Different Reactions to the Wheat Curl Mite and *Wheat streak mosaic virus* in Various Wheat–*Haynaldia villosa* 6V and 6VS Lines

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ABSTRACT

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Wheat curl mite (WCM), *Aceria tosichella*, is the vector of *Wheat streak mosaic virus* (WSMV), a destructive viral pathogen in wheat (*Triticum aestivum*). Genetic resistance to WCM colonization can reduce the incidence of wheat streak mosaic. Chromosome 6V in *Haynaldia villosa* is a new source of WCM resistance. We compared variation in resistance among different sources of *H. villosa* chromosome 6V and 6VS lines to WCM and WSMV and their effectiveness in controlling the incidence of WSMV following exposure to viruliferous WCM. WCM resistance varied among the 6V and 6VS lines depending on the *H. villosa* parent. The 6V substitution lines Yi80928, GN21, and GN22 derived from an accession of *H. villosa* from China, and the 6VS translocation lines 92R137, 92R178, and Sub6V from an *H. villosa* accession collected from the United Kingdom were uniformly resistant to WCM colonization. In contrast, the 6V substitution line RW15 and a 6VS translocation line Pm33 developed from an *H. villosa* collection from the former Union of Soviet Socialist Republics were susceptible to WCM. All 6V and 6VS lines were susceptible to WSMV when manually inoculated. However, symptom expression was delayed in the WCM-resistant 6V and 6VS lines after exposure to viruliferous WCM. The 6V and 6VS lines differed in their ability to control WSMV infection. WCM-susceptible lines RW15 and Pm33 had no effect on controlling the infection by WSMV. Lines GN21 and GN22 were the most effective of the three *H. villosa* sources in limiting the spread of WSMV. Their high yield potential and protein content, in combination with resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*), make GN21 and GN22 promising sources of WCM resistance.

Additional keywords: chromosome substitution, ELISA, GISH, SCAR

Wheat streak mosaic (WSM), one of the most serious viral diseases of winter wheat (*Triticum aestivum* L.) in the Great Plains of North America, is vectored by the wheat curl mite (WCM), *Aceria tosichella* (Keifer) (syn. *Aceria tulipae* Keifer and *Eriophyes tulipae* Keifer) (31). Outbreaks of WSM have resulted in large economic losses in some winter wheat growing areas

in the United States and Canada (1,3,14). The development of WCM-resistant cultivars offers an approach to control the spread of *Wheat streak mosaic virus* (WSMV), because virus epidemics are associated with the dynamics of the WCM population (11,14,24). However, there are few WCM-resistant common wheat cultivars (15,16). This lack of WCM resistance in wheat has made it difficult to realize genetic improvements in WCM resistance. Wild relatives of wheat provide a potential gene pool for improving the WCM and WSMV resistance of wheat (12). WCM resistance has been identified in rye (*Secale cereale* L.) (13), *Aegilops tauschii* Coss. (syn. *A. squarrosa* L.) (32), *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey (syn. *Agropyron elongatum* (Host) P. Beauv.) (34), and *Triticum timopheevii* var. *araraticum* (Jakubz.) (4). However, some WCM biotypes are able to multiply on certain genotypes previously identified as resistant (15,16).

A new WCM source of resistance has been identified in wheat–*Haynaldia villosa*

(L.) Schur. (syn. *Dasyphyrum villosum* (L.) Candargy) derivatives that carry chromosome 6V or 6VS (7). *H. villosa* is an annual diploid species ($2n = 14$, V genome) in the tribe Triticeae (29). Since it has multiple disease resistance and good potential for wheat improvement (29), a number of substitution and translocation lines involving chromosome 6V or 6VS of different origins have been independently developed (5,9,10,19,21,36). Resistance to powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal) in these 6V and 6VS lines has been well documented, but only limited information is available on their responses to WCM infestation (7). The objectives of this study were to determine the resistance of various sources of chromosome 6V and 6VS lines to WCM and their effectiveness in reducing the incidence of WSMV transmitted by viruliferous WCM.

