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ORIGINAL ARTICLE

Development of a diagnostic co-dominant marker for stem rust resistance gene *Sr47* introgressed from *Aegilops speltoides* into durum wheat

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Abstract

Key message **A robust and diagnostic STS marker for stem rust resistance gene *Sr47* was developed and validated for marker-assisted selection.**

Abstract Stem rust (caused by *Puccinia graminis* f. sp. *tritici*, *Pgt*) resistance gene *Sr47*, originally transferred from *Aegilops speltoides* to durum wheat (*Triticum turgidum* subsp. *durum*) line DAS15, confers a high level of resistance to *Pgt* race TTKSK (Ug99). Recently, the durum Rusty 5D(5B) substitution line was used to reduce the *Ae. speltoides* segment, and the resulting lines had *Sr47* on small *Ae. speltoides* segments on wheat chromosome arm 2BL. The objective of this study was to develop a robust marker for marker-assisted selection of *Sr47*. A 200-kb segment of the *Brachypodium distachyon* genome syntenic with the *Sr47* region was used to identify wheat expressed sequence tags (ESTs) homologous to the *B. distachyon* genes. The wheat EST sequences were then used to develop sequence-tagged site (STS) markers. By analyzing the markers for polymorphism between Rusty and DAS15, we identified a co-dominant STS marker, designated as *Xrwgs38*, which amplified 175 and 187 bp fragments from wheat chromosome 2B and *Ae. speltoides* chromosome 2S segments, respectively. The marker co-segregated with the *Ae. speltoides* segments carrying *Sr47* in the families from four BC_2F_1 plants, including the parent plants for durum lines RWG35 and RWG36 with the pedigree of Rusty/3/Rusty 5D(5B)/DAS15//47-1 5D(5B). Analysis of 62 durum and common wheat cultivars/lines lacking the *Sr47* segment indicated that they all possessed the 175-bp allele of *Xrwgs38*, indicating that it was diagnostic for the small *Ae. speltoides* segment carrying *Sr47*. This study demonstrated that *Xrwgs38* will facilitate the selection of *Sr47* in durum and common wheat breeding.

Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn. (*Pgt*), is one of the most devastating diseases of durum (*Triticum turgidum* L. subsp. *durum*, $2n = 4x = 28$, AABB) and common wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD). The *Pgt* race TTKSK, commonly known as Ug99, was found in Uganda in 1998 (Pretorius et al. 2000), and it poses a great threat to wheat production worldwide (Singh et al. 2011). Three strategies, including eradication of the alternate host (barberry; *Berberis vulgaris* L. and *B. canadensis* Mill.), chemical application, and utilization of resistant cultivars, can be used to control stem rust. Although eradication of the alternate host can reduce the chance of generating new virulent races and initial inoculum (Roelfs 1982), it would be less effective on races already present in the environment, including Ug99. Chemical control can be effective; however, there are long-term environmental concerns. The best strategy for controlling Ug99 is to develop cultivars carrying stacked (i.e., multiple) Ug99-effective resistance genes (Singh et al. 2006, 2011).

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To date, at least 29 stem rust resistance (*Sr*) genes, including *Sr2*, *Sr9h/SrWeb*, *Sr13*, *Sr21*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr40*, *Sr42*, *Sr43*, *Sr44, Sr45*, *Sr46*, *Sr47*, *Sr51*, *Sr52*, *Sr53*, *Sr55*, *Sr57*, and *Sr58*, have been found to confer resistance to Ug99 (see Yu et al. 2014; Niu et al. 2014; Yu et al. 2015). However, the levels of resistance conferred by these *Sr* genes vary. For genes conferring a high level of resistance such as *Sr26*, *Sr35*, *Sr36*, and *Sr39* (Jin et al. 2007), selection can be based solely on phenotype if there are not multiple *Sr* genes segregating in the population. For crosses in which single genes confer partial or adult resistance such as *Sr2*, *Sr55*, *Sr56*, and *Sr57* (Spielmeyer et al. 2003; Singh et al. 2013; Bansal et al. 2014; Herrera-Foessel et al. 2014), it is difficult to make selections without marker assistance. Selection without markers is also difficult if multiple genes are segregating in the population. Thus, development of diagnostic or tightly linked markers to the Ug99-effective *Sr* genes is essential for developing wheat cultivars with resistance to stem rust using a gene-pyramiding strategy in breeding programs.

