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Development of a PCR assay and marker-assisted transfer of leaf rust resistance gene *Lr58* into adapted winter wheats

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Abstract Leaf rust resistance gene *Lr58* derived from *Aegilops triuncialis* L. was transferred to the hard red winter wheat (HRWW) cultivars Jagger and Overley by standard backcrossing and marker-assisted selection (MAS). A co-dominant PCR-based sequence tagged site (STS) marker was developed based on the sequence information of the RFLP marker (*XksuH16*) diagnostically detecting the alien segment in T2BS·2BL-2^{t}L(0.95). STS marker *Xncw-Lr58-1* was used to select backcross F_1 plants with rust resistance. The co-dominant marker polymorphism detected by primer pair NCW-Lr58-1 efficiently identified the homozygous BC_3F_2 plants with rust resistance gene *Lr58*. The STS marker *Xncw-Lr58-1* showed consistent diagnostic polymorphism between the resistant source and the wheat cultivars selected by the US Wheat Coordinated Agricultural Project. The utility and compatibility of the STS marker in MAS programs involving robust genotyping platforms was demonstrated in both agarose-based and capillary-based platforms. Screening backcross derivatives carrying *Lr58* with various rust races at seedling stage suggested the transferred rust resistance in adapted winter wheats is stable in both cultivar backgrounds. *Lr58* in adapted winter wheat backgrounds could be used in combination with other resistance genes in wheat rust resistance breeding.

Keywords Wheat · Rust resistance · Marker-assisted selection · Mapping

Abbreviations

MAS	Marker assisted selection
STS	Sequence tagged sites
RFLP	Restriction fragment length polymorphism
HRWW	Hard red winter wheat
CAP	Co-ordinated agricultural project

Introduction

Rusts are severe diseases of wheat worldwide. Of the three rusts leaf rust, caused by *Puccinia triticina* (Eriks.), is a very damaging fungal disease of wheat (Kolmer 1996). Some yield losses from leaf rust are reported in all the wheat-growing areas of the world every year. Yield losses from leaf rust have been significant in the past decade especially in the Midwest and Pacific Northwest of the USA.

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Developing and deploying host genetic resistance through resistance breeding has been one of the most successful ways to protect wheat varieties against rusts. To date, about 59 leaf rust resistance genes are catalogued (McIntosh et al. 2010) but only a handful of these resistance genes have been deployed (McIntosh et al. 1995; R.A. McIntosh, personal communication 2006). Rapid changes in the virulence characteristics of rust populations pose a continuous threat to the effectiveness of the existing rust resistance genes deployed in agriculture. A constant search for new and effective sources of rust resistance and their transfer into wheat cultivars is needed to counterbalance the continuous evolution in rust populations.

Developing durable resistance to rusts was proposed as a breeding strategy to minimize yield losses from leaf rust (Johnson 1983, 1988). Deployment of different genes in different geographic regions and gene pyramiding are proposed strategies to increase the durability of resistance. These approaches depend upon characterization of spectrum of resistance genes in question and pyramiding them for effectiveness against the target pathogen population (Watson and Singh 1952). Pyramiding major genes for resistance in a single genotype by conventional breeding may be time-consuming and laborious when one or more of the genes are effective against all known isolates of the pathogen. The availability of DNA-based markers closely linked with resistance genes could make the transfer of multiple genes into the same plant feasible (Paterson et al. 1991). Molecular marker-assisted selection (MAS) offers the advantage that wheat breeders can follow marker-linked resistance genes during cultivar development (Dekkers and Hospital 2002; Dubcovsky 2004).

Previously, we transferred rust resistance gene *Lr58* from *Aegilops triuncialis* into wheat in the form of wheat-alien translocation T2BS·2BL-2^{t}L(0.95). This translocation with leaf rust resistance gene was mapped to the chromosome arm 2BL and tagged with RFLP markers *XksuH16*, *XksuF11* and *XksuD23* (Kuraparthy et al. 2007a). Translocation T2BS·2BL-2^{t}L(0.95) is genetically compensating due to its origin from homoeologous recombination between wheat and alien chromosome arms 2BL and 2^{t}L (Kuraparthy et al. 2007a). The objectives of the present study were to develop a PCR-based, co-dominant marker for *Lr58*, assess the cross-applicability of the STS marker, validate the utility of the STS marker by using it for marker-assisted back crossing and to confirm phenotypically the transfer of rust resistance in the backcross derivatives.

