Theor Appl Genet (2016) 129:2171–2177
DOI 10.1007/s00122-016-2765-4

ORIGINAL ARTICLE

Genetics and mapping of seedling resistance to Ug99 stem rust in winter wheat cultivar Triumph 64 and differentiation of *SrTmp*, *SrCad*, and *Sr42*

Colin W. Hiebert[1] · Mulualem T. Kassa[1,2] · Curt A. McCartney[1] · Frank M. You[1] · Matthew N. Rouse[3] · Pierre Fobert[2] · Tom G. Fetch[4]

Received: 22 April 2016 / Accepted: 2 August 2016 / Published online: 9 August 2016
© Her Majesty the Queen in Right of Canada 2016

Abstract
Key Message **Resistance to Ug99 stem rust in Triumph 64 was conferred by *SrTmp* on chromosome arm 6DS and was mapped to the same position as *SrCad* and *Sr42*, however, the three genes show functional differences.**
Abstract Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is an important disease of wheat that can be controlled by effective stem rust resistance (Sr) genes. The emergence of virulent *Pgt* races in Africa, namely Ug99 and its variants, has stimulated the search for new Sr genes and genetic characterization of known sources of resistance. Triumph 64 is a winter wheat cultivar that carries gene *SrTmp*, which confers resistance to Ug99. The goals of this study were to genetically map *SrTmp* and examine its relationship with other Sr genes occupying a similar chromosome location. A doubled haploid (DH) population from the cross LMPG-6S/Triumph 64 was inoculated with Ug99 at the seedling stage. A single gene conditioning resistance to Ug99 segregated in the population. Genetic mapping with SSR markers placed *SrTmp* on chromosome arm 6DS in a region similar to *SrCad* and *Sr42*. SNP markers developed for *SrCad* were used to further map *SrTmp* and were also added to a genetic map of *Sr42* using a DH population (LMPG-6S/Norin 40). Three SNP markers that co-segregated with *SrTmp* also co-segregated with *SrCad* and *Sr42*. The SNP markers showed no difference in the map locations of *SrTmp*, *SrCad*, and *Sr42*. Multi-race testing with DH lines from the Triumph 64 and Norin 40 populations and a recombinant inbred-line population from the cross LMPG-6S/AC Cadillac showed that *SrTmp*, *SrCad*, and *Sr42* confer different spectra of resistance. Markers closely linked to *SrTmp* are suitable for marker-assisted breeding and germplasm development.

Communicated by E. Lagudah.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-016-2765-4) contains supplementary material, which is available to authorized users.

✉ Colin W. Hiebert
colin.hiebert@agr.gc.ca

[1] Agriculture and Agri-Food Canada, Morden Research and Development Centre, 101 Route 100 Morden, Manitoba R6M 1Y5, Canada

[2] National Research Council, 110 Gymnasium Place, Saskatoon, SK S7N 0W9, Canada

[3] United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory, University of Minnesota Department of Plant Pathology, 1551 Lindig Street, St. Paul, MN, USA

[4] Agriculture and Agri-Food Canada, Brandon Research and Development Centre, Brandon MB R7A 5Y3, Canada

Introduction

Stem rust, caused by the fungus *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), is a destructive disease of wheat worldwide. Stem rust can be controlled by deploying wheat cultivars that carry effective stem rust resistance (Sr) genes. For example, the last major wheat stem rust epidemics in North America occurred in the 1950s and destroyed up to 40 % of the spring-sown wheat crop (Peturson 1958); however, resistant cultivars have since controlled stem rust. In 1999, a new *Pgt* race detected in Uganda was virulent to *Sr31* and many other Sr genes (Pretorius et al. 2000). This new race, commonly known as Ug99 and formally designated as TTKSK, continued to evolve and accumulate additional virulence (Jin

et al. 2007, 2008, 2009). The Ug99 race group was reported to be virulent to approximately 90 % of the world's contemporary wheat cultivars (Singh et al. 2008) and most Canadian wheat cultivars (Fetch et al. 2012). The emergence of the Ug99 race group stimulated resurgence in research to improve resistance to stem rust.

