

Temperature Sensitivity and Efficacy of Wheat Streak Mosaic Virus Resistance Derived from *Agropyron intermedium*

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ABSTRACT

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Agronomically promising wheat (*Triticum aestivum*) lines are now available that have the short arm of chromosome 4Ai-2 from *Agropyron intermedium* translocated onto the long arm of wheat chromosome 4D. This translocation confers a high level of resistance to wheat streak mosaic virus (WSMV). In growth chamber tests, we demonstrated that, when the translocation is present, the resistance is effective at 20 but not at 25°C. Lines with the entire Ai-2 chromosome remained symptom-free at both temperatures. In field tests, both naturally infested and mechanically inoculated lines carrying the 4Ai-2-S translocation were WSMV symptom free, and grain yields, test weights, and plant height were not reduced by WSMV. The grain yields of WSMV-susceptible cultivars were reduced by 21 to 45% in the same test. Although the WSMV resistance carried on the translocation is high-temperature sensitive, it was effective in the field and continues to be a very promising source for the development of WSMV-resistant cultivars.

Wheat streak mosaic (WSM), caused by wheat streak mosaic virus (WSMV) and vectored by the wheat curl mite, *Eriophyes tulipae* Keifer (15), is a very serious disease of wheat (*Triticum aestivum* L.) in western Kansas. Estimated losses from WSM in Kansas from 1976 to 1987 ranged from a trace to 7%, and averaged 1.6% for the period (14).

High levels of resistance to WSMV are not available in commercial cultivars. Although lower levels of resistance are available, these cultivars show significant losses under severe epiphytotic (13). High levels of WSMV resistance have been identified in *Agropyron elongatum* (Host) P. Beauv. and *A. intermedium* (Host) P. Beauv., and several wheat/*Agropyron* addition, substitution, or translocation lines and germ plasms have been released that carry the resistance (5,11,18,19). Several wheat-breeding programs have used these sources of resistance, but none has succeeded in developing WSMV-resistant lines free of serious agronomic or bread-making-quality defects.

CI15092 is a WSMV-resistant, disomic, substitution line in which the 4Ai-2 chromosome from *A. intermedium* is substituted for the 4A chromosome of wheat (3). It was developed and released by South Dakota State University in 1971 (4,19). Another substitution line was derived from the cross CI15092/*T. speltoides* (Tausch) Gren. ex Richter// 'Fletcher' and was crossed with cv. Centurk (18). The F₁ seed were irradiated with fast neutrons to induce translocations. The surviving plants were backcrossed to Centurk four times, with selection for the WSMV resistance prior to each backcross. CI17881 through CI17886 were selected and released from this material. All lines carried a high level of WSMV resistance. CI17885 was reported to be a substitution line, and the remainder were reported as translocation lines. No agronomically acceptable lines were derived from these releases.

Resistance to biotype E greenbug (*Schizaphis graminum* (Rondani)) was reported in CI17882, CI17884, and CI17885 (17). All parents of these lines were tested for greenbug resistance except the *T. speltoides* parent, which was not available. All tested parents proved to be susceptible to greenbugs; thus, the authors concluded that *T. speltoides* must have been the source of the resistance. This was the first evidence that these lines were still carrying either one or more whole chromosomes or parts of chromosomes from the wild wheat relative *T. speltoides*, which could partially explain the poor agronomic performance

of derivatives of these lines. The substitution of the 7S chromosome from *T. speltoides* for the 7A chromosome of wheat in the greenbug-resistant lines was verified by C-banding and in situ hybridization (3). The 7S chromosome probably is being preferentially transmitted. The expected frequency of an unselected trait occurring in lines selected after four backcrosses with a susceptible parent is only 3%. Of the six lines released, three carried the unselected greenbug resistance. Our own unpublished data, derived from segregating populations involving crosses with CI17-884, support this theory.

The *A. intermedium* chromosomal constitution of these lines also was determined with the use of C-banding and in situ hybridization (3). CI17881 is actually an addition line, while CI17882 and CI17885 are substitution lines with 4Ai-2 substituting for the wheat chromosome 4D. CI17883 is a translocation line with two translocations; the short arm of 4Ai-2 is translocated to the long arm of 6A, and the long arm of 4Ai-2 is translocated to the short arm of 6A; the 4D chromosome is missing. CI17884 is the only line that did not have the whole 4Ai-2 chromosome present in some form. CI17884 has the short arm of chromosome Ai-2 translocated to the long arm of wheat chromosome 4D (T4DL.4Ai-2S).

