Theor Appl Genet (2005) 111: 1651–1660
DOI 10.1007/s00122-005-0100-6

ORIGINAL PAPER

J. A. Mammadov · B. J. Steffenson · M. A. Saghai Maroof

High-resolution mapping of the barley leaf rust resistance gene *Rph5* using barley expressed sequence tags (ESTs) and synteny with rice

Received: 6 March 2005 / Accepted: 1 September 2005 / Published online: 30 September 2005

Abstract The rapidly growing expressed sequence tag (EST) resources of species representing the *Poacea* family and availability of comprehensive sequence information for the rice (*Oryza sativa*) genome create an excellent opportunity for comparative genome analysis. Extensive synteny between rice chromosome 1 and barley (*Hordeum vulgare L.*) chromosome 3 has proven extremely useful for saturation mapping of chromosomal regions containing target genes of large-genome barley with conserved orthologous genes from the syntenic regions of the rice genome. *Rph5* is a gene conferring resistance to the barley leaf rust pathogen *Puccinia hordei*. It was mapped to chromosome 3HS, which is syntenic with rice chromosome 1S. The objective of this study was to increase marker density within the sub-centimorgan region around *Rph5*, using sequence-tagged site (STS) markers that were developed based on barley ESTs syntenic to the phage (P1)-derived artificial chromosome (PAC) clones comprising the distal region of rice chromosome 1S. Five rice PAC clones were used as queries in a blastn search to screen 375,187 barley ESTs. Ninety-four non-redundant EST sequences were identified from the EST database and used as templates to design 174 pairs of primer combinations. As a result, 9 barley EST-based STS markers were incorporated into the 'Bowman' × 'Magnif 102' high-resolution map of the *Rph5* region. More importantly, six markers, including five EST-derived STS sequences, were found to co-segregate with *Rph5*. The results of this study demonstrate the usefulness of rice genomic resources for efficient deployment of barley ESTs for marker saturation of targeted barley genomic regions.

Communicated by Q. Zhang.

J. A. Mammadov · M. A. S. Maroof (✉)
Department of Crop Soil Environmental Sciences,
Virginia Tech, Blacksburg, VA, 24061-0404 USA
E-mail: smaroof@vt.edu
Tel.: +1-540-2319791
Fax: +1-540-2313431

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

Introduction

Comparative genetic mapping in several economically important crops, particularly rice and barley (Saghai Maroof et al. 1996), revealed the conservation of gene or marker order (colinearity) within large portions of the genomes of these two species due to low numbers of chromosomal rearrangements during their evolution. According to Moore (1995), individual rice chromosome arms or complete chromosomes can be assembled like "Lego" blocks to reconstitute the chromosomes of large-genome grass species. Comparison of sequences from putatively syntenic loci in rice and other grass species demonstrated that sequence conservation between the species is mainly restricted to coding regions (Avramova et al. 1996; Tikhonov et al. 1999; Dubcovsky et al. 2001). The extensive synteny reported between rice chromosome 1 and barley chromosome 3 has been extremely useful for saturation mapping of chromosomal regions containing target genes of large-genome barley (Smilde et al. 2001) with conserved orthologous genes from syntenic regions of the rice genome (Bennetzen and Freeling 1993). From this point of view, comprehensive datasets of 375,187 barley expressed sequence tags (ESTs), which represent portions of the coding regions (summary dbEST release 14 January 2005, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html), are rich resources for genome mapping (Holton et al 2002; Thiel et al. 2003; Varshney et al. 2005).

Draft genome sequences of two rice subspecies [*Oryza sativa ssp. indica* (Yu et al. 2002) and *Oryza sativa ssp. japonica* (Goff et al. 2002)] and high-quality sequences of rice chromosomes 1 (Sasaki et al. 2002) and 4 (Feng et al. 2002) have been recently reported. In contrast to rice, the large-genome crop of barley is unlikely to be targeted for full genome sequencing in the near future.

This is reflected by the limited availability of complete sequences of genomic bacterial artificial chromosome clones (BAC) of barley, which resulted from attempts of targeted gene isolation and studies of microcolinearity among grass species (Büschges et al. 1997; Lahaye et al. 1998; Wei et al. 1999; Druka et al. 2000; Collins et al. 2001; Dubcovsky et al. 2001). Hence, available rice genomic sequences as well as barley ESTs serve as excellent resources for the saturation mapping of the target region with gene-based markers and, consequently, may facilitate synteny-based positional cloning of agronomically important genes from large genome cereal species (Perovic et al. 2004).

Few studies have focused specifically on synteny-based marker saturation around genes of interest in the barley genome. Exploiting the synteny between rice chromosome 4 and barley chromosome 2, Schmierer et al. (2003) mapped 26 barley ESTs to the region containing a major effect QTL for Fusarium head blight resistance. These barley ESTs were chosen for mapping purposes based on their homology to the rice BAC clones comprising rice chromosome 4. Perovic et al. (2004) integrated 11 barley ESTs into an *rph16* (barley leaf rust resistance gene) high-resolution map using synteny between barley chromosome 2 and rice chromosomes 4 and 7 L (Dunford et al. 2002). In both studies, the target regions for marker saturation were quite large, 20 and 40 cM, respectively.

The objective of this study was to increase marker density within the sub-centimorgan region around the barley leaf rust resistance gene *Rph5*, using sequence-tagged site (STS) markers developed based on barley ESTs syntenic to BAC and PAC clones comprising rice chromosome 1. *Rph5* confers resistance to many of the most prevalent barley leaf rust races in the United States. Although virulence for *Rph5* in *P. hordei* is widely prevalent in Europe (Parlevliet 1976) and South America (Brodny and Rivadeneira 1996; Fetch et al. 1998), it has not been identified in North America. Thus, *Rph5* could be used to protect barley cultivars from leaf rust damage in North America. *Rph5* was recently mapped onto the extreme telomeric region of the short arm of barley chromosome 3H in a 'Bowman' (*rph5*) × 'Magnif 102' (*Rph5*) population (Mammadov et al. 2003). In this study, an initial F_2 population of 93 individuals, developed from a 'Bowman' × 'Magnif 102' cross, was expanded to 400 individuals. Rice clone C970, which mapped to the syntenous region in rice chromosome 1, was used as an anchor marker for saturation and construction of a high-resolution map around the *Rph5* locus.

