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ORIGINAL PAPER

# Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*

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**Abstract** Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is one of the most destructive diseases of wheat. A new race of the pathogen named TTKSK (syn. Ug99) and its derivatives detected in East Africa are virulent to many designated and undesignated stem rust resistance genes. The emergence and spread of those races pose an imminent threat to wheat production worldwide. Genes *Sr25* and *Sr26* transferred into wheat from *Thinopyrum ponticum* are effective against these new races. DNA markers for *Sr25* and *Sr26* are needed to pyramid both genes into adapted germplasm. The previously published dominant markers Gb for *Sr25* and Sr26#43 for *Sr26* were validated with eight wheat lines with or without *Sr25* or *Sr26*. We tested six published STS (sequence tagged site) markers amplifying diagnostic bands of *Th. ponticum*. Marker BF145935 consistently amplified well and can be used as a co-dominant marker for *Sr25*. Among 16 STS markers developed from wheat ESTs mapped to deletion bin 6AL8-0.90-1.00, none was co-dominant for tagging *Sr26*. However, five 6A-specific markers were identified. Multiplex PCR with marker Sr26#43 and 6A-specific marker BE518379 can be used as a co-dominant marker for *Sr26*. The co-dominant markers for *Sr25* and *Sr26* were validated with 37 lines with known stem rust resistance genes. A diverse set of germplasm consisting 170 lines from CIMMYT, China, USA and other counties were screened with the co-dominant markers for *Sr25* and *Sr26*. Five lines with the diagnostic fragment for *Sr25* were identified, and they all have 'Wheatear' in their pedigrees, which is known to carry *Sr25*. None of the 170 lines tested had *Sr26*, as expected.

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## Introduction

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has historically caused severe wheat (*Triticum aestivum*) production losses worldwide, and had been controlled effectively with the deployment of resistant wheat cultivars for the last several decades. However, a new race of stem rust pathogen, Ug99, with virulence to a widely used resistance gene *Sr31*, was detected in Uganda in 1999 (Pretorius et al. 2000), and was named TTKS based on the North American stem rust race nomenclature system (Wanyera et al. 2006, Jin et al. 2008). Most wheat cultivars currently grown are susceptible to TTKS (Jin and Singh 2006; Singh et al. 2006), and the stem rust population is evolving rapidly. Another race, TTKST, with virulence to the widely used gene *Sr24* was detected in Kenya in 2006 (Jin et al. 2008). Only 1 year later yet another race, TTTSK, with virulence to gene *Sr36* was discovered in Kenya (Jin et al. 2009). Emergence and spread of these new races of stem rust pose an imminent threat to wheat production worldwide

(Singh et al. 2006) and demand the rapid development of wheat cultivars with durable resistance to stem rust.

The durability of effective resistance genes can be enhanced by deploying them as pyramids in cultivars. Genes *Sr25* and *Sr26* are among the few major genes effective against the TTKS lineage that includes races TTKST and TTTSK (Singh et al. 2006; Jin et al. 2007). Both *Sr25* (Sharma and Knott 1966) and *Sr26* (Knott 1961) genes were transferred into wheat from *Thinopyrum* (*Th*) *ponticum* (Podp.) Barkworth and Dewey ($2n = 10x = 70$) [syn. *Agropyron elongatum* (Host) Beauvois and syn. *Lophopyrum ponticum* (Podp.) Löve]. Gene *Sr25* and the linked leaf rust resistance gene *Lr19* were translocated onto the long arm of wheat chromosome 7D (Friebe et al. 1994). Initial use of germplasm containing *Sr25*/*Lr19* was limited because of linkage with another *Th. ponticum* derived gene that resulted in undesirable yellow flour. Knott (1980) produced two mutant lines, Agatha-28 and Agatha-235, with reduced levels of yellow pigment in flour. The *Sr25* gene was lost in the mutant line Agatha-235 (Friebe et al. 1994). Agatha-28, which contains *Sr25*/*Lr19*, was backcrossed into the Australian wheat backgrounds and has been used in the CIMMYT breeding program (Bariana et al. 2007).

