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Validation of Quantitative Trait Loci for Multiple Disease Resistance in Barley Using Advanced Backcross Lines Developed with a Wild Barley

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ABSTRACT

Quantitative trait loci (QTLs) for resistance to spot blotch {*Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur [anamorph: Bipolaris sorokiniana (Sacc. in Sorok.) Shoem.]}, net type net blotch (NTNB, *Pyrenophora teres* f. *teres*), Septoria speckled leaf blotch (SSLB, primarily *Septoria passerinii* Sacc.), and leaf scald [caused by *Rhynchosporium secalis* (Oudem.) Davis] were previously detected in a recombinant inbred line (RIL) population derived from a cross between the wild barley [*Hordeum vulgare* subsp. *spontaneum* (K. Koch) Thell.] accession OUH602 and the two-rowed malting barley (*H. vulgare* L.) cultivar Harrington. Our objectives in this study were to develop and genetically characterize an advanced backcross (AB) population with OUH602 as the donor parent and Harrington as the recurrent parent, to validate seedling disease resistance QTLs detected in the OUH602/Harrington RIL population, and to identify adult plant spot blotch resistance QTLs. We developed an AB population ($BC_2F_{6:8}$) and genotyped the population with a set of 111 simple sequence repeat (SSR) markers. The estimated donor-parent genome introgression averaged 11.25%. A QTL analysis of the AB population validated all major QTLs previously identified in the OUH602/Harrington RIL population. No new QTLs for seedling resistance were identified in the AB population, indicating that AB populations do not provide an advantage compared with RIL populations for detecting seedling disease resistance QTLs. A previously unidentified QTL conferring adult plant spot blotch resistance was identified on chromosome 4(4H). The resistance alleles for each of the QTLs were contributed by OUH602. The AB population is a valuable resource for further genetic studies and breeding applications.

Quantitative trait locus analysis is a powerful approach to identify regions of the genome that impact complex traits (Tanksley, 1993). In general, QTL analysis is conducted on populations derived from diverse parental genotypes, providing the advantage of high levels of DNA polymorphisms for mapping and a high level of variation for the trait(s) of interest. The disadvantages of these populations are that they usually do not produce useful lines for breeding, and many of the markers that are polymorphic for diverse parents are not polymorphic in elite breeding germplasm (Tanksley and Nelson, 1996). Thus, QTL analysis and breeding are often separate processes. A paradox is that most crop plants have wild relatives that carry a wealth of untapped and useful allelic variation for agronomic disease resistance and quality traits of interest. This source of variation is important for crop improvement. To effectively utilize this allelic variation, an approach referred to as advanced backcross–quantitative trait loci (AB–QTL) analysis has been developed (e.g., Eshed and Zamir, 1994; Tanksley and Nelson, 1996).

Populations for AB–QTL analysis commonly use exotic or wild germplasm as the donor and elite germplasm as the recurrent parent. The QTL analysis is conducted in the BC_2 or BC_3 generation to reduce the frequency of deleterious donor alleles. The AB–QTL analysis has been successful in identifying and introgressing favorable QTL alleles from unadapted germplasm in many crop species (Xiao et al., 1996, 1998; Fulton et al., 2000; Poncet et al., 2000; Brondini et al., 2002; Matus et al., 2003; Von Korff et al., 2004; Pillen et al., 2004). Because many of the deleterious alleles from the donor parent have been eliminated, lines from AB–QTL analysis can be directly integrated into breeding programs. In addition, markers used to genotype the AB lines are more likely to be useful for future breeding efforts. Thus, this approach tightly couples QTL mapping with breeding.

Foliar diseases have been one of the limiting factors in barley production around the world. In the USA, leaf scald is a serious disease of barley in the Pacific Northwest, California, and the Mid Atlantic region, where yield losses can reach 40% (Mathre, 1997). In the Midwest, NTNB, spot blotch, and SSLB are important foliar diseases of barley because they result in yield reductions ranging from 5 to 35% each year (Mathre, 1997; Toubia-Rahme and Steffenson, 2004).

Numerous genetic studies have identified major genes and QTLs conferring resistance to spot blotch, NTNB, SSLB, and leaf scald (Williams, 2003). *Hordeum vulgare* subsp. *spontaneum*, the wild progenitor of barley, is a rich source of disease resistance genes (Metcalfe et al., 1977, 1978; Nevo, 1992; Sato and Takeda, 1997; Fetch et al., 2003; Genger et al., 2003; Williams, 2003). We previously identified QTLs for resistance to these foliar diseases in the wild barley accession OUH602 using OUH602/Harrington RILs (Yun et al., 2005). Six QTLs for which wild barley provided the resistance alleles were identified: one for spot blotch resistance on chromosome 1(7H); two for NTNB resistance on chromosomes 3(3H)

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Abbreviations: AB, advanced backcross; NTNB, net type net blotch; PCR, polymerase chain reaction; QTL, quantitative trait locus; RIL, recombinant inbred line; SSLB, Septoria speckled leaf blotch; SSR, simple sequence repeat.

and 4(4H); two for SSLB resistance on chromosomes 2(2H) and 6(6H); and one for leaf scald resistance on chromosome 5(1H).