Triumph 64 is an American winter wheat cultivar known to carry resistance to *Pgt* (McVey and Hamilton 1985). A resistance gene from Triumph 64, with an informal designation of *SrTmp*, has been included in wheat differential sets for pathotyping races of *Pgt* since the 1970s (Roelfs and McVey 1975, 1979). Early studies used Triumph 64 as a differential line, but subsequently putative single gene lines were used as the differential line (e.g. Jin et al. 2007). *SrTmp* was shown to confer resistance to the Ug99 group of *Pgt* races (Jin et al. 2007, 2008, 2009). Any gene conferring resistance to Ug99 and its variants has attracted the attention of the international wheat genetics, breeding, and pathology communities.

A previous genetic study employed monosomic analysis to identify putative chromosome locations of Sr genes derived from Triumph 64 (Williams et al. 1998). However, in that instance a spring-habit derivative of Triumph 64 was used as the resistant parent. The chromosome locations proposed by Williams et al. (1998) have not been confirmed. In an inheritance study, Knott (2000) suggested that Triumph 64 may carry as many as nine Sr genes. Again, these results have not been confirmed in subsequent studies.

Lopez-Vera et al. (2012) located a gene (*SrNiini*) for resistance to race TTKSK on chromosome arm 6DS. The gene was possibly derived from Triumph 64. The map position was similar to the map position of *SrCad* and *Sr42* (Hiebert et al. 2011; Ghazvini et al. 2012). *SrCad* is the informal designation of a Sr gene that was discovered in several Canadian spring wheat cultivars; it conferred resistance to the Ug99 group of *Pgt* races, and was mapped to chromosome arm 6DS with DNA markers (Hiebert et al. 2011). As *Sr42* was also reported to be carried on chromosome arm 6DS, a population derived from Norin 40, the reference stock of *Sr42*, was used to genetically map *Sr42* with DNA markers (Ghazvini et al. 2012). Both *SrCad* and *Sr42* mapped to a similar location on chromosome arm 6DS and their positions could not be unequivocally differentiated. More recently, single-nucleotide polymorphism (SNP) markers were identified or developed to saturate the genetic map in the *SrCad* region (Gao et al. 2015; Kassa et al. 2016). These new markers provide additional resources for mapping Sr genes that are located near *SrCad*.

Resistance genes from the primary gene pool are the most desirable for breeding purposes, as alien-derived resistance genes are often accompanied by deleterious characteristics (The et al. 1988). The Sr gene(s) in Triumph 64 that confer resistance to race TTKSK are thus of great interest given that few Sr genes from the primary gene pool of wheat confer resistance to Ug99 (Hiebert et al. 2011). The goals of this study were to determine the number of Sr genes conferring resistance to race TTKSK in Triumph 64, map the resistance genes with DNA markers, and test *SrTmp*, *SrCad*, and *Sr42* for functional differences using several different *Pgt* races.

Materials and methods

Plant material

A cross was made between LMPG-6S (Little Club//Prelude*8/Marquis/3/Gabo) and Triumph 64 (Danne Beardless-Blackhull//Kanred-Blackhull/Florence/3/Kanred-Blackhull/Triumph). LMPG-6S is a spring-type wheat line that is susceptible to all *Pgt* races used in this study. The F_1 progeny were used to generate a doubled haploid (DH) population using the maize pollination method to produce haploids followed by colchicine-induced chromosome doubling (Thomas et al. 1997). Only DH plants that showed a spring growth habit ($n = 135$) were retained for phenotyping with *Pgt* and genetic mapping.

A DH population from the cross LMPG-6S/Norin 40 (Ghazvini et al. 2012, 2013) was used to compare *SrTmp* (the Sr gene from Triumph 64 that confers resistance to Ug99) with *Sr42*. The LMPG-6S/Norin 40 DH population segregated for *Sr42* and *Sr54*. Additional genetic mapping was performed on this population to compare the map positions of *Sr42* and *SrTmp*.

An F_7-derived recombinant-inbred line (RIL) population was generated from the cross LMPG-6S/AC Cadillac. This population was previously used to map *SrCad* and was phenotyped for stem rust response using *Pgt* race TTKSK (Ug99) at the seedling stage (Kassa et al. 2016). In this study, a large subset ($n = 106$) of this population with sufficient amounts of seed was used to compare the resistances conferred by *SrCad* to *SrTmp* and *Sr42*.

