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ORIGINAL ARTICLE

A new 2DS·2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat

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Abstract
Key message **A new stem rust resistance gene *Sr59* from *Secale cereale* was introgressed into wheat as a 2DS·2RL Robertsonian translocation.**
Abstract Emerging new races of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*), from Africa threaten global wheat (*Triticum aestivum* L.) production. To broaden the resistance spectrum of wheat to these widely virulent African races, additional resistance genes must be identified from all possible gene pools. From the screening of a collection of wheat–rye (*Secale cereale* L.) chromosome substitution lines developed at the Swedish University of Agricultural Sciences, we described the line 'SLU238' 2R (2D) as possessing resistance to many races of *P. graminis* f. sp. *tritici*, including the widely virulent race TTKSK (isolate synonym Ug99) from Africa. The breakage-fusion mechanism of univalent chromosomes was used to produce a new Robertsonian translocation: T2DS·2RL. Molecular marker analysis and stem rust seedling assays at multiple generations confirmed that the stem rust resistance from 'SLU238' is present on the rye chromosome arm 2RL. Line TA5094 (#101) was derived from 'SLU238' and was found to be homozygous for the T2DS·2RL translocation. The stem rust resistance gene on chromosome 2RL arm was designated as *Sr59*. Although introgressions of rye chromosome arms into wheat have most often been facilitated by irradiation, this study highlights the utility of the breakage-fusion mechanism for rye chromatin introgression. *Sr59* provides an additional asset for wheat improvement to mitigate yield losses caused by stem rust.

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Introduction

Bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the most important food crops, serving as a daily staple for human consumption worldwide (International Wheat Genome Sequencing Consortium 2014). However, the lack of sufficient genetic diversity in bread wheat germplasm challenges geneticists and breeders to enhance the crop against various biotic and abiotic stresses in order to meet the increasing global demand for food security. To meet the future challenges of food security and sustainable wheat production, it is imperative to exploit new sources of diversity for valuable genes in wild relatives of wheat (Kole 2011). Stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn (*Pgt*), is one of the most devastating diseases of wheat crops globally (Szabo et al. 2014). The *Pgt* race TTKSK (also known as isolate Ug99) has broad virulence for currently deployed resistance genes in wheat, most notably *Sr31*, which was widely deployed in many cultivars around the world. The

discovery of race TTKSK has raised serious concerns regarding food security since it was first detected in 1998 in Uganda (Pretorius et al. 2000). Since that time, variants of TTKSK (i.e. the Ug99 lineage races) have been detected with virulence for additional resistance genes, including *Sr24*, *Sr36*, *Sr9h* and *SrTmp* (Jin et al. 2008, 2009; Rouse et al. 2014; Patpour et al. 2015b). More than eight variants in the Ug99 race group have been detected and are spreading across eastern, southern and northern Africa as well as the Middle East (Patpour et al. 2015a; Singh et al. 2015). The resistance genes *Sr13* and *Sr1RS*^{Amigo} are effective against TTKSK; however, they are not effective against an unrelated race (TRTTF) that was recently detected in Yemen and Ethiopia (Olivera et al. 2012). Furthermore, the recent severe epidemics (2013–2014) caused by race TKTTF on variety 'Digalu' carrying *SrTmp* in Ethiopia highlights the dynamic challenges of breeding for stem rust resistance (Olivera et al. 2015).

Rye (*Secale cereale* L., $2n = 2x = 14$, RR) is an important source of new genes for wheat improvement (Martis et al. 2013; Schlegel 2014). It has been a particularly rich genetic resource for genes conferring resistance to *Pgt*, including those emerging and widely virulent races from Africa (Mago et al. 2015). For example, *Sr27* from Imperial rye (Marais and Marais 1994), *Sr31* from Petkus rye (Mago et al. 2005b), *Sr50* from Imperial rye (Mago et al. 2004), and *Sr1RS*^{Amigo} from Insave rye (The et al. 1991) have been introgressed into wheat. Globally, *Sr31* provided a high level of resistance against all stem rust pathogen races for more than 30 years (Evanega et al. 2014) and still remains effective except to the Ug99 race group. Introgressing additional sources of resistance to stem rust from rye or other species in the tertiary gene pool of wheat could enhance the number of tools available for wheat breeders (Niu et al. 2011; Liu et al. 2013; Mago et al. 2015). Indeed, several TTKSK-effective resistance genes such as *Sr25*, *Sr26* (Mago et al. 2005a; Liu et al. 2010), *Sr43* (Niu et al. 2014), *Sr44* (Liu et al. 2013), and *Sr52* (Qi et al. 2011) have been transferred from the tertiary gene pool and are now being used in breeding.

