Theor Appl Genet (1996) 93:421–425

A. Graner · A. Tekauz

RFLP mapping in barely of a dominant gene conferring resistance to scald (*Rhynchosporium secalis*)

Received: 29 September 1995 / Accepted: 5 December 1995

Abstract A progeny consisting of 52 anther-derived doubled haploid barley lines from a F_1 between the winter cultivars 'Igri' (susceptible) and 'Triton' (resistant) was tested for resistance to *Rhynchosporium secalis*. A dominant gene was detected and tagged by a series of cosegregating RFLP markers located in the proximal portion of the long arm of chromosome 3, close to the centromere. One of the cosegregating RFLP markers, *cMWG680*, was converted into a codominant sequence tagged site marker. Polymerase chain reaction analysis with this marker of a series of accessions carrying known resistance genes provided evidence that scald resistance in cv 'Triton' is due to the presence of the *Rh* gene.

Key words Distored segregation · Doubled haploids · *Hordeum vulgare* · STS marker · PCR

Introduction

Leaf scald incited by the fungus *Rhynchosporium secalis* is an important disease that occurs in all of the major barley growing regions of the world. The pathogen is characterized by an extensive genetic variability which is apparently the result of a high mutation rate of the fungal DNA (Goodwin et al. 1994) and results in a broad pathogenic variation and continual changes in the population (Tekauz 1991). Breeding for resistance using conventional field testing procedures can be difficult due to considerable environmental variation. Conversely, the testing of seedling plants with several isolates of *R. secalis* requires a precisely controlled environment. Furthermore, individual isolates of *R. secalis* must be passed through the host regularly to maintain virulence, as repeated in vitro culturing can lead to physiological changes. Until now, at least 13 different genes, most of which confer seedling resistance, have been described (Søgaard and Wettstein-Knowles 1987; Barua et al. 1993; Abbott et al. 1995). Of these, the *Rh9* gene was assigned to chromosome 4 by trisomic analysis (Bockelman et al. 1977). Dyck and Schaller (1961a) used both morphological markers and translocation interchange stocks to localize the *Rh3* and *Rh4* genes on chromosome 3. Together with the *Rh* gene these two genes form a complex locus (Dyck and Schaller 1961b) whose detailed genetic structure remains unclear. The availability of dense molecular marker maps of the barely genome (Garner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993) allows for the rapid and accurate localization of genes. Using such maps and the random amplified polymorphic DNA (RAPD) technique, Barua et al. (1993) localized the Rh_y resistance gene on chromosome 3, and two additional genes, *Rh2* and *Rh13*, have been mapped by restriction fragment length polymorphism (RFLP) markers on chromosomes 1 and 6, respectively (Abbott et al. 1995; Schweizer et al. 1995). In addition, a major component of quantitative resistance to *R. secalis* has been identified on the long arm of chromosome 2 (Backes et al. 1995).

In this study we report RFLP mapping of an additional dominant resistance gene in the proximal portion of the long arm of chromosome 3 and the development of a polymerase chain reaction (PCR)-based codominant marker.

Communicated by F. Salamini

A. Graner (✉)
Institute for Resistance Genetics, Graf-Seinsheim-Str. 23, 85461 Grünbach, Germany

A. Tekauz
Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, Manitoba R3T 2M9, Canada

Material and methods

Plant material

Genetic analysis was performed in a progeny comprising 52 F_1 anther-derived doubled haploid (DH) lines of a cross between the winter barley cultivars 'Igri' (two-rowed) and 'Triton' (six-rowed), which were produced by Dr. B. Foroughi-Wehr (BAZ, Grünbach). In

vitro regeneration was as described by Kuhlmann and Foroughi-Wehr (1989). The spring barley assession E224 was kindly provided by Dr. R. Waugh (SCRI, UK). Pedigrees of the cultivars were obtained from Baumer and Göppel (1992) and checked by personal communication with barley breeders.

