GENETIC CHARACTERIZATION AND UTILIZATION OF MULTIPLE *AEGILOPS TAUSCHII* DERIVED PEST RESISTANCE GENES IN WHEAT

by

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B.S., University of Kentucky, 1998
M.S., University of Kentucky, 2002

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2006

Abstract

Aegilops tauschii, the D-genome donor of modern wheat, has served as an important source of genetic variation in wheat breeding. The objective of this research was to characterize and utilize multiple *Ae. tauschii*-derived pest resistance genes contained in the germplasm KS96WGRC40.

Two *Ae. tauschii*-derived genes, *H23* and *Cmc4,* provide resistance to the Hessian fly (HF) and wheat curl mite (WCM), respectively. A linkage analysis of a testcross population estimated 32.67% recombination between *H23* and *Cmc4* on chromosome 6DS in wheat indicating that the two genes are not tightly linked as previous mapping reports show. Haplotype data of recombinant lines and physical mapping of linked microsatellite markers located *Cmc4* distal to *H23*. Haplotype data indicated that both KS89WGRC04 and KS96WGRC40 have the distal portion of 6DS derived from *Ae. tauschii*. Microsatellite primer pairs BARC183 and GDM036 were more useful than the previously published linked markers in identifying lines carrying *Cmc4* and *H23,* respectively.

Through phenotypic selection and advancement within the testcross population, three $TC_1F_{2:4}$ lines were identified as homozygous resistant for *H23* and *Cmc4* and have the complete terminal segment of 6DS from *Ae. tauschii*. Two lines are more desirable than the original germplasm releases and can serve as a source of resistance to both HF and WCM in an elite background.

A linkage analysis of a segregating recombinant inbred line population identified an *Ae. tauschii*-derived gene of major effect conferring resistance to Septoria leaf blotch (STB) and another *Ae. tauschii*-derived gene of major effect conferring resistance to soil-borne wheat

mosaic virus (SBWMV) in the germplasm KS96WGRC40. The STB resistance gene in KS96WGRC40 is located in the distal 40% of the short arm of chromosome 7D flanked by microsatellite markers *Xgwm044* and *Xbarc126*. Two previously reported STB genes, *Stb4* and *Stb5*, have been mapped to 7DS in the same region as the STB resistance gene in KS96WGRC40. The uniqueness of the STB resistance genes on 7DS is questionable. The SBWMV resistance gene in KS96WGRC40 is located on chromosome 5DL linked to microsatellite marker *Xcfd010*. The SBWMV resistance gene within KS96WGRC40 was derived from TA2397 via KS95WGRC33.

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Table of Contents

List of Figures

List of Tables

Acknowledgements

As I worked to complete this work, God blessed me along the way with many helpful people. This dissertation would not be complete without acknowledging them.

First, I would like to thank my co-major professors, Dr. Gina Brown-Guedira and Dr. Allan Fritz, for their help and guidance in this work. I also appreciate the suggestions and advice from my other committee members, Dr. Ming-Shu Chen, Dr. Bikram Gill, and Dr. Bill Bockus. Thanks to Dr. Frank Arthur for serving as the outside chair.

Special thanks to Kim Howell, Elburn Parker, Andy Auld, Duane Wilson, Jon Raupp, Maria Mateos-Hernadez, Sally Herrmann, Beth Gillett, Heather Williams, Christy Baucom, and Jared Smith. I am grateful for their assistance in the lab, field, and greenhouse. I would also like to thank Dr. John Fellers for his constant support and willingness to lend a hand. I would like to acknowledge Dr. Tom Harvey for providing me with a collection of wheat curl mites.

As graduate students we learn a lot from each other. Francis Kirwigi, Maria Mateos-Hernandez, Peter Nyori, Leandro Perugini, and Corey Rozenboom have been great teachers and most of all friends.

I also want to acknowledge my family. My parents, sisters, aunts, cousins, and all the rest of the gang back in Kentucky kept me motivated and always seemed to know when I needed some encouragement. Finally, I want to express my deep appreciation to my loving husband Mike for his devotion. Simply put I could not have done it without you.

Dedication

for Lana

CHAPTER 1 LITERATURE REVIEW

Wheat Production

Common wheat, *Triticum aestivum* L., is a grass species belonging to the tribe Triticeae. Among all of the grain crops it possesses the unique ability to produce an elastic dough, which can be made into leavened bread. Due to its wide adaptation to many climates, wheat is grown all over the world. More land is committed to wheat production than any other commercial crop worldwide (Briggle and Curtis, 1987). The major wheat producing countries of the world include: Russia, China, India, Australia, Canada, and the United States.

In the US, six market classes of wheat are grown: hard red winter, soft red winter, hard red spring, hard white, soft white, and durum. The US market classes are defined by end use milling and baking characteristics and are associated with geographical production regions. Hard red winter wheat is milled to make bread flour and is produced throughout the Midwest. Soft red winter wheat is milled into flour traditionally used for biscuits, cookies, pastries, pancakes, and crackers. The majority of soft red winter wheat is produced in regions east of the Mississippi River. In the colder climates of the northern Great Plains, hard red spring wheat is produced. Spring wheat is the premier bread wheat flour and is also used to make pizza dough, croissants, and bagels. Along side both red wheat classes, two white wheat classes are grown. Hard white wheat is devoted to the production of fresh noodles, tortillas, and bulgur. A concentrated area for the production of hard white wheat is located in the Pacific Northwest. Soft white wheat is used to make whole grain extruded products and is grown in similar regions as soft red

wheat. Durum wheat (*Triticum turgidum* L. var. durum) is milled to make pasta flour; most US production of durum wheat is concentrated in North Dakota and the desert west.

Wheat Evolution

Common wheat (*Triticum aestivum* L.) has a very large genome. Possessing an estimated 16 billion base pairs of DNA (Arumuganathan and Earle, 1991), it has over five times as much DNA as humans (Venter et al., 2001). *Triticum aestivum* (2n=6x=42) is an allohexaploid consisting of three genomes (AABBDD) that came together through two separate natural amphiploidization events to create common wheat.

Approximately 0.5 million years ago, two wild diploid species created a wild tetraploid species through the process of hybridization and spontaneous chromosome doubling. One of the wild diploid species was *Triticum urartu* (genomes AA), the A-genome donor of common wheat (Dvorak et al., 1993). The other diploid species is considered to be *Aegilops speltoides* (genomes SS), the potential B genome donor of common wheat (Maestra and Naranjo, 1998). These two diploids crossed and produced the wild tetraploid species *Triticum turgidum* ssp. *dicoccoides* (genomes BBAA), also known as wild emmer wheat. Wild emmer wheat was domesticated into the cultivated emmer wheat species *Triticum turgidum* ssp. *dicoccon*.

The second amphiploidization event was explained by McFadden and Sears (1946). They determined that the wild diploid goatgrass species *Aegilops tauschii* was the D-genome donor of common wheat. *Ae. tauschii* (genomes DD) hybridized with the cultivated emmer wheat species *Triticum turgidum* ssp *dicoccon* (genomes BBAA), and after spontaneous chromosome doubling resulted in the formation of common hexaploid wheat (genomes BBAADD). Based on the variation at 55 loci, Dvorak et al. (1998)

suggested Transcaucasia and southwestern Caspian Iran as the birthplace of common hexaploid wheat.

Use of Progenitor Species in Wheat Improvement

The creation of common hexaploid wheat through the process of amphiploidization occurred naturally. Wheat geneticists have reproduced synthetic hexaploid wheats, but doing so requires embryo rescue techniques. Consequently, natural amphiploids must have been rare. The domestication of these rare natural amphiploids reduced the amount of genetic diversity within hexaploid wheat even more. Therefore, the genetic bottlenecks of amphiploidization and domestication severely limited the amount of variation within common wheat. For comparison, *Ae. tauschii*, the D-genome donor of common wheat, has a mean adjusted polymorphic index of 0.41, while common wheat has a mean adjusted polymorphic index of only 0.04 (Cox, 1998).

This lack of diversity within common wheat influences wheat breeding. In order to develop improved wheat varieties, breeders must have variation from which to select. To that end, wheat breeders and geneticists have turned to the genetic variation existing in wheat's progenitor species. Potentially all members of the Triticeae tribe can be utilized as a source of genetic variation in wheat (Feldman and Sears, 1981). There are numerous examples of gene transfers between various progenitor species and common wheat for many varied traits.

The D-genome donor *Ae*. *tauschii* has been heavily utilized as a source of useful traits for wheat improvement since the amount of recombination between *Ae*. *tauschii* chromosomes and D-genome wheat chromosomes can be very close to the amount of recombination between two wheat chromosomes (Jones et al., 1995). Most of the *Ae.*

tauschii introgression work has focused on improving resistance to biotic stresses such as diseases or pests (Table 1.1). A few gene transfers providing tolerance to abiotic stresses such as cold tolerance, salt tolerance, and pre-harvest sprouting have been reported (Schachtman et al., 1992; Limin and Fowler, 1993; Xiu-Jin et al., 1997). Beyond the reported improvements in qualitative traits, *Ae. tauschii* may also be a source of beneficial QTL effects for kernel weight and grain protein (Fritz et al., 1995; Cox et al., 1990). Clearly, *Ae. tauschii* is an important source of genetic variation for wheat improvement.

Table 1.1. Wheat breeding improvements derived from *Aegilops tauschii.*

Trait	**Gene**	**Location**	**Germplasm**	**Reference**
Salt tolerance				Schachtman et al., 1992
Cold hardiness				Limin and Fowler, 1993
Sprouting tolerance			RSP	Xiu-Jin et al., 1997
Leaf rust resistance	*Lr39*	2DS	KS86WGRC02	Raupp et al., 2001
	Lr41	1D	KS90WGRC10	Cox et al., 1994B
	Lr42	1D	KS91WGRC11	Cox et al., 1994B
	Lr21	1DS	KS89WGRC07	Huang et al., 2003
	Lr22a	2DS	RL5404	Rowland and Kerber, 1974
	Lr32	3D	RL5713	Kerber, 1987
			Syn101	Assefa and Fehrmann, 2000
			Syn701	Assefa and Fehrmann, 2000
			Syn901	Assefa and Fehrmann, 2000
			Syn301	Assefa and Fehrmann, 2000
				Manisterski et al., 1988
				Miller et al., 1992
				Tomerlin et al., 1983
Stem rust resistance	*Sr29*	6DL	Etoile de Choisy	Dyck and Kerber, 1977
	Sr5	6DS	Admonter Fruh	Kerber and Dyck, 1979
				Cox et al., 1992
Stripe rust resistance	*Yr18*	7DS	Opata85	Singh and Rajaram, 1994
	Yr28	4DS	Synthetic	Singh et al., 2000
				Ma et al., 1995
				Yildirim et al., 1995
				Yang et al., 1998

Table 1.1 continued. Wheat breeding improvements derived from *Aegilops tauschii*.

Trait	Gene	Location	Germplasm	Reference
Powdery mildew resistance	*Pm2*		Apollo	Lutz et al., 1995
	Pm19		Synthetic XX 186	Lutz et al., 1995
			NC96BGTD1	Murphy et al., 1998
			NC96BGTD2	Murphy et al., 1998
			NC96BGTD3	Murphy et al., 1998
				Gill et al., 1986
				Cox et al., 1992
				Lutz et al., 1994
				Tomerlin et al., 1983
Eyespot resistance				Yildirim et al., 1995
Septoria tritici resistance				McKendry and Henke, 1994
				May and Lagudah, 1992
Septoria nodourm resistance			RL5271	Murphy et al., 2000
				Tomerlin et al., 1983
Greenbug resistance	*Gbz*	7D	KSU97-85-3	Zhu et al., 2004
	Gb3	7D	Largo	Weng and Lazar, 2002
	Gb4	7D	CI17959	Martin et al., 1982
	Gba		TA4152L94	Smith and Starkey, 2003
	Gbx	7D	KS89WGRC04	Weng and Lazar, 2002
Hessian fly resistance	*H22*	1D	KS85WGRC01	Raupp et al., 1993
	H23	6D	KS89WGRC03	Raupp et al., 1993
	H24	3D	KS89WGRC06	Raupp et al., 1993
	H26	4D	KS92WGRC26	Cox and Hatchett, 1994
	H13	6D	Molly	Gill et al., 1987
	H32	3D	W-7984	Sardesai et al., 2005
				Gill and Raupp, 1987
				Hatchett and Gill, 1981
Russian wheat aphid resistance	*dn3*		CIMMYT Synthetic	Nkongolo et al., 1991

Table 1.1 continued. Wheat breeding improvements derived from *Aegilops tauschii.*

Trait	**Gene**	**Location**	**Germplasm**	**Reference**
Wheat curl mite resistance	*Cmc1*	6D	ACPGR16635	Whelan and Thomas, 1989
	Cmc4	6D	KS96WGRC40	Malik et al., 2003B
				Malik et al., 2003A
Cereal cyst nematode resistance				Eastwood et al., 1991
Spindle streak mosaic virus resistance			KS92WGRC21	Cox et al., 1994A
Soil-borne mosaic virus resistance			KS92WGRC21	Cox et al., 1994A
			KS92WGRC22	Cox et al., 1994A
			KS89WGRC04	Gill et al., 1991
Tan spot resistance				Siedler et al., 1993
			KS96WGRC39	Cox et al., 1992
Take-all resistance				Eastwood et al., 1993

Molecular Markers

Molecular markers reveal variation in alleles between individuals at the DNA level. Many different types of molecular markers are available. The first molecular marker system developed was isozymes. Isozymes are multiple forms of the same protein which can be separated based on size and conformation on a gel. The first molecular markers based on DNA sequences were restriction fragment length polymorphisms (RFLPs) which are hybridization-based markers that are revealed by DNA probes. The introduction of the polymerase chain reaction (PCR) led to the development of many different molecular marker systems including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), sequence tagged sites (STS), sequence characterized amplified regions (SCAR), and single nucleotide polymorphisms (SNP).

SSR markers, also known as microsatellites, use primers which match unique sequences that flank tandemly repeated di-, tri-, or tetra-nucleotide repeats. SSR markers display a co-dominant inheritance, are evenly distributed throughout the wheat genome (Roder et al., 1998), are highly polymorphic, are locus specific, and easy to use. Several genetic linkage maps of hexaploid wheat have been generated using different mapping populations and different molecular marker systems (Devos and Gale, 1997; Roder et al., 1998; Pestova et al. 2000; Gupta et al., 2002). Four of these maps have been consolidated into one high-density consensus map of hexaploid wheat (Somers et al., 2004) that contains 1,235 mircosatellite markers. The availability of a high-density consensus map and numerous PCR based molecular markers has greatly improved the genetic characterization of hexaploid wheat.

Molecular markers can be used to tag particular genes of interest through the process of marker-assisted selection (MAS). In MAS, markers linked to traits of interest are used to select desired alleles through the process of indirect selection. Plants containing undesirable alleles are discarded in early generations thus saving valuable resources. Molecular markers suitable for MAS in wheat have been identified that are linked to 24 fungal resistance genes, three viral resistance genes, nine insect resistance genes, seven quality traits, and three abiotic stress traits (http://maswheat.ucdavis.edu, verified 19 May 2006).

An example of MAS in wheat involves the selection of lines resistant to Fusarium head blight (*Fusarium graminearum* Schwabe). Anderson et al. (2001) identified a QTL for resistance to Fusarium head blight in the Chinese cultivar Sumai-3. This QTL, named *Qfhs.ndsu-3BS,* is flanked by microsatellite markers *Xgwm493* and *Xgwm533* (Anderson et al., 2001). Selecting for the resistant allele at the *Xgwm533* marker locus, Yang et al. (2003) was able to identify seven of the eight most resistant lines in a doubled haploid population. Phenotypic screening for Fusarium head blight is time consuming, laborious, requires plants be grown until flowering, and is heavily influenced by environmental conditions. Screening lines with the molecular markers *Xgwm493* and *Xgwm533* can be completed with relative ease at any stage of plant growth and is not influenced by environmental conditions.

Molecular markers can also be used to pyramid multiple desired genes into a single germplasm. Tar'an et al. (2003) successfully used three molecular markers to pyramid two ascochyta blight resistance genes and one anthracnose resistance gene in 11 lines of lentil (*Lens culinaris* Medik).

Biology of the Wheat Curl Mite

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is a microscopic arthropod pest previously identified as *Aceria tulipae*. The WCM belongs to the class Arachnida and thus is not an insect. The WCM has an elongated cylindrical soft body, forcep-like mouthparts, two pairs of legs, and no antennae.

Wheat plants infested with WCMs show distinctive leaf rolling or curling. Emerging wheat leaves can become trapped in tightly rolled leaves preventing the normal elongation of new leaves. Under high infestations on seedlings in the greenhouse, plant death can occur (Harvey et al., 2002). The more important threat from WCM infestations is the transmission of plant viruses. Two economically important viruses of wheat, the high plains virus (Seifers et al., 1997) and the wheat streak mosaic virus (Slykhuis, 1955), are vectored by the WCM. Wheat streak mosaic virus caused a 13% yield loss statewide in Kansas wheat in 1998 (Bockus et al., 2001), with losses in individual fields being much greater. In addition to vectoring yield-damaging viruses, nonviruliferous WCMs can also reduce yield by as much as 17% (Harvey et al., 2000) when compared to naturally infected control plots.

Female WCMs lay eggs longitudinally between the veins of wheat leaves. Females hatch from fertilized eggs while males hatch from unfertilized eggs. After hatching, WCMs develop through two nymphal instars into adults. Virus transmission does not occur through the egg and must be acquired by the WCM by feeding on virus infected plant tissue (Oldfield and Proeseler, 1996). The life cycle of the WCM is completed in 8-10 days. Travel is accomplished by wind and hitchhiking on insects and even birds (Jeppson et al., 1975). Distribution of the WCM is worldwide (Oldfield and Proeseler, 1996).

Different collections of the WCM vary in their ability to infest different varieties of wheat (Harvey et al, 1999). Malik (2001) reported that six collections of WCMs, identified as the Kansas, Montana, Alberta, Nebraska, Texas, and South Dakota strains, caused differential reactions on known WCM resistance genes and in accessions of *Ae. tauschii*.

The host range of the WCM is extremely wide. First identified from tulip (*Tulipa spp.*) bulbs by Keifer in 1938, the WCM has also been reported on onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) (Slykhuis, 1955). Various species of wild and cultivated grasses serve as reproductive hosts to the WCM including: wheat, barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), Sudan grass (*Sorghum vulgare* var. *sudanense*), and sorghum (*Sorghum bicolor*) (Connin, 1956). In the US Great Plains, the WCM has no problem finding other grass hosts on which to over-summer.

