Consensus Mapping and Identification of Markers for Marker-Assisted Selection of *Wsm2* in Wheat

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ABSTRACT

A recently identified *Wheat streak mosaic virus* (WSMV) resistance gene *Wsm2* confers a high level of resistance. Objective of this study was to identify closely linked DNA markers for *Wsm2* for use in marker-assisted selection (MAS) in wheat (*Triticum aestivum* L.). Two segregating populations (CO960293-2 × 'TAM 111' and CO960293-2 × 'Yuma') of $F_{2:3}$ families were evaluated for response to WSMV infection in growth chamber experiments. Forty-eight simple sequence repeat (SSR) or sequence-tagged site (STS) markers were screened for polymorphism between the parents of both populations. In the CO960293-2 × TAM 111 population, five markers were mapped to the region of *Wsm2* with *XSTS3B-55* being the closest marker (5.2 cM distal to *Wsm2*). In the CO960293-2 × Yuma population, eight markers were linked to *Wsm2* with the closest marker *Xbarc102* linked at 3.9 cM proximal to *Wsm2*. Results from consensus mapping of the two populations suggested that *Xbarc102* was distal to *Wsm2*. The marker *Xbarc102* was associated with *Wsm2* in all 22 wheat lines derived from crosses between susceptible parents and either CO960293-2 or 'RonL' (also carrying *Wsm2*). The marker allele *Xbarc102-219-bp* present in CO960293-2 was amplified in polymerase chain reaction (PCR) from *Wsm2*-carrying genotypes CO960293-w133, RonL, and 'Snowmass' but not from the resistant line KS96HW10-3 (carrying *Wsm1*) or the susceptible genotypes 'Karl 92', 'TAM 107', and 'N96L9970'. Therefore, this marker should be useful for MAS of *Wsm2* in breeding programs.

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Abbreviations: DPI, days post inoculation; FHB, Fusarium head blight; LOD, logarithm of the odds; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait loci/locus; RH, relative humidity; SSR, simple sequence repeat; STS, sequence-tagged site; WSMV, *Wheat streak mosaic virus*.

WHEAT STREAK MOSAIC VIRUS (WSMV) belongs to the genus *Tritimovirus* and is found in most wheat (*Triticum aestivum* L.) growing regions in the world. The virus, transmitted by the wheat curl mite (*Aceria tosichella* Keifer) is a serious pathogen of wheat, especially winter wheat, in the Great Plains of North America (French and Stenger, 2003). Typical symptoms caused by WSMV on wheat are a chlorotic, streaking mosaic. Yield loss estimates specific to WSMV in the region range from 2.6 (Christian and Willis, 1993) to 5% (French and Stenger, 2003), although local disease outbreaks can result in total crop failure.

Wsm1 has been reported to confer resistance to WSMV (Friebe et al., 1991) and has been used for improvement of wheat (Baley et al., 2001; Graybosch et al., 2009; Sharp et al., 2002). It originated

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from intermediate wheatgrass [*Thinopyrum intermedium* (Host) Backworth & D. R. Dewey] and was transferred to wheat via a compensating Robertsonian translocation, where the short arm of wheat chromosome 4D was replaced by the short arm of the intermediate wheatgrass chromosome 4Ai#2, forming T4DL.4Ai#2S (Friebe et al., 1991; Wells et al., 1973, 1982). *Wsm1* is a temperature-sensitive gene, conferring a high level of resistance up to 18°C but being ineffective at 24°C (Seifers et al., 1995). Because of the large fragment of an alien chromosome in the wheat background, the T4DL.4Ai#2S translocation has deleterious effects on yield and bread-making quality (Seifers et al., 1995), which limits its application in wheat improvement. Efforts have been made to reduce the size of the alien chromosomal fragment while retaining the *Wsm1* gene in wheat (Friebe et al., 2009). More recently, CO960293-2, a winter wheat germplasm line developed by Colorado State University in Fort Collins, CO, was identified to have a high level of resistance to WSMV (Haley et al., 2002; Seifers et al., 2006). Genetic study and mapping work showed that the resistance in CO960293-2 was controlled by a single dominant gene different from *Wsm1*. This newly identified gene has been designated as *Wsm2* and located to chromosome 3BS (Lu et al., 2011). *Wsm2* is flanked by two simple sequence repeat (SSR) markers *Xgwm389* and *Xgwm566* at a 76.2 cM interval, but these flanking markers are too far from the gene and therefore are not useful for marker-assisted selection (MAS). Like *Wsm1*, *Wsm2* functions as a temperature-sensitive gene, effective against WSMV at 19°C or below (Lu et al., 2011; Seifers et al., 2006) but ineffective at 24°C (Seifers et al., 2006).

