Plant Breeding **126**, 482—486 (2007)
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doi:10.1111/j.1439-0523.2007.01331.x

PCR-based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat–rye translocations in wheat background

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With 3 figures and 3 tables

Received September 27, 2006/Accepted October 6, 2006
Communicated by P. Wehling

Abstract

The rye (*Secale cereale* L.) chromosome arm 1RS is one of the most successfully used alien resources in wheat (*Triticum aestivum* L.) improvement, and it is still being widely utilized by many breeding programmes. With increasing application of marker-assisted selection in wheat breeding, development of an efficient molecular marker system to monitor and track 1AL.1RS and 1BL.1RS wheat–rye translocations is of practical value. In this study, we systematically evaluated the utility of eight rye-specific molecular markers in detecting 1RS chromatins with different origins in diverse wheat genetic backgrounds. Two such markers, PAWS5/S6 and SCM9 were identified that were able to differentiate multiple sources of wheat–rye translocations involving 1RS. A duplex polymerase chain reaction (PCR) procedure was developed with two rye-specific markers PAWS5/S6 and RIS and tested in a set of representative wheat lines. The two rye-specific markers and the duplex PCR procedure established in this study provided a useful tool in marker-assisted selection of materials containing desirable 1RS chromatin in wheat breeding.

Key words: *Triticum aestivum* — *Secale cereale* — wheat–rye translocation — rye-specific molecular markers — duplex polymerase chain reaction — marker-assisted selection

The wheat–rye translocations involving 1RS of rye are probably the most successfully used alien resources for wheat improvement. Hundreds of commercial wheat cultivars carrying either 1AL.1RS or 1BL.1RS wheat–rye translocations have been developed (Rabinovich 1998). However, when analysing the pedigrees of these cultivars, the sources of wheat–rye translocations are strikingly limited. The donor of 1RS in the majority of wheat cultivars with 1BL.1RS including the widely used 'Kavkaz' and 'Aurora' from Russia could be traced back to the rye variety 'Petkus' (Schlegel and Korzun 1997). The 1BL.1RS translocation derived from 'Kavkaz' possesses resistance genes for stem rust (*Puccinia graminis* Pers), stripe rust (*P. striiformis* Westend), leaf rust (*P. recondita* Rob. Ex Desm.) and powdery mildew (*Erysiphe graminis* DC). Another source of 1BL.1RS is 'Salmon' wheat (Tsunewaki 1964, Zeller 1973), but it was less characterized and rarely used in wheat breeding. The wheat cultivar 'Amigo' and germplasm line GRS1201 each carries a 1AL.1RS translocation with 1RS originated from the same rye variety 'Insave', which was obviously genetically heterogeneous (Porter et al. 1991, Sebesta et al. 1994). The 1RS in 'Amigo' bears the greenbug (*Schizaphis graminum* Rondani) biotype C resistance gene *Gb2*, whereas 1RS in GRS1201 carries *Gb6* that confers resistance to biotypes E, I and K greenbugs (Porter et al. 1994). The two lines also differ in secalin protein components and the fingerprints of a rye-specific molecular marker PAWS5/S6 (Graybosch et al. 1999).

Due to the importance of 1RS to wheat variety performance, the wheat–rye translocations continue to play significant roles in many wheat breeding programmes. In the USA, nearly 25% of entries in wheat performance trials in the Great Plains region contain either 1AL.1RS or 1BL.1RS wheat–rye translocation (Graybosch 2001, 2006). Development of methods for quick and reliable identification of 1AL.1RS or 1BL.1RS translocated chromosomes in wheat breeding is therefore of practical importance. With increased availability of molecular markers for many agronomically important genes, and the establishment of wheat genotyping centres, molecular marker-assisted selection is gaining more attention from wheat breeders in the USA. A fast and reliable marker system that is able to identify major sources of wheat–rye translocations involving 1RS will be very helpful for efficient selection of interested lines in wheat breeding. Although a number of rye-specific molecular markers have been reported (e.g. Koebner 1995, Shimizu et al. 1997, Nadella et al. 2002, Nagy and Lelley 2003), systemic evaluation of their utility for marker-assisted selection is lacking. The objectives of this study were to re-evaluate the usefulness of selected rye-specific molecular markers and develop a polymerase chain reaction (PCR)-based marker system for simultaneous identification of different sources of wheat–rye translocations involving 1RS in wheat.

