Cytological dissection and molecular characterization of chromosome 1R derived from ‘Burgas 2’ common wheat

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Rye chromosome 1R harbors many agronomically important genes such as resistance genes for rusts. Using the gametocidal system, we dissected the 1R chromosome substituted for wheat chromosome 1B in a common wheat cultivar ‘Burgas 2’. The gametocidal system induces chromosomal breakage in the 1R chromosome, as well as in wheat chromosomes. We cytologically examined a pool of prescreened common wheat plants that had been shown to have single or multiple rearranged 1R chromosomes and established 95 common wheat lines carrying single 1R segments. We conducted PCR analysis of these lines, termed ‘1R dissection lines’, using 10 PCR-based 1R-specific markers. We mapped the 10 PCR-based markers along the 1R chromosome with the breakpoints of the 1R dissection lines. Based on the PCR result and the positions of the primary and secondary constrictions, we could separate the breakpoints of the rearranged 1R chromosomes into 12 regions along the 1R chromosome. On the other hand, using the breakpoints, we could separate the PCR-based markers from each other except for two markers. These dissection lines are useful in mapping DNA markers and may facilitate the construction of contig maps.

Key words: rye, chromosome 1R, dissection line, gametocidal chromosome, chromosome map

INTRODUCTION

Rye is one of the major crops belonging to the tribe Triticeae. It grows in areas of adverse agro-climatic conditions, and therefore is cultivated in wide areas of Europe and North America (Loarce et al., 1996). The rye genome is large and complex (~7900 Mbp/1C, Doležel et al., 1998), 92% of which comprises repetitive sequences (Flavell et al., 1974), and repetitive sequences constitute about 60% of the rye genomic library (Philipp et al., 1994). This abundant existence of repetitive sequences would hamper researchers to assemble rye genomic DNA sequences into linear chromosome maps, as achieved in rice (Doležel et al., 2007). In common wheat, there are various series of aneuploid lines, such as nullisomic-tetrasomic lines, ditelosomic lines, and addition lines carrying alien chromosomes including rye chromosomes. These aneuploid lines are indispensable to the genome analysis of the Triticeae species and therefore have been used to determine the chromosomal location of genes and

DNA markers (Gale et al., 1993; Sears, 1954; McIntosh et al., 2003; Serizawa et al., 2001; Masoudi-Nejad et al., 2005; Nasuda et al., 2005; Nagy and Lelley, 2003). Aneuploid lines dissect a whole genome into its components, chromosomes and chromosome arms (Doležel et al., 2007). Aneuploid lines with sub-arm deficiency are even more useful for genome analysis. In common wheat, the most potential tool to cause sub-arm deficiency, i.e., to dissect a chromosome is the gametocidal (Gc) system in which the Gc chromosome introduced from wild species induces chromosome breaks in gametes lacking the Gc chromosome (Endo, 1988, 1990). Endo and Gill (1996) developed the deletion stocks of common wheat that carry deficient chromosomes in homozygous condition. The Gc system works for alien chromosomes added to common wheat as effectively as for wheat chromosomes (Endo et al., 1994; Shi and Endo, 1999, 2000; Friebe et al., 2000; Endo, 2007).

Using the Gc system of chromosome 2C derived from *Aegilops cylindrica*, Tsuchida et al. (2008) produced 55 dissection lines of common wheat carrying single rearranged chromosomes of the 1R chromosome derived from ‘Imperial’ rye. Endo et al. (1994) developed a line generating

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structural rearrangements in another rye 1R chromosome in Bulgarian winter wheat cultivar 'Burgas 2', using the Gc system of another Gc chromosome 3C derived from *Ae. triuncialis*. Endo et al. (1998) continued with screening for 3C-induced rearranged 1R chromosomes and obtained a large pool of lines carrying rearranged 1R chromosomes. However, most of the rearranged 1R chromosomes, except a few reported by Masoudi-Nejad et al. (2002), have not been isolated in established lines.

Undoubtedly, the more 1R dissection lines we have, the more precisely we can conduct genome analysis of chromosome 1R. Also, it was interesting to compare the structures of the two 1R chromosomes derived from 'Imperial' rye and 'Burgas 2' wheat. In this study, we planned to establish more 'Burgas 2' 1R dissection lines from the already available pool of common wheat lines produced by Endo et al. (1998). We conducted in situ hybridization to establish dissection lines that were homozygous or hemizygous for single rearranged 1R chromosomes, and then conducted PCR analysis with the dissection lines to characterize the rearranged 1R chromosomes.

