Introgression of Resistance to Powdery Mildew Conferred by Chromosome 2R by Crossing Wheat Nullisomic 2D with Rye

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Abstract

Using the nullisomic back-cross procedure, four wheat-rye chromosome substitution 2R (2D) lines with different agronomic performance, designated WR02-145-1, WR01-145-2, WR02-145-3, and WR02-145-4, were produced from a cross between 2D nullisomic wheat (*Triticum aestivum* L. cv. “Xiaoyan 6”) and rye (*Secale cereale* L. cv. “German White”). The chromosomal constitution of $2n=42=21$ in WR02-145 lines was confirmed by cytological and molecular cytogenetic methods. Using genomic *in situ* hybridization on root tip chromosome preparations, a pair of intact rye chromosomes was detected in the WR02-145 lines. PCR using chromosome-specific primers confirmed the presence of 2R chromosomes of rye in these wheat-rye lines, indicating that WR02-145 lines are disomic chromosome substitution lines 2R (2D). The WR02-145 lines are resistant to the powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* E. Marchal) isolates prevalent in northern China and may possess gene(s) for resistance to powdery mildew, which differ from the previously identified *Pm7* gene located on chromosome 2RL. The newly developed “Xiaoyan 6”- “German White” 2R (2D) chromosome substitution lines are genetically stable, show desirable agronomic traits, and are expected to be useful in wheat improvement.

Key words: genomic *in situ* hybridization; nullisomic back-cross procedure; PCR; powdery mildew resistance; wheat-rye chromosome substitution line.

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The introgression of rye (*Secale cereale* L.; $2n=2x=14$; R genome) chromatin has proved to be useful in wheat (*Triticum aestivum* L. em Thell) improvement (Zeller and Hsam 1983; Rabinovich 1998). Rye has been used extensively as a valuable

source of genes for resistance to various diseases and pests, such as powdery mildew (caused by *Erysiphe graminis* DC. f. sp. *tritici* E. Marchal; Heun and Friebe 1990; Friebe et al. 1994), stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.; Miller 1984), leaf rust (*P. triticina* Eriks.; McIntosh et al. 1995), stem rust (*P. graminis* Pers. f. sp. *tritici* Eriks. et Henn.; Stewart et al. 1968), Barley yellow dwarf virus (BYDV; Nkongolo et al. 1992), hessian fly (*Mayetiola destructor* Say; Friebe et al. 1990), greenbug (*Schizaphis graminum* Rondani; Sebesta et al. 1994), and cereal cyst nematode (*Heterodera avenae* Wollenweber; Asiedu et al. 1990). Since the first wheat-rye hybrid was obtained in 1875 (Wilson 1875), many wheat-rye chromosome addition, substitution, and translocation lines have been

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developed. The agronomic success of wheats with the T1BL·1RS wheat-rye chromosome translocation has prompted further attempts to transfer rye chromatin into wheat genome.

The introgression of genes from rye into wheat via chromosome substitution is a proven approach. Wheat-rye chromosome substitution lines are a source of genes for desirable traits in wheat improvement, which can be developed using monosomic (Sears 1968) and monotelosomic methods (Kota and Dvoák 1985). Chromosome substitution also occurs in progenies derived from anther and embryo *in vitro* cultures (Hu et al. 1988; Li et al. 1998). In order to quickly develop an alien chromosome substitution line, Li et al. (1982) developed a nullisomic back-crossing procedure in which nullisomic wheats were crossed with wild relatives of wheat. Using this method, chromosome 4R of rye was introduced into wheat as a replacement for chromosome 4D of wheat (Zhang et al. 1990). Xu et al. (1992) produced wheat-*Aegilops speltoides* Tausch ($2n=2x=14$) 4S(4D) chromosome substitution lines.

