

Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker-assisted selection

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Abstract The use of major resistance genes is a cost-effective strategy for preventing stem rust epidemics in wheat crops. The stem rust resistance gene *Sr39* provides resistance to all currently known pathotypes of *Puccinia graminis* f. sp. *tritici* (Pgt) including Ug99 (TTKSK) and was introgressed together with leaf rust resistance gene *Lr35* conferring adult plant resistance to *P. triticina* (Pt), into wheat from *Aegilops speltoides*. It has not been used extensively in wheat breeding because of the presumed but as yet undocumented negative agronomic effects associated with *Ae. speltoides* chromatin. This investigation reports the production of a set of recombinants with shortened *Ae. speltoides* segments through induction of homoeologous recombination between the wheat and the *Ae. speltoides* chromosome. Simple PCR-based DNA markers were developed for resistant and susceptible genotypes (Sr39#22r and Sr39#50s) and validated across a set of recombinant lines and wheat cultivars. These markers will facilitate the pyramiding of ameliorated sources of *Sr39*

with other stem rust resistance genes that are effective against the Pgt pathotype TTKSK and its variants.

Introduction

Puccinia graminis Pers. f. sp. *tritici* (Pgt), the causal agent of wheat stem rust, continues to threaten wheat crops worldwide. Stem rust has been effectively controlled in Australia through the deployment of diverse sources of resistance since the early 1980s (Bariana et al. 2004, 2007; Park 2007). Stem rust resistance in cultivars grown worldwide has been based primarily on major genes that are expected to follow gene-for-gene relationship. The sustainability of resistance is dependent upon the availability and careful management of resistance genes and continuous monitoring of Pgt populations for new virulence phenotypes. In 1999, a new pathotype of Pgt with virulence on the widely deployed rust resistance gene *Sr31* was detected in Uganda. It was initially named as Ug99 (Pretorius et al. 2000). The name TTKSK was given based on the North American stem rust nomenclature system of 16 differential lines (Jin et al. 2008). Tests have shown that TTKSK carries virulence for several race-specific stem rust resistance genes currently used across the world and variants with virulence against commonly used stem rust resistance genes *Sr24* (TTKST) and *Sr36* (TTTSK) have also been detected (Jin and Singh 2006; Wanyera et al. 2006; Jin et al. 2008). TTKSK has been detected in Iran (Nazari et al. 2009) and may soon threaten wheat production in the Indian sub-continent (Singh et al. 2006; Stokstad 2007). *Sr39* is effective against TTKSK (Jin et al. 2007) and its variants TTKST and TTTSK.

Sr39 was transferred to the hexaploid wheat cultivar Marquis from *Aegilops speltoides* (Kerber and Dyke 1990). The gene is located on a translocated segment of *Ae. speltoides*

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chromosome 2S to wheat chromosome 2B (translocation stock 2S#2; McIntosh et al. 1995; Friebe et al. 1996; Gill et al. 2008). The translocated segment carries the seedling stem rust resistance gene *Sr39* and an adult-plant hypersensitive leaf rust resistance gene *Lr35*. No current Australian wheat cultivar carries *Sr39*, presumably because of negative agronomic effects of this large translocation. Labuschagne et al. (2002) reported that a South African wheat genotype (Karee*6/RL6082) with *Lr35* (and also *Sr39*) showed significant increase in flour water absorption compared to the recurrent parent Karee. Recombination can be induced between the chromosome segment from a wild relative and a homoeologous wheat chromosome to remove deleterious effects associated with the alien chromatin and/or to restore wheat genes lost due to the translocation (Dundas and Shepherd 1994, 1996a, b, 1998; Dundas et al. 1999, 2001, 2004; Lukaszewski 2000; Anugrahwati et al. 2008). For example, Anugrahwati (2006) showed improvement in flour quality of wheat-1RS recombinants with shortened 1RS chromatin compared to the original 1DL.1RS translocation line. A preliminary report summarized recombination events between the wheat chromosome 2B and the *Sr39/Lr35* carrying *Ae. speltooides* chromosome 2S (Dundas et al. 2007). These recombinants are described in detail in this paper. A simple set of PCR markers (Gold et al. 1999; Seyfarth et al. 1999; this work) was used to identify the presence of recombinants with shortened *Sr39* carrying segments. These markers will serve as useful tools for transfer of the shortened *Sr39* carrying *Ae. speltooides* translocation into future wheat cultivars individually as well as a component in gene combinations effective against TTKSK and its variants.

Materials and methods

Plant materials

The *Sr39* translocation parent RL5711 was produced by Kerber and Dyke (1990) in the susceptible wheat ‘Marquis’ background. The original 2B-2S#2 translocation line with *Sr39/Lr35* has been generously supplied to the Australian Cereal Rust Control Program (ACRCP) by Dr. Taing Aung (formerly of Agriculture Canada). Recombinants between *Ae. speltooides* and the wheat chromosome were produced as part of the continuing germplasm improvement effort of the ACRCP (Dundas et al. 2007).

