Agronomic and end-use quality evaluation of wheat streak mosaic virus resistant spring wheat
by George James Baley, Jr

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
Development of wheat varieties that are resistant to wheat streak mosaic virus (WSMV) and remain productive under non-disease situations would be beneficial to wheat growers in Montana. Previous attempts at developing WSMV resistant germplasm resulted in poor agronomic and end-use quality. A new source of WSMV resistance carried on a Thinopyrum intermedium chromosome translocation was recently released by Kansas State University. The effects of the Thinopyrum translocation on agronomic performance and end-use quality have not been documented. A study was conducted to determine if this translocation, that prevents infection by WSMV, has detrimental effects that would deem it unsuitable for deployment in WSMV resistant spring wheat cultivars. Four populations, consisting of a total of twenty-two WSMV resistant, thirty-six susceptible lines, and eight parental lines, were grown in three replications at Bozeman and Conrad, MT in 1998 and 1999. The agronomic performance of resistant and susceptible lines was compared under disease and non-disease conditions to determine the effectiveness of resistance under disease pressure and to determine the effects of Thinopyrum translocation in the absence of disease. A significant decrease in yield was observed for non-inoculated resistant lines in contrast to susceptible lines. However, the yield range of resistant entries suggests that the recovery of parental yield is possible. Resistance was also found to be effective in limiting virus replication, resulting in only a 5% yield reduction under inoculated conditions compared to 32% for susceptible lines. In all instances where WSMV was introduced to field trials the Thinopyrum translocation provided a significant benefit for resistant lines when compared to susceptible lines. The T. intermedium translocation present in resistant lines had no effect on end quality factors. This study indicates that the release of WSMV resistant wheat that is yield competitive and meets industry standards for quality is achievable.

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A thesis submitted in partial fulfillment
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MONTANA STATE UNIVERSITY
Bozeman, Montana

November 1999

APPROVAL

of a thesis submitted by

George James Baley, Jr.

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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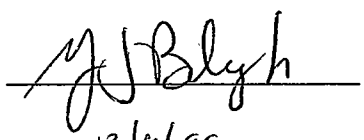
Signature 

Date 12/6/99

ACKNOWLEDGMENTS

I would like to express my thanks and sincere appreciation to the following: To my major advisor Dr. Phil Bruckner and the other members of my committee; Dr. Luther Talbert, Dr. Jack Martin, and Dr. Mark Young, for their guidance, patience, and friendship during my studies at Montana State University. And, the Wheat and Barley Committee for their support.

To all the "Winter Wheaties", especially Jim Berg, Christina Riesselman, Mary Hudson, and Eric Donaldson, for all the laughs on long road trips, assisting in the dirty work of harvest and inoculation, and most of all being good friends.

To all the Spring Wheat team, especially Susan Lanning, for her help in bridging the wheat barrier.

To every soul who "volunteered" to help spread WSMV around the state, especially Sue Brumfield and Debbie Willits for all their assistance in organizing the inoculation trials and to Dr. Greg Kushnak, Dr. Grant Jackson, John Miller, and Ron Thaut for all their help in running field tests in Conrad.

And, to the crew in the CQL. I appreciate all the help and the bread is a treat in itself.

To Doug Collins, for giving me someone to compete against in the thesis writing game and for all the distractions along the way.

And to my family, for supporting my desire to learn.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Development of wheat varieties that are resistant to wheat streak mosaic virus (WSMV) and remain productive under non-disease situations would be beneficial to wheat growers in Montana. Previous attempts at developing WSMV resistant germplasm resulted in poor agronomic and end-use quality. A new source of WSMV resistance carried on a *Thinopyrum intermedium* chromosome translocation was recently released by Kansas State University. The effects of the *Thinopyrum* translocation on agronomic performance and end-use quality have not been documented. A study was conducted to determine if this translocation, that prevents infection by WSMV, has detrimental effects that would deem it unsuitable for deployment in WSMV resistant spring wheat cultivars. Four populations, consisting of a total of twenty-two WSMV resistant, thirty-six susceptible lines, and eight parental lines, were grown in three replications at Bozeman and Conrad, MT in 1998 and 1999. The agronomic performance of resistant and susceptible lines was compared under disease and non-disease conditions to determine the effectiveness of resistance under disease pressure and to determine the effects of *Thinopyrum* translocation in the absence of disease. A significant decrease in yield was observed for non-inoculated resistant lines in contrast to susceptible lines. However, the yield range of resistant entries suggests that the recovery of parental yield is possible. Resistance was also found to be effective in limiting virus replication, resulting in only a 5% yield reduction under inoculated conditions compared to 32% for susceptible lines. In all instances where WSMV was introduced to field trials the *Thinopyrum* translocation provided a significant benefit for resistant lines when compared to susceptible lines. The *T. intermedium* translocation present in resistant lines had no effect on end quality factors. This study indicates that the release of WSMV resistant wheat that is yield competitive and meets industry standards for quality is achievable.

CHAPTER 1

INTRODUCTION

Wheat Streak Mosaic Virus (WSMV), which is vectored by the wheat curl mite (*Acer tulipae* Keifer), is an important and widely distributed wheat (*Triticum aestivum* L.) disease in North America. It is most prevalent in the central Great Plains of the United States, where it destroys a significant percentage of both the spring and winter wheat crop annually (Wiese, 1987).

The disease was first identified in Montana in 1954, and Montana growers have experienced major outbreaks in 1964, 1981, 1993, and 1994 (Bramford et al., 1996). Past WSMV infections in Montana caused significant winter wheat crop loss in 1964 and an estimated $12.7 million (US) damage in 1993 (Fowler, 1998). In 1988, an epidemic of WSMV in Kansas resulted in an estimated loss of about 13% of the winter wheat crop, corresponding to 1.1 billion kg (Sim et al., 1988).

Wheat and wheat products are the leading export in Montana, and accounted for 72% of the State's agricultural exports in 1997. These products sold by Montana farmers in 1997 accounted for nearly a quarter of a billion dollars, and nationwide wheat products grossed over 4 billion dollars (Montana Agricultural Statistics, 1999). Current agricultural trends show a $557 million decrease in value of wheat and wheat products exported from the United States between 1996 and 1997. Such trends make it even more

important for farmers to minimize the amount of crop lost to disease each year as wheat commodity prices remain low.

For this reason wheat breeders have worked to develop varieties of wheat that possess resistance to WSMV. The challenge begins here since no known wheat varieties are resistant, though some do show varying levels of tolerance (Seifers and Martin, 1988). Attempts at introducing WSMV resistance into wheat have been unsuccessful in producing adequate varieties for release. These attempts involved wide crosses with wild relatives of wheat and resulted in "wheat-like" lines whose end use qualities were deemed unsuitable for agricultural production.

As a result of these previous shortcomings, breeders have continued to utilize various gene transfer techniques involving wild relatives of wheat. These efforts focus on incorporating only alien resistance into cultivated wheat, in order to decrease any detrimental quality effects associated with non-disease resistance alien gene expression. The ultimate goal is the expression of only those alien genes conferring disease resistance while achieving the quality demands of both growers and consumers.

Recent cytogenetic studies have the shown the introgression of a WSMV resistance gene into wheat from *Thinopyrum intermedium*, a wild relative of wheat. *T. intermedium* lacks the milling and baking qualities of wheat, but does possess resistance to numerous wheat diseases and serves as an important germplasm pool for wheat breeders. Many decades of development have led to a promising line, KS93WGRC27, which contains a translocation from *T. intermedium* chromosomal arm $4J^s$ that carries the resistance gene *Wsm1* (Chen et al., 1998). This has resulted in WSMV resistance being introgressed into wheat, with minimal detrimental alien gene expression (Gill et al.,

1995). The line KS93WGRC27 is a BC_3F_2-derived line from the backcross of the hard red winter wheat cultivar Karl with CI 17884 (Gill et al., 1995). Because of the decreased size of alien gene translocation present as detected by *in situ* hybridization (compared to previous studies), and the lack of viral replication, KS93WGRC27 shows promise as a reliable source of resistance to WSMV.

Evaluating WSMV resistance in wheat can be performed by numerous techniques. Symptoms can be visually determined, but some varieties show decreased symptomology due to the presence of minor tolerance genes and may be misclassified as resistant to WSMV. Antibodies to WSMV are useful to evaluate viral accumulation via ELISA, but this technique involves a large input of labor needed for inoculating field trials and the possibility that plants may escape infection. The development of the sequence tagged site (STS) primer set J15 (STSJ15) has enabled breeders to easily detect the presence of *Wsm1* in wheat crossed with KS93WGRC27 (Talbert et al., 1996). This allows researchers to screen potential resistant progenies and design field trials accurately. The WSMV resistance present in KS93WGRC27 has now been crossed with Montana adapted spring and winter wheat varieties. The Montana spring wheat breeding program has developed advanced spring wheat progeny with and without the translocation marked by STSJ15. The progeny of these populations are suitable for evaluating the overall impact of the *T. intermedium* segment on WSMV resistance, and agronomic and end use quality.

The dichotomy between resistance and susceptible plants is at the core of this investigation. Evaluating the effects of the translocation from *T. intermedium* when incorporated into cultivated wheat is important to the development of agronomically

productive WSMV resistant wheat cultivars. Knowledge gained from experiments with spring wheat will serve as a template for parallel applications in winter wheat.

CHAPTER 2

LITERATURE REVIEW

Wheat streak mosaic was first recognized in Nebraska as "yellow mosaic" in 1922 (Wiese, 1987). Since that time the disease has been identified as a major cause of yield losses in wheat for many areas of North America and the world. Wheat streak mosaic virus can also infect barley (*Hordeum vulgare*), corn (*Zea mays*), rye (*Secale cereale*), oats (*Avena sativa*), and a number of annual and perennial grasses (Wiese, 1987). Infected wheat plants are normally stunted, with mottled and streaked leaves. Leaf streaks are green-yellow, parallel, and discontinuous. Heads, if formed, are partially or totally sterile. Immuno-electron microscopy has been used to describe the virus as a long filamentous rod about 700nm long and 19nm wide (Brakke, 1971). Traditionally wheat streak mosaic virus had been classified in the family *Potyviridae* of the Genus *Rymovirus*. Phylogenetic studies within the *Potyviridae* have shown that WSMV is not a member of the genus *Rymovirus*, and should be placed in a newly classified genus named *Tritimovirus* (Stegner et al., 1998).

Viruses, like any other organism, display genetic variation within species. Early studies into WSMV strains were performed on eight WSMV isolates collected throughout central and eastern Montana (Carroll et al., 1982). Seven isolates were found to be similar to previously classified mild strains and one resembled the type strain of the virus. All eight isolates were separated on Michigan Amber wheat and classified into

two groups, mild and severe, based on symptom expression. The strain isolated from Conrad, MT, was found to display severe symptoms in almost all cultivars it was inoculated to, as did the type strain and a Nebraska isolate. This Conrad isolate from 1982 was used to inoculate field trials for this experiment.

The field of molecular biology has also identified variability among WSMV isolates. Serological characterization of isolates can be performed by using numerous techniques. The differences among isolates can be determined by using enzyme-linked immunosorbent assay (ELISA), Western blot, protein fingerprinting, and serological specific electron microscopy (Montana et al., 1996). Genome sequencing can also be used to characterize genetic variability of WSMV isolates (McNeil et al., 1996). Because there is variation in severity of infection among isolates it is important to develop resistance that protects consistently against the most potent strains.