Seedling stem rust tests for mapping Ug99 resistance

Seedlings of the LMPG-6S/Triumph 64 DH population were inoculated after the first leaf had fully emerged. The population was inoculated with *Pgt* race TTKSK (Ug99; isolate SA31) inside a plant pest level 3 (PPC3) biocontainment laboratory. Inoculations were performed by suspending urediniospores in light mineral oil (Bayol 55, Imperial Oil Canada, Toronto, ON) and spraying the suspension onto the seedlings. Inoculated seedlings were incubated in a dew chamber for 16 h and then slowly dried under light. Plants were transferred to growth chambers and grown at

approximately 20 °C with 16 h of light daily. Two weeks after inoculation seedlings were rated for infection type (IT) following the 0–4 scale described by Stakman et al. (1962). Plants with ITs of 0–2 were classified as resistant, whereas plants with ITs of 3–4 were classified as susceptible.

Linkage mapping *Pgt* resistance with DNA markers

Tissue for DNA extraction was collected from young leaves of DH lines and parents that was lyophilized and macerated. DNA was extracted using a modified ammonium acetate extraction (Chao and Somers http://maswheat.ucdavis.edu/PDF/DNA0003.pdf, accessed July 2009) based on the procedures of Pallotta et al. (2003). PCR amplification and fragment analysis with an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, ON) were performed as described by Somers et al. (2004). Analysis with KASP markers (Supplemental Table S1) was performed following the procedures described by Kassa et al. (2016). The PCR marker *FSD_RSA* (Laroche et al. 2000) was also genotyped following protocols described by Hiebert et al. (2011).

As stated above, a mapping study found that a gene for resistance to race TTKSK and postulated to be derived from Triumph 64 was located on chromosome arm 6DS (Lopez-Vera et al. 2012). SSR markers known to be located on chromosome arm 6DS (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005) were targeted for initial mapping of resistance to race TTKSK using 94 DH lines and parents from the LMPG-6S/Triumph 64 population. After a chromosome location of the Sr gene was confirmed, 135 DH lines were used to construct a linkage map with the SSR markers. After determining the map position of *SrTmp*, SNP markers developed for mapping *SrCad* (Kassa et al. 2016) were tested for polymorphism between LMPG-6S and Triumph 64. Polymorphic SNP markers were tested on the DH population and added to the genetic map of *SrTmp*. These SNP markers were also tested on the parents of the LMPG-6S/Norin 40 DH population and polymorphic SNP markers were added to the genetic map of *Sr42*. Genetic maps were constructed using MapDisto (Lorieux 2012) and genetic distances were calculated using the Kosambi mapping function (Kosambi 1943).

Comparing *SrTmp*, *SrCad*, and *Sr42* with multiple *Pgt* races

SrTmp, *SrCad* and *Sr42* have similar map positions (see Results section) and all confer resistance to *Pgt* race TTKSK. The LMPG-6S/Triumph 64 DH population and subset of 16 DH lines from the LMPG-6S/Norin 40 population (Ghazvini et al. 2012, 2013) were used to assess functional differences between *SrTmp* and *Sr42*. DH lines from the LMPG-6S/Norin 40 population were selected to be either positive for *Sr42* and negative for *Sr54* or negative for *Sr42* and positive for *Sr54* based on phenotypic data and DNA marker haplotypes. The population subsets and the parental lines were tested with *Pgt* races TTKSK (isolate SA31), RKQSC (isolate 1375), QTHJF (isolate 1347), and QCCJB (isolate 1540) at the seedling stage as described above, except that races RKQSC, QTHJF, and QCCJB were not tested inside the PPC-3 facility as they were isolated in North America.

The LMPG-6S/AC Cadillac RIL population and the parents, and the LMPG-6S/Norin 40 population and parents, were grown and maintained in a biocontainment facility at the University of Minnesota in St. Paul, MN as described by Rouse et al. (2014) for testing with *Pgt* race TRTTF (isolate 06YEM34-1) from Africa (Olivera et al. 2012). Seedlings were inoculated after the first leaf had fully emerged following the procedures of Rouse et al. (2011). Fourteen days after inoculation the seedlings were rated for IT as described above. The populations and parents were tested in two replicates and the data were compared to the race TTKSK IT data collected previously (Ghazvini et al. 2012; Kassa et al. 2016).

