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Authors
Briggs, Jordan
Chen, Shisheng
Zhang, Wenjun
et al.

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Mapping of *SrTm4*, a Recessive Stem Rust Resistance Gene from Diploid Wheat Effective to Ug99

Jordan Briggs, Shisheng Chen, Wenjun Zhang, Sarah Nelson, Jorge Dubcovsky, and Matthew N. Rouse

First, fourth, and sixth authors: Department of Plant Pathology, University of Minnesota, St. Paul 55108; second, third, and fifth author: Department of Plant Sciences, University of California, Davis 95616; second author: Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu, Sichuan, 611130, P.R. China; fifth author: Howard Hughes Medical Institute, Chevy Chase, MD 20815; and sixth author: USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108.

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ABSTRACT

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Race TTKSK (or Ug99) of *Puccinia graminis* f. sp. *tritici*, the causal agent of wheat stem rust, is a serious threat to wheat production worldwide. Diploid wheat, *Triticum monococcum* (genome A^m), has been utilized previously for the introgression of stem rust resistance genes *Sr21*, *Sr22*, and *Sr35*. Multipathotype seedling tests of biparental populations demonstrated that *T. monococcum* accession PI 306540 collected in Romania contains a recessive resistance gene effective to all *P. graminis* f. sp. *tritici* races screened, including race TTKSK. We will refer to this gene as *SrTm4*, which is the fourth stem rust resistance gene characterized from *T. monococcum*. Using two mapping populations derived from crosses of PI 272557 × PI 306540 and G3116 × PI 306540, we mapped *SrTm4* on chromosome arm $2A^mL$ within a 2.1 cM interval flanked by sequence-tagged markers *BQ461276* and *DR732348*, which corresponds to a 240-kb region in *Brachypodium* chromosome 5. The eight microsatellite and nine sequence-tagged markers linked to *SrTm4* will facilitate the introgression and accelerate the deployment of *SrTm4*-mediated Ug99 resistance in wheat breeding programs.

Over 700 million metric tons of wheat, *Triticum aestivum* L. and *T. turgidum* ssp. *durum* (Desf.) Husn., are produced worldwide each year (FAO 2013), playing an important role in global food security. Reducing yield losses to pests and pathogens is critical to maintain and increase wheat productivity. Historically, stem rust has produced large yield losses, which can exceed 50% in susceptible wheat cultivars during *Puccinia graminis* f. sp. *tritici* epidemics (Leonard 2001; Roelfs 1978). Breeding efforts to increase genetic resistance to stem rust, development of early maturing varieties, and efforts to remove the alternate host *Berberis vulgaris* L. near wheat-growing regions provided significant control of *P. graminis* f. sp. *tritici* in the past and reduced both severity and incidence of the stem rust disease (Peterson et al. 2005; Roelfs 1982, 1985). However, the emergence of the *P. graminis* f. sp. *tritici* Ug99 race group, composed of race TTKSK and variant races, allowed *P. graminis* f. sp. *tritici* to overcome many of the deployed stem rust resistance genes, making *P. graminis* f. sp. *tritici* again a threat to wheat production worldwide (Pretorius et al. 2000; Singh et al. 2011). The adaptation of the Ug99 race group to previously resistant cultivars has rendered three resistance genes (*Sr24*, *Sr36*, *Sr9h*) ineffective since the initial characterization of race TTKSK (Jin et al. 2008, 2009; Pretorius et al. 2012; Rouse et al. 2014), although these three genes remain effective against many *P. graminis* f. sp. *tritici* races. Over 50 wheat stem rust resistance genes have been identified (McIntosh et al. 1995, 2013), yet few of the ones present in current commercial wheat varieties are effective to the Ug99 race group (Jin and Singh 2006; Sharma et al. 2013; Singh et al. 2011).

Corresponding author: M. N. Rouse; E-mail address: matthew.rouse@ars.usda.gov

The first two authors contributed equally to this work.

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Wheat wild relatives at different ploidy levels have been previously utilized for introgressing resistance genes effective against stem rust into cultivated varieties including *Sr21*, *Sr22*, and *Sr35* from *T. monococcum* L. (Gerechter-Amitai et al. 1971; McIntosh et al. 1984; The 1973); *Sr33*, *Sr45*, *Sr46*, *SrTA10171*, *SrTA10187*, and *SrTA1662* from *A. tauschii* Coss. (Kerber and Dyck 1979; Marais et al. 1998; McIntosh et al. 2013; Olson et al. 2013a,b); *Sr32*, *Sr39*, and *Sr47* from *A. speltoides* Tausch (Faris et al. 2008; Kerber and Dyck 1990; McIntosh et al. 1995); and *Sr36*, *Sr37*, and *Sr40* from *T. timopheevii* (Zhuk.) Zhuk. (Dyck 1992; McIntosh and Gyarfas 1971). Among these resistance genes only *Sr35* and *Sr33* have been cloned and both encode nucleotide-binding-site leucine-rich repeat proteins (NB-LRR) (Periyannan et al. 2013; Saintenac et al. 2013).