Resistance tests

Barley was tested for reaction to *R. secalis* using procedures similar to those described by Tekauz (1991). Inoculum of *R. secalis* was adjusted to $0.8–1.0 \times 10^6$ conidia per milliliter, and 20–25 ml were applied to each pot containing four clumps of six to eight barley seedlings. Pre-inoculation and post-inoculation temperatures were $18° +/- 1.0°C$, respectively, with 17 h of daily illumination. The isolates, and their provenance, included: WRS 1493, Tisdale, Saskatchewan; WRS 1824, Pilot Mound, Manitoba; WRS 1389, Arnell Station, Ontario; WRS 1395 Guelph, Ontario; WRS 1402, Ponoka, Alberta; WRS 1826, Sylvan Lake, Alberta, WRS 1860, Olds, Alberta; WRS1862, Brandon, Manitoba; WRS 1865, Lacombe, Alberta; WRS 1872, Olds, Alberta; WRS 1974, Medstead, Saskatchewan. The two Ontario isolates originated from winter barley, the remainder from spring barley.

DNA and linkage analysis

Isolation of genomic DNA, Southern analysis, and probe labelling were performed according to standard procedures, essentially as described by Graner et al. (1990), except that a solution containing 0,4 *M* NaOH, 0.6 *M* NaCl was used for the transfer of DNA onto membranes (Biodyne 'B'; Pall, Dreieich). PCR amplification was performed by using the following primer pair, designed from the sequence of the anonymous cDNA clone cMWG680:

primer 1 5′ GTT GCC TGC TTC ACC TTC TG 3′

primer 2 5′ GAG CTT CCA CGA ACA GTG TG 3′

PCR was performed in 20 µl volumes, containing 50 ng DNA, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris pH 9.0, 200 u*M* of each dNTP, 0.2 u*M* of each primer and 1 unit *Taq* polymerase (Pharmacia, Freiburg) using a PHC2 thermocycler (Techne, Cambridge). Samples were subjected to 41 repeats of the following cycle: 1 min 93°C, 1 min 60°C, 2.5 min 72°C. Upon completion, the entire reaction was loaded onto a 1.2% agarose gel. DNA fragments were stained by ethidium bromide and visualized under UV light ($\lambda = 302$ nm). Linkage analysis was performed using 'MAPMAKER' computer software (Lander et al. 1987). Recombination values were converted into map distances (centiMorgans, cM) by choosing the Kosambi function (Kosambi 1944). For designation of resistance genes at the *Rh*, *Rh3*, *Rh4* complex the nomenclature of Dyck and Schaller (1961b) was adopted.

Results

Resistance tests and genetic mapping

'Triton' is a six-rowed German winter barley released in 1986 that exhibits a high degree of field resistance to *R. secalis*. In a first screen, this cultivar was tested together with a set of German barley varieties and several accessions with known resistance genes for its reaction to 11 *R. secalis* isolates collected from several locations in Canada (Table 1). Cultivars 'Triton' and 'Franka' generally exhibited similar reaction patterns, except with isolates WSR 1389 and 1395. The enhanced resistance in 'Triton' (MR) compared to 'Franka' (S) is consistent with field data from Germany (Anonymous 1987). Compared to the reactions of barley cultivars with known resistance genes, the reaction pattern of 'Triton' is most similar to those of 'Hudson' and 'Atlas 46', especially when only major differences of more than one resistance category are considered (Table 1, lower panel). A major difference of more than one scoring level was only recorded for isolates WRS 1395 and WRS 1826. The cultivar 'Danilo' (two-rowed, winter) was susceptible to all of the isolates tested, while 'Igri' (two-rowed, winter) was moderately resistant to one and less susceptible to several other isolates. This slightly improved resistance of 'Igri' compared to 'Danilo' is again in accordance with field observations (Anonymous 1987).