The segment carrying *Sr26* was transferred to the long arm of wheat chromosome 6A (Friebe et al. 1994), and has been used as a source of resistance only in Australia where the first cultivar, Eagle, was released in 1971 (Martin 1971). Despite the reported yield penalty associated with the *Th. ponticum* segment (The et al. 1988), several cultivars with *Sr26* in addition to Eagle were developed and released (McIntosh et al. 1995). New lines with shortened alien segments have been developed and they do not suffer from the yield reduction of the original *Sr26* containing lines (Dundas et al. 2007).

Stacking two or more effective rust resistance genes into a common background using rust bioassays is challenging due to a lack of isolates with specific avirulence/virulence gene combinations that enable unambiguous assignments of resistance genotypes. This is particularly true for broadly effective genes such as *Sr25* and *Sr26* (Singh et al. 2006; Jin et al. 2007). Furthermore, field bioassays for the TTKS lineage and related races can only be conducted in regions where they are already present. So, molecular markers for *Sr25* and *Sr26* are needed to facilitate selection of desirable genotype combinations. Prins et al. (2001) converted an AFLP (amplified fragment length polymorphism) fragment specific for the *Th. ponticum* segment containing *Sr25*/*Lr19* into a dominant STS (sequence tagged site) marker Gb, which amplified a 130 bp fragment specific to *Sr25*/*Lr19* lines. Similarly, a dominant STS marker Sr26#43 for *Sr26* was developed (Mago et al. 2005). Both markers have been used for marker-assisted selection (MAS) in breeding programs (Bariana et al. 2007). The objectives of this study were to (1) test previously available markers for genes *Sr25* and *Sr26*; and (2) develop and validate co-dominant markers for *Sr25* and *Sr26*.

## Materials and methods

### Plant materials

Wheat cultivar 'Chinese Spring' and its chromosome group 6 and 7 nullisomic–tetrasomic lines (N6AT6D, N6BT6D, N6DT6B, N7AT7D, N7BT7A, and N7DT7A) (Sears 1966) were used for identifying DNA markers located on the targeted chromosomes 6A and 7D. Initially, eight wheat lines including two lines with *Sr25*, 'Wheatear' and CIMMYT line C80.1/3*Batavia//2*Weebil, the first *Sr26* cultivar 'Eagle', and five lines without genes *Sr25* or *Sr26*, 'Cranbrook', 'Weebil', MN02072-7, MN03130-1-62 and MN03148, were used to validate markers for *Sr25* and *Sr26*. Thirty-seven lines with known stem rust resistance genes and five genetic background cultivars (Table 1) were used to further validate the co-dominant markers for *Sr25* and *Sr26*. These lines were chosen because they are or are likely, resistant to races of the TTKS lineage (Jin et al. 2007). To test whether the co-dominant marker can be used to select for *Sr26* on shortened alien segments (Dundas et al. 2007), the recurrent parent 'Angas' and five lines with shortened alien segments, WA1, WA5, WA6, WA8 and WA9, were also genotyped with the co-dominant marker for *Sr26*. A total of 170 lines (Table 2) from several countries were screened with these co-dominant markers for *Sr25* and *Sr26*.

### DNA marker validation

Markers Gb (F: CATCCTTGGGGACCTC, R: CCAGC TCGCATACATCCA) (Prins et al. 2001) for *Sr25* and Sr26#43 (F: AATCGTCCACATTGGCTTCT, R: CGCA ACAAAATCATGCACTA) (Mago et al. 2005) for *Sr26* were used for initial tests. Ayala-Navarrete et al. (2007) developed STS markers from wheat ESTs mapped to chromosome 7DL (Qi et al. 2004), that is homoeologous to the translocated segment of *Th. ponticum* containing *Sr25* and *Lr19*. Six STS markers amplifying diagnostic bands of *Th. ponticum* were tested for use as co-dominant markers for *Sr25*. Dundas et al. (2007) reported that *Sr26* is located in the extreme distal portion of the 6Ae#1 chromosome. To develop DNA markers for *Sr26*, 16 wheat ESTs mapped to deletion bin 6AL8-0.90-1.00 (Qi et al. 2004) were chosen to design STS markers with Primer 3 software (Rozen and Skaletsky 2000). DNA extraction and PCR protocols were the same as described by Liu and Anderson (2003) with the exception of 400 nM instead of 100 nM for each primer,