Materials and methods

Genetic materials

An F_2 population derived from the cross 'Bowman' (PI 483237) × 'Magnif 102' (PI 337140) was used for high-resolution mapping of *Rph5*. Magnif 102 (Franckowiak et al. 1997) is the donor of *Rph5*, whereas Bowman is a cultivar susceptible to all known leaf rust races. The expanded F_2 mapping population (400 individuals) was developed by combining the original F_2 population (93 individuals) with an additional 307 F_2 individuals. The 400 F_2 individuals were used for high-resolution mapping of *Rph5*.

Disease screening

Leaf rust evaluations were done according to established protocols. Briefly, 15 F_3 seeds per F_2 plant were sown in plastic pots (12.7×12.7 cm^2) filled with a 50:50 mix of soil:potting media (Metro Mix 200: vermiculite, peat moss, perlite and sand; Scotts-Sierra Horticultural Products Company, Marysville, OH) and grown at 18–23°C in a greenhouse. Fertilizer was applied at planting, with water-soluble (15-0-15, N-P-K, 0.2 g per seedling) and controlled-release (14-14-14, N-P-K, 0.1 g per seedling) formulations. When the primary leaves of plants were fully expanded (7 days after planting), inoculations were made with heat-shocked urediniospores of isolate ND8702 of race 8 suspended in a light-weight mineral oil (3.5–5.0 mg spores/0.7 ml oil). Inoculum was applied using an atomizer pressured by an air pump at 27.5 kPa. Plants were then placed in mist (generated by ultrasonic humidifiers) chambers and incubated at 20–23°C for 16 h in the dark at near 100% RH. After the mist period, plants were allowed to slowly dry for at least 3 h before being returned to the greenhouse under the same conditions described above. Nine to twelve days after inoculation, the infection types on plants were rated according to the 0–4 scale of Levine and Cherewick (1952). Infection types of 0, 1, or 2 were considered indicative of host resistance, whereas infection types 3 or 4 were considered indicative of host susceptibility. Infection types of F_2 progeny were compared with infection types of the parental lines and host-differentials to ensure proper scoring and assignment into resistant and/or susceptible classes.

Development of barley STS markers

The entire sequences of 5 rice PAC [phage (P1)-derived artificial chromosome] clones syntenic to the *Rph5* region (see Results) were used as queries in a blastn (Altschul et al. 1997) search to screen 370,258 barley ESTs stored in the EST database (dbEST) of the National Center for Biotechnology Information (NCBI). Barley ESTs with an expected (*E*) value equal to or less than 1 e^{-10} were selected for the construction of temporary contigs (TCs), using the SeqMan tool of Lasergene software (DNASTAR, Madison, WI, USA). Consensus sequences of TCs, as well as sequences of the singletons, were used as a template to design EST-specific primers using the Primer Select tool of Lasergene software. Simple sequence repeats (SSRs) and indels

(insertions and deletions) were targeted for primer design. Indels were detected as a result of alignment of redundant EST sequences representing one TC, which originated from EST libraries of different barley cultivars, including cvs. 'Morex', 'Haruna Nijo', 'Sloop', 'Akashinriki', 'Golden Promise', 'Barke', 'CI16151', and 'Optic'. If SSRs and indels were absent, the entire consensus sequences of the TCs were used as queries to screen the GenBank database using the blastn program. The query and the best non-barley hit were then aligned using the Megalign tool of Lasergene software. If alignment showed large gaps between the two sequences, primers were designed to flank those gaps. PCR amplification was carried out according to published procedures (Saghai Maroof et al. 1994; Liu et al. 1996; Ramsay et al. 2000).

Mapping of other molecular markers to the *Rph5* region

In addition to barley ESTs, we tested 28 wheat STS markers (a gift from J.A. Anderson (University of Minnesota, St. Paul) in our Bowman × Magnif 102 mapping population. These STS markers were mapped by Liu and Anderson (2003) to chromosome 3BS of wheat as part of an effort to map a QTL conferring resistance to Fusarium head blight. Since this region is syntenic to barley chromosome 3 HS, an attempt was made to map these wheat STS markers.

The RFLP marker ABG70 is located in the telomeric region of chromosome 3HS based on A. Kleinhofs' Bins at http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html. Using sequence information provided at NCBI for ABG70 (Accession no. L44083), an STS marker was developed.

Linkage analysis

Disease and marker data were used to determine the chromosomal location of *Rph5*. For genetic mapping and linkage analysis, the computer program MAPMAKER version 3.0b was used (Lander et al. 1987). Linkage maps were constructed based on a LOD threshold of 3.0 and maximum Haldane distance of 50 cM.

Results

Identification of syntenic rice chromosomal region for *Rph5* target region

The barley leaf rust resistance gene *Rph5* was genetically mapped between two RFLP markers VT1 and C970 in an initial F_2 population of 93 individuals. The region between these two markers, flanking the locus, was selected as a target for saturation mapping with barley gene-based markers syntenic to rice chromosome 1S. Significantly, rice clone C970 also mapped to the telomeric region of rice chromosome 1S (http://www.gramene.org/japonica/contigview?chr=1&vc_start=645764&vc_end=646132&bottom=%7Cbump_Markers%3Aon). Thus, the locus of barley chromosome 3HS is syntenous to the distal region of chromosome 1S of rice.