The homoeologous transfer of stem rust resistance genes from the secondary (*Aegilops* spp.) and tertiary gene pools is difficult due to presence of the *Ph1* gene that prevents homoeologous pairing (Riley and Chapman 1958). Deletion of the *Ph1* gene allows homoeologous chromosome pairing and recombination (Sears 1977). A *ph1b* mutant line was used to facilitate the introgression of *Sr32* from *Ae. speltoides* (Mago et al. 2013), *Sr39* from *Ae. speltoides* (Niu et al. 2011), *Sr43* from *Thinopyrum ponticum* (Niu et al. 2014), *Sr47* from *Ae. speltoides* (Klindworth et al. 2012), *Sr51* from *Ae. searsii* (Liu et al. 2011a), and *Sr53* from *Ae. geniculata* (Liu et al. 2011b). Use of a *ph1b* mutant line (Lukaszewski 2000) produced a large number of wheat–rye recombination events with improved end use quality that do not carry the *Sec-1* (secalin) allele on chromosome arm 1RS in wheat. Moreover, several wheat-alien Robertsonian translocation lines have been developed that confer resistance to the three rust diseases of wheat (Dundas et al. 2007; Liu et al. 2011a; Mujeeb-Kazi et al. 2013).

A large number of wheat-alien introgression lines were developed from 1980 to 2000 by the late Professor Arnulf Merker at the Swedish University of Agricultural Sciences (SLU). From field and greenhouse screening of wheat–rye substitution lines developed by Merker, we described line 'SLU238' [2R (2D) wheat–rye disomic substitution] to confer effective resistance against a number of diverse African and North American races of *Pgt* (Rahmatov et al. 2016). The objectives of this study were to introgress the chromosome 2R stem rust resistance gene from 'SLU238' into the wheat genome and identify linked molecular markers that could facilitate marker-assisted selection of the trait in wheat breeding.

Materials and methods

Plant materials and stem rust seedling evaluations

The spring wheat disomic substitution line 2R (2D) 'SLU238' was developed from the hexaploid triticale breeding line 'VT828041' at the SLU (Merker 1984). The Chinese Spring *ph1b* mutant line (Sears 1977) was kindly provided by Dr. Steven Xu, United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Northern Crop Science Laboratory, Fargo, North Dakota. 'SLU238' was crossed with the Chinese Spring *ph1b* mutant, producing 49 F_1 seeds. The F_1 plants were selfed and produced 863 F_2 seeds (progeny). A total of 198 F_2 plants were assessed for their seedling response to *Pgt* race TTKSK at the USDA-ARS Cereal Disease Laboratory according to previously described methods (Stakman et al. 1962; Rouse et al. 2011). The remaining 665 F_2 plants were assessed for their seedling response to *Pgt* race TTTTF (Table 1). A total of 15 F_2 plants were selected for further study based on (1) seedling resistance to either TTKSK or TTTTF, (2) a marker haplotype indicative of homozygosity at *ph1b*, and (3) a marker haplotype indicative of the presence of both chromosomes 2R and 2D. A total of 208 F_3 seeds were derived from the F_2 plants. Of these 208, seven F_3 plants were selected based on (1) seedling resistance to TTTTF, (2) a marker haplotype indicative of the presence of some, but not all 2R chromatin, and (3) a marker haplotype indicative of the presence of some, but not all 2D chromatin. The seven F_3 plants were selfed to derive $F_{3:4}$ families. At least 20 plants from each of the seven families were assessed for their seedling response to TTTTF.

Table 1 The origin and virulence phenotype of *Puccinia graminis* f. sp. *tritici* races used in this study

