Mapping a Resistance Gene to *Puccinia graminis* f. sp. *tritici* in the Bread Wheat Cultivar ‘Matlabas’

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Abstract

Puccinia graminis f. sp. *tritici* race TTKSF+ was collected from the South African wheat cultivar ‘Matlabas’ in 2010. F₂ and F₃ populations derived from a Matlabas × Line 37-07 cross segregated for a single resistance gene to race TTKSF that is avirulent to Matlabas. In screening genomic DNA bulks of susceptible or resistant F₂ plants with simple sequence repeat (SSR) markers, three chromosome arm 2BS markers and one multilocus marker amplified alleles present only in the resistant bulks and Matlabas. Additional 2B-specific SSR markers, incorporating markers spanning regions containing *Sr9h*, *SrWLR*, *Sr28*, and *Sr47*, were screened in the parental lines and mapped in the F₂ population. Linkage and QTL mapping showed that the gene is located between *Xbarc160* in the centromeric region

and *Xgwm47* on the long arm of chromosome 2B. When 2B-specific SNP markers were mapped, the area of interest was delimited to a 15.3 cM region on chromosome arm 2BL, with *XIWA543-HRM* and *Xgwm47* as flanking loci. Matlabas, Webster, and related *Sr9h* lines all produced a similar, low infection type to race TTKSF, but were susceptible to race TTKSF+. Phenotypic data and allelic studies suggested that stem rust resistance in Matlabas was derived from an *Sr9h* source.

Keywords: fungi, field crops, cereals and grains, disease management, cultivar/resistance, epidemiology, disease development and spread, pathogen diversity

The detection of the African *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn race TTKSK (Ug99) stimulated research on wheat stem rust (Jin et al. 2008; McIntosh and Pretorius 2011; Pretorius et al. 2000; Singh et al. 2011, 2015). Given that Ug99 and its related races are virulent on most wheat (*Triticum aestivum* L.) cultivars (Singh et al. 2015), stem rust surveillance and race analyses have been a distinctive focus worldwide (Hodson 2011; Park et al. 2011). In addition to the original description of Ug99 (Pretorius et al. 2000), detection of related races with new virulence profiles raises serious concerns about the threat posed by this adaptable *P. graminis* f. sp. *tritici* race group. At present, 13 variants have been described with virulence gains for the major resistance genes *Sr9h*, *Sr24*, *Sr36*, and *SrTmp* (Patpour et al. 2016; Singh et al. 2015). According to <https://rusttracker.cimmyt.org> Ug99-lineage races have been detected in 11 African countries as well as Yemen and Iran.

Race TTKSF was first detected in South Africa in 2000 (Boshoff et al. 2002) and has since dominated the local *P. graminis* f. sp. *tritici* population (Terefe et al. 2016). Although TTKSF lacks virulence for *Sr31*, Visser et al. (2009) provided evidence that this race belongs to the Ug99 group. In 2010, a race classified as TTKSF+ was collected from the severely stem rust infected bread wheat cultivar ‘Matlabas’ in the eastern Free State, South Africa (Pretorius et al. 2012).

‘Matlabas’, developed by ARC-Small Grains, was released for winter wheat production under dryland conditions in the Free State in 2004 (Anonymous 2007; Smit et al. 2010). The cultivar was favored by growers due to its preharvest sprouting tolerance, excellent straw strength, and standability. Plantings of ‘Matlabas’ increased steadily and contributed an estimated 18.2% of winter wheat grain produced in the eastern Free State in 2010 (Anonymous 2011).

The objectives of this study were to use pathogenicity tests and a QTL mapping approach to locate the chromosome position and determine the identity of the ‘Matlabas’ stem rust (*Sr*) resistance gene. In addition to accurately documenting diversity in *P. graminis* f. sp. *tritici*, confirmation of the resistance gene which became ineffective in ‘Matlabas’ will allow its detection in other wheat germplasm and add to descriptions of stem rust vulnerability.

Materials and Methods

Plant material. An F₂ population and F₃ families were developed from a cross between ‘Matlabas’ (pedigree: Saulesku-28/Tugela-DN) and stem rust-susceptible Line 37-07. Line 37-07 was selected as entry 37 (pedigree: Kasyob/Genaro-81//Cham4) of the 2nd International Stem Rust Trap Nursery (ISRTN07) based on its susceptibility to stem rust in field trials in South Africa. All progeny were derived from a cross between a single plant of each parent. Due to the vernalization requirement of offspring, F₃ families were advanced from F₂ plants which were different than those phenotyped as seedlings. The F₃ families were selected without prior knowledge of their stem rust response, and each contained a minimum of 30 plants per family. To test for an allelic relationship between the resistance gene in ‘Matlabas’ and *Sr9h*, ‘Matlabas’ was crossed with ‘Webster’, CItr 4311, and an F₃ selection from a RL6071 × Webster cross. The presence of *Sr9h* has been confirmed in these lines (Babiker et al. 2016b; Hiebert et al. 2010; Rouse et al. 2014). F₂ populations were developed from a single F₁ spike for each cross. Additional *Sr* gene tester lines (Tables 1 and 2) were included for phenotypic and/or molecular assessments. These lines were selected because they carry *Sr* genes on either the short or long arm of wheat

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chromosome 2B, the target gene location suggested by initial mapping results.

