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Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm

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Abstract The use of major resistance genes is the most cost-effective strategy for preventing stem rust epidemics in Australian wheat crops. The long-term success of this strategy is dependent on combining resistance genes that are effective against all predominant races of the pathogen, a task greatly assisted by the use of molecular markers linked to individual resistance genes. The wheat stem rust resistance genes *Sr24* and *Sr26* (derived from *Agropyron elongatum*) and *SrR* and *Sr31* (derived from rye) are available in wheat as segments of alien chromosome translocated to wheat chromosomes. Each of these genes provides resistance to all races of wheat stem rust currently found in Australia .We have developed robust PCR markers for *Sr24* and *Sr26* (this study) and *SrR* and *Sr31* (previously reported) that are applicable across a wide selection of Australian wheat germplasm. Wheat lines have recently become available in which the size of the alien segments containing *Sr26*, *SrR* and *Sr31* has been reduced. Newly developed PCR-markers can be used to identify the presence of the shorter alien segment in all cases. Assuming that these genes have different gene-for-gene specificities and that the wheat industry will discourage the use of varieties carrying single genes only, the newly developed PCR markers will facilitate the incorporation of two or more of the genes *Sr24*, *Sr26*, *SrR* and *Sr31* into wheat lines and have the potential to provide durable control to stem rust in Australia and elsewhere.

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Introduction

Puccinia graminis f. sp. *tritici*, the causal agent of wheat stem rust, has threatened wheat crops in Australia since European settlement in the late 18th century. The development and cultivation of resistant varieties have been cost effective in protecting wheat from rust epidemics. At present, stem rust is mainly controlled by the deployment of major genes that confer race-specific resistance in a gene-for-gene manner. However, the sustainability of this method is dependent upon the availability of resistance genes and the non-appearance of the corresponding virulent pathotypes. The use of varieties carrying two or more stem rust resistance genes with different resistance specificities, each effective against current races in the stem rust populations, should contribute to the durability of resistance in Australia. Our aim is to develop markers that will assist breeders in incorporating two or more resistance genes into a wheat line, thereby resulting in greater protection for Australian wheat varieties. Our target genes have been *SrR* and *Sr31*, derived from *Imperial* and *Petkus* rye, respectively (Mago et al. 2002, 2004) and *Sr24* and *Sr26*, derived from *Agropyron elongatum* (this study). Each of these genes provides resistance to all of the current stem rust races in Australia (McIntosh et al. 1995). Furthermore, wheat-rye translocation lines are now available that carry *SrR* and *Sr31* on a reduced rye segment that may not effect dough quality (Rogowsky et al. 1991; Lukaszewski 2000, 2003; Dundas et al. 2004). Sources of *Sr26* with reduced *Agropyron* chromatin are also available (Dundas and Shepherd 1994, 1996), and these may lack the previously reported yield penalty associated with this gene (The et al. 1988).

The stem rust resistance gene *Sr24* has been introgressed into wheat from *Agropyron elongatum*. Smith

et al. (1968) described the stem rust-resistant variety *Agent* that carries a spontaneous translocation between chromosome 3Ag of *A. elongatum* and chromosome 3DL of bread wheat. The *Agropyron* chromosome segment (3Ag) in *Agent* also carries the leaf rust resistance gene *Lr24* but is closely associated with red grain color which has prevented this source of *Sr24*/*Lr24* being used in Australian wheat. Sears (1973) used the *ph1* mutant background to induce homoeologous recombination between wheat and *Agropyron* chromosomes resulting in additional transfers of *Sr24*/*Lr24*into wheat. Several recombinant lines were obtained including white-seeded lines from 3Ag/3D transfers nos. 3 and 14. Cytogenetic studies confirmed that the *Agropyron* chromosome segment of the white-seeded recombinants is smaller than the one in *Agent*-derived wheats (McIntosh et al. 1976, 1995; Friebe et al. 1996). Although white-grained wheats carrying the reduced *Sr24*/*Lr24* segment have been grown extensively in Australia, virulent pathotypes of stem rust have not yet been isolated. Virulence to *Sr24* has been reported in South Africa (Le Roux 1985) and India (Bhardwaj et al. 1990), and virulence to *Lr24* has been reported in Australia (Park et al. 2002).

Another wheat variety, *Amigo*, carrying a 1AL.1RS translocation that was derived from *Insave* rye, carries two stem rust resistance genes (Sebesta and Wood 1978). One of these genes was mapped to rye chromosome 1RS, while the second gene was postulated to be *Sr24* because it was associated with an *Agropyron*-derived chromosome segment. However, the *Agropyron* segment was not translocated to chromosome 3DL as in *Agent* but to the short arm of chromosome 1B; several Australian varieties possess this 1BS-*Agropyron* translocation (The et al. 1992). The chromosomal structures of these *Sr24* translocations are illustrated in Fig. 1.