Stem rust resistance gene *Sr47* was originally transferred from an accession (PI 369590) of *Aegilops speltoides* Tausch ($2n = 2x = 14$, SS) into durum wheat line DAS15 through *ph1b*-induced homoeologous recombination by Dr. L.R. Joppa (USDA-ARS, Fargo, ND, USA). It was located on a T2BL-2SL•2SS translocation chromosome where the distal 2BL segment accounted for less than 10 % of the long arm, with the rest of the chromosome originating from *Ae. speltoides* (Faris et al. 2008). Recently, the *Ae. speltoides* segment in DAS15 was reduced to small segments through crossing and backcrossing to a durum 5D(5B) substitution line to induce homoeologous recombination between chromosomes 2B and 2S (Klindworth et al. 2012). The characterization of recombinant lines containing shortened *Ae. speltoides* chromosomal segments showed that *Sr47* was located on chromosome arm 2BL. The seedling infection type (IT) conditioned by *Sr47* was IT 0; to most *Pgt* races, though to TTKSK the seedling IT varied from IT 0; to 0;2. Because the IT conditioned by *Sr* genes is highly correlated with field response (Jin et al. 2007), *Sr47* is considered to be highly effective against TTKSK. *Sr47* also confers a high level of resistance to many North American *Pgt* races including TPMKC, TPPKC, TMLKC, TCMJC, THTSC, RHTSC, RTQQC, QTHJC, QFCSC, QCCJB, MCCFC, HKHJC, and HPGJC (Klindworth et al. 2012).

To facilitate MAS for *Sr47*, five SSR markers mapped to 2BL were evaluated for their association with *Sr47* in the progenies developed from a cross between DAS15 and a durum 5D(5B) substitution line (Klindworth et al. 2012). Among them, *Xgpw4112*, *Xgpw4165*, and *Xgwm47* are dominant markers in repulsion, which produces a null allele from the *Ae. speltoides* segments. *Xgwm501* is the only dominant marker in coupling phase, which amplifies a 109-bp fragment from *Ae. speltoides*. *Xgpw4043* is a co-dominant marker but it is difficult to score. Therefore, the objective of the current study was to develop a robust co-dominant marker for MAS of *Sr47* in durum and common wheat breeding programs.

Materials and methods

Plant materials

Durum wheat-*Ae speltoides* chromosome translocation line DAS15 (Faris et al. 2008) was previously crossed to the 5D(5B) substitution of durum lines Rusty (Klindworth et al. 2006) and 47-1 to develop new allosyndetic recombinants carrying *Sr47* in small *Ae. speltoides* segments (Klindworth et al. 2012). Based on previous data of the stem rust resistance and fluorescence genomic in situ hybridization, four BC_2F_1 plants 0406, 0439, 0696, and 0735 having the pedigree of Rusty/3/Rusty 5D(5B)/DAS15//47-1 5D(5B) were identified as heterozygous Ti2BL-2SL-2BL•2BS allosyndetic recombinants carrying *Sr47* in small *Ae. speltoides* segments of similar sizes. The four plants were advanced to generate plants homozygous for *Sr47*, and 36 BC_2F_3 plants from the four families were used for marker analysis in the study. A subset of the BC_2F_3 families derived from the 36 BC_2F_3 plants were used to map the newly developed marker for *Sr47*. The common wheat landrace 'Chinese Spring' (CS) and three CS nulli-tetrasomic lines N2AT2D (nullisomic for 2A and tetrasomic for 2D, which was maintained with monosomic 2A), N2BT2A (nullisomic for 2B and tetrasomic for 2A), and N2DT2A (nullisomic for 2D and tetrasomic for 2A) were also included in the marker analysis.