Assessments of phenotype. ‘Matlabas’, Line 37-07, 247 Matlabas × Line 37-07 F₂ plants, 79 Matlabas × Line 37-07 F₃ families, and the *Sr* gene tester lines were evaluated in the seedling stage for their response to *P. graminis* f. sp. *tritici* race TTKSF (isolate UVPgt55_04/2002). This race is avirulent on ‘Matlabas’ and virulent on Line 37-07. Once assessment of F₃ families with TTKSF was completed, those families homozygous for resistance, and all tester lines, were also inoculated with *P. graminis* f. sp. *tritici* race TTKSF+ (isolate UVPgt61/1_13/07/2011) which is virulent on ‘Matlabas’. For the allelism tests, F₂ seedlings of Matlabas × Webster, Matlabas × CTr 4311, and Matlabas × RL6071/Webster together with the respective parents, were tested for resistance to race TTKSF. For all seedling tests, seed was sown in a sterilized soil-peat moss (50:50) mixture in 10-cm-diameter pots which were maintained in a growth chamber at 22°C until seedling emergence, before placement at 18 to 23°C under natural light in a greenhouse. Primary leaves of plants were inoculated 8 days after sowing with a 5 mg/ml suspension of urediniospores in Soltrol 130 (Chevron Phillips Chemical Company, The Woodlands, TX) light mineral oil. After drying for 1 h, plants were placed in a dark dew chamber for 16 h. Upon removal, seedlings were dried for 3 h under light in a growth chamber and returned to the greenhouse under the same conditions as before. Infection types (0 to 4 scale, Stakman et al. 1962) were scored 13 days postinoculation. All seedling tests were repeated except for the F₂ and F₃ Matlabas × Line 37-07 germplasm, for which limited genetic stocks were available.

Molecular analysis, linkage mapping, and QTL analysis. Following phenotyping, leaf samples were collected from the respective resistant or susceptible Matlabas × Line 37-07 F₂ plants and the parents at the three-leaf stage and freeze-dried for genomic DNA (gDNA) extraction. A CTAB (cetyltrimethyl ammonium bromide) DNA extraction method was used (Doyle and Doyle 1990). The gDNA was quantified with a Nanodrop Spectrophotometer ND-1000 (Thermo Scientific, Wilmington, DE) and diluted to a working concentration of 25 ng/μl. One susceptible (S) and two resistant (R) gDNA bulks, consisting of 10 randomly selected F₂ plants each, were constructed using equimolar amounts of DNA (Michelmore et al. 1991). The individuals in the R-bulks could have been either homozygous or heterozygous resistant.

The parental lines and the bulks were then screened with a low resolution genome scan (LRGS) marker set consisting of 104 microsatellite (SSR) markers (Wessels and Prins 2017). The polymerase chain reaction

(PCR) mixtures contained 0.08 mM of each deoxynucleotide, 1.5 mM MgCl₂, 0.25 U *Taq* polymerase, and 3 to 10 pmol of fluorescently labeled primer, depending on the primer’s amplification efficiency. PCR products were analyzed on a 3730xl DNA Analyzer (Applied Biosystems Inc., Foster City, CA) at the Central Analytical Facility of Stellenbosch University, South Africa. GeneScan 500 LIZ size standard (Applied Biosystems Inc.) was used as an internal size standard. The data were analyzed using GeneMapper V4.0 (Applied Biosystems Inc.).

Once marker-trait confirmation was obtained, the 2B linkage map for the Matlabas × Line 37-07 F₂ population was improved by screening additional chromosome 2B-specific SSRs in the parental lines with a focus to incorporate as many SSR markers as possible reported to span regions containing *Sr9h* (previously *SrWeb*), *SrWLR*, and *Sr28* (Appels 2003; Bansal et al. 2012; Hiebert et al. 2010; Rouse et al. 2012, 2014; Somers et al. 2004; Sourdille et al. 2004; Zurn et al. 2014). Furthermore, single nucleotide polymorphism (SNP) markers on the 9K Illumina Infinium iSelect wheat assay (Cavanagh et al. 2013) that previously mapped to the region of interest (Zurn et al. 2014) were converted to high resolution melt-curve (HRM) markers (Supplementary Table S1) using Primer3 V4.0.0 (Koressaar and Remm 2007; Untergasser et al. 2012). The parents were initially screened with these markers to confirm polymorphism. The real-time PCR reaction mixture contained 1× Kapa HRM Fast PCR Kit (Kapa Biosystems, Cape Town, South Africa), 2.5 mM MgCl₂, and 3 pmol primer. Reactions were performed in a Bio-Rad CFX96 real-time thermocycler (Bio-Rad Laboratories (Pty) Ltd., Hercules, CA), and the data were analyzed with Bio-Rad CFX Manager software V3.1 and Bio-Rad Precision Melt Analysis software V1.2 (Bio-Rad Laboratories (Pty) Ltd.).

