Temperature Sensitivity and Efficacy of *Wheat streak mosaic virus* Resistance Derived from CO960293 Wheat

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ABSTRACT

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Wheat yields often are limited by infection by *Wheat streak mosaic virus* (WSMV). Host plant resistance to WSMV can reduce losses. This study was conducted to characterize a new source of temperature-sensitive resistance found in CO960293 wheat. The source of the temperature-sensitive resistance in CO960293 is unknown. Parental and other wheat lines were tested for WSMV resistance using 51 WSMV isolates under different temperatures to determine the stability of the resistance, and yield trials were conducted in the field for 3 years. All parental wheat lines became infected by WSMV at all temperatures and were infective in back assay to 'Tomahawk' wheat. No WSMV isolate defeated the resistance of CO960293 at 18°C. Yield of CO960293 in field trials was reduced in only 1 of 3 years. Our data demonstrate that this wheat line can be a valuable source of resistance to WSMV in wheat programs, particularly in areas where temperatures are cool following planting in the fall.

Infection of wheat by *Wheat streak mosaic virus* (WSMV), vectored by the wheat curl mite (WCM) *Aceria tosichella* Keifer (15), causes wheat streak mosaic, a serious disease of wheat in Kansas causing losses as high as 13% reduction in yield of the wheat crop (5,14,18).

High levels of WSMV resistance are not available in commercial cultivars. Lower levels of resistance are available, but these cultivars suffer significant yield losses under high WSMV pressure (12). The KS96HW10-3 germ plasm derived from *Agropyron intermedium* has been shown to have a high level of resistance under field conditions (13). The resistance from *A. intermedium* has been shown to be tightly linked to the gene identified as *wsml*, and its presence can be verified using the J15 (SCAR) DNA marker (17). This source was demonstrated to be temperature-sensitive; however, under field conditions, this resistance still was effective in preventing losses to WSMV infection (13). To date, this is the only temperature-sensitive

source of resistance to WSMV that has been identified.

In 1999, wheat from the Hard Winter Wheat Regional Germplasm Observation Nursery was tested at Hays, KS under natural infestation with WCM viruliferous for WSMV in a WSMV resistance screening nursery (8). All plants of wheat line CO960293 (PI222668/TAM 107//CO850034 pedigree) remained symptomless in this field test. The symptomless plants tested negative in enzyme-linked immunosorbent assay (ELISA) against antisera to WSMV and the High Plains virus. A homozygous single-plant selection made from CO960293 has been released as germ plasm CO960293 (3).

The objective of this work was to characterize the type and effectiveness of the WSMV resistance of CO960293 and other wheat germ plasm under greenhouse, growth-chamber, and field conditions.

MATERIALS AND METHODS

Sources and maintenance of WSMV isolates. Fifty-one WSMV isolates were used in these studies (Table 1). The susceptible wheat cv. Tomahawk was mechanically inoculated on the first leaf using the finger-rub technique (11) and held in a growth chamber at 20 to 22°C for 2 weeks. The symptomatic plant tissue then was frozen at -80°C until needed. Inoculations in all experiments were done using thawed portions of such frozen tissue. Inoculum was prepared by grinding infected tissue in a mortar and pestle at a 1:10 (wt/vol) dilution in 0.02 M potassium phosphate buffer

(pH 7). The homogenate was filtered through cheesecloth, and 1 g of abrasive (Crystolon flour B, 600 mesh; Norton Co., Worcester, MA) was added for 100 ml of inoculum. This method of inoculum preparation and inoculation used throughout this investigation has been described previously (11).

Infectivity assays. Tomahawk wheat was mechanically inoculated as described above at the single-leaf stage with 1:5 (wt/vol) extracts prepared from different wheat lines. Inoculated plants were held in a greenhouse under natural lighting and rated for numbers of symptomatic plants at 14 days post inoculation (DPI).

Indirect ELISA. Leaf tissue was ground 1:30 (wt/vol) in 0.05 M carbonate buffer, pH 9.6 (1). Extracts (200 µl) were placed in wells of ELISA plates (Immulon 1; Dynatech Laboratories, Inc., Chantilly, VA) for 1 h at 37°C. Following rinsing, the wells were incubated for 1 h at 37°C with a WSMV antiviral antibody (12) dilution of 5 µg/ml in blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4). The plates then were rinsed and blocked for 1 h at 37°C. Then, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates, Birmingham, AL) in blocking buffer (1:3,000 vol/vol) was added per well. The plates were held at 37°C for 1 h. The plates then were rinsed, and 200 µl of substrate (p-nitrophenyl phosphate, 0.714 mg/ml) in substrate buffer (1) was added to each well. The plates then were held at room temperature (20 to 22°C) for 30 min. Absorbance was measured at 405 nm using a Titertek Multiscan plate reader (Flow Laboratories, Inc., McLean, VA). Absorbance values were considered positive if they were twice those of the equivalent mock-inoculated control.