Genetics of Resistance to the Wheat Curl Mite

Resistance to the WCM does exist within the wheat genome itself (Harvey and Martin, 1992). However, four named genes reported to confer resistance to the WCM have been transferred to wheat from related species (Table 1.2). Thomas and Conner (1986) transferred a single dominant gene from *Ae. squarrosa* L. (syn *Ae. tauschii* (Coss.). Schmal.) to wheat and named the gene *Cmc1*. Thomas and Whelan (1991) estimated that *Cmc1* was independent of the centromere of chromosome 6DS in monotelodisomic wheat plants. *Cmc2* was transferred to wheat from *Agropyron elongatum* (Host.) Beauv. (Whelan and Hart, 1988). Rye (*Secale cereale* L.) has also served as a source of WCM resistance. The T1AL·1RS translocation in the wheat cultivar Amigo (Sebesta et al., 1994) provides resistance to WCMs and has been given

the designation *Cmc3* (Malik et al., 2003B). The Amigo source of resistance has been utilized in such cultivars as TAM107, TAM200, and Century. Another resistance gene transferred from *Ae. tauschii* has been designated as *Cmc4* and was located in the distal region of chromosome 6DS by Malik et al. (2003B). *Cmc1* and *Cmc4* are both located on chromosome 6DS in wheat; allelism tests show that *Cmc1* and *Cmc4* segregate independently (Malik, 2001).

Other sources of WCM resistance have been reported in *Haynaldia villosa* L. Schur. (Chen et al., 1996) and the T1BL·1RS translocation from rye in the wheat cultivar Salmon (Zeller, 1973; Martin et al., 1976). A translocation from *Elytrigia pontica* (Podp.) Holub conferred resistance to WCMs as reported by Whelan et al. (1986).

Resistance to the WCM also provides protection against the high plains virus and the wheat streak mosaic virus, as the WCM is the only known vector for both viruses. Resistance to the WCM in wheat can breakdown over time as shown in the cultivar TAM107 by Harvey et al. (1997).

Table 1.2. Sources, chromosome locations, and markers linked to named wheat curl mite resistance genes in wheat.

Gene	Source	Linked Markers	Location	Reference
Cmc1	*Aegilops tauschii*		6DS	Thomas and Conner, 1986; Whelan and Thomas, 1989
Cmc2	*Agropyron elongatum*		6A	Whelan and Hart, 1988
Cmc3	'Amigo' wheat T1AL•1RS		1RS	Malik et al., 2003B
Cmc4	*Aegilops tauschii*	*Xgdm141*	6DS	Malik et al., 2003B

Biology of the Hessian Fly

The Hessian fly, *Mayetiola destructor* (Say), is an arthropod pest classified in the order Diptera, suborder Nematocera, and group Cecidomyiidae. Members of the

Cecidomyiidae or gall midge group are classified as having long antennae, one pair of wings with no crossveins, and phytophagous larvae. The larvae of the Hessian fly hatch from small glossy red eggs. Larvae grow through various stages of instar development feeding on wheat sheaths until pupating. The puparium is not active and referred to as the flaxseed stage because of its resemblance to the seed of flax (*Linum usitatissimum* L.). The flaxseed stage serves to overwinter and/or oversummer the Hessian fly. Adults emerge from the flaxseed stage, mate, and soon die. The adult Hessian fly does not feed. An adult female Hessian fly can lay between 200-300 eggs in her short 2-3 day lifespan. In winter wheat growing regions adults can be seen both in the fall and spring.

Hessian fly larvae feed on wheat seedlings at the crown. Wheat plants injured by larval feeding are stunted and have a broader, more erect, darker green appearance (Gallun, 1965). Under heavy infestations, young wheat plants can die. Typically, only infested wheat stems will die thus reducing tiller numbers per plant and yield per harvested area (Sadras et al., 1999).

The Hessian fly is distributed throughout the major wheat growing regions of the world including Europe, Northern Asia, North Africa, North America, and New Zealand. The most recent survey in 1997 states that it had not been reported in Japan, Mexico, South America, or Australia (Ratcliffe and Hatchett, 1997). The Hessian fly was named according to the generally believed oral history that it was first introduced in the US by infested straw brought with Hessian soldiers during the American Revolutionary war. Numerous other introductions of the pest have undoubtedly occurred throughout American history, closely following the paths of immigrants as they brought along with them their preferred wheat cultivars (Somsen and Oppenlander, 1975).

The pioneering entomologist R. H. Painter first described the existence of different biological races of the Hessian fly in 1930 while working at Kansas State University. Sixteen races (i.e. biotypes or strains) of Hessian fly have now been identified (Patterson et al., 1992) according to their ability to infest a set of differential wheat cultivars.

Genetics of Resistance to the Hessian Fly

Plant resistance to Hessian fly is controlled by the mechanism of antibiosis (Painter, 1951) in which larvae die after feeding on plants. Thirty-two Hessian fly resistance genes, *H1* to *H32*, have been named to date (Table 1.3) (McIntosh et al., 2003; Ratcliffe and Hatchett, 1997, Sardesai et al., 2005). The majority of Hessian fly resistance genes have been mapped to chromosome 5A in wheat (Ratcliffe and Hatchett, 1997) although their location on 5A is suspect due to linkage of these genes with *H9* which as recently determined to be located on chromosome 1A, along with *H10,* and *H11* (Liu et al., 2005A). Genes *H20* and *H21* are located on chromosome 2B (Amri et al., 1990; Friebe et al., 1990). Chromosome 6D contains two genes, *H13* and *H23* (Gill et al., 1987; Raupp et al., 1993). Raupp et al. (1993) demonstrated that *H13* and *H23* were separate genes based on allelism crosses and segregation ratios. They reported that while both *H13* and *H23* lie on chromosome 6D they are different genes and are approximately 25 ± 5 map units apart from each other. Other Hessian fly resistance genes have been mapped to chromosomes 1D, 3D, 4B, 4D, 5B, 5D, and 6B while seven named genes remain unmapped (Ratcliffe and Hatchett, 1997).

Genes conferring resistance to Hessian fly in wheat have been identified and transferred from the following species: *Triticum turgidum* L. var. durum, *Triticum*

turgidum ssp. *dicoccum*, *Ae. tauschii* (Coss.) Schmal., *Secale cereale* L., *Ae. ventricosa* and *Ae. triuncialis* (Ratcliffe and Hatchett, 1997; Liu et al, 2005B; Delibes et al., 1997; McIntosh et al., 2003). About half of the resistance genes have been identified in *Triticum turgidum* L. var. durum making this species an important source for Hessian fly resistance in wheat. *Ae. tauschii* is the source of resistance genes *H13, H22, H23, H24,* and *H26* (Ratcliffe and Hatchett, 1997). Two genes, *H21* and *H25*, were donated from *Secale cereale* L. germplasms and were transferred to wheat via wheat-rye translocations. Resistance to Hessian fly in common wheat itself does exist as eight named genes have been identified in *Triticum aestivum* germplasms.

Table 1.3. Sources and chromosome locations of named Hessian fly resistance genes in wheat (Summarized from Ratcliffe and Hatchett, 1997 with additions from McIntosh et al., 2003; Sardesai et al., 2005, Liu et al., 2005A and Liu et al., 2005B).

Gene	Source	Location
H1	*T. aestivum* L.	Unknown
H2	*T. aestivum* L.	Unknown
H3	*T. aestivum* L.	5A?
h4	*T. aestivum* L.	Unknown
H5	*T. aestivum* L.	1A
H6	*T. turgidum* L. var. *durum*	5A?
H7	*T. aestivum* L.	5D
H8	*T. aestivum* L.	Unknown
H9	*T. turgidum* L. var. *durum*	1A
H10	*T. turgidum* L. var. *durum*	1A
H11	*T. turgidum* L. var. *durum*	1A
H12	*T. aestivum* L.	5A?
H13	*Ae. tauschii*	6D
H14	*T. turgidum* L. var. *durum*	Unknown
H15	*T. turgidum* L. var. *durum*	5A?
H16	*T. turgidum* L. var. *durum*	5A?
H17	*T. turgidum* L. var. *durum*	5A?
H18	*T. turgidum* L. var. *durum*	Unknown
H19	*T. turgidum* L. var. *durum*	5A?
H20	*T. turgidum* L. var. *durum*	2B
H21	*Secale cereale* L.	2B
H22	*Ae. tauschii*	1D
H23	*Ae. tauschii*	6D
H24	*Ae. tauschii*	3D
H25	*Secale cereale* L.	4B, 6B
H26	*Ae. tauschii*	4D
H27	*Ae. ventricosa*	$4M^{V}$
H28	*T. turgidum* L. var. *durum*	5A?
H29	*T. turgidum* L. var. *durum*	5A?
H30	*Ae. triuncialis*	Unknown
H31	*T. turgidum* L. var. *durum*	5BS
H32	*Ae. tauschii*	3DL
Hdic	*T. turgidum* ssp. *dicoccum*	1A

Biology of Septoria Leaf Blotch

Septoria tritici Roberge in Desmaz. (telomorph *Mycosphaerella graminicola* (Fuckel) J. Schrot in Cohn) is an ascomycete fungal pathogen of wheat that produces

chlorotic and necrotic lesions on wheat leaves commonly referred to as Septoria leaf blotch (STB). STB causes wheat yield losses throughout every wheat-growing region on every continent (Eyal, 1999). Many grasses can serve as hosts to STB including but not limited to the genera: *Agropyron*, *Bromus*, *Festuca*, *Hordeum*, *Poa*, *Secale*, and *Triticum* (Eyal, 1999).

The disease cycle of STB in wheat has both a sexual and asexual stage. Doyle (2004) summarized the STB disease cycle in wheat. The sexual stage starts with the release of asci from within a pseudothecium fruiting structure. When the relative humidity is high these asci release ascospores that are dispersed by wind currents. Upon landing on a compatible host the ascospores germinate and cause disease. In the developing necrotic leaf tissue, pycnidia will form. Pycnidia are asexual fruiting bodies that extrude pycnidiospores. Pycnidiospores are rain-splashed onto adjacent leaves where they will germinate and cause more disease if conditions are favorable. Mycelium can develop on wheat leaves if environmental conditions are favorable. A lesion of STB can form the sexual pseudothecium or the asexual pycnidia. Pseudothecia serve as the overwintering structures and typically develop from older lesions on stubble. Pycnidia serve to rapidly produce more infectious pycnidiospores, which spread STB lesions throughout a growing crop.

McDonald et al. (1995) used DNA based molecular markers to study the population genetics of *M. graminicola*. From this work they concluded that many different genotypes of the fungus are present within a single wheat field. Fungal genotypes are distributed randomly throughout a wheat field with each lesion on a particular leaf typically belonging to a different fungal genotype. The researchers also

studied the population structure between different field populations of *M. graminicola* and found a high degree of similarity between two geographically diverse fungal populations within the United States. They suggest that the center of origin for *M. graminicola* is the Middle East based on measures of genetic diversity in an Israeli fungal isolate.

Genetics of Resistance to Septoria Leaf Blotch

Both qualitative and quantitative modes of inheritance of STB resistance have been identified in wheat depending on the source of resistance examined. Rosielle and Brown (1979) reported simple genetic control of resistance in genotypes 'Veranopolis' and 'IAS-20' while genotype 'Seabreeze' contained a more complex genetic control. Shaner and Finney (1982) found that resistant cultivars could be obtained from crosses of susceptible parents. In fact, the soft red winter wheat cultivars Caldwell, Knox, and Beau were developed from susceptible parents and contain quantitative resistance to STB (Shaner and Finney, 1982).

While quantitative gene effects for STB resistance are evident in some germplasms, qualitative effects are predominant. The presence of additive gene effects for STB resistance is evident in many experiments (Jlibene et al., 1994; Zhang et al., 2001; Rosielle and Brown, 1979; Shaner and Finney, 1982). The inheritance of seedling STB resistance as reported by Zhang et al. (2001) is greatly influenced by additive gene effects. In their eight parent diallel experiment, general combining ability (GCA) effects far outweighed specific combining ability effects. In an adult plant resistance diallel experiment, Jlibene et al. (1994) also reported highly significant GCA effects. Reciprocal maternal effects of STB resistance have also been observed (Zhang et al.,

2001; Jlibene et al., 1994). Cowger et al. (2000) clearly showed a gene-for-gene interaction between a *M. graminicola* isolate from Oregon and the wheat cultivar Gene. When released as a cultivar, Gene was resistant to STB; however, this resistance has since been defeated by virulent isolates of *M. graminicola* in Oregon.

Resistance has been found in several Australian varieties of *Triticum aestivum* L. while a large number of Australian *Triticum turgidum* L. var. durum varieties may be immune (Rosielle, 1972). McKendry and Henke (1994) evaluated 254 accessions of wheat progenitor species and found resistance to STB in accessions of *Triticum speltoides* and *Ae. tauschii*.

R. E. Wilson was the first to name specific genes controlling resistance to STB in wheat (Wilson, 1985). He designated *Stb1* for the resistance in 'Bulgaria', *Stb2* for the resistance in 'Veranopolis', and *Stb3* for the resistance in 'Israel493'. Five additional STB resistance genes have been named in wheat to date. The genes *Stb4*, *Stb6,* and *Stb7* were all designated in wheat cultivars. The genes *Stb5* and *Stb8* were designated in the synthetic hexaploid wheats 'Synthetic6X' and 'W7984', respectively. The original source of resistance for *Stb5* and *Stb8* was traced back to the donor species *Ae. tauschii* (Arraiano et al., 2001; Adhikari et al., 2003).

Chromosome locations and linked molecular markers have been published for all eight of the named STB genes (Table 1.4). Linkage analysis and physical mapping of *Stb1* placed SSR marker *Xbarc74* 2.8 cM distal from *Stb1* (Adhikari et al., 2004C). The SSR markers *Xgwm389* and *Xgwm533.1* were mapped approximately 1 cM distal to *Stb2* with marker *Xgwm493* located 3.7 cM proximal to *Stb2* (Adhikari et al., 2004B). The resistance gene *Stb3* has been located on chromosome 6DS with the marker *Xgdm132*

linked at a distance of 3.0 cM (Adhikari et al., 2004B), but there currently exists doubt as to the linkage of *Xgdm132* and *Stb3* (S. Goodwin, personal communication). Resistance genes *Stb4* and *Stb5* are both located on chromosome 7DS near the centromere (Adhikari et al., 2004A; Arraiano et al., 2001). Resistance gene *Stb4* is 0.7 cM distal to SSR marker *Xgwm111* (Adhikari et al., 2004A). Resistance gene *Stb5* was mapped 7.2 cM distal to SSR marker *Xgwm44* and 11.9 cM distal to *Xgwm111* (Arraiano et al., 2001). Brading et al. (2002) placed *Stb6* on chromosome 3AS and detected linkage with the SSR marker *Xgwm369* which was mapped at a distance of 2.0 cM. The resistance gene *Stb7* is located on the distal end of chromosome 4AL, 0.5 to 0.3 cM distal to *Xwmc313* (McCartney et al., 2003). The SSR markers *Xgwm146* and *Xgwm577* flank resistance gene *Stb8* at distances of 3.5 and 5.3 cM respectively on chromosome 7BL (Adhikari et al., 2003).

Table 1.4. Sources, chromosome locations, and markers linked to named Septoria leaf blotch resistance genes in wheat.

Gene	Source	Linked Markers	Location	Reference
Stb1	*T. aestivum* 'Bulgaria88'	*Xbarc74*	5BL	Adhikari et al., 2004C
Stb2	*T. aestivum* 'Veranopolis'	*Xgwm389, Xgwm533.1, Xgwm493*	3BS	Adhikari et al., 2004B
Stb3	*T. aestivum* 'Israel493'	*Xgdm132*	6DS	Adhikari et al., 2004B
Stb4	*T. aestivum* 'Tadinia'	*Xgwm111*	7DS	Adhikari et al., 2004A
Stb5	Synthetic hexaploid 'Syn6X'	*Xgwm44*	7DS	Arraiano et al., 2001
Stb6	*T. aestivum* 'Flame'	*Xgwm369*	3AS	Brading et al., 2002
Stb7	*T. aestivum* 'ST6'	*Xwmc313*	4AL	McCartney et al., 2003
Stb8	Synthetic hexaploid 'W7984'	*Xgwm146, Xgwm577*	7BL	Adhikari et al., 2003

Biology of Soil-borne Wheat Mosaic Virus

Soil-borne wheat mosaic virus (SBWMV) is a destructive pathogen of wheat that belongs to the viral group Furovirus. The disease was first described in Illinois by McKinney (1923) and can now be found in most winter wheat-growing regions throughout the world including most recently the United Kingdom (Clover et al., 2001). Shirako and Wilson (1993) determined the complete nucleotide sequences of RNAs one and two of a SBWMV isolate collected from Nebraska. Based on this sequence and other biological characteristics, Shirako et al. (2000) concluded that four strains of the virus exist – the American, Chinese, European, and Japanese.

The soil inhabiting fungus *Polymyxa graminis* vectors SBWMV (Rao and Brakke, 1969). Symptoms of SBWMV on wheat seedlings include mostly yellow to light green

leaves with darker green mottling and stunting. Depending on environmental conditions, infected seedlings may be able to recover a dark green appearance. Yield losses to SBWMV have been estimated as high as 45% in Kansas (Nykaza, 1978).

Genetics of Resistance to Soil-borne Wheat Mosaic Virus

During the late 1970's SBWMV was the most devastating disease of winter wheat in Kansas (Bockus et al., 2001). Following several epidemic years, breeding for resistance to SBWMV was considered a priority by Kansas State University wheat breeders. With the use of specific breeding nurseries in severely infested SBWMV fields, several SBWMV resistant cultivars were developed and released thus drastically reducing the losses due to SBWMV (Bockus et al., 2001). Although many Kansas cultivars contain resistance to SBWMV, the inheritance of resistance to this virus is still not well understood.

Upon investigating the resistance contained within the cultivars Shawnee, Centurk, and KS73256, Brunetta (1980) reported that resistance was simply inherited as a single dominant resistance gene. Merkle and Smith (1983) also reported that resistance to SBWMV was simply inherited as a single dominant gene. The Brazilian cultivar Embrapa 16 contains two SBWMV resistance genes (Barbosa et al., 2001). The hard red winter wheat cultivar Karl92 contains a putative SBWMV resistance QTL on chromosome 5D which explains 38% of the phenotypic variation (Narasimhamoorthy, 2003). One heritability estimate for SBWMV resistance in wheat can be obtained from the literature and is relatively high being over 40% (Barbosa et al., 2001).

Driskel et al. (2002) demonstrated that the SBWMV resistance in cultivars Tonkawa, Hawk, and Newton did not block the systemic spread of SBWMV within the

plant. The authors hypothesize that virus resistance functions in the plant roots and must block virus infection but not virus movement.