MATERIALS AND METHODS

Plant materials. The wheat–*H. villosa* lines GN21 and GN22 were developed from crosses of *Triticum durum* L. cv. Sauwne20/*H. villosa*/T. *aestivum* at Guizhou Agricultural University, Guiyang, China. The *H. villosa* parent was collected by the Institute of Crop Germplasm, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China (36). GN21 and GN22 are 6A/6V substitution lines (23). Line Yi80928 was developed from a cross of GN21 with the wheat line JYP3 at the Institute of Genetics, Chinese Academy of Sciences, Beijing, China (X. Jia, unpublished data). It was produced to eliminate the black awn trait of its parent GN21. RW15 is a 6D/6V substitution line developed from TH₃/Wan7107*2/Jimai 30*3///Shanxi859 (10). TH₃ is a hexaploid amphiploid line derived from *T. durum* cv. Mo75 × *H. villosa* (10). Pm33 is a 6DL/6VS translocation line derived from TH₃/Wan7107*4 (19). Both RW15 and Pm33 were developed at the Institute of Crop Cultivation and Breeding, CAAS, Beijing, China (10,19). The *H. villosa* parent of these two lines originated from the former Union of Soviet Socialist Republics (USSR) (9). Lines Sub6V, 92R137, and 92R178 were developed at the Cytogenetics Institute, Nanjing Agricultural

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University, Nanjing, China (5,21). Sub6V was identified as a wheat-6VS translocation (7). The lines 92R137 and 92R178 were characterized as 6AL/6VS translocations (5). The *H. villosa* parent of these three lines originated from the Cambridge Botanical Garden, in the United Kingdom (UK) (5,21). A wheat-*Thinopyrum ponticum* partial amphiploid line Agrotana, which is WCM and WSMV resistant (6,8), and a WCM- and WSMV-susceptible Chinese Spring wheat were used as resistant and susceptible controls, respectively. JYP3, the parental wheat line of Yi80928, was also included in this study.

Maintenance and transfer of WCM and WSMV. The WCM colony was collected from Lethbridge, Alberta, Canada, and was virulent to common wheat and T-Ai (18), a wheat-*Thinopyrum intermedium* substitution line (11). This nonviruliferous WCM population was started from individual eggs transferred to seedlings of T-Ai and was reared on T-Ai (32). Seedlings of T-Ai, colonized by the WCM, were grown in a locked Convion (Winnipeg, MB) E-7 growth cabinet set at 21°C with a 16/8 h light/dark photoperiod supplied by white fluorescent and incandescent lights at the intensity of 100 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. When infested with this WCM, the virus-susceptible wheat cultivars Rescue and Chinese Spring were free of symptoms of WSM.

Screening for WCM resistance. Six seeds for each line were seeded in Ferdinand-style Roottrainer trays (Spencer-Lemaire, Edmonton, AB) and arranged in a randomized complete block design with three replications, and the experiment was carried out twice. Before the seedlings emerged, the Roottrainer trays were exposed to WCM by placing them adjacent to colonized seedlings of T-Ai (32). Seedlings were individually rated for their reaction to WCM colonization on the basis of rolling and trapping of leaves at 24, 27, and 31 days after exposure to WCM (32). A combined analysis of variance of the data indicated that the results from both experiments could be pooled. Duncan's multiple range procedure generated by SAS general linear model (GLM) procedure (Version 6; SAS Institute, Cary, NC) was used to compare the percentage of WCM-resistant plants among the treatments ($P < 0.05$).

Reactions to WSMV following manual inoculation or exposure to viruliferous WCM. WSMV was increased on the hard red spring wheat cultivar Rescue. Lines were evaluated for resistance to WSMV following manual inoculation using the procedure described previously (11). The plot arrangement and growing conditions were the same as the previous experiments. At the two- to three-leaf stage, each plant was inoculated with WSMV using an artist's airbrush (Model AF-689, Paasche Airbrush Co., Harwood Heights, IL). The inoculum (1:10 wt/vol) was prepared by grinding the leaves of Rescue wheat plants

infected by the WSMV isolate in distilled water with 2% Carborundum (320 grit) with a mortar and pestle, and filtering through four layers of cheesecloth. The inoculated plants were grown in a greenhouse at $25 \pm 3^\circ\text{C}$ under natural light supplemented with fluorescent light at intensity of 1,000 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ set for a 16/8 h light/dark photoperiod to promote symptom development. Plants were rated for their reactions to WSMV at 4 weeks after inoculation. The experiment was carried out twice.

