Theor Appl Genet (2013) 126:2943–2955
DOI 10.1007/s00122-013-2184-8

ORIGINAL PAPER

Development of wheat–*Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2S#1 chromosome

Rohit Mago · Dawn Verlin · Peng Zhang · Urmil Bansal · Harbans Bariana · Yue Jin · Jeffrey Ellis · Sami Hoxha · Ian Dundas

Received: 25 April 2013 / Accepted: 16 August 2013 / Published online: 30 August 2013

Abstract
Key message **Wheat–*Aegilops speltoides* recombinants carrying stem rust resistance genes *Sr32* and *SrAes1t* effective against Ug99 and PCR markers for marker-assisted selection.**
Abstract Wild relatives of wheat are important resources for new rust resistance genes but underutilized because the valuable resistances are often linked to negative traits that prevent deployment of these genes in commercial wheats. Here, we report *ph1b*-induced recombinants with reduced alien chromatin derived from E.R. Sears' wheat–*Aegilops speltoides* 2D-2S#1 translocation line C82.2, which carries the widely effective stem rust resistance gene *Sr32*. Infection type assessments of the recombinants showed that the original translocation in fact carries two stem rust resistance genes, *Sr32* on the short arm and a previously undescribed gene *SrAes1t* on the long arm of chromosome 2S#1. Recombinants with substantially shortened alien chromatin were produced for both genes, which confer resistance to stem rust races in the TTKSK (Ug99) lineage and representative races of all Australian stem rust lineages. Selected recombinants were back crossed into adapted Australian cultivars and PCR markers were developed to facilitate the incorporation of these genes into future wheat varieties. Our recombinants and those from several other labs now show that *Sr32*, *Sr39*, and *SrAes7t* on the short arm and *Sr47* and *SrAes1t* on the long arm of 2S#1 form two linkage groups and at present no rust races are described that can distinguish these resistance specificities.

Germplasm requests should be addressed to I. Dundas.

Communicated by T. H. Tai.

R. Mago (✉) · J. Ellis
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
e-mail: rohit.mago@csiro.au

D. Verlin · I. Dundas
School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Private Mail Bag 1, Glen Osmond, SA 5064, Australia

P. Zhang · U. Bansal · H. Bariana · S. Hoxha
Plant Breeding Institute-Cobbitty, The University of Sydney, Private Mail Bag 4011, Narellan, NSW 2567, Australia

Y. Jin
USDA-ARS Cereal Disease Lab, University of Minnesota, Saint Paul, MN 55108, USA

Introduction

Stem rust of wheat, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn. (Pgt), has been controlled effectively in many parts of the world through deployment of resistance genes. The occurrence of a highly virulent stem rust pathotype (Ug99, formally named TTKS then TTKSK) in Africa in 1999 and its subsequent derivatives has, however, made the majority of current wheats vulnerable to stem rust (Pretorius et al. 2000; Wanyera et al. 2006; Jin et al. 2007, 2008a). More recently Pretorius et al. (2010) reported the appearance, in South Africa, of a variant of the Ug99 lineage PTKST which has virulence for over 21 stem rust resistance genes including *Sr31* and the widely used *Sr24*. Until this report, the original TTKSK strain and its derivatives carrying virulence for *Sr31* and *Sr24* (Jin et al. 2008b) or *Sr31* and *Sr36* (Jin et al. 2009) had only been found in East Africa, Yemen (Singh et al. 2008) and Iran (Nazari et al. 2009). The projected spread of Ug99 to the Indian subcontinent (Hodson et al. 2005)

and beyond to the Americas and Australia is of a great concern to the international wheat industries. For example, Park et al. (2009) postulated rust resistance genes in Australian wheat cultivars which showed that there is currently reliance in Australian wheat cultivars on the genes *Sr8a*, *Sr9b*, *Sr15*, *Sr24*, *Sr30*, *Sr36* and *Sr38* which are ineffective against Ug99 and its derivatives. Pathotype PTKST is avirulent on genes *Sr13, 14*, *21*, *22*, *25*, *26*, *27*, *29*, *32*, *33*, *35*, *37*, *39*, *42*, *43*, *44*, *Tmp*, *Em*, and *Satu* (Pretorius et al. 2010). Of these, only *Sr13*, *Sr26*, and *Sr33* are currently used in any Australian wheat cultivars. *Sr13* originates from *Triticum turgidum dicoccum* var. Khapli and virulent rust pathotypes have been reported to be common in the Indian subcontinent (McIntosh et al. 1995) and Ethiopia (Olivera et al. 2012). *Sr33* was transferred to wheat from *Aegilops tauschii* (Kerber and Dyck 1979) but has not yet been widely used in cultivars (McIntosh et al. 1995). The durable adult plant resistance gene *Sr2,* which is used extensively in Australia and elsewhere, was derived from *T. turgidum dicoccum* var. Yaroslav (Hare and McIntosh 1979) and provides only partial resistance against TTKSK and its derivatives.

The appearance and spread of these new strains of Pgt with virulence on many widely used resistance genes, have focussed research attention on the identification of new sources of effective resistance genes from wheat relatives. For example, several stem rust resistance genes had been transferred to wheat from *Ae. speltoides* Tausch where they occur as wheat–*Ae. speltoides* chromosome translocations. These include; *Sr32* (McIntosh et al. 1995; Friebe et al. 1996), *Sr39* (Kerber and Dyck 1990; McIntosh et al. 1995), *Sr47* (Faris et al. 2008), and *SrAes7t* (Klindworth et al. 2012). However, none of these genes, which are all effective against the Ug99 derivatives, have been used in the development of wheat cultivars either because of the presence of mostly undefined negative traits linked to the resistance genes or, lack of these genes in wheat backgrounds suitable for local breeding programs. As a consequence, several groups of researchers have established programs to use *ph1b*-enhanced recombination (homoeologous recombination) between wheat and *Ae. speltoides* chromatin to attempt to break the linkage between resistance and negative traits and develop markers to assist transfer of the genes into breeding programs. This paper focuses on the amelioration of sources of the *Sr32* gene.

E.R. Sears used homoeologous recombination to transfer *Sr32* from a group 2 *Ae. speltoides* chromosome 2S#1 to Chinese Spring wheat chromosomes 2A, 2B, and 2D (Friebe et al. 1996). Line W3531 (C95.24) is a translocation to chromosome 2A and has adherent glume fragments in the crease region of the grain (McIntosh et al. 1995). C77.19 is a translocation of a 2S chromosome segment carrying *Sr32* to chromosome 2B. Sears later produced four additional transfers of *Sr32* onto chromosomes 2B (C82.1) and 2D (C82.2, C82.3 and C82.4) (McIntosh et al. 1995). *Sr32* appears to be effective against all known stem rust pathotypes and produces a low infection type 1+ to 2C (McIntosh et al. 1995). Despite the availability of backcross derivatives carrying *Sr32* in different genetic backgrounds, no cultivars carry this gene, presumably because of the presence of deleterious gene(s) derived from the donor species that has led to selection against *Sr32* in new varieties. A preliminary summary of *ph1b*-enhanced homoeologous recombination between wheat and the 2S#1 chromosome from C82.2 was provided in Dundas et al. (2007). Initial stem rust response assays on the recombinants indicated the presence of two resistance genes (one on each arm) on the 2S#1 segment.

The purpose of our study was to (a) select new wheat–*Ae. speltoides* recombinant chromosomes carrying shortened segments of 2S#1 chromatin and (b) develop simple PCR-based markers to allow efficient selection of these new sources of resistance into wheat-breeding programs.