Development of new recombinants

The wheat–*Ae. speltooides* chromosome 2S#2 recombinants were produced in the Cereal Cytogenetics Laboratory, Waite Campus, University of Adelaide. The first step was

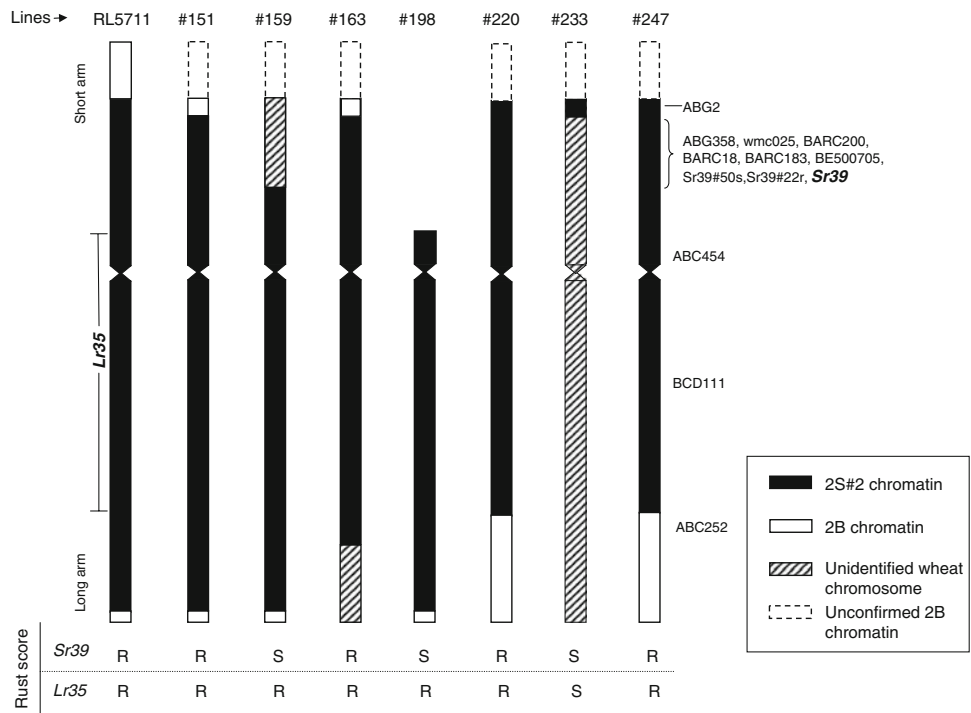
to determine the size of the 2S#2 chromosome segment in the translocation line RL5711. A range of group 2 DNA probes of both wheat and barley, previously mapped to the distal and proximal regions of short and long arms (GrainGenes: <http://wheat.pw.usda.gov/>), were obtained from the Australian Triticeae Mapping Initiative (ATMI) (maintained by Prof P. Langridge, University of Adelaide). They were labeled with ³²P-dCTP by the random primer method and hybridized to genomic DNA of RL5711 (+*Sr39*) and Marquis (-*Sr39*) digested with *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV or *Hind*III and transferred by blotting to Hybond N+ membrane (Amersham, UK). These probes were analyzed for the presence of polymorphic bands associated with the presence of the 2S#2 chromosome.

The second step was to introduce the *ph1b* mutant (Sears 1977) to induce homoeologous recombination. Line RL5711 (carrying the 2B-2S#2 translocation) was crossed as female with wheat line ‘Angas *ph1bph1b*’ (pedigree: Eagle/Chinese Spring *ph1bph1b/3/2**Angas) [the initial F₃ plant of Eagle x CS was screened before backcrossing to Angas for absence of the Eagle ‘6Ae#1’ chromosome (ensuring the absence of gene *Sr26*), the presence of normal 6A chromosomes, the absence of *Xpsr128-5B* and the absence of the rust resistance gene *Sr9g*]. F₂ seedlings were screened for a *ph1bph1b* genotype (negative for *Xpsr128-5B*), and also for the presence of both the normal 2B and 2B-2S#2 translocation chromosomes using probe MWG503 (*Dra*I). Sixty-five F₃ and 55 BC₁ (backcross with Angas *Ph1Ph1*) seedlings were screened for dissociation of the 2S#2 markers with probes ABC252 (most distal marker on 2S#2 long arm), ABG2 (most distal marker on 2S#2 short arm), BCD111 (proximal marker on 2B long arm) and ABC454 (proximal marker on 2B short arm) (*Eco*RI). RFLP probe ABG358 (interstitial on the short arm) (Fig. 1) was used to further define the sizes of the 2S#2 chromosome segments. Plants showing dissociation of 2S#2 markers were progeny-tested and crossed as females with cv. Angas up to BC₃.