The stem rust resistance gene *Sr26* was introduced by translocation of the long arm of a group 6 *A. elongatum* chromosome to wheat chromosome 6A (Knott 1961, 1968). No virulence toward the *Sr26* segment has been reported world-wide. The first Australian variety released carrying *Sr26* was *Eagle* (Martin 1971). Although several varieties have been produced with *Sr26*, the use of the gene has been limited due to the reported yield penalty associated with the *Agropyron* chromosome arm (The et al. 1988). This yield penalty has been possibly overcome by reducing the size of the *Agropyron* chromosome segment. Dundas and Shepherd (1994, 1996) used the *ph1b* mutant background to induce recombination between the $6Ag^{e}\alpha$ (or 6Ae#1L) chromosome segment in cv. *Eagle* and the wheat 6AL chromosome arm. In several of the resulting lines, the recombination event had occurred in the region proximal to *Sr26* and reduced the amount of *Agropyron* chromatin. In initial field tests, these recombinants produced yields similar to that of the recurrent parent, which is lacking *Sr26* (Dundas et al. 2001, 2004).

It is time-consuming to stack two or more rust resistance genes in a common background using rust bioassays. It is also often not possible due to a lack of isolates with specific avirulence/virulence genes combinations that enable unambiguous assignments of

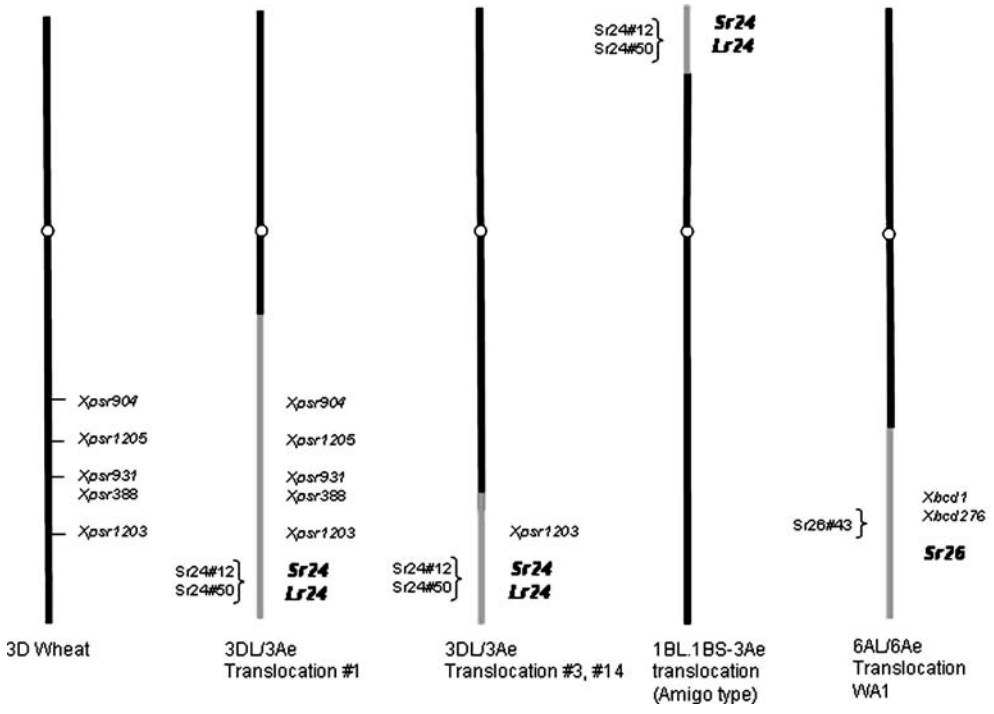

Fig. 1 Schematic representation of 3D/3Ae, 1BL.1BS-3Ae#1 and 6AL/6Ae translocation lines showing the position of restriction fragment length polymorphism (RFLP) markers (Schachermayr et al. 1995) and PCR markers identified in the current study. Wheat chromatin is represented by a *narrow black line* and *the gray portion* represents the *Agropyron* translocation. The exact location of marker *Xpsr388* has not been determined

resistance genotypes. This is particularly true for the broadly effective genes *Sr24*, *Sr26*, *SrR* and *Sr31*. The development of linked PCR-based markers is an efficient method to help identify and stack these genes. Previously, Schachermayr et al. (1995) and Dedryver et al. (1996) identified RFLP markers polymorphic for the 3Ag arm in the donor line *Agent* and also developed PCR-based markers for *Sr24*. These markers have not been useful in detecting the shorter *Agropyron* chromosome translocations carrying *Sr24*/*Lr24* (3Ae/3D#3 and 3Ae/3D#14) in Australian varieties (McIntosh et al. 1995).

We report here the development and validation of new PCR-based markers for the stem rust resistance genes *Sr24* and *Sr26* and show the usefulness of these markers along with markers previously developed for *SrR* and *Sr31* (Mago et al. 2002, 2004) for marker-assisted selection in Australian wheat varieties. These markers will enable the rapid development of near-isogenic lines to test whether the modified versions of *Sr26, Sr31* and *SrR* are devoid of the quality and agronomic defects associated with the original sources of these genes. The markers will also assist in the stacking of resistance genes with other broadly effective resistance genes, such as *Sr2*, for which PCR markers are available (Spielmeyer et al. 2003; Hayden et al. 2004) in order to develop wheat lines with potentially stable stem rust resistance. However, the durability of the gene stack will depend on careful management of the resistance resource and discouragement of the use of varieties that carry only single components of the resistance gene stack that could act as "stepping stones" for the rust pathogen to acquire virulence by stepwise mutations.