Materials and methods

Germplasm

Stocks carrying the 2A-2S#1 translocation (C95.24), the 2B-2S#1 translocations (C77.19 and C82.1) and the 2D-2S#1 translocations (C82.2, C82.3, and C82.4), developed by E.R. Sears in the 'Chinese Spring' background were kindly provided by R.A. McIntosh, University of Sydney. Eighteen *Ae. speltoides* accessions ($2n = 2\times = 14$) were obtained from the Australian Winter Cereal Collection (AWCC), Tamworth, NSW, Australia to examine the level of genetic polymorphism in this species using markers linked to 2S#1 rust resistance genes.

Isolation of homoeologous recombinants

Molecular analysis of wheat–*Ae. speltoides* chromosome 2S#1 translocations and isolation of recombinants derived from the translocation line C82.2 were conducted at the Cereal Cytogenetics Laboratory, University of Adelaide. The first step was to determine the translocation breakpoints in lines C95.24 (T2A-2S#1), C77.19 and C82.1 (T2B-2S#1), and C82.2, C82.3 and C82.4 (T2D-2S#1). In the absence of marker maps for *Ae. speltoides* chromosomes, we resorted to selecting markers from published barley maps for characterizing the 2S#1 chromosome. This presumes a degree of co-linearity of markers on the 2S#1 and 2H chromosomes. The wheat–barley addition lines have been used extensively to map new probes/markers to the

homoeologous groups. Sixteen group 2 DNA probes previously mapped across the distal to proximal segments of the short and long arms of barley chromosome 2H (Langridge et al. 1995; Qi et al. 1996; http://barleygenomics.wsu.edu/all-chr.pdf; http://wheat.pw.usda.gov/) were obtained from the Australian Triticeae Mapping Initiative (ATMI) (maintained by P. Langridge, University of Adelaide). The probes were labeled with ^{32}P-dCTP by the random primer method and hybridized to genomic DNA of C95.24, C77.19, C82.1, C82.2, C82.3, and C82.4 (+*Sr32*) and Chinese Spring (−*Sr32*) digested with *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV or *Hin*dIII and transferred by blotting to a Hybond N+ membrane (Amersham, UK). Analysis of these results focussed on the presence of polymorphic fragments associated with the presence of the 2S#1 chromosome. The probes, enzyme digests producing polymorphisms and the presence/absence of RFLPs in the translocations are shown in Tables 1 and 2. The next step involved inducing homoeologous recombination between the 2D–2S#1 translocation chromosome and a wheat chromosome with the *ph1b* mutant (Sears 1977). Line C82.2 was crossed as female with wheat line 'Angas *ph1bph1b*' (pedigree: Eagle/Chinese Spring *ph1bph1b*/3/2*Angas) (further details described in Mago et al. 2009). F_2 seedlings were screened for a *ph1bph1b* genotype (−ve for *Xpsr128-5B*) (*Hin*dIII), and also for the presence of both the normal 2D and 2D-2S#1 translocation chromosomes using probe ABC454 (*Hin*dIII). A total of 298 F_3 critical seedlings (defined as plants potentially containing wheat-2S#1 recombinant chromosomes of random origin and differing chromotypes; 228 from F_2 plant 553/97 and 70 from F_2 plant 564/97) were screened for dissociation of 2H probes ABG58 (*Bam*HI), ABC252, and ABG5 (*Dra*I), ABC454, ABG358, and BCD221 (*Eco*RI), and ABG2, ABG72 and BCD111 (*Hin*dIII). Plants showing dissociation of 2S#1-specific markers were crossed with wheat cultivars 'Aroona' or 'Angas' and then progeny-tested. Plants showing confirmed 2S#1 dissociations were crossed as females with cv. Angas up to BC_3 to replace the *ph1b* gene with its allele *Ph1*. Selected recombinants were also backcrossed into 'Westonia', a cultivar in which the presence of chromosome 2S#1-derived Sr genes could be assayed with available rust strains.

Rust assays

Recombinant lines were tested for rust resistance at the University of Sydney, Plant Breeding Institute, Cobbitty, using Pgt pathotype 34-1,2,3,4,5,6,7 (Culture no. 103), which is virulent on all the wheat background genotypes used in this work, according to Bariana and McIntosh (1993). The recombinants #102 (*Sr32*) and #122 Type II (*SrAes1t*) were also tested with additional nine Australian stem rust

Table 1 Chromosome-specific bands of *Aegilops speltoides* 2S#1 chromatin scored after digesting genomic DNA from 2A–2S#1 translocation line C95.24 and 2B–2S#1 translocation lines C77.19 and C82.1 with up to five restriction enzymes and hybridizing with 16 group 2 short and long arm Triticeae RFLP probes

Probe	Restriction Enzyme													
	*Bam*HI			*Dra*I			*Eco*RI			*Eco*RV			*Hin*dIII	
	C95.24	C77.19	C82.1	C95.24	C77.19	C82.1	C95.24	C77.19	C82.1	C95.24	C77.19	C82.1	C95.24	C77.19
ABC252	+	−	−	+	−		+	−		+	−		+	−
ABC454	−	−		+	+		+	+		+	+	+	+	+
ABG002	−	−		+	+		+	+		+	−		+	+
ABG005	+	+	+	+	+		−	−		+	+		+	+
ABG058	+	+	+	+	+		+	+		+	+		+	+
ABG072				+	−					+	−	−	+	−
ABG358	−	−		+	+		+	+	+	−	−			
BCD111	−	−		+	−		+	−	−	+	−		+	−
BCD135										−	−			
BCD221							+	+	+					
BCD292	−	−		−	−		−	−	−	−	−		−	−
BCD410	−	−		−	−									
CDO064	−	−		−	−		+	+	+	−	−			
MWG503	+	−	−	+	−		+	−		+	−		−	−
MWG892	+	−	−	+	−	−	+	−		+	−			
WG645	−	−	−	−	−	−	−	−	−	−	−			

+ 2S#1 chromosome-specific band present

− 2S#1 chromosome-specific band absent

Table 2 Chromosome-specific bands of *Aegilops speltoides* 2S#1 chromatin scored after digesting genomic DNA from three translocation lines of 2D-2S#1 (C82.2, C82.3, and C82.4) with up to five restriction enzymes and hybridizing with 16 group 2 short and long arm Triticeae RFLP probes

Probe	Restriction enzyme												
	*Bam*HI			*Dra*I			*Eco*RI			*Eco*RV			*Hin*dIII
	C82.2	C82.3	C82.4	C82.2	C82.3	C82.4	C82.2	C82.3	C82.4	C82.2	C82.3	C82.4	C82.2
ABC252	+	+	−	+			+			+			+
ABC454	−			+			+			+	+	+	+
ABG002	−			+			+	+	+	−			+
ABG005	+	+	+	+			−			+			+
ABG058	+	+	+	+			+			+			+
ABG072				+	+	−				+			+
ABG358	−			+			+	+	+	−			
BCD111	−			+			+	+	+	+			+
BCD135										−			
BCD221							+	+	+				
BCD292	−			−			−	−	−	−			−
BCD410	−			−									
CDO064	−			−			+	+	+	−			−
MWG503	+	+	+	+			+			+			−
MWG892	+	+	+	+			+			+			−
WG645	−	−	−	−	−	−	−	−	−	−			

\+ 2S#1 chromosome-specific band present

− 2S#1 chromosome-specific band absent

Table 3 Rust response of *Sr32* recombinants with Australian stem rust races and Ug99

Rust pathotype	Gene/infection type				
	Sr39	*Sr32*	*SrAes1t*	*Sr47*	*SrAes7t*
TTKSK	2−[a]	;2−	;2−	;2−[a]	2−[a]
TTKST		;2−	;2−		
TTTSK		;2−	;2−		
34-1,2,3,4,5,6,7 (103)	2=	2−	;1+		
98-1,2,3,5,6,7 (580)	2=	2−	2=		
343-1,2,3,4,5,6 (429)	2=	2=	1−		
34-1,2,7+Sr38 (565)		2−	1		
321-1,2,3,5,6 (436)		2−	11−		
11-1,2,3,4,5,6,7 (169)		2−	11−		
21-1,2,3,5,7 (364)		;12−	;12−		
222-1,2,3,5,6,7 (308)		2−	2=		
34-1,2,3,4,5,6,7,8,9,11 (171)		2:	;11−		
40-1,2,3,4,5,6,7 (383)		2=	;11−		

[a] Rust scores from Klindworth et al. 2012

pathotypes (Table 3). Infection type descriptions were based on the original scales proposed by Stakman et al. (1962). Stem rust tests with the Ug99 race rust group TTKSK (isolate 04KEN156/04), TTKST (isolate 06KEN19v3) and TTTSK (isolate 07KEN24-4) were conducted at the USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St. Paul, USA, according to Jin et al. (2007).

