Theor Appl Genet (2014) 127:231–239
DOI 10.1007/s00122-013-2212-8

ORIGINAL PAPER

Resistance to stem rust Ug99 in six bread wheat cultivars maps to chromosome 6DS

Eric E. Lopez-Vera · Sarah Nelson · Ravi P. Singh · Bhoja R. Basnet · Scott D. Haley · Sridhar Bhavani · Julio Huerta-Espino · Beatriz G. Xoconostle-Cazares · Roberto Ruiz-Medrano · Matthew N. Rouse · Sukhwinder Singh

Received: 25 June 2013 / Accepted: 4 October 2013 / Published online: 13 October 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Key message **Identified SSR markers (*Xcfd49* and *Xbarc183*) linked with stem rust resistance for efficient use in marker-assisted selection and stacking of resistance genes in wheat breeding programs.**

Abstract More than 80 % of the worldwide wheat (*Triticum aestivum* L.) area is currently sown with varieties susceptible to the Ug99 race group of stem rust fungus. However, wheat lines Niini, Tinkio, Coni, Pfunye, Blouk, and Ripper have demonstrated Ug99 resistance at the seedling and adult plant stages. We mapped stem rust resistance in populations derived from crosses of a susceptible parent with each of the resistant lines. The segregation of resistance in each population indicated the presence of a single gene. The resistance gene in Niini mapped to short arm of chromosome 6D and was flanked by SSR markers *Xcfd49* at distances of 3.9 cM proximal and *Xbarc183* 8.4 cM distal, respectively. The chromosome location of this resistance was validated in three other populations: PBW343/Coni, PBW343/Tinkio, and Cacuke/Pfunye. Resistance initially postulated to be conferred by the *SrTmp* gene in Blouk and Ripper was also linked to *Xcfd49* and *Xbarc183* on 6DS, but it was mapped proximal to *Xbarc183* at a similar position to previously mapped genes *Sr42* and *SrCad.* Based on the variation in diagnostic marker alleles, it is possible that Niini and Pfunye may carry different resistance genes/alleles. Further studies are needed to determine the allelic relationships between various genes located on chromosome arm 6DS. Our results provide valuable molecular marker and genetic information for developing Ug99 resistant wheat varieties in diverse germplasm and using these markers to tag the resistance genes in wheat breeding.

Communicated by B. Keller.

E. E. Lopez-Vera · R. P. Singh · B. R. Basnet · S. Singh (✉)
International Maize and Wheat Improvement Center, PO Box 6-641, 06600 Mexico, D.F., Mexico
e-mail: suk.singh@cgiar.org

E. E. Lopez-Vera · B. G. Xoconostle-Cazares · R. Ruiz-Medrano
Department of Biotechnology, CINVESTAV-IPN, Col. San Pedro Zacatenco, 07360 Mexico D.F., Mexico

S. Nelson
Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

S. D. Haley
Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, USA

S. Bhavani
International Maize and Wheat Improvement Center, Nairobi, Kenya

J. Huerta-Espino
INIFAP CEVAMEX, Chapingo, 56230 Edo. De Mexico, Mexico

M. N. Rouse
USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108, USA

Introduction

Stem rust (SR), also known as black rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is an important disease of wheat. During the twentieth century, severe yield losses due to SR epidemics were reported in Europe, Asia, Australia, and the USA (Nagarajan and Joshi 1975; Roelfs 1978; Leonard and Szabo 2005). Stem rust was controlled successfully by growing resistant semi-dwarf spring wheat varieties during and after the Green Revolution (Singh et al.

2008). The slow rusting, adult plant resistance (APR) gene *Sr2* and few major genes were transferred to hexaploid wheat Hope and H44-24 from tetraploid emmer wheat Yaroslav (McFadden 1930). This likely conferred durable resistance to SR (McIntosh 1988; Rajaram et al. 1988; Singh et al. 2011b).

The appearance in Uganda of race TTKSK of the SR fungus, commonly known as Ug99, and its evolution and spread outside eastern Africa were recognized as a serious threat to wheat production because of the susceptibility of numerous important varieties grown worldwide (Singh et al. 2011a; Sharma et al. 2013). This race possesses a unique virulence combination, including virulence to *Sr31*, *Sr38*, and a number of resistance genes used widely by breeding programs worldwide (Pretorius et al. 2000; Jin and Singh 2006). About 80–90 % of wheat varieties and other germplasm were found to be susceptible to Ug99 (Jin and Singh 2006; Fetch 2007; Singh et al. 2008). Variants of Ug99 possess additional virulence to the resistance genes *Sr24* and *Sr36* (Jin et al. 2008, 2009). These two genes were widely used in Australia, South America, and in winter wheat varieties of the USA. (Jin et al. 2009). Ug99, or its variants, is now known to occur in Kenya, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, and South Africa (Nazari et al. 2009; Pretorius et al. 2010; Singh et al. 2011a; Sharma et al. 2013).

For the economic and environmental friendly disease control, Ug99-resistant wheat varieties could be developed and deployed. Efforts have been made by several groups to counter the challenges and identified some effective resistance genes for Ug99 threat (Jin et al. 2007; Singh et al. 2008, 2011a, b). Most of these genes have been introgressed from wild relatives of wheat and some possess genetic linkages to undesirable traits (Singh et al. 2008). To achieve long-lasting resistance, combinations of multiple genes need to be deployed (Singh et al. 2008). Identifying molecular markers closely linked to resistance genes could enable us to incorporate multiple resistance genes in breeding materials. Molecular markers are available for genes conferring resistance to Ug99 that were transferred from alien wheat relatives or related species (Mago et al. 2002, 2005, 2011; Sambasivam et al. 2008; Tsilo et al. 2008; Qi et al. 2011; Simons et al. 2011), and also three genes native to bread wheat (Hiebert et al. 2010, 2011; Rouse et al. 2012).