Winter wheat cultivar "Xiaoyan 6" is derived from a wheat-*Thinopyrum ponticum* (Host) Liu et Wang ($2n=10x=70$; StStEeEbEx genomes) cross and possesses characteristics of promising quality and high yield, earliness, stress tolerance, and wide adaptation (Li et al. 1990; Wei et al. 2000). This cultivar has been grown for two decades, but has recently become vulnerable to certain diseases, such as powdery mildew. Rye is a valuable source of genes for resistance to powdery mildew, such as *Pm7* located on chromosome arm 2RL, *Pm8* and *Pm17* on chromosome arm 1RS, and *Pm20* on chromosome arm 6RL (McIntosh et al. 2003). Since resistance to powdery mildew conferred by *Pm7*, *Pm8*, and *Pm17* has been overcome in northern China (Zhuang and Li 1993; Zhuang 2003), there is a need to transfer other resistance genes from rye to improve the resistance of "Xiaoyan 6" to powdery mildew, because this cultivar is still grown in various wheat-producing regions in China.

Several methods can be used to detect rye chromatin transferred into wheat (Le et al. 1989; Schwarzacher et al. 1989; Mukai et al. 1992; Rogowsky et al. 1993; Brunell et al. 1999). Of these, *in situ* hybridization using probes either from genomic DNA (GISH) or cloned DNA sequences (fluorescence *in situ* hybridization (FISH); Mukai and Nakahara 1993) allows the detection of rye chromosomes or chromosome segments. PCR using DNA primers specific for the rye genome also is a valuable method for the identification of rye chromatin in wheat genetic backgrounds (Lee et al. 1993). In this way, rye chromosomes or chromosome segments can be discriminated in terms of chromosome substitution, addition, and translocation.

The present study dealt with the development of chromosome substitution lines involving rye chromosome 2R by the nullisomic back-crossing procedure. The wheat-rye hybrids produced were characterized by cytological and molecular cytogenetic methods and resistance to powdery mildew was

evaluated by inoculation with isolates that are prevalent in northern China.

Results

Production of WR02-145 wheat-rye lines

Nullisomic 2D wheat ($2n=40$) of "Xiaoyan 6" behaved 20 bivalents during meiosis and was crossed with "German White" rye. Of the 61 F_0 immature embryos, 28 plants were obtained by embryo rescue on MS medium. These regenerated F_1 plants were stored at 4 °C until field transplantation. Among them, 12 F_1 plants had a chromosome number $2n=27$ (Figure 1A). Because rye chromosomes rarely partnered with wheat homoeologous chromosomes of F_1 plants behaved 27 univalents (Figure 1B). After chromosome doubling, eight F_1 hybrid plants with $2n=54$ were obtained (Figure 1C). The spikes of these plants resembled the rye parent, showing longer spikes and more florets than wheat, whereas plant heights were similar to those of wheat. Plants were back-crossed with nullisomic 2D, resulting in 32 BC_1F_1 seeds. Although the progenies were morphologically intermediate between rye and wheat, plants that resembled the wheat parent grew well in the field. Segregation was observed in plant height and reactions to powdery mildew isolates. Six powdery mildew resistant plants ($2n=47$) were back-crossed as the maternal parent with nullisomic 2D, resulting in 26 BC_2F_1 seeds. Twenty-two BC_2F_1 seedlings were tested for their reaction to powdery mildew and resistant plants were examined cytologically. Four progenies with chromosome number $2n=41$ were self-pollinated. Of the BC_2F_2 , four plants having chromosome number $2n=42$ were candidate wheat-rye 2R(2D) substitution lines and were designated WR02-145-1, WR02-145-2, WR02-145-3, and WR02-145-4, which were self-pollinated for an additional three generations before molecular cytogenetic characterization.

Evaluation of agronomic performance and reaction to powdery mildew of the WR02-145 wheat-rye lines

Following three consecutive generations of selfing, no segregation was observed in the four WR02-145 wheat-rye lines, either in morphology or cytology. The plants resembled common wheat, except for a thin gray-green leaf color. Compared with nullisomic 2D wheat and rye, the WR02-145 lines grew vigorously, with an average fertility for the four WR02-145 lines of 93.2% based on 30 plants measured for each line. The seeds were plump and the average 1 000-kernel weight ranged from 38 to 40 g (Table 1; Figure 2A).

In a greenhouse test, 20 individual plants of the four WR02-145 lines each were tested for reaction to powdery mildew. "German White" rye and WR02-145 lines were resistant to the

Figure 1. Cytological analyses.

