Resistance to Scald (*Rhynchosporium secalis*) in Barley (*Hordeum vulgare*) Studied by Near-Isogenic Lines: I. Markers and Differential Isolates

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ABSTRACT

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Near-isogenic lines (NILs) with resistance to scald in seventh generation backcross with 'Ingrid' as recurrent parent (RP) were tested with seven differential isolates of *Rhynchosporium secalis* in Norway and Canada. NILs of 'Turk', 'Brier', 'CI 8162', 'La Mesita', 'Hispoint', 'Atlas 46', 'Modoc', 'Hudson', 'Abyssinian', 'Steudelli', and 'CI 2222' also were evaluated for field reactions. The genetic characterization of the NILs (degree of isogeneity with Ingrid) and with each other was carried out. The molecular marker pattern shows that the backcrossing program has resulted in from 86.3 to 100% RP genome in the NILs,

depending on the marker system. On an average, 96% RP genome was found in the NILs. There were certain consistent (pairwise) differences between the NILs and RP on chromosomes 3H and 7H. Both chromosomes are known to contain loci conferring resistance to *R. secalis*, indicating successful introgression from the donors into the NILs. Approximately two-thirds of the observed RP-NIL polymorphisms were linked to the assumed resistance in the NIL. Based on the marker and phenotypic analyses of the NILs, suggestions for a more appropriate and updated terminology of genes for resistance to *R. secalis* in barley are made. The proposed changes in nomenclature also indicate the differentials that are available as NILs and those lacking.

Additional keywords: differential varieties, molecular markers, NILs, PCA, resistance genes.

Leaf scald (*Rhynchosporium secalis* (Oudem.) J. J. Davis) of barley (*Hordeum vulgare* L.) is a serious disease in many barley-growing areas. Crop rotations, fungicides, or resistant cultivars may control it. The latter, however, depends critically on a clear perception of the interaction between host and pathogen, which in turn requires a well-characterized set of differential cultivars or differential isolates. As to this understanding, much ambiguity still persists. First, the pathogen populations are highly diverse in terms of virulence genes (19,43) as well as in restriction fragment length polymorphism (RFLP) markers (44). The origin of this diversity has been discussed, given the lack of a known sexual stage. Based on the finding of nearly perfect gametic disequilibrium in most populations, Salamati et al. (44) hypothesized that a sexual stage must exist. Second, there is no well-defined set of differential cultivars. The 23 standard differentials described by Goodwin et al. (13) have been helpful, and some of them will be described here, as a historical overview. It will be noted that many of the genetic studies were performed more than 30 years ago, after which there was a gap until the advent of molecular markers. Now knowledge about the genetic basis of resistance against scald is again growing. A short review will follow.

Since the early studies of inherited resistance against scald (30), at least 14 resistance genes or alleles against the fungus have been described. A single dominant gene designated *Rh_a* was responsible for resistance in cv. Brier (8), later amended to *Rh* (38). Graner and Tekauz (15) tagged this dominant gene by a series of cosegregating

RFLP markers located close to the centromere on chromosome 3H. One of the cosegregating RFLP markers, *cMWG680*, was converted into a codominant sequence tagged site marker (STS/MWG680) as a tool for marker assisted selection. Dyck and Schaller (9,10) described five resistance genes (*Rh2*, *Rh3*, *Rh4*, *Rh4²*, and *Rh5*) against scald by the use of morphological markers, segregation tests, or both. The gene in 'Atlas' was designated *Rh2*. Schweizer et al. (46) localized the *Rh2* gene on the distal part of chromosome arm 7HS, close to the RFLP locus *cMWG2018*. The simple sequence repeats (SSRs) Bmac0206 and Bmag0021 also map close to this locus (16,17).

Further, Dyck and Schaller (9) found that the three cvs. La Mesita, Trebi, and Osiris had the dominant *Rh4* in common. In 'Modoc' an allele at the *Rh4* locus was termed *Rh4²*, and in 'Atlas 46' and 'Turk', a gene called *Rh3* was found tightly linked to the allele in 'Modoc'. In addition to *Rh3*, Dyck and Schaller (9) designated a second resistance gene in Turk, *Rh5*, which was independent from *Rh3* and *Rh4*. *Rh3* and *Rh4* both were localized on chromosome 3H. The gene *Rh3* has also been renamed *Rrs3* (24) or *Rrs1* (39) in accord with a more recent terminology of resistance genes.

Through an extensive study, Habgood and Hayes (18) revealed that *Rh4* (9) was not a unique resistance gene, but another allele at the *Rh* complex locus, and therefore called it *Rh⁴*. They also found a complementary gene to this allele in Osiris, *Rh10* (should not be confused with the *Rh10* [19] in 'Pioneer'). The allele in Modoc (9) was renamed *Rh²*. An incompletely dominant gene in 'Cb 1084' also was found at the complex locus on chromosome 3H and was called *Rh³* (18).

The two complementary recessive resistance genes in cvs. Jet and Steudelli were designated *rh6* and *rh7* (4). Later, Habgood

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and Hayes (18) confirmed these findings, but again *rh6* was an allele at the *Rh* complex locus, and was therefore designated as *rh5* instead. Further, they assigned *rh11* in 'CI 4364'. Bockelman et al. (7) confirmed *rh7* that was on chromosome 4H, where they also located the gene designated *Rh9* (4) in 'Kitchin' and 'Abyssinian'. However, we were not able to verify these results in Jet, Steudelli, or Abyssinian. Similarly, Patil (34) did not find the recessive gene designated *rh8* by Wells and Skoropad (52) in 'Nigrinudum' ('CI 2222'). Both studies highlighted resistance genes at the 3H centromeric and 7HS telomeric regions. However, 'Ingrid' was shown to carry a previously unknown locus more proximal on 7HS, and 'CI 11549' a locus more distal on 3HL.