Field and greenhouse screenings of International Maize and Wheat Improvement Center (CIMMYT) and USA wheat germplasm with Ug99 identified some lines that appeared to possess uncharacterized resistance genes (Jin and Singh 2006; Njau et al. 2010; Rouse et al. 2011). Njau et al. (2010) identified 'Niini#1', 'Tinkio#1', and 'Coni#1' as possessing seedling and field resistance to the Ug99 race group derived from a Chinese resistance source. It was hypothesized that Blouk#1 possessed *SrTmp*, an SR gene derived from 'Triumph 64' (McVey and Hamilton 1985), based on seedling infection type and pedigree. CIMMYT line Pfunye#1 was also identified as possessing seedling and field resistance. Jin and Singh (2006) suggested that many USA hard red winter wheat varieties (HRWW) resistant to Ug99 possess *SrTmp*, based on seedling infection type, race specificity, and pedigree. The HRWW variety Ripper is also postulated to carry *SrTmp*, based on seedling infection type, race specificity, and pedigree (Haley et al. 2007). Several advanced lines possessing a combination of adult plant and race-specific resistance, derived from different sources such as Niini#1, Coni#1, Tinkio#1, Blouk#1, and Pfunye#1, have already been developed and distributed worldwide and are being utilized by the bread wheat improvement program (Singh et al. 2011b). Some varieties carrying race-specific resistance to Ug99 were released in different countries: 'Gambo' (Ethiopia) and 'Koshan 09' (Afghanistan) possess Ug99 resistance derived from Babax/Lr42/Babax, the resistant parent of Blouk#1 (Njau et al. 2010); similarly, Shanghai 7-derived Ug99 resistant varieties Digalu and Morvarid were released in Ethiopia and Iran, respectively. Digalu is now the predominant bread wheat variety in Ethiopia with over 0.5 million hectares being sown with the variety.

This study aimed to (1) determine the genetic basis of resistance to Ug99 in six bread wheats, (2) identify the chromosomal location(s) of the resistance gene(s), and (3) identify molecular markers linked to resistance genes for marker-assisted selection in breeding programs.

Materials and methods

Plant materials

Of the six Ug99-resistant parents, five (Niini#1, Pfunye#1, Blouk#1, Tinkio#1, and Coni#1) were spring wheats derived from CIMMYT crosses and selections (Table 1). For simplicity in this manuscript, the selection number (#1) will hereafter be omitted from the line name. The sixth parent was the Ug99-resistant USA HRWW variety Ripper (Haley et al. 2007). Ug99-susceptible parents Cacuke and PBW343 were used to develop mapping populations with the CIMMYT lines (Table 1), whereas the susceptible parent Bill Brown (Haley et al. 2008) was chosen for the Ripper mapping population.

Cacucke was crossed with Niini and Pfunye and 148 F_5 Recombinant inbred lines (RILs) were developed for each of the crosses, as described by Singh et al. (2013). PBW343 was crossed with Tinkio, Coni, and Blouk, and 148, 190, and 142 F_5 RILs, respectively, were included in the study.

Table 1 Parental lines used in mapping of resistance to Ug99 race group of stem rust fungus with their pedigrees and responses to Ug99 in seedlings and adult plants

Parents	Pedigree	GID[a]	Seedling reaction to Ug99	Field reaction to Ug99
Niini#1	Ningmai9558//Chilero/Chuanmai18	4902635	2	5R-15RMR
Phunye#1	Pfau/Milan/3/Skauz/KS94U215//Skauz	5534349	22+	5R-10R
Blouk#1	Babax/LR42//Babax*2/3/Kuruku	5398610	22+	10RMR-40MR
Tinkio#1	Ningmai9415.16//Shanghai#4/Chilero/3/Ningmai50	4942852	2+	5R-30RMR
Coni#1	Ningmai9415/3/Ures/Bow//Opata/4/Ningmai7	4911181	2+	5R-10RMR
Ripper	CO940606/TAM107R-2	–	22+	–
Cacuke#1	Canadian/Cunningham//Kennedy	5347441	3+	80S-100S
PBW343	Nord Deprez/VG9144//Kalyansona/Bluebird/3/Yaco/4/Veery#5	2430154	3+	60MSS-90S
Bill Brown	Yumar/Arlin	–	3+	–

[a] Germplasm identification number in CIMYYT database

A population of 139 $F_{2:3}$ families, obtained by harvesting individual F_2 plants from the cross between Ripper and Bill Brown, were used in mapping.

