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ORIGINAL PAPER

Identification of markers linked to the race Ug99 effective stem rust resistance gene *Sr28* in wheat (*Triticum aestivum* L.)

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Abstract Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* can cause devastating yield losses in wheat. Over the past several decades, stem rust has been controlled worldwide through the use of genetic resistance. Stem rust race TTKSK (Ug99), first detected in Uganda in 1998, threatens global wheat production because of its unique virulence combination. As the majority of the currently grown cultivars and advanced breeding lines are susceptible to race TTKSK, sources of resistance need to be identified and characterized to facilitate their use in agriculture. South Dakota breeding line SD 1691 displayed resistance to race TTKSK in the international wheat stem rust nursery in Njoro, Kenya. Seedling screening of progeny derived from SD 1691 crossed to susceptible LMPG-6 indicated that a single resistance gene was present. Allelism and race-specificity tests indicated the stem rust resistance gene in SD 1691 was *Sr28*. The chromosome arm location of *Sr28* was previously demonstrated to be 2BL. We identified molecular markers linked to *Sr28* and validated this linkage in two additional populations. Common spring wheat cultivars in the central United States displayed allelic diversity for markers flanking *Sr28*. These markers could be used to select for *Sr28* in breeding populations and for combining *Sr28* with other stem rust resistance genes.

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Introduction

Stem rust of wheat (*Triticum aestivum* L.), caused by the basidiomycete fungus *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.; *Pgt*, is a very destructive disease of wheat. Tetraploid durum wheat (*Triticum turgidum* var. *durum*), barley (*Hordeum vulgare* L.), triticale (X *Triticosecale* Wittmack), and wheat progenitors are also primary hosts of the stem rust fungus (Roelfs et al. 1992). Stem rust has historically been a major problem in most wheat-growing areas of the world, causing devastating yield losses. In years conducive for disease development and presence of susceptible varieties such as 1919, 1920, 1923, 1927, 1935, 1953, and 1954 average statewide wheat yield losses due to stem rust were 25.4 % for Minnesota, 28.4 % for North Dakota, and 19.3 % for South Dakota (Roelfs 1978). During the first two-thirds of the twentieth century, an emphasis on breeding resistant varieties of wheat coupled with the removal of the alternate host of stem rust, barberry (*Berberis vulgaris* L.), and monitoring the pathogen population successfully resulted in the control of wheat stem rust in North America.

Wheat stem rust has re-emerged as a major threat to global wheat production. In 1998 a race of *Pgt* was identified in Uganda with virulence to stem rust resistance gene *Sr31* (Pretorius et al. 2000; Jin et al. 2007). This race, commonly known as Ug99, was characterized as race TTKSK based upon the North American *Pgt* nomenclature system (Roelfs and Martens 1988; Jin et al. 2008). Race TTKSK was demonstrated to be virulent on nearly all Asian cultivars and the majority of North American cultivars (Jin and Singh 2006; Fetch 2007; Singh et al. 2008). The majority of resistant cultivars in the United States were reported to possess resistance genes *Sr24* and *Sr36* (Jin and Singh 2006). Variants of race TTKSK have been detected with virulence to these genes, increasing the devastative potential of the TTKSK group of races (Jin et al. 2008, 2009). Since its discovery, race TTKSK and related races have been detected in Kenya, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, and South Africa (Wanyera et al. 2006; Nazari et al. 2009; Pretorius et al. 2010; Singh et al. 2011).

Resistant varieties of wheat to race TTKSK and its variants are needed. Several resistance genes have been identified that are effective to race TTKSK (Jin et al. 2007). Most of these genes were introgressed from wild relatives of wheat and some possess genetic linkage to undesirable traits. In order to achieve long-lasting resistance to stem rust, and race TTKSK in particular, combinations of multiple genes will need to be deployed in cultivars. Combining multiple resistance genes resulted in the control of stem rust in North America for several decades (Kolmer et al. 1991). Identification of molecular markers closely linked to resistance genes could facilitate the combining of genes in breeding lines. Molecular markers linked to at least 18 race TTKSK-effective resistance genes are available for genes transferred from diploid and tetraploid bread wheat relatives (Saal and Wricke 1999; Mago et al. 2002, 2005, 2011; Faris et al. 2008; Sambasivam et al. 2008; Tsilo et al. 2008; Wu et al. 2009; Liu et al. 2010, 2011a, b; Olson et al. 2010; Zhang et al. 2010; Niu et al. 2011; Qi et al. 2011; Simons et al. 2011) and two genes native to bread wheat (Hiebert et al. 2010, 2011). Markers linked to additional stem rust resistance genes native to bread wheat and effective to race TTKSK are desirable to provide breeders with several genes for combining in adapted varieties.

