Theor Appl Genet (2009) 119:663–673
DOI 10.1007/s00122-009-1077-3

ORIGINAL PAPER

Development and functional assessment of EST-derived 2RL-specific markers for 2BS.2RL translocations

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Received: 14 January 2009 / Accepted: 15 May 2009 / Published online: 20 June 2009

Abstract ESTs-derived markers are useful for comparative genomic analysis and can also serve as phenotype-linked functional markers. Here, we report the development of EST-derived 2RL-specific markers and the evaluation of the possibility of functional assessment of markers tagging 2RL, which carries Hessian fly resistance genes (loci). To identify transcripts specific to 2RL, unigene sequences in combination with wheat progenitor genomes were used. Total 275 contigs mapped to the long arms of homoeologous group 2 chromosomes were downloaded. To obtain a cluster corresponding to each of the wheat 275 contigs, unigene sequences of wheat, rice, barley, and rye were pooled for cross-species clusters. Out of 275 clusters examined, it was possible to design 112 cross-species primer pairs for genome-specific amplifications. Out of 112 cross-species primer pairs, 45 primer pairs (40%) produced amplicons from at least one species (three wheat progenitors or rye). Among the 45 contigs, 73% were associated with one of known functions and 82% of the contigs associated with known functions were also associated with one of the GO categories. On the basis of the oligonucleotide sequence alignment of each of 45 genome-specific amplifications, 21 amplifications (47%) were suitable for designing RR genome-specific primers, which are specific to translocated rye chromatin 2RL. Six primer pairs (13%) successfully produced amplicons in the 2BS.2RL translocation lines and not in the non-2RLs. Functional assessment of one of the 2RL-specific markers, NSFT03P2_Contig4445, was performed on Hessian fly infested NILs. Under Hessian fly infestation, significantly high expression of a gene tagged by a 2RL-specific marker (NSFT03P2_Contig4445) was observed 1 day after infestation. EST-derived 2RL-specific marker development from this study provides a basis for the development of ESTs-derived markers for detecting wheat–rye translocations. In addition, these markers could be employed in elucidating functional analysis of genes on 2RL.

Communicated by B. Keller.

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Introduction

Wheat–rye translocation lines in the form of 1AL.1RS, 1BL.1RS, and 2BS.2RL have been developed for an important genetic source of disease and pest resistance, and superior performance in the unfavorable environment for crop production (Friebe et al. 1996). The long arm of 'Chaupon' rye chromosome 2R carries a gene or gene complex that conditions antibiosis to the Hessian fly larvae (Friebe et al. 1990). Gene *H21*, derived from the 2RL of 'Chaupon' rye (Hatchett et al. 1993), conferred stable resistance to biotype L of the Hessian fly (Friebe et al. 1990; Hatchett et al. 1993). To date, several resistance genes related to the Hessian fly infestation have been mapped (*H13* in Liu et al. 2005; *H32* in Sardesai et al. 2005; *H22* in Zhao et al. 2006), but *H21* was not one of them.

Several markers derived from repetitive rye DNA or amplified fragment of wheat genomic DNA have been developed for identifying 2RL (Lee et al. 1996; Brunell et al. 1999; Saal and Wricke 1999; Seo et al. 1997, 2001).

Unlike these previous markers, gene-derived markers such as ESTs-derived markers can be served as phenotype-linked functional markers, and are also more useful for comparative genomics (Anderson and Lübberstedt 2003; Gupta and Rustgi 2004). More than 1.05 million wheat EST sequences have been registered in public databases (as of August 2008, http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucest&cmd=search&term=triticum+aestivum). However, the marker development, which is supported by these databases, is still emerging.

There has been progress in dealing with useful ideas that could be applied to develop markers (especially based on ESTs) specifically detecting translocated rye chromatins in wheat genomes. Nomura et al. (2005) designed primers that are specific to each homoeologous chromosome group (AA, BB, or DD) using the hexaploid wheat 'Chinese Spring' and confirmed the differentially expressed transcripts for each respective gene on three homoeologs. Numerous studies reported on the cloning of specific sequences which are based on ESTs. Feltus et al. (2006) developed conserved-intron scanning primers that included relatively conserved exons located near exon–intron boundaries for targeted discovery of SNPs. To effectively assign homoeologous genes to each of the three wheat genomes, PCR-based unique gene marker system has shown the chromosome locations of ESTs based on orthologous gene conservation in hexaploid wheat 'Chinese Spring' (Ishikawa et al. 2007). These marker systems were directly applied in dissection of rye chromosome 1R that was translocated in common wheat lines (Tsuchida et al. 2008).

Comparative approaches by using public sequence databases and understanding orthology between rye and rice for mapping of self-incompatibility locus Z on 2RL (Hackauf and Wehling 2005), and synteny analysis between wheat and rice for the mapping of the rust resistance genes on the short arm of rye chromosome 1 (Mago et al. 2005) have given new perspectives for marker developments in wheat–rye translocation lines. Currently, there is little usage of wheat progenitors (AA, SS≒BB, and DD) AA, SS≒BB, and DD for marker development. Thus, integrated approaches, which encompass comparative genomics using public Triticeae sequence databases such as TIGR and Graingenes with a better understanding of evolutionary history of common wheat and pedigrees of breeding lines, would be useful tools for the successful development of marker systems for identifying genes that reside on the translocated chromatin such as 2RL.

The objectives of this study were to develop EST-derived 2RL-specific markers and to evaluate the possibility of functional assessment of markers tagging 2RL, which carries Hessian fly resistance gene(s).

Materials and methods

Plant materials

Diploid progenitors ($2n = 14$) of hexaploid wheat, *T. uratu* (Kyoto university seed stock accession no. KU199-6, AA genome), *A. speltoides* (KU5727, SS≒BB genome), and *A. squarrosa* (KU20-9, DD genome), were used as surrogates for the common wheat genome (AABBDD). Three diploid progenitors have been used for the analysis of homoeologous gene expressions (Nomura et al. 2005).

Near-isogenic lines (NILs) were developed by backcross introgression $BC_3F_{3:4}$ ('Coker 797' *4/'Hamlet') and differed by the presence or absence of *H21* (on 2RL) derived from 'Chaupon' rye (*Secale cereale* L.) (Seo et al. 1997). In 'Hamlet' ('ND 7532'/'Chaupon'//4* 'ND 7532'), 2RL segment is present in a cytologically stable form as a 2BS.2RL (Friebe et al. 1990). NIL possessing *H21*, 'Coker 797' (Cltr 17722), 'Hamlet' (PI 549276, 2BS.2RL), 'ND 7532' (none 2RL parent), diploid rye 'Chaupon' (TA 9002, 2RL donor of NIL possessing *H21* and 'Hamlet'), and 'Chinese Spring' (common wheat) were used for 2RL specific marker development and confirmation. Presence or absence of 2RLs in plant materials (NIL with *H21*, NIL without *H21*, and 'Hamlet') were confirmed by fluorescence in situ hybridization (FISH) using rye genomic DNA as a probe. FISH was performed as described by Jang et al. (2003).