- (A) Chromosome numbers of F_1 hybrids derived from a cross between nullisomic 2D of “Xiaoyan 6” wheat and “German White” rye, $2n=27$.
 (B) Chromosome pairing configuration of the F_1 hybrid derived from a cross between nullisomic 2D of “Xiaoyan 6” wheat and “German White” rye, $2n=20W+7R$.
 (C) Chromosome number of an F_1 plant following treatment with colchicine, $2n=54$.
 (D) Chromosome pairing patterns at meiotic metaphase I. WR02-145 lines $\times$ “Chinese Spring”, showing 20 bivalents and two univalents, one from “Chinese Spring” wheat and the other from rye.

Table 1. Agronomic traits of the WR02-145 wheat-rye chromosome substitution lines

Lines or parents	Plant height (cm)	No. of spikes per plant	Spike length (cm)	No. kernels per spike	No. spikelets per spike	1 000-kernel weight (g)	% Seed setting
WR02-145-1	92	17	10.5	65	23	38.9	94.0
WR02-145-2	88	20	11.5	67	24	40.2	93.0
WR02-145-3	90	18	10.5	60	21	38.5	95.2
WR02-145-4	87	21	11.0	68	25	40.6	90.4
Nullisomics 2D	59	12	7.0	37	15		60.5
"German White" rye	133.5	26	13.2	83	42		96.6

mixture of powdery mildew isolates collected from northern China, with an infection type (IT) score of 0 for "German White" and scores of 1 or 2 for the WR02-145 lines. All plants of "Xiaoyan 6", nullisomic 2D, and susceptible check "Mingxian 169" were susceptible (IT scores of 4).

In field experiments, plants of the WR02-145 lines and "German White" developed no symptoms of powdery mildew or necrotic flecks (IT 0 or 0;) with a disease severity score of 1 and these were regarded as highly resistant. In contrast, the leaves of "Xiaoyan 6" and nullisomic 2D were covered by powdery mildew spores (IT 4) and the disease severity score was 9; these plants were considered susceptible (Figure 2B).

PCR analysis of WR02-145 wheat-rye lines

Using the chromosome 2R-specific primers R1 and R2, the 473 bp PCR diagnostic fragment was produced in the four WR02-145 lines and "Chinese Spring" wheat-"Imperial" rye 2R(2D) chromosome substitution line, which indicated the presence chromatin 2R in WR02-145. This 473-bp band was also amplified in the rye parent, but was not amplified in "Xiaoyan 6" or nullisomic 2D, "Chinese Spring", "Mingxian 169", Lovrin 10, and Lovrin 13 (T1BL·1RS translocation lines), indicating that they did not contain 2RS chromatin of rye (Figure 3).

Cytological and GISH analyses of WR02-145 wheat-rye lines

To determine the mitotic and meiotic chromosome constitutions

of the wheat-rye derivatives, karyotypes were examined on at least 20 metaphase chromosome spreads from eight seeds for each genotype. The WR02-145 lines had 42 chromosomes and most pollen mother cells (PMCs; average 96.5%) had 21 bivalents at the first meiotic metaphase. When crossed with Chinese Spring wheat, most cells of the F₁ plants had 20 bivalents and two univalents (Table 2; Figure 1D), indicating that a pair of wheat chromosomes had been replaced by a pair of rye chromosomes in the WR02-145 lines.

Eight to 10 individual plants from the four WR02-145 lines were tested for the presence of rye chromosomes by GISH. Using rye genomic DNA as a probe and wheat genomic DNA as a blocker, GISH on mitotic metaphase chromosomes permitted the identification of a pair of rye chromosomes in the four WR02-145 lines as replacements for the two wheat chromosomes. The rye chromosomes were easily distinguished from the wheat chromosomes by their bright-green hybridization signals along the entire length of the chromosomes, whereas the wheat chromosomes were counterstained red with propidium iodide (PI; Figure 2C). Taking results from the PCR, cytological and GISH investigations together, the WR02-145 wheat-rye hybrids were determined to be 2R(2D) wheat-rye chromosome substitution lines.