Materials and Methods

Plant materials: Two sets of wheat materials were used for systematic evaluation of rye-specific markers in detecting 1AL.1RS and 1BL.1RS wheat–rye translocations. The first set (Set 1) included 20 entries representing four sources of 1AL.1RS and 1BL.1RS translocations (Table 1). For convenience, wheat lines carrying 'Amigo'-derived and GRS1201-derived 1AL.1RS translocations will be referred 1AL.1RS-A and 1AL.1RS-G, respectively, hereinafter. Similarly, wheat lines with 1BL.1RS translocations originated from 'Kavkaz' and 'Salmon' will be designated as 1BL.1RS-K and 1BL.1RS-S, respectively. KS80H4200 was the only entry belonging to 1BL.1RS-S in this study. Three non-1RS wheat lines, 'Chinese Spring', TAM 105 and TX00D1390 were used as controls in marker analysis.

The second set (Set 2) of materials included 15 wheat genotypes. Of them, five contain 1AL.1RS-A ('Ogallala', 'Prairie Red', TAM 112, TAM 200 and TAM 202) and five carry 1BL.1RS-K ('Clement', 'Longhorn', 'Rawhide', TAM 300 and TAM 302). The remaining five (KS00F5-20-3-2, TX99A0153-1, TX03M1004, TX03M1179 and

Table 1: Plant materials (Set 1) used for evaluating rye-specific markers

Number	Materials	Seed source	Source of translocation	Reference
1	Insave	GRIN[1]	Rye line, 1RS donor of Amigo	Sebesta and Wood (1978)
2	Amigo	This study	1AL.1RS, original line	Sebesta et al. (1994)
3	TAM 107	This study	1AL.1RS from Amigo	Porter et al. (1987)
4	TAM 110	This study	1AL.1RS from Amigo	Lazar et al. (1997)
5	TAM 303	This study	1AL.1RS from Amigo	J C. Rudd (unpublished data)
6	Fannin	GRIN	1AL.1RS from Amigo	Inferred from pedigree
7	Nekota	GRIN	1AL.1RS from Amigo, heterogeneous	Haley et al. (1996)
8	GRS1201	D. Porter	1AL.1RS independent of Amigo	Porter et al. (1991)
9	N96L9970	R. Graybosch	1AL.1RS from GRS1201	Graybosch et al. (2004)
10	Kavkaz	GRIN	1BL.1RS with 1RS from Petkus	Schlegel and Korzun (1997)
11	Cougar	GRIN	1BL.1RS from Kavkaz	Baenziger et al. (2001)
12	Custer	GRIN	1BL.1RS from Kavkaz	Inferred from pedigree
13	Freedom	GRIN	1BL.1RS from Kavkaz	Gooding et al. (1997)
14	KS91HWGRC14	GRIN	1BL.1RS from Kavkaz, durum wheat	Friebe et al. (1993)
15	Siouxland	GRIN	1BL.1RS from Kavkaz	Schmidt et al. (1985)
16	Veery 'S'	GRIN	1BL.1RS from Kavkaz	Rabinovich (1998)
17	KS80H4200	GRIN	1BL.1RS from Salmon wheat	Martin et al. (1983)
18	TX00D1390	This study	Non-1RS wheat line	J. Rudd (unpublished data)
19	Chinese Spring	This study	Wheat cultivar, non-1RS	
20	TAM 105	This study	Wheat cultivar, non-1RS	

[1]USDA Germplasm Resources Information Network (http://www.ars-grin.gov/index.html).

TX01M5009-28) were advanced breeding lines with unknown status of wheat–rye translocations. Set 2 materials were used to test the duplex PCR procedure for simultaneous detection of different sources of wheat–rye translocations.