**Table 1** Wheat lines with known stem rust resistance genes used to validate co-dominant markers for *Sr25* and *Sr26*

| Name | *Sr* gene | Background | Fragments amplified[a] | |
|---|---|---|---|---|
| | | | BF145935 | Sr26#43/ BE518379 (bp) |
| Vernstein | 9e | CS | 7A, 7D | 6A (303) |
| K253/3*Steinwell//8*LMPG | 9e | LMPG-6 | 7A, 7D | 6A (303) |
| Combination VII | 13(+17) | W2691 | 7A, 7D | 6A (303) |
| Khapstein/9*LMPG | 13 | LMPG-6 | 7A, 7D | 6A (303) |
| Line A sel | 14 | W2691 | 7A, 7D | 6A (303) |
| CS_T_mono_deriv | 21 | CS | 7A, 7D | 6A (303) |
| T. mono. Deriv./8*LMPG | 21 | LMPG-6 | 7A, 7D | 6A (303) |
| Sr22TB | 22 | | 7B?, 7D | 6A (303) |
| T. momoc.Deriv./9*LMPG | 22 | LMPG-6 | 7A, 7D | 6A (303) |
| LcSr24Ag | 24 | Little Club | 7A, 7D | 6A (303) |
| Agent/9*LMPG | 24 | LMPG-6 | 7A, 7D | 6A (303) |
| LcSr25Ars | 25 | Little Club | 7Ae#1, 7A, 7D | 6A (303) |
| Agatha/9*LMPG | 25 | LMPG-6 | 7Ae#1, 7A | 6A (303) |
| Eagle (Aus) | 26 | | 7A, 7D | 6Ae#1 (207) |
| PW327/4*Tc//9*LMPG | 26 | LMPG-6 | 7A, 7D | 6Ae#1 (207) |
| 73,214,3-1/9*LMPG | 27 | LMPG-6 | 7A, 7D | 6A (303) |
| W2691/Sr28Kt | 28 | W2691 | 7A, 7D | 6A (303) |
| Pusa/Etoile de Choisy | 29 | | 7A, 7D | 6A (303) |
| Pld*8/Et. de Choi//6*LMPG | 29 | LMPG-6 | 7A, 7D | 6A (303) |
| CnsSr32 A.s. | 32 | CS | 7A, 7D | 6A (303) |
| C82,1CS+Sr32/6*LMPG | 32 | LMPG-6 | 7A, 7D | 6A (303) |
| RL 5405 | 33 | | 7A, 7D | 6A (303) |
| Tetra Canthatch/7*LMPG | 33 | LMPG-6 | 7A, 7D | 6A (303) |
| Mq(2)5*G2919 | 35 | Marquis | 7A, 7D | 6A (303) |
| W2691SrTt-1 | 36 | W2691 | 7B?, 7D | 6A (303) |
| CI12632/8*LMPG | 36 | LMPG-6 | 7A, 7D | 6A (303) |
| W3563 | 37 | W2691 | 7A, 7D | 6A (303) |
| RL 6082 | 39 | | 7A, 7D | 6A (303) |
| RL 6088 | 40 | | 7A, 7D | 6A (303) |
| TAF-2 | 44 | | 7Ai#1?, 7A | 6A (303) |
| CnsSrTmp | Tmp | CS | 7B?, 7D | 6A (303) |
| Triumph 64 | Tmp | | 7A, 7D | 6A (303) |
| Thatcher | Thatch | | 7A, 7D | 6A (303) |
| TAM 107 | 1A.1R | | 7A, 7D | 6A (303) |
| Amigo | 1A.1R | | 7A, 7D | 6A (303) |
| W199/Tt113*W199 | Tt-3 | | 7B?, 7D | 6A (303) |
| Federation SrTt-3/6*LMPG | Tt-3 | LMPG-6 | 7A, 7D | 6A (303) |
| LMPG-6 | | | 7A, 7D | 6A (303) |
| Chinese Spring | | | 7A, 7D | 6A (303) |
| Little Club | | | 7A, 7D | 6A (303) |
| Marquis | | | 7A, 7D | 6A (303) |
| W2691 | | | 7A, 7D | 6A (303) |

