

Genetic mapping of stem rust resistance gene *Sr13* in tetraploid wheat (*Triticum turgidum* ssp. *durum* L.)

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Abstract Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici*, can cause significant yield losses. To combat the disease, breeders have deployed resistance genes both individually and in combinations to increase resistance durability. A new race, TTKSK (Ug99), identified in Uganda in 1999 is virulent on most of the resistance genes currently deployed, and is rapidly spreading to other regions of the world. It is therefore important to identify, map, and deploy resistance genes that are still effective

against TTKSK. One of these resistance genes, *Sr13*, was previously assigned to the long arm of chromosome 6A, but its precise map location was not known. In this study, the genome location of *Sr13* was determined in four tetraploid wheat (*T. turgidum* ssp. *durum*) mapping populations involving the TTKSK resistant varieties Kronos, Kofa, Medora and Sceptre. Our results showed that resistance was linked to common molecular markers in all four populations, suggesting that these durum lines carry the same resistance gene. Based on its chromosome location and infection types against different races of stem rust, this gene is postulated to be *Sr13*. *Sr13* was mapped within a 1.2–2.8 cM interval (depending on the mapping population) between EST markers *CD926040* and *BE471213*, which corresponds to a 285-kb region in rice chromosome 2, and a 3.1-Mb region in *Brachypodium* chromosome 3. These maps will be the foundation for developing high-density maps, identifying diagnostic markers, and positional cloning of *Sr13*.

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Introduction

Puccinia graminis Pers.:Pers f. sp. *tritici* Eriks. & E. Henn., the causal agent of wheat stem rust, is found in all major wheat growing areas and has been responsible for widespread yield losses during the first half of the previous century. A large-scale campaign to eradicate the alternate host, barberry (*Berberis vulgaris* L), in the early 1920s was later found insufficient to end the stem rust epidemics (Leonard 2001). The barberry eradication efforts were then complemented by the deployment of stem rust resistance genes in the released wheat varieties bringing the stem rust under control during the last half of the twentieth century.

In 1999, a new race of stem rust virulent on most previously resistant lines was identified in Uganda (Pretorius et al. 2000). This new race, designated Ug99 or TTKS (Wanyera et al. 2006), spread to Kenya in 2001 and to Ethiopia in 2003 (Singh et al. 2006). By 2006, TTKS was identified in Sudan and Yemen (<http://www.fao.org>), and in 2008 its presence was confirmed in Iran (Nazari et al. 2009). Three different races within the TTKS lineage were since identified from isolates in Kenya that led to the re-designation of the original race as TTKSK, and the other two races as TTKST (with additional virulence on *Sr24*) (Jin et al. 2008) and TTTSK (with additional virulence on *Sr36*) (Jin et al. 2009).

Several resistance genes are still effective against the three TTKS lineages in both tetraploid (*T. turgidum* ssp. *durum* L.) and hexaploid wheat (*T. aestivum* L.) cultivars (Jin et al. 2007). Among them, *Sr13* is the only known gene with effective resistance to the TTKS races present within the US durum wheat adapted cultivars. The Ethiopian land race ST464, and the domesticated emmer wheat (*T. turgidum* ssp. *dicoccon* L.) Khapli are the two major sources of *Sr13* in durum (Knott 1962; Klindworth et al. 2007). The *Sr13* resistance gene from Khapli was transferred to the common wheat variety Khapstein from the cross Steinwedel × Khapli, and was subsequently mapped on the distal region of the long arm of chromosome 6A by McIntosh (1972). Tests of Khapstein with several North American races of stem rust showed that the transferred gene was a useful source of resistance to the prevalent races of stem rust at that time, but that Khapstein did not carry all of the resistance of Khapli (Watson and Stewart 1956), in particular the *Sr7a* gene as stated by McIntosh et al. (1995).

The moderate resistance of *Sr13* to TTKS makes it a good candidate for gene pyramiding with other stem rust resistance genes. Gene pyramiding is a strategy commonly used by wheat breeders to extend the durability of the deployed resistance genes. However, the generation of these resistance gene pyramids based on marker selection will require the development of markers closely linked to the target genes. To date, molecular markers have been identified for several stem rust resistance genes including *Sr2* (Spielmeyer et al. 2003), *Sr6* (Tsilo et al. 2009), *Sr9a* (Tsilo et al. 2007), *Sr24* and *Sr26* (Mago et al. 2005), *Sr25* and *Sr26* (Liu et al. 2009), *Sr31* (Mago et al. 2002), *Sr36* (Tsilo et al. 2008), *Sr38* (Seah et al. 2001), *Sr39* (Mago et al. 2009), and *Sr40* (Wu et al. 2009), but many more stem rust resistance genes including *Sr13* remain to be mapped. The objectives of this research were to map *Sr13* in a durum wheat genetic background and to identify closely linked markers that can be used to screen germplasm carrying the resistance gene.