Stem rust resistance evaluation

The 36 BC_2F_3 plants derived from the four BC_2F_1 plants 0406, 0439, 0696, and 0735 were tested against *Pgt* race TMLKC at the seedling stage. Following the procedures of Williams et al. (1992), individual plants were tested by planting one seed per super-cell cone (Stuewe and Sons, Inc., Corvallis, OR, USA) filled with Sunshine SB100 mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA) supplemented with Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Marysville, OH, USA). The seedlings were grown in the greenhouse at 20–23 °C with 16/8 h (day/night) photoperiod. Seven-day-old seedlings were inoculated with urediniospores of *P. graminis* f. sp. *tritici* suspended in light mineral oil. The plants remained in a subdued light mist chamber for 24 h after inoculation. Seedlings were

then moved to a greenhouse at 20–23 °C with supplemental fluorescent light to maintain a 16/8 h (day/night) photoperiod. Plants were scored for infection types 14 days after inoculation, using the scale of Stakman et al. (1962), where 0 = immune, ; = necrotic flecks, 1 = small necrotic pustules, 2 = small to medium-sized chlorotic pustules with green island, 3 = medium-sized chlorotic pustules, and 4 = large pustules without chlorosis. The plants with IT 2 or lower were considered resistant while the plants with IT scores of 3 or greater were considered susceptible. For progeny tests, a subset of 30 BC_2F_3 families derived from the 36 BC_2F_3 plants were also tested with TMLKC at the seedling stage, with 25–30 plants per family being tested. The inoculation and scoring procedure was similar to those described above except that each family was planted in a single 9.0 cm clay pot and primary leaves were excised for scoring.

Sequence-tagged site (STS) marker development and validation

We used Blastn to search the GrainGenes database with the sequences of the SSR marker amplicons around *Sr47* (Somers et al. 2004; Klindworth et al. 2012) with an *e* value <e-1 and 100 % identity to the primer sequences, and identified an *Aegilops tauschii* genomic sequence, AX462329, corresponding to *Xcfd73-2B*. We then searched the *Brachypodium distachyon* genome sequence with AX462329, and identified a 200-kb syntenic segment of *B. distachyon* chromosome 5 (http://www.phytozome.org/cgi-bin/gbrowse/brachy/). Using this *B. distachyon* genomic sequence, we searched the NCBI Mapped Wheat EST database (http://wheat.pw.usda.gov/GG2/blast.shtml) for the EST sequences with *e* value <e-40. Based on the EST sequences, 11 pairs of primers were designed using the program Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3) (Koressaar and Remm 2007; Untergrasser et al. 2012).

For marker analysis, a leaf sample was taken from each plant and DNA was extracted from freeze-dried leaf samples using the procedure of Dellaporta et al. (1983). Parental lines Rusty and DAS15 were used to identify polymorphisms between the two parents. After the polymorphic STS markers were identified, they were analyzed on the four BC_2F_1 plants (0406, 0439, 0696 and 0735) and their derived 36 BC_2F_3 individuals, along with Rusty, DAS15, and three CS nulli-tetrasomic lines N2AT2D, N2BT2A, and N2DT2A. The polymerase chain reaction (PCR) amplification was carried out as described by Yu et al. (2009). The PCR products were electrophoresed on 6 % polyacrylamide gels and stained with Gel-Red, then scanned with a Typhoon 9410 imager (GE Healthcare, Waukesha, WI, USA).