Previous studies identified SD 1691 (CI 12499) as possessing seedling and adult plant resistance to race TTKSK (Rouse et al. 2011). SD 1691 is a South Dakota breeding line developed during the 1940s. Though SD 1691 displayed adult plant resistance to a bulk of stem rust isolates in a field nursery in Saint Paul, Minnesota, it was susceptible to several races screened at the seedling stage except races BCCBC, TTKSK, and TTKST (*Sr24*-virulent variant of race TTKSK; Rouse et al. 2011). The genes conferring the adult plant resistance in SD 1691 to North American isolates are not known, but *Sr2* is a candidate as indicated by the presence of an *Sr2* cultivar, Hope, in the pedigree of SD 1691 (listed as "Hope/Reliance/Reward//Mercury" in USDA 2012). Since SD 1691 was resistant to race TTKSK at the seedling stage, but not to most other races of stem rust, we suspected that SD 1691 possessed *Sr28*. SD 1691 has the variety Ceres in the pedigree (pedigree of Mercury is Ceres//Hope/Florence) and Ceres is known to possess *Sr28* (McIntosh 1978). Gene *Sr28* was previously described as providing an intermediate level of resistance in a field nursery in Kenya to race TTKSK (Jin et al. 2007). The objectives of this research were to (1) test the hypothesis that SD 1691 possesses resistance gene *Sr28*, (2) identify molecular markers linked to seedling stem rust resistance in SD 1691, and (3) validate markers linked to *Sr28* in multiple populations and spring wheat germplasm.

Materials and methods

Plant materials

In order to accomplish our objectives, we first obtained seed of (1) lines known to possess *Sr28* including Kota, Ceres, and Line AD (W2691*5/Kota) in addition to (2) lines without *Sr28*, LMPG-6 and W2691, (3) a line we suspected to possess *Sr28*, CI 7611, and (4) a line we postulated not to possess *Sr28* though it is resistant to *Pgt* race TTKSK, Gabo 56. We derived four populations. In order to determine the genetics of resistance in SD 1691, a cross between SD 1691 and susceptible LMPG-6 was made (104 $F_{2:3}$ families derived). In order to test for allelism between *Sr28* and resistance in SD 1691, a cross between Line AD and SD 1691 was made (639 F_2 plants derived). In order to test if resistance in CI 7611 mapped to a similar location as resistance in SD 1691, potentially validating the SD 1691 mapping results, a cross between CI 7611 and LMPG-6 was made (172 $BC_1F_{1:2}$ families derived). In addition, a cross between SD 1691 and Gabo 56 was made (194 $F_{2:3}$ families derived) to differentiate resistance in SD 1691 and Gabo 56 and to map resistance in SD 1691 in a second population. Seeds of the cultivars SD 1691 (CI 12499), Gabo 56 (CI 14035), CI 7611, Ceres (PI 35118), and Kota (CI 5878) were obtained from the United States Department of Agriculture National Small Grains Collection (Aberdeen, ID). W2691 and Line AD, a monogenic line carrying the *Sr28* gene, were originally obtained from the University of Sydney (McIntosh 1978). LMPG-6 is a selection of LMPG (Little Club//Prelude/8*Marquis/3/Gabo), a stem rust susceptible spring wheat line developed by Knott (1990). W2691, LMPG-6, and a Line AD selection were obtained from the United States Department of Agriculture Cereal Disease Laboratory (St. Paul, MN, USA). A total of 24 wheat varieties adapted to Minnesota and North Dakota were obtained from the 2011 University of Minnesota spring wheat advanced yield nursery for the purpose of testing marker haplotypes in this germplasm.