Materials and methods

Jagger (PI 593688) and Overley (PI 634974), popular winter wheat cultivars of the Southern Great Plains, were selected as recurrent parents for backcrossing. Both Jagger and Overley are brown-chaffed, early maturing, semi-dwarf cultivars. Jagger is characterized by high grain protein content and good baking quality. Overley shows excellent yield and is characterized by large seed and outstanding milling and baking quality. Although Jagger carries leaf rust resistance genes, *Yr17/Lr37/Sr38* complex from *Ae. ventricosa* and *Lr39*, it is now fully susceptible to the prevalent leaf rust races in the Southern Great Plains (Allan Fritz-personal communication). Overley carries *Lr39*, originally transferred from *Ae. tauschii* Coss. and is moderately resistant to stem rust (http://www.oznet.ksu.edu/library/crpsl2/L924.pdf). Virulence for *Lr39* has been reported in USA (Singh et al. 2004).

The wheat germplasm line T2BS·2BL-2^{t}L(0.95) (TA5605) derived from *Ae. triuncialis* (TA10438) in WL711 background (Kuraparthy et al. 2007a) was used. Germplasm line TA5605 is the source of leaf rust resistance gene *Lr58* as WL711, an Indian soft white spring wheat cultivar, is highly susceptible to leaf rust. However, WL711 carries leaf rust resistance gene *Lr13,* which is effective against race PNMQ of leaf rust at adult plant stage (Kuraparthy et al. 2007a).

Development of sequence tagged site (STS) markers

For developing STS marker(s), primer pairs were designed from the DNA sequences of RFLP probes KSUH16, KSUF11 and KSUD23 diagnostically detecting the *Ae. triuncialis* segment in the translocation T2BS·2BL-2^{t}L(0.95) (TA5605) (Kuraparthy et al. 2007a). Sequence information of these RFLP probes was kindly provided by Dr. John Fellers (USDA-ARS, Manhattan, KS). Primer design was done with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and amplicons of 110–400 bp were targeted.

Gel-based STS marker assay

Polymerase chain reaction (PCR) amplifications were performed in 25 µl reactions with 2.5 µl magnesium-free 10× PCR buffer, 1.5 µl magnesium chloride (25 mM), 2.5 µl dNTPs (2.5 mM each dNTP), 1 µl each forward and reverse primer (100 pmol/µl), 0.75–1 unit of Taq DNA polymerase (Bioline, Taunton, MA, USA) and 75 ng DNA in a gradient Mastercycler thermal cycler (Eppendorf AG, Hamburg, Germany). PCR conditions were: initial denaturation of 94°C for 8 min; 35 cycles of three consecutive steps 94°C for 1 min, 52°C for 1 min, 72°C for 2 min, 72°C for 10 min, and 4°C to end. Primer sequences of the STS marker *Xncw-Lr58-1* are; forward primer: TCACTTTGGTCAGGGTAGG G, reverse primer: CGACGAGGTCCTGATATGGT. Amplicons were resolved in 1% agarose gel with electrophoresis carried out at 65 V for 2.5 h.

Validation of the STS marker in US wheat germplasm

The validation of the STS marker for MAS was performed on a set of 44 wheat cultivars/lines that were selected by the wheat Coordinated Agricultural Project (CAP) of the USDA-CSREES (Table 1). These cultivars/lines were used for developing mapping populations in wheat breeding and genetic analysis in US. The details about these cultivars and the derived mapping populations can be found at http://maswheat.ucdavis.edu/Mapping/index.htm.

For capillary based marker assay, PCR amplifications were performed in 12 µl reactions with 1.2 µl 10× PCR buffer (final 1.5 mM magnesium chloride), 0.96 µl dNTPs (2.5 mM), 0.3 µl of 10 µm forward primer labeled with 6-FAM (Integrated DNA Technologies, IA, USA), 0.3 µl reverse primer (10 µM) 0.09 µl of Taq DNA polymerase (5 units/µl) (New England BioLabs), 7.15 µl H_2O and 2 µl of DNA (25 ng/µl). PCR conditions were an initial denaturation of 94°C for 3 min; 41 cycles of three consecutive steps of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, 72°C for 10 min, and 4°C to end.

ABI 3130XL Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using 36 cm capillaries was used to resolve the PCR products at the USDA-ARS Regional Small Grain Genotyping Laboratory, North Carolina State University, Raleigh, NC. The ABI generated profiles were screened using GeneMarker V. 1.5 software (SoftGenetics, State College, PA).