To ensure a linkage map of high quality, only codominant polymorphic SSR and HRM markers were then screened in the F₂ population. Several markers previously reported to be associated with stem rust resistance on chromosome 2BL were included in the parental screens, but were not screened in the Matlabas × Line 37-07 F₂ population for various reasons: GWM388, BARC101, CFD73, WMC332, BARC128, WMC477 (not polymorphic), GWM120 (dominant marker), and WMC360 (complex profile; Akbari et al. 2006; Bansal et al. 2012; Hiebert et al. 2010; Somers et al. 2004; Tsilo et al. 2007). Nine markers spanning the chromosome 2B region in which the Matlabas gene was mapped were also used to genotype selected chromosome 2B control lines and resistant and susceptible Matlabas × Line 37-07 F₃ families.

Based on the results obtained from the bulk and parental screen, three chromosome 2B markers, putatively associated with the

Table 1. Infection types of wheat lines with *Sr* genes known to occur on chromosome 2B to *Puccinia graminis* f. sp. *tritici* races TTKSF and TTKSF+

Line	<i>Sr</i> gene	Chrom. location	Source	TTKSF	TTKSF+
ISr9a-Ra CI14169	<i>9a</i>	2BL	Seventh ISRTN#8	4	4
Prelude*4/2/Mq*6/K117A	<i>9b</i>	2BL	Seventh ISRTN#9	4	4
ISr9d-Ra CI14177	<i>9d</i>	2BL	Seventh ISRTN#10	4	4
Vernstein PI 442914	<i>9e</i>	2BL	Seventh ISRTN#11	4	4
Acme	<i>9g</i>	2BL	UFS II-25	4	4
W2691Sr10 CI 17388	<i>10</i>	2BL	Seventh ISRTN#13	4	4
Thatcher/CS CI 14173	<i>16</i>	2BL	Seventh ISRTN#19	3+	3++
94A 236-1Marquis “B”	<i>19</i>	2BS	Seventh ISRTN#23	4	4
94A 237-1 Marquis “C”	<i>20</i>	2BL	Seventh ISRTN#24	4	4
Exchange	<i>23</i>	2BS	Seventh ISRTN#28	3+	4
Kota RL471	<i>28</i>	2BL	Seventh ISRTN#33	;12	;1
W2691SrTt-1	<i>36</i>	2BS	UFS II-74	0;	0;
2S#2/163	<i>39</i>	2BS	UFS II-111	;	1 =
RL5711	<i>39</i>	2BS	Seventh ISRTN#46	1	1
RL6087	<i>40</i>	2BS	Seventh ISRTN#47	1	1
DAS15	<i>47</i>	2BS	CDL	;1-	;1-
LCSrWst-2Wst	<i>Wst-2</i>	- ^a	UFS II-80	1	4
Matlabas	?	2BL	UFS CV2011#84	1+	4
Webster	<i>9h</i>	2BL	CRC CM661	2	3+
RL6071/Webster F3	<i>9h</i>	2BL	CRC CM664	2	3++
RL6071	<i>Susc. contr.</i>	-	CRC CM354	3+	3++
PI 626573-2	<i>SrWLR</i>	2BL	PI 626573-2	2-	33+

^a Not yet confirmed.

resistance gene, were screened on 186 individual F₂ plants to create a preliminary, partial linkage map of chromosome 2B with JoinMap V4.1 using an independence test LOD score of 10 for linkage group formation (Van Ooijen 2006). The map was constructed using the Monte Carlo Maximum likelihood (ML) mapping algorithm (Van Ooijen 2011) and the Kosambi mapping function (Kosambi 1943). A final linkage map was constructed with the complete dataset (including additional polymorphic SSR and HRM markers). To avoid using the trait as a dominant marker in an F₂ population, a QTL mapping approach was followed. For QTL analysis, phenotypic data were converted to “1” (for susceptible plants) and “0” (for resistant plants). Kruskal-Wallis single marker trait associations were calculated and interval mapping (IM) was performed using MapQTL V6 (Van Ooijen 2009). Default parameters were used with the number of permutations set at 10,000 to determine QTL significance.

Results

Assessments of phenotype. Matlabas × Line 37-07 F₂ plants and F₃ families segregated for a single resistance gene to TTKSF. In the F₂ generation, 74% of the plants were resistant, showing a clear 1+ to

2 infection type, whereas 26% were susceptible (Supplementary Fig. S1). ‘Matlabas’ produced infection type 1+ to TTKSF and Line 37-07 a distinctively more susceptible 3++ infection type. The χ^2 value (χ^2 of 0.228 ($P = 0.663$)) did not deviate from the expected ratio for single, dominant gene inheritance (3:1) in the F₂ generation. F₃ families segregated in a 15 homozygous resistant, 52 segregating, and 12 homozygous susceptible ratio ($\chi^2 = 8.139$, $P = 0.017$) which deviated from the expected 1:2:1 ratio. Both parents and all F₃ families homozygous for resistance to TTKSF were susceptible to TTKSF+ (infection types 3+ to 4). All F₂ progeny from each of the crosses Matlabas × Webster ($n = 527$ seedlings), Matlabas × Citr 4311 ($n = 628$ seedlings), and Matlabas × RL6071/Webster ($n = 601$ seedlings) as well as the parents were resistant to race TTKSF. In these populations, resistance was uniformly expressed as a 1 to 2- infection type, which was clearly different from the susceptible control (Supplementary Fig. S2).