Many hard red winter wheat germplasms have been released by the Wheat Genetics Resource Center at Kansas State University that have resistance to SBWMV (Cox et al., 1994A; Gill et al., 1991). However, the inheritance of this resistance has not been characterized for these or any other SBWMV resistance sources.

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CHAPTER 2 ANALYSIS OF RESISTANCE TO HESSIAN FLY AND WHEAT CURL MITE ON THE SHORT ARM OF CHROMOSOME 6D OF WHEAT

Introduction

Wheat curl mites (WCMs), *Aceria tosichella* Keifer, and Hessian fly (HF), *Mayetiola destructor* (Say), are two pests of common wheat that reduce yield. In the US nonviruliferous WCMs can directly reduce yield by as much as 30% when compared to naturally infected control plots (Harvey et al., 2002). Along with direct reductions in yield, the WCM can also cause further damage by vectoring two important viruses, the high plains virus and the wheat streak mosaic virus (Seifers et al., 1997; Slykhuis, 1955). Wheat streak mosaic virus caused a 13% yield loss statewide in Kansas wheat in 1998 (Bockus et al., 2001), with losses in individual fields being much greater.

The Hessian fly is distributed throughout the major wheat growing regions of the world including Europe, Northern Asia, North Africa, North America, and New Zealand. The HF can cause total crop failure of susceptible wheat varieties in regions of North Africa (Amri et al., 1992). In the Pacific Northwest region of the US, growing HF resistant wheat cultivars can increase grain yields by as much as 68% in comparison with susceptible cultivars (Smiley et al., 2004). Host plant resistance to the WCM and to the HF has been identified in wheat and is considered to be the most reliable and economical means of control (Hatchett et al., 1987).

Two major resistance genes, *Cmc4* and *H23*, provide protection against WCMs and HF, respectively. Both of these pest resistance genes were derived from *Aegilops tauschii* and have been mapped to chromosome 6D in wheat (Ma et al., 1993; Malik et al., 2003). The WCM resistance gene *Cmc4* was mapped to the terminal portion of

chromosome 6DS linked to microsattelite marker *Xgdm141* at a distance of 4.1 cM (Malik et al., 2003). HF resistance gene *H23* was also located on chromosome 6DS in the distal region (Ma et al., 1993).

The *Cmc4* gene was transferred to wheat germplasm KS96WGRC40 from the *Ae. tauschii* accession TA2397. TA2397 belongs to the *Ae. tauschii* subspecies *typica* and was originally collected in Afghanistan. The *Cmc4* gene is widely effective in the United States, providing resistance to collections of the WCM from Kansas, Montana, and Nebraska (Malik et al., 2003). Wheat germplasm KS96WGRC40 contains another WCM resistance gene designated as *Cmc3* located on chromosome 1RS of the wheat-rye translocation T1AL·1RS, derived from the wheat cultivar TAM107 background of the germplasm. The *Cmc3* gene is not effective against WCMs in Kansas. The rye specific microsatellite primer SCM09 can be used to test for the presence of the 1RS segment (Malik et al., 2003).

The *H23* gene was transferred to wheat germplasm KS89WGRC03 from the *Ae. tauschii* accession TA1642 (Raupp et al., 1993). TA1642 was collected in Iran and belongs to the *strangulata* subspecies. The *H23* gene provides resistance to HF biotype D, GP, and L (Gill et al., 1991). Wheat germplasm KS89WGRC04 has a gene transferred from *Ae*. *tauschii* accession TA1695 that also provides resistance to HF biotype D, GP, and L. No segregation was observed in a population of more than 200 F_2 individuals from a cross between KS89WGRC03 and KS89WGRC04 inoculated with HF biotype L. This indicates that KS89WGRC04 also has the *H23* gene (Singh, unpublished).

Previous mapping studies suggest that *Cmc4* and *H23* should be in close proximity to each other (Liu et al., 2005, Malik et al., 2003, Ma et al., 1993). The published linkage maps for *Cmc4* and *H23* (Figures 2.1 and 2.2) both contain the RFLP marker *XksuG48.a*. Malik et al. (2003) located *Cmc4* 11.4 cM proximal to *XksuG48.a* (Figure 2.1). Ma et al. (1993) placed the *H23* gene 15.6 cM from *XksuG48.a,* but the small number of markers in their map of 6DS did not allow them to determine if the resistance gene was distal or proximal (Figure 2.2). Based on these two linkage maps, the distance between *Cmc4* and *H23* ranges from 4.2 cM to 27.0 cM, depending on the orientation of the genes with respect to *XksuG48.a*.

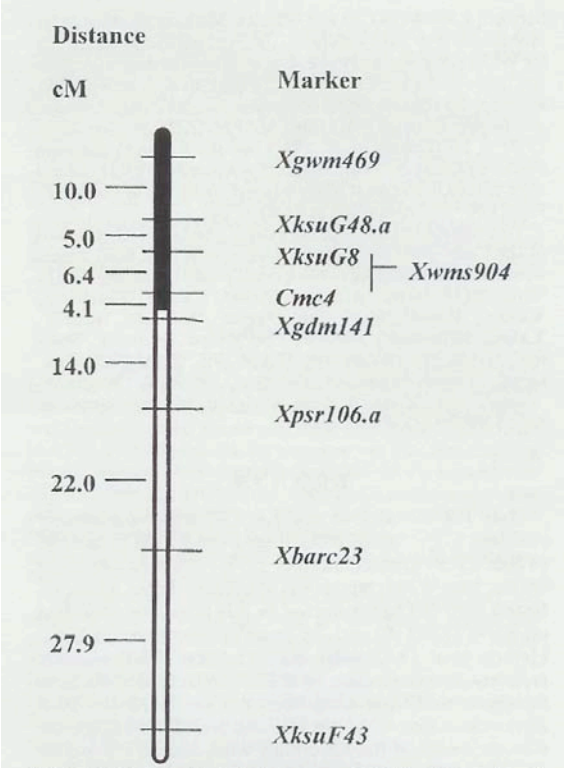

Figure 2.1. Linkage map of *Cmc4* on wheat chromosome 6DS from Malik et al. 2003.

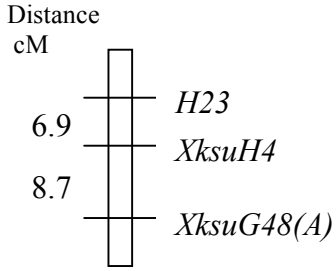

Figure 2.2. Linkage map of *H23* on wheat chromosome 6DS from Ma et al. 1993.

Combining *Cmc4* and *H23* together in one germplasm would facilitate breeding efforts. The objectives of this experiment were to estimate linkage between *Cmc4* and *H23*, to combine *Cmc4* and *H23* in coupling within an agronomically acceptable background, and to validate microsatellite markers for the selection of derived lines containing *Cmc4* and *H23*.

Materials and Methods

Plant Materials

A testcross (TC) population was constructed from the cross KS89WGRC04/KS96WGRC40//Jagger. The pedigree of KS89WGRC04 is Wichita*3/TA1695 (Gill et al., 1991). KS96WGRC40 has a mostly TAM107 background; the pedigree of KS96WGRC40 is TAM107*3/TA2460//TA2397/3/TAM107*3/TA2460 (Cox et al., 1999). The F_1 between KS89WGRC04/KS96WGRC40 was crossed to the cultivar Jagger because it is susceptible to both the WCM and HF and allowed for the recombinant gametes to be individually examined in the TC_1F_2 families. In addition to serving as a tester for the

genetic analysis, Jagger is a widely adapted cultivar that could serve as a donor of genes for yield and quality to germplasm resulting from the cross.

The *H23* and *Cmc4* loci can be followed at each generation during the development of the testcross population (Figure 2.3). Each TC_1F_1 individual receives a susceptible allele for both *Cmc4* and *H23* from the tester genotype Jagger. The other allele in each TC_1F_1 individual was inherited from the KS89WGRC04/KS96WGRC40 F_1. The F_1 could produce four different gametes: recombinant gametes *Cmc4H23* or *cmc4h23* or parental gametes *cmc4H23* or *Cmc4h23*. Each F_1 gamete is combined with a Jagger gamete (*cmc4h23*) to produce four possible TC_1F_1 genotypes: *Cmc4cmc4H23h23*, *cmc4cmc4h23h23*, *cmc4cmc4H23h23* or *Cmc4cmc4h23h23*. The TC_1F_1 individuals were selfed to produce 115 TC_1F_2 families.

The TC_1F_2 families were evaluated for reaction to HF and WCMs in separate screenings. Due to germination problems only 101 families had data recorded from both screenings. Based on the results from the TC_1F_2 screenings, each TC_1F_2 family was classified into one of the four possible TC_1F_1 genotypes.

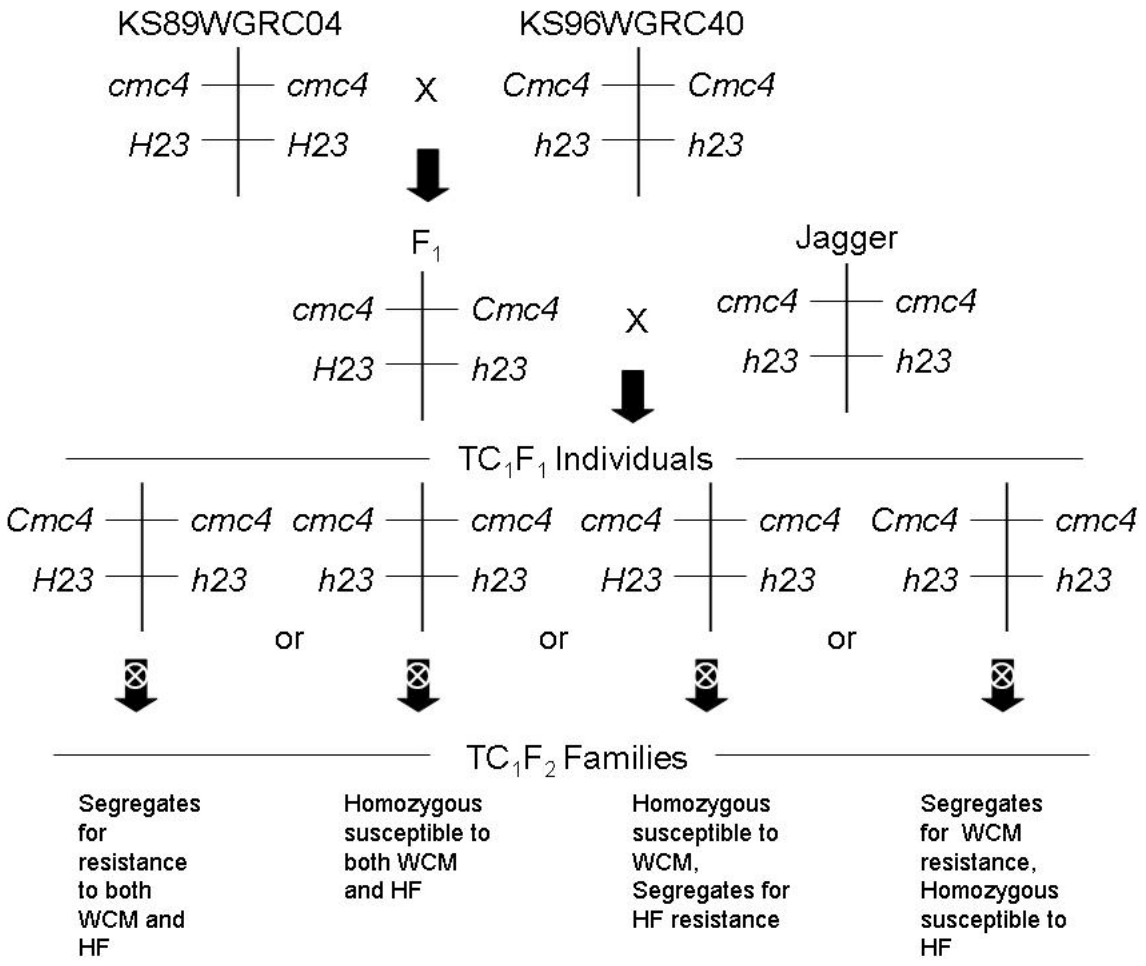

Figure 2.3. Representation of the chromosome 6DS segment containing *Cmc4* and *H23* during the development of TC_1F_2 families.

Hessian Fly Screening

Twelve seeds of each family from the TC_1F_2 population were planted in flats and infested at the two-leaf stage with mature HF of the Great Plains (GP) biotype. The HF resistant (KS89WGRC04) and HF susceptible (Jagger) controls were included in the screening. Plants were scored 14 days after infestation. Families were classified as either susceptible or segregating and the number of resistant and susceptible plants in each family were recorded. Resistant individuals from segregating families were saved and served as the source of seed of the next generation.

Wheat Curl Mite Screening

Eight seeds of each family within the TC_1F_2 population were planted in 2" pots and infested with mature nonviruliferous WCMs collected in Kansas following the procedure of Harvey et al. (1999). The WCM resistant (KS96WGRC40) and WCM susceptible (TAM107) controls were included in the screening. Plants were scored 11 days after infestation. Families were classified as either susceptible or segregating and the number of resistant and susceptible plants in each family were recorded. Resistant individuals from segregating families were saved and served as the source of seed of the next generation.

Selection and Advancement

Compiling the data from the HF and WCM screenings identified 16 families that segregated for resistance to both pests. Resistant individuals from these families were saved and selfed to produce $TC_1F_{2:3}$ lines. Forty-eight $TC_1F_{2:3}$ lines from 16 families were screened for HF in a similar fashion as described above with 25 seed per line

planted. Fifteen lines homozygous resistant to HF were identified from this screening. These 15 homozygous HF resistant lines came from nine TC_1F_2 families.

The $TC_1F_{2:3}$ lines that had enough seed were grown in the field during the 2004-2005 growing season at the Kansas State University Department of Agronomy Ashland Bottoms Research Farm in Manhattan, KS. Lines were each grown in a single one meter row. Reaction to naturally occurring epidemics of leaf rust (caused by *Puccinia triticinia* Eriks.) and powdery mildew [caused by *Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici*] were recorded for each line. Overall adaptation and plant type was also noted. These rows were harvested in bulk and served as the source of seed of the $TC_1F_{2:4}$ generation.

The 15 homozygous HF resistant $TC_1F_{2:4}$ lines were screened for WCMs in a similar fashion as described above with 25 seed per line planted. Three lines were identified as homozygous resistant for WCMs. The three lines, U5287-(8)-24-7-1, U5287-(8)-42-1-1, and U5287-(4-5)-47-1-1, are homozygous for *H23* and *Cmc4*.

The 15 selected $TC_1F_{2:4}$ lines, Jagger, KS89WGRC04, and KS96WGRC40 were grown in the field during the 2005-2006 growing season at Ashland Bottoms Research Farm in Manhattan, KS. A portion of the seed of each $TC_1F_{2:4}$ line was planted in a single one meter row in a soil-borne wheat mosaic virus (SBWMV) infected area. Reaction to SBWMV and to leaf rust was recorded for each line in this nursery. The 15 selected $TC_1F_{2:4}$ lines, Jagger, KS89WGRC04, and KS96WGRC40 were also grown in 3-row, 3-meter plots in a randomized complete block design with two replications at Ashland Bottoms Research Farm in Manhattan, KS during the 2005-2006 growing season. These plots were harvested with a research plot combine to estimate yield

potential. Plant height, heading date, test weight, and thousand kernel weight was recorded for each plot. Plant height was measured from the ground to the top of the inflorescence not including the awns. Plots were considered headed when at least 50% of the plants within a plot had an entire inflorescence clear from the boot. Test weights were measured with a grain analysis computer (Dickey John GAC 2000). A Seedburo 801 Count-A-Pak (Seedburo Equipment Co., Chicago, IL) was used to mechanically count a sample of 1000 kernels which was weighed to obtain thousand kernel weight. Genotype means from the randomized complete block experiment were generated and analyzed with SAS v8.0 for Windows (SAS Institute Inc., Cary, NC).

DNA Extractions

Leaf tissue was collected from 7-day old germinated seedlings from each of the 15 selected $TC_1F_{2:4}$ lines, Jagger, Wichita, TAM107, KS96WGRC40, KS89WGRC04, TA1695 and TA2397. Leaf tissue was placed in 1.5 mL microcentrifuge tubes and stored at -80°C and then ground to a fine powder in liquid nitrogen using a mortar and pestle and stored -80°C. Small scale DNA extractions were performed by adding 500 µl extraction buffer [100 mM glycine, 50 mM NaCl, 10 mM EDTA, 2% SDS, and 30 mM sodium lauryl sarsosine] and 500 µl phenol:chloroform:isoamyl alcohol [50:49:1] to the ground tissue and mixing for 10 minutes at room temperature. Samples were centrifuged at 8000 rpm for 10 minutes and the supernatant transferred to a new microcentrifuge tube with a micropipette. An equal volume of chloroform:isoamyl alcohol [24:1] was added and samples were centrifuged again at 8000 rpm for 10 minutes. The supernatant was transferred to a new microcentrifuge tube and mixed with 1/10 volume of 3M sodium acetate and one volume isopropanol for DNA precipitation. DNA precipitation occurred

overnight at 4°C. The DNA was pelleted by centrifugation at 8000 rpm for 10 minutes after which the isopropanol/sodium acetate mixture was poured off. The DNA was washed with 70% ethyl alcohol and pelleted by centrifugation at 8000 rpm for 10 minutes. The ethyl alcohol was poured off and the DNA was re-suspended in 50 µl of 1X TE buffer. This method is adapted from Malik et al. (2003).

Marker Analysis

Microsatellite primer pairs located in the distal portion of wheat chromosome 6DS, based on the wheat consensus map of Somers et al. (2004), were screened for polymorphisms. Selected germplasm lines were evaluated with polymorphic markers from chromosome arm 6DS and primer SCM09 that is specific for the short arm of rye chromosome 1R. Each 25µl polymerase chain reaction contained 2.5 µl of 10X Mg free PCR buffer, 3.0 µl of 2.5mM dNTP's, 0.25 µl of Taq DNA polymerase (5 units/µl), 2.5 µl of 25 mM magnesium chloride, 5.0 µl of genomic DNA, 10.75 µl of sterile molecular grade water, and 1.0 µl of 10 µM primers [0.5 µl of forward primer and 0.5 µl of reverse primer]. Reactions were carried out in a PTC-200 Thermal Cycler (MJ Research, Watertown, MA, USA). A 10 µl aliquot of PCR product was separated by electrophoresis in 2.3% agarose gels to check for amplification and estimate fragment size. Agarose gels were stained with ethidium bromide and visualized with an ultraviolet transilluminator. From the remaining PCR product, 5 µl was separated by electrophoresis in 7% denaturing polyacrylamide gels. The polyacrylamide gels were visualized through a silver nitrate staining process following the protocol of Bassam et al. (1991).

