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ORIGINAL PAPER

Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99

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Abstract

Key message **Wheat stem rust resistance gene *SrWeb* is an allele at the *Sr9* locus that confers resistance to Ug99.**

Abstract Race TTKSK (Ug99) of *Puccinia graminis* f. sp. *tritici*, the causal fungus of stem rust, threatens global wheat production because of its broad virulence to current wheat cultivars. A recently identified Ug99 resistance gene from cultivar Webster, temporarily designated as *SrWeb*, mapped near the stem rust resistance gene locus *Sr9*. We determined that *SrWeb* is also present in Ug99 resistant cultivar Gabo 56 by comparative mapping and an allelism test. Analysis of resistance in a population segregating for both *Sr9e* and *SrWeb* demonstrated that *SrWeb* is an allele at the *Sr9* locus, which subsequently was designated as *Sr9h*. Webster and Gabo 56 were susceptible to the Ug99-related race TTKSF+ from South Africa. Race TTKSF+ possesses unique virulence to uncharacterized Ug99 resistance in cultivar Matlabas. This result validated that resistance to Ug99 in Webster and Gabo 56 is conferred by the same gene: *Sr9h*. The emergence of pathogen virulence to several resistance genes that are effective to the original Ug99 race TTKSK, including *Sr9h*, suggests that resistance genes should be used in combinations in order to increase resistance durability.

Introduction

Puccinia graminis Pers.:Pers f. sp. *tritici* Eriks. & E. Henn (*Pgt*) is the causal fungus of wheat stem rust, one of the most significant diseases of bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* var. *durum*), barley (*Hordeum vulgare* L.), and triticale (X *Triticosecale* Wittmack) (Leonard 2001; Roelfs et al. 1992). Wheat stem rust has become increasingly important since 1999 when an isolate of *Pgt* called Ug99 (race TTKSK; Jin et al. 2007, 2008) was shown to possess unique virulence to *Sr31* in addition to virulence to the majority of wheat cultivars around the world (Jin and Singh 2006; Pretorius et al. 2000; Singh et al. 2008). Variants of race TTKSK were identified with additional virulence to wheat resistance genes *Sr24* (Jin et al. 2008; Pretorius et al. 2010; Visser et al. 2011) and *Sr36* (Jin et al. 2009). In 2010, a *Pgt* isolate collected in South Africa possessed virulence to winter wheat cultivar Matlabas (Pretorius et al. 2012). Further studies demonstrated that Matlabas displayed resistance to several Ug99 race group variants, but not to the isolate collected from Matlabas described as race TTKSF+. The spread of race TTKSK and diverse variants throughout Africa and the Middle East (Nazari et al. 2009) demonstrated the need for identifying and utilizing multiple

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Ug99 resistance genes. There are at least 31 wheat stem rust resistance (*Sr*) genes with diverse origins that confer resistance to Ug99: *Sr2*, *Sr13*, *Sr21*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr40*, *Sr42*, *Sr44*, *Sr45*, *Sr46*, *Sr47*, *Sr51*, *Sr52*, *Sr53*, *Sr57*(*Lr34*), *SrTA10171*, *SrTA10187*, *SrTA1662*, *SrTmp*, *SrWeb*, $Sr1RS^{Amigo}$ (Faris et al. 2008; Ghazvini et al. 2012; Hiebert et al. 2010; Kolmer et al. 2011; Jin and Singh 2006; Jin et al. 2007; Liu et al. 2011a, b; Olson et al. 2013a, b; Qi et al. 2011; Rouse et al. 2011a; Rouse and Jin 2011; Singh et al. 2013). These 31 genes vary in their level of effectiveness to Ug99 and race specificity within the Ug99 race group and to diverse *Pgt* races. The five genes that originate from *T. aestivum*, *Sr28*, *Sr42*, *Sr57*, *SrTmp*, and *SrWeb*, are valuable resources for Ug99 resistance because they are not located on introgressed chromatin from alien species with possible linkage drag.