Wsm2 has been recently incorporated into the cultivars RonL (PI 648020; Seifers et al., 2007) and Snowmass (PI 658597; Haley et al., 2011). *Wsm2* appears to have originated from common wheat, and there is no evidence of deleterious impacts on yield and agronomic traits such as has been found with *Wsm1*. Identification of markers that are tightly linked to *Wsm2* will facilitate MAS to accelerate the transfer of *Wsm2* to improved wheat cultivars. This study was designed to (i) develop a saturated map in the 3BS chromosome region containing *Wsm2*, (ii) identify the closely linked markers to *Wsm2* for MAS, and (iii) validate the newly identified markers in selected advanced wheat breeding lines.

MATERIALS AND METHODS

Plant Materials

The population CO960293-2 × 'TAM 111' (PI 631352; Lazar et al., 2004) consisting of 188 $F_{2:3}$ families was previously used for mapping of WSMV resistance gene in CO960293-2, which resulted in identification of *Wsm2* and locating it to chromosome 3BS (Lu et al., 2011). TAM 111 is moderately susceptible and clearly distinguishable for its reaction to WSMV infection from CO960293-2. In the present study, the same population was used for saturation mapping of the *Wsm2* region. Another population of 142 $F_{2:3}$ families was developed from the cross of CO960293-2 × 'Yuma' (PI 559720) to verify the gene and the linked markers. Yuma is susceptible to WSMV. Molecular mapping in the CO960293-2 × TAM 111 population was conducted in Amarillo, TX, and the CO960293-2 × Yuma population was done in Fort Collins, CO.

Twenty-eight additional advanced breeding lines or wheat genotypes were used to validate the markers closely linked to *Wsm2* (Table 1). Among these, the WSMV resistant line KS96HW10-3 carries *Wsm1* (Seifers et al., 1995) while 'Karl 92', 'N96L9970', and 'TAM 107' are susceptible to WSMV. CO960293-w133 is a reselection of CO960293 (the line from which CO960293-2 was selected) and presumably contains *Wsm2* (T.J. Martin, unpublished data, 2010). The remaining 22 lines were advanced breeding lines containing either CO960293-2 or RonL in their pedigrees.

Inoculum Preparation

Wheat streak mosaic virus inoculum used in this study was the isolate Sidney 81, courtesy of Dr. D. Seifers (Kansas State University, Hays, KS). Preparation of the inoculum for screening the CO960293-2 × TAM 111 population and for use in validation of markers in Texas was described previously (Lu et al., 2011). Briefly, the leaf tissue was blended in a blender with 0.1 M potassium phosphate buffer (pH 7.4), filtered with cheesecloth, and mixed with silicon carbide powder. The prepared inoculum was used for mechanical inoculation of the Karl 92 wheat plants at two- to three-leaf stage using a spray paint gun. The infected plants were kept in a greenhouse for 2 wk and the plants showing symptoms were harvested for preparation of the inoculum for screening the population and for use in marker validation. The infected tissue was first tested by polymerase chain reaction (PCR) to confirm single infection of WSMV as described in Lu et al. (2011). Then 40 g of infected tissue were blended in a blender with 1 L of 0.1 M potassium phosphate buffer (pH 7.4), filtered with four layers of cheesecloth, and mixed with silicon carbide powder. The inoculum for screening the CO960293-2 × Yuma population in Colorado was prepared by propagating virus in 'Ankor' wheat (PI 632275; Haley et al., 2004). Briefly, isolate Sidney 81 was blended in a blender at a 1:10 (w/v) dilution in 0.02 M potassium phosphate buffer (pH 7.4), filtered through cheesecloth, and mixed with silicon carbide powder. The Ankor plants were mechanically inoculated at the one- to two-leaf stage with Sidney 81 inoculum using the finger-rub inoculation method (Seifers, 1992). Plants were kept in a growth chamber at 21°C with 12-h daylength. Symptomatic tissues were harvested at 28 d post inoculation (DPI) and frozen at −80°C until use. The tissues were tested by indirect enzyme-linked immunosorbent assay (ELISA) against Triticum mosaic virus and WSMV antiserum to confirm infection with WSMV using the procedure in Seifers et al. (2008). Inoculum was prepared from the thawed tissues of infected Ankor plants as described above and was applied to plants of the CO960293-2 × Yuma population using the finger-rub method.

Wheat Streak Mosaic Virus Screening

To screen the CO960293-2 × TAM 111 population, five seeds of each $F_{2:3}$ family, the parents, resistant checks KS96HW10-3

Table 1. Advanced wheat breeding lines and cultivars used for validation of markers in marker-assisted selection for *Wsm2*.