This study focused on a resistance gene from *T. monococcum*, a species closely related to *T. urartu*, the donor of the A genome to the polyploid wheat species. *T. monococcum* and *T. urartu* are endemic to the same geographic regions, have identical karyotypes (Giorgi and Bozzini 1969), and have normal bivalent chromosome pairing in meiosis (Dvorak 1976; Nath et al. 1984), but their hybrids are sterile confirming that they are different species (Johnson and Dhaliwal 1976). The DNA sequences of these diploid species are sufficiently differentiated to greatly reduce paring between the *T. monococcum* chromosomes and the A genome chromosomes from polyploid wheat when they are in the presence of the wild-type *Paring homeologous 1* (*Ph1*) gene (Dubcovsky et al. 1995; Luo et al. 1996).

The *T. monococcum* ssp. *monococcum* accession PI 306540 was identified in a previous study as possessing potentially new resistance genes based on seedling ITs to *P. graminis* f. sp. *tritici* races (Rouse and Jin 2011b). $F_{2:3}$ families derived from a cross between PI 306540 and stem rust susceptible *T. monococcum* ssp. *monococcum* PI 272557 were screened with race TTKSK and *Sr21*-virulent races QFCSC and TTTTF. Segregation of resistance to race TTKSK indicated the presence of three resistance genes

(Rouse and Jin 2011a). One of the three genes was predicted to be *Sr21*. The other two genes are different from *T. monococcum* known resistance genes *Sr22* and *Sr35* (Rouse and Jin 2011a). One of these two genes was effective to *P. graminis* f. sp. *tritici* races TTKSK and QFCSC, but ineffective to race TTTTF. The other gene we refer to as *SrTm4* produced a mesothetic (intermediate reaction with both resistant and susceptible ITs present) IT when present singly (Rouse and Jin 2011a). In a population derived from PI 306540, a low or mesothetic reaction was observed in environmentally controlled settings to races QFCSC and TTKSK in families predicted to carry *SrTm4* based on the presence of a mesothetic IT to race TTTTF. The objective of this study was to genetically map *SrTm4*, to identify the colinear regions in other sequenced genomes, and to develop molecular markers closely linked to the gene that can be effectively used to monitor the introgression of a small segment of *T. monococcum* chromosome $2A^m$ carrying *SrTm4* into common wheat.

MATERIALS AND METHODS

Plant materials. Two F_2 mapping populations, including 89 individuals from the cross of PI 272557 × PI 306540 and 190 individuals from the cross of G3116 × PI 306540, were used to map *SrTm4* in diploid wheat. PI 306540 and PI 272557 are cultivated spring *T. monococcum* ssp. *monococcum* accessions obtained from the U.S. Department of Agriculture (USDA) National Small Grains Collection. PI 272557 was characterized as susceptible to five races of *P. graminis* f. sp. *tritici* in a previous study (Rouse and Jin 2011b). G3116 (PI 427992) is a wild *T. monococcum* ssp. *aegilopoides* and was selected as a parent of the second cross because of its high level of polymorphism compared with cultivated *T. monococcum* ssp. *monococcum* (Dubcovsky et al. 1996). Based on seedling tests with multiple *P. graminis* f. sp. *tritici* races, G3116 was postulated to possess resistance gene *Sr21* (Zhang et al. 2010). This hypothesis was confirmed in a recent study that mapped the *Sr21* resistance from G3116 to the long arm of chromosome $2A^m$, roughly 50 cM from the centromere (Chen et al. 2015). Since both G3116 and PI 306540 carry *Sr21*, a *P. graminis* f. sp. *tritici* race virulent to *Sr21* (e.g., TTTTF) was used to map *SrTm4* in this population. The Swiss winter wheat cultivar Arina (obtained from the Australian Winter Cereals Collection, Tamworth, AUS-21732) was utilized as a check line for *Sr48* in some of our experiments.

Evaluation of stem rust resistance. Inoculation of seedlings with *P. graminis* f. sp. *tritici* isolates was performed at the USDA-Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory according to previously described methods (Rouse et al. 2011). *P. graminis* f. sp. *tritici* race TTTTF (isolate 01MN84A-1-2) was used to inoculate the parents and 25 individuals from each $F_{2:3}$ family. At least two replications were performed for each $F_{2:3}$ family. After inoculation, plants were incubated in growth chambers maintained at 18°C day, 15°C night with a 16 h photoperiod. The lower incubation temperature was necessary to ensure consistent expression of *SrTm4* resistance. Infection types (ITs) were assessed 14 days after inoculation on the primary leaves using a 0 to 4 scale described by Stakman et al. (1962). ITs of 2 and less including mesothetic (;3, 13, or ;23) ITs denote resistant reactions and ITs of 3 and greater denote susceptible reactions. The F_2 *SrTm4* genotypes were based on the phenotypes of their $F_{2:3}$ families and classified as homozygous resistant (HR), heterozygous (Het), or homozygous susceptible (HS).

We also inoculated PI 306540, PI 272557, G3116, Einkorn (PI 10474), and wheat cultivar Arina with diverse *P. graminis* f. sp. *tritici* races that are virulent on *Sr21* including TTTTF, TPMKC, RKQQC, RCRSC, QTHJC, QFCSC, and SCCSC (isolates 01MN84A-1-2, 74MN1409, 99KS76A, 77ND82A, 75ND717C, 06ND76C, and 09ID73-2) to determine the effectiveness of *SrTm4* to different *P. graminis* f. sp. *tritici* races. The five lines were also inoculated with *Sr21*-avirulent race MCCFC (isolate 59KS19). Presence of ITs characteristic of *SrTm4* (IT ;3, 13, or ;23) were considered indicative of the effectiveness of *SrTm4* to each *P. graminis* f. sp. *tritici* race.