To investigate the genetic basis of scald resistance in 'Triton' we performed resistance tests on a set of 52 DH lines using isolates WRS 1824, as this isolate provided good differentiation between the parental lines 'Igri' (S) and 'Triton' (R). The resulting disease reactions are summarized in Fig. 1. The majority (62%) of progeny lines were either fully resistant (R) or completely susceptible (S). Of the remaining lines, 15 (29%) were moderately susceptible (MS), while 5 lines (10%) were moderately resistant (MR). Four F_1 seedlings, which were also tested, showed the R phenotype, indicating a dominant inheritance of the resistance gene.

Table 1 Reaction of four German winter cultivars (upper panel) and four resistant accessions (lower panel) to 11 isolates of *Rhynchosporium secalis* (*R* resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible)

Cultivar	*R. secalis* isolate										
	WRS 1493[a]	WRS 1824[a,b]	WRS 1389	WRS 1395	WRS 1402	WRS 1826	WRS 1860	WRS 1862	WRS 1865	WRS 1872	WRS 1874
Triton	MR	R	MR	MR	R	MS	MR	R	R	MS	MS
Franka	MR; R	R	S	S	R	S	MS	R	R	MS	S
Igri	S	MS; S	S	S	MR	MS	MS	MS	MS	MS	MS
Danilo	S	MS; S	S	S	S	MS	S	S	MS	S	S
Hudson (*Rh*)	R	MR; R	MS	S	R	R	R	R	R	MS	MS
Atlas 46 (*Rh2*, *Rh3*)	R	MR; R	MR	MS	R	MR	MR	R	R	MR	MR
Johnson (*Rh4*)	MR	MR	S	R	MR	R	R	MR	R	R	R
CI3515 (*Rh4*, *Rh10*)	R	R; MR	MR	R	R	R	R	R	R	R	R

[a] Isolate was tested in two independent replications; in the case of divergent ratings, individual scores are given for each replication
[b] Isolate used for tests of 'Igri' × 'Triton' DH progeny

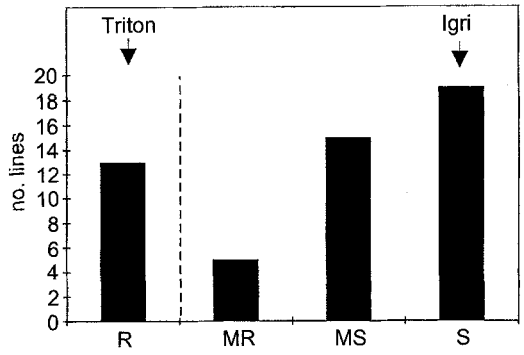


Fig. 1 Reaction to *Rynchosporium secalis* of 52 DH progeny lines and their parents. The resistant and susceptible groups are delineated by a *dashed line*

