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ORIGINAL PAPER

Molecular mapping of a recessive gene for resistance to stripe rust in barley

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Abstract Barley stripe rust, caused by *Puccinia striiformis* f. sp. *hordei*, is one of the most important barley (*Hordeum vulgare*) diseases in the United States. The disease is best controlled using resistant cultivars. Barley genotype Grannenlose Zweizeilige (GZ) has a recessive gene (*rpsGZ*) that is effective against all races of *P. striiformis* f. sp. *hordei* identified so far in the USA. To develop a molecular map for mapping the gene, F_8 recombinant inbred lines (RILs) were developed from the Steptoe X GZ cross through single-seed descent. Seedlings of the parents and RILs were evaluated for resistance to races PSH-14 and PSH-54 of *P. striiformis* f. sp. *hordei* under controlled greenhouse conditions. Genomic DNA was extracted from the parents and 182 F_8 RILs and used for linkage analysis. The resistance gene analog polymorphism (RGAP) technique was used to identify molecular markers for *rpsGZ*. A linkage group for the gene was constructed with 12 RGAP markers, of which two markers co-segregated with the resistance locus, and two markers were closely linked to the locus with a genetic distance of 0.9 and 2.0 cM, respectively. These four markers were present only in the susceptible parent. The closest marker to the resistance allele was 11.7 cM away. Analyses of two sets of barley chromosome addition lines of wheat with the two RGAP markers that were cosegregating with the susceptibility allele showed that *rpsGZ* and the markers were located on the long arm of barley chromosome 4H. Further, tests with four simple sequence repeat (SSR) markers confirmed the chromosomal location of the *rpsGZ* gene and also integrated the RGAP markers into the known SSR-based linkage map of barley. The closest SSR marker EBmac0679 had a genetic distance of 7.5 cM with the gene in the integrated linkage map constructed with the 12 RGAP markers and 4 SSR markers. The information on chromosomal location and molecular markers for *rpsGZ* should be useful for incorporating this gene into commercial cultivars and combining it with other resistance genes for durable resistance.

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Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *hordei* (*P. s. hordei*), is an important disease of barley in many parts of the world (Stubbs 1985). In the USA, the disease was first detected in Texas in 1991 (Roelfs et al. 1992). Since then, the pathogen has become established in the south central and western USA and the disease has caused considerable yield losses in the western US, especially in California and some locations in the Pacific Northwest (Chen et al. 1995; Brown et al. 2001; Chen 2004).

Growing resistant cultivars is the best approach to control stripe rust. Resistance to stripe rust mainly includes all-stage (also called seedling) resistance, which is expressed in all growth stages, and high-tem-

perature, adult-plant (HTAP) resistance, which is expressed in the adult-plant stage at high temperature (Qayoum and Line 1985; Chen et al. 1995; Chen and Moore 2003). All-stage resistance is race-specific and therefore, it is not durable because new virulent races of the pathogen can develop rapidly and circumvent the resistance (Line and Qayoum 1991; Chen 2004, 2005). HTAP resistance is durable and non-race specific (Chen and Line 1995), but it is more difficult to incorporate into commercial cultivars than all-stage resistance due to its multigenic or quantitative inheritance. Ideally, cultivars with a combination of effective all-stage resistance genes and HTAP resistance quantitative trait loci (QTL) should provide durable and high level resistance. However, it is time consuming and difficult to combine both all-stage and HTAP resistance genes with conventional breeding methods. Molecular markers may accelerate the process of developing barley cultivars with superior durable resistance.

Chen and Line (1999, 2003) identified at least 26 different genes in 18 barley genotypes for resistance to stripe rust. Of these genotypes, Grannenlose Zweizeilige (GZ) (PI 548740), an awnless, hulless, two-row barley from Ethiopia, has a recessive gene, temporarily designated as *rpsGZ*, conferring resistance to all races identified so far in the USA (Chen and Line 2003; X. M. Chen 2005, unpublished data). Therefore, it would be useful in combination with other all-stage resistance genes and HTAP resistance genes to obtain durable and superior resistance to *P. s. hordei* in barley breeding programs. However, the chromosomal location of *rpsGZ* was not determined. The population of F_8 recombinant inbred lines (RILs) from the Steptoe x GZ cross made in the previous study (Chen and Line 1999) is suitable for molecular mapping of this resistance gene.

