New Sources of Scald (*Rhynchosporium secalis* Davis) Resistance for Western Canadian Barley

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Abstract

Scald, caused by *Rhynchosporium secalis* Davis, is an important fungal foliar disease of barley which can cause significant losses of yield and quality in western Canada. Scald can be controlled by fungicides and/or cultural methods, however, the use of genetic resistance is most desirable control strategy. The objectives of this study were to evaluate scald resistance in two New Zealand barley genotypes; to study the inheritance of that resistance and to test its novelty relative to a number of existing resistance sources available to Canadian breeding programs. New Zealand genotypes 145L2 and 4176/n, which showed scald resistance in NZ nurseries and in Alberta scald screening nurseries in 1998, were evaluated in 1999 and 2000 Alberta nurseries. To determine the genetic control of resistance, these resistant lines were each crossed with scald susceptible CDC McGwire; and resistant versus susceptible progeny ratios from F_2 populations and F_5 recombinant inbred lines (RILs) were tested for chi-square goodness of fit for one or two gene control. To determine the source of the resistance, 'known' *H. vulgare* parents of these NZ lines were evaluated in the Alberta scald nurseries. In addition, 145L2 was crossed with 4176/n and four local resistant lines to determine allelic relationships between '145L2' resistance and the resistance in the local lines. In 1999 and 2000, both NZ lines expressed good scald resistance. Inheritance studies indicated that resistance in both NZ lines is governed by a single dominant gene. '145L2' resistance is different from resistance in 4176/n and the other barley lines tested. All 'known' progenitors of these lines were susceptible suggesting that resistance is a result of mutation and/or introgression(s) from what is described as an 'unknown' parent in their pedigrees. The NZ lines provide new sources of scald resistance that can be incorporated into local breeding lines.

Introduction

Scald, caused by *Rhynchosporium secalis* Davis, can be a serious foliar disease of barley in cool, moist environments with yield losses of 10 – 40% commonly encountered (Shipton et al. 1974). It is a major disease in the prairie provinces of Canada (Tekauz 1991). Common control measures include cultural practices, application of fungicides and the use of resistant cultivars. The use of resistant cultivars is the most economical method for the control of major fungal diseases. Sources of scald resistance have been extensively studied and can be readily acquired with resistance selected for in breeding populations (Penner et al. 1996).

Screening germplasm for new sources of resistance gene(s) and studying inheritance of that resistance enables plant breeders to decide on appropriate breeding strategies to improve local breeding populations. Species of the primary, secondary and to some extent the tertiary gene

pool of cultivated crops can be exploited as sources of disease resistance, and may give a wider spectrum of genetic defense than that contained in the primary gene pool (Harlan 1976).

The main objectives of this study were to:

1. evaluate scald resistance in two NZ lines and determine the genetic control of that resistance.
2. assess the novelty of scald resistance in these lines.

Materials and Methods

Evaluation and Inheritance of resistance

Two New Zealand breeding lines, 145L2 and 4176/n, were chosen as potentially "novel" resistant lines for this study. These lines expressed scald resistance in NZ and in Alberta screening nurseries in 1998. The lines were each crossed with CDC McGwire, a scald susceptible line, and the subsequent F_1 and F_2 generations, and single seed derived F_5 recombinant inbred lines (RILs) were tested for scald reaction at the Agriculture and Agri-Food Canada Research Center, Lacombe, AB in 1999 and 2000; and at the University of Alberta, Edmonton, AB in 2000. F_1 plants and F_2 single plant populations were evaluated in 1999 at Lacombe, while F_5 RILs were evaluated in replicated hill plots at both sites in 2000. One hundred F_5 RILs per hybrid combination were replicated four times at Lacombe and twice at Edmonton. The final analysis was performed on combined F_5 RIL data from five or more replications out of a possible six, pooled across sites.

Source of scald resistance in NZ lines

To determine the source of resistance, the 'known' parents of 145L2 (Emir, Vada, Valetta, 907-12, and 908-11) and 4176/n (Emir and Vada) were evaluated. The pedigree of 145L2 and 4176/n also include an 'unknown' parent(s), possibly *H. spontaneum* lines(s), which was not available for evaluation in this study.

