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ORIGINAL PAPER

Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42*

Habibollah Ghazvini · Colin W. Hiebert · Taye Zegeye · Sixin Liu · Mridull Dilawari · Toi Tsilo · James A. Anderson · Matthew N. Rouse · Yue Jin · Tom Fetch

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Abstract Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a devastating disease of wheat. The emergence of race TTKSK (Ug99) and new variants in Africa threatens wheat production worldwide. The best method of controlling stem rust is to deploy effective resistance genes in wheat cultivars. Few stem rust resistance (Sr) genes derived from the primary gene pool of wheat confer resistance to TTKSK. Norin 40, which carries *Sr42*, is resistant to TTKSK and variants TTKST and TTTSK. The goal of this study was to elucidate the inheritance of resistance to Ug99 in Norin 40 and map the Sr gene(s). A doubled haploid (DH) population of LMPG-6/Norin 40 was evaluated for resistance to the race TTKST. Segregation of 248 DH lines fitted a 1:1 ratio (χ^2 1:1= 0.58, $p = 0.45$), indicating a single gene in Norin 40 conditioned resistance to Ug99. This was confirmed by an independent $F_{2:3}$ population also derived from the cross LMPG-6/Norin 40 where a 1:2:1 ratio ($\chi^2$1:2:1 = 0.69, $p = 0.71$) was observed following the inoculation with race TTKSK. Mapping with DNA markers located this gene to chromosome 6DS, the known location of *Sr42*. PCR marker FSD_RSA co-segregated with *Sr42*, and simple sequence repeat (SSR) marker BARC183 was closely linked (0.5 cM) to *Sr42*. A previous study found close linkage between FSD_RSA and *SrCad*, a temporarily designated gene that also confers resistance to Ug99, thus *Sr42* may be the same gene or allelic. Marker FSD_RSA is suitable for marker-assisted selection (MAS) in wheat breeding programs to improve stem rust resistance, including Ug99.

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H. Ghazvini · C. W. Hiebert (✉) · T. Zegeye · T. Fetch
Cereal Research Centre, Agriculture & Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada
e-mail: colin.hiebert@agr.gc.ca

Present Address:
S. Liu
USDA-ARS, National Center for Cool and Cold Water Aquaculture, 11861 Leetown Rd, Kearneysville, WV 25430, USA

S. Liu · M. Dilawari · J. A. Anderson
Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

T. Tsilo
Agricultural Research Council, Small Grain Institute, Private Bag X29, Bethlehem, South Africa

M. N. Rouse · Y. Jin
United States Department of Agriculture–Agricultural Research Service (USDA-ARS), Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA

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. The last devastating epidemic in North America occurred in the early 1950s, when stem rust race 15B became prevalent. Race 15B destroyed up to 40 % of the continent's spring wheat (Peturson 1958) and 80 % of durum wheat crops (Stakman and Harrar 1957). Soon thereafter, deployment of effective stem rust resistance (Sr) genes in wheat breeding programs controlled the damage caused by *Pgt* in North America as well as other parts of the world. Utilization of alien resistance genes such as *Sr24*, *Sr26*, *Sr31*, *Sr36*, or *Sr38* from wild relatives

of wheat or rye cultivars, along with *Sr2* (from *Triticum turgidum*), has had a significant impact on reducing incidence of stem rust in various countries for over three decades (Singh et al. 2008). However, in Canada no alien Sr genes have been deployed in spring wheat cultivars, thus Sr genes from the primary gene pool are a critical resource.

In 1999, virulence to *Sr31* was detected in nurseries in Uganda (Pretorius et al. 2000) and the isolate was designated as *Pgt*-Ug99. Wanyera et al. (2006) designated this strain as race TTKS using the letter code stem rust nomenclature system (Roelfs and Martens 1988). New variants of Ug99 were found in Kenya in 2006 and 2007. A fifth set of differential lines was added, thus *Pgt*-Ug99 is race TTKSK and variants with added virulence to *Sr24* and *Sr36* are TTKST and TTTSK, respectively (Jin et al. 2008, 2009). The pathogen has continued to evolve in Africa. Four other variants of the Ug99 race lineage (TTKSF, TTKSP, PTKSK, and PTKST) are present in different parts of Africa (Park et al. 2011).