The chromosome 2B resistance genes *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr10*, *Sr16*, *Sr19*, *Sr20*, and *Sr23* were ineffective to TTKSF and TTKSF+ (Table 1). *Sr28* (2BL), *Sr36* (2BS), *Sr39* (2BS), *Sr40*, and *Sr47* (2BL) were effective to both races, indicating that the

Table 2. Selected simple sequence repeat (SSR) marker profiles of lines with *Sr* genes known to occur on the chromosome 2B region of interest

Line	<i>Sr</i> gene	Chrom. location	Source	GWM148	BARC230	WMC592	GWM319	BARC167	BARC160	WMC441	IWA543-HRM ^{a,b}	GWM47
Line 37-07	<i>Susc. control</i>	-	UFS ^c sel 6A	166	198	272	172	248	105	166	L	160 ^d
Matlabas ^e	?	2BL	AK2010 ^f	143	201	278	174	267	116	176	M	183
Vernstein	<i>9e</i>	2BL	Seventh	162	200	270	174	257	110	158	L	-
PI 442914			ISRTN#11 ^g									
LC5rWst-2Wst	<i>Wst-2</i>	?	UFS IL-80	145	175	270	174	260	110	162	L	189
Thatcher	<i>5, 9g, 12, 16, & APR^h</i>	9g, 16 & APR on 2BL	Seventh ISRTN#83	164	209	276	172	236	105	160	L	null
Webster CM661	<i>9h</i>	2BL	CRC Winnipeg ⁱ	143	175	270	174	260	110	162	L	189
RL6071/ Webster F3	<i>9h</i>	2BL	CRC Winnipeg	164	207	272	174	257	110	162	L	189
RL6071	<i>Susc. control</i>	-	CRC Winnipeg	154	201	272	174	257	110	174	M	null
Gabo56	<i>9h</i>	2BL	CRC Winnipeg	166	198	272	172	248	105	158	L	null
PI 626573-2	<i>WLR</i>	2BL	NDSU ^j	143	201	270	174	267	116	153	M	187
Citr-4311-1 ^k	<i>9h</i>	2BL	USDA ^l Aberdeen	143	201	270	174	267	119	182	L	177
PI 178188-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	143	201	270	172	250	110	148	L	null
PI 623118-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	143	201	270	174	260,267	110,119	174,181	L	181
PI 623582-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	143	201	270	174	267	119	182	L	179
PI 623785-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	143	201	270	172	250	110	148	L	null
PI 625315-1 ^m	<i>9h+</i>	2BL	USDA Aberdeen	139	201	278	174	263	116	178	L	179
PI 625348-1 ^m	<i>9h+</i>	2BL	USDA Aberdeen	143	201	270	174	267	119	181	L	181
PI 626255-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	143	199	279	174	244	110	155	D ⁿ	175, 183
PI 626409-1 ^m	<i>9h+</i>	2BL	USDA Aberdeen	143	201	276	174	267	116	176	L	183
PI 626491-1 ^m	<i>9h</i>	2BL	USDA Aberdeen	156	199	273	174	267	116	176	L	189

^a Marker with the highest significant LOD score.

^b qPCR marker results cluster as “M” (Matlabas allele) or “L” (Line 37-07 allele).

^c University of the Free State.

^d The third locus of the multilocus marker GWM47 mapped to the Matlabas gene location.

^e The Matlabas alleles are in bold.

^f The AK2010 seed source refers to the particular Matlabas plant from which TTKSF+ was isolated in 2010.

^g International Stem Rust Trap Nursery.

^h Adult plant resistance.

ⁱ Cereal Research Centre Winnipeg.

^j North Dakota State University.

^k According to Babiker et al. (2016b).

^l United States Department of Agriculture.

^m According to Babiker et al. (2016a).

ⁿ Different allele to those in Matlabas and Line 37-07.

defeated Matlabas gene is different (Table 1). Similar to 'Matlabas', Line LCSrWst-2Wst with *SrWst-2* produced a low infection type to TTKSF, but a high infection type to TTKSF+. 'Webster', PI 626573-2, and an RL6071/Webster F_3 line fixed for *Sr9h*, but lacking *Sr30*, showed comparable low and high infection types to races TTKSF and TTKSF+, respectively (Table 1).

