Evaluation of Wheat Germ Plasm for Resistance to Wheat Streak Mosaic Virus by Symptomatology, ELISA, and Slot-Blot Hybridization

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) and a slot-blot hybridization assay were developed to evaluate a large number of accessions of wild wheat species, in conjunction with a symptomatology assay, for resistance to wheat streak mosaic virus (WSMV). No resistance was found among the *Triticum* or the *Aegilops* species tested. Five wheat/wheatgrass (*Thinopyrum* = *Agropyron* species) amphiploids were found resistant to WSMV. Nine registered germ plasms resistant to WSMV were evaluated; eight were immune to the virus and one gave a susceptible reaction. Both ELISA and slot-blot hybridization assays were sensitive in virus detection. The three assays showed good correlation.

Wheat streak mosaic (WSM) is an important disease in the Great Plains of the United States and Canada as well as many other wheat-producing countries. In Kansas, losses have been estimated at 7% in 1981 and have averaged 1.8% during the past 5 yr (21). This figure is more significant when considering that only about half of the wheat is grown in WSMV endemic areas and that up to 460 million bushels of wheat are produced annually in Kansas. Although other controls exist, breeding for resistant cultivars of wheat (*Triticum aestivum* L. em. Thell) will provide the best means of controlling WSM. As yet, there are no commercial cultivars highly resistant to the virus.

The more distant relatives of wheat are the most promising sources of WSMV resistance. McKinney and Sando (14) tested 17 species of wheatgrass (*Thinopyrum* = *Agropyron* species) for WSMV resistance. None of the 17 species of wheatgrass showed systemic symptoms, although a few developed local lesions. The virus was not detected in any of the wheatgrass species tested, and four species segregated for resistance. Recently, WSMV-resistant wheat germ plasm derived from *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey (*Agropyron intermedium* (Host) Beauvois) has been developed (8,23). Although none of the lines are desirable agronomically, they appear promising for use in wheat breeding programs.

Apart from identifying new sources of resistance, finding suitable methods for characterizing WSMV resistance is also important. Two virus diagnostic techniques, enzyme-linked immunosorbent assay (ELISA) and slot-blot hybridization, in conjunction with a symptomatology assay, appear promising for screening wild relatives of wheat and for characterizing WSMV-resistant wheat germ plasm.

The symptomatology assay, which involves visual inspection of symptom development, is currently the most common method of screening germ plasm for resistance to WSMV. Observation of symptom expression in an assay for viral diseases should not be used exclusively because, as with many viral diseases, the degree of symptom severity is not always proportional to viral replication within the host. Also, many wild wheats are darkly pigmented and thus mask virus symptoms. As a result, making use of assays that determine the viral nucleic acid content in the host (slot-blot assays) and viral capsid protein content (ELISA), in addition to symptomatology assays, will provide a more accurate method of screening wheat lines and germ plasm for WSMV resistance.

ELISA was used to determine the presence of viral capsid protein. The slot-blot hybridization procedure employed is a modification of the dot hybridization method developed by Kafatos et al (8). It was used to rapidly determine the relative concentrations of viral nucleic acid in host tissue samples.

The first objective of this study was to determine if symptomatology, viral protein production as detected by ELISA, and viral RNA replication as detected by slot-blot hybridization were correlated and what assay or combination of assays best determined resistant germ plasm. The second objective was to determine the value of slot-blot hybridization in monitoring the ability of WSMV to cause systemic infection in registered germ plasm known to be resistant to WSMV.

MATERIALS AND METHODS

A large selection of wild wheat germ plasm obtained from the Wheat Genetics Resource Center (WGRC), Kansas State University, was screened for WSMV resistance. The germ plasm included 158 accessions of *Aegilops* species and 325 accessions of *Triticum* species. In addition, 14 amphiploids of various origins, as well as nine registered germ plasms resistant to WSMV, were tested. Nomenclature was based on Terrell et al (22) for the *Triticum* and *Aegilops* species. The genomically based nomenclature of Dewey (5) was used for wheatgrass. All seeds from the WGRC were assumed to be homogenous. Every accession is periodically grown out and selfed as a part of the germ plasm maintenance program. Twenty or fewer seeds, depending on availability, were used in evaluating each accession for reaction to WSMV. All accessions were evaluated by symptomatology and by ELISA. Most accessions were also tested by the slot-blot hybridization assay.