In this study, we developed an AB population with the wild barley accession OUH602 as a resistance donor and Harrington as a recurrent parent to (i) validate resistance QTLs for the foliar diseases identified in our OUH602/Harrington RIL population, (ii) determine the genomic location of adult plant spot blotch resistance, (iii) assess the genetic characteristics of the AB population, and (iv) provide access to novel OUH602 alleles in an adapted genetic background for further breeding and genetics applications.

MATERIALS AND METHODS

Development of the Advanced Backcross Population

An AB population ($BC_2F_{6:8}$) was developed with the wild barley accession OUH602 as the donor parent and the cultivated barley (*H. vulgare* L.) cv. Harrington as the recurrent parent. The accession OUH602 was collected in the Middle East, but the exact geographical origin is unknown (Sato and Takeda, 1997). Harrington is the North American industry standard for two-rowed malting barley. OUH602 is resistant to spot blotch, NTNB, SSLB, and leaf scald (Yun et al., 2005). The recurrent parent was used as the female and the donor as the male to obtain the F_1 plants. For the BC_1 and BC_2 generations, the recurrent parent was used as the male. Ninety-eight random BC_1 plants were backcrossed to Harrington to generate 98 BC_2 generation plants, one BC_2F_1 tracing back to one BC_1F_1. From each BC_2F_1 plant, single seed decent was used to advance progeny to the BC_2F_6 generation. Seed for disease evaluations was bulk harvested from BC_2F_7 plants derived from each BC_2F_6 plant.

DNA Marker Analysis

DNA was extracted from the leaves of each of the 98 BC_2F_6 plants using the methods described by Mesfin et al. (1999). A total of 111 polymorphic SSR markers were used to genotype the AB population. Sixteen, 16, 17, 15, 12, 17, and 18 markers from chromosomes 1(7H) to 7(5H), respectively, were selected from the previously generated linkage map for the wild barley OUH602 × Harrington RIL population (Yun et al., 2005). The polymerase chain reaction (PCR) was performed according to the procedures of Ramsay et al. (2000). Amplified products were separated on 5% (w/v) polyacrylamide gels and visualized by silver staining as described by Bassam et al. (1991). The PCR products amplified using fluorescent-labeled primers were separated and detected using the IR^2 DNA analyzer (Global ed., LI-COR, Lincoln, NE).

JoinMap (v. 3.0; Van Ooijen and Voorrips, 2001) was used to develop a linkage map of SSR markers for the RIL (Yun et al., 2005) and AB (BC_2F_6) populations. Marker locations in both populations were compared to confirm their chromosomal locations. Linkage map positions of the markers determined for the RIL population were used for graphical genotyping and QTL analysis of the AB population. Bin information for the markers was inferred following the definitions of Kleinhofs and Graner (2001), the barley genomics website (Washington State University, 2005), and the Oregon Wolfe Barley population data (Hayes and Filichkin, 2004).

Graphical Genotype Analysis

Graphical genotyping of the AB population was conducted using GGT software (Van Berloo, 1999). The size and number of introgressions were also determined using GGT software. The parental genome ratio was calculated by determining the total genome derived from the donor parent (OUH602) as a proportion of the recurrent parent (Harrington) genome, as described by Young and Tanksley (1989).

Greenhouse Evaluations

Six seeds of each AB line were planted individually into six 8- by 8-cm plastic pots filled with Metro Mix 200 growing media (Scotts-Sierra Horticulture Products, Marysville, OH). After planting, all pots were moistened and then moved to a cold room at 4°C for 7 d to break any seed dormancy. After the 4°C exposure period, pots were moved to a greenhouse, and plants were grown at approximately 22°C (range from 18–25°C) with a 16-h photoperiod using sodium vapor lights. The plants were fertilized with a 20:20:20 (N–P–K) water-soluble fertilizer (Scotts-Sierra Horticulture Products, Marysville, OH) weekly at the recommended rate.

All greenhouse disease evaluation experiments for seedling diseases were arranged in a randomized complete block design with three replications per line. At least two evaluation experiments were conducted for each disease. Each block included the AB lines, the parents, and a set of controls that were susceptible or resistant to the evaluated pathogens. The parents and progeny from the AB population were evaluated for resistance to spot blotch, NTNB, SSLB, and leaf scald as seedlings by artificial inoculation in the greenhouse at the University of Minnesota in St. Paul. The following pathogen isolates were used for disease evaluation: isolate ND85F of spot blotch, isolate 30199013 of NTNB (from R. Dill-Macky), isolate SP97–15 of SSLB, and isolate LA94–1A of leaf scald. All isolates except *P. teres* were provided by B. Steffenson. The pathogen isolates used represent common virulence spectra found in the Upper Midwest region of the USA.

