Mol Breeding (2014) 34:871–881
DOI 10.1007/s11032-014-0081-8

High-density mapping of a resistance gene to Ug99 from the Iranian landrace PI 626573

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Received: 12 December 2013 / Accepted: 25 March 2014 / Published online: 3 April 2014

Abstract Managing wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is imperative for the preservation of global food security. The most effective strategy is pyramiding several resistance genes into adapted wheat cultivars. A search for new resistance sources to *Pgt* race TTKSK resistance identified a spring wheat landrace, accession PI 626573, as a potentially novel source of resistance. A cross was made between LMPG-6, a susceptible spring wheat line, and PI 626573 and used to develop a recombinant inbred population to map the resistance. Bulk segregant analysis (BSA) of LMPG-6/PI 626573 F_2 progeny determined resistance was conferred by a single dominant gene given the provisional designation *SrWLR*. The BSA identified nine microsatellite (SSR) markers on the long arm of chromosome 2B associated with the resistant phenotype. Fifteen polymorphic SSRs, including the nine identified in the BSA, were used to produce a linkage map of chromosome 2B, positioning *SrWLR* in an 8.8 cM region between the SSRs GWM47 and WMC332. This region has been reported to contain the wheat stem rust resistance genes *Sr9* and *SrWeb*, the latter conferring resistance to *Pgt* race TTKSK. The 9,000 marker Illumina Infinium iSelect SNP assay was used to further saturate the *SrWLR* region. The cosegregating SNP markers IWA6121, IWA6122, IWA7620, IWA8295, and IWA8362 further delimited the *SrWLR* region distally to a 1.9 cM region. The present study demonstrates the iSelect assay to be an efficient tool to

Electronic supplementary material The online version of this article (doi:10.1007/s11032-014-0081-8) contains supplementary material, which is available to authorized users.

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delimit the region of a mapping population and establish syntenic relationships between closely related species.

Keywords Wheat · Stem rust · *SrWLR* · iSelect · Infinium · SNP

Introduction

Wheat stem rust has historically been a major disease affecting multiple cereal crops resulting in severe yield losses (Leonard and Szabo 2005; Roelfs 1985; Singh et al. 2008, 2011). The causal organism of wheat stem rust, *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a heteroecious macrocyclic fungus that can persist in many parts of the world in the uredinial stage of its life cycle (Roelfs 1982; Singh et al. 2008, 2011). In the United States, *Pgt* overwinters as uredinia on susceptible winter habit wheat varieties in southern states and Mexico and is blown north to wheat and barley fields along the "*Puccinia* pathway" in the summer (Kolmer 2001; Kolmer et al. 2007). In Africa, fields of different maturity exist due to the long growing season and elevation variability allowing *Pgt* to persist in the uredinial stage (Singh et al. 2008, 2011).

The use of resistance genes in common wheat, *Triticum aestivum*, has been shown to be an effective disease management strategy in North America since the 1950s (Roelfs 1982). The long-term effectiveness of resistance genes in controlling the North American *Pgt* population is partially due to the removal of the sexual cycle of the pathogen through the eradication of the common barberry, resulting in the stabilization of the Midwestern United States rust population from year to year (Jin 2005; Roelfs 1982). The durability of this management strategy has caused the use of resistance genes to be the primary means for the control of *Pgt* (Kolmer 2001; Kolmer et al. 2007; Singh et al. 2008; Singh et al. 2011) The emergence of the highly virulent *Pgt* race TTKSK (Ug99) and its variants has stimulated the need to identify new resistance genes (Pretorius et al. 2000; Singh et al. 2011). Ug99 was first observed in Uganda in 1998, when susceptible infection types were observed on cultivars containing *Sr31* (Singh et al. 2011). Since then, Ug99 has migrated out of Uganda to countries along the Eastern African coast, Yemen, and Iran (Hale et al. 2013, Nazari et al. 2009; Pretorius et al. 2000; Singh et al. 2011). Currently, 23 named resistance genes are effective against the Ug99 lineage; however, many of the genes are not in adapted germplasm or are ineffective against other common races (Rouse et al. 2011; Singh et al. 2011). Many Ug99 resistance genes originated from *T. aestivum,* including *Sr28, Sr29, Sr48, SrTmp, SrCad, SrSha, SrHuw234, SrND643,* and *SrWeb* (Hiebert et al. 2010, 2011; Rouse et al. 2012; Singh et al. 2011). Only two resistance genes effective against Ug99, *Sr33*, *and Sr35* have been cloned. However, neither of these genes are naturally found in *T. aestivum*. *Sr33* and *Sr35* are both members of the NBS-LRR gene family commonly associated with disease resistance (Dubcovsky et al. 2012; Periyannan et al. 2013a, b; Saintenac et al. 2013).

The high selection pressure imposed upon many crop species through modern breeding practices has created diversity bottlenecks, leaving current varieties with limited resistance sources and vulnerable to diseases that have long been under control (Tanksley and McCouch 1997). Landraces are a useful tool for many wheat breeding programs due to their association with traditional farming systems (Dreisigacker et al. 2005; Reif et al. 2005; Villa et al. 2005; Warburton et al. 2006). Landraces allow for easier incorporation of new and under-utilized sources of resistance into adapted material through traditional crossing techniques compared to using resistance from wild wheat relatives (Reif et al. 2005). The screening of landraces to identify new sources of disease resistance and the subsequent mapping of these genes has become a common practice in a number of crops, including wheat (Bonman et al. 2007; Fu et al. 2013; Gurung et al. 2011; Xiao et al. 2013).

