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Leaf-rust resistance in rye (*Secale cereale* L.). 1. Genetic analysis and mapping of resistance genes *Pr1* and *Pr2*

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Abstract Genetic analysis of resistance to leaf rust in rye (*Puccinia recondita* f. sp. *secalis*) led to the identification of two dominant resistance genes, *Pr1* and *Pr2*. Both genes proved to be effective against a local leaf-rust population as well as a subset of single-pustule isolates (SPIs) the latter of which comprised SPIs with very high virulence complexity. Resistance conferred by *Pr1* and *Pr2* was expressed in detached-leaf tests of seedlings as well as in field tests of adult plants. Molecular marker analysis allowed us to map *Pr1* in the proximal part of rye chromosome 6RL, whereas *Pr2* was assigned to the distal part of chromosome 7RL. These results are discussed in view of homoeology relationships among Triticeae. A proposal is submitted for the designation of resistance genes to rye leaf rust which would avoid interference with existing gene-symboling in respect to wheat leaf-rust resistances introgressed from rye into wheat or triticale.

Keywords *Secale cereale* · *Puccinia recondita* · Resistance genes · Mapping

Introduction

Leaf rust caused by *Puccinia recondita* Rob. ex Desm. f. sp. *secalis* is the most important windspread pathogen in rye and is endemic in all rye growing regions. Epidemic incidence of leaf rust has the potential to result in distinct yield reduction. Under natural infection yield-losses were reported to mount up to 40% under continental climate conditions (Kobylansky and Solodukhina 1983). Frauenstein (1985) observed increased yield reductions in the range of 8–20% to be associated with increasing areas of infection of 5–65% on the two uppermost leaves among a sample of 1,400 single culms in an open-pollinated cultivar. Miedaner and Sperling (1995) found mean reductions in 1,000-kernel weight of 14%, ranging from 11 to 27% among ten single crosses upon artificial inoculation in three natural environments.

Compared to major cereals like wheat and barley there is little information available on the inheritance of leaf-rust resistance in rye, the number of resistance genes, their genomic location and effectiveness. In six rye inbred lines the presence of a total of ten genes conferring resistance to a *P. recondita* f. sp. *secalis* isolate was inferred from tests for allelism (Musa et al. 1984). Four rye leaf-rust resistance genes were found by segregation analysis in the Russian rye cultivars 'Sanim' (from 'Sangaste' × 'Immunaya1'), 'Immunaya 1' which had been selected from a population of *Secale montanum*, 'Chulpan', and 'Novozybkovskaya 4', respectively (Solodukhina 1994; Kobylanski and Solodukhina 1996). Designation of these four genes as *Lr4, Lr5, Lr6*, and *Lr7* was based on the assumption that three rye genes, *Lr25, Lr26*, and Lr_{Satu}, which were known to confer resistance to wheat leaf rust in wheat and triticale would also represent resistance genes to *P. recondita* f. sp. *secalis* in the orginal rye parents (Solodukhina 1994).

Besides resistance governed by single genes and postulated to be race-specific, quantitatively inherited leaf-rust resistance in rye was also addressed. Investigation on 44 inbred lines selected for their resistance to leaf rust, and their testcrosses with a susceptible tester revealed high entry mean heritability of resistance (h^2 = 0.96 and 0.92, respectively), negligible genotype × environment interaction and heterosis for race-specific but not for nonrace-specific resistance. The majority of these lines displayed race-specific resistance. Only two or three lines exhibited leaf-rusting scores resembling a

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quantitative resistance, i.e. medium infestation with leaf rust (Sperling et al. 1996; Miedaner et al. 2002). Hence, a qualitative, simply inherited type of host/pathogen interaction appears to be predominant also with leaf-rust resistance in rye. In a programme to unlock genetic resources of rye for resistance breeding we have started a systematic evaluation of genebank accessions to identify novel resistance genes by genetic analysis and molecular mapping (Ruge et al. 1999; Roux et al. 2000). In the present study, an analysis of two genes conferring resistance of rye to leaf rust is reported.

Materials and methods

Plant material

As resistance donor, the S6 inbred line *L2527* was used which originated in a cross of '*345* × *sf*' at the former Institute of Plant Breeding in Gülzow/Güstrow (East Germany). Further parentage of the plant material is unknown. *L2527* had been selected due to its field resistance to leaf rust. The resistance donor was crossed to the susceptible S1 inbred line *L2635* which originated in the cross *Fix msG* × *L360* (G. Melz, personal communication) to give rise to F1 offspring. Five different F1 individuals were selfed to the F2 families *BAZ-1060, BAZ-3293.0, BAZ-3293.1, BAZ-3293.2*, and *BAZ-6025*, respectively. Selfing of an individual from *BAZ-3293.2* gave rise to the F3 family *BAZ-2137*. The latter family and *BAZ-1060* were used for mapping purposes. Plants of the selfed generation S4 which had been derived from these mapping populations were tested in their reactions to the local leaf-rust population as well as to 23 single-pustule isolates (SPIs) of different line origin.