In order to delimit the syntenic region in rice, nucleotide sequences of C970 (GenBank accession no. D15622) and VT1 were used as queries to screen the GenBank database using the blastn program (Altschul et al. 1997). The blastn search was limited to the *Oryza sativa* non-redundant (NR) database. C970 detected two rice PAC clones AP003219 and AP002882. VT1 did not reveal any significant similarities with rice sequences. In order to determine the location of the C970 clone with respect to the retrieved PAC clones, the entire sequences of the two PAC clones and C970 were used to construct TCs. The results indicated that C970 is located at the intersection of the two rice PAC clones (Fig. 1). In addition to C970 and VT1, the sequence of RFLP clone CDO549, which was previously mapped 0.2 cM distal from VT1 in the Bowman × Magnif 102 population, was used as a query to screen the rice NR database using the blastn program. CDO549 retrieved the rice PAC clones AP002845 and AP002863 with a significant E value (9e-33) (Table 1). Thus, the *Rph5* locus, spanning the interval between markers C970 and CDO549 in barley, was syntenic to the segment of rice chromosome 1 S bordered by the proximal and distal rice PAC clones AP003219 and AP002863, respectively. Apart from these two PAC clones, this region of the rice genome is represented by AP002882, AP002845, and AP002818, which are contiguous to AP003219 and AP002863 (http://www.gramene.org/japonica/SeqTable?chr=1). The genomic sequences of these five rice PAC clones were blastned against the barley dbEST. These five rice PAC clones detected a total of 3,962 barley ESTs. The level of redundancy among the blastn hits was quite high. In order to remove the redundancy, the EST sequences were assembled into TCs. This approach reduced the final number of candidate gene fragments to 94, which were either represented by EST singletons or EST TCs.

Saturation of the *Rph5* region with gene-based markers

In total, 94 non-redundant barley EST sequences were used to design primers for PCR amplification. Primers were designed to flank the source of potential polymorphisms, including microsatellites and indels. We targeted PCR products smaller than 300 bp to enable polyacrylamide gel electrophoresis (PAGE) assays. When the sources of polymorphism were scattered within one TC or singleton of 1 kb + size, we designed several primer combinations in order to cover the whole span of the target sequence. We designed 174 primer combinations to amplify the 94 non-redundant EST sequences syntenic to the five rice PAC clones. Out of the