Molecular marker validation for marker-assisted selection

A set of 62 durum and common wheat cultivars and breeding lines were used to validate the usefulness of the

Table 1 Primer information for sequence-tagged site marker *Xrwgs38* linked to stem rust resistance gene *Sr47* introgressed from *Aegilops speltoides* into durum wheat

Marker	Forward primer	Reverse primer	T_m, °C[a]	EST accession[b]
Xrwgs38	AGTGGCTGCAGTGGAATTG	ACCGAGAACAAGGAGAAGCA	60	BF484929

[a] Melting temperature at the condition of 50 mM Na^+

[b] Wheat ESTs mapped to deletion bin C-2AL1-0.85 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi)

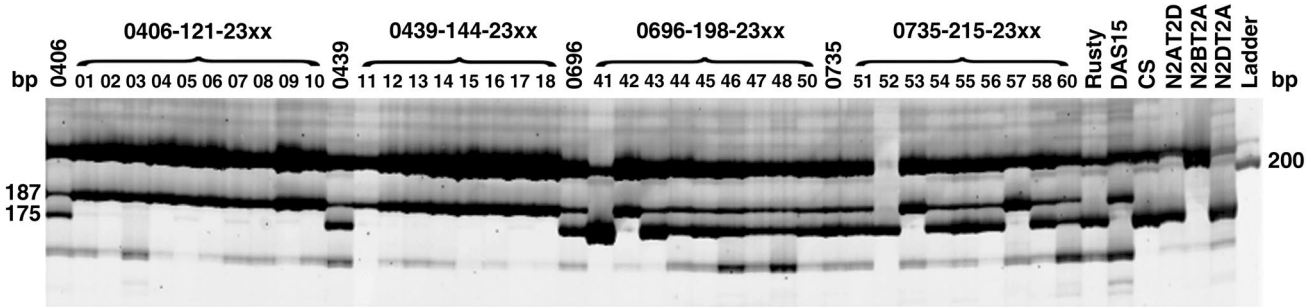

Fig. 1 Gel image of the co-dominant sequence-tagged site marker *Xrwgs38* linked to *Sr47*. Amplicons were separated on 6 % non-denaturing polyacrylamide gels. *Lanes* are identified by Plant ID No. The Plant ID no., which corresponds to Table 2, shows genetic relationships, e.g., 0406-121-23xx is a family composed of plants 01 through 10. Plant 0406-121-2301 was a BC_2F_3 progeny of BC_2F_1 Plant 0406. CS represents Chinese Spring, N2AT2D, N2BT2A, and N2DT2A represent the CS nullisomic–tetrasomic stocks in which the N-designation indicates the nullisomic chromosome and the T-designation indicates the tetrasomic chromosome. *bp* base pair

Table 2 Marker *Xrwgs38* genotypes of 36 BC_2F_3 plants derived from four BC_2F_1 plants having the pedigree of Rusty/3/Rusty 5D(5B)/DAS15//47-1 5D(5B) and infection type (IT) of the 36 BC_2F_3 plants and 30 BC_2F_3 derived families to race TMLKC of *Puccinia graminis* f. sp. *tritici*