Molecular analysis, linkage mapping, and QTL analysis. A total of 68% of the 104 LRGS markers used to screen the Matlabas $\times$ Line 37-07 gDNA bulks and parents were polymorphic and represented all 21 chromosomes. This served as a sound basis to identify chromosomes that may be associated with the single gene trait under investigation. Three polymorphic chromosome 2B markers (GWM148, WMC35, and BARC160) and one multilocus (2B, 5B) marker (WMC27) amplified alleles that were present in the R-bulks and 'Matlabas', but were absent in the S-bulk and susceptible parent (Supplementary Fig. S3). This result indicated that the markers were associated with the resistance gene. All other markers amplified both the parental alleles in the R- as well as the S-bulks and were thus not associated with the resistance gene. A subset of 186 Matlabas $\times$ Line 37-07 F_2 individuals was screened with the three single locus 2B markers. Kruskal-Wallis single marker regression analysis indicated significant associations between the trait and chromosome 2B markers ($P < 0.05$). The parental lines were screened with an additional 74 chromosome 2B-specific SSR and HRM markers of which 32% were polymorphic. The Matlabas $\times$ Line 37-07 F_2 individuals were subsequently screened with these markers, bringing the total number of polymorphic 2B markers to 27.

Seven markers remained unlinked, of which three, WMC175, CFA2043 and GWM382, were multiloci markers, while 20 markers were assigned to a 77.5 centimorgan (cM) linkage group partially covering the short arm, centromeric region, and long arm of chromosome 2B (Fig. 1).

The order of markers between the chromosome 2B linkage group from the present study and the 2B consensus maps of Appels (2003) and Somers et al. (2004) was similar with minor rearrangements between the three maps (Fig. 2). All markers where minor rearrangements

were observed occurred within a 6.4 cM interval (*Xwmc344* to *Xbarc160*) on the Matlabas-derived 2B map and span the region surrounding the centromere (Appels 2003; Somers et al. 2004), which was located approximately 19 cM from the region of interest in Matlabas (Fig. 2). Additionally, the order of the two markers on the distal end of 2B was inverted between the Somers et al. (2004) and Matlabas-derived maps. One locus mapped here (*Xbarc230*) was not included on either of the consensus maps, but was placed on the short arm distal to *Xcfa2278* and *Xgwm47* by Sourdille et al. (2004). The decision to utilize only codominant markers resulted in a 2B map comparable with other reliable maps.

All chromosome 2B markers indicated a significant association ($P < 0.05$) with the trait using Kruskal-Wallis single marker regression analysis. Interval mapping performed with MapQTL V6 in the Matlabas $\times$ Line 37-07 F_2 population indicated that the gene was located between *XIWA543-HRM* and *Xgwm47* on chromosome arm 2BL (Fig. 1) with *IWA543-HRM* being the marker closest to the gene of interest explaining 92.6% of the phenotypic variance observed. The gene was thus defined to a region spanning 15.3 cM. For multilocus marker GWM47, both parental lines (Matlabas and Line 37-07) amplified a 126-bp product for the first locus (thus nonpolymorphic) and a dominant profile for the second (null/145 bp). Therefore, only the codominant alleles identified for the third locus of *Xgwm47* (183 bp/160 bp) were mapped in the F_2 population and scored in 20 Matlabas $\times$ Line 37-07 F_3 families represented by a single F_3 plant each.

Analysis of individual resistant and susceptible Matlabas $\times$ Line 37-07 F_3 plants representing 20 different F_3 families with markers spanning the gene region (GWM148, BARC230, WMC592, GWM319, BARC167, BARC160, WMC441, IWA543-HRM, and GWM47) validated the marker-trait associations identified in the F_2 population (Table 3). The 10 resistant Matlabas $\times$ Line 37-07 F_3 plants, representing families homozygous for resistance to TTKSF, amplified the same allele as in 'Matlabas' with all markers except for GWM148, which was the marker most distant (30.8 cM) to the resistance gene. Of the 10 susceptible Matlabas $\times$ Line 37-07 F_3 plants, eight amplified only the susceptible allele for GWM148.

The marker which was most significantly linked to the Matlabas *Sr* gene (*IWA543-HRM*) amplified the Matlabas allele in the susceptible control line that lacks *Sr9h* (RL6071) and in PI 626573-2 (*SrWLR*), but not in any of the *Sr9h* carrying control lines including RL6071/Webster and 'Webster', all of which produced the Line 37-07 allele (Table 2). *IWA543-HRM* detected a different allele to either 'Matlabas' or Line 37-07 in the *Sr9h* control line PI 626255-1. GWM47 is a multilocus marker and appeared to produce two amplicons for the third locus (175 bp and the Matlabas allele of 183 bp) in PI 626255-1. The two flanking SSR Matlabas alleles of *Xwmc441* (176 bp) and *Xgwm47* (183 bp) were not detected in either PI 626573-2 (*SrWLR* control) or in any of the *Sr9h*-postulated lines except for PI 626409-1 (*Sr9h*+) . In addition, the *Xwmc441*-176 bp Matlabas allele was detected in PI 626491-1 (*Sr9h*), but with the Matlabas alleles for *IWA543-HRM* and GWM47 being absent.