GISH analysis of recombinant lines

The original source 2B-2S#2 translocation line and the recombinants were also characterized by the genomic in situ hybridization (GISH). GISH followed the procedure described by Zhang et al. (2001). Total genomic DNA from *Ae. speltooides* (University of Sydney cytogenetics collection accession # C64.69) was labeled with biotin-14-dATP using BioNick DNA Labeling System (Invitrogen Life Science, Carlsbad, CA). Unlabeled total genomic DNA of wheat was used as a blocker. The probe:blocker ratio was ~1:50. Signals were detected with fluorescein avidin DN (Vector Laboratories, Burlingame, CA). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole

Fig. 1 Schematic representation of wheat–*Aegilops speltoides* chromosome 2S#2 recombinant lines showing the position of RFLP markers. *Ae. speltoides* chromatin is represented in black. Wheat chromatin is represented in white. Recombinant numbers 159, 198 and 233 were susceptible to stem rust and numbers 151, 163, 220 and 247 were resistant



(DAPI) (Invitrogen Life Science) and pseudocolored red. Slides were analyzed with a Zeiss Axio Imager epifluorescence microscope. Images were captured with a Retiga EXi CCD (charge-coupled device) camera (QImaging, Surrey, BC) operated with Image-Pro Plus 6.2 software (Media Cybernetics Inc., Bethesda, MD) and processed with Photoshop version 8.0 software (Adobe Systems, San Jose, CA).

Rust assays

The recombinant lines were rust tested for both stem rust and leaf rust at the University of Sydney Plant Breeding Institute, Cobbitty. Pathotype 34-1,2,3,4,5,6,7 (Culture no. 103) of Pgt and 104-1,2,3,(6),(7),11,13 (Culture no. 547) of *P. triticina* (Pt) were used. Rust test procedures were described in Bariana and McIntosh (1993).

PCR marker development

Development of new PCR-based markers for *Sr39* was undertaken at CSIRO, Plant Industry, Canberra. 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, Lithuania; NEB, USA).

SSR and EST analyses of *Sr39* lines

Primer sequences for the SSR markers from chromosome 2B were obtained from GrainGenes (<http://wheat.pw.usda.gov/GG2/quickquery.shtml#microsats>).

ESTs in the deletion bin maps of chromosome 2B were identified from <http://wheat.pw.usda.gov/cgi-bin/westsq/locus.cgi> and the primers for amplification were designed using the Primer3 software (<http://frodo.wi.mit.edu/>).

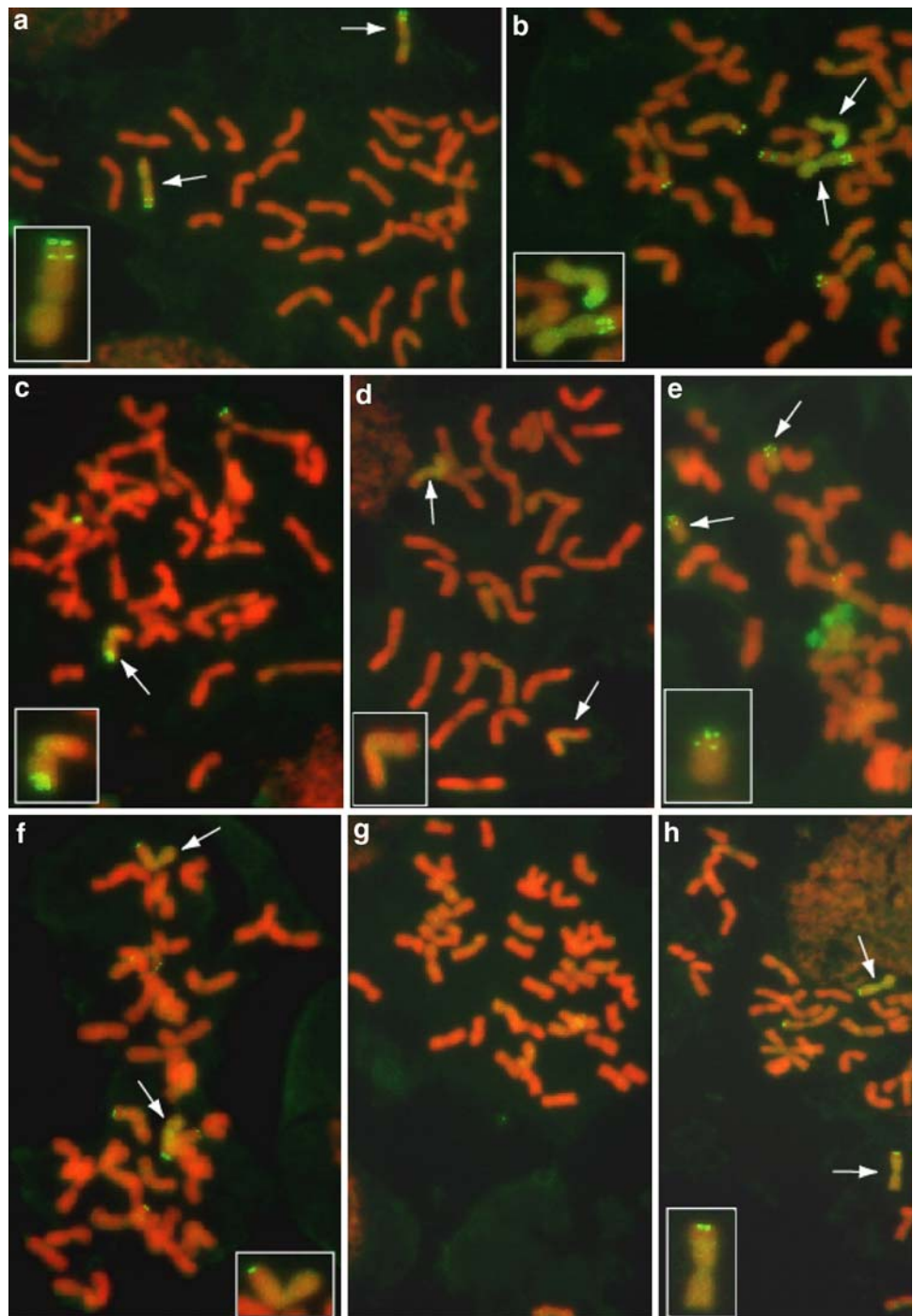
Isolation of AFLP markers linked to *Sr39*