Race	Isolate	Origin	Virulence profile																	
			5	*21*	*9e*	*7b*	*11*	*6*	*8a*	*9 g*	*36*	*9b*	*30*	*17*	*9a*	*9d*	*10*	*Tmp*	*24*	*31*	*38*	*McN*
TTKSK	04KEN156/04	Kenya	*5*	*21*	*9e*	*7b*	*11*	*6*	*8a*	*9 g*	–	*9b*	*30*	*17*	*9a*	*9d*	*10*	–	–	*31*	*38*	*McN*
TTTSK	07KEN24-4	Kenya	*5*	*21*	*9e*	*7b*	*11*	*6*	*8a*	*9 g*	*36*	*9b*	30	–	*9a*	*9d*	*10*	*Tmp*	–	*31*	*38*	*McN*
TTTTF	01MN84A-1-2	USA	*5*	*21*	*9e*	*7b*	*11*	*6*	*8a*	*9 g*	*36*	*9b*	30	*17*	*9a*	*9d*	*10*	*Tmp*	–	–	*38*	*McN*
RKQQC	99KS76A-1	USA	*5*	*21*	–	*7b*	–	6	*8a*	*9 g*	*36*	*9b*	–	–	*9a*	*9d*	–	–	–	–	–	*McN*
TPMKC	74MN1409	USA	*5*	*21*	*9e*	*7b*	*11*	–	*8a*	*9 g*	*36*	–	–	*17*	–	*9d*	*10*	*Tmp*	–	–	–	*McN*
RCRSC	77ND82A	USA	*5*	*21*	–	*7b*	–	–	–	*9 g*	*37*	*9b*	–	*17*	*9a*	*9d*	*11*	–	–	–	–	*McN*

"–" Indication of avirulence

Table 2 List of molecular markers used in the study to detect 2D and 2R chromosomes

Type of marker	Primers	Crop	Markers tested	Chromosome	Polymorphic markers	References
SSR	*Xgwm*	Wheat	6	2D	2	Röder et al. (1998)
	Xwmc	Wheat	9	2D	3	Somers et al. (2004)
	Xscm	Rye	5	2R	2	Saal and Wricke (1999)
	Xgwm	Rye	13	2R	0	Khlestkina et al. (2004)
EST-SSR	*Xrems*	Rye	9	2R	2	Khlestkina et al. (2004)
	Xgrm	Rye	20	2R	4	Martis et al. (2013)
	Xcgg	Rye	3	2R	2	Xu et al. (2012)
PLUG	*Xtnac*	Rye	4	2R	2	Li et al. (2013)
EST-SNP	KASP	Rye	34	2R	3	Martis et al. (2013)

In addition, 5–10 plants from each of the seven families were also tested for their seedling response to *Pgt* races TPMKC, RKQQC, and RCRSC. Finally, three families that were homozygous for resistance to race TTTTF were evaluated for seedling response to *Pgt* races TTKSK and TTTSK (Table 1).

Molecular marker analyses

Genomic DNA was isolated from young leaf tissue according to Edwards et al. (1991) with some slight modifications. The F_2 plants were analyzed with touchdown molecular markers *Xpsr128*, *Xpsr574* and *Xawjl3* to detect homozygous *ph1b* plants (Roberts et al. 1999; Niu et al. 2011). We tested 15 Simple Sequence Repeat (SSR) markers previously mapped to chromosome 2D, 36 SSR markers previously mapped to chromosome 2R, and 4 PCR-based Landmark Unique Gene (PLUG) markers previously mapped to chromosome 2R for polymorphism between the two parents (Table 2). The polymorphic markers were used to select plants with the desired haplotypes at the F_2 and F_3 generations. The polymerase chain reaction (PCR) master mix for all markers consisted of 2 µl of 25–50 ng genomic DNA template, 0.6 µl of a 10 µM mixture of each forward and reverse primers, 0.075 µl (0.5 U) of Taq polymerase, 1.75 µl of 10× Ex Taq Buffer (10 mM Tris–HCL, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 1.75 µl of a 2.5 mM Mixture of dNTPs, and 9 µl of water, bringing the total of each reaction volume to 15 µl. The cycling conditions of the PCR were based on particular primer sets for correct product amplification (Röder et al. 1998; Saal and Wricke 1999; Khlestkina et al. 2004; Somers et al. 2004; Xu et al. 2012; Martis et al. 2013). The PCR products were resolved on 2–4 % agarose gels and visualized under UV light after ethidium bromide staining.