Line or cultivar	Pedigree	Response to WSMV infection†	*Xbarc102* 219 bp (VIC)‡	*Xbarc87* 119 bp (FAM)	*Xgwm493* 214 bp (NED)	Reference
CO960293-2	PI 222668/TAM 107//CO850034	R	+	+	+	Haley et al., 2002
CO960293-w133	PI 222668/TAM 107//CO850034	R	+	+	–	T.J. Martin, unpublished data, 2010
Snowmass	KS96HW94//Trego/CO960293-2	R	+	+	+	Haley et al., 2011
KS96HW10-3 (*Wsm1*)	KS97HW29/KS97HW131//KS96HW100-5	R	–	+	–	Seifers et al., 2003
Karl 92	Plainsman VV3/Kaw/Atlas 50V//Parker/Agent	S	–	+	–	Sears et al., 1997
N96L9970	GRS1201/TAM 202	S	–	–	–	Graybosch et al., 2004
TAM 107	TAM 105*4/Amigo	S	–	–	–	Porter et al., 1987
7001	KS025580(TREGO/CO960293-2)/KS02HW119 (95HW431(RB/89H33)/JGR8W//LAKIN SIB)	R	+	+	+	T.J. Martin, this study
7004	KS025580(TREGO/CO960293-2)/KS02HW25 (TGO/JGR 8W)	R	+	+	+	T.J. Martin, this study
7007	KS025597(TREGO/CO960293-2)//KS00HW152-1-4(94H871//VTA/94HW301)/KS02HW174 (FIDEL/KS97HW153//KS97HW349)	R	+	+	+	T.J. Martin, this study
7010	KS025597(TREGO/CO960293-2)//KS00HW152-1-4(94H871//VTA/94HW301)/KS02HW174(FIDEL/KS97HW153//KS97HW349)	R	+	+	+	T.J. Martin, this study
7019	KS03HW154(TREGO/CO960293-2)/KS03HW1(FIDEL/97HW150//97HW349/3/TGO)	R	+	+	+	T.J. Martin, this study
7022	KS03HW154(TREGO/CO960293-2)/KS03HW1(FIDEL/97HW150//97HW349/3/TGO)	R	+	+	+	T.J. Martin, this study
7025	KS03HW154(TREGO/CO960293-2)/KS03HW1(FIDEL/97HW150//97HW349/3/TGO)	R	+	+	+	T.J. Martin, this study
7028	DANBY/KS03HW149-1(TREGO/CO960293-2)	R	+	+	+	T.J. Martin, this study
7031	DANBY/KS03HW149-1(TREGO/CO960293-2)	R	+	+	–	T.J. Martin, this study
7046	JAGALENE/KS01HW163-4(TREGO/BTY SIB)// KS03HW149(TREGO/CO960293-2)	R	+	+	–	T.J. Martin, this study
6030	04-5421(99-5011/3/FIDEL/KS97HW153// KS97HW349)//KS03HW157-4(TREGO/CO960293-2)/KS02HW118-1(95HW431/JGR8W//TREGO)	R	+	+	+	T.J. Martin, this study
5141	KS04HW101-3(98HW423(JGR/93HW242)/98HW170 (ARL/WGRC15))/RonL	R	+	+	+	T.J. Martin, this study
5147	KS04HW101-3(98HW423(JGR/93HW242)/98HW170 (ARL/WGRC15))/RonL	R	+	+	+	T.J. Martin, this study
CO07W380	Burchett/CO960293-2//Stanton	R	+	+	+	S.D. Haley, this study
CO07W607	JAGALENE/KS03HW149-1 (TREGO/CO960293-2)	R	+	+	+	S.D. Haley, this study
CO07W614	JAGALENE/KS03HW122 (LAKIN/ TGO//96HW71)//03-6149 (TREGO/CO960293-2)	R	+	+	+	S.D. Haley, this study
CO07W607-F1	JAGALENE/KS03HW149-1 (TREGO/CO960293-2)	R	+	+	+	S.D. Haley, this study
CO07W607-F2	JAGALENE/KS03HW149-1 (TREGO/CO960293-2)	R	+	+	+	S.D. Haley, this study
6029	04-5421 (99-5011/3/FIDEL/KS97HW153// KS97HW349)//KS03HW157-4 (TREGO/CO960293-2)/KS02HW118-1 (95HW431/JGR8W//TREGO)	S	–	–	–	T.J. Martin, this study
5143	KS04HW101-3 (98HW423(JGR/93HW242)/ 98HW170 (ARL/WGRC15))/RonL	S	–	+	–	T.J. Martin, this study
5148	KS04HW101-3 (98HW423(JGR/93HW242)/98HW170(ARL/WGRC15))/RonL	S	–	+	–	T.J. Martin, this study
5150	KS04HW101-3(98HW423(JGR/93HW242)/98HW170 (ARL/WGRC15))/RonL	S	–	+	–	T.J. Martin, this study

†R, resistance to *Wheat streak mosaic virus* (WSMV); S, susceptibility to WSMV.

