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ORIGINAL PAPER

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Leaf-rust resistance in rye (*Secale cereale* L.). 2. Genetic analysis and mapping of resistance genes *Pr3*, *Pr4*, and *Pr5*

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Abstract Three dominant resistance genes, *Pr3*, *Pr4*, and *Pr5*, were identified by genetic analysis of resistance to leaf rust in rye (*Puccinia recondita* f. sp. *secalis*). Each of the three genes confers resistance to a broad scale of single-pustule isolates (SPIs), but differences could be observed for specific *Pr* gene/SPI combinations. Resistance conferred by the three genes was effective in both detached-leaf tests carried out on seedlings and in field tests of adult plants. Molecular marker analysis mapped *Pr3* to the centromeric region of rye chromosome arm 1RS, whereas *Pr4* and *Pr5* were assigned to the centromeric region of 1RL. Chromosomal localization and reaction patterns to specific SPIs provide evidence that the three *Pr* genes represent distinct and novel leaf-rust resistance genes in rye. The contributions of these genes to resistance breeding in rye and wheat are discussed.

Introduction

Rust diseases of the genus *Puccinia* are some of the most economially important diseases of cereal and grass crops. In rye (*Secale cereale* L.), leaf rust (*P. recondita* f. sp. *secalis*) is the most frequent disease endemic to rye-cultivating regions of Germany. In European rye collections, only a minor genetic variance for leaf-rust (LR) resistance is present. The occurrence of epidemic forms of LR is associated to appropriate weather conditions and causes significant yield losses (Kobylanski and Solodukhina 1983; Frauenstein 1985; Miedaner and Sperling 1995). Rye-leaf and other cereal rusts are favored by climatic scenarios in which the average air temperature increases while precipitation remains about equal (Jahn et al. 1995). Although LR may be controlled by fungicide treatment, decreasing market prices for rye, increasing producer's prices, and an altered ecological basis of assessment in agriculture stress the future need of a genetically based plant protection involving a range of resistance genes and their combination.

While the level of resistance to LR in modern rye varieties is low, resistance genes from rye have constituted a valuable genetic resource for resistances to wheat rusts both in durum and soft red winter wheat. Translocations involving the short arm of rye chromosome 1R and carrying the resistance gene cluster *Lr26/Sr31/Yr9* have been of particular interest and are widely used in winter and spring wheat breeding programs worldwide (Zeller and Hsam 1983; Sawhney and Sharma 1999). The 1RS fragment present in most of the T1BL.1RS translocations presumably originates from a Petkus source (Bartoš and Bareš 1971; Mettin et al. 1973; Zeller 1973; Zeller and Hsam 1983) and, thus, represents a very narrow fraction of the genetic variability for rust resistances which should exist for an outbreeding species such as rye.

There has been a number of studies on the genetical analysis of qualitative as well as quantitative rye LR resistance (Parlevliet 1977, 1989; Kobylanski and Solodukhina 1983, 1996; Musa et al. 1984; Solodukhina 1994, 2002; Miedaner et al. 2002). To obtain a more comprehensive overview of genes for disease resistances in rye, we began a systematic evaluation and genetic analysis of a "world collection" of genebank accessions (Ruge et al. 1999; Roux et al. 2000). Resistance genes for rye LR are denoted with the *Lr* gene symbol, which is also used for resistance genes effective in wheat against wheat LR (*P. triticina*). In a previous paper, we proposed using the gene

The authors dedicate this paper to Prof. Dr. H.H. Geiger, University of Hohenheim, on the occasion of his 65th birthday.

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designation *Pr* for genes conferring resistance to rye LR and reported the mapping of two such genes, *Pr1* and *Pr2*, on chromosomes 6R and 7R, respectively (Wehling et al. 2003). We report here the genomic localization and effectiveness of three additional *Pr* genes, namely *Pr3*, *Pr4*, and *Pr5*.

Materials and methods

Plant material

The plant materials that were used and the genetic activities carried out are summarized in Table 1. A self-incompatible BC_9 family of Russian origin—Jaroslavna (resistant) and Ilmen (susceptible; recurrent parent)—was kindly provided by O. Solodukhina (VIR, St. Petersburg, Russia) and A.V. Voylokov (St. Petersburg State University, Russia). Crossing the Russian inbred line L7 (Voylokov et al. 1993) to a resistant plant of the BC_9 family resulted in the segregating, self-compatible BC_9F_1 family BAZ-60003. The F_2 families BAZ-1086 and BAZ-1089 were obtained by selfing two resistant individuals from BAZ-60003.

In 1995, a group of 117 populations obtained from a world-wide rye collection was tested for LR resistance under natural field conditions as non-vernalized, vegetatively growing plants. In 1996, a single plant of population Turkey of Canadian origin was selected based on its field resistance at the adult-plant stage and crossed to the susceptible inbred line L301-N. The F_2 family BAZ-97-206-6 was generated by selfing an individual F_1 plant of this cross.

More than 700 additional genebank accessions, kindly provided by the genebanks located at Warsaw, Gatersleben, and Braunschweig as well as by the State Plant Breeding Institute, Stuttgart-Hohenheim, and populations developed at the Institute of Agricultural Crops in Gross Lüsewitz were tested between 1996 and 2003 for LR resistance in the field under conditions of natural infection. Among the potential resistance donors, population WSR of German origin was identified as being resistant based on the non-linear rating scheme (1–9) of Miedaner and Sperling (1995). Crossing a resistant plant from the WSR population to L301-N and selfing a derived F_1 plant resulted in the F_2 family BAZ-97-211-7.

The three populations—BAZ-60003, BAZ-97-206-6 and BAZ-97-211-7—were employed for mapping studies.

Plants of BAZ-1089 and of six F_3 families obtained from BAZ-97-211-7 individuals as well as most of the plants of mapping population BAZ-97-206-6 were tested under field conditions with artificial inoculation to assess adult-plant resistance. In addition, F_3 plants derived from the mapping populations (resistance from Turkey and WSR) or BC_3S_2 derived from the backcross program (resistance from Jaroslavna) and carrying alleles of either of the three resistance genes were tested in their reaction to 23 single-pustule isolates (SPIs).