Race TTKSK and its variants are virulent to about 90 % of the world's wheat cultivars (Singh et al. 2008) and to most Canadian wheat cultivars (Fetch 2007). A number of stem rust resistance genes (*Sr6, 7a, 7b, 9a, 9b, 10, 11, 12, 16, 17,* and *Wld-1*) that are present in wheat cultivars in North America, often in combinations, have been shown to be ineffective against race TTKS (Jin and Singh 2006). Screening of North American wheat cultivars indicated that only 16 % of hard red spring wheat, 48 % of hard red winter wheat, and 28 % of soft winter wheat had resistance to race TTKS (Jin and Singh 2006). In Canada, the most widely grown Canadian western spring wheat (CWRS) cultivar (Lillian) is susceptible to Ug99 (DePauw et al. 2009), and all of the popular spring wheat varieties are susceptible (T. Fetch, unpublished data). Thus, Ug99 and its derivatives pose a serious threat to wheat production in North America.

Host resistance is the best control method for stem rust and has been used worldwide for over 50 years, but TTKSK is virulent on most Sr genes (Jin et al. 2007). Among 56 designated and a few undesignated stem rust resistance genes in wheat, only eight designated genes in the primary gene pool (*Sr13*, *Sr14*, *Sr22*, *Sr28*, *Sr33*, *Sr35*, *Sr42*, and *Sr45*) confer resistance to TTKSK (Pretorius et al. 2000; Jin et al. 2007; Hiebert et al. 2011). It should be noted that Norin 40 was used as the tester line for *Sr42* and it was assumed that the resistance of Norin 40 to TTKSK was due to *Sr42*. *Sr42*, initially designated as *SrNorin 40*, is derived from winter wheat cultivar Norin 40 and is on the short arm of chromosome 6D (McIntosh et al. 1995). The gene *SrCad* (temporary designation of a Sr gene in the Canadian cultivar AC Cadillac), which confers resistance to Ug99, was recently mapped on the short arm of chromosome 6D (Hiebert et al. 2011). The relationship between *SrCad* and *Sr42* is unknown. Until the present study, neither the genetics nor exact chromosome location of *Sr42* in Norin 40 has been published. The objectives of this study were to determine the inheritance of seedling resistance to Ug99-type *Pgt* in Norin 40, map the gene(s) using molecular markers, and compare the location of *Sr42* to *SrCad* on chromosome 6DS. Populations derived from Norin 40 were independently produced and analyzed by research groups at the AAFC Cereal Research Centre (CRC), Winnipeg, Canada, and the University of Minnesota (U of M) and the USDA Cereal Disease Lab (CDL), St. Paul, USA. The results from both of these studies are presented here.

Materials and methods

Experiments conducted at the CRC, Winnipeg, Canada

Evaluation of stem rust resistance in Norin 40

Norin 40 (accession CN30674 obtained from the Germplasm Resource Centre, Saskatoon, Canada) was evaluated for stem rust reaction to 12 domestic *Pgt* races and race TTKST (isolate 09-Ken-28) to assess its resistance against North American isolates as well as Ug99. The domestic races used were previously stored in the stem rust collection of the Cereal Research Centre, Winnipeg, MB. Seedlings were inoculated at the first-leaf stage. Inoculations were performed by suspending urediospores in light mineral oil (Bayol 55, Imperial Oil, Toronto, ON, Canada) and spraying the inoculum onto the seedlings using methods described by Fetch (2009). Inoculated plants were incubated in a dew chamber in the dark for 16 h and then were kept under 2 h of light while plants slowly dried off. Plants were grown at approximately 20 °C with 16 h of light daily. 14 days after inoculation, seedlings were rated for their infection type (IT) using a 0–4 scale (Stakman et al. 1962).

Inheritance of resistance in Norin 40 to *Pgt* race TTKST

A population of 248 doubled haploid (DH) lines with a spring growth habit was generated from the cross between line LMPG-6 (Little Club//Prelude*8/Marquis/3/Gabo, susceptible parent; obtained from the late Dr. D. Knott) and Norin 40. To develop DH lines, the maize pollination method was used to generate haploids, which were then treated with colchicine to induce chromosome doubling (Thomas et al. 1997). For each DH line, three seeds were sown into a cone-tainer (Stuewe and Sons., Tangent, OR) filled with Sunshine Mix #5 (Sun Gro Horticulture, Inc., Bellevue, WA). DH lines were inoculated with race TTKST (isolate 09-Ken-28) and stem rust rating was carried out as

described above. Infection types 0, 1, 2, or combinations thereof were considered as a resistant response, whereas ITs 3–4 were considered a susceptible response. Observed segregation ratios (resistant vs. susceptible) were evaluated using Chi-square analysis to test the probability of their goodness of fit to various theoretical ratios. Plants were grown to the adult plant stage for increasing the seeds and also to collect fresh leaves for DNA extraction.

