RAPD MARKERS FOR ASCOCHYTA RESISTANCE IN LENTIL

C.P. Andrahennadi, A.E. Slinkard and A. Vandenberg
Crop Development Centre
University of Saskatchewan, Saskatoon, S7N OWO, Canada.

Genetic linkage of RAPD markers with genes for resistance to ascochyta blight was studied in two crosses of lentil. DNA was extracted from F_2 plants of both crosses (ILL 5588 x Eston and Indianhead x Eston). Screening for ascochyta was performed on F_2-derived F_3 lines in a disease nursery with a susceptible check. Bulked segregant analysis was used to identify polymorphic RAPD bands in four bulks for 60 primers. Two polymorphic RAPD bands (one each in primer #333 and 362 in the resistant bulk) were detected in ILL 5588 x Eston population, indicating linkage with the dominant gene, *Ral*$_1$ for ascochyta resistance. Another polymorphic RAPD band (#338 in the susceptible bulk) was detected in Indianhead x Eston population, indicating linkage with the recessive gene, *ra12* *These* marker linkages must be confirmed by studying individual plants so that they can be useful in the selection program for ascochyta resistance in lentil.

Key words: Ascochyta, Lentil, RAPD markers, Bulked segregant analysis

Introduction

Ascochyta blight, caused by *Ascochytu 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 can progress quickly and develop up to the epidemic levels. It is seedbome as well as stubbleborne 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 infection. 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 use of a foliar fungicide have been proven useful in managing this disease (Russell *et al.* 1987). However, the use of resistant lentil varieties 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 imperative to the lentil breeding program for ascochyta resistance. Recent attempts have been reported by many scientists to utilize molecular markers as tools for indirect selection. The basic principle behind this idea is to use known molecular marker and disease resistance gene linkages in the selection process. Accordingly, resistant genotypes can be selected by screening the segregating plants for the linked molecular marker.

Random amplified polymorphic DNA (RAPD or "Rapids" as they are popularly known) is mainly a method of amplifying 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 general process that results in the RAPD bands is known as the 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 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 recent 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 RAPD marker linkages with genes for resistance to ascochyta blight in two lentil crosses using bulked segregant analysis.

Materials and Methods

Crosses were made between two lentil varieties with high levels of resistance to ascochyta (ILL 5588 and Indianhead) and the susceptible variety Eston lentil. ILL 5588 lentil has a single dominant gene *(Ral_1)* and Indianhead lentil has one recessive gene (razz) 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 F2 plants using leaf samples and by modified CTAB (Murray and Thompson 1980). DNA samples were stored in a freezer at -200C. Seventy F_2-derived F, 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, plant lines were harvested separately at the end of the season. One hundred seeds from each line from both populations were plated on potato dextrose agar (PDA) to determine percent seedbome ascochyta infection.

The DNA from highly resistant and susceptible plants in each population was pooled (bulked) separately. 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/ul).

RAPD assays

PCR buffer xl		
KC1	5omM	
Tris-HC1	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 m M	4 m M
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		94
annealing	30	36
extension	60	72

Using the above PCR reaction mixture and the PCR program four bulks of template DNA were screened for 60 random primers (10-mer) (from Biotechnology Laboratory, University of British Columbia) in 1.2% agarose gel electrophoresis.

Results and Discussion

Disease screening experiment resulted in a heavy infection of ascochyta (susceptible check, Spanish Brown lentil had 37% seedborne ascochyta infection level). Parental lentil varieties clearly indicated their differences in resistance and susceptibility. ILL 5588 and Indianhead had 2.3 and 6.1%, and Eston lentil had 3 1.2% seedborne ascochyta infection levels. The results of the ascochyta screening experiment is summarized in Table 1. Both populations showed high variances (36.0 and 55.0), indicating that these populations were segregating for the genes for resistance to seedbome ascochyta infection. In the BSA, two primers [#333 (GAATGCGACG) and 362 (CCGCCTTACA)] produced one polymorphic band in each primer. These DNA products were approximately 2.1 and 0.9 kb in size, respectively. Thus, this indicates that these two markers may be linked with the dominant gene *Rul,* for ascochyta resistance in ILL 5588 lentil. One primer, #338 (CTGTGGCGGT), produced one polymorphic band (approximately 0.8 kb in size) in the susceptible bulk in the Indianhead x Eston population. This indicates that this marker may be linked with the susceptible allele in Eston lentil for ral_2 gene for ascochyta resistance.