To isolate AFLP markers from the *Sr39* carrying region, the seven dissociation lines with shortened *Ae. speltoides* chromosome segments (Dundas et al. 2007) along with Marquis (susceptible) and the resistant parent RL5711 were used. AFLP analysis was performed using the standard protocol (Vos et al. 1995). For selective amplification *Pst*I and *Mse*I primers with three additional nucleotides were used. Cloning and analysis of AFLP fragments were done as described in Mago et al. (2002).

Sequence-tagged site analysis

Three AFLP fragments P-ACA/M-GCT-842 (*Sr39*#22r), ACA/M-GAG-360 (*Sr39*#8r) and P-AAG/M-GTA-743 (*Sr39*#3r) associated with the presence of *Sr39*, and three AFLP markers P-ACA/M-GCT-911 (*Sr39*#2s), P-AAG/M-GTA-350 (*Sr39*#26s) and P-ACC/M-GCC-284 (*Sr39*#50s) associated with the susceptible genotype were sequenced using the dye terminator sequencing system and analyzed on an ABI prism system. Specific primers were designed for amplification of each of these fragments and used for PCR amplification of the diagnostic product. Primer

Fig. 2 In situ hybridization using *Aegilops speltoides* genomic DNA as probe on: **a** the original wheat–*Ae. speltoides* 2B-2S#2 translocation (+*Sr39*) from RL5711, and 2S#2 dissociation lines **b** #151, **c** #159, **d** #163, **e** #198, **f** #220, **g** #233 and **h** #247. The magnified images on chromosomes (all lines except #233) show the *Ae. speltoides* chromosome segment (fluorescent section). **b–h** Hybridization signals on the SAT chromosomes due to the highly similar repetitive rDNA sequences between wheat and *Ae. speltoides*. **e** The loss of most or the entire short arm of the translocation chromosome in RL5711



The original 2B-2S#2 translocation consists of a large segment of *Ae. speltoides* 2S#2 chromatin with a segment of non-labeled wheat chromatin on the distal end of the short arm (Fig. 2a). Approximately 80% of the physical length of the short arm is 2S#2 chromatin. Interpretation of structure of the long arm is more difficult because of the presence of labeled telomeres. However, chromosome pairing observations during backcrossing showed that ring bivalents were formed with this chromosome (T. Aung,

personal communication), indicating that these labeled long-arm telomeres probably represent 2BL chromatin (Fig. 2a). If this assumption is correct, and the sub-terminal band on the long arm is of *Ae. speltoides* origin, then at least 77% of the long arm is 2S#2 chromatin.

Line #151 had lost the 2S#2 allele for the probe ABG2. The 2B allele for this probe was present indicating that recombination had occurred in the distal region of the short arm. The in situ photograph of this line shows labeling

along most of the length of the chromosome (Fig. 2b). This line is resistant to stem rust (+*Sr39*) and leaf rust (+*Lr35*).

Line #159 had lost two 2S#2 RFLP markers ABG2 and ABG358 (Fig. 1). This line was susceptible to stem rust (–*Sr39*) and resistant to leaf rust (+*Lr35*). The in situ photograph shows that the distal end of the short arm has been replaced with non-labeled wheat chromatin extending to approximately 30% of the length of the arm (Fig. 2c). We have been unable to determine which wheat chromosome was involved in the recombination event.