Stem rust resistance genes *Sr9*, *Sr16*, *Sr28*, and *Sr47* have been located on chromosome arm 2BL. In addition, Ug99 resistance gene *SrWeb* was previously mapped to chromosome arm 2BL near the *Sr9* locus (Hiebert et al. 2010; Tsilo et al. 2007). There are six characterized alleles at the *Sr9* locus, and each demonstrates unique race specificities to *Pgt* races: *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f*, and *Sr9g* (Green et al. 1960; Knott 1966; Loegering 1975; McIntosh and Luig 1973). The gene designation *Sr9c* was originally reserved for a gene that was subsequently designated as *Sr36* on chromosome arm 2BS (McIntosh et al. 1995). The previously characterized *Sr9* alleles and *Sr16* are ineffective to Ug99, whereas *Sr28* and *Sr47* confer resistance (Jin et al. 2007). Molecular markers linked to *Sr28* were identified on chromosome arm 2BL (Rouse et al. 2012). Molecular markers linked to *Sr47* and a shortened *Aegilops speltoides* Tausch introgression possessing *Sr47* were recently derived (Klindworth et al. 2012). The potential allelism of *SrWeb* and other stem rust resistance genes on chromosome arm 2BL has not been tested.

We observed seedling resistance to *Pgt* race TTKSK in 1956 Rockefeller Foundation cultivar Gabo 56. The pedigree of Gabo 56 is Timstein/Kenya 58//Gabo (CI 14035). Previous studies determined that Timstein and Gabo possessed stem rust resistance gene *Sr11* (Knott and Anderson 1956). A second stem rust resistance gene was initially described in Timstein and Gabo (Knott and Anderson 1956), but subsequent studies demonstrated that abnormal transmission of gametes and *Sr11* explained the observed segregation of resistance (Luig 1960; Sears and Loegering 1961). Watson and Stewart (1956) suggested that Timstein and Gabo are both derived from the cross Bobin W39*2/Gaza based on observed race specificity of resistance to stem and leaf rust and dissimilarity between resistance in Timstein and that of its originally proposed parents Steinwedel (CI 1702) and *T. timopheevi* Zhuk. (Boasso and Levine 1951; Levine et al. 1951). Kenya 58 was demonstrated to possess *Sr6* and *Sr7a* (Knott and Anderson 1956; Loegering and Sears 1966). Genes *Sr6*, *Sr7a*, and *Sr11* are all ineffective to Ug99 race TTKSK (Jin et al. 2007). The genetics and source of Ug99 resistance in Gabo 56 were not known.

Our objectives were (1) to determine the genetics of resistance to Ug99 in cultivar Gabo 56 and (2) to determine the allelic relationship between *SrWeb* and other stem rust resistance genes on chromosome arm 2BL.

Materials and methods

Wheat germplasm

We derived a total of five wheat populations from six parental lines. We crossed cultivar Gabo 56 (accession CI 14035, from the United States Department of Agriculture National Small Grains Collection) with susceptible cultivar Chinese Spring in order to characterize the stem rust resistance in Gabo 56. A total of 104 $F_{2:3}$ families were derived. Phenotyping the $F_{2:3}$ families with race TTKSK and mapping the resistance with molecular markers indicated that Gabo 56 resistance could be conferred by *SrWeb* (see "Results"). In order to test this hypothesis, we crossed *SrWeb*-containing wheat line Webster obtained from the Cereal Research Centre (RL6201; Agriculture and Agri-Food Canada) with Gabo 56 in order to conduct an allelism test. A total of 356 doubled haploid lines were derived from this population at the Cereal Research Centre using a maize pollination method (Thomas et al. 1997). To test for the allelic relationship between the resistance gene in Gabo 56 and other resistance genes on chromosome arm 2BL, Gabo 56 was crossed to *Sr9e* line Vernstein (Jin et al. 2007) and also *Sr28* line SD 1691 (CI 12499; Rouse et al. 2012). For the Gabo 56/Vernstein population, 347 $F_{2:3}$ families were derived. A total of 82 $F_{2:3}$ families were utilized from a previously derived SD 1691/Gabo 56 population (Rouse et al. 2012). A single F_3 plant from a SD 1691/Gabo 56 $F_{2:3}$ family that was fixed for both *SrWeb* and *Sr28* (see "Results") was increased for two subsequent generations and deposited at the USDA-ARS National Small Grains Collection as CDL001 (PI 670015). To validate the presence of both *SrWeb* and *Sr28* in CDL001, we derived a total of 292 F_2 seeds from CDL001/LMPG-6. Wheat line LMPG-6 is a stem rust susceptible hard red spring wheat (Knott 1990). We obtained the parents of Gabo 56, Timstein (CI 12347), Kenya 58 (CI 12471), and Gabo (CI 12795), to assess the origin of resistance to Ug99 in Gabo 56.