[a] Please refer to Figs. 1 and 2 for the designation of each DNA fragment amplified with co-dominant markers for *Sr25* and *Sr26*

and annealing temperature 60°C was used for all markers. The PCR products were separated on 3% agarose gels and visualized with ethidium bromide under UV light. Due to the small size differences among alleles, 5% standard polyacrylamide gels were used for marker BF145935 (F: CT TCACCTCCAAGGAGTTCCAC, R: GCGTACCTGATC ACCACCTTGAAGG) instead of agarose gels. Heterozygotes for *Sr26* were simulated by mixing equal of amounts

**Table 2** Wheat germplasm screened with co-dominant markers for *Sr25* and *Sr26*

| Country/institution | Growth habit | Number of lines | | |
|---|---|---|---|---|
| | | Total | *Sr25* | *Sr26* |
| CIMMYT | Spring | 89 | 5 | 0 |
| China | 24 winter/ 19 spring | 43 | 0 | 0 |
| Cornell University | Winter | 7 | 0 | 0 |
| University of Minnesota | Spring | 6 | 0 | 0 |
| India | Spring | 1 | 0 | 0 |
| Kenya | Spring | 3 | 0 | 0 |
| Kazakhstan | Winter | 4 | 0 | 0 |
| Kyrgystan | Winter | 4 | 0 | 0 |
| Tadjikistan | Winter | 1 | 0 | 0 |
| Turkmenistan | Winter | 3 | 0 | 0 |
| Uzbekistan | Winter | 5 | 0 | 0 |
| Azerbaijan | Winter | 2 | 0 | 0 |
| Russia | Winter | 1 | 0 | 0 |
| Turkey | Winter | 1 | 0 | 0 |

of DNA of lines with and without this gene prior to PCR, and using 400 nM of primer for Sr26#43 and 400 or 800 nM of primer for BE518379 (F: AGCCGCGAAATCT ACTTTGA, R: TTAAACGGACAGAGCACACG).

## Results

### Validation of previously published dominant markers for genes *Sr25* and *Sr26*

Marker Gb for *Sr25* and Sr26#43 for *Sr26* were validated with eight wheat lines. As expected, a faint 130 bp fragment was amplified with marker Gb in the two lines with *Sr25*, Wheatear and C80.1/3*Batavia//2*Weebil (data not shown). The other six lines without *Sr25* did not amplify any detectable fragment with primers of marker Gb. Only the cultivar Eagle was positive for marker Sr26#43 and no PCR product was observed for the other seven lines (data not shown).

### Development and testing of co-dominant markers for *Sr25*

Since co-dominant markers are needed to distinguish homozygotes from heterozygotes, we developed and tested co-dominant markers for genes *Sr25* and *Sr26*. Among the six STS markers tested on the eight wheat lines, BE404744 and BF145935 were co-dominant in marking *Sr25*. We focused on marker BF145935 because it consistently worked well and was easier to score. Marker BF145935 amplified two DNA fragments from most lines tested (Fig. 1). Based on aneuploid analysis, the lower band of Chinese Spring is located on chromosome 7A, and the top band is on chromosome 7D. The highest molecular weight fragment found in *Sr25*-containing lines, such as Wheatear, are located on the 7Ae#1 segment that is translocated onto wheat chromosome 7DL.