Genetic mapping of resistance to *Pgt* race TTKST

Genomic DNA of the parents and each DH line was extracted from fresh leaves of healthy plants with no disease symptoms. Detached leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C before lyophilization. DNA was extracted using an ammonium acetate method (Chao and Somers, http://maswheat.ucdavis.edu/PDF/DNA0003.pdf, accessed Dec. 2010) based on the procedures of Pallotta et al. (2003). Concentrations of extracted DNAs were determined by fluorimetry using Hoechst 33258 stain. Simple sequence repeats (SSRs, also known as microsatellite markers) previously mapped on chromosome 6D (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004) were initially used since *Sr42* was previously described to be in Norin 40 and on 6D. Polymerase chain reaction (PCR), thermal cycling, and fragment analysis of PCR products with an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, ON, Canada) were performed as described by Somers et al. (2004) and Sourdille et al. (2004). Norin 40, LMPG, and the DH population were also screened with the PCR marker FSD_RSA (Laroche et al. 2000), which is the closest marker to *SrCad* (Hiebert et al. 2011). The PCR conditions used for FSD_RSA were 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 12 pmol FSD forward primer, 3.5 pmol RSA reverse primer, 2U *Taq* DNA polymerase, and 120 ng of genomic DNA. The PCR program was conducted for 3 min (94 °C), then 35 cycles of 30 s (94 °C), 1 min 45 s (44 °C) and 2 min (72 °C), followed by 10 min (72 °C). PCR products were separated on 1.5 % agarose gels that were run at 80 V for 1.5 h. Genetic maps were constructed using JOINMAP version 3.0 software package (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl). Map distances in centiMorgan (cM) were calculated using the Kosambi mapping function (Kosambi 1944).

Survey of SSR alleles for markers linked to *Sr42*

One hundred and eighty-seven Canadian varieties, breeding lines, and exotic wheat germplasm with diverse genetic backgrounds were surveyed for the presence of two tightly linked SSR markers to *Sr42*. Marker screening was performed as described above. Allele sizes were determined by processing the output data from the ABI genetic analyzer using Genographer (available at http://hordeum.oscs.montana.edu/genographer). The FSD_RSA marker was also surveyed with the same set of germplams.

Experiments conducted at the U of M and USDA-CDL, St. Paul, USA

Evaluation of stem rust resistance in Norin 40 to African *Pgt* isolates.

Norin 40 was inoculated with nine African *Pgt* isolates to further test the breadth of resistance. This included two isolates of TTKSK, two isolates of TTKST, two isolates of TTTSK, one isolate of TTKSF, and two isolates of TRTTF. Inoculations and rating were performed as described above.

Inheritance of resistance in Norin 40 to *Pgt* race TTKSK

Evaluation of seedling infection types (ITs) of an $F_{2:3}$ population derived from Norin 40 and LMPG-6 was performed at the USDA-ARS Cereal Disease Laboratory as described above. From a total of 190 $F_{2:3}$ families, 20–24 seedlings from each family were grown in 2.5 inch square plastic pots filled with vermiculite (Sun Gro Horticulture, Bellevue, WA). The ITs of the parents and families were scored using race TTKSK, isolate 04KEN156 (Jin et al. 2007), at 14 days after inoculation. Families with at least 15 resistant plants and zero susceptible plants were classified as resistant. Families with at least 15 susceptible plants and zero resistant plants were classified as susceptible. Families with both resistant and susceptible plants were classified as segregating. The inheritance of resistance was tested for goodness to fit against various theoretical segregation ratios using Chi-square analyses.

Genetic mapping of resistance to *Pgt* race TTKSK

DNA was extracted from at least 15 bulked $F_{2:3}$ seedlings for each family using the protocol described by Riede and Anderson (1996) with modifications described by Liu et al. (2006). Resistance to TTKSK was mapped using SSR markers. PCR and polyacrylamide gel electrophoresis were performed as described by Tsilo et al. (2007). A linkage map was constructed using MapMaker version 3.0 (Lander et al. 1987) and the Kosambi mapping function was applied (Kosambi 1944).