With the presence of the whole 4Ai-2 chromosome in all but one of these germ plasms, coupled with the presence of the 7S chromosome from *T. speltoides*, which may be preferentially transmitted, it is not surprising that little progress has been made in developing a WSMV-resistant cultivar from these lines. Probably very few lines derived from crosses with this material and tested by breeding programs have carried only the 4DL.4Ai-2S translocation.

We used CI17884 as a parent in a randomly mated, recurrently selected population. CI17884 was crossed with 32 WSMV-susceptible cultivars and advanced experimental lines. The resulting F₁s were grown and randomly inter-mated. This population then was screened in the seedling stage for resistance to WSMV, leaf rust (*Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici*), and Hessian fly (*Mayetiola destructor* Say). The survivors were randomly inter-mated. From this population, two lines, KS91H174 and KS91-

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H184, eventually were selected. Neither line has 7S, but both have 4DL.4Ai-2S. These lines survived 2 years of preliminary yield tests in western Kansas and were used in replicated advanced yield tests in 1992. Although neither line performed well enough to be a candidate for cultivar release, their performance was much improved over that of CI17884 and equal to that of Larned, which is still being grown on about 20% of the hectare in the western third of Kansas. The bread-making characteristics of these lines also have been good, which was not the case with lines derived from other *Agropyron* sources (11). Both lines appear to be very promising parents for the continued improvement of this source of resistance to WSMV.

In July of 1993, a time of year when maintaining temperatures below 25°C in a greenhouse is difficult, seedlings of KS91-H184 were infested with WCM in an attempt to develop a culture of WCM that was free of WSMV. Two weeks later, mosaic symptoms were noted on some of the plants. The symptomatic tissue tested positive in the enzyme-linked immunosorbent assay (ELISA) for WSMV. This was unexpected, because the resistance in CI15092 is effective at 27°C (8). Systemic symptoms did develop in CI15092 seedlings when held at 35°C for the first 3 days after inoculation and then at 25°C for 11 days.

The objective of this investigation was to use both symptom expression and ELISA to determine the temperature stability of resistance to WSMV in the newly developed lines that carry T4DL.4Ai-2s relative to other previously released germ plasm with WSMV resistance derived from *A. intermedium*. The level of protection against WSMV provided by this translocation also was determined in both naturally infested and mechanically inoculated field studies.

Changes have been suggested for the Latin binomials of *A. elongatum* (Host) P. Beauv. (to *Thinopyrum ponticum* (Podp.) Barkw. and D. R. Dewey) and *A. intermedium* (Host) P. Beauv. (to *T. intermedium* (Host) Barkw. & D. R. Dewey subsp. *intermedium*) (1). Because of the complexity in following the sources of genetic material involved from the original crosses described in the literature to those of the wheat used in this study, the original binomials will be used in this paper to prevent confusion.

MATERIALS AND METHODS

Virus isolate source and virus maintenance. The Sidney 81 isolate of wheat streak mosaic virus (collected at Sidney, Nebr., in 1981 and obtained from W. G. Langenberg, USDA-ARS, Lincoln, Nebr.) was used in this study. Virus isolate purity was verified by indicator plants (16) and diagnosis against antiserum to WSMV,

Agropyron mosaic virus, and brome mosaic virus (PVAS 178-ATCC). WSMV was maintained in Arkan wheat in growth chambers (Warren/Sherer model E138-15) at 22°C with a 12-h photoperiod of fluorescent light (approximately 500 $\mu\text{E s}^{-1}\text{m}^{-2}$). At 14 days following inoculation, systemically infected leaves were harvested and frozen at -120°C. All inoculations in the experiments were made using thawed portions of this frozen tissue. Inoculum preparation and inoculation procedures have been described previously (6).

Antiserum. Antiserum to WSMV-Sidney-81 was prepared by injecting rabbits intramuscularly with preparations of WSMV purified from wheat according to previously described procedures (12). Approximately 1 mg of virus, emulsified with Freund's complete adjuvant (1:1), was used for the first injection followed by two such injections with Freund's incomplete adjuvant at 1-week intervals. Serum used in this study was collected 2 weeks following the last injection.