The same lines were screened for WSMV infection using viruliferous WCM reared on the infected spring wheat cultivar Rescue in a Convion growth cabinet set at 21°C with a 16/8 h light/dark photoperiod (140 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Plots consisting of six seeds per line were arranged in a randomized block design with three replications, and the experiment was carried out twice. Plants at the two- to three-leaf stage were exposed to viruliferous WCM by setting the Roottrainer trays next to Rescue wheat plants that were colonized by WCM viruliferous for WSMV for 24 h in the growth cabinet (11). The plants were then dusted with sulfur to eliminate the viruliferous WCM before they were transferred to a greenhouse with the same conditions as the previous experiments. The numbers of plants with WSM symptoms were rated at weekly intervals for 4 weeks following exposure to the viruliferous WCM. Transmission of WSMV by viruliferous WCM was confirmed by enzyme-linked immunosorbent assay (ELISA) readings of 0.480 and 0.646 in exposed plants of the susceptible wheat cultivars Rescue and Norstar, respectively.

Double antibody sandwich (DAS)-ELISA. ELISA was conducted using the DAS-ELISA PathoScreen kit for WSMV with an alkaline phosphatase label (Agdia Inc., Elkhart, IN). Four weeks after inoculation, the newly developed leaves were harvested from all plants in a plot, and equal-sized portions of the central parts of each leaf were bulked. One hundred milligrams of the bulked leaves was ground in 1.0 ml of extraction buffer (bovine serum albumin, 2.0 g; polyvinyl pyrrolidone, 20.0 g; and sodium azide, 0.2 g, in 1,000 ml of phosphate-buffered saline (PBS)-Tween 20, pH 7.4). One hundred microliters of the sap was further diluted with 900 μl of extraction buffer to make the ELISA sample for each plot. Negative controls consisted of mock-inoculated (buffer and abrasive only) Chinese Spring wheat plants. The WSMV positive control was provided by the manufacturer of ELISA kit (Agdia Inc., Elkhart, IN). For ELISA, a 100- μl sample of the sap was incubated overnight in the ELISA wells precoated with rabbit polyclonal antibody (0.4 μg per well) at 4°C in a refrigerator. Then, 100 μl of rabbit polyclonal antibody conjugated to alkaline phosphatase enzyme conjugate (1/100

dilution with PBS-Tween 20) was added to each well, which was followed by a 2-h incubation at 21°C in a humid plastic bag. The substrate solution was prepared by dissolving *p*-nitrophenyl phosphate tablet in buffer (magnesium chloride, 0.1 g; sodium azide, 0.2 g; and diethanolamine, 97.0 ml, in 1,000 ml of distilled water, pH 9.8) at a concentration of 1 mg/ml. One hundred microliters of the substrate solution was loaded into each well. After incubation for 1 h at 21°C, the color intensity of each well was assessed at 410 nm using a DYNATECH MR 5000 Microplate reader (Dynatech Laboratories, Chantilly, VA). The ELISA value of the Chinese Spring negative control was 0.075, and the grinding buffer was 0.066. The ELISA reading of the positive control provided by the manufacturer of the ELISA kit was 0.273. In the present study, the resistant control of Agrotana gave an ELISA value of 0.140. Therefore, the lines with ELISA values above the resistant control were considered susceptible to WSMV.

ELISA was also used to detect WSMV in individual plants of GN21 and GN22 at 4 weeks after exposure to the viruliferous WCM. Plants with ELISA values comparable to the Chinese Spring negative control (0.075) were rated as negative to WSMV, whereas plants having ELISA values higher than 0.140 for Agrotana were classified as positive to WSMV.

Screening for stripe rust and powdery mildew resistance. A randomized complete block design with three replications and eight seeds per plot was used to evaluate resistance to stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) and powdery mildew in the 6V and 6VS lines. Stripe rust isolate SRC 4-84 was used and is designated as race 44E14 (R. L. Conner, unpublished data) according to its reactions on the World and European race differentials (17). At the two- to three-leaf stage, the plants were sprayed with spores of the stripe rust fungus. One hour later, the plants received a light misting of water and were then kept in the dark in a moist plastic bag at 10°C for 2 days. The plants were returned to a growth cabinet set at 12°C with a 16/8 h light/dark photoperiod. Stripe rust infection types (IT) of 0 (immunity) to 4 (susceptible) (33) were rated 17 days after inoculation. Plants with IT less than 2 were classified as resistant, while those with IT of 3 and 4 were susceptible.