Field evaluation for SR resistance

The parents and four RIL populations (Cacuke/Niini, Cacuke/Pfunye, PBW343/Tinkio, and PBW343/Coni) were phenotyped for SR infection responses in field trials at the Kenya Agricultural Research Institute (KARI), Njoro, Kenya, during two seasons (off season: December 2009-April 2010, and main season: June-October 2010). Approximately 4 g seed of each line was planted in 0.7 m long paired-row plots with 0.3 m row spacing and 0.3 m wide pathways between plots. To create artificial epidemics in the field, spreader rows, consisting of a mixture of Ug99 susceptible lines, were planted as 1 m borders around the experimental area. Spreaders were planted as hill plots on one side of the entry plots in the middle of 0.3 m pathways to ensure the uniform disease development and spread within the field. A lightweight mineral oil suspension of freshly collected urediniospores of Pgt race TTKST, the *Sr24* virulent variant of Ug99, was injected/sprayed on the spreaders as described in Njau et al. (2013). Disease severity and infection responses were recorded when the plants reached flowering to soft dough stages. The susceptible parent Cacuke displayed about 60–80 % rust severity in both seasons. Infection responses are based on Pgt uredinia size and shape (Roelfs et al. 1992), where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible. Plants displaying a mixture of infection responses were described using multiple categories, such as MR-MS. RILs were classified as resistant if all plants displayed low infection responses of R, MR, or MR-MS. They were considered susceptible if all plants displayed MS or S infection responses, and segregating (Seg) if plants segregated for resistant and susceptible infection responses. Disease severity was determined using the modified Cobb Scale (Peterson et al. 1948), but these data were not useful in mapping the major effect resistance genes in this study. To avoid complication in linkage map construction, the RILs with Seg or ambiguous responses were removed from analyses. The final disease scores for each RIL were based on the consistent expression of resistance and susceptible responses across seasons.

Seedling evaluation with Pgt race TTKSK

The parents and RIL populations (Cacuke/Pfunye, PBW343/Blouk,) and the $F_{2:3}$ lines derived from Bill Brown/Ripper were assayed at the seedling stage with race TTKSK (isolate 04KEN156/04). For the PBW343/Blouk F_5 RIL population, five seeds of each family were sown. For the Bill Brown/Ripper and Cacuke/Pfunye populations, 20 seeds of each family were tested. Once the plants reached the 2-leaf stage, 7–10 days after planting, seedlings were inoculated at a Biosafety Level 3 greenhouse facility in St. Paul, MN, USA (Rouse et al. 2011). Fourteen days after inoculation, each plant was rated for seedling infection type on a 0–4 scale, where ≤ 2 corresponded to resistance and ≥ 3 corresponded to susceptibility, following Stakman et al. (1962). Families were characterized as resistant, segregating, or susceptible. After preliminary mapping, all families that displayed allelic disassociation with linked markers were re-phenotyped. For the PBW343/Blouk population, families identified as segregating in the first screening were retested (20 seedling for each) and confirm their reaction to stem rust pathogen.

Molecular marker analysis

Genomic DNA of the parents of four mapping populations (Cacuke/Niini, Cacuke/Pfunye, PBW343/Tinkio, and PBW343/Coni) and the corresponding derived families was extracted using a hexadecyltrimethyl ammonium bromide (CTAB) method, according to laboratory protocol (CIMMYT 2005). Bulked segregant analysis was used to ascertain the chromosome location of resistance in these populations (Michelmore et al. 1991; Lowe et al. 2011). Genomic DNA from 15 homozygous resistant and 15 homozygous susceptible families was mixed in equivalent concentrations and volumes to constitute the resistant and susceptible bulks, respectively. DNA concentrations of each of the susceptible and resistant families were diluted to 30 ng/μL and then equal amounts were mixed in the respective bulks. The parents and the resistant and susceptible bulks were screened with 372 SSR markers (*Xwmc, Xgwm, Xbarc, Xcfd* and *Xcfa*) covering the whole wheat genome. Primer sequences and annealing temperatures for each SSR marker were obtained from the GrainGenes web database (http://wheat.pw.usda.gov). The PCR reaction was performed in a volume of 10 μL reaction mix containing 1X green GoTaq® Flexi buffer, 2.5 mM $MgCl_2$, 100 mM dNTPs, 1 pmol forward primer, 1 pmol reverse primer, 1 unit of GoTaq® DNA Polymerase, and 100 ng template DNA. PCR products were separated on 12 % polyacrylamide (29:1) gel at 300 V for 3 h. After electrophoresis, the gel was silver-stained to observe and score amplified alleles for polymorphism (CIMMYT 2005).

Four single nucleotide polymorphism (SNP) markers (*XBS00009514*, *XBS00010742*, *XBS00009806*, *XBS00021867*), previously mapped on chromosome 6DS (http://www.cerealsdb.uk.net), were also screened on the parents. Marker *XBS00010742* was polymorphic among the parents of the four populations and was assayed on the RILs. For genotyping RILs with SNP markers, 100 ng DNA of each RIL was dried at 60 °C for 1 h to avoid differences in DNA concentration between distinct samples. The technique used for SNP genotyping was based on the KASPar genotyping system as described on the KBiosciences portal (http://www.kbioscience.co.uk/). The PCR program used was a touchdown program with initial denaturation at 94 °C for 15 min; followed by 11 cycles of 94 °C for 30 s, 65 °C for 1 min with 0.8 °C decrease per cycle, 72 °C for 30 s; followed by 26 cycles of 94 °C for 30 s, 57 °C for 1 min, 72 °C for 30 s; and the last extension step at 72 °C for 5 min. The alleles were visualized with a Pherastar Plus plate reader equipment® (BMG labtech company) using 30 % of gain and 5.7 focus. The software for analyzing the SNPs markers was SNPviewer2 (http://www.lgcgenomics.com).