WSMV testing of wheat lines. Seed of wheat lines CO960255, CO960223, CO960293, CO960315, CI6501, KS96H10-3, plant introduction (PI)222651, PI222655, PI222661, PI222666, PI222668, PI222671, PI222679, PI222680, PI222681, and PI222682 were planted into 30-by-50-cm soil-filled metal flats at 10 seed per row for each entry with eight rows per flat for a total of four flats (two for each temperature). Plants were mechanically inoculated as described above with WSMV isolate

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Sidney 81 at the single-leaf stage. One set of plants was held at 18°C in a growth chamber for 14 days with 8 h of illumination (250 $\mu\text{E sec}^{-1} \text{m}^{-2}$) per day and one set of plants was held for 14 days at 24°C in a growth chamber with the same light conditions. Symptom expression of both sets was recorded at 14 DPI. The experiment was conducted twice. All plantings in these and other experiments conducted in the course of this investigation used a Harney clay loam (fine montmorillonitic, mesic Typic Argiustoll) soil. Lines PI222651 to PI222682 were tested to determine whether they might have resistance to WSMV because preliminary tests showed that the parental lines PI222668, TAM 107, and CO850034 were susceptible to infection by WSMV (D. L. Seifers, *unpublished*).

WSMV infectivity testing of CO960293 and parental wheat lines. Wheat lines CO960293, CO850034, PI222668, and TAM107 were planted into 30-by-50-cm soil-filled (soil type as described above) metal flats at 10 seed per row for each entry so that each flat contained one row for each wheat line. Two sets of this planting were done so that plants were available for the 18 and 24°C temperature treatments. The plants were held at 18°C until they were at the single-leaf stage and then were mechanically inoculated as described above on the first leaf with a 1:10 wt/vol dilution of the Sidney 81 isolate of WSMV. The plants then were placed immediately in growth chambers at either the 18 or 24°C temperature treatment. A randomized complete block

design was used with the location of wheat lines within flats randomized, as were the positions of the flats within the growth chambers. At 21 DPI, all symptomatic tissues of the third leaves were bulked (within a wheat line) and ground in 0.02 M KPO_4 , pH 7, buffer at a 1:25 wt/vol dilution. From this preparation, serial dilutions of 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200 were prepared. The extracts were used to inoculate Tomahawk wheat plants (7 to 10 plants per dilution at the single-leaf stage) as described above. The inoculated Tomahawk infectivity assay plants were held in a greenhouse under natural lighting (temperatures ranged from 19 to 28°C) prior to determining the number of symptomatic plants at 14 DPI. The experiment was conducted three times. Infection percentage data were transformed with the arcsine transformation before analysis of variance because the range of percentages among treatments was greater than 40 (6); untransformed data will be presented. The analysis of variance (ANOVA) for the data was conducted using SAS (version 8; SAS Institute, Cary, NC) and significant treatment effects were determined using the least significance difference (LSD) test at $P = 0.05$.

Wsm1 gene probe. Healthy wheat leaves of CO960293, CO960315, CO960255, CI15092, KS96HW10-3, TAM 107, and the Canadian spring wheat cvs. AC Elsa and AC Superb were tested for the presence of the J15 (SCAR) DNA marker, which has been shown to be tightly linked to the presence of the resistance gene, *wsm1*, derived from *Thinopyrum intermedium* (17).