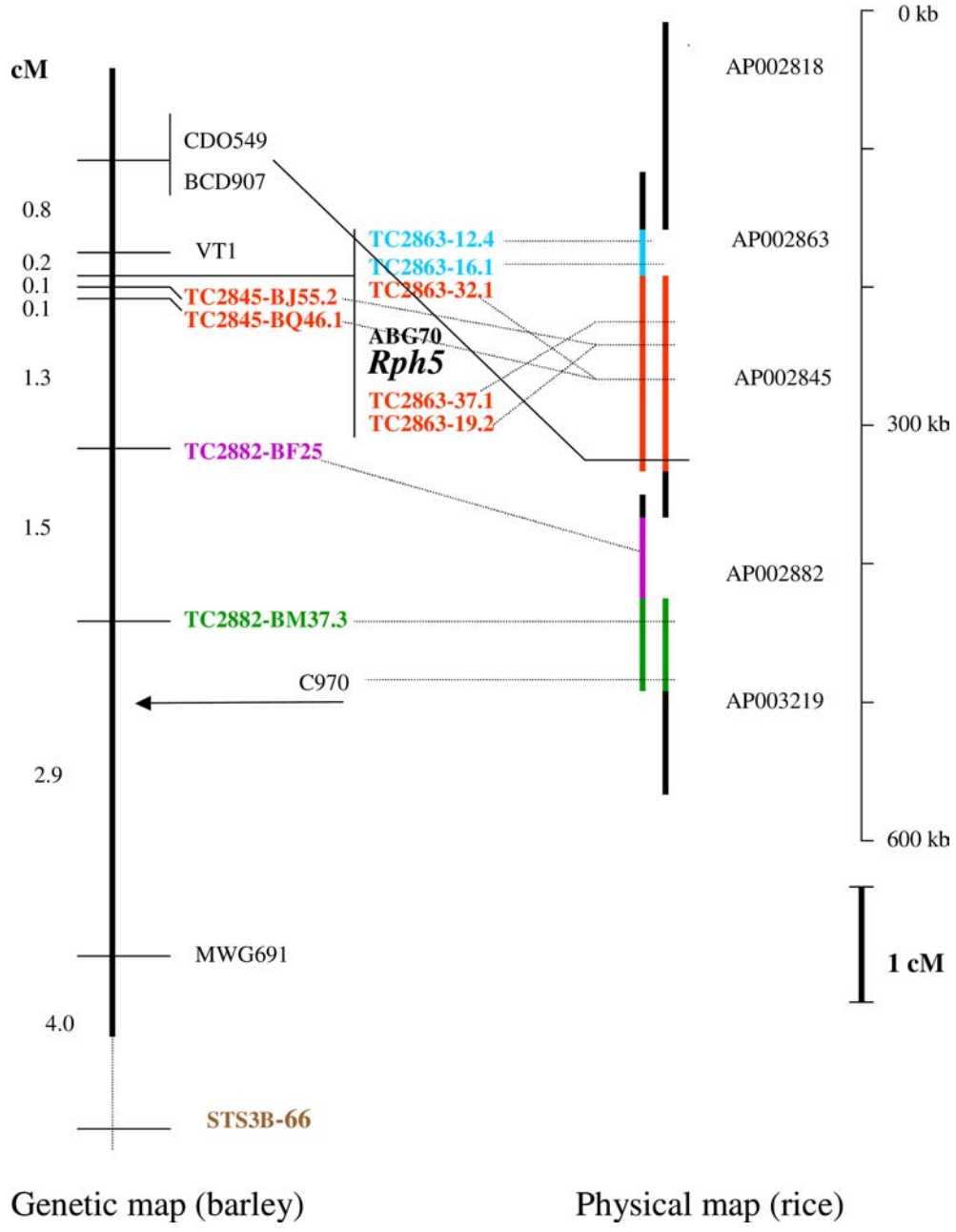

Fig. 1 Comparative mapping of the region in barley and rice. High-resolution genetic map of the *Rph5* region resulting from the 'Bowman' × 'Magnif 102' population. Molecular markers shown in *color* are barley EST-based STS sequences. Markers, previously mapped in Mammadov et al. (2003), are shown in regular font. The approximate location of the rice RFLP clone C970 is indicated by an *arrow*. This clone was previously tested in 93 F_2 individuals (Mammadov et al. 2003) only, and was not used in this study for linkage analysis. Since C970 is 12 kb proximal from the segment of the rice clone AP003219, which is syntenic to TC2882-BM-37.3 (http://www.gramene.org/japonica/contigview?chr=1&vc_start=645764&vc_end=646132&bottom=%7Cbump_Markers%3Aon), we assumed that, genetically, this clone is also located proximal to TC2882-BM-37.3. Physical map of the 585-kb-length distal portion of the rice chromosome 1S syntenic to the *Rph5* region of barley chromosome 3HS, represented by overlapping rice PAC clones, is depicted on the right side of Fig. 1. The length of the rice contig was calculated using PAC coordinates on the rice chromosome 1S (http://www.gramene.org/japonica/SeqTable?chr=1). The *color code* of the rice PAC clones corresponds to the *color code* of the syntenic polymorphic EST-based STS markers. Portions of the PAC clones shown in *black* are syntenic to the barley ESTs, which were monomorphic in our mapping population. The distal part of each PAC clone is its 5′ end and proximal 3′ end. *Dotted lines* connecting barley genetic map with rice physical map indicate colinear markers between barley and rice, while *solid lines* show deviation from microcolinearity. Several EST-derived STS markers are syntenic to the same region within the rice PAC clone. Hence, lines from these markers are projected to the single point on the rice PAC clone

94 EST sequences, 9 were polymorphic between the two parental lines and were subsequently mapped to a region of 6.1 cM between markers VT1 and MWG691 (Fig. 1). EST sequences syntenic to a portion of rice PAC clone AP003219 (but non-overlapping with AP002882), as well as to the entire AP002818 clone (Fig. 1, shown in

Table 1 *Rph5* markers and syntenous rice PAC [phage (P1)-derived artificial chromosome] clones

Rph5 markers	Rice PAC clones	Sequences within rice PAC clones syntenic to *Rph5* region		Score	*E*-value
		Beginning	End		
TC2863-12.4	AP002863	70888	70947	224	4e-55
TC2863-16.1	AP002863	83022	82966	93	3e-17
TC2863-32.1	AP002863	165652	165712	80	5e-13
	AP002845	54075	54122	80	5e-13
TC2845-BQ46.1	AP002863	165652	165759	80	5e-13
	AP002845	54015	54122	80	5e-13
TC2845-BJ55.2	AP002863	169412	169662	246	5e-62
	AP002845	57775	58025	246	5e-62
TC2863-19.2	AP002863	169412	170059	355	1e-95
	AP002845	57775	58422	355	1e-95
CDO549	AP002863	177705	177828	143	9e-33
	AP002845	66068	66191	143	9e-33
TC2882-BF25	AP002882	24711	24807	129	7e-28
TC2882-BM37.3	AP002882	105798	105907	74	8e-12
	AP003219	14937	15046	74	8e-12
C970	AP002882	121902	122935	2008	0.0
	AP003219	31041	32061	2008	0.0

black color) were monomorphic between the two parents. Two markers TC2882-BF25 and TC2882-BM37.3 were mapped 1.5 and 3.0 cM proximal to *Rph5*, respectively (Fig. 1). The former is syntenic to the rice PAC clone AP002882, while the latter is syntenic to the region of the rice genome located at the intersection of AP002882 and AP003219 (Table 1 and Fig. 1). Markers TC2845-BJ55.2 and TC2845-BQ46.1 were syntenic to both rice PAC clones AP002845 and AP002863 (Table 1 and Fig. 1) and were located 0.1 and 0.2 cM, respectively, proximal to *Rph5* (Fig. 1). Remarkably, five EST markers, TC2863-37.1, TC2863-16.1, TC2863-12.4, TC2863-32.1 and TC2863-19.2, syntenic to both AP002863 and AP002845, co-segregated with *Rph5* (Fig. 1). In addition to EST markers, we mapped the barley STS marker ABG70, which also co-segregates with *Rph5* (Fig. 1). With respect to the wheat STS markers, only one out of 28 (STS3B-66) was polymorphic in the Bowman × Magnif 102 population. It mapped 9.9 cM proximal to *Rph5* (Fig. 1). In total, we saturated the *Rph5* region with 11 new molecular markers (ten barley and one wheat STS markers), including six co-segregating sequences (Fig. 1 and Table 2).

Discussion

In this study, synteny-based marker saturation of the *Rph5* leaf rust resistance gene region was achieved by utilizing the abundant rice genomic information available and the rapidly growing barley EST sequence resources. Rice RFLP clone C970 was previously mapped 0.5 cM proximal to *Rph5* in barley (Mammadov et al. 2003). Since this marker was also mapped onto the distal region of rice chromosome 1S, it was used as an anchor marker to identify the syntenic region in the rice genome. As a result, a 585 kb region of rice chromosome 1S was identified as being syntenic to the *Rph5* region spanning from CDO549 to C970. This segment of rice chromosome 1S is comprised of 5 PAC clones (Fig. 1), which were used as queries to blastn the barley EST database. An important aspect of this analysis was the reduction in redundancy of the retrieved EST sequences.

