Identification of a Quantitative Trait Locus for High-Temperature Adult-Plant Resistance Against *Puccinia striiformis* f. sp. *hordei* in 'Bancroft' Barley

Guiping Yan and Xianming Chen

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

Accepted for publication 8 September 2007.

ABSTRACT

Yan, G. P., and Chen, X. M. 2008. Identification of a quantitative trait locus for high-temperature adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* in 'Bancroft' barley. *Phytopathology* 98:120-127.

Sustainable control of plant diseases can be achieved by developing cultivars with durable resistance. 'Bancroft' barley has durable high-temperature, adult-plant (HTAP) resistance to stripe rust caused by *Puccinia striiformis* f. sp. *hordei*. The objectives of this study were to determine the inheritance of the HTAP resistance in Bancroft, develop molecular markers for the HTAP resistance using the resistance gene analog polymorphism (RGAP) technique, map the HTAP resistance quantitative trait locus or loci (QTL) on barley chromosomes, and determine the usefulness of the RGAP markers in other barley cultivars for marker-assisted selection. The parents and F₄ recombinant inbred lines (RIL) and the parents and F₅ RIL were evaluated in 2004 and 2005 in one and three field sites, respectively, in Washington State. Infection type (IT) and disease severity (DS) were recorded three times at each location during each growing season. Area under the disease progress curve (AUDPC) was calculated for each parent and RIL based on the DS

data. Genetic analyses of IT data of the parents, F₁, and F₂ tested in the adult-plant stage under controlled high-temperature cycle in the greenhouse and the parents, F₄, and F₅ RIL in the field indicated that one dominant gene controlled the HTAP resistance in Bancroft. Using 119 F_{5,6} RIL and IT data, a linkage map on chromosome arm 3HL was constructed with eight RGAP markers and three simple sequence repeat (SSR) markers. Using the QTL analysis, a QTL for HTAP resistance was mapped with the DS and AUDPC data on the same chromosome location as with the IT data. The QTL explained >70% of the total phenotypic variation for the DS and AUDPC. The heritability of the HTAP resistance based on the AUDPC data was 76%. The two markers most close to the QTL peak detected polymorphisms in 84 and 88% of 25 barley genotypes that do not have the Bancroft HTAP resistance when used individually, and detected polymorphism in 100% of the genotypes when used in combination, indicating that the markers could be used in incorporating the HTAP resistance into these barley genotypes to improve the level and durability of resistance to stripe rust.

Additional keywords: *Hordeum vulgare*.

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend. f. sp. *hordei* Erikss., is an important disease in the barley (*Hordeum vulgare* L. emend. Bowden) production region in the western United States (2,6,15). Growing resistant cultivars is the best approach to control barley stripe rust. After the first report of barley stripe rust in the United States in 1991 in Texas, numerous barley cultivars and germplasms were identified with different levels of resistance (3,11,13,37). New cultivars with resistance to stripe rust recently were released in the Pacific Northwest of the United States (12,41). However, most malting barley cultivars that currently are grown in the United States are susceptible to barley stripe rust (2).

'Bancroft' was released by Wesenberg et al. (42) in 2000 as one of the first barley cultivars developed in the United States with resistance to stripe rust. Soon after release, Bancroft was found to be infected by stripe rust and, therefore, was added to the set of barley differential cultivars in 2001 to differentiate races of *P. striiformis* f. sp. *hordei*. New races that were virulent on seedlings of Bancroft were identified on the samples collected from Texas, California, Oregon, and Washington (17,18). However, Bancroft

has remained resistant in the fields even when predominant races in the fields were virulent on seedlings of the cultivar. Chen and Moore (18) characterized the Bancroft resistance in both seedling and adult-plant stages at high and low diurnal temperature cycles and identified Bancroft to have high-temperature adult-plant (HTAP) resistance.

HTAP resistance in wheat has been demonstrated to be durable and successfully used to control wheat stripe rust caused by *P. striiformis* f. sp. *tritici* in the Pacific Northwest and other regions of the United States (7,25). HTAP resistance is most effective at the adult-plant stage at high temperatures (9,10,34). In the field, expression of HTAP resistance commences at stem elongation and increases as plants grow older and temperatures become higher (9,10,31). Sporulation of the fungus is inhibited at high temperatures but can resume at low temperatures. Generally, HTAP resistance is more evident in the flag leaves than in the lower leaves. HTAP resistance protects the crop from stripe rust damage by reducing the infection type, the number of new infections, and the amount of inoculum (7). Compared with race-specific all-stage resistance (also called seedling resistance), HTAP resistance is considered to be non-race specific, durable, and controlled by quantitative trait loci (QTL) (7). However, HTAP resistance is more difficult to incorporate into commercial cultivars than all-stage resistance because of its quantitative inheritance under the influence of the environmental conditions. Because HTAP resistance expresses in the adult-plant stage, it is time consuming to evaluate. Molecular markers can be useful in overcoming the dif-

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

doi:10.1094/PHYTO-98-1-0120

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, 2008.

difficulties and in accelerating the introgression of the HTAP resistance into commercial cultivars. Markers tightly linked to the resistance genes or QTL for HTAP resistance in Bancroft can be essential for combining the durable resistance with all-stage resistance genes to develop new barley cultivars with high-level and durable resistance to control stripe rust.

The objectives of this study were to determine the inheritance of the HTAP resistance in Bancroft, develop molecular markers for the HTAP resistance using the resistance gene analog polymorphism (RGAP) technique (16,38), map the HTAP resistance QTL on barley chromosomes, and determine the usefulness of the RGAP markers in other barley cultivars for marker-assisted selection.