DNA extraction. Wheat leaf pieces (approximately 3 by 1 cm each) were placed in individual 15-ml tubes and lyophilized overnight. The tissue in each tube was pulverized by vigorous abrasion with acid-washed sand (2 ml of sand per tube, 5 min of agitation on a commercial paint shaker), then extracted with 6 ml of DNA extraction buffer (0.1 M Tris-HCl, pH 8.7; 0.05 M EDTA, pH 8.0; 1.5 M NaCl; and 1% cetyltrimethylammonium bromide) to which 30 μl of proteinase K (10 mg/ml in Tris-EDTA buffer [10 mM Tris-HCl, pH 7.6, and 1 mM EDTA] pH 7.6) and 660 μl of 20% (wt/vol) sodium dodecyl sulfate were added (2 h of incubation at 65°C in a water bath), followed by addition in a fume hood of 6 ml of chloroform/isoamyl alcohol (24:1). After slow shaking for 30 min, the tubes were centrifuged at $5,000 \times g$ for 10 min. The supernatant (approximately 3 ml) from each tube was removed to a fresh tube and 3 ml of ice-cold isopropanol was added. The DNA which precipitated at the interface between the two phases was removed (1 ml) to a microcentrifuge tube, then pelleted by centrifugation at $15,000 \times g$ for 20 min. After washing with 70% (vol/vol) ethanol, DNA

Table 1. Wheat streak mosaic virus (WSMV) isolate designation, origin, isolation host, and year of isolation

WSMV isolate	Location of origin	Isolation host	Year isolated
BT95	Kansas	Wheat	1995
CK93	Kansas	Wheat	1993
CL93	Kansas	Wheat	1993
CM93	Kansas	Wheat	1993
CO85	Kansas	Wheat	1985
CO87	Colorado	Wheat	1987
El Batán 3	Mexico	Wheat	1996
EW95	Kansas	Wheat	1995
FO93	Kansas	Wheat	1993
GH95	Kansas	Wheat	1995
GO93	Kansas	Wheat	1993
GY93	Kansas	Wheat	1993
H81	Kansas	Wheat	1981
H88	Kansas	Wheat	1988
H94PM	Kansas	Wheat	1994
H94S	Kansas	Wheat	1994
H94USDA	Kansas	Wheat	1994
H95LB	Kansas	Wheat	1995
H95S	Kansas	Wheat	1995
H98	Kansas	Wheat	1998
HM93	Kansas	Wheat	1993
HV91	Kansas	Wheat	1991
ID96	Idaho	Wheat	1996
ID99	Idaho	Maize	1999
IHC	Canada	Wheat	1989
KM93	Kansas	Wheat	1993
KY00	Kentucky	Wheat	2000
KY0083SV	Kentucky	Wheat	2000
LC95	Kansas	Wheat	1995
LG92	Kansas	Wheat	1992
MO00	Missouri	Wheat	2000
MO99	Missouri	Wheat	1999
MON96	Montana	Wheat	1996
ND	North Dakota	Wheat	1969
NE96	Nebraska	Wheat	1996
OK98	Oklahoma	Wheat	1998
OSU	Unknown	Unknown	Unknown
PL95	Kansas	Wheat	1995
PN95	Kansas	Wheat	1995
PV106	Ohio	Maize	1964
PV106JM	Ohio	Maize	1964
PV57	Kansas	Wheat	1932
PV91	Kansas	Wheat	1949
RO95	Kansas	Wheat	1995
SD96	South Dakota	Wheat	1996
Sidney 81	Nebraska	Wheat	1981
TK1	Turkey	Wheat	1999
TK2	Turkey	Wheat	1999
TX96	Texas	Wheat	1996
WA99	Washington	Maize	1999
WO93	Ohio	Maize	1993

pellets were left in 70% ethanol overnight at -20°C, centrifuged at 15,000 × g for 20 min, and the ethanol decanted. The pellets were allowed to air dry; then, each pellet was dissolved in 250 µl of autoclaved, deionized water.

Amplification of marker DNA by polymerase chain reaction. Amplifications of the 241-bp J15 marker DNA sequence were carried out using a polymerase chain reaction (PCR) DNA amplification kit (cat. no. N808-0130; Perkin Elmer—Roche Diagnostics, Nutley, NJ) and the custom primers left primer (5'–3') GTA GCA GGG GAA GCT GAA GA and right primer (5'–3') CCG AGC TCA CAC GCT AAT TT (10/µl). From the kit, the following reagents were added to a microcentrifuge tube: 32 µl of deionized water, 7.5 µl of reverse-transcription PCR buffer, 5 µl of MgCl₂, and 5 µl of 10× dNTPs. To this mixture, 2.5 µl of each of the left and right primer DNA, 1 µl of the target DNA sample, and 0.75 µl of Taq DNA polymerase (5 units/µl) were added. After mixing the

reagents by gently flicking each microcentrifuge tube, thermal cycling was carried out in a PTC 100-MJ thermocycler (MJ Research, Watertown, MA) for 3 min at 94°C, followed by 34 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min. at 72°C; a 10-min period of annealing at 72°C; and concluding with cooling overnight at 4°C.

