A Dominant Gene for Resistance to *Wheat Streak Mosaic Virus* in Winter Wheat Line CO960293-2

Huangjun Lu, Jacob Price, Ravindra Devkota, Charlie Rush, and Jackie Rudd*

ABSTRACT

Wheat streak mosaic (WSM), caused by *Wheat streak mosaic virus* (WSMV), is a devastating disease in wheat (*Triticum aestivum* L.) in the Great Plains of North America. Use of resistance is an effective and environmentally sound method to control the disease. In this study, six wheat genotypes were compared for their responses to WSMV infection under growth chamber conditions. The three resistant genotypes, KS96HW10-3 (*Wsm1*), Mace (*Wsm1*), and CO960293-2, had disease scores significantly lower than the remaining three genotypes without major resistance. Disease in TAM 111 and TAM 112 was consistently less severe than Karl 92. A population consisting of 188 $F_{2:3}$ families derived from the cross CO960293-2 × TAM 111 was used for determining inheritance of the WSMV resistance and for molecular mapping of the resistance in CO960293-2. Data on segregation of resistance indicated that the resistance in CO960293-2 is conditioned by a single dominant gene, which was named *Wsm2*. Transgressive segregation toward susceptibility occurred in the population suggesting a minor gene in the moderately susceptible parent TAM 111, which was not allelic to *Wsm2*. *Wsm2* was mapped to the short arm of chromosome 3B with two flanking simple sequence repeat markers. The single dominant gene inheritance for WSMV resistance in CO960293-2 has been consistent with the observations that the resistance can be readily transferred to adapted cultivars.

Texas AgriLife Research and Extension Center at Amarillo, 6500 Amarillo Blvd. West, Amarillo, TX 79106. Received 26 Jan. 2010. *Corresponding author (jcrudd@ag.tamu.edu).

Abbreviations: ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; LSD, least significant difference; MAS, marker-assisted selection; PCR, polymerase chain reaction; SSR, simple sequence repeat; WSM, wheat streak mosaic; WSMV, *Wheat streak mosaic virus*.

Wheat streak mosaic (WSM) is an economically important disease of wheat (*Triticum aestivum* L.) in the Great Plains of North America (Burrows et al., 2009). This disease is caused by *Wheat streak mosaic virus* (WSMV), which is transmitted by the wheat curl mite (*Aceria tosichella* Keifer). There is no chemical treatment available for the disease once the virus infects the plant. Infection can occur throughout the wheat growing season. Fall infection is a problem for winter wheat and results in more yield reduction than spring infection (Hunger, 2004). Annual yield losses caused by WSM were estimated to range from 2.6% (Christian and Willis, 1993) to 5% (French and Stenger, 2003). An outbreak of WSM in Alberta in 1964 resulted in 18% reduction in winter wheat yield (Atkinson and Grant, 1967) and the 1988 epidemic in Kansas caused an estimated loss of 13% of the wheat crop (Sim et al., 1988). It is not rare that crop damage in individual fields exceeds 30%, and in the most severe cases entire fields can be lost. *Wheat streak mosaic virus* also has significantly adverse effects on forage yields and water use efficiency of the infected plants (Price et al., 2010; Workneh et al., 2010).

The use of genetic resistance can be an effective means of controlling WSM. There are primarily two resistance sources

Published in Crop Sci. 51:5–12 (2011).
doi: 10.2135/cropsci2010.01.0038
Published online 18 Oct. 2010.

that are being used by wheat breeders for development of WSMV resistant wheat cultivars. The gene *Wsm1*, transferred to wheat from intermediate wheatgrass {*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey [*Agropyron intermedium* (Host) P. Beauv.]} (Friebe et al., 1991; Gill et al., 1995; Wells et al., 1973, 1982), confers resistance to WSMV. *Wsm1* has been introduced into the cultivar Mace (Graybosch et al., 2009) and a number of germplasm lines. The other source of resistance was identified in germplasm line CO960293-2 (Haley et al., 2002) and has been incorporated into the hard winter wheat cultivars RonL (Seifers et al., 2007) and Snowmass (Haley et al., 2010). CO960293-2 was developed from the cross PI 222668/TAM 107//CO850034. The origin of WSMV resistance in CO960293-2 is unclear because none of its parents were found to be resistant to WSMV in greenhouse or growth chamber experiments (Haley et al., 2002; Seifers et al., 2006). Some data indicated that this resistance was different from that conferred by *Wsm1* (Haley et al., 2002), but inheritance of WSMV resistance in CO960293-2 has not been determined. A recent report (Seifers et al., 2007) suggested a new source of resistance present in KS03HW12.

In addition to resistance, tolerance and disease escape are also useful in breeding programs for development of cultivars that have less yield reduction when WSM is epidemic. Divis et al. (2006) found that the wheat cultivar Millennium, which is susceptible to WSMV, had significantly higher yield than other cultivars under natural epidemic of WSM. Because WSM frequently becomes epidemic in the major wheat growing areas of Texas, such as the Panhandle and Rolling Plains, development of wheat cultivars with resistance or tolerance is an important breeding objective in the Texas Wheat Improvement Program. Among the cultivars released from this program, TAM 111 and TAM 112 consistently have reduced incidence and severity of WSM symptoms than other cultivars in field trials. It is unknown if this phenomenon is due to disease tolerance, partial resistance or tolerance to the vector, or some other mechanism.

The objectives of the present study were to (i) compare the virus resistance of TAM 111 and TAM 112 with CO960293-2 and the genotypes carrying *Wsm1*, (ii) determine the inheritance of WSMV resistance present in germplasm line CO960293-2, and (iii) identify the chromosome location of the resistance gene in CO960293-2.