Genomic in situ hybridization (GISH) analysis

The original 2D–2S#1 translocation line C82.2 and the recombinants were characterized by GISH following the procedure of Zhang et al. (2001). Total genomic DNA from *Ae. speltoides* (University of Sydney cytogenetics collection accession # C64.69) was labeled with Biotin-16-dUTP

(Roche Diagnostic Australia, Castle Hill, NSW, Australia) using nick translation. Unlabeled total genomic DNA of wheat was used as a blocker. The probe to blocker ratio was ~1:100. Signals were detected with fluorescein-avidin DN (Vector Laboratories, Burlingame, CA). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (Invitrogen Life Science, Carlsbad, CA) 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, Canada) operated with Image-Pro Plus version 7.0 software (Media Cybernetics Inc., Bethesda, MD) and processed with Photoshop version CS5.1 software (Adobe Systems, San Jose, CA).

SSR and EST analyzes of Sr32 lines

SSR markers from group 2 chromosome deletion bins were identified using wheat Genoplante SSR mapping data (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/) and primer sequences were obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/quickquery.shtml#microsats). ESTs in the deletion bin map of chromosome 2D were identified using the graingenes EST database (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) and primer sequences were designed using the Primer3 software (http://frodo.wi.mit.edu/). The SSR and EST markers were tested for polymorphism on a wheat–*Ae. speltoides* translocation line C82.2 (R), resistant dissociation lines 70 Type I, 70 Type II, 102, 122 Type II and CS (S) (Figs. 2, 3).

Isolation of AFLP markers linked to *Sr32 and SrAes1t*

To isolate AFLP markers from the *Sr32* region, two dissociation lines with shortest *Ae. speltoides* chromosome segments showing stem rust resistance (Lines 70 Type II and 102) (Fig. 3) along with Chinese Spring (CS) (susceptible) and the resistant parent C82.2 were used. For *SrAes1t* marker development, recombinant #70 Type I was 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 performed according to Mago et al. (2002).

Sequence-tagged site (STS) analysis

Three AFLP fragments P-ACA/M-GTG-120 (csSrAes1t), ACA/M-GAT-184 (csSr32#1), and P-AGA/M-GAA-152 (csSr32#2) associated with the presence of *SrAes1t* and *Sr32*, respectively, were sequenced using the dye terminator sequencing system and analyzed on an ABI prism system (Applied Biosystems, Foster City, CA, USA). Specific primers were designed for amplification of each of these fragments and used for PCR amplification of the diagnostic product. Primer sequences of the markers which amplified diagnostic polymorphisms are listed in Table 4. PCR products were separated on 2 % agarose gels.

Validation of marker-trait associations

Marker-trait associations were validated on a set of backcrossed near-isogenic lines derived from two crosses involving C77.19 (Table 5) and an F_3 segregating family consisting of 118 lines derived from a cross between C77.19 × Yarralinka. The marker was also validated on other *Sr32* translocations, including C95.24 (T2AL·2S#1L-2S#1S), C82.1 (T2BL·2S#1S), C82.3 (T2DL-2S#1L·2S#1S), and C82.4 (T2DL-2S#1L·2S#1S).

Results

A study of Sears'wheat-2S#1 translocation lines was undertaken to define the translocation breakpoints. Of the 16 group 2 chromosome RFLP probes that hybridized to restriction enzyme digests of DNA from the wheat-2S#1 translocation lines, 12 detected an *Ae. speltoides*-specific restriction fragment, and among these 11 detected wheat- and *Ae. speltoides*-specific restriction fragments (codominant markers), and one (BCD221) detected *Ae. speltoides*-derived restriction fragments, but no corresponding wheat fragments. Four probes, BCD135, BCD292, BCD410, and WG645, derived from the distal region of the long arm of barley chromosome 2H, produced wheat-specific markers but no *Ae. speltoides*-derived restriction fragment (Tables 1 and 2).

Lines C95.24 (T2A-2S#1) (Table 1) and C82.2 and C82.3 (T2D-2S#1) (Table 2) showed 2S#1-specific markers for all seven short arm probes and for five proximal probes on the long arm. Line C82.4 (T2D-2S#1) (Table 2) showed *Ae. speltoides*-specific markers for all seven short arm probes and three proximal probes of the long arm. Lines C77.19 (Table 1) and C82.1 (T2B-2S#1) (Table 1) showed 2S#1-specific markers for the seven short arm probes only. Breakpoints are summarized below and translocation chromotypes are depicted in Fig. 1.

Isolation of shortened 2S#1 segments

According to McIntosh et al. (1995), Sears indicated that the wheat line C82.2 was "the most normal of the translocations" involving *Sr32* and hence this line was chosen for producing recombinants with shortened *Ae. speltoides* chromatin. Eight RFLP probes specific for 2S#1 (ABC252, ABC454, ABG2, ABG5, ABG58,

Table 4 PCR primers and conditions for amplification of *Sr32* and *SrAes1t* markers

Marker	Primers	PCR conditions	
csSrAes1t	F-5' TTCCACGATGCCAGATTTACA	95°C- 2min : 1 cycle	
	R-5' GGTTGTGTGAAGCCACATGAA	95°C- 30sec	30 cycles
		58°C- 30sec	
		72°C- 40sec	
		72°C- 5min: 1 cycle	
		20°C- 1min: 1 cycle	
csSr32#1	F-5' GGTTTGGTGGCAACTCAGGT	95°C- 2min : 1 cycle	
	R-5' CATAAGCCAAAGAGGCACCA	95°C- 30sec	30 cycles
		60°C- 40sec	
csSr32#2	F-5' CAAATGAATAGAAAAACCCGTGCT	72°C- 50sec	
	R-5' CACACACTGTTTTCCGTTGC	72°C- 5min: 1 cycle	
		20°C- 1min: 1 cycle	

Table 5 Marker analysis of *Aegilops speltoides* translocations including wheat–*Ae. speltoides* recombinants produced from C82.2

Line	Markers			
	csSr32#1	csSr32#2	csSrAes1t	gwm501
C95.24	+	+	+	+
C77.19/4*Spear	+	+	−	−
Spear	−	−	−	−
C77.19/4*Tatiara	+	+	−	−
Tatiara	−	−	−	−
C82.1	+	+	−	−
C82.2	+	+	+	+
C82.2-#102[a]	+	+	−	−
C82.2-#70 Type I[a]	−	−	+	+
C82.3	+	+	−	+
C82.4	+	+	−	+
Chinese Spring	−	−	−	−