Stem rust phenotyping

Seedling assays were performed at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota. Progeny of all crosses and germplasm used for marker haplotyping were evaluated at the seedling stage with race TTKSK isolate 04KEN156/04 (synonymous with Ug99; Jin et al. 2007, 2008). We also evaluated a subset of the genetic stocks (SD 1691, Ceres, Kota, CI 7611, W2691, LMPG-6, and Line AD) with *Pgt* race BCCBC, isolate 09CA115-2, in order to validate the *Sr28* phenotype with an additional *Pgt* race avirulent to *Sr28*. In the populations derived to $F_{2:3}$ or $BC_1F_{1:2}$ families, 15–20 seedlings per family were evaluated with race TTKSK. In order to evaluate seedlings with *P. graminis* f. sp. *tritici*, dried urediniospores stored in gelatin capsules were retrieved from −80 °C storage, heat shocked in a 45 °C water bath for 15 min, rehydrated for 2–4 h in a chamber maintained at 80 % relative humidity by a KOH solution (Rowell 1984), and suspended in a light-weight mineral oil (Soltrol 170, Phillips Petroleum, Borger, TX, USA) in preparation for inoculation. Spores were inoculated onto the primary leaves of seedling wheat plants 7–9 days following planting. The plants were placed under a fume hood to allow the oil to evaporate for approximately 30 min. The dried leaves were placed into a dark dew chamber for 14 h at 18 °C, followed by an additional 3–4 h with fluorescent light. After drying, the plants were then placed in a greenhouse maintained at 18 ± 2 °C with a day length of 16 h.

Stem rust infection types (ITs) on the primary leaves of seedlings were classified on a '0' to '4' scale 14 days post inoculation as described by Stakman et al. (1962). Infection types '0' to '2', including ';3' mesothetic ITs, were considered indicative of plant resistance and pathogen avirulence. Infection types '3' to '4' were considered high infection types corresponding to plant susceptibility. For the LMPG-6/SD 1691 and SD 1691/Gabo 56 populations, $F_{2:3}$ families were classified as homozygous resistant, segregating, or homozygous susceptible, whereas for the LMPG-6*2/CI 7611 population, $BC_1F_{1:2}$ families were classified as segregating or homozygous susceptible. For the SD 1691/Line AD allelism test, the numbers of resistant and susceptible F_2 plants were recorded. In each population, segregation of resistance was tested against expected ratios by Chi-square (χ^2) goodness-of-fit tests.

Molecular marker analyses

A total of 104 F_2 DNAs were extracted from the progeny of LMPG-6/SD 1691 corresponding to the $F_{2:3}$ families evaluated with race TTKSK. DNA was extracted for each F_2 plant following Riede and Anderson (1996) with modifications by Liu et al. (2006) and further modifications including the use of a bead grinder FastPrep®-24 from MP Biomedicals, Inc. to pulverize plant tissue. DNA of 92 $F_{2:3}$ families and DNA of the parents were genotyped with Diversity Arrays Technology (DArT) markers according to Akbari et al. (2006). Preliminary mapping results indicated linkage between stem rust resistance segregating in the LMPG-6/SD 1691 population and DArT markers previously mapped to the long arm of chromosome 2B (2BL) (Akbari et al. 2006).

In order to identify microsatellite (simple sequence repeat, SSR) markers linked to the race TTKSK resistance, we screened SD 1691, LMPG-6, and bulks of six homozygous resistant or susceptible plants (Michelmore et al. 1991) with a total of 32 gwm, wmc, and barc microsatellite markers previously mapped to chromosome arm 2BL (Roder et al. 1998; Somers et al. 2004; Song et al. 2005). The F_2 DNAs were then genotyped for the identified microsatellite markers. Microsatellite genotyping was performed in 10 µL volumes containing 45 ng genomic DNA, 0.4 pmol forward primer, 3.0 pmol reverse primer, 3.0 pmol of M13 primer (labeled with one of the following fluorescent dyes: 6-FAM, NEC, PET, and VIC), 0.125 mM dNTPs, 0.05 units/µL Taq DNA polymerase (New England Biolabs, Inc. Beverley, MA, USA), and 1× PCR buffer (as supplied by the manufacturer). Polymerase Chain Reactions (PCR) were performed in a GeneAmp® PCR System 9700 Thermal Cycler machine from Applied Biosystems (Foster City, CA, USA) under the following conditions: 10 min at 94 °C, 40 cycles of (1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C), 5 min at 72 °C, hold at 4 °C permanently. A total of 3 µL of each PCR reaction were combined with 0.14 µL of GeneScan™ -500 LIZ® Size Standard and 6.86 µL of Hi-Di™ Formamide from Applied Biosystems. The mixtures were denatured for 5 min at 94 °C and then placed on ice. Amplified fragments were analyzed using an ABI 3130xl Genetic Analyzer and GeneMapper® software v3.7 from Applied Biosystems.

Linkage mapping

For the DArT and microsatellite markers used in genotyping, Chi-square tests were conducted to test for segregation distortion. Genetic linkage maps based on the molecular markers and stem rust resistance were constructed using JoinMap software version 4.0 (Stam 1993; van Ooijen 2006). Genetic distances were calculated using the Kosambi function (Kosambi 1944), and linkage groups were formed at logarithm of odds (LOD) value of 5.0 and 40 % maximum recombination frequency.