Molecular marker analyses. Genomic DNA from the $F_{2:3}$ families was bulk extracted using a modified CTAB protocol (Yu et al. 2008) from a minimum of 10 $F_{2:3}$ plants per family. Single sequence repeats (SSR) markers were selected to screen for polymorphism in regions associated with *SrTm4* using protocols described before (Röder et al. 1998; Somers et al. 2004; Song et al. 2005). Wheat ESTs that were previously mapped to the long arm of chromosome 2A were also tested for sequence polymorphism to develop sequence-based markers. ESTs linked to *SrTm4* were then used to search for the orthologous region in the *Brachypodium* genome. *Brachypodium* genes found in the targeted region were then used to find wheat orthologs and to develop new markers.

Genetic mapping. The linkage map of *SrTm4* was constructed using MapMaker version 3.0b (Lander et al. 1987). The Kosambi mapping function was used to calculate map distances. The genetic linkage map was drawn with the software MapDraw V2.1 (Liu and Meng 2003).

RESULTS

Evaluation of *SrTm4* resistance. PI 306540 displayed resistant ITs characteristic of *SrTm4* to all of the *P. graminis* f. sp. *tritici* races tested (Table 1; Figs. 1 and 2). The ITs of G3116 and PI 10474 were consistent with the presence of *Sr21* in these lines (Table 1). PI 272557 was susceptible to all of the *P. graminis* f. sp. *tritici* races and wheat cultivar Arina was resistant to all of the races.

ITs in resistant $F_{2:3}$ families derived from the PI 272557 × PI 306540 cross ranged from ;3 to 31, whereas susceptible families exhibited ITs ranging from 3 to 4 in response to race TTTTF (Fig. 1). Among the 89 F_3 families, we detected 11 homozygous resistant, 49 segregating for resistance, and 29 homozygous susceptible. This ratio deviated slightly from the expected 1:2:1 segregation ratio ($\chi^2 = 8.19$, $P = 0.017$). However, the segregation of resistance among 1,370 plants from the progeny of the 49 segregating $F_{2:3}$ families was 346 resistant plants and 1,024 susceptible, which fit a 1:3 segregation ratio expected for a single recessive resistance gene ($\chi^2 = 0.048$, $P = 0.82$).

ITs in resistant $F_{2:3}$ families derived from the G3116 × PI 306540 cross ranged from ;3 to 31, whereas susceptible families exhibited

TABLE 1. Infection types of *Triticum monococcum* lines and wheat cultivar Arina to selected races of *Puccinia graminis* f. sp. *tritici*

	Line				
P. graminis f. sp. *tritici* race	PI 306540	PI 272557	G3116	PI 10474	Arina
TTTTF	;13	33+	3	33+	13-LIF
TPMKC	;	33+	3	33+	13-LIF
RKQQC	;13	33+	3	3	13-
RCRSC	;13	33+	3	33+	3-LIF
QTHJC	;12+	33+	3	33+	13-LIF
QFCSC	;1	33+	3	33+	13-LIF
SCCSC	;13	33+	3	33+	13-LIF
MCCFC	;1	33+	;1	;1	3-LIF

ITs ranging from 3 to 4 in response to race TTTTF. Among the 190 $F_{2:3}$ families analyzed for this population, we detected 39 homozygous resistant families, 108 families segregating for resistance to race TTTTF, and 43 homozygous susceptible families. This ratio did not deviate significantly from the expected 1:2:1 segregation ratio expected for a single gene ($\chi^2 = 3.72$, $P = 0.155$). Analysis of 1,318 plants from the progeny of 60 segregating $F_{2:3}$ families in this population resulted in 363 (27.5%) resistant plants and 955 (72.5%) susceptible plants, which is close to the 1:3 segregation ratio expected for a single recessive resistance gene ($\chi^2 = 4.54$, $P = 0.033$).

Identification of SSR markers linked with *SrTm4*. Bulked segregant analysis (BSA) was used to identify SSR markers linked with *SrTm4*. Among the initial polymorphic SSR markers tested in population PI 272557 × PI 306540, *gwm265* showed significant linkage to *SrTm4*. Marker *gwm265* was previously mapped to chromosome arm 2AL in wheat (Röder et al. 1998; Song et al. 2005). Therefore, additional SSR markers from 2AL were screened on the parents for polymorphisms and all polymorphic markers were screened on the $F_{2:3}$ population. A total of seven SSR markers in population PI 272557 × PI 306540 and six SSR markers in population G3116 × PI 306540 were found to be linked with *SrTm4* (Fig. 3; Table 2).

Development of sequenced-based markers linked to *SrTm4*. To saturate the chromosome region carrying *SrTm4* and to identify the collinear region in other cereal genomes like *Brachypodium*, we selected several wheat ESTs from the long arm of chromosome 2A to develop sequence based markers. Wheat EST *BG313738* was found to be polymorphic in both populations and was mapped proximal to *SrTm4*. The sequence of *BG313738* was used to identify a colinear region in chromosome 5 of *Brachypodium* where we selected thirty genes to develop additional markers for *SrTm4*. These efforts resulted in two additional sequence-tagged markers in the PI 272557 × PI 306540 population and eight in the G3116 × PI 306540 population linked with *SrTm4* (Fig. 3; Table 2). The two closest markers flanking *SrTm4* were *BQ461276* (1.6 cM proximal to *SrTm4*) and *DR732348* (0.5 cM distal to *SrTm4*, Fig. 2), which defined a 2.1 cM candidate region for *SrTm4* in the G3116 × PI 306540 population. Marker *DR732348* was developed from the same contig as SSR markers *gwm526* and *gdm93*, which amplify the same SSR locus. The flanking SSR sequences were used to identify the *T. aestivum* contig (IWGSC_2AL_contig 6401556, The International Wheat Genome Sequencing Consortium 2014), and then a gene within this contig was used to develop marker *DR732348*.