To assign diagnostic bands detected by marker PAWS5/S6 to specific rye chromosomes, a set of seven 'Imperial' – 'Chinese Spring' disomic addition lines were used, which was kindly provided by Dr Gary E. Hart of Texas A&M University, College Station. Additional materials used in this study included rye variety 'Petkus' and four greenbug-resistant wheat germplasm lines GRS1202, GRS1203, GRS1204 and GRS1205, which were sister lines of GRS1201 (Porter et al. 1991), and were kindly provided by Dr Dave Porter (USDS-ARS, Stillwater, OK). The five GRS lines all carry chromatins from 'Insave' and are resistant to greenbug biotypes B, C, E and G (Porter et al. 1991). Chromosome constitution of GRS1202 and GRS1203 is unknown. GRS1204 has $2n = 44$ chromosomes with two translocations T2AS-1RS.1RL and T2AL.2AS-1RS (Friebe et al. 1995). GRS1205 is an 1R(1A) substitution line (Lee et al. 1996). The GRS series were used to verify the band patterns of rye-specific molecular markers in 'Insave'.

Rye-specific primers and PCR analysis: Eight rye-specific, PCR-based molecular markers were selected. The details of these markers were listed in Table 2. As control, a wheat-specific genomic simple sequence repeat (SSR) marker, BARC53, was used as the positive control to monitor the DNA quality of tested materials. All primers were synthesized commercially.

Genomic DNA was extracted from young leaf tissues harvested from seedlings grown in the greenhouse except for the five advanced breeding lines which were collected directly from field nurseries of the Texas Wheat Breeding Program located at Bushland, Texas. Evaluation of the eight markers was performed by PCR amplification on Set 1 materials using 2x PCR master mix (Promega, Madison, WI, USA). A typical 10.0 µl PCR contained 25 ng of template DNA and 5.0 µl 2x master mix. The optimized final concentration of the forward and reverse primers for SCM9, PAWS5/S6 and the remaining six markers was 0.5, 1.0 and 0.375 µM, respectively. All primer sets used the same PCR programme: 3 min initial denaturation at 95°C followed by 30 cycles of 45 s at 94°C, 60 s at 60°C and 90 s at 72°C. Duplex PCR was performed on Set 2 materials using PAWS5/S6 and RIS. Each PCR mixture contained the same components as single primer pair but the final concentration for each left or right primer of RIS and PAWS5/S6 was 1.0 µM. A touchdown PCR programme was designed for duplex PCR: after 3 min initial denaturation at 95°C, five cycles were performed with 45 s at 94°C, 5 min at 68°C, 1 min at 72°C with the annealing temperature being reduced by 2°C per cycle; further, the

Table 2: Rye-specific molecular markers used in this study

Markers	Sequence similarity	Primer sequence (5′ → 3′)	Reference
SCM9	Rye microsatellite marker	F: TGACAACCCCCTTTCCCTCGT R: TCATCGACGCTAAGGAGGACCC	Saal and Wricke (1999)
Sec1Gene	Rye secalin gene, coding region	F: AACATGAAGACCTTCCTCATC R: CGTTACATTGAACACTCCATT	Yamamoto and Mukai (2005)
Sec1Pro	Rye secalin gene, promoter region	F: GGATCCAAATTTGCATGCGTA R: CAACTCTTGTTCGCTAGGGTT	Yamamoto and Mukai (2005)
RIS	Rye non-tandemly repeats	F: TAATTTCTGCTTGCTCCATGC R: ACTGGGGTGCACTGGATTAG	Koebner (1995)
NOR	Rye nucleolar organizer sequence	F: GCATGTAGCGACTAACTCATC R: CCCAGTTTTCCATGTCGC	Koebner (1995)
PAW161	Rye telomeric, tandemly repeated sequence	F: TGAGGGCCCAGACGGCCCTTTTTG R: TTATCGCAATTACAACTCAAATTT	Guidet et al. (1991)
PAWS5/S6	R173 family repeats	F: AACGAGGGGTTCGAGGCC R: GAGTGTCAAACCCAACGA	Rogowsky et al. (1992a)
RyeR3/F3	Rye-specific repeated sequence	F: GATCGCCTCTTTTGCCAAGA R: TCACTGATCACAAGAGCTTG	Katto et al. (2004)

F, forward primer; R, reverse primer.