Resistance tests

In situ testing for leaf-rust resistance was performed in detached-leaf tests using leaf segments from seedlings grown in the greenhouse at 18 °C. Leaf segments about 2-cm long were layed onto solid agar media containing 5.4% agar and 40 ppm of benzimidazole (Wolfe 1963). Tests comprised two independent, subsequent inoculation experiments using the seed leaves of 12–14-day old seedlings and the first-true leaves of 20–23-day old plantlets, respectively. In each experiment, two leaf segments per plant were placed onto separate plates and these were inoculated at a given time. Inoculation of leaf segments was done using an infection tower with an interchangeable, 5-bar air-pressured plexiglass device for uredospore dispersal. The device was charged with 34 mg of uredospores/shot for inoculation of 960 leaf segments. Following inoculation, agar plates were wrapped in damp clothes and held in the dark at 20 °C for 24 h, following incubation in a growth chamber at 20 °C/70–80% RH/16-h daylength with 4 kLx fluorescent lighting (Philips TLD 58W/25). As an inoculum, a local leaf-rust population (GL) was employed which had been sampled on the trial field in Groß Lüsewitz and propagated on susceptible rye plants cv 'Pluto' for several propagation cycles. In addition, 23 single-pustule isolates collected from 11 different locations in six regions of Germany and showing different levels of virulence were used for a closer characterization of the resistance. Seven of them had been developed from the local GL rust population and displayed virulence complexities of 8–11 while the remaining 16 SPIs had virulence complexities between 7 and 20. Virulence complexities of SPIs had been assessed on a differential set of 24 leaf rust-resistant rye inbred lines, including 17 lines reported by Lessner and Sperling (1995) and seven novel lines which were added in 2000. For instance, a virulence complexity of 20 means that the given SPI is virulent on 20 of the 24 inbred lines of the differential rye tester set. The 23 SPIs had been chosen for maximum virulence complexities among a total of 318 SPIs.

Table 1 Leaf-rust rating scheme with ITs *1–6* according to Frauenstein and Reichel (1978)

Infection type (IT)	Typical symptoms
1	No symptoms of infestation
2	Formation of chlorosis
3	Formation of very small pustules with chlorotic coronas
4	Formation of middle-sized pustules with chlorotic coronas
5	Formation of large pustules with chlorotic coronas
6	Formation of large pustules without chlorosis

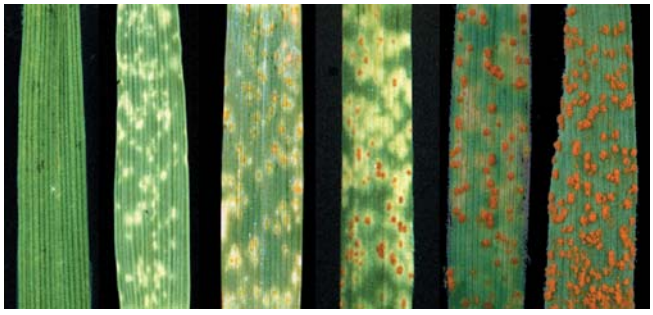

Fig. 1 Infection types IT *1* through IT *6* (from left to right) assessed in tests for leaf-rust resistance. The IT series was assembled using leaf segments from different resistance tests and from plants of different origin and genotype

Reactions of leaf segments to leaf rust were scored 8–10 days post infection (dpi) using infection types (ITs) on a scale from 1 to 6 according to Frauenstein and Reichel (1978) (Table 1, Fig. 1). For progeny tests, plants with given IT scores were selfed and 15–20 offspring individuals evaluated in detached-leaf tests for their resistance.

To test for adult-plant resistance, non-inoculated, 4-month-old vernalized plants were planted in the field in March, 1996. Following spontaneous infection with the GL leaf-rust population, infestation with leaf rust was assessed for F, F-1 and F-2 leaves approximately 7 days before flowering and during anthesis. Scoring was according to the scheme of Frauenstein and Reichel (1978).

