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ORIGINAL PAPER

Genetic analysis and molecular mapping of crown rust resistance in common wheat

Zhixia Niu · Krishna D. Puri · Shiaoman Chao · Yue Jin · Yongliang Sun · Brian J. Steffenson · Shivcharan S. Maan · Steven S. Xu · Shaobin Zhong

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Abstract
Key Message **This is the first report on genetic analysis and genome mapping of major dominant genes for near non-host resistance to barley crown rust (*Puccinia coronata* var. *hordei*) in common wheat.**
Abstract Barley crown rust, caused by *Puccinia coronata* var. *hordei*, primarily occurs on barley (*Hordeum vulgare* L.) in the Great Plain regions of the United States. However, a few genotypes of common wheat (*Triticum aestivum* L.) were susceptible to this pathogen among 750 wheat accessions evaluated. To investigate the genetics of crown rust resistance in wheat, a susceptible winter wheat accession PI 350005 was used in crosses with two resistant wheat varieties, Chinese Spring and Chris. Analysis of F_1 plants and F_2 populations from these two crosses indicated that crown rust resistance is controlled by one and two dominant genes in Chris and Chinese Spring, respectively. To determine the chromosome location of the resistance gene *Cr1* in Chris, a set of 21 monosomic lines derived from Chris was used as female parents to cross with a susceptible spring type selection (SSTS35) derived from the PI 350005/Chris cross. Monosomic analysis indicated that *Cr1* is located on chromosome 5D in Chris and one of the crown rust resistance genes is located on chromosome 2D in Chinese Spring. The other gene in Chinese Spring is not on 5D and thus is different from *Cr1*. Molecular linkage analysis and QTL mapping using a population of 136 doubled haploid lines derived from Chris/PI 350005 further positioned *Cr1* between SSR markers *Xwmc41-2* and *Xgdm63* located on the long arm of chromosome 5D. Our study suggests that near non-host resistance to crown rust in these different common wheat genotypes is simply inherited.

Introduction

Crown rust, caused by *Puccinia coronata* var. *avenae*, is a well-known and widely distributed rust disease of oats (*Avena sativa* L.) in many regions of the world (Simons 1985). In addition to attacking oats, other forms [i.e.,

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Z. Niu and K. D. Puri contribute equally to this research.

Shivcharan S. Maan: Retired.

Z. Niu · S. Chao · S. S. Xu
USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND 58102, USA

K. D. Puri · Y. Sun · S. Zhong (✉)
Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA
e-mail: shaobin.zhong@ndsu.edu

Y. Jin
USDA-Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA

Present Address:
Y. Sun
Monsanto Vegetable Seeds, 37437 State Highway 16, Woodland, CA 95695, USA

B. J. Steffenson
Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

S. S. Maan
Department of Plant Science, North Dakota State University, Fargo, ND 58108, USA

formae speciales (f. sp.) or varieties (var.) of *P. coronata sensu latu*] are known to infect many different gramineous species and several cereal crops as well (Szabo 2006). Crown rust was first observed on barley (*Hordeum vulgare* L.) in the 1950s (Lutey and Covey 1959), but the pathogen was not described as a new variety of *P. coronata* [i.e., var. *hordei* Jin & Steff (Jin and Steffenson 1999)] until a serious outbreak occurred in south central Nebraska in 1991 (Jin et al. 1992). This rust disease occurs annually on barley in the Upper Midwest region of the United States and Prairie Provinces of Canada (Jin and Steffenson 1999; unpublished data); however, the incidence and severity varies from year to year, with only occasional epidemics. The fact that most barley genotypes are susceptible to crown rust and the pathogen frequently undergoes sexual recombination on the alternate host (common buckthorn, *Rhamnus cathartica* L.) (Jin and Steffenson 1999) to generate new races raises some concerns for barley production. Thus, several investigations have been made to identify sources of resistance and determine the genetic basis of this trait in this crop (Jin et al. 1993a; Jin and Steffenson, unpublished data).

Among 526 barley accessions evaluated for reaction to isolate ND91-36 of *P. coronata* var. *hordei* at the seedling stage, only 10 (1.9 % of the total) were resistant [i.e., exhibited infection types (ITs) of 0–1] (Jin et al. 1993a). All other barley accessions were moderately susceptible to susceptible (ITs of 3–4) to the pathogen (Jin et al. 1993a). Genetic analyses of crosses between three (PI 356467, PI 356543, and Hor2596) of the ten resistant accessions and the susceptible barley cultivar Bowman (PI 383237) revealed that a single major gene controls resistance to crown rust in each of the three barley accessions (Jin and Steffenson 2002). The resistance gene *Rpc1* in Hor 2596 (CIho 1243) was later mapped to chromosome 3H using DNA markers [i.e., randomly amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and restriction fragment length polymorphism (RFLP) markers (Agrama et al. 2004)], although its allelic relationship to genes in other resistant barley accessions remains to be investigated.

Although barley is the primary cultivated host of *P. coronata* var. *hordei*, some accessions of other gramineous species, including common wheat (*Triticum aestivum* L.), foxtail barley (*H. jubatum* L.), rye (*Secale cereal* L.), quackgrass [*Elytrigia repens* (L.) Desv. ex Nevski], slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners], and western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve], were also susceptible to this rust fungus (Jin and Steffenson 1993; Jin et al. 1993b). Nevertheless, the frequency of susceptibility in these gramineous species was very low compared to that observed in barley. Jin et al. (1993b) screened 750 randomly selected accessions of *T. aestivum* for reaction to barley crown rust isolate ND91-36, and found most were immune or highly resistant; only 2 % showed a high level of susceptibility. Based on the definition of Heath (1981) and Niks (1988), barley crown rust resistance in wheat can be considered as "near non-host resistance," which is defined as the condition when nearly all genotypes of a plant species are resistant to nearly all isolates of a potential pathogen. Non-host/near non-host resistance is the most common form of resistance in plants and potentially could be more durable (Niks et al. 2011). However, information about the genetic basis of this type of host resistance is limited. Therefore, the objectives of this study were to investigate the genetics of crown rust resistance in wheat varieties Chris and Chinese Spring and to determine the chromosomal locations of resistance gene(s) in Chris through monosomic and genome mapping analyses.

