MOLECULAR MAPPING OF STEM RUST RESISTANCE GENES IN WHEAT

by

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Abstract

Stem rust, caused by *Puccinia graminis* f. sp. *tritici,* has successfully prevented rust epidemics by Deployment of resistant cultivars in the past several decades. Unfortunately, race TTKS (termed Ug99) has defeated most stem rust resistance genes existing in commercial cultivars. *Sr40,* a stem rust resistance gene from *Triticum timopheevii* ssp. *araraticum,* was transferred to wheat and provides effective levels of seedling and adult plant resistance against Ug99**.** To characterize *Sr40* in wheat, two mapping populations were developed from the crosses RL6088 / Lakin and RL6088 / 2174. RL6088 is an Ug99-resistant parent with *Sr40*. Since race TTKS is a quarantined pathogen, a US stem rust isolate RKQQ that is avirulent to *Sr40* was used to evaluate the rust resistance in the F_2 and $F_{2:3}$ populations at the seedling stage. A total of 83 simple sequence repeats (SSR) primers on chromosome 2B were used to screen the parents for polymorphism. Each F_2 population was analyzed with the markers polymorphic between two parents. Marker Xwmc344 was the most closely linked to *Sr40,* at 0.7 cM proximal, in the linkage map constructed from the population RL6088 / Lakin, while Xwmc474 and Xgwm374 were also tightly linked. Xwmc474 was mapped 2.5 cM proximal to *Sr40* in the RL6088 / 2174 population. Xwmc474 and Xwmc661 were flanking markers for *Sr40* in both populations. Markers linked to *Sr40* will be useful for marker-assisted integration of *Sr40* into elite wheat breeding lines. In addition, a unknown stem rust resistance gene from another source, OK01307, a breeding line from Oklahoma State University shows partial resistance to Ug99, and was characterized using SSRs in this study. Two mapping populations were developed from cross OK01307 / Chinese Spring and OK01307 / LMPG-6. A total of 1300 SSR primers were screened for polymorphism between OK01307 and Chinese spring, and 1000 SSR primers were screened for polymorphism between OK01307 and LMPG-6. Polymorphic primers between parents and between bulks were used to screen the corresponding population. One *Sr* gene in OK01307 was mapped on chromosome 1BS of the both populations, which was closely linked to *Sr24.* Whether the gene is *Sr24* per se or a new *Sr* gene that closely linked to *Sr24* needs further investigation.

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CHAPTER 1 - Introduction

1.1 Stem rust pathogen and disease cycles

Stem rust, also called black rust, historically was the most destructive disease of wheat worldwide. Stem rust of wheat is caused by a parasitic fungus *Puccinia graminis* f.sp. *tritici,* and the fungus is heteroecious which means that two unrelated hosts, such as wheat and barberry, are required to complete its life cycle (http://www.apsnet.org/education/lessonsPlantPath/StemRustWheat/default.htm; (Leonard and Szabo 2005). Wheat is the primary host where the pathogen spends most time, and barberry is the secondary host. The fungus can reproduce itself only in living host plants. The fungus has five types of spores at different developmental stages: pycniospores (speramatia), aeciospores, urediniospores (uredospores), teliospores, and basidiospores (Leonard 2001a). The disease cycle starts with the exposure of the new wheat crop to stem rust inoculum. The source of the inoculum is different under different environments. In regions with warm climates, the volunteer plants carry the spores over summer and urediniospores from the infected volunteer wheat are the source of initial inoculum for wheat planted in the next fall to start a disease cycle (Leonard and Szabo 2005).

In the regions with a cold winter, aeciospores are the main source of initial inocula for wheat stem rust (Figure 1.1). Aeciospores are produced on barberry, the most common alternate host in the United States (http://www.apsnet.org; Leonard and Szabo 2005). On barberry, *P. graminis* starts its life cycle by producing black thick walled, diploid teliospores that keep *P. graminis* dormant overwinter (Roelfs 1985). After karyogamy, which forms a diploid nucleus from the fusion of two haploid nuclei, meiosis begins and results in four haploid basidiospores. In spring, each teliospore germinates to produce two identical thin walled haploid basidiospores (Roelfs 1985; Leonard and Szabo 2005). Mature basidiospores are carried by air currents to reinfect barberry. Basidiospores germinate and form a haploid mycelium growing on the leaf surface. From the mycelium, pycnia are produced on the upper leaf surface of barberry. Pycnia produce receptive hyphae that serve as female gametes and pycniospores served as male gametes (Roelfs 1985). Pycniospores are produced in honeydew that is attractive to insects, and rain splashing helps disperse pycniospores. When pycniospores are paired with receptive hyphae,

cross-fertilization occurs successfully and dikaryotic mycelium forms. Then a cup-shaped dikaryotic aecium forms to release aeciospores. Normally aeciospores stalked in chains produced on barberry are transported by wind to start a new disease cycle in susceptible wheat cultivars (Roelfs 1985; Leonard and Szabo 2005).

In wheat, rust infection mainly occurs on stems and leaf sheaths. Within two weeks after inoculation, a brick-red structure, called a rust pustule also known as a uredium containing urediospores, appears at the point of inoculation. In heteroecious rusts, urediospores can reproduce themselves and re-infect wheat multiple times (Leonard and Szabo 2005), which can lead to severe damage on wheat production. In a later developmental stage, another type of spore called teliospore, which is a black overwintering spore, is produced in telia (Cummins and Hiratsuka 1983) to conclude the disease cycle of stem rust in wheat and to start a new life cycle in barberry (Figure 1.1).

In the United States, barberry eradication has significantly reduced the number of epidemics for many years. Since common barberry has been largely removed after the 1920s (Figure 1.2), barberry bushes have not been a major source of disease inoculum (Kolmer et al. 2007); http://www.ars.usda.gov/Main/docs.htm?docid=10755). In recent years, barberry plants have reappeared in a few original eradication sites in south-eastern Minnesota (Peterson et al. 2005), and it is still necessary to monitor the barberry's population and avoid rust infection from barberry (Kolmer et al. 2007).

However, since *P. graminis* can survive in wheat in southern part of USA and Mexico, stem rust epidemics still can occur without help of barberry. Urediniospores, as the major inoculum source can spread by wind along the coast of the Gulf of Mexico to infect the winter wheat in the central Great Plains, and eventually the spring wheat in the north Great Plains (Leonard and Szabo 2005) (Figure 1.3). In the southern areas, urediniospores from volunteer wheat plants infect local winter wheat seedlings in the fall to start a new disease cycle.

The development of stem rust in wheat is favored by warm temperature (18-30^{O}C) with high relative humidity. The symptoms of the disease are visualized as erumpent uredia pustules mainly on the stems and leaf sheaths about 7 to 10 days after infection (Leonard 2001a). The shape of pustules is oval or spindle-shaped, and the color is reddish brown (Figure 1.4). The symptoms on resistant plants range from no uredia to small or medium uredia surrounded by

chlorosis or necrosis, while the symptoms on susceptible plants include large uredia with or without chlorosis or necrosis (Roelfs and Martens 1988).

1.2 Damage of stem rust disease

Stem rust can cause severe yield losses in susceptible cultivars of wheat in environments favorable for disease development (Leonard and Szabo 2005). In the mid of 20th century, yield losses reached 20 - 30% in eastern and central Europe (Zadoks 1963) and many other countries, including Australia, China and India (Rees 1972; Joshi and Palmer 1973; Roelfs 1977; Leonard and Szabo 2005). In the United States, from 1920 to 1960, stem rust epidemics in Minnesota, North Dakota and South Dakota caused average yield losses over 20% and up to 50% in 1935 (Leonard 2001b) (Figure 1.5). Stem rust resistance genes were successfully deployed in commercial cultivars worldwide from the middle 1950s, effectively controlling the disease (http://www.seedquest.com/News/releases/2007/january/18117.htm). However, in 1999, a new race of stem rust, Ug99, also called TTKS, emerged in Uganda (Pretoruis et al. 2000). Later, it was also found in Kenya, Ethiopia and Yemen (Singh et al. 2006). More recently, Ug99 has spread to major wheat production areas of the Middle East, such as Iran, Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan (http://www.fao.org/newsroom/en/news/2008/1000805/). The possible route of stem rust spread is proposed as East Africa – Middle East – West Asia – South Asia (Singh et al. 2006). Field stem rust data from Kenya, a severe and frequent stem rust epidemic region between 2005 and 2006, showed that most current cultivars deployed in the potential risk zone were susceptible to Ug99 (Singh et al. 2006). The estimated annual losses due to race Ug99 reached to approximately USD $3 billions in Africa, the Middle East and South Asia (http://www.seedquest.com/News/releases/2007/january/18117.htm).

1.3 Pathogen races and resistance genes

Stem rust pathogen can be divided into many different races according to their reactions to different resistance genes in wheat. The first report on stem rust race (races 1 and 2) was in 1916 (Stakman and Piemeisel 1917). Classification of physiologic races of *Puccinia graminis* f.sp.*tritici* was proposed seven years later based on the pathogen's reactions on a set of differential wheat genotypes (Stakman and Levine 1922). After several revisions, the last version

of the race classification document was published in 1962 (Stakman 1962) and had been used till 1988 (Roelfs and Martens 1988). The changes for the classification of races of *P*. g. f. sp. *tritici* mainly followed two scientific events. The first was the single gene concept, and the second was differential host genotypes of single resistance genes (Roelfs and Martens 1988). The classification of different pathogen races greatly facilitated identification of corresponding resistance genes in wheat and wheat relatives and transfer of individual race-specific resistance genes into adapted wheat backgrounds. Following the changes in classification, a new international system of nomenclature was established (Roelfs and Martens 1988; Leonard and Szabo 2005) (Figure 1.6).

Recently, virulence surveys showed that several common races were found on wheat in the US, including TPMK, TTTT, QFCS, and MCCF (Kolmer et al. 2007; Jin et al. 2008) (Table 1.1). Race TPMK was the most common race from the late 1950s until the late 1980s and is virulent to *Sr9b*, *Sr36* and *SrTmp* (Table 1.2). Since the late 1980s, race TPMK has been successfully controlled by *Sr6* and other resistance genes deployed in hard red winter and spring wheat cultivars (Kolmer et al. 2007). In 2000, race TTTT was identified to be virulent to *Sr6*, *Sr9a, Sr9b*, *Sr30*, *Sr36* and *SrTmp* (Jin 2005) (Table 1.2). Fortunately, most spring wheat cultivars in the northern Great Plains are resistant to TTTT. In the hard red winter wheat regions, *Sr24* and *Sr31* and the resistance derived from the 1AL.1RS translocation in Amigo are successfully used to control race TTTT (Kolmer et al. 2007). Race QFCS has been prevailed in the US since the late 1980s (Jin 2005) and is virulent to *Sr5*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, *Sr17*, and *Sr21* (Table 1.2). Resistance genes *Sr6*, *Sr24*, *Sr31*, *SrTmp*, and the gene from the 1AL.1RS translocation in Amigo show effective resistance against race QFCS (Kolmer et al. 2007).