During a recent disease resistance screening of the USDA National Small Grains Collection (NSGC), Newcomb et al. (2013) identified 278 spring habit common wheat accessions resistant to Ug99 including the Iranian accession PI 626573. PI 626573 displayed resistance to *Pgt* race TTKSK during seedling tests at the USDA-ARS Cereal Disease Laboratory and *Pgt* race TTKST during adult tests at the Kenyan Agricultural Research Institute. PI 626573 often presents an infection type of 2 to 22+ during seedling tests and a severity of trace to 30 % severity and infection responses of MR to MR-MS during adult stage field tests (Newcomb et al. 2013; Roelfs et al. 1992; Stakman et al. 1962). Single-plant selections of PI

626573 were used as males and crossed with the susceptible Canadian line LMPG-6 (Little Club// Prelude/8*Marquis/3/Gabo) to evaluate the mode of inheritance (Acevedo et al. 2011; Knott 1990). Chi-square analysis of seedling tests at the F_3 generation fits a 3:1 segregation ratio, indicating that resistance is conferred by a single dominant nuclear gene (Acevedo et al. 2011). Due to the results of the chi-square analysis, the population was advanced via single seed decent, and the genetic region was identified using bulk segregant analysis (BSA) and fine mapped using microsatellite markers (SSRs) and the 9K Illumina Infinium iSelect wheat assay (Cavanagh et al. 2013). The current study provides information for the mapping of previously unmapped iSelect SNP markers and the fine mapping of the Ug99 resistance, gene, *SrWLR,* from the landrace PI 626573.

Materials and methods

Population development and phenotypic evaluation

The previously developed population was created by crossing single-plant selections of PI 626573, PI 626573-2, and PI 626573-3, to the female parent LMPG-6 (Acevedo et al. 2011; Knott 1990). The LMPG-6/PI 626573 population was advanced via single seed descent to the F_6 generation. Families with greater than 20 seeds were evaluated at the F_3, F_4, F_5, and F_6 generations. The number of families evaluated for the F_3, F_4, F_5, and F_6 generations were 166, 245, 240, and 240 individuals, respectively.

The population was phenotyped at seedling stages during the F_3 and F_4 generations in the biosafety level-three facility at the University of Minnesota in St. Paul, MN. Seedling infection types were determined using the 0–4 scale developed by Stakman et al. (1962). Twenty seedlings of each F_3 family and 2 replicates of 10 seedlings for each F_4 family were inoculated 7–10 days after planting with *Pgt* race TTKSK isolate 04KEN156/04 urediniospores retrieved from −80 °C storage. The spores were revitalized via a 45 °C heat shock for 15 min and a 2 to 4 h rehydration under 80 % relative humidity created with a KOH solution (Rowell 1984). The spores were suspended in a lightweight mineral oil (Sotrol 170, Phillips Petroleum, Borger, TX, USA) and disseminated onto the primary leaves of the plants using a spray inoculator. The newly inoculated plants were placed in a fume hood for 30 min to facilitate oil evaporation and then placed in a dark dew chamber for 14 h at 18 °C followed by a 3 to 4 h period under florescent light to allow for spore germination (Rouse et al. 2012). Plants were then grown for 14 days at 18 ± 2 °C in a greenhouse with a 16 h photo period prior to evaluating the disease. Individuals exhibiting an infection type of 2 or lower were considered resistant and those with infection types of 3 or higher were considered susceptible.

The population was also phenotyped in the field during the F_5 and F_6 generations at the International Stem Rust Nursery at the Kenyan Agricultural Research Institute in Njoro, Kenya. Field trials were planted in hill plots with 10–15 seeds per family and replicated two times. The parental lines were planted every 20 entries as checks to validate the distribution of disease pressure in the field. The hill plots were bordered by spreader rows consisting of a mixture of susceptible Kenyan cultivars containing *Sr31* and *Sr24* to select for *Pgt* race TTKST. The spreader rows were inoculated using a mixture of talc powder and urediniospores. Phenotyping was conducted by estimating the area of infection on the stem ranging from 0 to 100 percent, following a modified Cobb Scale, and using categorical scores to evaluate the infection response (Peterson et al. 1948; Roelfs et al. 1992). The infection response categories included resistant (R), moderately resistant (MR), intermediate (M), moderately susceptible (MS), and susceptible (S) and could be used individually or in combination to describe cases where a mixed response was observed (Roelfs et al. 1992). Individuals with an infection response of M or lower were considered to be resistant, whereas individuals with infection types of MS or higher were considered susceptible.