Discussion

The segregation ratio of the phenotypic scores on the Matlabas $\times$ Line 37-07 F_2 seedlings indicated that a dominant, single gene was responsible for the resistance in 'Matlabas'. The deviation from the 1:2:1 single gene segregation in the Matlabas $\times$ Line 37-07 F_3 families is most likely the result of outcrossing between families in the field where they were produced without covering the spikes during anthesis. Traditionally, the chromosomal position of a major gene can be determined using qualitative trait mapping where the phenotypic trait is imported as a dominant molecular marker into the mapping software. However, using dominant markers for genetic mapping in an F_2 population decreases the accuracy of a genetic map (Semagn et al. 2006). Since homozygous and heterozygous gene carriers could not be discriminated phenotypically, a QTL approach was used.

To accelerate and reduce the cost of the identification of the chromosome position of the unknown gene, a previously developed LRGS consisting of 104 SSRs located over a large genetic interval

Fig. 1. LOD profile obtained with interval mapping in MapQTL V6 of the stem rust resistance gene located on chromosome arm 2BL in 'Matlabas'. The marker most significantly associated with the gene is indicated in bold. The significance level shown was determined at LOD 4.8 with 10,000 permutations.

covering all 21 chromosomes was utilized in the present study. Although standard bulking approaches (Michelmore et al. 1991) to identify the chromosome locations of single major and partially dominant resistance genes have been used with varied success (Ghazvini et al. 2013), it was considered worthwhile to use the LRGS combined with a bulking approach as a first step to target the major *Sr* gene in 'Matlabas'. Despite the limitation of possibly working with heterozygous resistant bulks, the bulking strategy proved effective for the detection of the gene. The intermarker distances between *XIWA543-HRM*, closest to the gene of interest, and the three SSR marker loci initially associated with the trait in the bulks were 30.8

cM (*Xgwm148*), 29.5 cM (*Xwmc35*), and 18.9 cM (*Xbarc160*), respectively. Ghazvini et al. (2013) advocated the use of a multiple BSA, which they used effectively to locate *Sr54*, a major effect gene that was located on a large linkage block on chromosome 2D. The success of the standard BSA approach used in the present study can probably be attributed to the major resistance response of the single *Matlabas* *Sr* gene segregating in the population.

The region identified on chromosome arm 2BL has previously been shown to be rich in stem rust resistance, including *Sr28* (Rouse et al. 2012), *Sr47* (Klindworth et al. 2012), *Sr9h* (formerly *SrWeb*; Hiebert et al. 2010; Rouse et al. 2014), and *SrWLR* (Zurn et al.

Fig. 2. Comparison of published genetic maps pertaining to the chromosome region of interest in 'Matlabas'. Other stem rust resistance genes previously mapped in the area are indicated in gray.

Table 3. Genotypes of individual F_3 plants representing different F_3 families screened with simple sequence repeat (SSR) markers spanning the *Matlabas* *Sr* gene region

Line	GWM148		BARC230		WMC592		GWM319		BARC167		BARC160		WMC441		IWA543-HRM ^{a,b}		GWM47	
Matlabas ^c	143		201		278		174		267		116		176		M		183	
Line 37-07	166		198		272		172		248		105		166		L		160	
KW203_R ^d	166		201		278		174		267		116		176		M		183	
KW215_R	143		201		278		174		267		116		176		M		183	
KW216_R	166		201		278		174		267		116		176		M		183	
KW231_R	143		201		278		174		267		116		176		M		183	
KW233_R	143		201		278		174		267		116		176		M		183	
KW245_R	143		201		278		174		267		116		176		M		183	
KW256_R	143		201		278		174		267		116		176		M		183	
KW259_R	143		166 198		201 272		278 172		174 248		267 105		116 176		M		183	
KW261_R	143		166 198		201 272		278 172		174 248		267 105		116 166		176 M		183	
KW262_R	143				201 278		174		267		116		176		M		183	
KW200_S	166		198		272		172		248		105		166		L		160	
KW212_S	166		198		272		172		248		105		166		L		160	
KW214_S	166		198		272		172		248		105		166		L		160	
KW232_S	166		198		272		172		248		105		166		L		160	
KW241_S	166		198		201 272		278 172		174 248		267 105		116 166		L		160	
KW247_S	166		198		272		172		248		105		166		L		160	
KW249_S	166		198		272		172		248		105		166		L		160	
KW252_S	166		198		272		172		248		105		166		L		160	
KW258_S	143		166 198		272		172		248		105		166		L		160	
KW260_S	143		166		201 278		174		267		116		166		L		160	

^a Marker with the highest significant LOD score.

^b qPCR marker results cluster as "M" (*Matlabas* allele) or "L" (Line 37-07 allele).

^c The *Matlabas* alleles are in bold.

^d R = resistant and S = susceptible to *Puccinia graminis* f. sp. *tritici* race TTKSF.