For each pathogen race, host plants have a corresponding resistance gene to it. This is also known as gene-for-gene theory. In many cases, a single resistance gene can effectively control one or more strains of particular pathogen, and breeders have used resistance genes in conventional resistance breeding programs for decades (McDowell and Woffenden 2003). These rust resistance genes came from common wheat or wheat relatives (Table 1.3). Twenty resistance genes including *Sr6*, *Sr28, Sr29* and *SrTmp* were identified in common wheat, *T. aestivum*; seven including *Sr2* were from *T. turgidum*; three *Sr* genes (*Sr21*, *Sr22*, and *Sr35)* were from *T. monococcum*; four *Sr* genes including *Sr24*, *Sr25*, and *Sr26* were from *Agropyron elongatum*; *Sr31* and *Sr1A.1R* were from *Secale cereale; Sr36* and *Sr37* were from *T. timopheevi;* one each

was from *T. ventricosum (Sr38)* and *T. araraticum (Sr40*), respectively (Roelfs 1988; Dyck 1992; Singh et al. 2006) (Table 1.3). In addition, several *Sr* genes have also be found to show resistance to leaf and stripe rusts of wheat, including *Sr24 / Lr24*, *Sr25 / Lr19*, and *Sr31 / Lr26 / Yr9* (Roelfs 1988; Dyck and Sykes 1995; Margo et al. 2002; Mago et al. 2005a). All these catalogued *Sr* genes are race specific, except gene *Sr2*.

1.4 Deployment of stem rust resistance genes in common wheat

Some of the *Sr* genes have been widely deployed in commercial wheat cultivars. *Sr2*, derived from *T. turgidum* is on chromosome 3BS, and has conferred durable rust resistance against all virulent races of *P. graminis* worldwide for more than 50 years. It has been deployed in many wheat cultivars worldwide (McIntosh 1988; Rajaram et al. 1988; Roelfs 1988; McIntosh et al. 1995; McIntosh et al. 1998; Spielmeyer et al. 2003). *Sr2* as a slow rust resistance gene shows partial resistance with variable levels of disease on adult plants grown in the field when it is used alone (Singh et al. 2006). The effect of *Sr2* can be enhanced by adding race-specific *Sr* genes (Spielmeyer et al. 2003; Kota et al. 2006). Pseudo-black chaff, a dark pigmentation around the stem internodes and glumes is closely associated with *Sr2* (Hare and McIntosh 1979) and has been used as a morphological marker to select for the gene (Hayden et al. 2004). Unfortunately, high pseudo-black chaff expression may also cause yield reduction in wheat (Hare and McIntosh 1979). *Sr24* was originally derived from *A. elongatum* and has been integrated into many wheat lines in South America, Australia and CIMMYT (Mago et al. 2005a; Singh et al. 2006). *Sr24* has two types of translocations from chromosome 3Ag of *A. elongatum* to 3DL or 1BS of wheat (Mago et al. 2005a). The stem rust resistant variety *Agent* has a spontaneous translocation between chromosomes 3Ag of *A. elongatum* and 3DL of bread wheat (Smith et al. 1968). Amigo, another wheat variety that has a 1AL.1RS translocation from Insave rye, has two *Sr* genes*:* one on rye chromosome 1RS and the other, *Sr24,* on 1BS (Sebesta and Wood 1978; Mago et al. 2005a). The chromosome 3Ag also carries a leaf resistance gene *Lr24* (Sears 1973). Both *Sr24* and *Lr24* are tightly linked together in chromosome 3DL and 1BS translocations (Mago et al. 2005a). *Sr24* is effective against most stem rust races worldwide.

Sr26 is translocated from *A. elongatum* to chromosome 6AL of wheat (Knott 1961; Knott 1968). Although races virulent to *Sr26* have not been reported to date, *Sr26* has not been widely deployed in commercial wheat varieties due to the yield penalty associated with the gene (The et

al. 1988). *Eagle* was the first wheat variety carrying *Sr26* that was released in Australia (Martin 1971).

Sr31, a strong resistance gene derived from *Secale cereale* introduced to bread wheat through a 1B/1RS translocation, has been deployed in winter and spring wheat varieties in China, Europe, India, and USA (Zeller 1973; Zeller and Hsam 1983; McIntosh et al. 1995; Das et al. 2006). Enhanced stem rust resistance was reported when it was stacked with *Sr25* (Tomar and Menon 2001).

Sr36, derived from *T. timopheevi* was originally transferred into two hard red spring wheat lines, CI12632 and CI12633 (Allard and Shands 1954; Dyck 1992; Tsilo et al. 2008). *Sr36* is located on chromosome 2BS, and has been deployed in many Australian wheat cultivars (Bariana et al. 2001) and some soft winter wheat cultivars in the USA (Jin and Singh 2006).

The new race, Ug99, overcomes many known *Sr* genes. Genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30*, *Sr31*, *Sr34*, *Sr38* and *SrWld-1* (Jin et al. 2007) were all defeated by the Ug99 (Pretoruis et al. 2000; Jin and Singh 2006). Only a few known genes are still effective against Ug99, and some of them have ready been or are being deployed in commercial wheat cultivars. In Kenya, *Sr2* itself showed insufficient resistance to Ug99. When *Sr2* was combined with other *Sr* genes, such as *Sr23*, *Sr25*, or some unknown *Sr* genes, high resistant levels were displayed against Ug99 (Singh et al. 2007). Therefore, *Sr2* is recommended to be used in combination with other genes to enhance resistance levels (Singh et al. 2007). *Sr26*, another gene effective against Ug99, has been successfully used in Australia, but the associated yield penalty significantly discounts its popularity in breeding programs (Jin and Singh 2006; Singh et al. 2006; Singh et al. 2007). *SrTmp* derived from *T. aestivum* exists in several hard red winter wheat cultivars (Roelfs and McVey 1975). It also results in low infection type to race Ug99 (Singh et al. 2007). *Sr1A/1R* derived from *Secale cereale* provides resistant to Ug99 and has been detected in several hard red winter wheat cultivars (Sebesta et al. 1995; Jin and Singh 2006). *Sr24* has been an important resource deployed in the breeding programs to control Ug99 recent years (Singh et al. 2007; Jin et al. 2008).

A big concern is the ability of Ug99 to change. In 1999, Ug99 was found to be virulent to *Sr31*, which was deployed in many wheat cultivars (Jin and Singh 2006). Recently additional new virulence has been recognized to separate isolates of race TTKS into two different races:

TTKSK and TTKST (Jin et al. 2008). The effectiveness of *Sr24* to the new virulent isolates of Ug99 has been decreased (Jin et al. 2008). Combining *Sr24* with other resistance genes may still enhance resistance to Ug99. *Sr36* showed high to medium resistance to the original race Ug99 and has been successfully used in Africa to control Ug99 (Singh et al. 2007). Recent detection of new virulent isolates of Ug99, TTTSK, that gives a high infection type on *Sr36,* renders the *Sr36* ineffective. Therefore, *Sr36* is no longer recommended for use as useful source of stem rust resistance against Ug99 (http://maswheat.ucdavis.edu/protocols/StemRust/index.htm).

Several other genes, including *Sr22*, *Sr25*, *Sr35*, *Sr39*, *Sr40* and *Sr44* identified from different wheat relatives, are moderately to highly resistant to Ug99 in seedling tests (Jin et al. 2007; Singh et al. 2007). These genes have been transferred into wheat backgrounds, but not deployed in commercial cultivars. They are good sources of resistance genes for control of Ug99. Instead of traditional breeding strategy that is single gene deployment, pyramiding race-specific resistance genes in one cultivar has been one of the most efficient ways to control stem rust disease. DNA markers are essential tool to facilitate such gene pyramiding in breeding programs. Currently, DNA markers are available for only few stem rust resistance genes, such as *Sr2* (Nelson et al. 1995; Bariana et al. 1998; Johnston et al. 1998; Spielmeyer et al. 2003), *Sr22* (Paull et al. 1994), *Sr24* / *Lr24* (Autrique et al. 1995; Schachermayr et al. 1995; Prabhu et al. 2004; Mago et al. 2005a), *Sr31* (Das et al. 2006), *Sr39* (Gold et al. 1999). Markers for some important *Sr* genes are not available; therefore, more makers will be urgently needed for effective deployment of these genes.

Figure 1.1 Life cycle of stem rust pathogen (http://nu-distance.unl.edu/homer/disease/agron/wheat/WhStRst.html)

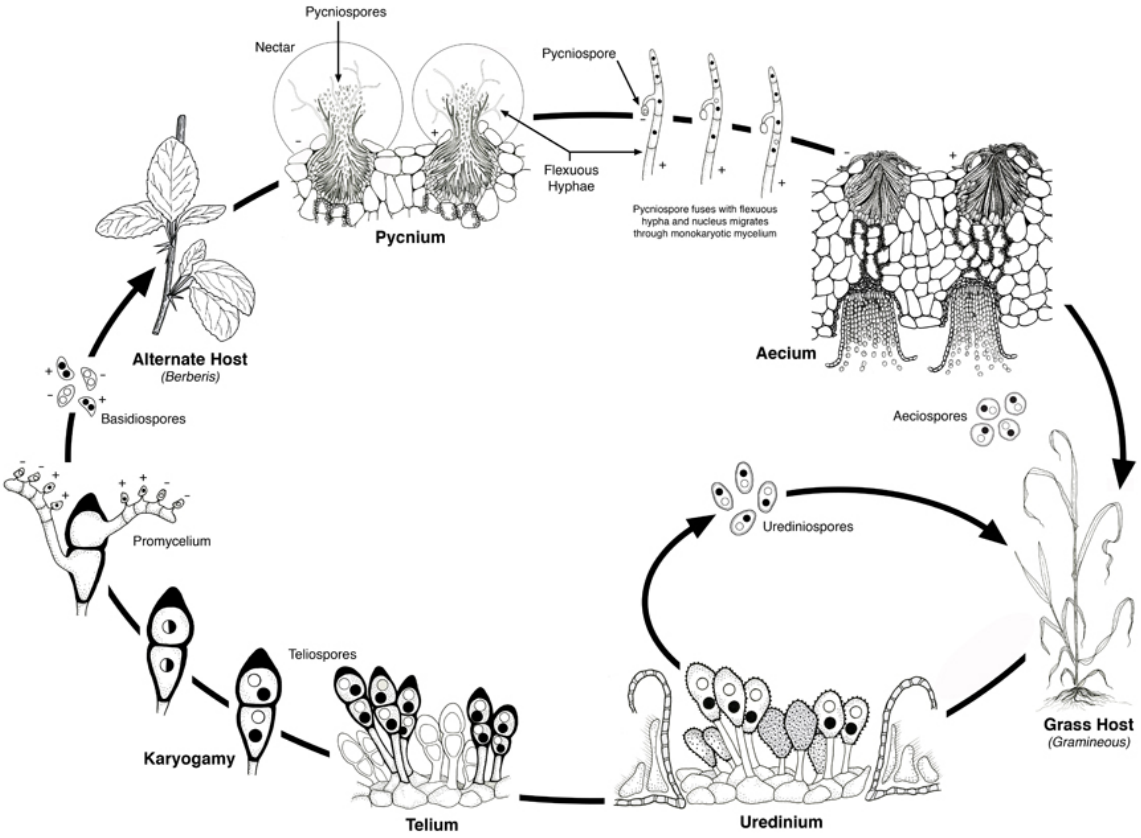


Figure 1.2 The impact of barberry eradication in USA. Average numbers of states with wheat yield losses exceeded 1% in any given year due to stem rust. (http://www.ars.usda.gov/Main/docs.htm?docid=10755).