During the last 10 years, important and new resistance genes have been detected in genotypes of wild barley, *H. vulgare* subsp. *spontaneum*, collected in Israel, Iran, and Turkey (1,2,11,12,47) (A. H. D. Brown, *personal communication*). Resistance genes found on chromosomes 1, 3, and 4 were designated as *Rrs12*, *Rrs13*, and *Rrs14*, respectively. Furthermore, a number of quantitative trait loci (QTL) have been mapped (3,26). Strikingly, many of them seem to map in the same areas of 7HS and 3HL as the loci described as major. In this context it is worth remembering that scald symptoms depend critically on environmental conditions, specifically cool temperatures and high humidity (42). Even in carefully controlled environments, scores of individual lines as well as presence or absence of minor QTL may vary considerably (16,17).

The present set of differential cultivars containing these genes is not optimal. The problems are (i) their genetic backgrounds are widely differing; (ii) in many cases, they have two or more known or assumed resistance genes; (iii) the allele/locus designations, as well as location, are unclear and call for revision. Claims have been made that recombination is possible between different loci close to the centromere of 3H (18). With due caution, therefore, Barua et al. (5) called the resistance gene they identified on chromosome 3H *Rh_y*. We will refer to this chromosomal region as the *Rrs* (*Rh*) complex. (iv) There are frequent identical or non-identical synonyms (e.g., Osiris [43] or Nigrinudum [34]); and (v) the accessions are frequently heterogeneous (17).

J. MacKey and H. Magnus developed a set of near-isogenic lines (NILs) in Ingrid; starting from 23 different donors in 1977, a strict backcrossing program has been performed. Since 1996, backcross (BC) progenies from different generations have been screened with two differential isolates. A set of nine BC₇ lines was selected and studied in a diallel design, using the same two isolates. Results indicated that seven of the NILs carried resistances that were allelic or linked, probably on chromosome 3H (34). The other two were unlinked to this region and to each other. In this article, we continue this analysis by means of (i) reactions of the nine NILs to seven differential isolates of *R. secalis* and in the field; (ii) a genetic marker characterization of the nine NILs (degree of isogeneity with Ingrid and with each other and NILs in Steudelli and CI 2222; and (iii) based on this, we will make some suggestions as to a more appropriate and updated terminology of genes for resistance to *R. secalis* in barley.

MATERIALS AND METHODS

Plant materials. NILs and donors. The nine scald-resistant NILs used in this study and their history have been described by Patil (34). In addition, NILs of Steudelli (16) and CI 2222 (34) have been included in this article. The names of donors and NILs are given in Table 1. In test 4, a number of Canadian differential cultivars also were used (not specified here).

Isolates and inoculation tests. The isolates are described in Table 2. The isolates 4004, 2, 16-6, 17, and 28-3 were chosen based on the study by Salamati and Tronsmo (43), and were provided by S. Salamati. All these isolates are of Norwegian origin, with the exception of 4004, which is of Danish origin. The latter was avirulent to all donors in this study and was used as a null isolate. At the other extreme, isolate 2 was virulent against all except Abyssinian and CI 2222. The remaining isolates were regularly used in screening breeding lines (37) and were later found to be rather similar. All were virulent against CI 8162, Modoc, Steudelli, and CI 2222 and avirulent against the others (except for virulence of 28-3 against Abyssinian). Isolates

TABLE 1. Summary of disease scores from the five inoculation tests of near-isogenic lines (NILs) in 'Ingrid' with isolates of *Rhynchosporium secalis*^a

Isolate test	Isolates										Field, test 5
	2, test 1	2, test 3	16-6, test 1	17, test 1	28-3, test 1	4004, test 1, rep1	4004, test 1, rep2	4004, test 2	WRS 1493, test 4	WRS 1824, test 4	
Ingrid	3.9	3.4 ± 0.1	5.0	5.0	5.0	5.0	5.0	4.7 ± 0.1	5.0	5.0	10.2
Abyssinian-NIL	3.0	1.4 ± 0.1	4.8	4.0	5.0	2.7	3.7	2.7 ± 0.1	1.0	1.3	4.0
Abyssinian	3.9	2.7 ± 0.1	2.8	2.8	3.0	1.8	1.5	1.7 ± 0.1	1.0	1.0	0.7
Atlas 46-NIL	2.0	1.7 ± 0.2	2.7	3.2	2.0	2.7	2.5	1.8 ± 0.1	...	...	0.0
Atlas-46	2.6	1.0 ± 0.1	1.3	1.0	1.0	1.0	1.0	1.0 ± 0.1	1.0	1.0	0.3
Brier-NIL	5.0	3.3 ± 0.2	2.0	0.5	2.1	1.5	1.8	1.4 ± 0.1	...	1.0	1.7
Brier	5.0	4.9 ± 0.1	0.7	1.0	1.3	2.0	1.8	1.6 ± 0.1	1.0	1.0	0.0
CI 8162-NIL	3.3	2.8 ± 0.1	4.0	2.5	3.5	2.7	2.3	2.2 ± 0.1	1.0	1.3	5.8
CI 8162	5.0	4.8 ± 0.1	2.5	2.7	5.0	3.0	2.3	1.6 ± 0.1	1.0	1.0	3.3
Hispont-NIL	0.8	0.9 ± 0.1	3.0	0.7	2.0	1.5	1.0	1.6 ± 0.2	1.0	1.0	0.7
Hispont	1.6	0.9 ± 0.2	1.5	0.3	1.7	2.0	...	1.4 ± 0.1	1.0	1.3	...
Hudson NIL	2.0	1.6 ± 0.1	5.0	4.7	5.0	5.0	1.0	2.1 ± 0.1	...	5.0	3.7
Hudson	3.5	2.8 ± 0.3	0.7	0.0	1.7	1.0	1.0	1.0 ± 0.1	1.0	1.0	...
La Mesita-NIL	2.0	1.7 ± 0.1	2.0	1.5	2.8	1.8	1.0	1.6 ± 0.1	1.0	1.76	3.3
La Mesita	4.1	1.6 ± 0.3	4.7	2.8	5.0	2.8	1.3	1.6 ± 0.3	1.0	1.0	9.0
Modoc-NIL	1.9	1.5 ± 0.2	4.7	3.0	4.7	2.7	2.7	2.4 ± 0.3	1.0	1.0	10.7
Modoc	5.0	3.1 ± 0.2	5.0	2.3	5.0	2.5	2.8	2.1 ± 0.2	1.0	2.0	5.0
Turk-NIL	3.9	2.9 ± 0.2	2.0	1.0	1.8	1.5	1.3	1.2 ± 0.1	...	1.0	0.7
Turk	5.0	4.6 ± 0.3	1.2	1.0	0.7	0.7	1.3	0.7 ± 0.1	1.0	1.0	0.3
CI 11549-NIL	...	...	...	...	...	1.0	...	...	2.0	3.3	...
CI 11549	2.5	...	2.8	2.0	2.7	2.2	...	...	1.0	1.0	1.7
CI 2222-NIL	...	...	...	...	...	1.8	...	...	1.0	1.0	...
CI 2222	2.5	...	4.3	2.0	3.0	2.3	...	...	1.3	1.0	...
Steudelli-NIL	2.9	...	5.0	5.0	5.0	5.0	5.0	...	1.0	1.0	...
Steudelli	2.0	...	3.0	2.5	5.0	2.8	2.0	...	1.0	1.0	4.0