A mixture of powdery mildew isolates was used to assess resistance in these lines. The isolates were virulent on powdery mildew resistance genes *Pm3b*, *Pm3c*, *Pm4*, *Pm5*, and *PmMA*, and avirulent on genes *Pm1*, *Pm2*, and *Pm3a* as determined by inoculation of the powdery mildew differential sets (27). At the two- to three-leaf stage, plants were dusted with the mixture of powdery mildew isolates from infected wheat plants and were then kept in

the dark overnight at 15°C in a humid plastic bag. The plants were grown at 15°C in a growth chamber adjacent to wheat plants heavily infected by powdery mildew. Powdery mildew symptoms on individual plants were recorded 2 weeks following inoculation.

Genomic in situ hybridization (GISH) and sequence characterized amplified regions (SCAR) analyses. GISH and SCAR techniques were used to confirm the chromosome composition of the 6V and 6VS lines. An accession of *H. villosa* was used to extract total genomic DNA (7). Total genomic DNA from *H. villosa* was used as a probe by labeling with biotin-14-dATP via nick translation (BioNick labeling System, GibcoBRL, Rockville, MD). Sheared genomic DNA extracted from *T. aestivum* cv. Chinese Spring was used as the blocking DNA. GISH was carried out following a published procedure (6).

A marker SCAR₁₄₀₀, linked to the gene *Pm21* on the chromosome arm 6VS (22), was used to detect chromosome 6V in line Yi80928. Polymerase chain reaction (PCR) was performed on Chinese Spring, JYP3, *H. villosa*, Yi80928, and GN21. The sequences of the two primers of SCAR₁₄₀₀ were as follows: Pm21C, 5'-CACTCTCCTCAAACCTTGCAAG-3' and Pm21D, 5'-CACTCTCCTCCACTAACAGAGG-3' (22). DNA extraction and PCR amplification were carried out as previously described (20,22).

RESULTS

Reaction of 6V and 6VS lines to WCM, WSMV, powdery mildew, and stripe rust. When exposed to the WCM, all Chinese Spring wheat plants developed typical rolling and trapping leaf symptoms by 31 days following infestation. The wheat-*Thinopyrum ponticum* partial amphiploid Agrotana displayed no symptoms associated with WCM colonization (Table 1). The 6V substitution lines GN21, GN22,

and Yi80928 did not develop any symptoms of WCM infestation, nor did the 6VS translocation lines Sub6V, 92R137, and 92R178 (Table 1). However, obvious WCM colonization symptoms were evident in the 6VS translocation line Pm33 (all plants), the 6V substitution line RW15, and the wheat line JYP3. Only 3.7 and 5.7% of the RW15 and JYP3 plants, respectively, escaped infestation by WCM at 31 days following exposure to the WCM (Table 1).

One week following manual inoculation with WSMV, all plants of Chinese Spring and JYP3 wheat showed WSM symptoms. After 4 weeks, symptoms of WSM were evident in all plants of the 6V and 6VS lines, indicating that no line with chromosome 6V or 6VS was resistant to WSMV. At 4 weeks after inoculation, WSMV infection was confirmed by ELISA (Table 1). ELISA values for all lines carrying 6V or 6VS were similar to the ELISA values of the WSMV-susceptible control Chinese Spring and the wheat line JYP3. The ELISA value for Agrotana, which was symptomless, was 0.140 (Table 1), which was higher than the noninoculated Chinese

Spring (0.075) but lower than the positive control (0.273).

All 6V and 6VS lines were resistant to powdery mildew isolates, while Chinese Spring and Agrotana were susceptible. JYP3 displayed segregation in its reaction to powdery mildew (Table 1). Pm33 was susceptible to the stripe rust isolate SRC 4-84, whereas all the other 6V and 6VS lines were resistant. Chinese Spring, JYP3, and Agrotana did not show any evidence of stripe rust resistance (Table 1).