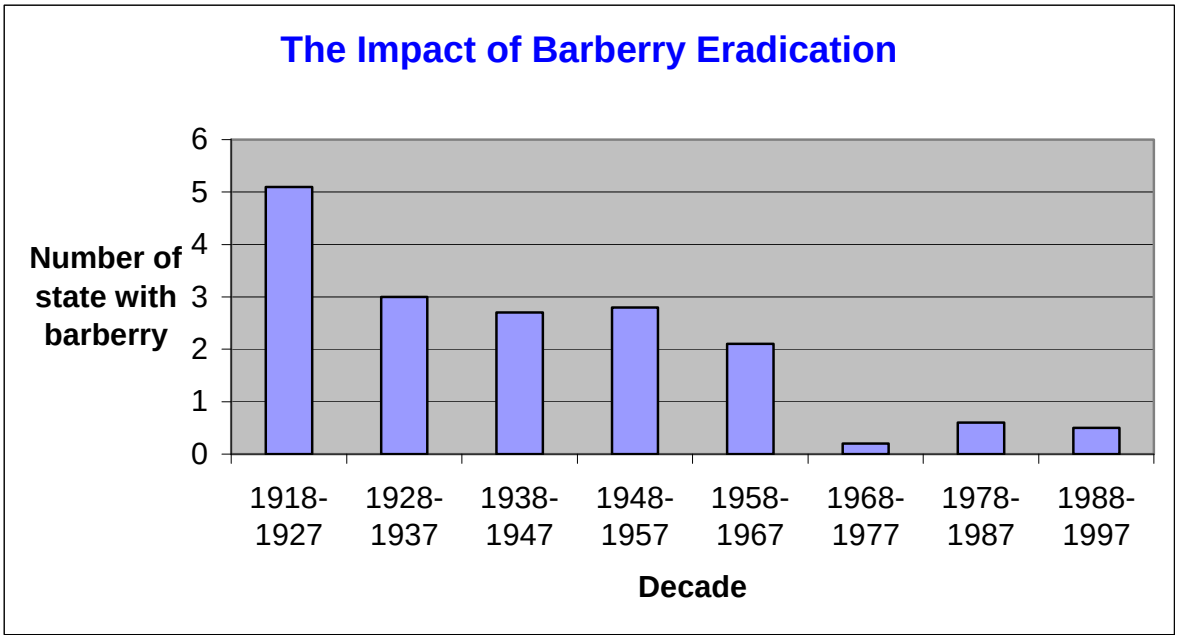


Figure 1.3 Urediniospores spread by wind from the Gulf Coast of Mexico to the north Great Plains (http://www.ars.usda.gov/Main/docs.htm?docid=10755).

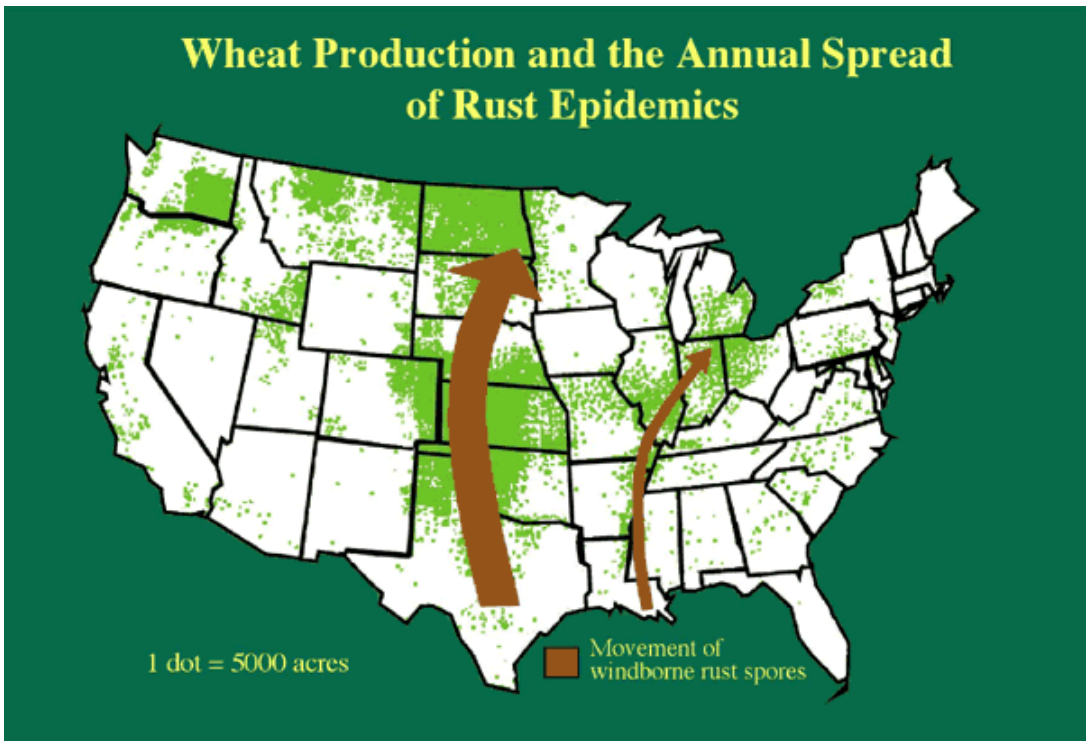


Figure 1.4 Stem rust symptoms on wheat stem and leaf (Photo by Cereal Disease Lab, USDA)

Figure 1.5 Yield loss caused by stem rust between 1920 and 2000 in MN, ND and SD (http://www.ars.usda.gov/Main/docs.htm?docid=10755)

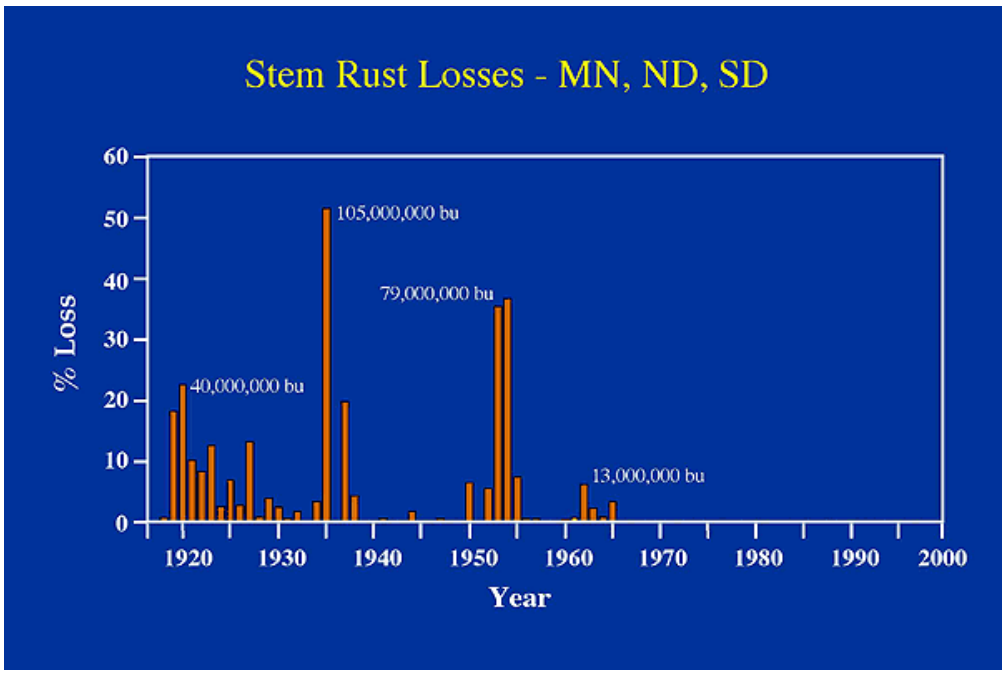


Figure 1.6 Infection types (ITs) of *Puccinia graminis* f. sp. *tritici*. at a 0 - 4 scale. A) IT=0 (no uredia); B) IT=0; (fleck); C) IT=1 (small uredia); D) IT=2 (small to medium uredia); E) IT=3 (medium uredia without chlorosis or necrosis) F) IT=4 (large uredia without chlorosis or necrosis) (Leonard and Szabo 2005)

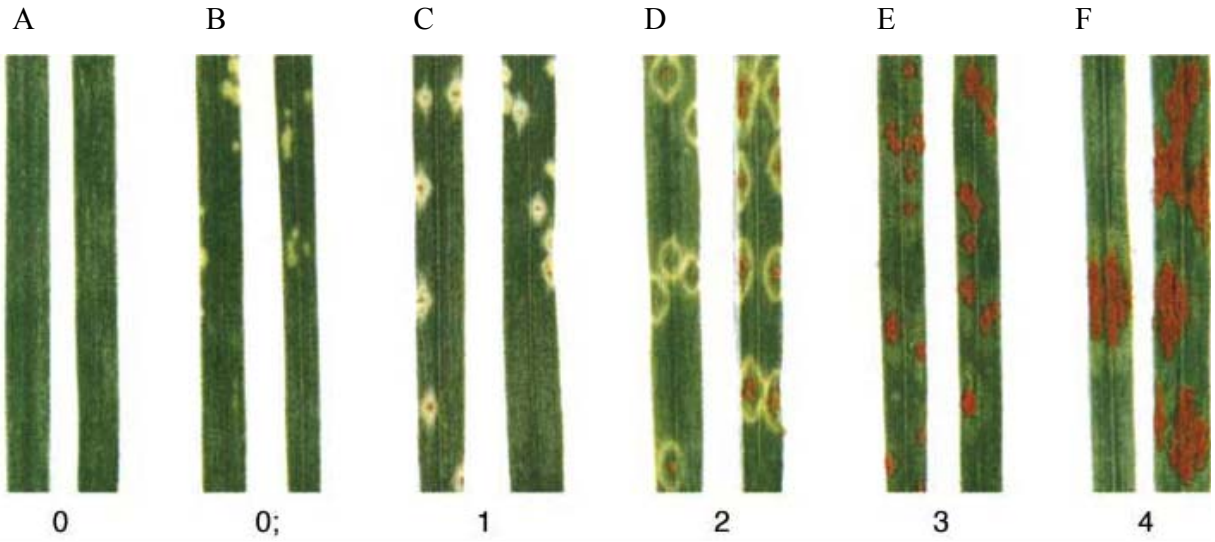


Table 1.1 Races of stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) on wheat in the US in 2004 (Kolmer et al. 2007)