During the fall of 2004 the initial crosses were made between the recurrent parents (Jagger and Overley) as females and the donor germplasm line (TA5605) as male parent in the greenhouse. In subsequent years, F_1 plants were backcrossed as male parents to the recurrent parents in the greenhouse. Diagnostic RFLP based DNA marker polymorphism detected by probe KSUH16 was used to select BC_1F_1 plants with rust resistance gene *Lr58* in both Jagger and Overley backgrounds. Rust resistant BC_2F_1 and BC_3F_1 plants and homozygous BC_3F_2 plants with rust resistance gene *Lr58* were selected through MAS using co-dominant diagnostic PCR based STS marker *Xncw-Lr58-1* (Fig. 1). After selecting rust resistant backcross F_1s and homozygous BC_3F_2 plants using MAS, backcross derivatives were vernalized for 6 weeks. After vernalization, two backcross F_1 plants were planted per pot filled with a regular greenhouse soil mix and grown in the greenhouse. The DNA isolation, Southern blotting, and hybridizations were as reported in Kuraparthy et al. (2007b). The donor line and all the other germplasm lines that were used and developed in the present study are maintained by the Wheat Genetic and Genomic Resources Center at Kansas State University.

Rust reaction of the recurrent parents (Jagger, Overley), backcrossed derivatives and the donor germplasm line TA5605 was tested by screening the plants using five pathotypes (PRTUS6, PRTUS25, PRTUS35, PNMQ, MCDSB) (for virulence/avirulence formulae see Long et al. 2000) of leaf rust at the two-leaf seedling stage. Rust inoculations, incubation of the infected plants, and rust scoring followed Browder (1971).

Results

Development of sequence tagged site (STS) marker

STS markers were developed from three diagnostic RFLP markers *XksuH16*, *XksuF11* and *XksuD23*. A total of 12 primer pairs, five each from KSUH16 and KSUF11 and two from KSUD23, were tested on WL711 DNA as template. All the primer pairs gave clear amplicons of the expected 150–400 bp size. Out

Table 1 Details of the CAP wheat parents and breeding lines used for capillary based STS marker validation and their marker pattern using primer pair NCW-Lr58-1

Parent/breeding line	Accession number	Origin	Market class	Amplicon(s) (bp)
TA10438 (*Ae. triuncialis*)	–	Kansas/Punjab	N/A	404
TA5605 (*Lr58*)	–	Kansas/Punjab	N/A	137, 404
UC1110	GSTR13501	California	HWS	184, 486
CIMMYT-2	PI 610750	CIMMYT	HRS	193, 218
Rio Blanco	GSTR 12901	Idaho	HWS	137, 218
IDO444	GSTR 12902	Idaho	HWS	204, 206
IDO556	PI 620633	Idaho	S Club S	147
Zak	PI 607839	Washington	SWS	137, 218
McNeal	PI 574642	Montana	HRS	137, 218
Thatcher	PI 168659	Minnesota	HRS	218
OS9 (Stephens)	CItr 17596	Oregon	SWW	136, 218
Q36 (OR9900553)	–	Oregon	SWW	183
Louise	PI 634865	Washington	SWS	136, 218
Penawawa	PI 495916	Washington	SWS	222, 256, 310
Finch	PI 628640	Washington	SWW	137, 218
Eltan	–	Washington	SWW	137, 218
GRN*5/ND614-A	N/A	Minnesota	HWS	218
NY18/CC40-1	GSTR 10402	New York	SWW	136, 218
Rugby	CItr 17284	North Dakota	durum	–
Maier	PI 607531	North Dakota	durum	137
Reeder/Bw-277 "R" Entry#5	–	North Dakota	HRS	137, 218
CO940610	GSTR 10702	Colorado	HWW	137, 218
Platte	GSTR 10701	Colorado	HWW	137, 218
TAM 105	CItr 17826	Kansas	HRW	137, 218
Heyne	PI 612577	Kansas	HWW	137, 205
Jagger	PI 593688	Kansas	HRW	137, 168, 218
KS01HW163-4	N/A	Kansas	HWW	137, 218
Harry	PI 632435	Nebraska	HRW	137, 218
Wesley	PI 605742	Nebraska	HRW	137, 218
2174	GSTR 12101	Oklahoma	HRW	137, 218
Weebil	GSTR 10502	Texas	HRS	137
Jupeteco	GSTR 10501	Texas	HRS	–
*P91193	GSTR 10001	Indiana	SRW	141, 218, 405
P92201	GSTR 10002	Indiana	SRW	137, 218
Cayuga	PI 595848	New York	SWW	137, 218
Caledonia	PI 610188	New York	SWW	137, 218
Pio 25R26	–	Ohio	SRW	218
Foster	PI 593689	Ohio	SRW	136, 137, 218
USG3209	PI 658508	Virginia	SRW	137, 218
Jaypee	PI 592760	Virginia	SRW	137, 218
McCormick	PI 632691	North Carolina	SRW	137
RSI5 Source 97039	–	–	HRS	137
Express	PI 573003	Arizona	HRS	137, 218