Development of kompetitive allele specific PCR (KASP) markers

To identify markers linked to stem rust resistance, we designed 34 KASP markers from the iSelect SNP markers that were mapped to chromosome 2R (Table 2) (Haseneyer et al. 2011; Martis et al. 2013). The primer sequences for the KASP markers are given in Table 3. Each KASP PCR consisted of 50 ng of DNA template and 5 µl of 2× KASP Master Mix and 0.14 µl of primer mixture. Thermal cycling conditions were 94 °C for 15 min; followed by 10 cycles of touchdown PCR: 94 °C for 20 s, 65–57 °C for 60 s (dropping 0.8 °C per cycle); and finally 36 cycles of regular PCR: 94 °C for 20 s, 57 °C for 60 s with the fluorescence

Table 3 KASP markers developed for the linked stem rust resistance gene on the introgressed 2RL chromosome

SNP ID	Primer name	Primer sequence
c25837_157	KASP_2RL_c25837A1	TAGTGTTTTGCTCGACCACTGTC
	KASP_2RL_c25837A2	GTTAGTGTTTTGCTCGACCACTGTT
	KASP_2RL_c25837C1	CACCAAACACTACCCACACCATCTA
c21825_230	KASP_2RL_c21825A1	ACATTTCGGTTGGTATTGATTCTAACG
	KASP_2RL_c21825A2	ACATTTCGGTTGGTATTGATTCTAACC
	KASP_2RL_c21825C1	CCAGCCATGAAGAAAATAACAATTCGAGAT
c20194_115	KASP_2RL_c20194A1	CCAGCTAGGACAAACTTTGCCTAAA
	KASP_2RL_c20194A2	CAGCTAGGACAAACTTTGCCTAAG
	KASP_2RL_c20194C2	CTTGTGGGCGCTCGTGGCTTT

reading at 20 °C. Both thermal cycling and fluorescence reading were performed on ABI Step One Plus Real Time PCR system.

Fluorescent in situ hybridization (FISH)

To visualize wheat–rye introgressions, the parents and lines from two selected $F_{3:4}$ families fixed for resistance to race TTTTF were analyzed by FISH with probes specific to rye and wheat repetitive DNA sequences. Rye chromosomes were painted using the combination of probes to dispersed repeat UCM600 (González-García et al. 2011) synthesized by SGI DNA, La Jolla, CA, USA; centromere specific pAWRC.1 (Francki 2001); and subtelomeric repeat pSc74 (Bedbrook et al. 1980; Lapitan et al. 1986) all labeled with Fluorescein-12-dUTP (PerkinElmer, cat. NEL413001EA). Wheat chromosomes were identified using Cy5-$(GAA)_9$ and TEX615-pAs1-2 oligonucleotide probes (Danilova et al. 2012) synthesized by IDT, Coralville, IA. Somatic chromosome preparations using the drop technique, direct probe labeling by nick translation and the FISH procedure were done according to Kato et al. (2006) with minor modifications as described in Danilova et al. (2012). Images were captured with a Zeiss Axioplan 2 microscope using a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics, Tuscon, AZ) and AxioVision 4.8 software (Carl Zeiss AG, Germany) and processed using the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA, USA).

Results

Stem rust resistance is conferred by chromosome 2R

Wheat SSR markers (*Xgwm349*, *Xgwm484*, *Xwmc18*, *Xwmc111,* and *Xwmc601*) for chromosome 2D (Röder et al. 1998; Somers et al. 2004) were identified as polymorphic between the parents (Table 2). 'SLU238' possessed a null allele for these chromosome 2D markers. The rye-specific SSR markers *Xscm43* and *Xscm75* (Saal and Wricke 1999), Expressed Sequence Tag (EST)-derived SSR markers *Xrems1251* and *Xrems1203* (Khlestkina et al. 2004), *Xgrm0676*, *Xgrm0837*, *Xgrm0315* and *Xgrm1265* (Martis et al. 2013), *Xcgg8*, and *Xcgg62* (Xu et al. 2012), and PLUG markers *Xtnac1142* and *Xtnac1383* (Li et al. 2013), previously mapped to chromosome 2R were identified as polymorphic between the parents (Table 2). The Chinese Spring *ph1b* mutant line possessed a null allele for the chromosome 2R markers.

Segregation for resistance to *Pgt* races TTKSK and TTTTF was observed in the F_2 population with infection types of 1; and 2− in resistant plants and 3 + 4 in susceptible plants. The observed segregation pattern did not fit a 3:1 ratio to either TTKSK (120 plants resistant, 78 plants susceptible, $\chi^2 = 21.9$, $P = \leq 0.005$) or TTTTF (428 plants resistant, 237 plants susceptible, $\chi^2 = 40.1$, $P \leq 0.005$), indicating the presence of segregation distortion with a preferential transmission of susceptibility, conferred by chromosome 2D.