DNA was extracted from the leaf tissue of each F_5 family of the PBW343/Blouk population, following the methods of Rouse et al. (2012). For the Bill Brown/Ripper population, DNA was extracted from the F_2 parent plant of each $F_{2:3}$ families. A total of ten resistant and ten susceptible families from each population were used to create resistant and susceptible bulks. The bulks and parents were screened for polymorphism with 23 SSR markers previously mapped to chromosome arm 6DS (Roder et al. 1998; Somers et al. 2004; Song et al. 2005). Identified polymorphic markers were assayed on the entire populations and we performed SSR genotyping using an ABI 3730, utilizing M13-tagged fluorescent dyes, as described by Rouse et al. (2012).

We found that resistance in these populations mapped to chromosome arm 6DS and, therefore, genotyped the DNA of resistant and susceptible lines with marker FSD_RSA (closely linked to *Sr42*), following the methods of Ghazvini et al. (2012), to test whether resistant lines carried the allele of FSD_RSA linked to *Sr42*.

Statistical analyses and genetic mapping

A Chi squared (χ^2) test for goodness of fit was used to test for deviation of observed data from the expected monogenic segregation ratios. For the Cacuke/Niini, Cacuke/Pfunye, PBW343/Tinkio, and PBW343/Coni populations, genetic mapping was performed using the MAPDisto program (Lorieux 2012) with LOD 3.0, algorithm SER, ripping SARF criterion, and Kosambi mapping function (Kosambi 1944). For the PBW343/Blouk and Bill Brown/Ripper populations, JoinMap software v4.0 (Stam 1993; Van Ooijen 2006) with an LOD of 5.0 and the Kosambi mapping function was used to create genetic maps.

Results

Phenotypic response and inheritance of SR resistance

Stem rust disease pressure was high during the crop season and field evaluations allowed discrimination of infection response between the parents and within the mapping populations. Parents Cacuke and PBW343 showed susceptible infection responses, whereas Niini, Pfunye, Tinkio, and Coni displayed R to MR responses (Table 1). The infection response in each RIL population varied from R to MS-S, corroborating previous knowledge that the resistance gene is influenced by genetic background (Singh et al. 2011a). The distribution of 70 homozygous resistant lines (R to MR) and 67 homozygous susceptible (MS or S) RILs of Cacuke/Niini conformed to the 1:1 segregation ratio expected for a single gene (Table 2). The Cacuke/Pfunye RIL also segregating for a single gene, with a distribution

Table 2 Phenotypic distribution of lines in the six mapping populations based of stem rust infection response evaluated in field and greenhouse trials

Population	Generation	Lines (no.)	Observed frequency[a]	Expected ratio[a]	χ^2 (1:1)	*P* value
Cakuke/Niini	F_5	110	50:60	1:1	0.91	0.34
Cakuke/Pfunye	F_5	116	57:59	1:1	0.03	0.85
PBW343/Tinkio	F_5	106	43:63	1:1	3.70	0.05
PBW343/Coni	F_5	190	106:84	1:1	2.52	0.11
PBW343/Blouk	F_5	117	58:59	1:1	0.01	0.93
Bill Brown/Ripper	F_3	130	36:69:25	1:2:1	2.35	0.31

[a] Expected frequency and ratio are for resistant: susceptible in F_5 RILs and resistant: segregating: susceptible in F_3 families

of 60 homozygous resistant and 58 homozygous susceptible. Seedling and field phenotypic data correlated in the population. The distribution of resistant and susceptible RILs in the other two populations also conformed to a 1:1 ratio; 63 homozygous resistant and 43 homozygous susceptible in PBW343/Tinkio and 84 homozygous resistant and 106 homozygous susceptible in PBW343/Coni (Table 2). For both Blouk and Ripper, seedling infection types to race TTKSK ranged from 2 to 2+ among replications. PBW343 and Bill Brown displayed a seedling infection type of 3+. Resistant progenies in the PBW343/Blouk and Bill Brown/Ripper populations displayed infection types ranging from 2 to 2+. A total of 117 F_5 families phenotyped from the PBW343/Blouk population segregated with 1:1 ratio expected for a single gene after excluding few segregating families (Table 2). From the phenotypic data available for 130 of the 139 Bill Brown/Ripper $F_{2:3}$ families, resistance to race TTKSK segregated as 36 resistant:69 segregating:25 susceptible, in accordance with the 1:2:1 ratio expected for a single gene (Table 2).

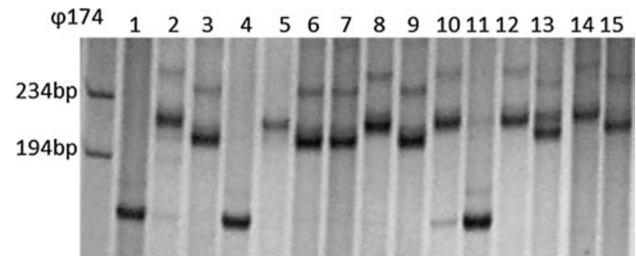

Fig. 1 Molecular polymorphism between susceptible and resistant parents and F_5 resistant and susceptible bulks detected with SSR marker *Xcfd49*. The *first column* is the size marker (φX174 digested with the enzyme *Hae*III). The numbers on the *left* indicate the molecular size in bp. The numbers on the *top* are DNA samples: *1* Norin40, *2* Cacuke, *3* Niini, *4* Pfunye, *5* PBW343, *6* Tinkio, *7* Coni, *8* Cacuke/Niini susceptible bulk, *9* Cacuke/Niini resistant bulk, *10* Cacuke/Pfunye susceptible bulk, *11* Cacuke/Pfunye resistant bulk, *12* PBW343/Tinkio susceptible bulk, *13* PBW343/Tinkio resistant bulk, *14* PBW343/Coni susceptible bulk, and *15* PBW343/Coni resistant bulk