Table 1 continued

Parent/breeding line	Accession number	Origin	Market class	Amplicon(s) (bp)
PIONEER 26R61	–	Pioneer	SRW	218
Kanqueen	CItr 12762	Kansas	HRW	137, 218
Clarks Cream	–	New York	HWW	218

* Accession showing marker fragment most similar to the diagnostic *Ae. triuancialis* fragment in TA5605

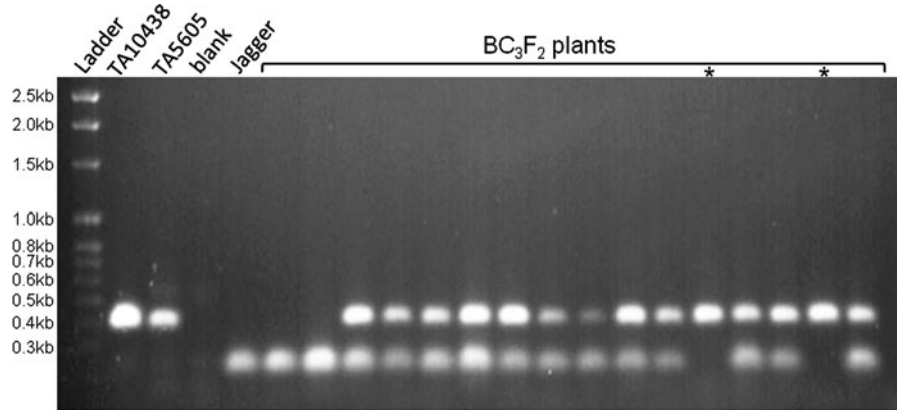

Fig. 1 Co-dominant sequence tagged site (STS) marker pattern of the parents and BC_3F_2 plants in Jagger background. The STS marker (*Xncw-Lr58-1*) was based on wheat RFLP marker *XksuH16*. PCR product was resolved on 0.9% agarose gel. Homozygous BC_3F_2 plants selected for the presence of *Ae. triuncialis*-derived resistance gene *Lr58* are indicated with an *asterisk*

of the total twelve primers tested, one primer pair NCW-Lr58-1, based on RFLP marker *XksuH16*, developed diagnostic polymorphism between the introgression line (TA5605) and recurrent parents Jagger and Overley in the agarose gel (0.9%) based assay (Fig. 1). Primer pair NCW-Lr58-1 amplified single low molecular weight monomorphic band (~200 bp) in WL711, Jagger and Overley. But substitution of this low molecular weight band by the ~400 bp band specific to *Ae. triuncialis* chromatin in TA5605 and its polymorphism with the recurrent parents Jagger and Overley suggests that *Xncw-Lr58-1* is a diagnostic co-dominant marker (Fig. 1).

Marker-assisted transfer of Lr58 from T2BS·2BL-2^tL(0.95) to Jagger and Overley

Screening of the source germplam line TA5605 with leaf rust isolates at both seedling and adult plant stage suggested that virulent races exist in North America to *Lr58* (Kuraparthy et al. 2007a). Furthermore, screening of the recurrent parents for leaf rust reaction at the seedling stage showed that races PRTUS25 and MCDL were virulent on Jagger (Table 2) and races PNMQ and PRTUS35 were virulent on Overley (Table 2).