Materials and methods

Plant materials

Four segregating populations were used to map *Sr13* resistance gene in durum wheat. The first population included 93 recombinant inbred lines (RILs) derived from the cross between UC1113 and Kofa (denoted as UK). Kofa is a Desert Durum[®] variety developed by WestBred (now owned by Monsanto) that is resistant to TTKSK and has excellent pasta quality. Kofa originated from a male sterile facilitated recurrent selection (MSFRS) population in which *T. dicoccon* lines with high protein were crossed onto Desert Durum[®] adapted MSFRS population. The exact pedigree of Kofa is not known. Thus, either *T. dicoccon* or the adapted Desert Durum lines included in the MSFRS could be the source of the observed resistance gene. UC1113 is a breeding line from the UC Davis wheat breeding program selected from a CIMMYT cross CD52600 (KIFS//RSS/BD1419/3/MEXIS-CP/4/WAHAS/5/YAV79) that is susceptible to TTKSK, and has excellent agronomic performance but intermediate pasta qualities (Zhang et al. 2008). Evaluations for adult plant stem rust resistance performed in Kenya in 2007 and 2008 showed that Kofa was resistant (10–50 R/MR) whereas UC1113 was highly susceptible (50–70 MS/S) to TTKSK. Seedling tests performed at the USDA-ARS Cereal Disease Laboratory and University of Minnesota confirmed that in Kofa resistance genes against TTKSK also confer resistance to the variants TTKST (*Sr24* virulence) and TTTSK (*Sr36* virulence) and five other races of stem rust (QTHJ, RCRS, RKQQ, TPMK, and TTTT). UC1113 was susceptible to all the three TTKS variants and to the races TPMK and TTTT, but was resistant to the North American races QTHJ, RCRS, and RKQQ.

The other two populations were F₂ progeny from crosses between Mindum × Medora (denoted as MM, 97 lines) and Mindum × Sceptre (denoted as MS, 80 lines). Mindum, susceptible to race TTKSK, was selected from a field in Turkey and released in 1917 from the University of Minnesota, whereas both Medora (Ward/Macoun) and Sceptre (D72110/Coulter, D72110 = D65150/Leeds//Ward) were Canadian cultivars released in 1981 and 1985, respectively, that showed resistance to TTKSK with infection type 2 when they were evaluated at the seedling stage. While the likely resistance source for Medora was Khapli, either ST464 or Khapli may have contributed to the resistance in Sceptre (Fig. S1).

A fourth population was developed from the cross Kronos × Rusty (denoted as KR) to link the previous three populations using common polymorphic markers. Rusty is a tetraploid genetic stock susceptible to almost all stem rust races (Klindworth et al. 2006), whereas Kronos is a Desert

Durum[®] variety developed by Arizona plant breeders that is resistant to TTKSK and TRTT (a virulent race from Yemen), and has excellent pasta quality and yield potential.

To determine the usefulness of the markers linked to *Sr13*, a set of 34 durum cultivars and breeding lines, mostly derived from North Dakota durum wheat breeding program and previously assessed for their resistance against TTKSK, was included in this study (Table 1). A set of 34 ND durum germplasm was selected for marker validation based on their similar breeding history and common resistance sources with the resistance parents, Medora and Sceptre (Fig. S1). To further validate the identification of the resistance gene mapped in this study as *Sr13*, one of the reference genetic stocks for *Sr13*, Khapstein/9*LMPG-6 (Knott 1990), along with Khapstein (PI 210125) and the original *Sr13* donor Khapli (Citr 4013), were genotyped with markers linked to *Sr13*.

Evaluation for resistance

All parents, RILs and F₃ lines of the UK, MM, and MS populations were evaluated for their reaction to race TTKSK isolate 04KEN156/04 collected in Kenya in 2004. The KR population was evaluated separately with stem rust races TTTTF and TPMKC. Urediniospores from long-term storage in a -80°C freezer were heat shocked at 40°C for 15 min and placed in a rehydration chamber for 2–4 h, where approximately 80% relative humidity was maintained by a KOH solution (Rowell 1984). The urediniospores were then suspended in a light mineral oil (Soltrol 170) and inoculated onto the fully expanded primary leaves of 7- to 9-day-old seedlings of wheat lines. Seedlings were incubated in a dew chamber for 12–16 h at 18°C in the dark, and then for an additional period of 3–4 h under fluorescent light. The inoculated plants were placed on a greenhouse bench at 18 ± 2°C with a photoperiod of 16 h. Infection types, described by Stakman et al. (1962), were assessed 14 days post inoculation. From each genotype, 12 seedlings were screened. Infection types 0, ;, 1, 2, or combinations thereof were considered low infections, indicating resistance, whereas infection types 3 and 4 were considered high infections and plants considered susceptible. Families were classified as homozygous resistant, segregating, or homozygous susceptible to race TTKSK.