[a] Lines produced in this study

ABG72, ABG358, and BCD111) were used to screen a total of 298 critical F_3 seedlings for dissociation of the linkage between *Ae. speltoides* group 2 markers. After repeating the marker assays or by progeny-testing, 82 plants (27.5 %) were confirmed to carry shortened *Ae. speltoides* 2S#1 segments. The dissociation chromotypes involved loss of short and/or long arm segments of chromosome 2S#1 and several of the confirmed dissociation lines carried chromosomes apparently involved in more than one cross-over event. A total of 96 cross-overs were detected along the length of the 2S#1 segment which equates to 32.2 % recombination between loci *Xabg58-2S#1* (distal short arm) and *Xabc252-2S#1* (distal long arm). Recombination was observed on either

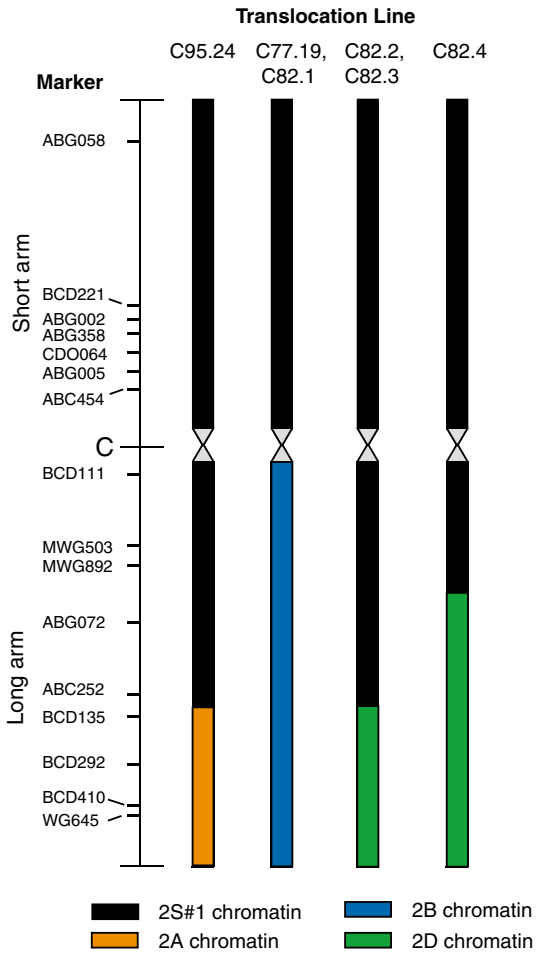

Fig. 1 Diagrammatic representation of marker positions and translocation breakpoints on wheat–*Aegilops speltoides* 2S#1 translocation lines C95.24 (T2AL–2S#1L·2S#1S), C77.19 and C82.1 (T2BL·2S#1S), C82.2, C82.3, and C82.4 (T2DL-2S#1L·2S#1S) based on published marker order for barley 2H chromosome (Langridge et al. 1995; Qi et al. 1996), assuming co-linearity with the current 2S#1 chromosome. The 2A–2S#1 translocation: Line C95.24 has the translocation breakpoint on the long arm between *Xabc252-2S#1* and *Xbcd292-2A*. The 2B–2S#1 translocations: Lines C77.19 and C82.1 have the breakpoint between the short arm locus *Xabc454-2S#1* and the long arm locus *Xbcd111-2B*; The 2D–2S#1 translocations: Lines C82.2 and C82.3 have the breakpoints on the long arm between *Xabc252-2S#1* and *Xbcd292-2D*, whereas C82.4 has the breakpoint on the long arm between *Xmwg892-2S#1* and *Xabg72-2D*

the short arm or long arm and occasionally on both arms. The highest recombination occurred in the distal segment of the short arm adjacent to *Xabg58-2S#1* (9.4 %) or in the distal region of the long arm adjacent to *Xabg72-2S#1* and *Xabc252-2S#1* (8.7 %). Within the progeny of several dissociation plants, chromotypes were observed which differed from the original dissociation type initially identified, probably resulting from a second meiotic cycle with the *ph1bph1b* genotype (Lines 70 Types I and II, 122 Types I and II, and 145 Types I and II; Figs. 2, 3).

Chromosome morphology

To simplify mapping of rust resistance, 17 different chromotypes were selected based on DNA marker analysis from the original 82 plants which were found to carry shortened 2S#1 chromosomes. These 17 chromotypes contained 2S#1 chromosome segments of varying sizes with recombination breakpoints across the short and long arms. The original T2DL-2S#1L·2S#1S chromosome from C82.2 and the 17 dissociation lines were examined cytologically and genomic in situ hybridizations (GISH) are shown in Fig. 2. Figure 3 portrays marker positions and the diagrammatic representations of each chromotype incorporating GISH and molecular marker data. The original T2DL-2S#1L·2S#1S chromosome in line C82.2 consists of a large segment of the *Ae. speltoides* 2S#1 chromosome on proximal and interstitial sections of the long arm and unlabeled wheat chromatin on the distal section (Fig. 2a). Approximately, 82.6 % ± 0.02 ($n = 7$) of the physical length of the long arm is 2S#1 chromatin and the short arm is a complete 2S#1S.

Short arm dissociations

Three types of alterations to the structure of chromosome 2S#1S were found: loss of either distal or proximal segments, or all the markers on the 2S#1S arm. Lines 70 Type II, 102, 107, and 137 retained distal 2S#1S chromatin (Figs. 2, 3). Lines 114, 122 Type I and 247 retained distal (but not terminal) segments of 2S#1S (Figs. 2, 3). Lines 52, 122 Type II, 142, 145 Types I and II and 287 retained only proximal short arm segments. Line 261 retained most of the short arm, except the terminal segment with *Xabg58-2S#1*. Recombinants 114, 122 Type I and 247 were apparently produced from double crossovers on chromosome 2S#1S. Line 70 Type I has the entire 2S#1S replaced by wheat chromatin, whereas line 45 contains a telocentric chromosome produced by deletion of 2S#1S.

Long arm dissociations

Four types of structural arrangements of 2S#1L were observed: no detectable alteration of the arm (Lines 44, 45, 52, 122 Type II, 142, 145 Type II, 261 and 287), complete loss of 2S#1L chromatin (Lines 70 Type II, 102, 107, 114, 122 Type I, 137 and 247), loss of distal 2S#1L markers only (Line 145 Type I) and loss of proximal 2S#1L markers (Line 70 Type I). Line 70 Type I apparently resulted from