Effectiveness of WCM resistance in 6V and 6VS lines on controlling WSMV infection. Viruliferous WCM infected all the Chinese Spring plants with WSMV within 1 week (Table 2). At the same time, 50% or more of the plants of RW15 and Pm33 susceptible to WCM developed symptoms of WSM following exposure to viruliferous WCM (Table 2). GN21, GN22, 92R137, and 92R178 did not exhibit any systemic symptoms a week following exposure. A few plants had WSM symptoms in Yi80928 and Sub6V after the first week. The percentage of WSMV-infected plants in these WCM-resistant lines increased over time, so that by fourth

Table 2. Percentage of *Wheat streak mosaic virus* (WSMV) infected plants of wheat-*Haynaldia villosa* lines at different periods following infestation by wheat curl mite viruliferous for WSMV

Lines	No. of plants observed	Weeks following inoculation			
		1	2	3	4
GN21	36	0.0 c ^z	11.1 fg	25.0 e	44.4 c
GN22	36	0.0 c	20.0 efg	33.3 e	44.4 c
Yi80928	32	8.9 c	33.9 def	61.7 cd	81.1 ab
Sub6V	35	3.3 c	22.2 efg	71.1 bc	79.4 ab
92R137	35	0.0 c	42.2 cde	80.6 abc	94.4 a
92R178	35	0.0 c	53.3 cd	83.3 abc	97.2 a
RW15	36	50.0 b	63.9 bc	83.3 abc	83.3 a
Pm33	36	61.1 b	77.8 ab	91.7 ab	94.4 a
JYP3	29	61.4 b	81.4 ab	84.2 abc	86.9 a
Chinese Spring	33	100.0 a	100.0 a	100.0 a	100.0 a
Agrotana	27	0.0 c	0.0 g	0.0 f	0.0 d

^z Mean values followed by a letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 1. Percentage of symptomless plants in wheat-*Haynaldia villosa* lines with resistance to the wheat curl mite (WCM), stripe rust, powdery mildew, and enzyme-linked immunosorbent assay (ELISA) values from wheat inoculated manually with *Wheat streak mosaic virus* (WSMV)

Lines	Sources of <i>H. villosa</i> parent	Chromosome constitution	Percentage of plants resistant to:				
			WCM ^v	Stripe rust isolate SRC 4-84	Powdery mildew	WSMV	ELISA ^w
GN21	China	6A/6V substitution	100.0 a ^x	100.0 a	100.0 a	0.0 b	0.646 a
GN22	China	6A/6V substitution	100.0 a	100.0 a	100.0 a	0.0 b	0.638 a
Yi80928	China	Wheat-6V substitution	100.0 a	100.0 a	100.0 a	0.0 b	0.726 a
Sub6V	Cambridge, UK ^y	Wheat-6VS translocation	100.0 a	100.0 a	100.0 a	0.0 b	0.685 a
92R137	Cambridge, UK	6AL/6VS translocation	100.0 a	100.0 a	100.0 a	0.0 b	0.741 a
92R178	Cambridge, UK	6AL/6VS translocation	100.0 a	100.0 a	100.0 a	0.0 b	0.716 a
RW15	Former USSR ^z	6D/6V substitution	3.7 bc	100.0 a	100.0 a	0.0 b	0.636 a
Pm33	Former USSR	6DL/6VS translocation	0.0 c	0.0 b	100.0 a	0.0 b	0.563 a
JYP3	...	<i>Triticum aestivum</i>	5.7 b	0.0b	55.6 b	0.0 b	0.720 a
Chinese Spring	...	<i>T. aestivum</i>	0.0 c	0.0 b	0.0 c	0.0 b	0.600 a
Agrotana	...	Wheat- <i>Thinopyrum ponticum</i> amphiploid	100.0 a	0.0 b	0.0 c	100.0 a	0.140 b

^v WCM reactions rated at 31 days following infestation.

^w ELISA values are means from six experiments.

^x Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

^y UK: the United Kingdom.

^z USSR: Union of Soviet Socialist Republics.

week, infestation ranged from 44.4 to 97.2%. However, WSM symptoms in the lines with WCM resistance developed much more slowly than in Chinese Spring. Only 44.4% of the plants in GN21 and GN22 produced symptoms of WSM after 4 weeks (Table 2). These results indicated that the WCM-resistant lines GN21 and GN22 were more effective in controlling the spread of WSM by viruliferous WCM than any of the other 6V or 6VS lines tested. Yi80928 showed a higher percentage of WSMV-infected plants than its parental line GN21 and GN22, which had the same *H. villosa* 6V chromosomes (Table 2). WSM symptoms never developed in

Agrotana following exposure to viruliferous WCM infestation. The wheat line JYP3 had little effect on limiting WSMV infection by viruliferous WCM (Table 2).