Deletion Mapping

The Wheat Genetics Resource Center at Kansas State University provided the genetic stocks of Chinese Spring used to physically map molecular markers in the chromosome 6DS region. The aneuploid stocks used in this study included: nullisomic 6D-tetrasomic 6A (N6D-T6A), nullisomic 6A-tetrasomic 6D (N6A-T6D) (Sears, 1966), ditelosomic 6DL (Dt6DL), ditelosomic 6DS (Dt6DS) (Sears and Sears, 1979), and three 6DS deletion stocks of Chinese Spring (Endo and Gill, 1996). Deletion stocks are identified by the chromosome arm carrying the deletion, the chromosome breakpoint, and the fraction length of the chromosome arm remaining. Deletion stocks used in this study included 6DS-2-0.45, 6DS-4-0.79 and 6DS-6-0.99. Together the aneuploid stocks and the deletion stocks were used to deletion bin map microsatellite markers previously reported to be in the distal portion of chromosome 6DS. DNA was extracted from Chinese Spring, the aneuploid stocks, and the deletion stocks following the DNA extraction protocol outlined above. PCR and fragment analysis was also performed following the protocol above.

Results and Discussion

Linkage Analysis of *H23* and *Cmc4*

The observed segregation for reaction to the HF and WCM of the TC_1F_2 families (Table 2.1) fit the segregation ratio of 1:1 expected for a single gene conferring resistance in each case (χ^2=2.14, p>0.90). The testcross population segregated for one dominant WCM resistance gene and one dominant HF resistance gene.

The parental germplasm KS89WGRC04 was resistant to HF and susceptible to WCMs as tested in the phenotypic screenings for both pests. KS96WGRC40 was susceptible to HF and resistant to WCMs. The HF susceptible control Jagger and the

WCM susceptible control TAM107 were susceptible in each respective phenotypic evaluation.

Table 2.1. Segregation for resistance to wheat curl mite and Hessian fly from greenhouse screenings of a TC_1F_2 KS89WGRC04/KS96WGRC40//Jagger population, Manhattan, KS 2004.

	Number of susceptible families	Number of segregating families	Total
WCM screening			
Observed	60	45	105
Expected	52.5	52.5	105
χ^2	1.07	1.07	2.14
HF screening			
Observed	48	63	111
Expected	55.5	55.5	111
χ^2	1.07	1.07	2.14

Analysis of the combined results of both screenings indicated that *H23* and *Cmc4* did not segregate independently and are linked (Table 2.2). The observed segregation ratio deviated significantly (χ^2=16.03, p>0.99) from a 1:1:1:1 ratio expected if the two genes segregate independently. The frequency of recombination between the two genes was estimated as 32.67% ± 4.7%. This estimate was obtained by dividing the number of recombinant families (33) by the total number of families (101). The standard error of recombination frequency in a testcross was calculated using the following formula: $\sqrt{p(1-p)/n}$, where p is the estimated recombination frequency (Allard, 1956).

Table 2.2. Linkage analysis between *Cmc4* and *H23* as tested in a TC_1F_2 KS89WGRC04/KS96WGRC40//Jagger population, Manhattan, KS 2004.

TC_1F_2 Phenotype	TC_1F_1 Genotype	Number of families observed	Number of families expected	χ^2
Susceptible to WCMs and HF	*cmc4cmc4 h23h23**	17	25.25	2.70
Segregating for WCMs and HF	*Cmc4cmc 4H23h23**	16	25.25	3.39
Segregating for WCMs and susceptible to HF	Cmc4cmc 4h23h23^	27	25.25	0.125
Susceptible to WCMs and segregating for HF	*cmc4cmc4 H23h23*^	41	25.25	9.82
Total		101	101	16.03

*Recombinant classes
^Parental classes

Marker Analysis

Thirteen microsatellite markers previously mapped to the short arm of chromosome 6D were screened for polymorphisms among KS89WGRC04, KS96WGRC40, Jagger, TAM107, Wichita, TA2397, and TA1695. Seven polymorphic markers were used to haplotype the 6DS segment in 15 selected $TC_1F_{2:4}$ lines from the KS89WGRC04/KS96WGRC40//Jagger population (Table 2.3). All of these lines were homozygous resistant to HF and three lines were homozygous resistant to both HF and WCMs. The order of the markers in Table 2.3 is subjective; the latest hexaploid wheat consensus map of chromosome 6D does not contain *Xgdm141* or *Xgdm036* (Somers et al., 2004). The positions of *Xgdm141* and *Xgdm036* in Table 2.4 were inferred from the linkage map of Liu et al. (2005).

The physical location of each microsatellite marker given in Table 2.3 was determined by deletion mapping. The D-genome specific fragments amplified by microsatellite primer pairs BARC183, GDM132, GDM141, CFD042, and GWM469

were not present in any of the 6DS deletion lines. This places these SSR markers (*Xbarc183*, *Xgdm132*, *Xgdm141*, *Xcfd042*, and *Xgwm469)* in the most distal bin 6DS-6-0.99-1. The D-genome specific fragments amplified by microsatellite primer pairs GDM036 and BARC054 were present in the deletion line 6DS-6-0.99 and absent in all other deletion lines. This indicates that deletion bin 6DS-4-0.79-0.99 contains the SSR markers *Xgdm036* and *Xbarc054*. Malik et al. (2003) placed *Cmc4* in deletion bin 6DS-6-0.99-1 which agrees with the marker analysis presented here.

From the haplotypes of the 15 selected lines it is evident the size of the *Ae. tauschii* segment transferred to each derived line varied (Table 2.3). Crossing over must have occurred at different points so that lines having a mosaic of 6DS segments derived from TA1695, TA2397, and Jagger were identified. However, all three derived lines homozygous for both *Cmc4* and *H23* [U5287-(8)-24-7-1, U5287-(8)-42-1-1, and U5287-(4-5)-47-1-1] posses the entire distal portion of 6DS from *Ae. tauschii.* Lines U5287-(8)-42-1-1 and U5287-(4-5)-47-1-1 have most of the terminal region derived from TA1695, with only the most distal marker *Xbarc183* derived from TA2397. Line U5287-(8)-24-7-1 has a larger distal segment derived from TA2397, with only the more proximal marker *Xgdm036* originating from TA1695.

Our haplotype data indicates *Cmc4* is distal to marker *Xgdm141,* as reported by Malik et al. (2003). The KS96WGRC40 used as a control in this study contains *Cmc4* but carries the 150 base pair TAM107 allele at the marker locus *Xgdm141*, consistent with the results of Malik et al. (2003). However, the 145 base pair TA2397 allele was amplified by primer pair GDM141 in eight of the 15 lines analyzed from the testcross population and no lines amplified the 150 base pair allele. This suggests that the

KS96WGRC40 used to produce our population differed at this locus from the one used as a control in the study. KS96WGRC40 was a bulk of several WCM resistant plants selected from KS95WGRC33 (Cox et al., 1999). Although these plants were homozygous resistant to the WCM, they may have been heterogeneous for the size of the chromosome segment transferred from TA2397.

Microsatellite primer pair BARC183 was more useful than GDM141 in identifying lines carrying *Cmc4* in this set of 15 derived lines. Two fragments were amplified by BARC183 from DNA of each of the hexaploid derived lines that were susceptiple to WCM, as well as Jagger, TAM107, KS89WGRC04, and Wichita (Figure 2.4). No amplification was observed for DNA of TA2397 with primer pair BARC183. For the WCM resistant germplasm KS96WGRC40, the TA2397-derived null allele for *Xbarc183* replaced the larger fragment of approximately 170 base pairs (Figure 2.4). All of the WCM resistant lines selected from the testcross population were also null for the BARC183 D-genome fragment (Table 2.3). Malik et al. (2003) did not report linkage between *Xbarc183* and *Cmc4*, presumably because the complete set of BARC microsatellite primers was not available at the time the mapping work was done.

The published linkage map of *H23* contains only RFLP markers that were not included in this marker analysis (Ma et al., 1993). However, in a mapping population from the cross KS89WGRC04/Wichita, microsatellite marker *Xbarc054* was located 7.3 cM from the HF resistance gene (Singh, personal communication). In the haplotype analysis reported here, the TA1695 allele amplified by BARC054 was present in only seven of the 15 lines homozygous resistant to HF. However, 11 of the 15 lines had the 160 base pair allele derived from TA1695 for marker *Xgdm036*, suggesting this

microsatellite marker may be more closely linked the *H23* gene (Table 2.3). Lines U5287-(8)-24-2-1 and U5287-(8)-14-3-1 are heterozygous at marker locus *Xgdm036* having both the 160 base pair allele from KS89WGRC04 and the 130 base pair allele from KS96WGRC40 (Table 2.3).

Table 2.3. Haplotypes of 15 $TC_1F_{2:4}$ lines from a KS89WGRC04/KS96WGRC40//Jagger population based on seven microsatellite markers mapped to chromosome 6DS. Primer pairs are in order based on the updated Somers et al., 2004 consensus map of hexaploid wheat chromosome 6DS posted on the GrainGenes website (http://wheat.pw.usda.gov, verified 10 April 2006) with primer pairs *gdm141* and *gdm036* inserted based on the linkage map of Liu et al., 2005.

		Germplasm										
		U5287-(8)-24-6-1	U5287-(4-5)-53-2-1	U5287-(4-5)-47-1-1	U5287-(4-5)-45-1-1	U5287-(8)-24-4-1	U5287-(8)-24-2-1	U5287-(8)-10-1-1	U5287-(8)-9-4-1	U5287-(8)-9-6-1	U5287-(8)-10-2-1	U5287-(8)-14-1-1
Markers	Deletion Bin	*H23*	*H23*	*Cmc04, H23*	*H23*	*H23*	*H23*	*H23*	*H23*	*H23*	*H23*	*H23*
Xbarc183	6DS-6-0.99-1	170	170	Null	170	170	170	175	170	175	175	170
Xgdm132	6DS-6-0.99-1	145	145	140	145	145	145	140	140	140	140	145
Xgdm141	6DS-6-0.99-1	145	175	175	175	145	145	175	175	175	175	145
Xcfd042	6DS-6-0.99-1	Null	200	200	200	Null	197	200	200	200	200	197
Xgwm469	6DS-6-0.99-1	155	155	170	160	155	155	170	170	170	170	155
Xgdm036	6DS-4-0.79-0.99	155	160	160	160	160	160 130	160	160	160	160	160
Xbarc054	6DS-4-0.79-0.99	190	200	200	200	190	190	190	200	200	190	190

		Germplasm										
		U5287-(8)-24-7-1	U5287-(8)-14-3-1	U5287-(8)-42-1	U5287-(4-5)-56-1-1	TA1695	KS89WGRC04	TA2397	KS96WGRC40	TAM107	Wichita	Jagger
Markers	Deletion Bin	*Cmc4, H23*	*H23*	*Cmc4, H23*	*H23*	*H23*	*H23*	*Cmc04*	*Cmc03, Cmc04*	*Cmc03*		
Xbarc183	6DS-6-0.99-1	Null	170	Null	170	165	175	Null	Null	170	170	170
Xgdm132	6DS-6-0.99-1	145	145	140	145	140	140	145	350	150	150	145
Xgdm141	6DS-6-0.99-1	145	145	175	145	175	175	145	150	150	125	125
Xcfd042	6DS-6-0.99-1	Null	197	200	Null	200	200	Null	190	190	220	197
Xgwm469	6DS-6-0.99-1	155	155	170	155	170	170	155	155	175	175	160
Xgdm036	6DS-4-0.79-0.99	160	160 130	160	155	160	160	155	130	130	130	130
Xbarc054	6DS-4-0.79-0.99	190	190	200	200	200	200	185	190	190	180	190

Notes: TA1695 alleles are in yellow. TA2397 alleles are in green.
Monomorphic markers are in pink.

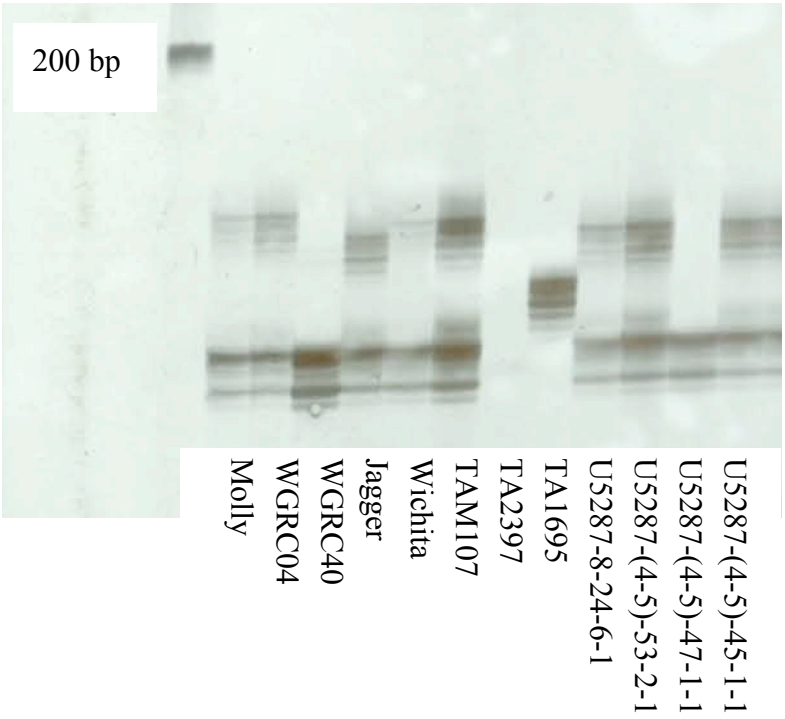

Figure 2.4. Banding pattern of microsatellite primer pair BARC183 separated on a 7% denaturing polyacrylamide gel visualized by silver staining.

Engineering a *H23*/*Cmc4* Linkage Block

Fifteen $TC_1F_{2:3}$ families were identified as homozygous resistant for *H23*. Three $TC_1F_{2:4}$ lines were identified as homozygous resistant for *H23* and *Cmc4* following the selection and advancement scheme as outlined above. These three lines, U5287-(8)-24-7-1, U5287-(8)-42-1-1, and U5287-(4-5)-47-1-1, are potential germplasm releases carrying *H23* and *Cmc4* in a very acceptable Jagger background. These three lines also carry resistance to SBWMV (Table 2.4), presumably derived from Jagger or KS96WGRC40 which are both resistant to SBWMV.

The 15 derived lines evaluated in the field varied agronomically (Table 2.4). The germplasm U5287-8-42-1-1, resistant to both HF and WCM, was late maturing, heading eight days after Jagger (Table 2.4). Although the other two lines that were resistant to

both HF and WCM, U5287-8-24-7-1 and U5287-(4-5)-47-1-1, had a more acceptable maturity, heading one day after Jagger, U5287-(4-5)-47-1-1 had black glumes at maturity. This trait is often associated with poor threshability. The three selected lines have acceptable heights, test weights, thousand kernel weights, and grain yields similar to Jagger (Table 2.4). All three of the potential germplasm releases surpass the KS89WGRC04 parent in grain yield (Table 2.4). However, none were significantly different from KS96WGRC40 for yield.

KS96WGRC40 contains the rye derived WMC resistance gene *Cmc3* as well as *Cmc4*. The rye specific microsatellite marker *Xscm09* can be used to test for the presence of the 1RS segment. Line U5287-(8)-24-7-1 contained the *Xscm09* locus (Table 2.4), indicating that it also has the *Cmc3* gene as well as *Pm17*. Although the translocation T1AL·1RS has been heavily used by soft winter wheat breeding programs, the translocation is not desirable for bread-making quality.

Table 2.4. Characterization of 15 selected lines derived from the population KS89WGRC04/KS96WGRC40//Jagger.

	Greenhouse Characterizations		Field Characterizations					
Trait	Hessian Fly	WCM	Trait	Leaf Rust		Powdery Mildew	SBWMV†	Height (cm) *
Race	Biotype GP	KS Strain	Location	Kansas		Kansas	Kansas	Kansas
Year	2005	2005	Year	2005	2006	2005	2006	2006
Genotype								
U5287-(8)-9-4-1	R	S		S	50S	R	S	92.5 ced
U5287-(8)-9-6-1	R	S		S	10-30S	R	R	92.5 ced
U5287-(8)-10-1-1	R	S		S	60S	S	S	87.5 ed
U5287-(8)-10-2-1	R	S		S	50S	S	R	100 bc
U5287-(8)-14-1-1	R	S		S	5-50S	H	S	100 bc
U5287-(8)-14-3-1	R	S		R	10-50S	R	S	85 e
U5287-(8)-24-2-1	R	S		S	70S	S	R	97.5 bc
U5287-(8)-24-4-1	R	S		S	30S	R	R	100 bc
U5287-(8)-24-6-1	R	S		S	60S	MR	R	95 bcd
U5287-(8)-24-7-1^	R	R		S	5-50S	R	R	95 bcd
U5287-(8)-42-1-1^	R	R		-	5MR-30S	-	R	92.5 ced
U5287-(4-5)-47-1-1^	R	R		S	40S	S	R	102.5 b
U5287-(4-5)-45-1-1	R	S		10S	10-30S	R	R	117.5 a
U5287-(4-5)-53-2-1	R	S		S	50S	S	R	102.5 b
U5287-(4-5)-56-1-1	R	S		-	5S	-	R	85 e
Jagger	S	S		20S	10S	S	R	95 bcd
KS89WGRC04	R	S		S	30-40S	S	R	92.5 ced
KS96WGRC40	S	R		S	60S	R	R	92.5 ced

- Line was not rated.

^ Potential germplasm release resistant to both HF and WCM.

* Means followed by different letters are significantly different ($p<0.05$).

† Soil-borne wheat mosaic virus

Table 2.4 cont. Characterization of 15 selected lines derived from the population KS89WGRC04/KS96WGRC40//Jagger.

