Tagging and Validation of a Major Quantitative Trait Locus for Leaf Rust Resistance and Leaf Tip Necrosis in Winter Wheat Cultivar Forno

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ABSTRACT

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A major leaf rust (*Puccinia triticina*) resistance quantitative trait locus (QTL) (*QLrP.sfr-7DS*) previously has been described on chromosome 7DS in the winter wheat (*Triticum aestivum*) cv. Forno. It was detected in a population of single-seed descent (SSD) lines derived from the cross Arina × Forno. *QLrP.sfr-7DS* conferred a durable and slow-rusting resistance phenotype, co-segregated with a QTL for leaf tip necrosis (LTN) and was mapped close to *Xgwm295* at a very similar location as the adult plant leaf rust resistance gene *Lr34* found in some spring wheat lines. Here, we describe the validation of this QTL by mapping it to the same chromosomal region close to *Xgwm295* on chromosome 7DS in a population of SSD lines from the winter wheat × spelt (*T. spelta*) cross Forno ×

Oberkulmer. In both populations, the log of the likelihood ratio curves for leaf rust resistance and LTN peaked at identical or very similar locations, indicating that both traits are due to the same gene. We have improved the genetic map in the target region of *QLrP.sfr-7DS* using microsatellite and expressed sequence tag (EST) markers. Two EST loci (*Xsfr.BF473324* and *Xsfr.BE493812*) define a genetic interval of 7.6 centimorgans containing *QLrP.sfr-7DS*, a considerably more precise genetic location for this QTL than previously described both in spring and winter wheat. The identified genetic interval is physically located in the distal 39% of chromosome 7DS. Single-marker analysis identified *Xsfr.BF473324* and *Xgwm1220* as the most informative loci for *QLrP.sfr-7DS* and *QLtn.sfr-7DS*. In the rice genome, the two ESTs flanking the *QLrP.sfr-7DS*/*QLtn.sfr-7DS* chromosomal segment in wheat are conserved on chromosome 6S in a region colinear with wheat chromosome 7DS. There, they define a physical region of three rice bacterial artificial chromosomes spanning ≈300 kb.

From the 50 known leaf rust (*Puccinia triticina* Eriks.) resistance genes, two of them, *Lr34* and *Lr46*, have been classified as slow-rusting genes (37,49,50,57). The origin of *Lr34* can be traced back to the Brazilian wheat (*Triticum aestivum* L., $2n = 6x = 42$) cv. Frontana, which was released in 1943 (51). In contrast to many race-specific resistance genes, *Lr34* proved to confer durable resistance over a long period of time, in different environments as well as against diverse pathotypes of the fungus. Genetic studies using lines from CIMMYT (34,48), Japanese (55), or Indian (22) spring wheat germ plasm confirmed the involvement of the gene in the expression of durable leaf rust resistance. Recently, *Lr34* or a winter wheat allele of it, *QLrP.sfr-7DS*, also was detected in the Swiss winter wheat cv. Forno, which has shown durable field resistance against leaf rust (44).

During the seedling stage, wheat lines with *Lr34* show a susceptible infection type (40). In the adult plant stage, the *Lr34* phenotype typically exhibits a rust pustule gradient on the flag leaf, with more and larger pustules at the leaf base and fewer and smaller pustules toward the leaf tip. Usually, the observation of a pustule gradient goes along with the presence of leaf tip necrosis (LTN) (26,45). From the CIMMYT spring wheat germ plasm, it is known that the *Ltn* gene conferring LTN is associated with the slow-rusting and durable adult plant resistance gene *Lr34* (45). However, it still is not yet clear whether *Ltn* and *Lr34* are due to effects of the same gene; however, there are strong indications for

the pleiotropic action of a single gene (33,44). In addition, the *Ltn/Lr34* chromosomal segment also confers a durable resistance to stripe (yellow) rust (*Yr18* gene) (46) and stem rust (8,31), as well as tolerance against the *Barley yellow dwarf virus* (*Bdvl* gene) (2,47), and inactivates a stem rust-resistance suppressor (24). Therefore, the *Ltn/Lr34* chromosomal segment is unique and a very valuable source of broad-spectrum and durable disease resistance in wheat breeding.