Table 2 Primer sequences of the barley STS markers mapped to the *Rph5* region

Marker	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	PCR product size, bp
ABG70	AAACAGGAGACAACGGGACCAA	GCAATGCAACGCCACGAG	244
TC2863-19.2	GCTGTTGATGGCACGGACGACGAC	GGATATGCCAAGGACACCGATGAC	236
TC2863-12.4	TACAAATACGTGCCCTTCAACATC	AGCCTCGTCGACTCTATCTTCC	154
TC2863-32.1	CCATGACTGGGGACCTTACACGACTA	TTGAGAAGGACAGGCCGAACACG	249
TC2863-16.1	AGCAGCCCCCTTTTCTTTGTCCGTCTCTC	CCCTTGGCCTTGAGCAGCGAGCAC	128
TC2863-37.1	CGGCTTCAACGGCATCATCAT	TGCTTCCCTCGGGTCAAATCC	233
TC2845-BJ55.2	AACCAGGCTGCCATCTTTCTAT	CGCCTTACGCTTCCCGAGTGA	227
TC2845-BQ46.1	TACGAGGAACATACATAAGTCTGC	GTTCACCTCATACCCCATTG	259
TC2882-BM37.3	GGTGGAGGAGGTGGCGGGAACTAC	AAGAATTGATGGACTACGAACTGA	184
TC2882-BF25	GGACAGCGGCGCGTTTGATT	GGCTTTCTCCCCACTGACTA	300

Table 3 Putative function of the EST-based STS markers mapped to *Rph5* region in barley

N	Contig ID	ID of EST comprising minimum tilling path in TC	Putative function	*E*-value
1	TC2863-1	BI958114	Sulfur starvation-induced isoflavone reductase-like IRL	3e-64
2	TC2863-2	BI956632	Sulfur starvation-induced isoflavone reductase-like IRL	2e-49
3	TC2863-3	BI958199	Sulfur starvation-induced isoflavone reductase-like IRL	3e-42
4	TC2863-4	BI957783	Sulfur starvation-induced isoflavone reductase-like IRL	7e-32
5	TC2863-5	BI957029	Sulfur starvation-induced isoflavone reductase-like IRL	9e-46
6	TC2863-6	BI956898	Sulfur starvation-induced isoflavone reductase-like IRL	3e-44
7	TC2863-7	BI958092	Sulfur starvation-induced isoflavone reductase-like IRL	6e-32
8	TC2863-8	BI957384	Sulfur starvation-induced isoflavone reductase-like IRL	2e-32
9	TC2863-9	BI956210	Sulfur starvation-induced isoflavone reductase-like IRL	1e-31
10	TC2863-10	AV924577	Ring-box protein	4e-53
		BF626717	Ring-box protein	6e-53
11	TC2863-11[a]	AJ472815	Ring-box protein	2e-23
		AJ434693	Ring-box protein	4e-25
12	**TC2863-12 [b]**	**BE422237**	**Putative endoplasmatic reticulum retrieval protein Rer1A**	2e-47
13	TC2863-13	CA031324	Putative endoplasmatic reticulum retrieval protein Rer1B	6e-61
		AV932674	Putative Rer1A protein	8e-18
14	TC2863-14	CB882366	PEX14-like	3e-41
		BF267192	PEX14-like	6e-54
15	TC2863-15	BI952238	PEX14-like	1e-56
16	**TC2863-16**	**CB883833**	**Hypothetical protein**	2e-06
17	TC2863-17	BG343857	AtATM-like protein	8e-47
18	TC2863-18	BE214169	Putative Rer1A protein	5e-14
19	TC2863-19	BJ551992	Unknown protein	1e-52
		BJ472847	Unknown protein	1e-104
		BJ481223	Unknown protein	3e-59
20	TC2863-20	BJ469711	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	5e-108
		BG343005	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	3e-83
21	TC2863-21	CB866109	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	2e-51
22	TC2863-22	CB882502	Putative ribokinase	4e-81
23	TC2863-23	BF262021	Putative ribokinase	5e-70
24	TC2863-24	BJ484270	Putative tDET1 protein	1e-82
		CB861571	Putative tDET1 protein	4e-30
		BU986751	Putative tDET1 protein	4e-97
25	TC2863-25	BE213967	Putative tDET1 protein	2e-51
26	TC2863-26	AV928160	Hypothetical protein	3e-62
		BU993189	Hypothetical protein	3e-94
27	TC2863-27	AV836513	Unknown protein	2e-83
28	TC2863-28	BG343969	Unknown protein	3e-45
29	TC2863-29	AV917523	IscA-like	9e-32
		AV912788	IscA-like	8e-32
30	TC2863-30	AJ484119	Hsr201-like	7e-30
31	TC2863-31	CA009100	Unknown protein	1e-69
		CB878221	Unknown protein	1e-72
32	**TC2863-32**	**BQ461715**	**Pectin-glucuronyltransferase -like**	9e-86
33	TC2863-33	BM371996	ARF GAP-like zinc finger-containing protein-like	0.30
34	TC2863-34	CA010719	Serine-protein kinase ATM	3e-62
35	TC2863-35	BQ468579	Putative cellulose synthase catalytic subunit	6e-12
36	TC2863-36	CB861487	Putative protein kinase	8e-28
37	**TC2863-37**	**BJ466219**	**Putative protein kinase**	2e-26
38	TC2863-38	BF259187	Hypothetical protein	8e-06
39	TC2863-39	BQ464240	Unknown protein	5e-64
40	TC2863-40	BF255637	Lipase-like protein	6e-85
41	TC2818-1	BE216478	Stress responsive protein	5e-112
42	TC2818-2	BF264439	R40g2 protein	2e-88
		BG417700	R40g2 protein	8e-74
43	TC2818-3	BG366978	Putative r40c1 protein	1e-122
		BE060338	Putative r40c1 protein	1e-120
44	TC2818-4	BG343528	Putative r40c1 protein	9e-70
45	TC2818-5	BF259842	Unknown protein	3e-43
46		BF254530	Unknown protein	9e-77
47	TC2818-6	BU416822	Pancreatic alpha-amylase precursor	7e-130
		BG416822	Pancreatic alpha-amylase precursor	5e-118
48	TC2818-7	AV922789	Unknown protein	2e-24
49	TC2818-8	BF267513	Putative phenylalkylamine binding protein	5e-82
50	TC2818-9	AV941778	Putative NAM (no apical meristem) protein	7e-69

Table 3 (Contd.)