‡VIC, FAM, and NED are dyes used to visualize the marker alleles in the ABI PRISM 3730 DNA Analyzer system (Applied Biosystems, Foster City, CA). +, presence of the marker allele; –, absence of the marker allele.

and 'Mace' (both carrying *Wsm1*), susceptible check Karl 92, and the Texas cultivar TAM 112 (PI 643143) were germinated in half of the 30-cm row in 30 × 50 cm plastic flats with 11 rows per flat. The families along with the six genotypes were grown in flats with a RCBD with two replications. The experiments were performed twice with a total of 20 seeds grown per F_3 family. The flats were placed in a greenhouse at Texas AgriLife Research, Bushland, TX. At the two- to three-leaf

stage, the plants were mechanically inoculated with inoculum using a spray paint gun at 4 kg cm^{-1}. The flats were moved into a growth chamber and kept at 19°C day time, 17°C night time, 15 h daylength, and 40% relative humidity (RH). Each plant was scored as resistant or susceptible at 28 DPI using the method described in Lu et al. (2011).

For screening the CO960293-2 × Yuma population, each $F_{2:3}$ family, parents, resistant controls RonL (*Wsm2*) and KS96HW10-3 (*Wsm1*), and susceptible controls 'Tomahawk' (PI 552814) and Ankor were planted in 30 by 50 cm plastic flats. Twelve seeds of each family and control were sown in each row with 12 rows per flat. The experiment was arranged in a RCBD design with two replications. Flats were held in growth chambers at 17°C with 12 h daylength and 40% RH at Colorado State University in Fort Collins, CO. At the one- to two-leaf stage, plants were inoculated with WSMV inoculum using the finger-rub method described in Seifers (1992). Plants were scored as resistant or susceptible at 28 DPI using the rating scales in Lu et al. (2011).

In the marker validation experiment, 10 seeds per entry (Table 1) were germinated in a 30-cm row in 30 by 50 plastic flats. Each flat held 11 rows. The experiment was conducted using a RCBD design with three replications. Therefore, a total of 30 seeds of each line or cultivar were tested. Flats were placed in a greenhouse at Texas AgriLife Research, Bushland, TX. At the two- to three-leaf stage, plants were mechanically inoculated using a spray paint gun at 4 kg cm^{-1}. The flats were moved to a growth chamber with the environmental conditions the same as those used in Lu et al. (2011).

Molecular Marker Analysis

Deoxyribonucleic acid extraction of the CO960293-2 × TAM 111 population was described previously (Lu et al., 2011). For the CO960293-2 × Yuma population, leaf tissues were collected from each F_2 plant used to develop the $F_{2:3}$ families. Approximately 10 cm of leaf tissue from each F_2 individual was placed into a single well in a 96-deep-well plate. Tissues were lyophilized for approximately 48 h and ground to a fine powder using a 4.5 mm steel bead with 2 min of agitation at 30 cycles per sec on a Mini Bead-Beater (Biospec Products, Bartlesville, OK). Total genomic DNA was extracted from the ground tissues using a sodium dodecyl sulfate (SDS)-based DNA extraction procedure (KS-USDA Genotyping Laboratory, Manhattan, KS). The DNA pellets were resuspended in 200 μL autoclaved double distilled H_2O and allowed to sit at 4°C overnight. The DNA concentrations were adjusted to 100 ng μL^{-1} for use in PCR. For marker validation, 1 cm of leaf tissue was cut from each of 10 seedlings of each cultivar and advanced breeding line at the two-leaf stage. Leaf tissues from the 10 seedlings were bulked and placed into a single well of a 96-deep-well plate. Deoxyribonucleic acid was extracted as described in Lu et al. (2011) and diluted to 100 ng μL^{-1} with sterile water.

Bulked segregant analysis (Michelmore et al., 1991) was conducted in both populations. The WSMV-resistant bulk samples were produced by pooling equal amounts of DNA from five to six F_2 plants whose F_3 families were homozygous resistant for WSMV while equal amount of DNA from five to six F_2 plants producing homozygous susceptible F_3 families were pooled to form the WSMV-susceptible bulk samples. Simple sequence repeat and sequence-tagged site (STS) markers between the flanking SSR markers *Xgwm389* and *Xgwm566* were selected for screening the parents and the bulks for polymorphism. A total of 48 SSR and STS markers (Supplemental Table S1) were tested. The primers information was obtained from the USDA website (USDA-ARS, 2010) and primers were commercially synthesized. Each 10 μL PCR reaction contained 1x PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphate (dNTP) mix, 200 nM reverse primer, 100 nM of M13-tailed forward primer, 1U *Taq* DNA polymerase, 100 ng template DNA, and 100 nM of M13 primers labeled with one of the following dyes: 6-FAM, NED, PET, or VIC (Applied Biosystems, Foster City, CA). Reactions were performed in a PTC-100 MJ Thermal Cycler (MJ Research, Watertown, MA) with a touchdown program starting with 5 min at 95°C; five cycles of 1 min at 96°C, 5 min at 68°C with the annealing temperature being decreased by 2°C per cycle, and 1 min at 72°C; followed by five cycles of 1 min at 96°C, 2 min at 58°C with annealing temperature decreased by 2°C per cycle, and 1 min at 72°C; and then 25 cycles of 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C with a final extension of 5 min at 72°C. Three microliters of the PCR products labeled with four different fluorescent dyes were pooled together with formamide plus GeneScan500 LIZ sizing standard (Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