Three plants per line were inoculated when their second leaf was fully expanded. Inoculations were performed according to Fetch et al. (2003). Disease reactions were assessed after specific incubation periods using published assessment guides for each respective pathogen (Fetch et al., 2003). Spot blotch reactions were rated 10 to 12 d after incubation using a 1-to-9 scale (Fetch and Steffenson, 1999). The NTNB reactions were evaluated 18 d after inoculation using a 0-to-10 scale (Tekauz, 1985). Leaf scald and SSLB were evaluated 23 d after inoculation using a 0-to-4 scale for leaf scald (Jackson and Webster, 1976) and a 0-to-5 scale for SSLB (Toubia-Rahme and Steffenson, 2004). For each disease scale, the higher number indicates greater susceptibility.

Field Evaluations

For the field evaluations of adult plant spot blotch reaction, seeds of AB lines were planted in paired rows of hill plots (10–12 seeds $plot^{-1}$) spaced 0.3 m apart. Two experiments were conducted in St. Paul in 2002 and 2003. Inoculations were performed according to Fetch and Steffenson (1999). The AB lines were sown in a randomized complete block design with two replications. Each block included the AB lines, the parents, and a set of reference barley cultivars susceptible or resistant to spot blotch. Disease reactions were evaluated on a 1-to-9 scale (Fetch and Steffenson, 1999) on the second and third leaf below the flag leaf.

Statistical Analysis

Analysis of variance for the disease data from the greenhouse and field evaluations were conducted using Statistix 8 (Analytical Software, Tallahassee, FL). Single marker analysis

was conducted by simple linear regression (Jiang and Zeng, 1997) between the disease phenotypes and the marker genotypes for the AB lines. QTL Cartographer version 1.17 (Basten et al., 2003), Window QTL Cartographer 2.0 (Wang et al., 2004), and Statistix 8 (Analytical Software, Tallahassee, FL) were used in the analysis.

RESULTS

Genetic Characterization of the AB Lines

Ninety-eight AB ($BC_2F_{6:8}$) lines were developed with the wild barley OUH602 accession as the donor parent and Harrington as the recurrent parent. We used 111 SSR markers spread evenly over the entire barley genome to genotype the population. These markers were previously mapped in our OUH602/Harrington RIL population (Yun et al., 2005) and shown to cover 974 cM of the barley genome. The calculated percentage of the donor-parent genome introgressed as homozygous segments into Harrington ranged from 0 to 28%, with an average of 11.25% (Fig. 1). One line with 0% homozygous donor introgressed segments contained 2.9% heterozygous alleles. In addition, the average percentage introgression for chromosomes 1(7H) to 7(5H) was 11.4, 11.3, 11.5, 10.5, 11.3, 12.3, and 10.5%, respectively. The average length of donor chromosome fragments was 11.3, 16.2, 16.2, 11.5, 14.7, 15.7, and 16.0 cM for chromosomes 1(7H) to 7(5H), respectively, with an overall average of 14.5 cM. The introgressed donor fragments per chromosome were mostly none to three and at most four. Lines 22 and 84 showed the highest and lowest percentage introgression of 28 and 0%, respectively (Fig. 1).

Most markers on each chromosome had an OUH602 allele frequency of between 7 and 17% on the 98 AB lines. Marker GBM1055 on the short arm of chromosome 4(4H) had a donor frequency of 32%, and UMB101 on chromosome 1(7H), EBmac0701 on chromosome 4(4H), GBM1003 on chromosome 4(4H), and HVM30 on chromosome 7(5H) each had an OUH602 allele frequency of 5.1%.

Disease Response of the Parents and AB Lines

OUH602 exhibited very high levels of seedling resistance to spot blotch, NTNB, SSLB, and leaf scald, and adult plant resistance to spot blotch. In contrast, Harrington was highly susceptible to these diseases (Table 1). Disease resistance levels between the AB lines were significantly different for all of the diseases evaluated. No significant interactions between lines and experiments were detected, except for seedling spot blotch in the two greenhouse experiments (Table 1). Therefore, QTL analyses for each disease phenotype, except for seedling spot blotch resistance, were conducted using the mean of the two experiments.