Resistance tests and disease assessment

Different types of resistance tests were employed.

Detached-leaf test

A detached-leaf test was carried out as described (Wehling et al. 2003). The first and the second true leaf of 14- to 15- and 21- to 23-day-old plantlets, respectively, were inoculated in two successive inoculation experiments. A local LR population sampled from the trial field carried out in Gross Lüsewitz (GL) was used. We subjected the plants to a set of 23 SPIs for a more precise characterization of the resistance. The SPIs were obtained from 18 different locations in eight regions of Germany and display different levels of virulence. The virulence complexity of this set of SPIs has been estimated on a differential set of 23 rye inbred lines and varies from 7 to 21 (Welz 1986; B. Klocke, unpublished). For example, a given SPI with a virulence complexity of 16 reacts compatibly with 16 of the 23 resistant rye inbred lines of the differential set. As described previously (Roux et al. 2000; Wehling et al. 2003), infection types (ITs) 1 through 4 indicate resistance, while 5 and 6 denote susceptibility. Mixed types were also identified and defined as IT 2(5), IT 3(5), or IT 4(5). These mixed types showed up sporadically and were characterized by the occurrence of one predominant IT (2, 3, or 4) associated with one to three pustules per leaf of IT 5.

Mapping populations BAZ-97-206-6 and BAZ-97-211-7 were genotyped by progeny testing via the detached-leaf test. For the progeny test, at least 12 offspring per selfed

Table 1 Plant materials used in the study

Source of resistance	Family	Generation	Activity carried out:			
			Segregation analysis	Mapping	Field test	SPI test[a]
Jaroslavna	BAZ-60003	BC_9F_1	x	x		
	BAZ-1086	F_2	x			
	BAZ-1089	F_2			x	
	BAZ-829-20	BC_3S_2				x
Turkey	BAZ-97-206-6	F_2[b]	x	x	x	
		F_3[c]				x
WSR	BAZ-97-211-7	F_2[b]	x	x		
		F_3[c]			x	x

[a]Resistance reactions in detached-leaf tests to single-pustule isolates (SPIs)
[b]F_2 populations genotyped by progeny test
[c]F_3 progenies derived from the F_2 families

individual were assessed. In contrast, plants of mapping population BAZ-60003 were evaluated per se, and progeny tests were limited to a random sample of 24 mapping individuals to validate the single plant classification.

Field tests

Segregating generations were tested in the field for adult-plant resistance following artificial inoculation (slightly modified according to Miedaner and Sperling 1995). Five-month-old vernalized plants that had been evaluated by means of detached-leaf tests for their resistance at the seedling stage were transferred to the field in March 2000 (BAZ-1089 and BAZ-97-206-6) and 2001 (F_3 families from BAZ-97-211-7). The GL leaf rust inoculum was used following propagation on plants of susceptible rye cultivar Pluto. The uredospores were first dried over silica gel for 4 h, then placed in long-term storage at −80°C. A suspension of LR uredospores (0.14 g dry LR uredospores per liter 0.02% aqueous agar solution) was then applied to single plants by inoculating each plant with approximately 7 ml uredospore suspension using a hand-operated sprayer. Using the beginning of the anthesis (EC 61, according to Zadoks et al. 1974) as the starting point, we assessed infestation of the second and the first leaf (F-2, F-1) below the flag leaf twice—at intervals of 10–12 days—utilizing the system of Frauenstein and Reichel (1978).

Marker analysis

The analysis of genomic (Saal and Wricke 1999) and expressed sequence tag (EST)-derived (Hackauf and Wehling 2002) rye microsatellite (SCM) markers was carried out as reported by Hackauf and Wehling (2002). Chromosomal localization of EST-derived SCM markers was determined using wheat-rye addition lines kindly provided by S.M. Reader (Department of Crop Genetics, John Innes Centre, Norwich). The *Sec-1* locus of rye was amplified using the primers o-sec-5′/a and o-sec-3′/r according to Shimizu et al. (1997). The primers IAG95-1 and IAG95-2 were used for the marker locus *Xiag95*, as described (Mohler et al. 2001). Based on sequence information available for a wheat receptor-like kinase gene encoded at the *Lr10* disease resistance locus (Feuillet et al. 1997) and for cDNA anchor markers *Xbcd98*, *Xpsr596*, *Xbcd762*, and *Xbcd921* localized in gene-containing regions of group 1S chromosomes in wheat (Boyko et al. 1999), we developed additional sequence-tagged site (STS) markers (Table 2) using assemblies of barley and wheat ESTs [tentative consensus (TC) sequences] provided by The Institute for Genomic Research (Rockville, Md., http://www.tigr.org).

For each STS assay, 50–100 ng of genomic DNA was used in a solution containing 1× reaction buffer (Qiagen, Valencia, Calif.), 1.5 m*M* $MgCl_2$, 200 μ*M* of each dNTP, 5 pmol of primers, and 0.5 U *Taq* DNA polymerase (Qiagen) in a 25-μl volume. Restriction patterns of cleaved amplicons were visualized on agarose gels by ethidium bromide staining.

Linkage analysis

Linkage analysis was according to the JOINMAP ver. 3. program (Van Ooijen and Voorrips 2001). Linkage groups were determined with a LOD score of 3.0, and the Kosambi function was applied to convert recombination values to genetic distances (centiMorgans). The confidence interval error for the recombination value between *Pr3* and *Prx7* was calculated by the method of Stevens (1942).

Results

Inheritance and mapping of resistances

Pr3 resistance derived from Jaroslavna

In BC_9F_1 family BAZ-60003 carrying Jaroslavna resistance, we observed a monogenic 1:1 segregation (Table 3). This is expected when a plant heterozygous for a single,

Table 2 Primer sequences for sequence-tagged site (STS) markers located on chromosome 1R of rye (*Secale cereale* L.)