Wheat–rye addition lines ('Chinese Spring'/'Imperial' rye) were kindly provided by S. M. Reader (Department of Crop Genetics, John Innes Centre, UK). Seven wheat–rye addition lines (21″+1R″, 21″+2R″+2RL″, 21″+3R″, 21″+4R″, 21″+5R″, 21″+6R″, and 21″+7R″) were used in this study. Several wheat–rye translocation germplasms differing for presence of translocated rye sources such as 1AL.1RS, 1BL.1RS, 6BS.6RL were provided by National Plant Germplasm System (http://www.ars-grin.gov). Six wheat–rye translocation lines [TAM 202 (1AL.1RS), GRS1201 (PI 561948, 1AL.1RS), SIOUXLAND (PI 483469, 1BL.1RS), GABO (PI 410845, 1DL.1RS), KS93WGRC28 (PI 583795, T6BS.6RL), and KS92WGRC17 (PI 592729, T6BS-6BL-6RL)] and three rye varieties ['Blanco' (PI 542470, *Secale cereale*), 'Chilbohomil' (*Secale cereale*), and 'Jochunhomil' (*Secale cereale*)] were used in this study. Most of wheat–rye translocation germplasms and rye varieties were used by independent researches (Lee et al. 1994, 1996; Seo et al. 2001).

Genomic DNA for PCR was isolated from seeds (all samples except for 'Chaupon' rye and wheat–rye addition lines) or leaf tissues ('Chaupon' rye and wheat–rye addition lines) by using *i*-genomic PLANT DNA Extraction Mini Kit (INTRON, Korea) according to the manufacturer's instructions.

Marker development

In silico data mining for 2RL-specific marker

A total of 275 contig sequences mapped on long arms of wheat homoeologous group 2 were downloaded from GrainGenes (http://wheat.pw.usda.gov/cgi-bin/westsql/contig.cgi; Conley et al. 2004). Unigene databases (1,359,497 wheat unigenes and 1,554,216 rice unigenes from NCBI and/or TIGR) and 405,326 barley unigenes and 5,013 rye unigenes, which were assembled by downloaded barley or rye ESTs from NCBI, were pooled for unigene-clusters. A unigene-cluster is defined in this study as the group of a contig sequence and unigene sequences that were from at least one species among wheat, rice, barley, and rye. Each unigene-cluster was defined if any unigene(s) of different species (wheat, rice, barley, and rye) was best matched with wheat contigs with cutoff value of E value $\leq 1 \times 10^{-10}$ using TBLASTX, which identifies the orthologous encoded by unigenes of each unigene-cluster. Unigene-clusters containing unigenes from at least two species were further analyzed for designing primer pairs, while unigene-clusters containing only one unigene were excluded.

Preparation of cross-species markers

The common primer pairs for three wheat progenitors (AA, SS≒BB, and DD genome) and rye (RR genome) were designed. Because the common primer pairs designate unigene-clusters specifically mapped on wheat bin and each unigene-cluster contains unigene sequences representing a unique gene, PCR primer pairs produced amplified products among species are defined here as cross-species markers. Conserved exon regions or exonic regions flanking introns were used for the design of cross-species primer pairs. To avoid non-specific amplification and increase the number of scanable genome species (AA, BB, DD, and RR), highly conserved (max. 0–2 nucleotide mismatch) exonic regions within unigene sequences aligned in each unigene-cluster were used in principle. The primer pairs (forward and reverse) were designed to avoid exon/intron junctions. The exon/intron junctions within unigene sequences were predicted by aligning rice unigene sequence with rice genomic DNA sequences that were obtained from TIGR using the ClustalW program (http://www.ebi.ac.uk/clustalw). The primer sites were mainly focused on the conserved regions in wheat and rye rather than barley and/or rice. Primer pairs were designed manually under the guidelines of 19–21 bp primer length, 40–60% GC content, 300–1,500 bp amplicon size. If there were SNPs in the conserved region among the unigenes within a unigene-cluster, primers containing mixed oligonucleotide bases for SNPs were designed.

A total of 112 cross-species primer pairs were designed, and screened for amplification using three wheat progenitors (AA, SS≒BB, and DD) and 'Chaupon' rye (RR). PCRs were carried out in a 25-μl solution containing 50 ng of genomic DNA template at annealing temperatures (50, 55, or 60°C) under conditions as follows: 5 min at 94°C, followed by 35 cycles of amplification (30 s at 94°C, annealing for 30 s). *Ex* Taq polymerase (Takara, Japan) were used for PCR. PCR products were run on agarose gels for the confirmation of successful amplification, and then amplicons showing expected size from annealing temperature range (50–60°C) were cloned for designing 2RL-specific primer pairs.

2RL-specific primer pairs

In order to develop 2RL-specific primer pairs, each amplified product that was generated from wheat progenitors and 'Chaupon' rye using cross-species markers were sequenced. 2RL-specific primer pairs, specific for targeting the oligonucleotide sequence on translocated 2RL chromatin, were designed based on the inter-homoeologous SNP sites within sequences generated by each cross-species markers. 2RL-specific primer pairs were designed for both RR and SS≒BB sequences within sequences generated by each cross-species markers where sufficient sequence conservation was observed between two sequences, not on either side of the AA and DD.

To achieve translocated rye chromatin-specific amplification, we conducted PCR under highly stringent conditions as follows: 5 min at 94°C, followed by 23–29 cycles of amplification (30 s at 94°C, 30 s at 60–72°C). Genomic DNA (50–150 ng) in a 25-μl reaction mixture and *Ex* Taq polymerase (Takara, Japan) were used for PCR. The 2RL-specific primer pairs were initially confirmed on 2BS.2RL translocation lines, and then applied on wheat–rye addition lines and other types of translocation lines. The cross-species markers were tested on barley (*Hordeum vulgare* L.) cv. 'Karl' (CI 15487), and rice (*Oryza sativa* L.) cv. 'Dongan-byeo'. PCR products were run on 3% w/v agarose gels for the confirmation of unique amplicon.

Evaluation of genomic colinearity

Sequences obtained from cross-species markers were compared to the rice pseudomolecules (version 4.0) by using the BLASTN algorithm with an E value $\leq 1 \times 10^{-15}$ cutoff for estimating genomic colinearity between wheat (or rye) and rice. Description and GO term for each sequence were identified based on the contig sequences at the TIGR Rice Database.

Hessian fly infestation and transcriptional expression analysis of a 2RL-specific marker in NILs

Biotype L of Hessian fly, which was reared on the susceptible wheat line, was kindly provided by Dr. Sue Cambron (USDA-ARS, Department of Entomology, Purdue University, IN, USA). Seeds of NILs were sown in Sunshine Mix #1 (Sun Gro, Canada) and grown in a Growth Chamber with an optimized condition. When plants reached the third leaf stage, plants were transferred to a growth chamber filled with adult Hessian flies. The beginning of larvae infestation, 0 day after infestation (0 DAI), was estimated by closely monitoring egg hatches on the leaf blade and examining the base of plants to determine the time when larvae had reached the crown of the plant. After 1 and 3 DAI, plant tissues [coleoptile, first leaf sheath (from cotyledonary node to auricle) around the second and third leaf, leaf blade or sheath surrounded by first leaf sheath of NILs] were collected and kept in RNA*later* solution (Ambion, USA) at 4°C until RNA isolation. Hessian fly larvae were removed before RNA isolation under LEICA EZ4 D microscope (Leica Microsystems, Switzerland). Total RNA was initially isolated from samples using TRIzol (Invitrogen, USA) reagent according to the manufacturer's protocol. The RNA was further purified using an RNeasy spin column (Qiagen, USA). Total RNA of 2 μg was used for cDNA synthesis. RT-PCR and real-time RT-PCR was conducted as described by Choi et al. (2007). The gene-specific primer pairs for each amplification were: 5′-CGCATGCTCTGCTATGAACA-3′ and 5′-CATTCCTCCAGATTTGGTAG-3′ for 2RL-specific marker no. 2 (NSFT03P2_Contig4445), and 5′-CAGCA ACTGGGATGATATGG-3′ and 5′-ATTTCGCTTTCAG CAGTGGT-3′ for *Actin*. For real-time RT-PCR analysis, the SYBR Green Supermix (Bio-Rad, USA) was used according to the manufacturer's instructions.