The RGAP technique, developed by Chen et al. (1998), has proven to be an efficient technique in identification of molecular markers for disease resistance, and has been used successfully to develop molecular markers for resistance genes in wheat and barley against stripe rust, leaf rust, scald, net blotch, barley yellow dwarf, and scab (Chen et al. 1999; Toojinda et al. 2000; Shi et al. 2001; Yan et al. 2003; Pahalawatta and Chen 2005a, b). The objectives of this study were to identify RGAP markers for the resistance gene *rpsGZ* and to map the gene to a barley chromosome using wheat-barley addition lines and chromosome-specific SSR markers. The information of chromosomal location will lead to a better understanding of the plant-pathogen interaction. Molecular markers should be useful in marker-assisted selection to incorporate this gene into commercial cultivars and to combine with other genes to achieve durable resistance.

Materials and methods

The mapping population and evaluation for stripe rust resistance

The resistant barley genotype, GZ, was crossed with susceptible cultivar Steptoe using Steptoe as the female parent (Chen and Line 1999). F_8 RILs were developed from this cross through single-seed descent.

Races PSH-14 and PSH-54 of *P. s. hordei* were used to evaluate the parents and 182 F_8 RILs for resistance in the seedling stage under the controlled greenhouse conditions described by Chen and Line (1992). PSH-14 first identified in 1993 was virulent on Topper, Heils Franken, Emir, Astrix, Hiproly, Varunda, Abed Binder 12, and Trumpf; and PSH-54 first identified in 2001 was virulent on Topper, Abed Binder 12, Trumpf, and Bancroft of the 12 barley differential genotypes (Chen et al. 1995; Chen and Line 2001; Chen 2004). For each of the parents and F_8 progeny, 15–20 plants were tested. Seedlings at the two-leaf stage were inoculated with fresh spores of the two races in separate tests. Inoculated plants were placed in a dew chamber at 10°C for 18–24 h and then grown in a growth chamber at a diurnal temperature cycle gradually changing from 4°C at 2 am to 20°C at 2 pm with a 16 h light/8 h dark diurnal cycle. For each line, infection type (IT) based on a 0–9 scale (Line and Qayoum 1991) was recorded 20 days after inoculation. The RILs were classified as resistant (IT 0–3), susceptible (IT 7–9) and segregating based on the infection data.

DNA Extraction

Three grams of fresh seedling leaves from 15 or more plants for each line were ground in liquid nitrogen. DNA was extracted from the leaf powder following the protocol described by Riede and Anderson (1996), dissolved in 1X TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0), and stored at −20°C. DNA was quantified using the mini-gel method (Maniatis et al. 1982).

Bulk segregant analysis and testing markers with the RIL population

To quickly identify RGAP and SSR markers potentially associated with the resistance locus, bulk segregant analysis was used. Genomic DNA samples from GZ, Steptoe, and resistant and susceptible DNA bulks were used for screening primers. Each bulk DNA consisted of equal amounts of DNA from 20 F_8 homozygous RILs. Polymorphic bands specific to GZ and the resistant bulk or to Steptoe and the susceptible bulk

were tested in a subpopulation with 20 individual RILs. Bands that matched the phenotypic disease data were tested further in the remaining 162 of the 182 F_8 RILs. The segregation data of RGAP and SSR markers and the disease data obtained from the F_8 population were used to estimate the genetic linkage between the markers and the resistance locus. The M series was used to designate the RGAP markers.

RGAP analysis

The RGA primers (Chen et al. 1998; Shi et al. 2001; Yan et al. 2003) used in this study were designed based on conserved domains of cloned plant resistance genes (Table 1) and synthesized by Invitrogen (Carlsbad, California). The RGAP procedure described by Chen et al. (1998b) was used with a modification to the reaction volume and ingredient amount. For each PCR reaction, the 15 µl volume contained 30 ng of template DNA; 0.2 mM each of dCTP, dGTP, dTTP, and dATP (Promega, Madison, WI); 0.6 U of *Taq* DNA polymerase (Promega), 4 µM of each primer, 1.5 µL of 10× PCR buffer (Promega, Mg-free), and 5mM $MgCl_2$ (Promega). Amplification was performed in GeneAmp PCR system 9700 DNA thermocycler (Applied Biosystems, Forster City, California) programmed for 5 min at 94°C for initial denaturation and 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 7 min extension at 72°C.

After amplification, 7 µl of formamide loading buffer (98% formamide, 10 mM EDTA (pH 8.0), 0.5% (w/v) bromophenol blue, and 0.5% (w/v) xylene cyanol) was added to the PCR product. A 5 µl mix of PCR product and loading buffer was electrophoresed in a 1% agarose gel to check the success of the amplification.