Marker analysis

Isozyme marker loci *Aco1* and *Est8* were assessed by polyacrylamide electrophoresis and isoelectric focussing, respectively (Schmidt-Stohn and Wehling 1983; Wehling 1985). For molecular marker analysis, genomic DNA was isolated from leaves with a modified CTAB protocol (Wilkie 1989). RFLP analysis was performed using genomic probes from rye (Xiag). RAPD fragments were assessed by use of 300 decamer primers (Operon Technologies, Alameda, USA) and analyzed on standard agarose gels with ethidium bromide staining. Genomic DNA fingerprints with primers derived from resistance-gene analogues were performed as described previously (Chen et al. 1998).

Rye SSR marker analysis was performed as reported elsewhere (Hackauf and Wehling 2002a) by screening of a total of 78 SCM markers of known map positions (Hackauf and Wehling 2002b).

A STS version of the marker locus *cMWG682* was established in rye using the forward and reverse primers 5′CTGCAG-CGACAGGGAGTATGT3′ and 5′TGATGGCCATGTCTCAG-

Table 2 Reaction of inbred lines *L2527* and *L2635* to GL inoculum

Inbreds, F1	N	No. of individuals falling in IT classes[a]											
		Detached-leaf test						Field Test					
		1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*
L2527	12	12	–	–	–	–	–	12	–	–	–	–	–
	24	6	17	1	–	–	–	nd					
L2635	33	–	–	–	–	33	–	–	–	–	13	20	–
L2635 × L2527	20	2	18	–	–	–	–	nd					
	10	nd						10[b]				0[b]	

[a] Entries refer to the highest of four IT scores obtained in two experiments with two repetitions/individual, respectively
[b] Numbers pooled for IT classes 1–4 and 5–6, respectively
nd, not determined

CAT3′, respectively, at 60 °C annealing temperature. Sequence specificity of the amplified subgenomic rye fragments was verified by Southern hybridization using *cMWG682* as a probe. Chromosomal localization of the STS marker was determined using disomic wheat-rye addition lines kindly provided by T. Miller (Department of Crop Genetics, John Innes Centre, Norwich). In an attempt to substitute the isozyme marker locus *Aco1* by a STS marker, a BLAST search of a cytosolic aconitase peptide sequence from *Nicotiana tabacum* (AAG28426) against translated rye ESTs was performed using the TBLASTN algorithm (Altschul et al. 1990) and the WWW interface of the BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/) provided by the NCBI (National Centre for Biotechnology Information, Bethesda, Md.). A subgenomic fragment of approximately 1,100 bp was amplified using the forward and reverse primers 5′AGCGTGAGTGACCGAAAGACT3′ and 5′ACCAAAAGATGAGCATCAGGT3′, respectively, derived from the rye EST BE704858 at 50 °C annealing temperature. For each STS assay, 50 ng of genomic DNA was used in a solution containing a 1 × reaction buffer (Qiagen), 1.5 mM of $MgCl_2$, 200 μM of each dNTP, 5 pmol of primers and 0.5 U of HotStar*Taq* DNA Polymerase (Qiagen). Restriction patterns of cleaved amplicons were visualized on agarose gels by ethidium-bromide staining.

Linkage analysis

Linkage analysis was performed using the software package JoinMap v.3 (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 (cM).

Results

Resistance donor *L2527*, susceptible parent *L2635* and their F1

Resistant and susceptible parent inbreds and their F1 were tested both in the seedling stage and as adult plants using the GL leaf-rust population as inoculum (Table 2). All individuals of *L2527* were assessed as being resistant. There was some minor variation in the resistance reaction of individual plants. In a detached-leaf test of another 24 plants, chloroses together with very small uredospore pustules (IT *3*) were observed with one individual. Pustules did, however, not further develop in this case. In contrast, *L2635* invariantly reacted susceptible with IT *5* when tested in situ. Based on the results obtained in detached-leaf tests, a preliminary grouping of ITs *1–4* and ITs *5–6* as resistant and susceptible reaction patterns, respectively, was applied in subsequent analyses. The F1 offspring from *L2527 × L2635* was invariantly resistant, demonstrating that resistance derived from *L2527* was dominantly expressed. F1 individuals were also tested in the field and proved to be resistant.