Results

Seedling stem rust tests and linkage mapping *Pgt* resistance with DNA markers

The LMPG-6S/Triumph 64 DH population was tested with *Pgt* race TTKSK and the resistant DH lines showed ITs 11^{-} to 22^{-}, whereas the susceptible lines showed ITs 33^{+} to 4. Of 135 DH lines tested with TTKSK 65 were resistant and 70 were susceptible, which fitted a single gene ratio ($\chi^2_{1:1} = 0.185$, 1 *d.f.*, $P = 0.667$).

LMPG-6S and Triumph 64 were tested with SSR and SNP markers on chromosome arm 6DS. Three SSR and seven SNP markers that were polymorphic between LMPG-6S and Triumph 64, while the dominant marker FSD_RSA was null in both of the parents. The polymorphic markers on chromosome arm 6DS were tested with the DH population and showed linkage to the Sr gene conferring resistance to TTKSK (*SrTmp*). Four SNP markers co-segregated with *SrTmp* (Fig. 1). Additionally, SSR marker *gpw5182* and SNP markers *kwm864* and *kwm929* were 0.8 cM distal to *SrTmp* and SNP markers *kwm71* and *kwm217* were 2.27 cM proximal to *SrTmp*.

The genetic map of *Sr42* from the LMPG-6S population shared seven markers with the map of *SrTmp* (Fig. 1). The genetic maps showed identical marker orders. SNP marker kwm871 co-segregated with *SrTmp* and *Sr42* in their respective maps. Similarly, markers gpw5182 and kwm864 co-segregated in both maps and are distal to *Sr42*

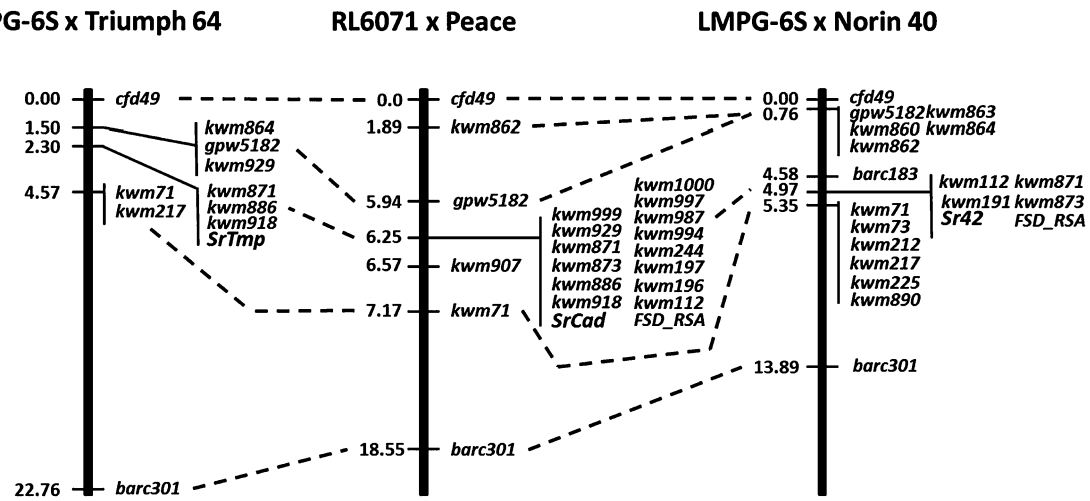

Fig. 1 Genetic maps of *SrTmp*, *SrCad*, and *Sr42* using SNP and SSR markers. Genetic distances are in centi-Morgans (cM)

Table 1 Reactions of *SrTmp*, *Sr42*, and *SrCad* to multiple *Pgt* races

Gene	Material tested	Reaction to *Pgt* races[a]				
		TTKSK	RKQSC	QCCJB	QTHJF	TRTTF
SrTmp	DH lines	R	R	R	R	S[b]
Sr42	DH lines	R	R	S	S	S
SrCad	RIL population	R	R[c]	–	–	R

[a] Reaction "R" = resistant reaction, "S" = susceptible reaction, and "–" = *Pgt* race not tested

[b] This reaction is based on the data of Olivera et al. (2012)

[c] This reaction is based on the data of Hiebert et al. (2011)

and *SrTmp*. The map positions of *SrTmp*, *Sr42*, and *SrCad* could not be differentiated.