Amplified DNA fragments were separated in a 5% denaturing polyacrylamide gel (398 mm × 338 mm × 0.4 mm), prepared as recommended by the manufacturer (Promega). A polymerized gel was pre-run in 1× TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0) for 50 min at 1,500 V, to bring the gel to 50°C. After urea and gel debris were removed from the well area using a 20 cc syringe, 6–7 µl of denatured PCR samples were loaded and the gel was run at 1,300 V for 2.5–3.5 h, depending on the approximate size of the bands of interest. After electrophoresis, the gel was silver-stained according to the manufacturer's recommendation (Promega, Madison, USA). The gel was dried at the room temperature overnight. A photograph of the gel was produced in a dark room using a silver-sequence automatic processor compatible film (Promega, Madison, USA).

Determination of chromosomal location of RGAP markers linked to the resistance locus

Wheat-barley disomic addition lines containing the entire wheat genome (cv Chinese Spring) with a single barley chromosome (cv Betzes) (Islam et al. 1981) were used to determine the chromosome location of the resistance gene. Ditelosomic addition lines in the wheat background (cv Chinese Spring) with a single barley chromosome arm (cv Betzes) (Islam 1983) were used to determine the specific chromosome arm for the gene. Six disomic addition lines for the barley chromosomes 1, 2, 3, 4, 6 and 7, and 13 ditelosomic addition lines of the 14 barley chromosome arms including 1α, 1β, 2L, 2S, 3L, 3S, 4L, 4S, 5S, 6L, 6S, 7L, and 7S were used to determine chromosomal location of the resistance gene to a barley chromosome using RGAP markers linked in repulsion to the resistance allele. The chromosomal location of the resistance locus determined by RGAP markers was confirmed with polymorphic SSR markers specific to the chromosome.

Table 1 Sequences, gene sources and conservative domains of the primers that produced polymorphic resistance gene analog polymorphic (RGAP) markers for the *rpsGZ* locus for resistance to *Puccinia striiformis* f. sp. *hordei*

RGAP primer	Sequence (5′→3′)	Gene	Domain[a]	Reference
XLRR-Rev	CCCATAGACCGGACTGTT	*Xa21*	LRR	Yan et al. 2003
RLRR-For	CGCAACCACTAGAGTAAC	*Rps2*	LRR	Chen et al. 1998
Wlrk-As	TGAGGGTCAGGCATGCAG	*Lr10*	Kinase	This study
Pto kin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Yan et al. 2003
Pto kin2	AGGGGGACCACCACGTAG	*Pto*	Kinase	Yan et al. 2003
Pto kin3	TAGTTCGGACGTTTACAT	*Pto*	Kinase	This study
NLRR-For	TAGGGCCTCTTGCATCGT	*N*	LRR	Chen et al. 1998
NLRR-Rev	TATAAAAAGTGCCGGACT	*N*	LRR	Chen et al. 1998
XLRR-INV1	TTGTCAGGCCAGATACCC	*Xa21*	LRR	Shi et al. 2001
XLRR-INV2	GAGGAAGGACAGGTTGCC	*Xa21*	LRR	Shi et al. 2001
Xa1NBS-F	GGCAATGGAGGGATAGG	*Xa1*	NBS	Yan et al. 2003
Cre3LR-R	CAGGAGCCAAAAATACGTAAG	*Cre3*	LRR	Yan et al. 2003

[a] *LRR* Leucine-rich repeat, *NBS* nucleotide-binding site

SSR analysis

Based on the RGAP data for the chromosome location of the resistance gene obtained with the wheat-barley addition lines tested with selected RGAP markers, 26 SSR markers of known map locations on chromosomes 4 (4H) and 5 (1H) were tested for polymorphism with genomic DNA from the parents and the F_8 RILs (Liu et al. 1996; Ramsay et al. 2000) to identify more markers and to confirm chromosomal locations of the resistance gene. SSR markers specific to chromosome 5 were tested because the wheat-barley addition lines for 5H and the long arm of 5H were not available. The PCR reaction mixture was the same as for the RGAP technique described above except that 2.5 mM of $MgCl_2$ was used in SSR analysis. Information on primer sequence, repeat motif, marker size and PCR amplification conditions for these SSR markers are available on line at http://www.genetics.org/cgi/content/full/156/4/1997/DC1 (Ramsay et al. 2000) and in Liu et al (1996). PCR products amplified by the SSR primers were first checked in a 1% agarose gel and then separated in a 5% denaturing polyacrylamide gel as described above for the RGAP technique. The polyacrylamide gel was run at 1,300 V for 2.0–2.5 h, which was shorter than that for the RGAP technique because the sizes of the bands of interest ranging from 116 to 249 bp are smaller than the RGAP bands.