Planting method. Seeds of each accession were planted in standard glasshouse flats containing a sandy loam soil mix (14 rows per flat). The flats were kept in a growth chamber at 14 hr in light at 21 C and 10 hr in darkness at 16–18 C. Fluorescent lighting (10,800 lux) was the sole source of light. Plants were watered regularly; fertilizer was not applied.

Inoculum preparation. A Kansas isolate of WSMV obtained from the Fort Hays Branch Agricultural Experiment Station (Hays, KS) was maintained on a susceptible corn hybrid, N28Ht, with frequent transfers (24). The inoculum preparation procedure followed was that of Martin (12). Plants were examined for symptoms 7–8 days after inoculation and every other day thereafter for 7–9 days. Parker wheat was the susceptible control in all tests (12).

Tissue samples. Leaf samples were harvested from each accession after 15–17 days. The middle third of the youngest leaves with symptoms (when

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present) were pooled from all plants on each line. The leaves were evenly divided into two labeled 1.5-ml microcentrifuge tubes (0.3 g of tissue per tube), one to be used for the ELISA, the other for the slot-blot hybridization assay. The tubes were stored at −20 C until assayed.

Bioassay. A bioassay was performed on registered germ plasms known to be resistant to WSMV. Two seeds of Parker, a susceptible wheat cultivar, were planted for each germ plasm line and grown under growth chamber conditions as previously described. Parker wheat was dusted with Carborundum 9 days after planting, and germ plasm samples previously inoculated with WSMV were ground and sap-rub-inoculated with cotton-tipped swabs. The plants were observed for systemic symptoms up to 2 wk postinoculation. Parker plants that showed systemic symptoms were rated positive, meaning the germ plasm inoculated to Parker was susceptible.

Those plants that did not show symptoms were rated negative, indicating that the germ plasm was immune. These plants were subsequently analyzed by ELISA for confirmation. Several of the resistant germ plasm lines showed a hypersensitive response on the inoculated leaves. This type of symptom was not used for the evaluation.

Antiserum and immunoglobulin preparation. WSMV was purified for serum production and ELISA following the procedure of Lommel et al (11). Antiserum against CsCl gradient-purified WSMV was produced in a New Zealand white rabbit. Virus in 1× PBS (phosphate-buffered saline) was injected intramuscularly into the thighs after emulsification with Freund's complete adjuvant on days 1, 14, 37, and 83. The rabbit was bled from the ear 1 day before initial injection and on days 14, 27, 37, 52, 59, 66, 73, 89, 96, and 103. In this report, the serum from day 37 was used because it had the highest titer. The immunoglobulin (Ig) fraction was purified from antisera as described by Clark and Adams (3). Alkaline phosphatase (Type VII-S; Sigma Chemical Co., St. Louis, MO) was coupled to purified Ig by the glutaraldehyde method of Avrameas (1).

Double-antibody sandwich ELISA. The sandwich ELISA procedure followed was as described by Clark and Adams (3). Coating of the Ig was at a 1:500 dilution in 200 µl of 50 mM carbonate buffer, pH 9.6. Incubation for all steps was for 1 hr at 37 C. Fivefold serial dilutions were made with purified virus in one column on all plates as a control. Infected tissue samples were ground with a wooden applicator stick in 250 µl of PBS and centrifuged at low speed for 5 min. Fifty microliters of sap was added to 200 µl of ELISA virus buffer (3), constituting a 1:5 dilution. Each sample was also diluted and tested at a 1:25 dilution. Each dilution was duplicated on each plate. Incubation with the enzyme-conjugated Ig was at a 1:100 dilution in ELISA buffer. Reactions were recorded at 15-min intervals for 45 min after the addition of substrate (200 µl of 1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine, pH 9.8) at A_{405nm} in a Titertek Multiskan photometer (Flow Laboratories, McLean, VA).