MATERIALS AND METHODS

Plant material We used 201 lines of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, genome formula AABBDD) in the available pool of lines carrying a single or multiple rearranged 1R chromosomes (Endo et al., 1998; Masoudi-Nejad et al., 2002). The prescreened lines were the progeny of a common wheat line carrying a pair of 1R chromosomes and one Gc chromosome 3C; this line had been derived from a cross between a Bulgarian winter wheat cultivar 'Burgas 2' and the 3C Gc line of common wheat 'Norin 26' (Endo et al., 1994). 'Burgas 2' has a disomic substitution of chromosome 1R for wheat chromosome 1B, and the 1R chromosome is most likely derived from 'Petkus' rye (Rabinovich, 1998). From the selfed progeny of the cross, Endo et al. (1998) had established a disomic 1R substitution line that we used as a control in this study. Chromosome 3C induces semi-lethal chromosomal breakage in the presence of *Igc1* of 'Norin 26' (Tsujimoto and Tsunewaki, 1985). Thanks to hybrid origin, the prescreened lines had heterozygous genotypes. In this study we backcrossed the prescreened lines to 'Chinese Spring' one to three times in order to establish 1R dissection lines in the standard cultivar for wheat genetic studies. We obtained a common wheat cultivar 'Chinese Spring' and a rye (*Secale cereale* L., $2n = 2x = 14$, genome formula RR) cultivar 'Imperial' from National BioResource Project (<http://www.nbrp.jp/report/reportProject.jsp>) and used them as controls.

Cytological screening We identified the rearranged 1R chromosomes by a combination of FISH (fluorescence

in situ hybridization) and GISH (genomic in situ hybridization). As a FISH probe we used the 521 bp tandemly repeated satellite DNA of rye pSc200 (Vershinin et al., 1995) that was amplified by PCR with a primer set, 5'-GAGTCTCGATCAATTTCGGACT-3'/5'-GCAAGTGAGAG-AACAAGCAAC-3' and was labeled with DIG-11-dUTP (Roche Diagnostics, Germany). The pSc200 probe detects the 1R telomeric heterochromatin (Vershinin et al., 1996). The GISH probe was the rye total genomic DNA labeled with biotin-16-dUTP (Roche Diagnostics, Germany). We used another FISH probe, the rye centromere-specific sequence pAWRC.1 (Francki, 2001), to examine the centromeres of some of the rearranged 1R chromosomes with breakpoints within or close to the centromere. We used two primer sets to amplify the pAWRC.1 sequence. The first set (5'-AGAACAAGATGCCGAGGCTA-3'/5'-TCTTGCTTGTCGCCCTAAGT-3') amplified approximately 950 bp and the second set (5'-ACGAGGTTCTCTTCCGTGTC-3'/5'-GTGGTCTCCTTCA-CACCTGA-3') amplified approximately 1100 bp major PCR products, as expected from the previously reported sequence (Francki, 2001). We labeled the PCR products separately with DIG-11-dUTP (Roche Diagnostics, Germany) and mixed the two probes for FISH. We conducted FISH and GISH as described by Sakai et al. (2009). Whenever possible, we estimated the relative chromosome lengths of the rearranged 1R chromosomes, as described by Endo and Gill (1996), and used them to draw a chromosome map.

PCR analysis We used 10 of the 1R-specific 11 PCR-based markers which Tsuchida et al. (2008) had mapped to the 'Imperial' 1R chromosome; one marker did not amplify well in the addition line carrying the 'Burgas 2' 1R chromosome. DNA was extracted from young leaves by the CTAB method. PCR was conducted in a 30 µl reaction mixture containing 1 µl template DNA (100 ng/µl), 2.5 µl of 10× PCR buffer (TaKaRa, Japan), 1.5 µl MgCl₂ (25 mM), 2 µl dNTP (2.5 mM), 1 µl each of the forward and reverse primers (10 pmol/µl) and 0.24 µl Ex Taq polymerase (5 U/µl, TaKaRa, Japan). We used the same PCR conditions as described by Tsuchida et al. (2008) for the EST and PLUG primers. The PCR conditions for the rye-specific centromeric sequence were as follows: 5 min initial denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 30 sec annealing at 67°C and 3 min extension at 72°C. We run the 5 µl aliquot of the PCR products on 1% agarose gels in TAE buffer at 100 V for 40 min.