Thirteen to twenty F_1 plants were grown in each backcross F_1 generation. First backcross F_1s with *Lr58* were selected using RFLP marker *XksuH16* in MAS. Rust resistant BC_2F_1s, BC_3F_1s and homozygous BC_3F_2 were selected by using PCR based STS marker *Xncw-Lr58-1* in both cultivar backgrounds. In the RFLP analysis, six enzymes (*Eco*RI, *Eco*RV, *Bam*HI, *Dra*I, *Xba*I, *Hind*III) were tested between donor line (TA5605) and Jagger and Overley for polymorphism using the KSUH16 probe. While restriction enzyme *Bam*HI produced polymorphism between TA5605 and Jagger, *Eco*RV detected polymorphism between TA5605 and Overley. Rust resistant BC_1F_1 plants were selected based on the presence of 2^tL specific *XksuH16* marker alleles (data not shown). BC_2F_1s and BC_3F_1 plants with resistant gene *Lr58* were selected based on the presence of 2^tL specific allele of the STS marker *Xncw-Lr58-1* in both the recurrent parent

Table 2 Infection types (ITs) of BC_3F_4 plants homozygous for the presence and absence of the *Ae. triuncialis* segment along with the parents of backcrossing. All lines were tested for co dominant RFLP marker *XksuH16* and STS marker *Xncw-Lr58-1*. At least 4 plants from each line were inoculated with *Puccinia triticina* races PRTUS6, PRTUS25, PRTUS35, PNMQ and MCDL. Jagger derivatives: 07-370; Overley derivatives: 07-371

Cultivar/line/BC3F3 plant	Diagnostic *Ae. triuncialis* specific marker allele		Infection type				
			Race				
	Xncw-Lr58-1	*XksuH16*	PNMR(PNMQ)	MCDSB (MCDL)	PRTUS6	PRTUS25	PRTUS35
Wichita	−	−	4	3+	4	4−	3+
Jagger	−	−	33+	3+	3+	3−	3+
Overley	−	−	3−	NT	;1+	0;	3
TA5605	+	+	;1	;1	;	0	33+
WL711	−	−	33+	3+	3+	3+	3+
07-370-2A-S2	−	−	3+	3+	3	3+	NT
07-370-9B-5	+	+	1	;	1−	0	NT
07-370-9B-8	+	+	1+	;	;	2	NT
07-371-1-7	+	+	NT	0	;	0	3
07-371-1-16	+	+	NT	0	1	0	NT
07-371-2-9	+	+	1+	;	NT	;	3

ITs of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). Seedling ITs are *0* no uredinia or other macroscopic sign of infection, *;* no uredinia but small hypersensitive necrotic or chlorotic flecks present, *N* necrotic areas without sporulation, *1* small uredinia surrounded by necrosis, *2* small to medium uredinia surrounded by necrosis or chlorosis (green islands may be surrounded by necrotic or chlorotic border), *3* medium uredinia with or without chlorosis, *4* large uredinia without chlorosis, X heterogeneous, similarly distributed over the leaves, C more chlorosis than normal for the IT, ± uredinia somewhat larger than normal for the IT, NT not tested. A range of variation between ITs was recorded with the most prevalent IT listed first

backgrounds. The BC_3F_2 plants homozygous for *Lr58* were selected from selfed progeny of a single BC_3F_1 plant, based on the co-dominant marker alleles of *Xncw-Lr58-1* (Fig. 1). Selected plants are being advanced to develop homozygous BC_3F_6 lines. Because the rust resistant donor line TA5605 was in a spring wheat background, resistant backcross F_1s were selected for winter type. Even after vernalization, winter types were easily identified because they flowered much later than the spring types. Rust resistant derivatives in both backgrounds were also selected for flowering time similar to their respective recurrent parents during the BC as well as selfed generations.

Validation of STS marker in the US wheat germplasm

To test the applicability of the STS marker in the US wheat-breeding programs, we validated the marker *Xncw-Lr58-1* on a set of wheat cultivars/lines selected by the wheat CAP in both gel-based and capillary-based platforms. In the gel-based assay this marker was polymorphic for all the CAP parents except for P91193, a soft red winter wheat from Indiana (data not shown). Resolving the PCR products of CAP parents along with the donor accession of *Ae. triuncialis* (TA10438) and donor germplasm line TA5605 on an ABI 3130XL sequencing platform suggested that most of the cultivars/lines including WL711 had a 137 and 218 bp amplicons of the marker *Xncw-Lr58-1*. While the donor *Ae. triuncialis* accession (TA10438) showed 404 bp fragment, germplasm line (TA5605) showed 137 and 404 bp fragments suggesting that 404 bp fragment is diagnostic for the alien segment carrying *Lr58* in capillary platforms. None of the CAP parents showed the 404 bp fragment except P91193 from Indiana, which showed 405 bp fragment suggesting that marker *Xncw-Lr58-1* could be used for MAS involving capillary platforms in all the major wheat breeding programs in the United States (Table 1).