STS marker	Primer sequence (5′→3′)	Expected amplicon (bp)	Observed amplicon (bp)	Annealing temperature (°C)
Lrk10	F: GGCCACTGTGCTTTATCTTTC R: ATAGCTCCGGTGCAATGTAGCC-CATCG	661	Approxi-mately 650	55
TC68078	F: GCAGCAAGATAAGTGGACTGG R: ATCTGCGATCCAACATTGAAC	934	Approxi-mately 1,250	55
TC72745	F: GATCATGAAGCAAACCTACCG R: CAATTGTGTTGATTCCACAGG	748	Approximately 900	55
TC76051	F: GCTAGGGGTTTGAAGAAGCAT R: AAACGACTCAATGCCTCAGAA	816	Approxi-mately 1,400	55
TC77841	F: TGGGGACAAGAATCCAATGTA R: TATGGCAGCAAATGTTCCTCT	506	Approxi-mately 550	50

dominant resistance gene is crossed to a homozygous susceptible individual. Upon selfing a resistant individual of BAZ-60003, the F_2 offspring (BAZ-1086) obtained significantly deviated from the expected ratio of 3:1, with the resistant class being under-represented.

To verify the classification of resistant versus susceptible individuals, we conducted progeny tests with 12–19 plants using a random sample of 24 offsprings of the mapping population BAZ-60003. Twelve plants of BAZ-60003, which had been classified as resistant with IT 1 or IT 2, resulted in segregating F_2 offspring. Of the remaining 12 individuals, which had been recognized as susceptible (IT scores 5 or 6), 11 gave rise to homogeneously susceptible progenies, whereas one plant was reclassified as heterozygous resistant.

The resistance gene segregating in BAZ-60003 and derived from Jaroslavna was named *Pr3*.

Pr4 and Pr5 resistances, derived respectively from Turkey and WSR

F_2 populations BAZ-97-206-6 and BAZ-97-211-7 were progeny-tested, which allowed us to distinguish homozygous- and heterozygous-resistant plants. For each population, the results of the progeny tests on individual plants indicated the segregation of a single dominant resistance, with a segregation in agreement with a monogenic 1:2:1 ratio as well as with the 3:1 ratio when heterozygous- and homozygous-resistant plants were pooled (Table 3). The two resistance genes derived from Turkey and WSR were designated *Pr4* and *Pr5*, respectively.

Of the 95 individuals of the *Pr4* family BAZ-97-206-6, four plants proved to be misclassified when tested per se. Two of these tested as resistant, having IT 3 or IT 4, but generated a homogeneously susceptible F_3 progeny, whereas two presumed susceptible plants with IT values of 5 or 6 of this F_2 family gave rise to segregating progenies. Of the remaining 91 F_2 individuals of BAZ-97-206-6, the initial classification was confirmed by progeny testing. Of the 107 individuals of *Pr5* family BAZ-97-211-7, only one F_2 plant was scored as susceptible, having an IT value of 5 but generating resistant offspring. Progeny tests of the remaining 106 individuals confirmed the initial classification carried out on single plants.

To conclude, as previously demonstrated for genes *Pr1* and *Pr2* (Wehling et al. 2003), the grouping of ITs 1-4 and 5-6 to represent resistant (*PrPr*, *Prpr*) and susceptible (*prpr*) genotypic classes, respectively, is also valid for the LR resistance genes *Pr3*, *Pr4* and *Pr5* studied in the present investigation.

Mapping of Pr3, Pr4, and Pr5

In addition to using previously published rye chromosome 1R PCR-based markers, we utilized sequence information on cDNA anchor markers to develop novel STS markers for this chromosome. Comparing selected cDNAs against barley and wheat ESTs (Table 4), we identified conserved DNA sequences that could be used to design primers suitable for gene amplification across species. The selected primers yielded single amplicons from rye genomic DNA that were sufficiently variable in the selected plant materials to develop and map cleaved amplified polymorphic sequence (CAPS)-based markers.

Pr3 was mapped in 60 BC_9F_1 individuals. The *Pr4* and *Pr5* maps were based on 91 and 94 individuals, respectively, of F_2 mapping families classified using a progeny test into the genotypic classes *PrPr*, *Prpr*, and *prpr*. *Pr4* and *Pr5* mapped on genetic intervals of chromosome 1R—71.6 cM and 67.6 cM, respectively (Fig. 1). The linear order of the markers compares well among the genetic rye maps and the physical consensus map of wheat (Fig. 1). An exception is the BC_9F_1 map including *Pr3*, where the order of markers in the interval *SCM9-Prx7-SCM39-Xscm1* is changed and the genetic distance between *Prx7* and *Xpsr162* is comparatively small. The accuracy of this map is not maximized due to its low statistical information content, as determined by the number of informative gametes in the underlying back-

Table 3 Genetic characterization of leaf-rust (LR) resistance derived from populations Jaroslavna, Turkey, and WSR

Source of resistance	Family		Segregation observed			Expected segregation ratio	χ^2 value
	BC	F_2	Resistant		Susceptible		
			Homozygous	Heterozygous			
Jaroslavna	BAZ-60003[a]			30	31	1:1	0.02 ns
		BAZ-1086[b]		66	43	3:1	12.14***
Turkey		BAZ-97-206-6[c]	23	51	21	1:2:1	0.60 ns
						3:1[b]	0.42 ns
WSR		BAZ-97-211-7[c]	28	58	21	1:2:1	1.68 ns
						3:1[b]	1.65 ns

ns, Non-significant; ***highly significant (α<0.001) with respect to deviation of the observed segregation from the expected segregation ratio

[a] BC_9F_1 (BAZ-60003)

[b] Selfing of BC_9F_1

[c] Classification based on progeny test

Table 4 BLASTN similarity comparison of selected cDNA anchor markers with barley (HvGI) and wheat (TaGI) expressed sequence tagged (EST) sequences (*ND* Not determined)