Genetic map construction

A complete genetic map of the UK segregating population including over 269 simple sequence repeat (SSR), single nucleotide polymorphism (SNP), restriction fragment length polymorphism (RFLP), and sequence tagged site

(STS) markers has already been published by Zhang et al. (2008).

Genomic DNA of Mindum, Medora and Sceptre were first screened with 1,440 wheat SSRs to detect polymorphic markers. Twelve wheat EST-derived SNP markers previously mapped to chromosome 6A (Chao et al. 2009) were screened and polymorphic ones were also included in the map. Additionally, primers for STS markers designed from the *Sr13* gene region by aligning unmapped wheat ESTs with rice genome sequences were also used. Genotyping of SSR and STS markers followed the same protocols previously developed for SSR markers using a capillary gel system from Applied Biosystems (Foster City, CA, USA) (Chao et al. 2007). The method used for SNP genotyping was based on template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI) (Chen et al. 1999). The KR population was used to correlate the UK population with the MM and MS populations and, therefore, was screened only with molecular markers previously mapped in these three populations.

Genetic maps of the *Sr13* region were constructed for the three populations using MAPMAKER software version 3.0b (Lander et al. 1987). Genetic distances were calculated using the Kosambi function. Initial maps were assembled at LOD 3. Additional markers were then placed in the most likely location using the TRY command. Regions where groups of markers ordered at LOD scores lower than 2 were denoted as asterisks (*) shown on the maps. The order was refined using the RIPPLE command.

Results

Gene postulation

Domesticated emmer wheat Khapli has been previously shown to carry *Sr7a*, *Sr13*, and *Sr14* and at least one additional undescribed stem rust resistance gene (Knott 1962; Williams and Gough 1965). Khapli is shown here to be resistant to race TTKSK (Table 2). Since *Sr7a* and *Sr14* are known to be susceptible to TTKSK (Table 2; Jin et al. 2007), the resistance in Khapli to this race is likely conferred by *Sr13*.

Sr13 is the only known stem rust resistance gene effective against race TTKSK in the US adapted durum wheat cultivars. Typical TTKSK infection types of lines carrying *Sr13* range from 2 to 2+ (Table 2). We observed similar infection types to race TTKSK in the durum lines Kofa, Kronos, Medora, and Sceptre (Table 2), and postulated that these low infections to TTKSK were conferred by *Sr13*. This gene postulation is consistent with the map location on chromosome 6AL described below.

Table 1 A list of durum wheat germplasm used for haplotype analysis in this study

Accession	Origin	<i>wmc580</i>	<i>CK207347</i>	<i>CD926040</i>	<i>BE403950</i>	<i>dupw167</i>	TTKSK
D98529	ND	317	1000	855	723	230	;
D98530	ND	317	1000	–	713	230	;
D99983	ND	317	1000	855	723	230	;
DH01039	ND	317	1000	855	723	230	;
Lloyd	ND	317	1000/1135	851	691/723	230	;
D00624	ND	317	1000	855	723	230	1
DH01060	ND	317	1000	855	723	249	1
Munich	ND	317	1000	855	723	230	1
Pierce	ND	317	1000	855	723	230	1
Plaza	ND	317	1000	855	723	230	1
D99073	ND	317	1000	855	723	245	1+
D99541	ND	317	1000	855	723	230	1+
D00534	ND	317	1000	855	723	230	12
D00095	ND	317	1000	855	723	230	;12
D00752	ND	317	1135	855	–	219	2
D00969	ND	317	1000	855	723	230	2
D99637	ND	317	1000	855	723	230	2
D99656	ND	319	1000	–	713	230	2
DH01066	ND	317	1000	855	723	230	2
Khapli (Citr4013) ^a	NSGC ^a	293	1000	851	691	230	;2–
W2691Sr13 ^a	NSGC	326	1000	851	691	245	2
Langdon ^a	ND	293	1000	–	691	249	2
Kofa ^a	WestBred	293	1000	855	723	230	2
Kronos ^a	CA-APB	293	1135	855	–	245	2+
Medora ^a	Canada	317	1000	855	723	230	2
Sceptre ^a	Canada	317	1000	855	723	230	2
ST464-C1 ^a	ND	293	1000	–	691	249	2+
Ben	ND	317	1000	855	723	230	2–
Maier	ND	317	1135	855	–	245	2–
Renville	ND	293	1135	855	–	252	2–
Leeds	ND	317	1000	855	723	230	2+
D00622	ND	317	1000	855	723	230	3
Grenora	ND	317	1000	855	723	245	3
Alkabo	ND	317	1000	–	723	249	3–
Belzer	ND	317	1000	855	723	249	3+
D001097	ND	317	1000	855	723	230	3+
D00767	ND	317	1000	855	723	249	4
D97643	ND	317	1000	855	723	249	4
Dilse	ND	317	1000	855	723	249	4
Divide	ND	317	1000	855	723	249	4
Rusty	ND	293	993	845	–	230	4
Mindum	MN	335	1135	851	691	230	4
UC1113	CA	317	1000	855	723	249	4