Results

Confirmation of 2RL translocation by FISH

Translocation of 2RL chromatin in wheat–rye translocation lines was confirmed by FISH. Translocated 2RLs were detected in NIL possessing *H21* and 'Hamlet', but no signals were detected in NIL lacking 2RL ('Coker 797') (Fig. 1). The result of FISH on NIL possessing *H21* and NIL lacking 2RL ('Coker 797') showed the same results with the previous work (Jang et al. 2003).

EST sequence analysis

From the 275 contigs that were downloaded from the GrainGenes database and mapped to the long arms of homoeologous group 2 chromosomes (2BL bin and missing specific 2BL bins), 49 contigs (17.8%) were mapped to only 2BL bin and 181 contigs (65.8%) were mapped to more than two homoeologus groups of 2AL, 2BL, and 2DL. The remaining 45 (16.4%) that were mapped to not only the long arms of homoeologous group 2 chromosomes, but also other wheat chromosomes were excluded. The unigenes from each species were pooled and clustered to construct 275 unigene-clusters. Out of 275 unigene-clusters examined, it was possible to design cross-species primer pairs for 112 unigene-clusters. Cross-species primer pairs could not be designed for 163 unigene-clusters because of insufficient sequence data from unigene-clusters or no sequence differences within the aligned unigene sequences.

Cross-species amplifications

Out of 112 cross-species primer pairs, 45 primer pairs (40%) produced amplicons from at least one species (AA, SS≒BB, DD or RR) (Table 1). A total of 18 primer pairs (16.1%) were identified to be produced from all four

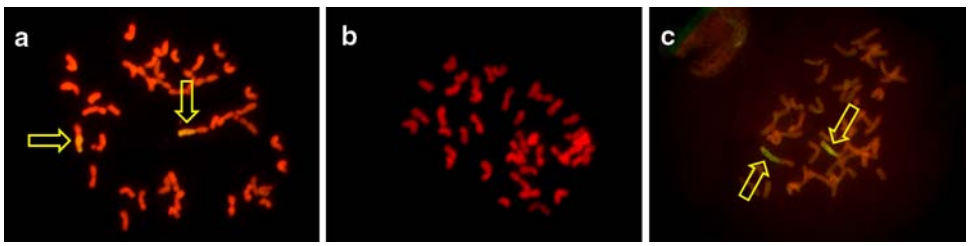


Fig. 1 Fluorescence in situ hybridization of 2BS.2RL translocation lines. **a** FISH of NIL carrying one pair of translocated chromosomes, where the entire long arm of rye 2R has replaced the entire long arm of 2B (2BS.2RL). **b** FISH of normal hexaploid wheat 'Coker 797' (with normal 2BL). **c** FISH of 'Hamlet' carrying one pair of translocated chromosomes, where the entire long arm of rye 'Chaupon' 2R has replaced the entire long arm of 2B (2BS.2RL). Two open *yellow arrows* in **a** and **c** represent one pair of translocated rye long arms (2RL) with a short wheat chromosome arm (2BS). Wheat chromosomes are *colored red* (**b**) or *orange* (**a** and **c**) and translocated rye segments are *yellow* (**a**) or *green* (**c**) from fluorescence in situ hybridization (Color figure online)

genomes. All 45 cross-species amplifications were sequenced and compared to those of the targeted unigene sequences in unigene-clusters. Of the 45 cross-species amplifications, 16 were comprised from the template of one exon region only, while 29 were from exonic regions flanking the exon–intron boundaries (containing at least one intron). Amplicon numbers that were obtained from each genome species were 31 for AA genome, 27 for SS≒BB genome, 28 for DD genome, and 35 for RR genome.

Cross-species amplifications detected from three wheat progenitors were compared with the contig datasets downloaded from GrainGenes (long arm of homoeologous group 2, which contains contigs mapped on 2AL, 2BL, and/or 2DL) to evaluate the efficacy of using contig information and wheat progenitors. Of the contigs that produced amplicons from at least one genome species, 8 (17.8%) are in agreement with the datasets. A total of 30 contigs among 45 (66.7%) matched with datasets; a part, 13 among 18 contigs from AA, 19 among 30 contigs from BB, and 15 among 23 from DD matched with Southern blot datasets. Out of those 30 contigs, 13 contigs newly produced amplicons within three wheat progenitors (11 from AA, none from SS≒BB, and 6 from DD). In contrast to Southern blot analysis, 7 (15.5%) failed to produce amplicons within three wheat progenitors. Those 7 contigs were cloned from rye genome only. The description of amplicons was displayed on Table 1.

Using BLASTN queries (E value $\leq 1 \times 10^{-15}$), putative functions were assigned to 45 cross-species amplifications. A total of 73% of cross-species amplifications were associated with one of the known functions and 82% of them were associated with one of the GO categories.

2RL-specific marker development and confirmation of specificity

On the basis of the oligonucleotide sequence alignment of each of 45 cross-species amplifications, 21 (47%) were suitable for designing 2RL-specific primer pairs. Seven primer pairs produced amplicons in the 2BS.2RL translocation lines (NIL possessing *H21* and 'Hamlet') and not in the non-2RLs ('Coker 797' and 'ND 7532'). Specificity of the amplicons was further confirmed using wheat–rye addition lines and various wheat–rye translocation lines. Six among seven primer pairs showed amplicons that were identical to expected target sizes in a wheat–rye addition line (21″+2R″+2RL″) and three rye cultivars ('Blanco', 'Chilbohomil', and 'Jochunhomil'), but none of the amplicons were generated in other wheat–rye addition lines (21″+1R″, 21″+3R″, 21″+4R″, 21″+5R″, 21″+6R″, and 21″+7R″) and other translocations (Fig. 2; Table 2, PCR results of three rye cultivars and other translocations were not shown). Representative sequence alignment of 2RL-specific marker no. 2 (NSFT03P2_Contig4445) was shown in Fig. 3.

According to the contig sequences mapped on long arms of homoeologous group 2 based on public database (GrainGenes 2.0), six 2RL-specific amplicons were confirmed to be resided on 2BL (4 on 1L+2L, 1 on 3L+4L+5L, 1 on 6L, and none on CL). The consensus bin designations represent the bin fraction as follows: CL stands for C-2BL2-0.36, 1L+2L stands for 2BL2-0.36-0.50, 3L+4L+5L stands for 2BL4-0.50-0.89, and 6L stands for 2BL6-0.89-1.00 (Conley et al. 2004). Cloned contig numbers follow the distribution of EST loci on the group 2 consensus map relatively well [Conley et al. 2004; 47 (12.6%) on CL, 142 (38.0%) on 1L+2L, 95 (25.4%) on 3L+4L+5L, and 90 (24.1%) on 6L].

Genomic colinearity of cross-species amplifications

Cross-species amplifications were grouped into seven rice chromosome groups according to their BLAST result. The sequences of these amplifications showed a highly significant relationship with rice chromosome 4 [23 in rice chromosome 4 (R4), 3 in R1, 1 in R2, 1 in R3, 2 in R5, 1 in R6, and 4 in R7 based on the sequences cloned from rye] (Table 1). Among the 45, three had each of their sub-genomic (AA, SS≒BB, DD, or RR) PCR products that did not belong to the same rice chromosome group.

Amplification on the Triticeae tribe

The cross-species markers were tested on barley and rice. No significant differences in patterns of amplicons were found (data not shown). Investigation of the transferability of 45 cross-species markers to the Triticeae tribe would be valuable steps for the enhancement of EST marker-based comparative maps in crops.