RESULTS

Cytological screening We checked the progeny, up to four plants for each line, of the prescreened 201 lines numbered consecutively in the order of identification.

Table 1. 1R dissection lines and rearranged 1R chromosomes

Line	Rearranged 1R chromosome						
1R-	State	Type of rearrangement	Breakpoint*				
1	homozygous	translocation	centromere (L)	96a	hemizygous	translocation	centromere (S)
6	hemizygous	translocation	centromere (L)	96b	hemizygous	translocation	long arm
7a	hemizygous	translocation	short arm	99	hemizygous	deletion	short arm
7b	hemizygous	translocation	long arm	102	hemizygous	deletion	short arm
9	hemizygous	deletion	short arm	103a	hemizygous	deletion	long arm
10	hemizygous	deletion	short arm	103b	hemizygous	translocation	centromere (S)
11	hemizygous	deletion	long arm	104	hemizygous	deletion/translocation	satellite/centromere (S)
14	hemizygous	translocation	long arm	105	hemizygous	translocation	satellite
16	hemizygous	deletion	satellite	106	hemizygous	translocation	long arm
17	hemizygous	deletion	centromere (S)	107	hemizygous	deletion	centromere (L)
19	hemizygous	translocation	short arm	108a	hemizygous	translocation	long arm
20	hemizygous	translocation	satellite	108b	hemizygous	translocation	long arm
21	hemizygous	translocation	short arm	109	hemizygous	translocation	long arm
24	hemizygous	deletion	long arm	110a	hemizygous	translocation	short arm
26	hemizygous	translocation	long arm	110b	hemizygous	translocation	short arm
27	hemizygous	deletion	long arm	112	hemizygous	translocation	centromere (S)
30	hemizygous	deletion	centromere (S)	113a	hemizygous	deletion	long arm
31	hemizygous	translocation	short arm	113b	hemizygous	translocation	long arm
32	hemizygous	translocation	short arm	115	homozygous	translocation	long arm
33	hemizygous	translocation	long arm	118	hemizygous	deletion/translocation	long arm/long arm
39	hemizygous	translocation	long arm	119	hemizygous	translocation (interstitial)	long arm/long arm
40	hemizygous	translocation	satellite	121	hemizygous	translocation	long arm
42	hemizygous	deletion	long arm	122	hemizygous	deletion	long arm
45	hemizygous	translocation	long arm	126	hemizygous	deletion	long arm
48	homozygous	translocation	long arm	131a	hemizygous	deletion	satellite
50	hemizygous	translocation	long arm	131b	hemizygous	deletion/translocation	satellite/centromere (S)
51	hemizygous	deletion	long arm	134	hemizygous	translocation	long arm
55	hemizygous	translocation	long arm	135	hemizygous	translocation	long arm
56	hemizygous	deletion	long arm	140	hemizygous	translocation	short arm
57	hemizygous	deletion	short arm	146	hemizygous	translocation	short arm
58	hemizygous	deletion	long arm	147	homozygous	deletion	long arm
59	hemizygous	translocation	long arm	152	homozygous	translocation	short arm
60	hemizygous	translocation	short arm	154	hemizygous	translocation	long arm
62	hemizygous	deletion	satellite	155	hemizygous	translocation	centromere (L)
64	hemizygous	translocation	short arm	157	homozygous	translocation	long arm
66	hemizygous	translocation	long arm	163	hemizygous	translocation	short arm
68	hemizygous	deletion	short arm	165	hemizygous	translocation	long arm
71	hemizygous	translocation	centromere (L)	167	hemizygous	translocation	long arm
72	homozygous	translocation	?	168	hemizygous	deletion	short arm
73	hemizygous	translocation	short arm	170	hemizygous	translocation	short arm
74	hemizygous	translocation	long arm	172	hemizygous	translocation	long arm
75	hemizygous	deletion	short arm	173	hemizygous	translocation	long arm
76	hemizygous	deletion	short arm	180	hemizygous	deletion	long arm
77	hemizygous	translocation	centromere (L)	182	hemizygous	deletion	short arm
79	hemizygous	translocation	satellite	184	hemizygous	deletion/translocation	long arm/long arm
82	hemizygous	deletion	long arm	187	hemizygous	translocation	long arm
84a	hemizygous	deletion	long arm	* (L) and (S) indicate the rearranged 1R chromosomes with breakpoints in the centromere (telocentric chromosomes) retained the long arm and the short arm, respectively. All the telocentric chromosomes had the rye-specific repetitive sequences.			
84b	homozygous	translocation	long arm				

*(L) and (S) indicate the rearranged 1R chromosomes with breakpoints in the centromere (telocentric chromosomes) retained the long arm and the short arm, respectively. All the telocentric chromosomes had the rye-specific repetitive sequences.