Bulk segregant analysis

A BSA was performed to identify the genetic region of interest. Before advancing to the F_3 generation, DNA was extracted from the F_2 individuals using a CTAB extraction method as described by Stewart and Via (1993). Homozygous resistant and homozygous susceptible $F_{2:3}$ families were identified by phenotyping the F_2-derived F_3 seedlings. The DNA from the F_2 generation of ten homozygous resistant and ten

homozygous susceptible plants was combined in equal amounts to create resistant and susceptible bulks. The homozygous resistant and susceptible F_2 bulks and parents were genotyped with 1,037 SSRs previously used in the wheat consensus map to confirm the inheritance of the trait and identify genetic regions responsible for the resistance (Somers et al. 2004). The polymerase chain reactions (PCRs) were accomplished in 10 μL volumes containing 0.4 pmol of forward primer, 0.3 pmol of the reverse primer, 0.3 pmol of a M13 primer, 0.125 mM dNTPs, 0.05 units/μL *Taq* DNA polymerase (New England Biolabs, Inc. Beverly, MA, USA), 1X PCR buffer, and 30 ng of DNA. The M13 primers were each labeled with a 6-FAM, NEC, PET, or VIC fluorescent dye to allow for multiplexing during fragment analysis. The PCRs were performed in Applied Biosystems (Foster City, CA, USA) GeneAmp® 9700 thermal cyclers programed to denature the DNA at 94 °C for 10 min, followed by 40 cycles of a 1-min 94 °C denaturation step, a 1-min 50 °C annealing step, and a 1 min 72 °C extension step; the program was then concluded with a final 5 min 72 °C extension step and a 4 °C permanent hold. A 3 μL aliquot of each reaction was combined with 0.14 μL of GeneScan™—500 LIZ® size standard (Applied Biosystems) and 6.86 μL of formamide, and then denatured for 5 min at 94 °C and placed on ice. The PCR amplicon sizes were evaluated using an ABI 3130xL genetic analyzer and GeneMapper® version 3.7 software (Applied Biosystems).

Molecular marker analysis and linkage mapping

Linkage mapping was done using SSRs at the F_4 generation and using SSRs and SNPs at the F_5 generation. The DNA from the F_4 and F_5 generations was extracted using the protocol described by Riede and Anderson (1996) with modifications by Liu et al. (2006) and the additional modification of lyophilizing the tissue and grinding it using a Retsch mm301 mixer mill (Retsch GmbH, Haan, Germany) as described in Rouse et al. (2012). Microsatellite markers that were identified from the bulk segregant analysis were used as a foundation for the mapping of the resistance gene at the F_4 generation. Additional microsatellite markers from the region were identified from the consensus map and used to create a skeletal map of the genetic region (Somers et al. 2004). PCR for the SSRs was performed as described previously.

The large number of SNP markers on the Infinium assay allowed for the development of a genetic map for the population. For the creation of this map, polymorphic SSRs from each chromosome were selected from markers identified during the bulk segregant analysis for use as chromosomal anchoring points. PCR was performed as previously described for the chromosome 2B SSRs and additional polymorphic SSRs on the F_5 generation. The F_5 generation was genotyped on the Illumina BeadStation and iScan instruments according to the manufacturer's protocol (Illumina, Inc. San Diego, CA, USA). The proprietary Infinium assay involves whole-genome amplification, targeted SNP region capture on a custom iSelect bead chip array as described by Cavanagh et al. (2013), array primer extension, and then a signal amplification that is read by the iScan platform (Steemers and Gunderson 2005). The markers were scored using the Illumina GenomeStudio® software. The markers used for mapping were polymorphic and had less than five percent missing data. The linkage maps were constructed using JMP Genomics 6.0 (SAS Institute, Cary, NC, USA) using the interactive hierarchal clustering algorithm to establish linkage groups and the map order optimization algorithm to establish markers positions within a linkage group. The Kosambi mapping function was used to calculate genetic distance between markers (Kosambi 1944). Due to the SSR mapping position of *SrWLR*, the *Sr9* differential lines and cultivar "Webster" containing *SrWeb* was also included in the Infinium iSelect assay in order to evaluate their similarity to PI 626573 in the genetic region of interest.

Results

Phenotypic evaluation

During seedling tests at the F_3 and F_4 generations, the lowest infection type observed was a 2- and the highest infection type observed was a 4. The line PI 626573 exhibited a resistant infection type of 2 to 2+, and LMPG-6 exhibited a susceptible infection type of 3 to 34. The lowest observed infection response for the population during the F_5 field trial was 10 M and the highest was 40S. The median observed infection responses for the resistant parent PI 626573 and susceptible parent LMPG-6 were 20MRMS and 20S,

respectively. The lowest observed infection response for the population during the F_6 field trial was 10MR and the highest infection response was 60S. The median observed infection responses for PI 626573 and LMPG-6 were 10MR and 60S, respectively. Disease reactions observed for the field and seedling trials were consistent; families that were considered susceptible at seedling were also considered susceptible in field evaluations.

Bulk segregant analysis

The BSA identified a single region of interest and was consistent with the segregation ratio observed by Acevedo et al. (2011), suggesting resistance is conferred by a single gene. Of the 1037 SSRs evaluated 640 were polymorphic between the parents, LMPG-6 and PI 626573, and nine markers cosegregated with the resistance phenotype. These nine markers were located on the long arm of chromosome 2B and consisted of BARC167, CFA2043, GWM47, GWM55, GWM120, GWM388, WMC27, WMC175, and WMC441 (Röder et al. 1998; Somers et al. 2004; Song et al. 2005). These SSR markers were used for the initial mapping of the gene.

Linkage mapping

Initial mapping of the gene utilized 15 SSRs selected from both the long and short arms of chromosome 2B and the phenotype from the F_5 generation (Fig. 1). The map produced had a total length of 127.3 cM with a marker density of 0.13 markers per cM. The resistance gene, temporarily designated as *SrWLR*, mapped to an 8.8 cM region delimited between the markers GWM47 and WMC332. Colinearity of this map was conserved with the wheat consensus map with the exception of a micro-rearrangement of GWM47 and WMC175 (Fig. 2; Somers et al. 2004). This micro-rearrangement has also been observed in the RL6071/Webster population (Hiebert et al. 2010).