2014). Rouse et al. (2012) mapped *Sr28* 5.9 cM distal to *Xgwm332*, which, according to the consensus map of Appels (2003), is located 2 cM distal to *Xgwm47*. Since *Xgwm47* is located 15.3 cM distal from the *Matlabas Sr* gene, it implies an even further map distance of 23.2 cM between *Sr28* and the gene of interest (Fig. 2). Evidence that *Sr28* is effective against TTKSF+ and other members of the Ug99 race group (Singh et al. 2015) supports the results of the genetic map comparisons that the *Sr* gene in 'Matlabas' is not *Sr28*.

Sr47 derived from *Aegilops speltoides* has previously been mapped close to *Xgwm501* and *Xgwm47* in durum wheat, placing it in the region of the *Matlabas Sr* gene (Klindworth et al. 2012; Fig. 2). *Sr47* is derived from a wild relative of wheat not in the pedigree of 'Matlabas'. Also, line DAS15, which has *Sr47*, showed low infection types to both TTKSF and TTKSF+ (Table 1). These results support the hypothesis that the stem rust resistance in 'Matlabas' is not due to *Sr47*.

Other Ug99 effective genes on 2BL (i.e., *Sr9h*, *SrWLR*, and the *Sr* gene in 'Matlabas') were susceptible to TTKSF+ (Table 1; Babiker et al. 2016a; Rouse et al. 2012; Zurn et al. 2014) and mapped to the same 2BL region (Yu et al. 2014; Fig. 2). The 1 to 2 low infection type of 'Webster' (*Sr9h/SrWeb*) and PI 626573 (*SrWLR*) to TTKSK (Hiebert et al. 2010; Zurn et al. 2014) closely resembles the *Matlabas* TTKSF phenotype (TTKSF is similar to TTKSK except for avirulence to *Sr31*). Based on infection type data the gene in 'Webster', LCSrWst-2Wst, and the line RL6071/Webster (Table 1), appears to be the same resistance gene (*Sr9h/SrWeb*) which broke down in 'Matlabas'. The specificity of *SrWst-2* toward TTKSF and TTKSF+, together with the fact that this gene was also transferred from 'Webster' (Roelfs et al. 1992), indicate it may be *Sr9h*. Infection type data with TTKSF and TTKSF+ from Rouse et al. (2014) and Babiker et al. (2016a) correspond to this postulation. Results from the allelism tests performed by test crossing 'Matlabas' with 'Webster', Ctr 4311 and line RL6071/Webster previously confirmed to carry *Sr9h* (Babiker et al. 2016b; Hiebert et al. 2010; Rouse et al. 2014) further supported the hypothesis based on infection types that the gene in 'Matlabas' is *Sr9h*. All progeny from the three crosses were resistant to race TTKSF, indicating the presence of the same dominant resistance gene in the respective parental lines.

Rouse et al. (2014) mapped *Sr9h* 2.8 cM distal to *Xgwm47* using a Gabo56 × Chinese Spring F₂ population (80 individuals for genotyping). They used seven SSRs and the stem rust resistance as a bridge to incorporate dominant markers in a combined map consisting of both dominant and codominant marker types. Hiebert et al. (2010) used a larger F₂ population (174) and placed *SrWeb* (*Sr9h*) 1.4 cM distal to *Xgwm47* using six SSRs, of which two (WMC175, GWM47) were inherited in a dominant fashion. Most of the markers on the 2BL *Matlabas*-derived map (Fig. 2) are in common with the various studies on genes in these regions except for the multiallelic markers (WMC175, CFA2043, and GWM382) of which the polymorphic allele remained unlinked, indicating a different chromosome position. Given the 15.3 cM interval between *XIWA543-HRM* and *Xgwm47* and the previously mapped distances of 1.4 cM (*SrWeb*) and 2.8 cM (*Sr9h*) distal from *Xgwm47*, distances of 16.7 cM and 18.1 cM, respectively, are implied between *XIWA543-HRM* and *SrWeb/Sr9h* (Fig. 2). However, HRM marker IWA543-HRM was derived from the SNP marker IWA543 on the 9K Illumina Infinium iSelect wheat assay, which is located on the long arm of chromosome 2B at 109.53 cM (Wang et al. 2014). This is the exact location of a gene hypothesized to be *Sr9h* (Babiker et al. 2016b), and overlaps with the map position of the gene in 'Matlabas'.

Zurn et al. (2014) mapped *SrWLR* in the same region as *Sr9h*, 1.5 cM distal to *Xgwm47*, in Iranian landrace accession PI 626573 (Fig. 2) using a large set of SSR and SNP markers. Marker analysis with 12 SNP markers flanking *SrWLR* revealed that PI 626573 and the cultivar 'Webster' (*Sr9h/SrWeb*) amplified different alleles, indicating that *SrWLR* and *Sr9h* may not be the same (Zurn et al. 2014). *SrWLR* provides resistance to North American race RKQCC, whereas it is unknown whether *Sr9h* in 'Webster' provides resistance to race RKQCC due the presence of *Sr30* (Zurn et al. 2014). However,

Ctr-4311-1 showed resistance to race RKQCC and likely carries *Sr9h* (Babiker et al. 2016b).