A total of 200 F_2 plants resistant to TTTTF or TTKSK were analyzed with *ph1b* markers *Xpsr128*, *Xpsr574* and *Xawjl3*, and a total of 46 plants were identified as homozygous for *ph1b*. These 46 plants were assessed for the presence of both rye alleles of markers *Xscm43*, *Xrems1251*, and *Xrems1203* and wheat alleles of markers *Xgwm349*, *Xgwm484*, *Xwmc601*, *Xwmc111*, and *Xwmc18* in order to identify 15 plants that appeared to possess both rye chromosome 2R and wheat chromosome 2D. Only 9 of these 15 F_2 plants produced total F_3 seeds.

Among the 208 F_3 plants, 146 were resistant to TTTTF at the seedling stage. Every one of these 146 plants possessed the chromosome 2R alleles for rye-derived markers *Xscm43*, *Xrems1251*, and *Xrems1203*, whereas the remaining 62 susceptible plants did not possess these rye-specific markers. Cosegregation of the resistance phenotype with the rye alleles indicated that stem rust resistance is conferred by rye chromatin.

Table 4 Infection types of the F_4 wheat–rye translocation lines to races of *Puccinia graminis* f. sp. *tritici*

Line or family#	1 Rep. TTKSK	2 Rep. TTKSK	TTTSK	TTTTF	TPMKC	RKQQC	RCRSC	Gene	Note
CS *ph1b*	4	4	4	4	4	4	4	None	Parents
SLU238	;1	;1	;1	2−	;1−	;1−	;0	New	Parents
#99	2−/3+	;1/3	3+/1;	11+	;1	11+	;1/4	New	Segregating
#100	12−	;1	12−	11+	;1	11+	;1	New	Fixed
#101 (TA5094)	;1	;1	;1	11+	;1	11+	;1	New	Fixed
#282 (S)	–	–	–	4	3+	4	3+	None	Susceptible
#284	–	–	–	2−/3+	;1/4	2−/4	2−/3+	New	Segregating
#311 (S)	–	–	–	3+	3+	3+	3+	None	Susceptible
#409	–	–	–	2−/3+	;1/3+	2−/3+	2−/4	New	Segregating
#505	–	–	–	2−/3+	;1/4	2+/3+	2−/3+	New	Segregating
#508	–	–	–	2−/3+	;1/4	2+/4	2−/3+	New	Segregating

Infection types observed based on 0-4 scale (Stakman et al. 1962). The plants with;0 to 2+ infection types were considered as resistant, and the plants with 3–4 infection types were considered as susceptible. The F_4 #282 (S) and #311 (S) lines were used as susceptible control

A Robertsonian translocation transfers *Sr59* to wheat

The 146 stem rust resistant F_3 plants were assessed with the remaining rye and wheat markers to identify seven F_3 plants that possessed rye alleles for some, but not all of the rye markers. From the initial screening of the derived $F_{3:4}$ families, we identified three (#99, #100 and #101) that were fixed for resistance to TTTTF, whereas the other four families segregated for resistance to TTTTF (Table 4). The families resistant and segregating for response to TTTTF displayed the same responses to TPMKC, RKQQC and RCRSC (except #99 to race RCRSC). Out of these three families, two were fixed for resistance to TTKSK and TTTSK (Table 4; Fig. 1). Based on the marker results, families #99, #100, #101, #505 and #508 were identified as potential wheat–rye 2DS·2RL Robertsonian translocations (Table 5). Families #284 and #409 were identified as possessing putative wheat-2R homoeologous recombinants resulting in shortened segments of rye chromatin. *Secale cereale* chromatin was not detected with rye (SSR, EST-SSR and PLUG) markers for two of the segregating families #284 and #409 (Table 5). DNA was collected from resistant and susceptible F_4 plants separately including susceptible plants from families #99, #284 and #409 in addition to two susceptible families, #282 (S) and #311 (S) (Table 5). FISH confirmed the phenotypic and marker results, indicating that lines #100, and #101 each are homozygous for a T2DS·2RL translocation chromosome and that line #99 had one T2DS·2RL chromosome. Line #100 possessed 41 chromosomes, including some rearranged wheat chromosomes (T6DL·5BL and T6DS·6DL/?W), whereas line #101 had 42 chromosomes (Fig. 2b). The resistant line #101 with the 2DS·2RL Robertsonian translocation was deposited at the Kansas State University Wheat Genetics and Genomics Resource Center as accession TA5094. Since no stem rust resistance genes have been described from rye chromosome arm 2RL, accession TA5094 (#101) was designated as the source of the gene *Sr59*.