Filter-bound hybridization. The slot-blot hybridization procedure was performed in a manner similar to that described by Brown et al (2) with several modifications. Nitrocellulose filters were prewetted in boiling water and allowed to soak in 20× SSC (3 M NaCl and 0.3 M Na citrate, pH 7.0) until ready for use. The slot-blot template (BRL) was incubated for 30 min in 20× SSC with 100 µg/ml denatured salmon sperm DNA and rinsed thoroughly with 20× SSC (4). Tissue samples were prepared as for the ELISA procedure. Two concentrations of infected sap were spotted on the nitrocellulose: 25 and 10 µl in 175 and 190 µl of ELISA virus buffer, respectively. Fivefold serial dilutions of WSMV RNA were made in one column. WSMV RNA used as a control in the slot-blot hybridization procedure was isolated by extraction of purified virus according to the method of Morris et al (15). Slots were postrinsed with 200 µl of 20× SSC. After the filter was rinsed in 5× SSC for 5 min and air-dried for 30 min, it was baked for 2 hr at 80 C. Prehybridization and hybridization procedures used were as described by Thomas (23).

A WSMV cDNA clone, pWSM-8 (10), was labeled with ^{32}P-ATP by nick translation (17) and used as the hybridization probe. Kodak XAR-5 film was exposed to the hybridized filters for 48 hr at −70 C with intensifying screens. A densitometer was used to scan the developed autoradiographs.

Data analysis. ELISA absorbance values were measured 30 min after the addition of substrate. All ELISA data were obtained from the samples at a 1:25 dilution. Peak heights of the autoradiograph densitometric readings obtained from the slot-blot hybridization assays were measured in millimeters for the 1:10 dilution only. Negative control (uninoculated Parker wheat) values ranged between 0.000 and 0.019 A_{405nm} in all the ELISA analyses for the germ plasm accessions, amphiploids, and registered germ plasms. In all instances, the slot-blot values for the same samples were 0 mm. Uninoculated accession samples were also analyzed by ELISA and slot-blot hybridization. These values were in no instance higher than the Parker negative controls. Therefore, for both assays, values greater than the negative controls were considered positive for WSMV. Each experiment was repeated twice, and for each experiment, two replicates of each sample were performed for both assays. Values presented in the data tables are the average values obtained from each replicate from both experiments. For the linear regression analysis of ELISA vs. symptoms only, the presence or absence of WSMV symptoms was noted for each individual plant, and these were averaged among the plants for each accession. A quantitative measure of symptom severity was not attempted. In the context of this paper, susceptible is the presence of virus within inoculated tissue as detected by hybridization and/or ELISA and the observation of systemic symptoms, resistance is a significant reduction in virus as determined by ELISA and hybridization and the absence of viral symptoms, and immunity is the absence of virus in leaf tissue as determined by a bioassay.

RESULTS

Obtaining high yields of purified WSMV has been difficult because of the length and instability of the virions. The purification procedure originally used was that described by Uyemoto and Ferguson (24), which consisted of grinding infected tissue with phosphate buffer followed by chloroform clarification. Because of the unsatisfactory yields of purified virus obtained using this method, as well as the user danger involved in using chloroform as a clarifying agent, the low-pH sodium acetate buffer procedure was adopted (11). Better yields and cleaner preparations were obtained with this method, and it eliminated the need to use organic solvents as a clarifying agent.

The antiserum produced against WSMV purified by elution from CsCl gradients had no cross-reactivity with healthy wheat or healthy corn leaf extracts (the virus was purified from infected corn leaves); therefore, cross-absorption of the antisera with healthy wheat protein was not necessary.

A 2.1 Kb cDNA clone (pWSM-8) representing more than 25% of the WSMV genome was used as the hybridization probe. The cDNA was cloned in pBR322 at the Pst-1 site using a modification of the RNase H-DNA polymerase 1-mediated second-strand synthesis of Gübler and Hoffman (7). The viral origin of the fragment was confirmed by southern blot hybridization using a randomly primed WSMV cDNA probe (10). It was determined that the pWSMV represented the 3′ terminal one-quarter of the WSMV genome. pWSM-8 has been shown to possess no homology to DNA from uninfected wheat or corn.