Results

Evaluation of stem rust resistance in Norin 40

Norin 40 exhibited differential infection responses at the seedling stage when inoculated with 22 *Pgt* isolates

Table 1 Infection types of cultivar Norin 40 to Canadian domestic *Puccinia graminis* f. sp. *tritici* races and TTKSK (Ug99) related races, and race TRTTF

Puccinia graminis f.sp. *tritici* races[a]	Laboratory[b]	Isolate	IT[c]
MCCF	Winnipeg	1541	33^+
RHTS	Winnipeg	1345	3
RKQS	Winnipeg	1312	33^+
TPMK	Winnipeg	1373	33^+
QTHJ	Winnipeg	1347	3^+
TMRT	Winnipeg	1311	33^+
RTHJ	Winnipeg	1561	33^+
TTTT	Winnipeg	1567	33^+
QFCS	Winnipeg	1418	33^+
LBBB	Winnipeg	360	1^-1
LCBB	Winnipeg	1386	;1
RTQS	Winnipeg	1375	;1
TTKST	Winnipeg	09-Ken-28	;1
TTKSK	CDL	04KEN156/04	2^-
TTKSK	CDL	09KEN09-2	2^-
TTKST	CDL	06KEN19-v3	2^-
TTKST	CDL	09KEN08-2	2^-
TTTSK	CDL	07KEN24-4	2^-
TTTSK	CDL	09TAN08-19	2^-
TTKSF	CDL	09ZIM01-2	2^-
TRTTF	CDL	06YEM34-1	4
TRTTF	CDL	09ETH06-1	4

[a] Race nomenclature as described by Roelfs and Martens (1988) and Jin et al. (2008)

[b] Seedling testing was done independently at Winnipeg and CDL

[c] Infection type (IT) was based on the scale described by Stakman et al. (1962), with IT 3 or higher considered susceptible

representing 17 races (Table 1). Norin 40 was susceptible (IT = 3 or higher) to nine North American races and TRTTF. Norin 40 was resistant (IT = ; to 2^-) to North American races LBBB, LCBB, and RTQS, and race TTKSK and its variants. Races LBBB, LCBB, and RTQS are not currently prevalent in North America (Fetch 2009).

Inheritance of resistance to *Pgt* races TTKST and TTKSK

Infection responses of 248 DH lines inoculated with *Pgt* race TTKST were distinct and could be clearly discriminated into resistant and susceptible phenotypes. All resistant lines exhibited IT = ; to 1, which corresponded to Norin 40. With the exception of a few lines with ITs from 3^- to 3, the majority (92 %) of susceptible lines exhibited ITs 33^+ to 4; these infection types corresponded to those observed in the LMPG-6 parent. The DH population segregated for a single gene with 118 resistant and 130 susceptible lines (χ^2 1 : 1 = 0.45, $p = 0.45$).

At the CDL, Norin 40 displayed IT 2^- to TTKSK whereas LMPG-6 displayed IT 3^+. $F_{2:3}$ plants displayed low (2^- or 2) and high (3 or 3^+) ITs. There were 43 resistant, 100 segregating, and 47 susceptible families, which also fitted the expected ratio for a single gene (χ^2 1 : 2 : 1 = 0.69, $p = 0.71$).

Mapping resistance to *Pgt* race TTKST and TTKSK

Norin 40 and LMPG-6 were screened with 66 SSR markers that were previously mapped on chromosome 6D of wheat. Forty markers were polymorphic, and 12 that provided well-spaced coverage of chromosome 6D were used to map the resistance gene in Norin 40. Initial linkage analysis of 96 DH lines showed that the gene mapped to the short arm of chromosome 6D, close to 3 terminal SSR markers (BARC183, GPW5182, and CFD49) on the genetic map of 6DS (data not shown). Thus, the gene conferring resistance to TTKSK in Norin 40 was presumed to be *Sr42* and this chromosome region was targeted for additional mapping. To improve resolution of the linkage map in the targeted region, all 248 DH lines were tested with BARC183, GPW5182, and CFD49 and five proximal SSR markers on the short arm of chromosome 6D. The presence (275 bp) or absence of PCR marker FSD_RSA was also scored on the DH population. The FSD_RSA marker co-segregated with *Sr42*, and BARC183 was tightly linked (0.5 cM distal) to *Sr42* (Figs. 1a, 2). The next closest markers were GPW5182 (1.8 cM distal; Figs. 1a, 2) and BARC301 (11.8 cM proximal). No other SSR markers between BARC301 and *Sr42* were informative in this population.