NSGC USDA-ARS, National Small Grains Collection, Aberdeen, ID; CA-APB California–Arizona plant breeders

^a Lines are known to carry *Sr13* gene

Genetic maps

The ratio of phenotypic scores against race TTKSK agreed with single gene segregation in all the three populations ($\chi^2 = 0.10$, $P = 0.76$ for UK; $\chi^2 = 0.22$, $P = 0.90$ for

MS; and $\chi^2 = 0.84$, $P = 0.66$ for MM). Mapping of resistance in the crosses UK, MM, and MS resulted in the localization of a single resistance gene against TTKSK on the distal region of the long arm of chromosome 6A (Fig. 1). These results agree with the previously reported

Table 2 Twelve wheat lines with previously determined stem rust resistance genes and infection types to race TTKSK of *Puccinia graminis* f. sp. *tritici*

Line	Known <i>Sr</i> genes	TTKSK
UC1113		4
Kofa		2
Kronos		2
Mindum	9d, X, + ^a	4
Medora		2
Sceptre		2
Khapli	7a, 13, 14, + ^a	2
Kenya Governor/10*MQ//8*LMPG	7a	3+
W2691Sr13	13	2+
St464Sr13 (PI 192334)	13	2+
Khapstein/9*LMPG	13	2+
Line A selection	14	4

^a + indicates additional undescribed stem rust resistance genes are present in these lines

location of *Sr13* (McIntosh 1972). Therefore, this resistance gene will be referred to as *Sr13* hereafter.

In the UK segregating population, resistance to TTKSK was initially mapped on the distal region of chromosome

arm 6AL between SSR markers *gwm617* and *dupw167*, more than 85 cM from the centromere (Fig. 1). Later, an STS marker derived from EST *BE471213* (see Table 3 for primers and PCR conditions) was mapped between *Sr13* and *dupw167*. The 25 RILs showing recombination between the flanking markers and *Sr13* were sent to the USDA-ARS Cereal Disease Laboratory for a second blind evaluation. All 25 RILs showed identical TTKSK infection types as observed in the first evaluation, confirming the location of *Sr13*. In summary, in the UK segregating population *Sr13* was mapped to a 20-cM interval defined by *gwm617* and *BE471213*.

In the two other crosses involving Mindum as the susceptible parent, the screening with SSR markers revealed low levels of polymorphism for markers previously mapped in chromosome 6A. The linkage mapping analysis showed that *Sr13* was located 2.8 cM distal to *wmc580* in the MS population (Fig. 1). A similar result was found in the MM population where *wmc580* was mapped 5.7 cM proximal to *Sr13* (Fig. 1). SNP markers developed from 12 ESTs previously bin-mapped to chromosome 6AL were further evaluated, and the polymorphic ones were all mapped proximal to, or tightly linked to the *Sr13* gene in both MM and MS populations (Fig. 1). All the ESTs distal to *BE403154* (Fig. 1) have been previously assigned to the