***SrTm4* colinear region in *Brachypodium*.** The proximal marker *BQ461276* was identified to be orthologous to *Brachypodium* gene *Bradi5g25720* (Bd5: 26,907,758 bp to 26,910,587 bp), whereas the distal marker *DR732348* was 91% identical at the DNA level with *Bradi5g26020* (Bd5: 27,127,725 bp to 27,147,892 bp) (Fig. 2C). The *SrTm4* flanking markers define a 240-kb genomic region in *Brachypodium* chromosome 5 (Bd5: 26,907,758 bp to 27,147,892 bp) that includes 29 putative *Brachypodium* genes (*Bradi5g25730* to *Bradi5g26010*). The functional annotation of these 29 genes (Supplementary Table S1) does not include any NB-LRR resistance genes.

The relative map position of *SrTm4* with other *Sr* genes on chromosome 2AL. Two previously characterized wheat stem rust resistance genes, *Sr21* and *Sr48*, have been mapped before on chromosome arm 2AL (Bansal et al. 2008, 2009; Chen et al. 2015). *Sr21* was mapped roughly 50 cM from the centromere and 29.1 cM proximal to marker BG313738, whereas *SrTm4* was mapped 5.8 cM distal to the same sequence-tagged marker and ~85 cM from the centromere. This result clearly indicates that *SrTm4* and *Sr21* are two different loci, located ~35 cM apart (Fig. 4).

Sr48 has been previously mapped 22.1 cM distal to marker *gwm382* (Bansal et al. 2008, 2009; Fig. 3B) on the long arm of chromosome 2A from 'Arina'. Initially, no common markers were available between the *Sr48* and *SrTm4* maps, complicating the comparative analysis. To generate a common reference point, we first sequenced the PCR product of *gwm382* and identified *T. aestivum* contig IWGSC_2AL_contig 6435092 (The International Wheat Genome Sequencing Consortium 2014) that includes the *gwm382* SSR flanking sequences. From this contig we developed marker *BG907495* that was mapped 11.7 cM distal to *SrTm4*. Based on these distances, *SrTm4* is located 29.9 cM proximal to *Sr48* (Fig. 4).

DISCUSSION

A previous survey of *T. monococcum* for resistance to Ug99 (Rouse and Jin 2011b) postulated that most of the resistant accessions carry the mapped genes *Sr21* (Chen et al. 2015), *Sr22* (Periyannan et al. 2011),

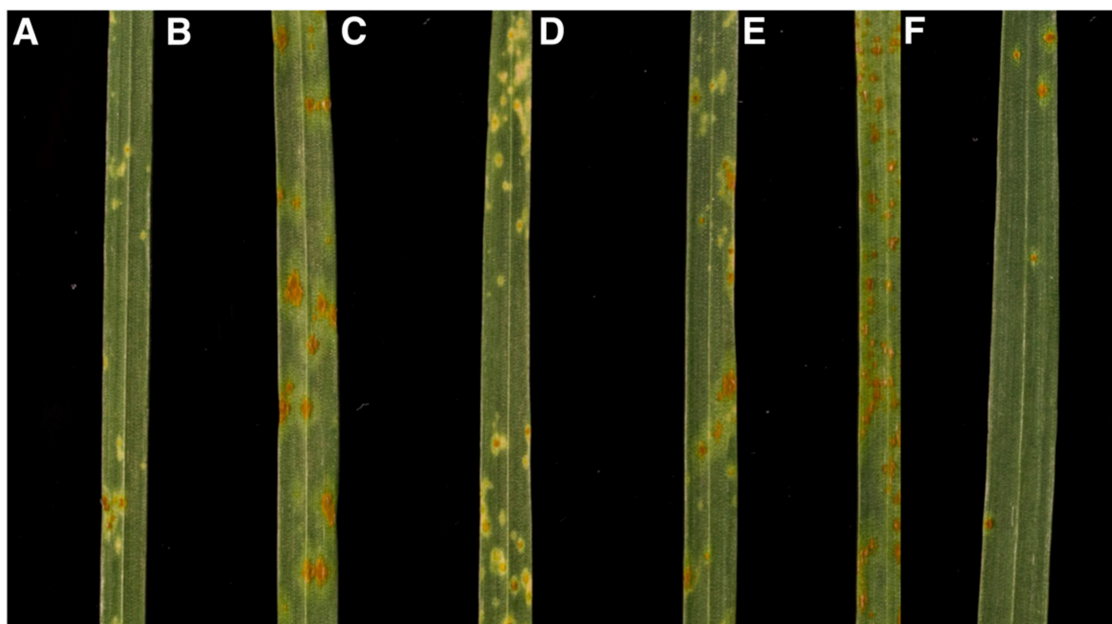

Fig. 1. Infection types of *Triticum monococcum* and *T. aestivum* lines **A,** PI 306540 (*SrTm4*); **B,** PI 272557; **C,** PI 272557/PI 306540 $F_{2:3}$ family 42 (*SrTm4*); **D,** PI 272557/PI 306540 $F_{2:3}$ family 70 (*SrTm4*); **E,** PI 10474 (*Sr21*); and **F,** Arina (*Sr48*) in response to physiologic race TTTTF of *Puccinia graminis* f. sp. *tritici*.

or *Sr35* (Saintenac et al. 2013). However, accession PI 306540, a cultivated *T. monococcum* ssp. *monococcum* collected in Romania, possessed two different resistance genes (Rouse and Jin 2011a).