PCR DNA products were analyzed in a 1.5% agarose gel containing ethidium bromide (2). The PCR product (10 µl) was mixed with 1.5 µl of PCR Loading Buffer (Perkin Elmer—Roche Diagnostics) and loaded into the gel, and electrophoresis was conducted for 2 h at 40 V. The presence or absence of the J15 SCAR DNA marker was noted compared with the 241-bp single band produced by the PCR reaction with DNA extracted from the positive control wheat lines CI15092 and KS96H10-3.

Temperature and WSMV isolate interactions for CO960293 resistance. Seed of CO960293 wheat was sieved to uniform size and planted in 30-by-50-cm soil-filled (11 kg of soil) metal flats, each with 22 rows (12 cm long). Six flats were used in each of three runs of the experiment (three flats for the 18°C treatment and three flats for the 24°C treatment). When the plants were at the single-leaf stage, each row was thinned to 10 plants of uniform size. The plants in a row then were mechanically inoculated (as described above) with one of the 51 WSMV isolates. One row in each flat was mock inoculated with buffer and abrasive as the healthy control. A randomized complete block design was used and the location of WSMV isolates and the healthy wheat control within flats was randomized, as were the locations of the flats in the growth chambers. The outer two rows of each flat were planted with wheat that was not inoculated so that all inoculated plants were surrounded by wheat on each side. Plants were held in a growth chamber at either 18 or 24°C. Plants were rated on a daily basis for first-symptom expression and for severity (whole plant and averaged for all plants in a row) at 21 DPI according to the following symptom scale: 1 = no symptoms, 2 = faint mosaic, 3 = moderate mosaic, 4 = severe mosaic, and 5 = severe

mosaic with yellowing of leaves. At 21 DPI, the plants within a row were cut at the soil surface, bulked for a given WSMV isolate or healthy control, and placed in labeled paper bags, and the plant tissue was dried to a constant weight.

ANOVAs (first-symptom expression, symptom severity, and dry weight) were performed using the general linear models procedure of SAS. In the analysis, the uninoculated, symptomless controls and plants inoculated with the El Batán 3 WSMV isolate were assigned a 15-day value to facilitate data analysis. Treatment means were separated using the Tukey's Studentized Range test.

Confirmation of infectivity of selected WSMV isolates on CO960293 grown at 18 and 24°C. The WSMV isolates LG92, CM93, El Batán 3, ND, WO93, and IHC were used to mechanically inoculate CO960293 and Tomahawk wheat. Wheat was planted in two 30-by-50-cm soil-filled metal flats each flat with six rows. Each row of plants was inoculated (as described above) at the single-leaf stage and then one of the two flats was placed at 18°C and the other at 24°C. After 2 weeks at 24°C, the third leaf was harvested from each plant and bulked within a row inoculated with a given WSMV isolate and a 1:5 wt/vol dilution of extract was prepared (as described above). The extract from plants infected with each virus isolate was used to inoculate a separate row of Tomahawk wheat seedlings (10 plants) planted 7 days previously. After 3 weeks at 18°C, the third leaves of plants inoculated with a respective WSMV isolate were bulked and used to make a 1:5 wt/vol dilution of extract. This extract was used to mechanically inoculate Tomahawk wheat seedlings planted 1 week previously, as described for the 24°C treatment. The experiment was conducted three times. The WSMV isolates were chosen from among the 51 isolates because of varied reaction for CO960293 wheat observed in the 18 and 24°C isolate interaction study.

Yield trials. Yield trials were conducted to compare the effectiveness of the resistance of CO960293 to that of KS96HW10-3 that carries resistance from *A. intermedium* (13). Several wheat cultivars with

Table 2. Numbers of symptomatic wheat plants from different lines following mechanical inoculation with *Wheat streak mosaic virus* (WSMV) and being held at 18 or 24°C for 2 weeks following inoculation

Wheat line	Temperature (°C) ^z	
	18	24
CO960255	8/8	8/8
CO960293	0/10	9/10
CO960315	10/10	10/10
CO960223	15/16	16/16
CI6501	15/15	12/12
KS96HW10-3	0/10	9/9
PI222651	4/4	NT
PI222655	7/7	NT
PI222661	6/6	NT
PI222666	9/9	NT
PI222668	14/14	NT
PI222671	10/10	NT
PI222679	4/4	NT
PI222680	5/5	NT
PI222681	5/5	NT
PI222682	4/4	NT

^z Numerator represents the total number of symptomatic plants and the denominator the total number of plants mechanically inoculated with WSMV in two experiments; NT = not tested.

