Molecular Mapping of the *rps1.a* Recessive Gene for Resistance to Stripe Rust in BBA 2890 Barley

Guiping Yan and Xianming Chen

First author: Department of Plant Pathology, Washington State University, Pullman 99164-6430; and second author: United States Department of Agriculture–Agricultural Research Service, Wheat Genetics, Quality, Physiology, and Disease Research Unit and Department of Plant Pathology, Washington State University, Pullman.

Accepted for publication 8 January 2007.

ABSTRACT

Yan, G. P., and Chen, X. M. 2007. Molecular mapping of the *rps1.a* recessive gene for resistance to stripe rust in BBA 2890 barley. *Phytopathology* 97:668-673.

Stripe rust, caused by *Puccinia striiformis* f. sp. *hordei*, is one of the most important diseases of barley in the south-central and western United States. Growing resistant cultivars is the best approach for controlling the disease. The barley genotype BBA 2890 has all-stage resistance against all races of *P. striiformis* f. sp. *hordei* (PSH) identified thus far in the United States. The resistance in BBA 2890 is controlled by a single recessive gene, *rps1.a*. The objectives of this study were to identify resistance gene analog polymorphism (RGAP) markers for the all-stage resistance gene *rps1.a*, to map the gene on a barley chromosome using chromosome-specific simple sequence repeat (SSR) markers, and to determine the presence or absence of the flanking RGAP markers for the gene in 24 barley genotypes. Seedlings of the parents and 200 F₈ recombinant inbred lines (RILs) were tested for resistance to pathogen races PSH-14, PSH-48, and PSH-54 in the greenhouse in 2005. Genomic DNA was extracted

from the parents and 150 F₈ RILs. The RGAP technique was used to identify molecular markers for the *rps1.a* gene. Twelve primer pairs generating repeatable polymorphic bands were selected for genotyping the 150 F₈ RILs. A genetic linkage group was constructed for the resistance gene with 13 RGAP markers and four chromosome-specific SSR markers. The four SSR markers mapped the gene on the long arm of barley chromosome 3H. The closest RGAP marker for the resistant allele was within a genetic distance of 2.1 centimorgans (cM). The closest marker for the susceptible allele was 6.8 cM away from the locus. The two closest RGAP markers for the resistant allele detected polymorphisms in 67 and 71% of the 24 barley genotypes when used individually, and detected polymorphism in 88% of the genotypes when used in combination. This information should be useful in incorporating the resistance gene into barley cultivars and in pyramiding the gene with other resistance genes for superior stripe rust resistance.

Additional keywords: *Hordeum vulgare*, marker-assisted selection, yellow rust.

Stripe rust (also called yellow rust) of barley (*Hordeum vulgare* L. emend. Bowden), caused by the fungus *Puccinia striiformis* Westend. f. sp. *hordei* Eriks., is an important disease in many barley-producing regions of the world. The disease first was reported in the Western Hemisphere in 1975 near Bogota, Colombia and was thought to be introduced from Europe (16). Since then, the pathogen spread south to Argentina by 1982 (16) and moved north to Mexico by 1987 (4). Barley stripe rust first was reported in the United States in Texas in 1991 (32). Within 5 years, the disease spread northward and westward to the states of Oklahoma, New Mexico, Arizona, Colorado, Utah, California, Idaho, Montana, Oregon, and Washington (13). Barley stripe rust has caused severe yield losses in the western United States, especially in California and the Pacific Northwest (13).

Although chemical control can be effective, it adds extra cost to barley production. Using genetic resistance is the preferred approach for control of stripe rust. Stripe rust resistance primarily consists of all-stage (also called seedling) resistance and high-temperature, adult-plant (HTAP) resistance (7). All-stage resistance can be detected at the seedling stage, but is expressed throughout all growth stages. In contrast, HTAP resistance is expressed at adult-plant stages when the weather is warm (7,13,29).

All-stage resistance conferred by a single gene usually is race specific and, hence, not durable because new virulent races of the pathogen, which can develop rapidly from mutation, asexual recombination, and reassortment of nuclei in the dikaryotic rust fungus, circumvent the resistance (7,24). In contrast, HTAP resistance is non-race-specific and, therefore, durable (9,26), but it is more difficult to incorporate into commercial cultivars than all-stage resistance because it often is controlled by quantitative trait loci (QTLs). Approaches for prolonging the life of all-stage resistance include pyramiding a number of all-stage resistance genes in a single cultivar and combining effective all-stage resistance genes with HTAP resistance QTLs to obtain durable and high-level resistance. However, it is time consuming and difficult to develop resistant cultivars based on gene pyramiding with conventional breeding methods. Molecular markers, closely linked to the resistance genes, can be used to select for progeny with pyramided resistance genes. Marker-assisted selection should accelerate the process of developing barley cultivars with durable resistance (6).