MATERIALS AND METHODS

Plant Materials

Wheat genotypes used for evaluation of responses to WSMV infection included 'Karl 92' (Sears et al., 1997), TAM 111 (Lazar et al., 2004), TAM 112 (PI 643143), CO960293-2, KS96HW10-3 (Seifers et al., 2003), and Mace. KS96HW10-3 and Mace carry the WSMV resistance gene *Wsm1*. Karl 92 is highly susceptible to WSMV. A population for genetic study and molecular mapping of WSMV resistance in CO960293-2 consisted of 188 $F_{2:3}$ families derived from a cross between CO960293-2 (female parent) and TAM 111. Although TAM 111 shows fewer symptoms of WSM than the susceptible cultivars like Karl 96 in field trials, CO960293-2 and TAM 111 are clearly distinguishable from each other for their responses to WSMV.

Preparation of WSMV Inoculum

The Sidney 81 isolate (infected wheat leaf tissue) of WSMV, kindly provided by Dr. D. Seifers (Kansas State University, Manhattan, KS), was used for virus propagation. 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 Karl 92 wheat plants at two- to three-leaf stage were mechanically inoculated with the above prepared virus using a spray paint gun. The infected plants were kept in a greenhouse for disease to develop. About 2 wk after inoculation, the plants showing symptoms were harvested for preparation of the virus inoculum for the experiments. Inoculum was prepared as described in Martin (1978) with minor modifications. Briefly, 40 g of infected wheat tissue was blended in a blender with 1 L of 0.1 M potassium phosphate buffer (pH 7.4). The inoculum was filtered with four layers of cheesecloth and mixed well with silicon carbide powder.

A subsample of infected tissue was tested by polymerase chain reaction (PCR) to confirm infection by WSMV. The primers used in PCR were specific for the Nib region of the WSMV RNA genome: forward primer WSF2 (5′-GCGCCATAGTAGCAGTAGCAGACC-3′) and reverse primer WSR3 (5′-CACGCCTCTCGTGGAGAAGT-3′). Virus RNA was extracted from infected tissue using an RNeasy mini kit from Qiagen (Valencia, CA). The sample was then quantified and brought to 500 μg concentration for reverse transcription using an Omniscript RT kit also from Qiagen, following the manufacturer's suggested protocol. Polymerase chain reaction amplification was performed using an EX *Taq* polymerase PCR kit from Takara (Shiga, Japan) in a 25-μL volume including 1× EX *Taq* PCR buffer, 0.2 μM dNTP, 0.02 U of Takara *Taq* polymerase, and 2.0 μL of RT product. Amplification was performed on a DNA Engine PTC-200 Thermo Cycler (MJ Research, Ramsey, MN) as follows: an initial denaturation at 95°C for 3 min; then 35 cycles of 15 s at 95°C, 30 s at 68°C, and 3 min at 72°C; and a final extension at 72° for 10 min. The amplified product (2.8 kb) was resolved in a 1% agarose gel and visualized with ethidium bromide under UV light.

WSMV Infection and Disease Scoring

The six wheat genotypes were evaluated for their responses to WSMV infection using a randomized complete block design. The experiments with three replications in each experiment were conducted two times (referred to as time effect in ANOVA), one from 6 Jan. to 9 Mar. 2009 and the other from 20 Feb. to 21 Apr. 2009 at Texas AgriLife Research, Bushland, TX. Ten seeds per replication of each genotype except for CO960293-2 were germinated in two 15-cm rows (five seeds per row), with 22 rows in 30 by 50 cm plastic flats. Due to seed limitation, only five seeds of CO960293-2 were planted in a single row in one of the two experiments. The flats were placed in a greenhouse with the temperature between 18 and 25°C and light intensity between 300 and 800 μmol m^{-2} s^{-1}.

For the segregating population, five seeds of each F_3 family, the parents, resistant checks KS96HW10-3 and Mace, susceptible check Karl 92, and the Texas cultivar TAM 112 were planted in half of the 30-cm row in the plastic flats. The families along with the six genotypes were randomly grown in the flats. The same group of seeds was planted in two replications. The experiments for the segregating population were also performed for two times with a total of 20 seeds grown per F_3 family.

After 3 wk, the plants (at two- to three-leaf stage) were mechanically inoculated with inoculum using a spray paint gun (at 60 psi). The flats were moved into a growth chamber after inoculation. Infected plant materials were kept in the growth chamber at 19°C day time, 17°C night time, 15-h daylength (a light intensity of 600 μmol m^{-2} s^{-1}), and 40% relative humidity.

To understand progression and development of disease, each plant in the experiments, involving the six genotypes, was rated for disease severity 3, 4, 5, and 6 wk after inoculation. The plants were scored as described in Thomas et al. (2004) with modifications: 0 (no symptoms), 1 (one to a few chlorotic streaks on only one leaf of a plant), 2 (symptoms on <25% leaf area of a plant), 3 (symptoms on 25–50% of leaf area), or 4 (symptoms on >50% leaf area). Plants with scores 0 or 1 were categorized as resistant, 2 as moderately susceptible, and 3 or 4 as susceptible.

For the segregating population, the rating for the severity of WSM was done using the same scoring method at 4 and 6 wk after inoculation. The raw data suggested qualitative inheritance of the resistance in CO960293-2. Thus the plants having scores 0 or 1 were categorized as resistant and scores ≥2 as susceptible.