Stem rust phenotyping

Procedures in inoculation, incubation, and disease assessment were followed as described previously (Rouse et al. 2011b). Infection types (ITs) were classified as in Stakman et al. (1962). Infection types ‘0’ to ‘2’ were considered low infection types indicating host resistance, whereas ITs ‘3’ to ‘4’ were considered high infection types indicating host susceptibility. For each seedling experiment, we inoculated the population, the parents of the population, and the International stem rust differential set (Roelfs and Martens 1988; Jin et al. 2008). For $F_{2:3}$ families, 20–25 plants from each family were screened. Five plants for each DH line were inoculated. The phenotypes of the $F_{2:3}$ families were used to classify the genotypes of the parent F_2 plants as homozygous resistant, homozygous susceptible, or heterozygous in reaction to the *Pgt* race tested. For $F_{2:3}$ families with <15 viable plants and for families with ambiguous ITs, the assay was repeated. All populations and families derived in this study were evaluated for reaction to *Pgt* race TTKSK (isolate 04KEN156/04). The Gabo 56/Vernstein population was tested for reaction to both race TTKSK and race QTHJC (isolate 75ND717C) in independent experiments. Race QTHJC is virulent to *SrWeb* and avirulent to *Sr9e*, whereas race TTKSK is avirulent to *SrWeb* and virulent to *Sr9e*. *Pgt* races were classified according to the International letter code nomenclature system (Jin et al. 2008; Roelfs and Martens 1988). For the Gabo 56/Chinese Spring population, the test for reaction to race TTKSK was replicated for all families. Assays of reaction to race TTKSK and QTHJC were conducted at the USDA-ARS Cereal Disease Laboratory, USA.

Webster, Gabo 56, and Matlabas were tested for seedling ITs in response to race TTKSF+ (isolate UVPgt61/1) at the University of the Free State, South Africa, according to previously described methods (Pretorius et al. 2000). Race TTKSF+ is avirulent to *Sr31* and virulent to resistance in Matlabas, whereas race TTKSK is virulent to *Sr31* and avirulent to resistance in Matlabas.

Molecular marker analyses

Tissue was harvested from the 104 Gabo 56/Chinese Spring F_2 parents of the corresponding $F_{2:3}$ families. DNA was extracted for each F_2 plant using a modified CTAB method (Rouse et al. 2012). A total of 80 F_2 DNAs and DNA of the parents were genotyped with diversity arrays technology (DArT) markers according to Akbari et al. (2006). Initial mapping of resistance in Gabo 56 with DArT markers indicated linkage of a resistance gene to DArT markers on chromosome arm 2BL. Therefore, SSR markers previously mapped to chromosome arm 2BL (Röder et al. 1998; Somers et al. 2004; Song et al. 2005) were tested for polymorphism among Chinese Spring and Gabo 56. Resistant and susceptible bulk DNAs of ten plants each were used to select, from the polymorphic markers, SSRs linked to resistance (Michelmore et al. 1991). The Gabo 56/Chinese Spring F_2 DNAs were then genotyped for the identified SSR markers. SSR genotyping was conducted using an ABI 3130xl Genetic Analyzer (Applied Biosystems) and GeneMapper software version 3.7 (Applied Biosystems) as described in Rouse et al. (2012).

Segregation and genetic linkage

Chi-squared tests were performed to test for deviation of observed segregation ratios compared to expected segregation ratios for stem rust phenotypes and molecular markers. In order to construct linkage maps, Joinmap version 4.0 (Stam 1993; Van Ooijen 2006) was used. Genetic distances were calculated using Kosambi’s distance estimate (Kosambi 1944). For the Gabo 56/Chinese Spring population, DArT markers were divided into maternal and paternal classes (maternal markers were scored ‘*a*’ or ‘*c*’, whereas paternal markers were scored as ‘*b*’ or ‘*d*’). A logarithm of odds threshold of 5.0 was used to identify maternal and paternal DArT markers linked to stem rust resistance. In order to map the dominant markers, we followed the technique used by Jing et al. (2009) where maternal DArT markers were initially mapped separately with the codominant markers, then the paternal markers. Using the ‘combine maps’ function in Joinmap, the two maps were then merged using the codominant markers and stem rust resistance as bridge markers.