Plant ID no.[a]	Generation	*Xrwgs38* genotype[b]	IT[c] to TMLKC	No. of progeny with IT[c]	
				0; or 0;1$^-$	34
0406	BC_2F_1	AB	0;	8	3
0439	BC_2F_1	AB	0;	11	1
0696	BC_2F_1	AB	0;	11	0
0735	BC_2F_1	AB	0;	4	0
0406-121	BC_2F_2	–	0;	9	0
0439-144	BC_2F_2	–	0;C	8	0
0696-198	BC_2F_2	–	0;C	7	2[d]
0735-215	BC_2F_2	–	0;	8	2[d]
0406-121-2301	BC_2F_3	BB	0;1$^=$	31	0
0406-121-2302	BC_2F_3	BB	0;1$^=$	27	0
0406-121-2303	BC_2F_3	BB	0;1$^=$	30	0
0406-121-2304	BC_2F_3	BB	Esc	30	0
0406-121-2305	BC_2F_3	BB	0;1$^=$	26	0
0406-121-2306	BC_2F_3	BB	0;1$^=$	–	–
0406-121-2307	BC_2F_3	BB	0;1$^=$	28	0
0406-121-2308	BC_2F_3	BB	0;1$^=$	29	0
0406-121-2309	BC_2F_3	BB	0;1$^=$	27	0
0406-121-2310	BC_2F_3	BB	0;1$^=$	–	–
0439-144-2311	BC_2F_3	BB	0;1$^=$	25	0
0439-144-2312	BC_2F_3	BB	0;1$^=$	30	0
0439-144-2313	BC_2F_3	BB	0;1$^=$	30	0
0439-144-2314	BC_2F_3	BB	0;1$^=$	27	0
0439-144-2315	BC_2F_3	BB	0;1$^=$	29	0
0439-144-2316	BC_2F_3	BB	0;1$^=$	30	0
0439-144-2317	BC_2F_3	BB	0;1$^=$	–	–
0439-144-2318	BC_2F_3	BB	0;1$^=$	30	0
0696-198-2341	BC_2F_3	AA	34	–	–
0696-198-2342	BC_2F_3	BB	0;1$^=$	31	0
0696-198-2343	BC_2F_3	AB	0;1$^=$	–	–
0696-198-2344	BC_2F_3	AB	0;1$^=$	27	3
0696-198-2345	BC_2F_3	AB	0;1$^=$	25	6
0696-198-2346	BC_2F_3	AB	0;1$^=$	26	3
0696-198-2347	BC_2F_3	AB	0;1$^=$	26	4
0696-198-2348	BC_2F_3	AB	0;1$^=$	24	4
0696-198-2350	BC_2F_3	AB	Esc	27	4
0735-215-2351	BC_2F_3	AB	0;1$^=$	21	9
0735-215-2352	BC_2F_3	AA	34	–	–
0735-215-2353	BC_2F_3	BB	0;1$^=$	30	0
0735-215-2354	BC_2F_3	AB	0;1$^=$	21	8
0735-215-2355	BC_2F_3	AB	0;1$^=$	24	5
0735-215-2356	BC_2F_3	AB	0;1$^=$	15	13
0735-215-2357	BC_2F_3	BB	0;1$^=$	29	0
0735-215-2358	BC_2F_3	AB	0;1$^=$	24	4
0735-215-2360	BC_2F_3	AB	0;1$^=$	23	5
Rusty		AA	34	0	15
DAS15		BB	0;	12	0

[a] Plant ID no. shows genetic relationships, e.g., the BC_2F_3 plant 0735-215-2360 was a progeny of the BC_2F_2 plant 0735-215, which in turn was a progeny of the BC_2F_1 plant 0735

[b] *Xrwgs38* genotypes: *AA* Rusty, *BB* DAS15, *AB* heterozygous

[c] The BC_2F_1 (0406, 0439, 0696, and 0735) stem rust data were previously reported by Klindworth et al. (2012), but all other data are unique to this study. Infection types follow Stakman et al. (1962) where 0, ;, 0;, 0, 1, 2, or combinations were considered low infection types, and 3–4 were considered high infection types; minus ($^-$) or double minus ($^=$) indicates pustules smaller or much smaller than normal, respectively, for that particular infection type; *C* chlorosis, – missing data, *Esc* escape

[d] Discrepancy in number of susceptible BC_2F_2 plants vs homozygous susceptible BC_2F_3 families is due to dropping some susceptible families from the analysis

markers closely linked to *Sr47* for marker-assisted selection. Among them, 42 cultivars were common wheat, 10 were durum wheat, and 10 were lines carrying stem rust resistance genes *Sr2*, *Sr13*, *Sr22*, *Sr25*, *Sr26*, *Sr35*, *Sr36*, *Sr40*, *Sr42/SrCad*, and *Sr46*. The original seed of the 62 cultivars and breeding lines was kindly provided by the wheat breeding and germplasm enhancement programs in Australia, Canada, China, and the United States. The DNA extraction and marker analysis were conducted using the procedures described above.