Allelic studies

Parents included in the allelic study were 145L2, 4176/n, Seebe, Senor, CDC Dolly and BM9216-4. Seebe, Senor, CDC Dolly and BM9216-4 represent scald resistant varieties or breeding lines available in western Canada. To determine the allelic relationship between scald resistance gene(s) in 145L2 and these other resistant lines, 145L2 was crossed with each resistant parent and the resulting F_1 plants and F_2 single plant populations were evaluated for segregation for resistance versus susceptibility in 2000 at Lacombe and Edmonton. There was a marked difference in the number of F_2 plants planted and evaluated at both sites, due to barley yellow dwarf virus infection and poor emergence. Data from 2000 Lacombe and 2000 Edmonton nurseries was combined.

Disease nursery

In 1999, four weeks after planting, the Lacombe nursery was inoculated with both a suspension of *R. secalis* isolate GF-80 at 1 x 10^5 spores/ml and infected barley residue from the previous growing season. In 2000, at Lacombe, the nursery was inoculated with a suspension of *R. secalis* isolate 99Rot35 spores at 1 x 10^5 spores/ml and infected straw was also spread in the nursery, three weeks after planting. In 2000 at Edmonton, in addition to infected straw application, the nursery was spray inoculated with a mixture of scald isolates WRS1860, WRS1824, 09-07 and 27-11, three weeks after planting.

Plants or hill plots were rated on a scale of 1 to 9 with 1 = no infection and 9 = complete infection. Resistant and susceptible checks were planted at frequent intervals.

F_2 single plants and F_5 RIL population resistant versus susceptible segregation data were tested for goodness of fit for one or two gene segregation ratios using the Chi-square test. In resistant x susceptible crosses, a resistant versus susceptible cutoff point was delineated at the lowest rating observed for the susceptible parent of the cross. In resistant x resistant crosses, a resistant versus susceptible cutoff point was delineated at the upper confidence limit of the higher rating parent. In F_1 and F_2 generations, ratings were recorded as whole numbers, therefore plants rating higher than the upper confidence limit were classed as susceptible. Confidence interval for parental data were calculated as follows:

Confidence Interval = Mean ± $(t_{df\,,\,\alpha/2})$*(Standard error)

Where,
t = t-value (Steel and Torrie 1980)
df = degrees of freedom (n – 1)
α = significance level (0.05)

The F_2 and F_5 population data from both crosses were tested for normality of distribution (Kolmogorov Smirnov test) and resistant versus susceptible classification was determined only if the populations were not normally distributed. The F_5 RIL data were tested for homogeneity using Bartlett's test before combining data over locations.

Results and Discussion

Evaluation and inheritance of scald resistance

The two NZ lines exhibited good resistance with occasional scald lesions confined to lower and middle leaves (Table 1).

Table 1. Mean and standard deviation of scald reaction for NZ lines and checks at Lacombe 1999 and Combined 2000 (Lacombe and Edmonton).

Line	Description	Mean ± Standard deviation	
		1999	2000[a]
145L2	NZ line	1.1 ± 0.3	1.0 ± 0.1
4176/n	NZ line	1.1 ± 0.3	2.0 ± 0.9
CDC McGwire	Susceptible line	5.4 ± 0.5	6.0 ± 0.9
Argyle	Susceptible line	5.4 ± 0.7	6.2 ± 0.9
Seebe	Resistant line	1.2 ± 0.5	1.3 ± 0.5
CDC Dolly	Moderately resistant line	2.8 ± 0.9	2.9 ± 1.0

[a] combined data (Lacombe + Edmonton)

The mean rating for susceptible lines, CDC McGwire and Argyle, was >5 in both years at both locations. Meanwhile, the resistant check Seebe, and moderately resistant check CDC Dolly, had mean ratings < 2 and < 3 respectively in both years at both sites.

In 1999, the F_1 and F_2 populations from the two inheritance study crosses were planted at Lacombe. A total of eight F_1 and 220 F_2 plants were planted per cross. Due to poor emergence and Barley Yellow Dwarf Virus infection, fewer plants were available for final evaluation. The four F_1 plants evaluated from the 145L2 x CDC McGwire cross were resistant (mean rating = 1.0). For the 4176/n x CDC McGwire cross, the five F_1 plants evaluated had a mean rating of 1.0 also indicating resistance. The resistant F_1 reaction in both crosses indicates that resistance from both NZ lines is inherited in a dominant fashion.