	Field Characterizations				Molecular characterizations
Trait	Heading Date*	Test Weight (lbs/bu)*	TKW (g) *	Yield (bu/acre)*	*SCM09* [§]
Location	Kansas	Kansas	Kansas	Kansas	locus
Year	2006	2006	2006	2006	2006
Genotype					
U5287-(8)-9-4-1	5 May dc	55.30 ba	23.4 f	44.43 fe	Present
U5287-(8)-9-6-1	8 May bc	54.00 bc	33.5 bac	59.83 fdec	Present
U5287-(8)-10-1-1	8 May bc	56.15 ba	29.1 dec	53.05 fde	Present
U5287-(8)-10-2-1	1 May de	56.30 ba	34.8 a	71.75 bdac	Absent
U5287-(8)-14-1-1	2 May de	56.35 ba	28.3 de	67.19 bdac	Present
U5287-(8)-14-3-1	12 May a	52.20 c	25.9 fe	55.44 fdec	Present
U5287-(8)-24-2-1	29 April f	55.00 ba	27.2 fe	77.71 bac	Absent
U5287-(8)-24-4-1	9 May ab	53.9 bc	26.3 fe	77.47 bac	Present
U5287-(8)-24-6-1	30 April ef	55.75 ba	35.2 a	72.83 bdac	Present
U5287-(8)-24-7-1^	1 May de	54.2 bc	35.7 a	88.55 a	Present
U5287-(8)-42-1-1^	8 May bc	56.5 ba	29.4 bdec	63.72 bdec	Absent
U5287-(4-5)-47-1-1^	1 May de	56.95 a	32.3 bdac	77.45 bac	Absent
U5287-(4-5)-45-1-1	12 May a	56.5 ba	31.9 bdac	64.40 bdec	Present
U5287-(4-5)-53-2-1	8 May bc	56.35 ba	30.1 bdec	83.87 ba	Absent
U5287-(4-5)-56-1-1	9 May ab	-	28.4 de	-	Absent
Jagger	30 April ef	55.45 ba	33.1 bac	72.49 bdac	Absent
KS89WGRC04	10 May ab	55.90 ba	33.7 ba	40.94 f	Absent
KS96WGRC40	1 May de	56.10 ba	35.3 a	84.41 ba	Present

- Line was not rated.

^ Potential germplasm release resistant to both HF and WCM.

* Means followed by different letters are significantly different ($p<0.05$). TKW = thousand kernel weight

[§] Primer pair *SCM09* tests for the presence of the T1AL·1RS translocation.

Conclusions

The recombination estimate of 32.67% observed in this study indicates that *H23* and *Cmc4* are not tightly linked. The observed frequency of recombination is close to the 27.0 cM expected from previous mapping work if marker *XksuG48.a* were distal to *Cmc4* and proximal to *H23*. However, the haplotype data of recombinant lines using SSR markers from 6DS located *Cmc4* distal to *H23*. Physical mapping of linked SSR markers also placed *Cmc4* distal to *H23*. The haplotype data indicated that in both KS89WGRC04 and KS96WGRC40 the distal portion of 6DS was derived from *Ae. tauschii* (Table 2.4). The *Ae. tauschii* origin of the chromosome segment may have influenced the amount of recombination observed between *H23* and *Cmc4* in this testcross population. Boyko et al. (2002) reported that the telomeric end of chromosome 6DS had the highest rate of recombination among all seven of the *Ae. tauschii* chromosomes. The maps of Ma et al. (1993) and Malik et al. (2003) were based on recombination between *T. aestivum* and *Ae. tauschii* derived chromosome segments. Further analysis of the amount of recombination between *H23* and *Cmc4* in crosses between germplasm lines having the distal portion of 6DS from *Ae. tauschii* and wheat breeding lines and/or cultivars will determine if *H23* and *Cmc4* will be inherited as a linkage block.

Our marker analysis determined that microsatellite primer pair BARC183 was useful in identifying lines carrying *Cmc4* and may be more closely linked to *Cmc4* than the previously published marker *Xgdm141*. However, the null allele for *Xbarc183* was in coupling with *Cmc4*, limiting the use of the marker to identification of homozygous resistant lines. In addition, we determined that the co-dominant microsatellite marker *Xgdm036* may be more closely linked to *H23* than previous reported markers.

Three $TC_1F_{2:4}$ lines were identified as homozygous resistant for *H23* and *Cmc4*. Although all three have the complete terminal segment of 6DS from *Ae*. *tauschii*, two of the lines are more agronomically desirable than the original germplasm releases. KS89WGRC04 is a tall, later maturing line in a Wichita background that is no longer desirable to wheat breeders. Although KS96WGRC40 has resistance to WCM, Septoria leaf blotch, and SBWMV, it is not in a background having desirable end-use quality. The germplasm lines developed in this study can serve as a source of resistance to both HF and WCM in an elite background.

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CHAPTER 3 GENETIC ANALYSIS OF RESISTANCE TO SEPTORIA LEAF BLOTCH IN KS96WGRC40

Introduction

Septoria leaf blotch (STB) is a fungal leaf disease of wheat caused by *Septoria tritici* [telomorph *Mycosphaerella graminicola* (Fuckel) J. Schrot.]. STB is routinely a problem in temperate high rainfall wheat (*Triticum aestivum* L.) growing regions such as the United Kingdom, western Australia, northwestern USA, and southeastern USA. Severe epidemics of STB have occurred in many Great Plains states in the USA whenever high rainfall occurs during the wheat growing season. There are fungicides available which control STB in wheat; however, *S. tritici* isolates resistant to the strobilurin chemistry have recently been identified in Europe (www.rothamsted.bbsrc.ac.uk, verified 28 April 2006). Fungicide applications are also expensive to apply. Utilizing resistant cultivars is a more economic and efficient means of control.

To date, eight STB resistance genes, *Stb1* through *Stb8*, have been characterized from common wheat and related species (Table 3.1). Molecular markers linked to these eight genes have been identified that may assist wheat breeders through marker assisted selection of lines carrying resistance genes. Genes for resistance to STB have been identified in all three genomes of wheat. Genes *Stb1*, *Stb2,* and *Stb8* are located in the B-genome. Genes *Stb6* and *Stb7* are located in the A-genome. Genes *Stb3*, *Stb4,* and *Stb5* are located in the D-genome. The *Stb5* and *Stb8* genes were transferred from *Aegilops tauschii* (2n=2x=14, DD genome) and *Triticum turgidum* (2n=4x=28, AABB genomes), respectively, via crosses with synthetic hexaploid wheat (AABBDD genomes).

KS96WGRC40 is a hard red winter wheat germplasm developed at the Wheat Genetics Resource Center (Manhattan, KS) that has resistance derived from *Ae*. *tauschii* to multiple pests including the wheat curl mite, leaf rust, soil-borne wheat mosaic virus, and STB. The pedigree of KS96WGRC40 is TAM107*3/TA2460//TA2397/3/TAM107*3/TA2460 (Cox et al., 1999). The wheat curl mite resistance gene *Cmc4* was characterized in KS96WGRC40 by Malik et al. (2003) and was derived from the *Ae. tauschii* accession TA2397. KS96WGRC40 also carries the leaf rust resistance gene *Lr39/Lr41* derived from the *Ae. tauschii* accession TA2460 (Cox et al., 1999). The STB resistance in KS96WGRC40 is derived from accession TA2397 of *Ae*. *tauschii*, but the inheritance of resistance has not been determined. The objective of this research was to characterize the inheritance and chromosome location of the gene(s) for STB resistance within KS96WGRC40.

Table 3.1. Sources, chromosome locations, and markers linked to previously reported Septoria leaf blotch resistance genes in wheat.

Gene	Source	Linked Markers	Location	Reference
Stb1	*T. aestivum* 'Bulgaria88'	*Xbarc74*	5BL	Adhikari et al., 2004C
Stb2	*T. aestivum* 'Veranopolis'	*Xgwm389, Xgwm533.1, Xgwm493*	3BS	Adhikari et al., 2004B
Stb3	*T. aestivum* 'Israel493'	*Xgdm132*	6DS	Adhikari et al., 2004B
Stb4	*T. aestivum* 'Tadinia'	*Xgwm111*	7DS	Adhikari et al., 2004A
Stb5	Synthetic hexaploid 'Syn6X'	*Xgwm44*	7DS	Arraiano et al., 2001
Stb6	*T. aestivum* 'Flame'	*Xgwm369*	3AS	Brading et al., 2002
Stb7	*T. aestivum* 'ST6'	*Xwmc313*	4AL	McCartney et al., 2003
Stb8	Synthetic hexaploid 'W7984'	*Xgwm146, Xgwm577*	7BL	Adhikari et al., 2003

Materials and Methods

Plant Material

A segregating population was created from the cross KS96WGRC40/Wichita. KS96WGRC40 is resistant to STB (Cox et al., 1999) while Wichita is susceptible. The population was advanced by single seed descent to the F_5 generation, then was harvested in bulk. Seventy-eight recombinant inbred lines (RILs) were used in this study.

Three sources of previously named STB resistance genes were included in this study. Dr. Steve Goodwin provided seed of 'Israel493', the *Stb3* source, and two susceptible lines, 'RAC875-2' and 'Taichung29'. Seed of 'Tadinia', the *Stb4* source, was provided by the USDA-ARS National Plant Germplasm Small Grains Collection, Aberdeen, ID. Seed of 'Chinese Spring-Syn6X', the material in which *Stb5* was mapped,

was provided by the John Innes Centre, Norwich, UK. Dr. William Bockus of Kansas State University provided seed of 'Newton' and 'Lakin', while Dr. Allan Fritz also of Kansas State University provided seed of 'Jagger'.

The Wheat Genetics Resource Center at Kansas State University provided the aneuploid genetic stocks of Chinese Spring used to physically map the genetic markers linked to the STB resistance gene within KS96WGRC40. The aneuploid stocks used in this study included: nullisomic 7D-tetrasomic 7A (N7D-T7A), nullisomic 7A-tetrasomic 7D (N7A-T7D) (Sears, 1966), ditelosomic 7DL (Dt7DL), ditelosomic 7DS (Dt7DS) (Sears and Sears, 1979), and two 7DS deletion stocks of Chinese Spring (Endo and Gill, 1996). Deletion stocks are identified by the chromosome arm carrying the deletion, the chromosome breakpoint, and the fraction length of the chromosome arm remaining. Deletion stocks used in this study included 7DS-1-0.37 and 7DS-4-0.61.

***Septoria tritici* phenotyping**

A parental STB phenotyping of germplasms was completed to confirm the resistance in KS96WGRC40 and test the inoculation procedures. Nine germplasms were included in this parental phenotyping: KS96WGRC40, KS89WGRC04, Wichita, Israel493, Jagger, RAC875-2, Newton, Lakin, and Taichung29. These nine germplasms were planted in plastic conical containers supported by plastic trays using a randomized complete block design with 20 replications. Each conical container had one plant and was considered the experimental unit. A conidial suspension was prepared from a mixture of two *Septoria tritici* isolates collected from infected wheat and represented the most virulent genotypes known to occur in Kansas. The conidial suspension contained about 5×10^6 conidia per mL as determined by hemacytometer counts. Knox gelatin was added to the conidial suspension to serve as a sticker. Plants were inoculated with the

conidial suspension (35 mL/100 seedlings) at the 3-leaf stage with the use of an atomizer. After inoculation, the conidial suspension was allowed to dry before the plants were placed in a moisture chamber to maintain leaf wetness. After 96 hours of leaf wetness, plants were removed from the moisture chamber and placed on a greenhouse bench. Eighteen days after inoculation, disease ratings on the first three leaves of each plant were recorded based on the percentage of leaf area covered by chlorotic/necrotic lesions. The disease ratings for the first three leaves were averaged for each experimental unit. Genotype least square means were separated by planned *t* tests using SAS v8.0 for Windows (SAS Institute Inc., Cary, NC). This phenotyping method is adapted from the methods reported by Zhang et al. (2001).

The 78 $F_{5:7}$ RILs from the KS96WGRC40/Wichita population were evaluated for STB resistance in the greenhouse using a similar method with some modifications. Each RIL was planted using a randomized complete block design with 10 replications. The parents of the population (KS96WGRC40 and Wichita) were included in the evaluation. The entire experiment was repeated a week later to give a total of 20 replications. The disease ratings for the first three leaves were averaged for each experimental unit. Data were analyzed as a randomized complete block experiment with a 2 by 80 factorial treatment structure using SAS v8.0 for Windows (SAS Institute Inc., Cary, NC).

DNA Extractions

Genomic DNA was extracted from the nine parental germplasms and the *Ae. tauschii* accession TA2397, which is in the pedigree of KS96WGRC40. Genomic DNA was also extracted from 78 $F_{5:7}$ RILs in the KS96WGRC40/Wichita population and the aneuploid and deletion line stocks used in the study.

Leaf tissue was collected from 7-day-old germinated seedlings and placed in 1.5 mL microcentrifuge tubes. The tissue was stored at -80°C and then ground to a fine powder in liquid nitrogen using a mortar and pestle. Ground tissue samples were stored at -80°C. Small scale DNA extractions were performed by adding 500 µl extraction buffer [100 mM glycine, 50 mM NaCl, 10 mM EDTA, 2% SDS, and 30 mM sodium lauryl sarsosine] and 500 µl phenol:chloroform:isoamyl alcohol [50:49:1] to the ground tissue and mixing for 10 minutes at room temperature. Samples were centrifuged at 8000 rpm for 10 minutes and the supernatant transferred to a new microcentrifuge tube with a micropipette. An equal volume of chloroform:isoamyl alcohol [24:1] was added and samples were centrifuged again at 8000 rpm for 10 minutes. The supernatant was transferred to a new microcentrifuge tube and mixed with 1/10 volume of 3M sodium acetate and one volume isopropanol for DNA precipitation. DNA precipitation occurred overnight at 4°C. The DNA was pelleted by centrifugation at 8000 rpm for 10 minutes after which the isopropanol/sodium acetate mixture was poured off. The DNA was washed with 70% ethyl alcohol and repelleted by centrifugation at 8000 rpm for 10 minutes. The ethyl alcohol was poured off and the DNA was resuspended in 50 µl of 1X TE buffer. DNA concentrations were adjusted to 10 ng/µl with the use of a spectrometer (Nanodrop ND-1000, Wilmington, DE). This method was adapted from Malik et al. (2003).

Molecular Marker Analysis

A bulk segregant analysis was used to identify polymorphic markers potentially linked to STB resistance (Michelmore et al., 1991). Bulk DNA samples were prepared by pooling equal amounts of DNA from the ten most susceptible RILs and the ten most

resistant RILs. The DNA samples of KS96WGRC40, Wichita, the resistant bulk, and the susceptible bulk were screened for polymorphisms with 249 D-genome specific microsatellite primer pairs. Only D-genome specific primer pairs were screened for polymorphism because the STB resistance within KS96WGRC40 is derived from accession TA2397 of *Ae. tauschii* (Cox et al., 1999), the donor of the D-genome of common wheat. The other germplasms within the pedigree of KS96WGRC40, TAM107 and TA2460, are susceptible to STB.

All PCR reactions were performed in 12 μl volumes and included 2.0 μl of genomic DNA, 1.2 μl of 10X PCR buffer with magnesium chloride, 0.96 μl of 10 mM dNTP's, 0.18 μl of Taq DNA polymerase (5units/μl), 5.26 μl of sterile molecular grade water, 0.96 μl of 1 μM forward primer, 0.72 μl of 10 μM reverse primer, and 0.72 μl of one of either FAM, PET, NED, or VIC 10 μM fluorescent labels. Reactions were carried out in either a PTC-200 Thermal Cycler (MJ Research, Watertown, MA,) or a Master Cycler EP384 System (Eppendorf, Westbury, NY). PCR products were multiplexed by pooling four differently labeled PCR products across the same genomic DNA sample with the use of a Hydra II 96 channel microdispenser (Matrix, Hudson, NH). PCR fragments were resolved in an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA) with GeneScan-500 LIZ as an internal size standard (Applied Biosystems, Foster City, CA). Fragment analysis was performed with GeneMarker v1.4 software (SoftGenetics, State College, PA).

Microsatellite primer pairs identified as polymorphic based on the bulk segregant analysis were screened on the entire mapping population. Linkage analysis was conducted with Mapmaker software (version 2.0 for Macintosh). Map distances were

converted to centimorgans using the Kosambi function (Kosambi, 1944). Linkage maps were generated using a maximum Kosambi distance of 50 and a minimum log of odds (LOD) of 3.0.

The linkage analysis identified microsatellite primer pairs linked to the STB resistance gene within KS96WGRC40. The chromosomal locations of these microsatellite primer pairs were previously determined in other mapping populations (Somers et al., 2004). This information was used to select more microsatellite primer pairs in the identified chromosomal region of interest. These additional microsatellite primer pairs were also screened on the population in similar fashion.

Five microsatellite primer pairs linked to the STB resistance in KS96WGRC40 were deletion bin mapped using the Chinese Spring aneuploid stocks. Five additional microsatellite primer pairs that were not identified as linked to the STB resistance in KS96WGRC40 but were proximal on chromosome 7D based on the Somers et al. (2004) consensus map were also deletion bin mapped to have a more complete physical map of 7D. PCR reactions and fragment analysis for the deletion bin mapping was performed as described above.

To further analyze the DNA fragments amplified by linked microsatellite primer pairs, several PCR fragments were also separated by electrophoresis in 7% denaturing polyacrylamide gels. The polyacrylamide gels were visualized through a silver nitrate staining process following the protocol of Bassam et al. (1991).

Results and Discussion

Phenotypic screening

Significant variation for reaction to STB was observed when the nine germplasms were inoculated with a mixture of isolates of *S. tritici* from Kansas. The germplasm line

KS96WGRC40 had the lowest mean STB severity (14.23%) of the genotypes tested. It had significantly higher levels for resistance in this study than the resistant lines Israel493 (*Stb3*) and Jagger (Table 3.2). Taichung29 was very susceptible to the STB isolates used in the screening and had the highest mean STB severity (94.17%). Wichita, the susceptible parent used in the mapping population, had an intermediate mean STB severity of 49.70% that was significantly greater ($p<0.05$) than that observed for KS96WGRC40. This confirms that KS96WGRC40 does contain resistance to STB as tested by a mixture of Kansas field isolates. The significant differences observed for the parents of the mapping population also suggest that the KS96WGRC40/Wichita population should adequately segregate for STB resistance under the same inoculation procedures.

Table 3.2. Least square means and standard deviations for Septoria leaf blotch (STB) severity for nine wheat germplasms (N = 20) screened with a mixture of Kansas *Septoria tritici* isolates in the greenhouse at Manhattan, KS 2004.

Genotype	STB Severity LS Mean*	Standard error of STB Severity LS Mean
KS96WGRC40	14.23 a	3.18
Israel 493	26.97 b	3.63
Jagger	27.97 b	3.27
Wichita	49.70 c	10.01
KS89WGRC04	57.18 c	3.03
Rac875-2	62.33 c	4.47
Newton	70.93 d	4.47
Lakin	72.67 d	4.49
Taichung29	94.17 e	1.82

*Means followed by different letters are significantly different ($p<0.05$).