Table 3. Percentages of 'Tomahawk' wheat plants infected by *Wheat streak mosaic virus* (WSMV) following mechanical inoculation with different dilutions of plant extract prepared from plants of different wheat lines grown at either 18 or 24°C^z

Wheat, temperature (°C)	Reciprocal of plant dilution						
	50	100	200	400	800	1,600	3,200
CO850034, 18	100 A	83 A-D	56 F-J	38 I-M	13 L-P	60 Q	0 Q
CO850034, 24	100 A	100 A	97 B	93 A-C	83 A-D	59 E-I	42 H-M
CO960293, 18	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q
CO960293, 24	100 A	100 A	93 A-C	81 B-D	60 E-I	23 K-O	7 N-Q
PI222668, 18	100 A	100 A	100 A	87 A-D	47 G-K	23 K-O	7 N-Q
PI222668, 24	100 A	100 A	100 A	87 A-C	73 B-F	42 H-M	20 L-P
TAM 107, 18	100 A	63 D-H	45 G-K	8 N-Q	0 Q	0 Q	0 Q
TAM 107, 24	100 A	93 A-C	87 A-D	80 B-E	70 C-G	40 H-M	17 M-Q

^z Percentages for the 1:25 dilution are identical to that of the 1:50 dilution and are not shown. Treatments not having a letter in common are significantly different using the least significance difference test ($P = 0.05$).

varying levels of susceptibility to WSMV infection were included: Trego, Jagger, 2145, Ike, Karl 92, and 2137. A randomized complete block experimental design was used for each of 3 years, with each treatment replicated four times. Plots (healthy or infected for each cultivar) con-

sisted of three rows, 2.9 m long with a 0.3-m row spacing, seeded at 50 kg/ha. Planting was done on 22 September 2000, 12 September 2002, and 25 September 2003. Plants were inoculated with the Sidney 81 isolate at the two-to-three leaf stage (13 October 2000, 26 September 2002, and 21

October 2003) using an air-blast inoculation technique (7). Harvest dates were 17 June 2000, 19 June 2002, and 28 June 2003. The ANOVA was conducted using SAS and significant treatment effects were determined using the LSD test at $P = 0.05$.

RESULTS

WSMV testing of wheat lines. Plants of all wheat lines except CO960293 and KS96HW10-3 developed mosaic symptoms when held at 18°C (Table 2). All lines tested at 24°C developed mosaic symptoms, including CO960293 and KS96HW10-3. Symptomatic tissue was positive in ELISA, and the symptomless tissue of CO960293 wheat and the KS96HW10-3 control held at 18°C all were negative in ELISA (*data not shown*).

WSMV infectivity assay of CO960293 and parental wheat lines. The parent lines of CO960293 (CO850034, PI 222668, and TAM 107), all became symptomatic and the tissue was infective for plants grown at both 18 and 24°C (Table 3). At 18°C, only CO960293 was not infective to Tomahawk wheat plants, although it was infective to Tomahawk assay plants when held at 24°C. TAM 107, held at 18°C, was infective only to the 1:400 dilution. At the 1:800 dilution, significant differences in percentages of infected Tomahawk wheat plants were observed between the 18 and 24°C treatments for each wheat line. We also tested wheat lines of similar origin to PI222668 (PI22651 to PI222682) and these also developed symptoms at 18°C (Table 2).

Wsm1 gene probe. The chromosome 4A substitution line CI15092 and the chromosome 4-DL translocation line KS96HW10-3 contained the identical 241-bp SCAR marker (*data not shown*). By contrast, no SCAR band was detected in PCR amplifications of DNA extracts of CO960293, CO960315, CO960255, TAM 107, or the spring wheat cvs. AC Elsa or AC Superb.

Temperature and WSMV isolate interactions on CO960293 resistance. At 18°C, none of the 51 WSMV isolates caused symptom expression in CO960293, nor did they cause any significant reduction in dry weight when compared with healthy controls (*data not shown*). However, at 24°C, all WSMV isolates except El Batán 3 induced symptoms (Table 4). First-symptom expression on CO960293 varied from 6.7 to 11.2 days and was significantly different for some isolates. All uninoculated controls and plants inoculated with the El Batán 3 isolate were symptomless; however, they were assigned a 15-day value to facilitate data analysis. Symptom severity varied among isolates and ranged from 2.2 to 4.5. The WO93 and the CM93 WSMV isolates were significantly different from each other for dry weight, whereas the other isolates were not different.