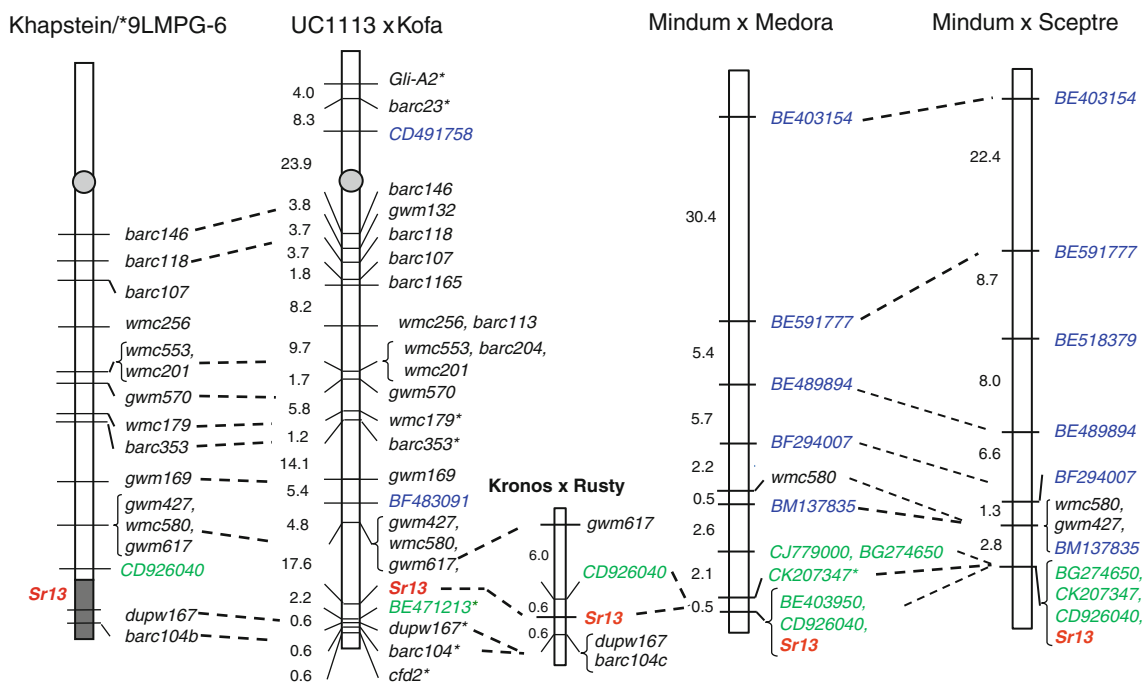


Fig. 1 Genetic maps of *Sr13* resistance gene on chromosome 6A developed from four mapping populations, UC1113 × Kofa, Rusty × Kronos, Mindum × Sceptre, and Mindum × Medora. DNA markers shown in blue were SNP-based, and those shown in green were STS markers. Asterisks indicate markers mapped at LOD scores lower than 2.0. The shaded area at the distal end of chromosome 6AL in *Khapstein*/*9LMPG-6 denotes the region transferred from Khapstein

into LMPG-6 including the *Sr13* gene. Markers outside the shaded area are not polymorphic between LMPG-6 and *Khapstein*/*9LMPG-6, but are polymorphic in *Khapstein* (PI 210125). Markers *barc104*, *barc104b* and *barc104c* represent different primers (Table 3) that amplify the same locus, but are more efficient to detect polymorphisms in different genetic backgrounds

Table 3 Primer pairs developed for SSR and STS markers, SNP primers, and annealing temperature used for PCR amplifications performed at either constant or touchdown (td) conditions

Wheat EST	Forward primer (SNP detection primer)	SNP	Reverse primer	Temperature
barc104b ^a	GCGCTTCCAAGGCTTAGAGGCT		GGACCAGGCATGTCTACCCT	50
barc104c ^a	GCATGTTTCCCATCCCTTA		GCCTTCCTCCCTTTTGAAAC	50
BE403950	GGAACATGTTGACGCTGTTG		AACACTGTTCCCGAAGTTGG	60–55 td
BE471213	GTCTTTCCTTGGCTGTCTG		TCATATCCTCTGCTTTCCTGAAA	58
BG274650	TCCTTCTTTCACCGTGGAG		ACTCTGAGCAGCGACCAAAT	60–55 td
CD491758	AGCTTGCCATGTTTATTATGTTAC		TGGACCATTACTATGTAGAGC	62
CD926040	GTTGGCTTGCTACTGCTTT		AGCATTGAGCTCTGTGAGCA	60–55 td
CJ779000	GATGTTGCCGCCAGAATAAT		TATGCAAAGGCCTCCACTTC	60–55 td
CK207347	TTACGGGCCACAAACAATCT		AGCTCTCATCCATCCAGGAA	60–55 td
BE294007	CACACCGTAGGAGAACCAGG		ATCCACAGGCTCCATAGCAC	60
	(GATAACTTCCTTTGTACTAGAAAAGTAGAT)	C/T		60
BE403154	CAGGTAAATCTGTAATTTTTTAAAGGGAAGAC		TGTTGTGAAGGCTCCACCA	60
	(GACCATCTTACCTGTGTAGCATAGATG)	C/T		60
BE489894	CAGTGGAAGGCAAGGTGTAC		TGGTTGTCAAGAGTCCCTAAGCCT	60
	(CATTGCCCCAGGGACCCGTCTCGACTT)	G/A		60
BE518379	AGGTGAGCACCCGTGCAGTT		TGTGTTTCATACTATTGAGCCAGTTCTAT	60
	(TCCGAACCATAACAATCACCAGGACAGGAG)	G/C		60
BE591777	CGTGCGTTCAGTATTCAAAA		CAGCGACAACAGACTCAAGCAAA	60
	(GGGAAAAAGATTACCACACTGCACAA)	C/T		60
BF483091	GCAAAAAATATCTGTTAGAACAAGACTG		CACCATTGCCATCACAAGAG	58
	(ATTGTGGCTTATGTTGCTATACAAGGCA)	G/A		60
BM137835	CAGGGCACAGTTCATGATTTA		GGAATATTTTTCTTAGGTACCAT	60
	(CTTTGGTAAGAAAATTGTGCATAGCAACT)	G/A		60

^a barc104b and barc104c amplify the same locus as the original barc104, but are optimized for different genetic backgrounds

distal 6AL-8 bin (*BE591777*, *BE518379*, *BE489894*, *BF294007*, *BM137835*, *BE403950*, and *BE471213*), thus, the *Sr13* region is included in the 6AL-8 bin.