PCR was continued by eight cycles of 45 s at 94°C, 2 min at 58°C, 1 min at 72°C with the annealing temperature reduced by 1°C per cycle, which was followed by 25 cycles of 45 s at 94°C, 2 min at 50°C and 1 min at 72°C.

Amplification products were separated in 2–4% high-resolution agarose gel. Gels were stained with ethidium bromide. DNA fragments were visualized under UV light and recorded using a MultiDoc-It Imaging System (UVP, Upland, CA, USA).

Results

PCR amplification with rye-specific molecular markers

PCR products were successfully amplified with all eight rye-specific primer pairs in Set 1 materials. The diagnostic bands for different sources of wheat–rye translocations were summarized in Table 3. Five (RIS, RyeR3/F3, PAW161, Sec1Gene and Sec1Pro) markers each indiscriminately amplified a monomorphic band in all 1RS-containing samples. Three bands, 400, 700 and 800 bp in size amplified by NOR could be easily recognized in Set 1 lines with 1RS. No product was amplified in the three non-1RS wheat lines.

PAWS5/S6 was the most polymorphic among the eight markers examined. In the range of 100–500 bp up to five bands could be recognized in agarose gels, but only two, 220 bp and 320 bp in size, respectively, were consistent and of diagnostic value (Fig. 1). As illustrated in Fig. 1, 'Insave' rye and all six wheat cultivars with 1AL.1RS-A had both bands, while GRS1201 and N96L9970 only had the 220 bp band. No 220 bp band was present in lines with either 1BL.1RS-K or 1BL.1RS-S. The 320 bp band could be seen in some 1BL.1RS lines, but not consistent.

Depending on PCR conditions, SCM9 could amplify up to five bands ranging from 100 to 500 bp in the agarose gel. Amplification in wheat renders two different products for 1AL.1RS and 1BL.1RS translocations: one 207 bp band associated with all 1BL.1RS lines, and the other 228 bp band which was always present in all materials carrying 1AL.1RS (Fig. 1). However, KS80H4200, the only wheat line carrying 1BL.1RS from 'Salmon' wheat had the same 228 bp band as found in all 1AL.1RS wheats. No amplification product by SCM9 could be detected in 'Chinese Spring', TAM 105 or TX00D1390.

Duplex touchdown PCR in Set 2 wheat lines

Duplex touchdown PCR was performed using PAWS5/S6 and RIS on Set 2 materials, and the result was illustrated in Fig. 2. As expected, the 220 and 320 bp bands amplified by PAWS5/S6 presented in all lines with 1AL.1RS-A, and absent or with weak intensity in all five lines with 1BL.1RS-K. The 111 bp band amplified by RIS was clearly recognizable in all 1RS-bearing lines. Among the five breeding lines, only TX99A0153-1 had diagnostic band patterns similar to lines with 1AL.1RS-A. No PCR products could be detected in other four lines suggesting TX99A0153-1 may carry the 1AL.1RS wheat–rye translocation derived from 'Amigo'.

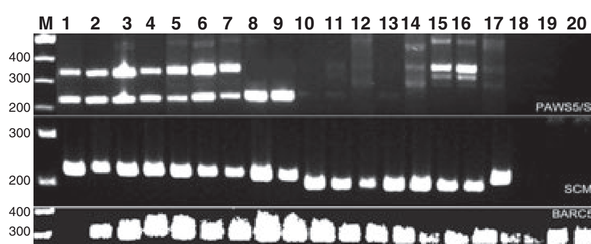

Fig. 1: Polymerase chain reaction-amplified diagnostic band patterns of rye-specific markers PAWS5/S6 and SCM9 among wheat lines carrying different sources of 1RS chromatin in wheat backgrounds. M = size marker lane. Numbers to the left of the marker lane are marker sizes in base pairs. The numbers at the top of panel correspond to Set 1 materials listed in Table 1. The bottom panel is amplification pattern of wheat-specific microsatellite marker BARC53 showing no product for 'Insave' rye (lane 1)