Comparing *SrTmp*, *Sr42*, and *SrCad* with multiple *Pgt* races

In the LMPG-6S/Triumph 64 DH population, resistance to *Pgt* races TTKSK, RKQSC, and QCCJB co-segregated and fitted a single gene ratio as above demonstrating that *SrTmp* on chromosome arm 6DS conferred resistance to all three of these races. All of the DH lines that were resistant to races TTKSK, RKQSC, and QCCJB were also resistant to race QTHJF. However, an additional independent Sr gene in Triumph 64 also conferred resistance to race QTHJF, and the population fitted a two-gene ratio ($\chi^2_{3:1} = 0.885$, $p = 0.347$). The DH lines from the LMPG-6S/Norin 40 population showed that *Sr42* conferred resistance to TTKSK and RKQSC as expected, but *Sr42* did not confer resistance to races QCCJB or QTHJF. The DH lines that carried *Sr54* singly were resistant to QCCJB and susceptible to the other three races.

According to Olivera et al. (2012) *Sr42* and *SrTmp* do not confer resistance to *Pgt* race TRTTF. Some preliminary testing of cultivars with and without *SrCad* suggested that *SrCad* may confer resistance to TRTTF (data not shown). This was further explored as race TRTTF could potentially differentiate *SrCad* from *Sr42* and *SrTmp*. The LMPG-6S/AC Cadillac RIL population, that had previously been tested with race TTKSK to genetically map *SrCad* (Kassa et al. 2016), was assayed with race TRTTF. There were 49 RILs with a resistant IT and 57 RILs with a susceptible IT, fitting a 1:1 ratio ($\chi^2_{1:1} = 0.604$, 1 *d.f.*, $P = 0.437$). Resistance to TRTTF perfectly co-segregated with resistance to Ug99, demonstrating that *SrCad* confers resistance to both races. The LMPG-6S/Norin 40 DH population and parents were all susceptible to *Pgt* race TRTTF, confirming that *Sr42* does not confer resistance to TRTTF. Taken together with the above data, *SrTmp*, *SrCad*, and *Sr42* showed unique functional differences (Table 1).

Discussion

While stem rust has historically been an important disease of wheat, resistance has effectively controlled stem rust in North America for several decades. With the evolution of

Pgt races that carry critical virulence combinations, namely race TTKSK and its variants, there have been renewed efforts globally to improve stem rust resistance in new wheat cultivars. This includes discovering new Sr genes, developing genetic tools to aid gene deployment, cloning high-value Sr genes, and characterizing known sources of resistance (e.g. Hiebert et al. 2010; Ghazvini et al. 2012; Saintenac et al. 2013). Resistance derived from Triumph 64, which has carried a long-term informal gene designation of *SrTmp* (Roelfs and McVey 1975), was claimed to confer resistance to race TTKSK (Jin et al. 2007). Triumph 64 has been reported to carry more than one Sr gene (Williams et al. 1998; Knott 2000), therefore, it was unknown as to which gene(s) was responsible for resistance to race TTKSK and its derivatives. None of the genes in Triumph 64 have been accurately genetically mapped and there are no reports of mapping populations where Triumph 64 was used as a parent. Thus, the primary goals of this study were to study the genetics of resistance to race TTKSK found in Triumph 64 and genetically map the resistance with DNA markers. After *SrTmp* was mapped in the population we endeavoured to compare *SrTmp* with *Sr42* and *SrCad* as all three genes mapped to the same region and conferred resistance to Ug99.