^a Data (tests 1 to 4) are replicate means (at least three plants) on a 0-to-5 scale. The field test (test 5) data are the percentage of leaf area infected on a 0-to-100 scale; ... indicates not included in test.

WRS1493 and WRS1824 are of Canadian origin and were used in test 4 in Canada from previous experience of differential virulence (50).

A number of inoculation tests were performed. In test 1, the donors and NILs were challenged with five different isolates (Table 2, except WRS1493 and WRS1824) during April and May 1998. Only one replicate was used, except for 4004, which was tested with two replicates. Individual scores (see scale below) or means of three plants were used as data. In test 2 (May and June 2000), all NILs and donors (except Steudelli and CI 2222) were inoculated with isolate 4004 as donors and parents in the NIL × NIL diallel crosses tested by Patil (34). Test 3 was identical to test 2, except that isolate 2 was used and it was carried out in December 2000 and January 2001 (34). In both cases, each genotype was scored on a replicate mean basis (mean of 27 plants), with three replicates in test 2 and two in test 3. In test 4, all NILs and donors (except CI 2222) were inoculated with isolates WRS 1493 (50)

TABLE 2. Overview of the different *Rhynchosporium secalis* isolates used in the study

Isolate	Octal number	Reference
4004 (Syn. 103)	...	23
2	76333777	43
16-6	72112313	43
17	73162313	43
28-3	73172317	43
WRS1493	...	50
WRS1824	...	50

and WRS 1824 (50), in November and December 2000. In test 5, all NILs and donors (except 'Hispoint' and 'Hudson') were tested in the field in summer 2000 at the Norwegian Crop Research Institute, Kvithamar station, Stjørdal, central Norway. Each genotype was sown in hill plots (30 seeds) at 40 cm distance between plots, in a randomized design with three replicates.

Growth conditions and scoring of symptoms. In tests 1 to 3, the plants were grown in Jiffy strips, in 30-by-20-cm plastic trays with 55 units in each tray and with 4 to 5 seeds per unit. They were grown at 16 to 18°C throughout the test period, with at least 18-h photoperiods (supplementary light) in test 3 and up to 22 h (natural day length) in test 2. The experiments were carried out either in a greenhouse (test 3) or in an air-conditioned daylight growth chamber during the spring and summer (tests 1 and 2). Inocula with an approximate concentration of 2.0×10^5 spores ml⁻¹ were made as described by Salamati and Tronsmo (43). Inoculation and scoring was as described by Salamati and Tronsmo (43). Briefly, a 0-to-5 scale was used, where 0 = symptom free, 1 = traces or small necrotic flecks, 2 = some chlorosis or necrosis along margins, 3 = necroses but less than 10% affected tissue, 4 = necroses on 10 to 40% of the lamina, and 5 = more than 40% and up to fully wilted leaf. Ingrid was used as susceptible control in all tests.

In test 4, accessions were tested as clumps of three to six plants, with four clumps planted per 15-cm-diameter pot. Inoculations were done at the three-leaf stage using inoculum concentration of 1×10^6 spores ml⁻¹. Inoculated plants were incubated for 48 h at 100% relative humidity in the dark with a 2-h light period after 24 h to allow some photosynthesis. Disease reactions based on

TABLE 3. List of restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and sequence tagged site markers (STS) showing polymorphism for 'Ingrid' and the different donors

	1H	2H	3H	4H	5H	6H	7H
RFLP probes	...	...	ABG396 ^a	...	...	...	MWG2018 ^b
	...	...	ABG471 ^a	...	...	...	MWG36B ^c
	...	...	CDO1174 ^d	...	...	...	...
	...	...	MWG555B ^c	...	...	...	...
	...	...	MWG680 ^c	...	...	...	...
SSRs	HVM6 ^e	HVM23 ^e	HVM09 ^e	HVM03 ^e	Bmac0113 ^f	HVM14 ^e	HVM4 ^e
	HVM20 ^e	HVM36 ^e	HVM15 ^e	HVM40 ^e	Bmag0218 ^f	HVM31 ^e	HVM5 ^e
	HVM43 ^e	HVM54 ^e	HVM33 ^e	HVM67 ^e	Bmag0222 ^f	HVM65 ^e	HVM49 ^e
	BMS32 ^g	HVBKAS1 ^h	HVM27 ^e	HVM68 ^e	Bmag0223 ^f	BMS40 ^g	HVM51 ^e
	Bmac0032 ^f	Bmac0093 ^f	HVM60 ^e	HvMLO3 ^f	EBmac0684 ^f	Bmac0018 ^f	HVCMA ^h
	Bmac0399 ^f	Bmac0134 ^f	HVM62 ^e	Bmag0353 ^f	HVLEU ^h	Bmac040 ^f	BMS64 ^g
	Bmag0211 ^f	Bmag0125 ^f	Bmac0067 ^f	EBmac0701 ^f	...	Bmac0316 ^f	Bmac0156 ^f
	Bmag0382 ^f	Bmag0378 ^f	Bmac0209 ^f	...	...	Bmag009 ^f	Bmac0273 ^f
	HvHVA1 ^f	EBmac0415 ^f	Bmag0013 ^f	...	...	Bmag0173 ^f	Bmag007 ^f
	WMC1E8 ^f	...	Bmag0112 ^f	...	...	EBmac0806 ^f	Bmag0021 ^f
	...	...	Bmag0136 ^f	...	...	...	Bmag0120 ^f
	...	...	Bmag0225 ^f	...	...	...	Bmag0135 ^f
	...	...	HvLTPPB ^f	...	...	...	Bmag0206 ^f
	...	...	EBMac0705 ^f	...	...	...	...
	...	...	GMS116 ⁱ	...	...	...	...
STS	KV ^j	...	SM1000 ^k	...	...	...	...
	...	...	agtc-17 ^l	...	...	...	...
	...	...	STS/MWG680 ^m	...	...	...	...
	...	...	YLM ⁿ	...	...	...	...
	...	...	Falcon666 ^k	...	...	...	...