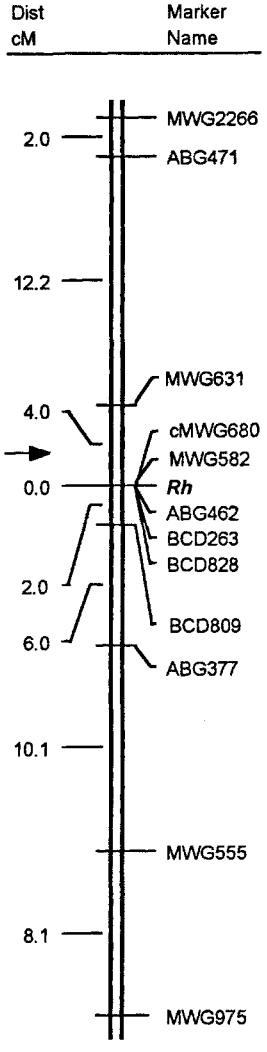


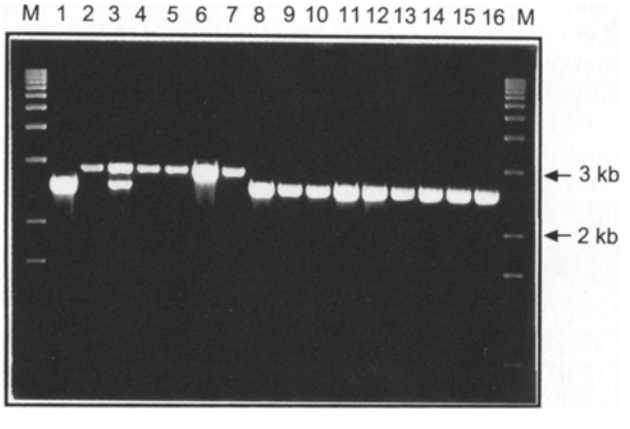
Fig. 2 Partial RFLP map of barley chromosome 3 describing the genetic location of the *Rh* resistance gene from cv 'Triton'. The approximate position of the centromere is indicated by an *arrow*. The long arm is oriented towards the *bottom*

Initial RFLP analysis of the DH progeny was performed with a set of RFLP probes distributed across all seven barley chromosomes. Only those markers located on chromosome 3 showed an association with disease resistance. In particular, a set of five RFLP markers showed a perfect fit within the subset of R and S lines. With respect to the MS and MR progeny lines, analysis of graphical genotypes of each individual DH line revealed that only in the case where both MR and MS lines were included into the susceptible group, i.e., the susceptible group being formed by MR, MS, and S lines, could a map without double crossovers between the gene and its most tightly linked markers be constructed [a simple computation shows that the likelihood of a double crossover within an interval of, e.g., 10 c*M* is $< 0.25\%$ (0.05×0.05); hence, a double crossover event can be expected with $P > 95\%$ only in a progeny of more than 1200 DH lines]. This line of evidence strongly favors the inclusion of both MS and MR phenotypes into the susceptible group, resulting in the map presented in Fig. 2. All of the five cosegregating RFLP markers were located by means of telosomic wheat barley addition lines (Islam et al. 1981) on the long arm of chromosome 3. Likewise, RFLP marker *MWG631* was assigned to the short arm of chromosome 3, placing the centromere into the corresponding 4-cM interval.

Development of a sequence-tagged site (STS) marker

As a basis for marker-assisted selection in breeding programs the anonymous cDNA probe *cMWG680* was converted into a codominant sequence-tagged site marker, which was subsequently used to analyze various barley genotypes. These included several accessions with known resistance genes as well as two progenitors, which are in common to 'Franka' and 'Triton' [cvs 'Ragusa' and 'Friedrichswerther Berg' (F. Berg)]. Amplification yielded two DNA fragments of different size. Cultivars 'Triton' and 'Franka' along with 'Hudson' (*Rh*), 'Dea' (*Rh*), and 'F. Berg' showed an identical DNA fragment of about 3 kb in size, whereas 'Igri', 'Ragusa' and a series of accessions carrying known resistance genes different from the *Rh* gene displayed a band that was clearly discernable by its smaller size (Fig. 3). The size differences of the amplification products are likely

Fig. 3 PCR analysis using STS marker *cMWG680*. *M*, Size marker (1-kb ladder; BRL Gaithersburg), *1* 'Igri', *2* 'Triton', *3* F_1 ('Igri' × 'Triton'), *4* 'Franka', *5* 'Dea' (*Rh*), *6* 'Hudson' (*Rh*), *7* 'F. Berg', *8* 'Ragusa', *9* 'CI8162' (*Rh3*), *10* 'Osiris' (*Rh4*), *11* 'Trebi' (*Rh4*), *12* 'Modoc' (*Rh4*), *13* 'Atlas 46' (*Rh3*, *Rh2*), *14* 'Atlas' (*Rh2*), *15* 'E224' (*Rhy*), *16* 'Abyssinian' (*Rh9*)



to be due to variation in the intron portion of the gene because the cDNA fragment, from the borders of which the primers were designed, is only about 700 bp in size.