The KS96WGRC40/Wichita population segregated for reaction to STB when inoculated in a similar fashion as was performed on the nine parental germplasms. There were significant differences among the genotype means ($p<0.01$) within the population (Table 3.3). In addition, there was a significant difference ($p<0.01$) between the two planting dates for the STB screening (Table 3.3). This was expected as the two screenings occurred at different times and thus were affected by different environmental conditions. The interaction between planting date and genotype was also significant ($p<0.01$) (Table 3.3). However, the mean squares for genotypes was much larger than genotype by planting date mean squares. The interaction was due to small, quantitative differences between planting dates and not qualitative differences; therefore, data of the two planting dates were combined.

Table 3.3. ANOVA for Septoria leaf blotch resistance in a $F_{5:7}$ KS96WGRC40/Wichita population, Manhattan, KS 2004.

Source	df	Mean Square	F value
Block	9	2352.3	7.72**
Planting Date	1	95601.5	313.73**
Genotype	79	7585.6	23.81**
Planting Date x Genotype	79	463.9	6.76**
Error	1352	68.6	
Total	1520		

**p<0.01

The significant variation between the two planting dates was reflected in the means of each planting date. The overall phenotypic mean from the first planting date was 36.10% STB severity, and the overall genotype mean from the second planting date was 21.03% STB severity. More disease occurred in the first planting date and was probably due to a more favorable environment.

A bimodial distribution of phenotypic means was observed for the 78 $F_{5:7}$ RILs, KS96WGRC40, and Wichita averaged across both planting dates (Figure 3.1) indicating a single major gene is segregating. This distribution is somewhat skewed toward resistance and is reflected in the overall STB severity mean of 28.56%. The mean STB severities for KS96WGRC40 and Wichita averaged across both planting dates were 8.82% and 78.83%, respectively. The mean STB severities for the RILs within the KS96WGRC40/Wichita population ranged from 4.15% to 72.53%.

The population was classified into two separate non-overlapping groups based on the least significant difference of the genotype means averaged across both planting dates (LSD=4.36, p<0.10). RILs with mean STB severities <32% were classified as resistant. Those RILs with mean STB severities >32% were classified as susceptible. Following

these criteria, 47 RILs were classified as resistant and 31 RILs were classified as susceptible. This segregation ratio was not significantly different from the 1:1 ratio expected if KS96WGRC40 contains a single STB resistance gene ($\chi^2 = 3.28$, $p > 0.95$).

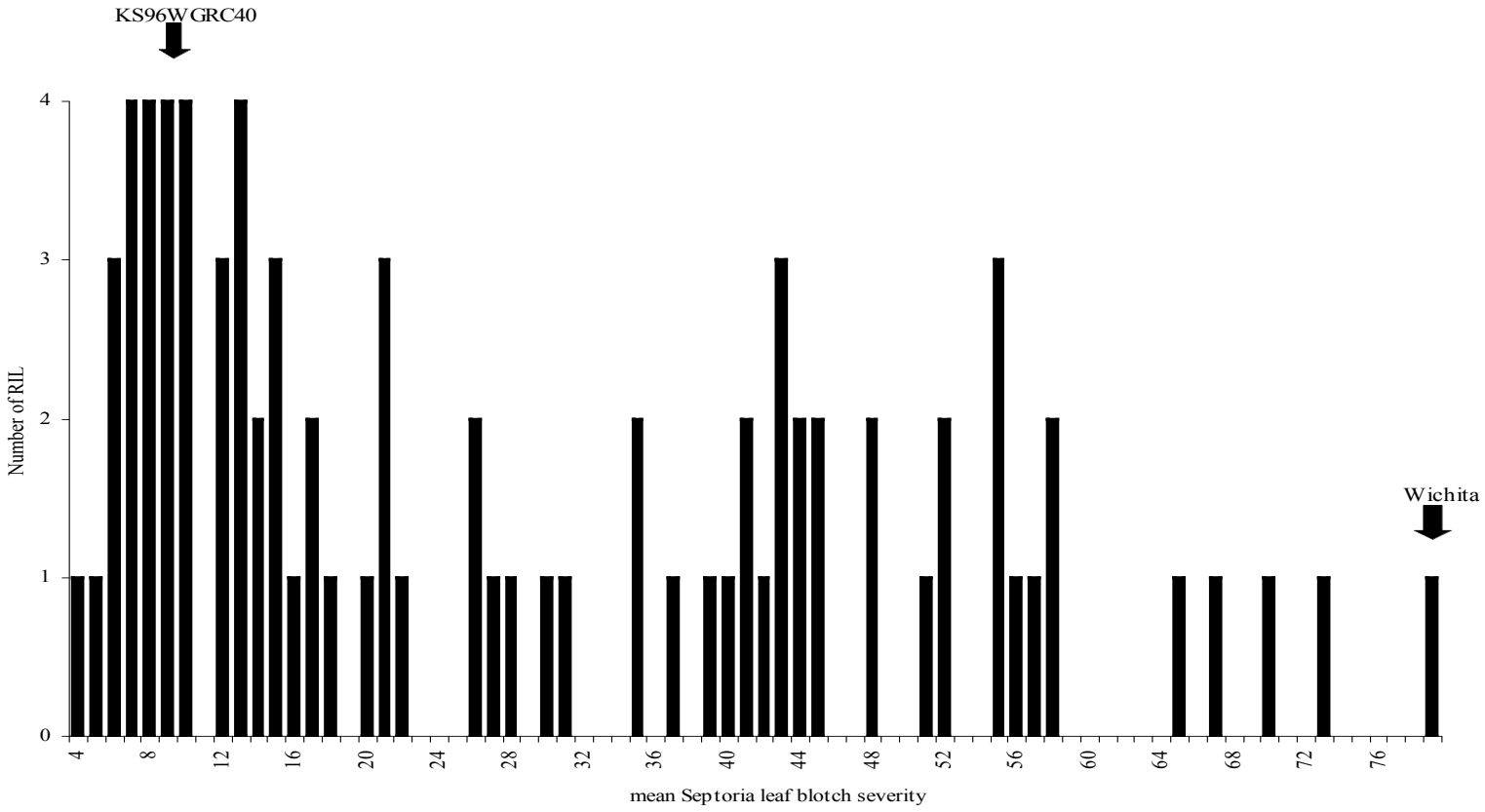


Figure 3.1. Segregation of resistance to Septoria leaf blotch in a $F_{5:7}$ KS96WGRC40/Wichita RIL population. Mean severity for each parent is indicated by an arrow.

Molecular Marker Analysis

From the 249 microsatellite primer pairs screened in the bulk segregant analysis, 48 were polymorphic between the parents. A preliminary linkage analysis identified one of these primer pairs, BARC126, to be associated with STB resistance. The most recent consensus map of hexaploid wheat (Somers et al., 2004) located the *Xbarc126* locus on the short arm of chromosome 7D. Additional microsatellite primer pairs located on chromosome 7DS near *Xbarc126* were used to further genotype the RIL population. Eight polymorphic microsatellite markers were used to construct a genetic linkage map of the region of the STB resistance gene (Figure 3.2A). The order of these markers is somewhat consistent with the reported consensus map. Markers *Xgwm295* and *Xwmc702* are distal to *Xgwm44* in the linkage map but are located proximal to *Xcfd021* in the consensus map (Somers et al., 2004).

A QTL analysis was performed using the quantitative data from the phenotypic screening of the population using QTL Cartographer software (QTL Cartographer V2.0, North Carolina State University). This analysis identified a QTL explaining 75.12% of the variation at marker locus *Xbarc126* with a LOD score of 21.0154. This QTL analysis supported our classification of resistant and susceptible lines used in the qualitative analysis.

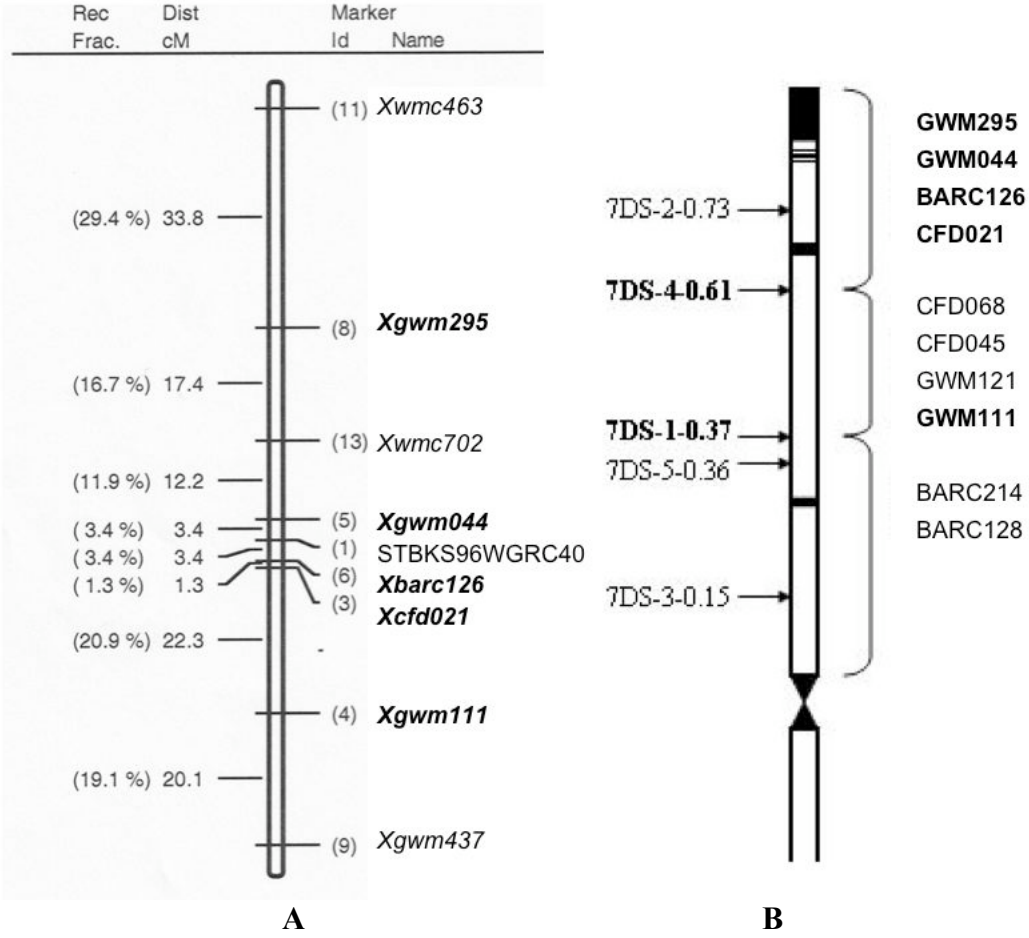


Figure 3.2. A.) Partial genetic linkage map of wheat chromosome 7D constructed from 78 $F_{5:7}$ recombinant inbred lines from a KS96WGRC40/Wichita population. B.) Physical map of wheat chromosome 7D. The centromere is indicated by the two triangles. The two deletion breakpoints used to physically map the microsatellite markers are shown in bold. Three deletion bins are delineated by brackets and contain the microsatellite markers noted to the right. Markers common to both maps are in bold.

The two microsatellite markers most closely linked to the STB resistance gene within KS96WGRC40, *Xgwm044* and *Xbarc126*, flanked the gene at distances of 3.4 cM distal and 3.4 cM proximal, respectively (Figure 3.2A). Primer pair BARC126 amplified a 130 base pair fragment from DNA of KS96WGRC40 and a 144 base pair fragment from Wichita. Primer pair GWM44 amplified three fragments; the fragment linked to the

STB resistance within KS96WGRC40 is approximately 150 base pairs in length (Figure 3.3).

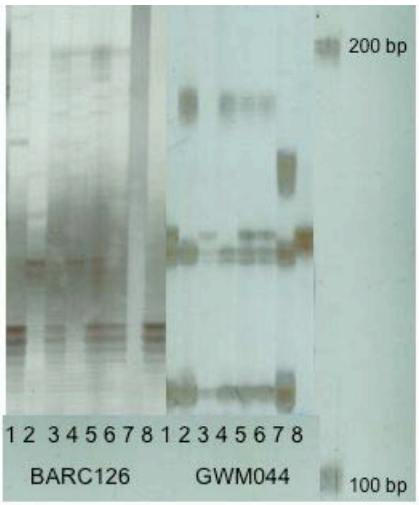


Figure 3.3. Banding pattern of microsatellite primer pairs BARC126 and GWM044 separated on a 7% denaturing polyacrylamide gel visualized by silver staining. Lane 1: KS96WGRC40, 2: Wichita, 3: Septoria leaf blotch resistant bulk, 4: Septoria leaf blotch susceptible bulk, 5: soil-borne wheat mosaic virus resistant bulk, 6: soil-borne wheat mosaic virus susceptible bulk, 7: TAM107, 8: TA2397.

The aneuploid stocks of Chinese Spring were used to physically locate the microsatellite markers linked to the STB resistance gene within KS96WGRC40. Only two deletion lines were used in this study (7DS-1-0.37 and 7DS-4-0.61). Although there is an additional terminal 7DS deletion line (7DS-2-0.73) available, the line is maintained in the hemizygous condition, limited amounts of seed were available, and no plants were identified having the deleted chromosome.

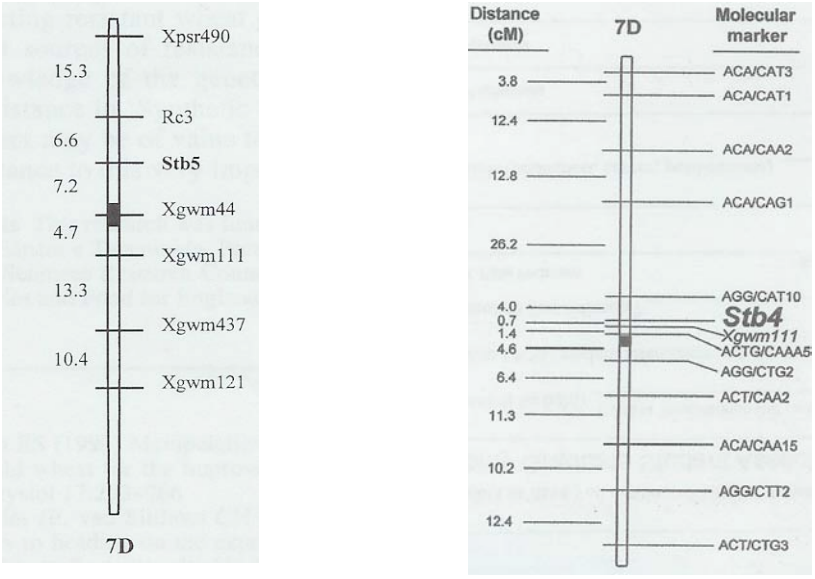
The STB resistance gene in KS96WGRC40 is located on the terminal portion of 7DS between markers *Xgwm44* and *Xbarc126* (Figure 3.2A). The flanking markers were physically located in the deletion bin 0.61-1.00 (Figure 3.2B) of chromosome 7DS.

Previous genetic linkage maps place *Xgwm44* near the centromere (Somers et al., 2004; Roder et al., 1998). However, our physical mapping of the fragment linked to STB resistance in the terminal region of 7DS agrees with the physical location of *Xgwm44* reported by Sourdille et al. (2004).

The *Ae. tauschii* segment transferred to KS96WGRC40 from TA2397 on chromosome 7D is a proximal segment. KS96WGRC40 has the TAM107 allele at locus *Xgwm295* (Figure 3.5) which was located distal to the STB resistance gene in KS96WGRC40 (Figure 3.2A). KS96WGRC40 has D-genome fragments derived from TA2397 at marker loci *Xwmc702*, *Xgwm044*, *Xbarc126*, and *Xcfd021*(Figure 3.5). At marker locus *Xgwm111* the 195 base pair fragment present in KS96WGRC40 is not polymorphic between TAM107 and TA2397. Therefore, the proximal breakpoint of the *Ae. tauschii* segment in KS96WGRC40 can not be delineated.

Comparison to Known STB Resistance Genes

Two genes conferring resistance to STB are located on the short arm of chromosome 7D. Arraiano et al. (2001) identified the STB resistance gene *Stb5* in a Chinese Spring substitution line of 'Syn6X' and located this gene on wheat chromosome arm 7DS linked to the microsatellite marker *Xgwm044* at a distance of 7.2 cM (Figure 3.4A). Adhikari et al. (2004A) located another STB resistance gene in the cultivar Tadinia, *Stb4*, on 7DS at a distance of 0.7 cM from the microsatellite marker *Xgwm111* (Figure 3.4B). Our linkage analysis suggests that the STB resistance gene within KS96WGRC40 is located in the same chromosomal region as *Stb4* and *Stb5* (Figure 3.2A).



A **B**

Figure 3.4. A). Genetic linkage map of *Stb5* on chromosome 7D in the wheat germplam Chinese Spring-Syn6X from Arraiano et al., 2001. B). Genetic linkage map of *Stb4* on chromosome 7D in the wheat germplasm Tadinia from Adhikari et al., 2004A.

To investigate the relationships between *Stb4*, *Stb5*, and the unnamed STB gene within KS96WGRC40, markers *Xgwm111, Xcfd021, Xgwm44*, and *Xbarc126* were used to genotype Tadinia, Chinese Spring-Syn6X, and KS96WGRC40, along with TAM107 (the wheat parent of KS96WGRC40) and TA2397. Primer pair GWM044 amplified multiple fragments from genomic DNA of the hexaploid wheat germplasms (Figure 3.5). The 150 base pair TA2397-derived allele in KS96WGRC40 that was linked to STB resistance was also present in Chinese Spring-Syn6X. No common TA2397-derived alleles were observed between Tadinia and KS96WGRC40 for other markers in this region (Figure 3.5).