TIGR database		HvGI[a]			TaGI[b]		
GenBank Accession	Marker	BLASTN hit	E value[c]	Identities[d]	BLASTN hit	E value[c]	Identities[d]
U51330	*Lrk10*	ND	ND	ND	ND	ND	ND
BE438849	*Xbcd98*	TC68078	1.2e-100	468/471 (99%)	TC68671	3.0e-89	441/471 (93%)
AJ440625	*Xpsr596*	TC72745	7.7e-40	218/240 (90%)	TC73156	5.3e-42	219/241 (90%)
BE438904	*Xbcd762*	TC76051	4.9e-66	314/317 (99%)	TC64300	1.2e-59	299/317 (94%)
BE438643	*Xbcd921*	TC77841	7.6e-84	392/393 (99%)	TC88045	4.4e-73	368/394 (93%)

[a]*Hordeum vulgare* gene index
[b]*Triticum aestivum* gene index
[c,d]For a description of the BLAST output parameters refer to http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/Blast_output.html

cross family. Both *Pr4* and *Pr5* mapped distal of *Xscm107*, an EST-derived SSR marker that could be assigned to the long arm of chromosome 1R (Fig. 2). Our conclusion is that *Pr4* and *Pr5* are located on chromosome arm 1RL, whereas *Pr3* co-segregates with the marker locus *Prx7* in a linkage group on chromosome arm 1RS.

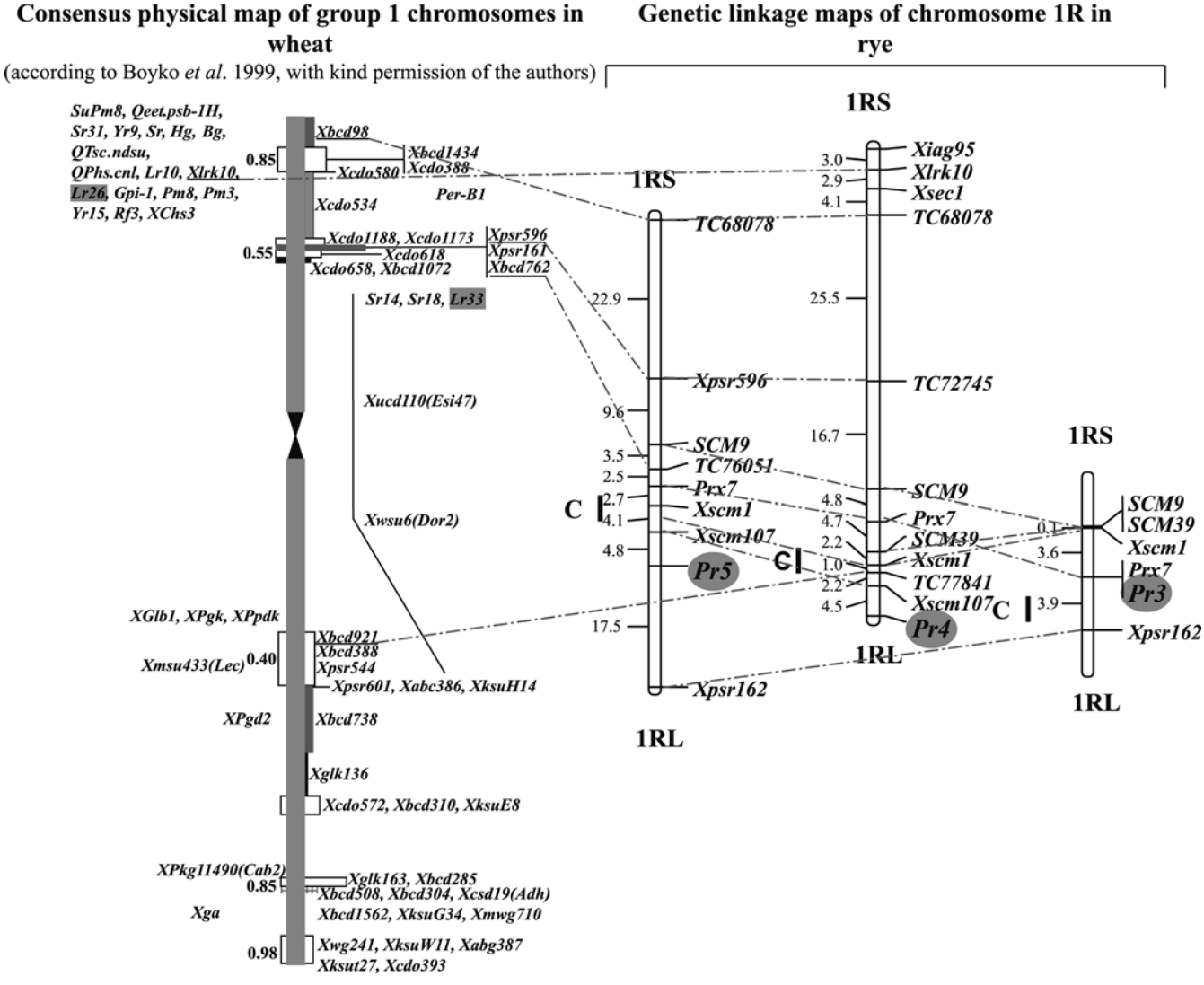

Fig. 1 Mapping of *Pr3*, *Pr4*, and *Pr5* on rye chromosome arms 1RS and 1RL, respectively. Genetic distances are given in centiMorgans. *Transversal lines* indicate markers in common to genetic rye maps and extend to markers present in the physical map of wheat. The three rye LR resistance genes *Pr3*, *Pr4*, and *Pr5* as well as the genes *Lr26* and *Lr33* conferring resistance to leaf rust in wheat are *highlighted*.