Fig. 1. FISH/GISH images of the normal 1R chromosome and rearranged 1R chromosomes induced by the Gc system. The rearranged 1R chromosomes were numbered in the order of initial identification; therefore some numbers are missing. Those with numerals followed by "a" and "b" originated from the same prescreened line. Except for the 1' image, the green FISH signals represented the pSc200 repeats and the pink GISH signals represented the rye chromatin. The green FISH signal of the 1' image shows the rye centromeric repeats on chromosome 1R-1 counter-stained with PI (propidium iodide) and the 1'' FISH/GISH image of the same chromosome proved that the chromosome was 1R-1. Bar = 10 μ m.

Table 2. Amplification (+) of the PCR markers in the 1R dissected lines

Line	Marker									
	KUR1001	TOP1078	TOP1088	KUR1007	TOP1094	KUR1047	TOP1075	TOP1017	<i>Sec1</i>	IAG95
131a	+	+	+	+	+	+	+	+	+	
10	+	+	+	+	+	+	+	+		
16	+	+	+	+	+	+	+	+		
21	+	+	+	+	+	+	+	+		
62	+	+	+	+	+	+	+	+		
64	+	+	+	+	+	+	+	+		
68	+	+	+	+	+	+	+	+		
73	+	+	+	+	+	+	+	+		
75	+	+	+	+	+	+	+	+		
79	+	+	+	+	+	+	+	+		
102	+	+	+	+	+	+	+	+		
110b	+	+	+	+	+	+	+	+		
168	+	+	+	+	+	+	+	+		
170	+	+	+	+	+	+	+	+		
9	+	+	+	+	+	+	+			
19	+	+	+	+	+	+	+			
57	+	+	+	+	+	+	+			
76	+	+	+	+	+	+	+			
99	+	+	+	+	+	+	+			
182	+	+	+	+	+	+	+			
17								+	+	+
30								+	+	+
32								+	+	+
60								+	+	+
96a								+	+	+
103b								+	+	+
110a								+	+	+
112								+	+	+
140								+	+	+
131b								+	+	
104								+		
7a									+	+
31									+	+
94									+	+
105									+	+
146									+	+
152									+	+
163									+	+
20										+
40										+
14	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+			
6	+	+	+	+	+	+	+			
50	+	+	+	+	+	+	+			
71	+	+	+	+	+	+	+			
77	+	+	+	+	+	+	+			

Continued

[illegible]

We found no rearranged 1R chromosome in the progeny of 46 lines and multiple or ambiguous rearranged 1R chromosomes in the progeny of 29 lines. From the remaining 126 lines, we successfully obtained plants with single rearranged 1R chromosomes. Among those lines, however, we omitted eight lines with an isochromosome (two lines for 1RS and six lines for 1RL) in this study. In the end, we established 95 dissection lines carrying single rearranged 1R chromosomes (Table 1, Fig. 1). Lines 1R-20, 1R-40, 1R-104 and 1R-105 were derived from the three prescreened lines that had been studied on rust response by Masoudi-Nejad et al. (2002). Out of the 95 dissection lines, 31 lines had terminal deletions with single breakpoints: 13 of them were in 1RS, 15 in 1RL and 3 in the centromere (1R-17 and 1R-30 retained the 1RS arm; 1R-107 retained the 1RL arm). Among the rest, 58 lines had translocations between chromosome 1R and wheat chromosomes with single translocation points: 20 of them had translocation points in the 1RS arm, 30 in the 1RL arm and 8 in the centromere. The remaining six lines had complex structures. Chromosome 1R-104 was a telosome derivative of the prescreened chromosome 1R-104 with a small wheat chromosomal segment translocated onto the 1R satellite. Chromosomes 1R-72 and 1R-

119 were wheat chromosomes with small rye chromosomal segments. Chromosomes 1R-118, 1R-131b and 1R-184 were 1R-wheat translocations with rye interstitial chromosomal segments. Chromosome 1R-131b seems to have been generated from a secondary restructuring of 1R-131a, namely centromeric breakage and centromeric fusion with a wheat chromosome arm. Three pairs of translocations (1R-84a and 1R-84b, 1R-104 and 1R-105, 1R-108a and 1R-108b) were derived from single plants and were probably reciprocal translocations. Supposing that the six translocations had two breakpoints, that the three reciprocal translocation had the same breakpoint, and that 1R-131a and -131b had the same breakpoint in the satellite, a total of 97 (= 95 + 6-3-1) breakpoints were available as landmarks of chromosome 1R. All the breakpoints in the centromere retained the rye-specific centromeric repeat (see Fig. 1, 1' and 1").