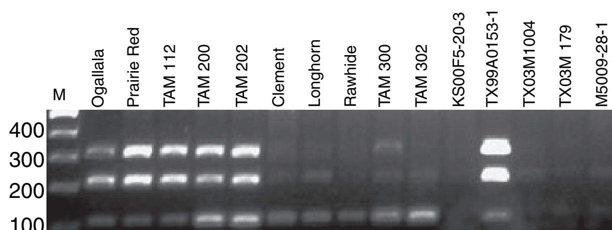

Fig. 2: Agarose gel profiles of duplex polymerase chain reaction with rye-specific markers RIS and PAWS5/S6 on 15 Set 2 wheat lines. M = size marker lane. Numbers to the left of the marker lane are marker sizes in base pairs

Assignment of PAWS5/S6 loci to rye chromosomes

The above experiment indicated that PAWS5/S6 was able to differentiate homoeologous group-1 wheat–rye translocations. It is not known if the 220 and 320 bp diagnostic bands detected by PAWS5/S6 (Fig. 1) are specific to rye chromosome arm 1RS. PCR amplification was performed with DNAs of

Table 3: Diagnostic markers to detect rye 1RS chromatin in wheat background

Markers	Diagnostic bands (bp)	1AL.1RS		1BL.1RS		Non-1RS wheat
		Amigo	GRS1201	Kavkaz	Salmon	
SCM9	207	–	–	+	–	–
	228	+	+	–	+	–
NOR	400, 700, 800	+	+	+	+	–
PAW161	350	+	+	+	+	–
PAWS5/S6	220	+	+	–	–	–
	320	+	–	+/–	–	–
RIS	111	+	+	+	+	–
Sec1Pro	1000	+	+	+	+	–
Sec1Gene	1160	+	+	+	+	–
RyeR3/F3	1400	+	+	+	+	–

–, band absent; +, band present.

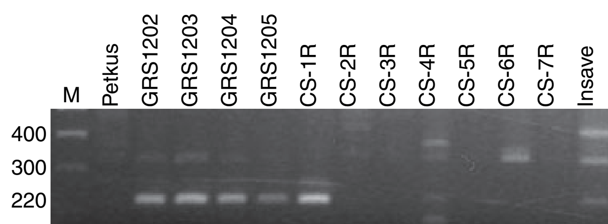

Fig. 3: Chromosomal assignment of 220 and 320 bp diagnostic bands amplified by rye-specific marker PAWS5/S6 in 'Insave' rye. M = size marker lane. Numbers to the left of the marker lane are marker sizes in base pairs. CS = 'Chinese Spring'; CS-1R to CS-7R = respective disomic addition lines of 'Imperial' chromosomes 1R to 7R of 'Chinese Spring'

seven 'Chinese Spring' – 'Imperial' alien disomic addition lines with PAWS5/S6, and the result was illustrated in Fig. 3. Both the 220 and 320 bp bands were present in 'Insave', but absent in 'Petkus'. As illustrated in Fig. 3, the 220 bp sequence was clearly located in rye chromosome arm 1RS, whereas the 320 bp marker could be assigned to at least two rye chromosomes (4R and 6R). In addition, consistent with the result in GRS1201 (Fig. 1), the other four GRS lines (GRS1202, GRS1203, GRS1204 and GRS1205) all had the 220 bp diagnostic band characteristic of 'Insave' rye.

Discussion

We evaluated the utility of eight rye-specific markers in detecting 1RS chromatins in different wheat genetic backgrounds. All markers were able to amplify diagnostic bands that distinguish 1RS-containing from non-1RS wheat lines (Table 3). However, for markers with rye repetitive sequence origins, it is clear that the specificity to the rye genome is not due to their exclusive presence, but rather to their relatively higher copy numbers in the rye genome than in wheat. For example, Sec1Pro and Sec1Gene are part of the *Sec-1* gene encoding the ω-secalin protein in rye. There are 15 copies of *Sec-1* gene in the rye genome (Hull et al. 1991, Yamamoto and Mukai 2005). The *Sec-1* gene sequence is highly homologous (> 80%) to its wheat counterpart, the ω-gliadin gene (Shimizu et al. 1997). We found that, under less stringent PCR conditions, multiple bands could be amplified with either Sec1Pro or Sec1Gene, which may include the wheat ω-gliadin gene product due to non-specific amplification (data not shown). Therefore, the specificity of these rye-specific markers may be affected by PCR conditions. Nevertheless, rye-specific, reproducible diagnostic bands could be easily produced from each marker in the present study. In high-throughput marker analysis to identify different sources of wheat–rye translocations, specificity should not be an issue in practice when the PCR conditions are optimized.