Data Analysis

Data files obtained from the ABI PRISM 3730 DNA Analyzer were analyzed with GeneMarker version 1.6 (SoftGenetics, 2010) and rechecked manually for accuracy. Individual linkage groups from the two populations and the consensus map were constructed with JoinMap 3.0 (Van Ooijen and Voorrips, 2001) using a logarithm of the odds (LOD) threshold of 3.0 and the Kosambi mapping function (Kosambi, 1944).

RESULTS

Genetic Linkage among Markers and *Wsm2*

In the previous study, F_3 families showed 1 (homozygous *Wsm2*):2 (segregating):1 (homozygous *wsm2*) segregation ratio ($\chi^2 = 1.86$, $p = 0.39$) in the CO960293-2 × TAM 111 population (Lu et al., 2011). In the CO960293-2 × Yuma population, only 106 $F_{2:3}$ families were used for disease scoring while the remaining 36 families grew poorly and the data were not usable. For 106 $F_{2:3}$ families scored for disease reaction, 30 showed a homozygous resistant reaction, 17 showed a homozygous susceptible reaction, and 59 showed a segregating reaction to WSMV infection. This segregation also fits a single gene inheritance ($\chi^2 = 4.83$, $p = 0.09$) in the CO960293-2 × Yuma population.

Among the 48 markers screened for polymorphism between the parents and the bulks, five showed polymorphism between CO960293-2 and TAM 111 and also between the bulks. The five markers were then used to genotype 188 F_2 individuals in the CO960293-2 × TAM 111 population. All five markers were mapped between *Xgwm389* and *Xgwm566*, the flanking markers for *Wsm2* locus. All five markers were distal to *Wsm2* with *XSTS55* being the closest (5.2 cM from *Wsm2*) (Fig. 1). In the CO960293-2

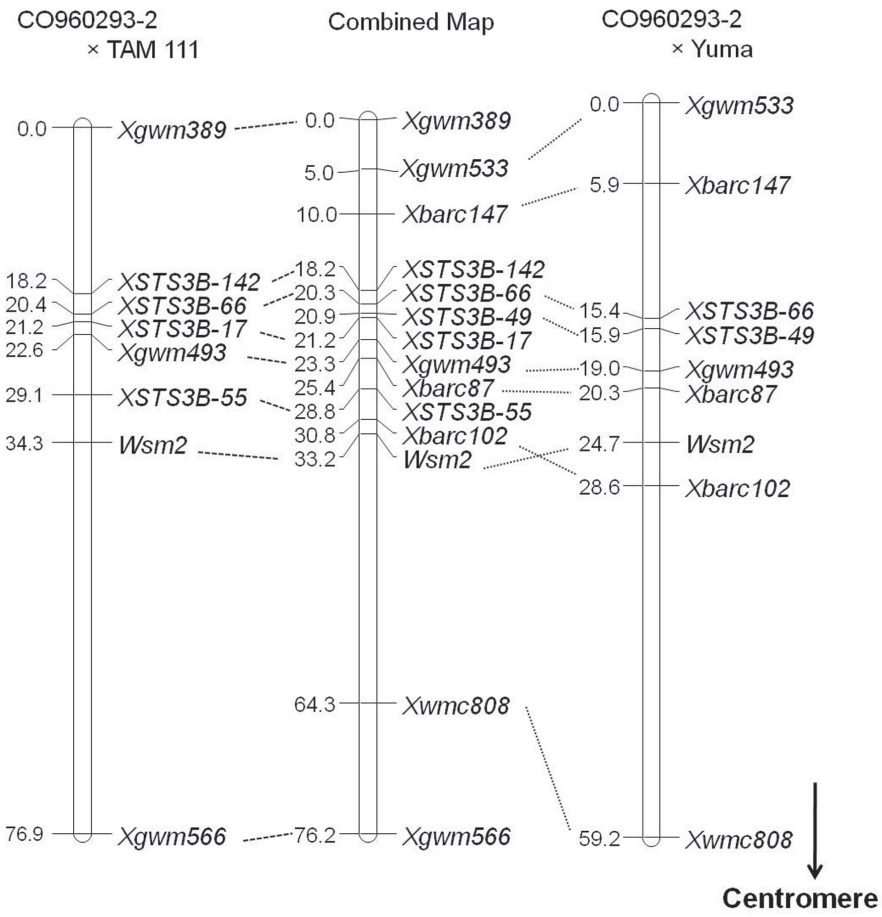

Figure 1. Genetic linkage maps constructed in wheat (*Triticum aestivum* L.) population CO960293-2 × TAM 111 consisting of 188 $F_{2:3}$ families and population CO960293-2 × 'Yuma' consisting of 142 $F_{2:3}$ families and the combined map. Numbers to the left of each linkage group are accumulated genetic distances in centimorgans. Markers and the gene *Wsm2* are shown to the right of each linkage map.