Results

Characterization of resistance to Ug99 in Gabo 56

Gabo 56 displayed a ‘2’ to ‘2^{+}’ seedling IT to race TTKSK in contrast to the ‘4’ IT observed for Chinese Spring. In the $F_{2:3}$ progeny, resistant plants displayed ‘2’ to ‘2^{+}’ ITs and susceptible plants displayed ‘3’ to ‘4’ ITs. In segregating families, sometimes ‘$2^{+}3$’ ITs were observed (possibly indicative of heterozygous individuals). Segregation of resistance did not deviate significantly from the expected 1:2:1 ratio (resistant:segregating:susceptible) for segregation at a single gene (Table 1). The parents of Gabo 56 (Timstein, Kenya 58, and Gabo) displayed ITs ‘2’, ‘3^{+}’, and ‘22^{+}’, respectively, to race TTKSK.

Hybridization of F_2 DNAs to the DArT wheat array indicated the presence of 293 polymorphic DArT markers. Five markers were linked to the resistance gene (logarithm of odds threshold of 5.0; *XwPt-1140*, *XwPt-3109*, *XwPt-3132*, *XwPt-4199*, and *XwPt-8460*). Markers *XwPt-3132*

Table 1 Segregation of reaction to *P. graminis* f. sp. *tritici* race TTKSK and linked markers among F_2 individuals or $F_{2:3}$ families used for mapping resistance in the Gabo 56/Chinese Spring population

Marker/gene	*a*[a]	*h*	*b*	*c*	*d*	Total	X^{2b}	*P* value
Xgwm319	23	35	14	1	6	79	2.72	0.26
Xgwm388	21	40	10	8	–	79	4.9	0.09
XwPt-4199	19	–	–	41	–	60	1.42	0.23
XwPt-3109	–	–	17	–	37	54	1.21	0.27
Xwmc175	22	–	–	58	–	80	0.27	0.61
XwPt-1140	–	–	14	–	45	59	0.05	0.82
Xbarc101	18	39	20	1	–	78	0.13	0.94
Xgwm120	19	40	21	–	–	80	0.1	0.95
Xgwm47	23	–	–	55	–	78	0.84	0.36
XwPt-3132	–	–	12	–	43	55	0.3	0.59
TTKSK	35	48	21	–	–	104	4.38	0.11
XwPt-8460	19	–	–	40	–	59	1.63	0.2
Xwmc332	20	43	16	–	–	79	1.03	0.6

[a] '*a*', homozygous for the Gabo 56 allele; '*h*', heterozygous, '*b*', homozygous for the Chinese Spring allele; '*c*', either heterozygous or homozygous for the Chinese Spring allele; '*d*', heterozygous or homozygous for the Gabo 56 allele

[b] X^2 values calculated for 1:2:1 segregation for codominant markers (2 *df*), or for 3:1 segregation for dominant markers (1 *df*). Occasional individuals classified as '*c*' or '*d*' were not included in calculation of X^2 values for codominant markers