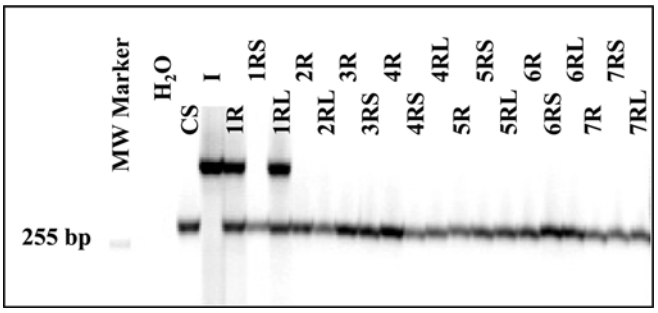

Fig. 2 Chromosomal localization of the EST-derived SSR marker *Xscm107* using Chinese Spring/Imperial (*CS/I*) wheat/rye addition lines. The added rye chromosomes are given at the *top* of each lane

Reaction of F_2 families to GL inoculum

The F_2 families segregating for *Pr3*, *Pr4*, and *Pr5*, respectively, showed different reactions to the GL inoculum when tested with detached leaves (Table 5). While resistant individuals of BAZ-1086 (*Pr3* from Jaroslavna) were predominantly classified with an IT score of 2, resistance phenotypes IT 2, 2(5), 3, 3(5), and 4 were shown by plants of BAZ-97-206-6 (*Pr4*, Turkey) and BAZ-97-211-7 (*Pr5*, WSR).

Effectiveness of *Pr3*, *Pr4*, and *Pr5* toward different SPIs

Individuals homozygous for either *Pr3*, *Pr4*, or *Pr5* were analyzed for their reaction toward 23 SPIs of different origins (Table 6). *Pr3* genotypes reacted resistant to 15 SPIs and susceptible to eight SPIs, the latter of which showed medium (SPI nos. 6, 11) to high (SPI nos. 2–16, 23) virulence complexity. Plants carrying *Pr4* or *Pr5* proved resistant to 16 SPIs and 17 SPIs, respectively. In the case of *Pr4*, only SPIs with high virulence complexities (SPI nos. 17–23) reacted compatible with *Pr4* carriers. In contrast, plants carrying *Pr5* showed susceptibility to some SPIs with low (SPI no. 2), medium (SPI nos. 9, 11) or high (SPI nos. 12, 15) virulence complexity. To summarize, each of the three genes *Pr3*, *Pr4*, and *Pr5* conferred resistance to a broad scale of SPIs, but differences could be observed for specific *Pr*-gene/SPI combinations, making each *Pr* gene unique with respect to its reaction pattern.

Effectiveness of *Pr3*, *Pr4*, and *Pr5* at the seedling and the adult-plant stage

In addition to carrying out the seedling detached-leaf test, we tested adult plants in the field under artificial infection conditions. A total of 97, 92, and 72 plants of segregating *Pr3*, *Pr4*, and *Pr5* families, respectively, were tested for their resistance to the GL rust inoculum both in detached-leaf tests and in the field.

Of the 50 plants of the *Pr3* family which had been classified as being resistant at the seedling stage, 40 also showed resistance in the field test; seedling resistance could not be confirmed in the remaining ten individuals at the adult plant stage. On the other hand, 9 of 47 individuals from the family segregating for *Pr3* and classified as susceptible at the seedling stage revealed resistance at the adult stage. Thus, for the *Pr 3* family the seedling and adult-stage evalution were 80.4% concordant (Table 7).

For the *Pr4* and *Pr5* populations, a higher conformity (96.7% and 97.2%, respectively) of resistance test data determined at the seedling and at the adult stage was observed. In both populations, all individuals resistant as seedlings proved also to be resistant as adult plants in the field. Among the plants susceptible at the seedling stage, 3 of 23 (*Pr4*) and 2 of 30 (*Pr5*) individuals did not show any infection as adult plants (Table 7). Correlation of the phenotypic classifications (resistant/susceptible) which were obtained from seedlings and adult plants was 0.61 for the *Pr3* population and above 0.9 for the *Pr4* and *Pr5* populations (Table 7). We therefore conclude that *Pr3*, *Pr4*, and *Pr5* contribute also to adult resistance to leaf rust.

Discussion

The LR resistance genes *Pr1* and *Pr2* are located on rye chromosome arms 6RL and 7RL, respectively (Wehling et al. 2003). In the present study, three additional genes conferring resistance to *P. recondita* in rye have been assigned and mapped to defined regions of the rye genome. The symbols *Pr3*, *Pr4*, and *Pr5* replace their previous names of *Lr-c*, *Lr-g*, and *Lr-h*, respectively (Ruge et al. 1999; Roux et al. 2000). The *Pr3* gene co-segregates without recombination with the isozyme marker locus *Prx7* (Fig. 1) mapping to the short arm of rye chromosome 1R (Voylokov et al. 1998). Thus, *Pr3*

Table 5 Reaction of F_2 families BAZ-1086, BAZ-97-206-6, and BAZ-97-211-7 to GL inoculum in detached-leaf tests

F_2 family (*Pr* gene)	Numbers of individuals falling in each IT class[a]							
	Resistant						Susceptible	
	1	2	2(5)	3	3(5)	4	5	6
BAZ-1086 (*Pr3*)	1	58	–	7	–	–	22	21
BAZ-97-206-6 (*Pr4*)	–	18	13	25	17	22	17	14
BAZ-97-211-7 (*Pr5*)	–	54	3	28	6	8	22	12

[a]Plants were classified in IT classes based on the highest score assigned in two experiments, each carried out with two repetitions/individual

Table 6 Reactions of homozygous *Pr3*, *Pr4*, and *Pr5* carriers to 23 single-pustule isolates (SPIs) in detached-leaf tests (*ND* Not determined)

SPI no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Virulence complexity	7	8	9	9	9	11	11	11	11	13	14	16	16	17	17	17	18	18	19	19	19	20	21
Pr Gene	IT scores																						
Pr3	**2**[a]	**2**	**2**	**2**	**2**	5	**2**	**2**	**2**	**2**	5	5	5	5	5	5	**2**	**2**	**2**	**2**	**2**	**2**	5
Pr4	**2**	**2**	**2**	**2**	**2**	**3**	**2**	**2**	**2**	**3**	**2**	**2**	**3**	**2**	**2**	**2**	5	5	5	5	5	5	5
Pr5	**2**	5	**3**	**3**	ND	**3**	**3**	**3**	5	**2**	5	5	**3**	**3**	5	**3**	**2**	**2**	**3**	**2**	**2**	**2**	**3**

[a]The IT score assigned corresponds to the highest of two IT scores recorded in two independent repetitions; the values in bold indicate a non-compatible host-pathogen reaction

appears to be localized on chromosome arm 1RS, even though the 95% confidence interval of the recombination value between *Pr3* and *Prx7* is relatively large, extending to 5 cM. Rye chromosome arm 1RS is known to carry the LR resistance gene *Lr26* (Singh et al. 1990) which confers resistance to wheat-LR in T1BL·1RS translocation wheat lines. The position of *Pr3* relative to *Xiag95* and *Xsec1* indicates that the *Pr3* gene is most probably not identical with *Lr26* because the latter is reported to be closely linked to *Xiag95* and to the seed-storage protein gene *Sec-1* near the end of the chromosome arm 1RS (Hsam et al. 2000).

