BROADENING THE WHEAT GENE POOL FOR STEM RUST RESISTANCE THROUGH GENOMIC-ASSISTED INTROGRESSIONS FROM AEGILOPS TAUSCHII

by

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B.S., University of Wisconsin Platteville, 2006
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AN ABSTRACT OF A DISSERTATION

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Abstract

The diploid D genome species, *Aegilops tauschii* Coss. (2n=2x=14,DD) has provided numerous genes for resistance to diseases and insect pests that attack bread wheat (*Triticum aestivum* L. (2n=6x=42, AABBDD). Wheat production is currently threatened by broadly virulent races of the 'Ug99' lineage of wheat stem rust caused by the fungus, *Puccinia graminis* f.sp. *tritici* Pers. & Eriks. Screening of a large set of *Ae. tauschii* germplasm for resistance to TTKSK (Ug99) identified potentially novel sources of resistance. To expedite utilization of TTKSK resistance from *Ae. tauschii*, a direct hybridization approach was established that integrates gene transfer, mapping and introgression into one process. Direct crossing of *Ae. tauschii* accessions with an elite wheat breeding was used to initiate transfer of resistance. Genetic mapping of resistance was accomplished during gene transfer through development of BC_2 mapping populations. Bulked segregant analysis of BC_2F_1 genotypes at 70 SSR loci across the D genome identified the chromosome locations of stem rust resistance genes and facilitated genetic mapping. Using this approach, TTKSK resistance from CDL4424 and TA1662 was mapped on chromosome arm 1DS flanked distally by *Xwmc432* and proximally by *Xwmc222* at 4.4 cM, TA10187 on 6DS linked to *Xcfd49* at 1.9 cM and TA10171 on 7DS linked to *Xwmc827* at 0.9 cM. TTKSK resistance from additional *Ae. tauschii* accessions CDL4366, TA1615, TA1642, TA1693 and TA1718 has been recovered in segregating populations but await mapping. Altogether, TTKSK resistance from eight *Ae. tauschii* accessions has been introgressed to a hard winter wheat genetic background. Three new stem resistance genes have been tagged with molecular markers for marker assisted breeding and will provide valuable material for stem rust resistance breeding and gene pyramids for effective control of stem rust.

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Table of Contents

List of Figures

List of Tables

List of Tables

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1 - Literature Review

Significance of wheat cultivation and origins of wheat

Significance of wheat cultivation

The Fertile Crescent region of Southwest Asia is the center of origin for many modern cultivated crop species including diploid Einkorn wheat, *Triticum monococcum* (*T. monococcum* ssp. *monococcum* L.) and tetraploid Emmer wheat, *T. dicoccum* (*T. turgidum* ssp. *dicoccum* Schübl.) (Zohary and Hopf, 2000). Before deliberate sowing and agricultural practices, humans were actively harvesting wild grain crops (Cox, 2009). The Fertile Crescent region encompasses modern-day Israel, Jordan, Lebanon and Western Syria, Southeast Turkey and reaches along the Tigris and Euphrates rivers into Iraq and Western Iran, early human societies began the first agricultural practices (Gopher et al., 2002). Near the end of the Pleistocene era the hunter-gatherer behaviors of human societies began to change resulting from climate change and increasing population densities (Diamond, 2002). The advent of agriculture lent to more sedentary populations and permanent settlements and an abundant food supply freed individuals from food acquisition and enabled the development of specialized trades, and the evolution of culture (Diamond, 1997).

In the modern era, wheat is cultivated on every continent of the globe except Antarctica and provides about 20% of calories consumed by the world population (Porter et al., 2007). In 2011 world wheat production reached the highest level of production in human history at 694.6 million tons. Global wheat consumption kept pace at 694.4 million tons (World wheat supply and disappearance, ERS, 2012). Current wheat yield increases are at 1% per year (Graybosch and Peterson, 2010). With the human population continuing to grow, the demand for calories and quality protein will increase while the availability of arable cropland continues to decrease, requiring the cultivation of wheat in more marginal land (Tester and Langridge, 2010). Increasing modernization and industrialization on a global scale has changed the diets of developing countries, leading to increased consumption of grains and an increased global demand (Brown, 2004).

The origin of cultivated wheat

Diploid and tetraploid wheat were the first to be domesticated and cultivated by early agriculturalists. The diploid A genome species, *T. boeoticum*, was domesticated to the cultivated form, *T. monococcum* (2n=2x=14, A^mA^m), in the mountains of Southeast Turkey (Heun et al., 1997). This species is presently under limited cultivation in the modern world.