We focused on one of these genes, namely *SrTm4*, that is effective to the virulent race TTTTF because of the interesting recessive nature of the resistance and its broad resistance to all the *P. graminis* f. sp. *tritici* races tested so far. The initial study showed that *SrTm4* confers resistance to Ug99 race TTKSK, and additional races TTTTF, TRTTF, QFCSC, and MCCFC (Rouse and Jin 2011a). We showed here that PI 306540 also displayed mesothetic resistant ITs to five additional races that are virulent on *Sr21* (TPMKC, RKQQC, RCRSC, QTHJC, and SCCSC) (Fig. 2). The mesothetic type of resistance observed to these races suggests that *SrTm4* is effective to these races. Though the presence of ITs characteristic of a particular gene is not necessarily diagnostic of the presence of that gene, the presence of the mesothetic ITs provides preliminary evidence of the spectrum of effectiveness of *SrTm4*. The broad-spectrum resistance of *SrTm4* makes it a valuable resource for breeding resistance to Ug99 and other virulent *P. graminis* f. sp. *tritici* races. However, *SrTm4* only confers partial resistance and therefore it should be deployed in combination with other *P. graminis* f. sp. *tritici* resistance to achieve economically useful levels of resistance.

We have initiated the introgression of *SrTm4* from PI 306540 into polyploid wheat. These crosses require the use of a tetraploid bridge because direct crosses between *T. monococcum* and hexaploid wheat are frequently sterile.

When the complete *T. monococcum* complement is present, some recombination is observed between the *T. monococcum* and the A genome chromosomes, but that recombination is reduced to very low levels when only single chromosomes or chromosome pieces of *T. monococcum* are present in hexaploid wheat (Dubcovsky et al. 1995, Luo et al. 1996). Fortunately, recombination between the A and A^m chromosomes is restored to normal levels in the presence of the *ph1b* mutation (Dubcovsky et al. 1995). Using the *ph1b* mutation and the multiple markers linked to *SrTm4* developed in this study, it will be possible to engineer a small *T. monococcum* introgression carrying only *SrTm4*.

Sr21 and *SrTm4* present very different resistance profiles that suggest that they are different genes. Resistance conferred by *Sr21* is dominant, ineffective at lower temperatures (16°C), and ineffective to several *P. graminis* f. sp. *tritici* races (TTTTF, QFCSC, TPMKC, RKQQC, RCRSC, QTHJC, and SCCSC). By contrast, resistance conferred by *SrTm4* is recessive, effective at lower temperatures, and screening data suggest *SrTm4* is effective against all 10 *P. graminis* f. sp. *tritici* races tested so far, including several that are virulent on *Sr21* (e.g., TTTTF). The genetic map of *SrTm4* presented here confirmed that this gene is different from *Sr21*, and that the two genes are roughly 38 cM apart (Fig. 4) (Chen et al. 2015).

The relationship of *SrTm4* and *Sr48* is not as clear as the relationship between *SrTm4* and *Sr21* described above. *Sr48* was mapped to chromosome arm 2AL of the bread wheat cultivar Arina (Bansal et al. 2008), and was first designated as *SrAn1*