Materials and methods

Plant material

Two pairs of near-isogenic lines (NILs) were used for marker development for *Sr24*: *Tincurrin* (susceptible)-*Datatine* (resistant) and *Westonia* (susceptible)-*Westonia**6/Sr24 (resistant). For *Sr26*, the two pairs of NILs were a wheat- *Agropyron* recombinant, WA1 (resistant) (Dundas and Shepherd 1998), and the recurrent parent, *Angas* (susceptible) (Wallwork et al. 1994) and, secondly, *Westonia* (susceptible) and a backcross-derived line *Westonia**4/*Currawong* (*Sr26*) (Penrose et al. 1998). WA1 carries a wheat 6AL- *Agropyron* recombinant chromosome with a shortened segment of 6Ae#1L chromatin developed from the 6AS.6AL/6Ae#1L translocation chromosome originally produced by Knott (1961). For validation of markers, wheat varieties and backcross-derived lines carrying *Sr24* and *Sr26* together with their recurrent parents were used. These lines were produced as part of the continuing germplasm effort of the Australian Cereal Rust Control Program (ACRCP).

Sixty-nine lines (scored for the presence/absence of *Sr24*) from a *Kukri*/*Janz* DH (doubled haploid) population were used for linkage analysis. This population was produced as part of the population development plan of the National Molecular Marker Program (Kammholz et al. 2001).

Plant DNA extraction and RFLP analysis

Genomic DNA was isolated from leaves, and DNA blot analysis was carried out according to Lagudah et al. (1991a, b). DNA was restricted with endonucleases under conditions recommended by the manufacturer (MBI Fermentas, Vilnius, Lithuania; NEB, Beverly, Mass.).

DNA probes used for hybridization to DNA blots were labeled with [^{32} P]-CTP using the megaprime DNA labeling system (Amersham Pharmacia, Piscataway, N.J.). RFLP probes PSR388, PSR931, PSR1203 and PSR1205 (Schachermayr et al. 1995) were obtained from Plant Biosciences, Norwich, UK, and probes bcd1 and bcd276 were obtained from the GrainGenes Probe Repository (http://wheat.pw.usda.gov/cgi-bin/graingenes).

Isolation of AFLP markers linked to *Sr24* and *Sr26*

To isolate amplified (A)FLP markers from the region carrying the stem rust gene *Sr24*, we used the NILs *Tincurrin-Datatine* and *Westonia-Westonia*/*Sr24* as templates; for *Sr26*, the NILs *Angas* -WA1 and *Westonia-Westonia*/*Currawong* were used. AFLP analysis was performed using the standard protocol of Vos et al. (1995). For selective amplification, the *Pst*I and *Mse*I primers with three additional nucleotides were used. Cloning and analysis of the AFLP fragments were carried out as described in Mago et al. (2002).

Sequence-tagged site analysis

Three AFLP fragments associated with the presence of the stem rust resistance gene *Sr24*—P-ACA/M-GCA-594 (Sr24#12), P-ACA/M-GTG-400 (Sr24#50) and P-ACC/M-GCG-754 (Sr24#59)—and one AFLP fragment associated with stem rust gene *Sr26*—P-AAG/M-GTC-550 (Sr26#43)—were sequenced using the dye terminator sequencing system and analyzed on an ABI Prism System (Foster City, Calif.). Specific primers were designed for the amplification of each of these fragments (Table 1). PCR products were separated on a 2% agarose gel. The microsatellite marker *barc71* was amplified using published primer sequences (http://wheat.pw.usda.gov/cgi-bin/graingenes; Somers et al. 2004) and amplified under the PCR conditions shown in Table 1. The *barc71* products were run on a 1.8% methaphor agarose gel (FMC Bioproducts, Rockland, Me.). PCR markers IB-262 and Iag95, which are diagnostic for the

Table 1 PCR primers and conditions for the amplification of the *Sr24* and *Sr26* markers

Marker	Primers	PCR conditions:	
		Temperature (°C)/time	Number of cycles
Sr24#12	F-5′CACCCGTGACATGCTCGTA	94/3 min	One
	R-5′AACAGGAAATGAGCAACGATGT	94/30 s; 65/30 s; 72/40 s	1°CReducing/cycle for seven cycles
		94/30 s; 58/30 s; 72/40 s	Thirty
		20/1 min	One
Sr24#50	F-5′ CCCAGCATCGGTGAAAGAA	94/3 min	One
	R-5′ ATGCGGAGCCTTCACATTTT	94/30 s; 57/30 s; 72/40 s	Thirty
		20/1 min	One
Barc71	F-5′ GCGCTTGTTCCTCACCTGCTCATA	94/3 min	One
	R-5′ GCGTATATTCTCTCGTCTTCTTGTTGGTT	94/30 s; 63/30 s; 72/40 s	Thirty
		20/1 min	One
Sr26#43	F-5′ AATCGTCCACATTGGCTTCT	94/3 min	One
	R-5′ CGCAACAAAATCATGCACTA	94/30 s; 56/30 s; 72/40 s	Thirty
		20/1 min	One

stem rust resistance genes *SrR* and *Sr31*, respectively, have been described previously (Mago et al. 2002, 2004). Marker IB-262 was amplified under the same PCR conditions as for Sr24#12.