None of the markers distal to *Sr13* in the UK population were polymorphic in the MM and MS populations. Similarly, attempts to transfer the EST markers mapped closely linked to *Sr13* in the MM and MS to the UK population were not successful due to lack of polymorphism. We were able to develop genome specific primers for seven 6AL ESTs (*BE403950*, *BE636872*, *BG274650*, *BQ802161*, *BQ841735*, *CK207347*, and *CJ779000*), but no polymorphisms were detected in the 6,790 bp sequenced from both UC1113 and Kofa. In summary, *gwm427* and *wmc580* are the closer common markers to *Sr13* between the UK and MM–MS populations.

To generate additional common markers closer to *Sr13* we developed a fourth population from the cross Kronos × Rusty (denoted as KR), which were polymorphic for both distal SSR markers, *barc104c* and *dupw167*, and the tightly linked proximal EST-derived marker, *CD926040*. The results confirmed segregation for a single gene conferring resistance to stem rust races TTTTF and TPMKC (16 homozygous resistant, 37 heterozygous, 29 homozygous

susceptible, 1:2:1 proportion χ^2 test $P = 0.08$) in the KR population. Resistance to both races was mapped within a 1.2 cM interval flanked by markers *CD926040* (0.6 cM) and *dupw167-barc104c* (0.6 cM), which were also closely linked to the resistance gene in the other three populations. Close linkage with common molecular makers suggests that these four resistant parents carry the same resistance gene, which is most likely *Sr13*. The *Sr13* gene postulation in the KR population was also supported by an infection type 2 in Kronos tested with stem rust races TTKSK and TRTT (virulent race from Yemen).

The identification of the homologous region in the sequenced grass species provided a starting point to discover additional markers in the *Sr13* region. The rice homologues to the wheat *Sr13* gene region flanked by ESTs *BF483091* and *BE471213* in the UK population defined a region on rice chromosome 2 (R2) of approximately 1 Mb (R2 34,885–35,805 kb). Wheat ESTs located within this region were identified and used to generate STS markers near the *Sr13* gene in the different populations. While EST *BE403950* (R2 35,512 kb) was completely linked to *Sr13* in the MM population, EST *CD926040* (R2 35,521 kb) was found co-segregating with *Sr13* in both

MM and MS populations, but was proximal to the resistance gene in the KR population determining a close proximal marker for *Sr13* (Fig. 1). Similarly, the *CK207347* EST (R2 35,501 kb) detected no recombination event with *Sr13* in the MS population but was mapped 0.5 cM proximal to *Sr13* in the MM population. EST *BG274650* (R2 35,300 kb) was closely linked to *Sr13* in the MS population, but was mapped 2.1 cM proximal to *Sr13* in the MM population and linked to EST *CJ779000* (R2 35,410 kb). Taken together, the results from the four mapping populations suggest that the wheat *Sr13* gene region on chromosome 6AL is delimited by flanking EST-derived markers *CD926040* and *BE471213*. The orthologues of these wheat genes correspond to the collinear regions of 285 kb on rice chromosome 2 (35,521–35,805 kb), and 3.1 Mb on *Brachypodium* chromosome 3 (56,534 to and 59,627) (Table 4).

Genetic stocks for *Sr13*

The *Sr13* gene name is defined by several genetic stocks, including Khapstein/9*LMPG-6 (Knott 1990; McIntosh et al. 2008). To test if the chromosome region where the resistance to TTKSK mapped in the four durum populations was also present in Khapstein/9*LMPG-6, markers from these four maps were evaluated in Khapstein/9*LMPG-6 and its recurrent susceptible parent LMPG-6 (Knott 1990), as well as in the tetraploid (Khapli) and hexaploid (Khapstein) *Sr13* donors (Fig. 2). Of the 19 SSR markers evaluated on 6AL, only *dupw167* and *barc104b* were polymorphic between LMPG-6 and Khapstein/9*LMPG-6, and the same alleles present in the latter were detected in Khapstein (PI 210125) (Fig. 2, *barc104b*; data

Table 4 Wheat EST markers mapped in this study and their corresponding collinear regions of rice and *Brachypodium* chromosomes