At 4 weeks after infestation with viruliferous WCM, 24 and 36 plants of GN21 and GN22, respectively, were analyzed by ELISA. Eight symptomatic GN21 and 16 GN22 plants tested positive in ELISA, as did six symptomless plants of each wheat line. The remaining 10 GN21 and 14 GN22 plants that were symptomless were negative in ELISA. Thus, six symptomless plants of each line were positive in ELISA for WSMV. The minimum and maximum ELISA values for these

symptomless plants were 0.181 and 0.654, respectively.

Characterization of chromosome 6V and 6VS arm of *H. villosa*. GISH revealed two distinguishable yellow-green colored chromosomes of *H. villosa* in Yi80928, RW15, GN21, and GN22, in addition to the 40 red wheat chromosomes (Fig. 1), indicating that these were chromosome substitution lines. Two wheat-*H. villosa* translocated chromosomes were also detected by GISH in Pm33, 92R137, 92R178, and Sub6V. No *H. villosa* chromatin was detected in JYP3 (data not shown).

PCR amplification with the primers Pm21C and Pm21D amplifying SCAR₁₄₀₀ that is linked to the gene *Pm21* on the 6VS chromosome arm resulted in a polymorphic DNA fragment of 1.4 kb in Yi80928 and its chromosome 6V donor GN21 (Fig. 2). This DNA fragment also was present in *H. villosa*, but was absent in Chinese Spring and JYP3, the wheat parent of Yi80928. The results clearly showed the presence of the gene *Pm21* and confirmed that both Yi80928 and GN21 carry chromosome 6V of *H. villosa*.

DISCUSSION

The short arm of chromosome 6V in *H. villosa* is a new source of WCM-resistance (7). We demonstrated that some wheat-*H. villosa* 6V substitution and 6VS translocation lines expressed high levels of resistance to colonization by WCM. However, the response to WCM infestation seemed to be related to the origins of *H. villosa* parents (Table 1). The 6V or 6VS lines developed from *H. villosa* accessions from China and the UK consistently had high levels of WCM resistance. The 6V or 6VS lines derived from an accession of *H. villosa* from the former USSR seemed to have little or no resistance to WCM. Variable reaction to WCM colonization was observed among different rye cultivars (13). This differential response to WCM colonization has also been shown in various chromosome 1R substitution and 1RS translocation lines (26). Being an open-pollinated species, *H. villosa* is expected to have high levels of genetic diversity. Heterogeneity has been reported for stripe rust resistance and other agronomic traits in *H. villosa* (35,37). Extensive examinations for WCM resistance should be carefully carried out before a 6V line is used as a source of resistance to the WCM.

Our data indicated that some plants of WCM-resistant 6V and 6VS lines developed WSM symptoms when exposed to viruliferous WCM (Table 2). A possible explanation would be that the viruliferous WCM probed the plants of the WCM-resistant 6V and 6VS lines and subsequently transferred WSMV to plants they probed, because in theory a single viruliferous WCM can transfer WSMV to any plant it feeds on (24). Another possi-

Fig. 1. Genomic in situ hybridization with total genomic DNA extracted from *Haynaldia villosa* as the probe blocked with wheat DNA on a mitotic metaphase preparation of line Yi80928. Arrows show the two chromosomes of *H. villosa*.

Fig. 2. Polymerase chain reaction (PCR) amplification patterns with sequence characterized amplified regions (SCAR)₁₄₀₀ primers Pm21C and Pm21D. Lane M, Lambda/HindIII DNA ladder (Promega, Madison, WI); lane 1, *Haynaldia villosa*; lane 2, GN21; lane 3, JYP3; lane 4, Yi80928; and lane 5, Chinese Spring. Arrow indicates the 1.4-kb diagnostic DNA fragment.

bility would be that some WCM might adapt on 6V and 6VS plants and overcome their resistance to WCM following prolonged exposure. If this were the case, it might endanger the effectiveness of WCM resistance derived from wheat-*H. villosa* 6V and 6VS lines on controlling the WSM incidence. Adaptation and survival of certain WCM biotypes on formerly resistant plants has been reported on WCM-resistant sources derived from rye, *Aegilops tauschii*, and *Thinopyrum ponticum* (15,16). However, plants with WCM resistance can greatly reduce WCM numbers, since following exposure to the WCM they maintain flat leaves rather than tightly rolling or trapping, as occurs for WCM-susceptible plants (3,15,16,26).