Development of the PCR-based marker wPt-7004-PCR

The sequence of DArT probe wPt-7004 was obtained from Eric Huttner (DArT P/L, Yarralumla, Australia). Primers were selected from this sequence information in order to provide a PCR based marker for the wPt-7004 probe. Several primer pairs were tested for repeatable polymorphism between LMPG-6 and SD 1691. Oligonucleotide sequences [5′CTCCCACCAAAACAGCCTAC3′ (forward) and 5′AGATGCGAATGGGCAGTTAG3′ (reverse)] differentially amplified fragments in the parental lines SD 1691 and LMPG-6. Amplification of this marker is successful using an annealing temperature of 60 °C. We designated this primer pair as corresponding to PCR marker wPt-7004-PCR.

Validation of markers linked to Sr28

Markers linked to *Sr28* were amplified from F_2 DNAs and $BC_1F_{1:2}$ DNA bulks for SD 1691/Gabo 56 and LMPG-6*2/CI 7611, respectively. Genomic DNA was isolated from 50–100 mg plant tissue from young seedlings. DNA extraction was performed for SD 1691/Gabo 56 as described for LMPG-6/SD 1691. Eight to ten plants for each $BC_1F_{1:2}$ family were pooled for LMPG-6*2/CI 7611. The DNA extraction of these pooled tissues was performed using the BioSprint 96 DNA Plant kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. DNAs were amplified with markers wmc332 and wPt-7004-PCR. A third microsatellite marker, cfd73, was also amplified for LMPG-6*2/CI 7611. Polymerase chain reactions (PCR) were performed in 10 µL of final reaction mixture containing 45 ng genomic DNA, 1 µmol/L of each primer, 0.125 mM dNTPs, 0.05 units/µL Taq DNA polymerase (Qiagen Inc., Valencia, CA, USA), and 1× PCR buffer (as supplied by the manufacturer). The PCR reactions were initially denatured at 94 °C for 7 min, followed by 35 cycles of (1 min at 94 °C, 2 min at 60 °C, and 1 min at 72 °C), 5 min at 72 °C, hold at 12 °C. The PCR thermal cycling was performed using the GeneAmp® PCR System 9700 Thermal Cycler machine from Applied Biosystems (Foster City, CA, USA). For the wmc332 and wPt-7004-PCR markers, the PCR products were separated by using the AdvanCE™ FS96 fluorescence system (Advanced Analytical Technologies Inc., Ames, IA, USA). Amplified fragments for cfd73 marker were separated in 2.0 % agarose gels and visualized using ethidium bromide staining. Chi-square tests and genetic linkage maps were performed as described above.

Haplotype analysis of hexaploid wheat germplasm

In order to determine if markers linked to *Sr28* allow the prediction of disease phenotype in hexaploid wheat germplasm, a set of 24 hard red spring wheat varieties were genotyped with the flanking markers wmc332 and wPt-7004-PCR. In addition to the 24 cultivars we included seven additional wheat lines: SD 1691 (+*Sr28*), Ceres (+*Sr28*), Kota (+*Sr28*), Line AD (+*Sr28*), LMPG-6 (−*Sr28*), W2691 (−*Sr28*), and CI 7611. Markers wmc332 and wPt-7004-PCR were amplified using the procedures described above, and amplicon sizes were determined using silver staining on an acrylamide gel.

Results

Stem rust phenotypic analyses and segregation of resistance

Both Line AD and SD 1691 displayed a ';3' seedling infection type to race TTKSK. Gabo 56 displayed a '2' to '2^+' infection type and LMPG-6 displayed infection type '3^+'. The $F_{2:3}$ progeny of LMPG-6/SD 1691 segregated for resistance to race TTKSK with resistant plants exhibiting ';3' to '3;' infection types and susceptible plants exhibiting infection types '3' to '4'. Segregation of resistance did not deviate significantly from that expected for a single dominant gene (Table 1). Generation F_2 progeny of the cross between Line AD and SD 1691 did not segregate for resistance and all plants evaluated (639) had infection type ';3'. The limited number of F_2 progeny plants assayed restricts an accurate allelism test. However, the lack of susceptible progeny observed provide no evidence that the gene in SD 1691 and the gene in Line AD are independent. These data indicate that the gene present in SD 1691 is *Sr28* or linked to *Sr28*. The $F_{2:3}$ progeny of the cross between SD 1691 and Gabo 56 displayed a range of infection types including ';3', '2', and '4' infection types. Infection type '2' plants likely inherited this uncharacterized resistance from Gabo 56. Segregation of the ';3' infection type derived from SD 1691 did not deviate significantly from the expected ratio for a single dominant gene (Table 1). Similarly, progeny of LMPG-6*2/CI 7611 segregated for what appeared to be a single gene (Table 1).