A number of provisionally designated genes conferring resistance to stripe rust have been identified from barley germplasms (5,27,35). In Europe, Nover and Scholz (27) reported that resistance in germplasm BBA 2890, an awned, hulled, six-row spring barley originating from Germany, to European race 24 of *P. striiformis* f. sp. *hordei* was controlled by a single recessive gene, designated *yr*. Chen and Line (10,11) investigated 18 barley lines and identified at least 26 different genes for resistance to stripe rust. Of these lines, BBA 2890 has a recessive gene conferring high-level resistance to all races identified thus far in the United

Corresponding author: X. M. Chen; E-mail address: xianming@wsu.edu

doi:10.1094/PHYTO-97-6-0668

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2007.

States, and the gene that was the same as *yr* was designated *rps1.a* using the current system for naming disease resistance genes in barley (10,11). Although the *rps1.a* resistance may not be durable by itself, the gene should be useful when pyramided with other all-stage resistance genes and HTAP resistance QTLs to obtain durable resistance to *P. striiformis* f. sp. *hordei* in barley cultivars. Because there is no pathogen race virulent to the gene, molecular markers tightly linked to the resistance gene should be extremely useful in marker-assisted introgression and pyramiding.

The resistance gene analog polymorphism (RGAP) technique, developed by Chen et al. (14), has been demonstrated to be efficient in identification of closely linked molecular markers for disease resistance, and has been used successfully to identify molecular markers for resistance genes in wheat (*Triticum aestivum* L.) and barley against stripe rust, leaf rust (caused by *P. hordei* G. Otth), scald (caused by *Rhynchosporium secalis* (Oudem.) J. J. Davis), net blotch (caused by *Pyrenophora teres* Drechs.), barley yellow dwarf (caused by *Barley yellow dwarf virus*), and scab (caused by *Gibberella zeae* (Schwein.) Petch) (35–37). Simple sequence repeats (SSRs), also called microsatellites, have been developed in many crop species, because they are abundant and display high levels of polymorphism. It has been shown that SSRs are useful genetic markers and have been applied to the construction of linkage maps, determination of genetic variation, and marker-assisted selection. SSR-based molecular linkage maps of barley (25,30) have played an important role for developing molecular markers and mapping genes of interest. The SSR markers have been used to identify stripe rust resistance genes in wheat (3,34) and determine chromosome location of the *rpsGZ* gene in barley for resistance to stripe rust (36). Chen and associates (22,36,37) have demonstrated that using the combination of RGAP and chromosome-specific SSR markers is an efficient approach to identify tightly linked markers and map resistance genes on chromosomal regions.

The objectives of this study were to identify RGAP markers for the all-stage resistance gene *rps1.a*, to map the gene on a barley chromosome using chromosome-specific SSR markers, and to determine the presence or absence of the flanking RGAP markers for the gene in 24 barley genotypes.

MATERIALS AND METHODS

Plant materials and the mapping population. Crosses were made in the greenhouse between BBA 2890 and the adapted spring barley cv. Steptoe. Steptoe is susceptible to all *Puccinia striiformis* f. sp. *hordei* (PSH) races and was used as the female parent. A total of 200 F₈ recombinant inbred lines (RILs) were de-

veloped through single-seed descent in the previous study (10) and used in the genetic analysis to confirm the resistance gene.

In addition to BBA 2890 and Steptoe, 24 barley genotypes were used to check the polymorphisms at the marker loci flanking the *rps1.a* locus. These barley genotypes consist of commercial cultivars currently grown in the U.S. Pacific Northwest and resistant stocks or stocks used for differentiating races of *P. striiformis* f. sp. *hordei* (10,11,13).

Pathogen isolates. Races PSH-14, PSH-48, and PSH-54 of *P. striiformis* f. sp. *hordei*, which were virulent on Steptoe but avirulent on BBA 2890, were used in phenotyping the 200 RILs. Single-pustule isolates were obtained and their avirulence or virulence reaction was tested on 12 differential barley genotypes (13,15). Race PSH-14 was virulent on eight genotypes (Topper, Heils Franken, Emir, Astrix, Hipoly, Varunda, Abed Binder 12, and Trumpf), PSH-48 was virulent on one genotype (Topper), and PSH-54 was virulent on four genotypes (Topper, Abed Binder 12, Trumpf, and Bancroft). Urediniospores of the single-pustule isolates that were increased on selected susceptible differential genotypes were collected using a vacuum collector and then stored in a desiccator at 4°C before use. Fresh urediniospores or those that had been stored in the desiccator for <2 months were used to inoculate the plants of the parents and F₈ RILs.