× Yuma population, eight markers were polymorphic and were linked to *Wsm2* (Fig. 1). Six markers were distal to the *Wsm2* locus and two markers were mapped proximally to *Wsm2*. Thus, two new flanking markers (*Xbarc102* and *Xbarc87*) were identified as 8.3 cM apart. The linkage maps from the two populations had two markers (*Xgwm493* and *XSTS3B-66*) in common. In the CO960293-2 × TAM 111 population, *XSTS3B-66* was 13.9 cM and *Xgwm493* was 11.7 cM from *Wsm2* whereas in the CO960293-2 × Yuma population, *XSTS3B-66* was 9.3 cM and *Xgwm493* was 5.7 cM from *Wsm2*. A consensus map of both populations constructed using JoinMap (Van Ooijen and Voorrips, 2001) contained 13 markers. *Xwmc808* was 31.1 cM and *Xgwm566* was 43 cM proximal to *Wsm2* whereas all other markers were distal to *Wsm2* with *Xbarc102* being the closest to *Wsm2* (2.4 cM). In the combined map, the order of *Xbarc102* and *Wsm2* was swapped in comparison with the map order in the CO960293-2 × Yuma population.

Validation of Markers in Different Genetic Backgrounds

Twenty-two advanced breeding lines developed by the wheat breeding programs at Agricultural Research Center, Kansas State University, Hays, KS, and at Colorado State University, Fort Collins, CO, were tested for their responses to WSMV infection. CO960-293-2 or RonL were ancestors in the pedigrees of these lines. Eighteen lines were resistant and four were susceptible. As expected, *Wsm2*-carrying genotypes CO960293-2, CO960293-w133, and Snowmass and *Wsm1*-carrying genotype KS96HW10-3 were resistant to WSMV while Karl 92, N96L9970, and TAM 107 were susceptible to WSMV (Table 1).

Four codominant markers were screened in the 22 advanced breeding lines. CO960293-2 had a 219-bp fragment at Xbarc102, a 202-bp fragment at XSTS3B-55, a 119-bp fragment at Xbarc87, and a 214-bp fragment at Xgwm493. The 219-bp fragment of *Xbarc102* was present in all resistant accessions except KS96HW10-3, in which *Wsm1* is responsible for the resistance (Fig. 2). All susceptible advanced breeding lines and controls lacked the 219-bp fragment of *Xbarc102* (Table 1). Therefore, *Xbarc102-219-bp* cosegregated with *Wsm2* among these accessions. *Xbarc87* correctly predicted presence or absence of *Wsm2* in 22 of 25 genotypes tested (88%) including 22 breeding lines, CO960293-2, CO960293-w133, and Snowmass. The 119-bp allele of *Xbarc87* was also present in KS97HW10-3 (*Wsm1*) and Karl 92 but not in N96L9970 or TAM 107. A similar result as *Xbarc87* was obtained for *Xgwm493*