Map order (population)	Rice Chr2 (kb) ^a	<i>Brachypodium</i> Chr3 (kb) ^b
BE518379 (MS)	34,632	55,278
BE489894 (MS & MM)	34,617	55,306
BE483091 (UK)	34,885	55,422
BF294007 (MS & MM)	35,006	55,579
BM137835 (MS & MM)	35,051	55,649
BG274650 (MS & MM)	35,300	56,091
CJ779000 (MM)	35,410	56,011
CK207347 (MS & MM)	35,501	56,148
BE403950 (MM)	35,512	56,554
CD926040 (MM & MS & KR)	35,521	56,534
BE471213 (UK)	35,805	59,627

^a Coordinates based on Gramene Gene build January 2009 (<http://www.gramene.org>)

^b Coordinates based on *Brachypodium* 8× release (<http://www.brachypodium.org>)

not shown for *dupw167*), indicating that the distal 6AL chromosome region in Khapstein/9*LMPG-6 was transferred from Khapstein (Fig. 1, shaded area). Among the other 17 non-polymorphic markers between LMPG-6 and Khapstein/9*LMPG-6, 13 (including *CD926040*) were polymorphic with Khapstein (PI 210125) (Fig. 1, non-shaded area), suggesting that this chromosome region was not transferred from Khapstein to Khapstein/9*LMPG-6. The other four markers, *gwm132*, *barc1165*, *barc113*, and *barc204*, were not polymorphic between Khapstein and LMPG-6 and were not included in the map since they were not informative.

It should be noted that heterogeneity was observed among the three different accessions of Khapstein available from the National Small Grains Collection (USDA-ARS, Aberdeen, Idaho). As a result, the actual donor source used to produce Khapstein/9*LMPG-6 is not certain. However, the fact that the accession PI 210125 shared the same *dupw167* and *barc104b* alleles with Khapstein/9*LMPG-6 suggests that this Khapstein accession is the most similar to the original source of *Sr13* in Khapstein/9*LMPG-6. Khapli accession C.I. 4013 showed the same *barc104b* allele as Khapstein and Khapstein/9*LMPG-6, but had a different *dupw167* allele (data not shown), implying that a different Khapli accession was used in the generation of Khapstein.

The *Sr13* resistant lines, Kofa, Medora, and Sceptre carry different *barc104b* alleles from those present in Khapli, Khapstein, Khapstein/9*LMPG-6 and Kronos (Fig. 2), but they share the same *dupw167* alleles with Khapli C.I. 4013, suggesting either different tetraploid sources of the *Sr13* gene or independent recombination events between the resistance gene and the markers used in this study. Characterization of EST markers mapped near the *Sr13* gene further revealed that *CD926040* is not

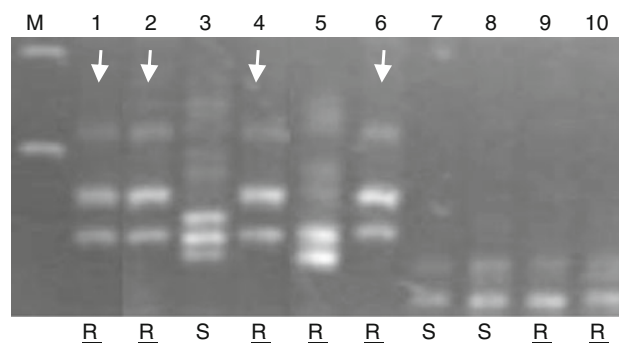


Fig. 2 Alleles of *barc104b* detected among ten wheat lines. 1 Khapstein (PI 210125), 2 Khapstein/9*LMPG-6, 3 LMPG-6, 4 Khapli (C.I. 4013), 5 Kofa, 6 Kronos, 7 Rusty, 8 Mindum, 9 Medora, and 10 Sceptre. *R* and *S* denote resistance and susceptibility to TTKSK, respectively. *R* indicates that the line is a known carrier of *Sr13*. Arrows indicate *Sr13* resistant genotypes carrying the same *barc104b* allele as Khapli (C.I. 4013)

polymorphic between Khapstein/9*LMPG-6 and LMPG-6, but detected a different allele in Khapstein, implying that this marker is located outside the region containing *Sr13* (Fig. 1). The proximal location of *CD926040* relative to *Sr13* is consistent with its map location in the KR population (Fig. 1).

