Ascochyta Resistance in Lentil (*Lens culinaris* Medikus).

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ABSTRACT

The genetic basis of resistance to ascochyta blight was determined for 20 lentil genotypes and six F_2 populations. Plants were space planted in rows in the field with adjacent spreader rows of the susceptible check Spanish Brown. Plants were inoculated by spreading infested crop debris and mist irrigation was used to create epiphytotic conditions. The disease reaction was measured by rating leaf symptoms on a 1-9 scale and by plating random samples of seed from each F_2 plant on PDA agar to estimate percent seed-borne infection. Lines PI 339283, PI 374118, ILL 5588 and PR 86-360 had high resistance levels with less than 7.5% seed-borne infection. Laird, Eston, CDC-Richlea and other lines were susceptible with seed-borne infection as high as 57%. Indianhead lentil was moderately resistant. Analysis of F_2 data from the six segregating populations suggests that ILL 5588 has one dominant gene and Indianhead has one recessive gene for resistance to ascochyta blight of lentil.

INTRODUCTION

Ascochyta blight, caused by *Ascochyta fabae* f.sp. *lentis*, is an important disease of lentil (*Lens culinaris* Medikus) in Western Canada. This disease has also been reported in Argentina, Brazil, Syria, Greece and Pakistan (Gossen 1985). Moreover, *A. fabae* f.sp. *lentis* has been isolated from seeds originating from Australia, Ethiopia, Hungary, India, Italy, Morocco, Russia, Spain, Turkey and Yugoslavia (Kaiser and Hannan 1982, Kaiser 1983, Kaiser and Hannan 1986). It was first reported in Canada in 1978 and now is the most serious disease of lentil in Western Canada (Morrall and Sheppard 1981).

Ascochyta blight is seed-borne, as well as stubble-borne, and attacks all above ground parts of the lentil plant. The symptoms can appear on all plant stages from seedling to the mature plant. Necrotic lesions on leaves and stems are the first visible symptoms. Lesions on leaves and stems initially are whitish to greyish, but later become light tan colored. These lesions reduce photosynthetic area, and premature defoliation occurs. Lentil requires a high photosynthetic capacity during the grain filling stage for high yields. Thus, heavy yield losses can occur in lentil fields that are heavily infected by ascochyta throughout the pod filling stage. Seedling death can occur due to girdling lesions of the lower stem.

Gossen and Morrall (1983) reported a comparison of ascochyta-inoculated ,uninoculated and with and without a foliar fungicide and observed yield losses of 25 to 40% in susceptible Common Chilean and Eston lentil. An overall income loss of more than 70%

was reported due to the combination of a 40% yield and 50% grade loss.

Ascochyta can be successfully controlled by an integrated approach to disease management. Crop rotation, use of clean seed, seed treatment and use of fungicides have proven useful in managing this disease (Russell et al. 1987). The use of resistant cultivars is by far the least expensive and most environmentally benign method of controlling this disease.

Cultivars with resistance to ascochyta blight are not available yet in Western Canada. The leading cultivar Laird is moderately resistant to foliage infection, but becomes susceptible at maturity resulting in a high incidence of seed-borne ascochyta infection if the plants are subjected to a heavy inoculum load in combination with free water on the plants during the late pod fill (Tay 1989). The other major lentil cultivar Eston is highly susceptible to ascochyta (Tay 1989).

Screening for ascochyta resistance is often complicated by the differences between foliage infection rating and percent seed-borne infection. Seed-borne ascochyta infection provides a consistent and reliable indication of the disease reaction of a lentil genotype. This approach is also practical since discoloration of heavily infected seed results in reduced quality. This approach was first suggested by Tay (1989) who showed that lines with low percent seed-borne ascochyta infection also had low foliage infection ratings.

The development of ascochyta resistant lentil cultivars is facilitated by the availability of resistant parents and knowledge of the mode of inheritance of these genes for resistance. Information on the inheritance of genes for ascochyta resistance also facilitates detection of molecular markers linked with the gene for resistance. This approach, known as molecular marker assisted selection (MMAS), can speed up the breeding program by greatly reducing the expensive and time consuming disease screening in the field and laboratory. The objectives of this study were to:

1) determine the availability of ascochyta-resistant parent lines, and
2) study the mode of inheritance of resistance to ascochyta in lentil.

MATERIALS AND METHODS

a. Development of segregating populations:

Six parental lines of lentil were grown in hydroponic tanks with 20 °C/ 16 hr days and 16 °C/ 8 hr nights. Crosses were made between known ascochyta resistant lines (ILL 5588 and Indianhead) susceptible lines(Eston, CDC-Richlea, Lo40 and PI 345635). F_1 plants were grown in hydroponic tanks in growth chambers during fall 1991 and winter 1992. F_2 seeds were ready for planting in spring 1992.

b. Disease screening of segregating populations:

The F_2 seeds of six selected crosses and their parents were space planted in rows at the North Seed Farm in Saskatoon in spring 1992. Each cross was planted in a 12-row plot with rows 30 cm apart and 4 m long. Odd-numbered rows were planted to the susceptible check (Spanish Brown lentil). The second and twelfth rows were planted to the two parents of each cross. Four inner rows (4,6,8, and 10) were planted with F_2 seeds with 15 cm within-row spacing. Seed was treated with inoculum of *Rhizobium leguminosarum* strain C.