Discussion

Analysis of the genetic data from this study revealed that resistance in 'Triton' is caused by a single, dominant gene. A considerable portion of the DH progeny (38%) displayed disease ratings intermediate (MR or MS) between that of the parents (Fig. 1). The occurrence of these phenotypes can be explained if one or both parents contibuted additional minor genes conferring a low level of resistance. This is suggested by the observation that 'Igri', serving as the susceptible parent of the mapping population, still displayed a higher level of resistance than the susceptible check, cv 'Danilo' (Table 1). Also, replicated test data with isolates WRS 1824 and 1493 showed that, despite uniform conditions, disease ratings can vary by one resistance category. Therefore, the MS genotypes of the DH progeny may also comprise "escapes" from the S group. Presence of the resistance gene from cv 'Triton' leads exclusively to the occurrence of R phenotypes, since only this constellation yielded haplotypes without any double crossover. Although seedling plants were used to determine reactions to *R. secalis* in this study, there is good evidence that the same genes which confer seedling resistance are still effective in the adult plant (Abbott et al. 1991; Schweizer et al. 1995).

The availability of DH or recombinant inbred lines appears to be crucial for an accurate genetic analysis of scald resistance. However, it is noteworthy that when anther-derived DH progenies are used large portions of the genome can become affected by distorted segregation (Graner et al. 1991). Therefore, segregation ratio is a poor predictor for the number of genes involved in the inheritance of a particular trait, as found in the present study, where the segregation pattern of both the resistance and adjacent RFLP markers showed a strong deviation from a 1:1 ratio ($Chi^2 = 13.0$, 1 *df*). The observed data fitted exactly the 3:1 segregation indicative of a digenic inheritance. However, the presence of a second gene is unlikely as RFLP survey with an additional set of 36 RFLP probes located on the remaining six chromosomes did not result in the detection of any other resistance gene (results not shown). An even higher degree of distortion was obseved for the same chromosomal region in an F_1 anther-derived DH progeny of a cross between cvs 'Igri' and 'Franka' (cMWG680, $Chi^2 = 35.7$, 1 *df*; Graner et al. 1991). In both cases 'Igri' alleles prevailed, which can be attributed to a superior response of this cultivar to in vitro regeneration. The reduced distortion in the current 'Igri' × 'Triton' progeny is likely the result of both a better in vitro response of 'Triton' in comparison to 'Franka' and the development of an improved regeneration procedure resulting in a decreased in vitro selection pressure (Kuhlmann and Foroughi-Wehr 1987; B. Foroughi-Wehr personal communication).

In this study several RFLP markers were found to cosegregate with the resistance gene. This finding is not unexpected since it is well-known that centromeric regions on wheat and barley chromosomes are characterized by drastically reduced recombination, resulting in an increased marker density in proximal regions of the corresponding genetic maps (Hohmann et al. 1995; Sorokin et al. 1994). Like the resistance gene from 'Triton' the *Rh*, *Rh3* and *Rh4* genes map to the long arm of chromosome 3 where they presumably form a complex locus consisting of closely linked genes (Dyck and Schaller 1961b; Starling et al. 1971). The *Rhy* gene derived from the Scottish breeding line E224 also maps on the long arm of chromosome 3 and might represent a further member of this complex (Barua et al. 1993).

When a series of *R. secalis* isolates were tested, the resistance patterns of both, 'Triton' and 'Franka' were very similar to that of 'Hudson' which is known to carry the *Rh* gene (Table 1). Analysis by PCR revealed a common DNA fragment to be present in 'Triton', 'Franka', 'Hudson', 'Dea', and 'F. Berg'. 'Dea' which is an ancestor of 'Franka' had previously been shown to carry the *Rh* gene (Habgood and Hayes 1971). Furthermore, analysis of the pedigree of 'Triton' revealed that this cultivar is related to 'Dea' and 'Franka' via the old cultivars 'Ragusa' and 'F. Berg'. In the PCR analysis 'F. Berg' displayed the "*Rh* allele", whereas 'Ragusa' showed the alternative fragment (Fig. 3). This line of evidence lends strength to the hypothesis that both 'Triton' and 'Franka' carry the *Rh* gene, which was bred into adapted barley germplasm from the six-rowed winter barley 'F. Berg'. This latter cultivar represents a selection from the Canadian landrace 'Mammuth' and was released in Germany back in 1904.