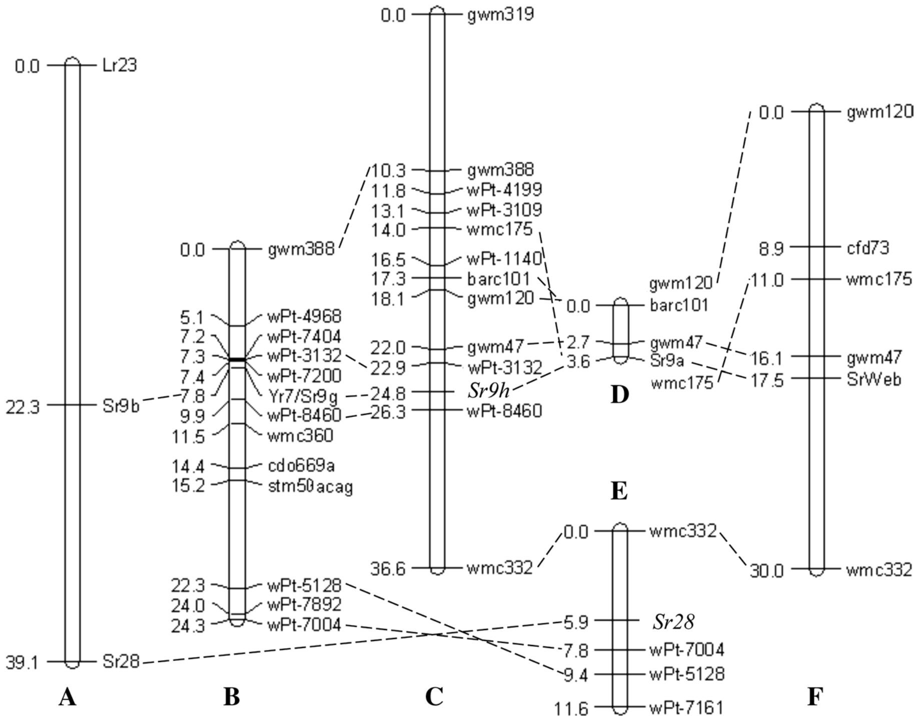

Fig. 1 Genetic maps of the *Sr9* region of chromosome 2B from our data and previously published studies: McIntosh (1978) (*A*), Akbari et al. (2006) (*B*), Gabo 56/Chinese Spring (*C*), Tsilo et al. (2007) (*D*), Rouse et al. (2012) (*E*), and Hiebert et al. (2010) (*F*)

and *XwPt-8460* were previously mapped to chromosome arm 2BL (Akbari et al. 2006). Seven SSR markers were identified that were polymorphic between Chinese Spring and Gabo 56 and segregated among resistant and susceptible bulks: *Xbarc101*, *Xgwm47*, *Xgwm120*, *Xgwm319*, *Xgwm388*, *Xwmc175*, and *Xwmc332*. None of the molecular markers deviated from expected segregation ratios (Table 1).

Linkage analyses identified that SSR marker *Xgwm47* was linked to the Ug99 resistance gene at a distance of 2.8 cM (Fig. 1C). *Xgwm47* was also linked to *Sr9a*, *Sr9e*, and *SrWeb* in previous studies (Fig. 1; Tsilo et al. 2007; Bhavani et al. 2008; Hiebert et al. 2010). Both *Xgwm47* and *Xwmc175* are dominant markers in this population and linked in repulsion with the resistance gene (Table 1). The closest DArT markers flanking the gene were *XwPt-3132* 1.9 cM proximal and *XwPt-8460* 1.5 cM distal (Fig. 1C). The correspondence between the mapped location of the Ug99 resistance gene in Gabo 56 and *Sr9a*, *Sr9e*, and *Yr7*/*Sr9g* provided preliminary evidence that the Ug99 resistance gene in Gabo 56 could be an allele of *Sr9*.

Allelic relationship between *SrWeb* and Gabo 56 resistance

Since the Ug99 resistance gene in Gabo 56 mapped to a similar location as *SrWeb*, we crossed Gabo 56 and Webster (RL6201) to test for allelism between the two genes. Gabo 56 displayed IT '2' to race TTKSK, whereas Webster displayed IT '2^+3^-'. The seedling ITs of the 356 DH lines varied from '2' to '2^+3'. A total of 26 DH lines with the highest ITs ranging from '2^+' to '2^+3' were assayed again for seedling IT to race TTKSK to validate the resistant ITs. The ITs in the second replication were resistant ranging from '2' to '22^+'. The 356 DH lines represent 356 gametes. The probability of not detecting susceptible recombinants utilizing 356 gametes and a single race (TTKSK) avirulent to both genes is $P = 0.028$ for two genes separated by 2 cM and $P = 0.168$ for genes separated by 1 cM. The maximum recombination values 'r' at $P = 0.05$ and $P = 0.01$ assuming two unique loci in this population are $r \leq 0.0083$ and $r \leq 0.013$, respectively (Hanson 1959). The allelism test indicates that the resistance genes in Gabo 56 and Webster are likely the same gene.