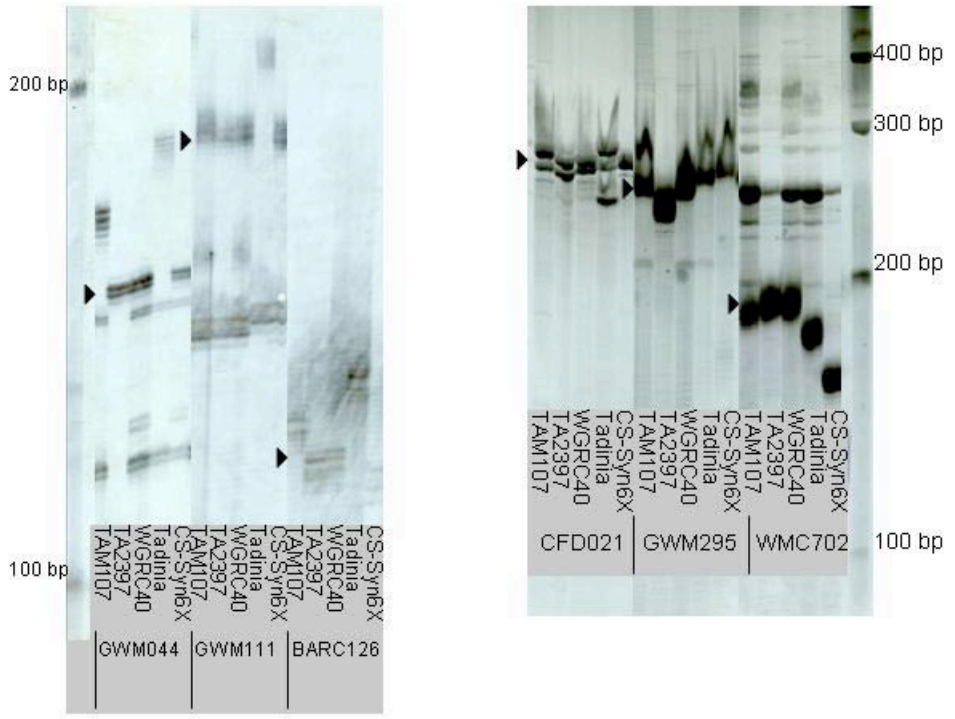


Figure 3.5. Banding pattern of microsatellite markers *Xgwm044*, *Xgwm111, Xbarc126* and *Xcfd021, Xgwm295,* and *Xwmc702* on eight wheat germplasms. Triangles indicate the bands associated with KS96WGRC40 Septoria leaf blotch resistance for each marker.

Conclusions

Hard red winter wheat germplasm KS96WGRC40 has an *Ae. tauschii*-derived gene of major effect conferring resistance to STB. Our analysis located this gene in the distal 40% of the short arm of chromosome 7D flanked by microsatellite markers *Xgwm044* and *Xbarc126.* These markers will be useful for marker-assisted selection (MAS) for STB resistance in hard red winter wheat since this is the first STB gene mapped in a germplasm adapted to the Southern Great Plains of the USA. Other genes for which markers are available can be selected for by MAS in crosses to KS96WGRC40 including *Pm17*, *Cmc3*, *Cmc4*, *Lr39*, and *Sbwmv1*.

Two previously reported STB genes, *Stb4* and *Stb5*, have been mapped to 7DS in the same region as the STB resistance gene within KS96WGRC40. The *Stb4* gene was

identified in the spring wheat cultivar Tadinia using a *S. tritici* isolate collected in Indiana and was useful for controlling STB in California from 1975 (Somasco et al., 1996) until recently as this resistance gene is no longer effective as evidenced by the cultivar Gene in Oregon (Cowger et al., 2000). The *Stb5* gene was derived from an unknown accession of *Ae. tauschii* which was used in the creation of Syn6X. This resistance was identified using *S. tritici* isolate IP094269 which was collected in the Netherlands. The STB resistance gene with KS96WGRC40 provides resistance to *S. tritici* isolates collected in Kansas and was also derived from *Ae. tauschii*. The relationship between the fungal isolates used to identify these genes is unknown.

The *Stb5* gene designation is based on map location and allelism analysis with *Stb4* has not been done. The map of *Stb4* was based primarily on AFLP markers and the only reference marker was *Xgwm111*. Although the *Stb4* gene was determined to be closely linked to *Xgwm111*, the lack of other landmarks makes it impossible to orient the map with reference to the centromere. Comparison of this map with the map constructed in the population segregating for *Stb5* is not reliable. Particularly problematic is the placement of *Xgwm111* in the long arm of chromosome 7D in the map of Arraiano et al. (2001). Other reports, including this study, have located this marker in the short arm of the chromosome 7D (Somers et al., 2004; Sourdille et al., 2004). The fragment generated by GWM111 that was determined to be linked to *Stb4* in Tadinia is in the same size range as the fragment mapped in the KS96WGRC40/Wichita population. Multiple fragments were amplified by this marker in the hexaploid germplasms including Chinese Spring. It is possible that the locus that was physically mapped on the short arm in our study differs from the locus mapped by Arraiano et al. (2001). However, the uniqueness of the STB

resistance genes on 7DS remains questionable. The haplotype analysis of the region was not conclusive. An analysis of populations from intercrossing these germplasms is necessary to clarify the allelic relationship of the STB genes.

Chromosome 7DS of wheat contains many other useful resistance genes. The durable leaf rust resistance gene *Lr34* and the durable stripe rust resistance gene *Yr18* are both located on chromosome 7DS (Nelson et al., 1997; Singh, 1992). Liu et al. (2002) mapped the Russian wheat aphid resistance genes *Dn1*, *Dn2*, *Dn5*, *Dn6,* and *Dnx* to chromosome 7DS. Therefore, this chromosome arm appears to be a common region of resistance gene transfer and may contain an important cluster of resistance genes for a wide range of pests and pathogens.

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CHAPTER 4 GENETIC ANALYSIS OF RESISTANCE TO SOIL-BORNE WHEAT MOSAIC VIRUS IN KS96WGRC40

Introduction

Soil-borne wheat mosaic virus (SBWMV) is a destructive pathogen of wheat that belongs to the viral group Furovirus. The disease was first described in Illinois by McKinney (1923) and can now be found in most winter wheat-growing regions throughout the world including most recently the United Kingdom (Clover et al., 2001). SBWMV is vectored by the soil inhabiting organism *Polymyxa graminis* (Rao and Brakke, 1969). Symptoms of SBWMV on wheat seedlings include mostly yellow to light green leaves with darker green mottling and stunting. Depending on environmental conditions, infected seedlings may be able to recover a dark green appearance. Yield losses to SBWMV have been estimated as high as 45% in Kansas (Nykaza, 1978).

During the late 1970's SBWMV was the most devastating disease of winter wheat in Kansas (Bockus et al., 2001). Following several epidemic years, breeding for resistance to SBWMV was considered a priority by Kansas State University wheat breeders. With the use of specific breeding nurseries in severely infested SBWMV fields, several SBWMV resistant cultivars were developed and released thus drastically reducing the losses due to SBWMV (Bockus et al., 2001). Although many winter wheat cultivars contain resistance to SBWMV, no single major resistance genes have been identified or named to date. A SBWMV resistance QTL on chromosome 5D linked to microsatellite markers *Xcfd10* and *Xcfd86* was identified in the hard red winter wheat cultivar Karl92 which explained 38% of the phenotypic variation (Narasimhamoorthy, 2003).

KS96WGRC40 is a hard red winter wheat germplasm developed and released by the USDA-ARS Plant Science and Entomology Research Unit, the Kansas Agricultural Experiment Station, and the Wheat Genetics Resource Center at Kansas State University. KS96WGRC40 contains resistance to SBWMV, wheat curl mite, Stagonospora leaf blotch, and Septoria leaf blotch. KS96WGRC40 was a reselection for wheat curl mite resistance out of the germplasm KS95WGRC33. The pedigree of KS96WGRC40 is TAM107*3/TA2460//TA2397/3/TAM107*3/TA2460 (Cox et al., 1999).

The objective of this research was to characterize the inheritance of SBWMV resistance contained in KS96WGRC40.

Materials and Methods

Plant Material

A segregating population was created from the cross KS96WGRC40 by Wichita. KS96WGRC40 is resistant to SBWMV while Wichita is susceptible. The population was advanced by single seed descent to the F_5 generation. After the F_5 generation, lines were harvested in bulk. Seventy-eight recombinant inbred lines (RILs) were used in this study.

SBWMV field phenotyping

The 78 $F_{5:7}$ RILs from the KS96WGRC40/Wichita population were evaluated for SBWMV resistance in the field at the Kansas State University Department of Agronomy Ashland Bottoms Research Farm during the 2004-2005 and 2005-2006 growing seasons. Each RIL was planted in a one-meter row using a randomized complete block design with two replications. The parents of the population (KS96WGRC40 and Wichita) were included in the experiments. Lines were scored as either resistant or susceptible based on SBWMV symptoms at Feekes growth stage three. Susceptible SBWMV checks (TAM107 and Wichita) were planted alongside each entry throughout the length of the

field to visualize SBWMV distribution. Symptomatic leaves of TAM107 and Wichita were collected and submitted to the Kansas State University Department of Plant Pathology Plant Disease Diagnostics Lab to verify SBWMV presence by ELISA.

SBWMV ELISA screening

Visual evaluations of SBWMV symptoms used in conjunction with SBWMV ELISA were recommended by Hunger and Sherwood (1985) as the best indicator of resistance. A 2.5 cm leaf sample from five random plants for each RIL and parent was collected from the first replication in the 2005-2006 field screening. The leaves from the five plants were combined for each RIL and parent sample. Leaf samples were collected at Feekes growth stage three, stored on ice in the field, and returned to the lab where they were stored at -80°C. The leaf samples were tested for the presence of SBWMV by compound direct labeled ELISA using the protocol and supplies in an Agdia SBWMV test kit (Agdia, Elkhart, Indiana). Absorbances were measured at 405 nm using a Bio-tek ELx800 microplate reader (Bio-tek, Winooski, Vermont). SBWMV positive and negative controls were included with the Agdia test kit. The ELISA was repeated using the same leaf extracts, and absorbance values from the two replicate SBWMV ELISA screenings were averaged.

DNA Extractions

Genomic DNA was extracted from the two parental germplasms (KS96WGRC40 and Wichita) and the two *Aegilops tauschii* accessions TA2460 and TA2397, both of which are in the pedigree of KS96WGRC40. Genomic DNA was also extracted from 78 $F_{5:7}$ RILs in the KS96WGRC40/Wichita population.

Leaf tissue was collected from 7-day old germinated seedlings and placed in 1.5 mL microcentrifuge tubes. The tissue was stored at -80°C and then ground to a fine

powder in liquid nitrogen using a mortar and pestle. Ground tissue samples were stored at -80°C. Small scale DNA extractions were performed by adding 500 µl extraction buffer [100 mM glycine, 50 mM NaCl, 10 mM EDTA, 2% SDS, and 30 mM sodium lauryl sarsosine] and 500 µl phenol:chloroform:isoamyl alcohol [50:49:1] to the ground tissue and mixing for 10 minutes at room temperature. Samples were centrifuged at 8000 rpm for 10 minutes and the supernatant transferred to a new microcentrifuge tube with a micropipette. An equal volume of chloroform:isoamyl alcohol [24:1] was added and samples were centrifuged again at 8000 rpm for 10 minutes. The supernatant was transferred to a new microcentrifuge tube and mixed with 1/10 volume of 3M sodium acetate and one volume isopropanol for DNA precipitation. DNA precipitation occurred overnight at 4°C. The DNA was pelleted by centrifugation at 8000 rpm for 10 minutes after which the isopropanol/sodium acetate mixture was poured off. The DNA was washed with 70% ethyl alcohol and repelleted by centrifugation at 8000 rpm for 10 minutes. The ethyl alcohol was poured off and the DNA was resuspended in 50 µl of 1X TE buffer. DNA concentrations were adjusted to 10 ng/µl with the use of a spectrometer (Nanodrop ND-1000, Wilmington, DE). This method was adapted from Malik et al. (2003).

Molecular Marker Analysis

A bulk segregant analysis was used to identify polymorphic markers potentially linked to SBWMV resistance (Michelmore et al., 1991). Bulk DNA samples were prepared by pooling equal amounts of DNA from ten susceptible RILs and ten resistant RILs. The DNA samples of KS96WGRC40, Wichita, the resistant bulk, and the susceptible bulk were screened for polymorphisms with 249 D-genome specific

microsatellite primer pairs. Only D-genome specific primer pairs were screened for polymorphism because the SBWMV resistance within KS96WGRC40 is derived from *Ae. tauschii*. The wheat cultivar in the pedigree of KS96WGRC40 (TAM107) is susceptible to SBWMV.

PCR reactions were preformed in 12 μl volumes and included 2.0 μl of genomic DNA, 1.2 μl of 10X PCR buffer with magnesium chloride, 0.96 μl of 10 mM dNTP's, 0.18 μl of Taq DNA polymerase (5units/μl), 5.26 μl of sterile molecular grade water, 0.96 μl of 1 μM forward primer, 0.72 μl of 10 μM reverse primer, and 0.72 μl of one of either FAM, PET, NED, or VIC 10 μM fluorescent labels. Reactions were carried out in either a PTC-200 Thermal Cycler (MJ Research, Watertown, MA,) or a Master Cycler EP384 System (Eppendorf, Westbury, NY). PCR products were multiplexed by pooling four differently labeled PCR products across the same genomic DNA sample with the use of a Hydra II 96 channel microdispenser (Matrix, Hudson, NH). PCR fragments were resolved in an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA) with GeneScan-500 LIZ as an internal size standard (Applied Biosystems, Foster City, CA). Fragment analysis was performed with GeneMarker v1.4 software (SoftGenetics, State College, PA).

Microsatellite primer pairs identified as polymorphic based on the bulk segregant analysis were screened on the entire mapping population. Linkage analysis was conducted with Mapmaker software (version 2.0 for Macintosh). Map distances were converted to centimorgans using the Kosambi function (Kosambi, 1944). Linkage maps were generated using a maximum Kosambi distance of 50 and a minimum log of odds (LOD) of 3.0.

The linkage analysis identified microsatellite primer pairs linked to the SBWMV resistance gene within KS96WGRC40. The chromosomal locations of these microsatellite primer pairs were previously determined in other mapping populations (Somers et al., 2004). This information was used to select more microsatellite primer pairs in the identified chromosomal region of interest. These additional microsatellite primer pairs were also screened on the population in similar fashion.

To further analyze the DNA fragments amplified by linked microsatellite primer pairs, several PCR products were separated by electrophoresis in 7% denaturing polyacrylamide gels. The polyacrylamide gels were visualized through a silver nitrate staining process following the protocol of Bassam et al. (1991).

Marker survey of other wheat and progenitor lines for SBWMV resistance

DNA was extracted following the above method from 23 WGRC germplasm releases, six hard red winter wheat cultivars, and two *Ae. tauschii* accessions. PCR was performed with the closest markers identified from the genetic linkage map constructed from the KS96WGRC40/Wichita population. Fragments were separated by electrophoresis in 7% denaturing polyacrylamide gels and visualized by silver staining.

Results and Discussion

Phenotypic screening

The distribution of SBWMV in the 2004-2005 field screening was sporadic. In those instances where the susceptible checks did not show SBWMV symptoms, phenotypic data were not recorded on the adjacent RIL. The SBWMV in the 2005-2006 field screening was evenly distributed (Figure 4.1) in the first replication. The second replication planted in 2005-2006 did not show any symptomatic expression of SBWMV on the susceptible checks TAM107 and Wichita. Therefore, phenotypic data were not

taken on the second replication in 2005-2006. ELISA testing confirmed the presence of SBWMV in the susceptible checks TAM 107 and Wichita from the first replication in 2005-2006.

Figure 4.1. Distribution of soil-borne wheat mosaic virus (SBWMV) infection in a field screening of 78 $F_{5:7}$ recombinant inbred lines, Ashland Bottoms Research Station, Manhattan KS, 2006. Dashed lines indicate the SBWMV susceptible varieties TAM107 and Wichita.

The classification of each RIL as resistant or susceptible based on the phenotypic evaluation was consistent between the two-year field screenings. Therefore, the phenotypic data were compiled across replications and years for each RIL. Thirty-nine RILs were classified as resistant; 38 RILs were classified as susceptible. One RIL did not germinate in either year and so the total number of lines phenotyped was 77. This segregation ratio is not significantly different from 1:1 ($\chi^2 = 0.01$, $p > 0.95$), as expected if KS96WGRC40 contains a single SBWMV resistance gene. It is proposed that this

SBWMV resistance gene in KS96WGRC40 be named soil-borne wheat mosaic virus 1 and abbreviated as *Sbwmv1*.

SBWMV ELISA screening

Average absorbance values for the RILs ranged from 0.1525 to 3.2520. SBWMV resistant and susceptible lines were easily separated based on average absorbance values. Lines with an average absorbance value <1 were resistant. Lines with an average absorbance value >2 were susceptible. No line had an average absorbance value >1 and < 2. Lines could also be easily separated by visually inspecting the microplate sample well color (Figure 4.2). A yellow sample well indicates the presence of SBWMV and a clear sample well indicates the absence of SBWMV. The resistant parent KS96WGRC40 produced a negative ELISA result; the susceptible parent Wichita produced a positive ELISA result. Separate positive and negative controls were included as provided in the SBWMV test kit. The positive control produced a positive result, and the negative control produced a negative result (Figure 4.2).

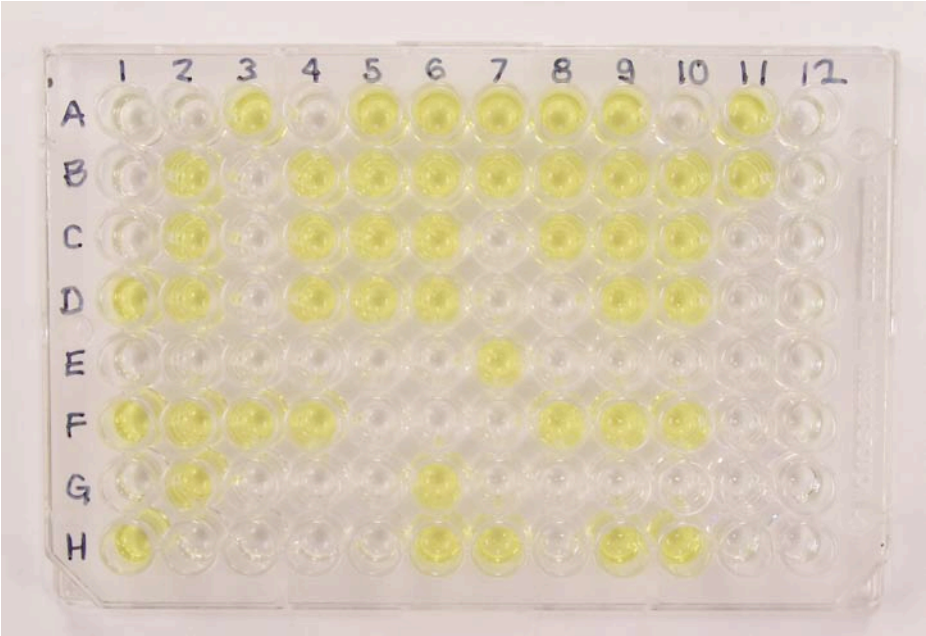

Figure 4.2. Soil-borne wheat mosaic virus (SBWMV) ELISA screening of a $F_{5:7}$ KS96WGRC40/Wichita recombinant inbred line population. A yellow sample well indicates a positive reaction and the presence of SBWMV. A clear sample well indicates a negative reaction and the absence of SBWMV. Sample well B11 is a positive control. Sample well C11 is a negative control. Sample well G10 and H10 are KS96WGRC40 and Wichita, respectively.