N	Contig ID	ID of EST comprising minimum tilling path in TC	Putative function	*E*-value
51	TC2818-10	AV922896	Putative NAM (no apical meristem) protein	2e-41
		BU980098	Putative NAM (no apical meristem) protein	3e-38
52	TC2818-11	AJ432912	Unknown protein	3e-92
		BE421520	Unknown protein	2e-89
53	TC2818-12	AJ466665	NAC domain transcription factor	4e-56
54	TC2818-13	BQ462040	Putative tDET1 protein	3e-60
		CB861571	Putative tDET1 protein	4e-30
		BJ479277	Putative tDET1 protein	6e-89
55	TC2818-14	BE213967	Putative tDET1 protein	2e-51
56	TC2818-15	CA015633	Putative protein kinase	1e-85
		BI950173	Putative protein kinase	2e-76
		BM373881	Putative protein kinase	6e-29
57	TC2818-16	BI953876	Pr1-like protein	5e-18
		BI953923	Pr1-like protein	4e-20
58	TC2818-17	BG416882	Retrotransposon protein, putative, Ty3-gypsy sub-class	3e-24
59	TC2818-18	AJ465772	Putative CGI-34 protein	3e-55
		BM816928	Putative CGI-34 protein	4e-51
60	TC2818-19	CA031517	Unknown protein	6e-26
		BF625028	Unknown protein	3e-32
		AV913562	Unknown protein	2e-24
61	TC2818-20	BF256699	Hypothetical protein	1e-04
62	TC2818-21	BG417079	Integrase core domain	2e-39
63	TC2818-22	BQ468579	Putative cellulose synthase catalytic subunit	6e-121
64	TC2818-23	BQ464240	Unknown protein	5e-64
65	TC3219-1	BM374358	Histone H2A.2	3e-46
66	TC3219-2	BI957307	Unknown protein	1e-10
67	TC3219-3	AV923355	Putative receptor kinase	2e-84
68	TC3219-4	AV934720	Putative receptor serine/threonine kinase	3e-84
69	TC2845-1	CA026509	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	2e-92
70	TC2845-2	CA012444	Transcriptional co-repressor-like	2e-66
71	TC2845-3	CA010719	Serine-protein kinase ATM	3e-62
72	TC2845-4	CA002439	Putative prolyl endopeptidase	4e-56
73	TC2845-5	BU972824	Transcriptional co-repressor-like	8e-54
74	**TC2845-BQ46.1**	**BQ461715**	**Pectin-glucuronyltransferase-like protein**	9e-86
75	TC2845- 7	BM440816	Putative ring-box protein	8e-45
76	**TC2845-BJ55.2**	**BJ552059**	**Unknown protein**	3e-35
77	TC2845-9	BJ548853	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	3e-59
78	TC2845-10	BJ471572	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	6e-98
79	TC2845-11	BG343005	Putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase	3e-83
80	TC2845-12	BF628427	PEX14-like protein	2e-59
81	TC2845-13	BF284227	Unnamed protein product	0.55
82	TC2845-14	BE412988	Unknown protein	9e-33
83	TC2845-15	AW983303	Unknown protein	2e-40
84	TC2845-16	AV938520	Putative prolyl endopeptidase	5e-83
85	TC2845-17	AJ486311	Metal-dependent phosphohydrolase HD domain-containing protein	1e-36
86	TC2882-1	BG367341	Unknown protein	8e-61
87	TC2882-2	BI957307	Unknown protein	1e-10
88	TC2882-3	BF264459	Unknown protein	5e-29
89	TC2882-4	BG414841	Putative SP2G	2e-67
90	TC2882-5	BG417617	Putative SP2G	6e-53
91	**TC2882-BM37.3**	**AW982176**	No similarity	
92	TC2882-6	BE455494	Putative acetoacetyl-coenzyme A thiolase	2e-74
93	TC2882-7	BF620560	Putative acetoacetyl-coenzyme A thiolase	1e-92
94	TC2882-BF25	**BF256599**	**Putative acetoacetyl-coenzyme A thiolase**	1e-126

[a]Some TCs are represented by several ESTs, which correspond to the minimum tiling path of the contigs
[b]Bolded are TCs, which were polymorphic in our study

These ESTs (3,962 sequences) were assembled into TCs that led to a reduction in the number of redundant sequences by 97.6%. The remaining 2.4% were represented by non-redundant EST sequences, which served as a template for the development of STS markers. By a similar approach, Perovic et al. (2004) were successful in the saturation of a 20 cM region around another barley leaf rust resistance gene, *rph16*, with barley EST sequences. Using a ''two-step *in silico* selection of candidate orthologous genes'', they reduced EST redundancy by 96%. In the present study, 8 EST-based STS markers were mapped between the VT1 and MWG691 markers,

covering a distance of 3.0 cM (Fig. 1). On the basis of the location of EST markers, the barley genomic region flanked by markers CDO549 and MWG691 is colinear with the corresponding region in rice, bracketed by PAC clones AP002863 and AP003219 (Fig. 1). However, small translocations were observed in the order of genes in barley related to syntenic rice clones AP002863 and AP002845 (Fig. 1). Brunner et al. (2003) and Perovic et al. (2004) also noted small translocations while saturating regions of the barley leaf rust resistance genes *Rph7* and *rph16*, respectively, with ESTs originating from rice chromosome 1S. In general, rearrangements in syntenic regions appear to be a common attribute at the DNA level between species (Tarchini et al. 2000; Dubcovsky et al. 2001; Song et al. 2002).