Arrangement of the break points of the rearranged 1R chromosomes Table 2 shows the result of PCR analysis of the 1R dissection lines. All three KUR markers and four TOP markers except TOP 1017 were located on the 1RL arm, TOP 1017 was on the 1RS arm, and *Sec-1* and IAG95 were located on the 1R satellite. Based on

Fig. 2. Cytological maps showing the breakpoints of the 94 rearranged 1R chromosomes except 1R-72 that contained no PCR marker site. Red bars stand for the position of the markers on the left and brackets and horizontal lines indicate the regions of the breakpoints on the right. The whole-arm translocation chromosomes with asterisks had the 1RL arm and those without asterisk had the 1RS arm. The vertical bars indicate approximate size of 1R chromosome segments of the 1R rearranged chromosomes with double breakpoints. Roman numerals I to XII on the right indicate the 12 bins separated by the PCR markers, NOR and centromere.

the data in Table 2, we arranged the breakpoints of the 95 rearranged 1R chromosomes along the 1R chromosome (Fig. 2). With the 10 PCR markers and two cytological landmarks, i.e. secondary constriction and centromere, the breakpoints were separated into 12 regions; there was no breakpoint distal to IAG95, and between TOP 1088 and KUR 1007. By the obvious difference in cytological position, we could divide the breakpoints in the pericentromeric region into three regions, namely the 1RS proximal, centromeric and 1RL proximal regions, and those in the 1RL distal region between TOP 1078 and KUR 1001 could be divided into two regions. Due to the absence of all the PCR markers in line 1R-72, we could not identify the chromosomal region of the inserted rye segment; therefore the breakpoints of 1R-72 were not indicated in Fig. 2. The PCR analysis suggested that the inserted rye segments of 1R-119 and 1R-184 were parts of 1RL containing TOP 1094 between KUR 1047 and KUR 1007. Three of the seven breakpoints in the satellite were located distal to *Sec-1* the remaining four proximal to locus. The breakpoints of the rearranged 1R chromosomes separated the 10 PCR markers from each other, except for TOP 1088 and KUR 1007. There was no PCR marker distal to the breakpoints of 1R-14, 1R-26, 1R-48, 1R-96b and 1R-134.

DISCUSSION

In this study, we developed 95 lines carrying single deletion or translocation chromosomes involving 1R, either in a hemizygous or homozygous condition. Since we checked only up to four plants for each line, we could undoubtedly have obtained many more dissection lines from the prescreened lines if we checked more progeny plants. We noticed that it is rather difficult to separate two rearranged 1R chromosomes resulting from a reciprocal translocation. This is probably because the two rearranged 1R chromosomes form a trivalent with a wheat chromosome and segregate alternatively.

In this study, the GISH/FISH and PCR analyses revealed that the 97 breakpoints of the 95 rearranged 1R chromosomes were distributed in the satellite (7), in the short arm excluding the satellite (26), in the centromere (13) and in the long arm (51). Tsuchida et al. (2008) reported that 55 rearranged 'Imperial' 1R chromosomes were induced by the Gc chromosome 2C and that their 58 breakpoints distributed in the satellite (4), in the short arm excluding the satellite (12), and in the centromere (14) and in the long arm (28). Considering that eight 1R isochromosomes were not included in the present study, the breakpoint distribution is not significantly different between the two studies. However, types of aberrations are different between the two studies. Tsuchida et al. (2008) reported that 25.5% of the rearranged 'Imperial' 1R chromosomes were translocations; on the other hand,

we found that 67.3% of the rearranged 'Burgas 2' 1R chromosomes were translocations. The Gc action of chromosome 3C seems to have the more potential for inducing translocations.

