# Inheritance of tolerance to wheat streak mosaic virus in an advanced spring wheat (*Triticum aestivum* L) line BW155, and combining BW155-derived tolerance and Wsm1 resistance.

by

**MANIKA PAKHRIN PRADHAN**

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**Inheritance of tolerance to wheat streak mosaic virus in an advanced spring wheat (*Triticum aestivum* L) line BW155, and combining BW155-derived tolerance and Wsm1 resistance**

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

## ABSTRACT

Wheat streak mosaic (WSM) caused by WSM virus (WSMV) is a serious disease of wheat which cannot be easily controlled using cultural practices or chemicals. Tolerance to WSMV exists in hexaploid spring wheat *(Triticum aestivum* L) germplasm. BW155 is an advanced spring bread wheat line with tolerance to WSMV. Canada Western Red Spring (CWRS) wheat cultivar, AC Elsa is moderately susceptible, while AC Barrie and Laura are highly susceptible to WSMV infection. Wsm1, a gene for resistance to WSMV has been transferred into winter wheat from *Agropyron intermedium.* The line 7166 is resistant to WSMV and carries the Wsm1 resistance gene. Both resistance and tolerance to WSMV contribute to the host's good performance. The overall objectives of this thesis were: (i) to determine the mode of inheritance of WSMV tolerance in BW155, and (ii) to combine BW155-derived tolerance and Wsm1 resistance derived from 7166. To study the genetic control of BW155-derived tolerance, crosses were made among plants from tolerant BW155 and susceptible Laura, followed by screening the $F_2$ and $F_2$ -derived $F_3$ families for segregation. The inheritance of BW155-derived tolerance was also determined by screening two $F_1$-derived doubled haploid (DH) populations generated from A18-4113 (BW155 descent)/AC Barrie and AC Elsa/A18-4113. Tolerance to WSMV in BW155 is controlled by three dominant genes with additive effects. To combine tolerance and resistance, 101 $F_1$-derived doubled haploid lines were generated from

reciprocal crosses of BW155/7166 and screened. A sequence-characterized-amplified region (SCAR) molecular marker (J15), linked to Wsm1, was used to identify the lines which carried Wsm1 gene. Wsm1-conferred resistance in 7166 is temperature sensitive and BW155-derived tolerance is stable over a range of temperatures. An elevated temperature treatment was applied to identify lines with both Wsm1 and temperature insensitivity. Lines which performed well at a higher temperature and contained the J15 SCAR marker were considered to possess both the tolerance and the resistance. Twenty five out of 101 DH lines, carried the J15 SCAR marker, and 12 out of those 25 lines with the marker performed well under disease pressure, at 27°C. The 12 lines which combine Wsm1 resistance with BW155-derived tolerance may form the basis of adapted, elite spring wheat germplasm that performs well under WSM pressure.

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## FOREWORD

This thesis is written in manuscript style. It begins with a general abstract, introduction, and literature review, followed by the presentation of two chapters of experimental research, each representing a particular research theme. The format of each paper is as follows: abstract, introduction, materials and methods, results and discussion. At the end of the thesis there is a general discussion including conclusions and ideas for future research, followed by a list of references cited throughout the thesis. The thesis is written to conform with the requirements of the Canadian Journal of Plant Pathology.

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## 1. Introduction

Wheat streak mosaic (WSM) is a systemic disease of wheat (*Triticum aestivum* L.) caused by wheat streak mosaic virus (WSMV) (Mckinney, 1949; Slykhuis, 1953) and vectored by the wheat curl mite (WCM), *Aceria tosichella* Keifer, (= *Aceria tulipae* Keifer) (Slykhuis, 1955; Wood et al, 1995). Wheat streak mosaic virus is a significant virus disease in parts of the prairie region of Canada, the Great Plain regions of the United States, Eastern Europe and Southwest Asia (Slykhuis and Bell, 1963; Wiese, 1987; Christian and Willis, 1993; Martens et al, 1994; Seifers et al, 1995; Haber et al., 1997).

Wheat streak mosaic reduces yield by producing chlorotic foliar lesions which proceed to necrosis of the tissue. It may also stunt the diseased plants, depending on the crop stage at the time of infection, and reduces tillering. Seed yield, plant height, test weight, 1000 seed weight and seeds per head can be reduced by WSM, depending on the severity of the disease. Yield losses due to WSM can range from trace to 100% (Haber & Townley-Smith, 1993; McMullen, 1995; Seifer et al, 1995). At times losses are so severe that affected fields are completely destroyed. Outbreaks of WSM resulted in yield loss of about 19000 tons in winter wheat in Alberta in 1964 (Atkinson and Grant, 1967) and an annual yield loss of about 421000 tons in Kansas, USA, between 1987-91 (Harvey et al., 1994). Haber and Townley-Smith (1993) reported that, in 1989, at Indian Head, Saskatchewan, where seed is increased for Agriculture and Agri-Food

Canada, yield losses due to WSM for some of the popular Canada Western Red Spring (CWRS) wheat cultivars were as high as 100%. Significant losses to the winter wheat crop as a result of WSMV infection in the Central Great Plains of the United States are reported annually (Wiese, 1987; McMullen, 1995).

Wheat streak mosaic virus and the mite vector survive through the winter on dormant wheat plants. The mites overwinter as eggs, nymphs or adults, and become active in the spring and spread the disease in a winter wheat growing region (Slykhuis, 1955; Somsen and Sill, 1970). However, the spread of the disease in a predominantly spring wheat region can be mostly attributed to increased winter wheat acreage in and around a spring wheat growing area (Conner et al, 1991; McMullen, 1995). The disease cycle is primarily maintained by volunteer winter wheat which provides a continuous green-bridge for survival of virus and the mite vector (Wiese, 1987; McMullen, 1995).

In the past, outbreaks of WSM have directly related to factors favoring a build up of mite population (Slykhuis, 1955; Somsen and Sill, 1970). The mite persists on living host material and cannot survive on ripened plants (Wiese, 1987). For this reason, outbreaks of WSM have been mainly limited by delaying the seeding of winter wheat until spring wheat in adjacent fields has been harvested and volunteer wheat destroyed (Conner et al., 1991; Hein and Mahmood,1999). Control based on time of fall seeding to prevent spread of viruliferous mites may not always be effective due to changes in environmental conditions (Atkison and Grant, 1967; Martin et al., 1984).

The importance of WSM as a disease of winter wheat has been well established, but the significance of WSM in spring wheat has attracted attention among wheat scientists only in recent years (Edwards and McMullen, 1988; Haber and Townley-Smith,1993). Introduction of mite resistant winter wheat cultivars, availability of better adapted winter wheat cultivars and effort to avoid other pests and diseases have increased winter wheat production in the United States and Canada (Harvey and Martin, 1988; Conner et al, 1991; Harvey et al., 1994; R.H. Elliot and L.W. Mann, 1996). There are currently no commercial spring wheat cultivars highly resistant to WSMV but a range of improved performance under WSM pressure has been detected in several spring wheat cultivars and advanced lines (Rahman et al., 1974; Timian and Lamey, 1985; Haber & Townley-Smith, 1993; McMullen, 1995; Seifers et al., 1995). Evaluation of the degree of resistance and tolerance in existing cultivars would be useful to plant breeders to provide potential sources of resistance and tolerance.

Host plant resistance is a highly desirable control strategy when dealing with WSM virus disease. Much effort has been applied to improve winter wheat by incorporating resistance to the mite vector, *Aceria tosichella* Keifer (Slykhuis, 1955; Somsen and Sill 1970; Martin et al 1976; Martin et al 1984; Seifers, 1984; Conner et al., 1991; Harvey et al., 1994) and to WSMV (Wang et al., 1980; Seifers and Martin, 1988; Friebe et al., 1991; Seifers et al., 1995). Considerable work has been done to develop WSMV resistant germplasm from crosses

between wheat and its wild relatives, mainly *Agropyron intermedium* (=*Thinopyrum intermdium*) and *A. elongatum* ( Lay et al, 1971; Sebesta et al, 1972; Wells et al, 1973; Liang et al, 1979). A resistance gene is located on the short arm of the *A. intermedium* chromosome (Wang and Liang 1977) designated as 4Ai-2 and the resistance gene is designated as Wsm1 (Friebe et, al., 1991) . However, the germplasm released initially (Liang et al, 1979) possesses some undesirable agronomic traits which have hindered development of new WSMV resistant cultivars(Seifers and Martins, 1988). However, the advent of biotechnological tools such as polymerase chain reaction and molecular markers have made it possible to bring improvement in agronomic and quality traits by eliminating undesirable traits (Seifers et al., 1995; Chen et al., 1998).

KS95H103 (KS91H184/KS89H20// TAM107), an advanced winter wheat line with improved agronomic and bread-making characteristics, has the short arm of chromosome 4Ai-2 from *A. intermedium* translocated onto the long arm of wheat chromosome 4D (T4DL.4Ai-2S) (Seifers et al., 1995). However, when the translocation is present, the resistance is not effective at temperatures above 25°C (Seifers et al., 1995; Haber, unpublished).

Although the wheat streak mosaic disease has been present in Canada since the early 1950s (Slykhuis, 1953; Atkinson and Grant, 1967), WSM became a greater concern in southwestern Manitoba and southeastern Saskatchewan

only after 1989, when there were massive losses of wheat due to what was determined to be WSM in an increase station in Indian Head, Saskatchewan, and in spring wheat fields growing in the vicinity of winter wheat (Haber and Townley-Smith, 1993). In spring wheat germplasm, an advanced wheat breeding line, BW155 (= ND640; 'Butte x Columbus') was found to possess tolerance to WSMV (Haber and Townley-Smith, 1995).

Information about the mode of inheritance of BW155-derived tolerance to WSMV can be useful in development of new tolerant lines. Both resistance and tolerance to WSMV can contribute to a host's good performance. Therefore, combining tolerance and resistance and understanding their combining ability, would facilitate the control of WSM. Few studies have been performed to examine the performance of spring wheat lines that possess WSMV tolerance under disease pressure. The following objectives were addressed in this study:

1. To determine the mode of inheritance to tolerance to wheat streak mosaic virus of the advanced spring bread wheat line BW155.
2. To combine resistance derived from KS95H103 (winter wheat line incorporating Wsm1 gene) and tolerance derived from BW155 to develop WSMV resistant germplasm that performs well under disease pressure.

# 2. Literature Review

## 2.1. Introduction to wheat streak mosaic disease of wheat.

Little is known about the origin of viruses. Gibbs and Harrison (1980) characterize the striped (infected) tulip flower painting, by Ambrousius Bosschaert (1619), 'as the most beautiful illustration of a virus infection'. For a considerable period of time, important diseases such as tobacco mosaic virus (TMV) were believed to be caused by bacteria. It was not until 1898 when Martinus W. Beijerinck described the pathogen, as *contagium vivum fluidum* because of the ultra-filterable nature (could pass through a porcelain filter), which was different from a bacterium, that the pathogen was recognized as a virus and not a bacterium.

Mosaic diseases of wheat (*Triticum aestivum* L) have been reported from various parts of the world for many years (Slykhuis and Bell, 1963; Wiese, 1987). In the early 1900s wheat mosaic diseases were observed in different parts of the United States of America. In the United States WSM was first reported from the fields of Kansas in 1932 (McKinney, 1937). By 1936 the mosaic disease was reported from most parts of the Great Plains regions of the United States (McKinney, 1937; Fellows, 1949). Since 1976, WSM is estimated to have caused an average wheat yield reduction of 2.6% per year in Kansas (Christian and Willis, 1993; Seifers et al., 1995; McNeil et.al,1996). In 1988, however, an epidemic of WSM resulted in an estimated yield loss of 13% in Kansas. This was

the largest yearly loss since 1959 and the second highest in the last 40 years (Christian and Willis, 1993).

Closer to Canada, WSM was reported from South Dakota in 1949, and serious winter wheat crop losses occurred in some counties in South Dakota in 1950 and 1951 (Slykhuis, 1952). In Canada, WSM was first reported in 1952, from fields in southern Alberta, where winter wheat crops have been grown regularly (Slykhuis, 1953). In 1964, yield losses based on an extensive survey in the winter wheat area of southern Alberta totalled more than 19000 tons or 18 % of the potential yield (Atkinson and Grant, 1967). Since then, low levels and localized yield losses from trace to 100% has been experienced yearly, especially in southwestern Manitoba, southeastern Saskatchewan and southern Alberta (Atkinson and Grant, 1967; Conner et al., 1991; Haber et al.,1997).

### 2.1.1. The causal agent

The disease wheat streak mosaic on wheat (*Triticum aestivum* L) is caused by wheat streak mosaic virus (Slykhuis, 1955). The acronym approved by the International Committee for Taxonomy of Viruses (ICTV) for this virus is WSMV. The virus has been characterized at the molecular level with ICTV Virus Code 57.0.2.0.006, and virus accession number 57020006. WSMV belongs to the genus *Rymovirus* (mite-borne) (VC 57.0.2.), family *Potyviridae* (rod- shaped) (VC 57.) (Niblett et al., 1991; Zagula et al., 1992). A single particle is called a virion. Morphologically, a virion consists of filamentous nucleocapsids with a

clear modal length of 700nm (Brakke, 1971, Niblett et al., 1991). One component in purified preparation has a sedimentation coefficient of 165 S with a buoyant density in CsCl of 1.37 g/cm$^3$ (Zagula et al., 1992); virions contain one molecule of single stranded RNA and the total genome size is 8.5 kb (Brakke and Ball, 1968). One structural virion coat protein has a size of 47000 Da (Brakke et al.,1990; Niblet et al., 1991). Virions are found in the cytoplasm of the infected cells with cylindrical (pinwheel) inclusions (CI) which are similar to other aphid transmitted potyviruses (Lagenberg, 1986 and 1989). The CI induced by WSMV is made up of a protein with size 66kDa (Brakke et al., 1987).

Stenger et al., (1998) conducted phylogenetic analyses with complete polyprotein sequences of 11 members of the family *Potyviridae*, including viruses of monocots or dicots and viruses transmitted by aphids, whiteflies, and mites. Their results have suggested that WSMV and BrSMV (brome streak mosaic virus) should be classified within a new genera of the family *Potyviridae* and should be separated from species of the genus *Rymovirus*.

### 2.1.2. The vector

Wheat streak mosaic virus is transmitted primarily by a very tiny eriophid mite, *Aceria tosichella* Keifer (= *Aceria tulipae* Keifer) (Slykhuis, 1955; Tosic, 1973; Armine and Stasny, 1994), in the family *Eriophydae*. This mite is also known as wheat curl mite (WCM). Evidence of an eriophid mite on wheat exhibiting its ability to transmit WSMV was described first by Slykhuis (1953). Its

economic importance is based on the ability to transmit wheat streak mosaic virus and High Plains Virus in the Great Plains and Pacific Northwest (Seifers et al., 1997; Mahmood et al.,1998).

The wheat curl mite is small, white and cigar-shaped with two pairs of legs near the head that can be seen under 10x magnification. It has no wings and is so tiny (0.25 mm) that it can be blown from plant to plant and field to field by the wind. The life cycle of the mite, from egg to an adult, is completed in eight to ten days (Wiese, 1987). Their numbers can increase very quickly under hot and dry weather. The mites reproduce most rapidly at temperatures from 24 to 29°C (McMullen, 1995). Slykhuis (1955), studied the mites and found that all stages except the eggs carried WSMV. Nymphs could acquire the virus and the older mites could not. The mite remained virulifereous for at least six days.

Independent of virus symptoms, plants infested by wheat curl mite exhibit leaf rolling and leaf trapping symptoms, caused entirely by the activity of the mites alone (Larson and Atkinson, 1973). However, the damage caused by WCM is not normally considered important because mite populations heavy enough to cause serious plant damage usually occur on plants that have serious virus infections, which generally limits the yield potential anyway.

### 2.1.3. Host Range

The information on host range of an economically important pathogen is of considerable significance since alternate hosts often serve as reservoirs. A high incidence of WSM is usually associated with the presence of volunteer wheat, which serves as a reservoir for both WSMV and WCM (Slykhuis, 1953). The major importance of the host lies in its ability to produce a large quantity of virus (McKinney 1947).

The wheat curl mite is widely distributed throughout North America and has a broad host range that includes most cereals, and annual and perennial grasses. Several scientists have identified plant hosts such as corn (*Zea mayes*), barley (*Hordium vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), and grasses such as foxtail millet (*Setaria italica*), giant foxtail (*Setaria faberi* Herm), cheat grass (*Bromus secalinus*), green foxtail (*Setaria viridis*), Barnyard grass (*Echinchloa crusgalli*), prairie cup-grass (*Eriochloa contracta*) and Canada wild-rye (*Elymus canadensis*) (Slykhuis,1952; Sill and Connin, 1953; Christian and Willis, 1993; McMullen, 1995).

Sill and Connin (1953) inoculated various monocot and dicot plants with WSMV to determine their reactions and found all species of the genus *Triticum* susceptible. A few barley and rye varieties were also reported susceptible and some rye varieties were reported to be symptomless carriers of the virus. Dicotyledons and some *Agropyron* species remained symptomless when inoculated. Different cereals and grasses exhibited various levels of mosaic

symptoms (McKinney, 1949; Slykhuis, 1952; Sill, 1953; Sill and Connin 1953; McMullen, 1995). Although there are several host plants which serve as reservoirs for the virus and mites for a long time, the major overwintering host of the virus is considered to be volunteer wheat. Several studies have shown that an array of different over-summering/over-wintering grass hosts may influence WSM epidemiology. A high incidence of WSM, however, is usually associated with the presence of volunteer winter wheat, which serves as a reservoir or a green bridge for the virus and the vector (Christian and Willis 1993; Bowden, 1995).

### 2.1.4. Disease symptoms

In field settings, the occurrence of WSMV depends on the distribution of the mite vector. WSMV can also be transmitted easily by mechanical inoculation (McKinney, 1949). Infections from manual transmission could be more consistent than from a vector when the objective is to determine the effects of the virus and not those of the vector. In order to obtain plants severely damaged by WSMV, wheat plants must be inoculated while young, prior to tillering or during the early tillering period. In general, the younger the plant when infected, the more severe the disease symptoms. However, inoculating too early, for example at the one to two leaf-stage, may produce symptoms so severe that it becomes impossible to identify useful levels of intermediate resistance or tolerance.