Table 3 Monohybrid and dihybrid segregations for leaf-rust resistance in F2 and F3 families of the cross *L2527 × L2635*

Family	N	Segregation		Ratio fit	χ^2
		Resistant	Susceptible		
BAZ-1060	139	97	39	3:1	0.98
	107	77	30	3:1	0.52
BAZ-2137	47	34	13	3:1	0.18
BAZ-6025					
1st test	95	71	24	3:1	0.00
2nd test	97	75	22	3:1	0.27
BAZ-3293.0					
1st test	90	83	7	15:1	0.37
2nd test	90	87	3	15:1	1.29
BAZ-3293.1	150	136	14	15:1	2.35
BAZ-3293.2					
1st test	101	97	4	15:1	0.89
2nd test	97	93	4	15:1	0.77

Segregation analysis of resistance conferred by *L2527*

The F1 offspring displayed homogeneity in respect to resistance. However, selfing of F1 individuals resulted in F2 families which segregated for resistance in different ratios (Table 3). In two F2 families (*BAZ-1060* and *BAZ-6025*) obtained by selfing different F1 individuals, a 3:1 ratio was observed which demonstrated that a single dominant resistance gene was segregating in each of the F2 families. In three other F2 families (*BAZ-3293.0, .1* and *.2*), segregation of resistant vs susceptible offspring was not consistent with 3:1 but fitted a 15:1 ratio. This suggested that two unlinked or loosely linked genes for leaf-rust resistance must have been transferred from resistance donor *L2527* into these F2 families, and that

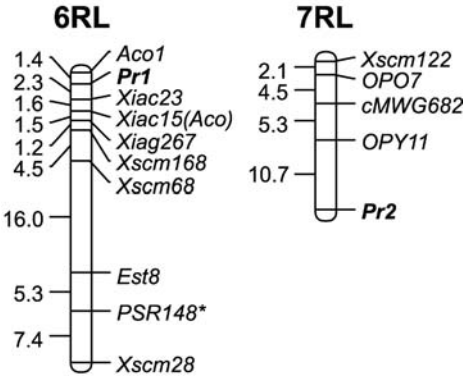

Fig. 2 Mapping of *Pr1* and *Pr2* on chromosomes 6RL and 7RL, respectively. Genetic distances are given in centiMorgans. **PSR148* was included in the linkage group at a LOD score of 2.0

these genes acted dominantly and independently from each other to confer resistance. Moreover, the resistant S_6 inbred *L2527* must have still been segregating for at least one of the genes, giving rise to F1 offspring with different resistance genotypes and, hence, to F2 families with different segregation ratios.

Except for *BAZ-3293.2* there were slight differences between the groupings of resistant vs susceptible when seed leaves and first-true leaves of identical plants were tested in two subsequent inoculation experiments. Two out of 95 and four out of 90 individuals underwent changed grouping in *BAZ-6025* and *BAZ-3293.0* families, respectively (Table 3).

Inheritance and mapping of *Pr1* resistance

A total of 18 individuals from F2 families *BAZ-3293.1* and *BAZ-3293.2* were selfed to F3 and 50 plants per F3 subjected to detached-leaf tests. Four out of the 18 F3 families displayed a 3:1 ratio of resistant and susceptible plants (data not shown), indicating that a single resistance gene was segregating. Of the four F3 families, *BAZ-2137* (Table 3) was used for further analysis.

The resistance gene segregating in *BAZ-2137* was submitted to linkage analysis using isozyme and molecular markers. Linkage was found between resistance and nine markers spanning an interval of 41 cM, of which isozyme loci *Aco1* and *Est8* as well as the RFLP marker *Xiag267* had previously been located on chromosome 6R and 6RL (Wehling 1985; Senft and Wricke 1996). As a conclusion, chromosome 6RL bears a gene for resistance to *P. recondita* f. sp. *secalis*. This gene was designated *Pr1*. The closest linkage with a 1.2-cM distance was observed between *Pr1* and *Aco1* (Fig. 2). In an attempt to convert the isozyme marker *Aco1* into a PCR-based STS marker, *Xiac15*(*Aco*) was obtained. This marker was found to be also linked closely to *Pr1*. However, since there was recombination between *Aco1* and *Xiac15*(*Aco*) the latter constituted a separate marker which mapped to the opposite side relative to *Pr1* in *BAZ-2137*. Another closely linked marker, *Xiac23* (Fig. 2), was tagged in a DNA fingerprint using the NLRR primer pair described by Chen et al. (1998).

Inheritance and mapping of *Pr2* resistance

F2 family *BAZ-1060* segregated for a single leaf-rust resistance gene (Table 3) but did not show joint segregation of *Aco1* and resistance. This family was, thus, chosen to address the second resistance gene. Of the initial 136 plants of *BAZ-1060* (Table 3), 39 individuals had been submitted to progeny tests (see below). For mapping studies, DNA samples of 107 individuals of *BAZ-1060* were available. Entries were based on the resistance as a dominant trait for all individuals of the mapping population. Molecular marker analysis indicated linkage between resistance, RAPD markers *OPY11* and *OPO7*, SSR marker *Xscm122* and *cMWG682*. While chromosomal localization of the RAPD markers was unknown, *Xscm122* and *cMWG682* had previously been mapped in two independent mapping populations on the distal end of chromosome 7RL (Korzun et al. 2001; Hackauf and Wehling 2002b). To conclude, chromosome 7RL carries a gene for resistance to rye leaf rust (Fig. 2). This gene was designated *Pr2*.