Results

We designed 11 pairs of primers based on syntenic information of the genomic region harboring *Sr47* in wheat chromosome 2B with *B. distachyon*, and identified four pairs that amplified polymorphic fragments between Rusty and DAS15. Among the four primer pairs, one amplified relatively large fragments (>900 bp) but with small size differences between the two parents; two amplified strong, medium-sized (400–600 bp) overlapping fragments, making them difficult to score; and one, designated as *Xrwgs38*, produced fragments of 187–175 bp from DAS15 and Rusty, respectively. The primer information for *Xrwgs38* is shown in Table 1. *Xrwgs38* amplified a 175-bp fragment from CS, N2AT2D, and N2DT2A but not from N2BT2A, confirming that the 175-bp fragment was derived from wheat chromosome 2B (Fig. 1). Heterozygosity at the *Xrwgs38* locus was detected in the BC_2F_1 plants 0406, 0439, 0696, and 0735 (Fig. 1), and

Table 3 Amplified fragment size (bp) from DAS15, Rusty and 62 wheat cultivars and breeding lines at the *Xrwgs38* locus linked to stem rust resistance gene *Sr47*

Cultivar/line	Origin[a]	Type	Fragment size (bp)
DAS15 (check)	ND	Durum wheat	187
Rusty (check)	USDA, ND	Durum wheat	175
Jimai 22	China	Common wheat	175
Yangmai 16	China	Common wheat	175
Shanrong 1	China	Common wheat	175
Shanrong 3	China	Common wheat	175
Jinan 17	China	Common wheat	175
Jinan 177	China	Common wheat	175
Zhengmai 9023	China	Common wheat	175
Amidon	ND	Common wheat	175
Howard	ND	Common wheat	175
Alsen	ND	Common wheat	175
Grandin	ND	Common wheat	175
Glenn	ND	Common wheat	175
Faller	ND	Common wheat	175
Glupro	ND	Common wheat	175
Ernest	ND	Common wheat	175
Steele-ND	ND	Common wheat	175
Reeder	ND	Common wheat	175
Mott	ND	Common wheat	175
Kulm	ND	Common wheat	175
Parshall	SD	Common wheat	175
Brick	SD	Common wheat	175
Russ	SD	Common wheat	175
Briggs	SD	Common wheat	175
Traverse	SD	Common wheat	175
Sabin	MN	Common wheat	175
Oklee	MN	Common wheat	175
Ulen	MN	Common wheat	175
Ada	MN	Common wheat	175
Tom	MN	Common wheat	175
Newton	KS	Common wheat	175
IL06-14262	IL	Common wheat	175
SD03028	SD	Common wheat	175
SD04581	SD	Common wheat	175
SD05085	SD	Common wheat	175
SD05W030	SD	Common wheat	175
ND495	ND	Common wheat	175
Lyman	SD	Common wheat	175
Granite	MN	Common wheat	175
Knudson	AgriPro	Common wheat	175
Norpro	MN	Common wheat	175
ND735	ND	Common wheat	175
Oxen	MN	Common wheat	175
Divide	ND	Durum wheat	175
Ben	ND	Durum wheat	175
Tioga	ND	Durum wheat	175
Grenora	ND	Durum wheat	175
Lebsock	ND	Durum wheat	175
Monroe	ND	Durum wheat	175

Table 3 continued

Cultivar/line	Origin[a]	Type	Fragment size (bp)
Alkabo	ND	Durum wheat	175
Mountrail	ND	Durum wheat	175
Snowmass	CO	Common wheat cultivar with *Sr2*	175
U5924-10-6	USDA, KS	Common wheat line with *Sr22*	175
Wheatear		Common wheat cultivar with *Sr25*	175
WA-1	Australia	Common wheat line with *Sr26*	175
U5930-13-5	USDA, KS	Common wheat line with *Sr35*	175
W2691SrTt-1		Common wheat line with *Sr36*	175
U5941-1-6	USDA, KS	Common wheat line with *Sr40*	175
HY438	Canada	Common wheat line with *SrCad*	175
W2691Sr13		Common wheat line with *Sr13*	175
TA4162-60	CIMMYT	Synthetic hexaploid wheat with *Sr13*	175
SW8	USDA, ND	Synthetic hexaploid wheat with *Sr46*	175

[a] Origin: *CO* Colorado, *KS* Kansas, *ND* North Dakota, *SD* South Dakota, *MN* Minnesota, *IL* Illinois

this result was in agreement with Klindworth et al. (2012) who found these plants to be heterozygous for *Sr47*.