Under mechanical inoculation, the symptoms of WSM include greenish-

yellow dashes and streaks parallel to the leaf axes, mottled (blotchy) leaves, and stunting (McKinney, 1947). The early symptoms are yellow mosaic patterns of discontinuous lines or blotches on the infected leaf followed by induction of symptoms on the young developing leaf. Later, these streaks or blotches become more extensive resulting in chlorosis covering much of the leaf followed by necrosis in severely infected plants (Martin, 1978).

In natural field conditions WSM usually appears at the field margins where mites are blown from adjacent, or more distant fields (Wiese, 1987). The edges of the streaked and mottled leaves roll up to the centre of the leaf (in case of mite infection). If the lower leaf is examined carefully, usually the wheat curl mite can be observed (Ashworth and Frutell, 1961; Atkinson and Grant, 1967). The disease usually remains restricted to the field margins but sometimes the disease spreads throughout the field.

In cases of mechanical or mite inoculation, early infection produces severely stunted plants and sometimes plants produce several small tillers (McKinney, 1949). The heads produced are usually wholly or partly sterile. If infection occurs in the late tillering stage seed set is poor and seeds have low test weight (Rahman et al., 1974). The symptoms induced by a virus often are influenced by interaction with environmental parameters such as soil fertility, temperature and light, as well as the stage of the plant at the time of infection, and the genotype of the wheat infected (Haunold, 1958; Stoddard et al., 1987; Seifers et al., 1995).

### 2.1.5. Disease Cycle

Development of WSM disease depends on the population of mite vector, the presence of virus-infected wheat plants, warm temperature, adequate moisture for good plant growth and rapid mite reproduction (Slykhuis, 1955; Ashworth and Frutell, 1961; Somsen and Sill, 1970; Wiese, 1987). The most severe infection occurs where volunteer wheat provides a green bridge through the fall between successive wheat crops.

Infective virus has not been obtained form leaves of brown dead plants or residues of either naturally or artificially infected plants, thus infected dead plants or crop residues are not responsible for overwintering or over-summering WSMV (Sill, 1953). However, leaves from a dormant, overwintering virus infected wheat plant consistently contained infective virus (Fellows and Schmidt,1953). The host ranges of WSMV and its mite vector differ but there are enough hosts in common that the association persists.

Infection of winter wheat can occur in the fall through any of the cereal crop/volunteer or grass hosts which have been infected by WSMV and WCM in the spring, and are still green when the winter wheat seedlings emerge, in the same or nearby fields. Warm temperature in early September enables mites to reproduce quickly and infect the winter wheat seedlings prior to the onset of winter. Although there is infection, symptoms do not appear on winter wheat in fall as the temperature declines (Sill and Fellows, 1953). Mosaic symptoms appear in spring and become increasingly severe with rising temperature as the

mites reproduce rapidly (Slykhuis, 1952; McMullen, 1995). Mites, which prefer green leaf tissues, leave leaves yellowed by virus infection, and are blown to other plants in spring and infect the spring seeded crops. If again the winter wheat emerges in good time, when the crops or volunteers/grasses are still green, then the winter wheat becomes infected and the cycle is complete (Wiese, 1987).

### 2.1.6. Management strategy

There are no chemicals registered to control either WCM or the WSMV. Losses due to WSM depend on the cultivar, the weather, the proportion of plants infected and the time of infection (Rahman et al., 1974; Edwards and McMullen, 1988). Although genetic control is the ultimate goal for any disease, some cultural practices may prove valuable in controlling WSM. Since WCM and WSMV need green plant tissue to survive and propagate, the first and most effective means of cultural control for WSM is to eliminate the "green bridge" created by volunteer wheat required by WCM to transfer the virus between wheat crops. At least a one week break in the green bridge is required to control the spread of WSMV (Christian and Willis, 1993; Fowler, 1998). However, this approach requires a community effort. Volunteer plants should be killed two-three weeks prior to the emergence of the new crop in the case of winter wheat (Bowden, 1995; Evans, 1996). In areas where no-till or minimum-till is followed,

volunteer/susceptible hosts should be destroyed with herbicides thus minimizing the source of WSMV.

Planting dates of winter wheat play an important role in controlling WSM continuity (Hunger et al., 1992). Therefore, if winter wheat needs to be seeded next to the spring wheat crop, one should wait until all the volunteers and grass hosts are killed (Bowden, 1995). Winter wheat planted early into a no-till situation is more vulnerable to WSM disease, if the field has not been sprayed with herbicide for volunteer control. Early planting of winter wheat allows enough time for the mites to move into the field, reproduce and spread the virus. However, late planting in western Canada may not be practical because of the late sown winter wheat may not get enough time for acclimation leading to poor winter survival. Hunger et al., (1992) reported that spring inoculation of wheat planted late in fall, causes significant reductions in yield and thousand kernel weight (TKW).

Elimination of the green bridge is effective in minimizing the sources of WSMV, and reducing the chance of WSM reaching epidemic proportions, but this will not prevent the occurrence of severe infections. Cultural methods may require coordination among neighbouring farmers with different priorities. Despite best efforts alternative host species for both WSMV, or WCM might survive.

Wheat varieties with resistance or high tolerance to WSMV or WCM could decrease the problem and provide more flexible options to farmers. Resistance

to WCM is effective in controlling WSM (Martin et al., 1984; Conner et al., 1991; Harvey et al., 1994) and is being used in commercial cultivars such as TAM 107, an American winter wheat. There are also spring or winter wheat accessions, derived from crossing various tolerant wheat that are adapted to western Canada and the US, that provide resistance or high levels of tolerance to WSMV. Attempts to exploit improved sources of resistance to WSMV and WCM and incorporate them into future cultivars are a part of ongoing crop improvement programs (McNeal and Carrol, 1968; Rahman et al., 1974; Martin et al., 1984; Seifers, 1984; McMullen, 1995; Haber et al., 1996).

Chromosome substitutions and translocations from *Agropyron* species into the desired wheat germplasm has become another promising source of WSMV resistance. Several such WSMV resistant wheat germplasms are available (Wells et al., 1973; Liang et al., 1979; Wells et al., 1982; Sebesta et al, 1995) but exploiting them to generate commercial cultivars poses a separate set of challenges.

## 2.2. Genetic variability of wheat streak mosaic virus

The host range for WSMV is primarily confined to the family *Gramineae* (wheat, maize, barley, oats and annual grasses) but little is known about virus isolates differentiated by their host ranges and/or symptomatology (McKinney, 1937; Slykhuis, 1952; Brakke, 1971; Christian and Willis, 1993; McNeil et al.,

1996; Montana et al., 1996). Because differences in antigenicity of distinct WSMV strains are associated with differences in vector transmissibility, host range, and virulence, it is also important to know the serological identity when breeding for resistance (Atreya et al., 1990; Montana et al., 1993).

### 2.2.1. Variability based on virus infectivity and symptom severity

Since the first report of WSMV infection in 1932 (McKinney 1937), virus isolates have been distinguished by their symptom severities on differential wheat hosts; some are of a highly virulent type and others are less so (McKinney, 1937; McKinney, 1956; Slykhuis and Bell, 1963; Slykhuis and Bell, 1966).

The mosaic viruses were preliminarily classified by McKinney (1937) into seven groups or strains: wheat virus 1 - 7 according to the percent infectivity, symptom expression, and the host. Amongst these, there were two streak mosaic viruses, 1) with mild green streak mosaic - *Marmor virgatum var. viride McKinney* or wheat virus 6, and 2) severe types with yellow streak mosaic virus *M. virgatum var. typicum McKinney* or wheat virus 7 (McKinney, 1944), and they were known as the western type of mosaic (Fellows, 1949). Combinations of these viruses were detected in Kansas in 1932, and in Alberta in 1952 (Slykhuis, 1953). Carroll et al. (1982), evaluated eight WSMV Montana isolates on Michigan amber wheat and categorized them into two type strains 1) mild type and 2) moderate type.

### 2.2.2. Variability according to serological characterization

WSMV isolates have also been classified into groups by differential virulence through serological assays using polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) on various cereal hosts (McNiel et al., 1996). Bottacin and Nassuth (1990) evaluated Ontario-grown cereals for susceptibility to WSMV using two isolates of WSMV, PV57 and PV106 and tested various lines of wheat, barley, oats, corn and triticale. Using the back-inoculation method, they found that some of the barley and oat varieties could not be infected by one isolate whereas the other virus isolate could be recovered from the plant material.

McNeil et al (1996) reported 32 distinct restriction fragment length polymorphism (RFLP) types derived from reverse transcriptase-polymerase chain reaction (RT-PCR) assay which was developed to amplify cDNA from the coat protein coding region and 3'-noncoding region of the WSMV genome. They used four already identified WSMV isolates (WSMV-T = PV57, mild mosaic ; WSMV-S = Sidney 81, intermediate; WSMV-W = Wyoming, intermediate; and WSMV-C = collected from infected corn, highly chlorotic) with diverse origin, producing different severity symptoms. Their results suggested that there are three main and many minor lineages of WSMV co-circulating in the region. Serological assays were also used by Montana et al. (1996) to detect WSMV isolate differences.

The virus isolate discovered in Indian Head (WSMV-IH), Saskatchewan,

Canada, in 1989 (Haber and Townley-Smith, 1993) is more virulent than the Sidney 81 isolate and among the most virulent described to date (Seifers, pers.comm.). Isolates collected since then in southeastern Saskatchewan and southwestern Manitoba have exhibited virulence similar to WSMV-IH when mechanically inoculated to susceptible spring wheat seedlings (Haber, pers. comm.).

## 2.3. Host resistance, tolerance and susceptibility

### 2.3.1. Definitions of resistance, susceptibility and tolerance

Disease resistance has been defined in numerous ways for different diseases. Parlevliet (1981) describes plant defence mechanisms in three general terms: 1) avoidance, which operates before parasitic contact between host and parasite is established and decreases the frequency of the contact; 2) resistance, where the host can resist the parasite by decreasing its growth (resistance) after parasitic contact has been established, and; 3) tolerance, where the host tolerates the parasite's presence by suffering relatively little damage. Nelson (1973) defined resistance and susceptibility in a non-genetic sense, such that resistance and susceptibility were relative terms, used to describe the amount of disease sustained by a plant or the amount of damage resulting from the disease.

Resistance in the WSMV-wheat system was defined in genetic terms by Sebesta and Bellingham (1963) where resistance has generally been taken to

describe "immunity" or high resistance where no systemic lesions appear. In contrast tolerance means "less disease", in which tolerant lines display less disease with lower chlorotic/necrotic tissue and sustain lower yield damage. In case of responses of plants to viruses, Cooper and Jones (1983) define the term resistance as characterized by the host's ability to restrict virus multiplication and movement after systemic infection and susceptibility is defined as inability of the host to restrict virus multiplication and movement while, tolerance is described as the ability of the host to endure specific virus multiplication and movement after systemic infection without causing severe symptoms or greatly diminishing plant growth or marketable yield.

### 2.3.2. Assessment of WSMV disease reaction

Studies of resistance in the WSMV-wheat system were initiated by McKinney in 1949 by inoculating virus isolates on winter wheats. Various qualitative and quantitative criteria have been used to assess resistance /susceptibility to WSMV: disease severity ratings (disease rating 1- 9 scale, which may take into account the growth stage of plant), amount of virus titre, and comparison of yield (including seed size, thousand kernel weight) of control and infected plants (McKinney and Sando, 1951; Atkinson and Grant, 1967; Rahman et al., 1974; Carrol et al., 1982; Christian and Willis, 1993; Haber and Townley-Smith, 1995). Apart from the symptamatology assays, other measurements such as ELISA and Slot -Blot Hybridization of WSMV reaction have also been

conducted to assess disease reaction (Stoddard et al., 1987). Because of the failure to find resistance among the commercial wheats tested, several species of *Triticum*, *Agropyron*, *Secale* and hybrids were explored (McKinney and Sando, 1951); in *Agropyron* species no systemic virus was detected. The genus *Secale* also showed a high resistance or immunity.

Fellows and Schmidt (1953) reported that *Agrotricum* hybrids were potential sources of resistance to losses from WSM disease, but they also observed that the more wheat-like the hybrid, the less resistance was retained. The wheat-like types, however, exhibited a high degree of variability, suggesting that it should be possible to select resistant wheat-like strains .

### 2.3.3. Factors affecting infection

Slykhuis (1952) observed that wheat could become infected with streak mosaic at various stages of development and differed in susceptibility at different growth stages. It was found that plants infected at the early seedling stage (1 -2 leaf stage) usually suffered more severe damage from WSM than plants that had been infected at a later stage (3 leaf) of development. Hunger et al.(1992) suggested that the maturity of plants at the time of infection might affect severity of WSMV because wheat planted in late November was less mature and suffered greater reductions in yield than wheat planted in September.

Sill and Fellows (1953) studied five wheat varieties to test the effect of different controlled temperatures upon incubation period and symptom severity.

They concluded that the incubation period lengthened at lower temperatures, the average being five days at an air temperature of 28°C, seven days at 24°C, nine days at 20°C, and 15 days at 16° C. During early stages of the disease the most severe leaf symptoms appeared at 28°C but symptoms became more severe at lower temperatures as the disease progressed. At lower temperatures disease symptoms were diffuse even though the plants contained infectious virus, explaining why symptoms are rarely seen on winter wheat in the field in fall.

Experiments with some *Agrotricum* lines by Pfannenstiel and Niblett (1978) have shown that resistance to WSMV is broken by high temperatures. Furthermore, they also detected that at 35°C, the longer the exposure to heat treatment the higher the percentage of plants becoming systemically infected. However, in the field, the lines would probably remain resistant as the heating cycle is interrupted by cool nights. Stoddard et al. (1987) reported that the resistance to WSMV present in wheat line TA 3426 appeared heat-sensitive. Seifers et al. (1995) demonstrated that some wheat lines with high level resistance (C.I.17884) due to translocated *Agropyron* chromatin, remained symptom-free at 20°C, although resistance broke down at 25°C.

**2.4. Host plant tolerance and resistance**

A broad range in tolerance to WSMV infection exists among the spring and winter wheat cultivars (Atkinson and Grant, 1967; Rahman et al., 1974; Timian and Lamey, 1985; Edwards and McMullen, 1987; Haber and Townley-

Smith 1993). A useful characteristic of many wheat-*Agropyron* derivatives is their resistance to diseases, such as wheat stem rust and wheat streak mosaic virus. In the early 1950s, it was reported that certain inter-generic hybrids like *Agrotricum*s (*Agropyron*/*Triticum*) could eventually provide a higher level of resistance to the virus, that *T. aestivum* alone could never demonstrate (McKinney and Sando, 1951; Andrews and Slykhuis, 1956; Schmidt et al., 1956). As a result, since the mid 1950s, attention has turned to the transfer of specific characters, such as disease resistance, from *Agropyron* to *T. aestivum*.

### 2.4.1. Screening of spring and winter wheat for WSMV tolerance

Several spring, winter wheat, and durum wheat cultivars show varietal differences for WSMV tolerance as measured by relative yield of inoculated and non-inoculated plants (Sill et al., 1964; Atkinson and Grant, 1967; McNeal and Carroll, 1968; Rahman et al., 1974; Timian and Lamey, 1985; Edwards and McMullen, 1987; Seifers and Martin, 1988; Bottacin and Nassuth, 1990; Haber and Townley-Smith 1993). The tolerance shown by some of the cultivars can make them economically important in the areas where the disease is epidemic and thus useful in breeding programs.

Field evaluation reports from the studies conducted by Timian and Lamey (1985) and Edwards and McMullen (1987) on reduction in yield in hard red spring wheats as a result of infection with WSMV show that American spring

wheat Butte was one of the most highly tolerant cultivars among those tested, with a 5 year average yield reduction of 40% compared with a 72% loss for popular cultivars such as Katepwa.

### 2.4.2. The inheritance of WSMV tolerance

Several reports indicate that no high degree of tolerance to WSMV exists among *Triticum aestivum* cultivars (McKinney and Sando, 1951; Slykhuis, 1955; Andrews and Slykhuis, 1956; Bellingham et al., 1957; Rahman et al., 1974; Edwards and McMullen, 1988; Haber and Townley-Smith, 1993). However, a certain degree of tolerance has been found in such cultivars as Triumph, Kiowa, Columbus, Butte, Oslo, advanced spring bread wheat lines BW122 and BW155 (Seifers and Martin, 1988; Haber and Townley-Smith, 1995). Seifers and Martin (1988) suggested that disease tolerance has not been used extensively in breeding programs because the results are less definitive than those obtained with WSMV resistance, and the screening process is accordingly time-consuming. As a result few inheritance studies of WSMV tolerance have been conducted.

### 2.4.3. Sources of WSMV resistance

Several distant relatives of wheat appear to be promising sources of WSMV resistance. Since McKinney and Sando (1951) reported WSMV resistance in *Agropyron* species and *Agropyron* and *Triticum* hybrids, considerable work has been done, particularly in Canada and the United States of America, to produce hybrids from the crosses between species of *Triticum* and *Agropyron* (Fellows and Schmidt, 1953; Andrews and Slykhuis, 1956). Among the several *Agropyron* species tested for WSMV resistance for several years, crosses between the wheat and *A. elongatum*, and wheat and *A. intermedium* were probably the most easily obtained hybrids and yielded the most fertile plants. Therefore, in North America, research has largely concentrated on these hybrids (Sharma and Gill, 1983; Sharma et al., 1984).

Many WSMV and WCM resistance genes have been transferred to common wheat from *A. intermedium* and *A. elongatum* (McKinney and Sando, 1951; Larson and Atkinson, 1972; Friebe et al., 1991; Jiang et al., 1993; Harvey et al., 1994; Seifers et al., 1995; Friebe et al., 1996).Two approaches are used to combat WSM: 1) direct control by developing resistance to virus in the wheat cultivars and, 2) indirect control by developing wheat cultivars resistant to the mite vector and diminishing the extent of virus infection.