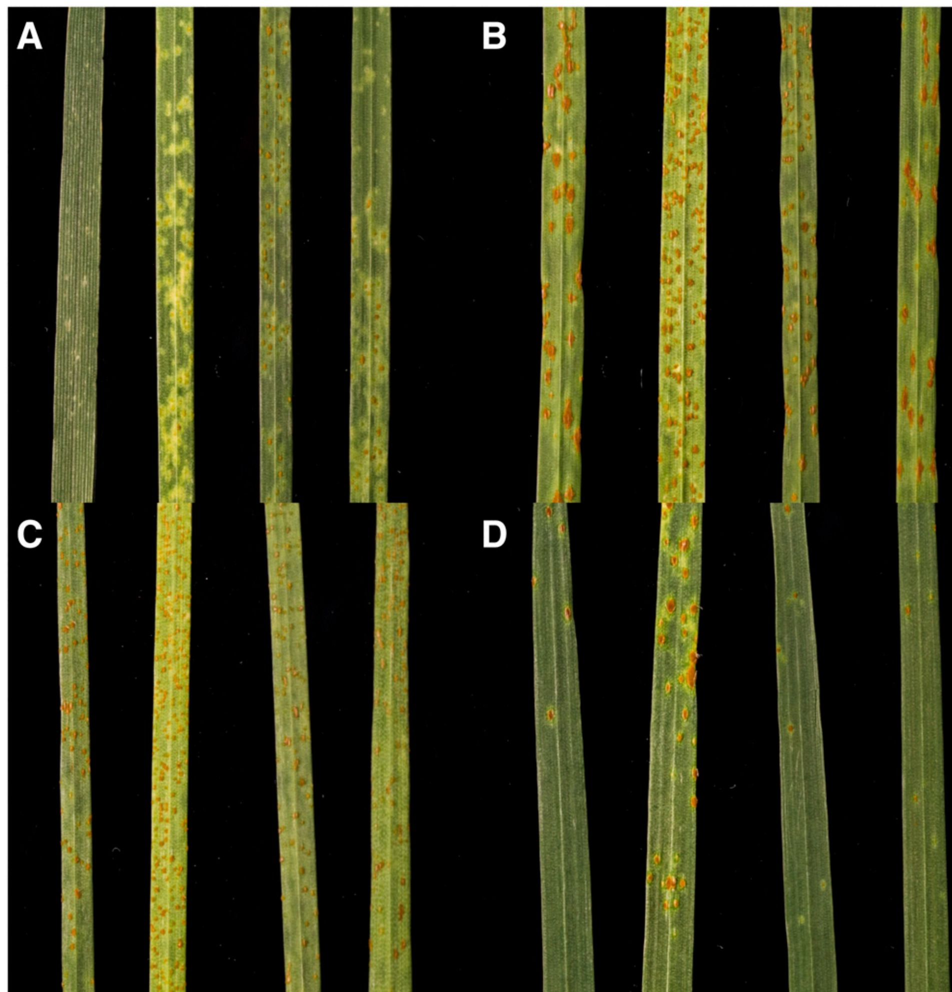

Fig. 2. Infection types of *Triticum monococcum* and *T. aestivum* lines **A,** PI 306540 (*SrTm4*); **B,** PI 272557; **C,** G3116 (*Sr21*); and **D,** Arina (*Sr48*) in response to physiologic races of *Puccinia graminis* f. sp. *tritici*: TPMKC, RKQQC, RCRSC, and SCCSC (from left to right within each frame).

using Australian *P. graminis* f. sp. *tritici* isolates 98-1,2,3,5,6 and 34-1,2,7+*Sr38*. Subsequent mapping placed *Sr48* 16.5 cM distal to the stripe rust resistance locus *Yr1* (Bansal et al. 2009). A comparative analysis of shared marker *gwm382* (*BG907495*, is from the same contig as *gwm382*) between the *T. monococcum* 2A^m genetic map in our present study and the hexaploid wheat 2A genetic map (Bansal et al. 2009) indicates *SrTm4* is located approximately 30 cM proximal to *Sr48* (Fig. 3A and B).