The difference in allele sizes between Norin 40 and LMPG-6 was small for some of the above SSR markers (Fig. 2). These markers were difficult to score reliably in the $F_{2:3}$ population and were omitted. Thus, fewer markers were mapped in the *Sr42* region. Nevertheless, *Sr42* mapped to the same region in the $F_{2:3}$ population compared to the DH population (Fig. 1b). The closest marker to *Sr42* was CFD49 with a genetic distance of 5.9 cM, which is similar to that observed in the DH population.

Survey of SSR alleles for markers linked to *Sr42*

Allele sizes (excluding the 19 bp for the M13 priming site) of SSR markers BARC183 and GPW5182 for Norin 40 were 169 bp and 161 bp, respectively (Fig. 2). One hundred and eighty-seven Canadian wheat varieties, breeding lines, and exotic wheat accessions were tested with these two markers to have an estimate of frequency of these alleles within Canadian wheat germplasm as well as in

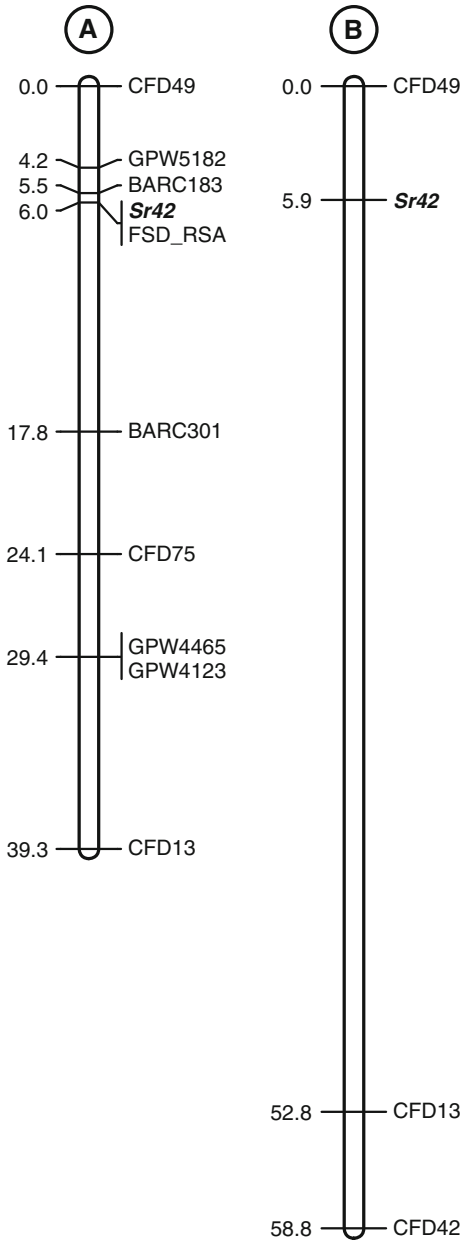

Fig. 1 **a** Genetic linkage map showing the position of *Sr42* on chromosome 6DS constructed using the LMPG/Norin 40 DH population. **b** Genetic linkage map showing the position of *Sr42* on chromosome 6DS constructed using the LMPG/Norin 40 $F_{2:3}$ population. All map positions are shown are in cM