Marker BF145935 was used to genotype each of the 42 lines in our validation set (Table 1). The Ae#1 fragment is unique and amplified only from Wheatear and the other two *Sr25*-containing lines LcSr25Ars and Agatha/9*LMPG (Fig. 1). Line Agatha/9*LMPG has the same marker genotype as *Sr25*-containing line Wheatear, and the recurrent parent LMPG has the same marker genotype as Chinese Spring. So, the top fragment of Wheatear and Agatha/9*LMPG was amplified from the 7Ae#1 segment carrying *Sr25/Lr19*. Instead of two DNA fragments, the 7D, 7Ae#1 and 7A fragments were amplified from line LcSr25Ars (Fig. 1), indicating that this line is heterozygous for marker BF145935. This result was confirmed with DNA extracted from two individual plants of this line. Both plants contained fragments located to 7A, 7D, and 7Ae#1. Among the 40 lines (Table 1) without *Sr25*, 35 lines have the same genotype for BF145935 as that of Chinese Spring. The marker genotypes of the other five lines, Sr22TB, W2691SrTt-1, CnsSrTmp, W199/Tt113*W199, and TAF-2, were different from that of Chinese Spring or Wheatear. Four of these lines, Sr22TB, W2691SrTt-1, CnsSrTmp and W199/Tt113*W199, have the same marker genotype for BF145935 (Fig. 1). Compared to the genotype of Chinese Spring, the Chinese Spring 7A fragment was replaced with a fragment larger than the Chinese Spring 7D fragment. We suspect that this larger fragment might be located on chro-

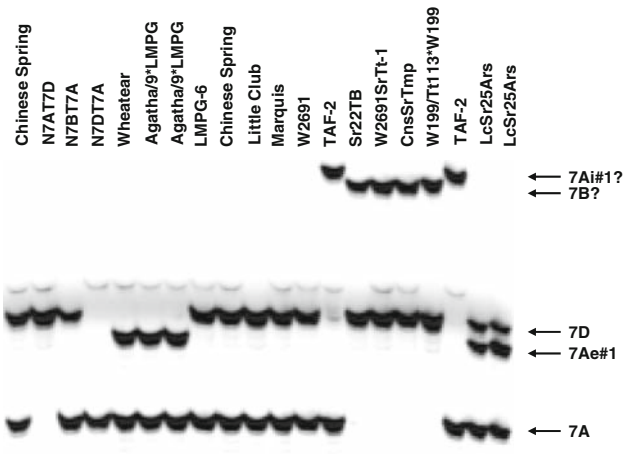


**Fig. 1** Genotypes of control lines and representative wheat lines with known stem rust resistance genes genotyped with marker BF145935 on a polyacrylamide gel. The chromosome assignment of each DNA fragment is indicated at the *right*

mosome 7B because wheat lines without a translocated group 7 chromosome and containing three DNA fragments for BF145935 were identified (see below). The larger fragment of line CnsSrTmp was not amplified from either of its two parental lines, Chinese Spring and Triumph 64, indicating cross contamination of plant material or DNA. The *Sr44*-containing line TAF-2, which is an addition line containing an extra pair of group 7 chromosomes from *Th. intermedium*, has a unique fragment (Fig. 1). The lower band has the same size as the Chinese Spring 7A fragment, however, the upper band is the largest among all fragments amplified with marker BF145935.

Among the 170 lines (Table 2) genotyped with marker BF145935, only five CIMMYT lines have the 7Ae#1 fragment. All these five lines have the *Sr25* line Wheatear in their pedigrees. Most of the lines have the same genotype as Chinese Spring. Three lines, one from CIMMYT, 'Tom' (a cultivar developed at the University of Minnesota) and 'Mirbashir-158' (a cultivar from Azerbaijan), have the same genotype as line Sr22TB. We did not identify any lines with the same genotype as line TAF-2 among this set of lines. However, two new genotypes were observed. Three CIMMYT lines amplified only one DNA fragment and this was the same size as the Chinese Spring 7A allele. These three lines may have a null 7D allele for marker BF145935. Another new genotype was observed in five winter wheat lines including 'Foster 159' and 'E0028' from Cornell University, 'Kupava' and 'Polovchanka' from Uzbekistan and the Russian cultivar 'Bezostaja'. Three DNA fragments, corresponding to fragments located on chromosomes 7A, 7B, and 7D in Fig. 1, were amplified from these five lines.