The Infinium assay produced 2,846 polymorphic SNP markers that had less than five percent missing data distributed across the entire wheat genome. The map produced had 25 linkage groups (Online Resource Table S1). The overall marker density across the 25 linkage groups was 1.28 markers per cM. Cosegregating loci accounted for 53.5 % of the mapped loci and ranged from two to 48 markers. The chromosome 2B linkage group was 122.7 cM and

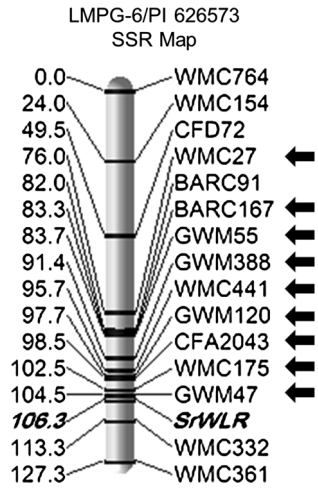

Fig. 1 Linkage map of *SrWLR* on chromosome 2B using SSR markers. *Arrows* denote SSRs identified during the BSA. BARC markers are from Song et al. (2005), GWM markers are from Röder et al. (1998), and CFD and WMC markers are from Somers et al. (2004)

was established using four of the SSRs from the skeletal map and the phenotype of *SrWLR* from the F_6 generation (Fig. 3). The genetic region delimiting *SrWLR* was reduced from 8.8 to 1.9 cM. The *SrWLR* region was delimited 1.5 cM proximally by the SSR GWM47 and 0.4 cM distally by the cosegregating SNPs IWA6121, IWA6122, IWA7620, IWA8295, and IWA8362. Numerous SNP marker rearrangements were observed between the consensus map and the linkage group in this study; however, the markers were in agreement with the assigned chromosomal linkage groups (Cavanagh et al. 2013). Synteny analysis was performed using the Wheat Zapper application to evaluate the accuracy of the map for the *SrWLR* region (Alnemer et al. 2013). The syntenic relationships between *Triticum aestivum*, *Oryza sativa*, *Sorghum bicolor*, and *Brachypodium distachyon* were evaluated around the *SrWLR* region to evaluate the mapping order (Table 1). Synteny was conserved for the linkage map produced in the present study allowing for the conclusion that the mapping order was correct despite the observed rearrangements. The cultivar Webster did not share any alleles with PI 626573 indicating there may not be any shared lineage for the region and that *SrWeb* may be different than *SrWLR* (Table 2). Furthermore, there were not any SNPs identified that could serve as diagnostic markers for *SrWLR, SrWeb,* or any of the *Sr9* alleles.

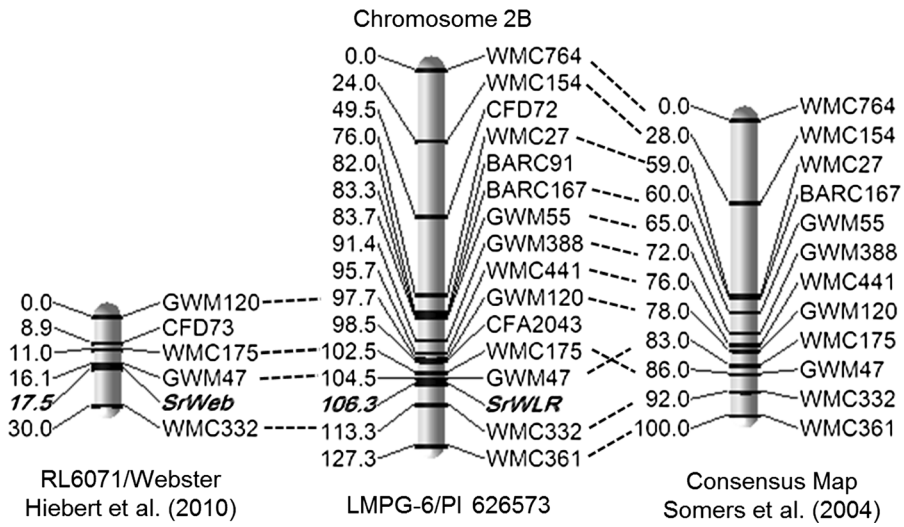

Fig. 2 Comparison of the LMPG-6/PI 626573 SSR map, wheat consensus map (Somers et al. 2004), and the RL6071/Webster chromosome 2B telocentric map (Hiebert et al. 2010). GWM markers are from Röder et al. (1998), BARC markers are from Song et al. (2005), and CFD and WMC markers are from Somers et al. (2004)

Discussion

The analysis of the segregation ratios at the F_3 generation, the BSA, and subsequent genetic mapping determined that the Ug99 resistance in accession PI 626573 is conferred by a single dominant gene as previously hypothesized by Acevedo et al. (2011). The ratio of observed polymorphic markers to total makers evaluated between PI 626573 and the line LMPG-6 during the BSA was higher than the typical observed ratio between two modern wheat cultivars. An increased amount of polymorphism between markers has been observed in other landrace populations (Dreisigacker et al. 2005; Reif et al. 2005). As such, the lack of selection pressure through modern breeding practices is the most likely explanation for the higher SSR polymorphism ration observed between the landrace PI 626573 and the line LMPG-6.