Area	State	No. of Collections	No. of isolates	no. of isolates					
				QFCS	MCCF	MCCD	TPMK	QCCN	TTTT
Great Plains	TX	1	1	1					
	OK	1	1	1					
	KS	3	3	3					
	NE	3	2	1			1		
	S.D.	3	3	3					
	ND	7	6	6					
Midwest	MO	1	1	1					
	IN	1	1		1				
	MN	15	19	15	2	1			1
Pacific NW	WA	1	2					2	
US Total		36	39	31	3	1	1	2	1

Table 1.2 Virulence of stem rust pathogen to stem rust resistance genes in 2004 (Kolmer et al. 2007)

Race	Ineffective *Sr* gene
QFCS	*Sr5, Sr21, Sr8, Sr9g, Sr17, Sr9e, Sr9b, Sr10*
MCCF	*Sr5, Sr7b, Sr9g, Sr17, Sr10, SrTmp*
MCCD	*Sr5, Sr7b, Sr9g, Sr17, Sr10*
TPMK	*Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr8, Sr9g, Sr36, Sr17, Sr9b, Sr10, SrTmp*
QCCN	*Sr5, Sr21, Sr9g, Sr17, Sr9a, Sr10*
TTTT	*Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr6, Sr8, Sr9g, Sr36, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, SrTmp*

Table 1.3 Origins of stem rust resistance genes to pathogen *P. graminis* f. sp. *tritici* (Roelfs 1988; Dyck 1992; Singh et al. 2006)

Origin of Sr gene	*Sr* genes
T. aestivum	*Sr5, Sr6, Sr7a, Sr8a, Sr8b, Sr9a, Sr9b, Sr9f, Sr10, Sr15, Sr16, Sr18, Sr19, Sr20, Sr23, Sr28, Sr29, Sr30, Sr41, Sr42, SrWld-1, SrTmp*
T. turgidum	*Sr2*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr13*, *Sr14*, *Sr17*
T. monococcum	*Sr21*, *Sr22*, *Sr35*
A. elongatum	*Sr24*, *Sr25*, *Sr26*, *Sr43*
S. cereale	*Sr27*, *Sr31, Sr1A/1R*
T. speltoides	*Sr32*, *Sr39*
T. tauschii	*Sr33, Sr45*
T. comosum	*Sr34*
T. timopheevi	*Sr36*, *Sr37*
T. ventricosum	*Sr38*
T. araraticum	*Sr40*

CHAPTER 2 - Molecular mapping of a stem rust resistance gene *Sr40* in wheat

2.1 Introduction

Stem rust, also known as black rust caused by *P. graminis* f. sp. *tritici* (pgt) used to be one of the most important wheat diseases worldwide. Severe wheat yield losses due to stem rust epidemics have been reported in Europe, Asia, Australia, and the United State before the middle of the 20th century (Zadoks 1963; Rees 1972; Roelfs 1977; Leonard 2001b; Leonard and Szabo 2005). In the United States, stem rust disease was severe in spring wheat from 1920 to 1960 and caused yield losses of 20% in average and up to 50% in some fields in some years (Leonard 2001b). Since 1970, yield losses caused by stem rust have been minimal due to successful deployment of stem rust resistance genes in commercial wheat cultivars (Leonard and Szabo 2005). In 1999, a new race of stem rust pathogen Ug99, also called race TTKS, was identified in Uganda. It spread to Ethiopia and some other east African countries in 2003 (Singh et al. 2006), and recently has dispersed to Mideast countries such as Iran. Therefore, stem rust is again becoming a threat to wheat production worldwide (http://www.fao.org/newsroom/en/news/2008/1000805/).

To date, about fifty stem rust resistance genes have been identified and some of them have been mapped on different chromosomes in wheat and its relatives (McIntosh et al. 1998). All these genes are race specific except *Sr2* that has provided durable non-race-specific slow-rusting adult plant resistance (McIntosh et al. 1995; Spielmeyer et al. 2003; Singh et al. 2006; Singh et al. 2007). Among these resistance genes, some genes deployed in commercial cultivars worldwide remained effective individually or in combination with other *Sr* genes until recently. *Sr2,* derived from *Triticum turgidum* and located on chromosome 3BS, has been widely used in the wheat program in CIMMYT, Mexico for global improvement of wheat stem rust resistance and hard winter wheat breeding programs in the USA (Spielmeyer et al. 2003). Moreover, the *Sr2* complex in combination with other resistance genes showed effective protection against Ug99 (Singh et al. 2006). Resistance gene *Sr26,* transferred from *Agropyron. elongatum* to chromosome 6AL, provides resistance to current stem rust races of wheat in Australia (McIntosh et al. 1995; Mago et al. 2005a). *Sr24,* a resistance gene located on chromosome 1BS and originally introduced from *A. elongatum*, has also been deployed in many U.S. wheat cultivars

(Margo et al. 2005b). *Sr36* from *Triticum timopheevii* has been widely used in Australia and several other countries (Bariana et al. 2001). *Sr31*, an important stem rust resistance gene from rye 1B/1R translocation has been deployed in many wheat cultivars worldwide. However, most deployed resistance genes are susceptible to Ug99 or overcome by virulence of Ug99 except few genes such as *Sr2*, *Sr1A/1R*, *Sr26*, and *SrTmp*. *Sr31* was identified to be defeated by a virulent isolate of Ug99 in 1999 (Das et al. 2006). *Sr36* was initially resistant to Ug99 initially, but a recent new isolate of Ug99 has been found to be virulent to *Sr36* (Jin, et al. 2007, http://maswheat.ucdavis.edu/protocols/StemRust/index.htm). *Sr24* also showed reduced level of resistance to Ug99 (Yue Jin, personal communication). Fortunately, several genes from different wheat relatives, including *Sr22*, *Sr25*, *Sr27, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40* and *Sr44,* are still resistant to Ug99 (Jin et al. 2007; Singh et al. 2007), and have been transferred into wheat backgrounds. However, deployment of these genes in commercial cultivars is still in its infancy (Singh et al. 2007).

Pyramiding of several genes into one cultivar can be an effective strategy to use these resistance genes to enhance durability of wheat resistance to stem rust (Leonard and Szabo 2005). Gene pyramiding using conventional method is difficult and time-consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before making selection. Usually, it is not feasible for a regular breeding program to maintain all necessary rust races needed for this type of work. Therefore, marker-assisted selection (MAS) is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars. Molecular markers are available for only few resistance genes such as *Sr2* (Hayden et al. 2004), *Sr22* (Paull et al. 1994; Kham et al. 2005), *Sr24* and *Sr26* (Margo et al. 2005b), *Sr31* (Das et al. 2006), *Sr36* (Bariana et al. 2001; Tsilo et al. 2008) and *Sr39* (Gold et al. 1999). Some of the markers have been used in MAS, but markers for some of the genes are not diagnostic for the genes and must be improved and markers for other genes are not available. At the present time, the research of stem rust in wheat is focusing on identifying more resistance genes to control Ug99. According to the Farm and Ranch Guide report, currently 50% of winter wheat and 70 to 80% spring wheat used in the USA are susceptible to Ug99. Moreover, 75-80% of the breeding materials are susceptible to Ug99 and most stem rust resistance genes deployed in breeding programs have been overcome by this new fungus. (http://www.farmandranchguide.com/articles/2008/03/13/ag_news/production_news/pro10.txt).

Therefore, new or better markers for stem rust resistance genes are urgently needed for improving wheat stem rust resistance.

Sr40 is a new stem rust resistance gene that originated from *Triticum araraticum,* a wild form of tetraploid wheat, *T. timopheevii* (Morris and Sears 1967; Dyck 1992). *T. timopheevii* has contributed several rust resistance genes such as *Sr36*, *Sr37* and *Lr18* to wheat (McIntosh and Gyarfas 1971; McIntosh 1988; Yamamori 1994). *Sr40* has been transferred into common wheat from *T. araraticum* accession PGR6195 collected from Turkey (Dyck 1992). *Sr40* was reported to be linked to rust resistance genes *Lr13, Lr23*, *Lr16*, and *Sr36*, therefore, it was tentatively located on the short arm of chromosome 2B (Dyck 1992). In wheat, *Sr40* exhibits stem rust infection types (IT) of 1+ to 2 to stem rust race RKQQ. Seedling test using race TTKS (Ug99) showed that *Sr40* is also resistant to Ug99 (Jin et al. 2007). Since race TTKS is a quarantine pathogen, we are not permitted to use this race, and the seedling test was done by USDA cereal disease laboratory in Minnesota. Therefore deployment of this gene in US commercial wheat cultivars may prevent stem rust epidemics to reduce the losses caused by the disease in future. Also, pyramiding *Sr40* with other stem rust resistance genes may enhance durability of stem rust resistance in a cultivar. The objectives of this study are to validate the chromosome location of *Sr40* through molecular mapping and identify closely linked markers to *Sr40* for MAS in breeding programs.

2.2 Materials and methods

2.2.1 Plant materials

The two mapping populations developed for this study are Rl6088 / Lakin and RL6088 / 2174. Rl6088 is a Ug99-resistant cultivar derived from the cross RL6071*7 / PGR6195 (Dyck 1992) (Figure 2.1), while Lakin is susceptible hard white winter wheat cultivar and 2174 is susceptible hard red winter wheat cultivar (http://www.oznet.ksu.edu/WheatPage/varieties.htm; http://jpr.scijournals.org/cgi/content/full/1/2/102).

2.2.2 Stem rust evaluation

Reaction of both populations to stem rust race RKQQ was evaluated at seedling stage in the greenhouse at Kansas State University, Manhattan, KS. The RKQQ isolate used in this study was provided by Dr. Yue Jin, increased at Kansas State University, and tested on the standard *Sr*

gene differential set to verify race identity. The population Rl6088 / Lakin with total of 202 F_2 plants and 88 randomly selected $F_{2:3}$ families and the population RL6088 / 2174 with total 107 F_2 plants and all their $F_{2:3}$ families were inoculated at 2-3 leaves stage by spraying urediniospores of the race RKQQ over the seedling plants. Before inoculation, urediniospores were recovered from a liquid N_2 tank and heat-shocked for 5 min in a water bath at 40˚C. Spores were then suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX) and heavily spray-inoculated. Plants were kept in a dark moist chamber at 21˚C with 100% relative humidity for 16 hours after inoculation, and then moved to a greenhouse bench at 20˚C with 16h day length by using supplemental growth light. The stem rust symptoms were scored on the 14th day after inoculation when the rust pustules fully erupted on the inoculated leaves (Jin et al. 2007). Rust ratings of three young leaves in a plant were averaged to reflect an overall rust score of the F_2 plant. Rust ratings of $F_{2:3}$ families was an average rust rating over 20 inoculated seedlings. Rust severity was determined based on infection types (ITs) using a 0 to 4 scale (Stakman et al. 1962; Roelfs and Martens 1988). ITs from 0 to 2 were rated as resistant and 3 to 4 as susceptible.