Discussion

The rye chromosome 2R carries genes for disease resistance,

Table 2. Chromosome pairing patterns of WR02-145 lines and F₁ plants of crosses between WR02-145 lines and *Triticum aestivum* "Chinese Spring" (CS) at meiotic metaphase I

Lines or crosses	Total no. cells observed	No. cells (% of total cells observed)			
		21II	20II+2I	19II+4I	Others
WR02-145-1	120	115 (95.8)	5 (4.2)		
WR02-145-2	101	99 (98.0)	2 (2.0)		
WR02-145-3	89	86 (96.6)	3 (3.4)		
WR02-145-4	68	65 (95.6)	2 (2.9)		1 (1.5)
WR02-145-1×CS	121		111 (91.7)	8 (6.6)	2 (1.7)
WR02-145-2×CS	80		64 (80.0)	11 (13.7)	5 (6.3)
WR02-145-3×CS	110		95 (86.4)	10 (9.1)	5 (4.5)
WR02-145-4×CS	62		52 (83.9)	6 (9.7)	4 (6.4)

Figure 2. Agronomic traits, powdery mildew reactions and genomic *in situ* hybridization.

(A) Spikes of “German White” rye (right), nullisomic 2D of “Xiaoyan 6” wheat (middle 2), chromosome substitution lines WR02-145 (left and middle 1), and seeds of WR02-145 line (above).

(B) Powdery mildew reactions of nullisomic 2D (left), “German White” (middle), and WR02-145-1 line (right).

(C) GISH of mitotic metaphase chromosomes of the WR02-145 lines, using rye genomic DNA as a probe in the presence of “Chinese Spring” genomic DNA as a blocker.

A chromosome pair of rye was visualized as bright-green fluorescein isothiocyanate hybridization signals (arrows).

Figure 3. PCR products amplified by primers R1 and R2 specific for chromosome 2R of rye.

Lane 1, "Chinese Spring"; lane 2, Lovrin 10; lane 3, Lovrin 13; lanes 4, 5, 15, "German White"; lanes 6, 7, "Chinese Spring" - "Imperial" 2R(2D) chromosome substitution line; lanes 8, 9, WR02-145-1; lanes 10, 11, WR02-145-2; lane 12, WR02-145-3; lane 13, WR02-145-4; lane 14, nullisomic 2D of "Xiaoyan 6"; lane 16, "Xiaoyan 6"; M, 100-bp DNA ladder (Dingguo Biotechnology, Beijing, China). The arrow indicates the 473-bp diagnostic DNA fragments.

such as powdery mildew (Heun and Friebe 1990), leaf rust (McIntosh et al. 1995), stem rust (Brunell et al. 1999), hessian fly (Friebe et al. 1990), and tolerance to BYDV and drought stress, as well as a gene for short straw (Schlegel et al. 1998; Merker and Forsström 2000), which are useful characteristics in wheat improvement. "Xiaoyan 6" was grown extensively in China for approximately 20 years, covering over 670 000 hm² (Zhuang 2003). However, this cultivar has recently become vulnerable to powdery mildew. Rye chromatin was not present in "Xiaoyan 6" (Li et al. 1990). In order to improve the resistance of "Xiaoyan 6" to powdery mildew and because of the multiple potentially useful genes on rye chromosome 2R, we transferred this chromosome to wheat "Xiaoyan 6" and produced new 2R(2D) "Xiaoyan 6" wheat-"German White" rye substitution lines.

Lind (1982) reported that chromosome 2R conditioned a high degree of powdery mildew resistance. Heun and Friebe (1990) found that chromosome 2R from "Prolific" rye was a source of resistance to powdery mildew and carried a more effective *Pm7* allele and/or some further powdery mildew resistance genes. In the present study, the new 2R(2D) wheat-rye chromosome substitution lines also conditioned a high level of resistance against a mixture of powdery mildew isolates, which was virulent on powdery mildew resistance genes, including *Pm7*, *Pm8*, and *Pm17* from rye. Together, these findings indicate that chromosome 2R of "German White" rye possesses a gene(s) for resistance to powdery mildew, which may differ from the previously identified *Pm7* located on chromosome 2RL (McIntosh et al. 2003). So far, the high levels of powdery

mildew resistance provided by rye chromosome 2R have not been used effectively in wheat-breeding programs. Compared with "Xiaoyan 6", the newly developed 2R (2D) chromosome substitution lines were free of powdery mildew symptoms in the field, were highly resistant to powdery mildew isolates prevalent in northern China, had a high seed set, and were cytologically stable, making them useful in wheat improvement.