The LMPG/Triumph 64 DH population showed that a single gene from Triumph 64 conferred resistance to race TTKSK. The suggestion that resistance in Triumph 64 may be located on chromosome 6D (Lopez-Vera et al. 2012) proved correct. Genetic mapping showed that resistance to TTKSK in Triumph 64 (*SrTmp*) mapped to the short arm of chromosome 6D. The location of *SrTmp* is proximal to SSR marker *gpw5182* and occupies a similar location to *SrCad* and *Sr42* (Hiebert et al. 2011; Ghazvini et al. 2012). The marker *FSD_RSA* is closely linked to *SrCad* and *Sr42* and has to this point only been found to produce a positive allele in carriers of *SrCad* and *Sr42* (i.e. the positive allele of this dominant marker appears to be rare), and suggested that *SrCad* and *Sr42* represented the same gene. Triumph 64 carries a null allele for *FSD_RSA* implying that the Sr gene on 6DS in Triumph 64 might not be the same as *SrCad*/*Sr42*. Furthermore, mapping with SNP markers developed for *SrCad* (Kassa et al. 2016) showed similar positions for *SrCad*, *Sr42*, and *SrTmp* (Fig. 1). In each genetic map there was a linkage bin of SNP markers that co-segregated with stem rust resistance, and SNP markers in these bins were shared across the maps (Fig. 1). Thus, genetic mapping was unable to resolve *SrCad*, *Sr42*, or *SrTmp* as distinct loci.

The breadth of resistance for an Sr gene can be assessed by testing near-isogenic lines with multiple or segregating populations with multiple *Pgt* races. As we possessed both the LMPG-6S/Norin 40 and LMPG-6S/Triumph 64 DH populations, we were able to compare *Sr42* and *SrTmp* using several *Pgt* races. The data revealed that *SrTmp* conferred resistance to races TTKSK, RKQSC, QCCJB, and QTHJF, while *Sr42* only conferred resistance to races TTKSK and RKQSC. Similarly, testing the LMPG-6S/AC Cadillac RIL population with *Pgt* race TRTTF showed that *SrCad* confers resistance to a *Pgt* race that is virulent to *SrTmp* and *Sr42* (Table 1). In addition, two new Ug99 races with virulence to *SrTmp* were recently detected and though these races were virulent to Triumph 64, they were avirulent to the two lines tested with *SrCad*: AC Cadillac and Peace (Newcomb et al. 2016). This demonstrates that even if *SrTmp*, *SrCad*, and *Sr42* occupy the same locus, the three genes are functionally different and thus must either represent different loci, unique alleles of the same locus, or a combination thereof.

While the above functional analysis shows that it is clear that *SrTmp*, *SrCad*, and *Sr42* are not the same, resolving their allelic relationship is not trivial. Testing for allelism by intercrossing lines carrying traits of interest can only give definitive results if recombinant progeny are identified that demonstrate that two genes are not allelic. The lack of recombinant progeny does not prove two genes occupy the same locus. There are practical limits to population sizes, and given the map positions of *Sr42*, *SrCad*, and *SrTmp* an allelism test would require analysis of a very large number of plants and could still lead to an erroneous conclusion. Thus, assigning formal gene names can be problematic. In the case of wheat leaf rust resistance gene *Lr14*, there are three alleles that are named, *Lr14a*, *Lr14b*, and *Lr14ab*. However, the first two "alleles" are distinct, closely linked (0.16 cM) loci (Dyck and Samborski 1970). This highlights potential inaccuracies that can occur when assigning gene names. At this time, no formal designation has been assigned to *SrTmp* or *SrCad*.

Lopez-Vera et al. (2014) suggested that they mapped *SrTmp* in six RIL populations and one $F_{2:3}$ population. However, four of the maps place the Sr gene in a different interval from *Sr42* while two maps place the Sr gene in the same interval as *Sr42*. The authors speculate that this could mean that they mapped two distinct loci, one of which could be allelic to *Sr42*. Given the additional SNP markers used in this study, we showed that *SrCad*, *Sr42*, and *SrTmp* occupy the same map position to the linkage resolution of the populations used in this study. For example, SNP marker *kwm871* co-segregated with *Pgt* resistance in all three populations (Fig. 1). The populations in the study by Lopez-Vera et al. (2014) that showed a different map position for the Sr gene conferring resistance to Ug99 stem rust were all phenotyped in the field, and there was no description of the materials (e.g. generation, single plants or bulks) used for DNA extraction. When genetic maps contain relatively few loci (as in Lopez-Vera et al. 2014), multiple map order solutions are possible that have similar likelihoods.

This could also explain the differences in the linkage maps among the seven populations in Lopez-Vera et al. (2014). Thus, it would be worthwhile to re-phenotype their populations, extract DNA from phenotyped plants, and test the populations with numerous SNP markers used in this study to better establish the relationship between the genes conferring resistance to race TTKSK on chromosome 6DS.

