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ORIGINAL PAPER

# Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster

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**Abstract** New races of wheat stem rust, namely TTKSK (Ug99) and its variants, pose a threat to wheat production in the regions where they are found. The accession of the wheat cultivar Webster (RL6201) maintained at the Cereal Research Centre in Winnipeg, Canada, shows resistance to TTKSK and other races of stem rust. The purpose of this study was to study the inheritance of seedling resistance to stem rust in RL6201 and genetically map the resistance genes using microsatellite (SSR) markers. A population was produced by crossing the stem rust susceptible line RL6071 with Webster. The $F_2$ and $F_3$ were tested with TPMK, a stem rust race native to North America. The $F_3$ was also tested with TTKSK. Two independently assorting genes were identified in RL6201. Resistance to TPMK was conferred by *Sr30*, which was mapped with microsatellites on chromosome 5DL. The second gene, temporarily designated *SrWeb*, conferred resistance to TTKSK. *SrWeb* was mapped to chromosome 2BL using SSR markers. Comparison with previous genetic maps showed that *SrWeb* occupies a locus near *Sr9*. Further analysis will be required to determine if *SrWeb* is a new gene or an allele of a previously identified gene.

## Introduction

Stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici*) is a disease of wheat that can cause devastating yield losses when susceptible cultivars are grown. In North America the last wheat stem rust epidemic occurred from 1953 to 1955 (Peturson 1958). Since then resistant cultivars have generally controlled the disease; annual virulence surveys of *P. graminis* show that stem rust population is relatively static in North America (Jin 2005; Fetch 2009). Recently, new virulence phenotypes have emerged in Africa, specifically race TTKSK (also known as Ug99) and its variants (Pretorius et al. 2000; Jin et al. 2008). These stem rust races are virulent to most North American wheat cultivars (TG Fetch unpublished data; Jin and Singh 2006). While the introduction of these races into North America poses a threat, previous experience has shown that long term control can be established using stem rust resistance genes. Thus, introducing new resistance will allow the development of elite cultivars with broadened resistance to stem rust. Currently, eight named seedling stem rust resistance (Sr) genes (*Sr13*, *Sr14*, *Sr22*, *Sr28*, *Sr33*, *Sr35*, *Sr42* and *Sr45*) from the primary gene pool of wheat confer resistance to TTKSK (Jin et al. 2007; TG Fetch unpublished data). However, most of the effective resistance postulated to be present in US cultivars resistant to TTKSK does not derive from the primary gene pool (Jin and Singh 2006). Introgressions from outside of the primary gene pool present a barrier to recombination, which can present challenges for wheat breeders. For example, there are no current Canadian wheat cultivars that carry any genes introduced from beyond the primary gene pool.

The accession (RL6201) of the wheat cultivar Webster maintained at the Cereal Research Centre (CRC) in Winnipeg, Canada, was resistant to TTKSK and its variants during indoor seedling testing. Webster is known to carry the recessive gene *Sr30* (Knott and McIntosh 1978); however, *Sr30* does not confer to resistance TTKSK (Jin et al. 2007). The CRC Webster accession (RL6201) also

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displayed resistant field reactions of 10R (2005) and 40 MR (2006) in stem rust nurseries in Njoro, Kenya, in which TTKSK was prominent. Subsequently, a different line carrying only *Sr30* (LMPG*6/Webster) displayed susceptible responses in the Kenya nurseries (50 MSS in 2007; 90S in 2008; TG Fetch unpublished). Therefore, RL6201 must carry other resistance in addition to *Sr30*. Analysis of this resistance in RL6201 could provide wheat breeders with an additional genetic resource for developing cultivars that are more broadly resistant to stem rust including TTKSK. The objectives of this research were to characterise and map the seedling resistance to stem rust found in the CRC accession of Webster (RL6201).