Using blastx algorithm, nucleotide sequences of EST-based STS markers were compared with the protein database at NCBI in order to identify their putative function. Apart from genes encoding putative acetoacyl-CoA-thiolase (TC2882-BF25), endoplasmatic reticulum retrieval protein *Rer1A* (TC2863-12.4), Pectin-glucuronyltransferase-like protein (TC2863-32.1 and TC2845-BQ46.1) and ATP-dependent metalloprotease *FtsH1* (STS3B-66), no function based on similarity with known proteins could be assigned to the other genes (Table 3). However, none of the mapped sequences belong to the NBS-LRR class, which contains the largest number of disease resistance genes in plants. Thus, *Rph5* may belong to a different class of R genes. For example, the wheat leaf rust resistance gene, *Lrk10* (GenBank accession no. U51330), encodes a receptor-like kinase (Feuillet et al. 1997). Interestingly, the STS marker TC2863-37.1, identified in this study, is similar to a putative protein kinase. Meanwhile, the barley STS marker ABG70, which also co-segregates with *Rph5*, does not exhibit similarity to any GenBank sequence, including rice. Thus, microcolinearity between rice and barley in the ABG70 locus is broken. The fact that segment of chromosome 3H containing ABG70 does not have an ortholog in rice can be explained by the fact that rice is apparently not a congenial host for any rust pathogen (Webster and Gunnell 1992) and, consequently, as a result of high selection pressure on this category of genes, rice must have lost the *Rph5* ortholog. Disruption in synteny among cereals at loci containing disease resistance gene homologs is not uncommon (Leister et al. 1998). For example, synteny-based cloning of the barley stem rust resistance gene *Rpg1* was unsuccessful because an ortholog of this gene was absent in the rice genome (Han et al. 1999). Later, *Rpg1* was isolated by a positional cloning approach, and it was confirmed that the rice genome does not possess the *Rpg1* orthologous gene (Brueggemann et al. 2002). Perovic et al. (2004) could not confirm colinearity among rice and barley at the barley *rph16* locus. Brunner et al. (2003) suggested that the barley leaf rust resistance gene *Rph7*, which is located on the same chromosome as *Rph5*, could belong to a new class of disease resistance genes, specifically HGA genes, which are very well conserved in rice. HGA genes represent an unknown protein family in rice. The acronym stands for the amino acids, namely, histidine (H), glycine (G) and alanine (A), ''which are the core of the most conserved domain located in the C terminus of the protein'' (C. Feuillet, JAM personal communication).

It should be noted that, in our study, only 9 out of 94 non-redundant TCs appeared to be polymorphic between the parental lines, which is not surprising taking into account the method used for developing STS markers and the relatively lower variation of EST sequences. However, the set of 86 monomorphic TCs should be a useful source for developing new molecular markers for the *Rph5* chromosomal region. For instance, they can be used as RFLP probes and for BAC library screening and physical mapping of the *Rph5* locus.

Acknowledgments This study was supported in part by the North American Barley Genome Mapping Project. We thank Stephanie Dahl for technical assistance in leaf rust phenotyping.

References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Liman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Avramova Z, Tikhonov A, SanMiguel P, Jin Y-K, Liu C, Woo S-S, Wing RA, Bennetzen JL (1996) Gene identification in a complex chromosomal continuum by local genomic cross-referencing. Plant J 10:1163–1168

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, colinearity and compatibility. Trends Genet 9:259–261

Brodny U, Rivadeneira M (1996) Physiological specialization of *Puccinia hordei* in Israel and Ecuador: 1992 to 1994. Can J Plant Pathol 18:375–378

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Brunner S, Keller B, Feuillet C (2003) A large rearrangement involving genes and low copy DNA interrupts the microcolinearity between rice and barley at the *Rph7* locus. Genetics 164:673–683

Büschges R, Hollrichter K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Töpsch S, Vos P, Salamini F, Schulze Lefert P (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Collins N, Park R, Spielmeyer W, Ellis J, Pryor AJ (2001) Resistance gene analogs in barley and their relationships to rust resistance genes. Genome 44:375–381

Druka A, Kudrna D, Han F, Kilian A, Steffenson B, Frisch D, Tomkins J, Wing R, Kleinhofs A (2000) Physical mapping of the barley stem rust resistance gene *rpg4*. Mol Gen Genet 264:283–290

Dubcovsky J, Ramakrishna W, San Miguel PJ, Busso CS, Yan L, Shiloff BA, Benetzen JL (2001) Comparative sequence analysis of colinear barley and rice bacterial artificial chromosomes. Plant Physiol 125:1342–1353

Dunford RP, Yano M, Kurata N, Sasaki T, Huestis G, Rocheford T, Laurie DA (2002) Comparative mapping of the barley *Ppd-H11* photoperiod response gene region, which lies close to a junction between two rice linkage segments. Genetics 161:825–834

Feng Q, Zhang Y, Hao P, Wang S, Fu G, Huang Y, Li Y, Zhu J, Liu Y, Hu , Jia P, Zhang Y, Zhao Q, Ying K, Yu S, Tang Y, Weng Q, Zhang L, Lu Y, Mu J, Lu Y, Zhang LS, Yu Z, Fan D, Liu X, Lu T, Li C, Wu Y, Sun T, Lei H, Li T, Hu H, Guan J, Wu M, Zhang R, Zhou B, Chen Z, Chen L, Jin Z, Wang R, Yin H, Cai Z, Ren S, Lv G, Gu W, Zhu G, Tu Y, Jia J, Zhang Y, Chen J, Kang H, Chen X, Shao C, Sun Y, Hu Q, Zhang X, Zhang W, Wang L, Ding C, Sheng H, Gu J, Chen S, Ni L, Zhu F, Chen W, Lan L, Lai Y, Cheng Z, Gu M, Jiang J, Li J, Hong G, Xue Y, Han B (2002) Sequence and analysis of rice chromosome 4. Nature 420:316–320