Progeny tests for validating *Pr1* and *Pr2* resistance scorings

A random sample of 21 plants from *Pr1* mapping family *BAZ-2137* was progeny tested by selfing, of which 18 plants had been recognized as resistant with scores of IT *2* to IT *4* and three plants as susceptible with IT scores *5* or *6*. The three susceptible plants gave rise to homogeneously susceptible F4 offspring, whereas the remainder led to either resistant or segregating progeny. The ratio of resistant, segregating and susceptible F4 offspring was 5:13:3 which is consistent with a monogenic 1:2:1 ratio ($\chi^2_{0.05;2} = 1.57$). The results were taken as evidence that grouping of IT scores *1–4* and *5–6* to represent resistant (*Pr1*.) and susceptible (*pr1pr1*) genotypic classes, respectively, was valid for the single resistance gene *Pr1* segregating in *BAZ-2137*.

Thirty nine randomly chosen F2 plants from *BAZ-1060* were progeny-tested in respect to *Pr2*. Seven F2 plants with IT scores of *5* or *6* led to susceptible F3 offspring. Of the remaining 32 F2 plants which had been scored with ITs of *1* to *4*, eight plants gave rise to resistant and 24 plants to segregating selfed offspring. The 8:24:7 ratio is consistent with a monogenic 1:2:1 segregation pattern ($\chi^2_{0.05;2} = 2.12$). To conclude, the grouping of ITs *1–4* vs ITs *5–6* into reaction patterns comprising resistant (*Pr2*.) and susceptible (*pr2pr2*) genotypes, respectively, was also applicable for the leaf-rust resistance gene *Pr2* segregating in *BAZ-1060*.

Table 4 Reactions of plants to GL inoculum among *Pr1*- and *Pr2*-segregating families at the seedling stage in detached-leaf tests and at the adult stage in the field in 1996

No. of plants	IT scores		
	Seedlings[a]	Adult plants[b] Field evaluation dates	
		19.06.	25.06[c]. 27.06[d].
BAZ-2137 (*Pr1*)			
24	*2*	*1–3*	*1–4*
1	*2*	*5*	*6*
6	*3*	*1–4*	*1–4*
1	*3*	*2*	*5*
2	*4*	*1*	*1*
11	*5*	*4–5*	*5–6*
2	*6*	*4*	*5*
BAZ-1060 (*Pr2*)			
4	*1*	*1*	*1–2*
13	*2*	*1–3*	*1–3*
9	*3*	*1–2*	*1–3*
6	*4*	*3–4*	*3–4*
5	*5*	*3–4*	*5*
2	*6*	*4–5*	*5*

[a] Entries give the highest of four IT scores obtained in two experiments with two repetitions/plant, respectively
[b] Where applicable, ranges of IT scores are given
[c,d] Second date of leaf-rust assessment for *Pr2* and *Pr1* families, respectively

Effectiveness of *Pr1* and *Pr2* to inocula and in developmental stages

Selfed offspring of the S4 generation derived from the original mapping populations and homozygous for either *Pr1* or *Pr2* were tested against the GL leaf-rust population as well as 23 SPIs of different origin. These comprised five SPIs with a virulence complexity of 19 and one SPI with the highest virulence complexity (20) found among 318 SPIs from Germany and Poland (B. Klocke, unpublished). Tests were done by use of detached leaves in two independent replications.

Both *Pr1* and *Pr2* conferred resistance via hypersensitivity, resulting in the development of chlorotic areas. Plants carrying *Pr1* proved to be resistant to the GL inoculum as well as to 22 of the 23 SPIs and reacted with IT *2*. One SPI, BSB GW 01 3.P12, derived from Bad Schönborn with a virulence complexity of 14, reacted compatible with *Pr1* carriers.

Plants carrying *Pr2* were invariantly resistant to all the 23 SPIs as well as the GL rust population and generally displayed ITs of *2–3*.