The distribution of WSMV is closely related to the dispersal of its mite vector, the wheat curl mite (*Acer tulipae* Keifer). The vector thrives on the lush, young growth of wheat and many grasses. The mites develop from eggs to adults within eight to ten days, and can only acquire the virus once they reach the instar stage. The mites are approximately 0.3mm long and are dispersed from plant to plant and from field to field via wind. The wheat curl mite and WSMV can persist from season to season on *T. aestivum*, *Z. mays*, and susceptible grasses, but the mites themselves remain viruliferous for only about 7 days (Orlob, 1966).

The movement of winter wheat into traditional spring wheat areas, and vice versa, provides a "green bridge" for the wheat curl mite. Conservation tillage also increases weedy hosts for the vector along with the presence of secondary host in non-cropped

areas (Wiese, 1987). The two most common cultural methods used to control outbreaks of WSMV are: the elimination of the "green bridge", and the alteration of planting dates. The absence of wheat host plants during the period between harvest and fall planting helps to ensure that the mite vectors do not survive. However, warm winter planting conditions, volunteer plants, and late spring wheat planting can often cause these methods to be unsuccessful (Bramford et al., 1996). For this reason, it is imperative to continue the development of WSMV resistant wheat.

Since no source of resistance to WSMV has been identified in cultivated wheat, evaluations of wild relatives of wheat were necessary to find a genetic source of resistance to WSMV. Early work explored several species of *Triticum*, *Agropyron*, and *Secale*, and certain hybrids between them called "Agrotricum", to test for sources of resistance (McKinney and Sando, 1951). *Agropyron* (also known as *Thinopyrum*) selections proved to offer the greatest and most consistent level of resistance and efforts began to transfer these genes to wheat.

Generally the transfer of desirable alien genes into a wheat background is not an easy process and requires a large input of labor and resources with mixed results (Lukaszewski and Gustatson, 1983). The facilitation of interspecific gene transfer in wheat can occur through four main methods: (1) ionizing radiation to break up alien and wheat chromosomes facilitating chromosomal translocations, (2) induced homeologous pairing and subsequent crossing over and genetic recombination by removing or suppressing chromosome 5B effect, (3) the uncontrolled misdivision of two univalents followed by reunion of two telocentrics from different univalents (Sears, 1972), and (4) genetic transformations. All four of these techniques have been utilized in efforts to

transfer genes conferring WSMV resistance.

Any addition of alien chromosomes into wheat are problematic. Many undesirable agronomic and quality characteristics can be transferred into wheat along with the ones desired for disease resistance. Previous examples of gene transfer from wild relatives of wheat have been shown to have mixed effects on quality and agronomic traits. An early experiment involved the transfer of a chromosome from a wheat-*Agropyron* derivative P.W. 27 carrying stem rust resistance to common wheat (Knott, 1964). Knott found that in 6A substitution lines there were no detrimental quality or agronomic effects. But, another study reported a linkage drag associated with resistance in near isogenic lines of wheat for stem, leaf, and stripe rust resistance derived from *Agropyron elongatum* that resulted in poor end-use quality (Zeven et al., 1983). Some alien translocations and additions have resulted in alien chromosome lines having higher yields and increased protein levels, but poor end-use quality and fertility (Soliman and Qualset, 1984; Forester and Ellis, 1990; Joppa et al., 1991; Espitia-Rangel et al., 1999). These studies address the difficulty breeders face when introducing alien genes of interest and the potential penalty associated with additional chromosome segments that contain them.

This was true of early attempts to breed for resistance to both WSMV and the wheat curl mite using the crosses made between wheat and *Thinopyrum intermedium*, (Lay et al., 1971; Wells et al., 1973; Wang and Liang, 1977; Liang et al., 1979; Wells et al., 1982) and wheat x *A. elongatum* hybrids (Sebesta and Bellingham, 1963; Larson and Atkinson, 1970; Martin et al., 1976; Sebesta et al., 1972). These amphiploids possessed

one or more undesirable agronomic traits such as poor protein levels or low yield and thus did not lead to the development of new cultivars (Friebe et al., 1991).

Lay et al. (1971) introduced WSMV immunity in wheat via irradiation of Agrotricum progenies. An octoploid derivative, TA25, of Carsten V (*Triticum aestivum* L. Em. Thell) was crossed to *T. intermedium* and their progeny were found to be resistant to WSMV. Seed obtained from the F_1 generation as a result of crosses of TA25 with common wheat were exposed to 1000 rads of fast neurons. After a process of backcrossing with 'Lathrop' spring wheat, one 42 chromosome substitution line (Figures 1,2 and 3) was obtained having resistance to WSMV that would later be registered as CI 15092 (Lay et al., 1971; Wells et al., 1973). Translocation of alien segments or arms have been the most promising way off reducing undesirable characteristics associated with amphiploids and alien chromosome expression in wheat, because half or more of the chromosome is wheat (Lukaszewski and Gustafson, 1983).

The next stage of WSMV resistant germplasm development involved the cross of the disomic substitution line, CI 15092/ *T. speltoides*// 'Fletcher', to 'Centurk' and 300 of the F_1 seeds were irradiated with fast neurons. After four backcrosses to Centurk, five translocation lines were classified as resistant to WSMV (Wells et al., 1982). These five lines displayed varying agronomic characteristics including tillers per plant, seed weight per 50 seeds, height, etc. (Table 1). Wells et al. (1982) reported that three of the five translocation lines showed increased flour protein levels versus Centurk. These variations in agronomic qualities suggested varying sizes of translocations. Line T_D (CI 17884), was found to be most similar overall to the recurrent parent, Centurk. The translocation line CI 17884 was found to have the same mixogram and other physical

Table 1.
Means for characters of lines resistant to WSMV and Centurk check at Brookings in a 1978 field test, a 1979 spring greenhouse test, and a 1979 field test.

GP no	Line or check	CI no.	Tillers/ plant	Seeds/ primary spike	Wt./50 seeds	Spikelets/ primary spike	Florets/ primary sprike	Seed set	Seeds/ plant	Length primary spike	Plant height	Days to flowering	Centromere	Meiotic index
			no.		g	no.		%	no.	mm	cm	no.	‡	%
199	TA	17881	5	39	1.11	14	54	75	132	78	84	80	Tae	86
200	TB	17882	7	42	1.06	14	52	80	187	81	89	76	Tae	84
201	TC	17883	6	45	1.05	14	59	73	160	104	118	80	Tae	71
202	**TD**	**17884**	8	**36**	**1.28**	**14**	**49**	**75**	**193**	**83**	**85**	**74**	**Ai**	**85**
203	TG	17886	5	42	1.2	14	55	75	132	94	85	80	Tae	93
204	DSF	17885	5	27	1.05	12	45	60	61	68	74	79	Ai	66
	Centurk	15075	8	50	1.14	14	57	89	252	78	88	85	Tae	97

Modified from Wells et al., 1982

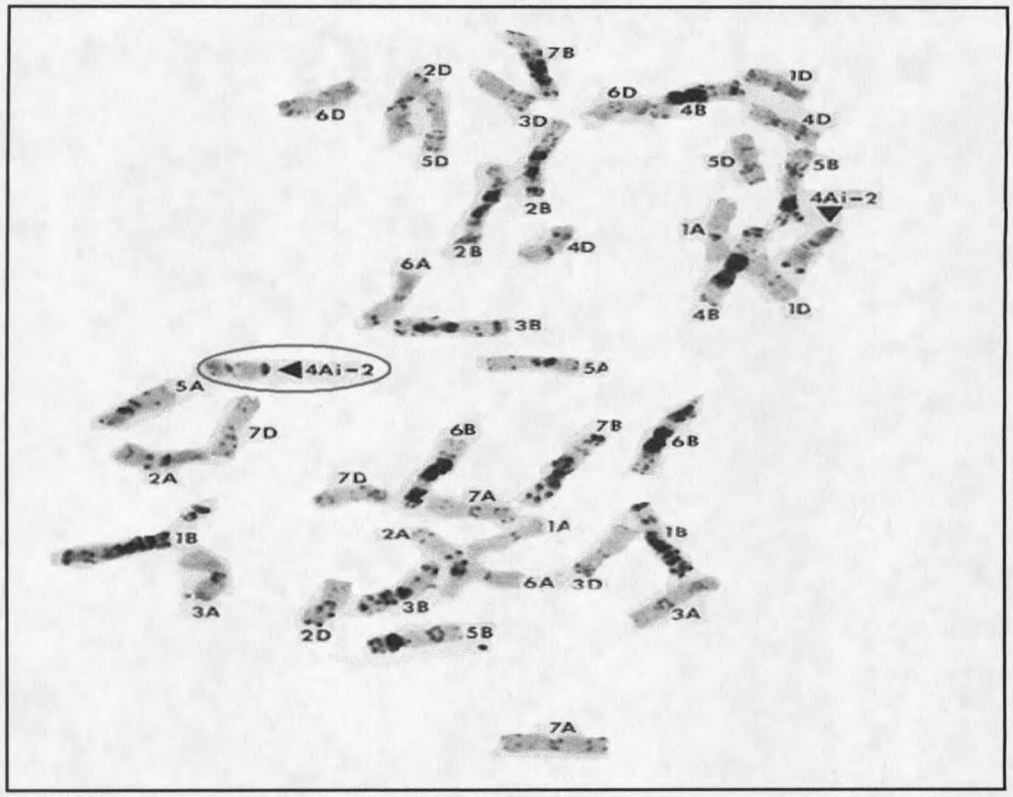


Figure 1. C-banded CI15092 (2n=42), 4 *Ai*-2 (4A) substitution line (Friebe et al., 1991)

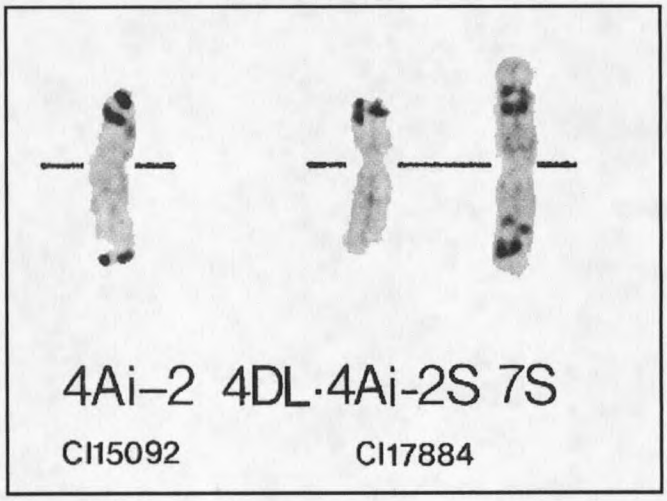


Figure 2. C-banding pattern of the critical chromosome arms present in WSMV resistant germplasm. (Friebe et al., 1991)

dough properties as Centurk which would predict good overall bread making properties (Wells et al., 1982).

C-banding and *in situ* hybridization analysis of CI 17884 identified one compensating translocation where a segment of chromosome $4J^s$ of *T. intermedium* was translocated to the short arm of wheat chromosome 4D (Friebe et al. 1992, 1996). The T4DL-4Ai#2S translocation line, CI17884, was also found to have a wheat *Aegilops speltoides* translocation conferring resistance to greenbug (Figures 2 and 4).

Unlike previous attempts to introduce resistance into wheat, only a chromosomal segment, $4J^s$, from *T. intermedium* replaces and thus compensates for the normal short arm of chromosome 4D in the wheat genome (Figure 2 and 4). These findings then led to the development of KS93WGRC27, a better agronomically adapted germplasm with only the T4DL-4Ai#2S translocation containing the *Wsm1* gene.

Studies have found that *Wsm1* loses its effectiveness and becomes unstable when temperatures exceed 25 degrees Celsius in the greenhouse, but resistance remains stable in field trials (Seifers et al., 1995). This should not pose a major problem since spring wheat is most susceptible to WSMV in early spring, and winter wheat is most susceptible in the fall when Montana seasonal temperatures tend to be low.

Upon the release of KS93WGRC27 (Gill et al., 1995), the *Wsm1* gene was introduced into Montana spring wheat varieties. One reason resistant varieties have not been available, besides the lack of useful resistance sources, has been the difficulty involved in screening for virus resistance. Field or greenhouse inoculations can prove to be very timely and laborious. For this reason, Talbert et al. (1996) developed a fast and

Figure 3. GISH. The two alien substitution chromosomes 4Ai-2 in CI15092 strongly hybridized at terminal centromeric regions by S genomic DNA probe.

(Chen et al., 1998)

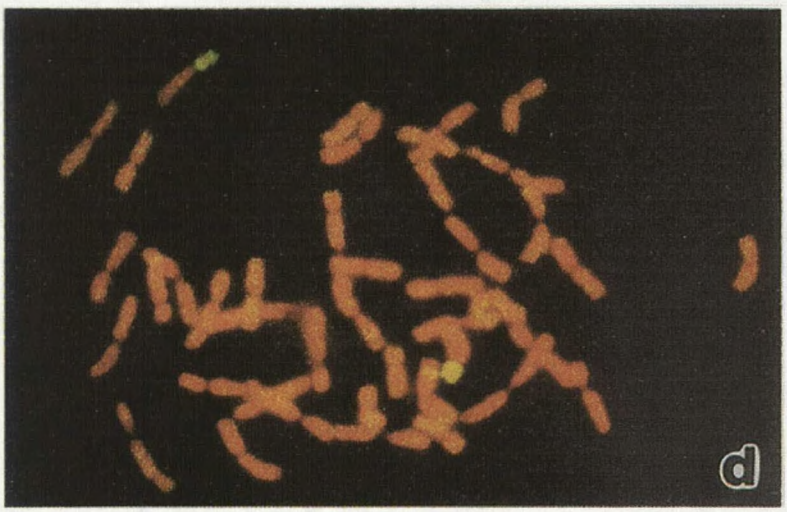


Figure 4. GISH. The two alien chromosome arm translocations in CI17884 detected by an S genomic DNA probe with break points at the centromere.

(Chen et al., 1998)

easy way to identify the presence of the *Wsm1* gene via a PCR primer, STSJ15, specific to the *T. intermedium*. The STSJ15 primer allowed for the evaluation and identification of resistant lines for this experiment without the need for direct inoculation to classify potential entries to test for resistance.

As mentioned earlier, efforts have also been made to introduce wheat curl mite resistance into a wheat background. Wheat with resistance to the wheat curl mite (WCM) has a translocation from rye (*Secale cereale* L.) that effectively reduces field infections of WSMV. Two cultivars have been developed, 'TAM 107' and 'TAM200', that have derived their resistance from 'Gaucho' a WCM resistant triticale (*X Triticosecale sp.)*. It has now been shown that mites are able to develop resistance-breaking strains in both the field and the laboratory (Harvey et al., 1995).

Breakdown of virus resistance in a proven resistant cultivar can occur. Because RNA viruses, like WSMV, lack an error-correcting mechanism during genome replication, they give rise to many new mutants involving one or a few nucleotide changes. Based on symptom morphology on varying cultivars, there are considered to be four main strains of WSMV (Carroll et al., 1982). Such variation can be attributed to mutations. The possibility exists, just as with the wheat curl mite, that strains of WSMV may develop that break the resistance provided by the *Wsm1* gene when mono-cultural conditions exist.

The mechanism of resistance conferred by the *T. intermedium* chromosome translocation is unknown, but there are numerous models proposed. *T. intermedium* displays non-host resistance to WSMV, since testing shows that the virus is incapable of supporting multiplication or symptom development (Pfannenstiel and Niblett, 1978). The

process of chromosomal translocation, as seen in KS93WGRC27, has transformed normally susceptible wheat and enabled WSMV resistance to be heritable.

The pathogenic cycle of a plant virus offer numerous targets which could be affected by viral resistance. These include the replication of virus particles during the primary infection in the initially infected cell, secondary cycles of replication resulting from the spread of virus from cell to cell and over long distances throughout the plant, and the spread of progeny virus particles by passive means or through vector transmissions to initiate fresh cycles of infection in a new plant (Fraser, 1998).

These large scale cycles of replication are composed of numerous smaller scale processes that are the likely target of the non-host resistance expressed in the germplasm investigated here. These molecular processes include: uncoating of the virus genome, expression of the virus genes, replication of the genome, synthesis of the viral proteins, assembly of the progeny particles, molecular interaction with host components involved in replication and protein synthesis, molecular interactions with host components involved in cell to cell and long distance movement and molecular interactions with components of the vectors. These can occur as positive mechanisms that actively or directly inhibit some phase of the viral replicative cycle. Or, as a negative mechanism where the resistant plant lacks some component required by the virus to complete its replicative cycle (Fraser, 1998).

As stated above, it is currently unknown what the exact mechanism responsible for the resistance derived from *T. intermedium*. Specifically, this study was conducted to determine the agronomic and end-use quality of lines containing the *T. intermedium* chromosome translocation relative to lines not containing the translocation. Preliminary

data for KS93WGRC27 derived WSMV resistant lines has been reported by Kansas State University (1998) from their 1998 Intra-state nurseries (Table 2). At this time, end-use quality data has not yet been reported. If there is found to be a cost associated with the translocation containing *Wsm1,* an understanding of the mechanism and the level of resistance from *T. intermedium* would potentially be beneficial in developing transgenic plants that contain this form of resistance.

Table 2. Summarized grain yields (bu/a) of selected entries for the 1998 Kansas IntraState Nursery for all wheat regions in Kansas.

Selection	Central	Irrigated	East	West	**Average**
KS90175-3	58	86	78	63	**71**
KS89180B-2-2-1	55	89	65	66	**69**
2137	53	80	66	61	**65**
JAGGER	53	86	61	59	**65**
KS95W62-6	48	85	52	67	**63**
HBK1064-5-1-1	55	72	67	54	**62**
KS96HW94	48	81	48	64	**60**
* **KS96HW10-3**	*45*	*79*	*49*	*61*	***58***
* **KS96HW10-1**	*46*	*77*	*44*	*63*	***57***
ORO BLANCO	46	74	46	57	**56**
KS95HW179-4	42	71	39	61	**53**
KS91W009-6-1	43	72	45	49	**52**

*** Identifies spring wheat lines containing *T. intermedium* translocation conferring resistance to WSMV.**

Adapted from Kansas State University (1998).

CHAPTER 3

MATERIALS AND METHODS

Molecular Marker Evaluation

Plant Material

The germplasm evaluated in this experiment was developed by the spring wheat breeding program at Montana State University. The WSMV resistant winter wheat line KS93WGRC27, originating from Kansas State University (Gill et al, 1995), was crossed with Montana adapted cultivars Amidon (North Dakota State University, 1988) and McNeal (Lanning et al., 1994). The resulting F1 progenies were topcrossed to various adapted Montana spring wheat lines to produce seven populations.

The F1 progenies were grown at the Post Farm in Bozeman, MT in 1995. The F_2 and F_3 generations were advanced via single seed descent at the Plant Growth Center at Montana State University from fall 1995 to spring 1996 and screened via PCR and greenhouse inoculation for resistance to WSMV. Germplasm was then grown as head rows for the F_4 generation in 1996 and as plant rows for the F_5 generation at the Post Farm in Bozeman in 1997. Resistant and susceptible selections from the F_6 generation was planted for the first year of this experiment, with the F_7 generation being grown in the second year of this two-year field study.

DNA Extraction

Young leaves from 80 entries were grown in the Plant Growth Center at Montana State University from seven F_6 populations. Total genomic DNA was extracted following the procedure of Lassner et al. (1989) from three to five individual plants. Aliquots of 5 µl of the DNA extracted were quantified on a 1.0% agarose gel by comparing the sample intensities to known standards. The concentrations of the samples were then adjusted to approximately 100 ng/µl.

PCR Reaction

PCR amplifications of each entry were performed in 50 µl reactions that consisted of 10X reaction buffer, 50 µM of dNTP's, 1.5mM $MgCl_2$, 400 nM of the left and right primers, 0.15 µl of Taq polymerase per reaction, and approximately 200ng of genomic DNA. The PCR was performed in a MJ Research, Inc. PTC-100 Programmable Thermocycler. The program consisted of the following conditions; an initial denaturation of the DNA at 94°C for 4 minutes, followed by 29 cycles of 94°C for 1 minute, 45°C for 1 minute, and 72°C for 1 minute and 20 seconds. And, a final 7 minutes at 72°C followed by an infinite hold at 4°C.

PCR Primer

The determination of WSMV disease resistance was established via PCR by using the STSJ15 primer reported by Talbert et al. (1996). A PCR product from a genotype carrying the *T. intermedium* translocation produces a band of approximately 500bp thus identifying genotypes containing the *Wsm1* gene. This allowed for the differentiation of resistant and susceptible genotypes for the populations tested.

PCR Product Evaluation

The PCR products were separated on either a 0.7% polyacrylamide gel with 0.5X EDTA running buffer or 1.0% agarose gel (containing ethidium bromide) with 1.0X EDTA running buffer. The polyacrylamide gels were stained with ethidium bromide and the DNA present in both types of gels were visualized with a UV light box and photographed. PCR product sizes were compared against RsaI/pUC18 marker (Figure 5).

Field Trial Evaluations

Plant Material

The criteria for the acceptance of entries into the study were a minimum of 300g of seed per entry harvested from F_5 plant rows grown at the Post Farm in Bozeman in 1997 and an adequate representation of resistant and susceptible lines from each population. This criteria resulted in the selection of four populations (Table 3).

Field Conditions and Location

In 1998 and 1999, field trials were conducted at the Western Triangle Agricultural Research Center north of Conrad, MT and at the Arthur H. Post Field Research Farm in Bozeman, MT. The soil type in Conrad is Scobey clay loam and in Bozeman Amsterdam silt loam. The elevation in Conrad is 1125m (3700 ft) and 1,439m (4,772 ft) in Bozeman. In 1998 the average temperature in Conrad during the field trial was 15.4°C (59.7°F),

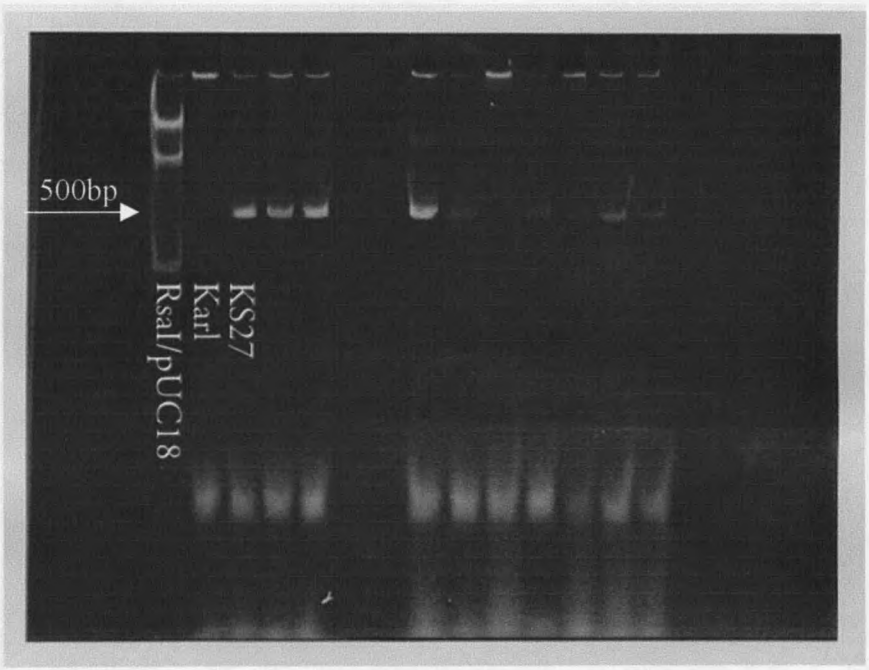


Figure 5. Example of 500bp WSMV resistant diagnostic PCR product for DNA extracted from plant tissue of resistant and susceptible entries and amplified by the STSJ15 primer for *Wsm1*.

Table 3. Resistant and susceptible entries from the four selected populations grown in Bozeman and Conrad in 1998 and 1999.

	Initial PCR Result	Re-screened PCR result
Population 1 McNeal/KS93WGRC27//MT9328		
Entry ID		
4138	S	S
4141	R	**S**
4142	R	R
4146	S	S
4156	S	S
4161	R	R
4165	R	R
4168	S	S
4170	S	S
4181	S	S
4182	R	R
4186	R	**S**
4189	S	S
4191	S	S
4195	R	R
4196	R	**S**
4197	S	S
4199	S	S
4211	R	R
4214	R	R
Population 2 AMIDON/KS93WGRC27//McNEAL		
Entry ID		
4228	S	S
4238	S	S
4241	S	S
4245	S	S
4248	R	R
4252	S	S
4259	R	R
4262	R	R
4266	R	R
4274	R	R

Table 3. (cont.) **Resistant and susceptible entries from the four selected populations grown in Bozeman and Conrad in 1998 and 1999.**

	Initial PCR Result	Re-screened PCR result
Population 3 AMIDON/KS93WGRC27//MT9328		
Entry ID		
4282	S	S
4288	R	R
4289	S	S
4292	R	R
4293	S	S
4295	R	R
4298	S	S
4303	S	S
4312	S	S
4316	S	S
4323	R	**S**
4330	R	**S**
4335	R	**S**
4336	R	R
4338	R	R
4343	S	S
Population 4 Amidon/KS93WGRC27//MT9419		
Entry ID		
4348	S	**R**
4359	S	S
4364	S	S
4366	S	**R**
4372	S	**R**
4378	R	R
4379	R	**S**
4387	R	**S**
4389	R	**S**
4392	S	S
4396	R	R
4398	R	**S**

with 23.3 cm (9.16 in.) of precipitation during the growing season. In 1999 the average temperature in Conrad during the field trial was 15.7 ° C (60.2° F), with 16.7 cm (6.6 in.) of precipitation during the growing season. In 1998 the average temperature in Bozeman during the field trial was 13.8° C (56.9° F), with 20 cm (7.85 in.) of precipitation during the growing season. In 1999 the average temperature in Bozeman during the field trial was 15.7 ° C (60.2° F), with 16.3 cm (6.4 in.) of precipitation during the growing season. The tests were grown in four row plots at eight grams per 3.0 meter row in Conrad and nine grams per 3.7 meter row in Bozeman.

Experimental Design

In 1998 the selected F_6 lines from four populations were planted in a randomized block with three replications of four rows each at both Bozeman and Conrad. Treatments were arranged as a split plot design with the four populations as main plots, and progeny and parents as subplots. In 1999, planted field experiments consisted of F_7 generation seed from one replication of non-treated entries grown at Bozeman in 1998, and planted using the same experimental design.

The planting dates for experiments in 1998, were April 21 in Conrad and May 5 in Bozeman. Experimental plots from 1998 were harvested on August 19-20 in Conrad, and on August 21 in Bozeman. In 1999, the experiments were planted on April 15 in Conrad and April 16 in Bozeman. The plots were harvested on August 24 and September 8 respectively.

Harvest Techniques

Once all the entries reached physiological maturity the inoculated rows were harvested via a single row rice binder and threshed by means of a Vogel (stationary

thresher) for the 1998 field trial. For the 1999 field trial, the two inoculated rows were harvested via a Wintersteiger small plot combine at both locations. The non-inoculated rows were harvested by a Wintersteiger small plot combine in 1998 and 1999 at both Conrad and Bozeman.

Inoculation and Evaluation of Viral Resistance

The inoculate prepared for both seasons consisted of greenhouse grown spring or winter wheat plants (~500g/L) that had been infected with the Conrad isolate reported by Carroll et al. (1982) and homogenized in a pH 6 phosphate buffer with silica or carborundum added to facilitate viral introduction. In 1998, the first row of each four row plot was manually inoculated at both Conrad and Bozeman with the hand rub method with plants at the two to four leaf stage (McKinney and Fellows, 1951). In 1999, two rows of each four row plot were inoculated.

At both 15 and 30 days after inoculation, two six-inch plant sections of each inoculated row were harvested in 1998. In 1999 14 to 16 leaves were randomly selected from the two inoculated rows. These harvested sections were used to measure the level of replicated virus by means of ELISA (Edwards and Cooper, 1985). Polyclonal antibodies utilized for the ELISA were made from WSMV purified from an adapted protocol described by Brakke and Ball (1968) and produced by rabbits at Montana State University.

Morphological Evaluation

Each non-inoculated subplot was evaluated for six morphological characteristics in both growing seasons. Each inoculated subplot was evaluated for three of the same morphological characteristics and two additional characteristics.

Heading Date – heading dates of non-inoculated rows were recorded in Julian days, the number of days from January 1, when 50% of the heads were completely emerged from the flag leaf.

Plant Height – height of both inoculated and non-inoculated rows were measured in centimeters from soil level to the top of the spike.

Physiological Maturity – physiological maturity of non-inoculated row was recorded in Julian days, the number of days from January 1 when glumes in non-inoculated rows were observed to have a complete loss of green color in 75% of the plants in a plot (Hanft and Wych, 1982).

Yield – grain from inoculated and non-inoculated rows was weighed in grams. Row lengths in Bozeman were variable necessitating measurement of individual plots. Rows in Conrad were end trimmed to 2.43 m. The number of rows harvested varied depending upon the treatment year. The calculated yield was expressed as kg ha^{-1}. Yield reduction was calculated by subtracting individual inoculated rows in a plot from the non-inoculated rows in the same plot. The mean yield reductions were then calculated for resistant and susceptible entries in each population for each location and year.

Test Weight –test weights were measured on a Seedburo Test Weight scale. Test weights from non-inoculated plots were measured in either quart or pint volumetric containers. Test weights from inoculated plots were measured in pint, ½ pint, or 1/8 pint containers dependent upon seed yield. Test weight was calculated from a standard curve and reported in kg m^{-3}.

Protein – the percent protein from non-inoculated plots was measured for whole grain samples using an Infratec 1225 grain analyzer in the Cereal Quality Lab at Montana

State University, Bozeman. The percent protein for inoculated rows was not measured and was predicted to be near 1% higher (Finney and Sill, 1963).

Disease Rating – the inoculated row were rated on a scale of 0 to 3 to measure disease response. This disease scale used 0 to indicate no affect, 1 to indicate mild chlorosis or possible rubbing injury, 2 to indicate moderate chlorosis, and 3 to indicate severe disease response or necrosis.

Viral Replication Levels – inoculated leaf tissue samples were weighed (2.5 – 5g) and pulverized in 50ml conical tubes by means of a Omni International TH hand grinder. 5ml/g of PBS-Tween solution (pH 7.4) was added to tube and vortexed for 1 min. A 1.5 ml sample was poured into a 1.5ml ependorf tube and spun at 10,000g for 45 seconds. Both the conical tubes and 1.5ml samples were then placed in -20° C until the ELISA procedure. The ELISA tests were performed as reported by Edwards and Cooper (1985). The absorbencies were measured on a Molecular Devices Kinetic Microplate Reader at 405nm, after a 20 minute incubation with p-nitrophenyl phosphate substrate at 1mg/ml in substrate buffer.

Cereal Quality Evaluation

Grain (650g) from one replication of non-inoculated plots from Conrad and Bozeman were cleaned and placed in pre-labeled cans for quality analysis. Cereal quality lab staff performed the procedures listed below according to approved methodology (AACC, 1995).

Milling and Flour Evaluation – one week prior to milling, the samples were processed through the first of a two-stage temper. The Dickey-John / Motomoco Model 919

Automatic Grain Moisture tester was used to determine the moisture content. Moisture was measured and the wheat was brought up to 12% moisture. Twenty-four hours before milling the wheat was tempered a second time to 15.5% moisture. Samples were next milled on a Brabender Automat Mill to obtain flour, bran and shorts/middlings.

Flour Yield – percent flour yield was calculated by dividing the flour by the total product (flour, bran, and shorts/middlings).

Flour Ash – the mineral content of the flour. Reported as the percent ash from three to five gram samples of flour ignited and heated for 18 hrs at 580° C in a muffle furnace (AACC Method 08-01).

Flour Moisture – two to three grams of flour were heated in an aluminum dish in a mechanical-convention oven for one hour at 130 ° C, allowed to cool in a desiccator and weighed (AACC Method 44-15A).

Flour Protein – 0.25 g of flour was combusted in a LECO FP-328 nitrogen analyzer in an oxygen atmosphere followed by a series of catalytic reaction that result in nitrogen gas. Percent crude protein = % nitrogen x 5.70 (AACC Method 46-30).

Mixogram – a test of the mixing properties of dough. Ten grams of flour, on a 14% moisture basis, was weighed and mixed at optimum water absorption. The mixograph curve suggests mixing time requirement, tolerance and optimum water absorption. The mixogram curves were then visually evaluated.

> **Mixogram Absorption** – optimum flour water absorption visually evaluated by the swings in the mixogram curve. Absorption was reported as percent by weight, corrected to a 14% flour moisture basis.

Mixogram Mixing Time – time in minutes required for optimum dough development. Measured as the maximum point on the mixing curve or slightly after the peak.

Mixogram Type – based on the quantity and quality of flour protein. Protein content/quality was divided into three categories: Low (less than 10% protein), Medium (10%-12.9% protein) and High (13% or greater protein). Tolerance was scored 1 – 8 (weak to strong).

Baking Evaluation – 100 g flour loaves were baked to determine the effect of environment, genotype, and wheat flour components. The method consisted of a 90 min., sugar-based, fermented dough system. The bread formula contained 100 g flour, 6 g sugar, 3.5 g shortening, 1.8 g yeast, 1.5 g salt, 180 ppm Doh-Tone (fungal alpha-amylase), and 150 ppm ascorbic acid as an oxidant (AACC Method 10-10B).

Baking Absorption – a measure of the amount of moisture required to make a dough of proper consistency for bread baking when mixed to optimum conditions based on the feel and appearance of the dough as judged by an experienced bread baker.

Bake Mixing Time – time required in minutes for dough to reach a minimum mobility. The test baker determines optimum mixing time.

Bake Volume – volume of a loaf of bread in cubic centimeters was determined by canola seed displacement. A good measure of protein quality.

Crumb Grain Score – bread is allowed to cool on racks 2 hrs. Before the appearance of the loaf was noted and the crumb grain graded on a scale of

(0-5). The higher the number the better the crumb grain. The general appearance and inside cellular structure determines the quality crumb grain. Subjective judgement of a crumb grain quality is determined by an experienced baker.

Statistical Analysis

An analysis of variance was computed for each trait. The analysis was first computed for each population-environment combination. The analysis was then combined across the environments for each population, and then the analysis was combined over populations. The mean of resistant lines was compared with the mean of susceptible lines for each trait. Replications and lines within resistant and susceptible classes were treated as random effects while other effects were considered fixed effects. Test of fixed effects in the model were constructed using linear combinations of mean squares to construct the appropriate error term (Satterthwaite, 1946).

CHAPTER 4

RESULTS

Molecular Marker Evaluation

PCR Product Evaluation

PCR products from DNA amplified with STSJ15 produced a 500-bp band as measured by RsaI digested pUC18 (Figure 5). This diagnostic band was used to identify entries resistant to WSMV as reported by Talbert et al.(1996). Entries were classified as susceptible if there was no PCR product present at 500-bp. Four of six populations were then selected for field trials based upon an adequate representation of resistant and susceptible entries.

PCR Product Field Results Comparison

Initially 29 resistant and 29 susceptible entries were selected via PCR, but first year field results suggested possible misclassifications. Re-evaluation of entries using PCR indicated some lines had been initially misclassified. Reclassification resulted in 22 resistant and 36 susceptible entries (Table 3). Theses changes were applied to the 1998 data set and were in place for the 1999 planting season (Table 4).

Table 4. Population Pedigrees and the number of resistant and susceptible entries for each population tested in each replication after reclassification.

Pedigree	Resistant Entries	Susceptible Entries
Population 1		
McNeal/KS93WGRC27//MT9328	7	13
Population 2		
Amidon/KS93WGRC27//McNeal	5	5
Population 3		
Amidon/KS93WGRC27//MT9328	5	11
Population 4		
Amidon/KS93WGRC27//MT9419	5	7

Field Trial Evaluations

Agronomic Trait Evaluation

The effects on lines containing the translocation from *T. intermedium* are reported below. The objective in evaluating these traits was to see if there are any consequences for resistant lines with and without WSMV. Mean performance of resistant and susceptible lines are reported: across environments and populations, for each of the four populations, for each individual population in each environment, and for each environment.

Yield

The overall mean yield of resistant lines for all environments and populations was significantly lower than yield of susceptible lines by 250 kg ha^{-1} (3.7 bu $acre^{-1}$) or 5% (Table 5). Mean yields of resistant lines of populations 1 and 3 were significantly lower in yield under non-inoculated conditions than susceptible lines in the same populations (Table 5). The yield reduction in population 1 occurred in all environments except Conrad in 1999 (Table 6). In population 3 resistant lines yielded significantly lower than susceptible lines only at Conrad in 1999, although showing a similar trend in all other environments (Table 6). Yields of resistant and susceptible lines in populations 2 and 4 were similar with the exception that population 4 resistant line yields were significantly less at Bozeman in 1999.

Yield Reduction

Yield reductions due to WSMV inoculation were significantly less for resistant lines for all populations over the entire experiment (Table 5). Overall means of resistant lines were 5% lower due to inoculation and mean yields of susceptible line were 32% lower. In 1998, resistant lines show significantly lower yield injury than susceptible lines in all populations and environments (Table 6). In, 1998, yield reductions at Bozeman, MT were 4% (3.5 bu acre^{-1}) for all resistant entries and 36% (33.4 bu acre^{-1}) for all susceptible entries. Yield reductions at Conrad in 1998 for resistant entries was 8% (6.2 bu acre^{-1}) and 55% (44.6 bu acre^{-1}) for susceptible entries.

In 1999 at Bozeman, populations 1, 3, and 4 showed significantly lower yield loss due to WSMV in resistant lines. Resistant and susceptible groups from population 2 did not differ at Bozeman in 1999. The yield reduction due to WSMV inoculation for resistant entries at Bozeman in 1999 was less than 1% (0.7 bu acre^{-1}) and for susceptible entries 13% (9.3 bu acre^{-1}). At Conrad in 1999, only populations 3 and 4 displayed a significant difference in the yield reductions between susceptible and resistant groups. At Conrad in 1999, the overall yield reduction for resistant lines was 4% (2.3 bu acre^{-1}) and for susceptible lines the yield reduction was 11% (6.6 bu acre^{-1}).

Test Weight

The overall mean test weights of resistant lines were significantly higher than susceptible lines. Resistant line means were 14.7 kg m^{-3} higher than susceptible line means (Table 5). Test weights of resistant lines were higher than test weights of susceptible lines in populations 1 and 4 but not in populations 2 and 3 (Table 5). Mean test weights of resistant lines were 13.4 and 25.4 kg m^{-3} higher than test weights of

susceptible lines in populations 1 and 4, respectively. Susceptible and resistant groups from population 1 differed at both locations in 1999 but neither location in 1998, although trends were similar in all environments (Table 6). In population 4 resistant and susceptible groups differed at both locations in 1998, but not 1999.

Test weight reduction

Test weight reductions due to WSMV inoculation were significantly greater in susceptible lines than resistant lines over all the field trials and in all populations (Table 5). Test weight reductions due to WSMV were substantial in susceptible lines of all populations except at Conrad in 1999 (Table 6). At Conrad in 1999 test weight reductions were minimal for susceptible entries and none of them were significantly different than the test weight reductions for resistant entries.

ELISA readings

Resistant and susceptible lines from all populations were significantly different for ELISA readings, a quantitative estimate of virus level in the plant (Tables 5 and 6). There was a three to ten fold difference between resistant and susceptible mean values for each population (Table 5).

Disease Rating

Consistent with ELISA data, susceptible lines showed significantly higher levels of disease symptoms than resistant lines in all populations (Table 5). Differences in visual disease ratings for resistant and susceptible groups were greatest for populations 1 and 3 and more moderate in populations 2 and 4.

Table 5. Mean and selected range values of various agronomic traits for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown in Montana from 1998 through 1999.

Source	Yield		Yield	Test Weight		Test Weight	ELISA	Disease
	mean	range	Reduction	mean	range	Reduction	readings	Ratings
	kg ha^{-1}		kg ha^{-1}	kg m^{-3}		kg m^{-3}	A	0-3
Resistant Lines	4892**	4226-5455	253.3**	851.0**	820-883	17.3**	0.27**	0.30**
Susceptible Lines	5142	4103-5984	1636.5	836.3	785-873	73.4	1.36	1.39
Parental Lines	5171		1578.8	827.3		102.0	1.47	1.12
Population 1								
McNeal	5140		1623.2	829.4		84.8	1.57	0.67
MT9328	4982		1847.3	818.4		85.2	1.57	1.58
Resistant Lines	4709**	4453-5092	254.0**	851.4*	834-870	17.1**	0.13**	0.09**
Susceptible Lines	5140	4830-5689	1677.7	836.1	818-856	66.6	1.42	1.44
PCR class x E	ns		**	ns		**	**	**
Population 2								
Amidon	5686		2294.7	836.9		125.8	1.81	1.33
McNeal	5196		1232.2	830.3		112.6	1.46	0.92
Resistant Lines	5312	5107-5455	452.0*	834.5	820-854	12.4*	0.23**	0.45*
Susceptible Lines	5327	4667-5576	1752.7	829.1	798-844	71.5	1.36	1.31
PCR class x E	ns		**	ns		ns	*	ns
Population 3								
Amidon	5222		1037.2	838.2		87.7	1.16	0.75
MT9328	5270		1379.8	825.5		71.5	1.25	1.00
Resistant Lines	4955*	4676-5308	37.8**	865.2	841-883	17.4**	0.16**	0.10**
Susceptible Lines	5316	4687-5984	1948.8	854.3	829-873	75.9	1.22	1.63
PCR class x E	ns		**	ns		ns	**	**
Population 4								
Amidon	5241		1905.7	836.2		168.9	1.61	1.17
MT9419	4629		1310.6	803.5		79.7	1.31	1.50
Resistant Lines	4592	4226-4884	269.6**	852.9*	843-860	22.1*	0.56**	0.57**
Susceptible Lines	4785	4103-5656	1166.8	825.8	785-850	79.7	1.43	1.18
PCR class x E	*		**	ns		**	*	*

***, **, means of resistant and susceptible groups differ or the PCR class x environment interaction is significant at the 0.05 and 0.01 probability levels, respectively, by analysis of variance. 'ns' denotes nonsignificant PCR class x environment interaction.**

Table 6. Means of selected agronomic traits reported for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown at four environments in Montana from 1998 through 1999.

Source	Yield		Yield Reduction		Test Weight	Test Weight Reduction		ELISA readings		Disease Ratings	
	kg ha^{-1}		kg ha^{-1}		kg m^{-3}	kg m^{-3}		A		0-3	
Population 1											
Bozeman 1998											
Resistant Lines	5534	**	419.8	**	850.8	40.4	**	0.29	**	0.14	**
Susceptible Lines	6123		2396.3		839.3	111.7		1.19		1.15	
Conrad 1998											
Resistant Lines	4999	**	303.1	**	852.0	28.7	*	0.13	**	0.14	**
Susceptible Lines	5470		2957.4		841.3	139.9		1.71		2.05	
Bozeman 1999											
Resistant Lines	4365	**	-48.7	**	844.0	0.4	**	0.04	**	0.00	**
Susceptible Lines	4807		842.7		829.6	12.1		1.48		1.51	
Conrad 1999											
Resistant Lines	3939		341.6		858.8	-1.0		0.06	**	0.09	**
Susceptible Lines	4210		514.3		841.9	2.5		1.19		1.26	
Population 2											
Bozeman 1998											
Resistant Lines	6516		525.8	**	812.2	11.9	*	0.09	*	0.33	
Susceptible Lines	6677		2872.9		825.9	87.2		0.70		1.13	
Conrad 1998											
Resistant Lines	5681		631.9	**	854.1	37.1		0.06	**	0.40	**
Susceptible Lines	5744		3118.3		835.8	174.1		0.98		1.93	
Bozeman 1999											
Resistant Lines	4891		420.9		827.1	2.1	*	0.18	**	0.67	
Susceptible Lines	4754		993.8		817.1	23.3		1.57		1.67	
Conrad 1999											
Resistant Lines	4159		229.5		844.5	-1.4		0.16	*	0.40	
Susceptible Lines	3999		35.4		830.3	1.4		1.17		0.80	

***, **, means of resistant and susceptible groups within populations and environments significantly differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance.**

Table 6. (continued) Means of selected agronomic traits reported for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown at four environments in Montana from 1998 through 1999.

Source	Yield	Yield Reduction	Test Weight	Test Weight Reduction	ELISA readings	Disease Ratings
	kg ha^{-1}	kg ha^{-1}	kg m^{-3}	kg m^{-3}	A	0-3
Population 3						
Bozeman 1998						
Resistant Lines	6163	313.2 **	857.2	38.0	0.01 **	0.00 **
Susceptible Lines	6516	2497.2	852.3	95.4	0.56	1.15
Conrad 1998						
Resistant Lines	5164	-21.8 **	868.4	27.5	0.31 **	0.20 **
Susceptible Lines	5391	2989.3	860.9	186.3	0.88	2.36
Bozeman 1999						
Resistant Lines	4659	-137.8 **	861.7	2.1 **	0.17 **	0.07 **
Susceptible Lines	4920	1425.9	853.4	19.4	1.51	2.06
Conrad 1999						
Resistant Lines	3828 **	-7.8 **	874.2	2.1	0.06 **	0.13 **
Susceptible Lines	4488	908.8	867.7	2.5	0.71	1.52
Population 4						
Bozeman 1998						
Resistant Lines	5820	262.8 **	852.3 **	47.1 **	0.04 *	0.13 **
Susceptible Lines	5651	1303.7	829.4	77.3	0.36	0.71
Conrad 1998						
Resistant Lines	4878	880.0 **	862.9 *	38.8 *	0.27 **	1.13 **
Susceptible Lines	5122	2768.2	844.6	243.5	1.30	2.19
Bozeman 1999						
Resistant Lines	4096 *	-79.0 *	838.7	3.6 **	0.69 *	0.60
Susceptible Lines	4655	307.8	829.4	14.0	1.57	0.90
Conrad 1999						
Resistant Lines	3573	14.5 *	857.5	-1.1	0.50 *	0.40
Susceptible Lines	3539	287.7	806.8	-15.8	1.33	0.71

***, **, means of resistant and susceptible groups within populations and environments significantly differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance.**

Protein

There were no significant differences among resistant and susceptible groups for grain protein in any population or environment (Tables 7 and 8). The overall mean protein levels were the same for resistant, susceptible, and parental lines at 13.3% (Table 7).

Height

No significant differences were observed for height among resistant and susceptible groups in populations 1, 3, and 4 (Tables 7 and 8). Resistant lines of population 2 were significantly taller than susceptible lines for both years at Conrad.

Height Reduction

The overall height reduction of WSMV inoculated susceptible lines were significantly greater than the height reduction of resistant lines over the entire experiment by 2.8 cm (Table 7). All populations except population 4 showed a significantly larger height reduction in susceptible lines than in resistant lines (Table 7). Population 4 resistant lines showed height reductions that were nearly the same as susceptible lines in all environments (Table 8).

Heading Date

Resistant lines headed one to three days later than susceptible lines depending on the population and environment (Tables 7 and 8), a non-significant difference in most cases. The overall heading date difference of one day later for resistant lines was significantly later than susceptible lines (Table 7).

Physiological Maturity

Physiological maturity dates were not available from Conrad. At Bozeman, resistant and susceptible groups did not vary for date of maturity (Tables 7 and 8).

Table 7. Means and selected ranges of various agronomic traits for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown in Montana from 1998 through 1999.

Source	Protein		Height		Height Reduction		Heading date		Physiological Maturity Date
	mean	range	mean	range					
	%		cm				Julian Days		
Resistant Lines	13.3	11.3-14.7	89.9	69.3-102.9	2.5	**	182	*	223
Susceptible Lines	13.3	11.9-15.1	92.4	68.7-112.7	5.3		181		223
Parental Lines	13.3		95.1		4.8		183		225
Population 1									
McNeal	13.8		88		5.5		183		224
MT9328	13.1		89		6.6		184		224
Resistant Lines	13.4	12.4-14.2	89	77.7-102.0	2.3	**	182	*	224
Susceptible Lines	13.0	12.5-13.9	92	84.7-101.0	5.8		181		222
PCR class x E	ns		ns		ns		ns		ns
Population 2									
Amidon	13.3		102		6.7		183		226
McNeal	13.4		87		2.2		184		226
Resistant Lines	12.8	11.6-13.5	92	* 81.8-102.4	2.4	**	183		224
Susceptible Lines	13.3	13.0-13.6	88	81.1-88.5	5.9		181		224
PCR class x E	ns		*		ns		ns		ns
Population 3									
Amidon	13.3		102		3.6		183		225
MT9328	12.7		91		3.8		184		225
Resistant Lines	13.0	11.3-13.8	82	69.3-99.8	2.5	*	183		224
Susceptible Lines	12.9	12.1-13.9	89	68.7-97.1	5.1		182		224
PCR class x E	**		ns		ns		ns		ns
Population 4									
Amidon	13.1		104		6.2		183		224
MT9419	13.9		99		3.5		179		224
Resistant Lines	14.0	13.6-14.7	96	92.8-102.9	2.8		180		221
Susceptible Lines	13.8	11.9-15.1	101	76.3-112.7	4.3		179		222
PCR class x E	ns		ns		ns		ns		*

***, **, means of resistant and susceptible groups differ or the PCR class x environment interaction is significant at the 0.05 and 0.01 probability levels, respectively, by analysis of variance. 'ns' denotes nonsignificant PCR class x environment interaction.**

Table 8. Means of selected agronomic traits reported for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown at four environments in Montana from 1998 through 1999.

Source	Protein	Height		Height Reduction		Heading Date		Physiological Maturity
	%	cm				Julian Days		
Population 1								
Bozeman 1998								
Resistant Lines	13.7	89		3.2	**	185.0	*	225.0
Susceptible Lines	13.6	94		7.4		183.0		223.0
Conrad 1998								
Resistant Lines	12.6	91		2.2		178.0		n/a
Susceptible Lines	12.4	96		6.7		176.0		n/a
Bozeman 1999								
Resistant Lines	15.1	92		1.6	**	184.0		223.0
Susceptible Lines	14.8	94		6.5		182.0		221.0
Conrad 1999								
Resistant Lines	12.1	85		2.3		183.0		n/a
Susceptible Lines	11.3	87		2.7		181.0		n/a
Population 2								
Bozeman 1998								
Resistant Lines	13.8	94		4.5		185.0		226.0
Susceptible Lines	14.1	87		5.8		183.0		225.0
Conrad 1998								
Resistant Lines	11.4	93	*	0.0		178.0		n/a
Susceptible Lines	12.0	84		8.1		175.0		n/a
Bozeman 1999								
Resistant Lines	14.8	95		2.3		183.0		222.0
Susceptible Lines	15.5	89		7.7		181.0		222.0
Conrad 1999								
Resistant Lines	11.1	86	*	2.9		184.0		n/a
Susceptible Lines	11.5	80		1.8		182.0		n/a

***, **, means of resistant and susceptible groups within populations and environments significantly differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance. The abbreviation 'n/a' denotes data is missing for lines reported.**

Table 8 (cont.) Means of selected agronomic traits reported for WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from four spring wheat populations grown at four environments in Montana from 1998 through 1999.

Source	Protein	Height	Height Reduction		Heading date	Physiological Maturity
	%	cm			Julian Days	
Population 3						
Bozeman 1998						
Resistant Lines	13.6	84	4.1		185.0	226.0
Susceptible Lines	13.5	90	5.5		184.0	225.0
Conrad 1998						
Resistant Lines	11.9	82	2.0		178.0	n/a
Susceptible Lines	12.2	89	5.8		177.0	n/a
Bozeman 1999						
Resistant Lines	14.6	86	2.6		184.0	223.0
Susceptible Lines	14.7	89	7.5		182.0	223.0
Conrad 1999						
Resistant Lines	11.7	76	1.2		183.0	n/a
Susceptible Lines	11.1	81	1.5		183.0	n/a
Population 4						
Bozeman 1998						
Resistant Lines	14.9	96	4.3		183.0	223.0
Susceptible Lines	15.1	101	5.4		183.0	222.0
Conrad 1998						
Resistant Lines	13.3	96	4.1		176.0	n/a
Susceptible Lines	13.0	100	5.1		175.0	n/a
Bozeman 1999						
Resistant Lines	15.7	99	3.2		181.0	219.0
Susceptible Lines	15.4	104	5.0		179.0	221.0
Conrad 1999						
Resistant Lines	12.30	93	-0.3	**	181.0	n/a
Susceptible Lines	12.1	97	1.7		181.0	n/a

*, **, means of resistant and susceptible groups within populations and environments significantly differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance. The abbreviation 'n/a' denotes data is missing for lines reported.

Cereal Quality Evaluation

The results from milling and baking tests are reported in tables 9 and 10. Statistical analysis of cereal quality data indicated no significant differences between resistant and susceptible groups except for a lower bake absorption for resistant lines in population 3 (Table 10).

Table 9. Mean milling and flour quality parameters for 22 WSMV Resistant and 36 Susceptible lines from four spring wheat populations derived from KS93WGRC27 and Montana adapted parental lines grown in two Montana environments in 1998.

Source	Pedigree	Flour protein content	Flour Yield	Flour Ash	Mixograph Absorption	Mixograph Mixing time	Mixograph Tolerance
		%	%	%	%	min	1-8
Resistant Lines		11.9	68.2	0.39	59.6	2.9	4.6
Susceptible Lines		12.1	68.9	0.40	60.6	3.1	4.5
Parental Lines		12.3	68.6	0.42	61.2	3.0	5.0
Population 1 R	**McNeal/KS27//MT9328**	11.7	67.4	0.38	60.2	3.0	4.9
Population 1 S		11.8	68.6	0.39	60.7	3.1	4.9
McNeal		12.9	66.3	0.41	62.4	4.2	6.0
MT9328		12.0	68.8	0.39	61.0	3.5	4.5
Population 2 R	**Amidon/KS27//McNeal**	11.5	67.2	0.40	60.5	3.6	5.5
Population 2 S		12.1	68.3	0.40	61.3	3.8	5.5
Amidon		12.3	70.0	0.42	62.1	2.7	5.0
McNeal		11.8	66.9	0.41	60.9	3.5	6.5
Population 3 R	**Amidon/KS27//MT9328**	11.5	67.9	0.37	58.3	2.9	4.4
Population 3 S		11.9	69.0	0.40	59.8	3.2	4.6
Amidon		12.7	70.2	0.42	61.0	2.5	4.5
MT9328		12.0	69.2	0.41	60.1	2.5	5.0
Population 4 R	**Amidon/KS27//MT9419**	12.7	70.2	0.42	59.4	2.2	3.4
Population 4 S		12.7	69.9	0.42	60.6	2.3	2.8
Amidon		12.0	69.5	0.44	60.1	2.7	4.5
MT9419		13.0	68.0	0.44	62.1	2.6	4.0

***, **, means of resistant and susceptible groups differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance.**

Table 10. Mean baking quality parameters for 22 WSMV Resistant and 36 Susceptible lines from four spring wheat populations derived from KS93WGRC27 and Montana adapted parental lines grown in two Montana environments in 1998.

Source	Pedigree	Bake Absorption	Bake Mixing Time	Loaf Volume	Crumb Grain Score
		%	min	cc	0-5
Resistant Lines		69.8	4.6	1011	2.7
Susceptible Lines		70.5	5.1	1041	2.7
Parental Lines		71.2	5.0	1044	2.5
Population 1 R	**McNeal/KS27//MT9328**	71.6	5.1	1029	2.8
Population 1 S		71.0	5.3	1054	2.8
McNeal		73.2	8.1	1138	2.5
MT9328		71.2	5.8	1045	2.5
Population 2 R	**Amidon/KS27//McNeal**	70.9	5.8	1053	3.0
Population 2 S		72.0	7.0	1089	2.6
Amidon		72.2	4.0	1010	2.5
McNeal		71.2	6.6	1090	3.0
Population 3 R	**Amidon/KS27//MT9328**	68.5*	4.6	978	2.4
Population 3 S		70.1	5.1	1049	2.8
Amidon		70.2	3.1	988	2.5
MT9328		70.2	5.4	1070	2.5
Population 4 R	**Amidon/KS27//MT9419**	68.1	3.0	983	2.4
Population 4 S		69.0	3.0	973	2.5
Amidon		69.7	3.6	973	2.5
MT9419		71.7	3.2	1038	2.0

***, **, means of resistant and susceptible groups differ at the 0.05 and 0.01 probability levels, respectively, by analysis of variance.**

CHAPTER 5

DISCUSSION

The molecular marker for WSMV resistance, detected by the STSJ15 PCR primer, was generally accurate in determining the presence of the *Wsm1* gene. Tables 3 and 4 show the changes made in the initial identification of resistance or susceptibility to WSMV. Since F_5 progeny rows were harvested and used as the seed source for this experiment, one can predict due to heterogeneity, that 12.5% of the plants in resistant lines were in fact susceptible to WSMV. This heterogeneity may have led to plants that were not truly representative of a entry being screened by PCR and thus misclassified. But the possibility of this being true is quite low since leaf samples from three to five plants were screened via PCR before reclassification.

Even considering possible heterogeneity, there were some entries that continued to be PCR positive for WSMV resistance yet displayed visual WSMV symptoms in the field and viral replication (ELISA) that is associated with susceptibility. It is important to note that the reverse scenario was not seen. If so, this could suggest that a cross over had occurred between the alien chromosome segment on the short arm of chromosome 4D and a wheat chromosome.

Dr. Luther Talbert (personal communication, 1999) has indicated that similar results were seen in about 5% of progeny when developing the STS J15 primer. In the current study, three lines that were identified as resistant by PCR but showed

susceptibility symptoms were not reclassified as susceptible for this study. This resulted in yield, test weight reductions, and higher ELISA and visual disease ratings for resistant lines in two populations (populations 2 and 4) due to the effects of inoculation with WSMV. But in general, no adverse effects were seen in tests of significance for these populations. The possibility remains that errors were made in gel loading and resulted in PCR misclassifications .

Mean yields of resistant lines in the absence of WSMV were 5% lower than susceptible lines in two of four populations and when averaged over populations (Table 5). The reduction in yield of resistant lines relative to susceptible lines occurred in six of sixteen of the possible environment-population combinations. In no cases did the resistant groups out yield the susceptible groups (Table 6). These findings show that there is an detrimental effect, at least for some populations, that results in a yield depression for WSMV resistant lines containing the *Thinopyrum intermedium* chromosomal segment. Previous investigations with the 1AL.1RS and 1BL.1RS alien chromosomal translocations from rye reported enhanced yield potential in addition to disease resistance in some genetic backgrounds (Villareal et al., 1996; Espitia-Rangel et al., 1999). Though the populations examined for WSMV resistance did not show enhanced yields this does not exclude the possibility of individual resistant entries yielding more than parental or susceptible lines, as seen in the range of resistant entry non-inoculated yields (Table 6). These results show that on average the replacement of the short arm of chromosome 4D in wheat by the $4J^s$ chromosomal segment from *T. intermedium* results in yields that are less than populations that do not carry the *T. intermedium* translocation.

As discussed in the literature review, at Kansas State University two WSMV resistant lines, KS96HW10-1 and KS96HW10-3, are currently being evaluated for potential release. Results for overall yield performance in their Interstate Nurseries at all locations show that these two lines tend to fall in the lower quarter of the lines being tested (Table 2). At Barton County, KS in 1998 there was a severe WSMV outbreak in the yield nursery. The mean yield of the 38 lines without WSMV resistance was 2465 kg ha^{-1} (36.7 bu/A), while the two WSMV resistant lines had the highest yields, 4172 kg ha^{-1} (62.1 bu/A) and 4522 kg ha^{-1} (67.3 bu/A) respectively (KSU, 1998). Based on Kansas results, one would predict that it is possible to select individual WSMV resistant entries from backgrounds that would yield at acceptable levels, with and without WSMV infection, for release as cultivars. This conclusion is consistent with results of this study and with observations made from Montana spring wheat yield trial results (L.E. Talbert, 1999, personal communication).

Yield reductions due to WSMV inoculation were 5% and 32% for resistant and susceptible groups respectively (Table 6). This indicates that *Wsm1* is a highly effective source of WSMV resistance. The minimal reduction reported for resistant lines may be due to a small level of heterogeneity in some resistant lines being tested, or silencing of the *Wsm1* gene in certain resistant entries resulting in viral replication and yield loss, or from the direct effects of manual inoculation. Regardless, the yield performance of resistant lines exceeds that of susceptible and parental lines under inoculated conditions for all populations in all environments.

Resistant lines had significantly higher test weights than susceptible lines and higher than parental lines (Table 5). Test weights were most pronounced in two of the

four populations (Tables 5 and 6). The trend towards higher test weights in resistant lines also was reported in the Kansas Intrastate nurseries. In the 1998 western Kansas nurseries, excluding Barton county, KS96HW10-1 and KS96HW10-3 were in the top five for mean test weights, 62.5 and 62.3 lb bu^{-1} respectively (KSU, 1998). Higher test weights are commonly associated with general health of plants during the grain fill period and higher output potential (flour) of wheat. The resistance to WSMV conferred by the alien chromosome segment may provide additional unidentified disease resistance factors resulting in the higher test weights reported in this study.

The effectiveness of the resistance gene, *Wsm1*, to minimize test weight reductions due to WSMV infection was readily apparent in all populations except at Conrad in 1999 (Tables 5 and 6). The average test weight reduction for resistant lines was 17.3 kg m^{-3} (1.4 lb bu^{-1}). The average test weight reduction due to WSMV for susceptible lines was 73.6 kg m^{-3} (5.7 lb bu^{-1}) and 102.0 kg m^{-3} (8.0 lb bu^{-1}) for the parental lines (Table 5). Similar findings were reported by Kansas State University for KS96HW10-1 and KS96HW10-3 at the Barton county test site. The average test weight of the susceptible varieties was 50.6 lb bu^{-1} while the test weights for the two WSMV resistant entries were 59.8 and 60.3 lb bu^{-1} respectively. So, not only did the resistance conferred by the *T. intermedium* chromosome translocation protect against yield reduction, it also minimized test weight reduction.

Average whole grain protein levels of harvested resistant lines were the same as those of susceptible and parental lines (Table 7). Protein content of wheat with alien chromosome disease resistance genes has long been a short-coming in developing varieties for agricultural production (Wells et al., 1982). It appears here that the presence

of an alien chromosome segment in resistant lines had no significant effect on protein levels, as seen in the average and range of resistant entries (Table 7).

Visual symptoms or disease ratings are not always accurate in accessing the level of WSMV replication. However, in this experiment patterns of symptom expression and ELISA were consistent, indicating a large decrease in visual WSMV symptoms and ELISA titer in resistant lines relative to susceptible lines. ELISA and WSMV symptomology were also consistent with data from inoculated treatments showing minimal yield and test weight reductions in resistant germplasm. The *Wsm1* resistance gene effectively limits viral replication and minimizes yield loss to WSMV.

Montana Agricultural Statistics (1999) lists McNeal as being moderately resistant to WSMV. This classification appears to be misleading in light of the findings of this study. Yield reductions for McNeal due to WSMV inoculation were 1623 kg ha^{-1} (24 bu $acre^{-1}$) and 1232 kg ha^{-1} (18 bu $acre^{-1}$) in populations 1 and 2 respectively (Table 6). The 28% yield reduction average for McNeal would suggest susceptibility not moderate resistance. Yield reduction due to WSMV inoculation for Amidon was similar, averaging 32% (Table 5). These reductions for Amidon and McNeal cannot be overlooked since these two varieties account for 14% and 40% of the total spring wheat acreage in Montana respectively (Montana Agricultural Statistic Service, 1999).

A report by Seifers and Martin (1988) investigated a low level of resistance to WSMV found in the wheat cultivar Triumph 64. They reported yield reductions of 18% and low virus titer for Triumph 64. The yield reductions of McNeal or Triumph 64, when inoculated with WSMV, do not compare to the minimal levels of yield reduction or virus titer seen for resistant lines from the KS93WGRC27 backgrounds in this study.

Misclassification of McNeal for WSMV resistance may give farmers a false sense of security resulting in an increase in the available pool of WSMV and potentially providing a green bridge for mite transmission of WSMV to spring and winter wheat.

It is important to note that symptom expression and agronomic reductions for field trials at both Bozeman and Conrad in 1999 were far less than what were reported for 1998 (Table 6). However, ELISA data shows that in fact virus was present and replicating in all field experiments. Decreased rainfall and higher mean temperatures at both locations in 1999 may have resulted in environmental conditions less conducive for severe symptom expression and yield loss. Similar levels of virus titer were present each year but differential symptom expression and response was seen at the whole plant level. This inconsistency and unpredictability of severity of WSMV infection from year to year and environment to environment strengthens the case for yield competitive WSMV resistant varieties which do not exhibit a yield penalty in the absence of disease.

A decreased height reduction for inoculated resistant lines reported here add to the previously mentioned results that support the resistance conferred by the *Wsm1* gene. The minimal height reduction that was seen in resistant inoculated lines may be simply the result of stress due to the abrasion of young leaf tissue when inoculum was introduced. The *T. intermedium* translocation was also found to have a minimal effect on heading date and physiological maturity.

McNeal and Amidon are the most widely planted spring wheat varieties in Montana. The reason for this is their consistent agronomic and end-use quality performance. Utilizing these varieties in breeding programs increases the likelihood that the resulting progeny will perform in a similar manner. These parental lines also serve as

an excellent measure of end-use quality for comparison of resistant and susceptible lines. The most telling indicators of end use quality in wheat are flour protein content, mixograph mixing time, mixograph mixing tolerance, water absorption, loaf volume, and crumb grain score (Finney et al., 1987).

Mean flour protein levels and flour yields in populations 1, 2 and 3, for resistant lines, tended to be lower than susceptible lines and their parents, but not significantly so. Population 4 resistant lines had the highest mean protein levels seen for resistant lines (12.7%) and flour yield (70.2%). Since whole grain protein levels are highly correlated to flour protein, whole grain protein analysis would detect resistant entries at the high end of the range reported in table 7 and provide an easy method of selecting resistant entries that are acceptable for industry needs.

Mixograph mixing time reported for the WSMV resistant predecessor CI17884 in 1982 was 3 ¾ minutes, the same time as the recurrent parent Centurk. A mixing time of 3 ¾ minutes is considered a medium-long time and is acceptable mixing time (Wells et al., 1982). Since CI17884 was advanced due to its end-use potential it comes as no surprise that the resistant lines in this experiment also display mixing times that are at accepted industry duration (Table 9).

The values reported in table 9 for mixograph tolerance show lower tolerance for resistant lines than susceptible or parental lines. The resistant group mixograph tolerances were not found to be significantly different from susceptible groups. The alien chromosome translocation was also not found to be detrimental for bake absorption, loaf volume, and crumb grain scores (Table 10). Replications from 1999 harvested populations will be evaluated in 2000. These results will help to better determine if there

is an end-use quality penalty associated with WSMV resistance from *Thinopyrum intermedium*.