^a Klienohfs et al. (27).

^b Schweizer et al. (46).

^c Graner et al. (15).

^d Heun et al. (20).

^e Liu et al. (28).

^f Ramsay et al. (36).

^g Russel et al. (40).

^h Becker and Heun (6).

ⁱ Struss and Plieske (49).

^j Kanazin et al. (25).

^k Penner et al. (35).

^l Grønnerød et al. (17).

^m Graner and Tekauz (14).

ⁿ Paltridge et al. (33).

lesion type were taken at 14 days using a resistant (R) to susceptible (S) qualitative scale. R and moderately resistant (MR) = no visible symptoms and lesions with dark margins only at lamina edges were regarded as the R phenotypes. In replication 1, the 3 to 6 plants per accession clump were given a single score, based on the mean visual lesion type. In replication 2, each plant was scored individually, and the mean of the 3 to 6 scores calculated as the reaction type.

In test 5, the plots were subjected to natural infection with *R. secalis*. All plots were scored for scald symptoms on 25 July, on a scale of 0 to 100% coverage of leaf area.

DNA extraction. Genomic DNA extraction from Ingrid, donors, and NILs was carried out from 2-week-old seedlings according to standard cetyltrimethylammonium bromide procedures (27). The seedlings were incubated in dark overnight before the DNA extraction to improve the quality.

Molecular markers. Molecular markers, such as RFLPs, simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and sequenced tagged sites (STS), were used.

RFLP analyses were performed using ³²P-dCTP (27). The RFLP probes, SSRs, and STS used, along with the references, are listed in Table 3. Segregation of the SSR alleles was analyzed by denatured polyacrylamide gel (5%) electrophoresis (41) followed by silver staining. The polymerase chain reaction (PCR) amplification condition for the SSR and STS markers was as described by the various authors. AFLP analysis (51) was performed with the modifications and selective primers as used by Grønnerød et al. (17).

SSRs and AFLPs were used to assess the degree of similarity between NILs and Ingrid, in order to have an even and random genome coverage. The expected proportions of recurrent parent (RP) genome in a NIL of a given generation are well known (32, 48) and may be tested by marker data. For chromosomes unlinked to the selected marker or trait, the RP fraction increases asymptotically according to the expression $\%RP = 1 - (0.5)^{n+1} \times 100$, where n equals backcross generations (in our case, seven) plus a final selfing (i.e., $n = 8$ in our case, which gives an expected value of <0.4% RP genome). Given an average chromosome length of 167 cM (36), this means 0.7 cM per chromosome and a total of 4 cM RP genome.

The expected “linkage drag” around the selected locus was estimated (from the above-mentioned papers) to be 0.17 or a total chromosome length of 29 ± 12 cM. The average total RP contribution in such a NIL would then expected to be approximately 33 cM or 2.8%. This means that approximately 9 out of 10 still polymorphic markers would be expected to be linked to the selected locus. Phenotypic selection for RP similarity (as practiced in our case) would be expected to reduce this further (32).

AFLP and SSR data were analyzed for each NIL, which was scored for all donor and RP polymorphisms. The number of poly-

morphic loci thus would vary in each comparison. The chromosomal location of polymorphic SSRs was assessed in all NILs. AFLPs were scored as homozygotes, on the basis of no observed heterozygosity in the SSRs included.

Donor segment lengths were estimated based on the distances between the same markers in the crosses between Ingrid and Abyssinian (17), Steudelli (16), and CI 11549 (34).

Statistical analysis. Results like the ones in Table 1 (25 genotypes $\times$ 11 inoculation matrix) may be easier to grasp when they are visualized. A useful way to do this is by principal components analysis (PCA). If there are strong covariances as to how genotypes react to inoculations, PCA will detect the main patterns of variance and reduce the 25×11 nominal number of dimensions to the real dimensions, the principal components (PCs), that are the latent variables in the data. In effect, differences and similarities among tests, donors, and NILs will be displayed. Using the software package “The Unscrambler” (version 7.6; Camo Ltd., Oslo, Norway), the various isolates were used as variables and genotypes as samples, based on scores from the individual plants or means (test 1), or individual replicates or overall means (tests 2 to 5). In all cases, PCAs were performed on mean-centered data, with full cross-validation and a jack-knife procedure to check the stability of the observed patterns (31). In addition, an analysis of variance (ANOVA) of the field data in test 5 was carried out using the software NM (Ø. Nissen, Agricultural University of Norway, unpublished data).

RESULTS

Average levels of molecular marker similarity between NILs and Ingrid. The results of NILs and RP analyzed for similarity with 70 SSRs covering all the barley chromosomes and randomly selected AFLP primers are presented in Table 4. The results show that the BC program has resulted in 86.3 to 100% RP genome in the NILs, depending on the marker system. The estimates based on SSRs and AFLPs were not significantly different and were reasonably correlated ($r = 0.65$). Among SSRs, the number of polymorphic loci varied from 45 (in Hispont-NIL) to 61 (in Atlas 46-NIL). There were certain consistent (pairwise) differences between the NILs and RP on chromosomes 3H and 7H. Both chromosomes contain known loci conferring resistance to *R. secalis*, indicating successful introgression from the donors into the NILs. Approximately two-thirds of the observed RP-NIL polymorphisms were linked to the assumed resistance in the NIL. These chromosomes are graphically summarized in Figure 1, including all SSRs, STS, and RFLPs. The marker similarity between donors and NILs will be discussed below.