Marker haplotypes of durum wheat germplasm

To determine if closely linked markers identified in MM and MS populations are robust enough to predict the disease phenotype in the durum germplasm, a set of 34 durum wheat cultivars from North Dakota that shared similar genetic background and *Sr13* resistance sources as Medora and Sceptre (Fig. S1) was genotyped with five flanking markers, *wmc580*, *CK207347*, *BE403950*, *CD926040*, and *dupw167*. This germplasm set included 10 and 24 lines susceptible and resistant to TTKSK, respectively. The results showed that a predominant allele was generally found present in both susceptible and resistant accessions for all five markers investigated, and that no particular haplotype was associated with the TTKSK susceptible or resistant phenotypes (Table 1). We confirmed the lack of association between stem rust resistance and haplotypes based on these five markers by examining additional genetic stocks known to carry *Sr13*, such as W2691Sr13 (Table 1). Therefore, the markers identified in this study, albeit tightly linked, are not diagnostic for the *Sr13* gene. It is worth noting that for the susceptible lines, the absence of *Sr13* can be predicted with a high level of confidence, but for the resistant lines that have not been analyzed genetically, we can't rule out the presence of stem rust resistance genes different from the prevalent *Sr13*.

Discussion

Sr13 mapping and gene designation

In this study, we present three lines of evidence to suggest that the resistance gene against TTKSK mapped in our study is *Sr13*. First, the infection type 2 against TTKSK obtained for the resistant parental lines Kronos, Kofa, Medora, and Sceptre used in this study was within the range of 2 to 2+ observed for typical infections in lines carrying *Sr13*. Second, consistent with a previous telocentric analysis that assigned *Sr13* to the long arm of chromosome 6A with no linkage with the centromere (McIntosh 1972), the resistance gene segregating in all four populations evaluated in this study was mapped in the distal region of chromosome arm 6AL. Finally, the

presence of different *dupw167* and *barc104b* alleles in LMPG-6 from those present in both Khapstein and Khapstein/9*LMPG-6 further supports the hypothesis that the gene mapped in this study is *Sr13*. The genetic stock Khapstein/9*LMPG-6, used to define the *Sr13* gene name, is a resistant isogenic line selected from backcrossing Khapstein nine generations into the susceptible line LMPG-6. During the backcross process, a recombination event between *CD926040* and *Sr13* likely restored most of the LMPG-6 in the 6AL proximal region, and reduced the Khapstein segment to the distal 6AL region in Khapstein/9*LMPG-6. Taken together, these three sources of evidence strongly indicate that the TTKSK resistance gene mapped in this study is *Sr13*.

Characterization of germplasm with *Sr13* linked markers

Khapli and ST464 were involved in the production of Langdon, Wells and Leeds (Fig. S1). Since these lines or their derivatives were present in most of the lineages of durum wheat cultivars released from North Dakota after 1978 (Klindworth et al. 2007), a large proportion of the ND germplasm is expected to carry the *Sr13* gene, which is in general agreement with the high proportion of TTKSK resistant lines found among the North Dakota germplasm included in this study (70%).

While the *Sr13* resistance source can be traced from the pedigree in Medora back to Khapli, the *Sr13* source for Sceptre can be either Khapli or ST464 (Fig. S1). Our mapping results also confirmed the presence of *Sr13* in Kofa and Kronos, but the use of a male sterile facilitated recurrent selection population with multiple parental lines complicates the identification of the *Sr13* donor in these two varieties. The presence of the same *barc104b* allele in Khapli and Kronos (Fig. 2) suggests that Khapli might have been the donor of *Sr13* in Kronos, but more closely linked diagnostic markers will be required for a more precise identification of the sources of *Sr13* in Kronos, and in the other three resistant parents as well.

Contrary to its wide distribution among ND durum wheat cultivars and germplasm, *Sr13* has not been exploited extensively in common wheat breeding programs (Knott 1989), with the exception of Australian wheats (McIntosh et al. 1995). Due to its moderate resistance and effectiveness against TTKSK, *Sr13* would be a valuable source of resistance for pyramiding with other resistance genes to provide durable resistance against stem rust. The use of molecular markers will assist breeders in the efforts of combining multiple resistance genes during the breeding process. The tightly linked markers identified in this study (e.g. *BE403950*, *CK207347*, or *CD926040*) would be useful for marker assisted selection efforts for *Sr13* only in

targeted populations generated from parental lines with known *Sr13* alleles.

The haplotype analysis of the set of 34 North Dakota durum lines revealed that the markers developed in this study are not diagnostic to predict the presence or absence of *Sr13* among germplasm, even though some of these markers were closely linked to *Sr13*. Low levels of polymorphism in the *Sr13* region in the UK, MM and MS populations delayed our initial efforts to develop closely linked markers. The recently developed KR mapping population showed higher levels of polymorphism than the previous populations, and will be the focus of future high-density mapping and positional cloning efforts. The collinear region from rice and *Brachypodium* will provide useful resources of new markers to saturate the wheat *Sr13* region. A dedicated positional cloning effort in wheat will be necessary not only to identify the *Sr13* gene, but also to understand the mechanisms by which it confers moderate resistance to TTKSK. A better understanding of the different stem rust resistance mechanisms and the different genes involved in each of them will be useful to design more durable gene combinations to control this devastating disease.

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