Phenotyping the mapping population. The procedures and conditions for growing plants prior to and post inoculation, inoculation, and note-taking were the same as those described by Chen and Line (8). In each race test, 15 to 20 plants were used for each of the parents and the 200 F₈ RILs in greenhouse in 2005. For each line, infection type (IT) was recorded 20 days after inoculation based on a 0-to-9 scale described by Line et al. (23). The RILs were classified as resistant (IT 0 to 3), susceptible (IT 7 to 9), and segregating (combination of IT 0 to 3 and IT 7 to 9). The χ^2 test was used to analyze the segregating population for stripe rust reactions and determine the goodness of fit of the observed ratio to expected segregation ratio of the F₈ generation. A *P* value for a race test was calculated using the “Chitest” analysis in the Microsoft Office Excel Data Analysis package (Microsoft, Redmond, WA).

DNA extraction and bulk segregant analysis. For convenience of running polyacrylamide gels, we used the first 150 of the 200 F₈ RILs for marker analysis. DNAs were extracted from the two parents and these F₈ RILs following the protocol described by Riede and Anderson (31). The extracted DNA was dissolved in 1× Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and stored at –20°C. DNA was quantified using the minigel method and DNA concentration for each line was adjusted to 30 ng μ l^{–1} for polymerase chain reaction (PCR) amplification.

TABLE 1. Sequences, gene sources, and conservative domains of primers that produced resistance gene analog (RGA) polymorphism markers for the *rps1.a* locus for resistance to *Puccinia striiformis* f. sp. *hordei*

RGA primer	Sequence (5'→3')	Gene	Domain ^a	Reference
AS3	IAGIGCIAGIGGIAGICC	N, Rps2	P-loop	21
CLRR-INV1	GCAGCAACTTGTGC	<i>Cf9</i>	LRR	33
CLRR Rev	TAACGTCTATCGACTTCT	<i>Cf9</i>	LRR	14
Cre3LR-F	CACACACTCGTCAGTCTGCC	<i>Cre3</i>	LRR	This study
Cre3LR-R	CAGGAGCCAAAAATACGTAAG	<i>Cre3</i>	LRR	37
LM637	ARIGCTARIGGIARICC	<i>L6, N, Rps2</i>	P-loop	18
NLRR-Rev	TATAAAAAGTGCCGACT	N	LRR	14
Pto kin1	GCATTGGAACAAGGTGAA	<i>Pto</i>	Kinase	14
Pto kin2	AGGGGGACCACCACGTAG	<i>Pto</i>	Kinase	14
Pto kin3	TAGTTCGGACGTTTACAT	<i>Pto</i>	Kinase	36
Pto kin4	AGTGCTTTGTAGGGTATC	<i>Pto</i>	Kinase	33
Pto-kin1IN	AAGTGGAACAAGGTACG	<i>Pto</i>	Kinase	33
Pto-kin2IN	GATGCACCACCAGGGGG	<i>Pto</i>	Kinase	33
RLK-For	GAYGTNAARCCIGARAA	LrK10	Kinase	17
RLK-Rev	TCYGGYGCRATRTANCCNGGITTGICC	LrK10	Kinase	17
RLRR-Rev	ACACTGGTCCATGAGGTT	<i>Rps2</i>	LRR	14
Xa1LR-R	GAGATTGCCAAGCAATTGC	Xa1	LRR	33

^a LRR = leucine-rich repeats.

Bulk segregant analysis was used to rapidly identify molecular markers closely linked to the resistance gene. Each of the resistant and susceptible bulks was made from an equal amount of DNA from 15 resistant and 15 susceptible homozygous RILs, respectively. Genomic DNA samples from BBA 2890, Steptoe, and the resistant and susceptible bulks were used for screening primers. Polymorphic bands specific to BBA 2890 and the resistant bulk or to Steptoe and the susceptible bulk first were tested in a subpopulation of 20 RILs that were included in the bulks. Bands that were strong, repeatable, and highly associated with the phenotypic data of the 20 RILs were tested further in the remaining 130 RILs.