This study unequivocally demonstrates that the resistance to race TTKSK derived from Triumph 64 (*SrTmp*) is located on chromosome arm 6DS. The SNP markers developed for use with the KASP assay may provide breeders with convenient methods for selecting *SrTmp* in breeding populations or for parent development. For programs not equipped to perform this assay, the SSR marker *gpw5182* provides an alternative for marker-assisted selection. In addition to genetic mapping of *SrTmp*, we have confirmed the presence of an additional resistance gene in Triumph 64 beyond *SrTmp*. It would be useful to further characterize this resistance and assess its value for wheat breeding.

Mapping *SrTmp*, *SrCad*, and *Sr42* with a common set of markers demonstrated that all three, while functionally distinct, occupy the same or a similar map location. Further dissection of this chromosome region would be useful to determine if it is possible to recover recombinants between these resistances which may be useful for breeding. While using a common set of markers allowed for accurate comparisons of the map positions, only *SrCad* has markers that are highly predictive of its presence (Kassa et al. 2016). Thus, it would be useful to develop additional markers that are more predictive of *SrTmp* and *Sr42*.

Author contribution statement CWH conceived the study, contributed to genetic mapping, population development, and wrote the initial draft of the manuscript. MTK contributed to DNA marker development, genotyping, and genetic mapping. CAM contributed to DNA marker development and genetic mapping. FMY contributed to DNA marker development. MNR conducted many of the stem rust assessments. PF contributed to experimental design. TGF phenotyped populations for stem rust reactions and contributed to population development. All authors contributed to and approved the final manuscript.

Acknowledgments The authors thank Taye Zegeye, Mira Popovic, Ghassan Mardli, Leslie Bezte, Tobi Malasiuk and Elaine Martineau for excellent technical assistance. We are grateful for the financial support from Agriculture and Agri-Food Canada Growing Forward, AAFC Agriflex project 2521, AAFC A-base project J-000006, the Canadian Wheat Alliance, and as part of CTAG and CTAG^2, Genome Prairie projects funded by Genome Canada, Saskatchewan Ministry of Agriculture, and Western Grain Research Foundation. M. Rouse also acknowledges the support from USDA-ARS appropriated project 5062-21220-021-00, USDA-ARS National Plant Disease Recovery System, and the Durable Rust Resistance in Wheat project. Based upon research contributions from the National Research Council of Canada, this publication was given the Contribution Number NRCC# 56224.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors have adhered to the ethical responsibilities outlined by Theoretical and Applied Genetics.

References

Dyck PL, Samborski DJ (1970) The genetics of two alleles for leaf rust resistance at the *Lr14* locus in wheat. Can J Genet Cytol 12:689–694

Fetch T, Zegeye T, Singh D, Wanyera R, Penner M, Rashid K (2012) Virulence of Ug99 (race TTKSK) and race TRTTF on Canadian wheat cultivars. Can J Plant Sci 92:602

Gao L, Kielsmeier-Cook J, Bajgain P, Zhang X, Chai S, Rouse MN, Anderson JA (2015) Development of genotyping by sequencing (GBS)- and array-derived SNP markers for stem rust resistance gene *Sr42*. Mol Breed 35:207. doi:10.1007/s11032-015-0404-4

Ghazvini H, Hiebert CW, Zegeye T, Liu S, Dilawari M, Tsilo T, Anderson JA, Rouse MN, Jin Y, Fetch T (2012) Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42*. Theor Appl Genet 125:817–824

Ghazvini H, Hiebert CW, Thomas JB, Fetch T (2013) Development of a multiple bulked segregant analysis (MBSA) method used to locate a new stem rust resistance gene (*Sr54*) in the winter wheat cultivar Norin 40. Theor Appl Genet 126:443–449

Hiebert CW, Fetch TG, Zegeye T (2010) Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theor Appl Genet 121:65–69

Hiebert CW, Fetch TG, Zegeye T, Thomas JB, Somers DJ, Humphreys DG, McCallum BD, Cloutier S, Singh D, Knott DR (2011) Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'. Theor Appl Genet 122:143–149

Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Fetch T, Pretorius ZA, Yahyaoui A (2007) Characterization of seedling infection types and adult plant infection responses of monogenic Sr gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096–1099

Jin Y, Szabo L, Pretorius Z, Singh R, Ward R, Fetch T (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926