Disease Resistance QTLs

Single marker analyses were conducted to identify marker-trait associations for seedling resistance to spot blotch, NTNB, SSLB, and leaf scald, and adult plant resistance to spot blotch (Table 2, Fig. 2). For QTLs that were found in the same chromosomal region as in the OUH602/Harrington RIL mapping study, the QTL names and BIN designations were based on those reported from the OUH602/Harrington RIL mapping

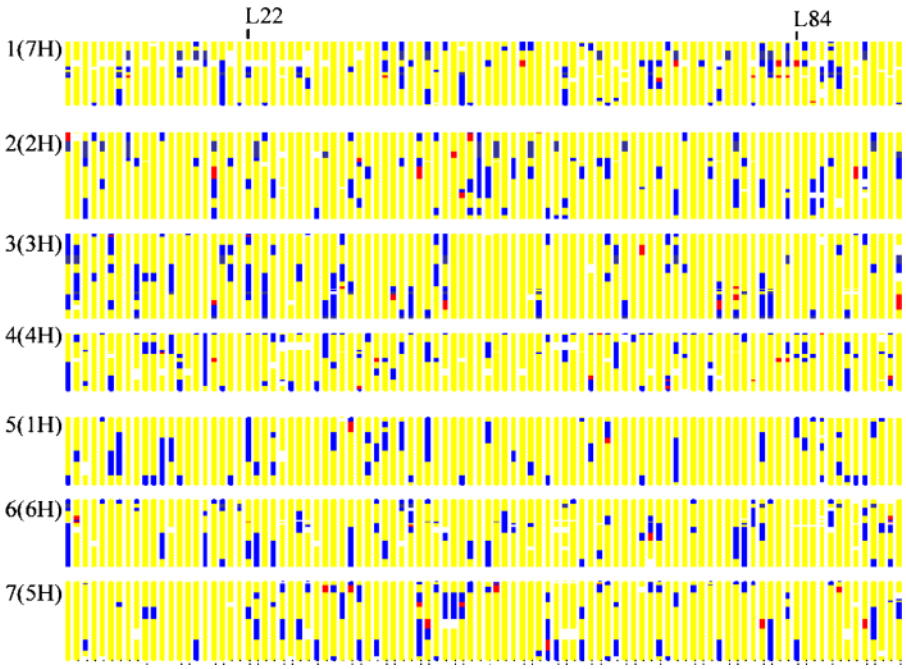

Fig. 1. Graphical genotypes of the seven barley chromosomes [chromosomes 1(7H) to 7(5H) from top to bottom] of 98 advanced backcross lines (lines 1 to 98 from left to right). Marker locations were based on the map positions from the OUH602/Harrington recombinant inbred line population (Yun et al., 2005). Advance backcross lines L22 and L84 have 28 and 0% introgressed donor fragments, respectively. The Harrington genome is shown in yellow, the OUH602 genome is in blue, heterozygotes are in red, and missing data are in white.

Table 1. Means, ranges, and variances for spot blotch (SB), net type net blotch (NTNB), Septoria speckled leaf blotch (SSLB), and leaf scald (LS) phenotypes of parents and lines from the OUH602 (OUH)/Harrington (HRT) $BC_2F_{6:8}$ lines.

						Population			*P* value	
Trait	Experiment†		Reading‡	HRT	OUH	Mean	Range	Error MS§	Line	L × E¶
SB	2002F	Exp. 1	F-2 & 3	7.4	1.5	6.8	3.5–8.0	1.91	<0.0001	
	2003F	Exp. 1	F-2 & 3	7.3	1.9	6.7	3.2–8.0	1.88	<0.0001	
	GH	Exp. 1	L2	6.3	1.7	5.0	1.3–8.0	1.46	<0.0001	
		Exp. 2		6.7	1.3	5.6	1.7–8.3	2.59	<0.0001	0.0378
NTNB	GH	Exp. 1	L2	5.3	0.7	4.6	1.0–9.0	3.08	<0.0001	
		Exp. 2		6.7	1.0	5.5	1.7–9.3	2.73	<0.0001	0.0708
SSLB	GH	Exp. 1	L2	4.7	0.0	3.5	0.7–5.0	0.39	<0.0001	
		Exp. 2		5.0	0.0	3.3	0.7–5.0	0.44	<0.0001	0.1468
LS	GH	Exp. 1	L2	3.3	0.0	3.2	0.7–4.0	0.30	<0.0001	
		Exp. 2		3.7	1.3	3.6	0.7–4.0	0.23	<0.0001	0.1299

† Field (F) experiments were conducted in St. Paul, MN, in 2002 and 2003. Greenhouse (GH) experiments were conducted in St. Paul in 2002. Each experiment was replicated at least two times.
‡ Disease readings were assessed on the second and third leaf below the flag leaf (F-2 & 3) of adult plants in the field, and the second leaf (L2) on the seedlings in greenhouse experiments. Disease reactions were evaluated using the following scales: spot blotch at a 1–9 scale (Fetch and Steffenson, 1999), NTNB at a 0–10 scale (Tekauz, 1985), SSLB at 0–5 scale (Toubia-Rahme and Steffenson, 2004), and leaf scald at a 0–4 scale (Jackson and Webster, 1976).
§ Mean square of the experiment.
¶ Interaction between lines and experiments.