Line #163 had lost the 2S#2 distal short arm allele for probe ABG2 (Fig. 1). This line was resistant to both stem rust and leaf rust (+*Sr39* and +*Lr35*). In situ photographs show a chromosome with labeling on proximal portions of both arms (Fig. 2d). The long-arm telomeres (thought to be of 2B origin) and sub-terminal dots (thought to be *Ae. speltoides* origin), which were present in the original translocation, are no longer on this chromosome. Approximately 70% of both the short and long arms remain as 2S#2 chromatin. The apparent loss of the labeled 2BL telomeres from this chromosome suggests that another as yet unidentified wheat chromosome may have been involved in the recombination event.

Line #198 had lost the 2S#2 alleles for probes ABG2 and ABG358 (Fig. 1). It was susceptible to stem rust (–*Sr39*) and resistant to leaf rust (+*Lr35*). The proximal short-arm probe for ABC454 shows a 2S#2 band. In situ photos appear to show a telocentric chromosome consisting of the long arm of the original 2B-2S#2 translocation chromosome (Fig. 2e). Because of the presence of the 2S#2 allele for the group 2 short-arm probe ABC454, it is possible that the breakpoint on the translocation chromosome in line #198 is not at the centromere and a sub-microscopic portion of the short arm is retained. Therefore, it is possible that this is an acrocentric chromosome instead of telocentric.

Lines #220 and #247 showed similar loss of the distal long arm 2S#2 allele for probe ABC252 and the presence of the 2B allele for this probe (Fig. 1). All short-arm markers tested appear to have been retained. These lines are resistant to stem rust and leaf rust (+*Sr39* and +*Lr35*). In situ photographs show loss of the sub-terminal dots on the long arm and also the insertion of non-labeling wheat chromatin so that the 2S#2 chromatin has been reduced from approximately 77 to 67% of the length of the long arm (Fig. 2f, h).

Line #233 retained only the 2S#2 allele for probe ABG2 (Fig. 1). All other 2S#2 alleles and resistance genes *Sr39* and *Lr35* were lost from this line. In situ photographs confirm loss of the large 2S#2 chromosome block of the original translocation (Fig. 2g). It is beyond the detection limit of the FISH technique to determine which wheat chromosome carries the remaining 2S#2 chromosome segment. Marker data suggest that the wheat 2B chromosome may not have been involved in the recombination event since

both loci, *Xabg2-2B* and *Xabg2-2S#2*, are present in a line homozygous for the 2S#2 chromosome segment.

Further markers for characterization of recombinants and marker-assisted selection of *Sr39/Lr35*

A range of SSR markers from chromosome 2B were tested on the *Sr39* parent and recombinants to look for polymorphisms. SSR markers BARC18, BARC183, BARC200 and *wmc025* each amplified products from all stem rust susceptible lines but not from stem rust resistant lines, consistent with replacement of wheat 2B segment by the translocation (Fig. 3 and data not shown). The PCR product for SSR marker *wmc025* in Chinese Spring (CS) (Fig. 3, lane 18) was smaller than in the other susceptible lines. SSR markers BARC7, BARC10, BARC45, BARC55, BARC101, BARC128, BARC160, *gwm55*, *gwm319*, *wmc261* and *wmc344* amplified monomorphic products from all lines (data not shown). Primers were also designed for chromosome 2B specific EST markers BF146002, BE443085, BE404432, BE500705, BE398939, BI479116 and BQ280506. Only BE500705 amplified a diagnostic PCR product from stem rust susceptible lines (data not shown) and none of the other EST markers amplified from any of the lines.

AFLP markers for *Sr39/Lr35*

We used the AFLP technique with *PstI/MseI* primer sets on the wheat near isogenic line (NIL) pairs Marquis (S)–RL5711 (R) and recombinants #151 (R) and #159 (S) (see Fig. 1) to develop additional markers. With 144 primer combinations, 6 AFLPs distinguished the stem rust resistant and susceptible recombinants. Three markers P-ACA/M-GAG-360 (*Sr39#8r*), P-ACA/M-GCT-842 (*Sr39#22r*) and P-AAG/M-GTA-743 (*Sr39#3r*) were linked with resistance and three markers P-ACA/M-GCT-911 (*Sr39#2s*), P-AAG/M-GTA-350 (*Sr39#26s*) and P-ACC/M-GCC-284 (*Sr39#50s*) were associated with susceptibility. The AFLP fragments were cloned, used as RFLP probes and mapped to chromosome 2B. Only, probes *Sr39#22r*, *Sr39#3r*,