General results from inoculation tests. The results from all five tests are given in Table 1. Ingrid was fully susceptible in all tests, with the exception of isolate 2, to which it was moderately susceptible and in accordance with previous experience (17).

TABLE 4. Similarity (%) of the near-isogenic lines (NILs) with cv. Ingrid the recurrent parent (RP)^a

Cultivar	AFLPs ^b	SSRs	Polymorphic SSRs: RP versus donor	Linkage	Estimate of introgressed donor segment in RP (cM)
Abyssinian-NIL	97.6	98.2	55 (5)	1	10
Atlas 46-NIL	92.7	96.7	61 (2)	1	6
Brier-NIL	90.3	96.2	53 (3)	2	3
CI 8162-NIL	100	100	48 (0)	0	1
Hispont-NIL	97.6	100	45 (0)	0	1
Hudson-NIL	97.6	97.2	49 (1)	?	?
La Mesita-NIL	92.7	98.2	53 (1)	0	2
Modoc-NIL	95.2	94.2	52 (2)	1	1
Turk-NIL	90.3	86.3	53 (8)	6	33
Mean	94.9 $\pm$ 1.2	96.3 $\pm$ 1.4	...	...	...

^a Polymorphic simple sequence repeats (SSRs) out of 70 tested between each donor and the RP are given, with polymorphisms remaining in the corresponding NIL in parenthesis, their linkage to the resistance locus, and an estimate of the introgressed donor segment.

^b Amplified fragment length polymorphisms.

In test 1, data are means of three plants. There were no replicates, except for inoculations with isolate 4004. In the latter case, the two replicates differed in that, in replicate 2, most symptoms were weaker. Hudson-NIL, was reversed from S to R. Otherwise, the correspondence was good.

In test 2, data (means of three replicates, approximately 60 to 70 plants) are from the donors and NIL entries in the diallel cross between NILs studied by Patil (34). In general, the precision was good, except for some crosses in one of the replicates.

In test 3, data (means of two replicates, approximately 40 plants) are also from Patil (34), but using isolate 2. In general, the ranking resembled those with the same isolate in test 1, but overall symptoms were less severe. This was most marked in La Mesita, which changed, by 2.5 units to an R phenotype.

In test 4, the Canadian isolates were much less virulent on both NILs and donors. However, Ingrid and other susceptible checks (mostly Canadian, not shown in the Table 1) included were consistently infected and in accord with previous experience. WRS1824 was virulent on Hudson-NIL.

In the field test (test 5), there were fewer symptoms in the two-rowed than in six-rowed barleys like 'Arve' (data not shown), with Ingrid reaching only 10% scalding of the leaf area. At this lower range of the 0-to-100 scale, differences are not expected to be measured accurately. Thus, in CI 8162, the scores ranged from 10 to 0 to 0 between the three replicates; whereas, in CI 8162-NIL, it was 10 to 5 to 2 and the coefficient of variation was as high as 62%. Still, the ANOVA detected significant differences between genotypes at the $P < 0.001$ levels, but no replicates effect (data not shown). The NILs ranged from complete absence of symptoms in Atlas 46-NIL to moderate susceptibility of Modoc-NIL. In a set of doubled haploids from the crosses Ingrid $\times$ Abyssinian and Ingrid $\times$ Jet, the correspondence with greenhouse inoculations was very good (data not shown).

No effect of individual replicates was observed on the PC pattern; hence, all calculations were done on a genotype mean basis. The PCA results are displayed in a graphical form in Figures 2 (biplot) and 3 (three-dimensional loadings plot). The axes here are the PCs, of which the first explains most of the variance, followed by the second, third, and so on, with a maximum of 11 possible PCs. However, in the validated PCA model, only three PCs, explaining 60, 24, and 7%, respectively, were necessary to account for the variation. Genotypes or variables located close to one PC axis are primarily explained by the PC, and the more distal, the greater the variance explained. The biplot (Fig. 2) shows that all isolates and tests except isolate 2 cluster along PC1; in other words, they were correlated and largely displayed similar resistance reactions. Genotypes with high scores (susceptibility) were found in the right or upper quadrants, with Steudelli-NIL the most susceptible and Hudson the most resistant along PC1. Similarly, PC2 differentiated isolate 2 from others, with CI 8162 as the most susceptible and Hispont-NIL the most resistant, respectively. As to genotypes in the intermediate positions, Modoc (upper right quadrant) was susceptible to both and Atlas 46-NIL resistant to both.

In Figure 3, the relationships between the different isolates may be seen in three dimensions. The PC3 differentiated the different scores with 4004 and 17 from 16-6, 28-3, WRS1824, and field data. However, this accounted for only 7% of the total variance.

Pairwise comparisons of donor and NIL. Both marker and inoculation data will be described for each pair, with reference to Figure 1 (left) and Table 1.

Abyssinian/Abyssinian-NIL. The 3H centromere markers Bmag0112, ABG396, MWG680, agtc-17, and YLM were all retained in the Abyssinian-NIL (5 out of 18 polymorphic markers are retained in the NIL [27%], whereas only 1 of 7 on 7H). While the donor allele of MWG680 had been retained, the corresponding STS marker for this locus did not amplify. As shown by Grønnerød et al. (17), the NIL has a crossover between the very

closely linked markers YLM and *Ylp*, indicating that the *Yd2* gene has been lost in the NIL.

The NIL was generally at least one unit weaker in resistance than the donor, except for isolate 2, in which it is about one unit stronger than the donor. Both results are in accordance with previous experience (17), the increased resistance to 2 being due to the additive effect of the genes conferring intermediate MR to MS reaction types in both the donor and RP. This is also clearly apparent in the relative positions of donor and NIL in Figure 2. Based on this, it is highly probable that the NIL contains the QTL conferring partial resistance in Abyssinian (17), within a donor segment of 10 cM around MWG680, from YLM to agtc-17. The MWG2018 Abyssinian allele on chromosome 7H may indicate another allele in the *Rh2* locus, mapped 1.2 cM away from this marker (46). But it is unlikely that this is an R allele due to the relative susceptibility detected here and the fact that no QTL was mapped in this area in the cross Ingrid $\times$ Abyssinian with the isolate 4004.