Sequence information on wheat and barley ESTs and on mapped cDNA anchor markers as well as the information available on the conserved genomic organization across grass species have facilitated the development of novel STS markers for rye chromosome 1R. These markers, together with previously published STS markers, enabled the mapping of resistance genes *Pr4* and *Pr5* on 1RL. Localization of the two genes on the long arm of 1R is demonstrated by the facts that (1) each of the two genes mapped distal to *Xscm107* and (2) using wheat-rye addition lines, *Xscm107* was localized on 1RL (Fig. 2). Adjacent to *Xscm107*, the marker *Xscm1* could be

Table 7 Reaction to the LR inoculum of plants of *Pr3*-, *Pr4*-, and *Pr5*-segregating families at the seedling stage in detached-leaf tests and at the adult stage in the field in 2000 (*Pr3*, *Pr4*) and 2001 (*Pr5*)

Number of plants	IT scores		Seedlings versus adult plants	
	Seedlings[a]	Adult plants	Conformity (%)	Correlation
Pr3 (*n*=97)			80.4	0.61
1	1	2(5)		
2[b]	1	6		
38	2	2, 2(5), 3, 3(5)		
7[b]	2	5, 6		
1	3	3		
1[b]	4	6		
2[c]	5	2(5)		
12	5	6		
1[c]	6	2		
4[c]	6	2(5)		
1[c]	6	3		
1[c]	6	4		
26	6	6		
Pr4 (*n*=92)			96.7	0.91
11	2	2, 2(5), 3		
9	2(5)	2, 2(5), 3		
18	3	2, 2(5), 3, 3(5)		
10	3(5)	2, 2(5), 3, 3(5), 4		
21	4	2, 2(5), 3, 3(5), 4, 4(5)		
3[c]	5	3		
6	5	5, 6		
14	6	5, 6		
Pr5 (*n*=72)			97.2	0.94
40	2	2, 2(5), 3, 3(5)		
2	3(5)	2, 4		
1[c]	5	2		
1[c]	5	3		
28	6	5, 6		

[a]The score assigned corresponds to the highest of four IT scores recorded in two experiments, each carried out with two repetitions/plant
[b]Plants classified resistant at the seedling stage but not at the adult stage
[c]Plants classified susceptible at the seedling stage but resistant at the adult stage

assigned to 1RS by means of wheat-rye addition lines (not shown). Thus, the centromere should be located between these two markers. The rye linkage maps presented in this paper are consistent with respect to the order of their markers with a recently published genetic linkage map of rye chromosome 1R (Korzun et al. 2001). The genetic distances relative to *Xscm107* compare well with that between *Pr4* and *Pr5* (Fig. 1), which suggests that the two resistance genes reside at closely adjacent sites on 1RL or even that they belong to the same genetic locus. The reaction patterns to specific SPIs, though, provide evidence that *Pr4* and *Pr5* as well as *Pr3* represent distinct LR resistance genes in rye.

The conservation of gene order across large sections of grass genomes has been demonstrated by comparative genetic mapping (for review see Gale and Devos 1998a, b; Devos and Gale 2000; Keller and Feuillet 2000; Laurie and Devos 2002). Our observation of LR resistance genes mapping to the centromeric region of rye chromosome arm 1RL is consistent with a previous report on the localization of the gene *Lr33* in the corresponding centromeric region of wheat chromosome arm 1BL (Hart et al. 1993). The large number of ESTs mapped in wheat (Sorrells et al. 2003) as well as two recently described disease resistance-gene analogs of the NBS-LRR class located on barley chromosome arm 1HL (Madsen et al. 2003) provide potential resources from which to develop further molecular markers for *Pr4* and *Pr5*.

F_2 segregation data for *Pr3* (BAZ-1086) deviated considerably from the 3:1 expected ratio, whereas in the F_1 family BAZ-60003 the expected 1:1 ratio was confirmed (Table 3). For *Pr3*, a close linkage to the peroxidase isozyme marker *Prx7* was found, and *Prx7* itself is tightly linked to the self-incompatibility locus *S* (Wehling and Wricke, 1985). On the basis of these linkage relations and because *Pr3* derives from the self-incompatible cv. Jaroslavna, the deviating F_2 segregation data for BAZ-1086 can be explained by tight linkage of *Pr3* with a functional allele (S_1) of the self-incompatibility locus *S*. This allele may either derive from the resistance donor (cv. Jaroslavna) or—less likely—from the recurrent backcross parent (cv. Ilmen). The allele *pr3* supporting susceptibility was probably linked to a non-functional mutant *S* allele (S_c) deriving from the inbred line L7 and conferring self-compatibility. Upon selfing, a strong selection against gametes bearing the resistance allele *Pr3* is expected. As a result, inbred lines homozygous for *Pr3* are not easy to select for. Meanwhile, a BC_3S_1 family segregating 3:1 for *Pr3* resistance versus susceptibility (not shown) was found which derives from a backcross program designed to create a set of near-isogenic lines. The family mentioned above possibly resulted from a recombination event between *S* and *Pr3* and should comprise one-third homozygous *Pr3Pr3* genotypes.