In addition to tests using leaves from seedlings, resistance genes *Pr1* and *Pr2* were also tested for their effectiveness in adult plants under field conditions. Fourty seven plants of the *Pr1* mapping population *BAZ-2137* and 39 plants of the *Pr2* mapping population *BAZ-1060* were tested for their field resistance. Among these samples, segregations of 34:13 and 32:7, respectively, had been observed for seedling resistance in detached-leaf tests. Of the 34 individuals with *Pr1* seedling resistance, 32 plants turned out to be resistant also in the field. The remaining two plants displayed infection types of IT *5* and *6*, respectively, at the second date of field evaluation and, thus, were regarded susceptible. All the 13 plants susceptible in the detached-leaf test were susceptible also in the field (Table 4). In the case of *Pr2* resistance, there was perfect congruency of resistance vs susceptibility in seedlings and in adult plants at the second evaluation date (Table 4). To conclude, both *Pr1* and *Pr2* confer seedling and adult resistance to leaf rust.

Discussion

Pr1 could be mapped on chromosome 6RL of rye. Due to translocational rearrangements this chromosome arm consists of segments homoeologous to group 6L, 3L, and 7L Triticeae chromosomes, respectively (Devos et al. 1993). *Pr1* was located in a linkage group together with markers *Xpsr148* and *Xscm28*. These anchor markers were previously mapped in the distal part of rye chromosome 6RL (Devos et al. 1993; Saal and Wricke 1999; Korzun et al. 2001) as well as barley chromosome 7HL (Laurie et al. 1995; Salvo-Garrido et al. 2001). The distant proximal position of *Pr1* from these two markers, together with its close linkage to *Aco1*, suggests that *Pr1* is located on the 6L segment. An aconitase gene was located on chromosome 6BL in wheat (Chenicek and Hart 1987). Wheat chromosome 6BL is also known to carry the leaf rust resistance genes *Lr3* (Sacco et al. 1998) and *Lr9* (Autrique et al. 1995).

In the present study, resistance gene *Pr2* was located on chromosome 7RL and mapped distally to markers *Xscm122* and *cMWG682*. Chromosome arm 7RL comprises a distal segment homoeologous to group 2S (Devos et al. 1993). In barley, *cMWG682* maps to the distal region of chromosome 2HS (Graner et al. 1991). Thus, this marker assigns a chromosomal region of 7RL which is homoeologous to Triticeae group 2S chromosomes. A number of wheat cultivars carry the rust-resistance gene cluster *Lr37*/*Yr17*/*Sr38* which had been introgressed via a 2S segment from *Triticum ventricosum* translocated to wheat chromosome 2AS (Bariana and McIntosh 1993, 1994). Our observation of a leaf-rust resistance gene linked to 2S markers in the distal part of rye chromosome 7RL is consistent with its homoeologous relationship to Triticeae group 2S.

Resistance genes *Pr1* and *Pr2* showed quite similar effectiveness to different leaf rust inocula. Both resistance genes were effective to the GL leaf-rust population as well as to SPIs displaying the highest virulence complexities of 19–20 among more than 300 SPIs tested on a set of 24 rye inbreds. *Pr1* and *Pr2* were recently reported to be also effective to a local leaf-rust population from St. Petersburg, Russia (Solodukhina 2002). To conclude, *Pr1* and *Pr2* represent resistance genes effective in the seedling as well as the adult plant stage to a broad range of leaf-rust virulence genes. The fact, though, that *Pr1* turned out to be susceptible to one out of 23 SPIs

demonstrates that virulence genes of the pathogen are already present in certain rye-growing areas like Bad Schönborn, Germany. Genetic vulnerability of *Pr1* resistance may be also reflected by the observation that in recent years when challenged with freshly collected GL leaf-rust samples, *Pr1* genotypes have started to develop a mixed infection type of 2(5), i.e. large chloroses together with sporadic medium-sized pustules (S.R. Roux, unpublished) which may be due to the occurrence of a *Pr1*-virulent race with initially low frequency.

To-date, four resistance genes (*Lr25, Lr26, Lr45*, and Lr_{Satu}) to wheat leaf rust have been derived from rye chromosomes and characterized in wheat or triticale. The gene *Lr25* from rye cv 'Rosen' was identified in the T4BS·4BL-5RL translocation wheat line 'Transec' (Driscoll and Anderson 1967; Friebe et al. 1994), while *Lr26* was derived from rye chromosome arm 1RS of cv 'Petkus' and was transferred to various wheat cultivars via a T1BL·1RS translocated chromosome descending from cv 'Salzmunder Bartweizen' and its sister lines (Bartoš and Bareš 1971; Mettin et al. 1973; Zeller 1973; Zeller and Hsam 1983). A third gene, *Lr45*, derived from cv 'Petkus' was found in a T2AS-2RS·2RL translocation line (McIntosh et al. 1995) while the fourth gene, Lr_{Satu}, was found to confer wheat leaf-rust resistance in triticale cultivars and assigned to chromosome 3R (Singh and McIntosh 1990). While these genes are effective against *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*; Anikster et al. 1997) no information is available on whether they are also effective against *P. recondita* f. sp. *secalis* in rye. Addressing this question may not be an easy task if, for instance, resistances to different rust species would be inherited by the same chromosomal region as is the case for *Lr26, Yr9, Sr31* and *SrR* in wheat (Singh et al. 1990).