The *SrWLR* locus originally mapped to an 8.8-cM region using SSRs and was reduced to a 1.9-cM region using a combination of SSRs and SNPs. As stated previously, rearrangements were observed when comparing the map produced in the present study to the consensus map. The rearrangements are not unexpected due to the method in which MergeMap group markers during consensus map construction (Endelman 2011; Wu et al. 2011). The MergeMap software arranges markers based on their association with nearby markers in multiple mapping populations. When two markers are grouped in at least two populations, the markers will be grouped in the consensus map regardless of ordering (Endelman 2011). As such, this grouping causes a loss of physical locality when mapping the markers and may cause an incorrect non-physical ordering that is greatly affected by increased marker density (Endelman 2011). When evaluating the syntenic relationships between *T. aestivum, O. sativa, B. distachyon,* and *S. bicolor*, the markers IWA933, IWA1534, IWA5415, and IWA8478 do not belong to the syntenic region (Table 1). These markers are associated with sequences homologous to genes annotated as transposable elements in *O. sativa, B. distachyon,* and *S. bicolor*. As such, the substantial change in syntenic location for these loci compared to surrounding loci is not unexpected.

Numerous rust resistance genes are present on chromosome 2B including leaf rust resistance genes (*Lr13*, *Lr16*, and *Lr23*), stem rust genes (*Sr9, Sr16*, *Sr19, Sr23*, *Sr28, Sr36*, *Sr40,* and *SrWeb*), and stripe rust resistance genes (*Yr5* and *Yr7*) (Hiebert et al. 2010; McIntosh et al. 1995). Of these resistance genes, only *Sr9*, *Sr16*, *Sr28*, *SrWeb*, *Yr5*, and *Yr7* are found on the long arm of chromosome 2B, with *Sr9*, *SrWeb*, *Yr5*, and *Yr7* being located near the SSR GWM47 (Hiebert et al. 2010; McIntosh et al. 1995). Based on the mapping data, there is a strong possibility that *SrWLR* may be a *SrWeb* or a *Sr9* allele (Hiebert et al. 2010; Tsilo et al. 2007). Currently, none of the *Sr9* alleles have been shown to provide resistance to *Pgt* race TTKSK (Jin et al. 2007). Both *SrWLR* and *SrWeb* provide similar infection types to Ug99 and do not appear to provide a broad resistance against other races. The races and number of races that *SrWeb* provides resistance to are