Bulk segregant analysis and polymorphic marker identification

For the Cacuke/Niini, Cacuke/Pfunye, PBW343/Tinkio, and PBW343/Coni populations, the number of markers screened varied by genome, with 113, 162, and 97 SSR marker loci from the A, B, and D genomes, respectively. Fifteen percent of the markers were polymorphic between parents. Only markers located on chromosome 6DS revealed linkages to Ug99 resistance, and this was, therefore, the chromosome region selected for additional mapping. Marker *Xcfd49*, positioned on chromosome 6DS, was polymorphic in all populations, producing an amplified fragment of 214 bp in Cacuke and PBW343 and a fragment of 202 bp in resistant lines Niini, Tinkio, and Coni. Bill Brown produced a 210-bp fragment. In contrast, Pfunye, and the Pfunye/Cacuke resistant bulk produced a 160-bp band with the marker *Xcfd49,* similar to that produced by 'Norin40' which is reported to carry *Sr42* (Fig. 1). This result was further confirmed on ten resistant and ten susceptible lines from the Cacuke/Pfunye population. However, it is not clear if Pfunye carries the *Sr42* gene, as the marker FSD_RSA, closely linked to *Sr42* (Hiebert et al. 2011), failed to amplify the fragment in Pfunye and the RILs. A 142-bp fragment was amplified for *Xcfd49* in Ripper and Blouk (excluding the 19 bp M13 tag). Marker *Xbarc183* was most closely linked to resistance in Ripper and Blouk. The alleles of both Ripper and Blouk were 151 bp (excluding M13), which is different from Norin 40 and AC Cadillac (Ghazvini et al. 2012). Both Bill Brown and PBW343 did not amplify a fragment for *Xbarc183*, and this marker was scored as a dominant marker in these populations. The other 6DS markers that showed polymorphism between resistant and susceptible bulks were *Xbarc173*, *Xcfd13*, *Xcfd42*, *Xcfd75*, *Xgdm132*, *Xgwm469*, *Xugwm61,* *Xwmc749*, and BS00010742. Only *Xcfd49* and *Xbarc183* were amplified in all six resistant parents.

Genetic maps of markers on chromosome arm 6DS linked to stem rust resistance

We evaluated polymorphic markers from 6DS on susceptible and resistant lines of all the populations and constructed linkage maps. Total map lengths for the populations Cacuke/Niini, Cacuke/Pfuney, PBW343/Tinkio, PBW343/Coni, PBW343/Blouk, and Bill Brown/Ripper were 55.3, 57.9, 50.2, 54.8, 38.0, and 33.3 cM, respectively. Markers *Xcfd49* and *Xbarc183* flanked the resistance gene in maps of the Cacuke/Niini, Cacuke/Pfunye, PBW343/Tinkio, and PBW343/Coni populations (Fig. 2). In the PBW343/Blouk and Bill Brown/Ripper populations, both *Xcfd49* and *Xbarc183* were distal to the mapped resistance gene. The genetic distances between closest distal and proximal markers to the resistance gene in the Cacuke/Niini population were 3.9 and 8.4 cM, respectively. In the Cacuke/Pfunye population, the distal marker *Xcfd49* was located 5.3 cM from the resistance gene, and proximal marker *Xbarc183* was located 6.2 cM from the gene (Fig. 2). In the PBW343/Tinkio population, the markers *Xcfd49* and *Xbarc183* were located at 6.6 and 27.5 cM from the gene, respectively (Fig. 2). In the PBW343/Coni population, the resistance gene was located 5.8 cM from the distal marker *Xcfd49* and 2.6 cM from the proximal marker *Xbarc183*. Resistance loci in the PBW343/Blouk and Bill Brown/Ripper populations were mapped 5.9 and 3.8 cM proximal to *Xbarc*183, respectively. These results indicate that the resistance loci mapped in six different populations on chromosome arm 6DS.

Molecular marker FSD_RSA reported to be closely linked to resistance gene *Sr42* in cultivar Norin 40 and *SrCad* in AC Cadillac (Ghazvini et al. 2012, Hiebert et al. 2011). However, the marker amplified same size fragment in Norin 40, all resistant and susceptible parents except Pfunye. Experiments conducted at the USDA-ARS Cereal Disease Laboratory to amplify the FSD_RSA marker in Norin 40, Bill Brown, Blouk, and PBW343 produced a band closer to 300 bp, as opposed to the expected size of 275 bp. In order to validate this difference, we used the FS and RS primers described in Laroche et al. (2000) to amplify the 604-bp fragment, within which the 275-bp FSD_RSA fragment was reported to be included (Qiagen PCR cloning kit; sanger sequencing at University of Minnesota Biomedical Genomics Center). Sequencing of the 5′ and 3′ ends of the amplified fragment from Ripper, Bill Brown, Blouk, and PBW343 identified that the sequence amplified by FSD_RSA was 292 bp and that there is a single-nucleotide polymorphism (SNP) within the FSD primer region of these wheat lines. The FSD primer 5′-gttttatcttttatttc-3′ corresponded to 5′-gttttatctttttattta-3′ in the wheat lines. The replacement of a cytosine with an adenine at the 3′ end is consistent with the sequence of wheat variety ‘Neepawa’ that did not produce the FSD_RSA fragment (Laroche et al. 2000). In fact, the FSD_RSA marker was designed to specifically amplify sequences with a cytosine at the 3′ end. This suggests that though we amplified a 292-bp fragment for these lines, the fragment does not share the SNP characteristic of the bunt resistant wheat line ‘BW553’, for which the FSD_RSA marker was developed. Therefore, the FSD_RSA marker produced false-positive amplification for Ripper, Bill Brown, Blouk, and PBW343.

