distinguish these pools. Markers that are polymorphic between pools will be genetically linked to the locus which was the basis for constructing these pools.

The objective of this paper is to present genetic linkage of RAPD markers with genes for resistance to ascochyta blight in two lentil crosses using bulked segregant analysis.

Materials and Methods

Crosses were made between two lentil cultivars with high levels of resistance to ascochyta (ILL 5588 and Indianhead) and the susceptible cultivar Eston lentil ILL 5588 lentil has a single dominant gene (Ral_1) and Indianhead lentil has a single recessive gene (ml,) for ascochyta resistance (Andrahennadi et al. 1993). The F_2 plants of these two crosses (ILL 5588 x Eston and Indianhead x Eston) were raised in a growth chamber (22/18°C, day/night) and DNA samples were extracted from individual F_2 plants using leaf samples and by modified CTAB method (Murray and Thompson 1980). The DNA samples were stored in a freezer at -20°C. Seventy F_2-derived F_3 family lines from each cross were screened in a disease nursery at the North Seed Farm in Saskatoon in summer 1994. Plant lines were space planted with the alternatively sown susceptible check, Spanish Brown lentil, in 4m long rows. These plants were inoculated by spreading ascochyta-infected lentil debris between the plant rows 40 days after sowing. In addition, a misting irrigation, twice each night, was used to create conditions suitable for ascochyta infection. The $F_{2:3}$ lines were harvested separately at the end of the season. One hundred seeds from each $F_{2:3}$ line from both populations were plated on potato dextrose agar (PDA) to determine percent seed-borne ascochyta infection (PSBAI).

The DNA from five highly resistant and five susceptible plants was pooled (bulked) separately for each F_2 population (bulked segregant analysis). Accordingly, each population had two bulks. one resistant and one susceptible bulk and each bulk was made of DNA samples from five F_2 plants (bulked DNA concentration was 30 ng/μl).

RAPD assays

PCR buffer xl	
KCI	50 mM
Tris-HCI	10 mM (pH=9.0)
Triton X- 100	0.1%

Reaction mixture	Per 25 μl	Conc. in reaction mixture
PCR buffer xl	2.5 μl	
$MgCl_2$	4 mM	4 mM
dNTP	4 μl	200 μM
Primer	4 μl	0.2 μM
Taq polymerase	1.5 units	
template DNA	30 ng	1.2 ng/μl

PCR program

Step	Time(s)	Temperature(°C)
denaturing	5	94
annealmg	30	36
extension	60	72

Using the above PCR reaction mixture and the PCR program, four bulks of template DNA were screened for polymorphisms using 300 random oligonucleotide primers (IO-mer) [from Biotechnology Laboratory, University of British Columbia (UBC)] in 1.2% agarose gel electrophoresis.

Results and Discussion

The disease screening experiment resulted in a heavy infection of ascochyta (susceptible check, Spanish Brown lentil had 37% seed-borne ascochyta infection) ILL 5588 and Indianhead lentil had 2.3 and 6.1% respectively and Eston lentil had 31.2% seed-borne ascochyta infection (Table 1). A wide range in percent seed-borne ascochyta infection (PSAI) was observed among $F_{2:3}$ families in both hybrid populations. The rating system considered resistant lines as <7.5 PSAI and susceptible lines as >17.5 PSAI, while the segregating lines were between 7.5 ≤ and ≤ 17.5 PSAI. Single gene inheritance in each population (Ral_1 in ILL 5588 and ***ml,*** in Indianhead lentil) was tested using chi squared test (Table 2.). Both populations resulted in a good fit to the expected 1.2: 1 ratio expected from the single gene inheritance of resistance to seed-borne ascochyta infection in these two resistant parental lines (ILL 5588 and Indianhead). The inheritance of resistance to ascochyta is due to a dominant gene (Ral_1) in ILL 5588 lentil and a recessive gene *(ml,)* in Indianhead lentil (Table 2).

After screening of 300 primers, only one primer, UBC #227 (CTAGAGGTCC), produced a distinct RAPD band (1.29 kb) between the resistant and susceptible pools in the Indianhead x Eston population. This primer was then used on the individual DNA samples of all F_2 plants in this population. The Linkage-l (Suiter et al. 1983) computer program was used to detect linkage and estimate the map distance This RAPD marker is linked to the recessive gene (ral_2) in Indianhead lentil in repulsion phase with a map distance of 14.1 ±4 4 cM. This marker can be a useful tool to screen for ascochyta resistance in breeding populations involving Indianhead lentil as the resistant parent. but a closer linkage would greatly increase the efficiency of selection. However. for gene pyramiding to be effective another marker, preferably a closely linked one, is required with the other resistance gene (Ral_1) in ILL 5588 lentil.

Table 1. Percent seed-borne ascochyta infection of the lentil parent lines and the susceptible check.

Lentil line	Seed-borne ascochyta infection (%)
Indianhead	6.1±0.21[z]
Eston	31.6±1.12
ILL 5588	2.3±0. I7
Spanish Brown (susceptible check)	37 6±1.19

'Standard error.

Table 2. Chi squared tests for goodness of fit to monogenic Inheritance of resistance to seed-borne ascochyta infection in two $F_{2,3}$ populations of lentil.

$F_{2,3}$ Population	Observed' R :H: S	Expected' R :H: S	df	χ^2	P
Indianhead x Eston	14' 38 17	l 2 1	2	0.97	0.62
ILL 5588 x Eston	16 35 12	l 2 l	2	1.29	0.53

[z] R=homozygous resistant, H=heterozygous and S=homozygous susceptible $F_{2,3}$ families.
'Resistant phenotype is recessive in Indianhead x Eston population and dominant in ILL 5588 x Eston population.

Conclusions

1. Ascochyta resistance in ILL 5588 lentil is governed by a dominant gene (Ral_1) and in Indianhead lentil by a recessive gene (ral_2).
2. UBC primer #227 (CTAGAGGTCC) produced a RAPD fragment (1.29 kb) which is linked to the recessive gene (ml,) in Indianhead lentil with a map distance of 14.l±4.4 cM.

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