Lr34 has been mapped to the short arm of chromosome 7D using cytogenetic analysis (8). Later, mapping studies revealed that *Lr34* is close (2 to 5 centimorgans [cM]) to the microsatellite marker *Xgwm295* (44,55) and the restriction fragment length polymorphism (RFLP) marker *Xwg834* (34) on chromosome arm 7DS. So far, there is no tightly linked (<1 cM) molecular marker available for *Lr34* or its winter wheat allele which could be used for marker-assisted selection (MAS). The sequence information of the rice genome (11,17,41) with its close relatedness and partial colinearity to wheat (14,53) enables efficient generation of new probes for genetic mapping in wheat. In addition, almost 10,000 wheat expressed sequence tags (ESTs) have been mapped physically using bin-mapping and chromosome-deletion lines (9, 38). This physical mapping information is very helpful to derive markers for specific chromosomal regions in wheat. In the last few years, there have been intensive efforts in wheat genomics with the goal of isolating agronomically important genes. Recently, it was shown that chromosomal walking in wheat is feasible (10,54). In addition, two major disease resistance genes against leaf rust, *Lr10* (12) and *Lr21* (21), have been isolated. So far, no durable or slow-rusting leaf rust resistance gene has been isolated in wheat.

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In this study, we defined the genetic interval containing the putative winter wheat allele of *Lr34* in cv. Forno to a region of 7.6 cM on chromosome 7DS. The flanking markers in wheat are colinear in the rice genome, representing a genomic region of ≈ 300 kb.

MATERIALS AND METHODS

Linkage analysis and physical mapping. Linkage analysis was carried out using the computer package MAPMAKER 3.0b for MS-DOS (28). All new simple-sequence repeat (SSR) markers, ESTs, or RFLP markers in the Arina $\times$ Forno population were mapped using 240 single-seed descent (SSD) lines as described in (36). In the Forno $\times$ Oberkulmer population, mapping was accomplished according to Messmer et al. (33) and based on 204 lines. The establishment of the new linkage group on the short arm of chromosome 7D in the Forno $\times$ Oberkulmer population was conducted using the procedures described in (36). For adding new markers to chromosome 7D in the Arina $\times$ Forno population, we used the “try” command and validated the new marker sequence by using the “ripple” command. Linkage groups were drawn with the software package MapChart (59). Physical mapping and bin assignment of 15 markers was conducted using the deletion line 7DS-4 (breakpoint 0.61) derived from cv. Chinese Spring, in which 39% of the short arm of chromosome 7D was missing (9,38).

Quantitative trait loci mapping. Interval quantitative trait loci (QTL) analysis was carried out with the composite interval mapping (CIM) program PLABQTL, version 1.1 (56) which is based on multiple regression. Model and covariate selection as well as determination of a significant log of the likelihood ratio (LOD) threshold were applied as described in Schnurbusch et al. (44). The phenotypic data for the QTL analysis in the Arina $\times$ Forno population were taken from the estimated genotypic values across seven environments as the area under the disease progress curve (AUDPC_%) for the percentage of infected leaf area, whereas the means of LTN were obtained by measuring in six field environments (44). In Forno $\times$ Oberkulmer, scoring across three field environments on a scale from one (fully resistant) to nine (fully susceptible) was used to estimate the phenotypic means of the population for leaf rust resistance, whereas LTN was measured quantitatively as the length of the necrotic leaf tip (33). Single-marker analysis was performed by a simple one-way analysis of variance using the SAS GLM procedure (42).

Sequence analysis and data sources. In order to identify rice bacterial artificial chromosome (BAC) clones with nucleotide sequence similarity to the wheat ESTs assigned to physical bin 7DS-4, we used a local BLAST server to perform BLASTn searches (1). The sequences of wheat ESTs were used as queries against local databases composed of the rice genome and the BAC clones assigned to rice chromosome 6. The sequences of *Oryza sativa* subsp. *japonica* BACs were downloaded from the RiceGAAS website as of May 2003 and the relative positions of BACs within the rice chromosome 6 were estimated using the IRGSP mapping data (available at the International Rice Genome Sequencing Project website). *Triticeae* EST sequences anchored within chromosome 7DS and assigned by chromosome bin mapping (38) were downloaded from the GrainGenes website. BLASTn results were parsed: a score of 50 or higher was set as threshold limit and the redundancies due to overlapping BAC clones were removed from raw data. Detailed sequence analysis were performed with the EMBOSS package (39).