**2.4.3.1 Direct control by developing resistance to virus in the wheat cultivars**

**a) WSMV resistance derived from *A. elongatum:*** Resistance to WSMV has been transferred into wheat from *A. elongatum* (Host) P. Beauv. using radiation treatment (Sebesta and Bellingham,1963; Sebesta et al.,1972; Martin et al., 1976; Pfannenstiel and Niblett, 1978). C-banding and genomic in situ hybridization (GISH) analyses revealed that the resistance gene came from the long arm of a group 1 *A. elongatum* chromosome, 1Ae#1, translocated to wheat chromosome 4D in germplasm C.I.15322 in the form of noncompensating T4DL.4DS-1Ae#1L translocation (Jiang et al., 1993; Friebe et al., 1996). C.I.15322 had a second complete *A. elongatum* chromosome, 1Ae#2, substituting for wheat chromosome 1D (Friebe et al., 1996). Wheat lines produced with only the T4DL.4DS-1Ae#1L did not share the same high level of resistance to WSMV compared with the parent line C.I.15322, therefore, this gene has not been used in wheat improvement (Friebe et al., 1996).

**b) WSMV resistance derived from *A. intermedium:*** Resistance to WSMV has also been transferred into wheat from *A. intermedium* (Host) Beauv. by irradiating $F_1$ seeds from the cross C.I.15092/Ae. Speltoides//Fletcher x Centurk with fast neutrons (Lay et al., 1971; Wells et al., 1982). Resistance to WSMV in *A. intermedium* has been shown to be controlled by the gene *Wsm1*, which is located on the short arm of chromosome 4Ai (Wang and Liang, 1977;

Seifers et al., 1995; Friebe et al., 1996). C-banding and GISH identified a compensating translocation, designated T4DL-4Ai-2S in germplasm C.I.117884 (derivative of C.I.15902) (Friebe et al., 1996; Seifers et al., 1995). Germplasms that contain only the T4DL.4Ai-2S translocation such as C.I.17884, KS93WGR27, performed better agronomically (Gill et al., 1995). To facilitate resistance screening, Talbert et al.(1996) developed a molecular marker closely linked to *Wsm1* resistance gene which is localized to the translocated alien chromatin. *Wsm1* is now being introduced into advanced breeding lines (Seifers et al., 1995; Friebe et al., 1996; Chen et al., 1998).

**Transfer of WSMV resistance by recombination.** WSMV resistance gene (*Wsm1*) was also transferred to wheat using induced homoeologous recombination (Wong et al., 1974; Wang and Liang,1977; Wang et al., 1980). They studied the cytogenetics and breeding behavior of C.I.15092 and reported that it has one *A. intermedium* chromosome pair carrying genes for immunity from WSMV. The short arm of the *A. intermedium* chromosome 4 carried the WSMV resistance gene. Immunity from WSMV is completely dominant, and the results also indicated some plants were highly fertile demonstrating that the whole *Agropyron* chromosome added to the wheat complement was not particularly harmful to self fertility. They suggested that C.I. 15092 could be made into a useful source of WSMV resistance genes for developing winter and spring wheats immune from WSMV infection. Wang and Liang (1977), reported

that C. I. 15092 is a disomic wheat substitution line in which chromosome 4B (later re-designated as 4A) has been replaced by a homoeologous chromosome from *A. intermedium*.

### 2.4.3.2. Indirect control by vector resistance

Andrews and Slykhuis (1956) were the first to report on the reactions of wheat and *Agrotricums* to both the mite vector and the WSMV. Larson and Atkinson (1972) showed that individual lines obtained from wheat x *A. elongatum* with a disomic - 6D substitution were both virus and mite resistant. They identified and isolated an *A. elongatum* chromosome conferring resistance to the wheat curl mite, vector of WSMV. Larson and Atkinson, (1973) further showed that immunity of the T-Ae line to virus was not confined to any one of the *A. elongatum* chromosomes substituting for wheat chromosomes 4D, 5D, 6D. However, they found that *Agropyron* substituting for wheat chromosome 6D (= 6Ag) carried almost the full resistance to the mite vector. Chromosome 6Ag replaced chromosome 6D in wheat and the gene designated Cmc2 was on the short arm of 6 Ag (Whelan and Hart, 1988). This was an additional benefit for control of WSMV because resistance to the mite vector could prevent or minimize the spread of the virus. But the translocation program with irradiation was not geared toward this, as single chromosome substitution line T-Ai immune to WSMV was available.

Martin et al. (1984) were the first to demonstrate field resistance to an eriophyid mite controlling a plant disease, such as WSM. They suggested that the reduced transmission of WSMV to wheat curl mite-resistant cultivars combined with wheat curl mite control in WCM-resistant volunteer wheat should give effective levels of WSM control. They reported that resistance in the wheat-rye translocation cultivar Salmon effectively reduced the incidence of WSMV compared to that in the susceptible winter wheat cultivar Sage. Similar studies were carried out and information was reported by Tyler et al. (1985) and Conner et al., (1991) working with WSMV resistant wheatgerm plasm. Further, Harvey and Martin (1988) demonstrated that the percentage of wheat streak mosaic infected plants was significantly lower in the wheat curl mite resistant wheat-rye translocation line TAM 107 than other wheat cultivars. TAM 107 is a popular winter wheat grown in Kansas and its popularity is largely due to its resistance to the wheat curl mite.

Harvey et al.(1995a), however, reported that some strains of wheat curl mite have overcome the resistance of TAM 107. Harvey et al. (1995b) and (1997) indicate that it has become necessary to deploy other sources of WCM resistance other than wheat-rye translocation in commercial cultivars.Thomas and Conner (1986) have introduced another source of wheat curl mite resistance transferred into wheat from *Aegilops squarrosa* L.

# 3. Inheritance of tolerance to wheat streak mosaic virus in an advanced spring wheat (*Triticum aestivum* L) line BW155.

## 3.1. Abstract

Wheat streak mosaic (WSM) is an important disease of wheat. Tolerance to WSM virus (WSMV) exists in spring wheat germplasm. The objective of this study was to determine the inheritance of tolerance to WSMV in BW155, an advanced spring bread wheat line. $F_1$, $F_2$ and $F_2$-derived $F_3$ progenies from reciprocal crosses between BW155 and Laura, a highly susceptible Canada Western Red Spring (CWRS) wheat cultivar, were evaluated under greenhouse conditions. The inheritance of BW155-derived tolerance to WSMV was also determined from two $F_1$-derived doubled haploid (DH) populations generated from A18-4113/AC Barrie and AC Elsa/A18-4113. AC Elsa and AC Barrie are moderately and highly susceptible to WSMV infections. Mechanically inoculated seedlings ($F_2$ and DH plants) were scored on a 1-9 disease rating scale. Segregation ratios for WSM disease expression showed that tolerance to WSMV was a dominant trait, and controlled by three genes. Several intermediate level reactions indicate that the inheritance to BW155 tolerance to WSMV is controlled by three dominant genes with additive effects.

## 3.2. Introduction

Wheat streak mosaic (WSM) caused by wheat streak mosaic virus (WSMV) is a continuing serious problem for wheat (*Triticum aestivum* L) production in the Great Plains of the USA and parts of the Prairie region of western Canada (Atkinson and Grant, 1967; Edwards and McMullen, 1987; Haber and Townley-Smith,1993). WSMV is transmitted by the wheat curl mite (WCM), *Aceria tosichella* Keifer (Slykhuis, 1955; Armine and Stasny, 1994) in natural field conditions, but the virus can also be transmitted mechanically (McKinney 1949; Martin, 1978).

Yield reduction in winter wheat caused by WSM reached 18% in southern Alberta in the early 1960s (Atkinson and Grant, 1967). Localized yield losses (trace to 100%) are an annual problem in individual winter and spring wheat fields, especially in southwestern Manitoba, southeastern Saskatchewan and southern Alberta (Conner et al., 1991; Haber et al., 1997 ). In the United States, it is estimated that the winter wheat yield loss due to WSM is about 2% per year (Christian and Willis, 1993; McNeil et.al.,1996). An increased incidence of WSM in spring wheat since the late 1980s is also reported from the Northern Plains of the United States (Edwards and McMullen, 1987 and1988; McMullen, 1995).

Historically, WSM disease has been of most concern in winter wheat (McKinney, 1949; Atkinson and Grant, 1967; Conner et al., 1991). However, winter wheat provides a natural green bridge for WSMV and WCM in areas

where spring wheat is grown. Therefore, WSM can also pose a threat to spring wheat crops (Edwards and McMullen, 1988; McMullen, 1995). Another important factor is that conservation tillage is increasing the prevalence of alternate hosts such as green foxtail, barnyard grass and volunteer cereals for over-summering (Christian and Willis 1993; Bowden, 1995). Infected plants can serve as an inoculum source for infection in fall-seeded winter wheat and volunteer winter wheat. Thus, conditions exist for outbreaks of WSM in spring wheat areas where spring wheat and winter wheat are grown in close proximity (Bowden 1995; McMullen, 1995).

The incidence of WSM can be minimized if agronomic practices such as proper crop-rotation, tillage, cultivation or mowing of headlands and use of herbicide summer fallows are carried out. However, breeding for disease resistance or tolerance to WSMV in wheat will provide a better means of controlling WSM as this would leave more options to producers and require fewer inputs.

Resistance is defined as a host's ability to restrict virus multiplication and movement after infection, and susceptibility as the inability of the host to restrict virus multiplication and movement. Tolerance differs from resistance, in that the host has the ability to endure specific virus multiplication and movement after systemic infection without suffering severe symptoms or greatly diminished plant growth or marketable yield (Cooper and Jones, 1983).

The response of a wheat host to WSMV infection can be assayed in several different ways. The two most common diagnostic techniques often used are symptomatology assay, which involves visual inspection of symptom development based on leaf chlorosis supported by reduction in plant height and yield, and enzyme-linked immunosorbent assay (ELISA) which determines the presence of viral capsid protien (McKinney and Sando, 1951; Stoddard et al., 1987; Haber and Townley-Smith, 1995; Seifers et al., 1995; Montana et al., 1996). A disease rating scale based on symptomatology assay, with a 1-5 disease rating scale (Stoddard et al., 1987; Seifers et al., 1995), or with a 1-9 disease rating scale based on leaf chlorosis and supported by height and yield information (Haber and TownleySmith, 1995), can be used to assess host reaction. New technologies such as the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) have facilitated detection and analysis of sequence polymorphisms among virus isolates, strains, and populations (McNeil et al., 1996; Montana et al., 1996).

There are no commercial spring wheat cultivars resistant to WSMV. High levels of resistance to WSMV are found in some wild relatives of wheat including the genera, *Agropyron* and *Secale* (McKinney and Sando, 1951; Sharma and Gill, 1983). This resistance has been transferred to common wheat by means of radiation treatment (Sebesta and Bellingham, 1963; Lay et al.; 1971; Larson and Atkinson, 1970 and 1972;; Sebesta et al., 1972; Wells et al., 1983; Friebe et al.,

1991; Jiang et al, 1993; Friebe et al., 1996). Alien derived resistance is advantageous but presents challenges by bringing in many undesirable agronomic traits along with the useful resistance gene(s) (Seifers et al., 1995). Therefore, exploiting the readily available tolerance to WSMV in advanced CWRS wheat lines, adapted to Western Canada, will help to develop superior tolerant breeding lines in shorter time than deriving tolerance / resistance from unadapted wild relatives.

Variation in tolerance to WSMV has been reported from several spring wheat cultivars (McKinney, 1956; McNeal and Carroll, 1968; Rahman et al., 1974; Timian and Lamey, 1985; Edward and McMullen, 1987and 1988; Haber and TownleySmith, 1993; McMullen, 1995), but no inheritance studies has been conducted on such tolerance. The American spring wheat cultivar Butte is one of the most highly tolerant cultivars with a five year average yield reduction of 40%, whereas popular cultivars such as Katepwa suffered 72% loss (Timian and Lamey,1985; Edwards and McMullen,1987). BW155, formerly known as ND640, was developed in North Dakota by crossing Butte and Columbus, a CWRS wheat cultivar, moderately tolerant to WSMV. Three years of field and indoor experiments indicated that BW155 could itself be used as a parent to provide WSMV tolerance in the CWRS wheat breeding program (Haber and Townley-Smith, 1995).

An understanding of the genetic basis of BW155-derived tolerance to

WSMV will help to exploit this tolerance. The primary objective of this study was to determine the mode of inheritance of tolerance of BW155 to WSMV using the qualitative disease assessment criteria of symptomatology assay (Haber and Townley- Smith, 1995).

## 3.3. Materials and methods

### 3.3.1. General Plant Growth Conditions

All experiments were conducted indoors in growth chambers and green houses under the controlled conditions of 14 hr light /10 hr dark and 20/16°C. Plants were grown in 15 cm fibre pots (parents, $F_1$ and DH lines), 60 x 90 cm flats ($F_2$) or 1 x 5m beds ($F_3$). The soil mixture used for the experiments was composed of 2:1:1 (v:v:v) soil, sand and peat. The plants were fertilized biweekly with 20:20:20 (N:P:K) fertilizer until heading, and with 15:28:15 after heading until maturity. Three weeks after inoculation, tillers were trimmed and only the main culm was allowed to grow and mature.

### 3.3.2. Parental materials

To study the inheritance of WSMV tolerance, three CWRS wheat cultivars (Laura, AC Elsa and AC Barrie), an advanced spring bread wheat line (BW155) and a DH line (A18-4113) were chosen as parents. The choice of Laura as susceptible and BW155 as tolerant parents was based on the disease ratings and yield reduction of each particular cultivar/line during disease screening and

selection processes conducted previously in field experiments (Haber et al., 1995). Reactions to WSMV under field conditions (1992-94 experiments) for the tolerant (BW155) and susceptible (Laura) parents are listed in Table 3.1. Previous laboratory and field experiments showed that AC Barrie is highly and AC Elsa is moderately susceptible to WSMV infections (Haber unpublished data). A18-4113 is a double haploid line derived from Laura/BW155. Reaction to WSMV under field conditions for DH line A18-4113 is not known. However, A18-4113 was repeatedly screened and scored for disease ratings (1-9 scale) indoors under WSM disease pressure and was consistently found to be equivalent to BW155 in disease ratings and yield performance (Haber unpublished).

### 3.3.3. Population development

Reciprocal crosses were made between BW155 (tolerant) and Laura (susceptible) to produce $F_1$ generations. Four $F_1$ seeds of each reciprocal crosses were grown individually in 15 cm pots to produce seeds to grow $F_2$ generations. The $F_1$ plants were bagged at anthesis to ensure self-pollination.

Two susceptible CWRS wheat cultivars, AC Elsa and AC Barrie, were crossed with A18-4113. Resulting $F_1$ seeds were grown and generated as DH populations through a maize pollen mediated doubled haploidy process (Laurie and Bennet, 1988; T. Aung personal communication). The two DH populations generated were DH96B01:28 lines (A18-4113/AC Barrie) and DH96B03:47 lines

(AC Elsa/A18-4113). Generation of these DH lines was conducted previously in the program.

All parental lines were included in all the tests as checks except for A18-4113, as there was no seed available. BW155, the tolerant parent was included as a non-inoculated control to compare with inoculated plants in $F_2$ and $F_2$ - derived $F_3$ experiments. Non-inoculated counterpart plants were used as controls for $F_1$ , DH96B01 and DH96B03 populations.

### 3.3.4. WSMV maintenance, inoculum preparation and inoculation procedure

WSMV isolate Indian Head (WSMV-IH) was used for all the experiments conducted for this study. WSMV-IH was obtained from Indian Head, Saskatchewan in 1990, and was maintained by continually growing the mechanically inoculated susceptible hosts in the growth chamber and greenhouse. In this study, BW155 plants were used to maintain the inoculum because BW155 leaves remained green longer and more virus could be extracted from these leaves, than from the dead tissues of other, more susceptible hosts. WSMV-IH isolate was periodically compared to WSMV Sidney81 isolate, whose relative virulence is well established (Seifers, unpublished), by inoculating on leaves of wheat seedlings to ensure the virulence.

Leaves with the distinct mosaic symptoms of WSMV were selected and

harvested for inoculum preparation. Freshly harvested leaves were ground with a mortar and pestle. The extracted sap was filtered to separate coarse tissues and diluted tenfold with distilled water (Haber and Townley-Smith, 1995). Individual plants from the experiment were inoculated mechanically, at the three-leaf stage, with freshly prepared virus extract. Carborundum (320 grit), an abrasive, was dusted on the leaves to facilitate virus entry. Leaves from individual plants were rubbed upward, three times, with a cotton ball soaked in virus extract.

### 3.3.5. Assessment of host reaction

WSMV infection caused leaf chlorosis, stunting, and decreased yield in susceptible host plants. Leaves that emerged after inoculation were visually rated on a 1-9 scale (Table 3.2), 20 days post-inoculation (dpi), based on leaf mosaic symptoms (chlorosis: blotch or thread type). Plant height and head mass measurements were taken at maturity to calculate percentage reduction relative to the uninoculated controls.

To provide a better assessment of the host reaction to WSMV infection, the chlorosis rating was supplemented by height and head mass data. Based on Table 3.2, a chlorosis rating of '5' for a particular cultivar or DH line, was expected to experience a height reduction of 20 - 30%, and head mass decrease of 50 - 60% compared to that of the non-inoculated control plants. WSM is a systemic disease and affects height and fertility thus the chlorosis rating may not

be consistent with the height and head mass ratings. In such cases available disease rating parameters were combined and averaged; a particular plant may have a chlorosis rating value of '5', a height reduction rating of '6' (30-40%) and yield reduction value of '7' (70-80%), or chlorosis rating of '5', height reduction of '6' and no head mass. In this study, a combined rating was calculated as the average of the three ratings (chlorosis, height and head mass), and if a rating was missing the remaining two ratings were averaged.