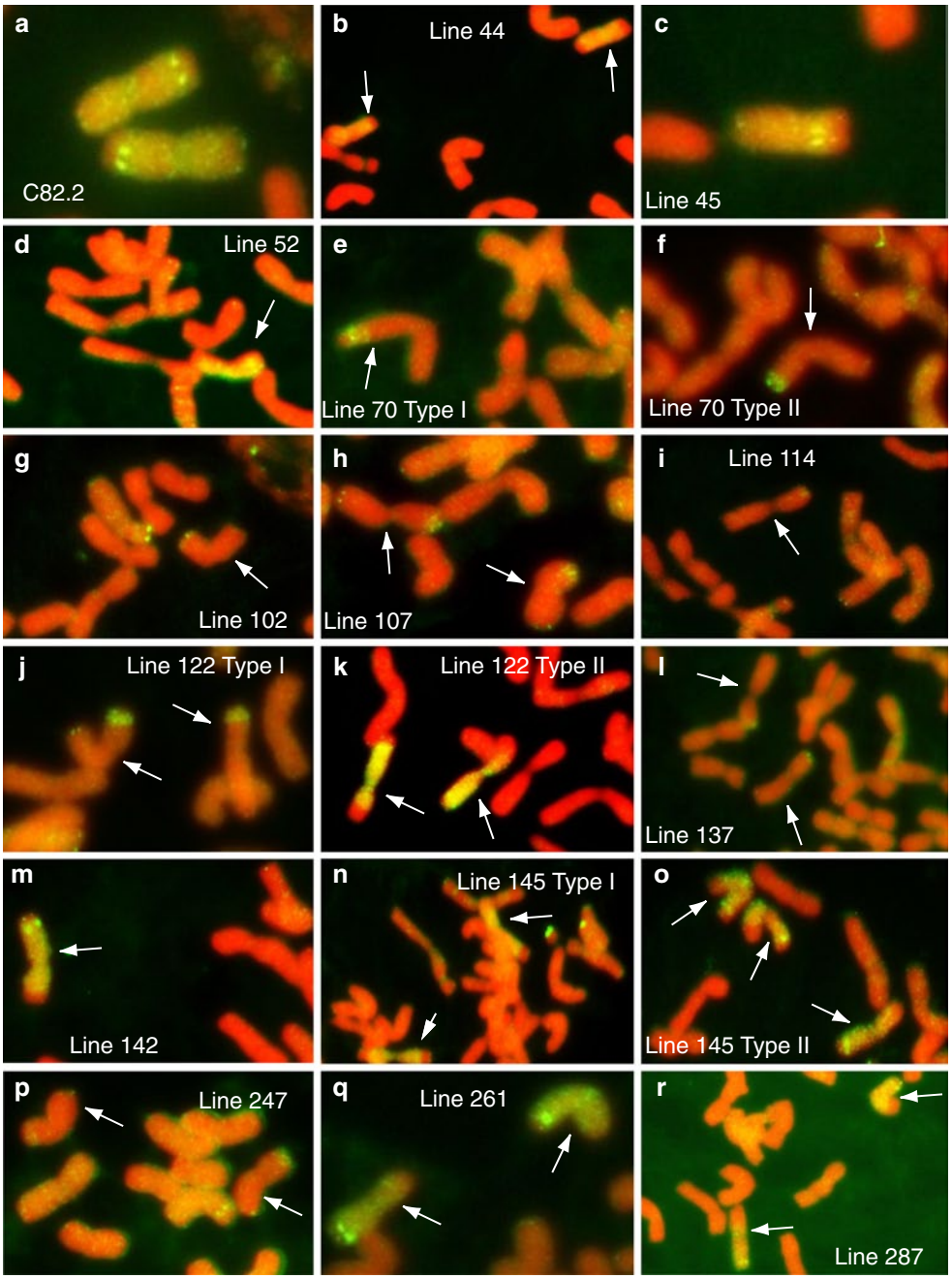

Fig. 2 Genomic in situ hybridization using *Aegilops speltoides* genomic DNA as probe (*yellow–green* signal) on **a** the original wheat–*Ae. speltoides* 2DL–2S#1L·2S#1S translocation (+*Sr32* and *SrAes1t*) from C82.2, and 2S#1 dissociation lines, **b** #44, **c** #45, **d** #52, **e** #70 Type 1, **f** #70 Type II, **g** #102, **h** #107, **i** #114, **j** #122 Type I, **k** #122 Type II, **l** #137, **m** #142, **n** #145 Type I, **o** #145 Type II, **p** #247, **q** #261, and **r** #287. *Arrow(s)* denote translocation chromosome(s) (color figure online)

a double cross-over and carries the shortest long arm segment from *Ae. speltoides*.

Stem rust testing and mapping of stem rust resistance

All lines, except 114, 137 and 145 Type I, were resistant to pathotype 34-1, 2, 3, 4, 5, 6, 7. Resistant lines with exclusively 2S#1S chromatin were designated 70 Type II, 102, 107, 122 Type I and 247. Given that *Sr32* was originally designated on the basis of the Sears' translocation lines (McIntosh 1988), the association of rust resistance with the short arm translocations C77.19 and C82.1 (Fig. 1) provides precedence for this gene being *Sr32*. We also tested the parental translocation and two recombinants (70

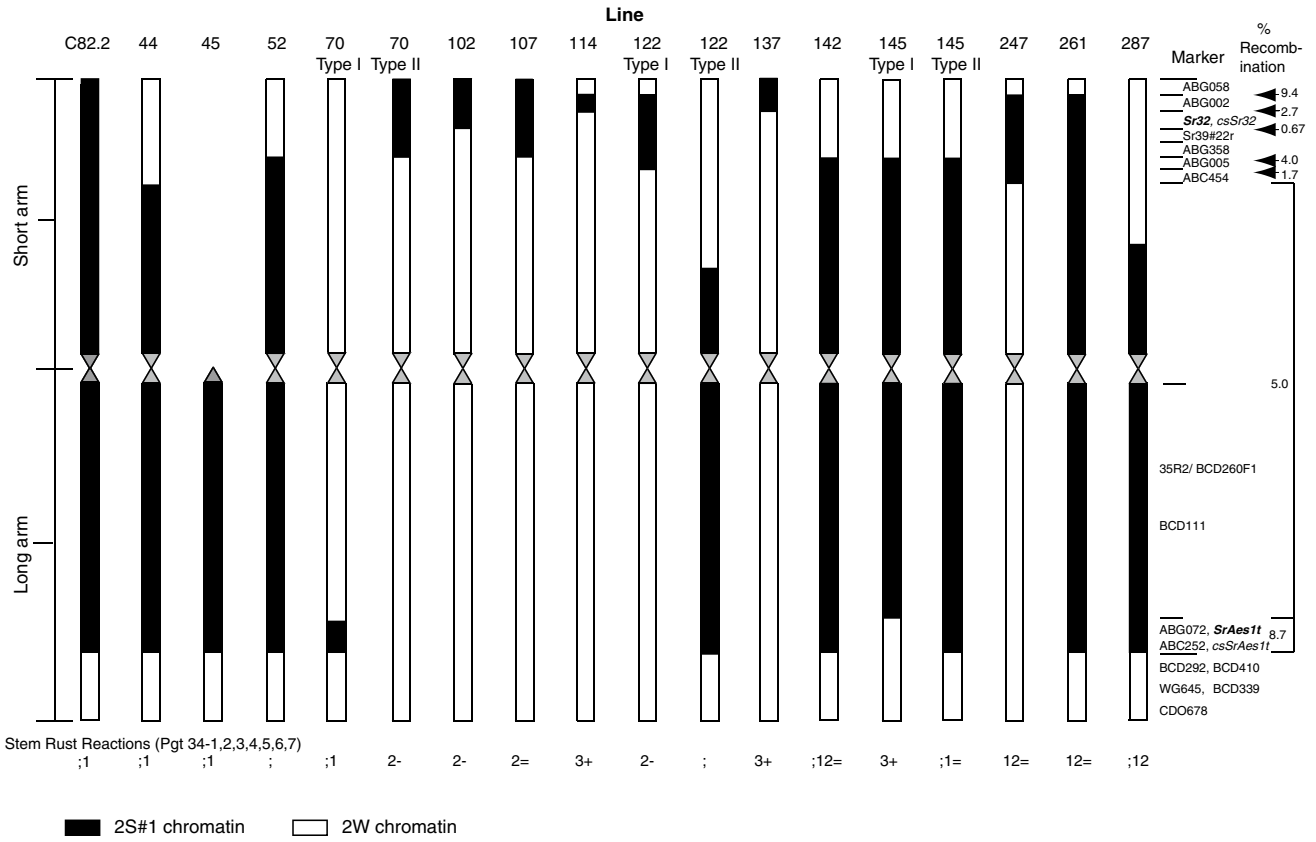

Fig. 3 Diagrammatic representation of wheat chromosome 2S#1 recombinant lines showing the positions of RFLP and PCR markers and stem rust resistance genes. *Aegilops speltoides* chromatin is represented in *black* and wheat chromatin is represented in *white*. Lines C82.2, 70 Type II, 102, 107, 122 Type I, 247 and 261 carry *Sr32*; lines C82.2, 44, 45, 52, 70 Type I, 122 Type II, 142, 145 Type II, 261 and 287 carry *SrAes1t*; and lines 114, 137 and 145 Type I are susceptible to stem rust

Type I and 102) with a previously developed PCR marker Sr39#22r linked to the *Ae. speltoides*-derived gene *Sr39* on chromosome 2S#2 (Mago et al. 2009; Niu et al. 2011). This marker is present on the C82.2 translocation but absent from the resistant recombinant #102 (data not shown). The *Sr32* gene was, therefore, located on the distal section of 2S#1 between RFLP marker *Xabg2-2S#1* and Sr39#22r (Fig. 3).