Using the monosomic method, Sears (1968) developed 2R (2B) and 2R(2D) "Chinese Spring" wheat-"Imperial" rye chromosome substitution lines. By substituting chromosome 2R of rye into commercial wheat, May and Appels (1978) produced a double monosomic substitution line 2R(2B). Chapman and Miller (1978) developed 2R(2A), 2R(2B), and 2R(2D) "Holdfast" wheat-"King II" rye chromosome substitution lines. Merker and Rogalska (1984) reported a double disomic 1R(1B), 2R(2B) wheat-rye chromosome substitution line that was free of powdery mildew symptoms in the field at the time, but this line was tall, susceptible to lodging, and low yielding (Merker and Forsström 2000). Friebe and Larter (1988) produced a disomic 2R(2D) "Thatcher" wheat-"Prolific" rye chromosome substitution line. Nevertheless, few wheat-rye chromosome substitution lines involving rye chromosomes 2R have been used extensively in wheat breeding because of agronomic disadvantages of the wheat genetic background, such as "Chinese Spring". The usefulness of wheat-alien hybrids depends, to some extent, on their wheat genetic backgrounds. In the present study, we considered not only the transfer of desirable genes of rye conferred by chromosome 2R, but also placing them in a valuable background of common wheat "Xiaoyan 6" (An et al. 2000). The 2R(2D) wheat-rye chromosome substitution lines in the present study have a favorable wheat genetic background, which makes the new 2R(2D) chromosome substitution lines more useful than the others.

Friebe and Larter (1988) suggested that rye chromosome 2R was homoeologous to group 2 chromosomes of wheat and only the replacement of genetically homoeologous chromosomes was effective in producing fertile vigorous plants. The 2R(2D) chromosome substitution lines described in the present study exhibited desirable agronomic characteristics, with vigorous vegetative growth and plump seeds with a high percentage of seed set (Table 1; Figure 2A).

The present study demonstrates the effectiveness of developing an alien substitution line using the nullisomic back-crossing procedure (Li et al. 1982). The wheat-rye 2R(2D) chromosome substitution lines were developed from BC₂F₂ plants that originated from the cross between nullisomics 2D wheat and rye. Compared with the monosomic method that needs at least eight to nine generations to produce an alien chromosome substitution line, the nullisomic back-crossing procedure shortened the duration to only four to five generations. In addition, the method used in the present study necessitates fewer cytological examinations (Zhang et al. 1989). The present study

provides an additional example of producing a chromosome substitution line using the nullisomic back-crossing method. However, the prerequisite of developing an alien chromosome substitution line using the nullisomic back-crossing procedure is the availability of nullisomic wheats that are fertile and have a high seed set. We developed fertile nullisomics 2D derived from monosomic 2D of "Xiaoyan 6", which grows vigorously with an average seed set of over 60%. By using this nullisomic line, chromosome 2R of rye was transferred into "Xiaoyan 6" wheat resulting in 2R(2D) chromosome substitution lines. Moreover, we had developed cytologically stable and fertile 1A, 1B, 1D, 2A, 2B, 3B, 4A, 5A, 5B, 5D, 6A, 6D, and 7D nullisomics of "Xiaoyan 6" (An et al., 2002, unpublished data). These nullisomic lines are useful for producing wheat-rye chromosome substitution lines by means of nullisomic backcrossing procedure.