Data analyses

The χ^2 test was used to analyze the segregating population for the stripe rust resistance obtained in this study. The probability of the goodness of fit (*P* value) for a test was obtained using the Chitest analysis in the Microsoft Office Excel Data analysis package. Linkage analyses and map construction of the RGAP and SSR markers and the resistance locus were performed with the computer program Mapmaker, version 3.0 (Lander et al. 1987). A logarithm of the likelihood ratio score of 3.0 or greater and Kosambi's mapping function (1944) were used to establish the linkage.

Results

Genetic and phenotypic analyses of stripe rust resistance in the Steptoe/GZ cross

In the tests with both races PSH-14 and PSH-54 of *P. s. hordei*, GZ was resistant with infection type (IT) 2 showing necrotic or chlorotic flecks without sporulation. Steptoe was susceptible with IT 8 showing abundant sporulation with chlorosis behind sporulating area. Of the 182 F_8 RILs tested with these races, 78 were homozygous resistant with IT 2, 1 was segregating with IT 2 and IT 8, and 103 were homozygous susceptible with IT 8, which fit into the expected ratio of 49.22, 1.56, and 49.22% ($\chi^2 = 1.28$, $P = 0.28$) of the resistant, heterozygous, and susceptible lines, respectively, for the F_8 generation developed through the single seed-descent method. Within the only one heterozygous line, the segregation of 3 resistant and 12 susceptible plants fit a ratio of 1:3 ($\chi^2 = 0.2$, $P = 0.65$). The results of the F_8 RIL population confirmed the previous report that GZ has a single recessive gene for stripe rust resistance using the plants of F_1, F_2 and F_3 generations of the same cross (Chen and Line 1999).

RGAP markers associated with the *rpsGZ* locus

Out of 73 primer pairs from random combinations of individual 48 RGAP primers that were screened in the bulk segregant analysis, 31 (42%) pairs produced 38 polymorphic bands that differentiated the susceptible parent (SP) and the susceptible bulk (SB) from the resistant parent (RP) and the resistant bulk (RB). Seven primer pairs that generated 12 strong and repeatable polymorphic bands were selected for linkage analysis. The fragment sizes and primer pairs of the 12 RGAP markers are shown in Table 2. The RGAP markers M2 and M3 were co-dominant, and the other markers were dominant. Of the 12 RGAP markers, eight markers were present in SP and SB but not in RP and RB, and only four markers were present in RP and RB but not in SP and SB (Table 2). As examples, the banding patterns of RGAP markers *M7* and *M8*, which co-segregated the *rpsGZ* locus, and *M5*, which was tightly linked to the resistance locus in polyacrylamide gels are shown in Fig. 1.

Mapping *rpsGZ* to the long arm of barley chromosome 4H using the wheat-barley addition line

To determine the chromosomal location of the resistance gene, RGAP markers, *M7*, *M8*, *M5* and *M6*, which were present in SP and SB but not RP and RB were tested with Chinese Spring, Betzes and two sets of wheat (Chinese Spring)-barley (Betzes) chromosome addition lines. The unique bands were detected with the markers *M5* and *M6* in Steptoe, Betzes, the wheat line with barley chromosome 4 (4H) and the wheat line with the long arm of the chromosome 4, but not in GZ, Chinese Spring and all the other wheat-barley addition lines. As an example, the banding pattern of two sets of the wheat-barley addition lines in