Based on the information found in the literature of the earlier experiments the WSM infected tolerant cultivar or line can experience a wide range of yield reduction (Butte 40%; BW155 range from -1 to 64 %). Therefore, for the purpose of analysis, plants segregating for reaction to WSMV infection were grouped as tolerant, intermediate or susceptible as follows:

| | Leaf chlorosis | Plant height[1] | Head mass[1] reduction | Rating scale |
|---|---|---|---|---|
| Tolerant | <50% leaf area | <30% stunting | <50% | 2-4 |
| Intermediate | < 75%leaf area | <50% stunting | <75 % | 5-7 |
| Susceptible | >75% leaf area | > 50% stunting | >75% | 8-9 |

[1] Plant height and head mass were calculated compared to the non-inoculated tolerant parent (BW155) in case of $F_1$, $F_2$ and $F_2$ -derived $F_3$ populations, and to their respective non-inoculated controls in case of DH96B01 and DH96B03

populations.

### 3.3.6. Screening parents for WSMV reaction

Twenty five seeds of each of Laura, the susceptible parent, and BW155, the tolerant parent, were grown in 15 cm fibre pots with five plants in each pot. One pot each of BW155 and Laura, with five plants each was grown as non-inoculated controls. The pots were placed in a growth cabinet under controlled conditions of 14 h photoperiod at 20/16°C. The plants were inoculated mechanically at the three-leaf stage using freshly prepared WSMV-IH extract. After rating for WSMV reaction, the plants were transferred to a greenhouse and kept until harvest.

### 3.3.7. Screening for $F_1$ hybrids

Ten $F_1$ seeds of each reciprocal cross between BW155 and Laura were grown in ten cm fibre pots with one plant in each pot. Four plants each of BW155 and Laura were grown as inoculated checks. Four plants each of $F_1$s, BW155 and Laura were grown as non-inoculated controls. The pots were placed in growth cabinet under controlled conditions of 14 h photoperiod at 20/16°C. The plants were inoculated mechanically at the three-leaf stage using freshly prepared WSMV-IH extract. The leaf symptoms were scored, on a 1-9 scale based on a visual estimate of leaf chlorosis, 20 days post inoculation. Tillers

were trimmed after rating and only the main culm was allowed to grow. After rating for WSMV reaction all plants were transferred to a greenhouse. Plant height and head mass were taken at maturity.

### 3.3.8. Screening $F_2$ seedlings for segregation of tolerance

A total of 513 $F_2$ seeds (BW155/Laura = 268; Laura/BW155 = 245) were grown in 60 x 90 cm flats in the greenhouse. Twenty eight seeds of each BW155 and Laura were included as checks. Plants were seeded at about 5 cm apart, leaving a 10 cm boarder at the edge of the flat. There were 148 plants including seven BW155 and seven Laura (seeded in different rows) in each of two flat for seeds from BW155/Laura cross, and. 136 plants (including parents) in one flat and 137 plants in the other for the seeds from Laura/BW155. Ten plants each of BW155 and Laura were grown as non-inoculated controls. These control plants were grown in 15 cm fibre pots with five plants in each pot, kept adjacent to the flats. Flats were placed in a green house under controlled conditions of 14 h photoperiod at 20/16°C.

The $F_2$ plants and parental checks were inoculated mechanically at the three-leaf stage using the freshly prepared WSMV-IH extract. The leaf symptoms were scored on a 1-9 scale based on visual estimate of leaf chlorosis, 20 days post inoculation. Tillers were trimmed after rating and only the main culm was allowed to grow. Plant height and head mass were taken at maturity.

### 3.3.9. Confirmation of mode of inheritance - $F_2$ derived $F_3$ plants (BW155/Laura)

The main heads produced by $F_2$ plants were harvested individually and the resultant seeds were collected. In total 408 $F_2$ plants (200 tolerant, 206 intermediate and 2 susceptible), produced, at least four seeds to give rise to $F_2$-derived $F_3$ plants. Since WSM is a systemic disease that affects fertility, not enough seed could be collected from individual $F_2$ plants to confirm specific $F_2$ reactions. However, four seeds from each available $F_2$ plant were grown in 1 x 5 m soil-filled beds (planted in 10-cm rows with four seeds per row) in the green house and screened under WSM pressure. Twenty seeds each of BW155 and Laura were seeded at random among the rows as checks. Ten plants of BW155 were grown on one side of the same bed as non-inoculated controls. Inoculation and rating of the plants were done as described above.

### 3.3.10. Screening DH96B01 and DH96B03 populations for segregation of tolerance

Two sets of four seeds from each DH line of the DH populations, DH96B01(28 lines) and DH96B03 (47 lines), were grown in 15 cm fibre pots filled with soil mixture in the greenhouse. One set of DH lines was treated as non-inoculated controls. Four plants each of BW155, Laura, AC Barrie, and AC Elsa were included as parental checks. The line 7166 ($BC_1$ $F_1$ Elsa/KS95H103//

AC Elsa) carrying WSMV resistance gene, Wsm1 was also included as check in the DH96B03 population experiment. Fertilizer and lighting regimes were followed as in other experiments. The plants of the DH lines and parental checks were inoculated mechanically at the three-leaf stage using freshly prepared WSMV-IH extract. The leaf symptoms were scored on a 1-9 scale based on visual estimate of leaf chlorosis, 20 days post inoculation. Tillers were trimmed after rating and only the main culm was allowed to grow. Plant height and head mass were taken at maturity. In the case of chlorosis rating, plants within a particular line had the same or similar symptom score in most cases and when it was not an average of the four plants were taken. An average of four plants was taken from the actual measurements of plant height and head mass for the analysis.

### 3.3.11. Statistical analyses

Segregation ratios of each ($F_2$, DH96B01 and DH96B03) population were tested for fit to several genetic models using chi-square analysis, and Yates' correction factor was used when there was one degree of freedom (Strickberger, 1985). Data from the reciprocal crosses for $F_2$ population (BW155/Laura) were tested for homogeneity prior to pooling. To assess the heritability a progeny-parent regression was used where $F_2$-derived $F_3$ plants (progeny) phenotypic values ($F_3$ means) were treated as dependent variable y, regressed against the

$F_2$ phenotypic values (parent), as the independent variable x. If the *b* is the regression coefficient (the slope of the line) of offspring ($F_3$) on mid parent ($F_2$) then $b_{o\,p} = h^2$ (Strickberger, 1985).

## 3.4. Results

### 3.4.1. Assessment of phenotypes

**Parent for WSMV reaction**. When 25 plants of the tolerant parent, BW155, and the susceptible parent, Laura, were inoculated with WSMV-IH, two types of symptoms were observed visually: blotch type (islands of chlorosis) in BW155 and thread type (streaks of bright chlorosis) in Laura. In Laura, symptoms appeared early (eight to ten days post inoculation), spread rapidly, and plant height was reduced greatly. Some of the severely infected susceptible plants died and others that survived produced no fertile heads. In BW155, symptoms appeared two to three days later than those of Laura. The symptoms induced in BW155 were less severe than those in Laura, blotchy rather than streaked, and progressed slowly. Later developing leaves were less chlorotic. The heights of the infected plants were not affected greatly compared to the non-inoculated controls.

BW155 is an advanced CWRS wheat line that is tolerant to WSMV while Laura is a susceptible cultivar (Figures 3.2, 3.3a and 3.3b). BW155 consistently

rated close to or less than 4, and Laura rated ≥8 to WSMV infection on a 1-9 disease rating scale (Fig. 3.1a).

**$F_1$ hybrids.** The $F_1$ hybrids of reciprocal (BW155/Laura) crosses, under WSM disease pressure, showed the phenotype of the tolerant parent BW155, indicating that BW155-derived tolerance to WSMV-IH is dominant. The $F_1$ hybrids exhibited a similar disease rating (2-4) at a 1-9 rating scale (Fig. 3.1b and 3.1c). No reciprocal differences were observed based on disease rating, reduction in plant height and head mass between the two $F_1$ populations studied, indicating that nuclear genes control reaction to WSMV-IH (Table 3.5).

### 3.4.2. Inheritance of tolerance to WSMV

**Population BW155/Laura.** The $F_2$ data from two BW155/Laura $F_1$ hybrids (cross and the reciprocal) were not significantly different at 0.05 probability level based on a chi-square test for homogeneity(Table 3.6). The data were pooled for further analysis. The $F_2$ plants segregated into several groups, when screened indoors under WSM disease pressure (Fig.3.1d and Tables 3.3 and 3.4). The response to WSMV of the inoculated plants ranged from highly tolerant to highly susceptible. Symptom scores ranged from 2-9. Plants with ratings of 2-4 were considered tolerant (T), 5-7 ratings were grouped as intermediate (I) and the plants rated 8 and 9 were treated as susceptible (S). The observed numbers fit a 27:36:1 (T:I:S) or 63:1 (T:S, if tolerant and intermediate were pooled)

segregation ratio, consistent with a three-gene complementary dominance model (Table 3.7).The mean disease rating score for the tolerant check, BW155 was 3.5 on the 1-9 scale. BW155 experienced a height reduction of 12% and head mass decrease of 38% when compared to non-inoculated BW155 control.

The $F_2$ result supported additive genetic effect at the genic level in the BW155-derived tolerance because many $F_2$ plants were intermediate in their response. The $F_1$ results showed clear dominance of the heterozygotes, and so the intermediate response in the $F_2$ probably resulted because tolerance increased as the number of dominant genes increased. For example, if three dominant genes condition BW155-like tolerance then all genotypes with at least a dominant allele from each gene would be expected to be tolerant; any genotype with a dominant allele from one or two gene(s) would be intermediate, and genotypes with no dominant allele (homozygous recessive) at all loci are susceptible.

### 3.4.3. Confirmation of $F_2$ phenotypes

**Analysis of $F_2$-derived $F_3$ population (BW155/Laura).** The $F_2$-derived $F_3$ plants from tolerant and intermediate $F_2$ seeds responded with tolerant and intermediate reactions under WSM pressure with disease ratings from 3-5. Therefore, individual plants classified indeed may have been either homozygous or heterozygous for tolerance, and not homozygous for susceptibility. The

expected homozygous susceptible plants from heterozygotes were not observed. Probably because only four plants were assessed for each $F_2$-derived $F_3$ families. With a three-gene additive dominant effect trait, intermediate phenotypes would rarely produce susceptibles. However, plants grown from the susceptible $F_2$ seeds (eight viable seeds from two plants) were all highly susceptible to WSMV infection, with greatly reduced height and heads dying inside the boot-leaf. This supported the $F_2$ result of susceptible reaction.

From the regression analysis of $F_2$ on $F_2$-derived $F_3$ plants, an average heritabilty of 0.63 was obtained. When the head mass and height were regressed independently, 0.60 from head mass and 0.66 heritability estimates were obtained. The narrow sense heritability estimate of 0.63 can be considered a high degree of heritabilty. This result supports the facts that there are major genes involved in inheritance of BW155 tolerance, and these genes are be qualitatively inherited. The similar heritability estimates for the three traits supported the conclusion that phenotypes were recorded accurately and also indicate the high repeatability of the results.

**Population DH96B01(DHA18-4113/AC Barrie).** The DH lines from DH96B01 population segregated into several groups, when screened indoors under WSM disease pressure (Tables 3.3 and 3.4). The response to WSMV inoculation of the different DH lines ranged from highly tolerant to highly susceptible. Symptom scores of the different lines ranged from 2-9. Lines with

ratings of 2-4 were considered tolerant (T), 5-7 ratings were grouped as intermediates (I) and the lines rated 8 and 9 were treated as susceptible (S). The observed number of lines in DH96B01 population fit a 1:6:1 (T:I:S) or 7:1 (T:S, if tolerant and intermediate lines were pooled) ratio, consistent with a three-gene dominant complimentary model (Table 3.7).The susceptible parent AC Barrie had a disease rating score of 7.75 with a height reduction of 67% and head mass decrease of 80% when compared to non-inoculated AC Barrie control.

The result from DH96B01 population supported an additive effect at the genic level in the BW155-derived tolerance, consistent with a tolerance increase as the number of dominant genes increased. For example, if three dominant genes condition BW155-like tolerance then all genotypes with at least a dominant allele from each gene would be expected to be tolerant; any genotype with a dominant allele from one or two gene(s) would be intermediate, and genotypes with no dominant allele (homozygous recessive) at all loci are susceptible.

**Population DH96B03(AC Elsa/DHA18-4113) .** The DH lines from DH96B03 population segregated into several groups, when screened indoors under WSM disease pressure (Tables 3.3 and 3.4). The response to WSMV inoculation of the dfferent lines ranged from highly tolerant to intermediates. Symptom scores of the different lines ranged from 2-7. Plants with ratings of 2-4 were considered tolerant (T), 5-7 ratings were grouped as intermediates (I) and

the plants rated 8 and 9 were treated as susceptible (S). No lines were observed with a rating of 8 or 9 (highly susceptible). The observed number in DH96B03 population fits a 2:6:0 (T:I:S) or 8:0 (T:S, if tolerant and intermediate are pooled) ratio (Table 3.7). The moderately susceptible parent AC Elsa had a disease rating score of 6 with a height reduction of 36% and head mass decrease of 70% when compared to non-inoculated AC Elsa control. AC Elsa may have performed as a moderately susceptible parent because it may have contained one dominant tolerance gene and susceptible alleles for the other two genes. The resistant check line 7166 scored 2 on a rating scale of 1-9, with height reduction of 3% and head mass reduction of 26% when compare to non-inoculated 7166 control.

The result from DH96B03 population supported an additive effect at the genic level in the BW155-derived tolerance, consistent with increased tolerance as the number of dominant genes increased. For example, if three dominant genes would condition BW155-like tolerance then all genotypes with at least a dominant allele from each gene would be expected to be tolerant; any genotype with a dominant allele from one or two gene(s) would be intermediate, and genotypes with no dominant allele (homozygous recessive) at all loci would be susceptible.

A chi-square analysis on leaf chlorosis alone, for the $F_2$ population, did not reject the hypothesis of three-gene control for inheritance of tolerance of

BW155-derived tolerance (Table 3.8). From the different models tested, segregation ratios for the $F_2$, DH96B01 and DH96B03 populations were consistent with a three-gene inheritance model for BW155-derived tolerance (Tables 3.7). The results also confirmed the dominance of the BW155-derived tolerance. Several intermediate level reactions (rating scale: 5,6 and 7) supported inheritance of BW155-derived tolerance to WSMV as controlled by three complementary dominant genes, with additive effect at genic level. For example, if AaBbCc are the three genes that condition full BW155 tolerance then degree of tolerance would be as follows A_B_C_ > A_B_cc > A_bbcc > aabbcc would represent genotypes with descending degrees of tolerance, regardless pf which dominant gene is present.

Some of the plants of the $F_2$ generation with an intermediate reactions (ratings: 5, 6 and 7) produced sterile heads, but this was not the case with the doubled haploid lines. WSMV may react differently in different cultivars such as the moderately susceptible AC Elsa, the susceptible AC Barrie, and the highly susceptible Laura background.

### 3.5. Discussion

Plants which possess specific genes for tolerance are usually less severely affected by pathogens. BW155 has displayed the same degree of tolerance in the greenhouse and in the field indicating full expression of the tolerance genes under both environmental regimes. It experienced a lower height and head mass reduction under disease pressure, producing more and better quality seeds than other susceptible cultivars. Laura and AC Barrie were highly susceptible to WSMV producing very little or no viable seeds. AC Elsa exhibited a moderate (intermediate) reaction to WSMV and produced some viable seeds. Some of the wheat cultivars commonly grown in the Midwest of the United States and western Canada such as Butte and Columbus have probably been selected for tolerance to WSMV in an incidental manner, because of the prevalence of WSMV (Timian and Lamey,1985; Edwards and McMullen 1987). The high tolerance to WSMV in BW155 could have been inherited from its parents (Butte and Columbus) in the same manner as it was developed in 1980s when the WSM disease pressure was severe (Edward and McMullen 1987 and 1988). The time taken for symptoms to develop following inoculation may be influenced by host genotype. Such effects on incubation period may be an important factor influencing the effects of virus. The fact that in BW155 expression of WSM disease symptom was delayed and the leaves remained green longer could contribute to its better yield under disease pressure.

The inheritance of BW155 tolerance to WSMV can be studied qualitatively with the distinction of two types of chlorotic symptoms (blotch type in BW155 and thread type in Laura and AC Barrie) produced by the hosts. When a chi-square analysis was done on leaf chorosis only, the hypothesis of inheritance of tolerance conditioned by three-gene control was not rejected (Table 3.8). The symptom reaction in tolerant plants could be visually distinguished from susceptible plants. It was easy to differentiate a rating scale of 3 from 6 when leaf chlorosis alone was considered. However, the difference between 4 and 5 or 7 from 8 was difficult to assess at times, therefore, a combined rating scale, averaging three parameters, which included plant height and head mass along with the chlorosis rating, provided a better assessment. The effects of WSM are systemic. Therefore, a rating system that takes effects of height and head mass into account will give a more complete assessment of host response and true tolerance.

In this study the inheritance of BW155 derived tolerance to WSMV reaction was consistent with control by three genes (Table 3.7). Although there are reports of screening for WSMV tolerance in winter and spring wheats (Rahman et al., 1974; Edwards and McMullen, 1988; Seifers and Martin, 1988; Martin and Harvey, 1992; Haber and Townley-Smith, 1993) and in the related grass species (*Agropyron* species) (Lay et al., 1971; Larson and Atkinson, 1973; Pfannentiel and Niblett, 1978; Seifers et al., 1995) there are no published reports

in the literature of the inheritance of WSMV tolerance in spring wheat for comparison. Unpublished studies conducted while generating doubled haploid lines from BW155/Laura and screening for WSMV tolerance, have produced results consistent with three-gene control (Haber and Czarnecki personal comm.). However, the previous task involved a breeding objective, and therefore, considered only the tolerant lines and were not confirmed with analyses of susceptible lines.

The analysis of the BW155/Laura $F_2$ population indicated an additive effect at genic level in the BW155-derived tolerance, however, dominance at allelic level for each locus can not be ignored as per the $F_1$ data of the reciprocal crosses. It appeared that the tolerance increased as the number of dominant genes increased. A dominant gene at all three loci would condition BW155-like performance. Two or one dominant genes would condition an intermediate performance and plants with no dominant gene for tolerance to WSMV would exhibit a highly susceptible reaction.