Atlas 46/Atlas 46-NIL. The Atlas 46-NIL contains donor alleles at MWG2018, but not at Bmag0021, indicating a small segment of maximum 6 cM. Moreover, on chromosome 3H, it contains Ingrid alleles at 20 out of 21 loci, inclusive of the MWG680 region, the percent RP genome being close to the average for the NIL (96.7%). Atlas 46 generally was more resistant than Atlas 46-NIL, except against isolate 2. This isolate has, in repeated tests, been avirulent against Atlas (37) and virulent against Turk (as in the present results). The donor closely resembled Turk in reaction to other isolates.

Atlas 46 has two dominant genes, *Rh3* and *Rh2*, the *Rh3* gene from Turk (CI 5611-2) being originally introgressed into the genetic background of Atlas (18). Our results here correspond well with the hypothesis that Atlas 46 is near isogenic with Atlas based on the diallel study (34) as well as the pedigree, and that the Atlas 46-NIL carries the gene *Rh2* but not *Rh3*. However, the 3H marker pattern of our Atlas 46 deviated markedly from our Turk, making it doubtful that the Atlas 46 used here actually contains *Rh3*. Alternatively, the confusion may derive from using Turk with different CI numbers (34). Thus, based on the 3H marker patterns, if the Atlas 46 used here does not contain *Rh3* from Turk, it does at least carry something beyond *Rh2*. It is noteworthy that Reitan et al. (37) observed that the Atlas they used was different from the one used to derive Atlas 46, illustrating the problems of the differential set in this pathosystem.

Turk/Turk-NIL. The Turk-NIL is the least isogenic of the nine, both on average and on 3H, on which 10 out of 13 loci polymorphic with Ingrid are retained, a minimum estimated RP region of 18 and up to 33 cM. Notably, the segment MWG680-YLM-Bmag0209 probably contains the *Rh3* gene, known to be allelic to *Rh1* (14,18). The more distal HVM60 to Bmag0225, linked at approximately 4 to 11 cM, corresponds to the *rrsx* locus region reported by Patil (34).

Both donor and NIL were resistant to all isolates except isolate 2, with the Turk-NIL only slightly weaker. The susceptibility of the Turk gene to isolate 2 was evident; the difference between the donor and its NIL may again be ascribed to the Ingrid gene in the latter. The NIL probably contains only one resistance gene, in accordance with previous reports. However, the *Rh5* gene seems to have been lost. Whether any of the markers HVM60 to Bmag0225 actually are linked to any functional resistance in the *rrx* locus region reported by Patil (34) seems doubtful.

Brier/Brier-NIL. The NIL contains donor segments on both 3H and 7H. On the former, the presence of the donor MWG680/YLM allele point to the *Rh1* gene previously mapped in Hudson and 'Dea' (15) and also present in Brier. A linkage drag of approximately 3 cM may be estimated in this region. The 7H marker Bmag0021 allele in the NIL differs from that in the donor as well as in Ingrid, indicating possible heterogeneity in one of the accessions used for backcrossing. Both donor and NIL resemble one

another closely in the inoculation results, indicating one locus in both. Moreover, they closely resemble the resistance in the Turk/Turk-NIL (Fig. 2). Indeed, from the present disease score data, we cannot differentiate the two donors or their NILs, but we may

from the marker data. Based on previous reports (9,10,18) we conclude that the Brier-NIL carries the gene *Rh1*.
Hudson/Hudson-NIL. Like Brier-NIL, Hudson-NIL is highly isogenic. Contrary to the latter, the only donor segment on chro-

Fig. 1. Graphical genotypes of chromosomes 3H and 7H in 'Ingrid' (recurrent parent), the nine different donors, and their near-isogenic lines (NILs). Gray boxes = recurrent parent alleles, black boxes = donor parent alleles, and white boxes = others.

mosome 3H is equivocal, although the MWG680 marker was present on some RFLP blots or extractions; the expected STS-MWG680 fragment was not amplified. No particular pattern can be discerned on the two chromosomes, or the others.

The NIL was generally more resistant than the donor against isolate 2, but highly susceptible against several of the isolates. Notably, the scores were inconsistent, as seen in the case of 4004. The diallel segregation data against 4004 indicated independent segregation, whereas the results with isolate 2 were unclear. In BC screenings over the years, this NIL usually has been susceptible, with a characteristic 1- to 2-day delay in symptom development before it became completely wilted. Also, screening of doubled haploids from this line has shown similar phenotype. The field test may also reflect this.

We have no clear conclusions about this NIL, but our hypothesis is that it is either heterogeneous for the *Rh1* gene, or, in addition, has another (unknown) weak resistance gene.

CI8162/CI8162-NIL. The NIL is highly isogenic with Ingrid on 3H, containing only ABG396 and MWG680 alleles from the donor, probably approximately 1 cM RP. The presence of the same Bmag0021 allele on 7H as in Brier reinforces the suspicion about possible heterogeneity in Ingrid.

While the donor was susceptible to isolate 2, the NIL resembled Ingrid. Both showed an R-MR phenotype against 4004 and the Canadian isolates, MR-MS(S) against the others. The resistance, therefore, was moderate to weak. The lack of segregation in the diallel analysis and good marker correspondence indicates the presence of one gene in both donor and NIL.

Modoc/Modoc-NIL. This NIL also is highly isogenic and contains the same MWG680 allele as in CI 8162, again approxi-

mately 1 cM RP. The Bmag0225 allele is in the same *rrsx* region as retained in Turk-NIL. On chromosome 7H, the NIL contains the same Bmag0021 allele as in six of the nine donors.

This NIL and donor closely resembled each other in resistance reactions. They may be differentiated from the previous pair through the stronger resistance in Modoc-NIL against isolate 2 and its weaker performance in the field. However, given that the diallel did not show segregation with 3H-containing NILs and that Modoc-NIL contains the same MWG680 allele as in CI 8162, the conclusion may tentatively be that the NILs carry allelic but not identical genes.

Hispont/Hispont-NIL. The presence of the donor MWG680 allele again pinpoints this region and the approximately 2 cM RP segment. The amplification of YLM in the NIL, not the donor, is conspicuous. The NIL and donor closely resembled one another and were highly resistant in all tests. From the lack of segregation with 3H containing NILs in the diallel analysis and the presence of the donor MWG680 allele, we conclude that the resistance is encoded by an allele at this locus.