Quantitative Enzyme-Linked Immunosorbent Assay

After the first rating, 5 cm of leaf tissue (~0.03–0.04 g) was cut from each plant in one experiment, including 188 F_3 families and the parents, KS96HW10-3, Mace, Karl 92, and TAM 112, and was put into 96-deep-well plates with a 5-mm steel bead in each well. The leaf tissue was frozen with liquid nitrogen and ground into fine powder in a high throughput homogenizer (Troemner LLC, Thorofare, NJ). After grinding 500 μL of sterile double-distilled water was added to each well in preparation for quantitative enzyme-linked immunosorbent assay (ELISA). Tests were conducted using a WSMV ELISA testing kit and the accompanying procedure from Agdia (Agdia Inc., Elkhart, IN) and included four standards of known virus concentrations to quantify virus content within each sample. Absorbance data from each sample was compared to that of the known standards to determine virus concentration of the sample.

Virus standards were prepared from infected plants of Karl 92. Seeds of Karl 92 were planted in a greenhouse. At Feeke's scale 3 (Large, 1954), plants were inoculated with WSMV inoculum prepared using the same method described above. Plants were grown in the greenhouse for approximately 12 d and then symptomatic leaf tissues were harvested and frozen at −80°C until virus purification. A subsample of infected tissue was tested by PCR to confirm single infection by WSMV. Virions were purified by a procedure similar to Lommel et al. (1982). One hundred grams of infected tissue was blended in 0.2 M sodium acetate buffer (pH 5.0, 4 mL g^{-1} of tissue) containing 0.25% of 2-mercaptoethanol. The filtered extract was centrifuged at 10,000 × *g* for 15 min. Virus particles were subsequently precipitated from the supernatant with 8% PEG-8000, 0.2 M NaCl, and 0.5% Triton X-100 by stirring for 1 h at 4°C. The pellet, collected after centrifugation at 10,000 × *g* for 15 min, was resuspended in 1/10 (v/v) of resuspension buffer (0.1 M Tris, pH 6.5, 32 mM sodium citrate, and 0.5% Triton X-100), and centrifuged through a 30% sucrose cushion at 100,000 *g* for 2 h. The final viral pellet was gently resuspended in 0.5 mL of resuspension buffer. Purifications were quantified and a serial dilution was created, with concentrations of 0.00267, 0.00173, 0.00133, and 0.00107 mg mL^{-1} virus particles to be used as standards for quantitative ELISA.

DNA Extraction

Leaf tissue from each F_2 plant used for development of the $F_{2:3}$ family was collected for extraction of DNA. Total genomic DNA was isolated as described in Stewart and Via (1993) with modifications. About 10 cm of leaf tissue was cut from each of 188 F_2 and the parents and was put into 96-deep-well plates with a 5-mm steel bead in each well. The leaf tissue was frozen with liquid nitrogen and ground into fine powder in a high throughput homogenizer (Troemner LLC, Thorofare, NJ). After grinding, 600 μL of DNA extraction buffer (0.2 M Tris-HCl [pH 8.0], 0.02 M EDTA [pH 8.0], 1.4 M of NaCl, 2% [w/v] CTAB [hexadecyl triammonium bromide], 0.2% [w/v] β-mercaptoethanol) was added to each well and mixed with the sample. The samples were incubated at 65°C for 1 h. After the samples were cooled for 5 min, an equal volume of chloroform/isoamyl alcohol (24:1 [v/v]) was added and mixed with each sample to remove proteins. The mixtures were centrifuged at 7500 × *g* for 10 min. After the supernatants were transferred to a new 96-well plate, 600 μL of ice-cold isopropanol was added and mixed with each sample to precipitate DNA. The DNA pellets were collected after centrifugation at 7500 × *g* for 10 min and washed using 75% ethanol for 1 h. The DNA pellets were air-dried and dissolved in 60 μL of TE (10 mM of Tris [pH 8.0] and 1 mM of EDTA [pH 8.0]) with RNase A (10 mg mL^{-1}) added. The concentration of the genomic DNA preparations was determined by spectrophotometry at 260 nm using a Gene Quant (Amersham Biosciences, Piscataway, NJ) and diluted to 50 ng μL^{-1} with sterile water.

Simple Sequence Repeat Marker Analysis

Simple sequence repeat (SSR) markers were selected from the publicly available sources (http://wheat.pw.usda.gov) for molecular mapping of the WSMV resistance in CO960293-2 in the population. Ten microliters of the PCR amplification contained 1 μL 10× PCR buffer, 1.0 mM $MgCl_2$, 0.4 mM dNTP mix, 2 pM each of the two primers, 50 ng template DNA, and 0.5 U *Taq* DNA polymerase. Amplifications were performed in an Applied Biosystems (Foster City, CA) 2720 thermocycler with a touchdown program: an initial 3 min denaturation at 95°C; six cycles of 45 s at 94°C, 5 min at 68°C, and 1 min at 72°C, with the annealing temperature being reduced by 2°C per cycle; followed by eight cycles of 45 s at 94°C, 2 min at 58°C, and 1 min at 72°C, with the annealing temperature being reduced by 1°C per cycle; then 25 cycles of 45 s at 94°C, 2 min at 50°C, and 1 min at 72°C; and ending with 5 min at 72°C. The PCR products were resolved in a 4% agarose gel in a 1× TBE buffer with ethidium bromide added to the gel.