Cultivar Matlabas previously displayed IT '4' to race TTKSF+, though Matlabas was resistant to other races in the Ug99 race group (Pretorius et al. 2012). Webster and Gabo 56 displayed susceptible ITs '3^+' and '3^{++}', respectively, to race TTKSF+. These data support our assertion that resistance in Gabo 56 and *SrWeb* are the same gene.

Allelic relationship between *SrWeb* and *Sr9e*

Gabo 56 and *Sr9e* line Vernstein were crossed to test for allelism between *SrWeb* and *Sr9e*. Gabo 56 displayed IT '2' to race TTKSK and '3^+' to race QTHJC. Vernstein displayed IT '3^+' to race TTKSK and '2^+' to race QTHJC. A total of 347 $F_{2:3}$ families were phenotyped for seedling reaction to race TTKSK and race QTHJC to postulate the presence of *SrWeb* and *Sr9e*, respectively. The population segregated for ITs '2' to '4' to race TTKSK and '2^+' to '4' to race QTHJC. We did not detect any recombination between *SrWeb* and *Sr9e* (Table 2). The 347 generation $F_{2:3}$ phenotypes representing F_2 genotypes correspond to 694 gametes. The probability of not detecting susceptible recombinants for this population size utilizing races to detect the two genes separately is $P = 0.001$ for two genes separated by 1 cM and $P = 0.125$ for genes separated by 0.3 cM. The maximum recombination values at $P = 0.05$ and $P = 0.01$ assuming two unique loci in this population are $r \leq 0.0043$ and $r \leq 0.0066$, respectively (Hanson 1959). Since *SrWeb* exhibits resistance to race TTKSK unlike any known *Sr9* allele and no recombination was detected in a robust *SrWeb/Sr9e* allelism test, the data suggest that *SrWeb* be designated as a seventh allele of *Sr9*: *Sr9h*.

Table 2 Genotypes at the *SrWeb* and *Sr9e* loci determined by testing $F_{2:3}$ families from Gabo 56/Vernstein

Sr9e locus	*SrWeb* locus[a]		
	SrWebSrweb	*SrwebsrWeb*	*srWebsrWeb*
Sr9eSr9e	0	0	81
Sr9esr9e	0	178	0
sr9esr9e	88	0	0

[a] *SrWeb* genotype was based on reaction to *Pgt* race TTKSK, whereas *Sr9e* genotype was based on reaction to *Pgt* race QTHJC; $X^2_{1:2:1} = 1.19$, $P_{2df} = 0.55$

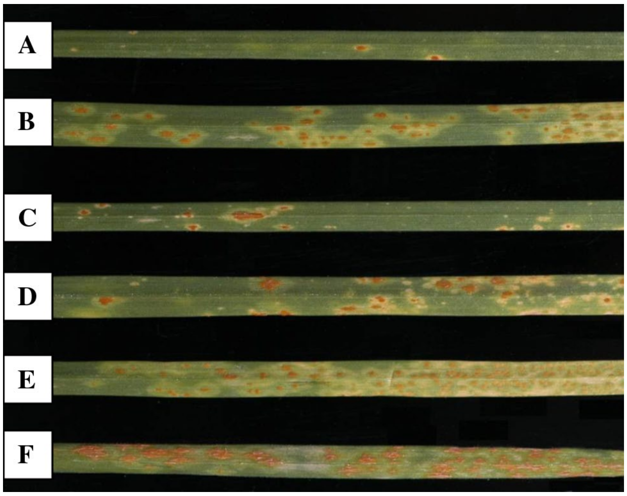

Fig. 2 *Puccinia graminis* f. sp. *tritici* race TTKSK seedling infection types of SD 1691 IT = ';13^-' (*A*), Gabo 56 IT = '22^+' (*B*), and SD 1691/Gabo 56 F_2 progeny ITs = ';13^-', ';13', '22^+', and '4', respectively (*C*–*F*)