The origin of the donor is unclear. In the testing of various BC lines with a Hispont pedigree, we have detected both S and R individuals in the accession used. The donor Hispont (PI 180687) from the United States Department of Agriculture National Small Grains Collection is susceptible to scald infection.

LaMesita/LaMesita-NIL. The donor and the NIL appear to have little in common. However, the NIL is identical with the Hispont-NIL in marker pattern, pointing to a possible admixture during the backcross process.

The donor and the NIL appeared different in many resistance reactions, except the diallel tests (which are the most reliable) and

Fig. 2. Principal component analysis biplot showing the positions of genotypes (scores) and isolates (variables) in the two first principal components (PCs). PC1 displays the reactions to a cluster of isolates, with Steudelli-NIL being the most susceptible (far right) and Hudson the most resistant (far left). PC2 displays the variation with regard to the two inoculations with isolate 2.

the Canadian test. The NIL, however, closely resembled Hispont-NIL, except (possibly) a weaker resistance in the field. Given their identity in marker pattern and lack of segregation in the diallel, we assume that LaMesita-NIL and Hispont-NIL are identical.

Steudelli/Steudelli-NIL. The *Steudelli-NIL* was analyzed by Grønnerød (16). Briefly, two QTL were mapped in the donor at the previously described loci on 3H and 7H. Both QTL had an individual effect, the 7H QTL being the stronger one. However, the expression of resistance in this donor was very sensitive to environmental conditions. During a disease test (using 4004) in 1996, four phenotypically resistant NILs were selected. The resistance has not been confirmed, and later it was found that the NIL was

susceptible to isolate 4004. Still, they found the NIL to be susceptible to isolate 4004. In the present tests, the NIL was susceptible, except in test 4 (the Canadian isolates). Also, the MR reaction against isolate 2 was as in Ingrid.

Due to the uncertain resistance, the *Steudelli-NIL* versions have not been as thoroughly analyzed as the others. However, three of the four NILs contained donor markers at MWG680, STS/MWG680, agtc-17, and HVM60, indicating a rather large segment (10 cM) on 3H, possibly including the 3H QTL. In addition, one line had the donor allele at the RFLP, MWG555 on 7H, but not the closely linked MWG2018. The apparent absence of resistance is difficult to explain.

X-loadings

RESULT2,X-expl: 60%,24%,7%

Fig. 3. Three-dimensional principal component analysis loadings plot showing the differences between different isolates (or replicates/tests). It may be seen that isolate 2 (rep. 1 and 2) is clearly differentiated from the others along the y axis. The x axis differentiates a lower cluster of isolate 17 and the different scores with isolate 4004 (test in 1998 [rep. 1 and 2] and test in 2000) from an upper cluster of isolates 16-6, 28-3, WRS1824, and field.

TABLE 5. Proposed nomenclature for resistance genes against *Rhynchosporium secalis* in barley

Proposed designation	Previous designation (reference)	Chromosome	Differential genotype	Other differentials ^a
<i>Rrs1</i> _{Brier}	<i>Rh</i> , <i>Rh1</i> (8,18)	3H	Brier	Hudson, Dea, Brier-NIL
<i>Rrs1</i> _{Turk}	<i>Rh3</i> (9)	3H	Turk	Turk-NIL
<i>Rrs1</i> _{La Mesita}	<i>Rh4</i> (9,18)	3H	La Mesita	Trebi, Osiris
<i>Rrs1</i> _{Modoc}	<i>Rh4</i> ² , <i>Rh2</i> (9,18)	3H	Modoc	Modoc-NIL
<i>rrs1</i> _{Jet}	<i>rh6</i> (4)	3H	Jet	Osiris
<i>Rrs1</i> _{CI 8162}	<i>Rh3</i> (18)	3H	CI 8162	CI 8162-NIL
<i>Rrs1</i> _{AB240}	None (13)	3H	AB - line 240	
<i>Rrs1</i> _{Abyssinian}	None (17)	3H	Abyssinian	Abyssinian-NIL
<i>Rrs1</i> _{Steudelli}	<i>rh6</i> (4)	3H	Steudelli	Steudelli-NIL
<i>Rrs1</i> _{B87/14}	None (53)	3H	B87/14	
<i>Rrs1</i> _{CI 11549}	None (34)	3H	CI 11549	None
<i>Rrs2</i> _{Atlas}	<i>Rh2</i> (9)	7H	Atlas	
<i>Rrs2</i> _{Steudelli}	None (16)	7H	Steudelli	CI 2222-NIL
<i>Rrs3</i>	<i>rh7</i> , <i>Rh9</i> (4,17)	4H	Abyssinian, Steudelli	Kitchin
<i>Rrs4</i> _{E224/3}	<i>Rhy</i> (5)	3H	E224/3	
<i>Rrs4</i> _{CI 11549}	<i>rrsx</i> (34)	3H	CI 11549	None
<i>Rrs12</i>	<i>Rrs12</i> (1)	7H	AB - line 200	Ingrid isolate 2
<i>Rrs13</i>	<i>Rrs13</i> (1)	6H	AB - line 30	
<i>Rrs14</i>	<i>Rrs14</i> (11,12)	1H	AB - line 208	None
Unconfirmed	<i>rh8</i> (18,52), <i>Rh5</i> (9), <i>Rh10</i> (18), <i>rh11</i> (18)	...	CI 2222, Turk, Osiris, CI 4364	CI 11797, CI 4368

^a NIL = near-isogenic line.

CI 2222/CI 2222-NIL. The CI 2222-NIL retained the markers agtc-17, YLM, and Bmag0021 (34). Two different resistant lines contained markers on 3H (YLM only, <1 cM) or 7H (up to 11 cM). The latter was similar to the two NILs in the pair CI 11549/CI 11549-NIL (BC₃ only). More extensive analysis is needed to draw conclusions about the gene they carry.