In the F_2 generation for both crosses, the Kolmogorov Smirnov test of normality indicated that populations were not normally distributed ($P < 0.01$), thus data were classed as resistant or susceptible and tested for fit to various segregation ratios. The Chi square test for goodness of fit for the F_2 population from the 4176/n x CDC McGwire cross gave a good fit for a single gene ratio of 3 resistant: 1 susceptible (Table 2). However, the 145L2 x CDC McGwire cross, did not give a good fit for a 3:1 ratio, but good fit for a 13:3 (two gene) segregation ratio was observed (Table 2). At least 800 to 900 plants are required to differentiate between a 3:1 and 13:3 ratio (Hansen, 1959), thus it was desirable to screen an additional generation to confirm the genetic control of resistance from 145L2.

Prior to performing Chi square goodness of fit tests, the F_5 RIL population data from the Lacombe and Edmonton nurseries were tested for homogeneity of variances. The test indicated homogeneous variances between locations ($P = 0.18$ and $P = 0.13$ for the two crosses) therefore data was combined over sites.

The F_5 RIL segregation data from each cross were tested against a 9 (resistant + segregating): 7 susceptible ratio which at F_5 indicates a single gene controlling resistance. The hypothesis for two epistatic genes controlling resistance at F_5 was also evaluated by testing three, two-gene ratios; 193 resistant: 63 susceptible; 81 resistant: 175 susceptible; and 207 resistant: 49 susceptible. For the 4176/n x CDC McGwire cross, only the single gene 9:7 ratio gave a good fit ($P = 0.08$) (Table 2). No two gene ratio tested gave a good fit (Table 2). For the 145L2 x CDC McGwire cross, a good fit ($P = 0.43$) to a 9:7 ratio was also observed (Table 2). However, the F_2

segregation data from that cross had given a good fit (P = 0.21) to a two gene 13:3 ratio. A 13:3 F_2 ratio would give a 193: 63 F_5 RIL ratio. The F_5 RIL population did not give a good fit to a 193:63 ratio and since advanced generation data is more reliable, it is concluded that resistance in 145L2 is governed by a single dominant gene.

Table 2. Chi-square tests for goodness of fit for reaction to scald in F_2 and F_5 populations from the crosses 145L2 and 4176/n with CDC McGwire.

Cross	Generation	Res. Obs.	Susc. Obs.	Ratio tested	Chi-square	P-value[a]
145L2 x CDC McGwire	F_2	132	23	3:1	8.54	0.00
	F_2	132	23	13:3	1.56	0.21
	F_5	52	34	9:7	0.62	0.43
4176/n x CDC McGwire	F_2	119	40	3:1	0.00	0.96
	F_5	43	48	9:7	2.94	0.08

[a] P-value > 0.05 indicate no difference in tested and observed genetic ratio

Novelty of resistance

To pinpoint the source of resistance in the NZ lines, the reaction of *H. vulgare* progenitors of these lines was evaluated in the 1999 Lacombe and 2000 Lacombe and Edmonton nurseries. The lines, 145L2 and 4176/n, were derived from same Vada x 'unknown' hybrid and resistant selections were subsequently crossed to different lines. The *H. vulgare* parents of 145L2 include Emir, Vada, Valetta, 907-12, and 908-11, while 4176/n has Emir and Vada as its *H. vulgare* parents. If all *H. vulgare* parents are susceptible, there is strong evidence that the resistance in the NZ lines is derived from the 'unknown' parent. This 'unknown' parent in 145L2 and 4176/n is possibly an *H. spontaneum* line(s) (Dr. R. Pickering, personal communication). Seed of the *H. spontaneum* parent(s) is no longer available, therefore, could not be evaluated in this study. The 'known' progenitor's of the two scald resistant NZ lines were all susceptible (Table 3) indicating that resistance in the NZ lines in either derived from the 'unknown' parent in their pedigrees or is a result of a mutation event.

Table 3. Summary of scald reaction in the parents of NZ lines and checks in 1999 and 2000.