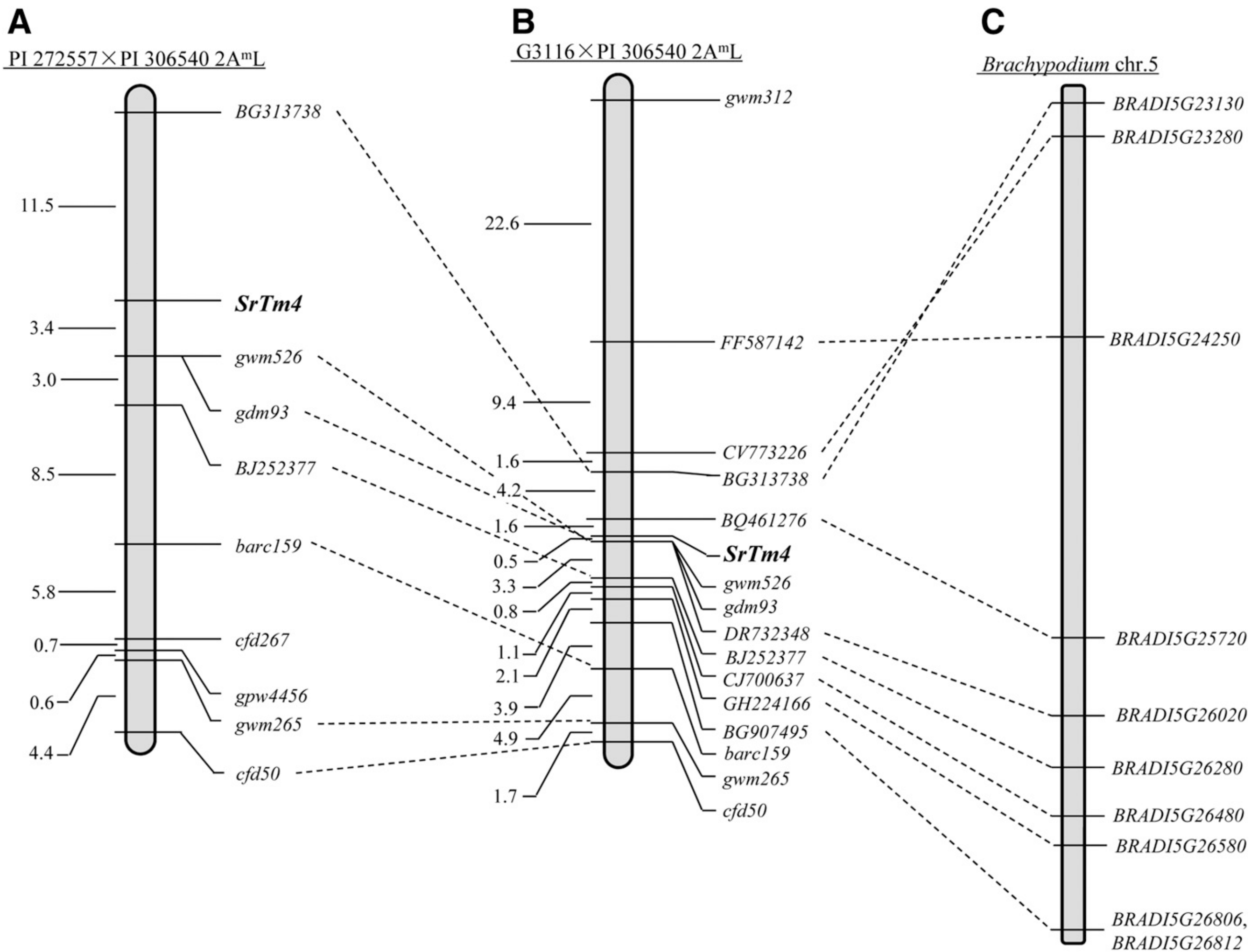

Fig. 3. Genetic maps of *SrTm4* on chromosome arm 2A^{m}L derived from two biparental mapping populations and their comparison with a physical map of *Brachypodium* chromosome 5: **A,** map based on the cross between cultivated *Triticum monococcum* ssp. *monococcum* accessions PI 272557 and PI 306540; **B,** map based on the cross between wild G3116 and cultivated PI 306540 *T. monococcum* accessions; and **C,** collinear region in the sequenced *Brachypodium* chromosome 5.

TABLE 2. Primer sequences, annealing temperatures, and restriction enzymes used for markers in the *SrTm4* region[a]

Markers	Marker type	Forward primer (5′-3′)	Reverse primer (5′-3′)	Restriction enzyme	Annealing temperature (°C)
gwm312	SSR	ATCGCATGATGCACGTAGAG	ACATGCATGCCTACCTAATGG	–	56
gwm526	SSR	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	–	55
gdm93	SSR	AAAAGCTGCTGGAGCATACA	GGAGCATGGCTACATCCTTC	–	55
barc159	SSR	CGCAATTTATTATCGGTTTTAGGAA	CGCCCGATAGTTTTTCTAATTTCTGA	–	50
gwm265	SSR	TGTTGCGGATGGTCACTATT	GAGTACACATTTGGCCTCTGC	–	55
gpw4456	SSR	ATTAGTCTCCTCCTCCCTTTGG	AGTAGCCGGGGCAGAAATAG	–	60
cfd267	SSR	GTGCGTCGTGTAGCAGCTC	CTCTCTGTCGTCCAGGTCGT	–	60
cfd50	SSR	TTCTGCAACATTTTGTCCCA	CGTATGATCCTAACGAGGGC	–	60
FF587142	Indel	TGGAGAAGGTTACGAGGAACGC	TTATGGGCTGTTAATGGGCAAA	–	59
CV773226	CAP	AGGTGCCCTGAGCAGCGAGAC	GCCATGTCCTTGCCGTAGCG	*Apo*I	56
BG313738	CAP	CTGACTGCGCCTTATGTTGA	GTGCCCATGGCTTGATGGAGCCG	*Sac*II	56
BQ461276	Indel	GAGGAAACTTCAATGTGGC	CAGTAATGCTTATCGGGTAAC	–	50
DR732348	CAP	TGAGACCATTCTACAGCGG	TCTTCATCATTTTGGACACCT	*Acc*I	54
BJ252377	Indel	ATCAGGGTAATCCCCAAGA	TTCAGAAATCAGATCAACAAGAC	–	50
CJ700637	CAP	CACCACCATCCAATCCTAC	CTTCATCACCCGAGACAAC	*Bsg*I	54
GH224166	CAP	AATTGTTTGTTCTGCTAGAC	AAATCACTGAAAGTCTTCTG	*Fok*I	48
BG907495	Indel	AAAGAAAGTTGTGCAGCAAA	TGTCATAGAGTTCAAACAGTGG	–	51

[a] SSR, simple sequence repeat; CAP, cleavage amplification polymorphism; Indel, insertion/deletion.

However, *P. graminis* f. sp. *tritici* isolates used to map *Sr48* do not possess comparable virulence to those used in this study or previously by Rouse et al. (2011). Therefore, further screening with *P. graminis* f. sp. *tritici* isolates covering a broad range of virulence phenotypes or a more precise map of *Sr48* sharing common markers with the *SrTm4* map will be necessary to determine if *SrTm4* and *Sr48* are different genes or different alleles of the same gene. Once *SrTm4* is transferred to hexaploid wheat, a direct allelism test in a *ph1b* background may provide a definitive answer to this question.

The fourth resistance gene from *T. monococcum*, *SrTm4*, confers resistance to diverse isolates of *P. graminis* f. sp. *tritici* and is an interesting target to be combined with other Ug99 effective stem rust resistance genes. Pyramids of broadly effective resistance genes are expected to confer more durable resistance, but only the test of time will confirm or reject this hypothesis. Since *SrTm4* and *Sr21* are on the same chromosome arm, it should be possible to introgress both genes simultaneously in hexaploid wheat. However, additional studies will be required to test if the large *T. monococcum* segment required to introgress both *T. monococcum* genes carries any detrimental gene to hexaploid or tetraploid wheat agronomic performance or quality. If *SrTm4* and *Sr48* are different genes, coupling both genes on chromosome arm 2AL should also be possible.