Fetch TG, Steffenson BJ, Jin Y (1998) Worldwide virulence of *Puccinia hord*ei on barley. Phytopathology 88:28

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Franckowiak JD, Jin Y, Steffenson BJ (1997) Recommended allele symbols for leaf rust resistance genes in barley. Barley Genet Newsl 27:36–44

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun WL, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa L. ssp. japonica*). Science 296:92–100

Han F, Kilian A, Chen JP, Kundrna D, Steffenson B, Yamamoto K, Matsumoto T, Sasaki T, Kleinhofs A (1999) Sequence analysis of a rice BAC covering the syntenous barley *Rpg1* region. Genome 42:1071–1076

Holton TA, Christopher JT, McClure L, Harker N, Henry RJ (2002) Identification and mapping of polymorphic SSR markers from expressed gene sequences of barley and wheat. Mol Breed 9:63–71

Lahaye T, Hartmann S, Töpsch S, Freialdenhoven A, Yano M, Schulze-Lefert P (1998) High-resolution genetic and physical mapping of the *Rar1* locus in barley. Theor Appl Genet 97:526–534

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

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologues in cereals genomes. Proc Natl Acad Sci USA 95:370–375

Levine MN, Cherewick WJ (1952) Studies on dwarf leaf rust of barley. U.S. Department of Agriculture Technical Bulletin. No. 1056, Washington

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu Z-W, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Mammadov JA, Zwonitzer JC, Biyashev RM, Griffey CA, Jin Y, Steffenson BJ, Saghai Maroof MA (2003) Molecular mapping of leaf rust resistance gene *Rph5* in barley. Crop Sci 43:388–393

Moore G (1995) Cereal genome evolution: pastoral pursuits with 'Lego' genomes. Curr Opin Genet Dev 5:717–724

Parlevliet JE (1976) The genetics of seedling resistance to leaf rust, *Puccinia hordei* Otth in some spring barley cultivars. Euphytica 25:249–254

Perovic D, Stein N, Zhang H, Drescher A, Prasad M, Kota R, Kopahnke D, Graner A (2004) An integrated approach for comparative mapping in rice and barley with special reference to the *Rph16* resistance locus. Funct Integr Genomics 4:74–83

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massarie A, Maestri E, Marmiroli N, Sjakste T, Ganalg M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Saghai Maroof MA, Biyashev RB, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Saghai Maroof MA, Yang GP, Biyashev MP, Maughan PJ, Zhang Q (1996) Analysis of the barley and rice genomes by comparative RFLP linkage mapping. Theor Appl Genet 92:541–551

Sasaki T, Matsumoto T, Yamamoto K, Sakata K, Baba T, Katayose Y, Wu J, Niimura Y, Cheng Z, Nagamura Y, Antonio BA, Kanamori H, Hosokawa S, Masukawa M, Arikawa K, Chiden Y, Hayashi M, Okamoto M, Ando T, Aoki H, Arita K, Hamada M, Harada C, Hijishita S, Honda M, Ichikawa Y, Idonuma A, Iijima M, Ikeda M, Ikeno M, Ito S, Ito T, Ito Y, Ito Y, Iwabuchi A, Kamiya K, Karasawa W, Katagiri S, Kikuta A, Kobayashi N, Kono I, Machita K, Maehara T, Mizuno H, Mizubayashi T, Mukai Y, Nagasaki H, Nakashima M, Nakama Y, Nakamichi Y, Nakamura M, Namiki N, Negishi M, Ohta I, Ono N, Saji S, Sakai K, Shibata M, Shimokawa T, Shomura A, Song J, Takazaki Y, Terasawa K, Tsuji K, Waki K, Yamagata H, Yamane H, Yoshiki S, Yoshihara R, Yukawa K, Zhong H, Iwama H, Endo T, Ito H, Hahn JH, Kim HI, Eun MY, Yano M, Jiang J, Gojobori T (2002) The genome sequence and structure of rice chromosome 1. Nature 420:312–316

Schmierer D, Drader T, Horsley R, Kleinhofs A (2003) Saturation mapping of a major Fusarium head blight QTL on barley chromosome 2H. In: Proceedings of 2003 National Fusarium head blight forum, pp 36–39

Smilde WD, Haluskova J, Sasaki T, Graner A (2001) New evidence for the synteny of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. Genome 44:361–367

Song R, Llaca V, Messing J (2002) Mosaic organization of orthologous sequences in grass genomes. Genome Res 12:1549–1555

Tarchini R, Biddle P, Wineland R, Tingey S, Rafalski A (2000) The complete sequence of the 340 kb of DNA around *Adh1-Adh2* region reveals interrupted colinearity with maize chromosome 4. Plant Cell 12:381–391

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

Tikhonov AP, SanMiguel PJ, Nakajima Y, Gorenstein NM, Bennetzen JL, Avramova Z (1999) Colinearity and its exceptions in orthologous *adh* regions of maize and sorghum. Proc Natl Acad Sci USA 96:7409–7414

Varshney RK, Sigmund R, Börner A, Korzun V, Stein N, Sorrells ME, Langridge P, Graner A (2005) Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci 168:195–202

Webster RK, Gunnell PS (1992) Compendium of rice diseases. APSPress, St. Paul

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosomal 5S (1HS) of barley. Genetics 153:1929–1948

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P,

Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L, Yang H (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). Science 296:79–92

Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor Appl Genet 101:1093–1099