GISH using total genomic DNA as a probe facilitates the identification of alien chromosomes and chromosome segments in wheat backgrounds. PCR using chromosome-specific primers proved to be a powerful tool for the identification of alien chromatin that had been introgressed into common wheat (Lee et al. 1993). In the present study, chromosome 2R was identified by PCR with primers specific for 2R (Figure 3). Combining results obtained by cytological, GISH, and PCR analyses, lines WR02-145 were determined to be a wheat-rye chromosome substitution line involving the rye chromosome 2R (Figure 2C).

Materials and Methods

Plant materials

Four wheat-rye WR02-145 lines, designated WR02-145-1, WR02-145-2, WR02-145-3, and WR02-145-4, and the winter rye cultivar "German White" (Sun 2002) were used in the present study. Winter wheat cultivar *Triticum aestivum* L. "Xiaoyan 6" was developed from a cross of St 2422/464/*T. aestivum* "Xiaoyan 96" in Shanxi Province, PR China, in 1981 (Zhuang 2003). Self-pollinated, fertile, and cytologically stable nullisomic 2D (2n=40, AABBDD-2(2D)) was derived from the selfed progeny of monosomic 2D of the wheat cultivar *T. aestivum* "Xiaoyan 6". The monosomic 2D line of "Xiaoyan 6" was produced by repeated back-crossing of the monosomic 2D line of wheat cultivar *T. aestivum* "Chinese Spring" to "Xiaoyan 6" as the male parent seven times. Wheat cultivar *T. aestivum* "Mingxian 169" was used as the susceptible control in powdery mildew tests. The "Chinese Spring" wheat-"Imperial" rye 2R(2D) chromosome substitution line, kindly provided by Dr S. Reader (John Innes Centre, Norwich, UK), was used as a control in the detection of 2R rye chromatin by PCR. Lovrin 10 and Lovrin 13, which are T1BL·1RS translocation lines (Rabinovich 1998), were also used as controls in the PCR

analysis.

Production of wheat-rye chromosome substitution lines by the nullisomic back-crossing procedure

The nullisomic back-crossing procedure (Li et al. 1982) was used to produce the wheat-rye chromosome substitution lines in the present study. "Xiaoyan 6" nullisomic 2D was crossed with "German White" rye and, 16–18 d following pollination, inflorescences were stored at 4 °C in a refrigerator for 48–72 h (Sharma 1995). Hybrid embryos were dissected from spikes and placed on MS medium (Murashige and Skoog 1962). Following chromosome doubling with a solution containing 0.05% colchicine, 1.5% dimethylsulfoxide, and 5% MS medium (An et al. 2003), amphidiploid plants were obtained and back-crossed as the female parent to the nullisomic 2D line. Plants of BC₁F₁ were screened for resistance to powdery mildew and resistant plants were again back-crossed as the female parent to the nullisomic 2D line. The BC₂F₁ plants were tested for resistance to powdery mildew and karyotyped by cytological examination. Plants with chromosome numbers 2n=41 were allowed to self-pollinate. Vigorous and fertile selfed progenies (BC₂F₂ plants) with somatic cell chromosome number 2n=42 were selected for further characterization. During development, each individual plant was selected for high seed set, wheat-like plant type, and resistance to powdery mildew. The subsequent generations were self-pollinated and planted in the field.

Evaluation of reaction to powdery mildew

Greenhouse tests

Powdery mildew inoculation and disease assessments were conducted in a greenhouse, as described by Sheng (1988). At the two- to three-leaf stage, plants of wheat-rye derivatives, "Xiaoyan 6", nullisomic 2D, "German White" rye, and "Mingxian 169" were artificially inoculated with a mixture of the powdery mildew isolates prevalent in northern China. The isolate mixture was virulent on powdery mildew resistance genes *Pm1*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3e*, *Pm3f*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm17*, and *Pm19*, and avirulent on genes *Pm2*, *Pm4a*, *Pm4b*, *Pm12*, *Pm13*, *Pm16*, *Pm20*, and *Pm21*, as determined by differential cultivars (Duan 1998). Infection types were scored 15 d after inoculation, when the susceptible wheat check "Mingxian 169" was heavily infected, using a scale of 0–4, where 0=no visible symptoms, 0+=necrotic flecks, and 1–4=highly resistant, resistant, susceptible, and highly susceptible reactions, respectively (Sheng 1988).