## Materials and methods

### Populations and stem rust testing

To avoid ambiguity, the CRC accession of Webster is hereafter identified as RL6201. The term Webster is reserved for other accessions which may or may not exhibit resistance to TTKSK. A cross was made between RL6071 (Prelude/8*Marquis*2/3/Prelude//Prelude/8*Marquis) and RL6201. The population was advanced to the $F_2$ and $F_3$ generations. $F_2$ seedlings ($n = 174$) were inoculated on the first leaf with *P. graminis* race TPMK, which is native to North America. $F_3$ families were tested twice, once with TTKSK ($n = 123$) and once with TPMK ($n = 135$). In each test, approximately 25 seedlings were grown per $F_3$ family. Inoculations were performed by suspending urediospores in light mineral oil (Bayol 55, Imperial Oil, Toronto, ON, Canada) and spraying the inoculum onto the seedlings using methods described by Fetch (2009). Inoculated seedlings were incubated in a dew chamber in the dark for 16 h and then were given 2 h of light while plants slowly dried off. Plants were grown at approximately 20°C with 16 h of light daily. Two weeks after inoculation, seedlings were rated for their infection type using a 0–4 scale (Stakman et al. 1962); ratings of three or greater were considered a susceptible response. Observed segregation ratios (resistant vs. susceptible) were evaluated using $\chi^2$ to test for the probability of their goodness of fit to various theoretical ratios.

### Genetic mapping

Young leaves were collected from $F_2$ plants and lyophilized for DNA extraction. DNA was extracted using an ammonium acetate method (Chao and Somers, http://maswheat.ucdavis.edu/PDF/DNA0003.pdf, accessed July 2009) based on the procedures of Pallotta et al. (2003). Microsatellite (SSR) markers (Röder et al. 1998; Somers et al. 2004; Song et al. 2005) were used for mapping seedling resistance to TPMK and TTKSK. PCR and fragment analysis of PCR products with an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, ON, Canada) were performed as described by Somers et al. (2004). Genetic maps were constructed using MapMaker version 3.0 (Lander et al. 1987) and genetic distances were calculated using the Kosambi mapping function (Kosambi 1944). Markers specific to chromosome 5DL were used to map resistance to TPMK and confirm the presence of *Sr30* since the location of this gene is already known (McIntosh 1978). Most Sr genes were previously characterized for their infection type in response to TPMK and TTKSK (Martens et al. 1989; Jin et al. 2007; TG Fetch unpublished data). As a starting point, in search for the location of the second gene, SSR markers were chosen based on the genome location of known Sr genes from the primary gene pool, namely *Sr28* and *SrTmp*, that gave pustule types similar to those exhibited by RL6201 and its resistant $F_3$ derivatives.

## Results

### Stem rust testing

RL6201 was resistant to stem rust races TPMK and TTKSK whereas RL6071 was susceptible to both races. Seedlings in the segregating population that were resistant to TPMK showed an infection type of 2 or 2+; seedlings resistant to TTKSK mostly showed an infection type between 1 and 2. These infection types corresponded to those observed in seedlings of RL6201 inoculated with the same isolates. Segregation of resistance to TPMK in $F_2$ fit the model for a single recessive gene (46 resistant, 128 susceptible; $\chi^2_{1:3} = 0.19$, $p = 0.66$). In $F_3$, segregation for resistance to TPMK and TTKSK individually fit a single gene model (Table 1) but the joint pattern of segregation was only just consistent with that expected for two independent genes ($p = 0.07$; Table 2). This distortion of the data could not be attributed to linkage. Among families tested with both races there was an under representation of families that were homozygous resistant to TPMK due to insufficiency of seed for testing, and it is for this reason that the $p$ value for rejecting a two-gene ratio approaches significance ($\alpha = 0.05$). Regardless of the observed ratios, a $\chi^2$ contingency test of the joint data showed that resistances to TPMK and TTKSK were not positively associated ($\chi^2 = 3.51$, $p = 0.90$). Thus, there were two unlinked seedling Sr genes that segregated in this population and both are carried by RL6201. These genes are *Sr30* and a second gene that confers resistance to TTKSK. We have temporarily designated the second gene *SrWeb*.