2.2.1 Marker analysis

Young wheat leaf tissue without rust symptom was collected in a 2ml micro tube for DNA isolation after rust evaluation. Collected leaf tissue was immediately dried in a freeze-dryer (ThermoSavant, Holbrook, NY) for 48 hours and ground to fine powder using a Mixer Mill (MM300, Rotsch, Germany). Genomic DNA was isolated using a revised CTAB protocol (Saghai et al. 1984). Because *Sr40* has been previously located on chromosome 2B based on genetic analysis (Dyck 1992), 83 SSR primers on chromosome 2B were selected to screen the parents and bulks for polymorphism (Somers et al. 2004). Both resistant and susceptible bulks were constructed separately by mixing genomic DNA of five homozygous resistant and five susceptible F_2 lines respectively. Polymorphic primers between bulks were then used to screen two mapping populations. For SSR analysis, a 13µl PCR mixture contained 3uL DNA template (20ng/uL), 100nM forward primer, 200nM of reverse primer, 200uM of dNTP, 1X ASB buffer (Bioline USA Inc. Taunton, MA), 2.5mM $MgCl_2$, 1U *Taq* polymerase, and 100nM of fluorescence-dye-labeled M13 primer compatible with ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

PCR amplification was conducted using a touch down program. Initially PCR mixture was denatured at 95°C for 5 min; the first five cycles started with 1 min of denaturing at 96°C, 5 min of annealing at 68°C with a decrease of 2°C for each subsequent cycle, and 1 min of extension at 72°C; in the next five cycles, the annealing temperature started at 58°C for 2 min with a decrease of 2°C for each subsequent cycle; the last 25 cycles ran 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C with a final extension at 72°C for 5 min and 4°C for 5 min. The PCR products were analyzed in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Marker data was scored using GeneMarker® version 1.5 (SoftGenetics LLC, State College, PA). All data were double checked to remove ambiguous data (Figure 2.2)

Genetic linkage map was constructed using JoinMap version 3.0 (Van Ooijen and Voorrips 2001) with a LOD (logarithm of odds) threshold at 3.0 and recombination fraction at 0.4. The genetic distance in centiMorgans (cM) was calculated according to Kosambi function (Kosambi 1944).

2.3 Results

About 10 days after inoculation with stem rust isolate RKQQ, wheat leaves of susceptible parents covered with medium to large oval or elongated reddish brown uredia pustules with infection types of 2+ for 2174 and 3 for Lakin, while resistant parent RL6088 had only very small size pustules surrounded by necrosis on the infected leaves with an infection type 1+ (Figure 2.1). Therefore, rust symptom in resistant plants was clearly distinct from that in susceptible parents.

The frequency distribution of stem rust ITs in the F_2 was continuous and showed two peaks with the major peak matching with the IT (1+) of resistant parent in both populations (Figure 2.3). Transgressive resistant (IT 1) and susceptible (IT 4) genotypes were observed in RL6088 / Lakin population, but not in RL6088 / 2174. To separate resistant genotypes from susceptible genotype in the F_2 generation, $F_{2:3}$ families were tested for rust resistance. When IT of an F_2 plant was equal to or less than 2, its F_3 family usually was either all resistant or segregating. When IT was larger than 2, its F_3 family was all susceptible. Therefore, the IT for separating resistant from susceptible F_2 genotypes was set at 2 and ITs of 2+ or higher were classified as susceptible genotypes. Based on this criteria, the segregation of resistant and susceptible genotypes in the population RL6088 / 2174 fit well to the expected 3:1 Mendelian

single gene ratio in F_2 generation and 1:2:1 (resistant: heterozygous: susceptible) ratio in the $F_{2:3}$ generation (Table 2.1). The result suggests that *Sr40* in a 2174 background is inherited as a single dominant gene. However, in the RL6068 / Lakin population, about 40% of the F_2 plants were susceptible. To verify the result, an additional 93 F_2 plants were inoculated with the same isolate and combined data from two F_2 sub-populations showed a similar result (Table 2.1). Therefore, the segregation ratio in both F_2 and $F_{2:3}$ generations of RL6068/Lakin population obviously deviates from single gene inheritance (Table 2.1). Segregation of marker alleles linked to *Sr40* significantly deviated from the expected Mendelian 1:2:1 ratio in the 88 F_3 families tested (Table 2.2), supporting phenotypic data observed for this population.

Marker analysis identified seven polymorphic SSRs between RL6088 and 2174. Those markers were mapped on the short arm of chromosome 2B using the F_2 population of 107 plants from the cross RL6088 / 2174 (Table 2.3). The linkage map covered a total genetic distance of 15.2 cM (Figure 2.4). Xwmc474 was the closed marker to *Sr40* at 2.5cM apart and Xwmc661 and Xwmc474 were flanking markers for *Sr40*. *Sr40* was located in the same position in the map constructed based on $F_{2:3}$ rust data (data not shown).

In the population RL6088 / Lakin, eight polymorphic markers were analyzed in 202 F_2 plants for map construction (Table 2.3). *Sr40* was located between the SSR markers Xwmc661 and Xwmc344 (Figure 2.4). Xwmc344 was the most closely linked marker to *Sr40* at 0.7cM. Xwmc344 was not polymorphic in the RL6088 / 2174 population (Table 2.3). Xwmc474 and two additional markers, Xgwm374 and Xbarc230, were also mapped at 1.6cM proximal to *Sr40*. To verify the rust data from the F_2 generation, 88 $F_{2:3}$ families were randomly selected from the 202 F_2 plants and further evaluated for rust resistance in a repeated experiment. The *Sr40* position in the map developed using $F_{2:3}$ rust data was almost the same as that in the map derived from F_2 rust data (Data not shown).

The linkage groups from two F_2 populations were similar with a slight variation in genetic distances between some SSRs and between SSR and *Sr40* (Figure 2.4). *Sr40* was flanked by Xwmc661 and Xwmc474 in both maps. Both markers were mapped closely to centromere on the short arm of chromosome 2B in a previous study. Therefore, *Sr40* was located on the short arm of chromosome 2B, close to centromere.

To verify the relationship between *Sr36* and *Sr40*, three markers Xgwm271, Xgwm319 and Xwmc477 closely linked to *Sr36* were also mapped and analyzed in the two

populations (Figure 2.4). Two markers, Xgwm319 and Xwmc477, were polymorphic between RL6088 and 2174, but only Xwmc477 was polymorphic between RL6088 and Lakin (Table 2.3). The two markers were mapped at 3.5 and 2.7 cM to *Sr40,* respectively, in the two populations, indicating *Sr40* is closely linked to *Sr36* using genetic mapping analysis.

2.4 Discussion

2.4.1 Phenotypes of parents and F_2 populations

Stem rust symptoms typically occur on the stem and leaf sheath of adult plants to cause damage to wheat, but the stem rust fungus also infects leaves of wheat plants to develop stem rust symptoms, especially in the seedling stage. Because seedling resistance is easy to evaluate on a large scale under environment-controlled conditions and is highly correlated with stem rust resistance at the adult plant stage, the method has been widely used to evaluate stem rust resistance of wheat materials (Jin et al. 2007). Therefore, it is the method of choice for evaluating mapping populations for stem rust resistance in this study.

Resistant parent RL6088 used in this study is the germplasm line that carries a single *Sr40* resistance gene to stem rust and showed a high level of resistance to Ug99 (TTKS) when it was evaluated in two field experiments in Kenya and one greenhouse test at Cereal rust laboratory, St Paul, MN, USA (Jin et al, 2007). Because Ug99 was not available for this study in Manhattan, KS, isolate RKQQ that is avirulent to *Sr40* was used to evaluate the parents and segregating progenies in this study. At 10 days after inoculation with race RKQQ, the resistant parent RL6088 showed very tiny pustules surrounded by necrosis on inoculated leaves and was scored as IT 1+ according to Roelfs' method (Roelfs and Martens 1988). Slight higher IT scores of RL6088 from this study (IT 1+) than that reported by Jin may be due to difference in isolates used in different studies or other non-genetic factors (Jin et al. 2007). Lakin and 2174 were susceptible and showed medium to large stem rust pustules with Lakin scored as IT3 and 2174 as IT 2+3. Although slight difference in pustule sizes were observed between the two susceptible parents, visual difference in rust symptoms was distinct between resistant and susceptible parents therefore they were appropriate parents for *Sr40* gene mapping.

The frequency distributions of F_2 ITs were continuous for both populations, indicating that modifier gene(s) from the susceptible parents might be involved and/or non-genetic factors might also affect expression of *Sr40*. The continuous distribution brings some ambiguity to

classification of susceptible genotypes from resistant genotypes. We set IT 2 or lower as resistant and IT 2+ or higher as susceptible genotypes based on parents and their F_3 segregation in this study. In RL6088 / 2174 population, the susceptible parent 2174 had IT of 2+, therefore, F_2 plants with IT 2+ in the population should be susceptible genotypes. This assumption was confirmed by the result from $F_{2:3}$ families. All 107 F_2 derived F_3 families segregated into 19 homozygous resistant: 57 heterozygous: 31 homozygous susceptible. The 30 susceptible F_2 plants were all homozygous susceptible in F_3. Only one plant that was resistant in F_2 was susceptible in F_3. This was likely due to misclassification between ITs 2 and 2+. All F_2 plants with 2+ were susceptible. In RL6088 / Lakin population, susceptible parent has IT 3, but the F_3 families from 8 F_2 plants with IT 2+ were all homozygous susceptible, thus, 2+ was also susceptible genotype in RL6088 / Lakin population, and the classification of rust reactions in two F_2 generations used in this study was adequate.

2.4.2 Inheritance of Sr40 in two wheat genetic backgrounds

In the population RL6088 / 2174, 107 F_2 plants showed 3 (resistant) to 1 (susceptible) Mendelian single gene segregation, indicating *Sr40* was the only gene that segregated for stem rust resistance in the population. Segregation analysis of all $F_{2:3}$ families derived from the F_2 population confirmed the result from F_2 (Table 2.1). The segregation ratio of 202 F_2 plants from RL6088 / Lakin population was about 2 (resistant) to 1 (susceptible), which was significantly deviated from the expected 3:1 ratio (Table 2.1). A similar result was obtained from analysis of $F_{2:3}$ families. A total of 88 F_2 plants with 58 resistant and 30 susceptible were randomly selected for F_3 test and the segregation ratio of the 88 $F_{2:3}$ families was 11 (homozygous resistant): 46 (heterozygous): 31 (homozygous susceptible) (Table 2.1). Therefore F_3 families also obviously deviated from single gene segregation as in F_2. Since *Sr40* was the only gene to be transferred to the population, this was likely due to preferential transmission of Lakin gametes in F_2. Preferential transmission was also observed from segregation of marker alleles that linked to *Sr40* significantly deviated from the expected Mendelian 1:2:1 ratio in the 88 F_3 families tested (Table 2.2), and the marker's segregation supported phenotypic data observed for this population. Beside *Sr40*, both *Sr36,* a stem rust resistance gene, and *Pm6*, a powdery mildew resistance gene, were transferred from *T. timopheevii* to the same region of chromosome 2B previously and preferential transmission was also observed for the two genes (Allard and Shands

1954; Brown-Guedira et al. 2003). However, we can't rule out the possibility that additional gene(s) in Lakin background might modify the expression of *Sr40*. This assumption can be supported by transgressive segregation. In this population, the plants with either IT 1 or IT 4 in the population were transgressive segregants because neither parents had IT 1 and IT 4. The results suggested that some modifying genes from elite wheat lines might be able to enhance resistance of *Sr40* and to create transgressive segregants in new cultivars.

2.4.3 Mapping Sr40 in two populations

A total of 10 markers showed linkage to *Sr40* in two populations. Among them, five markers were common between two populations and eleven markers were polymorphic in both populations (Table 2.3). Both linkage maps had similar length (15 cM) and most of common markers showed the same order in two maps (Figure 2.4). A slight variation in genetic distance between *Sr40* and several markers was observed between two maps. This may be due to segregation distortion in RL6088 / Lakin population, and difference in population size and recombination frequency between populations, missing data in different individual plants of two populations, or disease scoring error for some plants. Eight of 10 markers mapped in the current study were previously mapped on the short arm of chromosome 2B with similar marker order and genetic distances among markers (Somers et al. 2004). This confirmed that *Sr40* is on chromosome 2BS as reported previously based on cytogenetic study (Dyck 1992).

Comparing the linkage maps of *Sr40* to the Somers' reference map (Somers et al. 2004), the linkage maps of *Sr40* with 15cM is much shorter than the reference map with 60cM (Somers et al. 2004) . Because the populations that we used came from the cross between a wheat relative and common wheat, there was not two much recombination, and the chromosome should be short. The populations used in the reference map came from the cross between common wheat, and there were more recombination involved. As a result, the chromosome should be long. Consistent maps derived from two mapping populations and a previous study of several different populations indicated that the current maps are appropriate for mapping of *Sr40*.

In a previous study (Tsilo et al. 2008), Xwmc477 and Xgwm319 were mapped near the centromere of 2BS and Xwmc477 and Xgwm319 were about 3 cM away from *Sr40*, thus *Sr40* is likely near centromere of 2BS. In addition, Xbarc230 was located on the bin C-2BS1-0.53 on the deletion bin map of Chinese Spring 2BS (http://wheat.pw.usda.gov/GG2/index.shtml).

Xwmc474 was mapped at 0.6 cM apart from Xbarc230 in RL6088/Lakin population (Figure 2.4), indicating *Sr40* is likely in or very close to the deletion bin C-2BS1-0.53.

2.4.4 Deployment of Sr40

Although *Sr40* has been transferred into wheat backgrounds, it has not been deployed in commercial cultivars to date. Molecular markers are not yet available for marker-assisted selection of the gene in breeding programs. This study reports closely linked markers to *Sr40* and these markers should be effective tools for quick deployment of the gene in wheat cultivars.

A total of seven markers were mapped within 3.5 cM from *Sr40*. Among them, four were mapped in RL6088/ 2174 population and five were mapped in RL6088/ Larkin. They are all proximal to *Sr40*. Among the seven markers, SSR Xwmc474 and Xwmc477 were common in the two maps and are good markers for marker-assisted selection. Marker Xwmc661 was about 6-8 cM distal of Sr40 and not an ideal flanking marker for *Sr40*. Therefore, new markers between Xwmc661 and *Sr40* are still needed to close the gap.

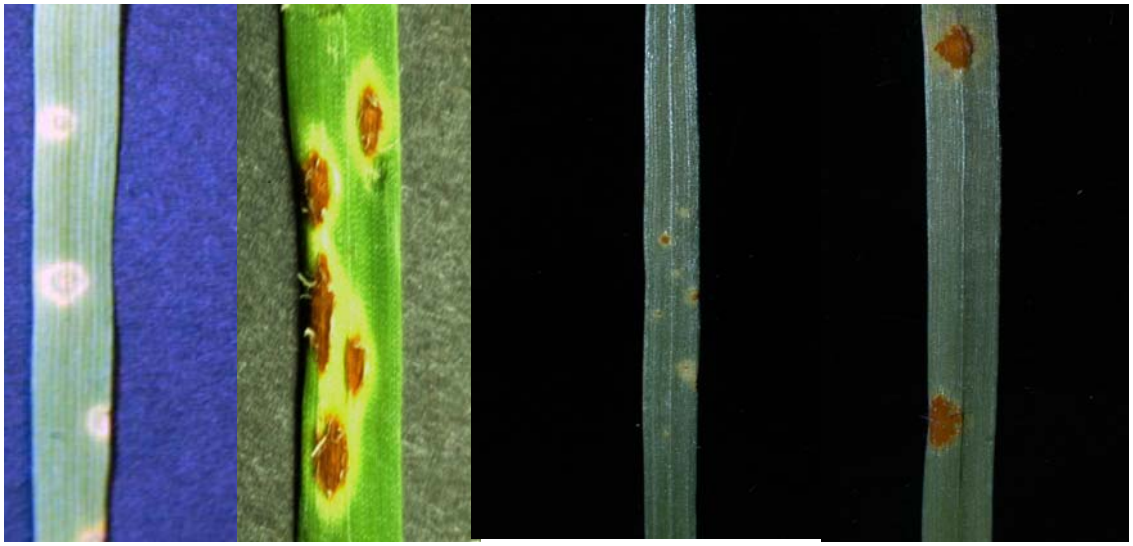
Results from comparative mapping of *Sr40* indicated that *Sr40* was closely linked to the gene cluster of *Lr13*, Lr23, and *Sr36* (Dyck 1992). Xwmc477 and Xgwm319, two diagnostic markers for *Sr36* (Tsilo et al. 2008), were mapped about 3 cM away from *Sr40*. This result disagrees with the result from a previous classic genetics study that genetic distance between *Sr40* and *Sr36* was 21.9cM (Dyck 1992). The discrepancy between two studies is likely due to different materials and methods were used. A previous study used cross between RL6088 having *Sr40* and a line having single gene *Sr36*. *Sr40* and *Sr36* were originally derived from two closely related tetraploid species where one would expect more recombinations (Dyck 1992). The current study used molecular markers to constructed two linkage maps from two populations and the genetic distance among markers in two maps is consistent and also consistent with the consensus map. Therefore this study more likely provided accurate assessment of performance in *Triticum aestivum* and showed that *Sr40* and *Sr36* are closely linked genes in chromosome 2B of wheat.

To enhance the single resistance gene durability, pyramiding genes from different loci is an important strategy in breeding programs. Combination of *Sr31, Sr24* or *Sr25* in a single genetic background has expressed a high level of resistance to stem rust (Tomar and Menon 2001). To control Ug99, test results showed that *Sr2* alone did not show sufficient resistance,

and *Sr2* alone was difficult to evaluate for resistance in both seedling and adult stages. Combining *Sr2* and *Sr23* in the cultivar 'Selkirk' gave moderate resistance against Ug99 (Singh et al. 2006). Combining *Sr40* with *Sr2* or other race specific genes probably is an effective strategy to control stem rust diseases.

Figure 2.1 Stem rust symptoms of *Sr40* after inoculation (a) *Sr40*-resistant parent with stem rust race RKQQ (b) susceptible parent with stem rust race RKQQ (c) *Sr40*-resistant parent with stem rust race TTKS (d) susceptible parent with stem rust race TTKS

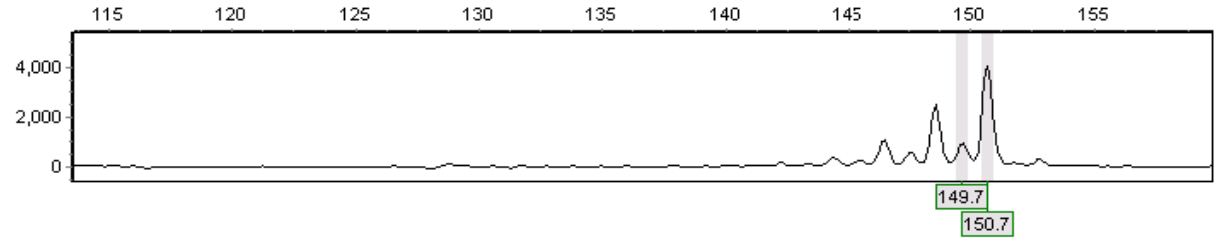
Race RKQQ Race TTKS (Ug99)



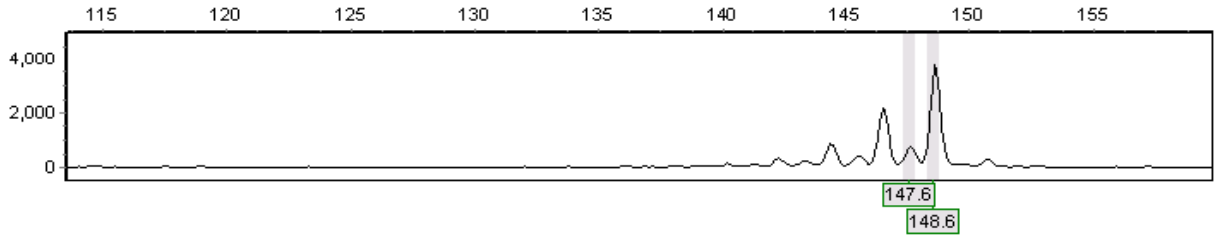
a b c d

Figure 2.2 ABI3730 electropherograms of SSR marker Xwmc474 on chromosome 2B showing polymorphism between RI6088 and Lakin or 2174. , The number on the left is PCR peak intensity, and the bottom number is the PCR peak size.

RL6088



Lakin



2174

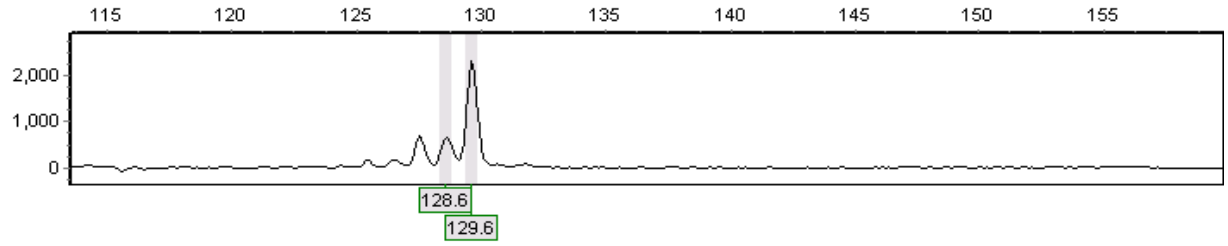


Figure 2.3 Frequency distribution of ITs in two F_2 populations (Rl6088/2174 and RL6088/Lakin) segregating for *Sr40* after inoculation with isolate RKQQ.