A total of 325 wild wheat accessions from the WGRC were screened by symptomatology for resistance to WSMV; 174 were *T. dicoccoides* (Koern. ex Asch. & Graebner) Aarons.: TA 51–68, 70–79, 81, 82, 84–92, 94–96, 98–100, 978–1005, 1008–1010, 1012–1015, 1018, 1021, 1022, 1024–1058, 1060–1079,

1081, 1082, 1084, 1086–1088, 1090–1092, 1094–1099, 1103, 1104, 1107, 1109, 1110, 1115, 1116, 1120, 1123–1126, 1129, 1130, 1132–1134, 1136, 1138, and 1165–1167; 136 were *T. araraticum* Jakubz.: TA 1–6, 8–32, 34–50, 102, 859, 861–864, 866–869, 871, 874, 875, 878, 882–893, 895–899, 901–910, 912–919, 923–933, 936, 938–940, 942–946, 949, 950, 952–957, 959, 960, 963–967, 970–973, and 976; 12 were *T. boeoticum* Boiss. accessions: TA 338, 368, 482, 491, 495, 496, 528, 534–536, 594, and 678; and three were *T. dicoccon* Schrank. All accessions showed symptoms, and no resistance to WSMV was detected among any of these lines.

Twenty-three accessions, TA 6, 9, 14, 16, 18, 24, 31, 52, 53, 63, 74, 79, 82, 96, 1001, 1018, 1058, 1062, 1063, 1076, 1120, 1129, and 1130, initially had very few plants showing symptoms after inoculation with WSMV. These lines were then replanted and again inoculated with WSMV; all lines had some infected plants that showed symptoms. In these lines and in many of the wild wheat species, symptoms were masked because of dark green to reddish pigments in the leaves. In addition, the slender leaves of some accessions made observation of symptoms difficult.

A variable number of accessions from six diploid and five polyploid species of *Aegilops* were screened by symptomatology for resistance to WSMV: *A. squarrosa* L. TA 1558–1561, 1563, 1564, 1566, 1568, 1570, 1571, 1573, 1576, 1578, 1580, 1582, 1584, 1586, 1588, 1590, 1592, 1594, 1597, 1618, 1620, 1622, 1624, 1626, 1629, 1631, 1634, 1638–1640, 1642–1651, 1656, 1657, 1659, 1661, 1662, 1665–1667, 1669, 1671–1673, 1675, 1677, 1679, 1681, 1684–1687, 1689, 1691, 1692, 1695, 1698, 1700, 1703–1709, 1712, 1715, 1717, and 1718; *A. cylindrica* Host TA 1843 and 1856; *A. ovata* L. TA 1813 and 1814; *A. triaristata* Willd. TA 1863; *A. triuncialis* L. TA 1719–1720, 1733, 1754, 1756, 1758, 1769; *A. bicornis* (Forsskal) Jaub. & Spach TA 1942, 1949, 1953, 1956, 1771, 1772, 1775, 1787, 1791, and 1796; *A. longissima* Schweinf. & Muschl. TA 1912, 1914, 1917, and 1924; *A. sharonensis* Eig TA 1999 and 2065; *A. speltoides* Tausch TA 1773, 1789, 1793, and 1795; and *A. umbellulata* Zhuk TA 1821 and 1826–1828. All accessions were screened by symptomatology. Most of these accessions were also tested by the ELISA and the slot-blot hybridization assays. All plants showed systemic symptoms, thus no resistance to WSMV was detected among these lines.

Two *Aegilops* accessions, however, gave variable results. One accession of *A. squarrosa*, TA 1645, had very dark green leaves that made observation of symptoms difficult. Although the symptomatology test was not a reliable way to evaluate this accession, ELISA gave a definite positive reading. The other line that gave variable results was TA 1863, the only accession of *A. triaristata* tested. Results from three separate symptomatology assays showed that only a small number of the inoculated plants showed symptoms. Results from ELISA were positive, but a negative slot-blot value was observed.

Five wheat/wheatgrass (*Thinopyrum* species) amphiploids were found resistant by symptomatology, ELISA, and slot-blot hybridization (Table 1). Five of the seven resistant plants of the amphiploid TA 3426 (*T. aestivum*/*Thinopyrum scirpeum* (K. Presl) R. Dewey) showed symptoms 10 wk after transfer to the glasshouse. The exact date of initial symptom expression was not known. The other amphiploid lines failed to show symptoms after transfer to the glasshouse.