**Table 1** Segregation of $F_3$ families inoculated with stem rust races TPMK and TTKSK

| Race | Resistant | Segregating | Susceptible | Ratio | $\chi^2$ | $p$ |
|---|---|---|---|---|---|---|
| TPMK | 30 | 62 | 43 | 1:2:1 | 3.40 | 0.18 |
| TTKSK | 28 | 60 | 35 | 1:2:1 | 0.87 | 0.65 |

**Table 2** Segregation of $F_3$ families that were tested with both TPMK and TTKSK

| | | TPMK | | | |
|---|---|---|---|---|---|
| | | Resistant | Segregating | Susceptible | Total |
| TTKSK | Resistant | 6 | 13 | 9 | 28 |
| | Segregating | 12 | 29 | 19 | 60 |
| | Susceptible | 4 | 12 | 16 | 32 |
| | Total | 22 | 54 | 44 | 120 |

1:2:1:2:4:2:1:2:1, $\chi^2 = 14.43$, $p = 0.07$

$\chi^2$ contingency, $\chi^2 = 3.51$, $p = 0.90$

The data shown here is a subset of the families from Table 1

## Genetic mapping

Telocentric mapping and DNA markers were used previously to map *Sr30* to chromosome 5DL (Knott and McIntosh 1978; Bariana et al. 2001). In the RL6071 × RL6201 population *Sr30* (resistance to TPMK) showed linkage to SSR markers on chromosome 5DL (Fig. 1). CFD12 and GWM292 were the closest SSR markers and flanked *Sr30* on 5DL with genetic distances of 9.0 and 16.6 cM respectively.

As described above, SSR markers from chromosomes 4B and 2BL were chosen for initial screening for *SrWeb* based on the location of *Sr28* and *SrTmp*; these were tested for linkage to *SrWeb* (resistance to TTKSK). Markers on chromosome 2BL showed linkage to *SrWeb* (Fig. 2); the closest proximal marker was GWM47 (1.4 cM) while on the distal side, the nearest marker was WMC332 (12.4 cM). All three of the closest SSR markers to *SrWeb* (GWM47, WMC175 and WMC332) were inherited as dominant markers in the RL6071 × RL6201 population. Positive alleles of GWM47 and WMC332 were linked in coupling to *SrWeb* while the positive allele of WMC175 was linked in repulsion.

## Discussion

RL6201 carries two independent seedling Sr genes as demonstrated in this study. One of these genes, *Sr30*, conferred resistance to stem rust race TPMK, which is native to North America. The other gene confers resistance to TTKSK and may represent either a new gene or a new allele.

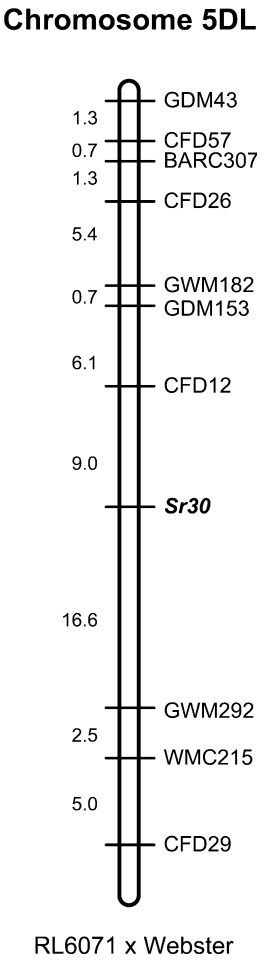


**Fig. 1** Genetic map of *Sr30* on chromosome 5DL using SSR markers. GWM markers are from Röder et al. (1998), WMC and CFD markers are from Somers et al. (2004) and BARC markers are from Song et al. (2005)