We arranged the 10 PCR markers based on the breakpoints of the 95 rearranged 1R chromosomes as shown in Fig. 2. Tsuchida et al. (2008) determined the order of the same PCR markers using the breakpoints of rearranged 'Imperial' rye 1R chromosomes. Although there was no rearranged 'Burgas 2' 1R chromosome with a breakpoint between KUR 1007 and TOP 1088, there was one rearranged 'Imperial' 1R chromosome with a breakpoint in that region; therefore KUR 1007 is placed proximal to TOP 1088 (Tsuchida et al., 2008). The order of the other markers was the same in the two 1R chromosomes. This fact suggests that the general structure of the 1R chromosomes in 'Burgas 2' wheat and 'Imperial' rye is the same, i.e., the 1R chromosomes have suffered no extensive structural changes such as inversions. On the other hand, the 95 breakpoints were grouped into 12 regions separated with the 10 PCR markers and two cytological landmarks, the secondary constriction and centromere. There was almost no contradiction between the cytological observation of the rearranged 1R chromosomes (Fig. 1) and the PCR analysis of the dissection lines except for one case; line 1R-33 did not have a marker that was supposed to be present (TOP 1088), which was probably due to a minor interstitial deletion (Table 2). This fact confirmed that the rearranged 1R chromosomes had no other structural changes such as interstitial deletions and intrachromosomal translocations.

The *Sec-1* gene encoding secalin, which has an adverse effect on dough quality, is located proximal to a cluster of genes for the resistance to rusts and powdery mildew on the satellite (Singh et al., 1990). The separation of the *Sec-1* locus from the resistance gene loci, which require an interstitial deletion, has been sought without success (Singh et al., 1990). Masoudi-Nejad et al. (2002) obtained seven 1R translocation chromosomes with a breakpoint in the satellite and found that two of the translocation chromosomes (1R-20 and 1R-40) lacked the *Sec-1* locus but conferred resistance to the rust on the plants carrying them. In this study we found six more translocations and deletions that have breakpoints in the satellite (1R-16, 1R-62, 1R-72, 1R-79, 1R-131a and 1R-131b). The breakpoints of 1R-20, 1R-40 and 1R-131a separated *Sec-1* and IAG95. These breakpoints might facilitate in future the construction of a contig map with BAC clones (Šimková et al., 2008) that would be allocated to the 1R satellite.

Some of the rearranged 1R chromosomes would be suitable for the isolation of 1R segments by flow sorting to construct a chromosome-region-specific BAC libraries, as achieved for a wheat chromosome 3B (Šafář et al., 2004). Six 1R dissection lines had small 1R segments that were

generated by double breakages (1R-72, 1R-104, 1R-118, 1R-119, 1R-131b and 1R-184). Telosomes of such rearranged 1R chromosomes could be flow-sorted. Further application of the Gc system to the 1R dissection lines would generate more lines carrying smaller 1R segments, and such lines would be useful in the analysis of the genes and genome structures in the corresponding regions, as Francki (2001) isolated centromeric sequences using midget rye chromosomes.

For the 'Burgas 2' 1R chromosome, Masoudi-Nejad et al. (2002) established seven lines, four of which (1R-20, 1R-40, 1R-104 and 1R-105) were included in the 95 dissection lines in this study, and Tsuchida et al. (2008) established 55 dissection lines for the 'Imperial' 1R chromosome. At present, there are a total of 153 dissection lines available for the genome analysis of chromosome 1R. They have single rearranged 1R chromosomes in homozygous or hemizygous condition. These dissection lines would be useful in the construction of detailed cytological maps of chromosome 1R when more markers like the ESTs and EST-based markers (e.g. SNPs) used for wheat and barley chromosome mapping (Varshney et al., 2007) become available. Although we need to select critical plants in the progeny of the hemizygous dissection lines, we can easily do so by PCR analysis using some of the markers used in this study, as well as by GISH/FISH. In this study, we cytologically checked all the rearranged 1R chromosomes for two or more generations and found no further structural changes revealed by GISH/FISH analysis, except for often breakage in the centromeric region. Telosomes of the rearranged 1R chromosomes generated by the centromeric breakage are even more useful because no more structural changes occur in the progeny and because we could flow-sort such telosomes carrying segments of the 1R chromosome arms to produce sub-arm BAC libraries. As Šimková et al. (2008) discussed, 1RS-specific BAC libraries, for example, would reduce the amount of genetic information to be studied by 18-fold because the 1RS arm (about 442 Mbp) represents only 5.6% of the whole rye genome (7900 Mbp/1C). A series of sub-arm BAC libraries will not only further reduce the amount of genetic information to be studied, but also facilitate the arrangement of the BAC libraries.

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