Use of isozyme markers to select for resistance to ascochyta blight in lentil

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

The possible linkages of isozyme loci with genes for resistance to ascochyta blight, caused by *Ascochyta fabae* f. sp. *lentis* Gossen et al., was investigated in lentil (*Lens culinaris* Medikus). The F_2 seeds of five crosses, their parents and five lentil lines (Du Puy, Laird, PI 339283, PI 374118 and PR 86-360) were space planted with a susceptible check, Spanish Brown lentil. Disease inoculation was performed by spreading ascochyta infected lentil debris and providing mist irrigation to enhance disease development. Gel electrophoresis was used to resolve eight isozyme loci using leaf tissue of F_2 plants in three gel and electrode buffer systems. Resistance to ascochyta is due to a dominant gene (Ral_1) in ILL 5588 lentil, and a recessive gene (ral_2) in Indianhead lentil. Significant contingency chi-squared values occurred between the Ral_1 gene and the isozyme locus *Aat-p* (29 cM) and between the ral_2 gene and the isozyme locus *Pgd-p* (28 cM). These markers are useless in selecting for resistance to ascochyta due to the large map distance from the genes for resistance. Thus, markers more closely linked with these genes are required. More powerful molecular markers, such as RAPD, can be used in this endeavour. Two RAPD markers, tightly linked with Ral_1 and ral_2 genes, respectively, will greatly facilitate "pyramiding" of these two genes into one lentil line and thus, produce a stable genetic resistance to ascochyta.

Key words: Lentil, ascochyta resistance, isozymes, genetics, linkage.

Introduction

Ascochyta blight, caused by *Ascochyta fabae* f. sp. *lentis*, is the most serious disease of lentil (*Lens culinaris* Medikus) in western Canada. It is seedborne as well as stubbleborne, and attacks all above ground parts of the lentil plant. Disease development is accelerated by rainy weather during pod filling and seed maturation. 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).

Ascochyta can be controlled successfully by following an integrated approach. Crop rotation, use of clean seed, seed treatment, and use of fungicides on the foliage have proven useful in managing this disease (Russell et al. 1987). However, the use of resistant cultivars is by far the least expensive, the most environmentally sound, and the most effective means of controlling this disease. The development of ascochyta-resistant lentil cultivars would be facilitated by an effective and efficient means of selecting resistant genotypes from segregating populations.

An isozyme marker locus, closely linked with the gene(s) for resistance to ascochyta, will greatly facilitate screening for resistance. This approach, known as molecular marker assisted selection (MMAS), can speed up the breeding program by reducing expensive, time consuming and highly subjective screening for ascochyta resistance in the field.

The objectives of this study were to:

1. Study the inheritance of resistance to ascochyta blight of lentil.
2. Detect isozyme markers linked with gene(s) for resistance to ascochyta.
3. Explore the genetic variation available for resistance to ascochyta blight.

Materials and Methods

Crosses were made between two lentil lines with high levels of resistance to ascochyta (ILL 5588 and Indianhead) and four susceptible lines [CDC Richlea, Eston, PI 345635 and Lo 40 (*Lens culinaris* ssp. *orientalis*, line 40)] that also carried variants for eight isozyme loci. The F_2 seeds of five crosses, their parents and five lentil lines (Du Puy, Laird, PI 339283, PI 374118 and PR 86-360) were space planted in alternate rows with a susceptible check, Spanish Brown lentil, in a disease nursery at the North Seed Farm in Saskatoon in spring 1992. Isozyme analyses on parents and individual F_2 plants were done, using enzyme extracts from leaf tissue (0.2 g), running starch gel electrophoresis and staining for the isozymes segregating in the F_2 (Selander et al. 1971, Cardy and Beversdorf 1984, Weeden and Emmo 1984).