Results

Testing previously identified RFLPs linked to *Sr24* and *Sr26*

Our aim is to develop simple DNA markers applicable across a wide range of wheat germplasm for the selection of *Sr24* and *Sr26* in wheat breeding. We tested the *Sr24*-linked RFLP markers PSR388, PSR931, PSR1203 and PSR1205 (Fig. 1) previously described by Schachermayr et al. (1995) for their efficiency in detecting the *Agropyron* segment in the NILs *Tincurrin-Datatine* and *Westonia-Westonia*/*Sr24*. Probes PSR388, PSR931 and PSR1205 did not detect polymorphism between either of the NIL pairs (data not shown), while PSR1203 identified several polymorphic fragments in DNA gel blots with hybridization patterns similar to those reported for European wheats (Schachermayr et al. 1995) (Fig. 2a). These results are consistent with the presence of the shorter *Agropyron* segment in Australian varieties, as suggested by previous reports (McIntosh et al. 1976, 1995; Friebe et al. 1996).

For *Sr26*, the presence of the *Agropyron* segment 6Ae#1L carrying *Sr26* was confirmed by RFLP analysis of the *Sr26* NILs (*Angas*-WA1 and *Westonia-Westonia*/*Currawong* with probes bcd1 and bcd276 (Dundas and Shepherd 1994, 1996, 1998). Both probes identified several polymorphic fragments specific to 6Ae#1L (data not shown). Figure 2b shows hybridization of RFLP probe bcd1 with the NILs. A Chinese Spring ditelo 6AS was included in the Southern analysis to differentiate between a wheat and *Agropyron* band.

PCR markers for *Sr24*

None of the PCR markers that were specific for the larger *Sr24* translocation (Schachermayr et al. 1995) were informative on lines which carried the reduced *Sr24* segment (data not shown). Attempts to design a PCR marker from the probe PSR1203, which was predicted to detect marker bands on the shorter *Sr24* translocation, were unsuccessful. Consequently, the AFLP technique and wheat NIL pairs *Westonia-Westonia*/*Sr24* and *Tincurrin-Datatine* were used to develop additional markers. Twenty-four primer combinations with *Pst*I/*Mse*I primer sets were used to amplify approximately 140 fragments per primer combination. Five AFLP fragments designated as Sr24#12,#21,#50,#59 and#79, were identified in the two *Sr24*-carrying lines (data not shown). The cloned AFLP fragments were used as RFLP probes in DNA gel blots to confirm their location on the 3Ag chromosome arm (data not shown). Two probes (Sr24#21 and#79) did not identify polymorphisms with 12 restriction enzymes (data not shown), while the remaining three probes clearly distinguished resistant and susceptible lines. Figure 3a shows the hybridization of marker Sr24#12 with the genomic DNA of the NILs.

The sequences of these three AFLP fragments, Sr24#12, -#50 and -#59, were used to design PCR primers (Table 1) for the *Sr24*/*Lr24*-linked segment. All of the PCR markers were dominant and were amplified from only the resistant NILs (Fig. 4a, b). Since Sr24#59

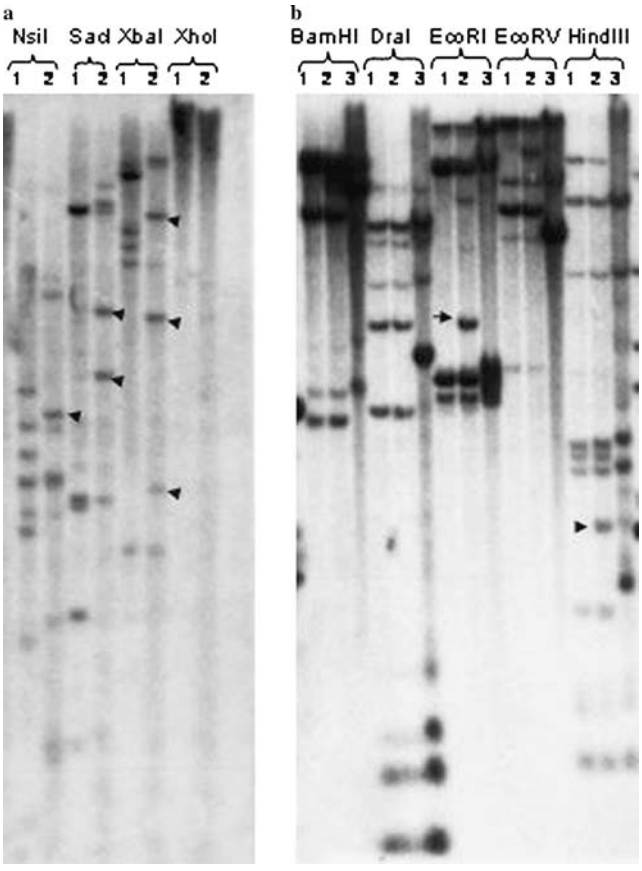

Fig. 2 **a** Hybridization of RFLP marker *Xpsr1203* (Schachermayr et al. 1995) with the genomic DNA of the susceptible and resistant NILs following digestion with various restriction enzymes. *Lanes*: *1 Westonia*, *2 Westonia*/*Sr24*. **b** Hybridization of RFLP marker *bcd1* with the genomic DNA of the susceptible and resistant NILs following digestion with various restriction enzymes. *Lanes*: *1 Angas*, *2* WA1, *3* CS.Dt 6AS. CS.Dt 6AS was included to enable differentiation between the wheat and *Agropyron* bands. Polymorphic fragments are indicated by *arrows*