MATERIALS AND METHODS

Plant materials. A cross was made in the greenhouse between susceptible barley ‘Harrington’ as the female parent and the resistant male parent Bancroft. Harrington is a two-rowed spring malting barley and serves as the industry standard in North America (46). Bancroft is a two-rowed spring feed barley and was selected from the cross Hector/60Ab1810-53 (42). In all, 197 F₂ seed originating from F₁ seed planted in the greenhouse were advanced to the F₄ generation in the greenhouse and then to the F₅ generation in the field by single-seed descent. F₁ seed from each crossed head and seed from each plant of the F₂ to F₅ progeny were kept separately. The F_{5,6} seed were bulk harvested from each F₅ line in the field. For convenience of running polyacrylamide gels, the first 119 F_{5,6} recombinant inbred lines (RIL) were used for the molecular mapping study.

Greenhouse tests for stripe rust resistance. Harrington and Bancroft were tested at the two-leaf stage in the greenhouse using a single race of *P. striiformis* f. sp. *hordei* in each test at the low diurnal temperature cycle changing from 4°C at 2:00 a.m. to 20°C at 2:00 p.m. at a rate of 1.3°C/h. Three races, PSH-54, PSH-56, and PSH-69, were selected for the tests based on their virulence on seedlings of Bancroft determined in previous studies (6,19). The adult plants at the heading stage were inoculated with fresh spores of the same races in the seedling test and were moved to the growth chamber at the high diurnal temperature cycle changing from 10°C at 2:00 a.m. to 35°C at 2:00 p.m. at a rate of 2.1°C/h following the procedure described by Chen and Line (10) for detecting HTAP resistance.

To determine the inheritance of HTAP resistance, eight F₁ and 150 F₂ plants of Harrington/Bancroft, as well as three plants each of the parents, were tested with race PSH-54 under the high diurnal temperature cycle at the adult-plant stage in the green-

house. Under the same conditions, 150 F₂ and 3 plants each of the parents also were tested in the greenhouse with race PSH-69.

Field tests for stripe rust resistance. Field tests were conducted in 2004 and 2005 (Table 1). In 2004, the parents and F₄ RIL were planted on 6 April at the Observatory (OB) Hill Farm near Pullman, WA, and inoculated at the stem-elongation stage (Zadoks growth stages 30 to 32). Urediniospores of races PSH-54 and PSH-56 of *P. striiformis* f. sp. *hordei* that were virulent on seedlings of Bancroft and both seedling and adult-plant stages of Harrington were mixed together with talc (Sigma-Aldrich, St. Louis) at a 1:50 ratio prior to inoculation. Spore production and storage were done following the procedures described by Chen et al. (15). The field inoculation was conducted using the procedure described by Chen and Line for testing wheat for resistance to stripe rust (9). Because the F₄ RIL grown in the field was mainly for advancing the mapping population to the F₅ and to obtain preliminary results on segregation of HTAP resistance, replications were not used in 2004. In 2005, the parents and F₅ RIL were planted at three field sites: OB Hill on 8 April and Tukey Farm on 10 April, both near Pullman in eastern Washington, and Mount Vernon in western Washington on 23 April. The plots near Pullman were inoculated with different races as listed in Table 1. To ensure adequate infection, inoculation was conducted four times for each field site starting at the five-leaf seedling stage (Zadoks growth stages 15 to 17), usually after light rain, in both 2004 and 2005. In addition, plants of the highly susceptible ‘Steptoe,’ which had been inoculated in the greenhouse and were producing spores, were transplanted in the fields with approximately five to seven plants/hill-plot and 2 m between hill-plots at the seedling stage to ensure the successful and uniform infection of PSH. The fields near Pullman were sprinkler irrigated for an hour each time and twice every week from 5 June to 15 July to increase infection of the stripe rust pathogen. Each plot in 2005 was planted using a randomized complete block design with three replications. For the two locations in Pullman, infection type (IT) based on a 0-to-9 scale (26) and disease severity (DS) (percentage of leaf area infected) based on the modified Cobb’s scale (33) were recorded three times at the dates and growth stages shown in Table 1. At Mount Vernon, IT and DS were recorded only once due to the interference of leaf rust (*P. hordei* G. H. Otth) that rapidly developed in the plots during the late growing season. The IT data were analyzed both qualitatively and quantitatively. When they were analyzed qualitatively, IT 0 to 3, 4 to 6, and 7 to 9 were considered to be resistant, intermediate, and susceptible reactions, respectively. The DS data of the three times at different stages of plant growth were used to calculate area under the disease pro-

TABLE 1. Year and progeny, field sites, number of replications, races of *Puccinia striiformis* f. sp. *hordei* used for inoculation, and dates and plant growth stages when stripe rust reaction was recorded in experiments for phenotyping recombinant inbred lines of the Harrington/Bancroft population to identify and map the gene or quantitative trait locus for high-temperature adult-plant resistance in Bancroft barley

Year, progeny, site	Replications	Pathogen race ^a	Reaction recorded	
			Date	Growth stage ^b
2004, F ₄ OB Hill	...	PSH-54 and PSH-56	30 June 8 July 15 July	Heading (51–55) Milk (71–73) Soft dough (81–85)
2005, F ₅ Tukey Farm	3	PSH-54	8 July 16 July 23 July	Milk (71–73) Soft dough (81–85) Dough (87–89)
OB Hill	3	PSH-69	6 July 13 July 19 July	Milk (71–73) Soft dough (81–85) Dough (87–89)
Mount Vernon	3	PSH-54, PSH-56, and PSH-69	29 June	Heading (51–55)

^a Races PSH-54, PSH-56, and PSH-69 of *P. striiformis* f. sp. *hordei* were virulent on seedlings of Bancroft under low temperatures and, therefore, detected high-temperature, adult-plant resistance expressed on Bancroft and recombinant inbred lines from the Harrington/Bancroft population at the adult plant stages in the field tests.