In addition, WCM may prefer to probe WCM-susceptible wheat lines to WCM-resistant lines. The preferential feeding by viruliferous WCM on susceptible plants resulted in a higher percentage of plants with WSM symptoms in lines susceptible to WCM (11,26). In the present study, WCM resistance of 6V substitution lines GN21 and GN22 resulted in a lower incidence of WSM than in the WCM-susceptible 6V or 6VS lines and Chinese Spring and JYP3 wheat (Table 2). Moreover, the WCM resistance of wheat-*H. villosa* lines appeared to be effective in delaying the expression of symptoms caused by WSMV infection even though they were probed by the viruliferous WCM and subsequently became infected by the virus. This was manifested by an absence or a low percentage of plants showing WSM symptoms in the 6V and 6VS lines resistant to WCM during the first 2 weeks following exposure to the viruliferous WCM (Table 2). A reduction in the spread of WSMV by viruliferous WCM has been reported for other sources of WCM resistance (11,14,24,25). The present study provides another source of WCM resistance from wheat-*H. villosa* lines that reduces and delays the expression of WSM, which may play a role in decreasing yield losses due to the WSMV infection. Further studies are needed to directly compare the wheat-*H. villosa* lines with other sources of WCM resistance to determine their relative effectiveness in controlling the spread of WSMV. It was noted that the WSMV-resistant line Agrotana had an ELISA value that was slightly higher than the noninoculated Chinese Spring when it was manually inoculated with WSMV. However, this wheat-*Thinopyrum ponticum* partial amphiploid did not show any systemic symptoms in this study or previous investigations (6,8). An infectivity assay from Agrotana to a susceptible wheat was not conducted; however, WSMV particles were not observed by examination of inoculated tissue from Agrotana with a transmission electronic microscope (F. L. Leggett, *personal communication*). Higher absorbance readings (ranging from 0.120

to 0.194) were again observed in 7 out of 27 ELISA reactions in Agrotana in various studies (H. J. Li and R. L. Conner, *unpublished data*), while the noninoculated Chinese Spring had ELISA values of less than 0.103. These findings indicated that WSMV might multiply in some plant samples of Agrotana. Variable reactions to WSMV infection were also reported in other *Thinopyrum ponticum* derivatives under greenhouse and growth cabinet conditions (28). More studies are needed to determine the resistance mechanism to WSMV in this wheat-*Thinopyrum ponticum* partial amphiploid line.

Some of the sources of WSMV and WCM resistance are sometimes linked to undesirable agronomic or quality traits (12,24,30). Wheat-*H. villosa* 6V substitution lines GN21 and GN22 are both resistant to WCM, reduce the infection by WSMV, and are resistant to stripe rust and powdery mildew. Furthermore, they have high yield potential and protein content (36). These characteristics make GN21 and GN22 promising WCM resistance sources for the control of WSMV. Powdery mildew and stripe rust are important diseases in most major wheat growing areas of the world. Since some of the other sources of WCM resistance, such as the partial amphiploid line Agrotana, are susceptible to these two fungal diseases, the powdery mildew and stripe rust resistance of these 6V substitution lines provides an extra benefit in addition to the WCM resistance. Resistance to WCM colonization and powdery mildew can be selected for simultaneously as the resistance genes conferring WCM and powdery mildew are both located on chromosome arm 6VS in these wheat-*H. villosa* lines. Additionally, most of the WCM-resistant 6V and 6VS lines in this study are black-awned. The new wheat-*H. villosa* substitution line Yi80928, derived from GN21, was the only white-awned 6V line with WCM resistance. However, the effectiveness of Yi80928 on limiting the WSMV infection seemed to be not comparable to its parent line GN21. The effectiveness of alien resistance might be influenced by the wheat background. Such an influence has been observed in wheat-*Thinopyrum intermedium* derivatives against the *Barley yellow dwarf virus* (2).

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