Parental line contributing resistance to Niini

The resistant parent Niini was derived from a three-way cross utilizing parents ‘Ningmai9558’, ‘Chilero’, and ‘Chuanmai18’. Genotyping of these parents showed that

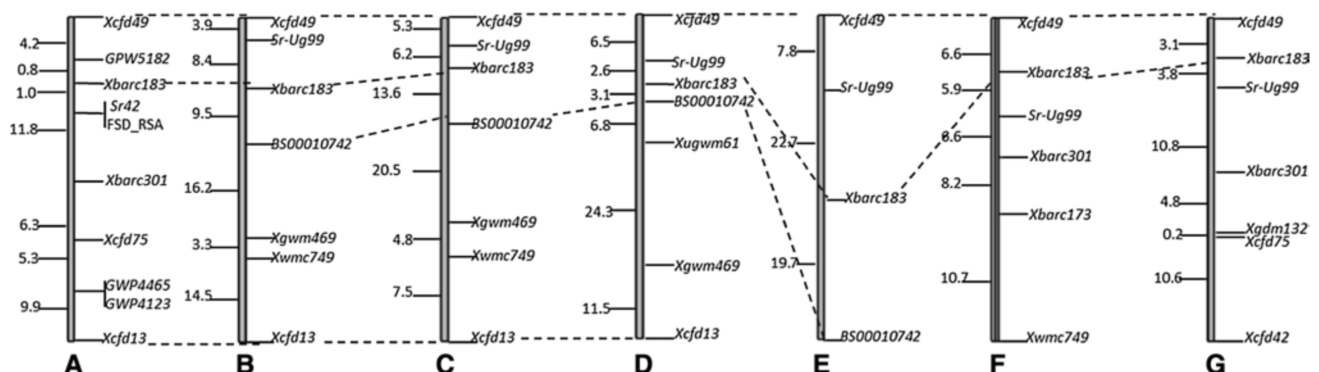

Fig. 2 Comparison of genetic maps showing the positions of *Sr* genes on chromosome 6D short arm. **a** 6DS map from double haploid population LMPG/Norin40 reconstructed from Ghazvini et al. (2012), **b** map constructed using Cacuke/Niini derived RIL population, **c** map constructed using Cacuke/Pfunye#1 derived RIL population, **d** map constructed using PBW343/Coni derived RIL population, **e** map constructed using PBW343/Tinkio derived RIL population and **f** map constructed using PBW343/Blouk derived RIL population, **g** map constructed using Bill Brown/Ripper derived F3 population. Loci names are indicated on the *right* and map distances are shown on the *left* in CentiMorgans

the resistance-linked allele of the marker *Xcfd49* was present in Ningmai9558 and absent in Chilero and Chuanmai18. However, Niini and Norin 40, donor of *Sr42*, had different allele sizes of 202 and 160 bp, respectively (Fig. 1).

Discussion

Niini, Pfunye, Tinkio, Coni, Blouk, and Ripper wheat were resistant to Ug99 and are being used in breeding for rust resistance at CIMMYT and Colorado State University. Race-specific resistance in all lines was conditioned by a single gene. Based on results for different mapping populations, the resistance gene was located on chromosome 6DS, 3.9–12.5 cM proximal to SSR marker *Xcfd49* (Fig. 2). *Xcfd49* is the terminal SSR marker on the genetic map of 6DS. Marker *Xbarc183* was located proximal or distal to SR loci in four and two of the populations, respectively (Fig. 2). Our data provide some evidence that the resistance locus in Niini, Coni, Tinkio, and Pfunye could be different from the resistance locus in Blouk and Ripper, based on marker order (Fig. 2). Further experiments, such as allelism tests, will be necessary to confirm the results of our mapping studies. The parents and resistant RILs also need to be tested with an array of SR fungus races that have diverse avirulence/virulence combinations including virulence for the mapped genes(s) on 6DS.

Phenotyping moderately effective race-specific resistance is challenging in field trials as disease severity data may not necessarily provide any demarcation point to distinguish between resistant and susceptible lines. The low phenotypic effect of moderately effective resistance genes on disease severity depends upon genetic background (Hiebert et al. 2011; Singh et al. 2011b). According to Singh et al. (2011b), *SrTmp* and *SrSha7* genes displayed a disease severity ranging from 5 to 60 % and 1 to 30 %, respectively, in CIMMYT derived advanced wheat breeding materials tested in Njoro, Kenya, in 2010. Similarly, *SrCad* has been shown to display a disease severity of 10–30 % depending upon the background presence or absence of APR gene *Lr34* (Hiebert et al. 2011). We too observed a wide range of disease severity, with incompatible reactions in the RILs and, therefore, chose to discretely classify RILs into resistant and susceptible classes, based on host infection responses, to map the single genes in the populations evaluated.