Some lines exclusively retaining 2S#1L chromatin (lines 45 and 70 Type I) also carried stem rust resistance. This gene is located on the distal segment between two loci *Xabg72-2S#1* and *Xabc252-2S#1* (Fig. 3). This gene is temporarily named *SrAes1t*. The parent C82.2 showed infection type (IT);1. The recombinants carrying only *Sr32* (lines 102 and 70 Type II) showed IT 2-, whereas the *SrAes1t* recombinant (lines 70 Type I and 122 Type II) exhibited a relatively lower IT; or 1. Rust tests of the recombinants #102 and #122 Type II carrying *Sr32* and *SrAes1t*, respectively, with nine other Australian isolates clearly showed that both genes are effective against all these stem rust pathotypes. Both the genes were also effective against three pathotypes of the Ug99 lineage TTKSK, TTKST, and TTTSK (Table 3).

Additional markers for characterization of recombinants and marker-assisted selection of *Sr32* and *SrAes1t*

A range of SSR markers from group 2 chromosomes were tested on the *Sr32*-carrying parent and recombinants to seek polymorphisms. SSR markers cfd36, cfd51, cfd56, gdm35, gpw4112, gwm47, and gwm157 were monomorphic (data not shown). Primers were also designed from EST markers specific for chromosome 2D, including BE423738, BE398553, BE424478, BE445465, BE445700, BE591772, BE442722, BE500833, BF145685, and BE423370. Only BE445465 amplified a polymorphic PCR product from stem rust susceptible lines (data not shown); however, the marker was not diagnostic in several other wheat lines. We used the AFLP technique with *Pst*I/*Mse*I primer sets on wheat near-isogenic line (NIL) pair CS (S) and C82.2 (R) and resistant recombinants #102 (*Sr32*) and #70 Type I (*SrAes1t*) (see Fig. 3) to develop additional

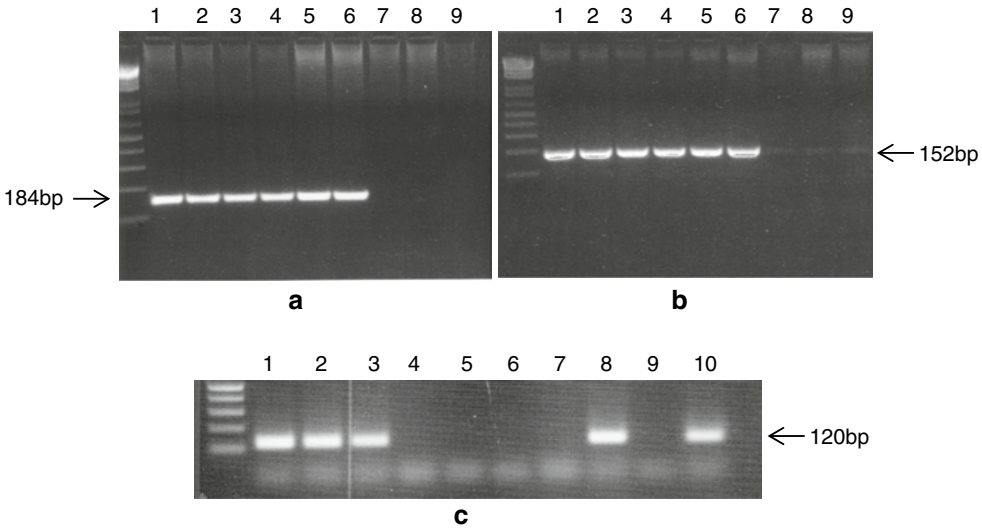

Fig. 4 Amplification from wheat-*Aegilops speltoides* translocation C82.2 and recombinants of **a** marker csSr32#1 and **b** marker csSr32#2. *Lanes 1*, C82.2 (resistant translocation), *2* #102, *3* #70 Type II, *4* #122 Type I, *5* #107, *6* #247, *7* CS (susceptible parent), *8* #70 Type I, *9* #122 Type II; **c** amplification of the csSrAes1t marker. *Lanes 1*, C82.2, *2* #44, *3* #70 Type I, *4* #107, *5* #70 Type II, *6* #122 Type I, *7* CS, *8* #142, *9* #145 Type I, *10* #52

markers. With 96 primer combinations, 13 AFLPs distinguished stem rust resistant and susceptible recombinants for *Sr32*. Five AFLPs (P-ACA/M-GAT, P-ACA/M-GCC, P-ACC/M-GAC, P-AAG/M-GCT, and P-AGA/M-GAA) were linked with resistance and eight were associated with susceptibility. For the stem rust resistance gene *SrAes1t* on the long arm, among 144 primer combinations, three AFLPs (P-AGT/M-GTT, P-ACA/M-GTG, and P-ACA/M-GAG) distinguished the stem rust resistant and susceptible recombinants. Two of these (P-ACA/M-GAG and P-ACA/M-GTG) were linked to the resistant and one (P-AGT/M-GTT) was linked to the susceptible phenotype. All polymorphic AFLP fragments were cloned and sequenced. The sequence information was used to design primers to develop simple PCR markers. Of the five AFLPs that were linked with *Sr32*, only two [P-ACA/M-GAT-184 (csSr32#1) and P-AGA/M-GAA-152 (csSr32#2)] could be converted to dominant PCR markers for *Sr32* (Fig. 4a, b). None of the AFLPs linked to the susceptible phenotype could be converted to PCR markers diagnostic for the absence of *Sr32*. One AFLP P-ACA/M-GTG-120 (csSrAes1t) was converted to a dominant PCR marker for *SrAes1t* on 2DL (Fig. 4c). Similar to our results using Sears' translocation line C82.2, Klindworth et al. (2012) also identified two stem rust resistance genes on an independently derived *Ae. speltoides* 2S translocation in the tetraploid wheat DAS15, *Sr47* on the long arm *SrAes7t* on the short arm and developed DNA markers. We tested their *Sr47* SSR markers gwm501, gwm47 and gpw4112 on the C82.2 recombinants carrying *SrAes1t* gene. Only marker gwm501 was diagnostic for *SrAes1t* in the germplasm used in this study and amplified a 109-bp fragment from C82.2 and the short recombinant 70 Type I (data not shown).