The gamma globulin (IgG) fractions of the antiserum were precipitated by adding one volume of saturated ammonium sulfate to two volumes of antiserum. The IgG was washed with cold (4°C) 33% ammonium sulfate and then extensively dialyzed against 0.01 M phosphate-buffered saline (PBS) (pH 7.0) at 4°C. The concentration of IgG used for ELISA was determined by reacting different dilutions of infected wheat (14 days postinoculation) or healthy plant material against IgG at 10, 5, 2.5, 1.25, and 0.75 $\mu\text{g/ml}$. The IgG did not react to healthy plant extract.

Indirect ELISA. Leaf tissue was ground in 0.05 M carbonate buffer, pH 9.6 (2). Samples were absorbed to ELISA plates (Immulon 1, Dynatech Laboratories, Inc., Chantilly, Va.) for 1 h at 37°C. Plates were rinsed and then incubated with a 5 $\mu\text{g/ml}$ dilution of antiviral antibody in blocking buffer (2) for 1 h at 37°C. Plates were rinsed and blocked with a solution containing 5% (wt/vol) nonfat dry milk and 0.01% (vol/vol) antifoaam A made in PBS, pH 7.0, for 1 h at 37°C. Then anti-rabbit antibody/alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, Mo.) in blocking buffer (1:1,000, vol/vol dilution) was added, and the plates were incubated for 1 h at 37°C. The plates were rinsed, substrate (p-nitrophenyl phosphate) in substrate buffer (2) was added at 0.714 mg/ml, and the plates were incubated for 30 min at room temperature. 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.

Effect of temperature on WSMV-resistant germ plasm inoculated with WSMV. Seed of CI15092, CI17766, CI17881, CI17882, CI17883, CI17884,

CI17885, KS91H174, KS91H184, and Triumph 64 were planted in 30- x 50-cm soil-filled metal flats (planted in 10-cm rows with 3 to 5 seeds per row). The flats were held in a growth chamber (chamber and light conditions described above) at 27°C. At 11 days following planting, the plants were inoculated on the second leaf with WSMV inoculum as previously described (6). The flats containing the inoculated wheat were placed in separate growth chambers and incubated for 2 weeks at a continuous 20 or 27°C with a 12-h photoperiod (chamber and light conditions described above).

The third leaf was harvested 14 days following inoculation (first and second leaves discarded) for indirect ELISA. Leaves from each entry were ground with a mortar and pestle (1:5, wt/vol) and two fivefold dilutions were made by transferring 0.05 ml from the first well to a well containing 0.2 ml of extraction buffer, and mixing. This process was repeated to make the last fivefold dilution. The experiment was conducted three times.

Efficacy of resistance in KS91H174 and KS91H184 under field conditions. KS91H174, KS91H184, and WSMV-susceptible check cultivars were tested in naturally infested WSMV screening nurseries from 1990 to 1994 (7). Notes on WSM severity were taken just after heading each year.

On 28 September 1992, a field test was planted to determine the relative yield loss from WSMV. A randomized complete block design was used with 10 cultivars or experimental lines plus a healthy treatment and a WSMV-inoculated treatment for each entry, and each treatment had three replications. Each plot consisted of four rows 3.9 m long with 0.3-m row spacing, seeded at 50 kg/ha⁻¹. Plants were inoculated with the Sidney isolate of WSMV on 14 October, 1994 using an air-blast inoculation technique (6). Plots were hand trimmed to 2.4 m just before harvest on 29 June, 1993. Yields were corrected to a 12% moisture basis. Data were analyzed with analysis of variance, and differences among means were compared with the Waller-Duncan multiple range test (10).

RESULTS AND DISCUSSION

Triumph 64, the WSMV susceptible-check, developed typical mosaic symptoms and tested positive in ELISA at both temperatures. CI15092 remained symptom free, as previously reported (8), and negative in ELISA to WSMV at both temperatures. Similar results were obtained for CI17881, CI17882, CI17883, and CI17885. CI17766, CI17884, KS91H174, and KS91H184 were all symptom free and were negative in ELISA at 20°C, but all developed symptoms and were positive in ELISA when incubated at 27°C (Table 1). All of the lines that developed WSMV symptoms at 27°C carry only the short arm

of the *A. intermedium* chromosome 4Ai-2. The WSMV resistance from CI15092 is reported to be on this short arm of 4Ai-2 (3), but apparently one or more genes occur on the long arm of 4Ai-2 that allow the resistance to remain effective at 27°C.