Materials and methods

Plant materials and crosses

PI 350005 was identified as the most susceptible wheat to *P. coronata* var. *hordei* from a preliminary screening of 750 accessions obtained from the USDA-ARS national small grains collection (NSGC) (Jin et al. 1993b). It is a winter type and was originally collected in Serbia in 1969. PI 350005 was used as a female parent in crosses with *T. aestivum* cvs., 'Chinese Spring' (CS) (CItr 14108) and 'Chris' (CItr 13751), which are immune and highly resistant to *P. coronata* var. *hordei*, respectively. F_1 and F_2 progenies were generated from these two crosses for genetic analysis. Since PI 350005 is a winter wheat, a susceptible spring type wheat line (SSTS35) (F_6), selected from progeny of a cross between PI 350005 and Chris, was used for monosomic analysis with a set of 21 monosomic lines of Chris (Carlson 1982). The monosomic lines 2D and 5D of CS also were used to determine if these respective chromosomes carried crown rust resistance genes in CS. To further map the crown rust resistance gene in Chris, a mapping population consisting of 136 doubled haploid (DH) lines from a cross between PI 350005 and Chris was developed using the methods described by Chu et al. (2008). Also included in the inoculation experiments were CS, barley cvs., Bowman (PI 383237) and Aim (CIho 3737) used as susceptible checks, and Hor 2596 (CIho 1243) used as resistant check.

Monosomic analysis

The monosomic lines of Chris or CS were used as females in crosses with the selected spring type susceptible line SSTS35. Monosomy ($2n = 41$) of the monosomic lines was determined by mitotic chromosome counts using the Feulgen staining method (Zeller et al. 2002). Bulked F_1

seeds from the same cross were planted in 20-cm-diameter plastic pots to produce F_2 progeny in the greenhouse. The frequencies of susceptible and resistant F_2 individuals were calculated for each cross after crown rust inoculation at the seedling stage (see below). The Chi-squared test was performed to determine the goodness of fit to standard Mendelian ratios.

Crown rust inoculation and infection type scoring

A single uredinial spore-derived isolate (ND91-36) of *P. coronata* var. *hordei* was used in all inoculation experiments. This isolate was originally collected from barley in Nebraska in 1991 and possesses a high level of virulence on many barley genotypes (Jin and Steffenson 1999) as well as a few wheat accessions including PI 350005 (Jin and Steffenson 1993). For rust inoculations, the wheat and barley materials were planted in plastic cones (3.8-cm diameter and 21 cm depth) filled with a peat moss/perlite (3:1) potting mix and grown in a greenhouse with a daily average temperature range of 20–24 °C and a 14–16-h photoperiod. Plants were inoculated 1 week after planting when the primary leaves were fully expanded. Urediniospores, suspended in Soltrol® oil (3 mg of urediniospores per 0.5 ml of oil), were applied with an atomizer at a rate of ~10 μg of urediniospores per plant. Plants were then placed in a dew chamber maintained near saturation by intermittent misting from ultrasonic humidifiers (Jin and Steffenson 2002). The plants were incubated at 20–21 °C for 16 h in the dark, and then allowed to dry slowly for several hours before being placed in a greenhouse under the conditions described above.

Crown rust ITs were assessed 12 days post-inoculation using a 0–4 qualitative scale adapted from that described for the wheat stem rust pathosystem by Stakman et al. (1962), where 0 = no visible infection; 0; = necrotic or chlorotic infection sites (flecks) without sporulation; 1 = minute uredinia, often surrounded by necrosis; 2 = small uredinia surrounded by extensive chlorosis; 3 = medium-sized uredinia with or without chlorosis; and 4 = large uredinia with or without chlorosis. ITs of 0, 0;, 1, or 2 were considered as an indication of a resistant host response, whereas ITs of 3 and 4 were considered as an indication of a susceptible host response.

Marker generation, linkage map construction and QTL analysis

A total of 1,500 primer pairs for simple sequence repeat (SSR) markers, previously reported by Röder et al. (1998), Stephenson et al. (1998), Pestsova et al. (2000), Eujayl et al. (2002), Guyomarc'h et al. (2002), Sourdille et al. (2003), Somers et al. (2004), Yu et al. (2004), Song et al. (2005), Barkley et al. (2006), were used to detect polymorphism between Chris and PI 350005. The primer sets amplifying polymorphic bands between the two parents were used to genotype the DH population. PCR conditions were the same as described by Somers et al. (2004). PCR products were separated by capillary electrophoresis using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following the procedures of Chao et al. (2007).

The computer program MAPMAKER (V2.1) for Macintosh (Lander et al. 1987) was used for linkage analysis and map construction. Segregation of each marker or locus in the DH population was subjected to a Chi-squared test. Markers showing highly significant segregation distortion ($P < 0.001$) from the expected 1:1 ratio were excluded from map construction. Linkage groups were determined with a minimum independence logarithm of odds (LOD) threshold of 3.0 using the two point/group command. This yielded large groups, which were subsequently regrouped with a minimum LOD of 10. Map construction was performed using the Kosambi mapping function (Kosambi 1944). The FIRST ORDER and RIPPLE (LOD >3.0) commands were used to determine the most plausible order of the markers within the linkage groups. The TRY command was used to add more markers which did not RIPPLE at an LOD >3 to construct the final map. The genetic linkage map was drawn by MapChart software (Voorrips 2002).