study (Yun et al., 2005). We consistently identified a seedling and adult plant spot blotch resistance QTL *Rcs-qtl-7H-2-4* (*Resistance to Cochliobolus sativus, Rcs* locus) on chromosome 1(7H) BIN 2 to 4 in the greenhouse and field experiments (Table 2). A new QTL for adult plant spot blotch resistance was identified on chromosome 4(4H) in the BIN 4 to 6 region (*Rcs-qtl-4H-4-6*), but only in the 2003 field experiment. Two seedling NTNB resistance QTLs, *Rpt-qtl-3H-4* and *Rpt-qtl-4H-5-7,* were identified on chromosome 3(3H) BIN 4 and chromosome 4(4H) BIN 5 to 7, respectively (Table 2). Two SSLB resistance (*Rsp*) QTLs were identified on chromosomes 2(2H) BIN 7 to 11 and 6(6H) BIN 10 to 14 (Table 2). One leaf scald resistance (*Rrs*) QTL was identified on chromosome 5(1H) BIN 3 to 4 (Table 2). The resistance alleles for all QTLs were derived from OUH602. The phenotypic variation explained by the associated markers were estimated by regression coefficient (R^2). The values were similar in the AB and RIL populations for most QTLs, except *Rrs-qtl-1H-1-4* whose associated

Table 2. Detection of resistance quantitative trait loci (QTLs) for spot blotch (SB), net type net blotch (NTNB), Septoria speckled leaf blotch (SSLB), and leaf scald (LS) in the 98 OUH602/Harrington advanced backcross ($BC_2F_{6:8}$) lines by single marker analysis with a significant threshold LOD value of 2.5.

								R^2#	
Trait	Exp.†	QTL‡	Chromosome	Est. BIN§	Associated marker	LOD	α¶	AB	RIL††
SB (adult)	2002F	*Rcs-qtl-7H-2-4*	1(7H)	3	Bmag0007	2.75	−0.58	8.2	–
				4	EBmac0603	8.72	−1.03	35.4	–
	2003F	*Rcs-qtl-7H-2-4*	1(7H)	3	Bmag0007	3.56	−0.57	18.9	–
				4	EBmac0603	2.84	−0.55	12.6	–
		Rcs-qtl-4H-4-6	4(4H)	4–5	Bmag0740	5.01	−0.67	9.0	–
				5–6	Bmag0381.1	3.61	−0.53	16.3	–
				6	GMS089	3.05	−0.55	6.0	–
				6	Bmag0808	3.83	−0.56	16.5	–
SB (seedling)	G1	*Rcs-qtl-7H-2-4*	1(7H)	2	GBM1060	3.06	−0.81	13.4	10.5
				3	Bmag0007	4.02	−0.83	14.2	19.4
				3	HVM04	3.46	−0.77	10.4	20.2
				4	EBmac0603	13.58	−1.46	39.7	40.5
	G2	*Rcs-qtl-7H-2-4*	1(7H)	4	EBmac0603	14.02	−1.55	48.6	25.0
NTNB	GC	*Rpt-qtl-3H-4*	3(3H)	4–5	Bmac0067	2.67	−0.99	11.9	7.7
				5–6	EBmac0558.2	2.50	−1.09	11.1	6.2
		Rpt-qtl-4H-5-7	4(4H)	5–6	Bmag0740	4.82	−1.16	5.8	15.1
				5–6	Bmag0381.1	7.14	−1.27	24.8	12.3
				6	GMS089	4.44	−1.05	8.4	9.1
				6	Bmag0808	3.76	−0.97	16.2	9.1
SSLB	GC	*Rsp-qtl-2H-7-11*	2(2H)	8–9	Bmag0140	5.54	−0.83	23.0	26.8
				8–9	GMS003	6.79	−1.01	9.2	27.0
				8–9	Bmag0518	7.22	−1.01	29.6	27.5
		Rsp-qtl-6H-10-14	6(6H)	13	Bmac0040	2.52	−0.65	11.2	6.2
				14	UMB603	4.39	−0.90	14.5	2.3ns‡‡
LS	GC	*Rrs-qtl-1H-1-4*	5(1H)	3–4	Bmac0213	5.87	−0.55	9.1	35.8
				3–4	GBM1007	4.50	−0.43	15.0	32.4

† 2002F and 2003F, 2002 and 2003 field experiments; G1 and G2, Exp. 1 and 2 in the greenhouse; GC, Exp. 1 and 2 in the greenhouse combined.
‡ The QTLs detected in the OUH602/Harrington RIL population were indicated as the corresponding locus name.
§ Estimated BIN based on the chromosome BIN location of barley markers (Washington State University, 2005).
¶ Average effect of substituting a Harrington allele with an OUH602 allele. Probability of all values were <0.001.
# Partial $R^2 \times 100$ based on simple linear regression. *P* values for regression were <0.01 except for UMB603 in RIL where $P = 0.129$.
†† Recombinant inbred lines (RIL) lines of the OUH602/Harrington population (Yun et al., 2005).
‡‡ ns, not significant.