Transcriptional expression of alleles located on 2RL during Hessian fly infestation

The 2RL specific marker no. 2 (NSFT03P2_Contig4445) which considered tagging an allele reside on 2RL was evaluated under the Hessian fly larvae attack (Fig. 4). Because the 2RL-specific marker no. 2 amplified only the exon region, the same primer pairs developed for 2RL-specific markers could be employed directly in RT-PCR and real-time RT-PCR analysis. Unique amplicon showing differential expressions in each NIL cDNA template of RT-PCR were cloned for sequence comparison in Fig. 3. No expression was observed in case of using 'Coker 797' cDNA as templates of RT-PCR. Under Hessian fly infestation, the expression was significantly enhanced (more than tenfold

Table 1 Primer sequences and description of cloned contigs

No.	Contig	Wheat chromosome bins[a]	Primer sequence (5′ → 3′)[b]		Amplicon size (bp)[c]				Rice gene model[d]	Description[e]	GO Term ID	GO Term Description
			Forward	Reverse	AA	SS≒	EDD	RR				
1	NSFT03P2_Contig10311	1L+2L	CACCGCTGCACAGTATCCACCCATAA	GAAGYTAGCGGTTGATCARCARGCAG	-	-	-	421	LOC_Os04g58790	B-type cyclin, putative	None found	-
2	NSFT03P2_Contig11534	CL	CAGCCATGGCTTCCCAGCTCT	TAGCCGATGATCTGGTCGAGG	492	-	-	486	LOC_Os07g05480	photosystem I reaction center subunit psaK, chloroplast precursor	None found	-
3	NSFT03P2_Contig11726	6L	GATGCYTGGCACGGTCTYGT	CGAGCATTCTGAAATCTGAA	-	528	-	526	LOC_Os04g54200	putative diacylglycerol kinase	GO:0007165	signal transduction
4	NSFT03P2_Contig11767	3L+4L+5L	TGCTKGTGCTGTCCGGGCTGA	YYAAGMCSCCTGCGCCTGGTC	314	314	314	314	LOC_Os04g46200	oleosin 2	GO:0003674	molecular function
5	NSFT03P2_Contig11886	6L	GCAATCAATAWRCARGAAATTG	CTAAATATTGACWGGAAGAGCG	-	-	-	341	LOC_Os04g43916	24 kDa vacuolar protein VP24	None found	-
6	NSFT03P2_Contig11083	6L	GAGGAAATGGTTATGAAGGA	GGCAATGACTGCATGTATGAT	601	-	603	-	-	unknown protein	None found	-
7	NSFT03P2_Contig11217	1L+2L	ATGCGAAGTACTGAAGTGAA	TGCCAWGTTTGWGWARCWTG	-	1,529	-	1,511	LOC_Os04g18200	dihydrodipicolinate synthase 1, chl oroplast precursor	GO:0003824	catalytic activity
8	NSFT03P2_Contig11959	6L	TCAAGCACARCAATMTKSAGG	SAGGAMTGGGTTCCCTTGATG	-	-	610	-	-	hypothetical protein	GO:0005515	protein binding
9	NSFT03P2_Contig16317	6L	TCCATCGAGGAYGCCTGCAA	TCTGGCACAGCTTCACCTCC	447	411	447	447	LOC_Os01g20970	unknown protein	None found	-
10	NSFT03P2_Contig4445	1L+2L	CGCATGCTCTGCTATGAACA	AGCTGCAAAGTRACCGTGCA	314	314	314	314	LOC_Os04g39660	hypothetical protein	None found	-
11	NSFT03P2_Contig17559	1L+2L	GTGCTCAAGAACCCGTTCCT	TACGCCTGCCCGATGAAGAT	443	443	443	443	LOC_Os04g38410	a/b-binding apoprotein CP24 precursor	GO:0005488	binding
12	NSFT03P2_Contig12802	1L+2L	ATGGCGTCTGCTGAAACAGGA	GCCAGGAACGCGTTGTAGCC	518	590	542	639	LOC_Os01g14550	putative pathogenesis related protein	None found	-
13	NSFT03P2_Contig15114	6L	ACAAGAGTGTCCCGATCTCG	GAGCATGAAGACGATGGAMC	344	344	344	344	LOC_Os04g34620	expressed protein	None found	-
14	NSFT03P2_Contig18149	1L+2L	AAGATCCAGGACAAGGAGGG	CCTTCTGGATGTTGTAGTC	557	-	-	-	-	polyubiquitin	GO:0005515	protein binding
15	NSFT03P2_Contig17755	1L+2L	CTCCTCACTCCTCAGGCCA	GATYGATCGTCTACATGAGG	566	561	577	577	LOC_Os04g33150	desiccation-related protein PCC13-6 2 precursor, putative, expressed	None found	-
16	NSFT03P2_Contig14078	3L+4L+5L	GAGAGTGAAGCTGGAGATGC	CTAGATCTTCTGCTTGAGCAC	567	567	567	567	LOC_Os04g53350	unknown protein	None found	-
17	NSFT03P2_Contig16472	3L+4L+5L	TGCACMGGTGGAGGAACCCT	GACGGCTTCTTTGAGSTCGA	557	559	-	529	LOC_Os04g48210	putative cytochrome P450	GO:0003824	catalytic activity
18	NSFT03P2_Contig10857	1L+2L	TACYTTCAACCTYACTTGGG	GAAACGGCATCGGCAAACTT	1,021	1,103	-	1,100	LOC_Os02g37590	(AF031231) S222 [*Triticum aestivum*]	GO:0008152	metabolic process
19	NSFT03P2_Contig7673	1L+2L	GGCMATGAAGARAGARGACA	GRATAAGTGTTGGTGCAGTA	880	878	-	887	LOC_Os04g33190	(AL607005) OSJNBb0118P14.2 [*Oryza sativa*]	GO:0003824	catalytic activity
20	NSFT03P2_Contig2406	CL	CCTACTGGCAATGGAGCTCC	ATGAACAGGGGCACCAGGGT	393	393	393	393	LOC_Os07g02090	diphosphonucleotide phosphatase-like protein	GO:0005618	cell wall
21	DSW02C4_Contig9625-1	CL+1L+2L+3L+4L+5L+6L	TCGCGGGAGAACTGGGACAA	ARCCTCYGGCTCCATAAGAG	-	-	-	376	LOC_Os04g31000	-	GO:0003824	catalytic activity
22	DSW03C5_Contig29629.1	3L+4L+5L+6L	TCTGTGAACTCCTCTCTTGC	GAAAGGTTGTCGCCGCTCTT	-	-	-	502	LOC_Os06g39600	protein phosphatase 2C	GO:0005488	binding
23	NSFT03P2_Contig10651	3L+4L+5L	TCCATGGAGCTGMACCGCYC	GGATGGATAGGCTCCAGCAT	-	-	-	279	LOC_Os04g52100	methionine aminopeptidase I (MAP1), putative	GO:0009987	cellular process
24	NSFT03P2_Contig12748	3L+4L+5L	GAAGCGGARGTCYAAAWCCG	GYTGGCGATCTTRTCTAAGC	905	914	911	903	LOC_Os04g49580	hypothetical protein	None found	-
25	NSFT03P2_Contig2515	1L+2L	AATGACCCATATGGAAGTTC	CTGTACCAACTACATGGATA	472	489	491	491	LOC_Os04g42370	AL606594) OSJNBa0029H02.1	None found	-
26	NSFT03P2_Contig16931	1L+2L	AAYCTTGAGCAGGCTCGGGA	ACTTCCAWATYTCCCGGTCA	656	647	656	656	LOC_Os04g41500	expressed protein	None found	-
27	NSFT03P2_Contig12661	3L+4L+5L	GACGAGTTATTGGAGAAGTC	ACATGCACATGCTACTTTGG	432	-	432	432	LOC_Os01g73234	thioredoxin-like protein	GO:0003824	catalytic activity
28	NSFT03P2_Contig17103	1L+2L	GTGATGAYAACCCYTATGTTA	AGCTCGGTTTGCTCCTGSACA	1,227	1,212	-	1,228	LOC_Os04g32020	2-oxoglutarate dehydrogenase , E1 component	GO:0003824	catalytic activity
29	NSFT03P2_Contig13757	6L	AACATGTTCTTACTGGTGG	AGGGCTATYTTGTTCTCRA	415	415	415	415	LOC_Os04g57560	AC007234) F1E22.18 [*Arabidopsis thaliana*]	GO:0003824	catalytic activity
30	NSFT03P2_Contig14669	3L+4L+5L	GAGGGTATATCAGARGCATC	AGAACCTTGTAKGGGTTYAG	797	810	796	796	LOC_Os07g08950	actin interacting protein	GO:0003824	catalytic activity
31	NSFT03P2_Contig2786	6L	CAGAGATCRTCAAAAGATTA	CAGCTTTAWRTCCAATATCC	440	440	440	440	LOC_Os04g55570	unknown protein	None found	-
32	NSFT03P2_Contig14233	3L+4L+5L	GCCGCTSATGCTSAGGTAYG	CCCAAAGAYACTCCRAGRGT	760	751	754	722	LOC_Os05g51610	calcium/proton exchanger CAX1-l ike protein	GO:0005215	transporter activity
33	NSFT03P2_Contig18090	1L+2L	TGCCAAGAAGGGCGATGCCAA	ATCTTGTTGGCCACGTCAAGA	434	-	434	434	LOC_Os04g42270	putative 60s Ribosomal protein L25	GO:0005622	intracellular
34	NSFT03P2_Contig13490	1L+2L	CTAGCTAATTCRAAGCCTTGT	AAGAACTGYCTTTTGGCATGT	989	-	952	-	-	hypothetical protein T8F5.21	GO:0003674	molecular_function
35	NSFT03P2_Contig11742	1L+2L	TCTCCATCAAAGGCWGTTGCY	GAAKGCCATCTCGATCACATC	-	-	838	-	-	putative protein	None found	-
36	NSFT03P2_Contig13352	1L+2L	AGAAYTGATGSAGCACATGG	GACAAGCCATYAACCTSCT	747	-	-	-	-	hypothetical protein	None found	-
37	NSFT03P2_Contig17859	1L+2L	TCCTGGAAGACGRTCACYCT	CRACCTCTCCTTTCTTCATG	338	349	-	-	-	probable peptidylprolyl isomerase (EC 5.2.1.8) FKBP 77	GO:0003824	catalytic activity
38	NSFT03P2_Contig8323	1L+2L	TTGATGCGGCCATTGCCAA	GTGTAGGCCTCGTAGTTTAT	757	753	753	746	LOC_Os04g51300	ascorbate peroxidase, putative	GO:0003824	catalytic activity
39	DSW03C5_Contig3555.1	1L+2L+3L+4L+5L	GATGGCGCYCTCTCGTCGCA	CTTGCAGAAGCACTTGCGCT	-	331	320	-	-	defensin	None found	-
40	NSFT03P2_Contig5258	1L+2L+3L+4L+5L	TGGTGGAGAAGGAGRARGAG	CAAGCTTCAAYMCAGARTTC	-	-	508	-	-	maize EST AI621709	GO:0000166	nucleotide binding
41	NSFT03P2_Contig17497	1L+2L	AGGCGTTCCTCACCATGCTSTG	CAGAAGAGAAACACACGGTT	-	-	-	363	LOC_Os07g01560	monosaccharide transporter 3	GO:0005215	transporter activity
42	NSFT03P2_Contig17374	1L+2L	ACATTACGATCACTGGAGCC	CTATGACGTCACCACCATCG	778	510	784	801	LOC_Os05g23740	heat shock protein 70	GO:0005739	mitochondrion
43	NSFT03P2_Contig14008	2AL, 2DL	TACCTCTTCAAGTACATCATC	GTTTTTGCAGATGCCTCCAT	567	567	567	567	LOC_Os04g39440	RAS-RELATED PROTEIN RAB-2-B	GO:0005634	nucleus
44	NSFT03P2_Contig16961	2AL, 2DL	TGTGCTTGAGGCATCTATGC	ACCATCCCATGTYRCAGGCT	-	-	-	499	LOC_Os03g45320	putative 3-isopropylmalate dehydrogenase	GO:0003824	catalytic activity
45	NSFT03P2_Contig18086	2AL, 2DL	GAAAGGGAGATGGAMTTGAA	GAASAGTTGCTGCCCAAGA	-	-	601	-	-	poly(A)-binding protein	GO:0003676	nucleic acid binding