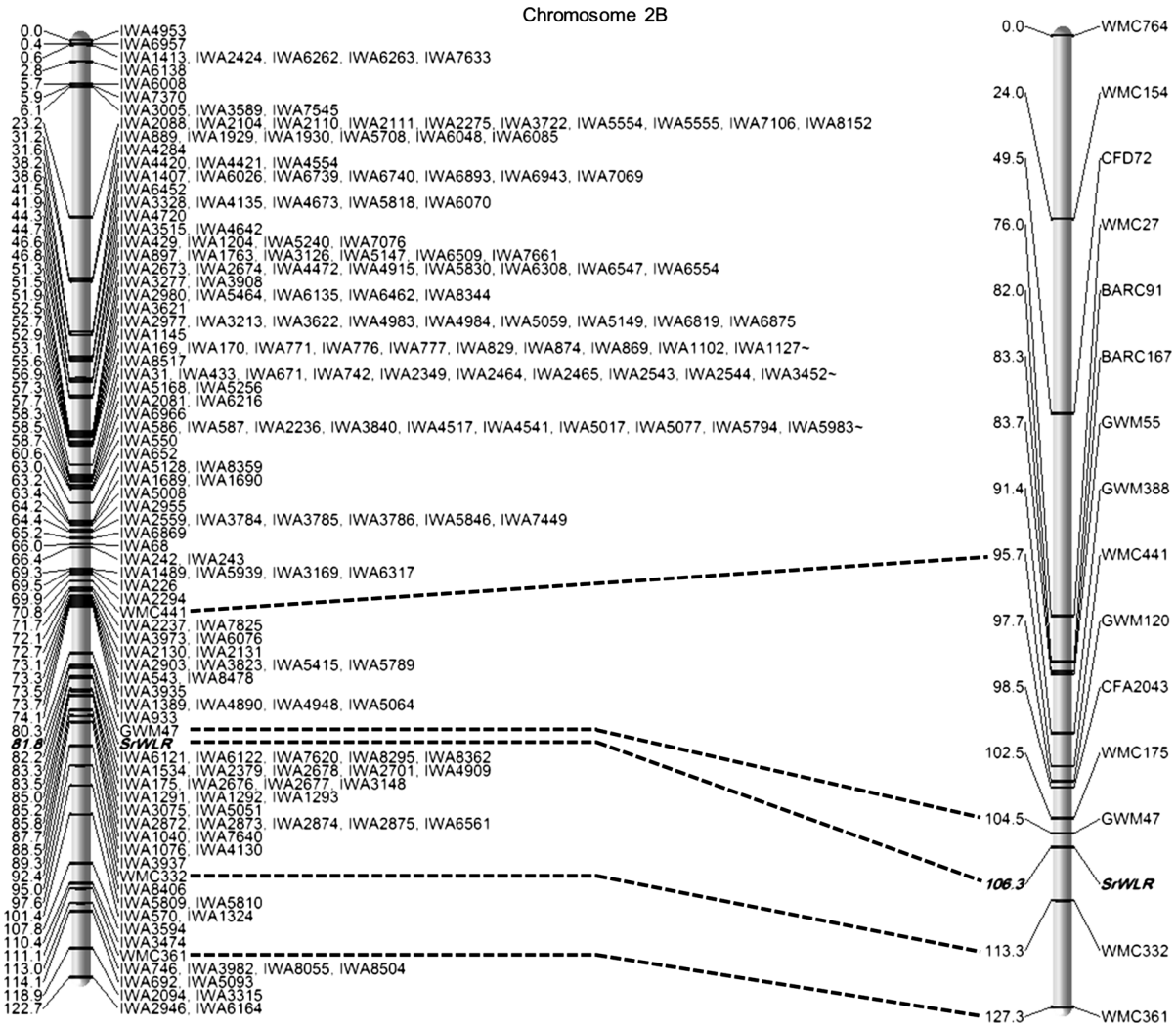

Fig. 3 Comparison of the chromosome 2B linkage maps produced using the iSelect assay and SSRs. GWM markers are from Röder et al. (1998), BARC markers are from Song et al. (2005), CFD and WMC markers are from Somers et al. (2004), and IWA markers are from Cavanagh et al. (2013). Loci containing more than 10 cosegregating markers are denoted with a tilde (~)

confounded by the presence of *Sr30* in the cultivar Webster (Hiebert et al. 2010). Also, the same micro-rearrangement of the SSRs GWM47 and WMC175 was observed in both the LMPG-6/PI 626573 and RL6071/Webster populations (Hiebert et al. 2010). Webster was also included as an entry for the Infinium assay in order to evaluate its similarity to PI 626573 in the genetic region of interest. Evaluation of 12 SNP markers flanking *SrWLR* showed that none of the markers had the same genotype for PI 626573 and Webster, suggesting variability in the region that may indicate *SrWLR* and *SrWeb* may be distinct genes. An allelism study is currently underway to determine whether *SrWLR*, *SrWeb*, and *Sr9* are allelic. However, considering that many resistance genes belong to multigene families in tandem arrays, allelism analysis may not determine whether *SrWLR*, *SrWeb*, and *Sr9* are truly distinct genes. This question may be answered by cloning the genes and characterizing the surrounding regions of the wheat genome.

Further testing of *SrWLR* has shown the gene is effective against the North American *Pgt* race RKQQC isolate 99KS76A. This was accomplished by mapping the resistance phenotype to the same location on

Table 1 Syntenic relationships for each SNP marker within 5 cM proximally and distally of *SrWLR* and their mapping positions on the map from the present study and the consensus map (Cavanagh et al. 2013)

Marker	Position on Chr. 2B (cM)	Consensus map position (cM)	*O. sativa* gene	*B. distachyon* gene	*S. bicolor* gene
IWA2130	72.7	270.15	Os04g52120	Bradi1g26560	Sb06g028130
IWA2131	72.7	270.48	Os04g52120	Bradi1g26560	Sb06g028130
IWA543	73.1	269.13	Os04g51070	Bradi1g02850	Sb06g022480
IWA5415*	73.1	269.63	Os11g31410	Bradi1g32110	Sb06g027770
IWA8478*	73.3	275.11	Os11g45650	Bradi1g04160	Sb06g001175
IWA3935	73.5	262.44	Os04g50970	Bradi5g20320	Sb06g027320
IWA4948	73.7	269.13	Os01g32800	Bradi5g20220	Sb01g027860
IWA1389	73.7	269.13	Os04g50860	Bradi5g20200	Sb06g027240
IWA5064	73.7	275.11	Os04g50790	Bradi5g20150	Sb06g027200
IWA4890	73.7	303.52	Os04g50790	Bradi5g20150	Sb06g027200
IWA933*	74.1	247.93	Os04g21960	Bradi4g04250	Sb01g049840
IWA6121	82.2	300.37	Os04g53440	Bradi5g22410	Sb06g029180
IWA6122	82.2	300.37	Os04g53440	Bradi5g22410	Sb06g029180
IWA8362	82.2	300.37	Os04g53440	Bradi5g22410	Sb06g029180
IWA7620	82.2	299.03	Os04g53440	Bradi5g22410	Sb06g029180
IWA8295	82.2	299.03	Os04g53450	Bradi5g22420	Sb06g029190
IWA2379	83.3	285.21	NA	NA	NA
IWA2701	83.3	285.21	NA	NA	NA
IWA4909	83.3	285.21	Os05g28510	Bradi1g12490	Sb07g001930
IWA1534*	83.3	289.87	Os11g30700	Bradi1g12270	Sb06g003360
IWA2678	83.3	286.72	Os11g11960	Bradi1g42010	Sb06g020600
IWA2676	83.5	291.52	Os11g11960	Bradi1g42010	Sb06g020600
IWA2677	83.5	291.52	Os11g11960	Bradi1g42010	Sb06g020600
IWA3148	83.5	291.52	Os04g53720	Bradi1g45620	Sb06g029476

Markers were organized by the map presented in the current study and then by syntenic ordering of the three related grass species. NA reflects markers where an orthologous gene was not identified for a particular species

*Markers associated with transposable elements

chromosome 2B (data not shown). It is unknown whether *SrWeb* provides resistance to race RKQQC due to the presence of *Sr30* in the cultivar Webster (Hiebert et al. 2010). Race RKQQC occurs in very low frequencies in the United States and is not of immediate concern; however, the threat of Ug99 does make *SrWLR* a useful gene for the incorporation into adapted germplasm. The iSelect markers in the *SrWLR* region are unsuitable for use as a diagnostic marker for *SrWLR* due to the presence of the PI 626573 allele being present in other cultivars (Table 2). Due to the narrow range of resistance, the pyramiding of additional effective stem rust resistance genes will be critical to reduce any future selection pressure induced by *SrWLR* as it is incorporated into wheat breeding programs.