Evaluating the backcross derivatives for rust resistance

Screening the backcross derivatives (BC_3F_4 plants) at seedling stage with leaf rust races showed that the infection types of the backcross derivatives were similar to the original source germplasm line to all the four rust pathotypes tested (Table 2) suggesting that the rust resistance due to *Lr58* was stably transferred to the adapted winter wheats during the MAS.

Discussion

Selecting backcross F_1 plants and their homozygous resistant derivatives based on the leaf rust resistance phenotype at seedling stage was not feasible in the present study because the recipient winter wheats carry known rust resistance genes. Thus the STS DNA marker (*Xncw-Lr58-1*) diagnostically identifying the *Ae. triuncialis* segment was used for the MAS of *Lr58* into Jagger and Overley.

Tight linkage between the target gene and the linked marker(s) increases the efficiency of MAS. If a robust polymorphic DNA marker is located in the alien segment the transfer of a target gene located in an alien segment is often efficient and accurate because alien segment do not recombine with wheat chromosomes, and inherit as single Mendelian factors. Therefore, marker *Xncw-Lr58-1* diagnostically detecting the rust resistant introgression T2BS·2BL-2^tL(0.95) acts almost like a 'perfect marker' for *Lr58*.

Although RFLP markers (*XksuH16, XksuF11 and XksuD23*) diagnostically detecting the alien segment in T2BS·2BL-2^tL(0.95) was efficient in genotyping the derivatives, it is cumbersome and expensive to use RFLP markers in wheat breeding programs. Further, one PCR based SSR marker *Xcfd50* was previously identified as diagnostic for the alien segment in T2BS·2BL-2^tL(0.95), but because of its dominant nature of inheritance it was not useful to identify homozygous resistant F_2 plants and consequently less suitable for MAS (Kuraparthy et al. 2007a). In the present study a PCR based co-dominant STS marker (*Xncw-Lr58-1*) was developed. The co-dominant nature of the STS marker could help discern the heterozygotes which is of great value in early generations of advancement (i.e. BC_1, BC_2, F_2 etc.) when transferring *Lr58* into adapted cultivars. Detection of diagnostic co-dominant polymorphism in both the agarose-based and capillary-based platforms suggests the utility and compatibility of the *Xncw-Lr58-1* in wheat MAS programs involving robust genotyping platforms in US. Although *Ae. triuncialis* specific diagnostic band of TA5605 is monomorphic with P91193 in gel based assay, MAS of *Lr58* using *Xncw-Lr58-1* could be possible in the capillary based platforms for marker assisted breeding of cultivars involving P91193 of Indiana.

Screening the backcross derivatives carrying *Lr58* with rust races at the seedling stage suggested that expression of the transferred rust resistance in adapted winter wheats is stable in both recurrent parent backgrounds (Table 2). Winter wheat germplasm with *Lr58* will provide breeders with adapted lines having an additional source of resistance that can be used in combination with other rust resistance genes in breeding for durable resistance. The availability of molecular markers linked with rust-resistance genes can facilitate pyramiding of more than one gene in new wheat cultivars. Since linked molecular markers are now available for *Lr34/Yr18* and *Lr46/Yr29* (Bossolini et al. 2006; Mateos-Hernandez et al. 2006; Lagudah et al. 2006, 2009, Dakouri et al. 2010), these slow rusting genes could be combined with major genes to extend the life of major genes.

DNA markers have been reported for several alien segments carrying rust resistance genes (Dubcovsky et al. 1998; Helguera et al. 2000, 2003; Mago et al. 2002; Seah et al. 2001; Kuraparthy et al. 2009). Few of these alien segments with rust resistance were transferred to adapted wheats (Helguera et al. 2003; Kuraparthy et al. 2009), but most germplasm lines/cultivars with alien segments have an agronomic penalty because of the associated linkage drag (Helguera et al. 2003; Knott 1968). To improve agronomic characteristics of these germplasm/cultivars with large alien segments further chromosome engineering is necessary (Lukaszewski 2006; Zhang et al. 2005). The adapted winter wheats with *Lr58* developed in the present study could be useful in agriculture because the introgressed alien segment with rust resistance was very small with apparently less linkage drag (Kuraparthy et al. 2007a). Furthermore, rust resistant backcross derivatives selected in the present study did not show any obvious effect on plant growth and development. The isolines will be used to further

assess the effect of the alien introgression on agronomic performance and end use quality.

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