Of 36 BC_2F_3 plants included in the marker analysis (Fig. 1), progeny tests for resistance to *Pgt* race TMLKC were conducted on 30 BC_2F_3 families (Table 2). Although the BC_2F_2 plants are not included in the marker analysis in Fig. 1, they were included in the rust tests to illustrate the parentage. Marker analysis indicated that the 18 BC_2F_3 plants derived from BC_2F_1 plants 0406 and 0439 were homozygous for the *Xrwgs38* amplicons from DAS15. Rust tests of 15 of the 18 BC_2F_3 families confirmed that these families were homozygous for resistance to race TMLKC (Table 2). The BC_2F_2 parental plants of each of these familial groups were 0406-121 and 0439-144, and no segregation was observed in the small progeny test that was conducted (Table 2). Therefore, BC_2F_2 plants 0406-121 and 0439-144 and all their progeny were homozygous for rust resistance and the DAS15 amplicon from *Xrwgs38*. In contrast, most of the families derived from plants 0696 and 0735 proved to segregate in BC_2F_2 and BC_2F_3 generations (Table 2). Two BC_2F_3 plants, 0696-198-2341 and 0735-215-2352 (Fig. 1), lacked amplicons from DAS15 for *Xrwgs38* and these plants were susceptible to TMLKC (Table 2). Seven BC_2F_3 plants from the 0696 family were heterozygous for marker *Xrwgs38* (Fig. 1), and progeny of six of these plants were tested with stem rust and all segregated for resistance (Table 2). Six BC_2F_3 plants from the 0735 family were heterozygous for marker *Xrwgs38* (Fig. 1), and progeny of all six families segregated for stem rust resistance (Table 2). Three BC_2F_3 families (0696-198-2342, 0735-215-2353, and 0735-215-2357) were observed to be derived from BC_2F_3 plants homozygous for DAS15 amplicons of marker *Xrwgs38* (Fig. 1) and these families were homozygous for resistance to TMLKC. The genotypes of all 30 BC_2F_3 plants matched the phenotypes of their families, indicating no recombination occurred between *Xrwgs38* and *Sr47*. Therefore, *Xrwgs38* was located on the small *Ae. speltoides* segments in the four translocation lines, and *Xrwgs38* can be used for MAS of *Sr47* on any of the small *Ae. speltoides* fragments present in 0406, 0439, 0696, and 0735 families.

Xrwgs38 was also tested on a panel of 52 durum and common wheat cultivars or breeding lines along with 10 lines that contain various *Sr* genes, including *Sr2*, *Sr13*, *Sr22*, *Sr25*, *Sr26*, *Sr35*, *Sr36*, *Sr40*, *Sr42/SrCad*, and *Sr46* (Table 3). All 62 lines tested possessed the 175-bp allele of *Xrwgs38,* and only DAS15 carried the 187-bp allele (Table 3).

Discussion

Selection in conventional plant breeding is made based on phenotypes, whereas MAS is conducted based on the marker genotypes associated with a trait. In theory, MAS has at least four advantages over phenotypic selection. First, for traits that are difficult, expensive, or time-consuming to evaluate, MAS is easier than phenotypic selection and can save time, resources, and effort. Fusarium head blight (caused by *Fusarium graminearum* Schwabe), cereal cyst nematode (*Heterodera avenae* Woll.), and root lesion nematode (*Pratylenchus* spp.) resistance in wheat are examples of traits that are difficult to evaluate (Buerstmayr et al. 2009; Miedaner and Korzun 2012; Eastwood et al. 1991; Eagles et al. 2001; Zwart et al. 2004). Secondly, MAS allows for pyramiding of multiple genes by screening multiple markers on individual plants of a population, thereby greatly expediting the production of breeding lines. Thirdly, for some traits such as dormancy, it is possible to do selection at the seedling stage using MAS, but not possible using phenotypic selection. Finally, in backcrossing a

recessive gene, phenotypic selection cannot be efficiently performed without one self-pollinated generation, while MAS can be performed on the immediate backcross progeny, reducing the breeding time by half.