Seven wheat amphiploids derived from crosses with distant wheat relatives such as *Elymus* sp., *Critesion* sp. (=*Hordeum*), *Aegilops* sp., and *A. squarrosa* (the presumed D genome donor of common wheat) were tested for reactions to WSMV (Table 1). All seven amphiploids were susceptible to WSMV as tested either by symptomatology (TA 3361 and TA 3397) or by symptomatology, ELISA, and slot-blot hybridization. Healthy controls gave negative results as expected.

Nine registered germ plasms resistant to WSMV were tested by symptomatology, ELISA, and slot-blot hybridization for reactions to WSMV (Table 2). WSMV-resistant germ plasms CI 15321 and CI 15322, 42-chromosome lines derived from crosses between wheat and *Thinopyrum elongatum* (Host) D. R. Dewey (*A. elongatum* (Host) Beauvois)

Table 1. Reactions of different wheat amphiploids to wheat streak mosaic virus (WSMV) as determined by symptomatology, ELISA, and slot-blot hybridization

WGRC accession no. (description of amphiploid)	Symptomatology	ELISA[a] (A_{405nm}) (mean ± SE)	Slot-blot[b] (mm) (mean ± SE)	Disease resistance evaluation[c]
Parker wheat (inoculated control)	9/9 7 PI[d]	0.249 ± 0.031	5 ± 1	S
Parker wheat (uninoculated control)	0/15 15 PI	0.003 ± 0.002	0	–
TA 3389 (*T. aestivum*/ *Thinopyrum podperae* (Nabelek) D. R. Dewey)	0/3 15 PI	0.019 ± 0.004	0	R
TA 3410 (*T. aestivum*/ *Thinopyrum podperae*)	0/10 15 PI	0.006 ± 0.002	0	R
TA 3411 (*T. aestivum*/ *Thinopyrum podperae*)	0/7 15 PI	0.012 ± 0.003	0	R
TA 3426 (*T. aestivum*/ *Thinopyrum scirpeum*)	0/7 15 PI[e]	0.003 ± 0.002	0	R
TA 3427 (*Elymus ciliaris* (Trin.) Tzvelev/ *T. aestivum*)	0/5 15 PI	0.002 ± 0.000	0	R
TA 3361 (*T. boeoticum*/ *A. squarrosa*)	12/12 13 PI	...	...	S
TA 3397 (*A. ovata*/ *T. durum* Desf.)	14/14 13 PI	...	...	S
TA 3404 (*A. ventricosa* Tausch/ *T. aestivum*)	18/18 15 PI	0.585 ± 0.146	170 ± 49	S
TA 3409 (*T. aestivum*/ *Thinopyrum districhum* (Thunb.) Löve	3/20 15 PI	0.344 ± 0.06	26 ± 6	S
TA 3412 (*T. timopheevii* (Zhuk.) Zhuk./ *Critesion bogdanii*) (Wilensky) Löve	11/12 13 PI	0.511 ± 0.048	170 ± 21	S
TA 3413 (*T. aestivum*/ *Leymus arenarius* (L.) Hochstetter	7/7 13 PI	0.583 ± 0.107	42 ± 8	S
TA 3414 (*T. aestivum*/ *Leymus arenarius*)	7/7 13 PI	0.281 ± 0.025	10 ± 2	S

[a] A = Average absorbance at 405 nm measured 30 min after the addition of substrate; mean and standard error (SE) of four replicates (two from each of two ELISA experiments).
[b] Peak heights of autoradiograph densitometric readings measured in millimeters; mean and standard error (SE) of four replicates (two from each of two slot-blot experiments).
[c] S = Susceptible and R = resistant to WSMV.
[d] No. of plants inoculated/number of plants showing symptoms; PI = number of days postinoculation that most of the plants showed symptoms before samples were collected.
[e] After 15 PI, the seven symptomless plants were grown in the glasshouse. Five of seven plants showed symptoms after 10 wk.