Validation of PCR markers for marker-assisted selection

Validation of the utility of markers across diverse genetic backgrounds is important for their successful implementation in breeding programs. The PCR markers were validated using seven Australian wheat cultivars or advanced breeding lines and their *Sr32*-carrying NILs derived from translocation C77.19 (Table 5; Fig. 5). Markers csSr32#1 and csSr32#2 amplified the diagnostic band from all the resistant lines, but not from any of the wheat background genotypes. Both markers were also diagnostic for

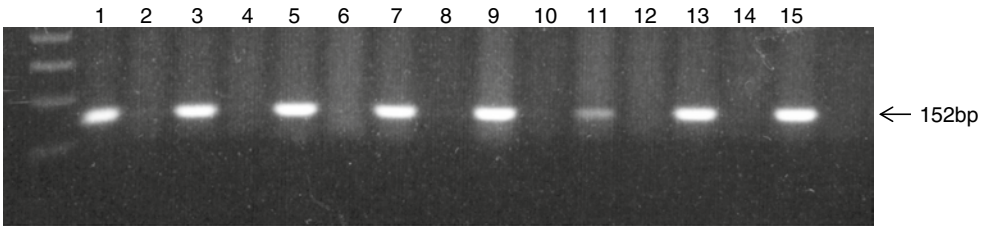

Fig. 5 Validation of csSr32#2 on breeding lines carrying *Sr32* translocation from C77.19. *Lanes 1* C77.19/4 × L23, *2* Warigal, *3* C77.19/4*Aroona/2*Warigal, *4* WT20/5, *5* C77.19/4*WT20/5, *6* WT14/20, *7* C77.19/4*WT14/20, *8* CO1568, *9* C77.19/4*CO1568, *10* Oxley, *11* C77.19/6*Oxley, *12* Cocamba, *13* C77.19/2*Cocamba, *14* CS, *15* C82.2

translocations C95.24, C82.2, C82.3, and C84.4. Both markers also co-segregated with stem rust resistance among 118 progeny lines from a cross between C77.19 and 'Yarralinka'.

The csSrAes1t marker was also validated with the same lines and *Sr32* translocations. The marker was not amplified from C77.19 derivatives, consistent with the RFLP results which showed this translocation had no detectable 2SL markers. Among other *Sr32* translocations, the csSrAes1t marker was amplified from C82.2 and C95.24, whereas the *Sr47* marker gwm501 (Klindworth et al. 2012) amplified from all translocations carrying 2SL RFLP markers, i.e., C82.2, C82.3, C82.4, and C95.24.

Marker screening of other *Ae. speltoides* accessions

The *Sr32* and *SrAes1t* loci in the *Ae speltoides* gene pool may be a source of further useful rust resistance genes with different specificities. Thus, new markers and also *Sr39*-linked markers (Mago et al. 2009) were used to screen 18 *Ae. speltoides* accessions from various parts of the Middle East (Table 6). Among these lines, 16 were fully resistant to two Australian Pgt strains, whereas two were partially resistant. Polymorphism was seen with the *Sr32* markers, which amplified from eight of the 18 accessions and the *SrAes1t*-linked marker was present in the majority (15) of the lines. The *Sr39* markers amplified from most of the *Ae speltoides* accessions. None of the markers amplified from the Turkish line AUS18813.

Table 6 Survey of csSr32, csSrAes1t and Sr39 markers in *Aegilops speltoides* accessions

AUS No.	Country of origin	Markers			
		csSr32#1	csSrAes1t	Sr39#22r	Sr39#50 s
AUS 18813	Turkey	−	−	−	−
AUS 18928	Unknown	+	+	+	+
AUS 18995	Israel	+	+	+	+
AUS 21636	Turkey	+[a]	+[a]	+	+
AUS 21637	Turkey	+	+	+	+
AUS 21638	Iraq	+*	+	−	+
AUS 21639	Iraq	+	+	+	+
AUS 21640	Syria	−	+	+	+
AUS 21641	Syria	+	+	+	+
AUS 21646	Turkey	−	+	+	+
AUS 21647	Syria	−	+	+	+
AUS 21648	Iraq	−	+	+	+
AUS 21649	Turkey	−	+[a]	+	+
AUS 21650	Turkey	−	+	+	+
AUS 26948	Israel	+	+	+	+
AUS 26949	Turkey	−	+	+	+
AUS 26955	Israel	−	−	+	+
AUS 26983	Israel	−	−	+	+

[a] Lines may be genetically heterogeneous as plants gave different PCR patterns

Discussion

New recombinants with *Sr32* and *SrAes1t*

This research was focused on producing new *Sr32* recombinants, more suitable than Sears' original translocations from which no commercial cultivar carrying this gene has been released (McIntosh 1991; McIntosh et al. 1995; Dundas et al. 2007; Brennan, personal communication). The failure of these translocations to be used in agriculture may be associated with the presence of deleterious genes introduced on the 2S#1 chromosome or with the loss of indispensible wheat genes displaced by the translocation. The 2A–2S#1 translocation for example, shows the presence of adherent glume fragments on the grain caused by the loss of part of the 2A chromosome. However, apart from that, no other obvious deleterious character(s) have been documented for the other three translocations (McIntosh et al. 1995). In the current study starting with translocation C82.2 we have produced recombinants carrying small segments of 2S#1 chromatin as determined by cytology and molecular markers. During the production of these, we determined that the translocation C82.2 carries two resistance genes either the short arm (e.g. 70 Type II) or long arm (e.g. 70 Type I). Because Sears' original translocation lines C77.19 and C82.1 involving the wheat 2B chromosome do not carry long arm 2S#1 chromatin, the gene on the short arm must be *Sr32*. We temporarily named the long arm gene *SrAes1t* (formerly called *Sr2S#1* by Dundas et al. 2007). Accurate PCR markers produced for both genes will allow introgression and assessment for quality and yield effects in wheat-breeding programs. Samples of stem rust resistant lines 70 Type I (AUS 91440), 70 Type II (AUS 91441), 102 (AUS 91442), 107 (AUS 91443), 122 Type I (AUS 91444), 122 Type II (AUS 91445), 247 (AUS 91446), and 287 (AUS 91447) have been lodged with the Australian Winter Cereals Collection, Tamworth, New South Wales.

The relationship of Sr genes on the chromosome 2S

Several independent translocations involving chromosome 2S derived from different accessions of *Ae. speltoides* carry stem rust resistance. Two named genes, viz. *Sr32* and *Sr39* (McIntosh et al. 1995) and the temporarily designated *SrAes7t* (Klindworth et al. 2012) have been mapped to the short arm of 2S#1. One named gene. *Sr47* (Klindworth et al. 2012) and temporarily designated *SrAes1t* have been mapped to the long arm of 2S#1 (Dundas et al.

2007 and this study). Although there are small quantitative differences in response among the short arm genes and among the long arm genes to different stem rust pathotypes, including Ug99 and its derivatives, it is not yet possible to separate the specificities because no virulent rust pathotypes have been reported that can distinguish between them. A similar situation had arisen with rye-derived genes *Sr31*, *SrR* and likely *Sr1RSAmigo* that occur in a similar region on chromosome 1RS (Mago et al. 2002). However, tests with Ug99 differentiated the susceptible *Sr31* from the resistant *SrR*, and *SrR* was given the formal designation *Sr50* (McIntosh et al. 2011). Stem rust isolates found recently in Yemen and Ethiopia (belonging to pathotype TRTTF) are virulent on *Sr1RSAmigo* (Olivera et al. 2012). Therefore, *Sr1RSAmigo* can also be differentiated from *Sr31 a*nd *Sr50*.

The occurrence of stem rust resistance effective against all tested Pgt strains derived from at least three independent translocations does raise a problem for wheat breeding. In the future, stem rust resistance introgressions from multiple accessions of *Ae. speltoides* and the extensive chromosome engineering required to make these genes suitable for use in wheat breeding runs the risk of expending large amounts of time and effort on recovering the same resistance specificity on multiple occasions. A similar problem has been encountered in developing late blight resistance in potato using resistance genes introgressed from wild species of *Solanum*. Methods based on tests using cloned pathogen effector (avirulence) genes have been developed for potato breeding that allow rapid tests to distinguish resistance gene specificities (Vleeshouwers et al. 2011). Similar tests are urgently needed for rust resistance breeding in wheat.