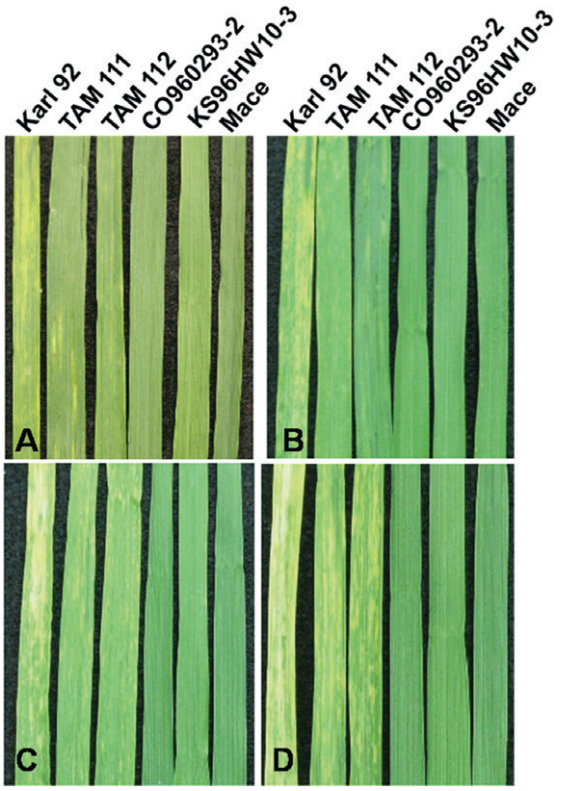

Figure 1. Symptoms of wheat streak mosaic (WSM) on leaves of six wheat genotypes kept in the growth chamber at 19°C day time, 17°C night time, 15-h daylength, and 40% relative humidity after inoculation with *Wheat streak mosaic virus* (WSMV). (A) 3 wk, (B) 4 wk, (C) 5 wk, and (D) 6 wk after inoculation.

Data Analysis

In the experiment of the six genotype trials, means and standard errors for disease severity scores at each rating time were computed for each genotype. Analyses of variance (ANOVA) using GLM procedure of SAS (SAS, 2003) was performed on the data of disease severity among the six genotypes at each rating time. The *F*-protected LSD test (Steel and Torrie, 1980) was used for comparison of the differences of means among the genotypes at the 5% level of significance.

For the population study, data from the quantitative ELISA assay and the disease scores were subjected to correlation analysis. Since WSMV resistance in CO960293-2 was qualitatively inherited, a chi-square (χ^2) test for goodness of fit to the theoretical ratio was performed on the segregation data of WSMV resistance in the 188 F_3 families. Linkage map was constructed with MAPMAKER 3.0 (Lander et al., 1987) using a LOD threshold of 3.0 and the Kosambi mapping function (Kosambi, 1944).

RESULTS

Disease Development and Severity among Genotypes

In the experiment involving six wheat genotypes, Karl 92, TAM 111, and TAM 112 started showing symptoms of WSM 2 wk after inoculation, while KS96HW10-3, Mace, and CO960293-2 had no symptoms, except for a few plants that also showed symptoms of disease at this time. The symptoms on these few plants were as severe as those on Karl 92, and this may have been due to seed contamination or heterogeneity. In week 3 (Fig. 1A), plants of Karl 92 were severely diseased with chlorosis and mosaic symptoms on the entire plant. Chlorotic streaks also began to appear on several leaves of each plant of TAM 111. TAM 112 had fewer symptoms than TAM 111 and only one to two leaves of TAM 112 exhibited streaking at this stage. There were no symptoms on most plants of KS96HW10-3, Mace, and CO960293-2. In week 4 (Fig. 1B), WSM became more severe on plants of Karl 92. Disease symptoms continued to spread to several leaves of each plant of TAM 111 and TAM 112, although there were still only a few chlorotic streaks on each leaf. Most plants of the three resistant genotypes had no symptoms of WSM. In week 5 (Fig. 1C), Karl 92 exhibited severe chlorosis, streaking, and necrotic leaf tissue. A faint mosaic began to appear on the entire leaf of several leaves on each plant in TAM 111 and TAM 112. No symptoms were seen on most plants of the three resistant genotypes. In week 6 (Fig. 1D), the leaves of all Karl 92 plants were highly symptomatic and many were dead. All leaves of each plant of TAM 111 and TAM 112 also exhibited severe symptoms, and at this stage TAM 111 could not be clearly distinguished from TAM 112 based on symptom severity. Most plants of the three resistant genotypes still had no symptoms.

The average scores of disease severity for Karl 92 were 2.28 at the first rating and 3.55 at the last (Fig. 2). Most of the Karl 92 plants were scored as 4 at the end of the experiments. The disease scores were between 0.1 (Mace in week 3) and 0.5 (KS96HW10-3 in week 6) and increased slightly from week 3 to week 6 for the three resistant genotypes. The averaged scores of disease for TAM 111 and TAM 112 were 1.28 and 0.82, respectively, in week 3, and 2.24 and 1.76 in week 6 after inoculation. Disease severity increased slightly faster in TAM 111 and TAM 112 than in Karl 92 during the last week of the experiments, but this was likely due to the symptoms in Karl 92 nearing maximum severity.

ANOVA revealed significant differences for disease among the genotypes (Table 1). Time effect was not significant except for the last week. There was no significant genotype × time interaction detected in the experiment. Comparisons of mean disease scores among the genotypes are presented in Fig. 2. Karl 92 had significantly higher disease scores than any other entry in this study from week 3 to 6. TAM 111 had the second highest disease scores, which were significantly higher than those of TAM 112, indicating that TAM 112 is less susceptible to WSM than TAM 111. The three resistant genotypes, KS96HW10-3, Mace, and CO960293-2, had similar low scores of disease, but KS96HW10-3 had slightly higher disease scores than Mace in week 4 and 5.