Wild tetraploid wheat *T. dicoccoides* was domesticated to the cultivated form, *T. turgidum* ssp *dicoccum* (2n=4x=28, AABB) (Ozkan et al., 2002). Tetraploid wheat came into being less than 0.5 million years ago (Huang et al., 2002) from the hybridization of the A genome species, *T. urartu* (2n=2x=14, AA) and an unidentified B genome species related to the extant S genome species, *Aegilops speltiodes* (2n=2x=14)(Dvorák and Zhang, 1990). In this hybridization, *T. urartu* has been identified as the male pollen donor and the B genome species as the female (Dvořák et al., 1993, Kilian et al., 2007).

During the course of cultivation of domesticated tetraploid wheat, crops were grown in close proximity to wild and weedy species. It was under this scenario that tetraploid wheat, *T. dicoccum* (AABB) hybridized with the diploid species *Aegilops tauschii* (2n=2x=14, DD) forming hexaploid wheat, *T. aestivum* (2n=6x=42, AABBDD) (Kihara, 1944a; McFadden and Sears, 1946). The addition of the D-genome from *Ae. tauschii* to the AB genomes of tetraploid wheat ~8000 years ago gave hexaploid wheat enhanced geographic and environmental adaptations and enhanced yield capacities leading wheat to its place as a modern staple crop (Zohary and Hopf, 1993). There were, however, limited hybridization events between *Ae. tauschii* and tetraploid wheat with possibly as few as two hybridization events leading to an extreme genetic bottleneck and limited genetic variation in the D-genome of cultivated wheat (Talbert et al., 1998). Further, intense selection during modern breeding has restricted genetic diversity.

Genetic transfers from the D genome of *Aegilops tauschii* to wheat

The diploid D genome donor species, *Ae. tauschii,* has been used extensively for the improvement of modern wheat cultivars. Since *Ae. tauschii* the D genome donor of wheat, its chromosomes are homologous to wheat D genome chromosomes. An array of genes for important traits critical to wheat production have been transferred from *Ae. tauschii*. Normal

pairing and recombination take place between D genome chromosomes of wheat and *Ae. tauschii* (Kihara, 1944b; McFadden and Sears, 1946) allowing for coupling linkages between alleles with a deleterious performance on agronomic performance and target alleles to be broken during normal meiosis. These recombinants with improved plant type can be identified through phenotypic selection in breeding.

Two approaches have been used to transfer alleles from the D-genome of *Ae. tauschii*: synthetic hexaploids and direct crossing between diploids and hexaploid wheat (Gill and Raupp, 1987). As many improved wheat cultivars have been developed in the United States and internationally using germplasm derived from direct crossing and synthetic wheat, it remains of great interest to know the particular genomic regions from the D-genome of *Ae. tauschii* contributing to superior agronomic performance. Most synthetic material was derived by artificial hybridization of durum wheat cultivars (AABB) with good yield potential and *Ae. tauschii* (DD). The contribution of the D-genome over the elite A and B genomes in synthetic derived breeding lines remains an important question in the development of new wheat germplasm.

Direct crossing between hexaploid wheat and Aegilops tauschii

The ability to directly transfer alleles to elite hexaploid wheat from *Ae. tauschii* was first demonstrated by Gill and Raupp (1987). In this study diploid *Ae. tauschii* (n=7, D) accessions were used as males to pollinate hexaploid wheat (n=21, ABD). The resulting ABDD (2n=28) embryos were rescued and F_1 plants were generated on artificial media. Alternatively, *Ae. tauschii* can be used as a female and *T. aestivum* as a male, resulting in a higher frequency of caryopsis formation (Sehgal et al., 2010).

Hybrids between *Ae. tauschii* and hexaploid wheat are male sterile. To restore fertility and recover target alleles, hybrids are first backcrossed as females to hexaploid wheat. Partially fertile BC_1F_1 plants display aneuploidy and are backcrossed again to hexaploid wheat as males generating a high frequency of euploid (n=21) gametes by certation. Target alleles can be recovered in BC_2 plants with restored chromosome number and fertility.

Endosperm formation is an epigentically controlled phenomenon in plants with parental imprinting determining allelic expression (Köhler and Makarevich, 2006). Crosses between diploid and hexaploid parents display altered allelic expression resulting in aberrant endosperm formation or abortion (Tiwari et al., 2010). The failure to develop functional endosperm after fertilization maintains a hybridization barrier between *Ae. tauschii* and *T. aestivum*.