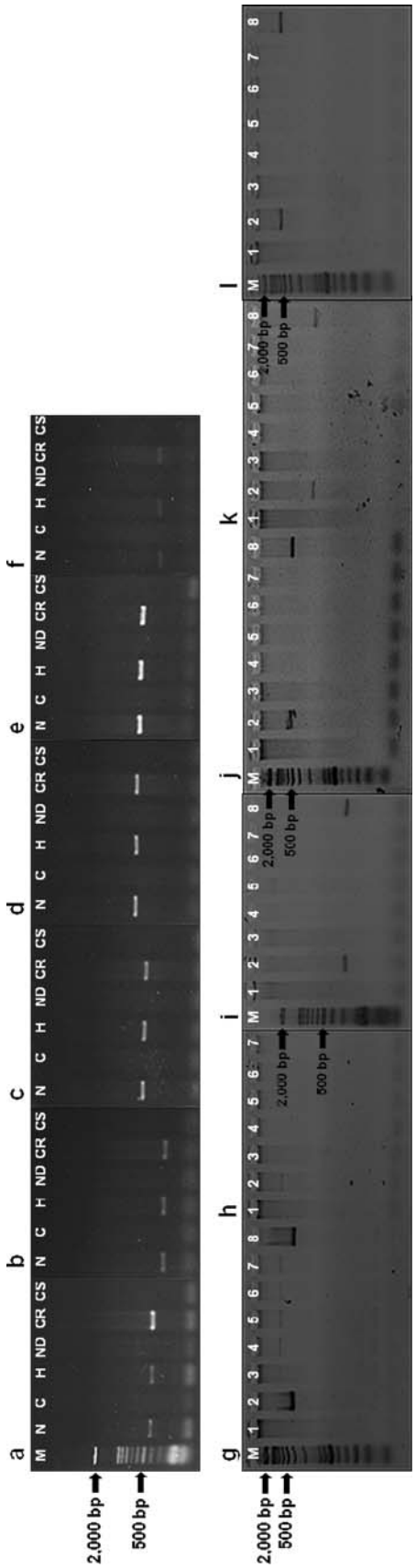
[a] Grain genes 2.0

[b] Mixed base code: R (A, G), Y (C, T), M (A, C), K (G, T), S (C, G), W (A, T)

[c] -: No amplification or did not match with target contig, yellow-colored rectangle: the same result as with Southern blot analysis, red-colored rectangle: newly cloned contigs in progenitors, blue-colored rectangle: failed to produce amplicons or did not match with target contigs

[d] Based on the sequences cloned from 'Chaupon' rye. Each rice gene model was color-coded according to the Fig. 2 of the Sorrells et al. 2003

[e] TIGR Rice Database (best match result)