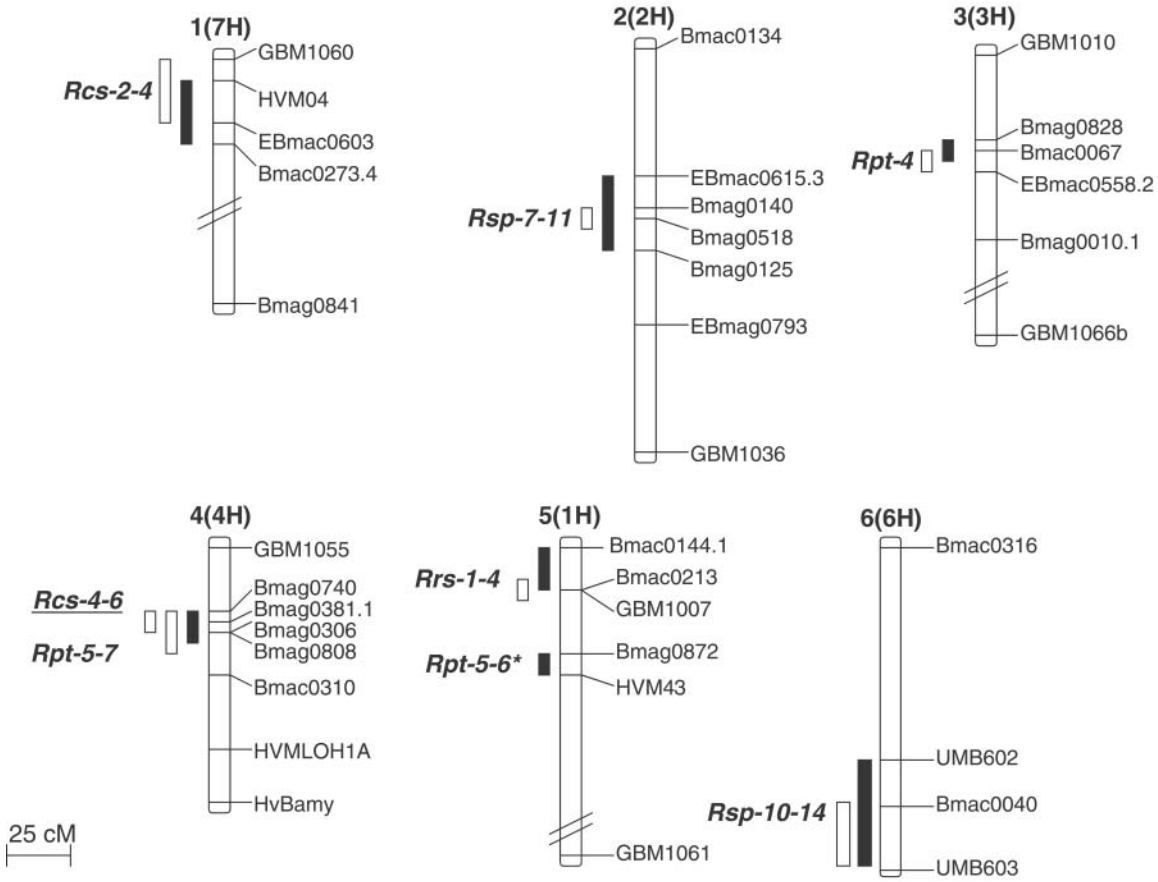

Fig. 2. Resistance QTLs for spot blotch (*Rcs*), net type net blotch (*Rpt*), Septoria speckled leaf blotch (*Rsp*), and leaf scald (*Rrs*) detected in the advanced backcross (AB, open bar) and recombinant inbred line (RIL, closed bar) populations of OUH602/Harrington. Marker locations were based on the map positions from the OUH602/Harrington RIL population (Yun et al., 2005). The QTL detected only in the AB population is underlined and the QTL detected only in the RIL population is designated with an asterisk. No QTL were detected on chromosome 7(5H).

markers had considerably higher values in the RIL than in the AB population (Table 2).

DISCUSSION

In this study, we developed an AB population with the wild barley accession OUH602 as the resistance donor and Harrington as the recurrent parent, and identified a total of seven QTL for resistances to spot blotch, NTNB, SSLB, and leaf scald. The AB population provided the opportunity to validate our previous QTL mapping in an OUH602/Harrington RIL population (Yun et al., 2005), to generate a population for future AB–QTL analysis, and to develop a resource for future barley breeding. The AB population was effective for QTL identification, and it will be valuable in accessing novel alleles from the OUH602 genome for barley improvement.