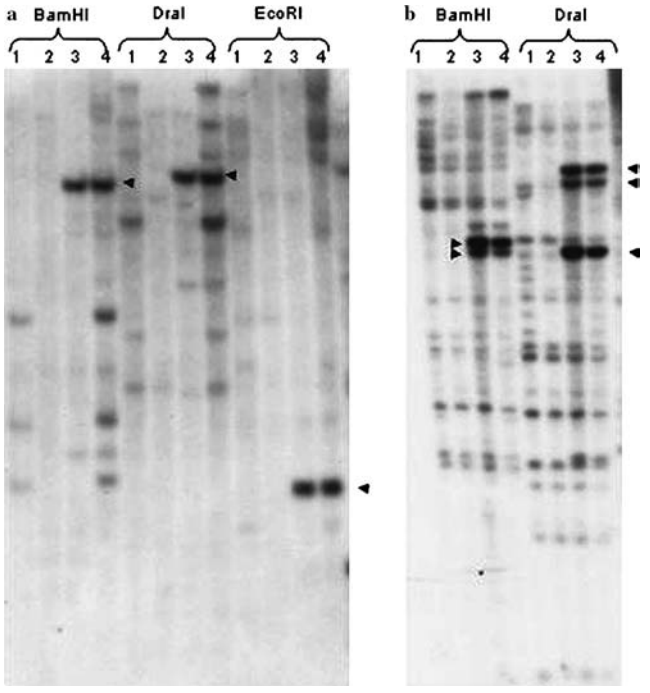

Fig. 3 Hybridization of AFLP markers. **a** Sr24#12 with the genomic DNA of the NILs following digestion with various restriction enzymes. *Lanes*: *1 Tincurrin*, *2 Westonia*, *3 Westonia*/*Sr24*, *4 Datatine*. **b** Sr26#43 with the genomic DNA of the NILs following digestion with various restriction enzymes. *Lanes*: *1 Westonia*, *2 Angas*, *3* WA1, *4 Westonia*/*Currawong*. *Arrow* shows the specific RFLPs

did not provide any additional information in comparison to that obtained by Sr24#12 and -#50, it was not used in subsequent analyses.

PCR markers for *Sr26*

As attempts to convert RFLP probes BCD1 and BCD276 to PCR-based markers linked to *Sr26* were unsuccessful, we used the AFLP technique with the *Pst*I/*Mse*I primer sets on wheat NIL pairs *Westonia*-*Westonia*/*Currawong* and *Angas*-WA1 to develop additional markers. Using 96 primer combinations, we identified ten polymorphic AFLPs between the resistant and susceptible NILs. The cloned AFLPs were used as RFLP probes to confirm their location on the 6Ag chromosome arm, but only two probes (Sr26#43 and #61) distinguished the susceptible and resistant NILs (Fig. 3b and data not shown) using a set of 12 restriction enzymes. These two AFLPs were sequenced to design PCR primers, and only one set of primers for Sr26#43 amplified a specific polymorphism (Fig. 4c).

Validation of PCR-markers for *Sr24* and *Sr26*

Wheat varieties and backcrossed derivatives that differed for the presence of *Sr24* and/or *Sr26* were used to validate the utility of the PCR markers. There was a complete association of markers Sr24#12 and Sr24#50 with *Sr24*, including *Sr24* that is associated with the 3Ag/1BS *Amigo*-type translocation (Table 2). The two PCR markers were also validated using 69 lines segregating for *Sr24* that derived from a *Kukri* × *Janz* DH family. While the Sr24#12 marker was completely linked to *Sr24*, the Sr24#50 marker failed to predict the presence of *Sr24*. Although the Sr24#50 marker amplified from *Janz* (the resistant parent), the same band amplified from only one of the 36 resistant DH lines and from none of the susceptible segregants. One possible explanation for this non-Mendelian inheritance could be that the mapping family was derived from more than one F_1 plant and that a deletion or a rare recombination event in one F_1 generated a shorter *Agropyron* segment (lacking the Sr24#50 marker) that was inherited by most of the progeny.