RESULTS

Genetic and physical mapping of wheat chromosome 7DS. Taking the map of chromosome 7D in the Arina $\times$ Forno population as a starting point (36), 11 new markers were added to this

chromosome (Fig. 1). The entire linkage group now spans 280.5 cM. Of the 11 additional markers, 7 were polymerase chain reaction (PCR)-based SSRs, three were ESTs, and one was an RFLP (CDO475). For many of these markers, only their chromosomal or physical bin location was known previously.

Due to lacking polymorphism on the short arm of chromosome 7D using only RFLP markers, this chromosomal region was not covered in the published Forno $\times$ Oberkulmer map (32). With the recent characterization of numerous wheat SSR markers, it was possible to establish a genetic linkage map for this chromosomal region in the Forno $\times$ Oberkulmer population (Fig. 1). Of 24 SSRs tested, 10 were polymorphic and formed a linkage group of 107.7 cM. Four SSRs were mapped in both populations with the same relative marker order (Fig. 1).

Physical mapping of 15 markers revealed that 14 markers failed to hybridize to or amplify from DNA of the 7DS-4 deletion line when compared with amplifications or hybridizations of DNA from cv. Chinese Spring, indicating that the 7DS-4 breakpoint 0.61 is distal to *Xcfd46* (Fig. 2). Two SSRs, BARC352 and BARC154 (the latter is monomorphic in both populations), revealed two loci being located in bin 7DS-4. Bin mapping located *Xgwm295*, previously the closest marker proximal to *Lr34* (55) and *QLrP.sfr-7DS* (44), being located in the 7DS4-0.61-1.00 physical bin of chromosome 7DS (Fig. 2). Therefore, this physical region also contains *QLrP.sfr-7DS*.

Identification of an orthologous chromosomal region in rice close to *QLrP.sfr-7DS* and its use to derive new markers. Southern blot analysis of a putative inorganic vacuolar pyrophosphatase (wheat EST accession nos. BE427461 and BE490643) revealed a single copy of this gene in each subgenome of hexaploid wheat. It was already mapped to chromosome 7BS as *Xsfr.BE427461* in the Arina $\times$ Forno population (36). The wheat

Fig. 1. Genetic linkage maps of wheat chromosome 7D for the Arina $\times$ Forno and Forno $\times$ Oberkulmer populations. Loci in bold represent newly added markers compared with the previously published linkage maps (32,36). Black bars beside the two linkage groups and underlined loci indicate the most probable marker interval of *QLrP.sfr-7DS/QLr.sfr-7DS*, whereas dotted lines show common markers for both populations.

EST BE490643 could be mapped approximately 50 cM proximal to *Xgwm295* to a homoeologous locus on the short arm of chromosome 7D (Fig. 1) using a different set of restriction enzymes cleaving DNA from cv. Chinese Spring and the 7DS-4 deletion line in order to identify the 7D polymorphism for southern mapping. Blast analysis (BLASTn) of this wheat EST in rice revealed two loci on rice chromosome 6.

Using the homoeologous relationships between group 7 chromosomes in wheat (35), the approximate map position for CDO475 was estimated to be close to *Lr34/QLrP.sfr-7DS*. CDO475 mapped 4 cM distal to *Xgwm295* in the Arina × Forno population (Fig. 1) and was closer to *QLrP.sfr-7DS* than *Xgwm295* (Fig. 3A). Sequencing of the anonymous cDNA probe CDO475 from oat (*Avena sativa* L.) revealed a putative carboxypeptidase. Its sequence gave a single BLASTn match on the short arm of rice chromosome 6. The rice ortholog of CDO475 was found on rice BAC AP003487 (Fig. 4).

