EVALUATION AND GENETIC ANALYSIS OF WHEAT STREAK MOSAIC VIRUS RESISTANCE IN WHEAT GERMPLASM BY SYMPTOMATOLOGY, ENZYME-LINKED IMMUNOSORBENT ASSAY, AND SLOT-BLOT HYBRIDIZATION

by

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TABLES

Introduction

Wheat Streak Mosaic (WSM) is historically an important virus disease in the Great Plains states and Canada, as well as many other wheat producing countries of the world. In Kansas, losses have been estimated up to 7% in 1981 and averaged 1.8% in the past 5 years (Sim & Willis, 1985). These losses are significant considering that up to 460 million bushels of wheat are produced annually in Kansas alone.

The wheat curl mite, Eriophyes tulipae (Keifer), efficiently vectors the virus from volunteer wheat, which serves as host to the vector and virus, to fall-seeded wheat. Destroying volunteer wheat and observing recommended late planting dates are practices that help, although not always effectively, to control WSM.

Breeding for resistant varieties of wheat (Triticum aestivum L.em. Thell) will provide the best control of WSM. As of yet there are no commercial varieties resistant to the virus, but many researchers are seriously addressing this problem in their breeding programs.

The purpose of this research was to adapt and evaluate two virus diagnostic techniques, the double antibody sandwich method of enzyme-linked immunosorbent assay (ELISA) and slot-blot hybridization, in screening wild wheat germplasm for resistance to WSMV. These assays were coupled with a symptomatology assay where wild wheat germplasm was screened for resistance to WSMV. Determining the ability of the virus to replicate and systemically invade the plant, as well as observing the symptoms caused by the virus, should be an accurate method for detecting resistant germplasm.

These diagnostic techniques were applied to the evaluation of wheat X Agropyron amphiploids and derived disomic addition lines (Cauderon,

Saigne, and Dauge, 1973 and Dvorak and Knott, 1974). These evaluations were performed to try to explain the mechanism(s) involved in conferring resistance to WSMV.

In addition to evaluating the Agropyron amphiploids and derived addition lines, crosses were made between the addition lines, derived from the amphiploid Chinese Spring X A. elongatum, for chromosomes 1E, 2E, 3E, 4E, 5E, and 7E as the female parents and the addition line for chromosome 6E as the male parent. The progeny from these crosses were inoculated with WSMV and screened for resistance by symptomatology, ELISA, and slot-blot hybridization. Results from these tests should help to elucidate the mechanism(s) involved in conferring resistance to WSMV. This information should be useful to breeders interested in developing WSMV-resistant cultivars.

Literature Review

Since its first recorded appearance in Kansas during the spring of 1929 (Melchers and Fellows, 1930), wheat streak mosaic (WSM) has received much attention. Severe crop losses from this disease occurred in the WSM epidemics of 1949, 1959, and 1981 (King & Sill, 1959 and Willis, 1981). Development of resistant commercial varieties of wheat has been a problem plant scientists and wheat breeders have been trying to solve for many years. Two Scout-derived lines, Eagle and Sage, have moderate tolerance to WSMV, but this tolerance is not stable and serious losses occur in years when conditions are favorable for disease (Martin, Harvey, and Livers, 1976). Follow-up work on these lines, however, has shown that they do not possess any tolerance to WSMV. Triumph is now the only known cultivar to possess tolerance to WSMV (Seifers, personal communication). Presently, there are no commercial wheat varieties available with high levels of resistance to WSMV, hence annual losses from this disease will be inevitable until resistant cultivars are produced.

There are several approaches being taken to develop commercially acceptable wheat varieties that are resistant to WSMV. Agropyron, otherwise known as western wheatgrass, is an important source of alien genetic variation and disease resistance in wheat. McKinney and Sando (1951) tested 17 species of Agropyron for WSMV resistance. None of the 17 species of Agropyron tested exhibited mosaic although a few showed local lesions. They were not able to detect the presence of virus in any of the Agropyron species tested. More recently, Sharma, Gill, and Uyemoto (1984) identified valuable germplasm among Agropyron species for resistance to WSMV. They found that 11 out of 16 Agropyron species tested remained

symptomless and 4 species contained only a few symptomatic plants. These segregants, they explained, could be attributed to mechanical mixture or outcrossing.

Intergeneric hybrids (Triticum X Agropyron) have been and are presently being used as sources of disease resistance in certain wheat breeding programs. McKinney and Sando (1951) performed a study with Agrotricum hybrids inoculated with WSMV. Those hybrids with Agropyron elongatum (Host) Beauv. were found to be resistant or immune to WSM. Later studies by Fellows and Schmidt (1953) and Schmidt, Sill, and Fellows (1956) showed that a wide range of reactions to WSMV, from complete resistance to complete susceptibility, could be observed in crosses between Agropyron species and wheat. The latter group concluded from their results that the immune reaction of A. elongatum may be controlled by a complex mechanism which, as a result, could complicate attempts to transfer a satisfactory level of resistance from A. elongatum to wheat.

Swarup et al. (1956) performed cytogenetical studies on various generations of hybrids derived from crosses of the susceptible variety Pawnee with a 56-chromosome wheat X Agropyron derivative which normally gave a local lesion type reaction to WSMV. The F_1 showed only the L (local lesion) reaction. The F_2 and F_3 segregated into three groups: S (systemic), L, and L/S (local lesion usually turning into a lethal systemic reaction). Segregation was random since no definite genetic ratios were observed. On the basis of the number and size of the chromosomes that were present in the different reaction types, they concluded that more than one Agropyron chromosome governed resistance to WSMV in the L plants. Furthermore, they concluded that genes for the L reaction were located on the Agropyron chromosomes that failed to pair

with wheat chromosomes.

The same group of researchers found that grass-like characters from the Agropyron parent are closely linked with the local lesion reaction exhibited by certain Agrotricum hybrids and are presumed to be carried on the same chromosome(s). They concluded from their findings that it will be difficult to produce a line of wheat with resistance to WSMV without the grass-like qualities of Agropyron.

From studies on advanced generation Agropyron X Triticum lines, Raj (1965), on the other hand, suggested that it was possible to select resistant wheat-like hybrids. He crossed one grass-like line (2n = 35-56) and five resistant wheat-like lines (2n = 35-44) with commercial cultivated wheat. The F_1 of the crosses involving the wheat-like lines had 42 chromosomes, but only one had 21 bivalent pairs. Since this Agrotricum line had considerable resistance, he concluded that at least one very small portion of the Agropyron chromosome must have been translocated to a wheat chromosome. He reasoned that pairing of homologous wheat chromosomes could take place because of the minute size of the translocated segment. A final conclusion Raj made from F_2 seedling segregation ratios of resistant and susceptible plants was that resistance was controlled by two recessive factors. When two lines had one of these factors in common, monohybrid ratios were observed.

Sebesta and Bellingham (1966) made observations on selections of the Sando-derived wheat X Agropyron hybrids which are wheat-like in nature yet still possess resistance to WSMV. In their two parental F_2 plants, which they regarded as alien addition types, it appeared that only one pair of chromosomes was involved in the genetic control of virus resistance. They concluded that if upon further testing they find that only one pair of

chromosomes is involved in governing resistance to WSMV, then genetic control of this virus would be much simpler.

Larson and Atkinson (1970) screened 286 Agrotricum lines for reaction to WSMV and identified one immune wheat-like line. These particular plants had 21 bivalent chromosomes, as does common wheat, but had seed and spike characteristics which resembled Agropyron. By making crosses with lines ditelosomic for known chromosomes, they determined that Agropyron chromosomes replaced wheat chromosomes 4D, 5D, and 6D in a triple substitution.

In a later paper, Larson and Atkinson (1973) isolated single chromosome substitution lines and showed that none were immune to WSMV. However, disomic substitution (ds) line 6D had considerable resistance, ds line 5D delayed development of the disease, and ds line 4D was susceptible. The ds line 6D was also resistant to the mite vector (Whelan et al., 1982). Germplasm LRS-1F193 was produced from this line and was released by the Lethbridge Research Station, Agriculture Canada, Lethbridge Alberta, in 1982 (Whelan et al., 1982). This germplasm was described as carrying resistance to the mite vector. This germplasm was evaluated for WSMV resistance in the present study by symptomatology, ELISA, and slot-blot hybridization.

Recently, there have been several wheat X Agropyron germplasm lines registered that are resistant to WSMV. Liang et al. (1978) at the Kansas Agricultural Experiment Station developed a wheat germplasm resistant to all four known strains of WSMV. It was a selection from the progeny of a cross between Chinese Spring monosomic 5B and CI 15092 (an Agropyron intermedium substitution line for chromosome 4B). This germplasm was further characterized in the present study by ELISA and slot-blot

hybridization.

Additional WSMV-resistant germplasm derived from CI 15092 was developed at the South Dakota Agricultural Experiment Station by Wells et al. (1982). Five translocation lines and one disomic substitution line were produced from the cross CI 15092/T. speltoides/Fletcher/3/5*Centurk. They tested these lines for seed and flour protein content and found that three of the translocation lines and the disomic substitution line exceeded that of Centurk, the recurrent parent. None of the lines, however, were as desirable agronomically as Centurk. Nevertheless, these lines look promising for use in wheat breeding programs, and were further characterized in this thesis.

Clearly, there is no general consensus on the genetic control of WSMV resistance in wheat X Agropyron hybrids. One of the goals of this thesis research it to try to explain the confusion in all the previous reports.

Another approach to control WSM is to incorporate vector resistance into common wheat. Martin et al. (1976) tested several WSMV-resistant lines that contained chromosome substitutions or translocations from A. elongatum. They found two lines that were resistant to both the wheat curl mite and WSMV when the plants were infested with viruliferous mites. Their findings indicated that WSMV resistance and mite resistance are genetically linked in both lines. These findings seemed promising in that mite resistance should be obtained simultaneously when selecting for WSMV resistance. In 1984, however, Martin et al. explain that adapted WSMV-resistant cultivars have not been developed because undesirable agronomic characteristics from Agropyron are genetically linked with WSMV resistance.

The main aspect of this thesis concerns the detection of

WSMV-resistant germplasm that could be used as potential sources of disease resistance in breeding programs. The wild relatives of the cultivated wheats have been shown to be a rich source of genetic variation and also to carry many genes of economic potential. With the increasing concern over the erosion of gene resources and genetic vulnerability of the world's wheat crop, the wild wheats show great promise for broadening the genetic base and reducing the genetic vulnerability of cultivated wheat (Gill *et al.*, 1984). Screening these wild wheats for increased resistance to WSMV is still in the initial stages.

McKinney and Sando (1951) screened several related species of *Triticum* for resistance to WSMV because none of the commercial wheats tested exhibited any resistance. Of the 14 species tested, 13 exhibited 100% infection. *T. dicoccum* had 93% mosaic infection. Gill *et al.* (1984) screened 176 accessions of wild wheats for resistance to WSMV by symptomatology and found 10 out of 69 accessions of *T. boeoticum* and 4 out of 21 accessions of *T. dicoccoides* to be resistant to WSMV. All of the *T. araraticum* or *T. urartu* accessions tested were susceptible to WSMV. They stated that the 4 resistant *T. dicoccoides* accessions did not show typical symptoms but that bioassay tests proved that the virus was present. They concluded that those 4 accessions were tolerant to WSMV. Since genes from *T. dicoccoides* can be readily transferred to durum and bread wheat, Gill *et al.* suggest that *T. dicoccoides* may provide a useful source of WSMV resistance.

Finding suitable methods of detecting WSMV resistance in wild wheats is important for the improvement of cultivated wheats and merits further consideration. One aspect of this thesis deals with adapting and evaluating two virus diagnostic techniques, the double antibody sandwich

method of ELISA and slot-blot hybridization, in conjunction with a symptomatology assay, in screening a large selection of wild wheats and wheat lines derived from crosses with Agropyron. All germplasm was obtained from the Wheat Genetics Resource Center, Kansas State University.

The symptomatology assay test, which involves the visual inspection of symptom development, is currently the most common method of screening germplasm for resistance to WSMV. McKinney and Fellows (1951) developed a successful spraying method to inoculate wheat with WSMV. Martin (1978) used this procedure, which involves spraying wheat seedlings using a DeVilbiss atomizer with prepared inoculum that contains an abrasive, in developing procedures for evaluating WSMV resistance in wheat lines derived from a wheat-Agropyron hybrid. It is a useful inoculation technique and has been used widely. Since WSMV does not overwinter in the soil and using the vector for inoculating large nurseries is impractical, spray inoculation and subsequent observation of symptom development has been the method of choice in most WSMV symptomatology assays.

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 with the ability of the virus to replicate within the host. In a susceptible host the virus is able to replicate and spread systemically, whereas in a resistant host the virus is not able to replicate and spread systemically. As a result, making use of assays which determine the replicative ability of the virus in the host, in addition to symptomatology assays, will provide a more accurate method of screening lines for WSMV resistance.

Serological diagnosis of plant viruses using enzyme-linked immunosorbent assay (ELISA) is a popular and widely used method for the

detection and quantitative assay of plant viruses. The ELISA procedure is simple, sensitive, requires small amounts of antiserum, and can be completed in several hours. ELISA was first used for the detection of virus antigens in animal tissues (Nakane and Pierce, 1966). Then Voller et al. (1976) succeeded in applying the double antibody sandwich ELISA to plant viruses. He showed that the technique could detect viruses at concentrations as low as 10 ng/ml in purified preparations as well as in crude plant extracts. Clark and Adams (1977) expanded the use of ELISA to several different plant viruses and were able to show that ELISA may be effective as a tool for virus detection and diagnosis.

Sherwood (1984), using purified preparations of WSMV from wheat, detected 2 ng/ml virus by ELISA and found that the degree of response was linear for 4 to 256 ng/ml. The sensitivity and applicability for quantitation of this test is obvious.

In this study, the double antibody sandwich form of ELISA was used in order to determine the presence of viral protein production. Initially rabbit antibodies, which are produced upon inoculation of pure virus (antigen), are coated to the surface of a polystyrene ELISA plate. The virus particles in the diseased plant sample are then trapped by the specific antibodies. In other words, the antibodies act as a probe to detect viral specific protein from host tissue. After washing, the trapped virus is reacted with a viral specific antibody with an attached enzyme 'tag'. The plate is washed to remove unbound enzyme-labeled antibody. Enzyme-labeled antibody that has complexed with the trapped virus is monitored by the addition of an enzyme-specific substrate.

The slot-blot hybridization procedure used in this experiment is a modification of the dot hybridization method developed by Kafatos, Jones,

and Efstratiadis (1979). The main purpose of the standard hybridization procedure is for rapidly determining the relative concentrations of nucleic acids in a mixture. Kafatos et al. spotted identical quantities of cloned linearized plasmid DNAs on a nitrocellulose filter in dots of uniform diameter. They hybridized the filter with a radioactive probe and evaluated the extent of hybridization of the probe with the filter-bound DNA after autoradiography. The method they employed to evaluate the amount of hybridization that occurred between the probe and the DNA dots was by visual comparison to a standard, which consisted of a dilution series of radioactive DNA spotted on a nitrocellulose filter in dots of the same diameter.

As with many other new advances in molecular virology, dot hybridization in animal systems has been studied much more extensively than in plant systems. In 1983, Brown, Tlsty, and Schimke modified the dot hybridization assay of Kafatos et al. (1979) to determine the presence of amplified dihydrofolate reductase (DHFR) genes in methotrexate (MTX)-resistant subclones. They designed a slot template which was machine made from 0.375-inch Lucite with parallel rows of polished conical slots tapering to 1 by 6 mm. Brown et al. were able to visually discriminate DHFR gene amplification from multiple exposures. They claimed that visual evaluation was as reliable as densitometric analysis of slot intensity.

Maule, Hull, and Donson (1983) described a dot hybridization technique for the detection of DNA and RNA viruses in plant tissues. They adapted the dot hybridization technique for the rapid screening of large numbers of leaf tissue samples for a range of DNA and RNA viruses, as well as for the application in the quantification of cauliflower mosaic virus

(CaMV) DNA from leaves and protoplasts of turnip. They determined that the limit of sensitivity is 5-20 pg virus/dot or approximately 5 ug/g leaf tissue, about the same order as that of ELISA. They also concluded that the dot-blot hybridization method is quantitative for DNA viruses in crude homogenates, but not for RNA viruses.

That same year, Sela, Reichman, and Weissbach (1983) described a dot hybridization method which could be used to detect 2.5 pg TMV-RNA in the crude saps of leaf tissue and in protoplasts derived from cultured cells. According to Sela et al., this technique would be useful in detecting early events of viral infection (before any measurable amount of virus protein is produced), as well as detecting unexpressed or latent infections. They concluded that the dot hybridization assay is very specific, easy to perform, and does not require the isolation of viral RNA. Furthermore, they claimed that dot hybridization is simpler and about twice as sensitive as ELISA, and that the radiolabeled cDNA probe can be rapidly prepared from TMV-RNA by reverse transcription with a commercially available enzyme.

Nucleic acid hybridization has become a popular technique for the detection and quantitation of DNA and RNA viruses in plant tissues. In this thesis, a large variety of wild wheat germplasm was inoculated with WSMV and examined by slot-blot hybridization for the occurrence of viral replication. The slot-blot assay utilizes a probe, a ^{32}P-labeled complementary copy of the viral nucleic acid (WSMV), which specifically binds to any viral nucleic acid in the sample. The infected leaf sample is ground in a buffer and spotted onto a nitrocellulose filter. The filter is exposed to the radioactive probe, put through stringent washings to remove unbound probe, and autoradiographed. The resultant slots are

evaluated by densitometric readings.

The first objective of this project is to determine if symptomatology, viral protein production as detected by ELISA, and viral replication as detected by slot-blot hybridization are correlated, and what assay or combination of assays best detects resistant germplasm. The second objective is to determine the value of slot-blot hybridization in detecting the ability of WSMV to cause systemic infection in registered germplasm known to be resistant to WSMV. The third objective is to develop a rapid and workable assay system using all three procedures (symptomatology, ELISA, and slot-blot hybridization) to analyze wild wheat germplasm for potentially useful WSMV resistance.

Finally, by applying ELISA and slot-blot hybridization to the evaluation of T. aestivum cv. Vilmorin X A. intermedium amphiploid and derived addition lines (Cauderon, Saigne, Dauge, 1973), and to the evaluation of T. aestivum cv. Chinese Spring X A. elongatum amphiploid and derived disomic and ditelosomic addition lines (Dvorak and Knott, 1974), it should be possible to determine the mechanism(s) involved in conferring WSMV resistance.

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Evaluation of Wheat Germplasm for Resistance to WSMV by Symptomatology, ELISA, and Slot-Blot Hybridization

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ABSTRACT

Stoddard, S. L., Lommel, S. A., and Gill, B. S. 1986. Evaluation of wheat germplasm for resistance to WSMV by symptomatology, ELISA, and slot-blot hybridization.

Double antibody sandwich enzyme-linked immunosorbent assay (ELISA) and slot-blot hybridization assays 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 X Agropyron amphiploids were found to be resistant to WSMV. Nine registered germplasms resistant to WSMV were evaluated; 8 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 overall good correlation, and the value of these procedures in detecting WSMV-resistant germplasm is discussed.

Wheat streak mosaic (WSM) is historically an important disease in the Great Plains states and Canada, as well as many other wheat producing countries of the world. In Kansas, losses have been estimated up to 7% in 1981 and averaged 1.8% in the past 5 years (20). These losses are significant considering that up to 460 million bushels of wheat are produced annually in Kansas. Breeding for resistant varieties of wheat (Triticum aestivum L.em. Thell) will provide the best control of WSM. As of yet there are no commercial varieties highly resistant to the virus.

McKinney and Sando (13), screened several related species of Triticum for resistance to WSMV because none of the commercial wheats tested exhibited any resistance. Of the 14 species tested, 13 exhibited 100% infection, while an accession of T. dicoccum had 93% infection. Gill et al (5) screened wild wheats for resistance to WSMV by symptomatology and found some accessions of T. boeoticum and T. dicoccoides to be tolerant to WSMV. The 4 tolerant T. dicoccoides accessions did not show typical symptoms but bioassay tests proved the virus was present. All of the T. araraticum or T. urartu accessions tested were susceptible to WSMV.

The more distant relatives of wheat appear to be more promising sources of WSMV resistance. McKinney and Sando (13) tested 17 species of Agropyron for WSMV resistance. None of the 17 species of Agropyron exhibited systemic symptoms although a few developed local lesions. They were not able to detect the presence of virus in any of the Agropyron species tested. Sharma et al (19) found that 11 of 16 Agropyron species tested remained symptomless and 4 species segregated for resistance. Recently, WSMV resistant wheat germplasm derived from A. intermedium has been developed (8, 23). Although none of the lines are desirable agronomically, they appear to be 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, the double antibody sandwich method of enzyme-linked immunosorbent assay (ELISA) and slot-blot hybridization, in conjunction with a symptomatology assay, appear to be promising in screening wild relatives of wheat and in characterizing WSMV resistant wheat germplasm.

The symptomatology assay, which involves the visual inspection of symptom development, is currently the most common method of screening germplasm 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 with the ability of the virus to replicate within the host. As a result, making use of assays which determine the replicative ability of the virus in the host, in addition to symptomatology assays, will provide a more accurate method of screening lines for WSMV resistance.

The double antibody sandwich form of ELISA was used in order to determine the presence of viral protein production. The slot-blot hybridization procedure is a modification of the dot hybridization method developed by Kafatos et al (7). It was used for rapidly determining the relative concentrations of viral nucleic acid replication 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 detected resistant germplasm. The second

objective was to determine the value of slot-blot hybridization in detecting the ability of WSMV to cause systemic infection in registered germplasm known to be resistant to WSMV. The third objective was to develop a rapid and workable assay system using all three procedures (symptomatology, ELISA, and slot-blot hybridization) to analyze wild wheat germplasm for potentially useful WSMV resistance.

MATERIALS AND METHODS

A large selection of wild wheat germplasm obtained from the Wheat Genetics Resource Center (WGRC), Kansas State University was screened for WSMV resistance. The germplasm included 158 accessions of Aegilops species and 325 accessions of Triticum species. In addition, 14 amphiploids of various origins, as well as 9 registered germplasms resistant to WSMV, were tested. Twenty or fewer seeds, depending upon 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 greenhouse flats containing a sandy loam soil mix (14 rows per flat). The flats were kept in a growth chamber at 21 C, 14-hr light, 16-18 C, dark. Flourescent 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 was maintained on a susceptible corn hybrid N28Ht with frequent transfers (21). The inoculation preparation procedure followed was similar to that of Martin (11). Ten-to 14-day old infected plants were triturated at a 1:20 dilution (w:v) in 0.2 M potassium phosphate buffer (pH 7.0) and strained through double-folded cheesecloth. Carborundum abrasive (600 mesh, 1.5%) was added to the filtrate. A DeVilbiss atomizer was used to spray 7-9 day old seedlings from a distance of 1 cm at 6.3 Kg/cm^2 until water-soaked lesions could be seen on inoculated leaves. Inoculated plants were rinsed with water to remove excess carborundum and returned to the growth chamber. The plants were examined for symptoms after 7-8 days and every

other day thereafter for 7-9 days. Parker wheat was the susceptible control in all tests (11).

Tissue Samples. Leaf samples were harvested from each accession after 15-17 days. The middle third of the youngest leaves were pooled from all plants in each line (Seifers, personal communication). The leaves were evenly divided into two labeled 1.5 ml microfuge tubes, one to be used for ELISA, the other for the slot-blot hybridization assay. Approximately 0.3 g tissue was placed in each tube. The tubes were stored at -20 C until both assays were performed.

Bioassay Test. A bioassay test was performed on all registered germplasms known to be resistant to WSMV (Table 4). Inoculated leaf tissue samples (0.3 g) were placed in labeled microfuge tubes and stored at -20 C until needed. Two seeds of Parker, the susceptible control, were planted for each germplasm line and grown under growth chamber conditions as described earlier. Parker wheat was dusted with carborundum 9 days after planting. Tissue samples were ground with a wooden applicator stick; sap was rub-inoculated to Parker with Q-tips. The plants were observed for symptoms up to 2 wk post-inoculation. Plants that expressed symptoms were given a positive reading. Those plants that did not show symptoms were given a negative reading.

Virus Preparation. WSMV was purified for serum production and ELISA following the procedure of Lommel et al (9). One hundred grams of infected N28Ht corn leaves were homogenized in 200 ml 0.2 M sodium acetate buffer, pH 5.0, 1% mercaptoethanol, strained through cheesecloth, and allowed to sit at room temperature for 15-20 min. The extract was centrifuged at 6,000g for 30 min in a Sorvall GSA rotor. One quarter volume of 40% polyethylene glycol (PEG 6000) in 1 M NaCl was added while

stirring on ice. The supernatant was stirred on ice for 1-2 hr and centrifuged at 6,000g for 30 min. The pellet was resuspended in 10 ml 0.1 M Tris, 0.032 M sodium citrate buffer, pH 6.5. The supernatant was centrifuged through 20% sucrose pads in a Sorvall AH-627 rotor at 25,000g for 2 hr. The pellet was resuspended at 4 C overnight in 1 ml 0.1 M Tris, 0.032 M Na citrate, pH 6.5, and further purified by one cycle of differential centrifugation and fractionation on linear log sucrose gradients in 0.1 M Tris, 0.032 M Na citrate, pH 6.5. Peaks collected from the sucrose gradients were then banded in CsCl equilibrium gradients. The CsCl gradients were prepared by adding 12.1 g CsCl to double distilled water and adjusting the volume to 30 ml. The CsCl gradients were centrifuged at 50,000g for 22 hr in a Beckman SW55Ti rotor and fractionated. Peaks were dialyzed against 3 changes of sterile 1X PBS (phosphate buffered saline) and centrifuged at 40,000g for 2 hr in a Beckman SW55Ti rotor. The pellet was resuspended in 400 ul sterile 1X PBS and assayed spectrophotometrically.

Antiserum production. Antiserum against CsCl gradient purified WSMV was produced in a white New Zealand rabbit. Virus in 1X PBS 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 prior to initial injection and on days 14, 27, 37, 52, 59, 66, 73, 89, 96, and 103. All serum used in this report was from day 37 which had the highest titer.

Immunoglobulin Preparation. The immunoglobulin (Ig) fraction was purified from antisera as described by Clark and Adams (3). Five ml of antiserum were diluted with 5 ml of saturated ammonium sulfate. The pellet resulting after centrifugation was dialyzed against 3 changes of

0.5X PBS. The Ig was passed through a 2-3 cm bed of DEAE cellulose in 0.5X PBS. It was then adjusted to approximately 1 mg/ml, diluted with a half volume of glycerol, and stored at -20 C.

Enzyme Antibody Preparation. Alkaline phosphatase (Type VII-S; Sigma Chemical Co., P.O. Box 14508, St. Louis, MO) was coupled to purified immunoglobulin by the glutaraldehyde method of Avrameas (1). The resuspended alkaline phosphatase pellet was dialyzed against 3 changes of 0.5X PBS at 4 C. Glutaraldehyde was added to a 0.0625% final concentration and removed by dialysis against 3 changes of 0.5X PBS. The conjugate was diluted with a half volume of glycerol and stored at -20 C.

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 ul of 0.05 M carbonate buffer, pH 9.6. Incubation for all steps was for 1 hr at 37 C. The plates were washed between steps with ELISA wash (0.15 M NaCl containing 0.1% Tween-20). Antigens were then added to the Ig-coated wells. Five-fold serial dilutions were made with pure virus in one column on all plates as a control. Infected tissue samples were ground with a wooden applicator stick in 250 ul 1X PBS and microfuged for 5 min. Fifty ul sap were added to 200 ul "ELISA buffer" (EB: 0.01 M PBS, pH 7.4, 0.05% Tween-20, 1% polyvinylpyrrolidone-40, and 0.2% ovalbumin). The remaining sap was transferred to a second labeled microfuge tube and frozen. Incubation with the enzyme-conjugated Ig was at a 1/100 dilution in EB. Reactions were recorded at 15 min intervals for 45 min after the addition of substrate (200 ul 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).

RNA Extraction Procedure. Pure WSMV viral nucleic acid to be 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 (14). Sucrose density gradient purified WSMV was incubated for 30 min at 37 C with Protease K and 10% SDS. Bentonite (40 mg/ml), 2X STE (pH 8.0), and 10% SDS were added to the virus preparation and the mixture was incubated at 60 C for 2 min. The nucleic acid was extracted with phenol, concentrated by ethanol precipitation, and analyzed by denaturing agar gel electrophoresis.

Filter-bound Hybridization. The slot-blot hybridization procedure was performed in a similar manner as described by Brown et al (2) with several modifications. Nitrocellulose filters were prewetted in boiling water and allowed to soak in 20X SSC (175.3 g NaCl and 88.2 g Na Citrate/l, pH 7.0) until ready for use. The slot-blot template was incubated for 30 min in 20X SSC with 100 ug/ml denatured salmon sperm DNA and rinsed thoroughly with 20X SSC (4). Tissue samples were prepared as for the ELISA procedure. Two concentrations of infected sap were spotted on the nitrocellulose: 25 ul and 10 ul in 175 ul and 190 ul buffer, respectively. Five-fold serial dilutions of pure WSMV RNA were made in one column as a control in all tests. Slots were post-rinsed with 200 ul 20X SSC. After the filter was rinsed in 5X SSC for 5 min and air dried for 30 min, it was baked for 2 hr at 80 C.

Prehybridization. Prehybridization of slot-blot filters was done by soaking filters in prehybridization buffer in a sealed bag overnight at 42 C in a shaking water bath. Prehybridization buffer consisted of 50% neutralized formamide, 20X SSPE (174 g NaCl, 27.6 g NaH_2PO_4 H_2O, and 7.4 g EDTA/L, pH 7.4), 10 ug/ml denatured salmon sperm DNA, 50X Denhardt's

solution, and distilled water.

Hybridization. Hybridization of slot-blot filters was done by soaking filters in hybridization buffer with labeled probe in a sealed bag overnight at 42 C in a shaking water bath. Hybridization buffer was identical to prehybridization buffer except it had 20% as much 50X Denhardt's solution and more distilled water to adjust the volume. After hybridization, the filters were washed with three changes of 2X SSC, 1% SDS for 15-20 min at room temperature with agitation, and three changes of 0.1X SSC, 1% SDS for 15-20 min at 60C with agitation.

Nick Translation. For hybridization to slot-blots, cloned probes were labeled by nick translation (16). Nick translation of WSMV cDNA was carried out by combining in a 1.5 ml microfuge tube 40 ul pWSM-8 (0.1 ug/ml), 2.5 ul ^{32}P (1 uCi/ml), 10 ul 10X reaction buffer (500 ul 1M Tris-HCL, pH 7.9, 50 ul 1 M $MgCl_2$, 100 ul 5 mg/ml bovine serum albumin, 8 ul mercaptoethanol, and 342 ul distilled water), 1 ul dNTPs (1 ul 20 mM dCTP, dTTP, dGTP, and 97 ul distilled water), and 1 ul DNAase solution (1 ml distilled water and 2 ul 50 ug/ml DNAase). This solution was mixed gently. Then 1 ul DNA polymerase I and 44.5 ul distilled water were added. The contents were again mixed gently and reacted for 4 hr at 15 C. The reaction was terminated by the addition of 1 ul of 10% SDS. At this point the RNA was counted for specific activity of ^{32}P in a scintillation counter. Phenol extraction and ethanol precipitation of the probe were then carried out in order to eliminate any free radioactive nucleotides.

Autoradiography. Kodak XAR-5 film was exposed to the hybridized filters for 48 hr at -70 C using Cronex Lightning Plus intensifying screens. A Kontes densitometer was used to scan the developed autoradiographs.

Data Analysis. For the ELISA tests, average absorbances at 405 nm, measured 30 min after the addition of substrate, were considered positive for test samples if the values were higher than the healthy wheat and healthy corn control samples in the same plate. Peak heights of the autoradiograph densitometric readings obtained from the slot-blot hybridization assays were measured in mm for the 10 ul dilution only. Results were considered positive for test samples if the peak heights were greater than the healthy wheat and healthy corn control samples in the same test.

RESULTS

Obtaining high yields of purified WSMV has historically been a difficult task for researchers due to the length of the rods and instability of the virus particles. The purification procedure originally used in this project was that of Uyemoto and Ferguson (21) which consisted of grinding infected tissue with phosphate buffer and clarifying with the organic solvent, chloroform. Due to the unsatisfactory yields of purified virus obtained using this method, as well as the danger involved in using chloroform as a clarifying agent, the low pH sodium acetate buffer procedure was adopted as the purification method of choice. Better yields and cleaner preparations were obtained with this method and it eliminated the need for using organic solvents, as well.

The antiserum produced against CsCl gradient purified WSMV showed no cross-reactivity with healthy wheat or healthy corn (the virus was purified from infected corn) therefore, cross-absorption of the antisera with healthy plant protein was not necessary (Table 3).

A 2.1 Kb fragment (pWSM-8) representing over 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 Gubler and Hoffman (6). 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. pWSMV-8 has been shown to possess no homology to DNA from uninfected wheat or corn.

A total of 325 wild wheat accessions were screened by symptomatology for resistance to WSMV (Table 1). One hundred seventy four *T. dicoccoides* accessions were tested. The remaining accessions with totals of 136, 12,

and 3 were T. araraticum, T. boeoticum, and T. dicoccum, respectively. No resistance to WSMV was detected among any of these lines (Table 1).

Twenty four accessions (those with superscript b in Table 1) initially had very few plants exhibiting symptoms upon inoculation with WSMV. These lines were then replanted and again inoculated with WSMV; all lines had some infected plants which expressed symptoms. In these lines and in many of the wild wheat species, symptoms were masked due to dark green to reddish pigments in the leaves. In addition, the extreme slenderness of the leaves of some accessions made observation of symptoms difficult. Upon retesting, TA 3 and TA 22 exhibited 1/7 12 PI and 2/9 15 PI, respectively. Although symptoms were not observed due to the masking phenomenon mentioned above, they were presumed to be present and, thus, these two lines were considered susceptible.

A variable number of accessions from 6 diploid and 5 polyploid species of Aegilops were screened by symptomatology for resistance to WSMV (Table 2). All accessions were screened by symptomatology. Most of these accessions were also tested by the ELISA and the slot-blot hybridization assays. No resistance to WSMV was detected among these lines.

Two Aegilops accessions, however, gave variable results. One accession of Aegilops squarrosa, TA 1645, had very dark green leaves which made observation of symptoms difficult. Although the symptomatology test was not a reliable way to evaluate this accession, the ELISA test gave a definite positive reading. The other line which gave variable results was TA 1863, the only accession of Aegilops triaristata tested. Results from three symptomatology assays never showed complete infection. Results from the ELISA test were positive, but a negative slot-blot value was observed.

Five wheat X Agropyron amphiploids were found to be resistant by

symptomatology, ELISA, and slot-blot hybridization (Table 3). Five of the seven resistant plants of the amphiploid TA 3426 (T. aestivum X A. scirpeum) exhibited symptoms 10 wks after being transferred to the greenhouse. The exact date of initial symptom expression was not known. The other amphiploid lines failed to show symptoms after being transferred to the greenhouse.

Seven different wheat amphiploids derived from crosses with distant wheat relatives such as Elymus sp. Hordeum sp., Aegilops sp., and T. tauschii (the presumed D genome donor of common wheat) were tested for reaction to WSMV (Table 3). All 7 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 germplasms resistant to WSMV were tested by symptomatology, ELISA, and slot-blot hybridization for reaction to WSMV (Table 4). WSMV-resistant germplasms CI 15321 and CI 15322, 42-chromosome lines derived from crosses between wheat and Agropyron elongatum (17), gave negative values for all 3 assays performed. The bioassay test, although not performed on CI 15322, was performed on CI 15321 which proved to be immune. CI 17766, a line derived from a cross with CI 15092 (an A. intermedium substitution line for chromosome 4B), was comprised of 4 selections: 4806, 4807, 4808, and 4809 (8). The one plant which exhibited symptoms in the original test was from selection 4806. Upon retesting, all plants tested from selections 4807 and 4808 exhibited symptoms, while no plants from selections 4806 and 4809 became infected.

Registered germplasms CI 17881-CI 17886 also were derived from crosses with CI 15092 (23). CI 17881, a translocation line, gave variable

results when tested by symptomatology. Some plants from this germplasm exhibited symptoms in two different symptomatology tests. Single plants from selections CI 17883 and CI 17885, translocation and disomic substitution lines respectively, also exhibited symptoms upon inoculation with WSMV. CI 17886, a translocation line, gave variable results in symptomatology assays. In one test, out of 8 plants inoculated none exhibited symptoms. However, in a second test, 4 out of 10 plants exhibited local lesions at the site of inoculation. ELISA, slot-blot hybridization, and bioassay test results were negative for all of the registered germplasms evaluated except for CI 17881, where all values were positive.

Germplasm LRS-1F193, released by the Lethbridge Research Station in Alberta, Canada (24) was produced from chromosome substitution line 6D which was originally derived from a cross between Triticum aestivum cv. Rescue X A. elongatum. This germplasm was described as carrying resistance to the mite vector, Eriophyes tulipae (Keifer). This line gave susceptible readings in the symptomatology, ELISA, and slot-blot assays.

DISCUSSION

Although the entire wild wheat germplasm collection 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. dicoccum were included in the evaluation. The conclusion that T. dicoccoides lacks WSMV resistance (Table 1) is not in agreement with the previous report (5), where they identified tolerance of WSMV in T. dicoccoides. Resistance is defined here as the lack of virus replication. In addition, Gill et al (5) identified WSMV resistance among accessions of T. boeoticum. In the present evaluation, albeit limited, no resistance was identified among T. boeoticum accessions. From our results, there does not appear to be any resistance to WSMV among the wild Triticum species.

From the results presented in Table 1 it is apparent that some Triticum accessions exhibit symptoms from 2-9 days earlier than other Triticum accessions. This delay in symptom expression may be due to the fact that leaf expansion occurs with plant growth. As the leaf expands, the expression 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.

Approximately half of the Aegilops species represented in the WGRC collection were tested for reaction to WSMV (Table 2). All accessions were found to be susceptible. Two accessions, TA 1645 (Ae. squarrosa) and TA 1863 (Ae. triaristata) produced variable results when retested by symptomatology. The leaves of TA 1645 were dark which masked the symptoms produced by WSMV. When the plants were allowed to mature in the

greenhouse, 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 and ELISA tests, yielded a negative slot-blot value. This is the only case where this phenomenon occured. It is possible that the nucleic acid was rapidly digested when the sample was processed.

The five wheat X Agropyron amphiploids resistant to WSMV (Table 3) derived their resistance from the Agropyron parent, since T. aestivum, when evaluated for resistance, was always susceptible. The Agropyron chromosomes carrying the genes which confer WSMV resistance must be stably transmitted from generation to generation in order to be a useful source of resistance in breeding programs. These 5 lines seem to be stable and good sources of WSMV resistance.

The resistance to WSMV present in TA 3426 appeared to be heat sensitive as 5 out of 7 plants expressed symptoms when allowed to grow in the greenhouse where daytime temperatures often reach 35 C. Experiments with some Agrotricum lines performed by Pfannenstiel and Niblett (15) have shown that 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 since, as described by Pfannenstiel and Niblett (15), 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 Agropyron species was demonstrated by the lack of resistance seen in the seven different wheat amphiploids derived from several different relatives of wheat (Table 3). All 7 amphiploids were susceptible to WSMV.

WSMV-resistant registered germplasms CI 15321 and CI 15322 appeared to be stable in eliciting a resistant reaction to WSMV in these tests (Table 4). These findings, however, were not in agreement with those of Pfannenstiel and Niblett (15), where they observed CI 15321 and CI 15322 (under both greenhouse and growth chamber conditions) to often develop mild WSMV symptoms on non-inoculated 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 greenhouse, CI 15321 remained symptomless throughout all growth stages, except for the typical hypersensitive response observed about 5 days post-inoculation.

WSMV-resistant registered germplasms CI 17766 and CI 17881-CI 17886 (Table 4) are all derived from CI 15092, a 42-chromosome line of wheat, T. aestivum L., that had a disomic substitution for resistance to WSMV obtained from Agropyron intermedium. Systemic symptoms were observed on some plants of CI 17766, CI 17881, CI 17883, and CI 17885. Wells et al (22) state that occasionally they would observe a susceptible plant of CI 15092; they attributed these findings to instability. Since all of these registered germplasms were derived from CI 15092, instability of the translocated segment of Agropyron chromosome in CI 17766, CI 17881, and CI 17883, as well as the substituted Agropyron chromosomes in CI 17885, is a plausible explanation for the few susceptible plants observed in these

lines.

The data in Table 4 suggest that the registered germplasms, with the exception of CI 17881, are essentially immune as determined by the bioassay test performed on Parker. Immunity as defined here is the absence of virus particles in the leaf tissue; therefore no infection can occur.

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 (12) described CI 15321 and CI 15322 to be unacceptable breeding material due to genetic linkage between WSMV resistance and undesirable agronomic characteristics from Agropyron. 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 Agropyron species than wild wheats, such as Triticum and Aegilops species. Presently, the most encouraging source of WSMV resistance seems to be among the Agropyron species. Sharma et al (19) found 11 Agropyron species which failed to express symptoms, and attempts to produce germplasm resistant to WSMV using hybrids between wheat and these Agropyron species is currently in progress (Gill et al, personal communication). Why these Agropyron species have more disease resistance than the wild wheats could be due to the fact that they share no homologous genomes with wheat (18) and, therefore, are not susceptible to infection by a virus adapted to genomes of Triticum species.

This is the first report of using the filter-bound hybridization technique in disease resistance studies; more specifically, for the

detection of WSMV nucleic acid replication in wheat. It has proven to be a successful method because of its high level of sensitivity to the presence of WSMV nucleic acid in infected plant tissues.

In conclusion, symptomatology, viral protein production as detected by ELISA, and viral replication as detected by slot-blot hybridization are generally proportional, except in a few isolated cases where the protein coat is successfully produced but the nucleic acid is not. Secondly, 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 germplasm. 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 critically analyze a wide variety of germplasm for potentially useful WSMV resistance.

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Table 1. Evaluation of Triticum species for resistance to WSMV by symptomatology

Inoc. Control	Symptomatology	Accession no.	Symptomatology
Parker	10/10 9 PI[a]	TA 21	8/8 15 PI
		TA 22	2/9 15 PI
Triticum araraticum		TA 23	6/8 15 PI
Accession no.		TA 24	6/6 9 PI[b]
TA 1	7/10 12 PI	TA 25	6/10 15 PI
TA 2	7/9 12 PI	TA 26	7/7 15 PI
TA 3	1/7 12 PI	TA 27	15/15 11 PI
TA 4	6/9 12 PI	TA 28	14/14 11 PI
TA 5	4/7 12 PI	TA 29	9/11 11 PI
TA 6	11/13 13 PI[b]	TA 30	2/4 11 PI
TA 8	6/9 12 PI	TA 31	5/6 13 PI[b]
TA 9	11/11 11 PI[b]	TA 32	11/11 11 PI
TA 10	5/8 12 PI	TA 34	13/13 11 PI
TA 11	3/7 13 PI	TA 35	11/12 11 PI
TA 12	7/8 13 PI	TA 36	10/13 11 PI
TA 13	4/5 13 PI	TA 37	6/10 11 PI
TA 14	6/6 13 PI[b]	TA 38	8/9 11 PI
TA 15	3/4 13 PI	TA 39	13/13 11 PI
TA 16	7/7 11 PI[b]	TA 40	8/9 11 PI
TA 17	4/7 13 PI	TA 41	6/8 11 PI
TA 18	1/1 7 PI[b]	TA 42	13/13 11 PI
TA 19	8/9 15 PI	TA 43	12/13 11 PI
TA 20	8/8 15 PI	TA 44	10/10 9 PI

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 45	12/12 9 PI	TA 885	13/13 11 PI
TA 46	13/13 11 PI	TA 886	13/13 11 PI
TA 47	2/3 11 PI	TA 887	13/13 11 PI
TA 48	10/11 11 PI	TA 888	15/15 11 PI
TA 49	2/2 7 PI	TA 889	13/13 9 PI
TA 50	6/6 11 PI	TA 890	14/14 11 PI
TA 102	11/11 7 PI	TA 891	13/13 9 PI
TA 859	14/14 9 PI	TA 892	15/15 11 PI
TA 861	14/14 11 PI	TA 893	5/5 11 PI
TA 862	15/15 11 PI	TA 895	14/14 9 PI
TA 863	14/14 7 PI	TA 896	11/11 11 PI
TA 864	12/12 11 PI	TA 897	13/13 11 PI
TA 866	6/6 7 PI	TA 898	13/13 11 PI
TA 867	10/10 11 PI	TA 899	12/12 11 PI
TA 868	14/14 9 PI	TA 901	13/13 11 PI
TA 869	15/15 9 PI	TA 902	13/13 9 PI
TA 871	11/11 9 PI	TA 903	11/11 9 PI
TA 874	13/13 9 PI	TA 904	11/12 11 PI
TA 875	14/15 11 PI	TA 905	15/15 11 PI
TA 878	14/14 11 PI	TA 906	6/6 11 PI
TA 882	14/14 11 PI	TA 907	13/13 11 PI
TA 883	15/15 9 PI	TA 908	15/15 11 PI
TA 884	15/15 11 PI	TA 909	14/14 11 PI

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 910	14/14 11 PI	TA 940	15/15 11 PI
TA 912	10/10 11 PI	TA 942	12/12 11 PI
TA 913	14/14 11 PI	TA 943	15/15 11 PI
TA 914	12/13 13 PI	TA 944	15/15 11 PI
TA 915	14/14 11 PI	TA 945	12/14 11 PI
TA 916	4/4 9 PI	TA 946	13/14 11 PI
TA 917	15/15 11 PI	TA 949	13/14 11 PI
TA 918	15/15 11 PI	TA 950	13/13 11 PI
TA 919	15/15 11 PI	TA 952	15/15 11 PI
TA 923	13/13 9 PI	TA 953	13/15 11 PI
TA 924	13/13 11 PI	TA 954	14/15 11 PI
TA 925	14/14 11 PI	TA 955	13/13 9 PI
TA 926	14/14 9 PI	TA 956	14/14 11 PI
TA 927	13/14 11 PI	TA 957	14/14 9 PI
TA 928	13/14 11 PI	TA 959	13/13 9 PI
TA 929	13/13 9 PI	TA 960	14/14 9 PI
TA 930	14/15 11 PI	TA 963	15/15 11 PI
TA 931	14/14 11 PI	TA 964	12/13 11 PI
TA 932	14/14 11 PI	TA 965	13/14 11 PI
TA 933	11/12 13 PI	TA 966	14/14 9 PI
TA 936	12/15 11 PI	TA 967	14/14 11 PI
TA 938	13/14 11 PI	TA 970	14/14 11 PI
TA 939	11/15 11 PI	TA 971	6/6 11 PI

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 973	15/15 9 PI	TA 71	5/7 11 PI
TA 976	15/15 7 PI	TA 72	9/9 11 PI
		TA 73	9/9 9 PI
Triticum dicoccoides		TA 74	11/13 13 PI[b]
TA 51	6/7 9 PI	TA 75	5/5 10 PI
TA 52	7/11 13 PI[b]	TA 76	7/7 12 PI
TA 53	2/2 12 PI[b]	TA 77	5/5 10 PI
TA 54	7/10 11 PI	TA 78	15/15 12 PI
TA 55	11/11 11 PI	TA 79	15/15 7 PI[b]
TA 56	6/7 11 PI	TA 81	2/2 12 PI
TA 57	10/10 7 PI	TA 82	12/12 12 PI[b]
TA 58	7/8 11 PI	TA 84	7/7 10 PI
TA 59	5/7 11 PI	TA 85	11/11 10 PI
TA 60	13/13 11 PI	TA 86	15/15 10 PI
TA 61	8/8 7 PI	TA 87	3/4 12 PI
TA 62	6/7 11 PI	TA 88	7/7 10 PI
TA 63	11/11 11 PI[b]	TA 89	14/14 10 PI
TA 64	10/12 11 PI	TA 90	13/13 12 PI
TA 65	7/7 11 PI	TA 91	2/2 8 PI
TA 66	11/11 11 PI	TA 92	1/1 12 PI
TA 67	8/8 9 PI	TA 94	2/3 12 PI
TA 68	7/7 11 PI	TA 95	8/9 12 PI
TA 70	5/5 11 PI	TA 96	5/5 9 PI[b]

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 98	9/9 10 PI	TA 997	5/5 10 PI
TA 99	12/12 12 PI	TA 998	11/11 10 PI
TA 100	12/12 12 PI	TA 999	12/12 10 PI
TA 977	15/15 7 PI	TA 1000	14/14 12 PI
TA 978	14/14 11 PI	TA 1001	12/15 13 PI[b]
TA 979	13/13 9 PI	TA 1002	10/10 12 PI
TA 980	15/15 9 PI	TA 1003	5/7 12 PI
TA 981	14/14 9 PI	TA 1004	8/10 12 PI
TA 982	12/12 11 PI	TA 1005	9/9 12 PI
TA 983	12/14 11 PI	TA 1008	11/11 12 PI
TA 984	13/14 11 PI	TA 1009	15/15 10 PI
TA 985	6/6 9 PI	TA 1010	9/9 10 PI
TA 986	10/10 9 PI	TA 1012	10/14 12 PI
TA 987	9/10 11 PI	TA 1013	11/12 12 PI
TA 988	10/10 9 PI	TA 1014	10/10 10 PI
TA 989	14/14 9 PI	TA 1015	8/9 12 PI
TA 990	14/14 9 PI	TA 1018	7/7 10 PI[b]
TA 991	13/13 9 PI	TA 1021	13/13 8 PI
TA 992	13/13 9 PI	TA 1022	14/14 12 PI
TA 993	12/12 9 PI	TA 1024	10/10 10 PI
TA 994	14/15 11 PI	TA 1025	3/3 10 PI
TA 995	12/14 11 PI	TA 1026	8/8 12 PI
TA 996	8/11 11 PI	TA 1027	11/11 10 PI

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 1028	9/9 10 PI	TA 1051	10/10 10 PI
TA 1029	11/11 12 PI	TA 1052	10/10 10 PI
TA 1030	7/7 8 PI	TA 1053	11/11 10 PI
TA 1031	13/13 10 PI	TA 1054	9/10 12 PI
TA 1032	11/11 10 PI	TA 1055	12/12 10 PI
TA 1033	12/12 10 PI	TA 1056	12/12 10 PI
TA 1034	8/8 10 PI	TA 1057	9/9 8 PI
TA 1035	10/10 10 PI	TA 1058	7/7 12 PI[b]
TA 1036	6/6 10 PI	TA 1060	13/13 12 PI
TA 1037	11/11 12 PI	TA 1061	12/12 12 PI
TA 1038	15/15 8 PI	TA 1062	12/12 13 PI[b]
TA 1039	8/8 8 PI	TA 1063	9/10 13 PI[b]
TA 1040	13/13 8 PI	TA 1064	6/8 13 PI
TA 1041	10/10 8 PI	TA 1065	5/9 13 PI
TA 1042	13/14 12 PI	TA 1066	5/9 13 PI
TA 1043	14/14 8 PI	TA 1067	6/6 13 PI
TA 1044	8/8 8 PI	TA 1068	3/5 13 PI
TA 1045	11/12 12 PI	TA 1069	8/9 13 PI
TA 1046	11/13 12 PI	TA 1070	7/10 13 PI
TA 1047	9/9 8 PI	TA 1071	7/10 13 PI
TA 1048	9/9 10 PI	TA 1072	2/2 11 PI
TA 1049	10/12 12 PI	TA 1073	6/9 13 PI
TA 1050	10/10 12 PI	TA 1074	14/14 13 PI

Table 1. (continued)

Accession no.	Symptomatology	Accession no.	Symptomatology
TA 1075	13/13 11 PI[b]	TA 1109	13/14 13 PI
TA 1076	11/14 13 PI[b]	TA 1110	12/12 11 PI
TA 1077	14/14 11 PI	TA 1115	8/10 13 PI
TA 1078	8/8 9 PI	TA 1116	7/7 11 PI
TA 1079	7/7 9 PI	TA 1119	12/12 13 PI
TA 1081	10/10 13 PI	TA 1120	10/10 11 PI[b]
TA 1082	12/12 11 PI	TA 1123	4/7 13 PI
TA 1084	8/8 11 PI	TA 1124	6/6 11 PI
TA 1086	10/10 13 PI	TA 1125	7/9 13 PI
TA 1087	14/15 13 PI	TA 1126	11/12 13 PI
TA 1088	10/12 13 PI	TA 1129	7/10 13 PI[b]
TA 1090	12/13 13 PI	TA 1130	14/14 13 PI[b]
TA 1091	10/11 13 PI	TA 1132	7/10 13 PI
TA 1092	9/9 11 PI	TA 1133	11/13 13 PI
TA 1094	11/14 13 PI	TA 1134	10/13 13 PI
TA 1095	13/13 11 PI	TA 1136	10/13 13 PI
TA 1096	8/8 13 PI	TA 1138	9/10 13 PI
TA 1097	7/7 11 PI	TA 1165	15/15 11 PI
TA 1098	13/14 13 PI	TA 1166	6/6 11 PI
TA 1099	9/9 11 PI	TA 1167	6/6 7 PI
TA 1103	9/9 9 PI		
TA 1104	12/12 11 PI	*Triticum boeoticum*	
TA 1107	11/11 9 PI	TA 338	8/8 16 PI

Table 1. (continued)

Accession no.	Symptomatology
TA 368	4/4 12 PI
TA 482	10/11 13 PI
TA 491	7/7 13 PI
TA 495	11/11 13 PI
TA 496	10/10 13 PI
TA 528	10/10 11 PI
TA 534	9/11 13 PI
TA 535	10/10 13 PI
TA 594	5/5 16 PI
TA 678	9/9 16 PI

[a]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

[b]These accessions were retested to verify questionable results obtained from original tests. Positive values obtained upon retesting are given in the table.

Table 2. Evaluation of diploid and polyploid Aegilops species accessions for resistance to WSMV by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

Aegilops species	Assays		
Accession no.	Symptomatology	ELISA (A)[a]	Slot-Blot (mm)[b]
Controls			
Parker (inoc.)	13/13 9 PI[c]	0.349	5
Parker (non-inoc.)	0/14 11 PI	0.016	0
N28Ht (inoc.)	---	0.687	24
N28Ht (non-inoc.)	---	0.004	0
Aegilops squarrosa			
TA 1558	4/5 15 PI	0.605	34[d]
TA 1559	14/15 15 PI	---	---
TA 1560	1/14 15 PI	---	---
TA 1561	7/11 16 PI	0.177	26
TA 1563	15/15 16 PI	0.371	124
TA 1564	7/15 16 PI	0.210	44
TA 1566	7/10 16 PI	0.331	36
TA 1568	15/15 14 PI	0.749	188
TA 1570	14/15 16 PI	0.699	192
TA 1571	6/15 16 PI	0.475	84
TA 1573	10/12 16 PI	0.471	144
TA 1576	14/15 16 PI	0.557	56

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
TA 1578	14/14 14 PI	0.628	162
TA 1580	15/15 14 PI	0.755	124
TA 1582	15/15 12 PI	0.785	98
TA 1584	12/12 12 PI	0.638	148
TA 1586	15/15 12 PI	0.662	136
TA 1588	13/13 14 PI	0.588	132
TA 1590	14/15 16 PI	0.572	103
TA 1592	15/15 14 PI	0.572	12
TA 1594	15/15 14 PI	0.725	8
TA 1597	15/15 12 PI	0.702	114
TA 1618	4/8 16 PI	0.484	34
TA 1620	14/14 16 PI	0.498	56
TA 1622	15/15 14 PI	0.664	174
TA 1624	15/15 14 PI	0.408	70
TA 1626	15/15 14 PI	0.878	178
TA 1629	10/20 15 PI	0.522	34[d]
TA 1631	9/11 16 PI	0.331	58
TA 1634	13/13 15 PI	---	---
TA 1638	14/15 16 PI	0.542	84
TA 1639	12/12 16 PI	0.219	180
TA 1640	10/14 15 PI	---	---
TA 1642	13/13 13 PI	---	---
TA 1643	3/4 15 PI	---	---

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
TA 1644	17/19 15 PI	0.557	16[d]
TA 1645	1/12 15 PI	0.278	---[e]
TA 1646	13/14 16 PI	0.256	18
TA 1647	6/15 15 PI	0.560	16[d]
TA 1648	13/13 13 PI	---	---
TA 1649	8/8 11 PI	---	---
TA 1650	8/10 16 PI	0.162	---
TA 1651	9/9 15 PI	---	---
TA 1656	11/11 11 PI	---	---
TA 1657	11/11 14 PI	0.297	190
TA 1659	14/15 16 PI	0.236	72
TA 1661	15/15 14 PI	0.230	158
TA 1662	12/14 16 PI	0.190	23
TA 1665	14/14 14 PI	0.273	43
TA 1666	14/15 15 PI	---	---
TA 1667	15/15 14 PI	0.368	49
TA 1669	15/15 14 PI	0.249	28
TA 1671	12/14 15 PI	---	---
TA 1672	15/15 15 PI	---	---
TA 1673	15/15 14 PI	0.585	93
TA 1675	14/14 14 PI	0.517	47
TA 1677	15/15 16 PI	0.232	167
TA 1679	9/12 15 PI	---	---

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
TA 1681	11/13 15 PI	---	---
TA 1684	17/17 13 PI	0.654	44[d]
TA 1685	15/17 15 PI	0.554	20[d]
TA 1686	16/18 15 PI	0.614	54[d]
TA 1687	15/15 14 PI	0.390	88
TA 1689	14/14 14 PI	0.524	139
TA 1691	13/13 16 PI	0.294	52
TA 1692	14/14 16 PI	0.336	33
TA 1695	15/15 16 PI	0.241	28
TA 1698	15/15 12 PI	0.478	62
TA 1700	15/15 14 PI	0.187	32
TA 1703	15/15 16 PI	0.368	108
TA 1704	14/15 16 PI	0.137	20
TA 1705	15/15 16 PI	0.237	98
TA 1706	15/15 16 PI	0.326	72
TA 1707	12/12 14 PI	0.325	162
TA 1708	13/15 16 PI	0.224	6
TA 1709	13/13 16 PI	0.278	28
TA 1712	15/15 12 PI	0.793	56
TA 1715	14/14 14 PI	0.457	30
TA 1717	12/12 14 PI	0.217	14
TA 1718	14/14 14 PI	0.296	148

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
Aegilops cylindrica			
TA 1843	14/15 15 PI	---	---
TA 1856	13/14 15 PI	---	---
Aegilops ovata			
TA 1813	13/13 15 PI	---	---
TA 1814	15/15 15 PI	---	---
Aegilops triaristata			
TA 1863	7/17 15 PI	0.240	0[f]
Aegilops triuncialis			
TA 1719	14/14 15 PI	---	---
TA 1720	7/11 15 PI	---	---
TA 1733	13/13 13 PI	---	---
TA 1754	5/7 15 PI	---	---
TA 1756	2/2 13 PI	0.541	36[d]
TA 1758	6/8 15 PI	0.397	2[d]
TA 1769	13/14 15 PI	---	---
Aegilops bicornis			
TA 1942	7/11 15 PI	0.259	182
TA 1949	5/13 15 PI	0.725	98[d]

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
TA 1953	5/16 15 PI	0.714	152[d]
TA 1956	8/17 15 PI	0.591	142[d]
Aegilops ligustica			
TA 1771	15/15 13 PI	---	---
TA 1772	5/17 15 PI	0.612	102[d]
TA 1775	11/12 15 PI	---	---
TA 1787	5/10 15 PI	0.656	78[d]
TA 1791	13/13 13 PI	---	---
TA 1796	15/15 13 PI	---	---
Aegilops longissima			
TA 1912	7/20 15 PI	0.735	66[d]
TA 1914	14/14 13 PI	---	---
TA 1917	13/15 15 PI	0.692	20[d]
TA 1924	17/19 15 PI	0.670	156[d]
Aegilops sharonensis			
TA 1999	7/15 15 PI+2 died	---	---
TA 2065	12/14 14 PI	---	---
Aegilops speltoides			
TA 1773	6/9 15 PI	---	---

Table 2. (continued)

Accession no.	Symptomatology	ELISA (A)	Slot-Blot (mm)
TA 1789	12/12 15 PI	---	---
TA 1793	7/9 15 PI	---	---
TA 1795	5/15 15 PI	0.693	58[d]
Aegilops umbellulata			
TA 1821	14/14 15 PI	---	---
TA 1826	14/15 15 PI	---	---
TA 1827	6/8 15 PI	---	---
TA 1828	13/13 13 PI	---	---

[a]A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

[b]mm = peak heights of autoradiograph densitometric readings measured in mm.

[c]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

[d]These accessions were retested to verify questionable results obtained from original tests. Positive values obtained upon retesting are given in the table.

[e]This accession gave variable results upon retesting several times.

Table 2. (continued)

Results from other symptomatology assays are: 0/14 15 PI, 1/12 14 PI, and 2/15 17 PI.

[f]This accession also gave variable results upon retesting. Results from other symptomatology assays are: 5/14 15 PI and 4/5 17 PI.

Table 3. Reaction of different wheat amphiploids to WSMV as determined by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

KSU Accession No. and Description of Amphiploid	Symptom-atology	ELISA (A)[a]	Slot-Blot (mm)
Parker (inoc.)	9/9 7 PI[c]	0.249	---
Parker (non-inoc.)	0/15 15 PI	0.003	0
TA 3389 (*T. aestivum* X *A. podperae*)	0/3 15 PI	0.019	0
TA 3410 (*T. aestivum* X *A. podperae*)	0/10 15 PI	0.006	0
TA 3411 (*T. aestivum* X *A. podperae*)	0/7 15 PI	0.012	0
TA 3426 (*T. aestivum* X *A. scirpeum*)	0/7 15 PI[d]	0.003	0
TA 3427 (*A. ciliare* X *T. aestivum*)	0/5 15 PI	0.002	0
TA 3361 (*T. boeoticum* X *Ae. squarrosa*)	12/12 13 PI	---	---
TA 3397 (*Ae. ovata* X *T. durum*)	14/14 13 PI	---	---
TA 3404 (*Ae. ventricosa* X *T. aestivum*)	18/18 15 PI	0.585	170
TA 3409 (*T. aestivum* X *Ae. distichum*)	3/20 15 PI	0.344	26

Table 3. (continued)

KSU Accession No. and Description of Amphiploid	Symptom-atology	ELISA (A)	Slot-Blot (mm)
TA 3412 (*T. timopheevi* X *Hordeum bogdanii*)	11/12 13 PI	0.511	170
TA 3413 (*T. aestivum* X *Elymus arenarius*)	7/7 13 PI	0.583	42
TA 3414 (*T. aestivum* X *Elymus arenarius*)	7/7 13 PI	0.281	10

[a]A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

[b]mm = peak heights of autoradiograph densitometric readings measured in mm.

[c]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

[d]After 15 PI, the 7 symptomless plants were grown in the greenhouse. Five out of seven plants exhibited symptoms after 10 wks.

Table 4. Evaluation of registered germplasms resistant to WSMV as determined by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

CI Number	Symptom-atology	ELISA (A)[a]	Slot-Blot (mm)[b]	Bioassay on Parker
Parker (inoc.)	12/12 10 PI[c]	0.249	5	not done
Parker (non-inoc.)	0/6 14 PI	0.003	0	not done
CI 15321	0/15 13 PI	0.012	0	-[g]
CI 15322	0/18 15 PI	0.005	0	not done
CI 17766	1/15 14 PI[d]	0.020	0	-
CI 17881	10/15 16 PI[e]	0.490	96	+
CI 17882	0/13 15 PI	0.013	0	-
CI 17883	1/6 11 PI	0.022	0	-
CI 17884	0/13 15 PI	0.010	0	-
CI 17885	1/11 11 PI	0.011	0	-
CI 17886	0/8 14 PI[f]	0.018	0	-
LRS-1F193	10/13 16 PI	0.373	25	not done

[a]A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

[b]mm = peak heights of autoradiograph densitometric readings measured in mm.

[c]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

Table 4. (continued)

[d]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. Upon retesting, selections 4807 and 4808 exhibited 3/3 16 PI and 2/2 14 PI, respectively.

[e]Upon retesting, 2/14 17 PI observed.

[f]Upon retesting, 4/10 gave a local lesion-like response 13 PI.

[g]Minus sign indicates symptoms were not observed on Parker when inoculated with previously inoculated germplasm. Plus sign indicates symptoms were observed on Parker when inoculated with previously inoculated germplasm.

Genetic Expression of WSMV Resistance in Two Wheat-*Agropyron* Hybrids

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ABSTRACT

Double antibody sandwich enzyme-linked immunosorbent assay ELISA and slot-blot hybridization, in conjunction with a symptomatology assay, were used to study genetic expression of resistance to wheat streak mosaic virus (WSMV) in two amphiploids, wheat X *Agropyron intermedium* and wheat X *A. elongatum*, and their derived disomic chromosome addition lines. The amphiploids showed virtual immunity to WSMV infection and stayed symptom-free throughout their growth under both growth chamber and greenhouse conditions. A single chromosome addition line isolated from each amphiploid was resistant to WSMV but the resistance broke down after the plants were transferred to the greenhouse. ELISA and slot-blot quantitation indicated a minor factor for genetic resistance in another *Agropyron* chromosome. Moreover, chromosome dosage effect also appeared to be involved in genetic expression of WSMV resistance. Apparently one major factor and at least one minor genetic factor are involved in determining the resistance observed in these two *Agropyron* species.

Additional index words: ELISA, slot-blot hybridization, amphiploid, disomic addition line, *Agropyron elongatum*, *Agropyron intermedium*.

Wheat streak mosaic (WSM) is historically an important virus disease in the Great Plains states and Canada, as well as many other wheat producing countries of the world. Kansas losses have been estimated up to 7% in 1981 and averaged 1.8% in the past 5 years (22). These losses are significant considering Kansas produces up to 460 million bushels of wheat annually.

The wheat curl mite, Eriophyes tulipae (Keifer), efficiently vectors the virus from volunteer wheat, which serves as the summer host to the vector and virus, to fall-seeded wheat. Destroying volunteer wheat and observing recommended late planting dates are practices that help control WSM, although not always effectively. Breeding for resistant varieties of wheat (Triticum aestivum L.em. Thell) will provide the best control of WSM. As of yet there are no commercial varieties highly resistant to the virus, but many researchers are seriously addressing this problem in their breeding programs.

Intergeneric hybrids (Triticum X Agropyron) have been and are presently being used as sources of disease resistance in certain wheat breeding programs. McKinney and Sando (14) performed a study with several Agrotricum hybrids inoculated with WSMV. Those hybrids with Agropyron elongatum (Host) Beauv. were found to be resistant or immune to WSM. Later studies by Fellows and Schmidt (7) and Schmidt et al., (18) showed that the immune reaction of A. elongatum may be controlled by a complex mechanism which could complicate attempts to transfer a satisfactory level of resistance from A. elongatum to wheat.

Swarup et al. (23) performed cytogenetical studies on various generations of hybrids derived from crosses of the susceptible variety Pawnee with a 56-chromosome wheat X Agropyron derivative which normally

gave a local lesion type reaction to WSMV. The F_1 showed only the L (local lesion) reaction. The F_2 and F_3 segregated into three groups: S (systemic), L, and L/S (local lesion usually turning into a lethal systemic reaction). On the basis of the number and size of the chromosomes that were present in the different reaction types, they concluded that more than one Agropyron chromosome governed resistance to WSMV in the L plants.

From studies on advanced generation Agropyron X Triticum lines, Raj (16), on the other hand, suggested that it was possible to select resistant wheat-like hybrids. The F_1 of a cross involving one resistant wheat-like line with a standard cultivar showed a meiotic pairing of 21 bivalents. Since this Agrotricum was resistant, he concluded that at least one very small portion of the Agropyron chromosome must have been translocated to a wheat chromosome. He also concluded on the basis of F_2 seedling segregation ratios of resistant and susceptible plants that resistance was controlled by two recessive factors.

Sebesta and Bellingham (19) observated selections of the Sando-derived wheat X Agropyron hybrids which were wheat-like in nature yet still possessed resistance to WSMV. In two parental F_2 plants, which they regarded as alien addition types, it appeared that only one pair of chromosomes was involved in the genetic control of virus resistance. They concluded that if upon further testing they find that only one pair of chromosomes is involved in governing resistance to WSMV, then genetic control of this virus would be much simpler.

Larson and Atkinson (8) screened 286 Agrotricum lines for reaction to WSMV and identified one immune wheat-like line, a derivative of Triticum aestivum cv. Rescue X A. elongatum. These plants had 21 bivalent

chromosomes, as does common wheat, but had seed and spike characteristics which resembled Agropyron. By making crosses with lines ditelosomic for known chromosomes, they determined that Agropyron chromosomes replaced wheat chromosomes 4D, 5D, and 6D in a triple substitution.

In a later paper (9), they isolated single chromosome substitution lines and showed that none were immune to WSMV. However, disomic substitution (ds) line 6D had considerable resistance, ds line 5D delayed development of the disease, and ds line 4D was susceptible. The ds line 6D was also resistant to the mite vector (26). Germplasm LRS-1F193 was produced from this line and was released by Lethbridge Research Station, Agriculture Canada, Lethbridge, Alberta, in 1982 (26). This germplasm was described as carrying resistance to the mite vector, Eriophyes tulipae (Keifer). This germplasm was evaluated for reaction to WSMV by symptomatology, ELISA, and slot-blot hybridization (Stoddard et al., manuscript in preparation). This line was found to be susceptible to WSMV by all three assays.

There is no general consensus on the genetic control of WSMV resistance in wheat X Agropyron hybrids. By applying ELISA and slot-blot hybridization to the evaluation of T. aestivum cv. Vilmorin X A. intermedium amphiploid and derived addition lines (3) and T. aestivum cv. Chinese Spring X A. elongatum amphiploid and derived disomic and ditelosomic addition lines (6), it should be possible to determine the mechanism(s) involved in conferring resistance to WSMV. Results on genetic expression of resistance to wheat streak mosaic virus in these two Agropyron species are reported here.

MATERIALS AND METHODS

Germplasm used in the genetic evaluations was obtained from the Wheat Genetics Resource Center, Kansas State University. This germplasm included Triticum aestivum cv. Vilmorin, the partial amphiploid Vilmorin X Agropyron intermedium, and 6 derived disomic addition lines (3). Germplasm also tested included T. aestivum cv. Chinese Spring, the amphiploid Chinese Spring X A. elongatum, 7 derived disomic addition lines, and 8 derived ditelocentric addition lines (6). Fifteen or fewer seeds (1-15), depending upon availability, were used in evaluating each accession for reaction to WSMV.

Planting Method. Seeds of each line were planted in standard greenhouse flats containing a sandy loam soil mix (8 rows per flat). The flats were kept in a growth chamber at 21C, 14-hr light, 16-18C, dark. Flouresoent lighting (10,800 lux) was the sole source of light. Plants were watered regularly; fertilizer was not applied.

Two seeds of each of the disomic addition lines, derived from the amphiploid Chinese Spring X A. elongatum, were germinated on filter paper in petri plates. Root tips (1-2 cm long) were collected, placed in vials of ice water for 24 hr, and stored at room temperature in a 3:1 dilution of 95% ethanol and glacial acetic acid for at least 5 days. Root tips were stained in acetocarmine 1 hr before squashing for chromosome counts.

Crosses were made between the addition lines for chromosomes 1E, 2E, 3E, 4E, 5E, and 7E as the female parents and the addition line for chromosome 6E as the male parent in the growth chamber. One to two top and bottom spikelets of the female parent were removed as well as the middle florets of the remaining spikelets. The two remaining florets were hand emasculated and pollinated. Fourteen days after pollination, 4-7

seeds were dissected and embryos were cultured on Murashige's minimal medium (Flow Laboratories, McLean, VA). Plantlets were later transferred to soil, allowed to grow to the 2-3 leaf stage, and inoculated with WSMV.

Inoculum preparation. A Kansas isolate of WSMV obtained from the Fort Hays Branch Agricultural Experiment Station was maintained on a susceptible corn hybrid N28Ht with frequent transfers (24). The inoculation preparation procedure followed was similar to that of Martin (13). Ten to 14-day old infected plants were triturated at a 1:20 dilution (w:v) in 0.2M potassium phosphate buffer (pH 7.0) and strained through double-folded cheesecloth. Carborundum abrasive (600 mesh, 1.5%) was added to the filtrate. A DeVilbiss atomizer was used to spray 7-9 day old seedlings from a distance of 1 cm at 6.3 Kg/cm until water-soaked lesions could be seen on inoculated leaves. Inoculated plants were rinsed with water to remove excess carborundum and returned to the growth chamber. The plants were examined for symptoms after 7-8 days and every other day thereafter for 7-9 days. Parker wheat was the susceptible control in all tests (13).

Tissue Samples. Leaf samples were harvested from each accession after 15-16 days. The middle third of the youngest leaves were pooled from all plants in each line (Seifers, 1983, personal communication). The leaves were divided into two labeled 1.5 ml microfuge tubes, one to be used for ELISA, the other for the slot-blot hybridization assay. Approximately 0.3 g tissue was placed in each tube. The tubes were stored at -20C until both assays were performed.

Virus Preparation. WSMV was purified for serum production and ELISA following the procedure of Lommel *et al.* (11). One hundred grams of infected N28Ht corn leaves were homogenized in 200 ml 0.2M sodium acetate

buffer, pH 5.0, 1% mercaptoethanol, strained through cheesecloth, and allowed to sit at room temperature for 15-20 min. The extract was centrifuged at 6,000g for 30 min in a Sorvall GSA rotor. One quarter volume of 40% polyethylene glycol (PEG 6000) in 1M NaCl was added while stirring on ice. The supernatant was stirred on ice for 1-2 hr and centrifuged at 6,000g for 30 min. The pellet was resuspended in 10 ml 0.1 M Tris, 32mM sodium citrate buffer, pH 6.5. The supernatant was centrifuged through 20% sucrose pads in a Sorvall AH-627 rotor at 25,000g for 2 hr. The pellet resuspended at 4C in 1 ml 0.1M Tris, 32mM Na Citrate, pH 6.5. Peaks collected from the sucrose gradients were then banded in CsCl equilibrium gradients. The CsCl gradients were prepared by adding 12.1 g CsCl to double distilled water and volume adjusted to 30 ml. The CsCl gradients were centrifuged at 50,000g for 22 hr in a Beckman SW55Ti rotor and fractionated. Peaks were dialyzed against 3 changes of sterile 1X PBS (phosphate buffered saline) and centrifuged at 40,000g for 2 hr in a Beckman SW55Ti rotor. The pellet was resuspended in 400 ul sterile 1X PBS and assayed spectrophotometrically.

Antiserum Production. Antiserum against CsCl gradient purified WSMV was produced in a white New Zealand rabbit. Virus in 1X PBS 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 prior to initial injection and on days 14, 27, 37, 52, 59, 66, 73, 89, 96, and 103. All serum used in this report was from day 37 which had the highest titer.

Immunoglobulin Preparation. The immunoglobulin (Ig) fraction was purified from antisera as described by Clark and Adams (4). Five ml of antiserum were diluted with 5 ml of saturated ammonium sulfate. The

pellet resulting after centrifugation was dialyzed against 3 changes of 0.5X PBS. The Ig was passed through a 2-3 cm bed of DEAE cellulose in 0.5X PBS. It was then adjusted to approximately 1 mg/ml, diluted with a half volume of glycerol, and stored at -20C.

Enzyme Antibody Preparation. Alkaline phosphatase (Type VII-S; Sigma Chemical Co., P.O. Box 14508, St. Louis, MO) was coupled to purified immunoglobulin by the glutaraldehyde method of Avrameas (1). The resuspended alkaline phosphatase pellet was dialyzed against 3 changes of 0.5X PBS at 4C. Glutaraldehyde was added to a 0.0625% final concentration and removed by dialysis against 3 changes of 0.5X PBS. The conjugate was diluted with a half volume of glycerol and stored at -20C.

Double Antibody Sandwich ELISA. The sandwich ELISA procedure followed was as described by Clark and Adams (4). Coating of the Ig was at a 1/500 dilution in 200 ul of 0.05M carbonate buffer, pH 9.6. Incubation for all steps was for 1 hr at 37C. The plates were washed between steps with ELISA wash (0.15M NaCl containing 0.1% Tween-20). Antigens were then added to the Ig-coated wells. Five-fold serial dilutions were made with pure virus in one column on all plates as a control. Infected tissue samples were ground with a wooden applicator stick in 250 ul 1X PBS and microfuged for 5 min. Fifty ul sap were added to 200 ul "ELISA buffer" (EB: 0.01M PBS, pH 7.4, 0.05% Tween-20, 1% polyvinylpyrrolidone-40, and 0.2% ovalbumin). The remaining sap was transferred to a second labeled microfuge tube and frozen. Incubation with the enzyme-conjugated Ig was at a 1/100 dilution in EB. Reactions were recorded at 15 min intervals for 45 min after the addition of substrate (200 ul 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).

RNA Extraction Procedure. WSMV viral nucleic acid to be 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). Sucrose density gradient purified WSMV was incubated for 30 min at 37C with Protease K and 10% SDS. Bentonite (40 mg/ml), 2X STE (pH 8.0), and 10% SDS were added to the virus preparation and the mixture was incubated at 60C for 2 min. The nucleic acid was extracted with phenol, concentrated by ethanol precipitation, and analyzed by denaturing agar gel electrophoresis.

Filter-bound Hybridization. The slot-blot hybridization procedure was performed in a similar manner as described by Brown et al. (2) with several modifications. Nitrocellulose filters were prewetted in boiling water and allowed to soak in 20X SSC (175.3 g NaCl and 88.2 g Na citrate/l, pH 7.0) until ready for use. The slot-blot template was incubated for 30 min in 20X SSC with 100 ug/ml denatured salmon sperm DNA and rinsed thoroughly with 20X SSC (5). Tissue samples were prepared as for the ELISA procedure. Two concentrations of infected sap were spotted on the nitrocellulose: 25 ul and 10 ul in 175 ul and 190 ul buffer, respectively. Five-fold serial dilutions of pure WSMV RNA were made in one column as a control in all tests. Slots were post-rinsed with 200 ul 20X SSC. After the filter was rinsed in 5X SSC for 5 min and air dried for 30 min, it was baked for 2 hr at 80C.

Prehybridization. Slot-blot filters were soaked in prehybridization buffer in a sealed bag overnight at 42C in a shaking water bath. Prehybridization buffer consisted of 50% neutralized formamide, 20X SSPE (174 g NaCl, 27.6 g NaH_2PO_4 H_2O, and 7.4 g EDTA/l, pH 7.4), 10 ug/ml

denatured salmon sperm DNA, 50X Denhardt's solution, and distilled water.

Hybridization. Slot-blot filters were soaked in hybridization buffer with labeled probe in a sealed bag overnight at 42C in a shaking water bath. Hybridization buffer was identical to prehybridization buffer except it had 20% as much 50X Denhardt's solution and more distilled water to adjust it to volume. After hybridization, the filters were washed with three changes of 2X SSC, 1% SDS for 15-20 min at room temperature with agitation, and three changes of 0.1X SSC, 1% SDS for 15-20 min at 60C with agitation.

Nick Translation. Cloned probes were labeled by nick translation (17) for hybridization to slot-blots. Nick translation was carried out by combining in a 1.5 ml microfuge tube 40 ul pWSM-8 (12) (0.1 ug/ml), 2.5 ul ^{32}P-ATP (1 uCi/ml), 10 ul 10X reaction buffer (500 ul 1M Tris-Hcl, pH 7.9, 50 ul 1M $MgCl_2$, 100 ul 5 mg/ml bovine serum albumin, 8 ul mercaptoethanol, and 342 ul distilled water), 1 ul dNTPs (1 ul 20mM dCTP, dTTP, dGTP, and 97 ul distilled water), and 1 ul DNAase solution (1 ml distilled water and 2 ul 50 ug/ml DNAase). The solution was mixed gently and 1 ul DNA polymerase I in 44.5 ul distilled water was added. The contents were mixed gently and incubated for 4 hr at 15C. The reaction was terminated by the addition of 1 ul of 10% SDS. RNA was counted for specific activity of ^{32}P in a scintillation counter. Phenol extraction and ethanol precipitation of the probe were then carried out in order to eliminate any free radioactive nucleotides.

Autoradiography. Kodak XAR-5 film was exposed to the hybridized filters for 48 hr at -70C using intensifying screens. A Kontes densitometer was used to scan the developed autoradiographs.

Data Analysis. For the ELISA tests, average absorbances at 405 nm,

measured 30 min after the addition of substrate, were considered positive for test samples if the values were higher than the healthy wheat and healthy corn control samples in the same plate. Peak heights of the autoradiograph densitometric readings obtained from the slot-blot hybridization assays were measured in mm for the 10 ul dilution samples only. Results were considered positive for test samples if the peak heights were greater than the healthy wheat control samples in the same test. Both the ELISA and slot-blot hybridization assays can be used quantitatively, but due to the lack of experimental replications, the assays in the three experiments were used to test solely for the presence or the absence of the virus.

RESULTS

Symptomatology, ELISA, and slot-blot hybridization results of the wheat X A. intermedium amphiploid and derived disomic addition lines are given in Table 1. The amphiploid is immune to WSMV. Two of the addition lines, L2 and L5, appeared to be more resistant than the other four addition lines. Not all of the plants inoculated in these 2 lines exhibited symptoms and the ELISA and slot-blot values were lower. L5 appears to be more resistant than L2 in that both the ELISA and slot-blot values were lower. The 3 plants of L5 that were transferred to the greenhouse (2 symptomless plants plus 1 of the plants exhibiting less severe symptoms) however, died after 10 wks. Addition lines L1, L3, L4, and L7 showed 100% infection by symptomatology and very high ELISA values. L1 & L7 and L3 & L4 had average slot-blot values of 178 mm and 48 mm, respectively. Both values are considered positive.

Symptomatology, ELISA, and slot-blot hybridization results of the wheat X A. elongatum amphiploid and derived disomic and ditelosomic addition lines are given in Table 2. The amphiploid is immune to WSMV, although 1 out of 3 plants inoculated exhibited symptoms 10 wks after being transferred to the greenhouse. The plant that became infected can be attributed to chromosome instability. The exact date of initial symptom expression is not known. All the addition lines except 6E had nearly 100% infection as determined by the symptomatology. ELISA values were higher than the healthy wheat controls (which ranged from .02-.05) and slot-blot hybridization values ranged from 14-258 mm, all positive for the presence of WSMV nucleic acid. Ditelosomic addition line 6ES gave variable results upon repeated symptomatology and ELISA assays (see footnote c in Table 2).

Disomic addition line 6E also gave variable results in repeated symptomatology assays. In one test, 20% of the plants exhibited symptoms, whereas in a second test 50% of the plants inoculated expressed symptoms. The 5 plants from this test that were free from symptoms plus a less severely infected plant were transferred to the greenhouse. All 6 plants expressed symptoms after 2.5 months, however the exact date of initial symptom expression is not known. The ELISA value for addition line 6E was negative and the slot-blot hybridization value was slightly positive (14 mm).

The results of the symptomatology, ELISA, and slot-blot hybridization assays performed on the F_1 hybrids derived from crosses between A. elongatum derived addition lines are given in Table 3. Addition line 6E gave slightly positive results and the wheat X A. elongatum amphiploid gave negative results as expected (see Table 2). None of the progeny from the crosses of the addition lines 1E, 2E, 3E, 4E, 5E, and 7E with 6E (as the male parent) gave ELISA or slot-blot readings as low as addition line 6E alone. The progeny from the cross between addition lines 5E and 6E appeared less susceptible than any of the other combinations. The ELISA and slot-blot hybridization assay results were lower than expected in comparison to the results of the symptomatology assay; all symptomatology values, except for addition line 6E, were comparable. The ELISA and slot-blot hybridization assay results were lower than 5E alone (see Table 2) but not as low as for 6E alone. The other progeny derived from crosses between the addition lines gave positive symptomatology, ELISA, and slot-blot hybridization results.