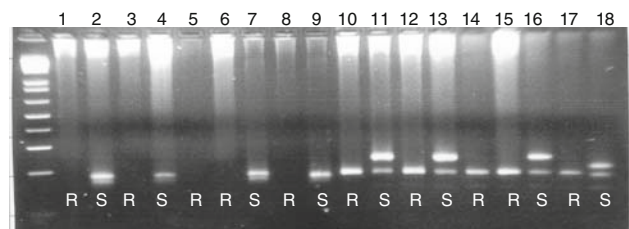
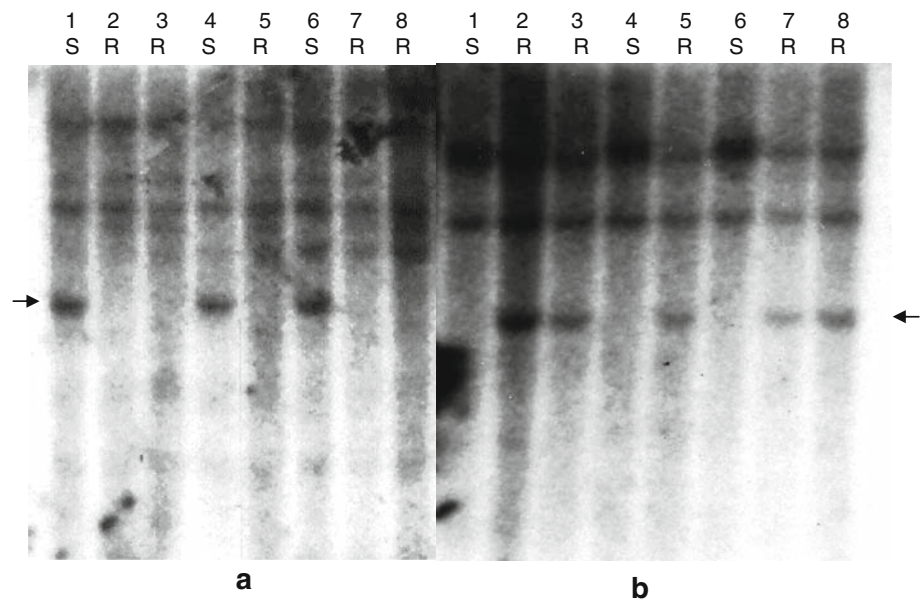


Fig. 3 PCR amplification of the SSR markers on wheat–*Aegilops speltoides* recombinants. Lanes 1–9 show BARC200 and 10–18 show *wmc025* amplification. Lanes 1 and 10, #151; 2 and 11, #159; 3 and 12, #163; 4 and 13, #198; 5 and 14, #220; 6 and 15, #247; 7 and 16, Marquis; 8 and 17, RL 5711; 9 and 18, Chinese Spring

Fig. 4 Hybridization of AFLP-derived markers (a) Sr39#50s, *Bam*HI (b) Sr39#3r, *Eco*RV, to wheat–*Ae. speltoides* recombinant lines. Lanes 1 Marquis (susceptible parent), 2 RL 5711 (resistant parent), 3 Recombinant #151, 4 #159, 5 #163, 6 #198, 7 #220, and 8 #247. Arrow indicates the position of polymorphic band



Sr39#2s and Sr39#50s showed polymorphism between the stem rust susceptible and resistant recombinants. Figure 4 shows the hybridization of two of the AFLP-derived markers Sr39#50s and Sr39#3r to genomic DNA of the wheat–*Ae. speltoides* recombinant lines. Similar results were seen for markers Sr39#2s and Sr39#22r (data not shown). The hybridization of Sr39#50s to line #198 is caused by background 2B chromosomes in this line.

PCR markers for Sr39/Lr35

AFLP fragments Sr39#22r, Sr39#3r, Sr39#2s and Sr39#50s that showed polymorphic bands were sequenced. The sequence information was used to design primers to develop simple PCR markers. Markers Sr39#3r and Sr39#2s which were polymorphic as RFLP probes did not amplify any PCR product diagnostic for the resistant and susceptible lines (not shown). Marker Sr39#22r amplified a diagnostic band from all the resistant recombinants (Fig. 5a). Interestingly Sr39#50s that was initially cloned from an AFLP marker for the susceptible #159 recombinant, amplified a single band from susceptible lines and two bands from the resistant recombinant lines (Fig. 5b). Both markers (Sr39#22r and Sr39#50s) were sequenced from the recombinant lines to confirm their identity. Figure 5c shows the amplification of a previously described PCR marker developed by Gold et al. (1999) for the parent and recombinant lines. This marker amplified PCR products from all recombinants including the susceptible recombinants #159 and #198 but not from #233. Similar results were also obtained using the marker of Seyfarth et al. (1999). Although these earlier markers were diagnostic for the translocations, they were more distantly linked to *Sr39* than Sr39#22r and Sr39#50s.