Markers such as #333 and 362 are always useful for indirect selection because they are linked to the resistance gene in the resistant parent (ILL 5588). However, the marker #338 is less useful as a selection agent primarily due its linkage to the susceptible parent. This type of marker can only

be satisfactorily used where the same cross (Indianhead x Eston, in this case) is screened in the breeding program.

However, the evidence for these markers is putative and confirmation is necessary for these findings. First, the individual plants that contributed to the bulks must be studied for the same marker pattern. If this is the case, secondly, individual plants of the whole population should be studied for the same primer for their RAPD pattern. Then, if the markers are linked, the distance between the marker gene and the resistance gene can be computed using a computer program such as Linkage- 1 or Map Maker.

Table 1. Percent seedbome ascochyta infection of F_2-derived F_3 families of ILL 5588 x Eston and Indianhead x Eston lentil.

Population	Min.	Max.	Mean	Variance	No. of lines
ILL 5588 x Eston	2.3	31.0	12.0	36.0	63
Indianhead x Eston	3.5	35.0	14.0	55.0	69

Conclusions

1. Two markers (#333 and 362) may be linked with the resistance gene *Ral_1* for resistance to ascochyta in ILL 5588 lentil.
2. One marker (#338) may be linked with the susceptible allele (in cv. Eston) of the resistance gene *ral_2* found in Indianhead lentil.
3. RAPD marker technology, when combined with BSA, is useful for marker assisted selection for resistance to ascochyta blight in lentil.

References

Andrahennadi, C. P., Slinkard, A. E. and Vandenberg, A. 1993. Inheritance of ascochyta resistance in lentil. Soils & Crops workshop'93. Extension Division, University of Saskatchewan. SK.

Gossen, B. D. and Morrall, R. A. A. 1983. Effect of ascochyta blight on seed yield and quality of lentils. Can. J. Plant Pathol. 5: 168-173.

Martin, G. B., Williams, J. G. K. and Tanksley, S. D. 1991. Rapid identification of markers linked to a ***Pseudomonas*** resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. U.S.A. 88:2336-2340.

Michelmore, R. W., Paran, I. and Kesseli, R. V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. U.S.A. 88:9828-9838.

Murray, M. G. and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4325.

Paran I., Kesseli, R. and Michelmore, R. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34: 1021 - 1027.

Parks, C., Chang, L. S. and Shenk. T. 1991. A polymerase chain reaction mediated by a single primer: Cloning of genomic sequences adjacent to a serotonin reporter portion coding region. Nucleic Acids Res. 19:7155-7 160.

Penner, G., Chong, J., Levesque, M., Molnar, S. J. and Fedak, G. 1993. Identification of a RAPD marker for the crown rust resistance gene PC 68 in oats. Genome 36:8 18-820.

Russell, A.C., Cromey, M.G. and Jermyn, W.A. 1987. Effect of seed treatment on seedborne ascochyta of lentil. Proc.Agron.Soc. New Zealand. 17: 15-18.

Sarfatti, M., Kata, J., Fluhr, R. and Zamir, D. 1989. An RFLP marker in tomato linked to the ***Fusarium exysporum*** resistance gene 12, Theor. Appl. Genet. 78:755-759.

Schuller, C., Backes, G., Fischbeck, G. and Jahoor, A. 1992. RFLP markers to identify the alleles on the ***Mla*** locus conferring powdery mildew resistance in barley. Theor. Appl. Genet. 84:330-338.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:653 l-6535.

Young, N. D., Zamir, D., Ganal, M. W. and Tanksley, S. D. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the ***Tm-2*** a gene in tomato. Genetics 120:579-585.