RGAP analysis. The primers (16,33,37) used in this study were designed based on conserved motifs of cloned plant resistance genes (Table 1) and synthesized by Invitrogen (Carlsbad, CA). The RGAP protocol described by Chen et al. (14) was used with modifications to the reaction volume, ingredient amounts, and number of PCR cycles. For each PCR reaction, a 15- μ l volume contained 60 ng of template DNA; 2.5 mM each dCTP, dGTP, dTTP, and dATP (Promega Corp., Madison, WI); 0.6 U of *Taq* DNA polymerase (Promega Corp.), 1.2 μ l of each primer (30 ng μ l⁻¹), 1.5 μ l of 10 $\times$ PCR buffer (Mg-free; Promega Corp.), and 5 mM MgCl₂ (Promega Corp.). PCR amplification was performed in the GeneAmp PCR system 9700 DNA thermal cycler (Applied Biosystems, Foster City, CA) programmed for 5 min at 94°C for initial denaturation; 40 cycles, each consisting of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C; followed by a final extension for 7 min at 72°C. Checking PCR amplification in agarose gel, separating fragments in denaturing polyacrylamide gel, and recording presence and absence of RGAP markers were performed following the procedures described by Chen et al. (14) and Yan and Chen (36). For convenience, the RGAP markers were temporarily designated the B series to represent markers developed for BBA 2890 barley.

SSR analysis. To determine chromosomal locations of the resistance gene and the RGAP markers, 83 SSR markers of known map locations covering the seven barley chromosomes (25,30) were screened with genomic DNA from the parents and the two bulks. The PCR reaction mixture was the same as for the RGAP technique described above, except that half the amount of MgCl₂ (2.5 mM) was used in SSR analysis. Annealing temperatures varied according to the primer pairs used. Information on primer sequence, repeat motif, marker size, and annealing temperature for these SSR markers is available online as described in Ramsay et al. (30), in Liu et al. (25), and in Becker and Heun (1). PCR products amplified with the SSR primers first were checked in a 1% agarose gel, and successfully amplified PCR products were separated in a 5% denaturing polyacrylamide (19:1 acrylamide/N, N'-methylene bisacrylamide) gel as described above for

the RGAP technique. The polyacrylamide gel was run at 1,300 V for 2.0 to 2.5 h because the sizes of the bands of interest usually ranged from 100 to 300 bp. The gel usually was dried at room temperature overnight. A picture of the gel was obtained by scanning the dried gel.

Linkage mapping. The segregation data of RGAP, SSR markers, and stripe rust phenotype obtained from the 150 F₈ RILs were used to estimate the genetic linkage between the markers and the resistance locus. Linkage analyses and map construction of the RGAP, SSR markers, and resistance locus were performed using the computer program Mapmaker, version 3.0 (20). A logarithm of the likelihood ratio score of ≥ 3.0 and Kosambi's mapping function that transformed recombination frequency to estimate the map distance in centimorgans (19) were used to establish linkage. Individual linkage groups, the most probable linkage order, and map distances of the markers within a linkage group were determined by the "group," "compare," and "map" commands, respectively.

RESULTS AND DISCUSSION

Stripe rust tests and inheritance of seedling resistance. BBA 2890 was resistant with IT 1, showing chlorotic flecks without sporulation to races PSH-14, PSH-48, and PSH-54 of *P. striiformis* f. sp. *hordei*, whereas Steptoe was susceptible with IT 8, showing abundant sporulation with chlorosis surrounding sporulating area. Of the 200 F₈ RILs tested with the three races, 87 were homozygous resistant (IT 1), 1 was segregated (IT 1 and IT 8), and 112 were homozygous susceptible (IT 8). The observed segregation ratio was not significantly different from the expected ratio of 49.22, 1.56, and 49.22% of homozygous resistance, heterozygous resistance, and homozygous susceptible, respectively, for monogenic inheritance in the F₈ generation ($\chi^2 = 3.2$, df = 2, $P = 0.2$). Within the heterozygous line, 16 plants segregated into 2:14 resistant:susceptible, which fit a ratio of 1:3 ($\chi^2 = 1.33$, df = 1, $P = 0.2$), suggesting that the stripe rust resistance in BBA 2890 was determined by a single recessive gene. These results from the F₈ RILs confirmed the previous report that BBA 2890 has a single recessive gene for resistance based on F₁, F₂, and F₃ of the same cross (10). Recessive genes controlling resistance to stripe rust have been reported in wheat (2) and also in other barley genotypes (10,27). Molecular markers for a recessive resistance gene are more useful than for a dominant gene because plants with the recessive gene in the heterozygous state that cannot be selected by resistant phenotype can be selected by molecular markers after each backcrossing (10).