Validation of PCR markers for marker-assisted selection

Validation of markers across diverse genetic backgrounds is important for their successful implementation in breeding programs. The PCR markers were validated using a set of Australian wheat cultivars and breeder's materials. Marker Sr39#50s amplified the susceptible allele (Fig. 6a) and Sr39#22r showed no amplification from any of the wheats lacking *Sr39* (not shown). The other susceptibility-linked markers wmc025 and BARC200 were not consistent in amplification from the susceptible wheats and thus proved useless. Figure 6b shows the amplification of markers Sr39#50s and Sr39#22r from several lines of a Cook/RL5711-derived F3 population segregating for the *Sr39/Lr35* translocation. Although Sr39#50s distinguished resistant and susceptible recombinants, it did not behave as a co-dominant marker and it failed to differentiate between heterozygous and homozygous susceptible families (Fig. 6b). However, using the two marker combination (Sr39#50s and Sr39#22r) it was possible to differentiate homozygous and heterozygous families. Since Sr39#50s is a wheat-derived marker, it is possible that it may recombine in some wheat backgrounds. The previously developed *Sr39* markers (Gold et al. 1999; Seyfarth et al. 1999) can be used to identify the resistant lines in this segregating population, but will not be able to distinguish between the homozygous and heterozygous lines.

Discussion

Two types of resistance genes have been used to control stem rust in Australia and other parts of the world. The predominant group includes race-specific or major genes

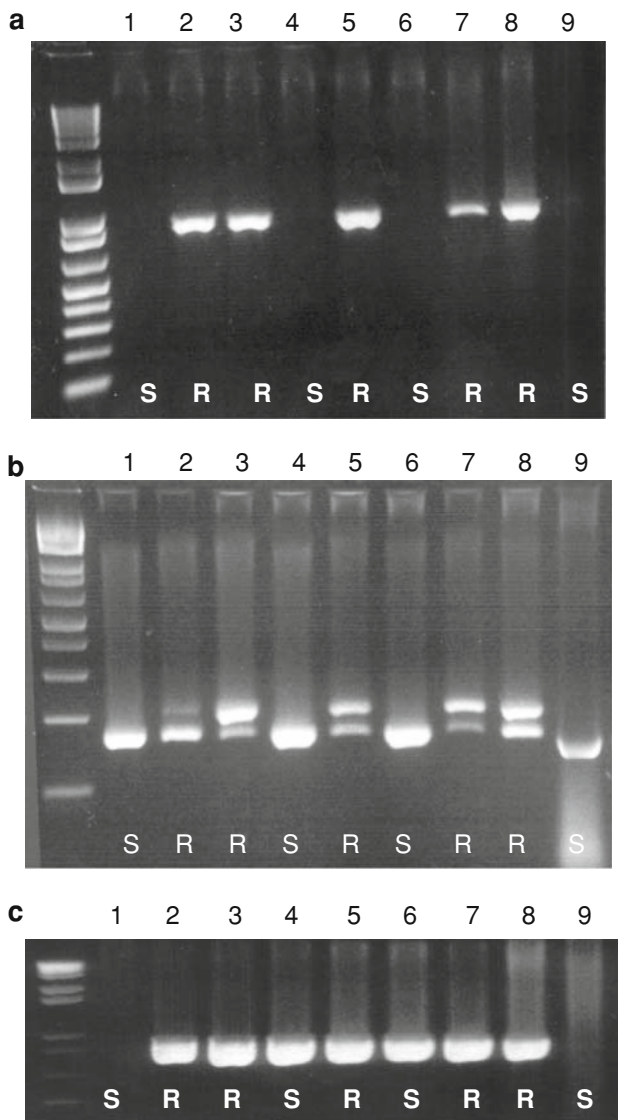


Fig. 5 Amplification of PCR-based markers developed in this study on wheat–*Aegilops speltoides* recombinants. **a** Marker Sr39#22r. **b** Sr39#50s. Lanes 1 Marquis (susceptible parent), 2 RL 5711 (resistant parent), 3 Recombinant #151, 4 #159, 5 #163, 6 #198, 7 #220, 8 #247 and 9 #233. **c** PCR amplification of the previously published marker (Gold et al. 1999) on wheat–*Ae. speltoides* recombinants. Lanes 1–9 Marquis; RL5711; wheat–*Ae. speltoides* recombinants #151; #159; #163; #198; #220; #247 and #233