Resistance genes *Pr3*, *Pr4*, and *Pr5* reacted in a comparably effective way to different LR inocula: in fact, the majority of the 23 SPIs tested showed non-compatible reactions to them. The 23 SPIs were selected out of 1,200 in the attempt to limit virulence redundance using a differential tester set of 23 rye inbreds. Of these 23 SPIs, 15 proved to be avirulent toward *Pr3*, 16 toward *Pr4*, and 17 toward *Pr5*. The remainder of the SPIs reacted compatibly to the resistance genes but deviated with respect to their virulence complexity. In contrast to *Pr3* and *Pr4*, which proved to be susceptible to SPIs with, respectively, medium-to-high and high complexity, *Pr5* was overcome by SPIs of low, medium, and high complexity. Broad effectiveness to a local LR population from St. Petersburg, Russia as well as to 17 out of 19 monopustule isolates was reported by Soludukhina (2002) for a resistance derived from Russian cv. Yaroslavna 3. Using the same sample of 23 SPIs as for *Pr3*, *P4*, and *Pr5*, detached-leaf tests revealed a lack of compatibility (data not shown) with the LR resistance genes *Pr1* and *Pr2* recently described (Wehling et al. 2003). It appears, thus, that unlike *Pr1* and *Pr2*, a considerable number of virulent fungal races compatible to the genes *Pr3*, *Pr4*, and *Pr5* are already present in German rye-growing areas. The broad-based effectiveness of the five *Pr* genes described so far as well as their unique and complementing specificity might open the prospect of a sustainable use of monogenic LR resistances in hybrid breeding combining lines with different dominant *Pr* genes in the hybrids.

The uniqueness of the LR resistance from Yaroslavna 3 (*Pr3*; syn. *Lr-c*) in relation to other resistances described so far has been underlined by Soludukhina (2002) who ruled out, based on the reaction to the LR inoculum, the identity of *Pr3* with LR resistance genes existing in the accessions Lovashpatonae 2, Gotor 2, Braunrostresistenz 2, Orlovskaya 9-2, and Talovskaya 12-2.

In the detached-leaf test with GL inoculum, plants carrying *Pr3* nearly exclusively displayed infection type 2. However, half of these revealed mixed infection types when tested as adult plants in the field. In contrast, plants carrying *Pr4* or *Pr5* showed mixed ITs both in detached-leaf tests and in the field. The mixed ITs can be interpreted as being due to the appearance of virulent races among the local GL rust population—races that were present either at low frequencies or characterized by a low aggressiveness. In agreement with the results of Soludukhina (2002), who noted heterogeneous resistance (type X) in field experiments, we found mixed ITs predominantly at the second date of disease assessment in adult plants. Assessment of infection type seems, therefore, to be less reliable when based on field experiments. Similar conclusions were drawn by Parlevliet (1989) for rusts in wheat and barley and Miedaner et al. (2002) for LR in rye.

In the investigation reported here and in the one reported by Wehling et al. (2003), a total of five dominant LR resistance genes have been genetically analyzed, mapped, and characterized in terms of effectiveness. As a result, marker-assisted use of *Pr* genes to improve adapted germplasm and the deliberate combination of resistance genes to increase LR resistance levels in rye cultivars is becoming feasible. Furthermore, the five LR resistance genes now available provide a novel opportunity to broaden the genetic base for LR resistance in wheat

introgression-breeding programs, provided that the *Pr* rye genes prove effective toward *P. triticina*.

Near-isogenic lines (NILs) provided with *Pr3*, *Pr4*, *Pr5* as well as additional *Pr* genes are currently being developed to compile a standard set of genetically characterized *Pr* genes. By means of this set of NILs the development of tightly linked molecular markers will be facilitated and investigations on the effectiveness of pyramiding different monogenic LR resistances as well as comparative studies on the influence of *Pr* genes on the yield potential of rye will become feasible.

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References

Bartoš P, Bareš I (1971) Leaf and stem rust resistance of hexaploid wheat cultivars Salzmünder Bartweizen and Weique. Euphytica 20:435–440

Boyko EV, Gill KS, Mickelson-Young L, Nasuda S, Raupp WJ, Ziegle JN, Singh S, Hassawi DS, Fritz AK, Namuth D, Lapitan NLV, Gill BS (1999) A high-density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. Theor Appl Genet 99:16–26

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell 12:637–646

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Frauenstein K (1985) Untersuchungen zur Schadwirkung des Braunrostes, *Puccinia recondita* Rob. ex Desm., an Winterroggen. Nachrichtenbl Dtsch Pflanzenschutz DDR 39:177–178

Frauenstein K, Reichel A (1978) Zum Erkennen von slow-rusting-Formen bei Roggenbraunrost (*Puccinia recondita* Rob. ex Desm.). 2. Symposium über Schaderreger in der industriemässigen Getreideproduktion, Martin-Luther-Universität Halle, Wissenschaftliche Beiträge 14 (S11), 403–411

Gale MD, Devos KM (1998a) Comparative mapping in the grasses. Proc Natl Acad Sci USA 95:1971–1974

Gale MD, Devos KM (1998b) Plant comparative genetics after 10 years. Science 282:656–659

Hackauf B, Wehling P (2002) Identification of microsatellite polymorphisms in an expressed portion of the rye genome. Plant Breed 121:17–25

Hart GE, Gale MD, McIntosh RA (1993) Linkage maps of *Triticum aestivum* hexaploid wheat, 2*n*=42, genomes A, B, and D) and *T. tauschii* (2*n*=14, genome D). In: O'Brien SJ (ed) Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 6204–6219

Hsam SLK, Mohler V, Hartl L, Wenzel G, Zeller FJ (2000) Mapping of powdery mildew and leaf rust resistance genes on the wheat-rye translocated chromosome T1BL·1RS using molecular and biochemical markers. Plant Breed 119:87–89

Jahn M, Freier B, Kluge E (1995) Zum Einfluss von Klimaveränderungen auf die phytosanitäre Situation im Agrarbereich. In: Weigel HJ, Dämmgen U, Scholz F (eds) Klimawirkungsforschung im Geschäftsbereich des BML. Reihe A: Angewandte Wissenschaft, vol 442. Landwirtschaftsverlag, Münster, pp 81–92