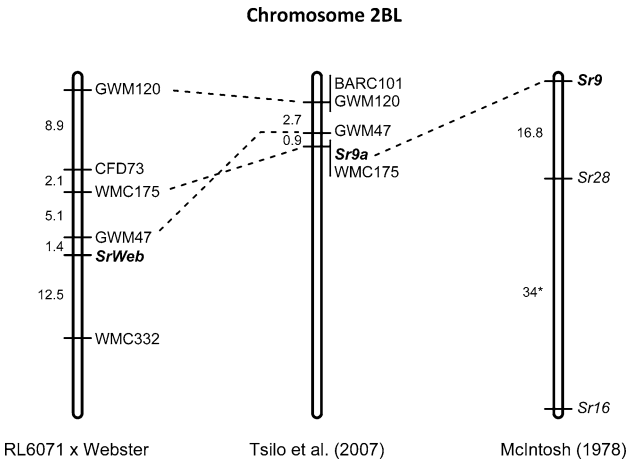


**Fig. 2** Genetic map of *SrWeb* from the cross of RL6071 × Webster using SSR markers. The map of *SrWeb* is compared to a genetic map of *Sr9a* (Tsilo et al. 2007) and a genetic map of *Sr9*, *Sr28* and *Sr16* (McIntosh 1978). GWM markers are from Röder et al. (1998), WMC and CFD markers are from Somers et al. (2004) and BARC markers are from Song et al. (2005). The "*" in the McIntosh (1978) map indicates a mean genetic distance from two estimates

Webster is listed as a reference stock for *Sr30* (McIntosh et al. 1995). The data presented here (mode of inheritance, mapped location and reaction to stem rust) confirm that it was *Sr30* that conferred resistance to TPMK in the RL6071 × RL6201 population. Knott and McIntosh (1978) reported *Sr30* as a "partially recessive" gene located on chromosome 5DL. Furthermore, *Sr30* was shown to confer resistance to TPMK (Martens et al. 1989), but not to TTKSK (Jin et al. 2007). The inheritance of *Sr30* in this study was generally recessive but the expression was variable. Of the 135 $F_2$ plants that were progeny tested in the $F_3$ with TPMK, 16 were misclassified; six $F_2$ plants scored susceptible proved homozygous resistant in the $F_3$ tests and ten $F_2$ plants resistant in the $F_2$ segregated in $F_3$. This represents an error rate of approximately 12% among segregating material. This is in keeping with previous reports that *Sr30* is variably expressed in segregating material (Knott and McIntosh 1978) and partly explains why the pooled ratio from segregating $F_3$ families did not fit 1:3; nevertheless, more plants were scored susceptible than resistant among the pooled segregating $F_3$ populations (data not shown). Most importantly, this underscores the importance of conducting progeny tests in genetic experiments, as misclassified phenotypes bring spurious recombinants into genetic maps and so disturb the locus order and genetic distances. Hence, $F_2$ Sr genotypes were assigned based on the $F_3$ data for all mapping purposes.

As expected, SSR markers located on chromosome 5DL (Röder et al. 1998; Somers et al. 2004; Song et al. 2005) showed linkage to *Sr30*. The expected differential response of *Sr30* to TPMK and TTKSK was also observed. Previously, *Sr30* has been mapped on chromosome 5DL using DNA markers (Bariana et al. 2001). Mapping with SSR markers in the RL6071 × RL6201 population placed *Sr30* in a relatively large genetic interval (≈25 cM) on chromosome 5DL (Fig. 1). While the map of *Sr30* produced by Bariana et al. (2001) and the map presented here share only one SSR marker, the two maps place *Sr30* in a similar position.

The second Sr gene in RL6201 (designated *SrWeb*) conferred resistance to stem rust race TTKSK (Ug99) (Table 1), and mapped to chromosome 2BL (Fig. 2). A second gene was not reported in Webster (Knott and McIntosh 1978; Luig 1983). The accession of Webster maintained at the Cereal Disease Laboratory (CDL), St. Paul, MN, is not resistant to TTKSK (Y. Jin, personal communication). Thus, it seems likely that the various accessions of Webster are genetically different. RL6201 (Webster) was previously used as the *Sr30* differential at CRC, (Canada) while a line derived from Webster (Baart/Webster; PI 442897) that carries *Sr30* is in use as the *Sr30* differential at the CDL (USA). During annual *P. graminis* surveys conducted in North America, little virulence was detected either on RL6201 (Harder et al. 2001) or on *Sr30* (Roelfs et al. 1993a, b, 1995). Thus, it is difficult to assess the effectiveness of *SrWeb* because *Sr30* is an effective gene in North America and the distribution of *SrWeb* in the several collections of Webster is unknown. Further testing will be required to determine the range of effectiveness of *SrWeb*. A single-gene line carrying *SrWeb* is being produced and will be available for distribution in the future.