KS91H174 and KS91H184 were rated resistant to WSMV in the screening nurseries in all 4 years tested. They remained symptom free and appeared to be unaffected by the virus. Larned checks throughout the nursery were rated susceptible in all years because of chlorosis, stunting, and delay in heading.

In the mechanically inoculated test plots of all entries, except KS91H174 and KS91H184, WSMV symptoms developed in 95 to 99% of the plants by May 1993. KS91H174 and KS91H184 remained symptom free with no significant reductions in yield (Table 2), test weight, or plant height. The other eight cultivars all had significantly reduced yields, test weights, and plant heights relative to the

healthy treatments of each cultivar. A significant delay in heading also occurred for the eight cultivars, but not for the WSMV-resistant lines. Among this set of cultivars, only Larned is considered completely susceptible to WSMV (9). The other cultivars tested have been traditionally rated as moderately susceptible or moderately resistant.

Although the resistance to WSMV carried on the short arm of 4Ai-2 is temperature sensitive, it appears stable enough to give complete protection from WSMV under field conditions. We have been using KS91H174 and KS91H184 as parents in the Kansas wheat-breeding program over the last 5 years. We tested over 150 F₄ lines with this resistance in preliminary yield trials in 1994 and will be testing an additional 500 lines in 1995. To date, we have been unable to identify any detrimental agronomic or bread quality characteristics associated with this source of WSMV resistance.

Table 1. Effect of temperature on symptom expression and enzyme-linked immunosorbent assay (ELISA) of wheat lines carrying resistance derived from *Agropyron intermedium* at 14 days postinoculation^a

Entry	Alien chromatin present ^b		Wheat chromatin missing	Temperature			
	Ai ^c	T. sp ^d		20°C		27°C	
				Symptoms	ELISA	Symptoms	ELISA
CI15092	4Ai-2	None	4A	- ^e	-	-	-
CI17766	4Ai-2S	None	4AS	-	-	+ ^f	-
CI17881	4Ai-2	None	None	-	-	-	-
CI17882	4Ai-2	7S	4D+7A	-	-	-	-
CI17883	4Ai-2S	None	4D	-	-	-	-
	+4Ai-2L						
CI17884	4Ai-2S	7S	4DS+7A	-	-	+	+
CI17885	4Ai-2	7S	4D+7A	-	-	-	-
KS91H174	4Ai2-S	None	4DS	-	-	+	+
KS91H184	4Ai2-S	None	4DS	-	-	+	+
Triumph 64	None	None	None	+	+	+	+

^a ELISA values (A_{405}) were considered positive if twice those of the healthy control. All samples that were positive in ELISA were so at all dilutions in all three replications.

^b Alien chromatin content based on C-banding and in situ hybridization (2).

^c Chromosomes derived from *Agropyron intermedium*.

^d Chromosomes derived from *Triticum speltoides*.

^e Indicates no symptoms or negative in ELISA.

^f Indicates symptoms present or positive in ELISA.

Table 2. Yield of 10 wheat cultivars or experimental lines either healthy or mechanically infected with Sidney isolate of wheat streak mosaic virus (WSMV) at Hays, Kans., in 1993

Entry	Yield (kg/ha ⁻¹)		Percent reduction due to WSMV
	Healthy	WSMV infected	
KS91H184	3,903	3,890	0.3
KS91H174	3,594	3,507	2.0
Mesa	4,306	3,406* ^a	21.0
Triumph 64	3,848	3,036*	21.0
Longhorn	4,286	3,332*	22.0
Thunderbird	3,782	2,801*	26.0
TAM 107	3,735	2,761*	26.0
Newton	3,628	2,546*	30.0
2163	4,656	3,131*	33.0
Larned	3,964	2,183*	45.0
Least significant difference	0.05		
Coefficient of variation	8.9 ^b		

^a Means followed by an asterisk were significantly lower ($P = 0.05$) than those of the healthy control based on separation by the Waller-Duncan multiple range test (10).

^b Values apply to both columns.

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