### Development and testing of co-dominant markers for *Sr26*

Among the 16 STS markers developed from wheat ESTs mapped to deletion bin 6AL8-0.90-1.00, none of them was co-dominant between lines with or without *Sr26*. However, five markers specific to chromosome 6AL amplified no PCR product from Eagle. We reasoned that multiplex PCR with the combination of one 6AL-specific marker and *Sr26*-specific marker Sr26#43 could be used to distinguish *Sr26* homozygotes from heterozygotes. Because the 6AL-specific marker BE518379 consistently worked well and the expected 303 bp allele can be unambiguously distinguished on agarose gels (Fig. 2) from the 207 bp fragment amplified by marker Sr26#43, we combined equal amounts of primers for marker BE518379 and Sr26#43 to genotype additional wheat lines. The 207 bp fragment was amplified from Eagle and lines without *Sr26* have the 303 bp allele (Fig. 2). The 303 bp allele was stronger after doubling the amount of primer for marker BE518379. Simulated heterozygotes for *Sr26*, consisting of a mixture of DNA from Eagle (contains

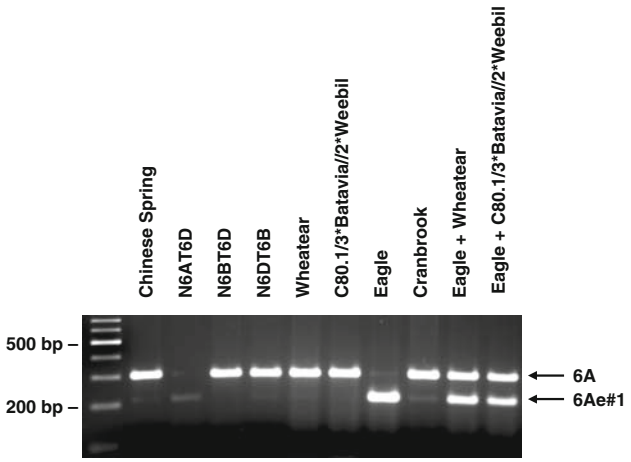


**Fig. 2** Banding patterns of multiplex PCR of markers Sr26#43 and BE518379 on an agarose gel. The *lanes* containing Eagle + Wheatear and Eagle + C80.1/3*Batavia//2*Weebil were from equal mixtures of DNA from the two lines prior to PCR. The chromosome assignment of each DNA fragment is indicated at the *right*

*Sr26*) and Wheatear or C80.1/3*Batavia//2*Weebil (do not contain *Sr26*) prior to PCR produced the expected two bands.

Either the 207 bp band or the 303 bp band was observed for marker Sr26#43/BE518379 among the 42 lines listed in Table 1. The two *Sr26* lines, Eagle and PW327/4*Tc//9*LMPG, have the expected 207 bp allele and all the other lines have the 303 bp allele. The five *Sr26* lines with shortened alien segments, WA1, WA5, WA6, WA8 and WA9, have the expected 207 bp allele and recurrent parent Angas has the 303 bp allele. Therefore, marker Sr26#43/BE518379 is a co-dominant and diagnostic marker for *Sr26*.

None of the 170 lines genotyped with Sr26#43/BE518379 has the 207 bp allele and only the 303 bp allele was amplified from all lines. This result indicates that none of these lines has *Sr26*.

## Discussion

### Marker-assisted selection and postulation for genes *Sr25* and *Sr26*

Diagnostic and co-dominant markers for *Sr25* and *Sr26* reported in this study can facilitate MAS for *Sr25* and *Sr26*, which are effective against races of the TTKSK lineage (Singh et al. 2006; Jin et al. 2008, 2009). We were successful to use both markers to screen segregating populations for *Sr25* and *Sr26*. However, the 303 bp allele was weaker than the 207 bp allele for *Sr26* heterozygotes when equal amounts of primers of markers Sr26#43 and BE518379 were used for multiplex PCR. After doubling the primer

concentration for marker BE518379, the 6A-specific allele and the *Sr26*-specific allele were amplified with similar intensities.