Previous studies evaluating chromosomal translocations between wheat and rye have shown have associated the translocation with increased grain yield and decreased end-use quality in wheat (Espititia-Rangel et al., 1999). However, studies evaluating the 1AL.1RS and 1BL.1RS translocation from rye have shown that many of these detrimental effects on end-use quality can be decreased by rigorous selection of the background genotype (Carver and Rayburn 1995; Lee et al., 1995). Yield depression was shown to be associated with WSMV resistance in this study, but such reductions should be overcome by selection as suggested by the ranges in resistant entry yields. The increase in test weights, lack of viral replication, and acceptable end-use quality, have been reported in this study for resistant lines. The resistance to WSMV present in KS93WGRC27 associated with the *Thinopyrum intermedium* translocation has shown that it is possible to have alien gene expression without a major penalty in agronomic and end-use quality. The use of KS93WGRC27 in winter and spring wheat breeding programs for wheat streak mosaic virus resistance should be further pursued in variety development which should result in reliable and productive WSMV resistant cultivars.

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APPENDIX

Appendix. Mean agronomic traits reported for individual WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from 4 spring wheat populations grown at four environments in Montana from 1998 to 1999.

Entry	Pedigree	PCR	Yield	Yield Reduction	Test Weight	Test Weight Reduction	Protein	ELISA	Disease rating	Height	Height Reduction	Heading Date	Physiological Maturity Date
			kg ha^{-1}	kg ha^{-1}	kg m^{-3}	kg m^{-3}	%	A	0-3	cm	cm	Julian Days	Julian Days
4138	MCNEAL/KS27//MT9328	S	5325	2039.3	850	11.6	12.7	1.73	1.50	84.7	7.2	184	224
4141	MCNEAL/KS27//MT9328	S	5238	1175.2	857	21.8	13.0	1.26	1.00	95.0	3.4	184	225
4142	MCNEAL/KS27//MT9328	R	4577	154.3	852	18.1	13.9	0.08	0.17	97.2	1.4	186	228
4146	MCNEAL/KS27//MT9328	S	5225	1681.8	847	17.6	13.2	1.60	1.42	90.7	7.9	184	225
4156	MCNEAL/KS27//MT9328	S	5291	1380	850	16.3	12.4	1.42	1.25	87.6	4.0	180	223
4161	MCNEAL/KS27//MT9328	R	4633	558.5	834	14.2	14.2	0.14	0.00	86.6	1.8	180	221
4165	MCNEAL/KS27//MT9328	R	4458	354.2	870	20.3	13.8	0.12	0.17	79.7	2.8	184	226
4168	MCNEAL/KS27//MT9328	S	5054	2012.2	823	87.4	13.8	1.55	2.25	94.6	9.2	182	225
4170	MCNEAL/KS27//MT9328	S	5689	1369.6	843	27.5	12.8	1.34	0.92	91.3	5.6	181	223
4181	MCNEAL/KS27//MT9328	S	5383	2621.2	835	81.5	12.6	1.07	2.25	100.9	5.8	180	220
4182	MCNEAL/KS27//MT9328	R	4898	86.6	847	38.1	13.1	0.07	0.08	10.0	2.9	184	226
4186	MCNEAL/KS27//MT9328	S	5039	1792.8	838	48.2	12.5	1.25	1.92	95.1	4.4	180	220
4189	MCNEAL/KS27//MT9328	S	4974	1433.4	854	26	13.6	1.60	0.75	98.0	4.5	180	219
4191	MCNEAL/KS27//MT9328	S	5084	1252.3	852	112.1	12.8	1.42	1.00	87.4	2.9	180	219
4195	MCNEAL/KS27//MT9328	R	4454	35.2	823	25.3	12.9	0.09	0.08	86.3	2.6	183	222
4196	MCNEAL/KS27//MT9328	S	4950	1921.3	856	77.5	13.5	1.51	2.00	87.4	4.9	183	222
4197	MCNEAL/KS27//MT9328	S	4831	1131.7	852	79.4	13.2	1.23	1.17	91.7	3.9	181	223
4199	MCNEAL/KS27//MT9328	S	4898	1998.8	823	74.3	12.6	1.10	2.00	100.1	3.4	184	221
4211	MCNEAL/KS27//MT9328	R	5093	295.5	831	114.1	12.4	0.27	0.08	77.7	1.9	184	225
4214	MCNEAL/KS27//MT9328	R	4825	193.2	818	63.8	13.2	0.14	0.08	96.1	2.9	183	223
4228	AMIDON/KS27//MCNEAL	S	5577	1023.7	820	13.3	13.0	1.29	0.92	88.5	3.6	182	224
4238	AMIDON/KS27//MCNEAL	S	4667	1364.2	842	25.3	13.6	1.50	1.33	86.1	5.4	178	221
4241	AMIDON/KS27//MCNEAL	S	5495	2035.1	822	-15.8	13.5	1.46	0.92	83.5	4.3	181	222
4245	AMIDON/KS27//MCNEAL	S	5113	1132.2	835	18.3	13.0	0.93	1.25	81.1	3.3	181	225
4248	AMIDON/KS27//MCNEAL	R	5455	645.9	854	21	11.6	0.47	0.92	93.0	4.6	186	228
4252	AMIDON/KS27//MCNEAL	S	5284	2955.4	838	26.6	13.3	1.17	2.50	85.0	40.4	184	226
4259	AMIDON/KS27//MCNEAL	R	5269	1368.4	832	45.3	13.1	0.29	1.08	94.2	3.4	180	220

Appendix. (cont.) Mean agronomic traits reported for individual WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from 4 spring wheat populations grown at four environments in Montana from 1998 to 1999.

Entry	Pedigree	PCR	Yield	Yield Reduction	Test Weight	Test Weight Reduction	Protein	ELISA	Disease rating	Height	Height Reduction	Heading Date	Physiological Maturity Date
			kg ha^{-1}	kg ha^{-1}	kg m^{-3}	kg m^{-3}	%	A	0-3	cm	cm	Julian Days	Julian Days
4262	AMIDON/KS27//MCNEAL	R	5205	179.9	823.3	125.6	12.8	0.09	0.08	90.4	3.8	184	225
4266	AMIDON/KS27//MCNEAL	R	5523	-3.4	844.3	24.4	12.7	0.16	0.08	81.8	2.5	185	223
4274	AMIDON/KS27//MCNEAL	R	5108	69.3	798.3	139.0	13.5	0.16	0.08	102.4	3.3	183	224
4282	AMIDON/KS27//MT9328	S	5752	2584.3	870	17.1	12.1	1.56	2.25	92.6	8.4	184	225
4288	AMIDON/KS27//MT9328	R	4682	-499.7	862.5	20.2	12.8	0.10	0.33	69.3	1.9	182	223
4289	AMIDON/KS27//MT9328	S	4937	2373.3	840.9	12.58	13.7	1.27	2.17	92.7	9.4	182	225
4292	AMIDON/KS27//MT9328	R	4749	123.4	869.8	18.7	13.6	0.22	0.00	86.8	3.7	184	225
4293	AMIDON/KS27//MT9328	S	5220	1913.7	883.1	19.8	13.2	0.92	1.75	73.2	5.2	182	225
4295	AMIDON/KS27//MT9328	R	5308	-208.5	844.1	76.9	11.3	0.11	0.00	72.5	1.9	186	227
4298	AMIDON/KS27//MT9328	S	5428	2632.5	856.2	115.7	13.1	1.07	2.17	94.8	5.3	181	225
4303	AMIDON/KS27//MT9328	S	5300	2352.1	849.2	38.2	13.2	1.29	2.17	95.5	5.9	181	224
4312	AMIDON/KS27//MT9328	S	5235	1931.5	864	125.6	13.0	1.24	1.83	88.8	3.6	180	219
4316	AMIDON/KS27//MT9328	S	5984	2651.9	873.2	115.7	12.1	1.37	2.00	97.0	7.8	185	225
4323	AMIDON/KS27//MT9328	S	5280	2323.2	867.8	122.8	12.6	0.85	2.25	68.7	0.6	181	221
4330	AMIDON/KS27//MT9328	S	4819	924.8	867.2	76.5	13.9	1.35	1.25	94.2	2.8	182	226
4335	AMIDON/KS27//MT9328	S	5599	945.8	854.8	82.3	12.5	1.27	0.58	87.6	2.1	183	224
4336	AMIDON/KS27//MT9328	R	4677	141.2	865.3	23.6	13.8	0.14	0.08	99.8	3.0	182	221
4338	AMIDON/KS27//MT9328	R	5034	190	872	25.3	13.3	0.20	0.08	82.2	2.4	184	226
4343	AMIDON/KS27//MT9328	S	4687	398.5	829.9	34.7	12.3	1.24	1.08	73.9	3.2	185	225
4348	AMIDON/KS27//MT9419	R	4398	311.2	860.5	17.0	14.7	0.13	0.17	95.5	2.2	182	223
4359	AMIDON/KS27//MT9419	R	4103	1287.6	842.6	23.7	15.0	1.58	1.42	103.7	5.5	178	222
4364	AMIDON/KS27//MT9419	S	4646	977.5	857.9	22.6	14.6	1.01	0.92	105.0	3.6	179	220
4366	AMIDON/KS27//MT9419	S	4884	489.4	844.1	26.5	13.7	0.97	0.75	102.9	4.6	183	222
4372	AMIDON/KS27//MT9419	S	4227	-125.1	859.2	20.6	14.1	0.14	0.50	92.6	1.0	181	219
4378	AMIDON/KS27//MT9419	S	4673	623.6	824.4	480.2	14.0	1.32	1.08	92.7	2.4	181	220
4379	AMIDON/KS27//MT9419	R	4273	985.9	843.4	38.5	15.1	1.41	0.92	112.7	3.9	179	221
4387	AMIDON/KS27//MT9419	S	4987	1342.5	831.2	108.4	13.2	1.37	0.92	102.3	4.7	181	220

Appendix. Mean agronomic traits reported for individual WSMV resistant and susceptible $F_{6 \text{ and } 7}$ lines from 4 spring wheat populations grown at four environments in Montana from 1998 to 1999.

Entry	Pedigree	PCR	Yield	Yield Reduction	Test Weight	Test Weight Reduction	Protein	ELISA	Disease rating	Height	Height Reduction	Heading Date	Physiological Maturity Date
			kg ha^{-1}	kg ha^{-1}	kg m^{-3}	kg m^{-3}	%	A	0-3	cm	cm	Julian Days	Julian Days
4389	AMIDON/KS27//MT9419	R	5202	1232.5	842.5	77.8	13.2	1.29	1.00	97.7	4.3	180	221
4392	AMIDON/KS27//MT9419	S	4325	795.6	850.3	79.7	14.3	1.43	1.08	105.9	4.8	180	224
4396	AMIDON/KS27//MT9419	R	4777	48.27	785.4	39.3	13.5	0.26	0.30	95.9	2.6	181	221
4398	AMIDON/KS27//MT9419	R	5656	1546.2	815.6	34.1	11.9	1.58	1.67	76.2	2.3	185	224
Amidon	Amidon	S	5374	1745.8	837.1	127.5	13.2	1.61	1.08	102.6	5.5	183	225
McNeal	McNeal	S	5168	1427.95	829.9	98.7	13.5	1.51	0.80	87.3	3.9	184	225
MT9328	MT9328	S	5126	1613.55	821.9	78.4	12.9	1.41	1.29	89.8	5.3	184	224
MT9419	MT9419	S	4629	1310.6	803.5	79.7	13.9	1.46	1.50	98.5	3.6	179	224