◀ **Fig. 2** Confirmation of 2RL-specific markers. PCR products of 2RL-specific markers using 2BS.2RLs (NIL possessing *H21* and 'Hamlet') and non-2RLs ('Coker 797', 'ND 7532', and 'Chinese Spring') (**a–f**), and wheat–rye addition lines (**g–l**). 2RL-specific marker no. 1 (NSFT03P2_Contig17755) PCR products (**a**, **g**), 2RL-specific marker no. 2 (NSFT03P2_Contig4445) PCR products (**b**, **i**), 2RL-specific marker no. 3 (NSFT03P2_Contig14078) PCR products (**c**, **j**), 2RL-specific marker no. 4 (NSFT03P2_Contig17103) PCR products (**d**, **h**), 2RL-specific marker no. 5 (NSFT03P2_Contig16931) PCR products (**e**, **l**), 2RL-specific marker no. 6 (NSFT03P2_Contig16317) PCR products (**f**, **k**). Lane, *N* NIL (2BS.2RL, 'Coker 797' *4/'Hamlet'), *C* 'Coker 797' (non-2RL), *H* 'Hamlet' (2BS.2RL, 'ND 7532'/'Chaupon'//4* 'ND 7532'), *ND* 'ND 7532' (non-2RL), *CR* 'Chaupon' (TA 9002, *Secale cereale*), *CS* 'Chinese Spring' (common wheat), *1* 21″1R″, *2* 21″+2R′+2RL′, *3* 21″+3R″, *4* 21″+4R″, *5* 21″+5R″, *6* 21″+6R″, *7* 21″+7R″, *8* 'Chaupon' (TA 9002, *Secale cereale*). *M* 25/100 bp Mixed DNA Ladder (Bioneer, Korea)

compared with NIL non-infested samples) 1 DAI. The expression was reduced at 3 DAI compared to 1 DAI.

Discussion

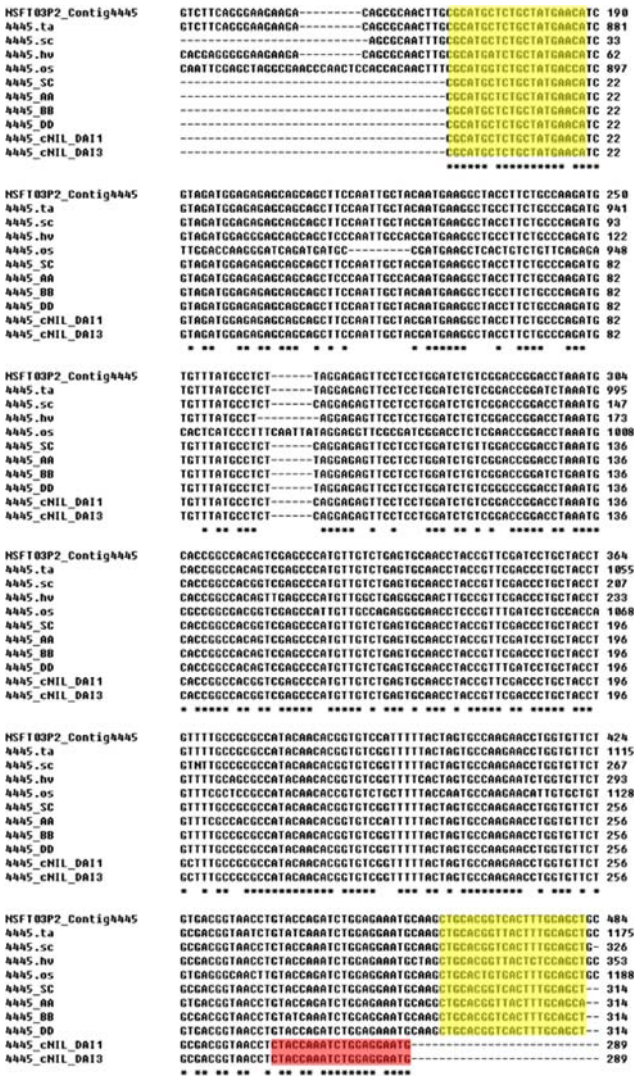
In order to identify transcripts specific to 2RL, unigene sequences in combination with wheat progenitor genomes were used. Considering the evolutionary history of the Triticeae tribe, there are close relationships among diploid genomes, e.g., wheat progenitors (AA, SS≒BB, and DD), barley (HH), and rye (RR) (Monte et al. 1993; Kellogg 1998; Paterson et al. 2003; Chantret et al. 2005). To facilitate the collection of interspecies-conserved regions across four species (wheat, rye, barley, and rice) securely, overcome the inaccuracy of rye EST sequences which showed relatively short length of sequences and less number of available sequence data, and avoid the possibility of INS/DEL of loci during translocation events, rice and barley unigenes were pooled for this study.

Rye chromatin 2RL showed homoeologous relationships to the long arms of wheat chromosome 2 (Naranjo and Fernández-Rueda 1991; Devos et al. 1995), suggesting a high sequence homology of homoeologous genes such as genes distributed on 2AL, 2DL, and 2RL (translocated rye chromatin) in homoeologous relationships. This would be one of the reasons why the homoeologous chromosomes are difficult to be discriminated by marker systems based on the differences of oligonucleotide sequences. To discriminate sequence polymorphisms between 2BL and 2RL, and to increase the specific marker numbers, three wheat progenitors and 'Chaupon' rye were used as surrogates for three common wheat genomes (AABBDD) and translocated rye arms (RR). Furthermore, contigs mapped on both long arms of homoeologous chromosome 2 and other wheat chromosome arms were excluded in order to reduce the misamplification introduced by multiple loci.

Table 2 2RL-specific primer pairs

No.	Contig	Primer sequence (5′ → 3′)[a]		Template DNA quantity for PCR (ng)	Annealing temp. (°C)	Product size (bp)
		Forward	Reverse			
1	NSFT03P2_Contig17755	CCTACCACCACTTGCCCATA	TTTCCCGTGCCGTAGACGAT	50	60	439
2	NSFT03P2_Contig4445	CGCATGCTCTGCTATGAACA	CATTCCTCCAGATTTGGTAG	50	60	289
3	NSFT03P2_Contig14078	CCAGCAGCTGGGAAACAATC	CTAGATCTTCTGCTTGAGCAC	50	65	548
4	NSFT03P2_Contig17103	CATTCCCCACGACCTCATC	AGCTCGGTTTGCTCCTGSACA	50	68	612
5	NSFT03P2_Contig16931	TGTTACACCATCCAGGCAGT	TCAAGAAGTGACCACCAAGA	50	65	579
6	NSFT03P2_Contig16317	AGACAGCCACGTACCTCTCA	TCTGGCACAGCTTCACCTCC	150	70	305

[a] Mixed base code: S (C, G)