Identification of RGAP and SSR markers and construction of a linkage map for the *rps1.a* locus. From a total of 346 ran-

TABLE 2. Resistance gene analog polymorphism (RGAP) markers, primer pairs, and sizes of markers used for mapping the *rps1.a* gene conferring resistance to *Puccinia striiformis* f. sp. *hordei* and their presence or absence in the resistant and susceptible parents and resistant and susceptible bulks

RGAP marker	Primer pair	<i>P</i> for a 1:1 ratio	Size (bp) ^b	Presence or absence ^a			
				SP	SB	RB	RP
B1	Cre3LR-R/ Pto kin2	0.06	480	—	—	+	+
B2	Cre3LR-R/ Pto kin2	0.25	450	—	—	+	+
B3	Pto kin2/ RLK-For	0.07	470	—	—	+	+
B4	Pto kin4/ CLRR Rev	0.41	275	+	+	—	—
B5	Pto kin1/AS3	0.87	250	+	+	—	—
B6	LM637/ Pto kin4	0.07	150	+	+	—	—
B7	AS3/ Cre3LR-R	0.10	240	+	+	—	—
B8	Pto-kin1IN/ NLRR-Rev	0.14	200	+	+	—	—
B9	Cre3LR-F/ RLRR Rev	0.10	500	+	+	—	—
B10	Pto-kin1IN/ CLRR-INV1	0.10	250	+	+	—	—
B11	Pto-kin2IN/ Pto kin3	0.74	120	+	+	—	—
B12	LM637/ Xa1LR-R	0.87	400	—	—	+	+
B13	Pto-kin2IN/ RLK-Rev	0.74	300	—	—	+	+

^a SP = susceptible parent, SB = susceptible bulk, RB = resistant bulk, RP = resistant parent, + = presence, and — = absence.

^b Sizes of all markers were estimated based on 1-kb plus DNA marker.

dom pairs of 48 RGA primers screened by bulk segregant analysis, 124 bands generated by 90 primer pairs showed polymorphisms between the resistant parent (RP) and the susceptible parent (SP), and 70 bands produced by 64 primer pairs showed polymorphisms between the resistant bulk (RB) and the susceptible bulk (SB). In all, 13 strong and repeatable markers produced by 12 primer pairs in the bulk segregant analysis were selected for genotyping the 150 RILs. The primer pairs, fragment sizes, and their presence or absence in SP, SB, RB, and RP for 13 dominant RGAP markers are shown in Table 2. Of the 13 RGAP markers, 8 were present in SP and SB but absent in RP and RB, and 5 were present in RP and RB but absent in SP and SB (Table 2).

The finding of the majority of the RGAP markers (8 of the 13 markers) associated with the susceptible allele may be due to the recessiveness of the *rps1.a* resistance gene. A similar phenomenon also was observed for the RGAP markers linked to the recessive *rpsGZ* gene for resistance to stripe rust (36). The possible reasons for the phenomenon were discussed in our previous publication (36). However, further cloning studies are needed to compare sequences and structures of recessive resistance and dominant susceptible alleles. The linkage group established in this study will serve as a starting point for cloning the *rps1.a* gene.

To determine the chromosomal location of the *rps1.a* gene, 83 SSR markers covering both long and short arms of the seven barley chromosomes (25,30) were scored in the RP, SP, RB, and SB. Of the 83 screened markers, 22 were polymorphic between the RP and SP. Of these 22 markers, 7 also were polymorphic between the RB and SB. After linkage analysis using the 150 F₈ RILs, only four of the seven SSR markers, Bmag0877, Bmag0013, EBmac0708, and EBmac0541, were linked to the resistance locus. The primer sequences, dominance or co-dominance, and fragment sizes of these SSR markers and their presence or absence in the parents are shown in Table 3.

A linkage map of the *rps1.a* locus was constructed using 13 RGAP and four SSR markers, which spanned a total of 171.8 centimorgans (cM) (Fig. 1). One flanking marker, B13, which was linked to the resistance allele, was at a distance of 2.1 cM from the resistance locus. Another flanking marker, B11, which was linked to the susceptible allele was at a distance of 6.8 cM. The marker B12 was linked to the resistance locus at a distance of 7.1 cM. The closest SSR marker, Bmag0877, had a genetic distance of 12.7 cM from the *rps1.a* locus. The marker Bmag0013 was linked to the gene with a genetic distance of 18.3 cM. Because the four SSR markers all were located on the long arm of chromosome 3 (3H) (30), the *rps1.a* gene was mapped to the same chromosomal location.

The *rps1.a* resistance gene was mapped to the long arm of barley chromosome 3H using SSR markers. This approach has been reported in previous studies to map genes to chromosomal regions. Using a linked SSR marker, HVM68, the dominant resistance gene against *P. striiformis* f. sp. *tritici* races, *RpstS1*, was

mapped in Steptoe barley chromosome 4H (28). Yan and Chen (36) confirmed the location of the *rpsGZ* resistance gene in Gran-nenlose Zweizeilige barley on the long arm of chromosome 4H using four linked SSR markers. In this study, the four SSR markers linked to the resistance gene had a sequential order similar to that presented in the linkage map of Ramsay et al. (30). Bmag0877 was in the same map location as Bmag0013 in Ramsay's map, whereas it had a genetic distance of 5.6 cM with Bmag0013 in our map. The genetic distances between Bmag0013 and EBmac0708

Fig. 1. Linkage map for the resistance gene *rps1.a* constructed with 13 resistance gene analog polymorphism (RGAP) markers (B1 to B13) and four simple sequence repeat (SSR) markers (Bmag0877, Bmag0013, EBmac0708, and EBmac0541). The RGAP markers linked to the susceptibility allele at the *rps1.a* locus are shown on the left, and the markers linked to the resistance allele are shown on the right. The linkage on the long arm of chromosome 3 (3H) was determined by the four chromosome-specific SSR markers; cM = centimorgans.

TABLE 3. Simple sequence repeat (SSR) markers, primer sequence, dominant or codominant nature, probability (*P*) value for a 1:1 segregation ratio in the 150 F₈ recombinant inbred lines, fragment sizes of the markers used to map the *rps1.a* locus on the long arm of chromosome 3H, and their presence or absence in BBA 2890 and Steptoe barley

SSR marker	Primer sequence (5'→3') ^a	Nature	<i>P</i> for a 1:1 ratio	Size (bp)	Presence (+) or absence (–)	
					BBA 2890	Steptoe
Bmag0877	F: AAAGCTCATGGTAGATCAAGA	Co-dominant	0.19	153	+	–
	R: TAGTTTTCCTCAAAAGCTTCTA			150	–	+
Bmag0013	F: AAGGGGAATCAAAATGGGAG	Co-dominant	0.61	155	+	–
	R: TCGAATAGGTCTCCGAAGAAA			150	–	+
EBmac0708	F: AATTTTGTGTTCCCATGC	Co-dominant	0.62	138	+	–
	R: AGCCCTATTGTACAGTTTT			132	–	+
EBmac0541	F: ACGGATCTACTTTAGCTAGCA	Dominant	0.32	106	–	+
	R: AAACAACCCACACAATC					

^a F = forward primer and R = reverse primer.

were similar (16.6 cM in our map and 15 cM in Ramsay's map), whereas the distance between EBmac0708 and EBmac0541 increased from 5 cM (30) to 10 cM. Similar differences were reported and discussed in our previous publication (36), and might be due to the use of different barley genotypes, chromosomal rearrangements, or deletions or insertions within the mapped region.

Genes for stripe rust resistance have been reported on chromosome 3H in other barley genotypes. Chen et al. (12) reported a QTL on chromosome 3H for stripe rust resistance in the barley doubled-haploid lines of Gobernadora/CMB 643. Toojinda et al. (35) mapped a QTL for adult-plant resistance against barley stripe rust to barley chromosome 3H in a doubled-haploid population derived from the Shyri/Galena cross. The *rps1.a* gene on chromosome 3H is likely to be different from the above resistance QTLs because *rps1.a* confers seedling resistance and is inherited as a qualitative trait, whereas the QTLs in the Shyri/Galena and Gobernadora/CMB 643 confer adult-plant resistance and exhibit quantitative inheritance (12,35). Further studies should be conducted to determine the physical relationships of these genes on chromosome 3H. Such information is important to determine the feasibility of pyramiding *rps1.a* with those genes on chromosome 3H.

Testing RGAP markers for polymorphism in barley genotypes. In addition to BBA 2890 and Steptoe, 24 barley genotypes were tested with RGAP markers B12 and B13 to determine their usefulness in marker-assisted selection. These genotypes do not have *rps1.a*, based on the previous studies (11). When tested with B12, 16 genotypes did not have the specific band (Table 4), indicating that this marker detected polymorphism in 67% of the tested genotypes. When tested with B13, 17 genotypes did not have the band (Table 4), detecting polymorphism in 71% of the tested genotypes. When these two markers were used in combination, they detected polymorphism in 88% of the tested genotypes. Only three genotypes, BBA 809, Stauffers Obersulzer, and Bigo,

did not show polymorphism with BBA 2890 when tested with both markers.

One of the factors determining the usefulness of a molecular marker in introgression of a resistance gene is the polymorphism of the marker in a broad range of genotypes. The amplification of genomic DNAs from the 24 barley genotypes was done to determine the polymorphism of the flanking markers, B12 and B13. The 24 barley genotypes that are either resistant or susceptible to stripe rust did not have *rps1.a* (10,11,13) and, therefore, we expected that the markers should be present in the majority of the tested genotypes. The 88% of the genotypes without at least one of the flanking markers indicated that the markers should be useful in introgressing the resistance gene into these genotypes. However, eight genotypes for B12 and seven genotypes for B13 did not show the expected polymorphism, which may be due to the fact that these markers are not tightly linked to the gene. Another possible explanation is that the bands in these genotypes have similar sizes but different sequence composition and, thus, they could be different DNA fragments. Cloning and sequencing of these DNA fragments and the marker bands in BBA 2890 are needed to determine the latter possibility. The markers should be tested before using them to incorporate the gene into other barley cultivars.

ACKNOWLEDGMENTS

This research was supported by the United States Department of Agriculture–Agricultural Research Service and the United States Barley Genome Project. PPNS No. 0439, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences Research Center, Project No. 11W-3061-5824, Washington State University, Pullman 99164-6430. We thank S. Ullrich, D. Johnson, and P. Okubara for their critical review of the manuscript.

LITERATURE CITED

- Becker, J., and Heun, M. 1995. Barley microsatellites: allele variation and mapping. *Plant Mol. Biol.* 27:835-845.
- Biffen, R. H. 1905. Mendel's law of inheritance and wheat breeding. *J. Agric. Sci.* 1:4-48.
- Börner, A., Röder, M. S., Unger, O., and Meinel, A. 2000. The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. *Theor. Appl. Genet.* 100:1095-1099.
- Calhoun, D. S., Arhana, S., and Vivar, V. H. 1988. Chemical control of barley stripe rust, a new disease for North America. *Barley Newsl.* 32:109-112.
- Castro, A. J., Capettini, F., Corey, A. E., Fillichkina, T., Hayes, P. M., Kleinhofs, A., Kudrna, D., Richardson, K., Sandoval-Islas, S., Rossi, C., and Vivar, H. 2003. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theor. Appl. Genet.* 107:922-930.
- Castro, A. Chen, X. M., Hayes, P. M., and Johnston, M. 2003. Pyramiding quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. *Crop Sci.* 43:651-659.
- Chen, X. M. 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. *Can. J. Plant Pathol.* 27:314-337.
- Chen, X. M., and Line, R. F. 1992. Inheritance of stripe rust resistance in wheat cultivars used to differentiate races of *Puccinia striiformis* in North America. *Phytopathology* 82:633-637.
- Chen, X. M., and Line, R. F. 1995. Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. *Phytopathology* 85:573-578.
- Chen, X. M., and Line, R. F. 1999. Recessive genes for resistance to *Puccinia striiformis* f. sp. *hordei* in Barley. *Phytopathology* 89:226-232.
- Chen, X. M., and Line, R. F. 2003. Identification of genes for resistance to *Puccinia striiformis* f. sp. *hordei* in 18 barley genotypes. *Euphytica* 129:127-145.
- Chen, X. M., Line, R. F., Hayes, P. M., Toojinda, T., Vivar, H., Kleinhofs, A., and Kudrna, D. 1999. Mapping barley genes for resistance to stripe rust, leaf rust, and scab using resistance gene analog polymorphism and restriction fragment length polymorphism. (Abstr.) *Phytopathology* 89:S15.

TABLE 4. Stripe rust reactions and presence (+) or absence (–) of resistance gene analog polymorphism (RGAP) markers B12 and B13 in 26 barley genotypes

Cultivars	Stripe rust response ^a		RGAP markers	
	IT	Severity (%)	B12	B13
Abed Binder 12	8	90	–	–
Abyssinian 14	2	5	–	–
Astrix	0	0	+	–
Bancroft	2	10	–	–
Baronesse	8	30	+	–
BBA 809	2	2	+	+
BBA 2890	2	2	+	+
Bigo	2	5	+	+
Cambrinus	8	10	–	–
Emir	8	5	–	–
Grannelose Zweizeilige	2	5	–	–
Harrington	8	60	–	–
Heils Franken	8	60	–	+
Hiproly	8	70	+	–
I 5	2	5	–	+
Mazurka	2	5	–	–
Nakai Zumizairai	8	60	–	–
PI 548708	0	0	–	–
PI 548747	2	5	–	–
Russell	8	100	+	–
Stauffers Obersulzer	0	0	+	+
Steptoe	8	100	–	–
Topper	8	100	+	–
Trumpf	8	20	–	–
Varunda	8	50	–	+
Zephyr	8	5	–	+

^a Stripe rust infection type (IT) based on a 0-to-9 scale (23) and severity data were from spring barley nurseries tested in Mount Vernon, WA in 2006 under natural infection.