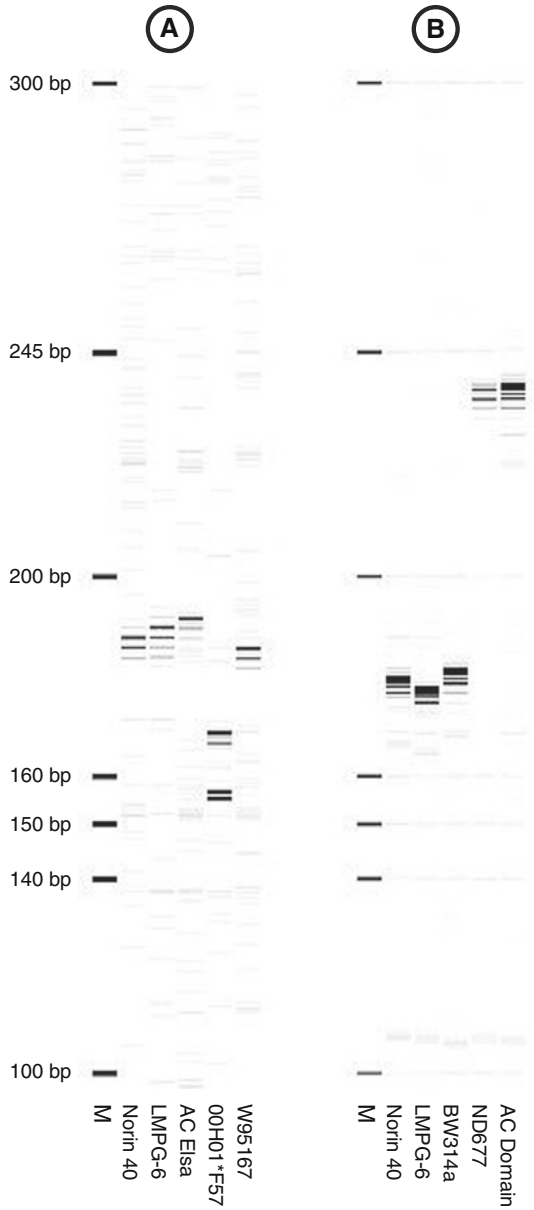

Fig. 2 Representative genotypes for the five different alleles found for SSR markers **a** BARC183 and **b** GPW5182. The parents of the both populations, Norin 40 and LMPG-6, are included. Both SSR markers were closely linked to *Sr42*. The molecular weight marker is labeled as *M*

exotic accessions. BARC183 amplified a 169-bp size allele in 86 accessions, while GPW5182 amplified a 161-bp allele in 88 accessions (Table S1). Four lines produced two alleles (161 + 220 bp or 161 + 221 bp) when tested with GPW5182, indicating heterozygosity for that marker. Simultaneous presence of both 169-bp (BARC183) and 161-bp (GPW5182) alleles was found in 66 accessions. However, this was not indicative of the presence of *Sr42* since only four of these lines were resistant to TTKS (Fetch, unpublished data).

The PCR marker FSD_RSA is highly cross-applicable for marker-assisted selection. FSD_RSA was developed as a marker for the common bunt resistance gene *Bt10* which is closely linked to *SrCad* (Hiebert et al. 2011). The only genotypes that were positive for FSD_RSA were cultivars or breeding lines known to carry *Bt10*. Of those that were tested with TTKSK, all showed seedling resistance (Table S1).

Discussion

Stem rust resistance in Norin 40 was first detected in 1980 and later (1983) located on the short arm of chromosome 6D (McIntosh et al. 1995). The gene in Norin 40 on chromosome 6DS was subsequently given the gene designation *Sr42* in 1995 (http://wheat.pw.usda.gov/ggpages/awn/41/awn41f4.html#f29, accessed July 2011). Although *Sr42* was effective against most Australasian isolates of *P. graminis* f. sp. *tritici* (McIntosh et al. 1995), it is not effective against the majority of North American *Pgt* races (Table 1). The lack of resistance of TTKSK and related races in the North American spring wheat germplasm suggests that *Sr42* is not present or common in the spring wheat cultivars in the United States or Canada. With the advent of Ug99 and its variants, there has been a renewed interest in breeding for stem rust resistance and using different undeployed sources of resistance, including *Sr42*.

Our research shows that resistance to Ug99 in Norin 40 was conditioned by a gene (*Sr42*) on chromosome 6DS and was based on the two independent mapping populations from two research groups. Previous reports of the effectiveness of *Sr42* to Ug99 (Pretorius et al. 2000; Hiebert et al. 2011) were based on the assumption that the low reaction of Norin 40 was caused by *Sr42*. Such conclusions may be erroneous in the event that Norin 40 carries additional Sr genes. For example, Webster is the reference line for *Sr30*, but one accession of Webster was recently shown to carry an additional gene (*SrWeb*; Hiebert et al. 2010). In that instance it was the unknown gene (*SrWeb*) that conferred resistance to Ug99, not *Sr30*. Norin 40 also carries a second Sr gene (Ghazvini et al. unpublished data). This second gene does not condition the resistance to *Pgt* race TTKST or TTKSK (data not shown).