Tolerance of virus infection in some barley genotypes against barley yellow dwarf virus is heritable and conditioned by major genes (Catherall et al., 1970). WSMV is also known to infect certain corn (*Zea mays* L) inbreds (How, 1963; McKinney et al., 1966 ). The inheritance of WSMV resistance in corn line Pa405 which was at first believed to be one-gene (McMullen and Raymond,

1991) has since been demonstrated to be controlled by three genes (McMullen et al., 1998).

Tolerant varieties yield better than susceptible cultivars, where virus infection causes severe crop losses. Tolerance to WSMV found in BW155 could be incorporated into other high yielding, but susceptible wheat cultivars. Additional inheritance studies are required to determine whether the three genes, identified in the BW155-derived tolerance, have a similar dosage strength. There could be a difference in number of genes involved for WSM tolerance in adult plants from seedlings since yield reduction depends on the crop stage at the time of infection. Therefore, it would be an additional benefit to the breeding program if studies were to be conducted comparing seedling tolerance to adult plant tolerance.

Fig3.1 Numbers of plants with different WSM disease ratings for BW155 (tolerant) and Laura (susceptible) parents and progenies of the cross

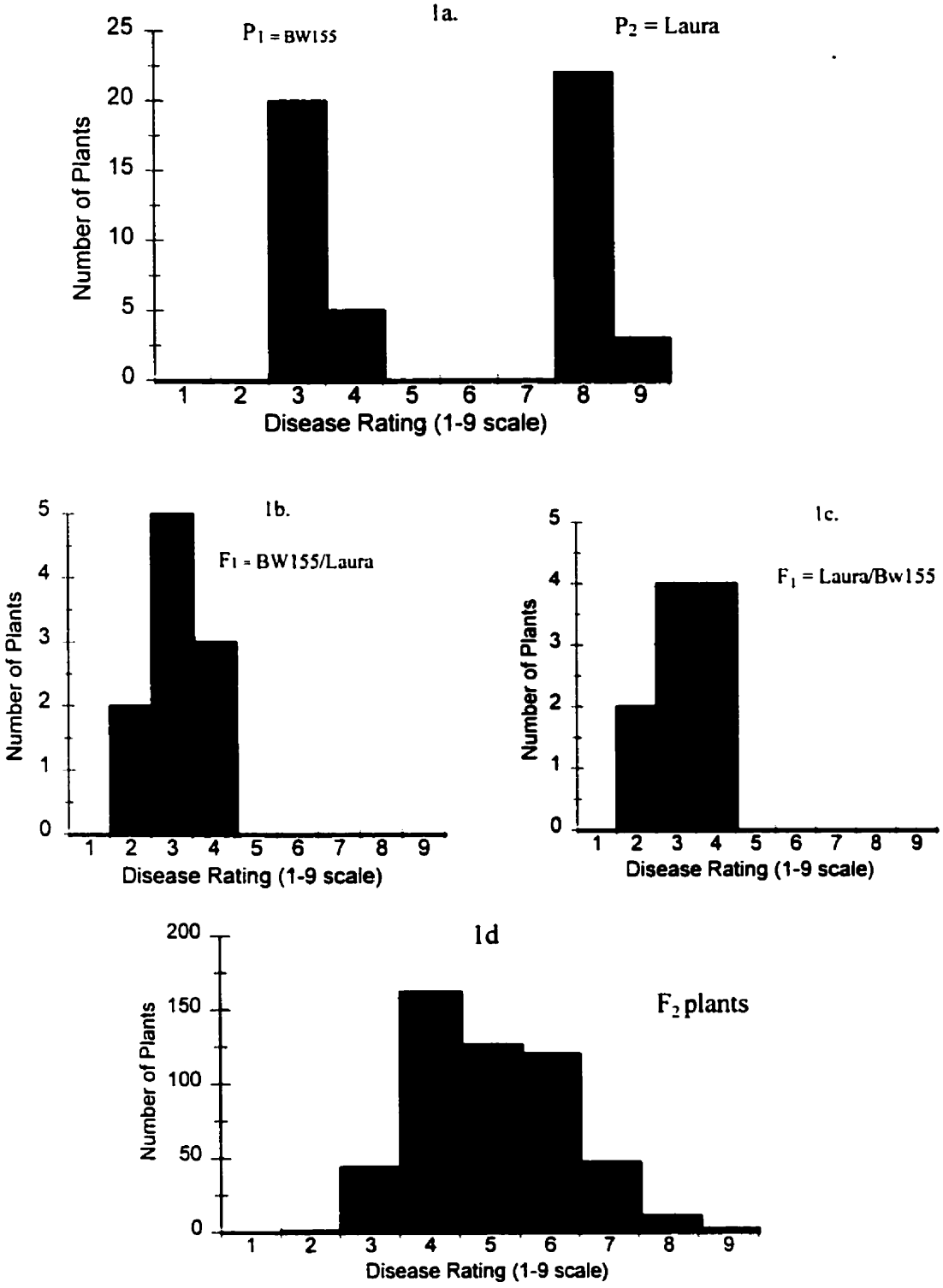
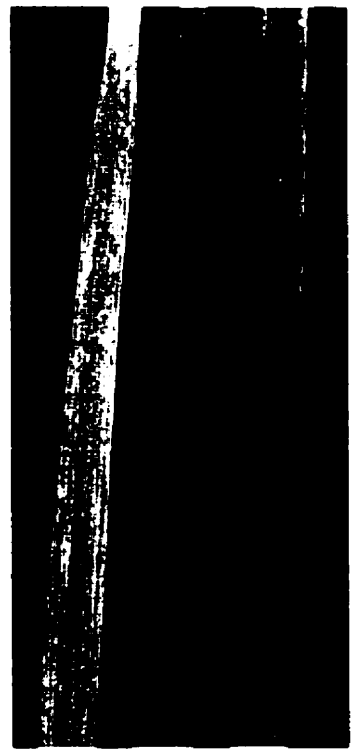


Figure3.2 Symptom expression on susceptible (Laura) and tolerant (BW155) leaves harvested 15 days post inoculation of WSMV.

1= Laura infected

2= Laura healthy

3= BW155 infected



1 2 3

Laura has more symptoms with bright chlorosis while BW155 has milder symptoms with more green.

Figure 3.3a. Healthy Laura (susceptible parent) and BW155 (tolerant parent) plants.


Healthy control plants

Figure 3.3b. BW155 and Laura under Wheat streak mosaic disease pressure.


Height and head production are severely affected in infected Laura while infected BW155 shows lesser symptoms and height reduction.

Table 3.1. Percentage yield loss under WSM disease pressure, calculated for inoculated vs non-inoculated controls, during 1992-94 field experiments conducted at Glenlea, Manitoba.

| Line | Disease rating[a] | | | Yield loss (%) | | |
|---|---|---|---|---|---|---|
| | '92 | '93 | '94 | '92 | '93 | '94 |
| Laura[b] | 8.0 | 6.5 | 7.6 | 98.1 | 29.4 | 56.7 |
| BW155[c] | 3.5 | 3.2 | 3.2 | 64.4 | 7.6 | -1.1 |
| Katepwa | 6.4 | 5.3 | 5.8 | 76.5 | 22.5 | 31.8 |
| Columbus[d] | 4.4 | 3.9 | 4.3 | 91.9 | 18.6 | 18.9 |
| Butte[d] | 4.9 | 4.6 | 4.8 | 69.9 | 20.7 | 25.9 |

(Haber and Townley-Smith, 1995)

[a]Disease rating was done at early heading stage (about 5 weeks after inoculation) on a disease rating scale of 1-9 (1 being best and 9 worst)

[b] Laura = susceptible parent used in this study.

[c] BW155 = tolerant parent used in this study.

[d] Columbus/Butte = parent for BW155.

Table 3.2. Phenotypic assessment of WSM disease reaction under 1-9 disease rating scale based on different parameters.

| Rating scale | Leaf chlorosis | Height reduction[a] | Head mass[a] reduction |
|---|---|---|---|
| 1 | no visual symptoms | none | none |
| 2 | small chlorotic areas (<5%) | negligible | ≤20% |
| 3 | ≤20%, blotchy | ≤10% | ≤40% |
| 4 | ≤30%, blotchy | ≤20% | ≤50% |
| 5 | ≤40%, short, intermittent streaks | ≤30% | ≤60% |
| 6 | ≤50%, longer streaks | ≤40% | ≤70% |
| 7 | <75% continuous streaks | ≤50% | ≤80% |
| 8 | >75% continuous streaks | severe | > 80% |
| 9 | complete | severe | plants die |

[a] height and head mass reduction were calculated against uninoculated tolerant check BW155 for $F_2$ plants, and DH and $F_1$ populations were compared to uninoculated DH and $F_1$ plants.

Table 3.3. Range of reduction in height and head mass for different populations, $F_2$ (BW155/Laura), DH96B01and DH96B03 under 1- 9 rating scale.

| | Head mass reduction range (%)[a] | | | Height reduction range (%)[a] | | |
|---|---|---|---|---|---|---|
| combined rating | $F_2$ | DH96B01 [b] | DH96B03 [b] | $F_2$ | DH96B01[b] | DH96B03[b] |
| 1 | - | - | - | - | - | |
| 2 | 12 | 22 | 16 | -5 | 12 | 5 |
| 3 | -11-46(28) | 19 -30 | 32 -37 | 1 - 22(10) | 11 - 13 | 12 -19 |
| 4 | 9 - 70 (44) | 46 | 41 - 45 | 7 - 36 (22) | 21 - 29 | 23 - 29 |
| 5 | 41 - 100 (60) | 51 -69 | 51 - 59 | 18 - 45 (32) | 30 - 39 | 32 - 39 |
| 6 | 48 - 100 (71) | 64 - 68 | 61 - 69 | 24 - 48 (36) | 40 - 45 | 43 - 49 |
| 7 | 60 - 100 (80) | 75 - 78 | 71 - 78 | 34 - 50 (44) | 51 - 58 | 50 - 56 |
| 8 | 80 - 100 (95) | 80 -83 | 80 - 84 | 53 - 62 (56) | - | - |
| 9 | 100 | 100 | - | 100 | 100 | - |

[a] $F_2$ (N = 513): Head mass (g) and height (cm) calculated against BW155 non-inoculated control. DH96B01 and DH96B03 populations: Head mass and height calculated against their respective controls (% reduction = control - infected/control x 100).

Values in the parentheses are average number per class given for $F_2$ plants

[b]DH96B01 (N = 28 lines) and DH98B03 (N = 47 lines) - percentage calculated on mean of 4 plants per line compared to corresponding non-inoculated check.

Table 3.4. Number of inoculated plants of different populations scored under different parameters using a 1-9 disease rating scale.

| Rating scale[a] | By chlorosis [b] | By height [c] | By head mass [c] | Combined[d] |
|---|---|---|---|---|
| | | **$F_2$ plants** | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1 | 1 | 1 | 1 |
| 3 | 50 | 37 | 66 | 44 |
| 4 | 174 | 101 | 143 | 162 |
| 5 | 129 | 134 | 110 | 126 |
| 6 | 112 | 162 | 90 | 120 |
| 7 | 34 | 65 | 24 | 47 |
| 8 | 11 | 11 | 29 | 10 |
| 9 | 2 | 2 | 50 | 2 |
| | | **DH96B01population** | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1 | 0 | 2 | 1 |
| 3 | 3 | 4 | 1 | 2 |
| 4 | 2 | 6 | 2 | 2 |
| 5 | 9 | 8 | 11 | 11 |
| 6 | 8 | 7 | 5 | 7 |
| 7 | 2 | 3 | 3 | 0 |
| 8 | 4 | 0 | 3 | 4 |
| 9 | 1 | 1 | 1 | 1 |
| | | **DH96B03 population** | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1 | 1 | 1 | 1 |
| 3 | 6 | 7 | 3 | 5 |
| 4 | 8 | 8 | 5 | 7 |
| 5 | 12 | 9 | 7 | 9 |
| 6 | 11 | 10 | 9 | 13 |
| 7 | 9 | 12 | 14 | 12 |
| 8 | 0 | 0 | 8 | 0 |
| 9 | 0 | 0 | 0 | 0 |

[a]Rating scale is based on chlorotic symptoms on the leaves.

[b]Number of plants categorized based on chlorotic symptoms on the leaves.

[c]Number of plants categorized based on height or head mass.

[d] Number of plants obtained form combining three parameters. Combined was calculated as the average of the three ratings, and if a rating was missing the remaining two ratings were averaged and used for chi-square analysis.

Table 3.5. Disease ratings, head mass and height of parental lines (BW155 and Laura) and $F_1$ population in presence or absence of WSMV.

| Population | No. plants | Disease rating[a] (1-9) | H. M Control[b] (g) | H. M infected[c] (g) | H. M reduction (%)[d] | Height control (cm) | Height infect (cm) | Height reduction (%)[d] |
|---|---|---|---|---|---|---|---|---|
| BW155 | 4 | 3.25 | 1.22 | 0.83 | 32 | 68.5 | 62.5 | 9 |
| Laura | 4 | 8.25 | 1.41 | 0.12 | 92 | 70.5 | 22.5 | 68 |
| BW155\Laura ($F_1$) | 10 | 3.10 | 1.88 | 1.30 | 31 | 70.0 | 68.0 | 3 |
| Laura\BW155 ($F_1$) | 10 | 3.10 | 1.90 | 1.33 | 30 | 69.5 | 68.5 | 1 |

[a] Disease rating scored on a 1-9 scale (1 =best, 9= worst) based on chlorotic symptoms on leaves (mean disease rating).
[b] H. M. control = head mass (g) of non-inoculated plants (single head).
[c] H. M . infected = head mass (g) of infected plant (single head).
[d] % reduction = non inoculated -inoculated / non-inoculated x 100.

Table 3.6. Homogeneity of chi-square analysis for two $F_2$ (BW155/Laura) population segregation classes tolerant (T), intermediate (I) and susceptible (S), for reaction to WSMV infection.

| | | | | | | |
|---|---|---|---|---|---|---|
| Laura/BW155 | | | | | | |
| | T | I | S | Total | df | $X^2$ |
| Obs. | 99 | 140 | 6 | 245 | | |
| Exp | 103 | 138 | 4 | 245 | | |
| | 0.16 | 0.029 | 1 | | 2 | 1.19 |
| BW155/Laura | | | | | | |
| Obs | 108 | 153 | 7 | 268 | | |
| Exp. | 113 | 151 | 4 | 268 | | |
| | 0.22 | 0.01 | 2.25 | | 2 | 2.48 |
| | | | | | --- | ----- |
| | | | | | 4 | 3.67 |
| Pooled | | | | | | |
| | 207 | 293 | 13 | | | |
| | 216 | 289 | 8 | | 2 | 3.56 |

$X^2$(Hom) = 3.67 - 3.56 = 0.11 $P$ = 0.57

A probability value greater than 0.05 indicates that the observed population does not differ significantly from the expected hypothesis. Data can be validly pooled.

Table 3.7. Segregation ratio and chi-square analysis for different populations, classified into three phenotypic classes based on all three parameters: tolerant (T), intermediate (I) and susceptible (S), for reaction to WSMV infection, under different models tested.

| Cross | Observed | | | Tested | $X^2$ | $P$ | Tested | $X^2$ | $P$ | Tested | $X^2$ | $P$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T[a]: | I[b]: | S[c] | **2-gene** | | | **3-gene** | | | **4gene** | | |
| BW155/Laura ($F_2$) | 207 | 293 | 13 | 9:6:1[d] | 87.68 | 0.00 | **27:36:1[e]** | 2.47 | 0.29 | 81:174:1[f] | 81.99 | 0.0 |
| A18- 4113/AC Barrie (DH96B01) | 5 | 18 | 5 | 1:2:1[d] | 2.28 | 0.32 | **1:6:1[e]** | 1.71 | 0.43 | 1:14:1[f] | 14.38 | 0.0 |
| AC Elsa /A18-4113 (DH96B03) | 13 | 34 | 0 | 2:2:0[dx] | 8.52 | 0.00 | **2:6:0[b]** | 0.07 | 0.79 | 2:14:0[f] | 8.52 | 0.00 |

[a] T =rating scale 2- 4; [b] I = rating scale 5-7; [c] S = rating scale 8-9.

[d] two gene model; [e] three gene model; [f] four gene model

[dx] Doubled haploid parent AC Elsa identified as moderately tolerant/susceptible with at least one dominant gene.
Segregation ratio rejected one-gene hypothesis . A probability value greater than 0.05 indicates that the observed population does not differ significantly from the expected hypothesis.

Table 3.8. Chi-square analyses **based on chlorosis**, blotch and thread (streak) type segregation ratio for different populations, classified in to three phenotypic classes: tolerant (T), intermediate (I) and susceptible (S).

| Cross | Observed T: | I: | S | Tested **3-gene** | $X^2$ | $P$ [a] |
|---|---|---|---|---|---|---|
| BW155/Laura ($F_2$) | 225: | 275: | 13 | 27:36:1 | 4.19 | 0.12 |
| A18- 4113/Barrie (DH96B01) | 6: | 19: | 5 | 1:6:1 | 2.62 | 0.27 |
| Elsa /A18-4113 (DH96B03) | 15 | 32 | 0 | 2:6:0 | 0.71 | 0.40 |

[a] A probability value greater than 0.05 indicates that the observed population does not differ significantly from the expected hypothesis. The chi-square values do not reject a three-gene inheritance model based on leaf chlorosis (blotch and thread type).