Although the D-genome of *Ae. tauschii* is the most accessible source of novel genetic variation for wheat, ploidy level and wild growth habit have limited the evaluation of *Ae. tauschii* directly for quantitative agronomic traits important to sustainable wheat production. However, insightful studies to characterize agronomic effects of the *Ae. tauschii* D genome have been done using lines derived from direct crosses. (Fritz et al., 1995a) characterized genomic introgressions from *Ae. tauschii* accession TA2567 and Fritz et al., (1995b) characterized the phenotypic effects of D-genome introgressions. Studies by Cox et al. (1995a,b) and Murphy et al., (1997) have evaluated phenotypic effects on yield and quality traits from multiple *Ae. tauschii* D-genome sources in different hexaploid wheat backgrounds in a germplasm development context. The majority of characterized *Ae. tauschii* genes transferred to hexaploid wheat confer resistance to insects or fungal diseases and can be easily selected based on phenotype in early generations.

Advanced Backcross QTL Analysis

Hexaploid wheat can be reconstituted in the hybridization of a tetraploid wheat carrying the AB genomes with the D genome of *Ae. tauschii* (Kihara et al., 1957a). Doubling the chromosome number of ABD amphiploids yields a fertile diploid synthetic wheat that is readily crossed with *T. aestivum*.

To identify QTL for yield and other agronomic traits in synthetic hexaploid wheat, the technique of advanced backcross QTL analysis has been implemented. Huang et al. (2003b) utilized this approach using 72 BC_2F_3 derived familes, and 210 SSR markers to evaluate grain yield, heading date, tiller number and plant height. QTL were identified in A, B and D-genomes with a QTL in the D-genome explaining 6% of the variation for grain yield. Narasimhamoorthy et al. (2006) used 190 backcross derived lines for analysis of agronomic traits and virus resistance with alleles contributed from A, B and D-genomes of a synthetic wheat using 154 SSR markers. Agronomic

and end use traits as well as leaf rust resistance were evaluated by Kunert et al. (2007) and Naz et al. (2008) utilizing the same synthetic genotype and identified QTL across the A, B and D genomes. Pestsova et al. (2006) backcrossed D-genome whole chromosome substitution lines to develop 84, D-genome-specific introgression lines in a Chinese Spring background. This method isolated the effects of a single *Ae. tauschii* accession. Using 88 SSR markers, QTLs were identified with significant effects on flowering time, plant height, spike length, spikelet number, fertility, and grain weight per spike. Remarkably, several introgression lines were found to have 33% higher spikelet number and 42% higher grain weight per ear than the recurrent parent. These studies indicate that valuable allelic variation can be transferred from the D-genome and evaluated in hexaploid wheat. However, improvements can be made in population size, structure and marker density to increase mapping resolution of QTL and maximize the D-genome allelic diversity sampled.

Resistance to wheat rust pathogens derived from *Ae. tauschii*

The most widely utilized genes from the D genome confer resistance to an array of fungal pathogens and insect pests of wheat. Direct hybridization and primary synthetics have been used to transfer resistance genes from *Ae. tauschii* to wheat. Some genes have been used extensively in wheat breeding while most have not been widely deployed.

Stem Rust

From previous work, three genes for resistance to wheat stem rust, caused by the basidiomycete fungus *Puccinia graminis* f.sp. *tritici* (*Pgt*), have been transferred from the D genome of *Ae. tauschii* to hexaploid wheat including *Sr33*, *Sr45* and *Sr46*. Kerber and Dyck (1979) developed synthetic hexaploids with multiple *Ae. tauschi* accessions and the extracted tetraploid of the spring wheat cultivar Canthatch, known as Tetracanthatch (Kerber, 1964). In this study, the *Ae. tauschii* accessions RL5289 (TA1600), carrying *Sr33* and RL5288 (TA1599), carrying *Sr45*, were hybridized with Tetracanthatch to generate synthetic hexaploids carrying the entire D genomes of these accessions. Results of studies on *Sr46* remain unpublished.

Sr33 is found across geographical ranges within the *Ae. tauschii* center of diversity and across the *strangulata* and *tauschii* subspecies of *Ae. tauschii* (Innes and Kerber, 1994a)(Olson et al.,

2012) and confers resistance to all known races of stem rust (Matt Rouse, personal communication). The chromosome location of *Sr33* was determined to be 1DS, located distally and linked closely in repulsion with *Lr21* (Jones et al., 1991). *Sr33* has been identified in multiple accessions of *Ae. tauschii* subspecies *strangulata* from diverse geographies in Azerbaijan, Iran and Turkmenistan (Innes and Kerber, 1994a). *Sr33* confers resistance to all known races of *Pgt* (Rouse et al., 2011) so selection pressures posed by stem rust populations in locations with climatic conditions favoring epidemics could maintain this gene at high frequency in natural populations of *Ae. tauschii.* Olson et al. (2012) transferred an additional allele of *Sr33* from *Ae. tauschii* ssp. *tauschii* accession CDL4424 from Turkmenistan by direct hyridization. This demonstrates *Sr33* is present across divergent subspecies of *Ae. tauschii* and suggests that *Sr33* arose early within *Ae. tauschii* and, perhaps due to it's broad spectrum resistance, has persisted in the gene pool.