For QTL analysis, composite interval mapping (CIM) was performed using the software Windows QTL Cartographer Version 2.5 (Wang et al. 2007; http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) to determine the genomic regions (marker intervals) associated with crown rust resistance. The software parameters were set as follows: a standard Model 6 with a window size of 10 cM around the test interval and a walk speed of 1 cM, 'forward and backward regression' method as cofactors. The empirical LOD thresholds for the trait were determined by 1,000 permutations at the $P < 0.05$ level (Churchill and Doerge 1994). A QTL was declared when the LOD score was greater than the threshold value in both experiments. Estimates of additive effect and coefficient of determination (R^2), explaining the percentage of phenotypic variance conferred by the putative QTL, were obtained from the CIM analysis.

Results

Infection types of wheat accessions and barley checks to *P. coronata* var. *hordei*

Rust inoculation experiments verified that PI 350005 was one of the most susceptible wheat accessions; it exhibited an IT ranging from 3 to 4 in two independent experiments

Table 1 Reactions of selected wheat and barley accessions to *Puccinia coronata* var. *hordei* at the seedling stage in the greenhouse

Genotype	Origin	Infection type (IT)[a]	
		Exp. 1	Exp. 2
Triticum aestivum			
Chinese Spring (CItr 14108)	China	00; small blotch	0; small blotch
Chris (CItr 13751)	Minnesota, USA	0; small blotch	0; small blotch
PI 350005	Serbia	3,3-	3,4
SSTS35[b]	Selection from Chris/PI 350005	3	3,4
Hordeum vulgare[c]			
Hor 2596 (CIho1243)	Ethiopia	0;1-	0;
Aim (CIho 3737)	Egypt	3,4	3,3-
Bowman (PI 383237)	North Dakota, USA	3,3-	3-,3

[a] A 0–4 qualitative scale was used for assessing ITs on barley and wheat. This scale was adapted from the one developed for the wheat stem rust pathosystem by Stakman et al. (1962), where 0 = no visible infection; 0; = necrotic or chlorotic infection sites (flecks) without sporulation; 1 = minute uredinia, often surrounded by necrosis; 2 = small uredinia surrounded by extensive chlorosis; 3 = medium-sized uredinia with or without chlorosis; and 4 = large uredinia with or without chlorosis

[b] A susceptible line selected from the F_6 progeny of the cross between Chris and PI 350005

[c] Hor 2596 was used as resistant check, and Aim and Bowman were used as susceptible checks

(Table 1). The spring type wheat line SSTS35, derived from a cross between PI 350005 and Chris, had the same level of susceptibility as PI 350005 to crown rust isolate ND91-36. The reactions of two resistant parents CS and Chris as well as the three barley accessions used as checks to the barley crown rust pathogen are given in Table 1.

Genetic analysis and chromosome locations of crown rust resistance loci

F_1 plants from both crosses (PI 350005/CS and PI 350005/Chris) were resistant (IT 0;) to isolate ND91-36, indicating dominant gene action. A close fit to a 3:1 ratio of resistant to susceptible plants was observed in the F_2 population derived from cross PI 350005/Chris (Table 2), indicating that a single gene controlled resistance in Chris. This gene was designated *Cr1*. In the F_2 population derived from cross PI 350005/CS, the segregation of resistant to susceptible plants fitted a 15:1 ratio (Table 2), indicating that two independent dominant genes conferred resistance in CS.

To determine the chromosome location of *Cr1* in Chris, a set of 21 monosomic lines derived from Chris were used as female parents in crosses with the susceptible spring type line SSTS35. A control cross between SSTS35 and Chris also was made for comparison. Segregation of resistant to susceptible plants in the F_2 population derived from cross Chris/SSTS35 fitted closely to a 3:1 ratio (Table 3), further confirming that a single dominant gene controlled crown rust resistance in Chris. Among the 21 monosomic crosses, 19 produced a segregation ratios of 3 resistant:1 susceptible in the F_2 generation at $P > 0.01$, whereas two (Chris mono-5D/SSTS35 and Chris mono-2D/SSTS35)

Table 2 Segregation of resistant and susceptible seedlings in F_2 populations from crosses between PI 350005 and Chinese Spring (CS) or Chris tested with the crown rust isolate ND91-36

Crosses	Number of F_2 plants			
	Resistant	Susceptible	χ^2	*P* value
CS/PI 350005	126	6	0.532 (for 15:1)	0.4657
Chris/PI 350005	84	24	0.444 (for 3:1)	0.5050
CS mono-2D/PI 350005	118	37	80.589 (for 15:1)	<0.0001**
CS mono-5D/PI 350005	99	4	1.026 (for 15:1)	0.3110

** Indicates a significance level at $P < 0.01$

deviated from this ratio at $P < 0.001$ (Table 3). In all three independent crosses involving monosomic 5D, the F_2 segregation ratios significantly deviated from a 3:1 ratio (resistant:susceptible) with susceptible plants markedly outnumbering resistant ones (35:65, 45:85 and 10:116 for resistant:susceptible plants, respectively), indicating that chromosome 5D was the location of the crown rust resistance gene *Cr1* in Chris. With respect to the cross involving Chris mono-2D and SSTS35, although the segregation did not fit a 3:1 ratio, the number of resistant plants was greater (1.6×) than the number of susceptible plants, thus suggesting that 2D was not the critical chromosome carrying a resistance gene.

To determine if the chromosome 5D and/or 2D carry crown rust resistance genes in CS, monosomics of 5D and 2D from CS were crossed with SSTS35 and the responses of derived F_2 plants were evaluated. In the cross of CS mono-5D/SSTS35, the segregation of resistant to

Table 3 Segregation of resistant and susceptible seedlings in F_2 populations from crosses between Chris monosomic lines and SSTS35 tested with the crown rust isolate ND91-36