Disease inoculation was performed by spreading infected lentil debris between the rows 40 days after sowing. A misting irrigation, twice each night for half an hour, was used to create artificial epiphytotic conditions. Leaf symptoms started to develop 9 to 10 days after inoculation. Foliage infection of parental lines was rated using the ICARDA scale (ICARDA 1989) for rating ascochyta diseases (1-no lesions, 3-few scattered lesions seen after careful searching, 5-lesions common and easily observed, but little defoliation, 7-lesions very common and damaging, 9-lesions extensive, many plants killed). Individual F_2 plants and parental plants were harvested, threshed and assayed for percent seed-borne ascochyta infection.

Fifty seeds from each F_2 plant and 100 seeds from each parental plant were plated on potato dextrose agar (PDA). Seed lots were surface sterilized with 0.6% NaOCl for 10 minutes. Then they were transferred to a pre-sterilized glass petri-dish with a filter paper in it to absorb excess surface sterilant from the seeds. Ten seeds were plated on each PDA plate under a laminar flow hood. The plates were illuminated with cool white fluorescent light about 12 hrs per day during incubation. These petri dishes were incubated on laboratory benches in a layer two plates deep for 9 to 10 days and percent ascochyta infected seeds determined for each plant.

RESULTS AND DISCUSSION

Parents and Other Lentil Lines:

The results of foliage infection rating and percent seed-borne ascochyta reaction are presented in Table 1 for 20 lines including the six parents. The wet weather prevailing during summer 1992 was favourable for the spread of ascochyta and it enhanced the epiphytotic conditions. The susceptible check Spanish Brown lentil showed a clear susceptible disease reaction (40% seed-borne ascochyta infection). Laird, Eston, and CDC-Richlea lentil showed both high foliage infection rating and high percent seed-borne ascochyta while ILL 5588, PR 86-360, and PI 339283 showed very low levels for both ratings (Table 1). Indianhead lentil had a low foliage rating (1) and a moderate level of percent seed-borne ascochyta reaction (12%). Laird had a moderately resistant foliage rating (5), but a high percent seed-borne ascochyta infection. This confirms the results of Tay (1989) who reported that the resistance in Laird lentil disappeared at maturity. Both wild lentil lines

Lo40 and Ld61 showed susceptible disease reactions.

Table 1. Foliage infection rating and percent seed-borne ascochyta infection for the parents and other lines of lentil.

Line	Foliage infection rating[1]	Seed-borne infection[1] (%)
PI 339283	1	0.0
ILL 5588	1	1.7
PR 86-360	1	2.6
PI 374118	3	6.6
Indianhead	1	12.0
Rose	3	12.0
74TA262	NA	12.2
ILL 5684	3	17.9
ILL 5588x Laird,line 27	5	20.9
ILL 5588x Eston,line 48	5	21.4
Ld61(*Lens odemensis*, line 61)	7	26.7
Lo40(*Lens orientalis*, line 40)	7	31.3
Laird	5	37.2
Spanish Brown	9	40.0
PI 297746	NA	51.0
Du Puy	9	51.0
Eston	7	56.7
CDC-Richlea	7	57.4
PI 345634	3	NA
PI 345635	9	NA

1-Foliage rating is the mean of ten plants(ICARDA scale) and percent seed-borne infection is the mean of three plants(100 seeds per plant assayed).

F_2 Populations of Crosses with ILL 5588 Lentil:

The frequency distributions of percent seed-borne ascochyta infection effectively differentiated between resistant and susceptible genotypes. Four F_2 populations of crosses with ILL 5588 namely, ILL 5588 x Eston, ILL 5588 x CDC-Richlea, ILL 5588 x PI 345635 and ILL 5588 x Lo40 showed clear bimodal frequency distributions of percent seed-borne ascochyta infection. Chi-squared test was used to test for goodness-of-fit to a 3 resistant : 1 susceptible ratio (Table 2.)

Table 2. Chi-squared tests for goodness-of-fit to 3 resistant : 1 susceptible ratio in four F_2 populations of lentil segregating for ascochyta resistance.