As of March 2003, 201 loci corresponding to 164 wheat ESTs were physically assigned to bin 7DS-4 (available online at the Grain Genes website), which is partially orthologous to rice chromosome 6 (14,53). We have blasted (BLASTn) the 164 wheat ESTs against rice chromosome 6, and blast results with a cut-off score value below 50 were discarded. The remaining wheat ESTs were arranged physically according to blast analysis on BACs/PACs of rice chromosome 6. Wheat ESTs that had rice orthologs closely linked to the rice CDO475 gene (carboxypeptidase) were selected based on low gene copy number in their Southern blot analysis (available online at the Grain Genes website). The rice ortholog of the wheat EST BF473324 (putative GTP-binding membrane protein) was directly adjacent to the rice carboxypeptidase gene on the same rice BAC AP003487 (Fig. 4). In wheat, *Xcdo475b* and EST *Xsfr.BF473324* were genetically tightly linked (0.3 cM) (Fig. 1). The wheat EST BE493812 (hypothetical protein) represents the distal end of the *QLrP.sfr-7DS* and *QLtn.sfr-7DS* interval in wheat (described below). Its sequence blasted to BAC AP003708 in the same region on rice chromosome 6 (Fig. 4). Thus, the two loci *Xsfr.BF473324* and *Xsfr.BE493812*, delimiting *QLrP.sfr-7DS* and *QLtn.sfr-7DS* in a 7.6-cM interval in hexaploid wheat (described below), correspond to approximately three rice BACs or ≈300 kb in the orthologous region in rice (Fig. 4).

Mapping QTL for leaf rust resistance and LTN in the improved genetic map of chromosome 7DS. QTL mapping (CIM and LOD > 4.4) using the improved map of chromosome 7DS in

the Arina × Forno population resulted in the definition of a small QTL interval (Fig. 3A) for leaf rust resistance (*QLrP.sfr-7DS*) and LTN (*QLtn.sfr-7DS*). Both QTL were flanked by *Xsfr.BE493812* and *Xsfr.BF473324*, reducing the marker interval from 60 cM (44) to 7.6 cM. Still, both LOD curves were completely overlapping and peaked at 3.1 cM (±2 cM for 1-LOD-support-interval) distal to *Xsfr.BF473324*, indicating pleiotropism between *QLrP.sfr-7DS* and *QLtn.sfr-7DS* (33,44). Due to the closer flanking markers, the percentage of the phenotypic variation explained increased to 59.7% with a corresponding LOD value of 47.4 for *QLrP.sfr-7DS* and to 72.2% with a LOD value of 66.7 for *QLtn.sfr-7DS*. Single-marker analysis for this chromosomal region identified *Xsfr.BF473324* as the most informative locus (Table 1), giving a coefficient of determination (R^2) of 29.4% for leaf rust resistance and 61.8% for LTN, respectively. The marker locus *Xcdo475b* was tightly linked to *Xsfr.BF473324* and, therefore, it also explained a high percentage of the observed phenotypic variation for both traits within the Arina × Forno population (Table 1).

QTL analysis of the linkage group representing chromosome 7DS in the Forno × Oberkulmer population revealed a major QTL for leaf rust resistance (designated as *QLr.sfr-7DS*) and LTN (Fig. 3B). Also in the Forno × Oberkulmer population, the two LOD curves for leaf rust resistance and LTN largely overlapped and were very similar, but did not peak at the identical position. *QLr.sfr-7DS* was located 2.7 cM (±3 cM) proximal to *Xgwm1220*, whereas *QLtn.sfr-7DS* was 4.7 cM (±3 cM) proximal to *Xgwm1220*. *QLr.sfr-7DS* accounted for 39% of the observed phenotypic variation for leaf rust resistance in the Forno × Oberkulmer population with a corresponding LOD value of 21.8 (Fig. 3B). *QLtn.sfr-7DS* reached a LOD value of 43.2, explaining 62.8% of the phenotypic variance for LTN. Both QTL most likely were localized within the *Xgwm1220-Xgwm130* interval (Fig. 3B). Single marker analysis of this linkage group in the Forno × Oberkulmer population supported this hypothesis by identifying *Xgwm1220* as the best (R^2 of 22.7% for leaf rust resistance and 65.4% for LTN) and *Xgwm130* as the second best locus (21.9 and 46% for leaf rust resistance and LTN, respectively) within this linkage group (Table 1).