Line	Description	Mean ± Standard deviation	
		1999	2000[a]
Emir	Progenitor	6.1 ± 0.4	6.0 ± 0.9
Vada	Progenitor	6.3 ± 0.6	6.3 ± 0.8
Valetta	Progenitor	6.1 ± 0.3	5.4 ± 1.3
907-12	Progenitor	5.3 ± 0.6	4.5 ± 1.3
908-11	Progenitor	6.4 ± 0.7	6.4 ± 0.6
Seebe	Resistant line	1.2 ± 0.5	1.3 ± 0.5
CDC Dolly	Moderately resistant line	2.8 ± 0.7	2.9 ± 1.0
Argyle	Susceptible line	5.4 ± 0.5	6.2 ± 0.9
CDC McGwire	Susceptible line	5.4 ± 0.5	6.0 ± 0.9

[a] Combined data (Lacombe + Edmonton)

Line 145L2 was crossed with five local scald resistant lines and disease reaction was observed in F_1 and F_2 generations in 2000 Lacombe and Edmonton nurseries. There was a marked difference in the number of F_1 and F_2 plants planted and evaluated due to poor emergence and/or barley yellow dwarf virus infection, therefore data from two locations was combined. A resistant versus susceptible cutoff was determined after calculating confidence intervals for the parent line data (Table 4). For crosses involving 145L2 with 4176/n, Senor, and BM9216-4, the R vs. S cutoff was 3, i.e. plants rating ≥3 were considered susceptible. For the 145L2 x Seebe cross F_2 plants rating ≥2 were considered susceptible. Finally, for the 145L2 x CDC Dolly cross, plants which rated ≥4 were considered susceptible.

Table 4: Mean, standard error and upper confidence limit of scald reaction of parental lines and cutoff point used in allelic study crosses.

Line	Description	Mean ± Std. Dev.	Upper confidence limit	Cut point used
145L2	NZ line	1.0 ± 0.2	1.1	
4176/n	NZ line	2.0 ± 0.9	2.2	3
Seebe	Resistant line	1.3 ± 0.5	1.3	2
Senor	Resistant line	2.7 ± 1.2	2.9	3
CDC Dolly	Resistant line	2.9 ± 1.0	3.1	4
BM9216-4	Resistant line	2.0 ± 1.1	2.4	3
Argyle	Susceptible line	6.2 ± 0.9		
CDC McGwire	Susceptible line	6.0 ± 0.9		

F_1 plants from all crosses expressed complete scald resistance and the F_2 populations from all five crosses segregated for resistance (Table 5).

Table 5: Number of resistant and susceptible F_1 and F_2 plants in Res. x Res. crosses.

Cross	Combined F_1		Combined F_2	
	Resistant	Susceptible	Resistant	Susceptible
145L2 x 4176/n	11	0	87	6
145L2 x Senor	14	0	74	7
145L2 x Seebe	8	0	72	10
145L2 x CDC Dolly	8	0	76	8
145L2 x BM9216-4	9	0	83	10

Segregation for resistance in a cross indicates that 145L2 carries a different source of resistance than the other parent.

Along with Senor, CDC Dolly and BM9216-4, Seebe is among the few commercial barley lines currently available with effective scald resistance for western Canada. The apparently 'novel' scald resistance in NZ line 145L2 is another source barley breeders in western Canada can utilize. It would be informative if line 4176/n was also crossed with Senor, Seebe, CDC Dolly and BM9216-4, to confirm if this line also contains an additional 'novel' source of resistance.

Conclusions

145L2 and 4176/n express good scald resistance in western Canada and that resistance is controlled by single dominant genes. The 145L2 and 4176/n resistance loci are different and possibly come from a *Hordeum spontaneum* introgression. The 145L2 resistance is different from that in four commercial western Canadian barley lines. These novel resistance source(s) can be incorporated into local barley breeding lines and provide plant breeders with an additional option for scald resistance breeding in western Canada.

References

Hansen, W.D. 1959. Minimum family sizes for the planning of genetic experiments. Agron. J. 51: 711 – 715.

Harlan, J.R. 1976. Genetic resources in wild relative of crops. Crop Science. 16: 329 – 333.

Penner, G.A., Tekauz, A., Reimer, E., Scoles, G.J., Rossnagel, B.G., Eckstein, P.E., Legge, W.G., Burnett, P.A., Ferguson, T., Helm, J.F. 1996. The genetic basis of scald resistance in western Canadian barley cultivars. Euphytica. 92: 367 – 374.

Steel, R.G.D., Torrie, J.H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. Second Edition. McGraw-Hill. New York. p 577.

Tekauz, A. 1991. Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. Canadian J. Plant Patho. 13: 298 – 304.