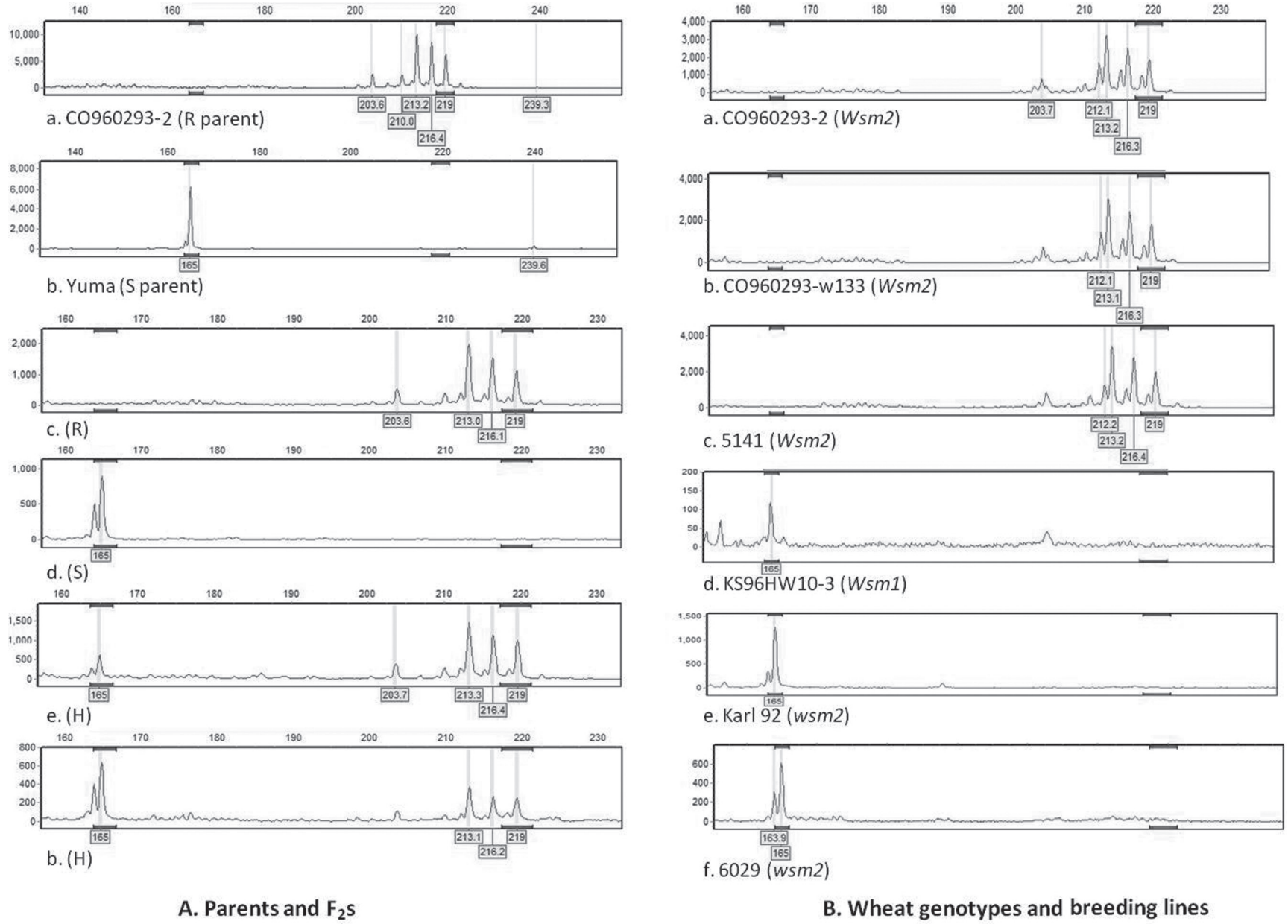

Figure 2. ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) electrophorograms of simple sequence repeat marker *Xbarc102* on chromosome 3BS showing (A) segregation in the CO960293-2 × 'Yuma' population and (B) polymorphism among different wheat genotypes and breeding lines. The allele sizes in the figure were 219 bp for *Wsm2* and 165 bp for *wsm2*. R, resistant; S, susceptible; H, heterozygous.

except that the 214-bp fragment of *Xgwm493* was absent in KS96HW10-3, Karl 92, and CO960293-w133. Although RonL was not included in the marker validation experiment, it amplified the marker alleles *Xbarc102-219-bp*, *Xbarc87-119-bp*, and *Xgwm493-214-bp* in a separate PCR run. Primer STS3B-55 for *XSTS3B-55* failed to amplify the targeted fragment in these accessions and was therefore excluded from further analysis.

DISCUSSION

CO960293-2 was a selection from the cross PI 222668/TAM 107//CO850034 and released as a germplasm source for resistance to Russian wheat aphid (*Diuraphis noxia* Kurdjumov) and WSMV (Haley et al., 2002). Russian wheat aphid resistance was presumably inherited from PI 222668 but the origin of the WSMV resistance is unknown since none of the parents of CO960293-2 are known to have resistance to WSMV (Haley et al., 2002). Some early preliminary studies showed that WSMV resistance in CO960293-2 might be controlled by a different gene than *Wsm1* (Haley et al., 2002; Seifers et al., 2006). Initial genetic study of resistance using the CO960293-2 × TAM 111 population confirmed this assumption and found that the WSMV resistance was conditioned by a dominant gene on wheat chromosome 3BS designated as *Wsm2* (Lu et al., 2011). In this study, the segregation ratio of resistance to susceptibility in the CO960293-2 × Yuma population also supported the previous results and confirms that *Wsm2* is responsible for resistance in CO960293-2.

In the previous map, *Wsm2* was flanked by two remote SSR markers *Xgwm389* and *Xgwm566* at an interval of 76.2 cM (Lu et al., 2011). Here, we added 11 more markers to the linkage group (Fig. 1). Although the markers *Xgwm533*, *Xbarc147*, and *Xwmc808* were mapped at genetic distances greater than 20 cM from *Wsm2* in the CO960293-2 × Yuma population and also in the combined linkage map, most of the markers identified in this study were close to *Wsm2*. The closest marker to *Wsm2* was *Xbarc102*, which was mapped proximally to *Wsm2* at 3.9 cM in the CO960293-2 × Yuma population but placed 2.4 cM distally to *Wsm2* in the consensus map. The discrepancy in marker order between the two maps may have been caused by the populations used and the position of crossovers along chromosomes within the progeny lines as explained in Somers et al. (2004). Nevertheless, the order change between *Wsm2* and *Xbarc102* in different maps should not adversely affect the application of the marker in MAS.