The use of high-throughput assays has been demonstrated to be a cost-effective way to saturate a region with a large number of markers in wheat (Akhunov et al. 2009, Würschum et al. 2013). Previous assays, such as the Illumina GoldenGate assay, were limited by the number of SNPs that could be run simultaneously. This limitation was overcome with the creation of the Illumina Infinium iSelect assay (Akhunov et al. 2009, Würschum et al. 2013). In the present study, the 9 K iSelect assay was able to reduce the region to a fraction of what was previously mapped using SSRs and the future advancement of this assay may provide even higher resolution. The markers IWA6121, IWA6122, IWA7620, and IWA8362 were found to be homologous to the *O. sativa* locus 04g53440, the *S. bicolor*

Table 2 The allele present for markers in the *SrWLR* region for Webster and lines containing *Sr9* alleles from the differential set

Marker	Position on chr. 2B (cM)	Webster (*SrWeb*)	Isr9a-Ra (*Sr9a*)	W2691Sr9b (*Sr9b*)	Vernstein (*Sr9e*)	CnSSr9g (*Sr9 g*)
IWA8478	73.3	L	P	L	L	L
IWA3935	73.5	L	P	L	L	L
IWA1389	73.7	L	P	L	L	L
IWA4948	73.5	L	P	L	L	L
IWA4890	73.7	L	P	L	L	L
IWA5064	73.7	L	P	P	P	LP
IWA933	74.1	L	P	P	L	LP
IWA8362	82.2	L	P	P	L	LP
IWA7620	82.2	L	P	P	L	LP
IWA6121	82.2	L	P	P	L	LP
IWA6122	82.2	L	P	P	L	LP
IWA8295	82.2	L	P	P	L	P

The DNA genotyped consisted of a bulk of five plants. The presence of the LMPG-6 or PI 626573 allele at a locus is indicated by an L of P, respectively. Loci where both alleles were present for a line are denoted by LP

locus 06g029180, and the *B. distachyon* locus 05g22410. There are numerous gene families that have been associated with disease resistance distally located from these loci in *O. sativa, S. bicolor,* and *B. distachyon*. Three of the syntenic genes of interest belong to the NBS-LRR gene family, which is commonly associated with race-specific resistance (Dubcovsky et al. 2012; Periyannan et al. 2013a, b; Saintenac et al. 2013). As such, future work will be focused on the positional cloning of *SrWLR.* To facilitate the positional cloning of *SrWLR,* a new population consisting of 6,382 recombinant gametes has been developed, and new markers based on syntenic sequences to *O. sativa*, *S. bicolor*, and *B. distachyon* are being mapped. The present study demonstrates the iSelect assay may not be appropriate for marker-assisted selection in the *SrWLR* region but is capable of efficiently fine mapping genes and establishing syntenic relationships in hexaploid wheat.

Acknowledgments The authors would like to thank Matt Breiland, Amanda Swank, Mary Osenga, Dawn Feltus, Richard Sonju, and Taylor Morgan for their technical support. This project was funded through the North Dakota Wheat Commission and USDA-ARS.

References

Acevedo M, Newcomb M, Rouse M, Bockelman HE, Goates BJ, Jackson EW, Jin Y, Brown-Guedira G, Kilian A, Njau P, Singh D, Wanyera R, Bonman JM (2011) Looking for a needle in a haystack: screening of the international stem rust nursery in Kenya for new sources of resistance in spring wheat expo landraces. In: Proceedings of the Borlaug Global Rust Initiative 2011 Technical Workshop, St. Paul, Minnesota, pp 140–143

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploidy wheat with the Illumina GoldenGate assay. Theor Appl Genet 119:507–517

Alnemer LM, Seetan RI, Bassi FM, Chitraranjan C, Helsene A, Loree P, Goshn SB, Gu YQ, Luo M, Iqbal J, Lazo GR, Denton AM, Kianian SF (2013) Wheat Zapper: a flexible online tool for colinearity studies in grass genomes. Funct Integr Genomics 13:11–17

Bonman JM, Bockelman HE, Jin Y, Hijmans RJ, Gironella A (2007) Geographic distribution of stem rust resistance in wheat landraces. Crop Sci 47:1955–1963

Cavanagh C, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira G, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson J, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell P, Dubcovsky J, Morell M, Sorrells M, Hayden M, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landrace and cultivars. PNAS 110:8057–8062

Dreisigacker S, Zhang P, Warburton ML, Skovmand B, Hoisington D, Melchinger AE (2005) Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. Crop Sci 45:653–661

Dubcovsky J, Saintenac C, Zhang W, Li C, Cantu D, Akunova A, Liang H, Rouse M, Akunov E (2012) New approaches to rust resistance in wheat. In: Book of abstracts for Plant and Animal Genome XX Conference, San Diego, CA 159