Previously, resistance genes *Pr1* and *Pr2* had preliminarily been named *Lr-a* and *Lr-b*, respectively (Linz and Wehling 1998; Ruge et al. 1999; Roux et al. 2000). In the present study we have re-named these genes as *Pr1* and *Pr2*, following a proposal by K.J. Leonard (Cereal Disease Laboratory, USDA-ARS, personal communication) who suggests to use a system analogous to that used for rust resistance genes in oat. Following this system, resistance genes would be named according to the rust fungus they are effective against, e.g. *Pr* and *Pg* for resistance genes to rye leaf rust and rye stem rust, respectively. According to K.J. Leonard (personal communication) this would leave open the possibility of calling genes for resistance to wheat leaf rust in rye *Pt* genes until they are given an official *Lr* number after they have been transferred into wheat and characterized. We propose, thus, to apply K.J. Leonard's suggestion to rye leaf-rust resistance genes rather than to continue in using the *Lr* nomenclature, the latter of which may give rise to confusion with leaf-rust resistance genes effective in wheat. The gene symbol *Pr* was previously used for a 55 kDa storage protein gene in rye, *Pr-3* (Benito et al. 1990). Since, however, the synonymous designation *Sec4* is preferred for this gene (Melz and Sybenga 1994) there would be no interference of the proposal made above with current gene designations in rye. Also, there would be no inconsistency in respect to the *Lr* genes listed in the rye gene inventory (Schlegel et al. 1998) since these genes have been defined according to their effectiveness to wheat leaf rust in a wheat or triticale genetic background.

In a comparative study of leaf-rust resistance genes, Solodukhina (2002) reported that *Pr1* (syn. *Lr-a*) and *Pr2* (syn. *Lr-b*) displayed reactions to the leaf rust inoculum which ruled out their identity with resistance genes present in the accessions 'Yaroslavna 3', 'Gotor 2', 'Braunrostresistenz 2' and 'Lovashpatonae 2'.

In the present study, the genes *Pr1* and *Pr2* have comprehensively been characterized in terms of inheritance, effectiveness, chromosomal localization as well as genomic map position in relation to molecular markers, and may, thus, be unequivocally identified among the variety of leaf rust resistance genes in rye. Furthermore, it was demonstrated that for these two genes, there was a perfect agreement among random samples between phenological grouping of resistant vs susceptible plants as based on the scoring scheme of Frauenstein and Reichel (1978) and the underlying resistance genotypes of *Pr.* vs *prpr* as deduced by progeny testing. Not surprisingly, though, repetitive testing of plants in independent inoculation experiments indicated that slight inconsistencies in the grouping of resistant vs susceptible phenotypes may occur which should be encountered for certain purposes, e.g. fine-mapping studies preferably by progeny testing or by confining the final data set to consensus test data.

Pr1 and *Pr2* as well as additional *Pr* genes are currently being introduced into self-fertile, near-isogenic lines to build up a standard set of genetically characterized *Pr* genes for resistance genetics and breeding in rye.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Anikster Y, Bushnell WR, Eilam T, Manisterski J, Roelfs AP (1997) *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats and rye. Can J Bot 75:2082–2096

Autrique E, Singh RP, Tanksley SD, Sorrells ME (1995) Molecular markers for four leaf rust resistance genes introgressed into wheats from wild relatives. Genome 38:75–83

Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36:476–482

Bariana HS, McIntosh RA (1994) Characterisation and origin of rust and powdery mildew resistance genes in VPM1 wheat. Euphytica 76:53–61

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

Benito C, Frade IM, Orellana J, Carrillo JM (1990) Linkage and cytogenetic maps of genes controlling endosperm storage proteins and isozymes in rye (*Secale cereale* L.). Theor Appl Genet 79:347–353

Chen XM, Line RF, Leung H (1998) Genome scanning for resistance-gene analogs in rice, barley and wheat by high-resolution electrophoresis. Theor Appl Genet 97:345–355