Comparing the SBWMV ELISA results with the phenotypic data provided some interesting results. In all instances where a RIL was classified as susceptible based on the phenotypic expression of symptoms, the ELISA test result was positive for the presence of SBWMV. Five lines (RIL #57, #76, #91, #98, and #103) were classified as resistant based on phenotypic expression of SBWMV symptoms, but were positive for the presence of SBWMV based on ELISA. These five RILs showed no symptoms of SBWMV, yet contained SBWMV coat protein within their leaves (Table 4.1). Heterozygous RILs, sampling error in the field, or cross contamination of samples could all explain the presence of SBWMV coat protein in these samples. However, no entries

in the SBWMV field screening appeared to be segregating for resistance. Furthermore, cross contamination or sampling error would have been diluted in the sample of five leaves. The absorbance values for these five RILs are all above 2.3000 and comparable to the absorbance value of the positive control (3.1910) used in the ELISA test kit (Table 4.1).

Table 4.1. Phenotypic expression of soil-borne wheat mosaic virus (SBWMV) symptoms, SBWMV ELISA absorbance values, and size of fragment amplified with microsatellite marker *Xcfd10* for nine recombinant inbred lines (RILs) from a KS96WRC40/Wichita population.

Sample	Phenotypic Expression of SBWMV Symptoms*	SBWMV ELISA Absorbance values (405 nm)^	*Xcfd10* fragment (base pairs)
RIL #57	R	3.1355	290
RIL #76	R	2.3960	NOAMP
RIL #91	R	3.0920	290
RIL #98	R	3.1960	290
RIL #103	R	3.0635	290
RIL #13	S	3.1635	290
RIL #42	S	2.8565	290
RIL #4	R	0.1770	280
RIL #21	R	0.1745	280
KS96WGRC40	R	0.1845	280
Wichita	S	3.2520	290
Positive Control†	-	3.1910	-
Negative Control†	-	0.1570	-

*Phenotypic expression of SBWMV symptoms at Feekes growth stage 3 in a naturally infected SBWMV field in Manhattan, KS, 2005 and 2006. R, resistant; S, susceptible; -, not rated.
^Mean absorbance values from two replicate tests, each consisting of combined leaf extracts from five random plants of the same genotype grown in a naturally infected SBWMV field in Manhattan, KS, 2006.
†Positive and negative controls as supplied in the SBWMV ELISA test kit.

Molecular Marker Analysis

From the 249 microsatellite primer pairs screened in the bulk segregant analysis, 48 were polymorphic between the parents. A preliminary linkage analysis identified one

of these primer pairs, CFD10, to be associated with SBWMV resistance. Marker *Xcfd10* is located on chromosome 5DL according to the most recent consensus map of hexaploid wheat (Somers et al., 2004). Additional microsatellite primer pairs located on chromosome 5DL near *Xcfd10* were used to further genotype the RIL population. Four polymorphic microsatellite markers were used to construct a genetic linkage map in the region of the *Sbwmv1* (Figure 4.3). The closest linked marker to *Sbwmv1* was *Xcfd10* and mapped 10.1 cM away from the resistance gene (Figure 4.3). Marker *Xcfd10* amplified a 280 base pair fragment in KS96WGRC40 and a 290 base pair fragment in Wichita (Figure 4.4). Unfortunately, no microsatellite markers flanking *Sbwmv1* were identified.

Marker *Xcfd10* was more closely linked to the SBWMV ELISA screening results rather than the SBWMV phenotypic field screening results (Figure 4.3). The observed recombination between *Sbwmv1* and the ELISA screening results is explained by the five RILs that were scored as resistant in the field screening yet contained SBWMV coat protein as tested by ELISA. These five RILs contain the susceptible allele at the marker locus *Xcfd10* (Table 4.1). The chromosomal region distal to *Xcfd10* on chromosome 5DL of wheat appears to be important for SBWMV resistance. This agrees with the results of Narasimhamoorthy (2003), who identified a QTL in the wheat cultivar Karl92 on chromosome 5DL linked to *Xcfd10* and *Xcfd86*. Marker *Xcfd86* was polymorphic in the KS96WGRC40/Wichita population but was not linked to *Sbwmv1*.

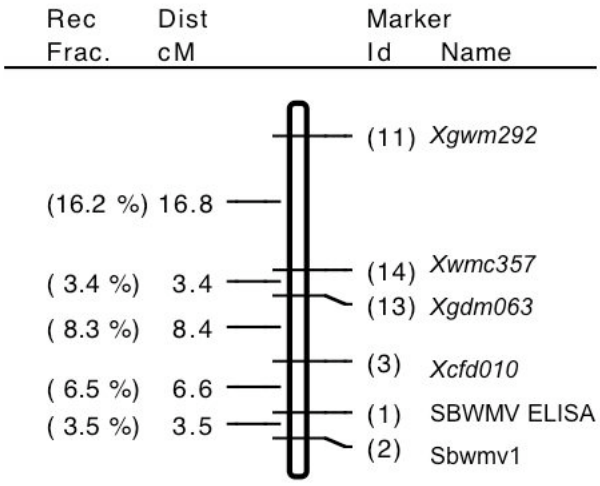

Figure 4.3. Genetic linkage map of wheat chromosome 5DL constructed from 78 $F_{5:7}$ recombinant inbred lines from a KS96WGRC40/Wichita population.

Marker survey of other wheat and progenitor lines for SBWMV resistance

The closest marker to *Sbwmv1* was *Xcfd10* (Figure 4.3). KS96WGRC40, KS95WGRC33, and the *Ae. tauschii* accession TA2397 amplified the same sized fragment with microsatellite marker *Xcfd10* (Figure 4.4). This indicated that KS96WGRC40 and KS95WGRC33 contain the same SBWMV resistance gene derived from TA2397 and not TA2460. KS96WRC40 and KS95WGRC33 contain the same SBWMV resistance gene as evidenced by both germplasms having the same *Xcfd10* resistance fragment. KS96WGRC40 was a reselection for wheat curl mite resistance out of the SBWMV resistant germplasm KS95WGRC33.

None of the other WGRC germplasm releases and wheat varieties screened had the same allele as KS96WGRC40 and KS95WGRC33 at the marker locus *Xcfd10* (Figure 4.4). The WGRC germplasm releases KS92WGRC23, KS96WGRC34, KS96WGRC38, KS96WGRC39, KS96WGRC35, KS93WGRC26, KS92WGRC22, KS92WGRC21, and

KS92WGRC16 are resistant to SBWMV (G. Brown-Guedira, unpublished data). These WGRC releases may contain additional SBWMV resistance genes. 'Heyne', 'Jagger', 'Overley', and 'Karl92' are all hard red winter wheat cultivars with resistance to SBWMV (A. Fritz, personal communication). These cultivars may also contain additional SBWMV resistance genes different from *Sbwmv1*.

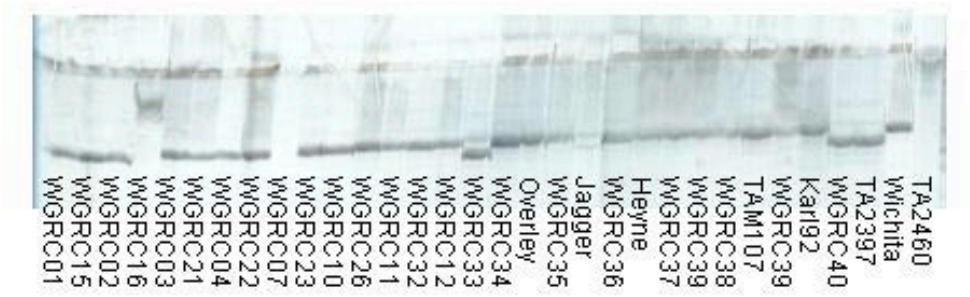

Figure 4.4. Banding pattern of microsatellite marker *Xcfd10* on 23 WGRC wheat germplasms, six hard red winter wheat cultivars, and two *Aegliops tauschii* accessions.

Conclusions

KS96WGRC40 contains one major SBWMV resistance gene located on chromosome 5DL. It is proposed that this SBWMV resistance gene be named resistance to soil-borne wheat mosaic virus 1 and abbreviated as *Sbwmv1*. Microsatellite marker *Xcfd10* is linked to this resistance gene. Our marker data suggest that the SBWMV resistance gene within KS96WGRC40 was derived from *Ae. tauschii* accession TA2397.

Kanyuka et al. (2004) identified a soil-borne cereal mosaic virus resistance gene in the UK cultivar Cadenza and proposed to name this gene *SbmCz1*. Soil-borne cereal mosaic virus (SBCMV) is the approved species name for a European mosaic virus which causes symptoms similar to those caused by SBWMV in the US. Some authors consider SBCMV to be a European strain of SBWMV; however, Diao et al. (1999) found only

70% homology between the two viruses. It is unclear if European cultivars with resistance to SBCMV would also contain resistance to SBWMV or vice versa. For these reasons, it is proposed that genes providing resistance to SBWMV be identified differently from those providing resistance to SBCMV.

SBWMV phenotyping can be difficult in field situations and is dependent on adequate distribution of the virus within a field. Planting susceptible checks throughout a field screening can aid in the visualization of the virus distribution. SBWMV ELISA screening can be useful to strengthen phenotypic scoring. Five RILs from an $F_{5:7}$ KS96WGRC40/Wichita population showed no visual symptoms of SBWMV yet contained high levels of SBWMV protein as evidenced by SBWMV ELISA absorbance values. Other researchers have reported positive SBWMV ELISA absorbance values in resistant cultivars (Armitiage et al., 1990; Hunger and Sherwood, 1985; Hunger et al., 1989). To gain a better understanding of the SBWMV resistance mechanism in wheat, the observations on these five RILs should be repeated and sampled throughout an entire growing season to investigate the accumulation of SBWMV protein within leaf tissue.

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APPENDIX 1. PHENOTYPIC DATA COLLECTED ON KS96WGRC40 BY WICHITA POPULATION

Table A1.1. Phenotypic data collected on 78 $F_{5:7}$ recombinant inbred lines from a KS96WGRC40/Wichita population, Manhattan, KS 2004-2006.

Genotype	Mean STB Severity *	STB Severity Planting Date 1 ^	STB Severity Planting Date 2 ^	SBWMV ELISA Absorbance (405 nm) †	Phenotypic Expression of SBWMV Symptoms §
RIL 1	6.49	11.97	1.27	0.2205	R
RIL 2	16.94	24.76	9.17	-	S
RIL 4	28.26	37.46	19.09	0.1770	R
RIL 5	10.38	15.50	5.27	3.2050	S
RIL 6	14.96	19.64	10.00	0.1675	R
RIL 7	9.60	12.43	7.27	3.2040	S
RIL 8	10.17	13.20	7.13	0.1985	R
RIL 9	4.93	8.80	1.98	3.2200	S
RIL 10	12.95	10.94	14.00	0.2095	R
RIL 13	51.58	63.67	39.50	3.1635	S
RIL 15	12.05	22.53	1.27	3.1495	S
RIL 16	42.62	46.40	38.83	2.4120	S
RIL 17	20.75	31.63	9.87	0.1895	R
RIL 19	9.27	16.63	1.90	3.1730	S
RIL 20	21.00	30.43	11.57	3.2160	S
RIL 21	15.40	26.57	4.23	0.1745	R
RIL 22	43.24	60.39	22.88	3.1825	S
RIL 23	15.80	23.93	7.66	0.1680	R
RIL 24	57.23	63.83	50.91	0.1525	R
RIL 26	42.52	59.33	25.70	-	-
RIL 27	45.43	59.40	31.47	0.1830	R
RIL 28	5.70	5.23	6.17	2.3915	S
RIL 30	9.44	14.20	5.40	0.1715	R
RIL 31	64.50	71.17	57.83	0.1720	R
RIL 32	69.71	75.27	82.59	0.1760	R
RIL 35	66.92	76.00	57.83	3.1970	S
RIL 37	7.93	11.57	4.31	3.1715	S

* Mean *Septoria tritici* leaf blotch (STB) severity averaged across two planting dates.
^ STB severity averaged from three seedling leaves tested with Kansas isolates of *Septoria tritici*.
† Mean absorbance values from two replicate tests, each consisting of combined leaf extracts from five random plants of the same genotype grown in a naturally infected SBWMV field in Manhattan, KS, 2006.
§ Phenotypic expression of SBWMV symptoms at Feekes growth stage 3 in a naturally infected SBWMV field in Manhattan, KS, 2005 and 2006. R, resistant; S, susceptible; -, not rated.

Table A1.1 cont. Phenotypic data collected on 78 $F_{5:7}$ recombinant inbred lines from a KS96WGRC40/Wichita population, Manhattan, KS 2004-2006.

Genotype	Mean STB Severity *	STB Severity Planting Date 1 ^	STB Severity Planting Date 2 ^	SBWMV ELISA Absorbance (405 nm) †	Phenotypic Expression of SBWMV Symptoms §
RIL 38	40.79	47.61	34.05	3.1620	S
RIL 41	72.53	72.83	73.03	0.1680	R
RIL 42	30.37	40.13	20.60	2.8565	S
RIL 43	39.12	53.03	25.20	0.1735	R
RIL 46	37.12	45.17	28.92	0.1570	R
RIL 47	44.03	52.87	35.20	3.2365	S
RIL 48	7.54	11.05	3.63	3.1690	S
RIL 49	6.44	8.96	3.43	3.1580	S
RIL 50	56.35	64.31	48.24	3.1035	S
RIL 51	9.74	14.53	4.59	0.1970	R
RIL 52	35.48	41.53	29.43	0.1570	R
RIL 53	15.11	18.77	11.88	0.1825	R
RIL 55	26.55	33.00	20.10	0.1980	R
RIL 56	42.24	53.89	30.09	3.1790	S
RIL 57	22.17	30.97	13.37	3.1355	R
RIL 58	17.30	32.50	2.10	2.3410	S
RIL 59	25.79	36.90	14.27	2.8610	S
RIL 60	54.60	70.00	39.20	0.1835	R
RIL 61	52.37	63.70	41.03	0.1860	R
RIL 62	12.07	21.27	2.71	3.1820	S
RIL 63	12.60	16.73	8.47	3.0695	S
RIL 64	35.13	33.10	37.17	3.1645	S
RIL 67	13.50	20.43	6.57	3.1340	S
RIL 68	45.12	61.37	28.87	0.1845	R
RIL 70	6.53	10.13	2.93	0.2170	R
RIL 71	18.28	29.37	7.20	2.8790	S
RIL 72	54.76	66.17	42.90	0.1860	R
RIL 73	12.76	20.08	5.37	0.2100	R
RIL 76	48.42	52.17	44.67	2.3960	R
RIL 77	40.77	43.67	37.87	3.0350	S
RIL 78	7.47	12.93	2.00	3.1645	S
RIL 79	11.87	20.70	3.03	3.1440	S
RIL 80	13.98	13.31	15.81	0.1645	R

* Mean *Septoria tritici* leaf blotch (STB) severity averaged across two planting dates.
^ STB severity averaged from three seedling leaves tested with Kansas isolates of *Septoria tritici*.
† Mean absorbance values from two replicate tests, each consisting of combined leaf extracts from five random plants of the same genotype grown in a naturally infected SBWMV field in Manhattan, KS, 2006.
§ Phenotypic expression of SBWMV symptoms at Feekes growth stage 3 in a naturally infected SBWMV field in Manhattan, KS, 2005 and 2006. R, resistant; S, susceptible; -, not rated.

Table A1.1 cont. Phenotypic data collected on 78 $F_{5:7}$ recombinant inbred lines from a KS96WGRC40/Wichita population, Manhattan, KS 2004-2006.

Genotype	Mean STB Severity *	STB Severity Planting Date 1 ^	STB Severity Planting Date 2 ^	SBWMV ELISA Absorbance (405 nm) †	Phenotypic Expression of SBWMV Symptoms §
RIL 81	4.15	7.50	0.80	0.1555	R
RIL 82	20.88	30.97	10.80	3.1990	S
RIL 83	50.52	53.20	47.83	0.1830	R
RIL 85	8.57	12.20	4.93	0.1585	R
RIL 89	20.13	25.37	15.16	3.1505	S
RIL 90	14.10	23.27	4.93	3.0935	S
RIL 91	58.20	75.00	41.40	3.0920	R
RIL 92	7.08	10.93	3.23	3.1555	S
RIL 93	54.58	77.50	29.95	0.1910	R
RIL 94	30.65	40.43	20.87	3.2150	S
RIL 97	47.60	58.50	36.70	0.1740	R
RIL 98	58.32	66.17	50.43	3.1960	R
RIL 99	7.20	10.94	3.46	0.1720	R
RIL 102	7.65	11.73	3.57	3.1615	S
RIL 103	40.08	50.83	29.33	3.0635	R
RIL 106	26.05	28.60	23.50	3.1480	S
RIL 107	8.15	15.30	1.00	0.1860	R
RIL 109	44.50	62.89	20.63	3.1960	S
KS96WGRC40	8.82	12.00	5.63	0.1845	R
Wichita	78.83	88.33	69.33	3.2520	S
Overall mean	28.56	36.10	21.03	1.7732	

* Mean *Septoria tritici* leaf blotch (STB) severity averaged across two planting dates.
^ STB severity averaged from three seedling leaves tested with Kansas isolates of *Septoria tritici*.
† Mean absorbance values from two replicate tests, each consisting of combined leaf extracts from five random plants of the same genotype grown in a naturally infected SBWMV field in Manhattan, KS, 2006.
-, not tested.
§ Phenotypic expression of SBWMV symptoms at Feekes growth stage 3 in a naturally infected SBWMV field in Manhattan, KS, 2005 and 2006. R, resistant; S, susceptible; -, not rated.

APPENDIX 2. FRAGMENT SIZE OF BANDS PRODUCED WITH VARIOUS 6DS PRIMER PAIRS IN THE REGION OF *H23* AND *Cmc4*.

Table A2.1. Estimated base pair size of amplified DNA fragments produced with various 6DS microsatellite primer pairs in the region of *H23* and *Cmc4*.

Primer pair	Jagger	KS96WGRC40	TA2397	KS89WGRC04	TA1695
CFD049	180	175	300	175 200	175 190 200
BARC183	160 170	160	null	160 175	165
BARC173	210	210	null	210	200
CFD075	200 400 410	390 400 410 420	200 420	190 390 400 410	190
GDM132	145 350 400	350	145 350	140	140 400
CFD013	300	275	285	250 290	290
CFD001	240	235	Null	230	230
CFD042	197	190	Null	200	200
GWM469	150 160 178	150 155 165 178	155	150 170	150 170 180
BARC054	190	190	185	200	200
GDM141	125	125 150	145	175	175
GDM036	120 130	120 130	Null	120 160	120 160