(18), gave negative values for all three assays performed. The bioassay, though not performed on CI 15322, was performed on CI 15321, which in our hands was immune. CI 17766, a line derived from a cross with CI 15092 (a *T. intermedium* substitution line for chromosome 4B), comprised four selections: 4806, 4807, 4808, and 4809 (9). The one plant that showed symptoms in the original test was from selection 4806. When retested, all plants tested from selections 4807 and 4808 showed symptoms, whereas no plants from selections 4806 and 4809 became infected.

Registered germ plasms CI 17881–17886 also were derived from crosses with CI 15092 (26). CI 17881, a translocation line, gave variable results when tested by symptomatology. Some plants from this germ plasm showed symptoms in two symptomatology tests. Single plants from selections CI 17883 and CI 17885, translocation and disomic substitution lines, respectively, also showed symptoms after inoculation with WSMV. CI 17886, a translocation line, gave variable results in symptomatology assays. In one test, of eight plants inoculated, none showed symptoms; however, in a second test, four of 10 plants showed local lesions at the site of inoculation. ELISA, slot-blot hybridization, and bioassay results were negative for all of the registered germ plasms evaluated except CI 17881, where all values were positive.

Germ plasm LRS-1F193, released by the Lethbridge Research Station, Agriculture Canada, Alberta (25), was produced from chromosome substitution line 6D, which was originally derived from a cross between *T. aestivum* cv. Rescue and *Thinopyrum elongatum*. This germ plasm was described as carrying resistance to the mite vector, *Eriophyes tulipae* Keifer (27). This line gave susceptible readings in the symptomatology, ELISA, and slot-blot assays.

The three assays used were compared for their ability to detect WSMV. A linear regression analysis was performed using all accessions and germ plasms evaluated by all three assays. Regression analysis of symptomatology vs. ELISA yielded a relatively low correlation (r^2 = 0.262, significant at $P = 0.0001$). Analysis of slot-blot hybridization vs. ELISA also yielded a low correlation (r^2 = 0.259, significant at $P = 0.0001$).

DISCUSSION

Although the entire wild wheat germ plasm collection (WGRC) of *Triticum* species was not evaluated for WSMV resistance, more than half of the *T. araraticum* and *T. dicoccoides* accessions represented were tested. Only small samples of *T. boeoticum* and *T. dicoccon* were included in the evaluation. The conclusion that *T. dicoccoides* lacks WSMV resistance does not agree with a previous report (6), where tolerance to WSMV in *T. dicoccoides* was identified. In addition, Gill et al (6) identified WSMV resistance among accessions of *T. boeoticum*. In the present evaluation, no resistance was identified among 11 *T. boeoticum* accessions. From our results, there does not appear to be any resistance to WSMV among the wild *Triticum* species.

From the WSMV symptomatology assay, it is apparent that some *Triticum* accessions show symptoms 2–9 days earlier than other *Triticum* accessions. This delay in symptom expression may result from leaf expansion; i.e., as the leaf expands, the espression of symptoms becomes more obvious. Another explanation for this delayed reaction of symptom expression could be that the masking effects of the dark plant pigments are decreased as the plant matures, or else, the rate of virus replication is very slow.

About half of the *Aegilops* species represented in the WGRC collection were tested for reaction to WSMV. All accessions were found susceptible. Two accessions, TA 1645 (*A. squarrosa*) and TA 1863 (*A. triaristata*), produced variable results when retested by symptomatology. The leaves of TA 1645 were dark, masking the symptoms produced by WSMV. When the plants were allowed to mature in the glasshouse, symptom expression became obvious. Coupling the symptomatology assay with the ELISA and slot-blot hybridization assays overcomes the problems associated with scoring the dark green leaves of many wild wheats for WSMV resistance. TA 1863, positive for both symptomatology tests and ELISA, was negative by slot-blot. This is the only case where this phenomenon occurred. It is possible that the nucleic acid was rapidly digested when the sample was processed.

The five wheat/wheatgrass amphiploids resistant to WSMV (Table 1) derived their resistance from the wheatgrass parent, because *T. aestivum*, when evaluated for resistance, was always susceptible. The wheatgrass chromosomes carrying the genes that confer WSMV resistance must be transmitted stably from generation to generation to be a useful source of resistance in breeding program. These five lines seem to be stable and good sources of WSMV resistance.