Chromosome 6D has been reported to be a gene-rich region of the wheat genome, containing resistance to powdery mildew (Ma et al. 2011), leaf rust (Mebrate et al. 2008), common bunt (Laroche et al. 2000), and stem rust (Hiebert et al. 2011; Ghazvini et al. 2012). Four *Sr* genes were previously mapped to chromosome 6D. These are *Sr5*, *Sr29*, *Sr42* (McIntosh et al. 1995; Ghazvini et al. 2012), and *SrCad* (Hiebert et al. 2011). Of these, *Sr29* is reported to be located on the long arm of chromosome 6D. The remaining three genes (*Sr5*, *Sr42*, and *SrCad*) were mapped to the short arm of chromosome 6D (McIntosh et al. 1995; Hiebert et al. 2011; Ghazvini et al. 2012). However, *Sr5* does not confer resistance to Ug99 (Jin et al. 2009), whilst both *Sr42* and *SrCad* confer resistance to Ug99 but produce a low seedling infection type similar to the resistant lines. Further, comparison of the maps in the present study with the published map of *Sr42* and *SrCad* (Ghazvini et al. 2012) reveals high similarity in the marker order and genetic distance between linked SSRs (Fig. 2). We, therefore, cannot rule out the possibility that the resistance loci reported in this study are the same as *Sr42* or *SrCad*.

The sequence of FSD_RSA, the PCR based marker which is the closest marker to both *SrCad* (Hiebert et al. 2011) and *Sr42* (Ghazvini et al. 2012), was used to evaluate the relationship between the mapped *Sr* gene(s) in the present study. FSD_RSA was developed as a marker for the common bunt resistance gene *Bt10*, which is closely linked to *SrCad* (Hiebert et al. 2011). Ghazvini et al. (2012) reported that *Sr42* (in Norin40) co-segregated with FSD_RSA that mapped 1.5 cM from *SrCad* (Hiebert et al. 2011). It has also been hypothesized that *Sr42* and *SrCad* represent the same allele, or different alleles of the same locus (Ghazvini et al. 2012). We evaluated the presence and absence of FSD_RSA in six resistant (Niini, Pfunye, Blouk, Ripper, Tinkio, and Coni) and three susceptible (Cacuke, PBW343, and Bill Brown) parents. No differences were found between susceptible and resistant parents except for Pfunye in which the band was not present (picture not shown). This result was further confirmed by evaluating FSD_RSA on the Cacuke/Pfunye RIL population, and no linkage to stem rust resistance was found. This could indicate that resistance derived from Pfunye could be different from *Sr42/SrCad* and also from resistance derived from Niini, Coni, and Tinkio. In sequencing the FSD_RSA region, Ripper, Bill Brown, Blouk, and PBW343 amplified the fragment but did not possess the diagnostic SNP, therefore, indicating that this marker may not be robust in diverse germplasm. Allelism tests will be required to determine if the resistance gene in Pfunye is different from resistance in other parents and *Sr42* or *SrCad*.

The closest SSR markers (*Xcfd49* and *Xbarc183*) identified in this study will expedite marker-assisted selection and stacking of resistance on 6DS in CIMMYT and Colorado State University wheat breeding germplasm. If further testing identifies more than one gene present on 6DS, recombinants could be selected to create a desirable linkage block of two or more disease resistance genes that would be easy to track and select in wheat breeding populations. When desirable genes are linked in coupling, as is

the case for *SrCad* and *Bt10* (Hiebert et al. 2011), breeding populations can be easily enriched with the linked genes.

Based on linked marker alleles, map location, pedigree information, and field infection response, it is likely that resistance in Niini, Coni, and Tinkio represents the same gene. The Chinese wheat lines Ningmai9558, Ningmai9415.16 (Ningmai50), and Ningmai9415 (Ningmai7) could share the common resistance gene identified in Niini, Tinkio, and Coni. On the other hand, it is also possible that Pfunye possesses a distinct gene based on the diagnostic marker allele and pedigree. Blouk and Ripper mapped to the same location as *Sr42*, and displayed seedling infection types similar to *Sr42*; thus it is possible that Blouk, Ripper, Norin 40, and AC Cadillac share a common resistance gene. Since Blouk and Ripper were postulated to possess *SrTmp,* our data indicate that *SrTmp* may be the same gene as *Sr42* and *SrCad*. Studies examining the SR resistance in the *SrTmp* donor 'Triumph64', and allelism tests among these resistance sources, will be needed for clarifying the relationships between *SrTmp*, *SrCad*, *Sr42* and the resistance in the six lines included in this study.

Acknowledgments This research work was supported by the Durable Rust Resistance in Wheat (DRRW) project managed by Cornell University with the Bill & Melinda Gates Foundation and the UK Agency for International Development (DFID). E.E. Lopez-Vera appreciates the Ph.D. fellowship from Mexico's Consejo Nacional de Ciencia y Tecnología (CONACYT). Editing assistance was received from E. Quilligan (CIMMYT).

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

References

CIMMYT (2005) Laboratory protocols: CIMMYT applied molecular genetics laboratory, 3rd edn. CIMMYT, Mexico

Fetch TG Jr (2007) Virulence of stem rust race TTKS on Canadian wheat cultivars. Can J Plant Pathol 29:441

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

Haley SD, Johnson JJ, Peairs FB, Quick JS, Stromberger JA, Clayshulte SR, Butler JD, Rudolph JB, Seabourn BW, Bai GH, Jin Y, Kolmer J (2007) Registration of 'Ripper' wheat. J Plant Regist 1:1–6

Haley SD, Johnson JJ, Peairs FB, Quick JS, Stromberger JA, Butler JD, Miller HR, Heaton EE, Rudolph JB, Seabourn BW, Bai GH, Jin Y, Kolmer J, Chen XM (2008) Registration of 'Bill Brown' wheat. J Plant Regist 2:218–223