Table 4. Days to first symptom expression (FSE), symptom severity, and dry weight, of CO960293 wheat mechanically inoculated with different *Wheat streak mosaic virus* (WSMV) isolates and held at 24°C for 3 weeks^w

Virus isolate or control	FSE ^x	Symptom severity ^y	Dry weight (mg) ^z
Uninoculated control 1	15.0 A	1.0 D	594 AB
Uninoculated control 2	15.0 A	1.0 D	643 AB
Uninoculated control 3	15.0 A	1.0 D	659 AB
El Batán 3	15.0 A	1.0 D	642 AB
WO93	11.2 B	2.2 CD	688 A
CO87	9.7 BC	2.2 CD	611 AB
PV106	9.2 BCD	2.2 CD	582 AB
KY00	9.0 BCD	2.5 BCD	663 AB
RO95	8.5 BCD	3.5 ABC	479 AB
IHC	8.5 BCD	3.0 ABC	530 AB
OSU	8.5 BCD	2.7 BC	550 AB
GO93	8.2 BCD	3.0 ABC	525 AB
H95LB	8.0 CD	2.7 BC	616 AB
H88	8.0 CD	3.0 ABC	574 AB
PV57	8.0 CD	2.7 BC	577 AB
ID96	8.0 CD	2.5 BCD	591 AB
Sidney 81	7.7 CD	3.0 ABC	625 AB
TK1	7.7 CD	3.0 ABC	469 AB
ID99	7.7 CD	3.0 ABC	606 AB
TX96	7.7 CD	3.2 ABC	519 AB
MON96	7.7 CD	3.7 ABC	515 AB
WA99	7.7 CD	3.5 ABC	525 AB
ND	7.7 CD	3.5 ABC	450 AB
PV91	7.5 CD	3.2 ABC	575 AB
CK93	7.5 CD	3.0 ABC	560 AB
MO00	7.2 CD	3.5 ABC	511 AB
BT95	7.2 CD	3.2 ABC	580 AB
CL93	7.2 CD	3.7 ABC	573 AB
HV91	7.2 CD	3.5 ABC	495 AB
GH95	7.2 CD	3.0 ABC	523 AB
SD96	7.2 CD	3.5 ABC	503 AB
EW95	7.2 CD	3.5 ABC	623 AB
GY93	7.2 CD	3.5 ABC	504 AB
H98	7.0 CD	3.0 ABC	568 AB
H81	7.0 CD	2.7 BC	591 AB
KY0083SV	7.0 CD	2.7 BC	629 AB
PN95	7.0 CD	3.5 ABC	480 AB
LG92	7.0 CD	4.5 A	549 AB
PV106JM	7.0 CD	3.5 ABC	548 AB
H95S	6.7 CD	3.5 ABC	497 AB
KM93	6.7 CD	3.2 ABC	575 AB
OK98	6.7 CD	3.2 ABC	611 AB
TK2	6.7 CD	3.0 ABC	570 AB
CM93	6.7 CD	4.0 AB	419 B
H94PM	6.7 CD	3.5 ABC	530 AB
HM93	6.7 CD	3.7 ABC	552 AB
FO93	6.7 CD	3.7 ABC	558 AB
PL95	6.7 CD	3.2 ABC	566 AB
LC95	6.7 CD	3.0 ABC	546 AB
NE96	6.5 D	3.0 ABC	562 AB
H94USDA	6.5 D	3.2 ABC	515 AB
H94S	6.5 D	3.5 ABC	524 AB
CO85	6.5 D	3.2 ABC	537 AB
MO99	6.5 D	3.2 ABC	561 AB

^w Treatments not having a letter in common are significantly different using the Tukey's Studentized Range test ($P = 0.05$).

^x FSE values represent average days post inoculation for appearance of first symptoms for a given treatment averaged over the three experiments.

^y Symptom severity ratings: 1 = no symptoms, 2 = faint mosaic, 3 = moderate mosaic, 4 = severe mosaic, and 5 = severe mosaic with yellowing of leaves. The ratings were based on the top three leaves collectively of all plants in a row for each treatment for each experiment.

^z Dry weight values represent average value from three experiments. The dry weight of all plants in a row for a given treatment was combined in each experiment.