DISCUSSION

Tagging and validation of *QLrP.sfr-7DS* and *QLtn.sfr-7DS*.

The major leaf rust resistance QTL of cv. Forno, *QLrP.sfr-7DS*, was physically mapped to the distal 39% of the short arm of chro-

Fig. 2. Physical bin-mapping of molecular markers using the deletion line 7DS-4 (breakpoint 0.61). Markers which failed to hybridize to or amplify from DNA of this deletion line, but hybridized to or amplified from DNA of cv. Chinese Spring, were assigned to the chromosomal bin 7DS4-0.61-1.00.

mosome 7D. In the Arina × Forno population, it was flanked by two wheat ESTs defining a 7.6-cM interval. Now, *QLrP.sfr-7DS* was localized genetically ≈7.0 cM distal to *Xgwm295* in a considerably more precise interval for this QTL than previously described in both spring (55) and winter wheat (44). The contribution of *QLrP.sfr-7DS* to leaf rust resistance was validated in another mapping population of cv. Forno and a winter spelt (cv. Oberkulmer). The map location of *QLrP.sfr-7DS* and *QLr.sfr-7DS* in the winter wheat cv. Forno is very similar to previously reported mapping data for the durable resistance gene *Lr34* in several spring wheat lines (34,55). The *Lr34* gene acts as a quantitative gene conferring durable leaf rust resistance, particularly in combination with other major resistance genes. Its activity has been validated in several populations derived from different spring wheat lines (16,22,25,26,43). We have found a complete linkage between *QLrP.sfr-7DS* and LTN. Similarly, it was found that the slow-rusting phenotype of *Lr34* and leaf tip necrosis were cosegregating in spring wheat lines carrying *Lr34* (45).

Molecular analysis of *QLrP.sfr-7DS/QLtn.sfr-7DS* showed that it was localized genetically in the same chromosomal region as *Lr34* (34,55). Phenotypically, *QLrP.sfr-7DS/QLtn.sfr-7DS* also was very similar to *Lr34*, because we found lines in our mapping populations exhibiting the typical “*Lr34*-phenotype” during the adult plant stage in the field (44). There, flag leaves of infected lines in the field showed a rust pustule gradient on the leaf with more and larger pustules at the leaf base and fewer and smaller pustules towards the leaf tip. Usually, the pustule gradient went

along with the presence of LTN (26,45). However, the *Lr34* gene initially was defined within the CIMMYT spring wheat germ plasm, because it was derived from cv. Frontana (51). To date, there is no pedigree link between cvs. Forno and Frontana indicating either another source for *Lr34* different from Frontana or the occurrence of an independent winter wheat allele of the *Lr34* gene within the winter wheat germ plasm.

Merging the mapping data from both Swiss winter wheat populations, one can conclude that the position of the *QLrP.sfr-7DS* chromosomal segment is most likely between *Xgwm1220* and *Xsfr.BF473324*. Thus, the SSR marker *Xgwm1220*, ≈2 to 3 cM away from *QLrP.sfr-7DS*, is the closest PCR-based marker currently available. However, *GWM1220* was not polymorphic in the

Fig. 3. Log of the likelihood ratio (LOD) curves for leaf rust resistance and leaf tip necrosis on chromosome 7DS. **A**, Population Arina × Forno and **B**, population Forno × Oberkulmer. Underlined loci indicate the most probable marker interval of *QLrP.sfr-7DS/QLr.sfr-7DS*. One dash on the vertical axis corresponds to 2 centimorgans.

Fig. 4. Genetic and physical relationships between rice chromosome 6S and wheat chromosome 7DS. The target interval in the genetic map of Arina × Forno (7.6 centimorgans) of *QLrP.sfr-7DS* was flanked by two wheat expressed sequence tag loci, *Xsfr.BE493812* and *Xsfr.BF473324*. Their orthologous loci in rice were detected on BACs/PACs AP003708 and AP003487, which flank a chromosomal region of ≈300 kb on rice chromosome 6S.