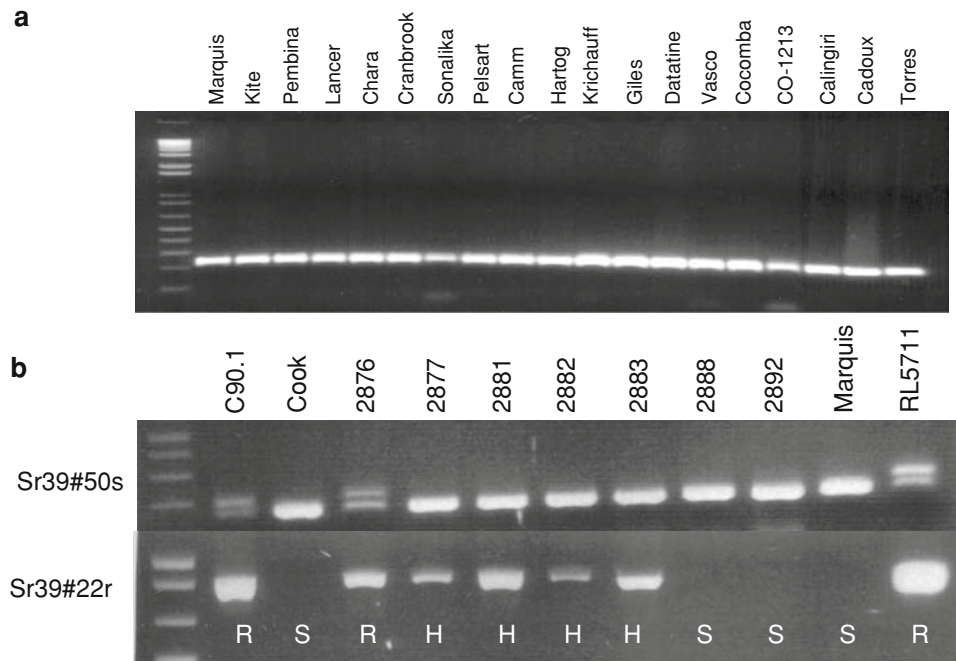
that provide high levels of resistance at both seedling and adult plant stages. The expression of resistance in this group is often associated with hypersensitivity. The other type involves adult plant resistance (APR) genes that are seedling susceptible and provide partial resistance and, *Sr2* belongs to this group. The success of stem rust control in Australian wheats relied on deployment of various combinations of stem rust resistance genes including *Sr2* (Bariana et al. 2007). The availability of diverse sources of resistance is essential to achieve long-

term protection against the stem rust pathogen. Such sources of stem rust resistance have been transferred from wild relatives of wheat to commercially acceptable genetic backgrounds (McIntosh et al. 1995). Some of these sources require amelioration of negative effects prior to their use in breeding programs (Dundas et al. 2007).

This investigation reports the cytogenetic characterization of the shortened *Ae. speltoides*/wheat 2S-2B translocation chromosome carrying linked rust resistance genes *Sr39* and *Lr35* and development of DNA markers that can differentiate homozygotes from heterozygotes. DNA markers and rust bioassays were used to select recombinants with reduced *Ae. speltoides* chromatin and retention of one or both of the rust resistance genes. Comparison of recombinants #151 and #159 that appeared to differ in the distal part of the short arm of chromosome 2 and by the presence and absence of *Sr39*, respectively, allowed the development of a PCR marker Sr39#22r for the shortened chromosome segment carrying *Sr39* (Fig. 1). These markers were validated on a selected set of F₃ lines segregating for *Sr39*. Although a relatively high frequency of wheat–*Ae. speltoides* recombination was observed in this study, the lines retaining *Sr39* still carry large segments of *Ae. speltoides* chromatin. Recombination between the 2B-2S#2 translocation chromosome and wheat homoeologues may have been influenced by the distal short- and long-arm segments of the translocation chromosome which are thought to be 2B chromatin. Further shortening of *Sr39*-carrying wheat–*Ae. speltoides* recombinants with smaller 2S#2 chromatin might be possible through the combined use of markers developed in this study and those previously reported (Gold et al. 1999; Seyfarth et al. 1999), however, it would require a large population.

The shortest recombinant from the current study, #247, which carries both *Sr39* and *Lr35*, has been released to Australian wheat breeders for assessment of its potential as a new source of rust resistance. The markers Sr39#50s and Sr39#22r used together will allow the recovery of homozygous lines in early generations. In addition, it is now possible to stack shortened versions of *Sr39*, *Sr26* and *SrR*, all of which are effective against the Ug99 (TTKSK group) and all other known Pgt pathotypes using the PCR markers developed in this study and earlier studies by Mago et al. (2002, 2005). The presence of three effective stem rust resistance genes should ensure durability for resistance to stem rust. However, durability of these sources will depend on the international wheat breeding community adhering to a hitherto unachieved level of cooperation to prevent the widespread release of these genes singly in future

Fig. 6 Validation of PCR markers. **a** *Sr39#50s* showing monomorphic amplification in a set of Australian and three exotic wheat varieties. **b** PCR products of *Sr39#50s* and *Sr39#22r* on Cook/RL5711—F3 families ($R = Sr39Sr39$, $H = Sr39sr39$, $S = sr39sr39$)



cultivars to avoid the step-wise accumulation of virulence in the stem rust pathogen.

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