Chenicek KJ, Hart GE (1987) Identification and chromosomal locations of aconitase gene loci in Triticeae species. Theor Appl Genet 74:261–268

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojic P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome realtive to that of wheat. Theor Appl Genet 85:673–680

Driscoll CJ, Anderson LM (1967) Cytogenetic studies of Transec – a wheat-rye translocation line. Can J Genet Cytol 9:375–380

Frauenstein K (1985) Untersuchungen zur Schadwirkung des Braunrostes, *Puccinia recondita* Rob. ex Desm., an Winterroggen. Nachrichtenbl Deut Pflanzenschutzdienst (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, Wiss Beitr 14 (S11):403–411

Friebe B, Jiang J, Raupp WJ, Gill BS (1994) Molecular cytogenetic analysis of radiation-induced alien genetic transfers in wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp Beijing, China. China Agriculture Scientech Press, Beijing, pp 519–529

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

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

Hackauf B, Wehling P (2002b) Development of microsatellite markers in rye: map construction. Proc EUCARPIA Rye Meeting, July 4–7, 2001, Radzików, Polen, pp 333–340

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

Kobylanski VD, Solodukhina OV (1996) Genetic bases and practical breeding utilization of heterogenous resistance of rye to brown rust. Vortr Pflanzenzüchtg 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, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Lessner B, Sperling U (1995) Charakterisierung von Roggen-Braunrost-Isolaten mit Hilfe von Roggen-Inzuchtlinien. 46. Tagung der Vereinigung österreichischer Pflanzenzüchter, Gumpenstein, pp 127–132

Linz A, Wehling P (1998) Identification and mapping of major leaf rust resistance genes in rye. Beitr Züchtungsforsch 4:23–24

McIntosh RA, Friebe B, Jiang J, The D, Gill BS (1995) Cytogenetical studies in wheat. XVI. Chromosome location of a new gene for resistance to leaf rust in a Japanese wheat-rye translocation line. Euphytica 82:141–147

Melz G, Sybenga J (1994) The 3rd workshop of rye genetics and cytogenetics – revision and completion of the genetic map of rye, October 25–28, 1993, Groß Lüsewitz, Germany. Genet Polon 35:131–132

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 Columbia, Missouri, USA, 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

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

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 Pflanzenzüchtg 46:169–176

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

Sacco F, Suarez EY, Naranjo T (1998) Mapping of the leaf rust resistance gene *Lr3* on chromosome 6B of Sinvalocho MA wheat. Genome 41:686–690

Salvo-Garrido H, Laurie DA, Jaffe B, Snape JW (2001) An RFLP map of diploid *Hordeum bulbosum* L. and comparison with maps of barley (*H. vulgare* L.) and wheat (*Triticum aestivum* L.). Theor Appl Genet 103:869–880

Schlegel R, Melz G, Korzun V (1998) Genes, marker and linkage data of rye (*Secale cereale* L.): 5th updated inventory. Euphytica 101:23–67

Schmidt-Stohn G, Wehling P (1983) Genetic control of esterase isoenzymes in rye (*Secale cereale* L.). Theor Appl Genet 64:109–115

Senft P, Wricke G (1996) An extended map of rye (*Secale cereale* L.). Plant Breed 115:508–510

Singh SJ, McIntosh RA (1990) Linkage and expression of genes for resistance to leaf rust and stem rust in triticale. Genome 33:115–118

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. Russian J Genet 38:399–407

Sperling U, Lessner B, Scholz M, Wehling P, Gey A-K, Geiger HH, Miedaner T (1996) Qualitative and quantitative variation for resistance of winter rye to leaf rust. Vortr Pflanzenzüchtg 35:175

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

Wehling P (1985) Electrophoretic analysis of ten enzyme systems in rye: linkage relationships and chromosomal location of isozyme loci. EUCARPIA Meeting of the Cereal Section on Rye, June 11th–13th 1985, Svalöf, Sweden, pp 101–124

Wilkie S (1989) Isolation of genomic DNA. In: Clark MS (ed) Plant molecular biology – a laboratory manual. Springer Verlag Berlin, Heidelberg New York, pp 3–14

Wolfe MS (1963) Use of benzimidazole in the study of wheat powdery mildew. Trans British Mycol Soc 46:620

Zeller FJ (1973) 1B/1R wheat-rye chromosome substitutions and translocations. Proc 4th Int Wheat Genet Symp, Missouri, Agric Exp Station Columbia, Missouri USA, pp 209–222

Zeller FJ, Hsam SLK (1983) Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 161–173