Crosses	Number of F_2 plants			
	Resistant	Susceptible	χ^2 (3:1)	*P* value
Chris/SSTS35	75	25	0.000	1.0000
Chris mono-1A/SSTS35	73	26	0.084	0.7719
Chris mono-1B/SSTS35	81	16	3.742	0.0531*
Chris mono-1D/SSTS35	74	21	0.425	0.5145
Chris mono-2A/SSTS35	79	17	2.722	0.0990
Chris mono-2B/SSTS35	68	20	0.242	0.6228
Chris mono-2D/SSTS35	61	38	9.458	0.0021**
Chris mono-3A/SSTS35	69	29	1.102	0.2938
Chris mono-3B/SSTS35	37	12	0.007	0.9333
Chris mono-3D/SSTS35	72	25	0.031	0.8602
Chris mono-4A/SSTS35	73	21	0.355	0.5513
Chris mono-4B/SSTS35	69	24	0.032	0.8580
Chris mono-4D/SSTS35	68	23	0.004	0.9496
Chris mono-5A/SSTS35	74	24	0.014	0.9058
Chris mono-5B/SSTS35	79	16	3.372	0.0663
Chris mono-5D/SSTS35	35	65	85.333	<0.0001**
Chris mono-5D/SSTS35	45	85	113.077	<0.0001**
Chris mono-5D/SSTS35	10	116	302.233	<0.0001**
Chris mono-6A/SSTS35	37	12	0.007	0.9333
Chris mono-6B/SSTS35	72	24	0.000	1.0000
Chris mono-6D/SSTS35	75	24	0.030	0.8625
Chris mono-7A/SSTS35	44	6	4.507	0.0338*
Chris mono-7B/SSTS35	76	23	0.165	0.6846
Chris mono-7DL/SSTS35	44	6	4.507	0.0338*

* Indicates a significant level at $P < 0.05$

** Indicates a significance level at $P < 0.01$

susceptible F_2 plants did not deviate significantly from a 15:1 ratio (Table 2), indicating that chromosome 5D of CS does not carry the gene(s) for crown rust resistance. However, in the cross between CS mono-2D and SSTS35, the F_2 population segregated in a 3 resistant:1 susceptible instead of the expected 15:1 ratio, suggesting that chromosome 2D is one of the chromosomes carrying a gene conferring crown rust resistance. The two crown rust resistance genes in CS were provisionally named as *CrCs1* (located on chromosome 2D) and *CrCs2* (with chromosome location to be determined), respectively.

Molecular mapping of the crown rust resistance gene *Cr1* in Chris

To map *Cr1* on chromosome 5D in Chris, 1,500 SSR primer pairs were screened and 702 (49.4 %) amplified polymorphic loci between PI 350005 and Chris. From these polymorphic primer pairs, 583 were chosen to genotype the DH population and 697 SSR markers were generated. These SSRs comprised 140 GWM (Röder et al. 1998), 1 PSP (Stephenson et al. 1998), 15 GDM (Pestsova et al. 2000), 7 DuPw (Eujayl et al. 2002), 63 CFD (Guyomarc'h et al. 2002), 22 CFA (Sourdille et al. 2003), 169 WMC (Somers et al. 2004), 15 KSM, 1CNL (Yu et al. 2004), 149 BARC (Song et al. 2005), and 1 AC (Barkley et al. 2006). Among these 697 markers, 29 showed significant segregation distortion ($P < 0.001$) from the expected ratio of 1:1 in the DH population and were excluded from map construction. The remaining 668 SSR markers along with the crown rust resistance gene *Cr1* were used for linkage analysis (MAPMAKER 2.1), and 438 markers were assigned to 21 linkage groups (Fig. 1) while 230 markers did not RIPPLE at a LOD >3 or were ungrouped after using the TRY command and not further mapped. All 21 linkage groups were assigned to specific chromosomes according to the SSR-based consensus genetic map of Somers et al. (2004). The total length of the maps spanned 2,559.7 cM with an average marker density of 8.1 cM per marker (Table 4). Overall, the orders and distances of most SSR markers were consistent with those in the genetic maps developed by Somers et al. (2004).

The total map lengths for the A, B, and D genomes were 1,103.5, 1,379.4 and 1,076.8 cM, respectively. The average distances between two markers for the respective genomes were 8.7, 8.0 and 7.8 cM, respectively. Markers