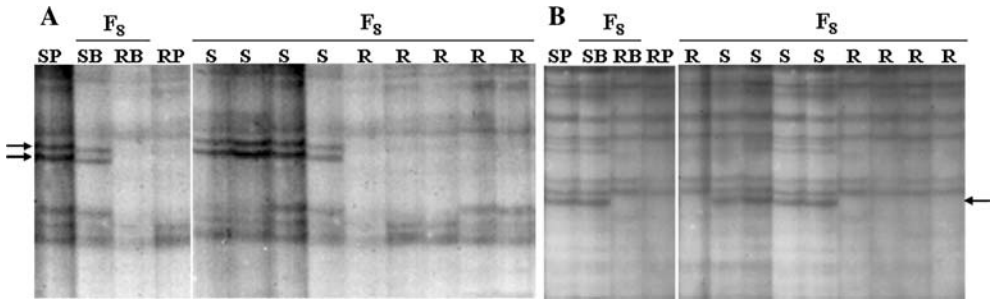

Fig. 1 Silver stained polyacrylamide gels showing the resistance gene analog polymorphism (RGAP) markers. **a** Markers *M7* (indicated by the *left upper arrow*, 259 bp) and *M8* (indicated by the *left lower arrow*, 257 bp) amplified with RGA primers NLRR-For (5′-TAGGGCCTCTTGCATCGT-3′) and NLRR-Rev (5′-TATAAAAAGTGCCGGACT-3′) were completely co-segregated with the *rpsGZ* locus in182 F_8 lines. **b** Marker *M5* (indicated be the *right arrow*, 350 bp) amplified with RGA primers Wlrk-As (5′-TGAGGGTCAGGCATGCAG -3′) and Pto kin1(5′- GCATTGGAACAAGGTGAA- 3′) was closely linked to the *rpsGZ* gene with a genetic distance of 2.0 cM in the 182 F_8 lines. *SP* the susceptible parent, Steptoe; *SB* the susceptible bulk; *RB* the resistant bulk; *RP* the resistant parent, GZ; *S* individual homozygous susceptible F_8 line; and *R* individual homozygous resistant F_8 line

Table 2 Resistance gene analog polymorphism (RGAP) markers associated with the *rpsGZ* locus for resistance to *Puccinia striiformis* f. sp. *hordei*

RGAP			Present/absent[b]			
Marker	Primer pair	Size (bp)[a]	SP	SB	RB	RP
M1	XLRR-Rev/RLRR-For	200	–	–	+	+
M2	XLRR-Rev/RLRR-For	198	–	–	+	+
M3	XLRR-Rev/RLRR-For	195	+	+	–	–
M4	XLRR-Rev/RLRR-For	193	+	+	–	–
M5	Wlrk-AS/Pto kin1	350	+	+	–	–
M6	Pto kin1/Pto kin3	188	+	+	–	–
M7	NLRR-For/NLRR-Rev	259	+	+	–	–
M8	NLRR-For/NLRR-Rev	257	+	+	–	–
M9	XLRR-INV1/XLRR-INV2	450	–	–	+	+
M10	XLRR-INV1/XLRR-INV2	448	–	–	+	+
M11	Pto kin1/Pto kin2	400	+	+	–	–
M12	Xa1NBS-F/Cre3LR-R	480	+	+	–	–

[a] Sizes of all markers were estimated based on 1-kb plus DNA marker

[b] *SP* the susceptible parent, *SB* the susceptible bulk, *RB* the resistant bulk, *RP* the resistant parent, (+) present, (−) absent

polyacrylamide gels tested with the RGAP marker *M6* is shown in Fig. 2. These results indicate that the RGAP markers and therefore the linked resistance gene are located on the long arm of chromosome 4.

Identification of SSR markers and confirmation of chromosomal location of the resistance gene

To identify more markers for and confirm the chromosomal location of the resistance gene, a total of 26 SSR markers covering both long arm and short arm of chromosomes 4H and 1H (Liu et al. 1996; Ramsay et al. 2000) were screened using the two parents and the two bulks. None of the screened SSR markers specific to chromosome 1H was associated with the resistance locus. The results excluded the possibility of the resistance gene on chromosome 1H. Four markers *EBmac0679* (amplified with primer pair F: 5′-ATTGGAGCGGATTAGGAT-3′ and R: 5′-CCCTATGTCATGTAGGAGATG-3′), *EBmac0701* (amplified with primer pair F: 5′-ATGATGAGAACTCTTCACCC-3′ and R: 5′-TGGCACTAAAGCAAAAGAC-3′), *WMS6* (amplified with primer pair F: 5′-CGTATCACCTCCTAGCTAAACTAG-3′ and R: 5′-AGCCTTATCATGACCCTACCTT-3′) and *Bmag0138* (amplified with primer pair F: 5′-ACCAGGAGGAATGAGAGAG-3′ and R: 5′-AATAAACCTTGAGACGATGG-3′) produced polymorphic bands that were specific to both resistant parent and resistant bulk or both susceptible parent and susceptible bulk. *EBmac0679* (148 and 142 bp), *WMS6* (205 and 201 bp) and *Bmag0138* (169 and 167 bp) were co-dominant markers and *EBmac0701* (149 bp) was a dominant marker. Linkage analysis using the four markers with the 182 F_8 RILs indicated that they were linked to the *rpsGZ* gene. As an example, the banding pattern of the closest marker *EBmac0679* in polyacrylamide gels tested with a set of F_8 lines is shown in Fig. 3. Because these four SSR markers are associated with the long arm of barley chromosome 4H (Ramsay et al. 2000) and linked to the *rpsGZ* locus, these results further confirmed that the *rpsGZ* gene was on the long arm of chromosome 4 (4H).