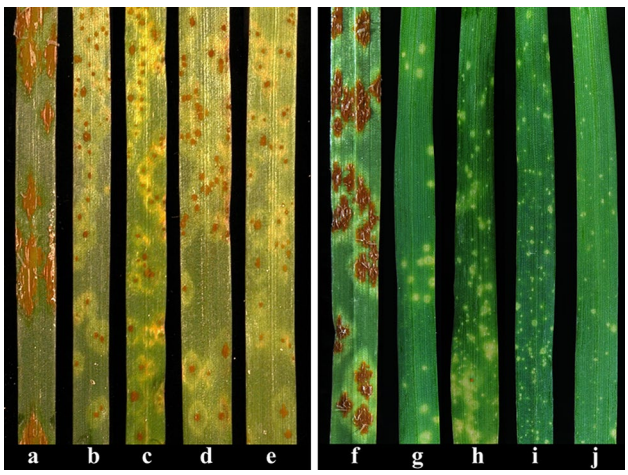

Fig. 1 Reaction to *Puccinia graminis* f. sp. *tritici* race TTTTF of **a** CS *ph1b* mutant; **b** SLU238; **c** #99; **d** #100; **e** #101 (TA5094), and TTKSK race of **f** CS *ph1b* mutant; **g** SLU238; **h** #99; **i** #100; **j** #101 (TA5094)

Development and validation of KASPar markers for 2R

By evaluating the 34 KASP assay SNP markers, we identified three (KASP_2RL_c25837C1, KASP_2RL_c21825C1 and KASP_2RL_c20194C2) that were able to clearly distinguish the Chinese Spring *ph1b* mutant line from 'SLU238' 2R (2D). These three markers were subsequently used to analyze F_4 resistant plants from families #99 (R), #100, #101, #284 (R), #409 (R), #505 and #508 as well as susceptible plants from families #99 (S), #284 (S), #409 (S), #282 (S) and #311 (S) (Table 5). Subsequent analysis

Table 5 Wheat and rye specific primers were used to detect the breakpoints of the 2D and 2R chromosome translocation in the F_4 generation

Primers	Chromosome	CS *ph1b*	SLU238	#99 (R)	#99 (S)[c]	#100	#101	#282 (S)[a]	#284 (R)	#284 (S)[b]	#311 (S)[a]	#409 (R)	#409 (S)[b]	#505	#508
Xgwm484	2DS	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Xwmc111	2DS	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Xwmc18	2DS	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Xgwm349	2DL	+	−	−	+	−	−	+	−	+	+	+	+	+	+
Xwmc601	2DL	+	−	−	+	−	−	+	+	+	+	+	+	+	−
Xgrm0676	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xgrm0837	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xgrm0315	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xgrm1265	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xcgg62	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xtnac1383	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xrems1203	2RS	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Xscm43	2RL	−	+	+	−	+	+	−	−	−	−	−	−	+	+
Xscm75	2RL	−	+	+	−	+	+	−	−	−	−	−	−	+	+
Xrems1251	2RL	−	+	+	−	+	+	−	−	−	−	−	−	+	+
Xcgg8	2RL	−	+	+	−	+	+	−	−	−	−	−	−	+	+
Xtnac1142	2RL	−	+	+	−	+	+	−	−	−	−	−	−	+	+
KASP_2RL_c25837C1	2RL	−	+	+	−	+	+	−	+	−	−	+	−	+	+
KASP_2RL_c21825C1	2RL	−	+	+	−	+	+	−	+	−	−	+	−	+	+
KASP_2RL_c20194C2	2RL	−	+	+	−	+	+	−	+	−	−	+	−	+	+

The signs "+" and "−" indicate presence and absence of the alleles of corresponding markers, respectively. Susceptible [a] lines and [b] plants based on TTTTF, and [c] susceptible plant based on RCRSC. All of the plants in this table were F_4, except parents