The resistance to WSMV present in TA 3426 appeared heat-sensitive, because five of seven plants showed symptoms when allowed to grow in the glasshouse, where daytime temperatures often reached 35 C. Experiments with some *Agrotricum* lines performed by Pfannenstiel and Niblett (16) have shown that

Table 2. Evaluation of registered germ plasms resistant to wheat streak mosaic virus (WSMV) as determined by symptomatology, ELISA, and slot-blot hybridization

CI number	Symptomatology	ELISA[a] (A_{405nm}) (mean ± SE)	Slot-blot[b] (mm) (mean ± SE)	Bioassay on parker	Disease resistance evaluation[c]
Parker wheat (inoculated control)	12/12 10 PI[d]	0.249 ± 0.031	5 ± 1	Not done	S
Parker wheat (uninoculated control)	0/6 14 PI	0.003 ± 0.002	0	Not done	–[e]
CI 15321	0/15 13 PI	0.012 ± 0.002	0	–	I
CI 15322	0/18 15 PI	0.005 ± 0.002	0	Not done	–
CI 17766	1/15 14 PI[f]	0.020 ± 0.006	0	–	
CI 17881	10/15 16 PI[g]	0.490 ± 0.092	96 ± 25	+	S
CI 17882	0/13 15 PI	0.013 ± 0.002	0	–	I
CI 17883	1/6 11 PI	0.022 ± 0.005	0	–	I
CI 17884	0/13 15 PI	0.010 ± 0.001	0	–	I
CI 17885	1/11 11 PI	0.011 ± 0.002	0	–	I
CI 17886	0/8 14 PI[h]	0.018 ± 0.006	0	–	I
LRS-1F193	10/13 16 PI	0.373 ± 0.017	25 ± 6	Not done	S

[a] A = absorbance at 405 nm measured 30 min after the addition of substrate; mean and standard error (SE) of four replicates (two from each of two ELISA experiments).

[b] Peak heights of autoradiograph densitometric readings measured in milliliters; mean and standard error (SE) of four replicates (two from each of two slot-blot experiments).

[c] S = Susceptible, R = resistant, and I = immune to WSMV. An immune designation is only applied if a bioassay is performed and shown to be negative.

[d] Number of plants inoculated/number of plants showing symptoms; PI = no. of days postinoculation that most of the plants showed symptoms before samples were collected.

[e] Minus sign indicates symptoms were not observed on Parker when inoculated with previously inoculated germ plasm. Plus sign indicates symptoms were observed on Parker when inoculated with previously inoculated germ plasm.

[f] Four selections were evaluated for WSMV resistance: 4806, 4807, 4808, and 4809. Results from all selections were combined and the ELISA values were averaged together. When retested, selections 4807 and 4808 exhibited 3/3 16 PI and 2/2 14 PI, respectively.

[g] When retested, 2/14 17 PI observed.

[h] When retested, 4/10 gave a local-lesion-like response 13 PI.

resistance to WSMV is broken by high temperatures. Additionally, they discovered that, at 35 C, the longer the exposure to heat treatment the higher the percentage of plants becoming systemically infected. Progeny derived from amphiploid TA 3426 would probably remain resistant under field conditions because, as described by Pfannenstiel and Niblett (16), the plants were exposed to high temperatures for only relatively short periods and the heating cycle was interrupted by long, cool nights. None of the remaining amphiploids appeared to possess this heat-sensitive mechanism.

Further evidence that WSMV resistance can be found mainly among the wheatgrass species was demonstrated by the lack of resistance seen in the seven wheat amphiploids derived from several relatives of wheat (Table 1). All seven amphiploids not containing wheatgrass chromosomes were susceptible to WSMV.

WSMV-resistant registered germ plasms CI 15321 and CI 15322 appeared to be stable in eliciting a resistant reaction to WSMV in these tests (Table 2). These findings, however, did not agree with those of Pfannenstiel and Niblett (16), who observed (under both glasshouse and growth chamber conditions) that CI 15321 and CI 15322 often developed mild WSMV symptoms on uninoculated leaves following the death of inoculated leaves. These workers concluded that they were not able to identify the critical environmental factor(s) necessary for the uniform expression of resistance. Although, in the present study, CI 15322 was never allowed to grow to maturity in the glasshouse, CI 15321 remained symptomless throughout all growth stages, except for the typical hypersensitive response associated with CI 15321 observed about 5 days postinoculation.