Several other Sr genes are located on chromosome 2B (McIntosh et al. 1995). Three of these genes, *Sr9*, *Sr16* and *Sr28*, were mapped to the long arm of chromosome 2B (McIntosh 1978). The relationship between *SrWeb* and other Sr genes located on chromosome 2BL is unknown. A comparison of genetic maps for *SrWeb* with previous maps containing *Sr9*, *Sr16* and *Sr28* suggests that *SrWeb* is not allelic to *Sr16* (Fig. 2). However, an allelic relationship may exist between *SrWeb* and either *Sr9* or *Sr28* (Fig. 2). If *SrWeb* is an allele of a previously named gene it appears that *Sr9* is the most likely locus; however, none of the several alleles of *Sr9* confer resistance to TTKSK (Jin et al. 2007). Alternatively, *SrWeb* may represent a new gene on chromosome 2BL.

The genetic map of chromosome 2BL presented here is in minor conflict with the previously published map of Tsilo et al. (2007; Fig. 2) and the consensus map of Somers et al. (2004). The markers in question, WMC175 and GWM47, were inherited as dominant markers in both the RL6071 × Webster population and in the population analyzed by Tsilo et al. (2007). Furthermore, $F_2$ populations were used for genetic mapping in both studies. The combination of $F_2$ populations and dominantly inherited markers decreases the robustness of the genetic map and the marker order compared to population types where each meiotic event is analyzed in isolation or where markers are co-dominant. Discrepancies in marker order on genetic maps are not uncommon. Other previous maps are also inconsistent with respect to the order of WMC175 and GWM47 (Somers et al. 2004; DJ Somers, unpublished data). Despite the disagreement in marker order, it appears that *SrWeb* and *Sr9* occupy similar positions on chromosome 2BL (Fig. 2).

Stacking multiple Sr genes in wheat cultivars would improve the durability of the resistance to stem rust. Selecting gene stacks by phenotype can be difficult or impossible. Marker-assisted selection (MAS) can facilitate reliable selection of lines carrying multiple resistance genes. The SSR markers linked to *Sr30* are not ideal for MAS singly. However flanking markers could be used to select the genetic interval carrying *Sr30* with an error rate of <1.5%. The closest marker to *SrWeb*, GWM47, is linked with a genetic distance of 1.4 cM (Fig. 2). Although this SSR marker was inherited as a dominant marker in the mapping population, the positive allele was linked in coupling to the resistance allele and may be useful for MAS depending on marker polymorphism.

Stem rust race TTKSK has a critical combination of virulence factors that makes this race a threat to wheat production, particularly in regions of the world that rely on *Sr31* (Jin et al. 2007). TTKSK was first found in Uganda (Pretorius et al. 2000) and concern about this race of stem rust has grown partly because it has migrated to other regions. TTKSK has been detected in Iran thereby placing an increasing amount of wheat production area at risk (Nazari et al. 2009). Currently eight named seedling Sr genes from the primary gene pool of wheat confer resistance to TTKSK (Jin et al. 2007; TG Fetch unpublished data). Here, we report an apparently different Sr gene conferring resistance to TTKSK. Identifying new sources of resistance to stem rust, including, but not exclusively, TTKSK, provides wheat breeders with an increased diversity of Sr genes to deploy in combinations in new wheat cultivars. With proper planning and accurate selection of Sr genes, long term, broad-spectrum resistance can be attained.

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