Disease inoculation was done by spreading ascochyta infected lentil debris between the plant rows 40 days after sowing. A misting irrigation, twice each night, was used to create conditions favourable for disease development. Foliage infection of the F_2 plants and other lentil lines was scored using the ICARDA scale (ICARDA 1989) for rating ascochyta (1-no lesions, 3-few scattered lesions, 5-lesions common, 7-lesions very common and damaging and 9-lesions extensive, many plants killed). In addition, 50 seeds from each F_2 plant and 100 seeds from each lentil line were plated on potato dextrose agar (PDA) to determine percent seedborne ascochyta infection. Single and contingency chi-squared tests were used to analyze inheritance and linkage of genes.

Results

The susceptible check, Spanish Brown lentil, was very susceptible to both foliar infection (9) and seedborne ascochyta infection (40%), indicating an effective disease screening procedure (Table 1). Du Puy, Eston and CDC Richlea lentils were highly susceptible to both foliar infection of ascochyta (7 to 9) and percent seedborne ascochyta infection (51 to 57%). Laird lentil was moderately resistant to the foliar infection (5), but was susceptible to seedborne ascochyta infection (37%).

The lentil lines, PI 339283, ILL 5588, PR 86-360 and PI 374118 were highly resistant to both foliar ascochyta infection (1 to 3) and seedborne ascochyta infection (0 to 6.6%). The foliar ascochyta rating of Indianhead lentil was resistant (1) with 12% seedborne ascochyta infection, indicating it had a moderately high level of resistance.

The frequency distributions for percent seedborne ascochyta infection in all four crosses between the resistant ILL 5588 and susceptible lentil lines were distinctly bimodal. All four F_2 populations gave a good fit to a 3 resistant : 1 susceptible ratio, indicating the presence of a single dominant gene (*Ral*$_1$) for resistance to ascochyta in ILL 5588 lentil (Table 2). The cross between the resistant Indianhead and susceptible lentil line, Lo 40 gave a good fit to a 1 resistant : 3 susceptible ratio, indicating the presence of a single recessive gene (*ral*$_2$) for resistance to ascochyta (Table 2).

Eight contingency chi-squared tests were performed to detect possible isozyme linkages with these two genes for resistance to ascochyta. Significant chi-squared values occurred for the *Ral*$_1$ */Aat-p* and *ral*$_2$*/Pgd-p* combinations in the F_2, indicating weak linkages between the dominant gene for resistance to ascochyta in ILL 5588 and the isozyme locus *Aat-p* (29 cM) and the recessive gene in Indianhead lentil and the isozyme locus *Pgd-p* (28 cM) (Table 3).

Table 1. Foliar ascochyta infection rating and percent seedborne ascochyta infection of parent and other lines of lentil.

Variety/line	Foliar ascochyta infection rating[z]	Seedborne ascochyta infection[y] (%)
PI 339283	1	0.0±0.0
ILL 5588	1	1.7±0.1
PR 86-360	1	2.6±0.1
PI 374118	3	6.6±0.2
Indianhead	1	12.0±0.4
Lo 40	7	31.3±1.0
Laird	5	37.2±1.5
Spanish Brown	9	40.0±0.7
Du Puy	9	51.0±0.5
Eston	7	56.7±1.2
CDC Richlea	7	57.4±1.0
PI 345635	9	NA[x]

[z]ICARDA scale, 1=no lesions to 9=lesions extensive, many plants killed (mean of 10 plants).
[y]Mean of 3 plants (100 seeds from each plated on PDA) and standard errors.
[x]Seed was not available for plating due to the complete destruction of this line by ascochyta.

Table 2. Chi-squared tests for a dominant gene in ILL 5588 and a recessive gene in Indianhead lentil for ascochyta resistance in five F_2 populations of lentil.