◀ **Fig. 3** Sequence alignment of 2RL-specific marker no. 2 (NSFT03P2_Contig4445). Oligonucleotide sequences of ESTs downloaded from rye, wheat, barley, and rice, cloned sequences of three wheat progenitors (AA, SS≒BB, DD), specifically amplified oligonucleotide sequence with 2RL-specific marker no. 2 (NSFT03P2_Contig4445), and RT-PCR products were aligned. NSFT03P2_Contig4445, contig sequence downloaded from GrainGenes; 4445.ta, unigene sequence downloaded from NCBI and/or TIGR; 4445.sc, unigene sequence assembled from NCBI; 4445.hv, unigene sequence assembled from NCBI; 4445.os, unigene sequence downloaded from NCBI and/or TIGR; 4445_sc, cloned sequence from 'Chaupon' rye; 4445_AA, cloned sequence from *T. uratu* (AA genome) genomic DNA; 4445_BB, cloned sequence from *A. speltoides* (SS≒BB) genomic DNA; 4445_DD, cloned sequence from *A. squarrosa* (DD) genomic DNA; 4445_cNIL_DAI1, RT-PCR products using cDNA conversed from DAI1 RNA of NIL possessing *H21*; 4445_cNIL_DAI3, RT-PCR products using cDNA conversed from DAI3 RNA of NIL possessing *H21*. Some parts of oligonucleotide sequences of NSFT03P2_Contig4445 (from 1 to 139 and 485 to 763), 4445.ta (from 1 to 827 and from 1,176 to 1,950), 4445.hv (from 1 to 8 and from 354 to 631), 4445.os (from 1 to 837 and from 1,189 to 1,910) were omitted for the efficient alignment, respectively. Sequence alignment was analyzed by ClustalW with the default value. *Yellow-colored rectangles* represent forward and reverse primer sequences of cross-species markers. *Red-colored rectangle* represents reverse primer sequence of 2RL-specific primer pairs (Color figure online)

Wheat chromosome group 2 was shown to share syntenous blocks with rice chromosomes 4 (R4) and 7 (R7) (Sorrells et al. 2003; Conley et al. 2004; La Rota and Sorrells 2004). The long arm of wheat chromosome 2 is generally related to R4 (Sorrells et al. 2003; Conley et al. 2004; La Rota and Sorrells 2004). Additionally, comparative mapping of DNA sequences in rye provided the syntenous status between the rye chromosome 2, and R4 and R7 from the rice genome point of view (Hackauf et al. 2009). Hackauf and Wehling (2005) revealed orthology between R4 and 2RL with rearrangements of the distal parts of rye chromosome in their mapping research. In the absence of sufficient number of translocated rye sequences that could be calculated statistically, orthology cannot be thoroughly evaluated. High density markers, which reside on translocated chromatins, along with other types of markers such as EST-SSRs in wheat may help to draw the comparative maps of homoeologous chromatins in wheat.

Cross-species amplifications detected from three wheat progenitors were compared with the contig datasets downloaded from GrainGenes to evaluate efficacy of use of

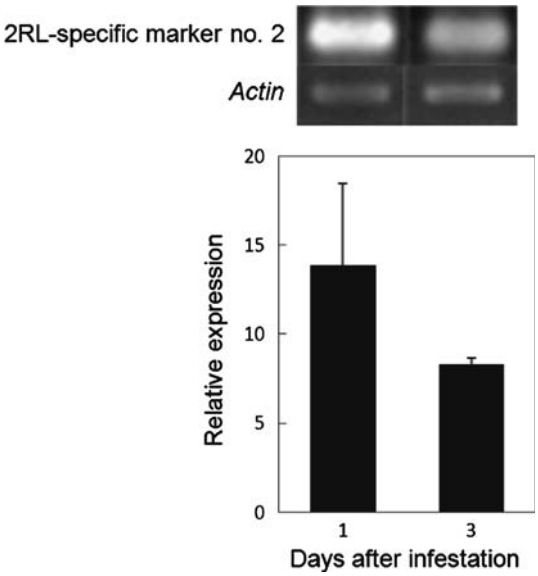


Fig. 4 Transcriptional expression of 2RL-specific marker no. 2 (NSFT03P2_Contig4445) in NIL possessing *H21*. RT-PCR analysis was performed in Hessian fly infested NIL (2BS.2RL). real-time RT-PCR was performed in Hessian fly infested and non-infested NIL (2BS.2RL). Expression of 2RL-specific marker no. 2 in Hessian fly non-infested samples was defined as 1. *Error bars* denote the standard deviation from the mean of at least two independent experiments. Expression of *Actin* gene served as a control in both experiments

contig information and wheat progenitors. Partial disagreement of evaluated clone numbers between detected amplicons and the contig datasets would be the results of, *in macro*:not all genomes (common wheat and wheat progenitors) are perfectly matched, *in micro*:genomic duplication or INS/DEL of primer pair tagging locus. A total of 67 cross-species primer pairs failed to produce amplicons, possibly because an intron occurred within the primer sequences interrupting amplification, a large intron disrupted PCR extension, or inaccurate unigene sequences occurred in unigene-clusters containing rye unigene sequences. Nevertheless, the obtained 45 cross-species amplifications that were cloned from three wheat progenitors and rye would be putative candidate genes (alleles) for comparative mapping in wheat and for traits of NILs containing 2RL. Seven cross-species amplifications (no. 1, 5, 21, 22, 23, 41, and 44 in Table 1) might be defined as being "rye-specific" at least under the assay conditions applied and could be evaluated for possible 2RL-specific markers. However, cross-species primer pairs for those seven cross-species amplifications produced multiple bands which were different from expected target size in each AA, BB, DD genome, which might hinder to develop primer pairs to generate clear single band of expected size. Therefore, those seven amplifications were not included in the further study.

Among 45 cross-species amplifications, six 2RL-specific markers (13%) specifically tagging 2RL were developed. These results could be explained partially by lesser polymorphism between homologous alleles due to a short time after divergence of wheat diploid progenitors and a lack of rye ESTs registered in public database. These primers amplified unique single amplified fragment in the 2BS.2RL translocation lines, one wheat–rye addition line (21″+2R′+2RL′) and rye cultivars, but not in the non-2BS.2RLs, and other types of wheat–rye addition lines and translocations. Confirmation of six 2RL-specific markers on 2BS.2RL translocation lines, wheat–rye addition lines, and various translocation lines established sufficient proof of specificity of the markers developed in this study. Based on public database (GrainGenes 2.0), six 2RL-specific markers were confirmed to be resided on specific wheat 2BL bin. Even though 2RL-specific amplicons that showed the best match with the targeted contigs via BLAST, direct hybridization on rye chromosome or PCR using 2RL deletion stock would be needed for the further localization of 2RL-specific markers. Intensive mapping researches using 1R or 6R deletion stocks were reported (Dundas et al. 2001; Tsuchida et al. 2008). Because the deletion stock of rye chromosome arm 2RL has not been developed yet, genetic mapping in a segregating population would be an alternative method for addressing the regions of 2RL-specific sequences in wheat–rye translocation lines.

For the functional assessment of developed 2RL-specific markers, 2RL-specific marker no. 2 (NSFT03P2_Contig4445) was applied on Hessian fly infested NILs. Under Hessian fly infestation, significantly high expression of gene was tagged by the 2RL-specific marker no. 2 at 1 day after infestation. Using BLASTN (E value $\leq 1 \times 10^{-15}$) queries, this contig did not match any known proteins in TIGR. We speculate that the early burst of gene expression at the initiation of larvae attack in the 2BS.2RL wheat–rye translocation might indicate its significant role in the antibiosis.

Wheat–rye translocation in the form of 2BS.2RL showed an improved agronomic performance that was related with biotic and/or abiotic stresses (Friebe et al. 1990; Heun and Friebe 1990; Hysing et al. 2007). It also has additional agronomic traits, such as powdery mildew resistance and has reddish glume and awn color. 2RL-specific markers primarily enhanced in quantity will facilitate to map not only Hessian fly resistance on 2RL, but also other important agronomic traits.

In this study: (1) 45 cross-species markers, which were derived from ESTs mapped to the long arms of homoeologous group 2 of common wheat, were identified in three wheat progenitors and rye, (2) six 2RL-specific markers discriminating 2BS.2RL wheat–rye translocation lines were developed, and (3) under Hessian fly infestation, the significant transcriptional increases of one of the 2RL-specific markers were functionally assessed.