Wild Barley Genome is Retained Normally in the Cultivated Barley Background

The AB population developed in this study exhibited a calculated average of 11.25% donor-parent (OUH602) genome introgression into Harrington. This introgression proportion is close to the expected amount of 12.5% for a BC_2 population in the absence of selection (Stam and Zeven, 1981). Similar amounts of donor chromosome introgression were reported in two wild barley × cultivated barley BC_2F_6 and BC_2DH populations (Matus et al., 2003; Von Korff et al., 2004).

The average length of donor chromosome fragments in the AB lines was 14.5 cM. This is significantly shorter than the average of 38.6 cM introgression fragments reported in the wild barley × cultivated barley BC_2F_6 populations (Matus et al., 2003), where 47 SSR markers were used to generate genotypes for 1067 cM of barley genome. In our study, 111 markers were used for the barley genome covering 974 cM. Thus, the shorter introgression regions in this study are likely due to the larger number of markers used to genotype the AB lines.

Segregation distortion was not observed for most markers, except for UMB101 on chromosome 1(7H), GBM1055, EBmac0701 and GBM1003 on chromosome 4(4H), and HVM30 on chromosome 7(5H). GBM1055 showed distorted segregation toward OUH602, while the others toward Harrington. This degree of segregation distortion is similar to that reported in the AB lines of wild barley Caesarea 26–24/Harrington (Matus et al., 2003). In our population, all markers exhibited transmission of donor alleles, indicating that there was no selection against the OUH602 alleles. However, unintentional selection against gene(s) on the chromosomal

regions not fully covered by markers used in this study might be undetected. Segregation distortion is a prominent feature in crosses between the cultivated and wild progenitor species in pearl millet (*Pennisetum glaucum* (L.) R. Br.; Poncet et al., 2000), rice (*Oryza sativa* L.; Brondini et al., 2002), and tomato (*Lycopersicon esculentum* Mill.; Foolad et al., 2002). Our results suggest that there is a high similarity in genome constitution between the accession OUH602 and Harrington. This interpretation is consistent with the consensus that malting barley and wild barley belong to the same species (Baum and Johnson, 1996).

Validation of OUH602-Derived Foliar Disease Resistance QTL

All the QTLs identified in the OUH602/Harrington RIL (Yun et al., 2005), except one NTNB QTL on chromosome 5(1H), were validated in the AB population. To maintain consistency between studies, QTL names and BIN numbers used were from the OUH602/Harrington RIL mapping study (Yun et al., 2005). All validated QTL marker positions in this study were in the same general region as detected by the OUH602/Harrington RIL mapping study. The QTLs detected in the OUH602/Harington RIL and AB populations are summarized in Fig. 2.

Spot blotch resistance QTLs have been localized on chromosomes 1(7H), 2(2H), 3(3H), 5(1H), and 7(5H) in studies of several cultivated barley mapping populations (Steffenson et al., 1996; Bilgic et al., 2005). The QTLs on chromosomes 1(7H) and 3(3H) conferred both seedling and adult plant resistance, but the QTLs on chromosomes 2(2H), 5(1H), and 7(5H) conferred adult plant resistance. Our results show that the QTL on chromosome 1(7H) confers both seedling and adult plant resistance to spot blotch, indicating that the OUH602 allele provides season-long resistance to spot blotch. The QTL *Rcs-qtl-4H-4-6* conferring adult plant resistance has not been reported previously, and may be a unique allele for use in cultivated barley.

Many QTLs conferring seedling resistance to NTNB have been localized on all chromosomes, except 1(7H) and 7(5H) (Steffenson et al., 1996; Richter et al., 1998). Two QTLs, *Rpt-qtl-4H-5-7* and *Rpt-qtl-3H-4*, identified in the OUH602/Harrington RIL population (Yun et al., 2005) were validated in the AB population in this study and located to the same chromosomal regions in other studies (Steffenson et al., 1996; Spaner et al., 1998). The QTL *Rpt-qtl-1H-5-6* identified in the OUH602/Harrington RIL population was not detected in the OUH602/Harrington AB lines. This result was not surprising since *Rpt-qtl-1H-5–6* is a minor effect QTL that was only detected in a single environment in the RIL population (Yun et al., 2005).

The two SSLB resistance QTLs (*Rsp-qtl-2H-7-11* and *Rsp-qtl-6H-10-14*) identified in this study validate the QTLs identified in the OUH602/Harrington RIL population (Yun et al., 2005). Many resistance sources have been identified in both cultivated and wild barley (Fetch et al., 2003; Metcalfe et al., 1977, 1978; Legge et al., 1996; Rasmusson and Rogers, 1963; Toubia-Rahme et al., 2003; Toubia-Rahme and Steffenson, 2004). Three major SSLB resistance genes have been mapped in barley. One derived from cultivated barley was localized on chromosome 4(4H) in a *H. v.* subsp. vulgare × *H. bulbosum* RIL population (Toubia-Rahme et al., 2003). The other two mapped genes (*Rsp2* and *Rsp3*) are very closely linked and were mapped to chromosome 5(1H) (S. Zhong, H. Toubia-Rahme, and B. Steffenson, 2004, unpublished data).