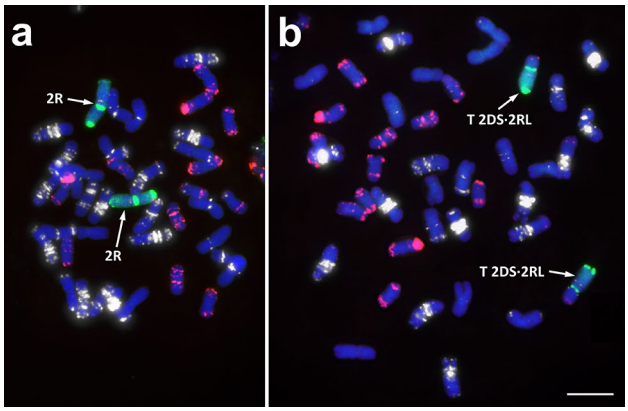

Fig. 2 FISH visualization of chromosomes from **a** resistant parent SLU238, disomic substitution line DS2R(2D) $2n = 42$ and **b** its resistant progeny line #101 (TA5094), homozygous on wheat–rye Robertsonian translocation T2DS·2RL, $2n = 42$. Rye chromatin is stained in green, probes GAA (*white*) and pAs1 (*red*) produce patterns that allow the identification of individual wheat chromosomes. *Bar* corresponds to 10 μm

of the F_4 plants with these three KASP markers identified the 'SLU238' allele in the resistant F_4 plants, but these markers failed to amplify any signal from the Chinese Spring *ph1b* and susceptible lines (Fig. 3). The three KASP markers also detected the 'SLU238' allele in the resistant plants derived from families #284 and #409, which did not have any rye alleles based on the SSR or PLUG markers. The three SNP (converted to KASP) markers were previously mapped to the distal end of 2RL (Martis et al. 2013). These three KASP assay SNP markers were perfectly predictive of the resistance phenotype among the F_4 plants.

Discussion

A new wheat–rye T2DS·2RL Robertsonian translocation line was developed through the breakage-fusion mechanism and verified by stem rust seedling tests, molecular marker assays, and FISH. Stem rust resistance corresponded with the presence of rye chromatin in the germplasm tested, indicating that a resistance gene introgressed from rye is responsible for the stem rust resistance. Since no stem rust resistance genes have been described from rye chromosome arm 2RL, the gene designation of *Sr59* was applied with accession TA5094 (#101) as its original source. Evaluation of 'SLU238' and derived lines #100 and #101 (TA5094) with the Robertsonian translocation carrying *Sr59* indicated that this gene is effective against the North American *Pgt* races TTTTF, RKQQC, TPMKC and RCRSC plus two African ones of the Ug99 lineage (TTKSK and TTTSK). Previously, the stem rust resistance genes *Sr27*, *Sr31*, *Sr50*, $Sr1RS^{Amigo}$, and *SrSatu* were described from *S. cereale* chromosomes 1R and 3R and found to be effective against many *Pgt* races (Marais and Marais 1994; Friebe et al. 1996; Mago et al. 2002; Singh et al. 2011; Olivera et al. 2013). This study is the first report of the transfer of *S. cereale* 2RL chromatin to wheat that confers stem rust resistance. Chromosome 2R from different rye genotypes has been described as a source of resistance to various wheat diseases and insects and also various agronomic traits (Hysing et al. 2007; Lei et al. 2013).

The reason for the different infection types observed for the reaction of 'SLU238' in response to TTTTF (IT = 2−) and TTKSK (IT = ;1) remains elusive with the available data. Hypotheses that could be tested to examine this