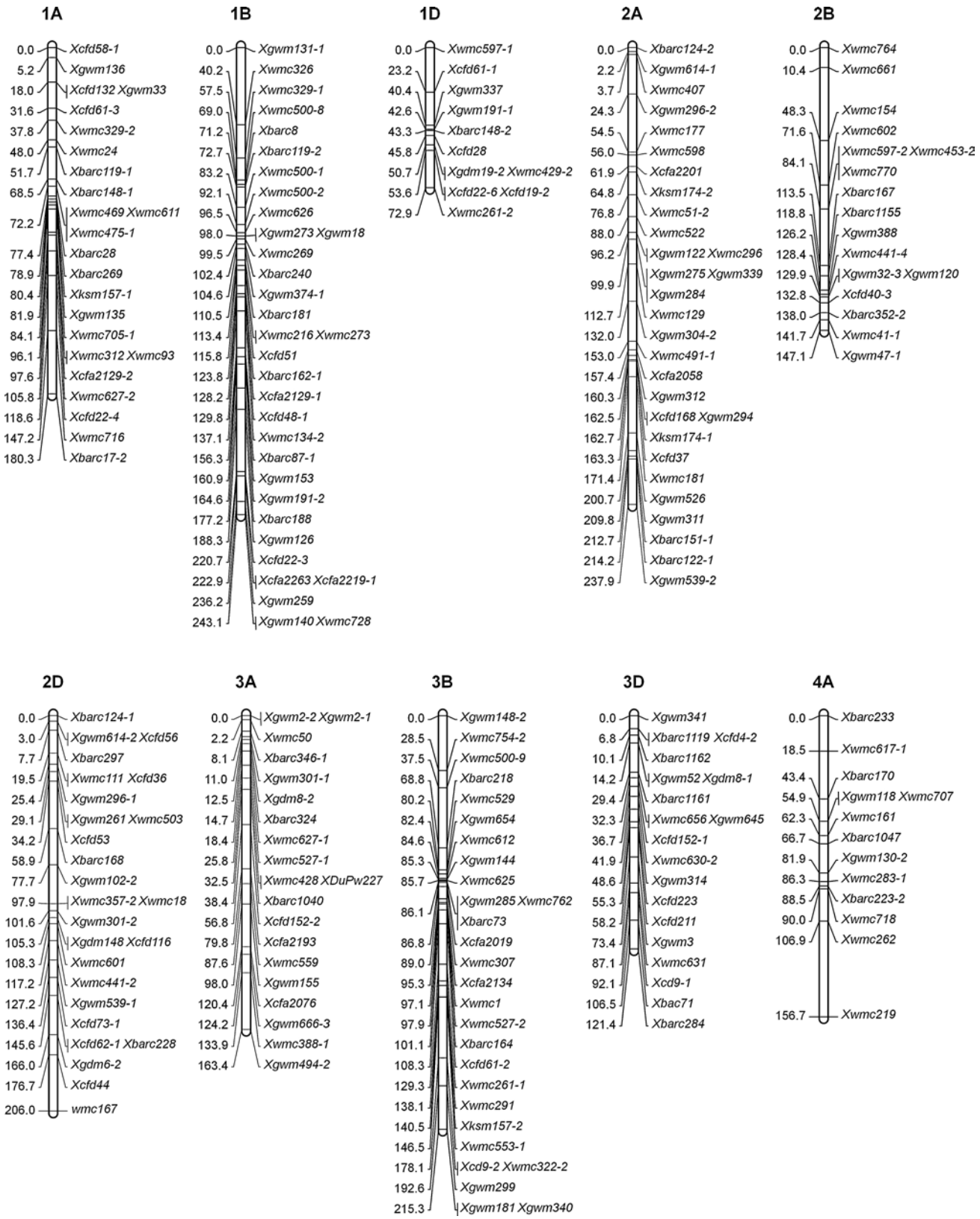

Fig. 1 Genetic linkage map developed from the P1350005/Chris doubled haploid population. The marker names are shown to the right of the linkage groups and the position (cM) of the markers is shown to the left. Linkage maps were constructed using the computer program MAPMAKER (V2.1) (Lander et al. 1987) with a minimum LOD threshold of 3.0 and the Kosambi mapping function (Kosambi 1944). The genetic linkage map was drawn by the MapChart (V2.2) software (Voorrips 2002)

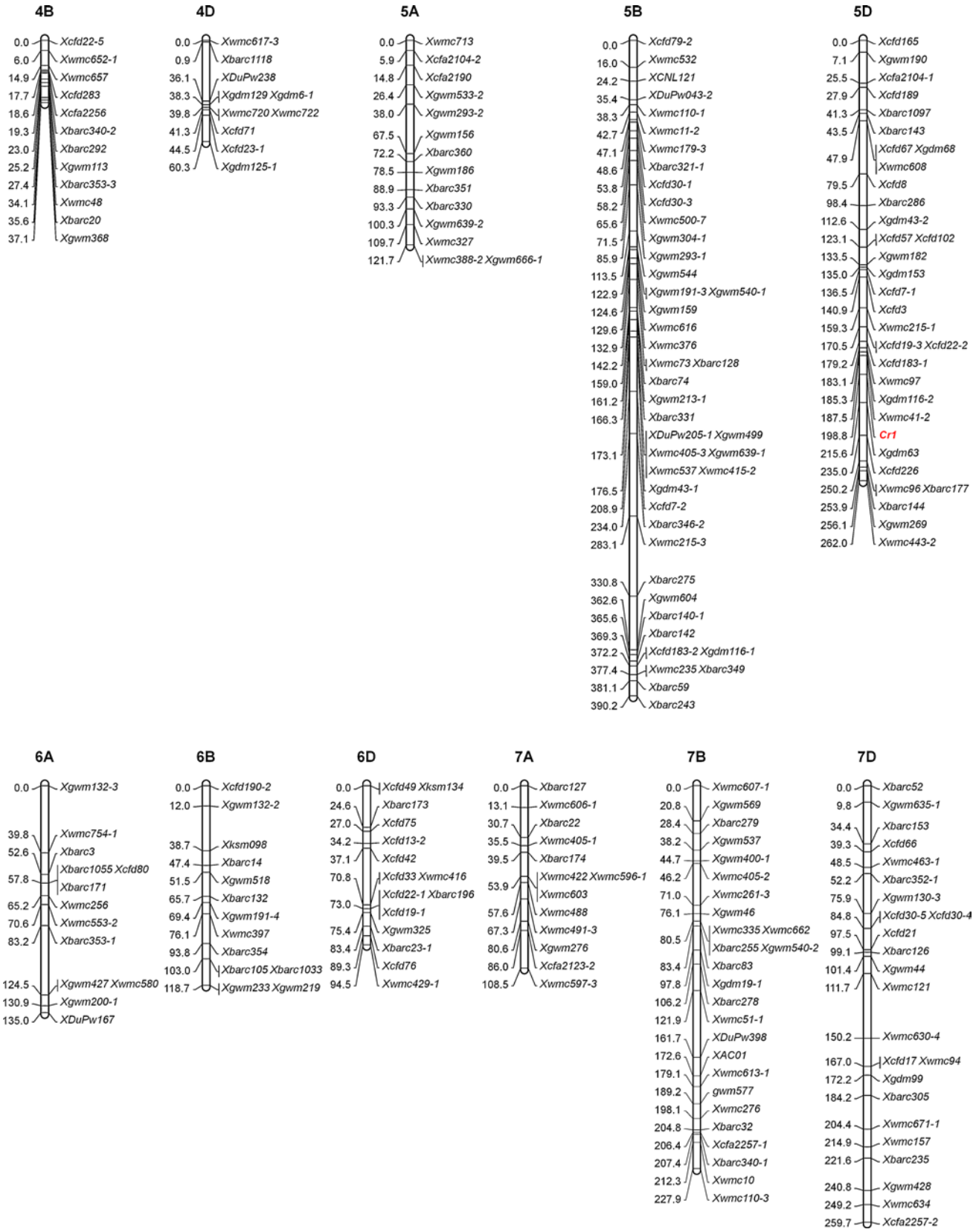

Fig. 1 continued

on the B genome were more dense than those on the A and D genomes. Map lengths for individual chromosomes also varied, ranging from 37.1 cM (4B) to 390.2 cM (5B). As expected, the crown rust resistance locus *Cr1* mapped to the long arm of chromosome 5D. The two flanking markers *Xwmc41-2* and *Xgdm63* were 11.3 cM proximal and 16.8 cM distal to the *Cr1* locus.