WSMV-resistant registered germ plasms CI 17766 and CI 17881–17886 (Table 2) are all derived from CI 15092, a 42-chromosome line of wheat (*T. aestivum*) that had a disomic substitution for resistance to WSMV obtained from *Thinopyrum intermedium*. Systemic symptoms were observed on some plants of CI 17766, CI 17881, CI 17883, and CI 17885. Wells et al (26) state that occasionally they would observe a susceptible plant of CI 15092. They attributed these findings to chromosomal instability. Because all of these registered germ plasms were derived from CI 15092, instability of the translocated segment of the *Thinopyrum* chromosome in CI 17766, CI 17881, and CI 17883, as well as the substituted *Thinopyrum* chromosomes in CI 17885, is a plausible explanation for the few susceptible plants observed in these lines.

The data in Table 2 suggest that the registered germ plasms, with the exception of CI 17881, are essentially immune to WSMV as determined by the bioassay performed on Parker.

For future wheat breeding programs involved in the production of WSMV-resistant cultivars, CI 17882, CI 17884, and CI 17886 seem to be the most promising sources of resistance. As stated earlier, Martin et al (13) described CI 15321 and CI 15322 as unacceptable breeding material because of genetic linkage between WSMV resistance and undesirable agronomic characteristics from *Thinopyrum*. Perhaps, with sophisticated cytogenetic techniques, this linkage can be broken. These lines may then be useful sources of resistance.

There apparently is more resistance to WSMV among wheatgrass species than among wild wheats, such as *Triticum* and *Aegilops* species. At present, the most encouraging source of WSMV resistance seems to be among the wheatgrass species. Sharma et al (20) found 11 wheatgrass species that failed to show symptoms, and attempts to produce germ plasm resistant to WSMV with hybrids between wheat and these wheatgrass species are currently in progress (B. S. Gill, *personal communication*). Why these wheatgrass species have more disease resistance than the wild wheats could be because they share no homologous genomes with wheat (19) and, therefore, are not susceptible to infection by a virus adapted to genomes of *Triticum* and *Aegilops* species.

Linear regression analysis was performed to determine the degree of correlation between the assays. Only a low level of correlation was observed between the ELISA and symptomatology. This is most likely due to the fact that a symptom severity rating was not used. Only the presence or absence of symptoms was noted. In addition, only a low correlation ($r^2 = 0.262$, significant at $P = 0.0001$) was observed between ELISA and slot-blot hybridization. Several factors may have contributed to a lack of high correlation. Upon grinding of tissue samples, nucleases are released that can quickly degrade the viral RNA. In general, RNA is highly susceptible to nuclease digestion. Also, a wide spectrum of host genotypes were evaluated. Upon graphing, a cluster of 16 samples all representing *T. dicoccoides* accessions all had relatively high slot-blot values (90–200 mm) and relatively low (0.120–0.480 A_{405nm}) but positive ELISA values compared with all the samples. It is possible that specific host genotypes may have an effect on virus replication, specifically, allowing virus replication to occur but inhibiting capsid protein synthesis. It is important to note that ELISA and the slot-blot hybridization assay correlated for the ability to detect virus. Both assays always rated positive or detected virus when present and were negative when virus was absent, except for the single false negative for the slot-blot assay previously noted.

In conclusion, symptomatology, viral protein production as detected by ELISA, and viral nucleic acid as detected by slot-blot hybridization are generally proportional, except in a few isolated cases where the capsid protein is synthesized but nucleic acid synthesis is greatly inhibited. Also, the slot-blot hybridization assay proved to be a valuable tool in determining the ability of WSMV to cause systemic infection in many wild wheat species as well as a previously designated resistant germ plasm. This information is useful to breeders interested in incorporating WSMV resistance into common wheat. Finally, a rapid, simple, and informative assay system using the symptomatology, ELISA, and slot-blot hybridization procedures was developed to analyze critically a wide variety of germ plasm for potentially useful WSMV resistance.

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