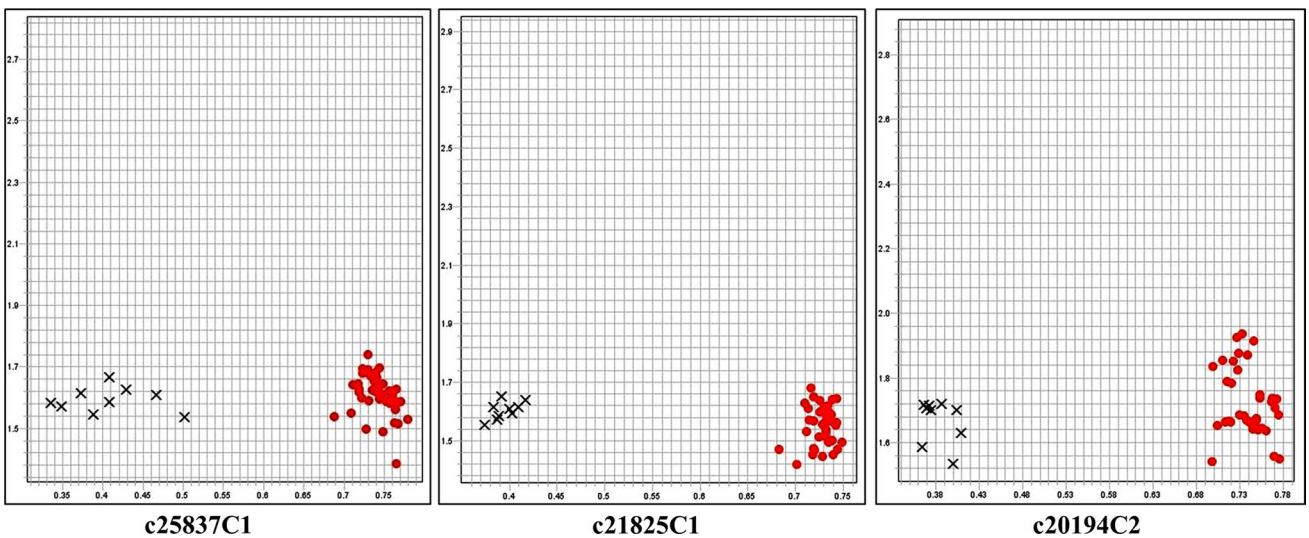

Fig. 3 Allele discrimination plots of the KASP markers used. For each of the three markers, 'SLU238' and resistant F_4 plants displayed the 'SLU238' allele indicated in red, and the CS *ph1b* mutant and susceptible plant displayed the null allele (indicated by *black* "x")

difference include: (1) the *Sr59* gene confers a stronger resistance effect to TTKSK compared to TTTTF, (2) chromosome arm 2RL possesses a resistance gene in addition to *Sr59* that explains the stronger resistance to TTKSK, and (3) the genetic background of 'SLU238' enhances the effect of *Sr59* in response to TTKSK. Interestingly, the infection types for TPMKC resembled those of TTKSK whereas the infection types of RKQQC resembled those of TTTTF (Table 4).

Although the wheat–rye Robertsonian translocation was produced in the *ph1b* background, this translocation is likely to have arisen from the breakage-fusion mechanism of univalent chromosomes at meiotic metaphase I. Univalents are known to misdivide at the centromeres and fusion of the broken ends will result in Robertsonian translocations (Friebe et al. 2005). The rye genome is known to be highly rearranged compared to that of wheat (Devos et al. 1993). However, the long arm of rye chromosome 2R is syntenic to wheat chromosome group 2 long arms and thus can compensate for the loss of the 2DL arm of wheat. 2RL is also the source of Hessian fly resistance gene *H21* present in the form of a T2BS·2RL Robertsonian translocation in the germplasm Hamlet (Friebe et al. 1990) and in derived T2BS·2BL-2RL recombinant stocks (Cainong et al. 2010). In addition, rye chromosome 2R is also the source of the leaf rust resistance gene *Lr45* that is present on a T2AS-2RS.2RL terminal translocation chromosome (McIntosh et al. 1995). Translocation lines with 2R are agronomically suitable for breeding because the protein genes associated with bread-making quality are found on the wheat chromosome groups 1 and 6; thus, the 2R translocations are not associated with reduced baking quality and neither with reduced yield (Friebe et al. 1996; Hysing et al. 2007; Johansson et al. 2013).

FISH and GISH are powerful and efficient tools for determining the amount of introgressed alien chromatin. Rye specific dispersed repeat UCM600, in combination with subtelomeric and centromeric repeats, were used to identify rye chromatin in the wheat genome by FISH (González-García et al. 2011). In this study, multicolor FISH with rye and wheat-specific repeats were used to confirm the T2DS·2RL Robertsonian translocations between *S. cereale* and wheat (Fig. 2). The FISH assays demonstrated that the translocated chromosomes in lines #100 and #101 were homozygous for T2DS·2RL. The putatively distal segment of 2RL in resistant plants from families #284 and #409 were detected by three KASP markers, but not by SSR markers on 2RL.

The characterization of *Sr59* by introgressing 2RL into wheat provides breeders with an additional genetic resource for developing stem rust resistant cultivars. *Sr59* can be pyramided with other stem rust resistance genes using the KASP markers identified in this study to select for wheat lines with multiple stem rust resistance genes. Research is currently underway to assess whether the rye chromosome arm 2RL segment confers resistance to other diseases. Additional studies are also in progress to backcross *Sr59* into adapted wheat cultivars to assess whether the 2RL introgression is associated with any deleterious effects on traits such as yield and quality.

Author contribution statement MR and MNR designed the study and were responsible for developing crosses, phenotyping, and genotyping. JN advised the genotyping. TD and BF were responsible for FISH analysis. MNR, BJS and EJ supervised the study. MR wrote the first draft of the manuscript and all authors contributed to writing and editing the manuscript.

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Compliance with ethical standards

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

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