Jin Y, Szabo L, Rouse M, Fetch T Jr, Pretorius ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene *Sr36* within race TTKS lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis 93:367–370

Kassa MT, You FM, Fetch TG, Fobert P, Sharpe A, Pozniak CJ, Menzies JG, Jordan MC, Humphreys G, Zhu T, Luo MC, McCartney CA, Hiebert CW (2016) Genetic mapping of *SrCad* and SNP marker development for marker-assisted selection of Ug99 stem rust resistance in wheat. Theor Appl Genet 129:1373–1382

Knott DR (2000) Inheritance of resistance to stem rust in 'Triumph 64′ winter wheat. Crop Sci 40:1237–1241

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, McKenzie R (2000) Development of a PCR marker for rapid identification

of the *Bt-10* gene for common bunt resistance in wheat. Genome 43:217–223

Lopez-Vera EE, Singh S, Singh RP, Bhavani S, Huerta-Espino J, Xoconostle-Cazares B, Ruiz-Medrano R (2012) Mapping gene SrNiini effective against the Pgt race Ug99 group. In: Borlaug Global Rust Initiative 2012 Technical Workshop, September 1–4, 2012, Beijing

Lopez-Vera EE, Nelson S, Singh RP, Basnet BR, Haley SD, Bhavani S, Huerta-Espino J, Xoconostle-Cazares G, Ruiz-Medrano R, Rouse MN, Singh S (2014) Resistance to stem rust Ug99 in six bread wheat cultivars maps to chromosome 6DS. Theor Appl Genet 127:231–239

Lorieux M (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breed 30:1231–1235

McVey DM, Hamilton K (1985) Stem rust resistance gene from Triumph 64 identified in four other winter wheats. Plant Dis 69:217–218

Newcomb M, Olivera PD, Rouse MN, Szabo LJ, Johnson J, Gale S, Luster DG, Wanyera R, Macharia G, Bhavani S, Hodson D, Patpour M, Hovmoller MS, Fetch TG, Jin Y (2016) Kenyan isolates of *Puccinia graminis* f. sp. *tritici* from 2008 to 2014: virulence to *SrTmp* in the Ug99 race group and implications for breeding programs. Phytopathology. doi:10.1094/PHYTO-12-15-0337-R

Olivera PD, Jin Y, Rouse M, Badebo A, Fetch T, Singh RP, Yahyaoui A (2012) Races of *Pucinnia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Dis 96:623–628

Pallotta MA, Warner P, Fox RL, Kuchel H, Jefferies SJ, Langridge P (2003) Marker assisted wheat breeding in the southern region of Australia. In: Pogna NE, Romano M, Pogna EA, Galerio G (eds) Proc 10th Int Wheat Genet Symp. Instituto Sperimentale per la Cerealcoltura, Rome, pp 789–791

Peturson B (1958) Wheat rust epidemics in western Canada in 1953, 1954, and 1955. Can J Plant Sci 38:16–28

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis 84:203

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roelfs AP, McVey DV (1975) Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1974. Plant Dis Report 59:681–685

Roelfs AP, McVey DV (1979) Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat line with designated genes for resistance. Phytopathology 69:722–730

Rouse MN, Wanyera R, Njau P, Jin Y (2011) Sources of resistance to stem rust race Ug99 in spring wheat germplasm. Plant Dis 95:762–766

Rouse MN, Nirmala J, Jin Y, Chao S, Fetch TG, Pretorius ZA, Hiebert CW (2014) Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99. Theor Appl Genet 127:1681–1688

Saintenac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E, Dubcovsky J (2013) Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341:783–786

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward WR (2008) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. USDA Agricultural Research Service E617, Washington DC

The TT, Latter BDH, McIntosh RA, Ellison FW, Brennan PS, Fisher J, Hollamby GJ, Rathjen AJ, Wilson RE (1988) Grain yields of near isogenic lines with added genes for stem rust resistance. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge, pp 901–906

Thomas J, Chen Q, Howes N (1997) Chromosome doubling of haploids of common wheat with caffeine. Genome 40:552–558

Williams ND, Miller JD, Klindworth DL, Joppa LR (1998) Genes for wheat stem rust resistance from Triumph 64. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp. University of Saskatchewan Extension Press, Saskatoon, pp 342–344