Leaf scald resistance QTLs have been localized on all chromosomes, except chromosome 7(5H) (Backes et al., 1995; Spaner et al., 1998; Garvin et al., 2000; Genger et al., 2003). Leaf scald resistance QTLs have been mapped to the same chromosomal regions as identified in this study (*Rrs-qtl-1H-1-4*), in cultivated and wild barley (Backes et al., 1995; Garvin et al., 2000), and in the OUH602/Harrington RIL population (Yun et al., 2005).

AB Population Did Not Detect Major New Disease Resistance Alleles

Advanced backcross–QTL analysis is a powerful approach to uncover novel alleles for traits that are masked in standard RIL populations by deleterious alleles in the donor genome. In this study, we did not identify any novel seedling-expressed disease resistance loci that were not detected in the OUH602/Harrington RIL population. Moreover, the phenotypic variation explained by the associated markers (R^2) was similar in AB and RIL populations for most QTLs, except *Rrs-qtl-1H-1-4* (Table 2). Markers associated with *Rrs-qtl-1H-1-4* had considerably higher values in the RIL than in the AB population. This is in contrast with the predicted superiority of the AB populations compared with selfing populations for detecting QTL (Tanksley and Nelson, 1996). Many new QTL for agronomic and quality traits were identified using AB lines developed from wild barley and Harrington (Matus et al., 2003). Among the 33 agronomic and quality trait QTLs detected in AB populations, 18 QTLs were not identified in the classical QTL analyses (Pillen et al., 2004). In tomato, more QTLs for soluble-solids and fruit weight were detected in an AB population compared with a BC_1 population created from the same parents, demonstrating the power of AB populations in detecting additional QTLs (Grandillo and Tanksley, 1996; Tanksley et al., 1996). These results indicate contrasting impact of wild germplasm-derived alleles on disease and agronomic traits. Therefore, our results suggest that the expression of seedling disease resistance to foliar pathogens is not constrained by the donor genome. There do not appear to be any alleles in the OUH602 genome that mask the expression of the foliar disease resistance we examined. Our interpretation is that beneficial alleles from wild germplasm that impact agronomic and quality traits can easily be masked by deleterious alleles in the wild germplasm, whereas alleles that impact foliar seedling disease resistance are in general not masked. Taken together, our results indicate that the OUH602/Harrington RIL population is equivalent to the OUH602/Harrington AB population in detecting QTL for seedling disease resistance. This suggests that while AB–QTL analysis may be very important for identifying wild genes for traits like yield in

rice (Xiao et al., 1996), it may not provide a substantial advantage to identify wild genes for resistance to foliar diseases in barley.

OUH602/Harrington AB Population for Future Barley Genetics and Breeding Applications

AB lines developed in this study will be useful for transferring favorable alleles to elite lines, dissection of QTLs, fine mapping of the regions where the resistance alleles were found, and cloning genes. The utility of AB lines for transferring favorable alleles from unadapted germplasms to elite lines has been demonstrated in many crops, including barley (Ho et al., 2002; Huang et al., 2003; Talamè et al., 2004; Wu et al., 2004). For instance, introgression lines with the gene *Ppd-H1* from exotic barley have a significantly shorter photoperiod response (Von Korff et al., 2004). Successful cloning of QTLs using AB lines has been reported for various traits (e.g., Fridman et al., 2000; Frary et al., 2000; Takahashi et al., 2001). Therefore, the AB lines developed in this study will have a variety of useful applications.

A major barrier to the use of exotic germplasm for crop improvement is that populations derived from wide crosses segregate for many undesirable alleles. This poses several problems. First, as was discussed earlier, donor parent alleles can mask potentially desirable alleles. This was not observed for disease resistance in our study, but has been observed for agronomic or quality traits. Also, the number of undesirable alleles segregating in wide crosses makes it unlikely to identify individuals that are fixed for the desirable allele at all or most of the important loci. This is particularly true when the size of the breeding populations is small. Lastly, the segregation for undesirable alleles can interfere with the measurement of traits important for breeding and hinder selection. For example, it is difficult to assess kernel plumpness in lines with substantial shattering. The AB lines developed in this study, therefore, provide good parents for developing breeding populations since the number of undesirable donor alleles in any cross will be reduced, each line has been genotyped with markers that are directly useful in marker-assisted selection, and several of the AB lines carry useful disease resistance genes.

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