Genetic mapping

Microsatellite marker wmc332 was identified as linked to *Sr28* based on amplification of different alleles from the resistant and susceptible bulked DNAs. Hybridization of F_2 DNAs to the DArT wheat array indicated the presence of 263 polymorphic DArT markers. Three markers were linked to *Sr28* (logarithm of odds value >5.0; wPt-7004, wPt-5128, and wPt-7161). All of the DArT markers identified linked to *Sr28* were dominant and in repulsion to *Sr28*. Markers wPt-5128 and wPt-7004 were previously mapped to chromosome arm 2BL (Akbari et al. 2006). The DArT markers did not deviate from expected segregation ratios (Table 1).

Mapping the markers linked to *Sr28* identified wmc332 and wPt-7004 as flanking *Sr28* at 5.9 and 1.9 cM away,

Table 1 Segregation of race TTKSK resistance and linked molecular markers in the *Triticum aestivum* populations 'LMPG-6/SD 1691', 'SD 1691/Gabo56', and 'LMPG-6*2/CI7611'

Population	Locus	*a*[a]	*h*	*b*	*c*	*d*	Total	χ^{2} [d]	*P* value
LMPG-6/SD 1691	wmc332	14	50	15	2	2	83	5.61	0.06
LMPG-6/SD 1691	*Sr28*	19	49	14	9	–	91	3.73	0.15
LMPG-6/SD 1691	wPt-7004	20	–	–	67	–	87	0.19	0.66
LMPG-6/SD 1691	wPt-5128	22	–	–	64	–	86	0.02	0.90
LMPG-6/SD 1691	wPt-7161	20	–	–	62	–	82	0.02	0.90
SD 1691/Gabo56	wmc332	32[b]	108	50	7	–	190	6.97	0.03
SD 1691/Gabo56	*Sr28*	18	44	21	–	–	83	0.51	0.77
SD 1691/Gabo56	wPt-7004-PCR	20	101	73	–	–	194	29.00	5.04E−07
LMPG-6*2/CI7611	wmc332	85[c]	86	–	–	–	171	0.01	0.92
LMPG-6*2/CI7611	*Sr28*	87	85	–	–	–	172	0.02	0.89
LMPG-6*2/CI7611	wPt-7004-PCR	87	82	–	–	–	169	0.15	0.70
LMPG-6*2/CI7611	cfd73	73	68	–	–	–	141	18.00	0.67

[a] '*a*' refers to homozygous for the SD 1691 allele for the SD 1691/Gabo56 population, 'h' refers to heterozygous, '*b*' refers to homozygous for the LMPG-6 allele for the LMPG-6*2/CI7611 population, '*c*' refers to either heterozygous or homozygous LMPG-6, and '*d*' refers to heterozygous or homozygous SD 1691

[b] '*a*' refers to homozygous for the SD 1691 allele for the SD 1691/Gabo56 population

[c] '*a*' refers to homozygous for the LMPG-6 allele for the LMPG-6*2/CI7611 population

[d] χ^2 values calculated for testing 1:2:1 segregation of codominant markers (2 *df*) or 3:1 segregation of dominant markers (1 *df*) in the F_2 populations and 1:1 segregation of markers (1 *df*) in the BC_1F_1 population. For codominant markers and *Sr28*, alleles classified as '*c*' or '*d*' were excluded from χ^2 analyses