Validation of the marker Sr26#43 was also carried out on several varieties and advanced breeders' lines

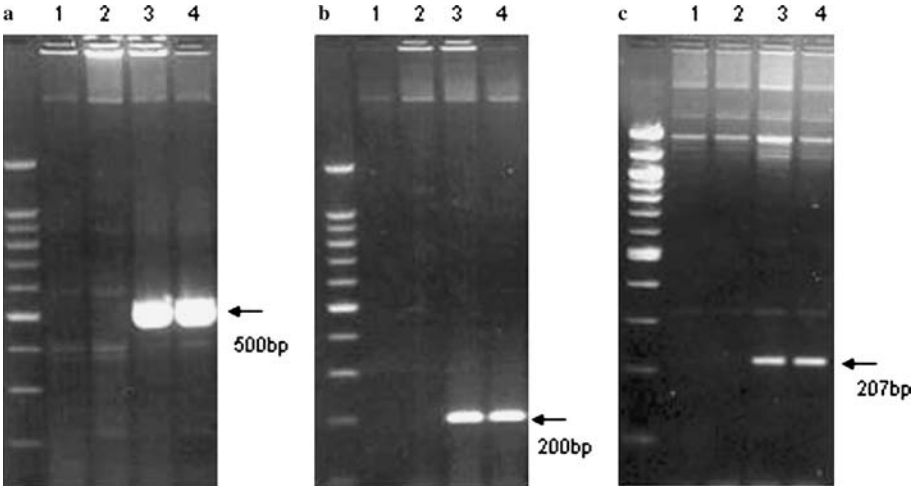

Fig. 4 PCR amplification of markers Sr24#12 (**a**), Sr24#50 (**b**) and Sr26#43 (c) from the susceptible and resistant NILs. **a, b** *Lanes*: *1 Westonia*, *2 Tincurrin*, *3 Datatine*, *4 Westonia/Sr24*. **c** *Lanes*: *1 Westonia*, *2 Angas*, *3* WA1, *4 Westonia/Currawong*. Arrow shows specific PCR product

(Table 3). The PCR marker was completely associated with the presence of *Sr26* resistance.

Development of co-dominant markers

Because all of the new *Sr24* and *Sr26* markers were dominant, we searched for co-dominant markers which could assist in differentiating between homozygous and heterozygous genotypes for either gene. For *Sr24*, we tested several microsatellite markers—*barc71*, *cfd4*, *cfd9*, *wmc3*, *wmc549*, *gdm72*—which had been mapped previously on the long arm of chromosome 3D of wheat (http://wheat.pw.usda.gov/cgi-bin/graingenes; Somers et al. 2004). Only *barc71* amplified a polymorphic fragment between the resistant and susceptible NILs. *Barc71* is the most distal marker mapped on the long arm of chromosome 3D of wheat, and all of the varieties carrying *Sr24* amplified two diagnostic bands of 103 bp and 85 bp (Fig. 5). While most of the *Sr24* susceptible lines amplified a 107-bp fragment, some non-*Sr24* wheat varieties carried different alleles (Fig. 5, lanes 6, 10 and 11). Among the lines tested, the *barc71* marker would provide a useful co-dominant marker in all backgrounds except for *Cunderdine* and *Spear* derivatives (Fig. 5, lanes 10, 11).

Three microsatellite markers *wmc59*, *wmc254* and *wmc621* that were previously mapped on the distal end of chromosome 6AL (http://wheat.pw.usda.gov/cgi-bin/graingenes; Somers et al. 2004) were tested on the *Sr26* susceptible and resistant NILs. None of the microsatellites primers amplified polymorphic fragments specific to the translocated segment carrying *Sr26* (data not shown).

Application of the PCR markers in gene stacking

To test the potential of these markers for stacking stem rust resistance genes in Australian wheats, we used DNA from existing varieties *Sunelg*, which contains *Sr24* and *Sr26* (*Sunelg*, AUS99109) and *Siouxland*, which carries *Sr24* and *Sr31* (*Siouxland*, AUS2204) and also from mixed DNA (1:1:1:1) from different wheats carrying the *SrR*, *Sr31*, *Sr24* and *Sr26* genes. *Sr24*-specific markers (Sr24#12 and -#50) and the *Sr26*-specific marker (Sr26#43) detected corresponding bands in wheat variety *Sunelg* (Tables 2, 3). The detection of *SrR* and *Sr31* was carried out previously using markers IB-262 and Iag95, respectively (Mago et al. 2002, 2004). *Sr24*- and *Sr31*-specific markers were amplified from wheat variety *Siouxland* (data not shown). Specific PCR markers for the resistance genes *Sr24*, *Sr26*, *Sr31* and *SrR* were amplified from a DNA mixture of *Datatine* (*Sr24*), WA1 (*Sr26*), *Federation*4/Kavkaz* (*Sr31*) and *Gabo* 1DL.1RS- Sr^{+} Sec^{-} (*SrR*) (Fig. 6). All of these markers amplified specific bands from the DNA mixture containing all four stem rust genes and thus could be used to follow and stack all of these stem rust resistance genes in breeding programs. *Sr24* and *SrR* markers could be amplified in a single PCR reaction, but all four genes could not be amplified simultaneously because of differences in the T_m of the primers or due to identical sizes of the different PCR products.