Previously, Hiebert et al. (2011) found highly effective resistance in the Canadian cultivars AC Cadillac (DePauw et al. 1998) and Peace (BW90*3/BW533//BW90′S′/Katepwa) conditioned by a combination of *Lr34* and a major gene temporarily designated as *SrCad*. In order to get a formal gene designation, the chromosome location and relationship to previously designated genes must be determined. *SrCad* was mapped to chromosome 6DS (Hiebert et al. 2011). Three other genes (*Sr5*, *Sr29*, and *Sr42*) are on 6D, but *Sr29* is on 6DL (Dyck and Kerber 1977). Neither *Sr5* nor *Sr42* had been previously mapped to a specific chromosome locus. However, *Sr5* is susceptible to TTKSK while *Sr42* and *SrCad* are resistant and have similar low ITs to TTKSK. In this study, *Sr42* co-segregated with a marker (FSD_RSA) that mapped 1.5 cM from *SrCad* (Hiebert et al. 2011). The similarity of infection type, marker order, and genetic distance between SSRs linked to resistance in the LMPG/Norin 40 and RL6071/Peace populations suggest that *Sr42* and *SrCad* may represent the same allele or different alleles of the same locus. A population is currently being developed to test allelism between *Sr42* and *SrCad*. Marker FSD_RSA is also closely linked to *Bt10* (Menzies et al. 2006), which confers resistance to common bunt, and appears to specifically mark genotypes carrying *Bt10*. *SrCad* and *Bt10* are linked in coupling. The similarities between the chromosome regions carrying *SrCad* and *Sr42*, including the presence of FSD_RSA, suggest that Norin 40 may carry bunt resistance. The reaction of Norin 40 to common bunt is currently unknown.

SSR markers BARC183 and GPW5182, which were closely linked to *Sr42,* may be useful for marker-assisted selection (MAS) depending on parental genotypes (Fig. 2). The results of this study showed that there is enough variation in allele sizes among Canadian and exotic wheat germplasm for both markers to facilitate MAS of *Sr42*. However, our data demonstrate that the presence of these marker alleles in an individual is not diagnostic for the presence of *Sr42*. For example, two widely grown CWRS cultivars (Lillian and AC Barrie) carry the allele sizes found in Norin 40 for both BARC183 and GPW5182, but were susceptible and moderately susceptible, respectively, to Ug99 when tested in Kenya (DePauw et al. 2009). While FSD_RSA is a dominant marker, it appears to be highly cross-applicable (Hiebert et al. 2011; Table S1) and is tightly linked to *Sr42*. The above markers will enable breeders to develop Ug99-resistant wheat varieties more efficiently.

Host resistance has been used for over 50 years to control stem rust. A stack of effective genes needs to be incorporated into modern wheat cultivars to produce long-term resistance to exotic races such as TTKSK and its variants. Although the exact number and type of genes that will produce durable resistance is debatable, a combination of major and minor resistance genes is likely to provide resistance that would be difficult for pathogen to overcome. Fetch (2007) found resistance to Ug99 in cultivars AC Cadillac and Peace that has been highly effective across several years of field trials in Kenya. This resistance has been attributed to a combination of *Lr34* and *SrCad* (Hiebert et al. 2011). While *Sr42* is ineffective against most North American races that were tested (Table 1), this "weak" gene may confer high levels of resistance in the field when in combination with other genes like *Lr34*. The gene *SrWeb*, which was recently found to be effective against Ug99 (Hiebert et al. 2010), is also a "weak" gene that is not effective against races common in North America. Although *Sr42* and *SrWeb* may be of little value against stem rust in North America, they have value when combined with other genes for use in resistance gene stacks. While the full composition of stem rust genes in AC Cadillac is yet to be determined, the effective combination

of *Sr42* and *Lr34* is a starting point to which other genes such as *Sr2* or *SrWeb* could be added. This will only be reliably achieved through use of molecular markers to predict the presence of desired genes, as rust races do not exist for testing of these gene stacks. Thus, flanking markers that are highly predictive of the presence of specific genes are desirable.

This study found that the resistance to Ug99 in Norin 40 was conditioned by a single gene, *Sr42*, located on chromosome 6DS. *Sr42* co-segregated with marker FSD_RSA, which previously has been linked closely to *Bt10* (Menzies et al. 2006) and gene *SrCad* Hiebert et al. (2011). Although it was hoped that *SrCad* was a newly described gene with resistance to Ug99, these results indicate that it is likely *Sr42* or an allele. Allelism test are in progress to ascertain the relationship between *Sr42* and *SrCad*. Marker FSD_RSA will be useful for combining *Sr42* with other Sr genes to breed wheat that is resistant to stem rust, including Ug99.

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