Confirmation of infectivity of selected WSMV isolates on CO960293 grown at 18 and 24°C. At 24°C, all WSMV isolates caused symptoms in Tomahawk wheat and extracts from these plants were infective to all Tomahawk wheat assay plants in all three experiments. However, at 24°C, all isolates except El Batán 3 caused symptoms in CO960293 and extracts from the symptomatic leaves were infective to Tomahawk assay plants. In contrast, extracts prepared from the symptomless leaves of CO960293 wheat inoculated with the El Batán 3 isolate were not infective to Tomahawk wheat. At 18°C, none of the CO960293 wheat plants inoculated with the six WSMV isolates developed symptoms, nor were extracts from these plants infective to Tomahawk wheat. In contrast, all WSMV isolates except El Batán 3 caused symptoms to develop in Tomahawk wheat held at 18°C and extracts from the symptomatic Tomahawk wheat was infective to all Tomahawk assay plants in all experiments. Extracts from the symptomless Tomahawk wheat inoculated with the El Batán 3 isolate held at 18°C were not infective to Tomahawk wheat.

Yield trials. In both 2000 and 2003, the grain yield and volume weight of entries inoculated with the Sidney 81 isolate of WSMV were not significantly different from healthy controls for both CO960293 and KS96HW10-3 (Table 5). For all other wheat lines, grain yield and volume weight of the inoculated treatment was significantly lower than its healthy control, except for the volume weight of Jagger in 2003. In 2002, all infected treatments except KS96HW10-3 were significantly lower in yield than their healthy controls.

DISCUSSION

Wheat line CO960293 was grown in the field and was infested naturally by WCM viruliferous for WSMV (8). In this setting,

it was observed that none of the plants of this line were symptomatic, whereas all plants (CO960255 and CO960315) in rows growing adjacent to it on either side were symptomatic. When plants grown from seed from these CO960293 plants were tested in replicated experiments mechanically inoculated with WSMV at 18 and 24°C, only the plants arising from seed of the symptomless CO960293 plants were symptomless at 18°C (Table 2). This confirmed that the symptomless plants in the field were not escapes and that CO960293 had temperature-sensitive resistance to WSMV as observed for KS96HW10-3 (13).

CO960293 was selected from the cross PI222668/TAM 107//CO850034. Thus, our assumption was that one of these lines was the source of the resistance seen in the CO960293 plants. When we tested these at 18 and 24°C, we found that all parents developed symptoms and were highly infective in back assay at 18°C. The observation that TAM 107 was infected was not unexpected because this wheat line has been demonstrated to be susceptible to mechanical inoculation by WSMV (13). TAM 107 was resistant to WCM infestation (10,19), but we have demonstrated that this resistance is not effective against new biotypes of WCM (4). No information is available in the literature about either CO850034 or PI222668 being resistant to WSMV. Because many PI sources are heterogeneous, it is possible that the WSMV resistance in CO960293 originated from a rare plant within PI222668 that was used in the original cross. We have evaluated many plants from several different samples of PI222668 and have failed to identify any plants with WSMV resistance. We have observed that the resistance from crosses with CO960293 has been recovered easily, although no extensive data are available concerning genetic control of the

temperature sensitive resistance derived from CO960293.

Temperature-sensitive WSMV resistance has been identified in KS96HW10-3 wheat (13). Because this line was grown in both breeding nurseries and greenhouses in both Colorado and Kansas, we explored the possibility that an outcross might have occurred and that CO960293 carries the resistance from *A. intermedium* (13). However, when tissue from CO960293 was tested for the presence of J15 (SCAR) DNA marker, no SCAR band was detected in PCR amplifications of DNA extracts of this line, CO960315, CO960255, TAM 107, or the spring wheat cvs. AC Elsa and AC Superb. By contrast, the chromosome 4A substitution line CI15092 and the chromosome 4-DL translocation line KS96HW10-3 (13) contained the identical 341-bp SCAR marker shown to be tightly linked to the *Wsm1* gene on chromatin derived from *A. intermedium* chromosome 4 (17). These observations suggest that CO960293 does not carry the resistance from *A. intermedium*.

When CO960293 was tested against 51 different WSMV isolates at 18°C, no plants developed symptoms, nor were any differences in dry weight noted among virus treatments at this temperature. Thus, the resistance present at 18°C was effective regardless of WSMV isolate source. However, at 24°C, all isolates except El Batán 3 incited symptoms. The El Batán 3 WSMV isolate was isolated in Mexico (9) and has a coat protein nucleic acid sequence that varies from the Sidney 81 isolate of WSMV by 20% (16). Such variation may be involved in the apparent lack of systemic infection of CO960293 we observed at 24°C.