Hiebert CW, Fetch TG Jr, 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 Appli Genet 122:143–149

Jin Y, Singh RP (2006) Resistance in US wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis 90:476–480

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 LJ, Pretorius ZA, Singh RP, Ward R, Fetch TG Jr (2008) Detection of virulence to resistance genes *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926

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

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenics 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 *Bt10* gene for common bunt resistance in wheat. Genome 43:217–223

Leonard KJ, Szabo LJS (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*. Mol Plant Pathol 6:99–111

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

Lowe I, Cantu D, Dubcovsky J (2011) Durable resistance to the wheat rust: integrating systems biology and traditional phenotype-based research methods to guide the deployment of resistance genes. Euphytica 179:69–79

Ma H, Kong Z, Fu B, Li N, Zhang L, Jia H, Ma Z (2011) Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theor Appl Genet 123:1099–1106

Mago R, Spielmeyer W, Lawrence GJ, Lagudah ES, Ellis JG, Pryor A (2002) Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theor Appl Genet 104:1317–1324

Mago R, Miah H, Lawrence GJ, Wellings CR, Spielmeyer W, Bariana HS, McIntosh RA, Pryor AJ, Ellis JG (2005) High-resolution mapping and mutation analysis separate the rust resistance genes *Sr31*, *Lr26* and *Yr9* on the short arm of rye chromosome 1. Theor Appl Genet 112:41–50

Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W (2011) An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. Theor Appl Genet 122:735–744

McFadden ES (1930) A successful transfer of emmer characters to vulgare wheat. Agron J 22:1020–1034

McIntosh RA (1988) The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 1–9

McIntosh RA, Wellings CR, Park RF (1995) Wheat rust an atlas of resistance genes. CSIRO, Australia

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

Mebrate SA, Oerke EC, Dehne HW, Pillen K (2008) Mapping of the leaf rust resistance gene *Lr38* on wheat chromosome arm 6DL using SSR markers. Euphytica 162:457–466

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nagarajan S, Joshi LM (1975) An historical account of wheat rust epidemics in India and their significance. Cereal Rust Bull 2:29–33

Nazari K, Mafi M, Yahyaoui A, Singh RP, Park RF (2009) Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK in Iran. Plant Dis 93:317

Njau P, Jin Y, Huerta-Espino J, Keller B, Singh RP (2010) Identification and evaluation of sources of resistance to stem rust race Ug99 in wheat. Plant Dis 94:413–419

Njau PN, Bhavani S, Huerta-Espino J, Keller B, Singh RP (2013) Identification of QTL associated with durable adult plant resistance to stem rust race Ug99 in wheat cultivar 'Pavon76'. Euphytica 190:33–44

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res Sect C26:496–500

Pretorius Z, 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

Pretorius ZA, Bender CM, Visser B, Terefe T (2010) First report of a *Puccinia graminis* f. sp. *tritici* race virulent to the *Sr24* and *Sr31* wheat stem rust resistance genes in South Africa. Plant Dis 94:784

Qi LL, Pumphrey MO, Friebe B, Zhang P, Qian C, Bowden RL, Rouse MN, Jin U, Gill BS (2011) A novel Robertsonian translocation event leads to transfer of a stem rust resistance gene (*Sr52*) effective against race Ug99 from *Dasypyrum villosum* into bread wheat. Theor Appl Genet 123:159–167

Rajaram S, Singh RP, Torres E (1988) Current CIMMYT approaches in breeding wheat for rust resistance. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rust of wheat. CIMMYT, Mexico, D.F, pp 101–118

Roder 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 (1978) Estimated losses caused by rust in small grains cereals in the United States—1918. Misc Publ US Dep Agric 1363:1–18

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico, p 81

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, Nava IC, Chao S, Anderson JA, Jin Y (2012) Identification of markers linked to the race Ug99 effective stem rust resistance gene *Sr28* in wheat (*Triticum aestivum* L.). Theor Appl Genet 125:877–885

Sambasivam PK, Bansal UK, Hayden MJ, Dvorak J, Lagudah ES, Bariana HS (2008) Identification of markers linked with stem rust resistance genes *Sr33* and *Sr45*. In: Appels R et al (eds) Proceedings of the 11th international wheat genetics symposium, vol 2. Sydney University Press, Australia, pp 351–353

Sharma RK, Singh PK, Vinod, Joshi AK, Bhardwaj SC, Bains NS, Sukwinder-Singh (2013) Protecting south Asia wheat production from stem rust (Ug99) epidemic. J Phytopathol 161:299–307

Simons K, Abate Z, Chao SM, Zhang WJ, Rouse M, Jin Y, Elias E, Dubcovsky J (2011) Genetic mapping of stem rust resistance gene *Sr13* in tetraploid wheat (*Triticum turgidum* ssp *durum* L.). Theor Appl Genet 122:649–658

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

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel SA, Singh PK, Singh S, Velu G (2011a) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol 49:465–481

Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D, Singh PK, Velu G, Mason RE, Jin Y, Njau P, Crossa J (2011b) Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175–186

Singh S, Singh RP, Bhavani S, Huerta-Espino J, Lopez-Vera EE (2013) QTL mapping of slow-rusting, adult plant resistance to race Ug99 of stem rust fungus in PBW343/Muu RIL population. Theor Appl Genet 126:1367–1375

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

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. Dep Agric Agric Res Serv E-617

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tsilo TJ, Jin Y, Anderson JA (2008) Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Sci 48:253–261

Van Ooijen JW (2006) JoinMap.4.0: Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen