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
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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.

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
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APPROVAL

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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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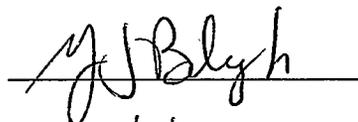
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ABSTRACT

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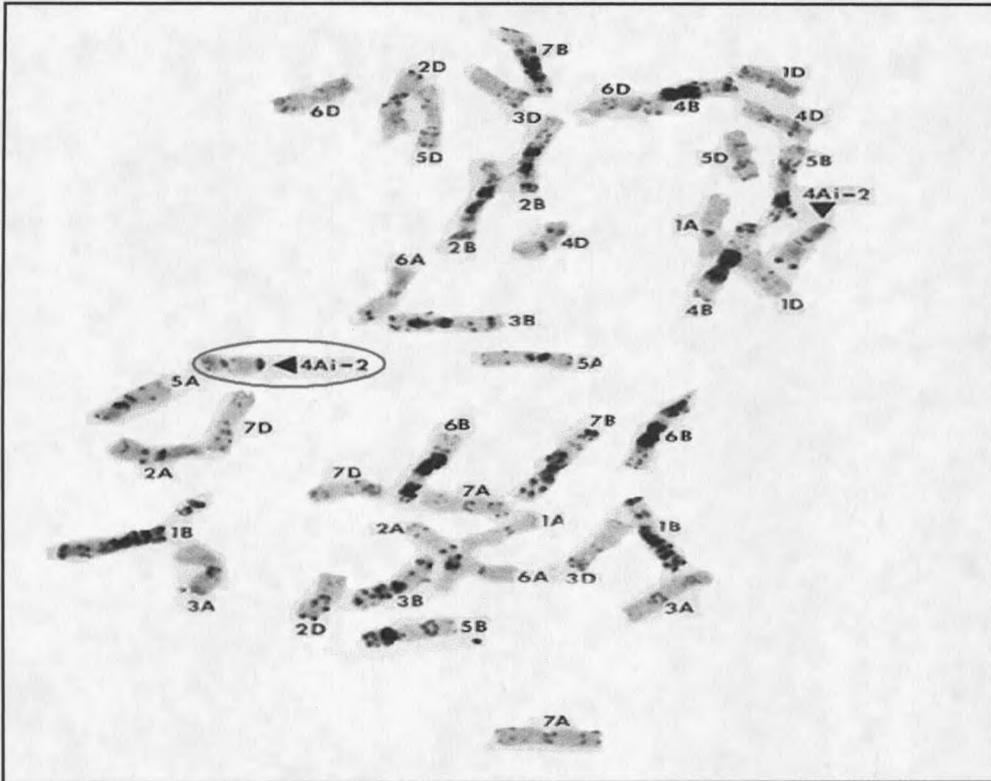
Figure 1. C-banded CI15092 (2n=42), 4 *Ai*-2 (4A) substitution line
(Friebe et al., 1991)

4Ai–2 4DL·4Ai-2S 7S
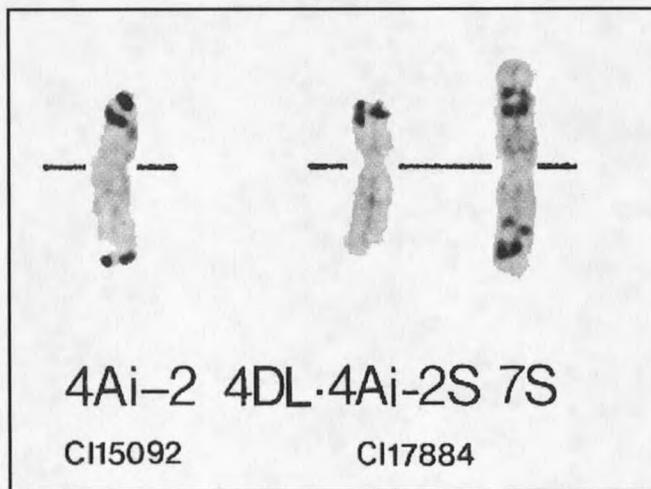
CI15092   CI17884

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.
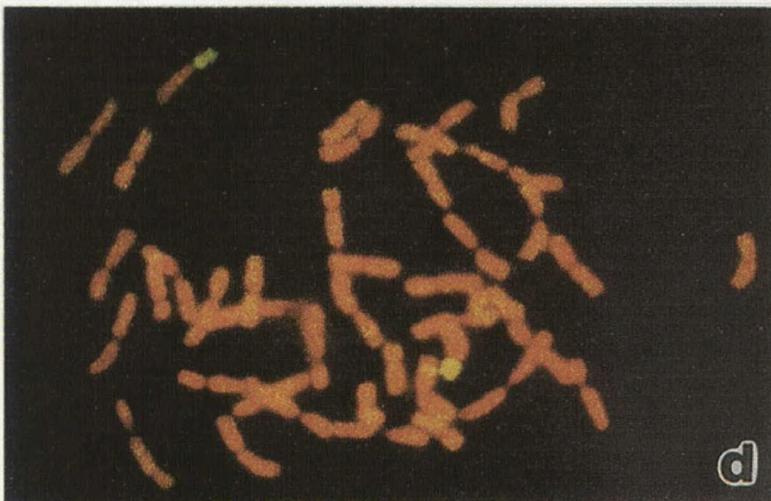
(Chen et al., 1998)

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