The conversion of an RFLP marker into a codominant, PCR-based sequence-tagged site (STS) marker provides a means for an efficient marker-assisted selection. This concept can now be effectuated as molecular markers are available for major scald resistance genes on chromosomes 1 (*Rh2*), 3 (*Rh*, *Rhy*), and 6 (*Rh13*) and 2 (quantitative trait locus). The development of corresponding STS markers and their combined deployment should facilitate the rapid selection of adapted genotypes carrying multiple resistances to *R. secalis* in barley breeding programs.

Acknowledgements The authors are indebted to Stephan Kirchner and Eric Mueller for skillful assistance. This work was supported in part by the German/Canadian Cooperation in Agriculture (BIO 16) and BMFT grant 0318990D.

References

Abbott DC, Burdon JJ, Jarosz AM, Brown AHD, Müller WJ, Read BJ (1991) The relationship between seedling infection types and field reactions of leaf scald in Clipper barley backcross lines. Aust J Agric Res 42:801–809

Abbott DC, Lagudah ES, Brown AHD (1995) Identification of RFLPs flanking a scald resistance gene on barley. J. Hered 86:152–154

Anonymous (1987) Beschreibende Sortenliste für Getreide, Mais, Ölfrüchte, Leguminosen, Hackfrüchte. Deutscher Fachverlag, Frankfurt

Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.) Theor Appl Genet 90:294–302

Barua UM, Chalmers KJ, Hackett CA, Thomas WTB, Powell W, Waugh R (1993) Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71:177–184

Baumer M, Göppel W (1992) Gerste: Sorten, Züchter, Ursprungsland, Zulassungsjahr. Bay Landesantalt Bodenkultur und Pflanzenbau, D-85354 Freising

Bockelman HE, Sharp EL, Eslick RF (1977) Trisomic analysis of several scald and net blotch resistance genes. Barley Genet Newsl 7:11–15

Dyck PL, Schaller CW (1961a) Association of two genes for scald resistance with a specific barley chromosome. Can J Genet Cytol 3:165–169

Dyck PL, Schaller CW (1961b) Inheritance of resistance in barley to several physiologic races of the scald fungs. Can J Genet Cytol 3:153–164

Goodwin SB, Webster RK, Allard RW (1994) Evidence for mutation and migration as sources of genetic variation in populations of *Rhynchosporium secalis*. Phytopathology 84:1047–1053

Graner A, Siedler H, Jahoor A, Hermann RG, Wenzel G (1990) Assessment of the degree and type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map in barley. Theor Appl Genet 83:250–256

Habgood RM, Hayes JD (1971) The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity 27:25–37

Heun M, Kennedy AE, Anderson JA, Lapitan N, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hohmann U, Graner A, Endo TR, Gill BS, Herrmann RG (1995) Gene synteny among *Triticeae*: Comparison of physical maps of wheat with genetic maps of barley. Theor Appl Genet 91:618–626

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes PM, Chen FQ, Latitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak J, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kuhlmann U, Foroughi-Wehr B (1989) Production of doubled haploid lines in frequencies sufficient for barley breeding programs. Plant Cell Rep 8:78–81

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Schweizer GF, Baumer M, Daniel G, Rugel H, Röder MS (1995) RFLP markers linked to scald (*Rhynchosporium secalis*) resistance *Rh2* in barley. Theor Appl Genet 90:920–924

Søgaard B, von Wettstein-Knowles P (1987) Barley: genes and chromosomes. Carlsberg Res Commun 52:123–196

Sorokin A, Marthe F, Houben A, Pich U, Graner A, Künzel G (1994) PCR-mediated localization of RFLP clones to microisolated translocation chromosomes of barley. Genome 37:550–555

Starling TM, Roane, CW, Chi KR (1971) Inheritance of reaction to *Rhynchosporium secalis* in winter barley cultivars. In: Nilan RA (ed) Barley genetics II. Washington State University Press, Seattle, Wash., pp. 513–519

Tekauz A (1991) Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. Can J Plant Pathol 13:298–304