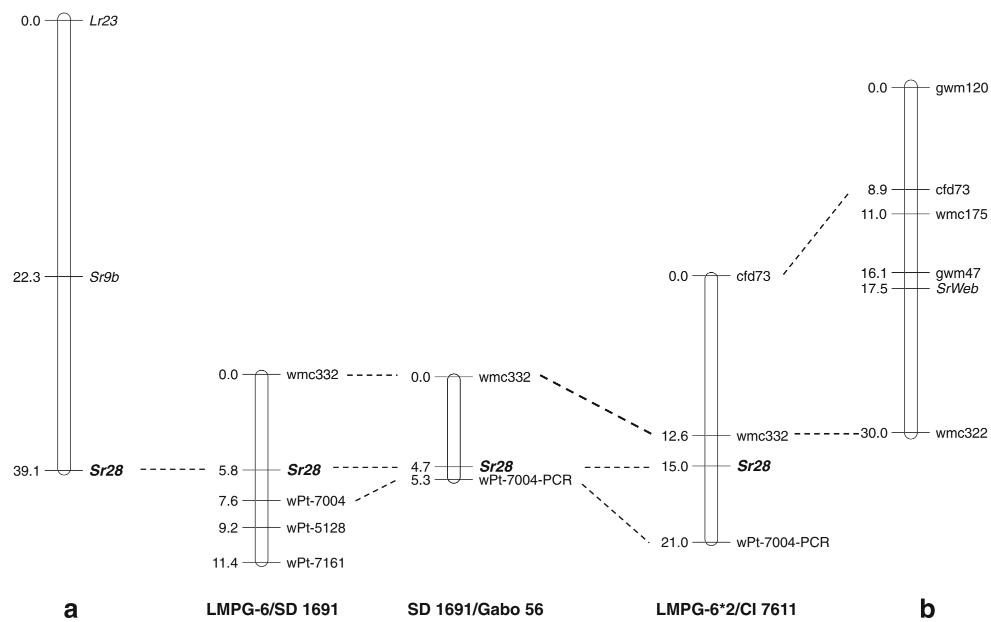

Fig. 1 Genetic maps of chromosome arm 2BL from LMPG-6/SD 1691, SD 1691/Gabo 56, and LMPG-6*2/CI 7611 and maps reconstructed from previous studies: **a** McIntosh (1978) and **b** Hiebert et al. (2010). Maps were constructed and visualized using JoinMap 4.0. Distances shown are in cM

respectively, in the LMPG-6/SD 1691 population (Fig. 1). These markers were confirmed to flank the locus segregating for stem rust resistance in the SD 1691/Gabo 56 and LMPG-6*2/CI 7611 populations (Fig. 1). The PCR-based marker wPt-7004-PCR was successful in differentiating alleles in the LMPG-6*2/CI 7611 population (Fig. 2).

Haplotype analysis

Markers wmc332 and wPt-7004-PCR were amplified from 24 hard red spring wheat varieties and seven additional wheat lines. Amplicon sizes of 214, 217, and 220 base pairs (bp) for marker wmc332 were associated with the presence

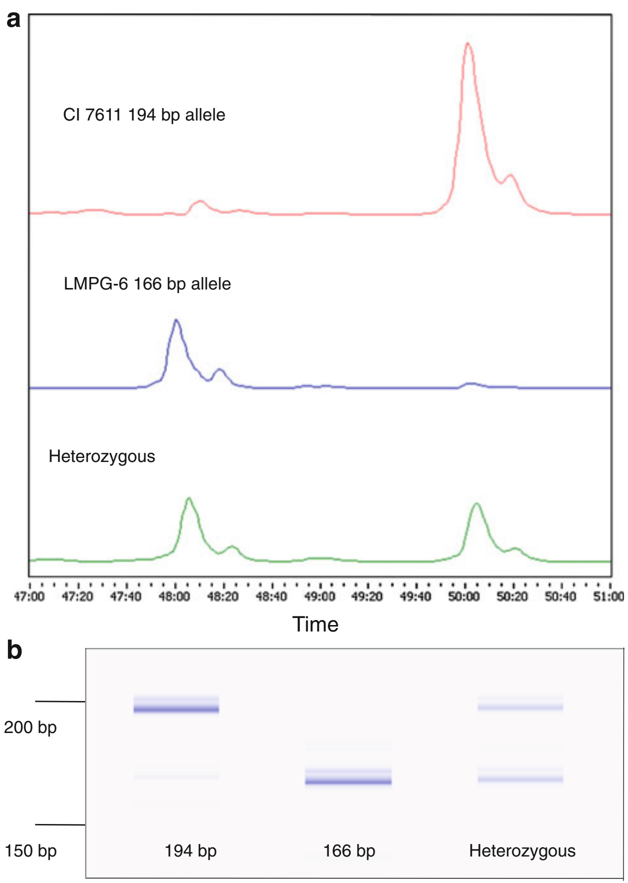

Fig. 2 Electropherograms displaying the amplified fragment sizes of marker wPt-7004-PCR visualized based on overlaid relative fluorescence units (**a**) and gel image (**b**) obtained using PROSize software