The two stem rust resistance genes cloned so far (Periyannan et al. 2013; Saintenac et al. 2013) are NB-LRR, and both confer resistance to Ug99 with dominant inheritance. The recessive nature of *SrTm4* and the mesothetic IT may be indicative of a different resistance mechanism, providing an additional incentive for the cloning of this gene. The absence of NB-LRR genes in the colinear region of *Brachypodium* provides indirect support for this hypothesis. However, the final identification of the *SrTm4* gene will be required to fully test this hypothesis.

The use of two diploid wheat populations with contrasting levels of polymorphism seems to be a viable strategy to clone this resistance gene. The more polymorphic cross between wild and cultivated *T. monococcum* can be used to rapidly develop markers in the initial stages of the high density mapping (as demonstrated in this study), whereas the cross between the two cultivated *T. monococcum* accessions can be used in the later stages of the project to identify the causal polymorphisms. A similar strategy was used successfully to clone the Ug99 resistance gene *Sr35* (Saintenac et al. 2013). The cloning of *SrTm4* will add additional tools to generate cisgenic resistance cassettes that can combine different resistance genes (e.g., *Sr33* and *Sr35*). In the meantime, the closely linked markers to *SrTm4* identified in this study will be useful tools to initiate the introgression of this gene into polyploid wheat and to deploy the natural *SrTm4* allele in wheat commercial varieties.

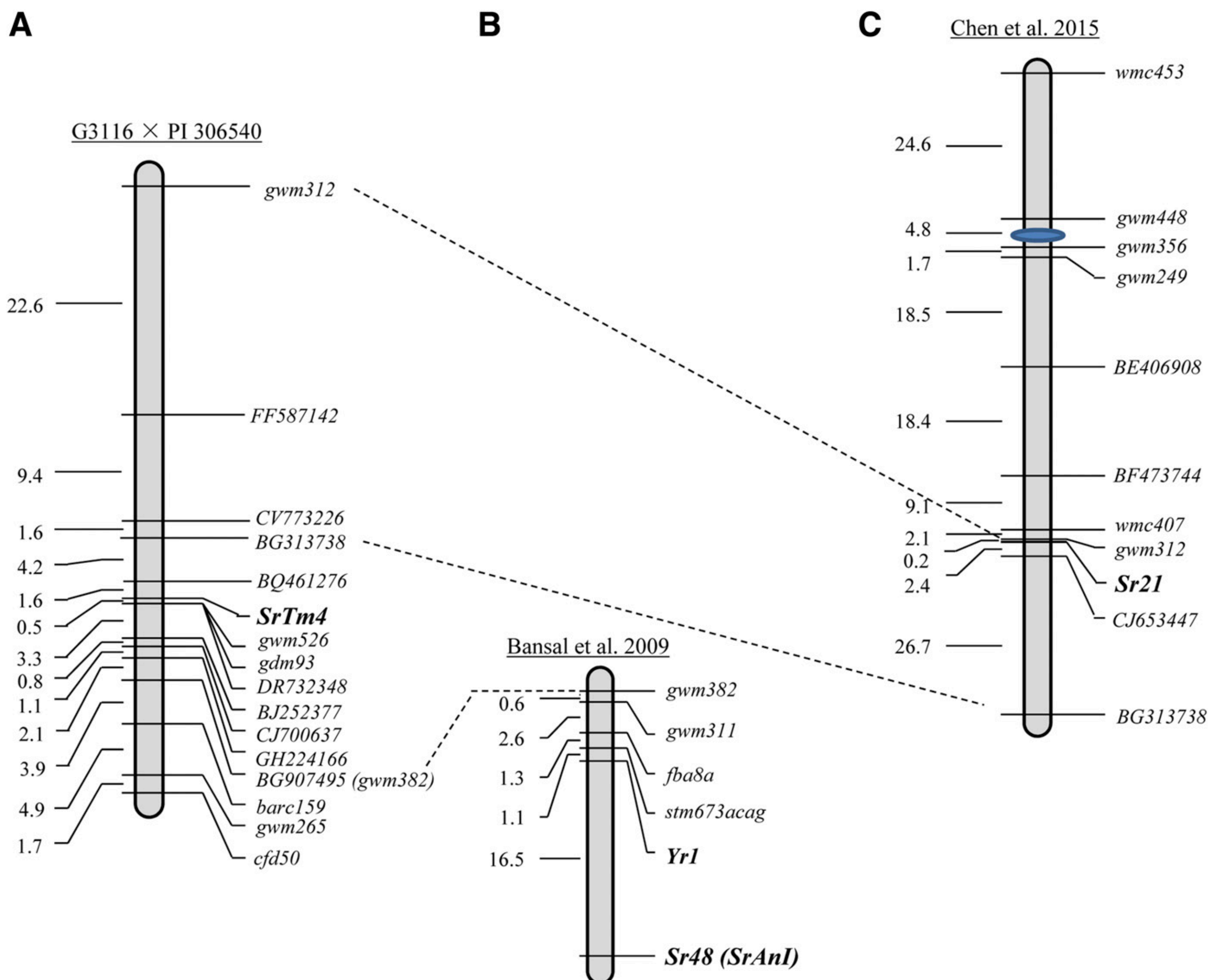

Fig. 4. Relative map position of *SrTm4*, *Sr21*, and *Sr48*. **A,** Genetic map of *SrTm4* derived from G3116 × PI 306540 in this study; **B,** genetic map of *Sr48* (*SrAnI*) from hexaploid wheat 2A (Bansal et al. 2009); and **C,** genetic map of *Sr21* from *Triticum monococcum* (Chen et al. 2015).

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