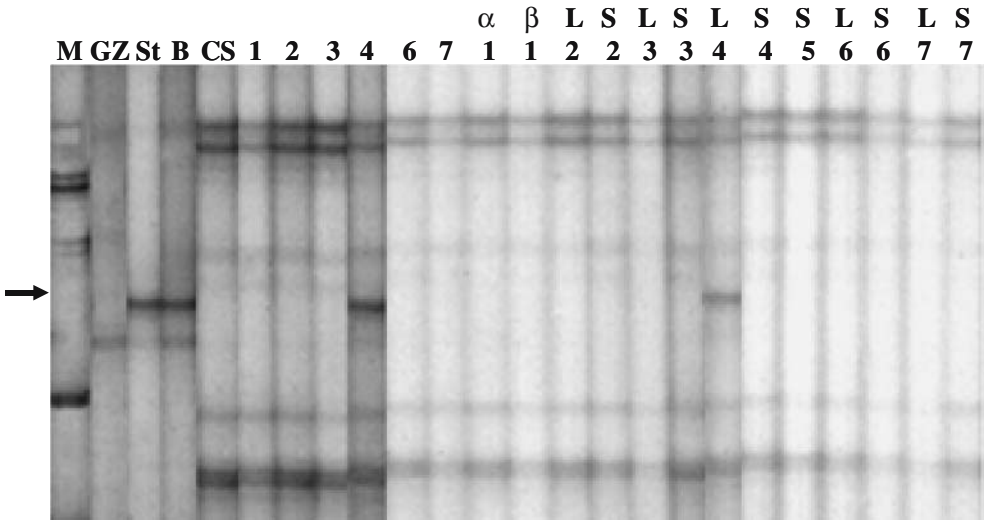

Fig. 2 Silver stained polyacrylamide gel showing the banding pattern of the wheat-barley chromosome addition lines tested with the resistance gene analog polymorphism (RGAP) marker *M6* (shown by *arrow*, 188 bp) amplified with Pto kin1 (5′-GCATTGGAACAAGGTGAA - 3′) and Pto kin3 (5′-TAGTTCGGACGTTTACAT-3′). The marker was present in the susceptible parent Steptoe (St), barley cultivar Betzes (B), the line with barley chromosome 4 (4H) and the line with the long arm of chromosome 4, but was absent in the resistant parent GZ, wheat cultivar Chinese Spring (CS) and all other wheat-barley chromosome addition lines. M stands for 1-kb plus DNA marker; 1- 6 represent disomic addition lines with barley chromosomes 1, 2, 3, 4, 6 and 7, respectively; 1α, 1β, 2L, 2S, 3L, 3S, 4L, 4S, 5S, 6L, 6S, 7L, 7S are 13 ditelosomic addition lines of the 14 barley chromosome arms

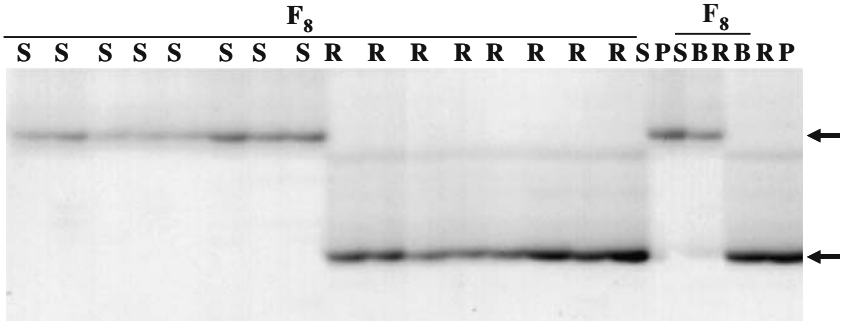

Fig. 3 Silver stained polyacrylamide gel showing the closest SSR marker *EBmac0679* (*upper arrow*, 148 bp; *lower arrow*, 142 bp) amplified with forward primer (5′-ATTGGAGCGGATTAGGAT-3′) and reverse primer (5′-CCCTATGTCATGTAGGAGATG-3′). *S* individual homozygous susceptible F_8 line; *R* individual homozygous resistant F_8 line; *SP* the susceptible parent, Steptoe; *SB* the susceptible bulk; *RB* the resistant bulk; and *RP* the resistant parent, GZ