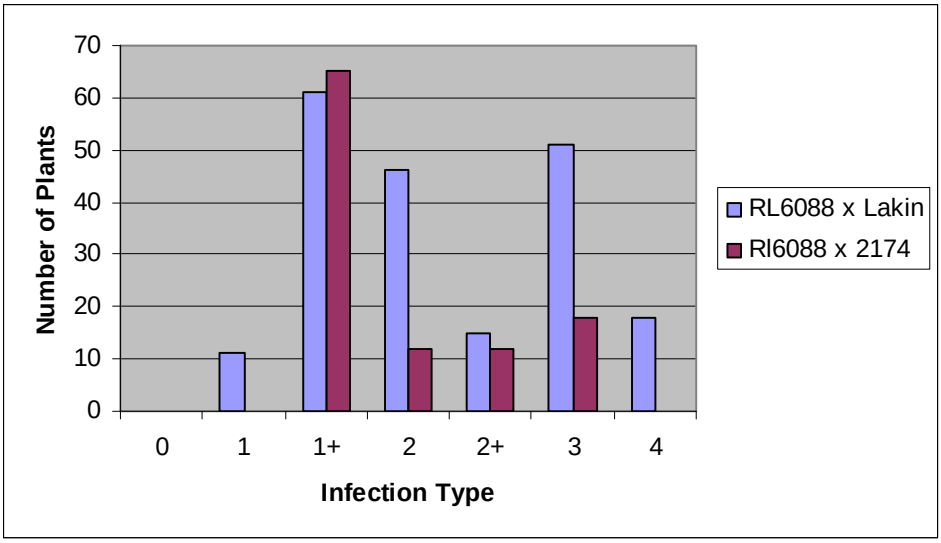


Figure 2.4 Location of *Sr40* on the wheat chromosome 2B map derived from the two mapping populations. Rust scores of F_2 populations were used to locate the *Sr40* on the linkage map. a) A linkage map of *Sr36* (Tsilo et al. 2008) b) Linkage map derived from the RL6088/2174 population, c) Linkage map derived from the RL6088/Lakin population, and d) A reference map of chromosome 2B (Somers et al. 2004). Genetic distance (cM) and marker name are listed on the left and right sites of each linkage map, respectively

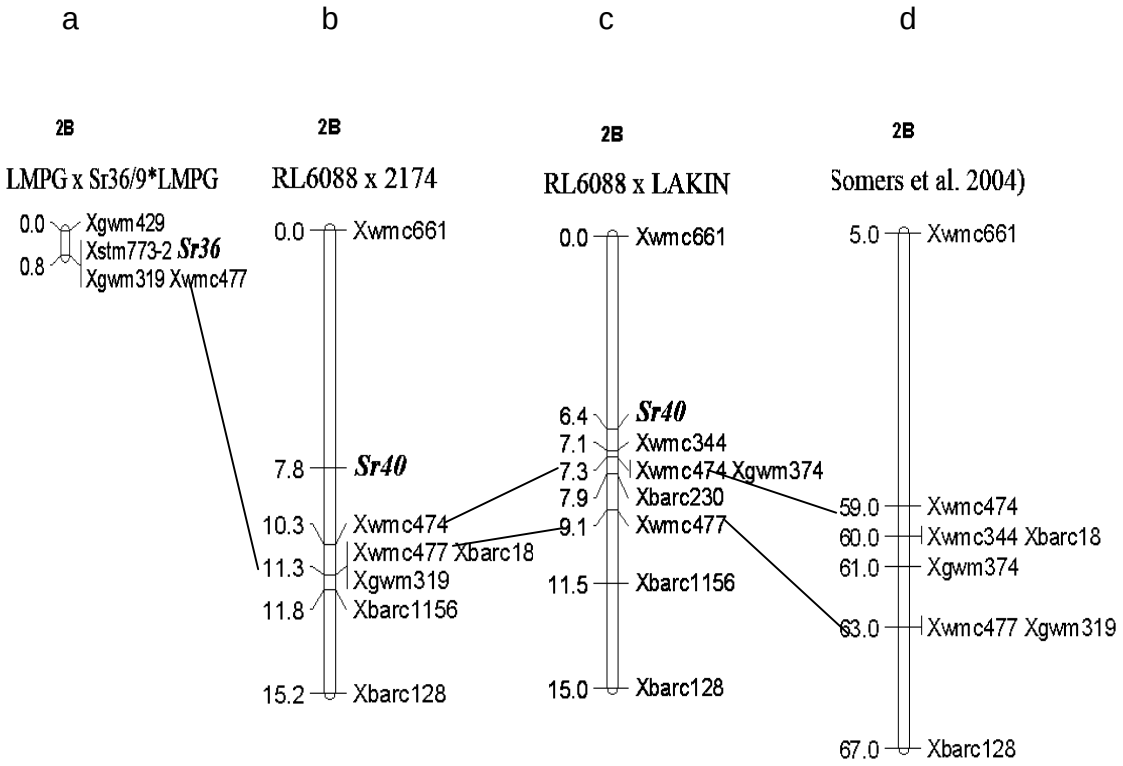


Table 2.1 Segregation of *Sr40* in F_2 and $F_{2:3}$ mapping populations derived RL6088 / 2174 and RL6088 / Lakin inoculated with stem rust isolate RKQQ at seedling stage

Population	Generation	Total #	Observed ratio [1]	Expected ratio	X^2	P-Value[2]
RL6088/2174	F_2	107	77:30	3:1	0.526	0.468
	F_3	107	19:57:31 (R:H:S)	1:2:1	3.15	0.207
RL6088/Lakin	F_2	202	133:69	3:1	9.036	0.0026**
	F_3	88	11:46:31(R:H:S)	1:2:1	9.273	0.0097**

[1] segregation ratio for F_2 populations was resistant plants (R): susceptable (S) and the ratio for F_3 was resistant plants (R): H= Heterozygous: susceptible (S)

[2] **=*P*-Value significant at 1% level.

Table 2.2 Segregation ratios of *Sr40* and SSR marker alleles in F_2 populations

Population	Gene/Marker	Total	RR	HS	SS	Expected Ratio	X^2	P-Value
RI6088/2174	Xwmc661	107	24	56	27	1:2:1	0.402	0.818
	Xwmc474	107	20	57	30	1:2:1	2.327	0.3124
	Xwmc477	107	20	57	30	1:2:1	2.327	0.3124
	Xgwm319	107	20	57	30	1:2:1	2.327	0.3124
	Xbarc18	107	20	57	30	1:2:1	2.327	0.3124
RL6088/Lakin	Xwmc661	88	15	46	27	1:2:1	3.455	0.178
	Xwmc344	88	12	46	30	1:2:1	7.545	0.023*
	Xwmc474	88	12	46	30	1:2:1	7.545	0.023*
	Xgwm374	88	12	46	30	1:2:1	7.545	0.023*
	Xbarc230	88	12	46	30	1:2:1	7.545	0.023*
	Xwmc477	88	12	45	31	1:2:1	8.25	0.0162*

RR = homozygous alleles of resistant parents; HS = heterozygous; SS = homozygous alleles of susceptible parents.

*=*P*-Value significant at 5% level.

Table 2.3 Amplified band size of SSR polymorphic markers between parents

RL6088	RL6088	Lakin	2174
Xwmc661	190	192	214/228
Xwmc474	150	148	130
Xgwm374	234	217	234
Xwmc344	264	248	264
Xbarc230	218	216	218
Xgwm319	192	192	196
Xwmc477	180	182	176
Xbarc18	260	260	236
Xbarc1156	133	127	130
Xbarc128	250	248	244
Xgwm271	171	171	171

CHAPTER 3 - Identification of a stem rust resistance gene in OK01307

3.1 Introduction

As described in the previous chapters, using resistant cultivars is the most effective strategy to control stem rust epidemics. To date, almost 50 stem rust resistance genes have been catalogued, most of them have already been defeated by Ug99 (Jin et al. 2007). Fortunately several stem rust resistance genes from wheat's wild relatives still are effective, but they have not been integrated into elite wheat lines and need further improvement of other agronomic traits before it can be used in commercial production. Stem rust resistance genes in elite breeding lines or commercial cultivars can be quickly transferred into other adapted wheat backgrounds without undesired traits from wild species. After screening wheat elite breeding lines from hard winter wheat regional nurseries, Dr Jin in USDA Cereal Disease Laboratory in St Paul, MN identified one of the breeding lines, OK01307 that showed resistance to many stem rust races including TTKS. However, the *Sr* gene could not be traced back to any known resistance genes from its pedigree. The objective of this study was to characterize *Sr* gene(s) in OK01307 and identify markers for the genes to facilitate marker-assisted selection of the genes.

3.2 Materials and methods

3.2.1 Plant materials and DNA isolation

Two mapping populations were developed from crosses of OK01307 / Chinese Spring and OK01307 / LMPG-6. OK01307 is an Ug99-resistant hard red winter wheat derived from the cross OK94406 / Jagger with excellent baking quality, and Chinese Spring and LMPG-6 are both susceptible to Ug99. Genomic DNA was extracted from young leaf tissue of the three parents and the two F_2 populations using CTAB protocol (see Chapter 2). After tissues were collected, the F_2 seedlings were transplanted into 4" x 4" tora pots and grown in a growth chamber to obtain F_3 seeds in Manhattan KS. All F_2 plants were harvested separately and 60 seeds per F_3 family were shipped to Cereal Rust Lab for rust evaluation.

3.2.2 Stem rust evaluation

Segregating $F_{2:3}$ families of OK01307 / Chinese Spring and OK01307 / LMPG-6 were inoculated with stem rust race TTKS at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN as described in Chapter 2. In brief, urediniospores were taken from a liquid nitrogen tank. After they were heat shocked at 40°C for 10 minutes, they were kept in a rehydration chamber maintained by a KOH solution for 2 to 4 hours with 80% relative humidity (Rowell 1984; Jin et al. 2007). The urediniospores then were mixed with light mineral oil (Soltrol 170) and sprayed onto the fully expanded young leaves of 7-9 days-old wheat seedlings. Inoculated seedling plants were incubated in a dew chamber for 14 hours at 18°C in the dark, and then placed under fluorescent light for extra 3 to 4 hours. The inoculated plants were kept on a greenhouse at 18 ± 2°C with a 16 hour photoperiod (Jin and Singh 2006; Jin et al. 2007). The infection types were estimated according to Stakman et al. (Stakman et al. 1962) at 14 d after inoculation. ITs 0, ;, 1, and 2, or their combinations were considered as resistant phenotypes and ITs 3 to 4 were susceptible phenotypes. A total of 20 seedlings per F_3 family were evaluated for both populations.

3.2.3 Marker analysis

Two DNA bulks contrasting in rust resistance were constructed using the same amounts of genomic DNA from 5 resistant and 5 susceptible F_2 plants. A total of 1300 pairs of simple sequence repeat (SSR) primers were screened for polymorphism between OK01307 and Chinese Spring, and 1000 primers were screened between OK01307 and LMPG-6. Polymorphic markers between the parents were used to screen the bulks from each population, and polymorphic markers between bulks from each population were used to screen the corresponding population.