of *Sr28* with the exception of susceptible genetic stock W2691 (Table 2). Marker wmc332 amplified a 214-bp fragment from CI 7611. Marker wmc332 amplified fragments of sizes of 208 bp or less from the susceptible genetic stocks and all the United States hard red spring wheat cultivars tested (Table 2). For wPt-7004-PCR, two amplicons of sizes 166 and 194 bp were amplified in all lines. However, polymorphism was observed as different size amplicons were sometimes preferentially amplified resulting in a repeatable, discriminatory marker (Fig. 3). Preferential amplification of the 194-bp amplicon was associated with the presence of *Sr28* including CI 7611. For the susceptible genetic stocks and all the United States hard red spring wheat cultivars tested, either no preferential amplification was observed, or the 166-bp amplicon was preferentially amplified. Only two of the hard red spring wheat cultivars displayed resistance to race TTKSK: Thatcher and Tom. Thatcher does not possess Kota in its pedigree and is believed to have resistance to race TTKSK derived from Iumillo. Tom resistance to race TTKSK could be similar to Thatcher resistance. As Thatcher and Tom are not known to be derived from Kota and do not possess marker haplotypes similar to *Sr28* lines, it is likely that their resistance to race TTKSK is independent of *Sr28*.

Discussion

Segregation of resistance indicated that the single dominant gene, *Sr28*, confers the seedling resistance to race TTKSK in SD 1691. We identified molecular markers linked to *Sr28* on chromosome arm 2BL (Fig. 1). Though only one microsatellite marker (wmc332) was identified as linked to *Sr28*, three DArT markers (wPt-7004, wPt-5128, and wPt-7161) were identified in the LMPG-6/SD 1691 mapping population. A PCR-based marker for wPt-7004 was developed. In the survey of Northern Great Plains hard red spring wheat cultivars, these markers amplified unique haplotypes compared with *Sr28* lines. Markers wmc332 and wPt-7004-PCR could be used for marker-assisted selection of *Sr28*.

The map location of resistance to race TTKSK segregating in the LMPG-6*2/CI 7611 population is consistent with that of *Sr28* (Fig. 1). This information combined with the similar seedling infection type and wPt-7004-PCR marker haplotype of CI 7611 compared with *Sr28* lines suggests that the race TTKSK resistance gene in CI 7611 is *Sr28*. The distance estimates between *Sr28* and flanking markers wPt-7004-PCR and wmc332 varied slightly among the three populations. These differences are likely due to differences in sampling given the limited population sizes or differences in population-specific recombination frequencies. CI 7611 was collected in the Former Soviet Union by N. I. Vavilov. As Kota was also developed in the Former Soviet Union, it is possible that these two cultivars are of common ancestry.

Hiebert et al. (2010) previously reported a stem rust resistance gene effective to race TTKSK on chromosome arm 2BL at a similar map location as *Sr9a* (Tsilo et al. 2007). In this study, *Sr28* was mapped distal to marker wmc332 as evidenced by the map for LMPG-6*2/CI 7611. Calculating the map distance between *Sr28* and *SrWeb* using marker wmc332 as a bridge between the *SrWeb* map (Hiebert et al. 2010) and our *Sr28* maps results in distances of 18.3, 17.2, and 14.9 cM. These distance estimates are comparable to the genetic distance estimate between *Sr28* and *Sr9b* (McIntosh 1978; Fig. 1). This supports both hypotheses that *Sr28* is distinct from *SrWeb* and that *SrWeb* is in the same region as the *Sr9* locus. The low IT of lines with *Sr28* (;3) is very different compared with the '1' to '2' IT of *SrWeb* to race TTKSK (Hiebert et al. 2010). An allelism test between *Sr28* and *SrWeb* would be necessary to confirm that these genes are indeed distinct, though neither map comparisons nor ITs suggest that they are the same gene or occupy the same locus.