Discussion

In this study we have developed PCR markers for stem rust resistance genes *Sr24* and *Sr26* that can be used in conjunction with those previously developed for *Sr31*, *SrR* (Mago et al. 2002, 2004), *Sr36* (Bariana et al. 2001), *Sr38* (Seah et al. 2001) and *Sr2* (Spielmeyer et al. 2003; Hayden et al. 2004) for marker-assisted stem rust resistance breeding. All of these markers are effective across a broad range of wheat germplasm. The identification of markers for *Sr24* and *Sr26* in this study was facilitated by the genes being on alien segments that do not recombine in wheat, and although markers on the alien segments may be physically distant from the resistance genes, they co-segregate with these genes. The efficient

Table 2 Marker differentiation of wheat varieties with and without *Sr24* using PCR markers Sr24#12, Sr24#50 and barc71

S. no.	Wheat variety/line	*Sr24* phenotype	Sr24#12 score	Sr24#50 score	Barc71 score
1	*Tincurrin*	–	–	–	–
2	*Datatine*	+	+	+	+
3	*Westonia*	–	–	–	–
4	*Westonia*/*Sr24*	+	+	+	+
5	*Cunderdine*	–	–	–	–
6	Ag/*Cunderdine*	+	+	+	+
7	*Agent*	+	+	+	+
8	Ag/163320	+	+	+	+
9	Ag/*Cocomba*	+	+	+	+
10	Coccomba	–	–	–	–
11	*Sunco*	+	+	+	+
12	*Tasman*	+	+	+	+
13	*Kukri*	–	–	–	–
14	*Janz*	+	+	+	+
15	*Cunningham*	+	+	+	+
16	*Annuello*	+	+	+	+
17	*Sunsoft98*	+	+	+	+
18	Co-1568	–	–	–	–
19	Co-1213	–	–	–	–
20	*Anlance1*	+	+	+	+
21	*Goroke*	+	+	+	+
22	*Vasco*	+	+	+	+
23	*Torres*	+	+	+	+
24	*Giles*	+	+	+	+
25	*Petrie*	+	+	+	+
26	*Krickauff*	+	+	+	+
27	*Nyabing*	+	+	+	+
28	*Yitpi*	–	–	–	–
29	*Chara*	–	–	–	–
30	*Spear*	–	–	–	–
31	*Amigo*	+	+	+	+
32	Ag/Am/576 W511	+	+	+	+
33	76 W511	–	–	–	–
34	*Amigo*/*Oxley*	+	No DNA	No DNA	No DNA
35	*Oxley*	–	–	–	–
36	*Mira*	+	+	+	+
37	C93.56	+	+	+	+
38	*Chinese Spring*	–	–	–	–
39	*Camm*	–	–	–	–
40	*Egret*	–	–	–	–
41	*Codoux*	–	–	–	–
42	Ag/*Codoux*	+	+	+	+
43	*Sunelg*	+	+	+	+
44	*Siouxland*	+	+	+	+

Table 3 Marker differentiation of wheat varieties with and without *Sr26* using PCR marker Sr26#43

S. No.	Wheat variety/line	*Sr26* phenotype	Sr26#43 score
1	*Angas*	–	–
2	*Angas* + *Sr26* (WA1)	+	+
3	*Westonia*	–	–
4	*Westonia* + *Sr26*	+	+
5	76W551	–	–
6	*Sr26*/3*76W551 -163437	+	+
7	-163438	+	+
8	-163439	+	+
9	-163440	+	+
10	-163441	+	+
11	-163442	+	+
12	-163443	+	+
13	-163444	+	+
14	-163445	+	+
15	*Vasco*	–	–
16	*Sr26*/3**Vasco* -163257	+	+
17	-163258	+	+
18	-163259	+	+
19	*Cranbrook*	–	–
20	*Sr26*/3**Cranbrook* -163406	+	+
21	-163407	+	+
22	-163408	+	+
23	K2001	–	–
24	*Sr26*/4*K2001-163115	+	+
25	*Lowan*	–	–
26	*Sr26*/4**Lowan*-163275	+	+
27	XL30	–	–
28	Sr26/4*×L30-163181	+	+
29	*Harrier*	+	+
30	*Sunelg*	+	+
31	*Apollo*	+	+
32	*Mercury* (Hybrid)	+	+
33	*Shrike*	+	+
34	*Petrel*	+	+
35	*Sunlin*	+	+
36	*Snipe*	+	+
37	*Wylah*	+	+
38	*Yitpi*	–	–
39	*Chara*	–	–
40	*Chinese Spring*	–	–
41	*Currawong*	+	+
42	*Kukri*	–	–
43	*Janz*	–	–

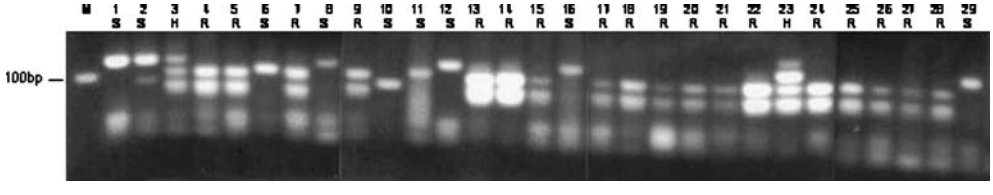

Fig. 5 PCR amplification of microsatellite marker *barc71* from various wheat varieties. *Lanes*: *1 Tincurrin*, *2 Westonia*, *3 Westonia* + *Westonia*/*Sr24* (mixed DNA), *4 Westonia*/*Sr24*, *5 Datatine*, *6 Cunderdine*, *7 Amigo*, *8 Cook*, *9 Sunco*, *10* CO-1568, *11* CO-1213, *12 Cocomba*, *13 Anlance1*, *14 Goroke*, *15* Ag/*Cocomba*, *16 Oxley*, *17 Vasco*, *18 Torres*, *19 Giles*, *20 Petrie*, *21 Krickauff*, *22 Sunsoft 98*, *23 Nyabing*, *24* Ag/*Cunderdine*, *25* Ag/163320, *26 Cunningham*, *27 Mira*, *28 Annuello*, *29 Chinese Spring*. *R* Resistant, *S* susceptible, *H* heterozygous