PCR was amplified using a touch-down program as described in chapter 2. The PCR products were analyzed in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Marker data was scored using GeneMarker® version 1.5 (SoftGenetics LLC, State College, PA). Genetic linkage map was constructed using JoinMap version 3.0 (Van Ooijen and Voorrips 2001) with a LOD (logarithm of odds) threshold at 3.0 and recombination fraction at 0.4. Genetic distance in centiMorgans (cM) was calculated according to Kosambi function (Kosambi 1944).

3.3 Results

In both populations, most resistant F_3 families had ITs of 2- to 2 with a few F_3 families showing ITs of 1 to 1+, while susceptible families had ITs of 3 to 4 in the OK01307 / LMPG-6

population and 4 in the OK01307 / Chinese Spring population. Moreover, in the OK01307 / Chinese Spring population, seven families had ITs of 2+ to 2++.

Segregation of $F_{2:3}$ families to race TTKS in both populations fit well to 1:2:1 monogenic inheritance ratio (Table 3.1), indicating that a single *Sr* gene from OK01307 showed major effect on stem rust resistance in the Chinese Spring and LMPG-6 backgrounds.

In OK01307 / Chinese Spring population, 450 primers were polymorphic between parents and used to screen the bulks. A total of 48 primers were polymorphic between bulks and they are from 14 different chromosomes. Those primers were used to analyze the F_2 population. Based on rust responses of F_3 population, F_2 plants were classified as homozygous resistance, heterozygous and homozygous susceptible genotypes and those data were used for construction of linkage map with marker data. The largest linkage group includes seven markers from chromosome 1B (Figure 3.1). The linkage group covered a 28 cM genetic distance. The targeted *Sr* gene from OK01307 was mapped between marker interval Xbarc312 and Sr24#50 and Sr24#12. Sr24#50 and Sr24#12 were linked tightly at 0.5cM apart. The closed marker for *Sr* gene was Sr24#50 at 3 cM apart. The other flanking marker Xbarc312 was about 4cM to the *Sr* gene.

In the OK01307 / LMPG-6 population, 350 polymorphic primers between parents and used to screen the bulks. Among them, 27 primers from 12 chromosomes were polymorphic between bulks and used to screen the population. Seven markers from chromosome 1B, including Xbarc194, Sr24#50, Sr24#12, Xwmc269, Xgwm18, Xwmc419, Xbarc301 were mapped in the same linkage group harboring the *Sr* gene (Figure 3.1). The linkage group covered a 49cM genetic distance and the *Sr* gene from OK01307 was flanked by markers Xbarc194 and Sr24#50 or Sr24#12 (Figure 3.1). Sr24#50 and Sr24#12 cosegregated with each other and were the most closely linked markers to *Sr* gene at 2.6cM apart. Another flanking marker Xbarc194 was 5.1cM away from the *Sr* gene.

3.4 Discussion

According to the stem rust data from 2005 southern Hard Winter Wheat Regional Nursery, OK01307 showed a high level of resistance to various stem rust races when it was evaluated at both seedling and adult plant stages (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/54402000HardWinterWheatRegionalNurseryPro

gram/05SRPN.pdf). At seedling stage, it had ITs of 0, ; or 1- for 5 races collected from USA. In the field tests, it showed high resistance in most of testing sites under natural infection conditions. ITs of Chinese Spring and LMPG-6 were 4 and 3, respectively, for both seedling and adult infection in 2005 and 2006 in Kenya (Jin et al. 2007). Therefore, parents of each population showed significant contrasts in stem rust resistance.

When $F_{2:3}$ families were inoculated with race TTKS, the ITs of resistant plants were mostly 2- to 2 and susceptible plants were 3 to 4 in the OK01307/Chinese Spring population. In the OK01307/LMPG6 population, resistant plants had ITs of ; 1, 2- and susceptible plants had ITs of 3 and 4. Chi-square test indicated that $F_{2:3}$ family segregation in both populations fit well to the expected 1:2:1 (resistant: heterozygous: susceptible) ratio (Tabel 3.1). Appearance of families with IT ;1 indicated transgressive segregation in OK01307 / LMPG-6 population. The transgressive segregation may be due to a minor gene(s) in LMPG-6 that interacted with the major gene in OK01307 to enhance the resistance in these families. In addition, seven families had ITs of 2+ to 2++, which are more resistant than susceptible family but more susceptible than that for resistant genotypes. Appearance of the unexpected genotypes provides another line of evidence to support that LMPG-6 may have a minor gene for stem rust resistance and combination of rust resistance genes among different sources can be a useful tool to enhance stem rust resistance in breeding systems (Jin et al. 2008).

A total 10 markers were mapped on the two linkage groups derived from the two populations. Seven markers were mapped in each population with four common markers, Sr24#12, Sr24#50, Xgwm18 and Xwmc419, in both populations. The common markers showed the same order in the two linkage maps from the two populations (Figure 3.1), although genetic distances among markers were slight different between the two maps. The linkage map from OK01307 / LMPG-6 population was longer than that from OK01307/Chinese Spring population. This may be due to the difference in recombination frequency between two backgrounds. Resistant parent OK01307 and susceptible parent LMPG-6 are U. S. cultivars, and another susceptible parent Chinese Spring is an Asian landrace. Therefore, OK01307 and Chinese Spring are more distant than that between OK01307 and LMPG-6, and fewer recombinations are expected between OK01307 and Chinese Spring than that between OK01307 and LMPG-6. In addition, missing marker data in different plants of two populations may also contribute to the genetic distance difference between two linkage groups. Compared the two linkage maps to the

previously published consensus map (Somers et al. 2004) (Figure 3.1), orders and genetic distances among markers were similar among the maps, indicating that the current maps are appropriate for mapping of *Sr* gene in Ok01307.

The *Sr* gene in OK01307 was mapped in the similar position in both populations. Two STS markers for *Sr24*, Sr24#12 and Sr24#50, were the most closely linked markers to the target genes in both populations (Mago et al. 2005a). The two *Sr24* markers are only 2.5 and 2.9 cM, respectively, away from the *Sr* gene in the two maps. Therefore, *Sr24* is a very closely linked gene to the Sr gene in OK01307. However, since both Sr24#50 and Sr24#12 are STS markers developed from AFLP and not gene markers per se, it is possible that the Sr gene in OK01037may be the same gene as *Sr24*. In either case, the markers for *Sr24* are useful markers for selection of the *Sr* gene in OK01307. In addition, flanking markers for the *Sr* gene in OK01307 are identified in both populations. Xbarc312 and Xbarc194 were mapped at 4 and 5cM distal to the targeted *Sr* gene in populations OK01307/Chinese Spring and OK01307/LMPG-6 respectively. Therefore, Sr24#12, Sr24#50, Xbarc312, and Xbarc194 can be used as flanking markers for marker-assisted selection of *Sr* gene in OK01307.

Figure 3.1 Linkage map showing the putative location of *Sr* gene in OK01307 from both populations: a) the linkage group containing the *Sr* gene derived from OK01307 / Chinese Spring, b) the linkage group containing the *Sr* gene derived from OK01307 / LMPG-6, c) a reference map of chromosome 1B (Somers et al. 2004). Genetic distances (cM) and marker names are listed on the left and right sites of each linkage map, respectively

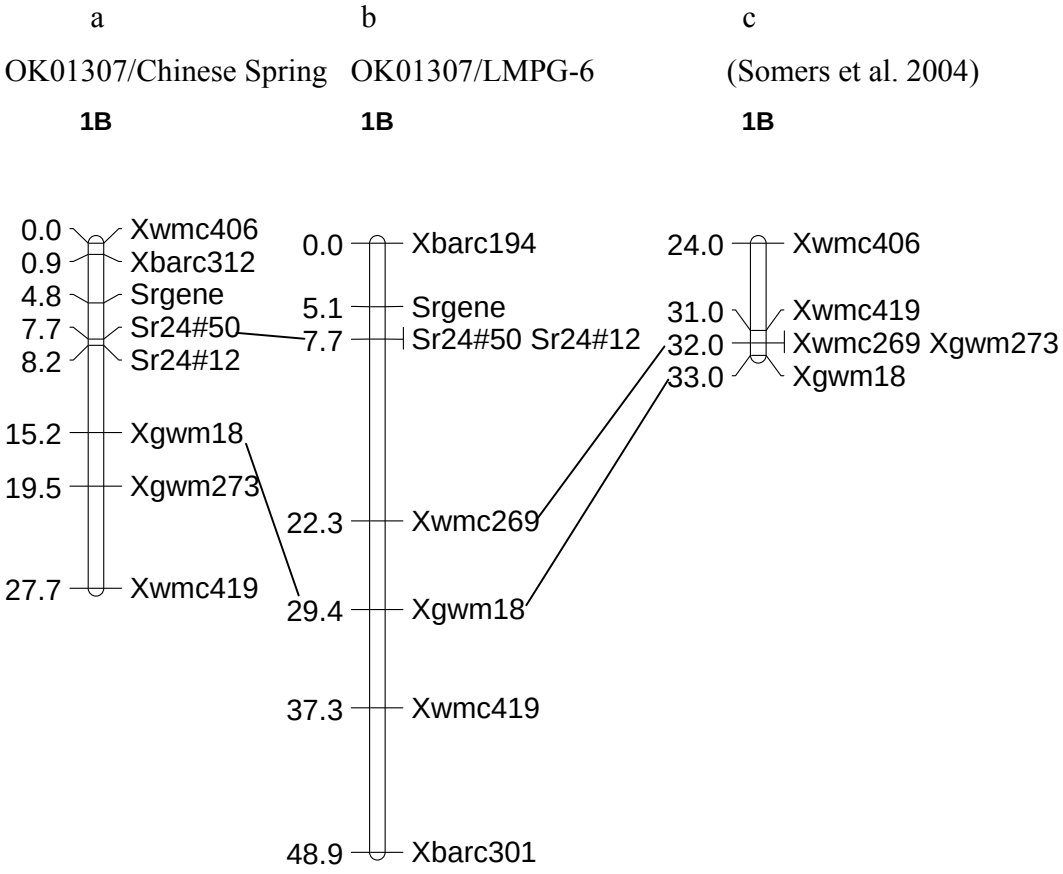


Table 3.1 Segregation of *Sr* gene evaluated at seedling stage by inoculating stem rust isolates TTKS (2006) in both populations

Population	Generation	Total #	Observed ratio [1]	Expected ratio	X^2	P-Value
OK01307 / Chinese Spring	F_3	120	35:62:23(R:H:S)	1:2:1(R:H:S)	2.53	0.28
OK01307 / LMPG-6	F_3	118	34:49:35(R:H:S)	1:2:1(R:H:S)	3.41	0.18

[1] Ratio of resistant plants (R): heterozygous plant (H): susceptible (S).

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