Combining *Sr9h* and *Sr28* in coupling

SD 1691 displayed IT ';13^-' to race TTKSK, whereas Gabo 56 displayed IT '22^+'. The F_2 progeny of the cross between SD 1691 and Gabo 56 displayed a range of ITs including ';13^-', '22^+', and '4' (Fig. 2). IT ';13^-' corresponded to the presence of *Sr28* from SD 1691, whereas IT '22^+' corresponded to the presence of *Sr9h* from Gabo 56. Of 422 progeny, 4 F_2 plants were identified with susceptible infection types. Segregation of resistance deviated significantly from a 15:1 ratio expected for two independent resistance genes ($X^2 = 20.25$, $P = 6.78 \times 10^{-6}$)

suggesting that *Sr28* and *Sr9h* are linked in repulsion. The Kosambi (1944) distance estimate between *Sr28* and *Sr9h* was 20.7 cM (r = 0.196). A total of 82 $F_{2:3}$ families were derived from this population and screened with race TTKSK to confirm the pattern observed at the F_2 generation. We considered ITs '0' to ';3' as indicative of *Sr28*, ITs '22^{-}' to '2^{+}' as indicative of *Sr9h*, and IT '3^{+}' as indicative of the absence of either gene. The *Sr28* ITs '0' to ';3' masked the presence of *Sr9h* ITs. The population segregated 17 *Sr28Sr28* : 44 *Sr28sr28* : 21 *sr28sr28* in good agreement with an expected single locus segregation ratio ($X^2 = 0.83$, $P = 0.36$). Among the 21 $F_{2:3}$ families without *Sr28*, segregation was as follows: 9 *Sr9hSr9h* : 11 *Sr9hsr9h* : 1 *sr9hsr9h*, a clear deviation from 1:2:1 ($X^2 = 6.14$, $P_{2df} = 0.013$), again indicating that *Sr9h* and *Sr28* are linked in repulsion.

In order to identify a plant homozygous for both *Sr28* and *Sr9h*, we haplotyped F_3 plants from SD 1691/Gabo 56 $F_{2:3}$ families that were homozygous for the presence of *Sr28*. Gabo 56 haplotypes at markers *Xwmc175*, *Xbarc101*, and *Xgwm120* were used to predict the presence of *Sr9h*, whereas the SD 1691 haplotype at marker *XwPt-7004-PCR* was used to predict the presence of *Sr28* (Rouse et al. 2012). Marker *Xgwm47* was not polymorphic in the SD 1691/Gabo 56 population. A single F_3 plant was identified with the marker haplotype indicating the presence of both *Sr28* and *Sr9h*. Seed of this plant was increased for two generations and deposited in the USDA-ARS National Small Grains Collection as CDL001 (PI 670015). In addition, 50 CDL001 plants were screened with *Pgt* race TTKSK and all plants displayed ITs '0' to '0;' indicating that *Sr28* is fixed in this line.

To confirm the presence of both genes in coupling, we crossed CDL001 to stem rust susceptible line LMPG-6 and assessed F_2 progeny with race TTKSK. Of 291 F_2 progeny, 205 displayed ITs '0;' to '31;' indicative of *Sr28*, 16 displayed ITs '2' to '$2^{+}3$' indicative of *Sr9h*, and 70 displayed IT '3^{+}' indicative of the absence of both genes. The presence of infection types indicative of both *Sr28* and *Sr9h* suggested that both genes are present in CDL001. Segregation of *Sr28* did not deviate from an expected 3:1 ratio (205:86, $X^2 = 3.22$, $P = 0.073$). However, among non-*Sr28* plants, segregation at the *Sr9h* locus deviated from 3:1 (16:70, $X^2 = 145.9$, $P = 1.38\text{x}10^{-33}$) with an abundance of susceptible plants confirming that *Sr9h* and *Sr28* are linked in coupling from CDL001.

Discussion

We demonstrated that a single resistance gene in cultivar Gabo 56 confers resistance to *Pgt* race TTKSK. A robust allelism test determined that the resistance gene in Gabo 56 is a new allele of *Sr9*: *Sr9h*. This same resolution in our test of 347 $F_{2:3}$ families could be achieved by screening 925,997 F_2 plants with a single *Pgt* race avirulent to both genes. Race specificity and an allelism test demonstrated that *SrWeb* from cultivar Webster is *Sr9h*. The same resolution in our test of 356 DH lines could be achieved by screening 126,738 F_2 plants with a single *Pgt* race avirulent to both genes. Though *Sr9h* is effective to Ug99 race TTKSK, our data demonstrated that race TTKSF+ detected from South Africa and Zimbabwe is virulent to *Sr9h* (Pretorius et al. 2012). *Sr9h* could be utilized in combination with other resistance genes to protect wheat from Ug99 in locations where *Sr9h* virulence has not been detected.