F_2 population	Observed ratio[z]		Expected ratio[z]		df	χ^2	P
	R	S	R	S			
ILL 5588 x CDC Richlea	30	5	3	1	1	2.10	14.73
ILL 5588 x Eston	65	23	3	1	1	0.06	80.65
ILL 5588 x Lo 40	32	17	3	1	1	2.45	11.75
ILL 5588 x PI 345635	29	9	3	1	1	0.03	86.25
Indianhead x Lo 40	14	30	1	3	1	1.10	29.43

[z]R=resistant and S=susceptible reaction to ascochyta.

Table 3. Contingency chi-squared tests for independent segregation of Ral_1 and ral_2 genes from seven co-segregating isozyme loci.

Paired loci	No. in each contingency cell[z]						df	χ^2	r±σ[y]
	R/F	R/H	R/S	S/F	S/H	S/S			
Ral_1/Aat-p	9	23	9	5	3	0	2	6.04*	28.9±7.4
Ral_1/Aco-1	10	22	9	5	2	1	2	4.59	-
Ral_1/Lap-1	13	15	13	0	5	3	2	3.71	-
ral_2/Aat-p	3	8	2	7	18	5	2	0.01	-
ral_2/Aat-m	2	9	1	7	15	8	2	2.46	-
ral_2/Me-2	1	10	2	9	14	7	2	3.70	-
ral_2/Pgd-p	6	6	1	4	16	10	2	6.62*	28.3±7.9
ral_2/Skdh-p	2	9	2	3	14	13	2	3.12	-

[z]R=resistant and S=susceptible reaction to ascochyta; F=fast, H=heterozygous and S=slow allozymes.
[y]r=recombination fraction (cM) and standard error.
*Significant at the 0.05 level of probability.

Discussion

The isozyme loci *Aat-p* and *Pgd-p* can be used to select resistant genotypes in populations segregating for both genes for ascochyta resistance and the *Aat-p* and *Pgd-p* isozyme loci. However, the practical value of this approach is limited by the relatively high map distances of 29 and 28 cM.

Thus, markers more closely linked with these two genes are needed. Detection of additional genetic markers tightly linked with the Ral_1 gene in ILL 5588 or the ral_2 gene in Indianhead will be much easier now that information is available on the Ral_1-*Aat-p* and ral_2-*Pgd-p* linkages. Thus, these segments of chromosomes can be studied using more powerful molecular markers, such as random amplified polymorphic DNA (RAPD), to find molecular markers tightly linked with the genes for resistance to ascochyta. Subsequently, these two RAPD markers can be used to "pyramid" these two genes for resistance into one genotype. Presumably, this two-gene resistance will be much more stable and durable than single gene resistance.

Conclusions

1. Resistance to ascochyta blight is determined by a single dominant gene in ILL 5588 lentil and a single recessive gene in Indianhead lentil.

2. The isozyme locus *Aat-p* is loosely linked (29 cM) with the dominant gene (Ral_1) for ascochyta resistance in ILL 5588 lentil and the isozyme locus *Pgd-p* is loosely linked (28 cM) with the recessive gene (ral_2) for ascochyta resistance in Indianhead lentil.

3. The lentil lines PI 339283, ILL 5588, PR 86-360, PI 374118 and Indianhead show valuable genetic variation for resistance to ascochyta that can be incorporated in the lentil breeding program for ascochyta blight resistance.

Literature cited

Cardy, B. J. and Beversdorf, W. D. 1984. A procedure for starch gel electrophoretic detection of isozymes of soybean (*Glycine max* L.). Dept. of Crop Science, Univ. of Guelph, Technical Bulletin No.119/8401.

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.

ICARDA. 1989. Food legume improvement program. International nurseries and trials. Lentil international ascochyta blight nurseries-1989. ICARDA, Aleppo, Syria.

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

Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. and Gentry, J. R. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. 1. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. Univ. of Texas Publ. 7103.

Weeden, N. F. and Emmo, A. C. 1984. Horizontal starch gel electrophoresis laboratory procedures. Dept. Hort. Science, New York State Agricultural Experiment Station, Geneva.