Successful MAS in plant breeding depends on several factors. One of the key factors is polymorphism. The restricted availability of diagnostic markers is one of the major constraints of MAS (Miedaner and Korzun 2012). Very few markers are polymorphic in all genetic backgrounds, such as the markers for stem rust resistance genes (http://maswheat.ucdavis.edu/protocols/StemRust/index.htm). Another limiting factor is the marker quality/robustness. Co-dominant markers are ideal for MAS because the genotype for the marker of any individual developed from a cross can be definitively determined. Dominant markers are useful if they are in coupling phase with the gene for selection. However, dominant markers in repulsion phase are of little use. Pairing two dominant markers, one in coupling and the other in repulsion phase, may improve the use of the markers, but it can be more difficult to identify the marker alleles in various parental lines. Some markers detect multiple fragments due to the homoeology among the three wheat genomes. Pairing two markers means doubling the number of the bands for these markers. Therefore, development of robust co-dominant markers for genes of interest is crucial for successful application of MAS in breeding.

Sr47 is an excellent *Sr* gene that confers a high level of resistance to multiple races of the stem rust pathogen, including TTKSK. Previously, one co-dominant marker and four dominant markers, with three and one being in repulsion and coupling phases, respectively, were used to characterize *Sr47* (Klindworth et al. 2012). The newly developed STS marker *Xrwgs38* is a co-dominant marker for *Sr47*, which makes it among the few TTKSK-effective *Sr* genes, such as *Sr2* (Hayden et al. 2004; Mago et al. 2011), *Sr25* (Liu et al. 2010); *Sr35* (Zhang et al. 2010); *Sr36* (Tsilo et al. 2008); and *Sr39* (Niu et al. 2011) that can be deployed using MAS with co-dominant markers. *Xrwgs38* amplifies fragments that are clear and easy to score. The distinct difference in size between polymorphic bands makes it possible to use agarose gels, which are cheap and widely used in wheat breeders' laboratories.

Four allosyndetic recombinants identified as 0406, 0439, 0696, and 0735 were used in this study. From our prior study, the co-dominant marker *Xgpw4043* can detect the *Ae. speltoides* segments carrying *Sr47* in three of these recombinants, the exception being recombinant 0406 (Klindworth et al. 2012). In contrast, *Xrwgs38* can detect the *Ae. speltoides* segments carrying *Sr47* in all four of the BC_2F_1 plants (0406, 0439, 0696, and 0735). Two of the four recombinants (0406 and 0696) were previously designated as durum germplasm lines RWG35 and RWG36, respectively (Klindworth et al. 2012). We are currently using *Xrwgs38* to develop durum and bread wheat breeding lines for *Sr47* for the Upper Midwest in the United States (unpublished data).

In conclusion, because *Xrwgs38* produces a 187-bp amplicon from the *Ae. speltoides* donor accession that is uniquely different from the 175-bp amplicon present in the 62 durum and bread wheat varieties and breeding lines, the marker is diagnostic for the *Ae. speltoides* segment carrying *Sr47* in both durum and common wheat. The size difference (12 bp) between the two polymorphic fragments will allow breeders to analyze the marker using agarose gels. This marker will greatly facilitate MAS for *Sr47* in various durum and bread wheat breeding programs.

Author contribution statement SSX, GY, and JBR initiated the project, designed the experiment, conducted the marker development and analysis and prepared the manuscript; DK performed all stem rust tests. TLF and SZ provided assistance with stem rust tests; JDF provided assistance with marker analysis; all authors provided comments and revisions of the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest.

Ethical standards The experiments were performed in compliment with the current laws of United States of America.

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