Table 2 *Sr28*-linked marker alleles in wheat genetic stocks and varieties and seedling reactions to *Puccinia graminis* f. sp. *tritici* races

Line	Origin	wmc332	wPt-7004-PCR	TTKSK	BCCBC
SD 1691 (CI 12499)[a]	SDSU	217	194[b]	;3	0;/;1 LIF/;13 LIF
Gabo 56 (CI 14035)	Rockefeller	197	166	2	–
Line AD[a]	USY	220	194	;3	0/0;/;1 LIF
W2691	USY	217	194/166	4	4
Ceres (PI 35118)[a]	NDSU	217	194	–	0;/;1 LIF
Kota (CI 5878)[a]	FSU	217	194	;13$^-$	0
CI 7611	FSU	214	194	;	0;13$^-$
LMPG-6	USK	199	166	3$^+$	4
Ada	UMN	208	194/166	4	–
Alsen	NDSU	–	194/166	4	–
Briggs	SDSU	175	166	4	–
Faller	NDSU	175	194/166	4	–
Freyr	Syngenta	175	166	4	–
Glenn	NDSU	208	194/166	3$^+$	–
Granger	SDSU	208	194/166	4	–
Hat Trick	Trigen	175	166	4	–
HJ98	UMN	205	194/166	–	–
Howard	NDSU	172	166	2$^+$3	–
Knudson	Syngenta	175	194/166	4	–
Marshall	UMN	175	194/166	3$^+$	–
Oklee	UMN	208	194/166	4	–
Oxen	UMN	208	194/166	3$^+$	–
RB07	UMN	175	194/166	4	–
Rollag	UMN	208	194/166	–	–
Sabin	UMN	175	194/166	4	–
Stecle-ND	NDSU	–	194/166	4	–
Thatcher	UMN	208	194/166	33$^+$;	–
Tom	UMN	175	194/166	3$^+$1;/;123$^-$	–
Traverse	SDSU	175	194/166	4	–
Ulen	UMN	175	194/166	33+	–
Verde	UMN	175	194/166	4	–
Wheaton	UMN	175	194/166	3+	–

FSU former Soviet Union, *USK* University of Saskatchewan, *SDSU* South Dakota State University, *NDSU* North Dakota State University, *USY* University of Sydney, *UMN* University of Minnesota

[a] Lines known to carry the Ug99 stem rust resistance gene *Sr28*

[b] Both 166- and 194-bp fragments amplified in all lines. Preferential amplification of the 194-bp fragment is indicated as 194 whereas preferential amplification of the 166-bp fragment is indicated as 166. Absence of preferential amplification is designated as 194/166

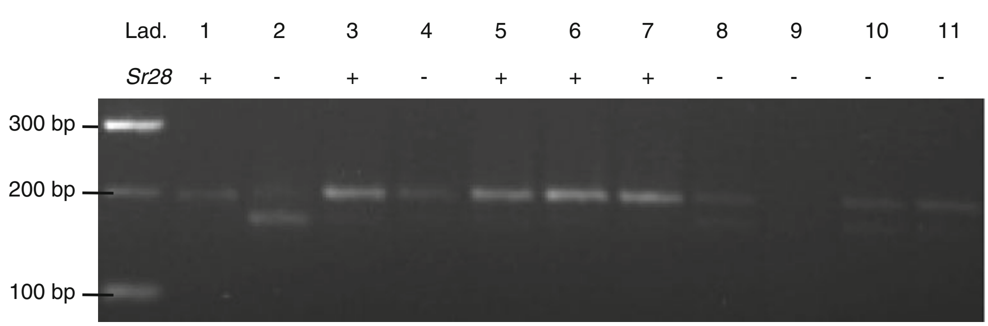

Fig. 3 Agarose gel displaying the amplified fragment sizes of marker wPt-7004-PCR for the following wheat lines: (*1*) CI 12499, (*2*) LMPG-6, (*3*) Line AD, (*4*) W2691, (*5*) Ceres, (*6*) Kota, (*7*) CI 7611, (*8*) Faller, (*9*) RB07, (*10*) Glenn, and (*11*) Briggs. Presence of *Sr28* is designated based on stem rust phenotype

The availability of effective resistance genes to race TTKSK in hard red spring wheat germplasm is limited (Jin and Singh 2006). Though *Sr28* is not effective to many common *Pgt* races present in the United States, it can be used in combination with genes that are effective and already present in breeding germplasm. The identification of flanking markers for the Ug99-effective resistance gene *Sr28* that will be selectable in United States hard red spring wheat germplasm may facilitate the efficient introgression and selection of *Sr28*. Since *Sr28* was previously used in breeding, it is unlikely that *Sr28* or closely linked genes have obvious deleterious attributes. Resistant lines SD 1691 (CI 12499) and CI 7611 are available from the USDA-ARS National Small Grains Collection in Aberdeen, ID. We are in the process of backcrossing *Sr28* into adapted lines 'Faller' (Mergoum et al. 2008) and 'RB07' (Anderson et al. 2009). In order to obtain highly effective and durable resistance, *Sr28* should only be used in combination with additional Ug99-effective resistance genes.

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