and unambiguous identification of markers using the AFLP technique was also facilitated by the availability of two pairs of near-isogenic lines for both *Sr24* and *Sr26*; all AFLPs common to the line possessing the resistance gene in both pairs were consistently associated with the target genes.

While *Sr24* is quite extensively deployed in Australian wheat varieties, *Sr26*, *SrR* and *Sr31* have not been used widely due to the associated agronomic and quality

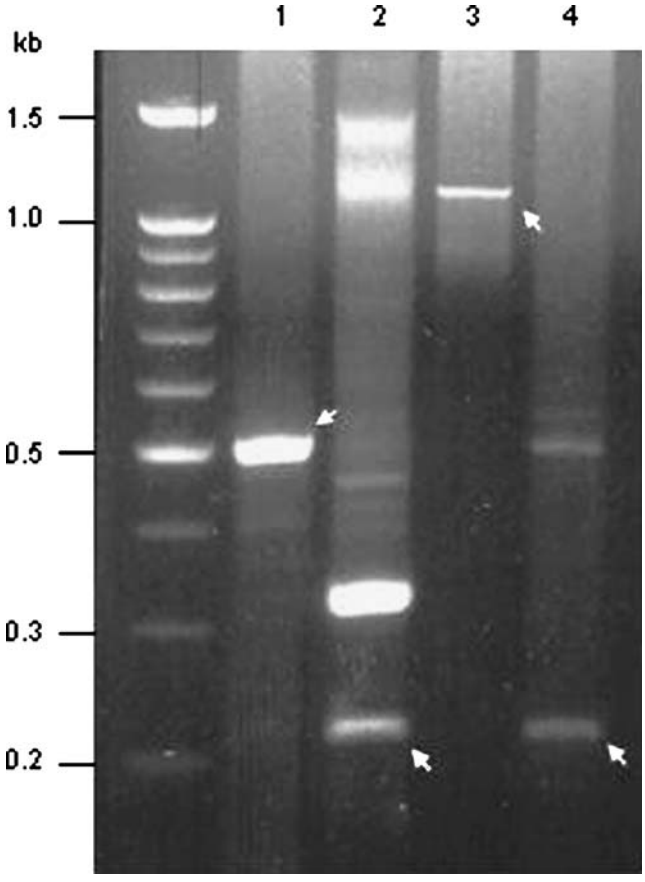

Fig. 6 Four stem rust gene markers detected in mixed DNA. A mixture of equal quantities of DNA samples from *Datatine* (*Sr24*), WA1 (*Sr26*), *Federation*4/Kavkaz* (*Sr31*) and *Gabo*1DL.1RS recombinant Sr^{+} Sec^{-} (*SrR*) was amplified in four separate PCR reactions to detect the presence of markers Sr24#12 (*lane 1*); Sr26#43 (*lane 2*); Iag95 (*lane 3*); IB-262 (*lane 4*)

defects. The recent development of wheat-rye recombinants that have reduced rye segments carrying rust resistance genes *SrR* and *Sr31* (Rogowsky et al. 1991; Lukaszewski 2000, 2003; Dundas et al. 2004) and wheat-*Agropyron* recombinants (Dundas and Shepherd 1994, 1996, 1998; Dundas et al. 2004) that have shorter *Agropyron* segments carrying *Sr26* are now available. It is now important that near-isogenic lines containing each of these genes singly be developed in several backgrounds to confirm the initial observations (Rogowsky et al. 1991; Lukaszewski 2003; Dundas et al. 2004) that the new translocations have lost the negative characters. The markers described herein will assist in the rapid development of these tester lines and allow breeders to stack *Sr24* and reduced alien segments containing *Sr26*, *Sr31* and *SrR* in any wheat background, while analysis of these tester lines would determine whether having several alien regions in a background will have yield or quality defects. To date, it has not been possible to combine *Sr24*, *Sr26*, *Sr31* and *SrR* because these genes each provide resistance to all known races of *P. graminis* f. sp. *tritici* in Australia. Combining three to four of these genes, each of which has gene-for gene specificity (Le Roux 1985; Bhardwaj et al. 1990; Pretorius et al. 2000; Mago and Kolmer, unpublished), should provide highly effective and potentially durable resistance to stem rust in Australia. This will, however, depend on the adoption of stem rust resistance gene management practices by the Australian wheat industry that discourages the use of varieties carrying these genes singly to avoid selection of sequential mutation events in *P. graminis* f. sp. *tritici* and on continued monitoring for introductions of new races that are virulent on one or more components of the resistance gene stack.

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