In infectivity assays, we showed that the extracts prepared from symptomless plants of CO960293 inoculated by the El Batán 3 isolate and held at either 18 or 24°C were

Table 5. Grain yield and volume weight of wheat cultivars either healthy (H) or infected (I) by *Wheat streak mosaic virus* over 3 years at Hays, KS^y

Cultivar ^z	Treatment	Yield (kg/ha) in year			Volume (kg/m ³) in year		
		2000	2002	2003	2000	2002	2003
CO960293	H	4,044	3,138	4,791	755	699	753
CO960293	I	4,172	2,392*	4,576	750	688	742
KS96HW10-3	H	4,051	2,573	3,769	795	754	790
KS96HW10-3	I	4,072	2,580	3,581	798	750	784
Trego	H	4,240	3,984	5,711	800	754	804
Trego	I	3,285*	2,761*	3,312*	742*	715*	696*
Jagger	H	3,036	2,633	5,597	772	712	775
Jagger	I	2,452*	1,236*	3,574*	709*	661*	665
2145	H	3,072	2,714	5,415	780	723	795
2145	I	2,035*	490*	1,841*	708*	668*	631*
Ike	H	3,547	2,909	5,308	777	712	790
Ike	I	2,465*	430*	2,997*	703*	654*	741*
Karl 92	H	3,487	2,250	5,006	784	721	800
Karl 92	I	2,606*	1,713*	2,385*	723*	695*	669*
2137	H	3,561	2,520	4,891	771	692	785
2137	I	2,885*	1,145*	3,003*	712*	651*	694*
CV ^z	...	9.7	14.5	9.7	2.0	1.7	2.0
LSD (0.05)	...	571	443	571	22	17	22

^y Asterisk (*) indicates that this value is significantly different from the healthy control for a given wheat line.

^z CV = coefficient of variation and LSD = least significant difference.

not infective. However, Tomahawk wheat developed symptoms inoculated with this isolate when held at 24°C and such tissue was infective to assay plants. This indicated that the inoculum was infective and that the lack of symptoms and infectivity in CO960293 at both temperatures was not because of poor-quality inoculum. The lack of symptoms and infectivity observed for CO960293 wheat at both temperatures and Tomahawk wheat at 18°C inoculated with this isolate may reflect the large difference in coat protein nucleic acid sequence observed for this isolate compared with others (16).

Only in fall 2001 were symptoms observed in the CO960293 plots following inoculation (Table 5). The significant reduction in yield of CO960293 in 2002 probably relates to the high temperatures in the week following inoculation that year. The average daily maximum temperature for the week following inoculation was 18.5°C for 2000, 31°C for 2002, and 18.8°C for 2003. This heat-induced breakdown in resistance in CO960293, but not in KS96HW10-3 during 2002, also supports our *Wsm1* gene analysis, where we could not demonstrate the presence of the J15 SCAR marker in CO960293. Although KS96HW10-3 resistance to WSMV is temperature sensitive (13), this wheat line did not suffer any yield losses in any year due to WSMV infection. This supports the hypothesis that the resistance in CO960293 is different than that of KS96HW10-3, and that KS96HW10-3 must require slightly higher temperatures of longer duration to induce loss of resistance. The exact temperature conditions (duration of high versus low temperatures) under field conditions at which the resistance is lost has not been determined. Although we demonstrated that the temperature-sensitive resistance was effective in 2 of 3 years for preventing losses to WSMV infection, the use of such resistance probably would be ineffective for growers who use wheat with the dual purpose of grazing and grain production because such wheat usually is planted early, when temperatures

are sufficiently high to prevent effective use of the temperature-sensitive resistance should viruliferous WCMs infest the early planted wheat.

In summary, we demonstrated that the temperature-sensitive resistance present in CO960293 was effective in field tests in 2 of 3 years. The temperature-sensitive resistance of this line was demonstrated to be effective at 18°C when inoculated by 51 WSMV isolates collected over time and representing a large geographic area. We also proved that the parental lines of CO960293 are susceptible to WSMV infection at both 18 and 24°C. This indicates that the source of the temperature-sensitive resistance in CO960293 is unknown, but its nature should be elucidated. However, regardless of the source, our data demonstrate that this wheat line can be a valuable source of resistance to WSMV, particularly in areas where temperatures are cool following planting in the fall.

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