Keller B, Feuillet C (2000) Colinearity and gene density in grass genomes. Trends Plant Sci 5:246–251

Kobylanski VD, Solodukhina OV (1983) Damage of important fungal diseases and methods for resistance breeding of short-straw rye (in Russian). In: Voprosy Sal. I Genetiki zernovych Kult, Moscow, pp 140–147

Kobylanski VD, Solodukhina OV (1996) Genetic bases and practical breeding utilization of heterogenous resistance of rye to brown rust. Vortr Pflanzenzuecht 35:155–163

Korzun V, Malyshev S, Voylokov AV, Börner A (2001) A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. Theor Appl Genet 102:709–717

Laurie DA, Devos KM (2002) Trends in comparative genetics and their potential impacts on wheat and barley research. Plant Mol Biol 48:729–740

Madsen LH, Collins NC, Rakwalska M, Backes G, Sandal N, Krusell L, Jensen J, Waterman EH, Jahoor A, Ayliffe M, Pryor AJ, Langridge P, Schulze-Lefert P, Stougaard J (2003) Barley disease resistance gene analogs of the NBS-LRR class: identification and mapping. Mol Genet Genomics 269:150–161

Mettin D, Blüthner WD, Schlegel R (1973) Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. Mo. Agric Exp Sta, Columbia, Mo., pp 179–184

Miedaner T, Sperling U (1995) Effect of leaf rust on yield components of winter rye hybrids and assessment of quantitative resistance. J Phytopathol 143:725–730

Miedaner T, Gey A-KM, Sperling U, Geiger HH (2002) Quantitative-genetic analysis of leaf-rust resistance in seedling and adult-plant stages of inbred lines and their testcrosses in winter rye. Plant Breed 121:475–479

Mohler V, Hsam SLK, Zeller FJ, Wenzel G (2001) An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8/Pm17* locus of common wheat. Plant Breed 120:448–450

Musa GLC, Dyck PL, Samborski DJ (1984) The inheritance of resistance in rye to *Puccinia recondita* f. sp. *secalis* and f. sp. *tritici*. Can J Plant Sci 64:511–519

Parlevliet JE (1977) Variation for partial resistance in a cultivar of rye, *Secale cereale*, to brown rust, *Puccinia recondita* f. sp. *secalis*. Cereal Rusts Bull 5:13–16

Parlevliet JE (1989) Identification and evaluation of quantitative resistance. In: Leonard KJ, Fry WE (eds) Plant disease epidemiology: genetics, resistance and management, vol 2. McGraw-Hill, New York, pp 215–248

Roux SR, Ruge B, Linz A, Wehling P (2000) Leaf rust resistance in rye—evaluation, genetic analysis and molecular mapping. Acta Phytopathol Entomol Hung 35:65–73

Ruge B, Roux SR, Linz A, Wehling P (1999) Erschließung und molekulare Charakterisierung von Resistenzen gegen Braunrost bei Roggen (*Secale cereale* L.). Vortr Pflanzenzuecht 46:169–176

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42:964–972

Sawhney RN, Sharma JB (1999) Novel complementary genes for adult plant leaf rust resistance in a wheat stock carrying the 1BL-1RS translocation. Plant Breed 118:269–271

Shimizu Y, Nasuda S, Endo TR (1997) Detection of the *Sec-1* locus of rye by a PCR-based method. Genes Genet Syst 72:197–203

Singh NK, Shepherd KW, McIntosh RA (1990) Linkage mapping of genes for resistance to leaf, stem and stripe rusts and ω-secalins on the short arm of rye chromosome 1R. Theor Appl Genet 80:609–616

Solodukhina OV (1994) Rye resistance to brown rust and powdery mildew: the potential of hereditary variability (in Russian). Genetika 30:616–618

Solodukhina OV (2002) Genetic characterization of rye accessions with regard to leaf rust resistance. Russ J Genet 38:399–407

Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin Mahmoud A, Ma X, Gustafson PJ, Qi LL, Echalier B, Gill BS, Matthews DE, Lazo GR, Chao S, Anderson OD, Edwards H, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvorak J, Zhang D, Nguyen HT, Peng J, Lapitan NLV, Gonzalez-Hernandez JL, Anderson JA, Hossain K, Kalavacharla V, Kianian SF, Choi DW, Close TJ, Dilbirligi M, Gill KS, Steber C, Walker-Simmons MK, McGuire PE, Qualset CO (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Stevens WL (1942) Accuracy of mutations rates. J Genet 43:301–307

Van Ooijen JW, Voorrips RE (2001) JOINMAP version 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voylokov AV, Fuong FT, Smirnov VG (1993) Genetic studies on self-fertility in rye (*Secale cereale* L.). 1. The identification of genotypes of self-fertile lines for the sf-alleles of self-incompatibility genes. Theor Appl Genet 87:616–618

Voylokov AV, Korzun V, Börner A (1998) Mapping of three self-fertility mutations in rye (*Secale cereale* L.) using RFLP, isozyme and morphological markers. Theor Appl Genet 97:147–153

Wehling P, Wricke G (1985) Linkage between an incompatibility locus and a peroxidase isozyme locus (*Prx7*) in rye. Theor Appl Genet 71:289–291

Wehling P, Linz A, Hackauf B, Roux SR, Ruge B, Klocke B (2003) Leaf-rust resistance in rye (*Secale cereale* L.). 1. Genetic analysis and mapping of resistance genes *Pr1* and *Pr2*. Theor Appl Genet 107:432–438

Welz G (1986) Struktur und Dynamik der Virulenz in Populationen von *Erysiphe graminis* f. sp. *hordei*. Marchal. PhD thesis, Justus-Liebig-Universität, Giessen, Germany

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 41:415–421

Zeller FJ (1973) 1B/1R wheat-rye chromosome substitutions and translocations. In: Proc 4th Int Wheat Genetics Symp. Mo. Agric Exp Sta, Columbia, Mo., pp 209–222

Zeller FJ, Hsam SLK (1983) Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Sakamoto S (ed) Proceedings of 6th international wheat genetics symposium, Kyoto, pp 161–173