Future applications

Translocations carrying *Sr32* were listed as being resistant to seven pathotypes of the Ug99 lineage [namely TTKSK (Ug99), TTKSF, TTKST (Ug99+*Sr24*), TTTSK (Ug99+*Sr36*), TTKSP, PTKST, and PTKSK)] (FAO 2013). However, it has been established in the current study that some of the original wheat–*Ae. speltoides* translocation lines carrying *Sr32* also carry an additional gene *SrAes1t*. We have now shown that both these genes are effective against the Ug99 lineage. With the markers and recombinants described in this study as well as in previous reports, it will be possible to develop monogenic near-isogenic lines in a highly Pgt susceptible recurrent parent carrying *Sr32* (line 102), *Sr39, Sr47*, *SrAes7t*, and *SrAes1t* (line 70 Type I) genes separately so that their resistance specificities and infection types could be simultaneously evaluated against a wide range of pathotypes of Pgt and also for pathogen monitoring purposes. Attempts should also be made to use markers to combine the shortened alien segments, for example *Sr32* and *SrAes1t*, into a single stock to enhance their durability when deployed into a pure-breeding variety. In that regard, our selections of the 2D translocation of *Sr32* will facilitate the effort in combining the stem rust resistance genes in group 2 chromosomes, for example *Sr32* (2D) and *Sr39* or *SrAes7t* (both 2B) if these genes indeed represent different resistance specificities. The utility of recombinant lines produced in the current study in agriculture will only be determined by their presence in future cultivars where selection is performed for yield and quality. Preliminary to this rigorous breeding test, we are currently evaluating the field performance of each recombinant in cultivars Westonia (Australia) and HUW234 (India).

Acknowledgments We thank the late Dr Paul Brennan, former Senior Wheat Breeder, Toowoomba, Australia, for initially suggesting *Sr32*-carrying germplasm as an objective for this research. We also thank Professor R.A. McIntosh (University of Sydney) for kindly providing the wheat-*Aegilops speltoides* translocation lines carrying *Sr32* and critically reviewing the manuscript. We are thankful to Xiaodi Xia and Hanif Miah for providing excellent technical assistance and to T.T. The (University of Sydney) for producing some of the backcross materials. Research involving the isolation of the wheat–*Ae. speltoides* recombinants was funded by the Grains Research and Development Corporation of Australia through the Australian Cereal Rust Control Program and was previously associated with the CRC for Molecular Plant Breeding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of Australia.

References

Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other resistance genes in chromosome 2A. Genome 36:476–482

Dundas IS, Anugrahwati DR, Verlin DC, Park RF, Bariana HS, Mago R, Islam AKMR (2007) New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust J Agric Res 58:545–549

FAO (2013) http://www.fao.org/agriculture/crops/rust/stem/stem-pathotypetracker/stem-effectivesrgenes/en/. Accessed 7 Feb 2013

Faris JD, Xu SS, Cai X, Friesen TL, Jin Y (2008) Molecular and cytogenetic characterization of a durum wheat-*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. Chromosome Res 16:1097–1105

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in 'Hope' and related cultivars to wheat rusts. Z Pflanz 83:350–367

Hodson DP, Singh RP, Dixon JM (2005) An initial assessment of the potential impact of stem rust (race UG99) on wheat producing regions of Africa and Asia using GIS. http://apps.cimmyt.org/gis/pdf/UG99postH.pdf. Accessed 7 Feb 2013

Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Fetch T, Pretorius ZA, Yahyaoui A (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096–1099

Jin Y, Szabo LJ, Pretorius ZA (2008a) Virulence variation within the Ug99 lineage. http://ses.library.usyd.edu.au/bitstream/2123/3435/1/O02.pdf. Accessed 7 Feb 2013

Jin Y, Szabo L, Pretorius ZA, Singh RP, Ward R, Fetch T (2008b) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926

Jin Y, Szabo L, Rouse M, Fetch T, Pretorius ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene *Sr36* within race TTKS lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis 93:367–370

Kerber ER, Dyck PL (1979) Resistance to stem and leaf rust of wheat in *Aegilops squarrosa* and transfer of a gene for stem rust resistance to hexaploid wheat. In: Proceedings of the 5th international wheat genetics symposium, pp 358–364

Kerber ER, Dyck PL (1990) Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from amphiploid of *Aegiolops speltoides* x *Triticum monoccum*. Genome 33:530–537

Klindworth DL, Niu Z, Chao S, Friesen TL, Jin Y, Faris JD, Cai X, Xu SS (2012) Introgression and characterization of a goatgrass gene for a high level of resistance to Ug99 stem rust in tetraploid wheat. G3 2:665–673

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. Mol Breed 1:389–395

Mago R, Spielmeyer W, Lawrence GJ, Lagudah ES, Ellis JG, Pryor A (2002) Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat–rye translocation lines. Theor Appl Genet 104:1317–1324

Mago R, Zhang P, Bariana HS, Verlin DC, Bansal UK, Ellis JG, Dundas IS (2009) Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker-assisted selection. Theor Appl Genet 124:65–70

McIntosh RA (1988) Catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proceedings of the 7th international wheat genetics symposium, vol 2. Institute of Plant Science Research, Cambridge, pp 1225–1323

McIntosh RA (1991) Alien sources of disease resistance in bread wheats. In: Sasakuma T, Kinoshita T (eds) Memorial international symposium on cytoplasmatic engineering in wheat. Nuclear and organellar genomes of wheat species. Hokkaido University, Sapporo, pp 320–332

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, Victoria

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2011) Catalogue of gene symbols for wheat: 2011 Supplement (http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2011.pdf). Accessed 7 Feb 2013

Nazari K, Ma WM, Yahyaoui A, Singh RP, Park RF (2009) Detection of wheat stem rust race (*Puccinia graminis* f. sp. *tritici*) TTKSK (Ug99) in Iran. Plant Dis 93:317

Niu Z, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, Xu SS (2011) Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021

Olivera P, Jin Y, Rouse M, Badebo A, Fetch T Jr, Singh RP, Yahyaoui A (2012) Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in field stem rust screening nursery in Ethiopia. Plant Dis 96:623–628

Park R, Wellings C, Bariana HS, Bansal U (2009) Cereal rust report 2009. 7(2). http://sydney.edu.au/agriculture/documents/pbi/cereal_rust_report_2009_vol_7_2.pdf. Accessed 7 Feb 2013

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis 84:203

Pretorius ZA, Bender CM, Visser B, Terefe T (2010) First report of a *Puccinia graminis* f. sp. *tritici* race virulent to the *Sr24* and *Sr31* wheat stem rust resistance genes in South Africa. Plant Dis 94:784

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39:379–394

Sears ER (1977) An induced mutant with homeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. United States Department of Agriculture), Washington DC

Vleeshouwers VG, Raffaele S, Vossen JH, Champouret N, Oliva R, Segretin ME, Rietman H, Cano LM, Lokossou A, Kessel G, Pel MA, Kamoun S (2011) Understanding and exploiting late blight resistance in the age of effectors. Annu Rev Phytopathol 49:507–531

Vos P, Hogers R, Bleeker M, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wanyera R, Kinyua MG, Jin Y, Singh RP (2006) The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis 90:113

Zhang P, Friebe B, Lukaszewski AJ, Gill BS (2001) The centromere structure in Robertsonian wheat–rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. Chromosoma 110:335–344