^b Growth stages in Zadoks codes are shown in parentheses.

gress curve (AUDPC) for each plant in 2004 and for each RIL in 2005 using the formula $AUDPC = \sum [(d_i + d_{i+1})/2](t_{i+1} - t_i)$, where d_i = the DS of the i th note, d_{i+1} = the DS of the $i + 1$ th note, and $(t_{i+1} - t_i)$ = the number of days from the i th note to the $i + 1$ th note (9,10). Relative AUDPC (%) was obtained for each RIL by comparison with the AUDPC data of the susceptible parent Harrington treated as 100%.

DNA extraction and bulk segregant analysis. For each RIL, approximately 3 g of fresh seedling leaves from 15 or more plants grown in a greenhouse were ground in liquid nitrogen. DNA was extracted from the fine leaf powder following the protocol described by Riede and Anderson (36), dissolved in 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and stored at -20°C . DNA concentrations were quantified using the mini-gel method (28) and spectrophotometer (Smartspec 3000; Bio-Rad, Hercules, CA), and DNA concentration for each RIL and parent was adjusted to the same amount (30 ng/μl). DNA quality was assessed by electrophoresis in 1% (wt/vol) agarose gels.

To quickly identify molecular markers potentially associated with the HTAP resistance, bulk segregant analysis (29) was used. Contrasting bulks were made from equal amounts of DNA from 10 resistant and 10 susceptible $F_{5,6}$ homozygous RIL. Genomic DNA samples from Bancroft, Harrington, and the resistant bulk (RB) and susceptible bulk (SB) were used for screening primers. All of the primer combinations first were screened to find the polymorphic bands specific to Bancroft and the RB or Harrington and the SB. The selected primer pairs that were strong and repeatable then were screened on a subset of 20 individual $F_{5,6}$ lines to select markers for mapping in the 119 $F_{5,6}$ RIL.

Identification of RGAP markers. The resistance gene analog (RGA) primers (16,32,45) used in this study were designed based on conserved motifs of many cloned plant resistance genes (Table 2) and synthesized by Invitrogen (Carlsbad, CA). The RGAP protocol described by Chen et al. (16) with modifications to the reaction volume and ingredient amount (43) was followed. After amplification, polymerase chain reaction (PCR) amplifications were checked on agarose gel and PCR fragments were separated on denaturing polyacrylamide gel, stained with silver nitrate, and scored following the procedures as previously described (16,43). For convenience, the RGAP markers were temporarily designated as an H series to represent markers developed for the HTAP resistance in Bancroft barley.

Identification of simple sequence repeat markers. To determine the chromosomal locations of the HTAP resistance gene and the RGAP markers, 83 simple sequence repeat (SSR) markers of known map locations covering all of the seven barley chromosomes were screened for the polymorphism with genomic DNA from the parents and the two bulks (27,35). The PCR reactions were the same as for the RGAP analysis described above, except that half the amount of MgCl_2 (2.5 mM) was used in the SSR

analysis. Information on marker name, sequences of forward and reverse primers, repeat motif, PCR conditions, and marker size are available in Becker and Heun (1), Liu et al. (27), and Ramsay et al. (35). The PCR amplification conditions for both primer pairs (Bmag0606 and EBmag0705) consisted of 1 cycle of 94°C for 1 min of denaturing, 55°C for 1 min of annealing, and 72°C for a 1-min extension, followed by 30 cycles of 1 min at 94°C , 1 min at 55°C , 1 min at 72°C , and a final extension step of 5 min at 72°C . The PCR condition for the Bmag0013 primer pair consisted of 1 cycle of 3 min at 94°C , 1 min at 58°C , and 1 min at 72°C , followed by 30 cycles of 30 s at 94°C , 30 s at 58°C , 30 s at 72°C , and a final extension step of 5 min at 72°C . The procedures and conditions after PCR amplification were the same as those described for the RGAP assay, except that the polyacrylamide gel was run at 1,500 V for approximate 2.0 h to separate products ranging from 100 to 300 bp.

Map construction, QTL and statistical analyses, and heritability estimation. The genotypic data of RGAP and SSR markers of the 119 $F_{5,6}$ RIL, as well as the phenotypic disease data obtained from their F_5 RIL, were used to estimate the genetic linkage between the markers and the HTAP resistance QTL. The computer program Mapmaker, version 3.0 (23) was used to obtain the map order of the linked markers at a logarithm of the likelihood (LOD) ratio score of ≥ 3.0 . Kosambi's mapping function that transformed recombination frequency to the map distance (centimorgans [cM]) (21) was used to establish the linkage. Mapmaker/QTL (22) was used for single marker analysis and interval mapping. Localization of the QTL that determines the HTAP resistance to barley stripe rust was revealed by the QTL scans. The Mapmaker/QTL program default LOD score of 3.0 was used as the conservative threshold for initially declaring the presence of a QTL.

Analyses of variance among three locations for IT, DS, and AUDPC in 2005 were performed using the general linear model (PROC GLM) in the SAS program (version 9.1; SAS Institute, Cary, NC). The χ^2 test that was used to analyze the F_2 , F_4 , and F_5 RIL for the stripe rust resistance was conducted using the "Chitest" analysis in the Microsoft Excel program of data analyses.

The heritability of the HTAP resistance was calculated based on the total variance of the AUDPC data of the F_5 RIL across the two experimental sites near Pullman using the equation $H = [VF_5 - (VP_1 + VP_2)/2]/VF_5$, where H denotes the heritability, VF_5 denotes the total variance of the F_5 RIL, and VP_1 denotes the variance of the resistant parent (RP) (Bancroft), and VP_2 denotes the variance of the susceptible parent (SP) (Harrington).