Since the accessions used in these tests are stable lines and, hence, are no longer segregating, it was not reasonable to expect that the plants

which failed to show symptoms were, in fact, resistant. This delayed response in symptom expression may be explained in several ways. As the leaves expand during growth of the plant, the symptoms become more obvious. Other explanations for this delayed reaction of symptom expression may be either that the masking effects of the dark plant pigments were decreased as the plant matured, or else the rate of virus replication was slower in these particular plants.

DISCUSSION

From the symptomatology, ELISA, and slot-blot hybridization assay results of T. aestivum cv. Vilmorin X A. intermedium amphiploid and derived addition lines (Table 1), it appears that chromosome L5 had a major genetic factor conferring WSMV resistance. A minor factor on chromosome L2 was also indicated. However, the level of resistance in the two addition lines was not comparable to the original amphiploid. Therefore, it can be postulated that at least two genetic factors were involved in conferring immunity observed in the wheat-A. intermedium amphiploid.

The symptomatology, ELISA, and slot-blot hybridization assay results of T. aestivum cv. Chinese Spring x A. elongatum amphiploid and derived disomic and ditelosomic addition lines (Table 2) indicate that the disomic addition line for chromosome 6E possessed the gene(s) for WSMV resistance. Neither ditelosomic addition line 6ES or 6EL were resistant, i.e. the factor(s) present on either/both arms were not expressed. But since disomic addition line 6E is resistant, we concluded that the factor(s) responsible for conferring WSMV resistance can only be effective when they are present in the form of the whole chromosome addition line 6E. The level of resistance in 6E was not comparable with the original amphiploid since some plants became infected and a low-level positive slot-blot hybridization value was observed. This could not be due to chromosome instability as all lines had 44 chromosomes as expected. Therefore, it may be speculated that an epistatic interaction between two A. elongatum chromosomes may be necessary for the expressed immunity in the wheat-A. elongatum amphiploid.

The lower than expected slot-blot values for addition lines 3ES, 4EL,

and 6E might be due to the fact that the narrow slots in the slot-blot template would occasionally become clogged with plant tissue debris that failed to sediment down upon centrifugation. This clogging prevents viral RNA to bind to the nitrocellulose filter, thus resulting in lower slot-blot hybridization values in relation to ELISA values.

Results from the evaluation of the progeny from crosses between A. elongatum derived disomic addition lines by symptomatology, ELISA, and slot-blot hybridization assays (Table 3) did not confirm or rule out this epistatic interaction for conferring WSMV immunity. None of the combinations of chromosome 6E with other E chromosomes conferred WSMV resistance. In fact, higher ELISA values were observed for all the hybrids except for 5E + 6E (2l"+ 5E'+ 6E') than for each line alone (Table 2). These results may be interpreted in several different ways. One interpretation may be that chromosome 6E must be in a homozygous condition in order for WSMV resistance to be observed. This would explain why none of the inter-addition line crosses showed WSMV reaction comparable to that observed in disomic 6E addition line plants.

These results are in agreement with Larson and Atkinson (9) who found that disomic substitution (ds) line 5D delayed development of the disease, ds line 6D had considerable resistance, and double substitution (dds) line 5D + 6D was highly resistant. In our tests (Tables 2 and 3), ds line 5D was susceptible, ds line 6E showed some resistance, and dds line 5E + 6E appeared to delay the development of disease. These findings again indicated an epistatic interaction between chromosomes 5E and 6E as was previously observed by Larson and Atkinson.

Yet another explanation could be allelic variation. Evidence of this can be seen in the WSMV-resistant registered germplasm CI 15321, a

42-chromosome line derived from crosses between wheat and A. elongatum (20). This line is immune to WSMV (Stoddard et al., manuscript in preparation). Cytogenetic analysis indicated that chromosome 6ES is responsible for conferring this immunity (B. S. Gill, unpublished). Further evidence of allelic variation can be seen in CI 17766, a WSMV-resistant selection from CI 15092, an A. intermedium substitution line for chromosome 4B (10). Although the same chromosomes (4D, 5D, and 6D) are not directly involved in the triple substitution line discussed earlier, the same chromosome groups are implicated and serve as further evidence that these chromosomes are indeed responsible for conferring resistance to WSMV.

Alternatively, the results of the hybrid formed between addition lines for chromosomes 5E and 6E may be spurious. Spurious ELISA and slot-blot hybridization assay results can be attributed to several factors. Small plant populations and some lines having plants which have very slender leaves limit the amount of tissue which can be used in the assays and, therefore, lower ELISA and slot-blot values are observed. Clogging of the slots in the slot-blot template, as described earlier, may also account for the lower slot-blot values. However, as reported in the literature, a valid interpretation of the observed results is the existence of an epistatic interaction between two genetic factors which is responsible for conferring WSMV immunity.

ELISA and slot-blot hybridization assays, used in conjunction with a symptomatology assay, proved to be useful in the genetic evaluation of WSMV resistance in two Agropyron species, A. intermedium and A. elongatum. Two different A. intermedium chromosomes, L2 and L5, were found to be responsible for conferring resistance in a set of disomic addition lines.

Similarly, one pair of A. elongatum chromosomes (6E) were found to confer resistance and an epistatic interaction between 5E + 6E may be required for an immune reaction to WSMV. This information should be useful to breeders interested in developing WSMV-resistant cultivars.

In order for this material to be useful to breeders, the gene(s) for resistance on the Agropyron chromosomes must be transferred to wheat chromosomes. The alien chromosomes of A. intermedium and A. elongatum are not known to pair with their wheat homoeologues, but cytogenetic techniques such as the chromosome 5B Ph mutant gene, which suppresses homologous pairing of wheat chromosomes, or ionizing radiation can be useful in inducing this transfer of genes (25, 21).

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Table 1. Evaluation of Triticum aestivum cv. Vilmorin X Agropyron intermedium amphiploid and Vilmorin X A. intermedium disomic chromosome addition lines for WSMV infection by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

Disomic Addition Lines	Symptomatology	ELISA (A)†	Slot-Blot (mm)‡
Parker (inoc.)	6/6 8 PI§	0.554	---
Parker (non-inoc.)	0/15 15 PI	0.018	0
Vilmorin	9/9 9 PI	0.690	188
Vilmorin X A. intermedium partial amphiploid	0/3 13 PI	0.017	0
Disomic addition L1	6/6 7 PI	0.484	176
Disomic addition L2	5/6 15 PI	0.106	2
Disomic addition L3	10/10 7 PI	0.491	46
Disomic addition L4	5/5 9 PI	0.419	50
Disomic addition L5	3/5 15 PI¶	0.033	0
Disomic addition L7	6/6 7 PI	0.423	180

†A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

‡mm = peak heights of autoradiograph densitometric readings measured in mm.

§Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

¶After 15 PI, the 2 symptomless plants plus 1 of the plants exhibiting

Table 1. (continued)

less severe symptoms were transferred to the greenhouse. After 10 wks, all 3 plants had died.

Table 2. Evaluation of Triticum aestivum cv. Chinese Spring X Agropyron elongatum and Chinese Spring X A. elongatum disomic and ditelosomic chromosome addition lines for WSMV infection by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

Disomic and Ditelosomic Addition Lines	Symptomatology	ELISA (A)[†]	Slot-Blot (mm)[‡]
Parker (inoc.)	12/12 12 PI[§]	0.349	5
Parker (non-inoc.)	0/14 15 PI	0.016	0
Chinese Spring (CS)	15/15 10 PI	0.928	266
CS X A. elongatum amphiploid	0/3 15 PI[¶]	0.017	0
Disomic addition 1E	3/3 7 PI	0.515	34
Ditelosomic addition 1ES	8/8 10 PI	0.505	52
Disomic addition 2E	5/5 7 PI	0.285	32
Ditelosomic addition 2EL	14/14 10 PI	0.524	72
Disomic addition 3E	7/7 11 PI	0.487	178
Ditelosomic addition 3ES	10/10 12 PI	0.444	14
Ditelosomic addition 3EL	1/1 9 PI	0.481	166
Disomic addition 4E	5/6 15 PI	0.460	104
Ditelosomic addition 4EL	8/8 12 PI	0.497	20
Disomic addition 5E	3/3 9 PI	0.282	258
Disomic addition 6E	5/10 15 PI[#]	0.009	14
Ditelosomic addition 6ES	14/15 16 PI[††]	0.277	74
Ditelosomic addition 6EL	2/2 10 PI	0.803	166
Disomic addition 7E	3/3 7 PI	0.386	140
Ditelosomic addition 7EL	10/11 14 PI	0.463	34

Table 2. (continued)

[†]A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

[‡]mm = peak heights of autoradiograph densitometric readings measured in mm.

[§]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

[¶]After 15 PI, the 3 symptomless plants were transferred to the greenhouse. After 10 wks, 1/3 exhibited symptoms.

[#]After 15 PI, the 5 symptomless plants plus 1 of the plants exhibiting less severe symptoms were transferred to the greenhouse. All 6 plants exhibited symptoms after 10 wks. In a second symptomatology assay, 1/5 exhibited symptoms 16 PI.

[††]Repeated symptomatology assay results: 6/15 13 PI, 1/8 14 PI, and 6/6 13 PI. ELISA values ranged from 0.021 to 0.103.

Table 3. Evaluation of F_1 hybrids from crosses between different Triticum aestivum cv. Chinese Spring X Agropyron elongatum disomic addition lines for WSMV infection by symptomatology, double antibody sandwich ELISA, and slot-blot hybridization

Double Monosomic Additions	Symptom-atology	ELISA (A)[†]	Slot-Blot (mm)[‡]
Parker (inoc.)	7/7 8 PI[§]	0.349	5
Parker (non-inoc.)	0/8 16 PI	0.047	0
Chinese Spring (CS)	15/15 10 PI	0.928	266
CS X A. elongatum amphiploid	0/15 16 PI	0.002	0
Disomic addition 6E	1/5 16 PI	0.052	6
1E + 6E	4/6 16 PI	0.662	92
2E + 6E	2/4 16 PI	0.699	46
3E + 6E	5/6 16 PI	0.758	62
4E + 6E	5/7 16 PI	0.509	110
5E + 6E	3/5 16 PI	0.148	18
7E + 6E	4/5 16 PI	0.453	96

[†]A = Average absorbance at 405 nm measured 30 min after the addition of substrate.

[‡]mm = peak heights of autoradiograph densitometric readings measured in mm.

[§]Denominator = no. of plants inoculated; Numerator = no. of plants exhibiting symptoms; PI = no. of days post-inoculation that the majority of plants exhibited symptoms before samples were collected.

EVALUATION AND GENETIC ANALYSIS OF WHEAT STREAK MOSAIC VIRUS RESISTANCE IN WHEAT GERMPLASM BY SYMPTOMATOLOGY, ENZYME-LINKED IMMUNOSORBENT ASSAY, AND SLOT-BLOT HYBRIDIZATION

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Double antibody sandwich ELISA and slot-blot hybridization assays 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 X Agropyron amphiploids were found to be resistant to WSMV. Nine registered germplasms resistant to WSMV were evaluated; eight were immune to the virus and one gave a susceptible reaction. The ELISA and slot-blot hybridization assays were sensitive in virus detection. Good overall correlation of the three assays was seen, and the value of these procedures in detecting WSMV-resistant germplasm is discussed.

All three assays were used in the evaluation of a wheat X Agropyron intermedium amphiploid and derived disomic addition lines, a wheat X A. elongatum amphiploid and derived disomic and ditelosomic addition lines, and the progeny derived from crosses between A. elongatum derived addition lines, for resistance to WSMV. The amphiploids showed virtual immunity to WSMV infection and stayed symptom-free throughout their growth under both growth chamber and greenhouse conditions. A single chromosome addition line isolated from each amphiploid was resistant to WSMV but the resistance broke down after the plants were transferred to the greenhouse. ELISA and slot-blot quantitation indicated a minor factor for genetic resistance in another Agropyron chromosome. Moreover, chromosome dosage effect also appeared to be involved in genetic expression of WSMV resistance. Apparently one major factor and at least one minor genetic factor are involved in determining the resistance observed in these two Agropyron species.