TABLE 1. Single-marker analysis for leaf rust resistance (rust) and leaf tip necrosis (LTN) on the short arm of chromosome 7D^a

Locus	<i>R</i> ² (%)			
	Arina × Forno		Forno × Oberkulmer	
	Rust	LTN	Rust	LTN
<i>Xcfd66</i>	5.2	20.1	6.9	22.9
<i>Xwmc463</i>	11.5	29.1	2.7	19.6
<i>Xbarc352</i>	13.0	35.7	2.7	20.3
<i>Xsfr.BE493812</i>	18.7	46.5	...	...
<i>Xgwm1220</i>	...	...	22.7	65.4
<i>Xsfr.BF473324</i>	29.4	61.8	...	...
<i>Xcdo475b</i>	27.6	59.4	...	...
<i>Xgwm295</i>	21.9	47.7	...	...
<i>Xgwm130</i>	...	...	21.9	46.0
<i>Xgwm1002</i>	3.1	4.1	17.6	16.8
<i>Xwmc405b</i>	...	...	14.2	7.2

^a Coefficients of determination (*R*²) in percent are given for the Arina × Forno and Forno × Oberkulmer populations and loci closely mapping to *QLrP.sfr-7DS* or *QLr.sfr-7DS*, respectively, and *QLtn.sfr-7DS*.

Arina × Forno population and, therefore, cannot be considered as a diagnostic marker. Nevertheless using SSR and wheat EST markers, we increased the marker density substantially on chromosome 7DS in both mapping populations although the low level of polymorphism, particularly for RFLP and EST markers on the D genome of hexaploid wheat, has been well documented (6, 32,36).

Colinearity between rice chromosome 6S and wheat chromosome 7DS. The rice genome sequence constitutes a model for other genomes of many related grass species, such as maize (*Zea mays* L.), sorghum (*Sorghum* sp. L.), barley (*Hordeum vulgare* L.), or wheat (29,53). We compared rice chromosome 6S and wheat chromosome 7DS at four orthologous loci and found colinearity of gene content and gene order in the region described. In wheat and rice, the putative pyrophosphatase gene (*Xsfr:BE490643*) mapped proximal to the target interval flanked by *Xsfr:BE493812* (hypothetical protein) and the putative GTP-binding membrane protein (*Xsfr:BF473324*). The orientation of the identified target interval containing *QLrP.sfr-7DS* also was conserved in both species, because *Xsfr:BE493812* mapped distal to the gene encoding a GTP-binding membrane protein, which itself was tightly linked to the putative carboxypeptidase (*Xcdo475b*). Despite the huge differences between the genomes of rice and wheat in chromosome number, ploidy level, and size, they often show a very good conservation of marker order (colinearity). Early reports found high colinearity between rice and the *Triticeae* spp. (wheat, barley, oat, and rye) (14,15,19,27,58). Detailed studies at the microlevel revealed rearrangements of gene content displaying a mosaic organization of the orthologous regions, with conserved sequences interspersed with nonconserved sequences (3,5,13,18,23,52). However, during the map-based cloning of the *Rpg1* stem rust resistance gene in barley, excellent colinearity between the distal end of the short arm of rice chromosome 6 and barley chromosome 7HS was demonstrated (4,20).

Therefore, in order to “mendelize” a quantitative trait or resistance into a single locus, rice sequence information in conjunction with mapped wheat ESTs will help to dissect the present target interval for fine-mapping. In addition, newly released BAC libraries from *Aegilops tauschii*, the D-genome donor of hexaploid wheat, also will be very valuable tools to conduct high-resolution mapping in wheat. The degree of conserved colinearity between rice chromosome 6S and wheat chromosome 7DS essentially will determine whether fine-mapping and cloning of *QLrP.sfr-7DS* will be rapidly possible.

Outlook. So far, despite enormous economic interest, no durable or slow-rusting leaf rust resistance gene has been isolated in wheat. The isolation and cloning of *QLrP.sfr-7DS* in wheat will be the first step of a physiological understanding of slow-rusting or partial disease resistance and also will clarify whether these slow-rusting genes are “defeated” major resistance genes (7,30). *QLrP.sfr-7DS* seems to be a very suitable candidate for a map-based cloning strategy of a durable and broad-spectrum disease resistance gene in hexaploid wheat.

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