We also conducted a QTL analysis using the mean ITs observed on individual DH lines of the mapping population. This analysis identified a single QTL for crown rust resistance, which mapped to the *Xcfd-22-2–Xgdm63* interval on the long arm of chromosome 5D (Fig. 2). This QTL (designated as *Qcr1.ndsu-5D* for its coincident position at the Mendelian locus) explained 80 % of the phenotypic variation with Chris contributing the positive allele.

Table 4 Description of 21 genetic linkage maps developed using a wheat doubled haploid population derived from a cross between Chris and PI 350005

Chromosome	No. of markers	Map length (cM)	Marker density (cM/marker)
1A	24	180.3	7.5
1B	33	243.1	7.4
1D	11	72.9	6.6
2A	30	237.9	7.9
2B	17	147.1	8.7
2D	26	206.0	7.9
3A	20	163.4	8.2
3B	28	215.3	7.7
3D	19	121.4	6.4
4A	13	156.7	12.1
4B	12	37.1	3.1
4D	10	60.3	6.0
5A	14	121.7	8.7
5B	44	390.2	8.9
5D	33	262.0	7.9
6A	13	135.0	10.4
6B	13	118.7	9.1
6D	15	94.5	6.3
7A	13	108.5	8.3
7B	26	227.9	8.8
7D	24	259.7	10.8
Total	438	3,559.7	8.1
Total group A	127	1,103.5	8.7
Total group B	173	1,379.4	8.0
Total group D	138	1,076.8	7.8

Discussion

In most cases, non-host/near non-host resistance is difficult to study because the pathogen is unable to form a basic, compatible association with the non-host plant and the mechanisms underlying the trait can be complex (Lipka et al. 2008; Niks et al. 2011). However, the existence of a few susceptible genotypes allowed us to study the genetics of near non-host resistance of wheat to barley crown rust. Our genetic analysis using crosses between the susceptible wheat accession PI 350005 and the two resistant varieties Chris and CS showed that crown rust resistance in these accessions is conferred by one or two dominant genes. This indicates that near non-host resistance of wheat is simply inherited and may operate on a gene-for-gene basis with the pathogen as previously demonstrated in other pathosystems (Heath 1991, 2001; Tosa 1996). For example, Tosa (1996) identified four genes that conferred non-host resistance in wheat to the powdery mildew *forma specialis* of *Agropyron* (*Blumeria graminis* f. sp. *agropyri*), with each of the genes individually triggering resistance to the pathogen. Rye is considered a non-host of the wheat stripe rust fungus (*P. striiformis* f. sp. *tritici*). The stripe rust R-gene on rye chromosome 1 has been transferred to many wheat varieties and was designated *Yr9*, which was shown to operate in a gene-for-gene manner (Niks 1988). Wheat is considered a near non-host of the barley stripe rust fungus (*P. striiformis* f. sp. *hordei*) while barley is considered a near non-host of *P. striiformis* f. sp. *tritici* because the two related pathogens are generally unable to cross infect the inappropriate hosts (Chen et al. 1995). Interestingly, both Pahalawatta and Chen (2005) and Sui et al. (2010) demonstrated that the near non-host resistance of wheat and barley to barley stripe rust (*P. striiformis* f. sp. *hordei*) and wheat stripe rust, respectively, is conferred by a single dominant gene.

However, some non-host resistance exhibits more complex quantitative inheritance. For example, Rodrigues et al. (2004) crossed the partially susceptible wheat line Chinese 166 to the resistant variety Lemhi and identified two major QTLs for resistance to *P. striiformis* f. sp. *hordei*: one on chromosome 1D and the other on chromosome 2B. These two QTLs accounted for 43.5 and 33.2 % of the phenotypic variance for resistance to barley stripe rust, respectively. In addition, they also identified two minor effect QTLs: one on chromosome 5A and the other on chromosome 6A, contributing 5.1 and 10.9 % of the phenotypic variance, respectively. Using an experimental barley line susPtrit, which is susceptible to several heterologous rust fungi, Jafary et al. (2006) identified multiple QTLs for non-host resistance, which were distributed across 18 chromosomal regions. Some of these loci conferred resistance to multiple heterologous rust pathogens, while others only conferred resistance to a single rust disease. These reports indicate that genetic mechanisms for non-host or near non-host resistance vary in different pathosystems, with some