# 4. Combining tolerance and resistance to wheat streak mosaic virus (WSMV)

## 4.1. Abstract

Wheat streak mosaic (WSM), an important disease of wheat, caused by wheat streak mosaic virus (WSMV) has the potential to cause serious losses in winter and spring wheats. The objective of this study was to combine tolerance and resistance to WSMV. Resistance means that the host restricts the multiplication or movement of the virus within the plant, while tolerance is the ability of a host to sustain a lower level of damage given a load of infectious virus similar to that of a susceptible plant. BW155 is an advanced spring bread wheat line with tolerance to WSM. The line 7166 is resistant to WSMV and carries the Wsm1 resistance gene. To combine tolerance and resistance, 101 doubled haploid (DH) lines were generated from BW155/7166 (51 lines) and 7166/BW155 (50 lines). These lines were screened indoors under WSM pressure with mechanical inoculation. A Sequence-Characterized-Amplified-Region (SCAR) molecular marker linked to Wsm1 was used to identify lines which carried the Wsm1 gene. Wsm1-conferred resistance in 7166 is temperature sensitive and becomes ineffective at 25°C, while BW155-derived tolerance is stable over a wide range of temperature. An elevated temperature treatment was applied to identify the lines with both Wsm1 and temperature

insensitivity. Lines which performed well in a higher temperature and contained the SCAR marker were considered to possess both the tolerance and the resistance. Twenty five out of 101 DH lines, carried the SCAR marker, and 12 out of those 25 lines with the marker performed well under disease pressure, at 27°C. The12 lines which combine Wsm1 resistance with BW155-derived tolerance may form the basis of adapted, elite spring wheat germplasm that performs well under WSM pressure.

## 4.2. Introduction

Wheat streak mosaic (WSM) is caused by wheat streak mosaic virus (WSMV) and vectored by the wheat curl mite (WCM), *Aceria tosichella* Keifer (Slykhuis, 1955; Armine and Stasny, 1994). WSM has the potential to be a serious problem to both winter and spring wheat (*Triticum aestivum* L) production, in parts of the Prairie region of western Canada, the Great Plains of the United States, eastern European countries and southwest Asia (Wiese, 1987; McMullen, 1995; Haber et al.,1997). WSMV can also be transmitted mechanically (McKinney, 1949).

Yield reduction in winter wheat caused by WSM reached 18% in southern Alberta in the early 1960s (Atkinson and Grant, 1967). Localized yield losses from trace to 100% are an annual problem in individual winter and spring wheat fields, especially in southwestern Manitoba, southeastern Saskatchewan and

southern Alberta (Conner et al., 1991; Haber et al., 1997). In the United States, it is estimated that the winter wheat yield loss due to WSM is about 2 % per year (Christian and Willis, 1993; McNeil et al., 1996). An increased incidence of WSM in spring wheat since the late 1980s has been reported from the Northern Plains of the United States (Edwards and McMullen, 1987 and 1988; McMullen, 1995).

Incidence of WSM can be minimized if agronomic practices such as crop-rotation and tillage are carried out. However, breeding for resistance or tolerance to WSMV or WCM in wheat will provide a better means of controlling WSM. Resistance is characterized by the host's ability to restrict virus multiplication and movement after systemic infection; susceptibility is defined as the inability of the host to restrict virus multiplication and movement; and, tolerance is described as the ability of the host to endure specific virus multiplication and movement after systemic infection without causing severe symptoms or greatly diminishing plant growth or marketable yield (Cooper and Jones, 1983).

Resistance to WSMV is not presently available in commercial wheat cultivars (Seifers et al., 1995). Variation in tolerance to WSMV is reported from several spring wheat cultivars (McKinney, 1956; McNeal and Carroll, 1968; Rahman et al., 1974; Timian and Lamey, 1985; Edward and McMullen, 1987 and 1988; Haber and TownleySmith, 1993; McMullen, 1995). However, resistance to WSMV and WCM has been found in some wild relatives of wheat in the genera *Agropyron* and *Secale* (McKinney and Sando, 1951; Sharma and Gill, 1983).

Considerable effort has been made to transfer resistance to WSMV into winter wheat from *A. elongatum* (Host) P. Beauv. (Sebesta and Bellingham, 1963; Larson and Atkinson, 1970 and 1972) and *A. intermedium* (Host) P. Beauv. (Lay et al., 1971; Wong et al., 1974; Pfannenstiel and Niblett, 1978; Seifers et al., 1995; Friebe et al., 1996). Resistance to the WCM has been transferred into wheat from rye (*Secale cereale* L.) and *A. ponticum* (Martin et al.,1976; Conner et al., 1991; Harvey et al., 1994). Several wheat/*Agropyron* lines with resistance have been released and these lines are utilized in several winter wheat breeding programs as sources of resistance, but it has always been difficult to develop WSMV resistant lines free of agronomic or bread-making quality defects (Sebesta and Bellingham, 1963; Wells et al., 1973; Liang et al, 1979; Wells et al., 1982; Seifers et al., 1995). However, with the advent of biotechnology and innovation of molecular markers with advanced and useful techniques such as polymerase chain reaction (PCR), it has become increasingly possible to combine effective resistance to WSMV with improved yield and quality in winter wheats (Seifers et al., 1995; Chen et al., 1998).

Accession C.I.15092, derived from wheat/*A. intermedium* (Lay et al., 1971), has been found to possess WSMV resistance (Martin et al., 1976; Pfannenstiel and Niblett, 1978). C.I.17884 is a translocation line derived from radiation treatment of $F_1$ seeds of C.I.15092/T. *Speltoides* (Tausch) Gren. ex Richt// 'Fletcher' and crossed with Centurk (Wells et al., 1982; Seifers et

al.,1995). In this translocation the short arm of chromosome 4Ai-2 is translocated to the long arm of wheat chromosome 4D and the *A. intermedium* segment contains the resistance gene Wsm1 (Friebe et al., 1991). This wheat chromosome containing the translocation is designated T4DL.4Ai-2S (Seifers et al., 1995). Seifers et al. (1995) further crossed C.I.17884 with 32 WSMV susceptible cultivars and advanced experimental lines and the $F_1$ were tested for reaction to several pathogens including WSMV; two lines, KS91H174 and KS91H184 were selected, which passed the repeated screening and preliminary yield tests and contained 4DL.4Ai-2S.

KS95H103 (KS91H184/KS89H20// TAM107) is an advanced winter wheat line with improved agronomic and bread-making characteristics and is derived from winter wheat KS91H184 which is WSMV resistant (Seifers et al., 1995). It has the short arm of chromosome 4Ai-2 from *A. intermedium* translocated onto the long arm of wheat chromosome 4D (T4DL.4Ai-2S) (Seifers et al., 1995). This major gene conferring a high level of resistance to WSMV is designated Wsm1 (Friebe et al., 1991). In indoor experiments under controlled conditions, it has been demonstrated that when the translocation (T4DL.4Ai-2S) is present the resistance derived from KS91H184 is effective at 20 but not at 25°C (Seifers et al., 1995).

A Sequence characterized amplified region (SCAR) (J15) molecular marker closely linked to Wsm1 resistance gene was developed by Talbert et al.

(1996). The J15 SCAR marker was expressed as a dominant phenotype, occurring in plants homozygous or heterozygous for the resistance gene. The association of marker and resistance phenotype indicated the marker was linked to the Wsm1 gene located on the *A. intermedium* chromosome segment.

BW155 (= ND640; Butte/Columbus), an advanced spring bread wheat line, has been identified as one of the best spring wheat lines possessing WSMV tolerance (Haber and Townley-Smith,1995; Haber et al.,1998). At higher temperatures, comparisons of early stage symptom development between BW155 and KS95H103, BW155 tolerance was stable over the range of temperature but KS95H103 showed trace symptoms below 20°C, anddeveloped moderate to high symptoms at 25°C indicating that BW155-derived tolerance is temperature-insensitive (Seifers et al.,1995; Seifers and Haber, unpublished). Therefore, tolerance from BW155 and resistance from KS95H103 may be distinguished at 25°C.

Both tolerance and resistance to WSMV infection can contribute to a host's good performance. The primary objective of this study was to determine if the resistance derived from KS95H103 and tolerance derived from BW155 could be combined.

## 4.3. Materials and Methods

### 4.3.1. Virus isolate maintenance, inoculum preparation and inoculation procedure

Wheat streak mosaic virus isolate Indian Head (WSMV-IH) was used for all the experiments conducted for this study. WSMV-IH was obtained from Indian Head, Saskatchewan in 1990 and was maintained by continually growing the mechanically inoculated susceptible hosts in the growth chamber and greenhouse. For this study BW155 plants were used to maintain the inoculum since BW155 leaves remained green longer and more virus could be extracted from these leaves than from the dead tissues of other, more susceptible hosts. WSMV-IH was periodically compared to WSMV Sidney 81 isolate, whose relative virulence is well established (Seifers unpublished), by inoculating leaves of young wheat seedlings.

Leaves with the distinct mosaic symptoms of WSMV were selected and harvested for inoculum preparation. Freshly harvested leaves were ground with a mortar and pestle. The extracted sap was filtered to separate coarse tissues and diluted tenfold with distilled water (Haber and Townley-Smith, 1995). Individual plants from the experiment were inoculated at the three-leaf stage with freshly prepared virus extract. Carborundum (320 grit), an abrasive, was dusted on the leaves to facilitate virus entry. Leaves from individual plants were rubbed upward, three times, with a cotton ball soaked in virus extract.

### 4.3.2. Assessment of phenotypic reactions

Two weeks after inoculation, plants were rated on a scale based on mosaic symptoms (Seifers et al., 1995), as follows: 1= no symptoms; 2= faint mosaic with discrete widely spaced lesions; 3= moderate mosaic; 4 = severe mosaic; 5= severe mosaic with bright chlorosis. In this study individual seedlings that responded to WSMV inoculation with small, discrete, widely spaced lesions (rating scale 2) on the next emerging leaf were considered resistant. These plants were expected to carry the Wsm1 gene which could be identified by detecting the presence of the J15 SCAR marker. Seedlings which, by contrast, expressed systemic chlorosis, with moderate to severe mosaic (rating scale 3, 4, 5) on later emerging leaves were considered susceptible or less resistant and therefore not expected to be positive for the Wsm1 marker. At 20°C, symptom from BW155 tolerance were not evaluated as it could have been masked by resistance effect.

### 4.3.3. Population development

Resistance was derived from KS95H103, a winter wheat line with the Wsm1 gene. KS95H103 was crossed with AC Elsa, a high quality Canada Western Red Spring (CWRS) wheat cultivar moderately susceptible to WSMV, and the $F_1$ backcrossed to AC Elsa (AC Elsa /KS95H103//AC Elsa) to produce a $BC_1$ $F_1$ population known as 7166. 7166 was selected for its resistant phenotype

and spring growth habit but was not selected for J15 SCAR marker as the marker was not available then. This 7166 with resistance was generated previously in the program and used for this study. BW155, formerly known as ND640, was developed in North Dakota by crossing Butte (an American spring wheat cultivar) and Columbus (CWRS wheat cultivar). BW155 was found tolerant to WSMV in field and green house tests (Haber and Townley-Smith, 1995)

A cross (BW155/7166) and its reciprocal (7166/ BW155) were made from BW155 and 7166. The $F_1$ plants of those crosses were used to generate doubled haploid lines. The $F_1$ plants were selected for the resistant phenotype before generating doubled haploid lines but were not tested for the presence of the marker. The two doubled haploid populations produced were: DH98B (51 lines) from BW155/7166 and DH98R (50 lines) from 7166/BW155.

### 4.3.4. Generation of doubled haploid (DH) lines $F_1$ of BW155/7166 and reciprocal

The method used to generate DH lines developed at the Cereal Research Centre, AAFC, Winnipeg, MB (Taing Aung personal comm), modified from Laurie and Bennet (1988) was followed in this program. Haploid production using maize pollen has been more efficient than the use of pollen from *Hordeum bulbosum* and is more easily applied in conventional breeding programs (Inagaki and Tahir, 1990; Suenaga et al.,1991).

The doubled haploid technology used in this research refers to the in vitro culturing of embryos obtained from crosses made from the maize (*Zea mays* L. cvs. Seneca 60, Golden Bantam, Manitoba Sweet and Indian Flint corn) and the wheat $F_1$ hybrids ( BW155/7166) considered for the development of WSMV tolerant germplasm. Pollen from the maize lines bred (Cereal Research Centre, Winnipeg, MB) for this particular technology for wheat and oats, was used to stimulate embryo development in the wheat caryopsis. Corn plants were grown continuously in growth cabinets set at 20°/18°C with a 16h photo period.

Five $F_1$ plants of BW155/7166 and its reciprocal were grown singly in 15 cm clay pots in growth cabinets set at 16/8 h light/dark and 16°/13°C . At the three-leaf stage the plants were inoculated with WSMV-IH. Three of the five inoculated plants that showed resistant phenotypes were allowed to grow and produce 6-8 tillers, and new emerging tillers were trimmed. The plants were fertilized biweekly with 20:20:20 (N:P:K) fertilizer until heading, and with 15:28:15 after heading until maturity.

At anthesis, wheat florets were emasculated 12 to 24 hours before anthers dehisced or when they still looked green, to avoid accidental selfing. The tools (forceps and scissors) were disinfected with 70% ethanol after each emasculation. Pollination was done 12 to 24 hours after the emasculation, when the stigmas became receptive. Pollen grains from the corn were applied manually by dusting recently matured (opened) corn anthers on top of the wheat

stigma. Large and plump anthers contained more pollen and could pollinate more than one floret. Availability of pollen depended on the timing of pollination, mornings (10:00 -12:00 noon) were always better than the afternoons, as corn pollen is usually shed during late mornings. After emasculation individual tillers were tagged with information on parental source and day of emasculation, and this information was updated after pollination. The pollinated wheat florets were sprayed with an aqueous 2,4-D (100mg/l) for two consecutive days after pollination.

The wheat spikes were harvested from plants 14-16 days after pollination, caryopses were removed from the florets and sterilized with 60% sodium hypochlorite solution for 2 minutes then for 40 seconds in 70% ethanol, washing twice with distilled water in between the sterilizing treatment. The developed embryos were rescued from the fertilized caryopses with the help of a microscope under sterile conditions in a laminar flow hood and placed in Gamborg's B-5 nutrient medium (Appendix 7.1) in a glass vial.

These developed embryos were treated with three days of cold conditions in the fridge (2-5°C) and with two days of darkness at room temperature. After the dark treatment, yellowish embryos growing in the nutrient media were placed on the nursery bench where they received 16 hour light and 8 hour dark at 21°C (constant), and eventually haploid plantlets grew. These plantlets were

transferred to a soil-less mixture (metromix) after the development of well formed root and shoot systems (about 3-4 cm long).

When the plantlets were at the two to three-tiller stage, they were treated with colchicine (Appendix 7.2) for chromosome doubling. Plantlets were treated with an aqueous 0.2% (v/v) solution of colchicine for 2.25 hours, and rinsed in running water for 2.5 hours. The treated plants were transferred to 8 cm pots and allowed to grow until roots were well developed. When the plants had revived after colchicine shock, they were transferred to 10 cm pots and allowed to mature. Plants that survive colchicine treatment produce fertile tillers and seeds, and these seeds are known as doubled haploid seeds. Although most of the regenerated doubled haploid plants produced healthy and vigorous plants with normal spikes and seeds, it was necessary to grow all seeds for one generation to produce uniformity in their seed shapes and sizes, and to accumulate sufficient amount of DH seeds for experiments.

### 4.3.5. Identification of lines with Wsm1

To identify the lines containing the Wsm1 gene, a Sequence Characterized Amplified Region (SCARs) (J15) marker derived from a polymerase chain reaction (PCR) generated DNA band, designed by Talbert et al. (1994 and 1996), was used. A characteristic band formed by the tightly-linked

J15 SCAR marker during gel electrophoresis should confirm the presence or absence of Wsm1 resistance gene in the young wheat plant.

### 4.3.6. DNA extraction procedure

This study used the DNA extraction procedure used by Kim et al. (1989) with one modification. In this study 5M NaCl was premixed in the extraction buffer. Leaves from young wheat plants (at the four-leaf stage, three pieces, about three centimetre long), growing in the green house at 20/16°C under 16 hour photoperiod, were harvested into 15 ml plastic tubes, labelled, frozen in liquid nitrogen and then lyophilized for 18 hours. Leaves were pulverized by adding glass beads (2ml - 3mm diameter) and silica sand (1 ml) to the tubes and shaking the tubes using a paint shaker (30 seconds). A mixture of 5 ml extraction buffer, 30 $\mu$l proteinase K and 660 $\mu$l SDS (Appendix 7.3) were added to each tube and shaken lightly. The samples were then incubated at 65 °C for 2 hours. The samples were removed from the water bath and cooled for 5 minutes. A chloroform/isoamyl alcohol mixture (24:1, v/v) was added to each tube (6 ml) and shaken for 20 minutes at low speed in the fume hood. The mixture was centrifuged for 10 minutes at 5000 rpm, and the upper supernatant was transferred into a clean test tube. The DNA was precipitated with 0.6 volumes of ice cold (-20 °C) isopropanol. The precipitated DNA in suspension was withdrawn using a pipette and transferred into micro tubes. The DNA was then

washed with 70 % ethanol and left at -20°C for 18 hours. The next morning the micro tubes containing the DNA were centrifuged at 15000 rpm for 20 minutes. The ethanol was decanted and then air dried for 1 hour. After the DNA was dissolved in 500 µl of autoclaved de-ionized distilled water (DDW) it was ready for PCR.

**4.3.7. Polymerase Chain Reaction (PCR)** (Appendix 7.4)

A PCR mixture (50 µl/tube) was prepared as follows:

27 µl DDW

7.5 µl 5x RT-PCR buffer

3.0 µl $MgCl^2$

5.0 µl 10x dNTP

2.5 µl right primer (CCGAGCTCACACGCTAATTT)

2.5 µl left primer (GTAGCAGGGGAAGCTGAAGA)

1.0 µl Chill-out 14°C liquid wax

1.0 µl extracted DNA

0.5 µl Taq

This study used the PCR procedures used by Haber et al. (1995) and Talbert et al. (1994) with some modifications (Appendix 7.5). The PCR reaction

mixture was prepared in a 0.5 ml micro tube on ice. Once all the ingredients were added the tubes were loaded in a PTC-100 thermocycler ( MJ Research Inc.) set on an amplification cycle specific to the Wsm1 marker. The typical temperature conditions for PCR were 94°C for 3 min, followed by 43 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min. The amplification cycle runs for 4 hours, with the final step chilling the sample to 4°C, thus halting all reactions in the tube.