Population	Observed ratio R : S	Expected ratio R : S	df	x^2	Prob.
ILL 5588 x Eston	65 : 23	3 : 1	1	0.06	0.75-0.90
ILL 5588 x CDC-Richlea	26 : 9	3 : 1	1	0.01	0.90-0.95
ILL 5588 x PI 345635	29 : 9	3 : 1	1	0.03	0.75-0.90
ILL 5588 x Lo40	37 : 12	3 : 1	1	0.01	0.90-0.95

R-Resistant, S-Susceptible

All four F_2 populations gave a good fit to 3:1 ratio (Table 2.) indicating the presence of a single dominant gene in ILL 5588 for ascochyta resistance. A few F_2 plants of the cross ILL 5588 x Lo40 were more susceptible than the susceptible parent Lo40. This parent is a wild lentil from sub species *orientalis* and matures very early. Thus, it can escape heavy pod infection in the later part of the season resulting in a moderately susceptible rating, rather than a susceptible rating.

F_2 Populations of Crosses with Indianhead Lentil:

The frequency distributions of the two F_2 populations of crosses with Indianhead lentil gave good fit to a 1:3 ratio, indicating the presence of one recessive gene for resistance to ascochyta blight in Indianhead lentil (Table 3).

Table. 3. Chi-squared tests for goodness-of-fit to a 1 resistant : 3 susceptible ratio in two F_2 populations of lentil segregating for ascochyta resistance.

Population	Observed ratio R : S	Expected ratio R : S	df	x^2	Prob.
Indianhead x PI 345635	12 : 32	1 : 3	1	0.12	0.50-0.75
Indianhead x Lo40	14 : 38	1 : 3	1	0.10	0.50-0.75

R-Resistant, S-Susceptible

According to Tay (1989), ILL 5588 has two dominant genes (*Ral*$_2$ and *Ral*$_3$) and one recessive gene (*ral*$_1$). Results from 1992 indicated the segregation of a single dominant gene in ILL 5588, at least in crosses with Eston, CDC-Richlea, PI 345635 and Lo40. Tay considered 31 F_2 plants and studied their F_3 families. It is questionable whether a three gene theory could be satisfactorily explained in such a small number of segregating plants. In addition, the subjectivity of the foliage rating method makes it difficult to reliably separate the resistant and susceptible genotypes in a segregating population. However, the level of resistance in ILL 5588 is very high and useful. This source of resistance can reliably be used in the development of ascochyta resistant lentil cultivars.

Resistance in Indianhead is controlled by a single recessive gene. The level of resistance available in Indianhead is moderate. This resistance can also be exploited in the lentil breeding program. Knowledge of the inheritance of resistance plays an important role in a genetic improvement program. It guides the breeder as to the type of disease segregation to expect in the breeding material. Knowing that a character of interest is monogenicaly controlled, rather than polygenically, is useful in determining the breeding method and type of disease screening to use.

The other important benefit of this information is that it provides the fundamental knowledge necessary for the use of MMAS for ascochyta resistance. Molecular markers have been linked with disease resistance genes in several species. Weeden et al. (1984) reported linkage between the gene for resistance for bean yellow mosaic virus and the isozyme locus phosphoglucomutase (*pgm-2*) in pea (*Pisum sativum*). Michelmore et al.(1991) used both restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) to screen segregating progenies for downy mildew resistance in lettuce and detected three RAPD markers linked to this gene. A molecular marker, closely linked with a gene for ascochyta resistance in lentil will increase the efficiency of selection for resistance by substituting a RAPD test on the DNA of a lentil seedling in the green house for the more expensive (time and money) process of selecting in the field under severe disease levels. However, disease rating of the final few selections must be confirmed by inoculation of the selections with the pathogen.

References

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.

Gossen, B.D. 1985. Ascochyta blight of lentil in Saskatchewan. Ph.D. Thesis, University of Saskatchewan, Saskatoon. 164 pp.

ICARDA. 1989. Food legume improvement program. International nurseries and trials. Lentil international ascochyta blight nurseries-1989. Aleppo, Syria. pp. 5-6.

Kaiser, W.J. 1983. Plant introduction and related seed pathology research in the United States. Seed Sci. and Technol. 11:1197-1212.

Kaiser, W.J., and Hannan, R.M. 1982. *Ascochyta lentis*: Incidence and transmission in imported lentil seed. Phytopathology 72:944 (Abstr.).

Kaiser, W.J., and Hannan, R.M. 1986. Incidence of seed-borne *Ascochyta lentis* in lentil germplasm. Phytopathology 76:355-360.

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 by using segregating populations. Proc. Natl. Acad. Sci.(USA) 88:9828-9832.

Morrall, R.A.A., and Sheppard, J.W. 1981. Ascochyta blight of lentils in Western Canada. 1978-1980. Can. Plant Dis. Sur. 61 (1):7-12.

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.

Tay, J. 1989. Inheritance of resistance to ascochyta blight in lentil. M.Sc. Thesis, University of Saskatchewan, Saskatoon. 70 pp.

Weeden, N.F., Provvidenti, R. and Marx, G.A. 1984. An isozyme marker for resistance to bean yellow mosaic virus in *Pisum sativum*. J. Hered. 75:411-412.