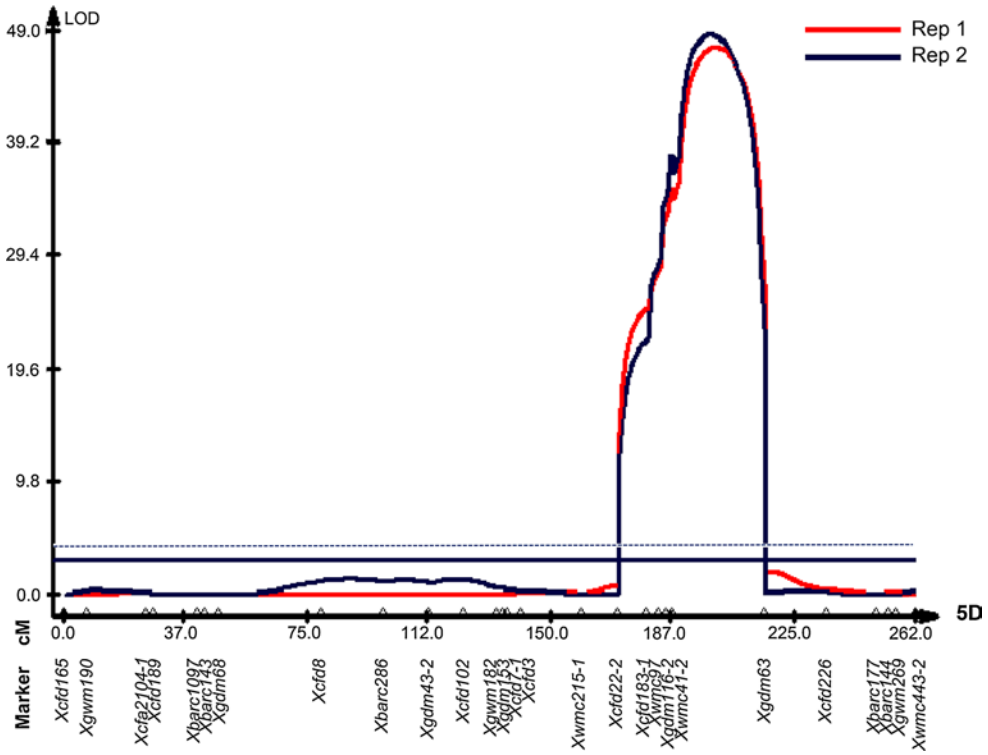

Fig. 2 The major effect QTL on chromosome 5D conferring resistance to barley crown rust in wheat variety 'Chris'. The computer software WinQTLCart V2.5 (Windows QTL Cartographer Version 2.5_009) was used for QTL analysis through composite interval mapping (CIM). The positions of marker loci are shown on the *horizontal axis*, and the logarithms of the odds (LOD) values are shown on the *vertical axis*. The *horizontal dotted line* indicates the LOD significant threshold of 4.25, which was obtained through 1,000 permutations and yielded an experiment-wise significance level of 0.05. The *horizontal solid line* indicates the LOD value of 3.0. Rep 1 and Rep 2 were two independent experiments, in which 3 to 4 individuals per line were rated for their reaction to the crown rust isolate ND91-36

fitting simple inheritance and others being genetically more complex.

Non-host resistance is considered as the most common form of resistance in plants and potentially more durable (Lipka et al. 2008; Niks et al. 2011). However, studies on the genetic basis of this resistance, including the present work, revealed that it can be conferred by a single major gene. Given that the barley crown rust pathogen is continuing to undergo sexual recombination on buckthorn in the USA and Canada (Carson 2011; Chong et al. 2011), it seems highly plausible that a variant could arise whose *avr* gene product(s) is not recognized by certain wheat accessions, resulting in a compatible rust infection. Thus, one may need to use caution in predicting the potential durability of such resistances.

Besides *Hordeum* species, some accessions of more than 70 gramineous species were susceptible to *P. coronata* var. *hordei* in greenhouse evaluations (Jin and Steffenson 1993). Susceptible species were mainly in the tribes Poaceae and Triticeae, including the genera of *Aegilops*, *Agropyron*, *Brachypodium*, *Bromus*, *Elytrigia*, *Festuca*, *Leymus*, *Lolium*, *Pascopyrum*, *Phalaris*, *Psathyrostachys*, and *Secale*. However, the frequencies of susceptible accessions varied with species. In *Triticum*, the vast majority of accessions evaluated were immune or highly resistant to *P. coronata* var. *hordei* and only a very small percentage (2 %) was susceptible (Jin and Steffenson 1993; Jin et al. 1993b). Interestingly, 11 % of the evaluated materials (549 accessions) derived from hybrids between wheat and *Agropyron*, *Elymus*, and *Secale* were susceptible. The frequency of susceptible accessions in the hybrids was, therefore, much higher than that observed in the *Triticum* species (Jin and Steffenson 1993; Jin et al. 1993b). These results suggest that crown rust susceptibility in hybrids could be due to alien chromosomes replacing wheat chromosomes carrying the crown rust resistance genes. PI 350005 is a wheat line of eastern European origin where many varieties are known to carry the 1B/1R translocation (Rabinovich 1998). Cytogenetic analyses revealed no evidence of any rye chromatin (Bernd Friebe, personal communication) in wheat accession PI 350005. Further investigations are needed to reveal the relationship between susceptibility and existence of the alien chromatin in these susceptible hybrids as well as susceptible wheat accessions.

'Chris' possesses several *Sr* genes for wheat stem rust resistance, including *Sr5, Sr7a, Sr8a, Sr9g* and *Sr12* (Singh and McIntosh 1987). Based on pedigree analysis, Chris may have *Sr5, Sr9g* and *Srl2* derived from 'Thatcher' (Sears et al. 1957; McIntosh 1983), *Sr8a* derived from 'Frontana' (Knott and Shen 1961) and *Sr7a* (=*Sr7*) derived from 'Kenya 58' (Knott and Anderson 1956). Although the chromosome locations of these genes have been determined by traditional monosomic analyses, fine mapping of these genes remains to be done using molecular markers. Recently, Chris was found to be moderately resistant to TTKSK (aka "isolate Ug99"), a race from Uganda that is virulent for most of the *Sr* genes deployed in common wheat varieties across the world (Jin

and Singh 2006). Seedling tests of the differential lines carrying individual *Sr* resistance genes indicated that race TTKSK is virulent for all the known *Sr* genes present in Chris. Thus, it appears that these known genes in Chris may not be the ones conferring resistance to TTKSK. The DH population and the molecular genetic linkage maps constructed here from cross PI 350005/Chris should be very useful in determining the precise positions of the wheat stem rust genes present in Chris and identifying DNA markers associated with the *Sr* genes, which can be used for marker-assisted selection in wheat breeding programs.