The marker order, *Xgwm493–Xbarc87–XSTS-3B55–Xbarc102*, in the consensus map is in agreement with that of Liu and Anderson (2003) except that in the latter *XSTS3B-55* and *Xbarc102* cosegregated. Based on the consensus map of Somers et al. (2004), *Xgwm493* and *Xbarc87*, which have the same order as in the present study, are located in the distal 30% portion of the short arm of chromosome 3B. Therefore, *Wsm2* is in the distal 30% portion of 3BS shown in the map of Somers et al. (2004).

In addition to *Wsm2*, chromosome 3BS harbors some important disease resistance genes such as the durable stem rust (*Puccinia graminis* f. sp. Tritici) gene *Sr2* and the Fusarium head blight (FHB) (*Fusarium graminearum*) resistance quantitative trait loci (QTL) *Fhb1*. *Sr2* was originally identified in cultivar Hope (CItr 8178; Hare and McIntosh, 1979) and was later mapped to the interval of *Xgwm389* and *Xgwm533* on 3BS (Spielmeyer et al., 2003). *Fhb1* is present in the Chinese cultivar Sumai 3 (PI 481542) and is a major QTL for FHB resistance. Using the recombinant inbred lines (RILs) derived from the cross of Sumai 3 × 'Stoa', Liu and Anderson (2003) found that the peak of LOD plots for FHB resistance was near the cosegregating markers *XSTS3B-138* and *XSTS3B-52*. In their map, *XSTS3B-138* and *XSTS3B-52* were proximal to *Xgwm389* and *Xgwm533* but distal to *XSTS3B-55* and *Xbarc102* (Liu and Anderson, 2003). These comparisons of map positions among the markers and genes suggest that *Fhb1* is proximal to the *Sr2* locus and the *Wsm2* locus is proximal to the *Fhb1* locus, giving a relative map order of *Sr2– Fhb1– Wsm2*–centromere. Therefore, it should be possible to pyramid the three genes into a single cultivar providing multiple diseases resistance. This would be highly desirable since stem rust, FHB, and wheat streak mosaic (WSM) may occur in the same wheat growing season in the Great Plains of North America.

Usefulness of a marker in MAS is dependent on the polymorphism level and genetic distance between the marker and the gene of interest. Markers tightly linked to target genes and having sufficient polymorphism have been identified and used for MAS in wheat. Examples include markers for the stem rust resistance gene *Sr2* (McNeil et al., 2008), leaf rust (*Puccinia triticina* Eriks.) resistance gene *Lr42* (Sun et al., 2010), Hessian fly (*Mayetiola destructor* Say) resistance gene *H32* (Yu et al., 2010), and the tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs.] susceptibility gene *Tsn1* (Lu et al., 2006). In this study, three of the four tested markers predicted presence or absence of *Wsm2* in advanced breeding lines at 88% or higher accuracies. The marker allele *Xbarc102-219-bp* was the best predictor for *Wsm2* with 100% accuracy. Furthermore, the 219-bp fragment was present in CO960293-2, CO960293-w133, Snowmass, and RonL but absent in KS96HW10-3, Karl 92, N96L9970, and TAM 107 indicating that polymorphism for *Xbarc102* exists in different germplasm lines. Marker *XSTS3B-55* was mapped 5.2 cM distal to *Wsm2* in the CO960293-2 × TAM 111 population. However, the primer STS3B-55 failed to amplify the targeted 202-bp fragment in our MAS test. This inconsistency indicates that STS3B-55 may require optimized PCR conditions for reliable use.

CONCLUSIONS

Genetic analysis in two segregating populations confirmed previous studies indicating that WSMV resistance in hard winter wheat line CO960293-2 is controlled by a single dominant gene *Wsm2*. *Wsm2* is located in the distal 30% portion of wheat chromosome 3BS. Of the 13 molecular markers mapped in the *Wsm2* region in two populations, *Xbarc102* and *XSTS3B-55* are the closest makers to *Wsm2*. In this study, *Xbarc102* predicted the presence of *Wsm2* at 100% accuracy among a collection of advanced breeding lines. *Wsm2* has been deployed in the recently released cultivars RonL and Snowmass to protect wheat crops from damage by *Wheat streak mosaic virus*. The markers closely linked to *Wsm2* identified in this study will help wheat breeders to accelerate the breeding process of transferring *Wsm2* to adapted cultivars.

Supplemental Information Available

Supplemental material is available at http://www.crops.org/publications/cs.

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