Construction of linkage group for the resistance locus

Genetic relationships of the RGAP and SSR markers with the *rpsGZ* resistance gene were determined by linkage analysis. The linkage group (Fig. 4) was constructed with 12 RGAP markers and four SSR markers tested with 182 F_8 lines. Two RGAP markers *M7* and *M8* completely co-segregated with the resistance locus, and *M5* and *M6* were closely linked to the locus with a genetic distance of 0.9 and 2.0 cM, respectively. However, these four markers were present in the susceptible parent but not in the resistance parent. The closest marker, *M9* that was linked to the resistance allele, had a genetic distance of 11.7 cM with the *rpsGZ* allele. The four SSR markers were linked to the resistance allele. The closest SSR marker to the gene was *EBmac0679* with a genetic distance of 7.5 cM. The other three SSR markers *EBmac0701*, *WMS6* and *Bmag0138* were linked to the resistance locus with a genetic distance of 9.9, 17.4 and 23.3 cM, respectively.

Discussion

The RGAP technique, which was first developed by Chen et al. (1998), has proven to be highly efficient to identify molecular markers for plant disease resistance genes. It has been used successfully to map barley

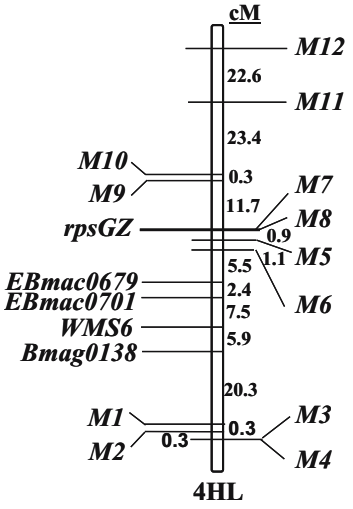

Fig. 4 Linkage map for the recessive resistance gene *rpsGZ* constructed with 12 resistance gene analog polymorphism (RGAP) markers (*M1* to *M12*) and four SSR markers (*EB mac0679*, *EB-mac0701*, *WMS6* and *Bmag0138*). The markers linked to the resistance allele at the *rpsGZ* locus are shown on the left and the RGAP markers co-segregating with or linked to the susceptibility allele at the *rpsGZ* locus are shown on the *right*. The linkage on chromosome 4L (4HL) was determined by analyzing the wheat-barley chromosome addition lines with RGAP markers *M5* and *M6*, and confirmed by the four SSR markers

genes for resistance to stripe rust, leaf rust, scald, net blotch, barley yellow dwarf, and scab (Chen et al. 1999; Toojinda et al. 2000; Pahalawatta and Chen 2005b), and to map wheat genes for all-stage resistance and QTL for durable HTAP resistance against stripe rust (Chen and Line 2000; Shi et al. 2001; Yan et al. 2003; Pahalawatta and Chen 2005a). Several RGAP markers that were coincident with resistance to different diseases were found in previous studies (Chen et al. 1999; Toojinda et al. 2000; Shi et al. 2001; Chen et al. 2003; Yan et al. 2003). In the present study, 38 potential RGAP markers were identified for the resistance gene *rpsGZ* in barley genotype GZ using 79 RGA primer pairs in bulked segregant analyses. Associations of 12 of the RGAP markers with *rpsGZ* were tested with the F_8 RIL population. Two of the markers co-segregated with the resistance locus, and another two markers were tightly linked to the locus. These results consistently demonstrate that the RGAP technique is highly efficient for identifying completely associated or tightly linked markers for plant disease resistance genes.

In this study, eight of the 12 RGAP markers mapped in the linkage group were detected only in the susceptible parent but not in the resistant parent. This phenomenon could be related to the factor that *rpsGZ* is a recessive resistance gene. Our hypothesis is that the dominant susceptibility allele may be a wild type gene and the recessive resistance allele may be a deletion at the locus. To date, many dominant resistance genes in plants have been cloned but few recessive genes have been cloned (Li et al. 2001). The products of dominant resistance genes are presumed to act as receptors which may interact directly or indirectly with the elicitors produced by pathogens to cause resistant responses. However, three characterized recessive resistance genes, *mlo*, *edr1* and *RRS1-R*, appear to produce different products and have different functions from most known dominant resistance genes that share conserved domains (Chu et al. 2004). More studies are needed in order to understand the formation, structure, and function of the recessive genes and the difference from dominant genes. Molecular cloning of the *rpsGZ* recessive resistance gene would lead a better understanding of the gene structure and organization. The two co-segregating markers and the two closely linked markers identified in this study should be a start point for cloning *rpsGZ*.