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References

Agrama HA, Dahleen L, Wentz M, Jin Y, Steffenson BJ (2004) Molecular mapping of the crown rust resistance gene *Rpc1* in barley. Phytopathology 94:858–861

Barkley NA, Roose ML, Krueger RR, Federici CT (2006) Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor Appl Genet 112:1519–1531

Carlson TB (1982) Meiotic stability and morphology of monosomic lines of *Triticum aestivum* CV Chris. M.S. thesis, North Dakota State University

Carson ML (2011) Virulence in oat crown rust (*Puccinia coronata* f. sp. *avenae*) in the United States from 2006 through 2009. Plant Dis 95:1528–1534

Chao S, Zhang W, Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Sci 47:1018–1030

Chen XM, Line RF, Leung H (1995) Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. Phytopathology 85:1335–1342

Chong J, Gruenke J, Dueck R, Mayert W, Fetch JM, Mccartney C (2011) Virulence of *Puccinia coronata* f. sp. *avenae* in the Eastern Prairie Region of Canada during 2007–2009. Can J Plant Pathol 33:77–87

Chu CG, Xu SS, Friesen TL, Faris JD (2008) Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. Mol Breeding 22:251–266

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 104:399–407

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops taushii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Heath MC (1981) A generalized concept of host-parasite specificity. Phytopathology 71:121–1123

Heath MC (1991) The role of gene-for-gene interactions in the determination of host species specificity. Phytopathology 81:127–130

Heath MC (2001) Non-host resistance to plant pathogens: nonspecific defense or the result of specific recognition events? Physiol Mol Plant Pathol 58:53–54

Jafary H, Szabo LJ, Niks RE (2006) Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with different and overlapping specificities. Mol Plant-Microbe Interact 19:1270–1279

Jin Y, Singh RP (2006) Resistance in US wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis 90:476–480

Jin Y, Steffenson BJ (1993) Natural occurrence of barley crown rust on forage grasses in North Dakota and its pathogenicity on gramineous species. Phytopathology 83:884 (Abstr.)

Jin Y, Steffenson BJ (1999) *Puccinia coronata* var. *hordei* var. nov.: morphology and pathogenicity. Mycologia 91:877–884

Jin Y, Steffenson BJ (2002) Sources and genetics of crown rust resistance in barley. Phytopathology 92:1064–1067

Jin Y, Steffenson BJ, Oberthur LE, Baenziger PS (1992) *Puccinia coronata* on barley. Plant Dis 76:1283

Jin Y, Steffenson BJ, Franckowiak JD (1993a) Resistance to crown rust in barley and its inheritance in CI 1243. Phytopathology 83:1336 (Abstr.)

Jin Y, Steffenson BJ, Wesenberg DM, Bockelman HE (1993b) Reaction of *Triticum* species and related genera to barley crown rust. Phytopathology 83:1387 (Abstr.)

Knott DR, Anderson RG (1956) The inheritance of rust resistance. 1. The inheritance of stern rust resistance in ten varieties of common wheat. Can J Agric Sci 36:174–195

Knott DR, Shen I (1961) The inheritance of rust resistance. 7. The inheritance of resistance to races 15B and 56 of stem rust in eleven common wheat varieties of diverse origin. Can J Plant Sci 41:587–601

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

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

Lipka U, Fuchs R, Lipka V (2008) Arabidopsis non-host resistance to powdery mildews. Curr Opin Plant Biol 11:404–411

Lutey RW, Covey RP (1959) Crown rust of barley in Minnesota. Plant Dis Rep 43:1287

McIntosh RA (1983) A catalogue of gene symbols for wheat. In: Proceedings of the 6th International Wheat Genetics Syrup, Kyoto, pp 1299–1309

Niks RE (1988) Non-host plant species as donors for resistance to pathogens with narrow host range. II. Concepts and evidence on the genetic basis of non-host resistance. Euphytica 37:89–99

Niks RE, Parlevliet JE, Lindhout P, Bai Y (2011) Breeding crops with resistance to diseases and pests. Wageningen Academic Publishers, Wageningen

Pahalawatta V, Chen XM (2005) Genetic analysis and molecular mapping of wheat genes conferring resistance to the wheat stripe rust and barley stripe rust pathogens. Phytopathology 95:427–432

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Rabinovich SV (1998) Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L. Euphytica 100:323–340

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rodrigues P, Garrood JM, Shen QH, Smith PH, Boyd LA (2004) The genetics of non-host disease resistance in wheat to barley yellow rust. Theor Appl Genet 109:425–432

Sears ER, Loegering WQ, Rodenhiser HA (1957) Identification of chromosomes carrying genes for stem rust resistance in four varieties of wheat. Agron J 49:208–212

Simons MD (1985) Crown rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts: vol. II. diseases, distribution, epidemiology, and control. Academic Press, Orlando, pp 131–172

Singh RP, McIntosh RA (1987) Genetics of resistance to *Puccinia graminis tritici* in 'Chris' and 'W3746' wheats. Theor Appl Genet 73:846–855

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot-Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. US Dep Agric Agric Res Serv E-617 (rev):1–53

Stephenson P, Bryan G, Kirby J, Collins A, Devos KM, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946–949

Sui X, He Z, Lu Y, Wang Z, Xia X (2010) Molecular mapping of a non-host resistance gene *YrpstY1* in barley (*Hordeum vulgare* L.) for resistance to wheat stripe rust. Hereditas 147:176–182

Szabo LJ (2006) Deciphering species complexes: *Puccinia andropogonis* and *Puccinia coronata*, examples of differing modes of speciation. Mycoscience 47:130–136

Tosa Y (1996) Gene-for-gene relationships in forma specialis-genus specificity of cereal powdery mildews. In: Mills D, Kunoh H, Keen NT, Mayama S (eds) Molecular aspects of pathogenicity and resistance: requirements for signal transduction. APS Press, St. Paul, pp 47–55

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Yu JK, Dake TM, Singh S, Benscher D, Li W, Gill B, Sorrells ME (2004) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. Genome 47:805–818

Zeller FJ, Kong L, Hartl L, Mohler V, Hsam SLK (2002) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 7. Gene *Pm29* in line Pova. Euphytica 123:187–194