Two markers, SCM9 and PAWS5/S6 showed polymorphisms among homoeologous group-1 wheat–rye translocations from different sources (Table 3). PAWS5/S6 was able to amplify one 220 bp and one 320 bp diagnostic bands in wheat lines with 1AL.1RS-A, but only the 220 bp band in lines with 1AL.1RS-G, and none in lines carrying 1BL.1RS-K or 1BL.1RS-S (Fig. 1). Consistent with this observation, both bands were present in 'Insave' and absent in 'Petkus' when amplified by PAWS5/S6 (Fig. 3). This 220 bp band was also absent in other rye varieties such as 'King II', 'Imperial' (Rogowsky et al. 1992a) and 'Chaupon' (Lee et al. 1996). This explained why the 220 bp band was found in all wheat lines with 1AL.1RS. Cytogenetic analysis suggested that the PAWS5/S6 locus represented by the 220 bp band was located only in 1RS of rye, whereas the sequence of the 320 bp band in 'Insave' may disperse in more than one chromosome of rye (Fig. 3). This is not unreasonable considering that PAWS5/S6 was part of the rye-specific R173 repetitive sequence family, which is structurally highly heterogeneous, and is dispersed in all seven chromosomes of rye (Rogowsky et al. 1992b).

The utility of rye-specific markers for simultaneous detection of homoeologous group-1 wheat–rye translocations was demonstrated on Set 2 materials by duplex PCR using PAWS5/S6 and RIS (Fig. 2). Of the five advance breeding lines in Set 2, only TX99A0153-1 was found to contain 'Amigo'-derived 1AL.1RS based on its duplex PCR profile (Fig. 2). This result was independently verified by infestation experiment with biotype C greenbugs of Set 2 materials (data not shown). This was expected because TX99A0153-1 was derived from a cross between 'Ogallala' and TAM 202, both of which carry the 1AL.1RS-A wheat–rye translocation.

The rye microsatellite marker SCM9 was also able to differentiate three different sources of wheat–rye translocations involving 1RS. Of the two diagnostic bands detected by SCM9, the 228 bp one was associated with all lines carrying 1AL.1RS and 1BL.1RS-S, and the 207 bp band was characteristic of 1BL.1RS-K. There is no amplification by SCM9 in the wheat genome (Fig. 1). Although PAWS5/S6 can amplify different products in 1AL.1RS-A and 1AL.1RS.G, it could not tell lines with 1BL.1RS from non-1RS wheats (Fig. 1), which might be more important in many cases. It seems that SCM9 may be a better choice when differentiation among wheat lines carrying 1AL.1RS and 1BL.1RS, and non-1RS genotypes is needed. In this case, a simple, regular PCR will be enough. On the other hand, if identification of the two sources of 1AL.1RS wheat–rye translocations (1AL.1RS-A and 1AL.1RS-G) is needed, PAWS5/S6 will be a marker of choice, and the duplex PCR procedure proposed here should be useful. To conclude, the two markers identified from this study provide a quick and reliable tool for identification of major sources of 1AL.1RS or 1BL.1RS wheat–rye translocations during marker-assisted selection in wheat breeding.

Acknowledgements

The authors thank Peihua Yan for technical help. We are indebt to Dr Bob Graybosch (USDA-ARS, Lincoln, NE) for his valuable comments on this manuscript. This research was supported by the Texas Agricultural Experiment Station (Cropping System Program) and the National Research Initiative of USDA's Cooperative State Research, Education and Extension Service, CAP grant number 2006-55606-16629.

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