Endelman JB (2011) New algorithm improves fine structure of the barley consensus SNP map. BMC Genom 12:407

Fu B, Chen Y, Li N, Ma H, Kong Z, Zhang L, Jia H, Ma Z (2013) *pmX*: a recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi. Theor Appl Genet 126:913–921

Gurung S, Mamidi S, Bonman JM, Jackson EW, Del Rio LE, Acevedo M, Mergoum M, Adhikari TB (2011) Identification of novel genomic regions associated with resistance to *Pyrenophora tritici-repentis* races 1 and 5 in spring wheat landraces using association analysis. Theor Appl Genet 123:1029–1041

Hale IL, Mamuya I, Singh D (2013) Sr31-Virulent races (TTKSK, TTKST, and TTTSK) of the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* are present in Tanzania. Plant Dis 97:557

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 D (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 (2005) Races of *Puccinia graminis* Identified in the United States during 2003. Plant Dis 89:1125–1127

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 TTKSK of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096–1099

Knott DR (1990) Near-isogenic lines of wheat carrying genes for stem rust resistance. Crop Sci 30:901–905

Kolmer JA (2001) Early research on the genetics of *Puccinia graminis* and stem rust resistance in wheat in Canada and the United States. In: Peterson PD (ed) Stem rust of wheat: from ancient enemy to modern foe. American Phytopathological Society, St. Paul, MN, pp 51–82

Kolmer JA, Jin Y, Long DL (2007) Wheat leaf and stem rust in the United States. Aust J Agric Res 58:631–638

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microlinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to fusarium head blight in wheat. Funct Integr Genomics 6:83–89

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, East Melbourne, Australia

Nazari K, Mafi M, Yahyaoui A, Singh RP, Park RF (2009) Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK (Ug99) in Iran. Plant Dis 93:317

Newcomb M, Acevedo M, Bockelman HE, Brown-Guedira G, Goates B, Jackson EW, Jin Y, Njau PN, Rouse MN, Singh D, Wanyera R, Bonman M (2013) Field resistance to the Ug99 race group of the stem rust pathogen in spring wheat landraces. Plant Dis 97:882–890

Periyannan S, Moore J, Bariana H, Deal KR, Wang X, Luo M, Huang L, Ayliffe M, Bansal U, Kong X, Dodds P, Dvorak J, Lagudah E (2013a) Cloning of a broad spectrum stem rust resistance gene in wheat. In: Book of abstracts for plant and animal genome XXI conference, San Diego, CA, p 215

Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong X, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J, Lagudah E (2013b) The gene *Sr33,* an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. Science 341:786–788

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res Sect 26c:496–500

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

Reif JC, Zhang P, Dreisigacker S, Warburton ML, van Ginkel M, Hoisington D, Bohn M, Melchinger AE (2005) Wheat genetic diversity trends during domestication and breeding. Theor Appl Genet 110:859–864

Riede CR, Anderson JA (1996) Linkage of RFLP markers to and aluminum tolerance gene in wheat. Crop Sci 36:905–909

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

Roelfs A (1982) Effects of barberry eradication on stem rust in the United States. Plant Dis 66:177–181

Roelfs AP (1985) Wheat and Rye stem rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts vol II: diseases, distribution, epidemiology and control. Academic Press, Florida, pp 3–27

Roelfs AP, Singh RP, Saari EE (1992) Diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico City, Mexico

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

Rowell JB (1984) Controlled infection by *Puccinia graminis* f. sp. *tritici* under artificial conditions. In: Bushnell WR, Roelfs AP (eds) The cereal rusts, origins, specificity, structure, and physiology, vol 1. Academic Press, Florida, USA, pp 292–332

Saintenac C, Zhang W, Salcedo A, Rouse RN, 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 D, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel S, Ward RW (2008) Will stem rust destroy the world's wheat crop. Adv Agron 98:272–309

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

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. US Department of Agriculture Agricultural Research Service E-617

Steemers FJ, Gunderson KL (2005) Company profile: Illumina, Inc. Pharmacogenomics 6:777–782

Stewart N, Via L (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. Biotechniques 14:748–749

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tsilo TJ, Jin Y, Anderson JA (2007) Microsatellite markers linked to stem rust resistance allele *Sr9a* in wheat. Crop Sci 47:2013–2020

Villa TCC, Maxted N, Scholten M, Ford-Lloyd B (2005) Defining and identifying crop landraces. Plant Genet Resour 3:373–384

Warburton ML, Crossa J, Franco J, Kazi M, Trethowan R, Rajaram S, Pfeiffer W, Zhang P, Dreisigacker S, van Ginkel M (2006) Bringing wild relatives back into the family: recovering genetic diversity in CIMMYT improved wheat germplasm. Euphytica 149:289–301

Wu Y, Close TJ, Lonardi S (2011) Accurate construction of consensus genetic maps via integer linear programming. IEEE/ACM Trans Comput Biol Bioinf 8:381–394

Würschum T, Langer SM, Longin CFH, Korzun V, Akhunov E, Ebmeyer E, Schachschneider R, Schacht J, Kazman E, Reif JC (2013) Population Structure, genetic diversity and linkage disequilibrium in elite winter wheat assessed with SNP and SSR markers. Theor Appl Genet. doi:10.1007/s00122-013-2065-1

Xiao M, Song F, Jiao J, Wang X, Xu H, Li H (2013) Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. Theor Appl Genet 126:1397–1403