RESULTS

Inheritance of HTAP resistance in Bancroft. In the greenhouse tests, Harrington showed a susceptible reaction with IT 8

TABLE 2. Sequences, gene sources, conservative domains, and references of the primers used to identify resistance gene analog polymorphism markers linked to the gene or quantitative trait locus for high-temperature adult-plant resistance to *Puccinia striiformis* f. sp. *hordei* in Bancroft barley

RGA primer ^a	Sequence (5'→3')	Gene	Domain ^b	Reference
AS1-INV	CCTAACGGTGATCGCAAC	<i>N, Rps2</i>	LRR	24
Cre3LR-F	CACACACTCGTCAGTCTGCC	<i>Cre3</i>	LRR	44
Cre3LR-R	CAGGAGCCAAAAATACGTAAG	<i>Cre3</i>	LRR	45
NLRR-Rev	TATAAAAAGTGCCGGACT	<i>N</i>	LRR	16
Pto-kin1IN	AAGTGGAAACAAGGTTACG	<i>Pto</i>	Kinase	38
Pto-kin2IN	GATGCACCACAGGGGG	<i>Pto</i>	Kinase	38
RLRR-Rev	ACACTGGTCCATGAGGTT	<i>Rps2</i>	LRR	16
S2-INV	CAICAIAAIGGITGIGGIGG	<i>N, Rps2</i>	P-loop	32
Xa1LR-F	CTCACTCTCTGAGAAAATTAC	<i>Xa1</i>	LRR	45
Xa1LR-R	GAGATTGCCAAGCAATTGC	<i>Xa1</i>	LRR	38
Xa1NBS-R	CTCTGTATACGAGTTGTC	<i>Xa1</i>	NBS	38
XLRR-For	CCGTGGACAGGAAGGAG	<i>Xa21</i>	LRR	16

^a RGA = resistance gene analog.

^b LRR = leucine-rich repeat and NBS = nucleotide-binding site.

in all tests with races PST-54, PST-56, and PST-69 of *P. striiformis* f. sp. *hordei* in the seedling and adult-plant stages under the low- and high-temperature conditions. Bancroft showed a susceptible reaction (IT 8) to the three races in seedling tests at the low-temperature cycle, but exhibited resistant reactions (IT 2 to 3) in the adult-plant tests under the high-temperature conditions, indicating that Bancroft has HTAP resistance to stripe rust.

When adult plants of the parents, F_1 , and F_2 were tested with PST-54 and PST-69 under the controlled greenhouse conditions with the high diurnal temperature cycle, all Bancroft plants had IT 2 or 3 and all Harrington plants had IT 8. When tested with race PST-54, all eight F_1 plants had IT 2 or 3, similar to those of Bancroft. Of 150 F_2 plants, 106 had IT 2 or 3 and 44 had IT 8. The segregation fit a 3:1 resistant (IT 2 and 3):susceptible (IT 8) ratio ($P = 0.47$) (Table 3). When tested with PST-69, 109 F_2 plants were resistant and 41 F_2 plants were susceptible. The segregation also fit a 3:1 ratio for resistant and susceptible plants ($P = 0.51$).

Disease responses of the parents and RIL in the field tests.

The three pathogen races that were virulent on the seedlings but avirulent on the adult plants of Bancroft in the greenhouse were used either individually or in combinations of two or three for inoculating the field plots. In all of these tests, the adult plants of Bancroft were resistant with IT 2 or IT 3. The adult plants of Harrington were susceptible with IT 8. The F_4 and F_5 RIL were classified into homozygous resistant (IT 2 or 3), segregating (combination of ITs 2, 3, and 8), and homozygous susceptible (IT 8). The three replications of each line in the F_5 did not differ in their classification. Of the 197 F_4 and F_5 RIL tested in 2004 and 2005, respectively, the number of homozygous resistant, segregating, and homozygous susceptible fit into the expected ratio of one gene model shown in Table 3, indicating a single gene for the HTAP resistance measured by IT in the Harrington/Bancroft population.

The mean relative AUDPC data for the F_4 lines in 2004 in the field of OB Hill, Pullman, WA, showed a continuous variation. In 2005, the relative AUDPC data showed a similar pattern for each replication in the two locations at Pullman, and still were distributed in a continuous manner with low to high AUDPC (Fig. 1A and B). The results indicated that the expression of the HTAP resistance in Bancroft as measured by DS or AUDPC demonstrated to be quantitatively inherited and under the influence of the environmental conditions in the fields. However, the bi-modal-like distribution suggested that the resistance was controlled by a single gene or a QTL with major effects.

Variance analyses among different locations. Based on the probability (P) value obtained by the general linear model (GLM) procedure in the SAS system, there was no significant difference

for the IT data among the three locations Tukey Farm, OB Hill, and Mount Vernon ($P = 0.52$). However, a significant difference ($P = 0.03$) was found for the DS data among the three locations. Pairwise tests between Tukey Farm and OB Hill near Pullman showed no significant differences for all the variables, IT, DS, and AUDPC (data not shown). There were significant differences for DS between the Mount Vernon and Tukey Farm sites, and between the Mount Vernon and OB Hill sites. The absence of significant differences in IT among three locations shows that IT is a reliable indicator of HTAP resistance and that the HTAP resistance in Bancroft may be non-race specific. The heritability was estimated to be 76% using the AUDPC data of the two experimental field sites near Pullman.

Construction of a linkage map for the HTAP resistance.

From a total of 120 RGA primer pairs used to screen for polymorphism in the bulk segregant analysis, 45 (38%) pairs produced polymorphic bands that differentiated the SP and SB from the RP and RB. In all, 19 RGA primer pairs were used for genotyping the 119 $F_{5,6}$ RIL. Eight primer pairs that produced eight strong and repeatable polymorphic bands were used for constructing a linkage map for the HTAP resistance after removing four ungrouped, four nonspecific, and three poorly amplified RGA primer pairs. The fragment sizes and primer pairs of the eight RGAP markers are listed in Table 4. The banding pattern of the marker H3 tested with a set of $F_{5,6}$ RIL is shown in Figure 2.

Fig. 1. Frequency distribution of relative area under the disease progress curve (AUDPC) for 197 F_5 recombinant inbred lines from the Harrington/Bancroft barley population evaluated for high-temperature adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* at the A, Tukey and B, Observatory Hill farms in Washington in 2005.

TABLE 3. Observed and expected ratio of resistant, segregating, and susceptible for the infection types among F_2 plants and F_4 and F_5 recombinant inbred lines from the Harrington/Bancroft barley population inoculated with races PST-54, PST-56, and PST-69 of *Puccinia striiformis* f. sp. *hordei* in a greenhouse under controlled conditions or at three field sites in Washington during the 2004 and 2005 growing seasons^a

Year, progeny, location	Pathogen races	Observed ratio (R:H:S)	Expected ratio (R:H:S)	P^b
2004, F_2				
Greenhouse ^c	PST-54	106:44	3:1	0.47
Greenhouse ^c	PST-69	109:41	3:1	0.51
2004, F_4				
OB Hill Farm, near Pullman, WA	PST-54 and PST-56	83:36:78	3:2:3	0.09
2005, F_5				
Tukey Farm, near Pullman, WA	PST-54	86:17:94	7:2:7	0.19
OB Hill Farm, near Pullman, WA	PST-69	92:17:88	7:2:7	0.22
Mount Vernon, WA	PST-54, PST-56, and PST-69	84:18:95	7:2:7	0.23

^a Ratios: R = homozygous resistant, H = heterozygous, and S = homozygous susceptible. The three classes were for the F_4 and F_5 field tests, whereas only R and S classes were used for the F_2 greenhouse tests. The classification of infection types was identical for the three replications of each F_5 recombinant inbred line.

^b P = probability of χ^2 test for goodness-of-fit of the observed ratio to the expected ratio.

^c In the greenhouse tests of the F_2 population, the plants were inoculated at the heading stage, placed in a dew chamber at 10°C for 24 h in the dark, and then grown in a greenhouse chamber under the high-temperature cycle, gradually changing from 10 to 30°C with a daily 16-h photoperiod.

Three SSR markers, Bmag0013, Bmag0606, and EBmag0705, associated with the long arm of chromosome 3H were categorized into the linkage group with the eight RGAP markers. The primer pairs and fragment sizes of the three SSR markers are shown in Table 4. Bmag0013 (amplified with the forward primer 5'-AAGGGGAATCAAAATGGGAG-3' and the reverse primer 5'-TCGAATAGGTCTCCGAAGAAA-3') and Bmag0606 (amplified with the forward primer 5'-CTATTTGTAATGTATGTATGTCCC-3' and the reverse primer 5'-TCATTGGTCCAGATAATACAA-3') were dominant markers and EBmag0705 (amplified with the forward primer 5'-TCCTGTAGTCCTCTTTGTTTC-3' and the reverse primer 5'-TATATTACACATGGAGAGAGAGG-3') was a co-dominant marker. The banding pattern of marker Bmag0013 associated with the resistant allele was shown in Figure 3. The three SSR markers mapped the resistance locus and the RGAP markers on the long arm of barley chromosome 3H based on the known SSR linkage map of barley (32).

The RGAP marker H2 was tightly linked to the resistance allele in repulsion and they did not show any recombination in the genetic linkage map (Fig. 4) constructed with the 119 F_{5,6} lines using the IT data obtained for 2 years and three locations. The RGAP marker H3 was closely linked to the resistance allele in coupling with a genetic distance of 0.4 cM. The closest SSR marker Bmag0013 for the resistance allele was within 1.3 cM.

Detection of HTAP resistance QTL. Scanning the linkage map with the eight RGAP and three SSR markers using Mapmaker/QTL for the DS and AUDPC data from the Tukey Farm revealed one QTL associated with the HTAP resistance in Ban-

croft (Fig. 4). Based on the LOD value, this QTL was located at the chromosome region between H2 to H3 with only a 0.4-cM interval. The highest LOD values for DS and AUDPC were 58 and 33, respectively, and they greatly exceeded the conservative LOD threshold 3, which strongly supported this QTL and its position. This QTL explained 76% of the total variance in DS and 71% of the total variance in AUDPC, indicating that it had a large effect on the HTAP resistance.

The data from the OB Hill and Mount Vernon supported the finding from the Tukey Farm. One QTL also was detected at the same region of the linkage map (Fig. 4). The QTL in OB Hill and Mount Vernon explained 70 and 79% of the total variance in DS with LOD scores of 31 and 41, respectively. The QTL in OB Hill explained 78% of the total variance in AUDPC, with a LOD score of 39.

Determination of polymorphism of tightly linked marker loci in other barley genotypes. The presence and absence of RGAP marker H3 and SSR marker Bmag0013 in 25 barley genotypes are shown in Table 5. Of the 25 genotypes, only Baroness is known to have intermediate HTAP resistance; however, it differs from the HTAP resistance in Bancroft with respect to high IT and DS (X. Chen, *unpublished data*). When tested with H3, 22 of 25 genotypes did not have the specific band (Table 5), indicating that this marker detected polymorphism in 88% of the genotypes. When tested with Bmag0013, 21 genotypes did not have the band, detecting polymorphism in 84% of the tested genotypes. When these two markers were used in combination, they detected polymorphism for at least one of the two markers in 100% of the tested genotypes.

Fig. 2. Silver-stained denaturing polyacrylamide gel showing the resistance gene analog polymorphism marker H3 (520 bp, arrows) that was associated with high-temperature adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* in the analysis of F₅ recombined inbred lines (RILs) derived from the Harrington/Bancroft barley population. P₁ = the susceptible parent, Harrington; P₂ = the resistant parent, Bancroft; PT = disease phenotypic data; GT = marker genotypic data; S = susceptible, R = resistant; + = presence, and - = absence.

Fig. 3. Silver-stained polyacrylamide gels showing the single-sequence repeat marker Bmag0013 (155 bp, arrows) that was associated with high-temperature adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* in **A**, the bulk segregant analysis and **B**, F₅ recombinant inbred lines derived from the Harrington/Bancroft barley population. PT = disease phenotypic data, GT = marker genotypic data, RP = resistant parent (Bancroft), RB = resistant bulk, SB = susceptible bulk, SP = susceptible parent (Harrington), M = 1-kb-plus DNA ladder, R = resistant, S = susceptible, H = heterozygous, + = presence, and - = absence of the polymorphic band.

TABLE 4. Resistance gene analog polymorphism and simple-sequence repeat markers linked to the high-temperature adult-plant resistance gene or quantitative trait locus against *Puccinia striiformis* f. sp. *hordei* and their size, probability value for segregating as a single locus, and presence or absence in Harrington and Bancroft barley

Marker ^a	Primer pair	Size (bp) ^b	P ^c	Present (+) or absent (-)	
				Bancroft	Harrington
H1	Xa1LR-F/Xa1LR-R	254	0.18	+	-
H2	S2-INV/Cre3LR-R	480	0.36	-	+
H3	S2-INV/NLRR-Rev	520	0.21	+	-
H4	Cre3LR-F/RLRR-Rev	535	0.12	+	-
H5	AS1-INV/Pto-kin2IN	200	0.14	+	-
H6	Pto-kin1IN/Xa1NBS-R	158	0.08	-	+
H7	Pto-kin2IN/XLRR-For	550	0.10	+	-
H8	Pto-kin2IN/Cre3LR-F	528	0.11	+	-
Bmag0013	Bmag0013-For/-Rev	155	0.14	+	-
Bmag0606	Bmag0606-For/-Rev	140	0.09	-	+
EBmag0705	EBmag0705-For/-Rev	155	0.12	+	-
EBmag0705	EBmag0705-For/-Rev	145	0.12	-	+

^a H1 to H8 are resistance gene analog polymorphism markers. Bmag0013, Bmag0606, and EBmag0705 are single-sequence repeat markers.

^b Sizes of all markers were estimated based on comparison with the 1-kb-plus DNA ladder bands.

^c P = probability of χ^2 test for goodness-of-fit of the observed ratio to the expected ratio of a single locus.

DISCUSSION

Both all-stage and HTAP resistance to stripe rust has been identified through previous studies in the barley cv. Bancroft (6, 17,18). The present study confirmed HTAP resistance in Bancroft.

HTAP resistance is non-race specific and more useful than all-stage resistance because the latter in Bancroft is no longer effective against the *P. striiformis* f. sp. *hordei* population present in the United States (6,7,25). The durability of HTAP resistance in wheat cultivars has been demonstrated in the Pacific Northwest

Fig. 4. Interval analysis of a quantitative trait locus for high-temperature, adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* mapped on the long arm of barley chromosome 3H using disease severity (DS) (dotted lines) and area under the disease progress curve (AUDPC) (solid lines) data of the F_5 recombinant inbred lines of the Harrington/Bancroft barley population from three field sites in the State of Washington in 2005. **A**, Tukey Farm for DS (H2–H3, $R^2 = 76\%$, logarithm of the likelihood ratio [LOD] = 58) and AUDPC (H2–H3, $R^2 = 71\%$, LOD = 33). **B**, Observatory Hill Farm for DS (H2–H3, $R^2 = 70\%$, LOD = 31) and AUDPC (H2–H3, $R^2 = 78\%$, LOD = 39). **C**, Mount Vernon for DS (H2–H3, $R^2 = 79\%$, LOD = 41). The location of the resistance (R) gene mapped qualitatively using the infection type data is indicated.

TABLE 5. Infection type (IT) and severity produced by *Puccinia striiformis* f. sp. *hordei* in seedling stage under controlled greenhouse conditions and in a field in 2004 and presence (+) or absence (–) of the resistance gene analog polymorphism marker H3 and the simple sequence repeat marker Bmag0013 in 25 barley genotypes without the high-temperature, adult-plant resistance of Bancroft to stripe rust to show polymorphism of the marker loci flanking the Bancroft resistance gene in various barley genotypes

		Reaction to stripe rust ^b						
		Greenhouse			Field		Markers	
No.	Cultivars ^a	PSH-54	PSH-56	PSH-69	IT	Percent	H3	Bmag0013
1	Topper	9	9	9	8	100	–	–
2	Heils Franken	2	2	2	2	2	–	+
3	Emir	2	2	2	0	0	–	–
4	Astrix	2	2	2	0	0	–	+
5	Hiproly	2	8	8	8	20	–	–
6	Varunda	2	2	8	8	10	–	–
7	Abed Binder 12	8	8	8	8	60	–	–
8	Trumpf	8	8	8	8	40	–	–
9	GZ	0	0	0	0	0	–	–
10	Steptoe	8	8	8	8	80	–	–
11	Cambrinus	2	2	2	0	0	–	–
12	BBA 809	2	2	8	8	10	+	–
13	Zephyr	8	8	8	8	60	–	–
14	Nakai Zumizairai	8	8	8	ND	ND	+	–
15	SO	2	2	8	8	20	–	–
16	Baronesse	8	8	8	5	20	–	+
17	Mazurka	2	2	8	8	30	–	–
18	Bigo	0	0	8	3	5	+	–
19	I 5	0	0	8	8	10	–	–
20	PI 548708	0	0	0	0	0	–	–
21	PI 548734	0	0	0	0	0	–	–
22	PI 548747	0	0	0	0	0	–	–
23	Abyssinian 14	0	0	0	0	0	–	–
24	Russell	9	9	9	8	100	–	+
25	BBA 2890	0	0	0	0	0	–	–

^a GZ = Grannelose Zweizeilige and SO = Stauffers Obersulzer.

^b Greenhouse = IT on seedlings in greenhouse test. The IT data were based on a 0-to-9 scale (26). The field data were recorded at the flowering stage and extracted from a barley nursery evaluated at Mount Vernon, WA in 2004. ND = no data.

and other regions of the United States (7,25). HTAP resistance in Bancroft and other barley cultivars also has shown long-lasting effectiveness.

This study demonstrated that HTAP resistance in Bancroft barley can be identified by both IT and DS. This is in agreement with previous reports of HTAP resistance in wheat cultivars (6,9,31,34). As measurements of HTAP resistance, IT and DS generally are highly correlated to each other, as shown in this study and previous studies with wheat genotypes (9,10,24). However, IT data were more consistent across different environments than DS data in this study because, at adult-plant stages and high-temperature conditions that allow HTAP resistance express, IT is not influenced by the inoculum pressure and disease cycles as DS is. This explains the absence of significant differences in the IT data but the presence of significant differences in DS data between locations in this study.

The environmental conditions at the three experimental locations did not affect the placement of the QTL mapping position, but did affect the magnitudes of LOD values and QTL effects. These results are similar to a previous study for mapping quantitative barley stripe rust resistance genes in the doubled haploid population of Shyri/Galena (40). The difference in QTL effect between the two locations near Pullman might be due to the effects of soil conditions and microenvironment, which might influence plant growth and rust development. Because HTAP resistance is non-race specific, infections from naturally occurring races should not change much DS value. In fact, natural infections in the Pullman region were very limited in both 2004 and 2005. The difference in QTL effects between Mount Vernon and the two locations near Pullman might be due to the effects of temperature, humidity, and rust pressure.

Unlike all-stage resistance that usually expresses as high-level to complete resistance and is qualitatively inherited (6), HTAP resistance in Bancroft barley is incomplete or partial. Therefore, we used both qualitative and quantitative approaches to identify and map the gene or QTL for HTAP resistance in Bancroft. Both these approaches identified a single gene for HTAP resistance in Bancroft and mapped the gene to the same chromosome locus region. These results were similar to reports of a single gene or QTL for HTAP resistances in wheat genotypes Gaines (30), Alpowa (24), and the AVS/6*Yr8 line (20) against *P. striiformis* f. sp. *tritici*. However, not all HTAP resistances are controlled by a single gene or QTL. For example, two to three genes or QTL controlled HTAP resistances in wheat genotypes Nugaines and Luke (30), Druchamp and Stephenes (10), and Express (8). Therefore, HTAP resistance can be controlled by one or more genes. Even though a qualitative approach may be useable for certain cases, as in this study, quantitative mapping is more appropriate for identifying genes for HTAP resistance. In this study, using both qualitative and quantitative analyses made the results of gene identification and chromosomal mapping more robust than any one type of analysis alone.

Mapping QTL for barley stripe rust resistance has been reported on different chromosomal regions in various crosses (4,5,39). Previous studies reported QTL on chromosome 3H for resistance to barley stripe rust. Chen et al. (14) detected a QTL on chromosome 3H in the doubled haploid lines of Gobernadora/CMB 643 evaluated in Mexican fields for resistance to stripe rust. In the population of Shyri/Galena, a QTL was mapped on the long arm of chromosome 3H, flanked by the restriction fragment length polymorphism marker *ABG004* and the amplified fragment length polymorphism marker *T22M3li* (40). The maximum percentage of variance explained by this QTL was only 12%. The difference in magnitude of effects between the Bancroft QTL and those reported in other genotypes on chromosome 3H suggests that they are different genes or alleles. However, further studies are needed to determine the relationship among the resistance QTL on chromosome 3H.

The sequential order of the three SSR markers in the linkage map (Fig. 4) was the same as the order in Ramsay et al. (35), but the genetic distances between markers were different. The genetic distance between Bmag0606 and Bmag0013 decreased from 16.0 cM in their map to 7.8 cM in our map while the distance between Bmag0013 and EBmag0705 increased from 28.0 cM in their map to 37.9 cM in our map. These differences might result from chromosome rearrangements and DNA insertions, substitutions, or deletions within this region of different barley germplasms, as we previously discussed (43,44). Other factors, such as mapping population size, scoring errors, and marker saturation, could contribute to the differences.

A major problem for marker-assisted selection is the lack of polymorphism across different genotypes at the locus of a marker identified in a particular cross. To determine how useful markers identified in the Harrington/Bancroft cross would be if used to intercross the HTAP resistance to other wheat genotypes, the RGAP marker H3 and the SSR marker Bmag0013 associated with the resistance allele were tested for their presence or absence in 25 barley genotypes. Based on their pedigrees and reactions to races of *P. striiformis* f. sp. *hordei*, these genotypes do not have the Bancroft HTAP resistance and, therefore, were expected not to have the bands of markers tightly linked to the HTAP QTL. When tested with H3, only three genotypes, BBA 809, Nakai Zumizairai, and Bigo, had the Bancroft marker band. When tested with Bmag0013, only four genotypes, Heils Franken, Astrix, Baronesse, and Russell, showed the Bancroft band. A possible reason for the monomorphic genotypes is that H3 and Bmag0013 are just markers linked to the resistance QTL. Another possibility is that the bands may be the same in size but different in their sequence composition. Further studies, such as cloning and sequencing of these DNA fragments and the marker band in Bancroft, are needed to determine sequences and sources of the amplified products in these genotypes with amplified bands. Nevertheless, none of the 25 barley genotypes had both H3 and Bmag0013 markers as Bancroft did. Therefore, these markers can be used in combination in marker-assisted selection to incorporate the Bancroft HTAP resistance gene into other barley genotypes.

ACKNOWLEDGMENTS

This research was supported by the United States Department of Agriculture, Agricultural Research Service and the United States Barley Genome Project. PPNS No. 0444, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences Research Center, Project No. 11W-3061-5824, Washington State University, Pullman. We thank D. Wood for field preparation; P. Cheng for technical assistance; and S. Ullrich, D. Johnson, and P. Okubara for their critical review of the manuscript.