Among the 170 lines screened with markers reported in this study, none had *Sr26* and only five lines carried *Sr25*. This is consistent with the limited use of both resistance genes in breeding programs (McIntosh et al. 1995). However, we anticipate the use of *Sr25* and *Sr26* will increase for several reasons. First, more wheat breeding programs are increasing efforts to develop cultivars with stem rust resistance due to the threat posed by races of the TTKS lineage. Genes *Sr25* and *Sr26* are among a few major genes effective against these races (Singh et al. 2006; Jin et al. 2007); second, *Sr25/Lr19* from the mutant line with white wheat flour has been recently transferred into Australian and CIMMYT wheat backgrounds (Bariana et al. 2007); third, the *Th. ponticum* segment carrying *Sr25/Lr19* can increase yield potential under irrigated condition (Singh et al. 1998; Monneveux et al. 2003), and the yield penalty observed in the original *Sr26* lines has been removed with shortened alien segments (Dundas et al. 2007); finally, the co-dominant markers reported in this study will improve the efficiency to select for *Sr25* and *Sr26* in wheat breeding programs.

The markers reported in this study are useful as a preliminary step to identify lines containing these genes. Many of the lines genotyped in this study have shown consistently high levels of resistance to stem rust for the last few years in Stem Rust Resistance Screening Nursery at Njoro, Kenya. We are genotyping these lines with additional markers associated with known stem rust resistance genes in order to identify lines that may have new stem rust resistance genes. Even though markers BF145935 and Sr26#43/BE518379 were diagnostic in this study for *Sr25* and *Sr26*, respectively, they may produce false positives with other genotypes, especially lines with alien chromosomes or fragments. Neither of the markers was derived from the sequences of resistance genes and the diagnostic genotypes reported in this study are associated with the *Th. ponticum* fragments carrying *Sr25* or *Sr26*. Some lines not included in this study may have the diagnostic marker genotypes but lack resistance genes *Sr25* or *Sr26*. Positive marker genotypes should be validated with rust bioassays and/or pedigrees.

## Converting dominant markers to co-dominant markers with multiplex PCR

Combinations of markers linked to a trait in coupling phase and in repulsion phase can mimic a co-dominant marker capable of differentiating homozygotes from heterozygotes. Mago et al. (2005) developed a robust dominant marker Sr26#43 for *Sr26*, but attempts to develop co-dominant markers failed. Fortunately, more genomic resources have become available to develop better DNA markers. For example, thousands of wheat ESTs have been mapped into chromosome deletion bins (Qi et al. 2004) and many genome-specific primers have been developed and validated during the process of single nucleotide polymorphism (SNP) discovery (http://wheat.pw.usda.gov/SNP/new/index.shtml). Taking advantage of these available genomic resources, we developed a chromosome 6A-specific marker based on the wheat EST BE518379. The *Th. ponticum* chromosome segment does not recombine with wheat chromosome 6A during meiosis (Knott 1980), and is inherited as a single linkage block. Thus, multiplex PCR with Sr26#43/BE518379 behaves like a single co-dominant marker. We believe this multiplex PCR strategy can be applied to other traits to convert dominant markers to co-dominant markers with multiplex PCR.

## Marker BF145935 may also be useful to study gene *Sr44*

The *Sr44* line TAF-2 used in this study is a chromosome addition line ($2n = 44$) with a pair of group 7 chromosomes from *Th. intermedium* (Cauderon et al. 1973). This line has a unique marker genotype among the 220 lines we genotyped with marker BF145935. We suspect the top band was amplified from the added *Th. intermedium* chromosomes. This is consistent with the report that marker BF145935 can amplify different sized bands from group 7 chromosomes of *T. aestivum*, *Th. ponticum* and *Th. intermedium* (Ayala-Navarrete et al. 2007). Thus, marker BF145935 may also be useful to study gene *Sr44*.

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