The resistance gene *rpsGZ* was mapped for the first time on the long arm of barley chromosome 4H using wheat-barley chromosome addition lines with the two closely linked RGAP markers in combination with the use of genetically mapped barley SSR markers. The use of wheat-barley addition lines is an efficient method for mapping genes to barley chromosomes (Muehlbauer et al. 2005; Liu et al. 1996; Nagy et al. 2002; Cannell et al. 1990). The two sets of addition lines used in this study showed consistent results that the unique bands for the gene were only detected on the addition lines with chromosome 4 and 4L, but not 4S and lines with other chromosomes and chromosomal arms added. For chromosome 5 (1H), only a ditelosomic addition line for 5L was available (Muehlbauer et al. 2005) in this study. The 26 SSR markers which covered both long and short arms of chromosomes 4 and 5 were tested. Four markers that are only associated with chromosome 4L (Liu et al. 1996; Ramsay et al. 2000) produced polymorphisms among SP, SB, RB and RP, and linked to the gene within genetic distances ranging from 7.5 to 23.3 cM, which excludes the possibility of the *rpsGZ* gene being located on chromosome 5. These results indicate that the combination of wheat-barley addition line assay and SSR analysis is an efficient way to determine the correct chromosome location for a resistance gene in barley. The use of addition line can narrow down the gene on a specific chromosome and save time in screening SSR primers. On the other hand, the use of SSR analysis can overcome the drawback of missing one line in wheat-barley addition lines and also allow

integrating the RGAP markers into the known SSR-based linkage map of barley.

The sequential order of the four SSR markers in the linkage map (Fig. 4) were generally similar to that presented in Ramsay et al. (2000), except that the orientation of EBmac 0679 and EBmac0701 was reversed in our linkage map. The genetic distance between WMS6 and Bmag0138 were similar (5.9 cM in our map and 7.0 cM in Ramsay et al.'s map) while the distance between EBmac0701 and WMS6 decreased from 32 cM in their map to 7.5 cM in our map and that between EBmac0701 and EBmac0679 decreased from 7 cM in their map to 2.4 cM in our map. These differences might be due to chromosomal rearrangement and DNA deletion or insertion within this region in different barley genotypes.

Chen et al. (1994) reported a gene for seedling resistance to stripe rust in Calicuchima-sib barley on chromosome 4 (4H). The gene was incorporated into barley cultivar Orca (Hayes et al. 2000). The *rpsGZ* gene on chromosome 4H is likely different from the gene originally described from Calicuchima-sib because Calicuchima-sib was susceptible in the seedling stage to race PSH-31 and Orca was susceptible to races PSH-1 and PSH-13, while GZ is resistant to all races identified so far in the USA (X. M. Chen, unpublished data). Further studies are needed to determine whether these genes are allelic or closely linked. It has been reported that GZ has the *mlo11* gene for resistance to powdery mildew and the *mlo* locus is near the centromeric region on chromosome 4HL (Jorgensen 1992; Simons et al. 1997). It is interesting to determine the genetic relationship of *rpsGZ* and *mlo11* because a close linkage of the two genes will facilitate the incorporation of resistance to both stripe rust and powdery mildew into barley cultivars.

Although the RGAP markers co-segregating with the *rpsGZ* locus may not be used to directly select the resistance allele, they are still useful for selection of homozygous resistant plants by the absence of the susceptibility-specific bands as long as a barley line with the *rpsGZ* resistance gene is used to incorporate the resistance into commercial cultivars. In this study, we only tested Bestzes barley in addition to the susceptible parent, Steptoe. For marker-assisted selection, the markers co-segregating with the susceptible allele at the *rpsGZ* locus should be further tested for their presence in cultivars, into which the gene is to be incorporated. Because GZ, is an Ethiopia landrace and does not have a desirable plant type, the F_8 progeny lines with the resistance gene and the well-adapted Steptoe plant type should serve as better *rpsGZ* sources for developing barley cultivars with resistance to stripe rust. Although *rpsGZ* is highly effective against all races of *P. s. hordei* identified so far, it may not provide long-lasting resistance because the pathogen may develop new virulent races overcoming the resistance. Therefore, the gene should be used in combination with other genes for developing cultivars with durable resistance.

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