Field tests

The wheat-rye derivatives were planted in six-row plots, each of which were 1.5 m long and spaced at 25 cm, in early October. The plots were arranged in a randomized block design with

three replications. "German White" rye was grown in a single row and the susceptible check cultivar "Mingxian 169" was grown in double rows beside the plots as a mildew spreader. In mid-November, and then in late March, the spreader rows were inoculated twice with a mixture of powdery mildew isolates, as described previously. Disease severity was assessed on a scale (0–9) as described by Sheng and Duan (1991). Infection types (scale 0–4) were scored on the upper four leaves on 5, 13, and 20 May. The field test was repeated in the following year's growing season using the same procedure.

PCR

In the present study, PCR amplification was used to detect the alien chromatin in wheat-rye derivatives. Total genomic DNA was isolated from seedling using the phenol/chloroform method (Sharp et al. 1988). The primers (R1: 5'-CCCAGCAACAACAAC-CGTCGATT-3'; R2: 5'-AATCGACGGTTGTTGTTGCTGGG-3'), which were derived from the partial cDNA clone pSC 503 of a rye gamma secaline gene (Kreis et al. 1985), were used. The 473-bp PCR product was detected in lines carrying either 2R or 2RS of rye (Lee et al. 1993). DNA amplification was conducted in a 25 µL reaction volume containing 1× *Taq* PCR buffer, 1.0 unit of *Taq* DNA polymerase, 0.25 µmol/L of each primer, 200 µmol/L of each deoxyribonucleotide, and 280 ng sample DNA. The PCR was performed in a PTC-200 Peltier Thermal Cycler (MJ Research, Ramsey, MN, USA) using a program of 95 °C for 5 min, 35 cycles at 94 °C for 1 min, 56 °C for 2 min, and 72 °C for 1 min, with an extension at 72 °C for 10 min. The PCR products were separated on 1.5% agarose gels in 1× TBE buffer (89 mmol/L Tris-borate, 2 mmol/L EDTA, pH 8.3) for 90 min at 110 V.

Cytological analyses

Root tips were removed from germinated seeds and pretreated in ice water for 24 h prior to fixation (ethyl alcohol : acetic acid = 3 : 1, v/v) for 24 h. The fixed root tips were hydrolyzed in 1 mol/L HCl for 3–4 min at 65 °C, prestained in 4% ferrovandium solution for 25 min, and then stained in 0.5% hematoxylin for 1 h. Mitotic chromosomes were prepared by squashing root tips in 45% acetic acid. Chromosomes were viewed with an Olympus BH-2 microscope (Olympus Optical, Tokyo, Japan). Anthers containing PMCs at the metaphase I stage were fixed in Carnoy solution (absolute alcohol : acetic acid : chloroform = 6 : 3 : 1) for 2 days and stored in 70% ethanol at 4 °C until use. The anthers were briefly stained with a drop of 2% (v/v) acetocarmine and the PMCs were squashed. Meiotic preparation slides were examined to analyze chromosome pairing configurations.

GISH analysis

To detect rye chromatin in the WR02-145 lines, GISH analysis with total genomic DNA from "German White" rye was

performed on mitotic metaphase chromosome preparations. Root tips were fixed and then squashed onto ethanol-washed microscope slides in 45% acetic acid and cover slips were removed after being frozen in liquid nitrogen. Slides were air-dried overnight at room temperature and then stored at –20 °C until GISH analysis.

Total genomic DNA of "German White" rye was sonicated into 500–800-bp fragments and labeled with DIG-Nick Translation Mix (Roche, Mannheim, Germany). The blocking DNA was prepared by shearing the total genomic DNA extracted from "Chinese Spring" wheat (ABD genomes) and was mixed with the DNA probe in a ratio of 30 : 1. The GISH was performed as described by Reader et al. (1994). The wheat chromosomes were counterstained red by PI. The bright-greenish fluorescein isothiocyanate (FITC) hybridization signals were then examined using a Nikon Eclipse E600 fluorescence microscope (Nikon, Tokyo, Japan). Photographs were taken with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA).

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