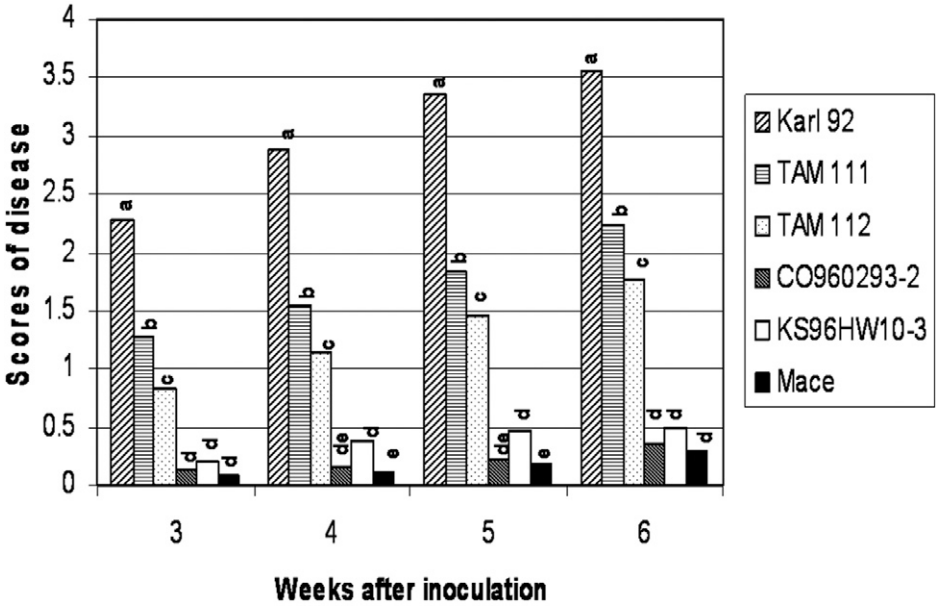

Figure 2. Mean wheat streak mosaic (WSM) scores of six wheat genotypes inoculated with *Wheat streak mosaic virus* (WSMV). The plants were kept in the growth chamber at 19°C day time, 17°C night time, 15-h daylength, and 40% relative humidity after inoculation. Scale is 0 = no symptoms and 4 = mosaic on >50% leaf area. Means with the same letter on a given rating time are not significantly different at $P = 0.05$.

Table 1. Analysis of variance for disease scores of wheat streak mosaic in six wheat genotypes 3, 4, 5, and 6 wk after inoculation. The experiment was conducted two times in a growth chamber in 2009.

		3 wk			4 wk			5 wk			6 wk		
Source of variance	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time	1	0.001	0.04	0.8371	0.022	0.7	0.4128	0.146	3.4	0.0801	0.284	4.98	0.0372
Rep (Time)	2	0.015	0.60	0.6693	0.040	1.21	0.3185	0.077	1.64	0.2164	0.144	2.52	0.1034
Genotype	5	4.391	171	<0.0001	6.87	213	<0.0001	9.085	210	<0.0001	10.11	177	<0.0001
Genotype × Time	5	0.015	0.33	0.889	0.012	0.39	0.8507	0.012	0.28	0.9163	0.015	0.28	0.9207
Error	22	0.025			0.033			0.047			0.057		

Inheritance of WSMV Resistance in CO960293-2

Three weeks after inoculation, all plants of some F_3 families were as severely diseased as the susceptible check Karl 92 (Fig. 3A). These families had more severe disease than TAM 111, the moderately susceptible parent of the population, which indicated that transgressive segregation had occurred in the population. In week 4 and 6, each plant of the 188 F_3 families and the parents, the resistant checks KS96HW10-3 and Mace, the susceptible check Karl 92, and TAM 112, was scored for disease using the previously described method (Fig. 3B and 3C). Although some numeric scores increased from week 4 to week 6, there were no differences in the classification of each family. Segregations among the 188 F_3 families conformed to 1:2:1 ratios ($\chi^2 = 1.86$, $P = 0.3945$) (Table 2). When the plants of all heterozygous families were pooled, the numbers of resistant and susceptible plants were 1293 and 421, respectively, fitting a 3:1 ratio ($\chi^2 = 0.17$, $P = 0.6801$). The homozygous resistant and heterozygous plants had the same disease responses. Therefore, a single completely dominant gene was responsible for WSMV resistance in CO960293-2.

Correlation between ELISA Data and Disease Scores

A total of 1624 plants from one experiment including the 188 $F_{2:3}$ families, the parents, the resistant checks KS96HW10-3 and Mace, the susceptible check Karl 92, and TAM 112 were subjected to quantitative ELISA analysis for virus accumulation in each plant. The ELISA data were significantly positively correlated with the scores of disease ($r = 0.82$, $P < 0.001$). The results from ELISA analysis were in good agreement with classification of each family based on disease scoring and there were no category changes of homozygous resistant, homozygous susceptible, or heterozygous families between the ELISA analysis and disease scoring in the 188 $F_{2:3}$ families. The ELISA thus confirmed that a single dominant gene confers WSMV resistance in CO960293-2. We designated the resistant gene in CO960293-2 as *Wsm2*.

Molecular Mapping of the Gene *Wsm2*

Among 672 SSR markers tested in TAM 111 and CO960293-2, 83 showed polymorphism and mapped in a subset (94 F_2s) of the population. Linkage analysis with MAPMAKER

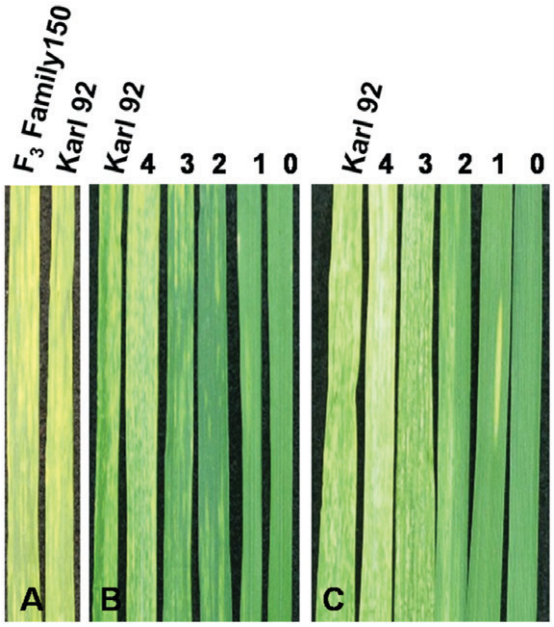

Figure 3. (A) Symptoms of wheat streak mosaic (WSM) on the leaves of the susceptible check Karl 92 and the most severely diseased F_3 families derived from the cross between CO960293-2 and TAM 111 at week 3 after inoculation with *Wheat streak mosaic virus* (WSMV). (B) WSM symptoms used as the criteria for determining phenotypes of the 188 $F_{2:3}$ families from the cross between CO960293-2 and TAM 111 at week 4 after inoculation. (C) WSM symptoms used as the criteria for determining phenotypes of the $F_{2:3}$ families at week 6 after inoculation