Marker analysis of the gene region of lines known to carry some of the known *Sr* genes on chromosome arm 2BL did not provide clear confirmation of the identity of the *Matlabas* gene (Table 2). The amplicon for the marker most significantly linked to the gene (IWA543-HRM) in 'Matlabas' was only in agreement with the *SrWLR* control (Iranian landrace PI 626573-2), while the two flanking loci (*Xgwm441* and *Xgwm47*) differed for this line. Furthermore, IWA543-HRM amplified the *Matlabas* allele in the line not carrying *Sr9h* (RL6071, pedigree Prelude/8*Marquis*2/3/Prelude//Prelude/8*Marquis). Neither IWA543-HRM, nor the flanking SSR markers WMC441 and GWM47, amplified the same alleles in 'Matlabas' as in the 13 *Sr9h* control lines. Among the haplotypes of these control lines, none was associated with *Sr9h* across all of the lines, even though most of these accessions are landraces from the same country of origin (Babiker et al. 2016b). A possible explanation is the distal map location of the gene where crossovers are more likely to occur.

The identity and relationships of the genes in this region of the long arm of chromosome 2B need further clarification. One of the main issues is the discrepancies in map positions reported for these genes. The area in which these genes are located remains contested in terms of marker order, which could be the result of different marker types and population types and sizes used by the various groups investigating these genes (Hiebert et al. 2010). The use of dominant markers such as GWM47, the marker linked to *Sr9h* (Babiker et al. 2016b; Hiebert et al. 2010; Rouse et al. 2014), decreases the accuracy of the genetic map, specifically in F₂ populations, in terms of marker order as 50% of the individuals are heterozygous and cannot be discriminated from the homozygous individuals (Semagn et al. 2006; Xu 2010).

Using competitive allele-specific PCR (KASP) assays, Babiker et al. (2016b) identified markers closely linked to a gene suspected to be *Sr9h*. However, Zurn et al. (2014) was not able to identify SNPs that could discriminate between and serve as diagnostic markers for either *SrWLR*, *Sr9h*, or any of the *Sr9* alleles despite mapping 2,846 polymorphic SNP markers (9K Illumina Infinium iSelect). Thus, diagnostic markers are needed for the stem rust resistance genes in this region of chromosome 2BL, which will most likely be achieved through cloning of the genes in this region (Zurn et al. 2014). With the availability of the annotated wheat genome (IWGSC 2018), this objective has become more feasible. Nonetheless, similar to Rouse et al. (2014), the allelism tests in the present study support the hypothesis that the *Matlabas* resistance gene is *Sr9h*.

Before the discovery of TTKSF+ and based on available data, 'Matlabas' was resistant to stem rust. However, more recent work with TTKSF isolates in the rust culture collection at University of the Free State determined that a 2003 isolate of *P. graminis* f. sp. *tritici* was mixed for avirulence and virulence on line LCSrWst-2Wst (Singh et al. 2015). A purified subculture confirmed virulence for 'Matlabas' and thus provided evidence that race TTKSF+ was present in South Africa before its collection in 2010. Since lines vulnerable to this race (such as *Sr9h*) were not part of the differential sets before 2010, the occurrence of TTKSF+ went unnoticed. Furthermore, TTKSF+ was also observed in a stem rust sample collected at Birchenough, Zimbabwe in September 2010 (Pretorius et al. 2012). Without additional testing of historic isolates, it is not possible to determine whether TTKSF+ survived in South Africa at low frequencies or whether it was reintroduced from Zimbabwe in 2010. From survey data, Terefe et al. (2016) reported that TTKSF+ was infrequently detected in South Africa. The race occurred in the Free State in 2010, whereas three *P. graminis* f. sp. *tritici* collections from the Western Cape were identified as TTKSF+ in 2013, the latter of which was unexpected since only spring types are grown in the Western Cape and none of the commercial spring wheats is known to carry *Sr9h* or *SrWLR*.

Considering published pedigree information on released South African wheat cultivars, 'Matlabas' is the only cultivar with Saulesku-28 in its pedigree (Smit et al. 2010). Since Tugela-DN is susceptible

to all members of the Ug99 race group, the Matlabas gene was likely transferred from Saulesku-28. Unfortunately, seed of Saulesku-28 could not be procured for stem rust screening in the present study, and the pedigree history of this line is not retrievable (N. Saulescu, *personal communication*). Annual seedling infection type data (not presented) for South African cultivars and advanced breeding lines showed that 'Matlabas' is the only commercial cultivar resistant to the original TTKSF isolate (accession no. 2013) collected in 2000 (Boshoff et al. 2002) and susceptible to isolate UVPgt61/1 of race TTKSF+ sampled from Matlabas plants. Based on phenotypic data less than 5% of breeding lines carry the Matlabas gene. It is thus unlikely that race TTKSF+ will threaten wheat production in South Africa at the present time. The downfall of the resistance in 'Matlabas' follows a similar pattern as *Sr24*, *Sr31*, *Sr36*, and *SrTmp*, confirming the nondurability of major-gene resistance to the Ug99 African race group. This unfortunate trend further exemplifies the need for continuous pathogen monitoring and importance of resistance gene stewardship.

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