LITERATURE CITED

1. Becker, J., and Heun, M. 1995. Barley microsatellites: Allele variation and mapping. *Plant Mol. Biol.* 27:835-845.
2. Brown, W. M., Jr., Hill, J. P., and Velasco, V. R. 2001. Barley yellow rust in North America. *Annu. Rev. Phytopathol.* 39:367-384.
3. Brown, W. M., Jr., Velasco, V. R., Hill, J. P., Wesenberg D. M., and Bockelman, H. E. 1993. International barley germplasm screening for stripe rust-race 24 resistance. (Abstr.) *Phytopathology* 83:1337.
4. Castro, A., Chen, X. M., Hayes, P. M., Knapp, S. J., Line, R. F., Toojinda, T., and Vivar, H. 2002. Coincident QTL that determine seedling and adult plant resistance to stripe rust in barley. *Crop Sci.* 42:1701-1708.
5. Chen, F. Q., Prehn, D., Hayes, P. M., Mulrooney, D., Corey, A., and Vivar, H. E. 1994. Mapping genes for resistance barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). *Theor. Appl. Genet.* 88:215-219.
6. Chen, X. M. 2004. Epidemiology of barley stripe rust and races of *Puccinia striiformis* f. sp. *hordei*: The first decade in the United States. *Cereal Rusts and Powdery Mildews Bulletin* 32. Online <http://www.crpmb.org/2004/1029chen>, accessed 8 July 2005.
7. Chen, X. M. 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. *Can. J. Plant Pathol.* 27:314-337.

8. Chen, X. M., and Lin, F. 2007. Identification and molecular mapping of genes for all-stage and high-temperature adult-plant resistance to stripe rust in 'Express' wheat. (Abstr.) *Phytopathology* 97:S21.
9. Chen, X. M. and Line, R. F. 1995. Gene action in wheat cultivars for durable, high-temperature, adult-plant resistance and interaction with race-specific, seedling resistance to *Puccinia striiformis*. *Phytopathology* 85:567-572.
10. 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.
11. Chen, X. M., and Line, R. F. 1999. Recessive genes for resistance to *Puccinia striiformis* f. sp. *hordei* in Barley. *Phytopathology* 89:226-232.
12. Chen, X. M., and Line, R. F. 2001. Races of barley stripe rust in the United States. *Barley Newsl.* 44. Online: <http://grain.jouy.inra.fr/ggpages/BarleyNewsletter/44/WashReport2.html>
13. 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.
14. 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.
15. 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.
16. 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.
17. Chen, X. M., and Moore, M. K. 2002. Epidemics and races of *Puccinia striiformis* in North America in 2001. (Abstr.) *Phytopathology* 92:S14.
18. Chen, X. M., and Moore, M. 2003. High-temperature, adult-plant resistance in the barley cultivar 'Bancroft' against stripe rust. *Barley Newsletter* 45. Online: http://wheat.pw.usda.gov/ggpages/BarleyNewsletter/45/Barley_temp_resist.html
19. 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.
20. Chen, X. M., and Zhao, J. 2007. Identification of molecular markers for Yr8 and a gene for high-temperature, adult-plant resistance against stripe rust in the AVS/6*Yr8 wheat line. (Abstr.) *Phytopathology* 97:S21.
21. Kosambi, D. D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.
22. Lander, E. S., and Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
23. 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.
24. Lin, F., and Chen, X. M. 2007. 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.* 114:1277-1287.
25. Line, R. F., and Chen, X. M. 1995. Successes in breeding for and managing durable resistance to wheat rusts. *Plant Dis.* 79:1254-1255.
26. 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.
27. 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.
28. Maniatis, T. A., Frisch, E. F., and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
29. Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Nat. Acad. Sci. USA* 88:9828-9832.
30. 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.
31. Milus, E. A., and Line, R. F. 1986. Gene action for inheritance of durable, high-temperature, adult-plant resistance to stripe rust in wheat. *Phytopathology* 76:435-441.
32. Pahalawatta, V., and Chen, X. M. 2005. Genetic analysis and molecular mapping of wheat genes conferring resistance to the wheat stripe rust and barley stripe rust pathogen. *Phytopathology* 95:427-432.
33. Peterson, R. F., Campbell, A. B., and Hannah, A. E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.* 26:496-500.
34. Qayoum, A., and Line, R. F. 1985. High-temperature, adult-plant resistance to stripe rust of wheat. *Phytopathology* 75:1121-1125.
35. 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.
36. Riede, C. R., and Anderson, J. A. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Sci.* 36:905-909.
37. Sandoval-Islas, J. S., Broers, L. H. M., Vivar, H. E., and Osada, K. S. 1998. Evaluation of quantitative resistance to yellow rust (*Puccinia striiformis* f. sp. *hordei*) in the ICARDA/CIMMYT barley breeding program. *Plant Breed.* 117:127-130.
38. 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.
39. Thomas, W. T. B., Powell, W., Waugh, R., Chalmers, K. J., Barua, U. M., Jack, P., Lea, V., Forster, B. P., Swanson, J. S., Ellis, R. P., Hanson, P. R., and Lance, R. C. M. 1995. Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 91:1037-1047.
40. 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.
41. Ullrich, S. E., Jitkov, V. A., Muir, C. E., Burns, J. W., Reisenauer, P. E., Line, R. F., Chen, X. M., Jones, B. L., Wesenberg, D. M., and Robertson, L. D. 2003. Registration of 'Bob' barley. *Crop Sci.* 43:1132-1134.
42. Wesenberg, D. M., Burrup, D. E., Brown, W. M., Jr., Velasco, V. R., Hill, J. P., Whitmore, J. C., Karow, R. S., and Hayes, P. M. 2001. Registration of 'Bancroft' barley. *Crop Sci.* 41:265-266.
43. 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.
44. 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.
45. 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.
46. Yun, S. J., Gyeon, L., Hayes, P. M., Matus, I., Smith, K. P., Steffenson, B. J., and Muehlbauer, G. J. 2005. Quantitative trait loci for multiple disease resistance in wild barley. *Crop Sci.* 45:2563-2572.