Table 2. Segregation of resistance to *Wheat streak mosaic virus* (WSMV) in 188 $F_{2:3}$ families from the cross of CO960293-2 × TAM 111.

Genotypes	Observed†	Expected
Wsm2Wsm2	53	47
Wsm2wsm2	95	94
wsm2wsm2	40	47
Total	188	188

†χ^2 = 1.86 for 1:2:1 ratio, P = 0.3945.

software assigned 74 markers to 23 linkage groups representing 18 wheat chromosomes and covering 1645 cM (about 64% of wheat genome as described in Somers et al., 2004), whereas nine markers were unlinked. One of the linkage groups contained *Wsm2* and two flanking SSR markers, *Xgwm389* and *Xgwm566*. The two markers were then mapped in the entire population. The resulting map distances between *Xgwm389* and *Wsm2* and between *Wsm2* and *Xgwm566* were 30.8 and 45.4 cM, respectively (Fig. 4). Because these two SSR markers were mapped on the short arm of chromosome 3B (Somers et al., 2004), we conclude that *Wsm2* is located on the short arm of chromosome 3B in CO960293-2.

DISCUSSION

The results of this study confirmed that responses of the six wheat genotypes to infection by WSMV were significantly different. Karl 92 had the highest disease scores, while the

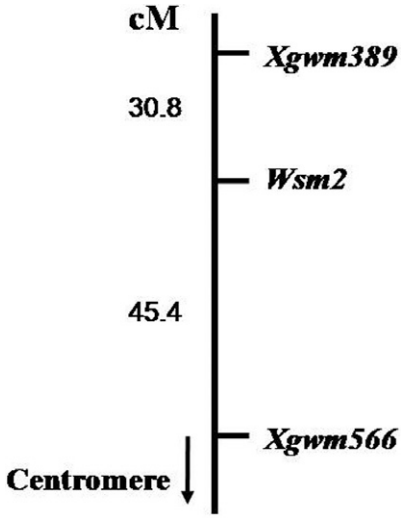

Figure 4. Linkage map of *Wsm2* and the simple sequence repeat markers *Xgwm389* and *Xgwm566* constructed in the population consisting of 188 F_2s derived from the cross between CO960293-2 and TAM 111.

three resistant genotypes had very low disease scores. TAM 111 and TAM 112 were consistently intermediate in their responses to WSMV infection, suggesting a partial resistance or tolerance in these two cultivars. Partial resistance or tolerance to WSMV has been reported for other wheat cultivars (Divis et al., 2006; Hakizimana et al., 2004; Rahman et al., 1974; Seifers and Martin 1988), but information on genetics of this type of resistance is lacking. In this study, we found that transgressive segregation toward susceptibility occurred in the segregating population. Eleven of 40 susceptible families were as susceptible to WSMV as Karl 92 and were more severely diseased than TAM 111, the moderately susceptible parent of the population. The remaining 29 susceptible families had disease scores similar to those of TAM 111, implying a partial resistance or tolerance in these families, which was most likely from TAM 111. The ratio of the two types of families was about 3:1. Based on this observation, there may be one minor gene in TAM 111 for the partial resistance that is not allelic to *Wsm2*.

A single dominant gene, which was designated as *Wsm2*, was responsible for the WSMV resistance in the winter wheat line CO960293-2. The origin of *Wsm2* and its relationship with another gene *Wsm1* is an interesting subject. Seifers et al. (2006) reported that the PCR primers J15 for the WSMV resistant gene *Wsm1* amplified the targeted 241-bp fragment from KS96HW10-3 (*Wsm1*) but did not amplify any fragment from CO960293-2. Furthermore, wheat cultivar Ron L inheriting the resistance from CO960293-2 was resistant to WSMV but susceptible to *Triticum* mosaic virus, whereas KS96HW10-3 and other genetic stocks carrying *Wsm1* were resistant to both WSMV and *Triticum* mosaic virus (Friebe et al., 2009). In the present study, *Wsm2* was mapped on the short arm of chromosome 3B, while *Wsm1* is present on the short arm of chromosome 4D as a 4DL.4Ai-2S translocation

(Friebe et al., 1991; Talbert et al., 1996). These results indicate that *Wsm2* and *Wsm1* are two different genes. An alternative explanation for the origin of *Wsm2* would be that *Wsm2* also originated in intermediate wheatgrass and was transferred to wheat as a translocation like *Wsm1*. If this is true, then the recombination frequencies between the two flanking markers *Xgwm389* and *Xgwm566* would have been reduced because the chromosome fragment from intermediate wheatgrass could not pair with the chromosome of wheat. But we found that the genetic distance between *Xgwm389* and *Xgwm566* in this population was similar to that of Somers et al. (2004). Thus, *Wsm2* should be derived from the primary gene pool of wheat and be excluded as a second instance of *Wsm1* introgression from intermediate wheatgrass.