### 4.3.8. Gel electrophoresis

A solidified 1% agarose gel (Appendix 7.6) was placed in a buffer tank containing 0.5 x TAE buffer. Each sample (10 $\mu$l) was prepared (2 $\mu$l stop solution + 8 $\mu$l PCR product) and loaded into predetermined wells on the gel. A 1 Kb DNA ladder (Appendix 7.6) was loaded in the first well to provide a stainable kilobase marker. The gel was run at 40 volts for 1-4 hours. A photograph of the gel was taken after checking it for the presence or absence of the marker under ultra violet light in the dark room. Ethidium bromide binds to DNA and fluoresces under ultraviolet light thus allowing visualization of the J15 SCAR marker bands.

### 4.3.9. Screening of DH lines under controlled environment

Two sets of four plants of each line of the doubled haploid populations (DH98B, DH98R) and the checks (AC Elsa, BW155, 7166; susceptible line

Laura) were grown in 15 cm fibre pots, filled with soil mixture composed of 2:1:1 (v:v:v) soil, sand and peat. Laura, a CWRS cultivar, has been identified as a highly susceptible to WSMV (Haber and Townley-Smith, 1993 and 1995). Individual plants from one set were mechanically inoculated at three-leaf stage (seedling stage) with freshly prepared virus (WSMV-IH) solution using the method described above. The other set was grown as the non-inoculated controls. All the experiments were conducted in a green house at 16 hour light / 8 hour dark and 20/16°C. Plants were fertilized biweekly with 20:20:20 (N:P:K) fertilizer until heading, and with 15:28:15 after heading until maturity. Two weeks after inoculation, plants were rated on a scale (1-5) based on mosaic symptoms (1= no symptoms; 2= faint mosaic with discrete, widely, spaced lesions; 3= moderate mosaic; 4 = severe mosaic; 5= severe mosaic with bright chlorosis) (Seifers et al., 1995). Individual seedlings that responded to WSMV inoculation with small discrete widely spaced lesions were considered resistant. Seedlings which, by contrast, expressed systemic chlorosis on later emerging leaves were considered susceptible.

### 4.3.10. Identifying lines with combined resistance and tolerance

Three sets of four plants of each of the 25 DH lines, (10 from DH98B, 15 from DH98R), with J15 SCAR marker, BW155 and 7166 were grown in 15 cm fibre pots in growth cabinets (one set) and green house (the other two sets).

Plants in the cabinet and one set in the green house were inoculated with WSMV-IH at the three-leaf stage. The other set in the greenhouse was grown as non-inoculated controls. Experiments for all the three sets were conducted in similar conditions until two weeks after inoculation. To identify the lines with combined tolerance and resistance, two weeks post-inoculation and after symptom reading, the temperature in the cabinet was elevated and maintained at 27°C for another two weeks. Two weeks later, the cabinet temperature was dropped back to normal (20/16°C). Plants were transferred to green house benches before head maturity. Plant height and single head mass were taken at maturity. This treatment of inoculated plants with higher temperature should have disabled the temperature-sensitive Wsm1-conferred resistance. DH Lines with systemic chlorosis on later emerging leaves but that suffered lower height and head mass reduction compared to the non-inoculated controls and inoculated plants at 20°C would be considered to have possessed both resistance and tolerance.

### 4.3.11. Statistical analyses

Segregation ratios for J15 SCAR marker and resistant phenotype (gene) in DH98B and DH98R population were tested for fit of several genetic models using chi-square analysis, and Yates' correction factor where appropriate (Strickberger, 1985). Height and head mass of inoculated plants, with Wsm1

marker, under two temperature treatments were compared with the non-inoculated control plants of each DH line, respectively.

## 4.4 Results

### 4.4.1. Generation of doubled haploid (DH) lines

A total of 870 caryopses were dissected 14 - 16 days after pollination of the wheat florets by maize pollen (Table 4.1). Of these, 272 (31.26 %) produced embryos (135/470 embryos for BW155 /7166, and 137/ 400 for 7166/BW155). Most of the fertilized caryopses did not contain any endosperm or the embryo. They resembled green sacs containing watery fluid, indicating an early abortion of the embryo.

Out of 145 haploids, 101 or 70% survived the colchicine treatment, developing into healthy DH plants (Table 4.1). These plants produced fertile florets bearing over 20 seeds per plant and these seeds were harvested as doubled haploid seeds. Cytological analyses of mitotic chromosome numbers, and chromosome pairing at meiosis were not conducted as the generated lines were further required to be screened under disease pressure which would eliminate lines with any abnormal phenotypes. Wheat haploids are generally sterile whereas doubled haploid plants are fertile and the aneuploid plants possess reduced fertility. About 98-99 % of a set of plants generated from wheat/

maize cross that survive colchicine treatment are doubled haploids (T. Aung personal communication).

### 4.4.2. Test for presence of J15 SCAR marker

DNA analysis of the plant samples, of DH98B and DH98R populations, taken at the seedling stage detected a total of 25 out of 101 lines showing the J15 diagnostic band. Ten out of fifty-one DH98B and fifteen out of fifty DH98R DH lines contained the J15 marker band. A picture of a minigel containing electrophoresed, PCR-amplified DNA of the parents, DH98B and DH98R lines showing presence or absence of the characteristic marker band is presented in Figure 4.1. An expected ratio of 1:1 (marker presence: marker absence ), from a tightly linked marker and the major gene was not obtained (Table 4.2). However, the observed frequency of the J15 SCAR marker in the DH98 population (25/101) clearly fit a 1:3 ratio of J15 SCAR marker present to marker absent.

### 4.4.3. Screening for phenotypic reaction

The phenotype of response to seedling infection was determined on the basis of visual symptoms. If an infected seedling responded by producing widely-spaced discrete chlorotic lesions or no lesions at all, it was scored as resistant; chlorotic streaking, mosaic or necrosis was scored as a susceptible response. Among the DH lines of the DH98B and DH98R populations, four combinations of

resistance phenotype and presence/absence of J15 DNA marker are possible (Table 4.3): a) phenotype susceptible / marker absent; b) phenotype resistant / marker present; c) phenotype resistant / marker absent; and d) phenotype susceptible / marker present.

In this study, 20 out of 51 DH98B lines showed a resistant phenotype. Only eight of these 20 lines possessed the J15 SCAR marker. Similarly, 24 out of 50 DH98R lines exhibited a resistant phenotype, of which only 12 possessed the marker. Chi-square analysis fails to reject the hypothesis of 1:1 ratio for resistant:susceptible phenotype in both the DH98B and DH98B populations (Table 4.4) but clearly rejects a 1:1 ratio for presence:absence of the J15 SCAR marker.

The SCAR marker J15 is located within the *A. intermedium* segment translocated in wheat chromatin (Talbert et al., 1996; Haber and Seifers unpublished). In an earlier study, Talbert et al. (1996) observed near-perfect correlation between the resistance gene Wsm1 and presence of the J15 SCAR marker, and concluded they were tightly linked. However, in a population of 81 [$F_3$-derived $F_4$ (KS93WGRC27/KS84063-9-12-1)] lines, one of the 81 lines had a susceptible reaction despite carrying the marker. Recombination between Wsm1 contained in the translocated *Agropyron* chromosome and the homeologous wheat chromosome was expected to occur rarely, if at all, as

pairing had almost never been observed (Friebe et al., 1991; Talbert et al., 1996).

In my investigation, by contrast, two DH98B and three DH98R lines containing the J15 SCAR marker (as detected by gel electrophoresis) had susceptible reactions (Table 4.3). The converse was also observed, as some of the DH lines (in both populations) that lacked the J15 SCAR marker exhibited a resistant phenotype (Table 4.3). In not exhibiting a 100% linkage between presence of the J15 SCAR marker and WSMV resistance, my DH98B and DH98R populations clearly differ from those examined in earlier studies (Friebe et al., 1991; Talbert et al., 1996).

### 4.4.4. Lines that combine tolerance and resistance (Wsm1)

Eighteen out of 25 DH lines which contained the J15 SCAR marker (seven from DH98B and eleven from DH98R) responded to WSMV-IH inoculation under similar temperature regimes (near-constant 20°C), in both growth cabinet and greenhouse experiments, with small, discrete, widely-spaced lesions (rating scale 2). Five DH lines in total from the two populations exhibited moderate to severe mosaic symptoms in both settings. Four weeks later, plants in the growth cabinet were again rated for symptom progression caused by temperature elevation, from 20 to 27°C, intended to disable Wsm1-conferred resistance. All plants that had experienced elevated temperatures in the growth

cabinet exhibited moderate to severe mosaic symptoms, whereas plants of the same lines that had remained in the green house at 20°C did not develop further symptoms on later emerging leaves.

In both experiments, some lines showed much smaller losses of height and yield (compared to uninoculated controls) than others. The lines with the smallest losses in both temperature regimes were clearly showing BW155-derived tolerance which could have been masked when resistance is not abolished by elevated temperature (Table 4.5 and 4.6). There were four lines from DH98B and eight lines from DH98R that experienced less reduction in height and yield (head mass) under both temperature regimes compared to their uninoculated counterparts and parental lines. These 12 lines in total from two populations were considered to have combined Wsm1 resistance with tolerance derived from BW155.

## 4.5. Discussion

Reaching the homozygous state is of primary importance in production of new breeding lines. The generation of doubled haploid (DH) lines provides one of the fastest methods of obtaining completely homozygous lines in a single generation (Poehlman,1987). Laurie and Bennett (1988) suggested that wheat/maize crosses have the potential to produce healthy haploid wheat plants in up to 33% of the florets. Therefore, 287 florets (33% of 870 total florets)

florets were anticipated to develop embryos upon fertilization close to the observed number of 272. Such a high success rate, approaching to the potential maximum number, may be due to the facts that the genotypes used in this study were derivatives of well- adapted wheat cultivars; the DH generation protocols were specifically developed for North American cultivars, and the efficacy of the methodology had been well tested. In this study a total of 101 doubled haploid lines were generated successfully.

The use of J15 SCAR marker was intended to facilitate the identification of DH lines containing the (supposedly) tightly-linked Wsm1 resistance gene early in the generation, avoiding a labourious, time consuming screening processes (Talbert et al.,1996). However, my observations with the DH98 population were not consistent with a 1:1 ratio, indicating the linkage between the J15 marker and the Wsm1 gene was broken for many of the lines. The findings of the current study suggest that it may not be advisable to depend entirely on marker-assisted selection to identify resistant lines. It is possible one will discard some of the highly resistant DH lines and select actually susceptible lines.

The recognition of the small, distinct, widely spaced mosaic symptoms produced by isolates of WSMV on wheat with resistant phenotypes and severe mosaic with susceptible, supported by height and yield data, has been used in breeding programs since the 1960s (Mckinney and Sando, 1951; Sebesta and

Bellingham, 1963; Seifers et al., 1995) and has helped analysis and interpretation of results from such studies. The development of a molecular marker has facilitated the identification of plants which incorporated the Wsm1 resistance gene from *A. intermedium* (Friebe et al., 1991; Talbert et al., 1996). In these studies both types of assessment were used to assess the DH lines which could have combined tolerance and resistance.

Earlier studies of Friebe et al. (1991) and Talbert et al. (1996) on Wsm1 gene and development of a linked J15 SCAR marker in the translocated *A. intermedium* chromatin indicated that recombination between wheat and *Agropyron* chromatin should not occur readily. However, results from this study show that recombination must have occurred in some cases. For example, DH98B70, a marker positive line, exhibited severe symptoms and DH98B108 and DH98R111, marker negative lines, showed a highly resistant phenotype. In the postulated process of recombination the DH lines could have also lost some portion of *A. intermedium* chromatin that contains Wsm1 and thus increased the temperature sensitivity of Wsm1. This postulated effect would be an extension of the effect observed by Seifers et al. (1995), where lines with shortened *A. intermedium* chromatin retained the near-immunity of substitution line CI.15092 at 20°C but showed susceptible responses at sustained temperature above 25°C.

Although temperature-sensitive, resistance derived from KH91H184 is considered to be highly useful, as this genotype exhibits better agronomic traits than many other lines that were screened (Seifers et al., 1995). Tolerance derived from BW155 has been shown to be useful source for WSMV tolerance in spring wheat germplasm. This study has shown that the combining ability between Wsm1 in the spring wheat back ground of 7166 and BW155 is excellent. Twenty-five lines incorporating the J15 SCAR marker which was used to identify the Wsm1 gene, plus those lines exhibiting a resistant phenotype but do not carry the marker, are available for further genetic/cytogenetic studies. Twelve DH lines which were identified with combined Wsm1 resistance and BW155 tolerance may form the basis of adapted, elite spring wheat germplasm that performs well under WSM pressure.

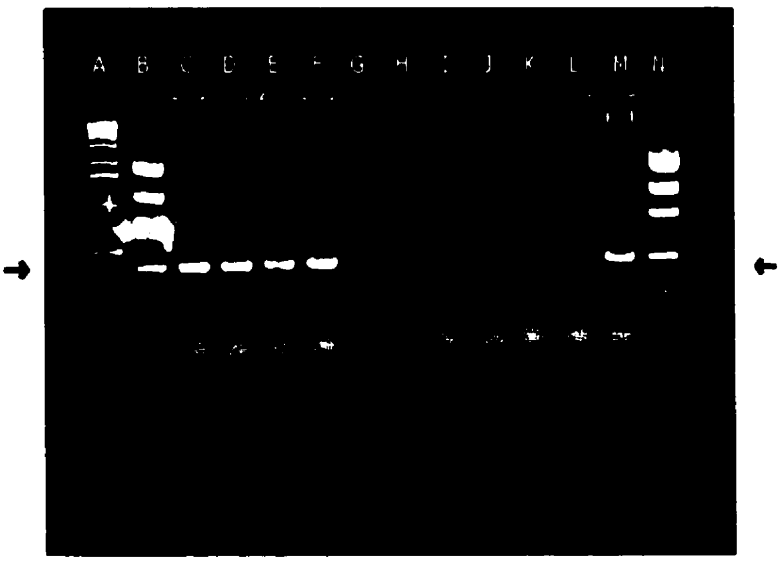



Fig. 4.1 Presence or absence of J15 SCAR marker on a 1.7% agarose gel stained with ethidium bromide. Lane A 1 Kb ladder; lane B low DNA mass ladder; lanes C and D DH98R lines positive for J15 SCAR marker; lanes E and F DH98B lines positive for J15 SCAR marker; lane G tolerant line BW155 containing no marker; lane H moderately susceptible spring wheat cultivar (AC Elsa) containing no marker; lanes I and J DH98R lines marker negative; lanes K and L DH98B lines marker negative; lane M KS95H103 containing J15 SCAR marker and lane N low DNA mass ladder. The arrow points to a band of approximately 450bp.

Table 4.1 Number of embryos, haploid and doubled haploids recovered from caryopses allowed (14 - 16 days) develop on plants after pollinating with maize pollen.

| | DH98B | DH98R | Total |
|---|---|---|---|
| No. of spikes | 26 | 24 | 50 |
| No. of florets pollinated with maize pollen | 470 | 400 | 870 |
| No. of embryos found | 135 | 137 | 272 |
| Haploid plantlets that survived colchicine | 70 | 75 | 145 |
| Doubled haploids generated | 51 | 50 | 101 |

Table 4.2 Segregation ratio and Chi-square analysis for two DH population DH98B and DH98R based on presence (M) or absence (m) of J15 SCAR marker.

| Cross | Class[a] | Obs. | Tested ratio | $X^2$ | P[b] | Tested ratio | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|
| BW155/7166 | M | 10 | | | | | | |
| (DH98B) | m | 41 | 1:1 | | | 1:3 | | |
| | | | | 17.64 | 0.00 | | 0.53 | 0.46 |
| 7166/BW155 | M | 15 | | | | | | |
| (DH98R) | m | 35 | 1:1 | | | 1:3 | | |
| | | | | 7.22 | 0.00 | | 0.43 | 0.47 |

[a] M = J 15 SCAR marker detected
m = J15 SCAR marker not detected in the gel electrophoresis

[b] A probability value greater than 0.05 indicates that the observed population does not differ significantly from the expected hypothesis. Yates correction factor was applied.

Table 4.3 Phenotypic response and J15 SCAR marker (presence / absence) for different combinations in DH98B and DH98R populations.

| Cross | No. of Lines tested | Phenotype susceptible marker absent | Phenotype resistant marker present | Phenotype resistant marker absent | Phenotype susceptible marker present |
|---|---|---|---|---|---|
| BW155/7166 (DH98B) | 51 | 29 | 8 | 12 | 2 |
| 7166/BW155 (DH98R) | 50 | 23 | 12 | 12 | 3 |
| Grand Total | 101 | 52 | 20 | 24 | 5 |

Table 4.4 Segregation ratio and Chi-square analysis for DH98B and DH98R population based on phenotypes. R = resistant phenotype; S = susceptible phenotype

| Cross | Class[a] | Obs. | Exp[b]. | $X^2$ | P[c] |
|---|---|---|---|---|---|
| BW155/7166 | R | 20 | 25.5 | | |
| (DH98B) | S | 31 | 25.5 | 1.96 | 0.51 |
| 7166/BW155 | R | 24 | 25 | | |
| (DH98R) | S | 26 | 25 | 0.02 | 0.7 |

[a] R = resistant phenotype rated 1-2 scale

S = susceptible phenotype rated 3-5 scale

[b] = tested ratio 1:1

[c] A probability value greater than 0.05 indicates that the observed population does not differ significantly from the expected hypothesis. Yates correction factor was applied.

Table 4.5. Effect of temperature on symptom expression and subsequent effect on heights (cm) of DH98B and DH98R lines (mean of four plants) carrying J15 SCAR marker.

| Lines | Height of Control plant (cm) (20°C) | Height of infected plant (cm) (20°C) | Height reduction [a] (%) | Height of infected plant (cm) (27°C) | Ht. reduction[a] (%) |
|---|---|---|---|---|---|
| B26 | 55.5 | 49.5 | 11 | 43.3 | 22 |
| B69 | 50 | 40 | 20 | 30 | 40 |
| B70 | 58.5 | 48.5 | 17 | 30.5 | 48 |
| B75 | 58.8 | 55.5 | 5 | 52.3 | 11* |
| B89 | 59.3 | 55.8 | 6 | 53 | 11* |
| B93 | 57.5 | 52 | 10 | 43 | 25 |
| B212 | 75.5 | 68.5 | 9 | 54.5 | 28 |
| B229 | 64 | 57 | 10 | 42.5 | 34 |
| B244 | 54.8 | 53 | 3 | 50 | 9* |
| B246 | 69.5 | 65.5 | 6 | 62 | 11* |
| R41 | 55.5 | 40 | 28 | 30 | 46 |
| R52 | 52 | 50 | 4 | 30 | 42 |
| R55 | 50.8 | 32 | 37 | 29 | 43 |
| R68 | 52.5 | 48 | 9 | 35 | 33 |
| R75 | 57 | 35 | 39 | 30 | 47 |
| R78 | 59.5 | 56 | 6 | 56.5 | 5* |
| R100 | 58.5 | 55.5 | 5 | 55 | 6* |
| R105 | 55.8 | 50.5 | 9 | 43.5 | 22 |
| R118 | 65 | 62.5 | 4 | 60.2 | 57 |
| R119 | 62 | 65 | -5 | 60 | 3* |
| R121 | 66 | 63.5 | 4 | 61.5 | 7* |
| R129 | 59.5 | 55 | 8 | 54 | 9* |
| R233 | 64 | 60 | 6 | 56.5 | 13* |
| R238 | 65 | 61.5 | 5 | 60 | 8* |
| R243 | 49.5 | 47 | 5 | 47 | 5* |
| 7166 | 64 | 60 | 6 | 50 | 22 |
| BW155 | 62.5 | 55 | 12 | 54 | 14 |

* = lines showed lower height reduction [a] Relative to non-inoculated controls

Table 4.6 Effect of temperature on symptom expression and subsequent effect on yields (single head mass in gram) of DH98B and DH98R lines carrying J15 SCAR marker (mean of four plants).

| Lines | Yield of Control plant(g) (20°C) | Yield of infected plant (g) (20°C) | Yield reduction [a] (%) | Yield of infected plant (g) (27°C) | Yield reduction [a] (%) |
|---|---|---|---|---|---|
| B26 | 0.75 | 0.65 | 13 | 0.35 | 53 |
| B69 | 0.85 | 0.26 | 69 | 0.20 | 76 |
| B70 | 0.95 | 0.35 | 63 | 0.25 | 74 |
| B75 | 0.75 | 0.65 | 13 | 0.55 | 27* |
| B89 | 1.01 | 0.99 | 2 | 0.85 | 16* |
| B93 | 0.95 | 0.90 | 5 | 0.47 | 51 |
| B212 | 1.11 | 0.1 | 10 | 0.55 | 51 |
| B229 | 1.06 | 0.95 | 10 | 0.60 | 43 |
| B244 | 0.85 | 0.90 | -5 | 0.75 | 12* |
| B246 | 1.00 | 0.95 | 5 | 0.72 | 28* |
| R41 | 0.49 | 0.42 | 14 | 0.25 | 50 |
| R52 | 0.61 | 0.50 | 18 | 0.30 | 51 |
| R55 | 0.65 | 0.34 | 51 | 0.29 | 55 |
| R68 | 0.82 | 0.65 | 21 | 0.35 | 57 |
| R75 | 0.78 | 0.34 | 56 | 0.30 | 63 |
| R78 | 0.77 | 0.73 | 5 | 0.65 | 16* |
| R100 | 0.64 | 0.6 | 6 | 0.51 | 20* |
| R105 | 0.67 | 0.50 | 25 | 0.35 | 48 |
| R118 | 0.77 | 0.72 | 7 | 0.65 | 16* |
| R119 | 0.79 | 0.77 | 3 | 0.68 | 14* |
| R121 | 0.79 | 0.79 | 0 | 0.65 | 18* |
| R129 | 0.69 | 0.65 | 6 | 0.55 | 20* |
| R233 | 0.70 | 0.67 | 4 | 0.55 | 21* |
| R238 | 0.71 | 0.65 | 9 | 0.60 | 16* |
| R243 | 0.77 | 0.65 | 16 | 0.47 | 39 |
| 7166 | 0.95 | 0.80 | 17 | 0.50 | 47 |
| BW155 | 1.05 | 0.85 | 19 | 0.82 | 22 |

* = DH lines showing lower yield reduction

[a] Relative to non-inoculated controls

## 5. General Discussion

While the importance of wheat streak mosaic as a disease of winter wheat is well established, its potential importance as a disease of spring wheat also should be recognized. Practices that increase the chances of overlap between spring and winter crops as well as observed increase in disease incidence in spring wheat make it crucial that we better understand the effects of WSMV on currently grown spring wheat cultivars.

Although there are other existing control measures for WSMV, breeding for resistant cultivars of wheat will provide the best means of controlling WSM. Clearly, a broad range in tolerance to WSMV infection exists among the tested spring wheat cultivars and lines (McKinney, 1956; McNeal and Carroll, 1968; Rahman et al., 1974; Timian and Lamey, 1985; Edward and McMullen 1987; Haber and TownleySmith, 1993). Experiments conducted by Edwards and McMullen (1987, 1988), Haber and Townley-Smith, (1993, 1995) clearly showed the performance of Butte and Columbus (parents to BW155), being better than many of the other tested cultivars. The level of tolerance possessed by spring bread wheat advanced lines such as BW155, BW250 and BW252 is among the best available (Haber, personal communication) and therefore, can be considered potentially useful in developing cultivars for commercial field use.

Periodic WSMV outbreaks in all the prairie provinces and high level of WSMV in1988-89 in Indian Head, Saskatchewan, serve to demonstrate the

vulnerability of existing spring wheat cultivars to WSMV (Haber et al., 1997). The importance of controlling WSMV is further under scored by the severity of losses it can cause when present in mixed infections with High Plain virus (Jardine et al., 1994). High plains virus disease of wheat and corn, also transmitted by wheat curl mite, the vector of WSMV, became an economically significant disease in the United States in the late 1990s (Jensen et al., 1996; Seifers et al., 1997; Mahmood et al., 1998; Marcon et al., 1999).

The present study was undertaken to determine the genetic inheritance of tolerance to WSMV in BW155, an advanced spring wheat line, identified as more tolerant than other contemporary CWRS wheat cultivars (Haber and TownleySmith, 1995). This is the first study conducted of the inheritance of spring wheat-derived tolerance to WSMV. Information on the number of genes, their allelic and genic interactions are important for breeding purposes. This research determined that the inheritance of BW155-derived tolerance in different populations, $F_1$, $F_2$ and $F_3$ (BW155/Laura), DH96B01 (A18-4113/AC Barrie) and DH96B03 (AC Elsa/A18-4113) is controlled by three complementary dominant genes with additive effect at genic level.

Natural epiphytotics of WSM are very unpredictable and there has been little success in inducing natural infection in the field (Martin et al 1984). Therefore, trials employing mechanical inoculation have been both necessary and convenient. The effects seen in tests employing mechanical inoculation are

usually more severe than those seem in outbreaks that arise under natural conditions. However, severe losses have been observed under environmental conditions conducive to vector reproduction and movement (Atkinson and Grant, 1967).

In this study, the result obtained from the use of qualitative disease assessment criterion, lesion type (blotch and thread type) followed by Haber and Townley-Smith (1993), was equally significant (shown by chi-square analysis) as the combined ratings which included plant height and head mass. Evidence of gene-dosage was shown from the analysis of different populations studied, as the plants were able to be grouped in three levels (tolerant, intermediate and susceptible).

Tolerant varieties yield better than susceptible cultivars, where virus infection causes severe crop losses. Tolerance to WSMV found in BW155 could be incorporated into other high yielding but susceptible wheat cultivars. Additional inheritance studies are required to determine whether the three genes, identified in the BW155-derived tolerance, have a similar dosage strength. There could be a difference in number of genes involved for WSM tolerance in adult plants from seedlings since yield reduction depends on the crop stage at the time of infection. Therefore, it would be an additional benefit to the breeding program if inheritance of tolerance is compared for seedling and adult plant tolerance. The only published studies of WSMV inheritance are

conducted with wheat -*Agropyron* derivatives. Schmidt et al., (1956), suggested that the immune reaction typical of *Agropyron elongatum* (host) Beauv. may be controlled by a complex mechanism, in which case it may be difficult to transfer a satisfactory level of resistance to wheat.

Further effort was initiated to combine BW155-derived tolerance and Wsm1 resistance derived from wheat line 7166 (AC Elsa/KS95H103//AC Elsa) using doubled haploid technology. The long term aim is to develop CWRS wheat cultivars incorporating both resistance and tolerance to WSMV. Generation of doubled haploid generation provided a fast method to achieve homozygous state. It is of primary importance to have non segregating populations while producing new breeding lines.

Cultivars carrying tolerance become infected and develop WSM symptoms, but yield is reduced less than in susceptible cultivars. Seifers and Martin (1988) argued that disease tolerance has not been used extensively in breeding programs because of the difficulty and often low accuracy of the time consuming screening processes. However, the benefits contributed by BW155-derived tolerance may be more effective in terms of overall WSMV disease control because of the fact that BW155-derived tolerance is not temperature sensitive.

Enzyme-linked immunosorbent assay (ELISA) assay is a valuable tool for estimating virus titre but it does not always explain the degree of tolerance

demonstrated by a particular plant/cultivar. ELISA shows how much viral antigen can be extracted from the tissue and not necessarily infectious virus. For example infected AC Elsa has lower infectious virus as determined by back assay titration than BW155 but at higher temperatures BW155 sustains less damage with higher infectious virus. ELISA and symptomatology had low correlation (Stoddard et al., 1987), and it could be even lower when we are dealing with tolerance. Therefore, more emphasis was given in this investigation on the overall performance of the lines.

The conclusion that the marker assisted identification is 100 % correlated with WSMV resistance was not confirmed. In the present evaluation, some of the DH lines carrying Wsm1 marker lacked WSMV resistance. Recombination may have caused loss of the J15 SCAR marker linked to Wsm1 in the translocated chromosome. In the process of recombination the DH lines could have also lost some portion of *A. intermedium* chromatin that contains Wsm1 increasing the temperature sensitivity of Wsm1. There might exist a threshold effect for temperature and the length of the translocated *A. intermedium*. From this result there does not appear to be an absolute correspondence between the presence of the J15 SCAR marker and the Wsm1 resistance gene. Evidence of some lines performing better than the parents with no marker also confirms that the linkage between the resistance gene and marker has been broken by apparent crossing over, perhaps by eliminating the alien chromosome portion that contains the

Wsm1 linked marker and retaining the piece with the actual resistance gene. Marker-assisted selection may create an unwanted situation where the less resistant ones are selected and resistant ones are eliminated. Therefore, in future spring wheat breeding programs involved in the production of WSMV-resistant cultivars, care should be taken not to depend only on the presence of J15 SCAR marker, as there seem to be recombination occurring between part of the translocated *Agropyron* chromosome and the wheat chromosome. Further studies are required on cytogenetics of the lines that performed well and did not have the marker to determine the presence of translocated *A. intermedium* genetic material. Additional studies of progenies derived by crossing 7166 and Laura, a highly susceptible CWRS cultivar, and comparisons with Elsa background, could also be beneficial to understand the nature of the genetic contribution from a moderately resistant/tolerant parents in the breeding program.

This study suggests that BW155-derived tolerance and resistance conditioned by Wsm1 can be combined and transferred into new backgrounds. It should therefore be feasible to develop commercial spring wheat cultivars with a higher level of resistance to WSMV than previously available. This study presents the first detailed examination of inheritance of tolerance to WSMV in spring wheat. This is also the first report of combining tolerance and resistance to WSMV in spring wheat.

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# 7. Appendix

## Appendix 7.1 Media preparation for haploid plant growth:

20 g of sucrose + 8 g of agar was added to 1 litre of distilled water. The mixture was then heated to 85° F, stirring until completely dissolved. The temperature was reduced to 75° and 2.5 g of Gamborg's B-5 basal medium (Manufacture: SIGMA) was added, stirring until completely dissolved. The pH level was adjusted to 6 by adding Sodium hydroxide(NaOH 1N). The medium was dispensed into glass vials (8 ml /35 ml vial), and sterilized in an autoclave at 15 psi, 121°C for 20 min.

## Appendix 7.2 Colchicine N–(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)acetamide:

### Colchicine stock solution:

Stock solution of 0.2 % by volume of colchicine was prepared by adding 1 g of colchicine (approx. 95% HPLC) to 500 ml of distilled water.

### Plant material preparation:

Hapliod plants at 2-3 tiller stage were uprooted from the growing medium (soil-less mix) and roots were washed throughly and trimmed in order to minimize metabolic activities. Any old leaves were scaled down and crown area was cleaned. The leaves were trimmed according to the height of the plant to minimize transpiration during colchicine treatment. The plants after washing were aligned at the crown area.

### Colchicine treatment:

60 ml of colchicine stock solution was transferred into 250 ml flat bottomed beaker and 6 drops of DimethylSulfoxide (DMSO) was added (1drop DMSO /10 ml 0.2 % colchicine solution or 1ml DMSO / 500ml of colchicine stock solution). Plants aligned by the crown area was immersed in the prepared solution for two and quarter hour, care was taken to assure the crown area is immersed in to the solution. After the cochicine treatment the plants were washed under running

water for two and half hours. Then the plants were potted in 4 x 4 cm plastic pots in metro mix.

**Appendix 7.3 Reagents for DNA extraction**:

**Proteinase K**

10 mg proteinase K / 1 ml TE buffer

**TE buffer**

10 mM Tris/HCl (pH 7.6)

1 mM EDTA (pH 8.0)

20 % **Sodium dodecyl sulfate** (SDS)

**Extraction buffer** (total volume = 1000ml)

110 ml 1.0 M Tris/HCl (pH 8.7)

110 ml 0.5 M EDTA (pH 8.0)

308 ml 5.0 M NaCl

110 ml 10 % CTAB

362 ml DDW

**10 % CTAB (Hexadecyltrimethylammoniumbromide)**

12.5g CTAB

62.5 ml 1.0 M Tris/HCl (pH 8.7)

25.0 ml 0.5 M EDTA (pH 8.0)

37.5 ml DDW (deionized distilled water)

**Chloroform/Isoamyl alcohol** (24:1 v/v)

**Isopropanol** (VWR)

(chilled at -20°C)

**Glass beads**

3mm diameter borosilicate (VWR)

**Silica sand** (Aqua tech)

**Appendix 7.4 Reagents for PCR:**

7.5 µl 5x RT-PCR buffer (Titan RT-PCR system - Roche)

3.0 µl $MgCl^2$ (25 mM)

5.0 µl 10 x dNTP (Roche)

2.5 µl each 10 ng/µl left and right primer(GIBCO BRL - Custom Primers)*

0.5 µl Taq (Cereal Research Centre, Winnipeg)

1.0 µl DNA

27 µl autoclaved distilled deionized water

1.0 µl Chill-out 14 Liquid wax (MJ Research)

50.0 µl total volume / tube

***Primer sequence:**

Right primer (5' to 3') CCG AGC TCA CAC GCT AAT TT

Left primer (5' to 3') GTA GCA GGG GAA GCT GAA GA

**Appendix 7.5 Thermocycler: (PCR):**

Thermocycler setting (PTC. 100™ programmable Thermal Controller, Peltier-Effect Cycling; M J Research, Inc.)

**Settings used in this study**

| | Temperature | Time |
|---|---|---|
| 1 | 94° C | 3:00 min |
| 2 | 94° C | 1:00 min |
| 3 | 60° C | 1:00 min |
| 4 | 72° C | 2:00 min |
| 5 | repeats cycle from #2 - 4 | for 34 times |
| 6 | 72 ° C | 10:00 min. |
| 7 | 4 ° C | 0:00 until the sample is removed |

**Settings used by Talbert et al. (1994)**

| | Temperature | Time |
|---|---|---|
| 1 | 94° C | 4:00 min |
| 2 | 94° C | 1:00 min |
| 3 | 45° C | 1:00 min |
| 4 | 72° C | 1.2 min |
| 5 | repeats cycle from #2 - 4 | for 30 times |
| 6 | 72 ° C | 10:00 min. |
| 7 | 4 ° C | 0:00 until the sample is removed |

**Settings used by Haber et al. (1995)**

| | Temperature | Time |
|---|---|---|
| 1 | 94° C | 1:00 min |
| 2 | 94° C | 1:00 min |
| 3 | 55° C | 1:00 min |
| 4 | 75° C | 1:00 min |
| 5 | repeats cycle from #2 - 4 | for 34 times |
| 6 | 75° C | 5:00 min. |
| 7 | 4 ° C | 0:00 until the sample is removed |

**Appendix 7.6 Agarose gel preparation**

800 ml 0.5 x **TAE running buffer**

**Gel**

0.5 g high temperature agarose

50 ml 0.5 TAE running buffer

Melt in microwave on medium for 5 minutes swirling every minute, until agarose is dissolved.

Add 7.5 µl ethidium bromide stock mix well, and allow to cool to approximately 60 °C.

Pour into moulding tray and allow to solidify.

Cover with plastic wrap and place in fridge for 5 minutes.

**10 x stock TAE** (pH 7.8)

48.4 g Tris base

7.4 g EDTA

16.4 g sodium acetate

pH with glacial acetic acid

DDW

total volume 1 litre

**Ethidium bromide stock** = 10 mg/ml

Ethidium bromide binds to DNA and fluoresces under ultraviolet light thus allowing visualization of the Wsm1 marker.

**1 Kb DNA ladder** (GIBCO BRL)

**Stop solution**

0.3 g Tris

0.9 g EDTA

0.5 g SDS

0.125 g Bromophenol blue

0.125 g x-cyanol

25 ml glycerol

DDW

total volume 50 ml