DISCUSSION

Degree of RP similarity in the NILs. A summary of the marker data is given in Table 4 and Figure 1. The average percent RP genome corresponds well with theory. However, with the exception of the Turk-NIL, all NILs had less linkage drag than expected from approximately 1 cM up to above 30 cM. This may reflect a highly efficient phenotypic selection during the backcross process. This is apparently contradictory to some of the results reported by Barua et al. (5), using four of the same NILs studied here (Modoc-NIL, CI8162-NIL, La Mesita-NIL, and Abyssinian-NIL). From 1,500 random amplified polymorphic DNA loci, they observed 60 (4%) NIL-RP polymorphisms, which compares well with our estimates. Moreover, NILs (with the exception of Abyssinian-NIL) showed great marker similarities. In order to estimate the size of the introgressed segments in NILs, they compared the relative distances between the same markers in the cross 'E224/3' × 'Blenheim', which segregated for a scald resistance locus they termed *Rhy*. The distance was estimated to be 34.7 cM. The closest marker to this locus was the multilocus RFLP probe CDO1174. However, on the background of our study using markers more closely linked to the centromeric *Rh* locus, one may speculate that '*Rhy*' may be identical to *rrsx* (34); this problem is further discussed below. Alternatively, due to long physical distances in the centromere region, variations in recombination between crosses or marker choice may give rise to rather different distance estimates.

In another study using NILs in Ingrid for *ml-o* resistance (also developed by J. MacKey), Hinze et al. (21) also found a lower RFLP marker linkage drag than anticipated (on average, 8.6 cM). They suggested this might be due to the single copy *Pst-I* probes being underrepresented in the *ml-o* region, or to successful phenotypic selection.

In the BC₅ NILs P01 and P10 in cv. Pallas, Schwarz et al. (45) found donor segments of 3.7 cM (<0.1% RP), which they ascribed to marker-assisted selection of the *MI-a* locus using the flanking *Hor1* and *Hor2* loci. Given linkage segments comparable with some of our NILs, the latter are remarkably near-isogenic.

A useful illustration may be found in an elegant study in maize (29), where two disease-resistant NILs in the BC₆ generation were 'revisited' by means of RFLPs. The total non-RP fraction was estimated to be as high as 13 and 11%, with only 2.0 and 3.5% (30 and 50 cM) linked to the disease resistance. Ma (29) concluded that NIL/donor/RP polymorphism comparisons should only be a first step in mapping and would often be misleading.

Disease resistance reactions. The results with different tests of 4004 clearly call for caution when using data from individual unreplicated tests. Still, we have found the PCA method useful to discriminate between noise or disturbance and information. The inclusion of replicates or individual plant scores had no effect on the pattern, which was also a great aid when inspecting the raw data. Different tests, isolates, and plants may thus be visualized in the same space and data summarized and interpreted.

Nomenclature of resistance genes. The present marker and phenotypic analyses of the NILs may allow some suggestions to be made as to the nomenclature of *R. secalis* resistance genes. The *Rh/rh* terminology was transposed to the recently suggested *Rrs/rrs* terminology (24), but without other alterations. Just as Jørgensen (24) pooled the various genes reported at the *MI-a* locus and renamed them alleles at *Reg1*, we have outlined some proposed changes in Table 5. This table makes explicit reference to

the previous nomenclature, following the changes proposed by Habgood and Hayes (18), naming all loci at the chromosome 3H centromere locus as alleles, a notation which has not been much followed. Also, we should have included QTL in the total picture, not only major genes, given the very variable phenotypic expressions of resistance in this pathosystem. However, we feel this is premature. We suggest a locus and allele numbering based on (i) historical priority and (ii) degree of verification of loci by marker or other chromosomal assignment techniques. We tend not to attach too much credibility to genes identified through mere segregation ratios, because skewed segregation seems so frequent around the centromeric region of 3H.

At the *Rrs1* locus, we have merged the previously described loci *Rh*, *Rh1*, *Rh3*, *Rh4*, and *rh7*. In all we have listed 11 alleles, including the ones from the classic differentials. Surely there are many more, which is evident because we have only included one from *H. spontaneum*. It will be seen that up to six NILs are represented as alleles, plus the ones from Steudelli and CI 2222.

At the *Rrs2* locus, we place the *Rh2* gene in Atlas. Few alleles have been described at this locus. However, we list the QTL explaining 29.7% of the variance in Steudelli, possibly identical to the locus in CI 2222. It is noteworthy that Kicherer et al. (26) reported that they repeatedly detected QTL in this chromosomal region.

We suggest the designation *Rrs3* for the loci on chromosome 4H, *rh6* and *Rh9*, described and located using addition lines (7). Although not confirmed by Grønnerød et al. (17) in the cross Ingrid × Abyssinian, this may be due to heterogeneity in the CI 668 accession. Indeed, scald resistance genes reported on this chromosome seem to have a habit of being elusive. Thus, the locus in *H. spontaneum* located on 4H (1) could not be confirmed later (A. H. D. Brown, *personal communication*), which is surprising given the diversity in these materials. For the same reason, we suggest only one locus for *rh6* and *Rh9*. Recently, Jensen et al. (22) have mapped a QTL (designated *Qryn4*) in the cross Alexis × Regatta, 11.3 cM distal from the *mlo* gene, giving an 8.8% reduction in scald severity.

We place two alleles at the *Rrs4* locus, *Rhy* (5) and *rrsx* (34). The designations for *H. spontaneum*-derived genes *Rrs12*, *Rrs13*, and *Rrs14* are retained. We suggest that the resistance in Ingrid to isolate 2 (17) is an allele at *Rrs4*. Similarly, the resistance locus *rrsx* in CI 11549 (34) also is an allele at *Rrs4* (V. Patil, *unpublished data*). Then comes a list of unconfirmed loci. The *Rh5* and *rh8* genes have been neither confirmed in our crosses (34) nor located on a chromosome previously. The locus *Rh10* has not been reanalyzed, but it is not identical to the *Rh10* (19) in the cv. Pioneer. The locus *rh11* in CI 4364 is currently being mapped in a cross with Ingrid, but seems difficult to trace (V. Patil, *unpublished data*).

The proposed changes in nomenclature also indicate the differentials available as NILs and those still missing. Notably, the loci on chromosomes other than 3H and 7H should be included with at least one allele, in order to use a differential set for pathogen surveys and as a tool for quick location of new germ plasm sources by means of segregation tests.

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