Due to the nature of single gene inheritance, the resistance can be easily transferred to adapted wheat cultivars to provide protection from WSM. Ron L and 'Snowmass' are the first commercially available cultivars with resistance from CO960293-2, and they recently were released for use in the southern Great Plains (Seifers et al., 2007; Haley et al., 2010). Identification of chromosome location of *Wsm2* provides opportunities to use marker-assisted selection (MAS) for incorporating *Wsm2* into adapted wheat cultivars. However, the two flanking SSR markers identified in this study are 30.8 and 45.4 cM from the gene and are not suitable for MAS. A saturated linkage map is under development in our laboratory and will be checked for usefulness of MAS in wheat breeding.

The resistance conditioned by *Wsm2* in CO960293-2, similar to that conferred by *Wsm1*, is temperature sensitive. *Wsm2* was effective against WSMV at 18°C but ineffective at 24°C (Seifers et al., 2006). The third source of resistance, recently identified in KS03HW12, is also temperature sensitive (Seifers et al., 2007). Therefore, when comparing resistance to WSMV in wheat cultivars based on observation of disease incidence and severity or studying inheritance of resistance in a population, it is important to minimize environmental effects, especially temperature variation, that could potentially confound the experiment. Significant environmental effects that resulted in inconsistency and unpredictability of the consequences of WSMV infection have been observed in field experiments (Baley et al., 2001; Thomas et al., 2004). In this study, which was conducted in a growth chamber where environmental factors such as temperature, light intensity, and relative humidity were well set and controlled, we were able to not only determine the inheritance of a single dominance gene for WMSV resistance in CO960293-2 but also demonstrate that partial resistance exists in TAM 111.

CONCLUSIONS

This study showed that wheat genotypes responded differently to WSMV infection. The wheat genotypes carrying *Wsm1* or *Wsm2* had significantly lower disease scores than the ones without major disease-resistance genes. The disease scores also varied among the genotypes that lack the major genes. Occurrence of the transgressive segregation toward the susceptibility in the mapping population derived from the cross between CO960293-2 × TAM 111 revealed the partial resistance in TAM 111. Another Texas wheat line, TAM 112, also showed lower disease scores compared to the highly susceptible genotype Karl 92. Further investigation is required to elucidate the genetic basis of the partial resistance in TAM 111 and TAM 112.

The resistance in CO960293-2 was found to be governed by the single dominant gene *Wsm2*. *Wsm2* was mapped on 3BS by two flanking SSR markers. Although the genetic distances between *Wsm2* and the SSR markers are relatively large, which makes them not suitable for MAS for incorporation of *Wsm2* into adapted cultivars, the information on chromosome location of *Wsm2* is useful for further saturation mapping within the region and for MAS in the future.

Acknowledgments

The authors thank Dr. Scott Haley (Colorado State University, Fort Collins, CO) for providing seed of CO960293-2 and Dr. Dallas Seifers (Kansas State University, Manhattan, KS) for providing Sidney 81 isolate of WSMV. We are also thankful to Gina Rudd, Peihua Yan, and Rex Brandon for their technical assistance. We thank the two reviewers for their valuable comments on the manuscript. This research was supported by the National Research Initiative of USDA's Cooperative State Research, Education and Extension Service, CAP grant number 2006-55606-16629 and grants from the Texas AgriLife Research Monocot Improvement Program, the Texas Cropping Systems Research Initiative, and the Texas Wheat Producers Board.

References

Atkinson, T.G., and M.N. Grant. 1967. An evaluation of streak mosaic losses in winter wheat. Phytopathology 57:188–192.

Baley, G.J., L.E. Talbert, J.M. Martin, M.J. Young, D.K. Habernicht, G.D. Kushnak, J.E. Berg, S.P. Lanning, and P.L. Bruckner. 2001. Agronomic and end-use qualities of *Wheat streak mosaic virus* resistant spring wheat. Crop Sci. 41:1779–1784.

Burrows, M., G. Franc, C. Rush, T. Blunt, D. Ito, K. Kinzer, J. Olson, J. O'Mara, J. Price, C. Tande, A. Ziems, and J. Stack. 2009. Occurrence of viruses in wheat in the Great Plains region. Available at www.plantmanagementnetwork.org. Plant Health Progr. DOI 10: 1094/PHP-1009-0706-01-RS.

Christian, M.L., and W.G. Willis. 1993. Survival of wheat streak mosaic virus in grass hosts in Kansas from wheat harvest to fall wheat emergence. Plant Dis. 77:239–242.

Divis, L.A., R.A. Graybosch, C.J. Peterson, P.S. Baenziger, G.L. Hein, B.B. Beecher, and T.J. Martin. 2006. Agronomic and quality effects in winter wheat of a gene conditioning resistance to wheat streak mosaic virus. Euphytica 152:41–49.

French, R., and D.C. Stenger. 2003. Evolution of wheat streak mosaic virus: Dynamics of population growth within plants may explain limited variation. Annu. Rev. Phytopathol. 41:199–214.

Friebe, B., Y. Mukai, H.S. Dhaliwal, T.J. Martin, and B.S. Gill. 1991. Identification of alien chromatin specifying resistance to wheat streak mosaic and greenbug in wheat germplasm by C-banding and in situ hybridization. Theor. Appl. Genet. 81:381–389.

Friebe, B., L.L. Qi, D.L. Wilson, Z.J. Chang, D.L. Seifers, T.J. Martin, A.K. Fritz, and B.S. Gill. 2009. Wheat-*Thinopyrum intermedium* recombinants resistant to *Wheat streak mosaic virus* and *Triticum* mosaic virus. Crop Sci. 49:1221–1226.