In conclusion, the approach using public sequence databases and the application of wheat progenitors to discriminate sequence polymorphisms provides a valuable tool for the development of translocated rye chromatin-specific markers, which corresponded directly to transcribed genes on 2RL and could be employed in elucidating functions of genes on 2RL. In addition, EST-derived 2RL-specific markers would be used for marker-aided selection in the efficient genomics-assisted breeding program.

Acknowledgments The study was supported by a grant (No. 20070301-034-016-009-01-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

References

Anderson JR, Lübberstedt T (2003) Functional markers in plants. Trends Plant Sci 8:554–560

Brunell MS, Lukaszewski AJ, Whitkus R (1999) Development of arm specific RAPD markers for rye chromosome 2R in wheat. Crop Sci 39:1702–1706

Chantret N, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, Dubois I, Dossat C, Sourdille P, Joudrier P, Gautier M-F, Cattolico L, Beckert M, Aubourg S, Weissenbach J, Caboche M, Bernard M, Leroy P, Chalhoub B (2005) Molecular basis of evolutionary events that shaped the *Hardness* locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*). Plant Cell 17:1033–1045

Choi HW, Kim YJ, Lee SC, Hong JK, Hwang BK (2007) Hydrogen peroxide generation by the pepper extracellular peroxidase $CaPO_2$ activates local and systemic cell death and defense response to bacterial pathogens. Plant Physiol 145:890–904

Conley EJ, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo GR, Hummel DD, Anderson OD, Qi LL, Gill BS, Echalier B, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvořák J, Peng JH, Lapitan NLV, Pathan MS, Nguyen HT, Ma X-F, Miftahudin, Gustafson JP, Greene RA, Sorrells ME, Hossain KG, Kalavacharla V, Kianian SF, Sidhu D, Dilbirligi M, Gill KS, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Anderson JA (2004) A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetics 168:625–637

Devos KM, Moore G, Gale MD (1995) Conservation of marker synteny during evolution. Euphytica 85:367–372

Dundas IS, Frappell DE, Crack DM, Fisher JM (2001) Deletion mapping of a nematode resistance gene on rye chromosome 6R in wheat. Crop Sci 41:1771–1778

Feltus FA, Singh HP, Lohithaswa HC, Schulze SR, Silva TD, Paterson AH (2006) A comparative genomics strategy for targeted discovery of single-nucleotide polymorphisms and conserved-noncoding sequences in orphan crops. Plant Physiol 140:1183–1191

Friebe B, Hatchett JH, Sears RG, Gill BS (1990) Transfer of Hessian fly resistance from 'Chaupon' rye to hexaploid wheat via a 2BS/2RL wheat–rye chromosome translocation. Theor Appl Genet 79:385–389

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat–alien translocations conferring resistance to diseases and pets: current status. Euphytica 91:59–87

Gupta PK, Rustgi S (2004) Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr Genomics 4:139–162

Hackauf B, Wehling P (2005) Approaching the self-incompatibility locus Z in rye (*Secale cereale* L.) via comparative genetics. Theor Appl Genet 110:832–845

Hackauf B, Rudd S, van der Voort JR, Miedaner T, Wehling P (2009) Comparative mapping of DNA sequences in rye (*Secale cereale* L.) in relation to the rice genome. Theor Appl Genet 118:371–384

Hatchett JH, Sears RG, Cox TS (1993) Inheritance of resistance to Hessian fly in rye and in wheat–rye translocation lines. Crop Sci 33:730–734

Heun M, Friebe B (1990) Introgression of powdery mildew resistance from rye into wheat. Phytopathology 80:242–245

Hysing SC, Hsam SLK, Singh RP, Huerta-Espino J, Boyd LA, Koebner RMD, Cambron S, Johnson JW, Bland DE, Liljeroth E, Merker A (2007) Agronomic performance and multiple disease resistance in T2BS.2RL wheat–rye translocation lines. Crop Sci 47:254–260

Ishikawa G, Yonemaru J, Saito M, Nakamura T (2007) PCR-based landmark unique gene (PLUG) markers effectively assign homoeologous wheat genes to A, B and D genomes. BMC Genomics 8:135

Jang CS, Kim JY, Haam JW, Lee MS, Kim DS, Li YW, Seo YW (2003) Expressed sequence tags from a wheat–rye translocation line (2BS/2RL) infested by larvae of Hessian fly [*Mayetiola destructor* (Say)]. Plant Cell Rep 22:150–158

Kellogg EA (1998) Relationships of cereal crops and other grasses. Proc Natl Acad Sci 95:2005–2010

La Rota M, Sorrells ME (2004) Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships between rice and wheat. Funct Integr Genomics 4:34–46

Lee JH, Graybosch RA, Lee DJ (1994) Detection of rye chromosome 2R-using the polymerase chain reaction and sequence-specific DNA primers. Genome 37:19–22

Lee JH, Graybosch RA, Kaeppler SM, Sears RG (1996) A PCR assay for detection of 2RL.2BS wheat–rye chromosome translocation. Genome 39:605–608

Liu XM, Gill BS, Chen M-S (2005) Hessian fly resistance gene *H13* is mapped to a distal cluster of resistance genes in chromosome 6DS of wheat. Theor Appl Genet 111:243–249

Mago R, Miah H, Lawrence GJ, Wellings CR, Spielmeyer W, Bariana HS, McIntosh RA, Pryor AJ, Ellis JG (2005) High-resolution mapping and mutation analysis separate the rust resistance genes *Sr31*, *Lr26* and *Yr9* on the short arm of rye chromosome 1. Theor Appl Genet 112:41–50

Monte JV, McIntyre CL, Gustafson JP (1993) Analysis of phylogenetic relationships in the *Triticeae tribe using* RFLPs. Theor Appl Genet 86:649–655

Naranjo T, Fernández-Rueda P (1991) Homoeology of rye chromosome arms to wheat. Theor Appl Genet 82:577–586

Nomura T, Ishihara A, Yanagita RC, Endo TR, Iwamura H (2005) Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat. PNAS 102:16490–16495

Paterson AH, Bowers JE, Peterson DG, Estill JC, Chapman BA (2003) Structure and evolution of cereal genomes. Curr Opin Genet Dev 13:644–650

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereal* L.). Genome 42:964–972

Sardesai N, Nemacheck JA, Subramanyam S, Williams CE (2005) Identification and mapping of *H32*, a new wheat gene conferring resistance to Hessian fly. Theor Appl Genet 111:1167–1173

Seo YW, Johnson JW, Jarret RL (1997) A molecular marker associated with the *H21* Hessian fly resistance gene in wheat. Mol Breed 3:177–181

Seo YW, Jang CS, Johnson JW (2001) Development of AFLP and STS markers for identifying wheat–rye translocations possessing 2RL. Euphytica 121:279–287

Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin Mahmoud A, Ma X, Gustafson PJ, Qi LL, Echalier B, Gill BS, Matthews DE, Lazo GR, Chao S, Anderson OD, Edwards H, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvorak J, Zhang D, Nguyen HT, Peng J, Lapitan NL, Gonzalez-Hernandez JL, Anderson JA, Hossain K, Kalavacharla V, Kianian SF, Choi DW, Close TJ, Dilbirligi M, Gill KS, Steber C, Walker-Simmons MK, McGuire PE, Qualset CO (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Tsuchida M, Fukushima T, Nasuda S, Masoudi-Nejad A, Ishikawa G, Nakamura T, Endo TR (2008) Dissection of rye chromosome 1R in common wheat. Genes Genet Syst 83:43–53

Zhao HX, Liu XM, Chen M-S (2006) *H22*, a major resistance gene to the Hessian fly (*Mayetiola destructor*), is mapped to the distal region of wheat chromosome 1DS. Theor Appl Genet 113:1491–1496