13. Chen, X. M., Line, R. F., and Leung, H. 1995. Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. *Phytopathology* 85:1335-1342.
14. Chen, X. M., Line, R. F., and Leung, H. 1998. Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis. *Theor. Appl. Genet.* 97:345-355.
15. Chen, X. M., and Penman, L. 2005. Races of *Puccinia striiformis* f. sp. *hordei*, the pathogen of barley stripe rust in the United States in 2004. *Barley Genet. Newsl.* 35:23-26.
16. Dubin, H. J., and Stubbs, R. W. 1986. Epidemic spread of barley stripe rust in South America. *Plant Dis.* 70: 141-144.
17. Feuillet, C., Schachermayr, G., and Keller, B. 1997. Molecular cloning of a new receptor-like-kinase gene encoded at the *Lr10* disease resistance locus of wheat. *Plant. J.* 11:45-52.
18. Kanazin, V., Marek, L. E., and Shoemaker, R. C. 1996. Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. USA* 93:11746-11750.
19. Kosambi, D. D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.
20. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, J. M., Lincoln, S. E., and Newberg, L. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
21. Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plant. *Nat. Genet.* 14:421-429.
22. Lin, F., and Chen, X. M. 2006. Genetics and molecular mapping of genes for race-specific all stage resistance and non-race-specific high-temperature, adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. *Theor. Appl. Genet.* Online publication no. <http://dx.doi.org/10.1007/s00122-007-0518-0>.
23. Line, R. F., Konzak, C. F., and Allan, R. E. 1974. Evaluating resistance to *Puccinia striiformis* in wheat. Pages 125-132 in: *Induced Mutations for Disease Resistance in Crop Plants*. Proc. Res. Coordination Meeting. 1973. Intl. Atomic Energy Agency, Vienna
24. Line, R. F., and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. *U. S. Dep. Agric. Tech. Bull.* No. 1788.
25. Liu, Z. W., Biyashev, R. M., and Saghai Maroof, M. A. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theor. Appl. Genet.* 93:869-876.
26. Milus, E. A., and Line, R. F. 1986. Number of genes controlling high-temperature, adult-plant resistance to stripe rust in wheat. *Phytopathology* 76:93-96.
27. Nover, I., and Scholz, F. 1969. Genetic studies on the resistance of barley to yellow rust (*Puccinia striiformis* West.). *Theor. Appl. Genet.* 39:150-155.
28. Pahalawatta, V., and Chen, X. M. 2005. Inheritance and molecular mapping of barley genes conferring resistance to wheat stripe rust. *Phytopathology* 95:884-889.
29. Qayoum, A., and Line, R. F. 1985. High temperature, adult-plant resistance to stripe rust of wheat. *Phytopathology* 75:1121-1125.
30. Ramsay, L., Macaulay, M., Ivanissevich, S. D., MacLean, K., Cardle, L., Fuller, J., Edwards, K. J., Tuveson, S., Morgante, M., Massari, A., Maestri, E., Marmiroli, N., Sjakste, T., Ganai, M., Powell, W., and Waugh, R. 2000. A simple sequence repeat-based linkage map of barley. *Genetics* 156:1997-2005.
31. Riede, C. R., and Anderson, J. A. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Sci.* 36:905-909.
32. Roelfs, A. P., Huerta-Espino, J., and Marshall, D. 1992. Barley stripe rust in Texas. *Plant Dis.* 76:538.
33. Shi, Z. X., Chen, X. M., Line, R. F., Leung, H., and Wellings, C. R. 2001. Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. *Genome* 44:509-516.
34. Suenaga, K., Singh, R. P., Huerta-Espino, J., and William, H. M. 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology* 93:881-890.
35. Toojinda, T., Broers, L. H., Chen, X. M., Hayes, P. M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R. F., Powell, W., Ramsay, L., Vivar, H. E., and Waugh, R. 2000. Mapping quantitative and qualitative disease genes in a double haploid population of barley (*Hordeum vulgare*). *Theor. Appl. Genet.* 101:580-589.
36. Yan, G. P., and Chen, X. M. 2006. Molecular mapping of a recessive barley gene for resistance to stripe rust. *Theor. Appl. Genet.* 113:529-537.
37. Yan, G. P., Chen, X. M., Line, R. F., and Wellings, C. R. 2003. Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. *Theor. Appl. Genet.* 106:636-643.