Gill, B.S., B. Friebe, D.L. Wilson, and T.S. Cox. 1995. Registration of KS93WGRC27 wheat streak mosaic virus resistant T4DL.4Ai#2S wheat germplasm. Crop Sci. 35:1236.

Graybosch, R.A., C.J. Peterson, P.S. Baenziger, D.D. Baltensperger, L.A. Nelson, Y. Jin, J. Kolmer, B. Seabourn, R. French, G. Hein, T.J. Martin, B. Beecher, T. Schwarzacher, and P. Heslop-Harrison. 2009. Registration of 'Mace' hard red winter wheat. J. Plant Reg. 3:51–56.

Hakizimana, F., A.M.H. Ibrahim, M.A.C. Langham, S.D. Haley, and J.C. Rudd. 2004. Diallel analysis of *Wheat streak mosaic virus* resistance in winter wheat. Crop Sci. 44:89–92.

Haley, S.D., J.J. Johnson, F.B. Peairs, J.A. Stromberger, E.E. Heaton, S.A. Seifert, R.A. Kottke, J.B. Rudolph, G. Bai, R.L. Bowden, M.-S. Chen, X. Chen, Y. Jin, J.A. Kolmer, and B.W. Seabourn. 2010. Registration of 'Snowmass' wheat. J. Plant Reg. (in press).

Haley, S.D., T.J. Martin, J.S. Quick, D.L. Seifers, J.A. Stromberger, S.R. Clayshulte, B.L. Clifford, F.B. Peairs, J.B. Rudolph, J.J. Johnson, B.S. Gill, and B. Friebe. 2002. Registration of CO960293-2 wheat germplasm resistant to *Wheat streak mosaic virus* and Russian wheat aphid. Crop Sci. 42:1381–1382.

Hunger, R. 2004. Wheat streak mosaic virus prevalent in Western Oklahoma and the Panhandle. Available at http://entoplp.okstate.edu/Pddl/advisory.htm. Plant Disease and Insect Advisory 3(7).

Kosambi, D. 1944. Estimation of map distances from recombination values. Ann. Eugen. 12:172–175.

Large, E. 1954. Growth stages in cereals: Illustration of the Feeke's scale. Plant Pathol. 3:128–129.

Lander, E., P. Green, A. Barlow, P. Daley, S. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181.

Lazar, M.D., W.D. Worrall, G.L. Peterson, A.K. Fritz, D. Marshall, L.R. Nelson, and L.W. Rooney. 2004. Registration of 'TAM 111' wheat. Crop Sci. 44:353–355.

Lommel, S.A., A.H. McCain, and T.J. Morris. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology 72.1018–1022.

Martin, J.T. 1978. Procedures for evaluating *Wheat streak mosaic virus* resistance. Plant Dis. Rep. 62:1062–1066.

Price, J.A., F. Workneh, S. Evett, D.C. Jones, J. Arthur, and C.M. Rush. 2010. Effects of *Wheat streak mosaic virus* on root development and water use efficiency of winter wheat. Plant Dis. 94:766–770.

Rahman, F., J.G. Ross, and W.S. Gardner. 1974. Tolerance to wheat streak mosaic virus in spring and winter wheat cultivars. Crop Sci. 14:178–180.

SAS. 2003. SAS/SAT user's guide, version 9.1. SAS Institute, Cary, NC.

Sears, R.G., T.J. Martin, T.S. Cox, O.K. Chung, S.P. Curran, W.F. Heer, and M.D. Witt. 1997. Registration of 'Karl 92' wheat. Crop Sci. 37:628.

Seifers, D.L., R.C. French, D.C. Stenger, and T.J. Martin. 2003. Biological variation among wheat streak mosaic virus isolates. American Phytopathological Society Annual Meeting. Phytopathology 93(suppl.):S78.

Seifers, D.L., and T.J. Martin. 1988. Correlation of low level wheat streak mosaic virus resistance in Triumph 64 wheat with low virus titer. Phytopathology 78:703–707.

Seifers, D.L., T.J. Martin, T.L. Harvey, and S. Haber. 2007. Temperature-sensitive *wheat streak mosaic virus* resistance identified in KS03HW12 wheat. Plant Dis. 91:1029–1033.

Seifers, D.L., T.J. Martin, T.L. Harvey, S. Haber, and S.D. Haley. 2006. Temperature sensitivity and efficacy of *wheat streak mosaic virus* resistance derived from CO960293-2 wheat. Plant Dis. 90:623–628.

Sim, T., W.G. Willis, and M.G. Eversmeyer. 1988. Kansas plant disease survey. Plant Dis. 72:832–836.

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

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York.

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

Talbert, L.E., P.L. Bruckner, L.Y. Smith, R. Sears, and T.J. Martin. 1996. Development of PCR markers linked to resistance to wheat streak mosaic virus in wheat. Theor. Appl. Genet. 93:463–467.

Thomas, J.B., R.L. Conner, and R.J. Graf. 2004. Comparison of different sources of vector resistance for controlling wheat streak mosaic in winter wheat. Crop Sci. 44:125–130.

Wells, D.G., R.S. Kota, H.S. Sandhu, W.S. Gardner, and K.F. Finney. 1982. Registration of one disomic substitution line and five translocation lines of winter wheat germ plasm resistant to wheat streak mosaic virus. Crop Sci. 22:1277–1278.

Wells, D.G., R. Wong, S. Chung, C.L. Lay, W.S. Gardner, and G.W. Buchenau. 1973. Registration of C.I. 15092 and C.I. 15093 wheat germ plasm. Crop Sci. 13:776.

Workneh, F., J.A. Price, D.C. Jones, and C.M. Rush. 2010. Wheat streak mosaic: A classic case of plant disease impact on soil water content and crop water-use efficiency. Plant Dis. 94:771–774.