Since both Timstein and Gabo displayed seedling resistance to race TTKSK, our data confirm earlier reports that Timstein and Gabo share the same *Pgt* race specificity (Boasso and Levine 1951; Levine et al. 1951; Watson and Stewart 1956). The reaction to race TTKSK of lines Bobin and Gaza, the parents of Gabo and proposed parents of Timstein (Watson and Stewart 1956), is not known. Given the low seedling reaction of both Timstein and Gabo to race TTKSK and the reported similarity of these two lines, the available data suggest that both Gabo and Timstein possess *Sr9h*. Although Webster carries *Sr30* in addition to *Sr9h* (Hiebert et al. 2010), line RL6203 (an F_3-derived line from Webster/RL6071) carries *Sr9h*, but not *Sr30* (Hiebert et al. 2010). RL6203 has been designated as the *Sr9h* reference line by the Catalogue of Gene Symbols for Wheat (McIntosh et al. 2012) and is available from the Cereal Research Centre, Agriculture and Agri-Food Canada.

Linkage of *Sr9h* to within 3 cM of *Xgwm47*, *XwPt-3132*, and *XwPt-8460* in the Gabo 56/Chinese Spring population is consistent with the previously mapped locations of *Sr9a*, *Sr9g*, and *SrWeb* (Fig. 1). DArT makers *XwPt-3132* and *XwPt-8460* flanked *Sr9h*. These markers also flanked the '*Yr7/Sr9g* locus' in a previous study (Fig. 1B; Akbari et al. 2006). Akbari et al. (2006) did not disclose how the '*Yr7/Sr9g* locus' was determined, but it is possible that the location of *Sr9g* in Akbari et al. (2006) is assumed based on the mapped location of *Yr7*. Although genes *Yr7* and *Sr9g* are closely linked, lines have been identified with *Sr9g*, but not *Yr7* (McIntosh et al. 1981, 1995). The marker order of microsatellite and DArT markers on the Gabo 56/Chinese Spring 2BL linkage map is consistent with previous studies with the exception of *Xwmc175*. This marker mapped to different locations in each population analyzed relative to *Xgwm47* and *Xgwm120* (Fig. 1). One reason for the inconsistency of the location of *Xwmc175* relative to the order of other markers may be caused by the dominant inheritance of this marker, which may result in less accurate map positions in F_2 populations compared to codominant inheritance.

Comparing distances between markers in the Gabo 56/Chinese Spring and the SD 1691/Gabo 56 maps indicated that *Sr9h* and *Sr28* are linked with a genetic distance of 17.7 cM (Fig. 1). This corresponds well with the 16.8 cM distance between *Sr9* and *Sr28* calculated by McIntosh (1978) and the estimated distance between *Sr9h* and *Sr28* based on segregation in SD 1691/Gabo 56 F_2 plants (20.7 cM). Unfortunately, we do not know of *Pgt* races that we could utilize to screen the SD 1691/Gabo 56 population to postulate the presence of *Sr28* and *Sr9h* in $F_{2:3}$ families independently.

The third Ug99 resistance gene on chromosome arm 2BL, *Sr47*, was available on a large *Ae. speltoides* chromatin introgression in tetraploid wheat at the time of the initiation of this study (Faris et al. 2008). This prevented testing of the potential allelism of *Sr47* with *Sr9h*. Recent work has reduced the size of the *Ae. speltoides* introgression possessing *Sr47* and identified linkage to markers *Xgwm501*, *Xgwm47*, and *Xgpw4165* (Klindworth et al. 2012). These data show that the breakpoint of the translocation carrying *Sr47* is linked to *Sr9* and *Sr28*.

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Conflict of interest The authors declare that there are no conflict of interest.

Ethical standard The authors declare that the experiments comply with the current laws of the countries in which the experiments were performed.

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