Both *Sr45* and *Sr46* confer race-specific resistance to *Pgt* (Rouse et al., 2011). The chromosome location of *Sr45* is 1DS (Marais et al., 1998) at a locus more proximal to *Sr33*. Additionally, the accession carrying *Sr45* also carries the wheat leaf rust resistance gene *Lr21* (Kerber and Dyck, 1979). 2DS is the chromosome location of *Sr46* (Lagudah ES, personal communication). *Sr45* has been transferred from the *Ae. tauschii* ssp. *tauschii* accession RL5288 (TA1599) (Kerber and Dyck, 1979), collected from North Western Iran (Jon Raupp, personal communication) and *Sr46* is derived from the *Ae. tauschii* ssp. *tauschii* accession AUS18913 (TA1708)(Rouse et al., 2011). The frequency of *Sr45* and *Sr46* in collections of *Ae. tauschii* is not readily known due to the race-specificity of resistance and the presence of other stem rust resistance genes in genetic backgrounds carrying *Sr45* and *Sr46*.

The three *Ae. tauschii*-derived stem rust resistance genes, *Sr33*, *Sr45* and *Sr46*, have become of great contemporary interest to wheat geneticists and stem rust pathologists as all three genes confer resistance to the *Pgt* race TTKSK (Ug99) (Rouse et al., 2011) that is virulent to most of the stem rust resistance genes derived from cultivated wheat (Singh et al., 2011a). Perhaps due to their presence in synthetics derived from extracted tetraploids carrying complex stem rust resistance and the limited contemporary impact of wheat stem rust, these genes have not been widely utilized in breeding for stem rust resistance.

Leaf Rust

Wheat leaf rust caused by the basidiomycete fungus *Puccinia triticina* Eriks. causes disease in wheat producing regions worldwide (Roelfs et al., 1992). Rapidly evolving leaf rust populations can quickly develop virulence to major gene resistance deployed over a large geographical scale leading to the classical boom and bust cycle (Eversmeyer and Kramer, 2000). The D genome of *Ae. tauschii* has been a valuable source of resistance to leaf rust (Cox, 1998; Gill et al., 2006). To date, six named *Lr* genes have been transferred from the D genome of *Ae. tauschii* for use in leaf rust resistance breeding efforts (McIntosh et al., 1995). Eight *Ae. tauschii*-derived *Lr* genes have been named, however, *Lr40* was found to be allelic to *Lr21* (Huang and Gill, 2001) and *Lr41* was found allelic to *Lr39* (Singh et al., 2004) reducing the number of recognized genes to six.

The first described *Lr* gene derived from *Ae. tauschii* is *Lr21* transferred by Rowland and Kerber (1974) from the *Ae. tauschii* accession, RL5289 which is the same source as *Sr45* (Kerber and Dyck, 1979). This gene located in the distal region of chromosome 1DS (Jones et al., 1990) has been positionally cloned and found to encode a unique NBS-LRR (nucleotide-binding site leucine rich repeat) protein (Huang et al., 2003a). *Lr21* is also unique in that the resistance gene represents the combination of two susceptible haplotypes that can be recombined to re-produce a the functional *Lr21* resistance gene (Huang et al., 2009) demonstrating that *Lr21* is a recombined allele of recent origin.

The *Lr22* locus is comprised of two alleles with *Lr22a* from the *Ae. tauschii* accession RL5271 (Dyck and Kerber, 1970) and *Lr22b* of common wheat origin (Dyck, 1979). Both *Lr22* alleles confer adult plant resistance, however, they differ in their race specificity and spectrum of resistance. No known races of wheat leaf rust are virulent on *Lr22a* and this gene has been utilized in leaf rust resistance breeding efforts (Hiebert et al., 2007). *Lr22b* is effective against only a few races of leaf rust and has limited utility in resistance breeding efforts (McIntosh et al., 1995). In fact, *Lr22b* was identified in the Thatcher background which is the susceptible recurrent parent used to develop *Lr* gene isolines (Dyck, 1979).

Kerber (1987) transferred *Lr32* from *Ae. tauschii* by way of a synthetic hexaploid between the *Ae. tauschii* accession RL5497 and the extracted tetraploid Tetracanthatch. The chromosome location of *Lr32* was determined to be 3DS (Kerber, 1988). *Lr32* is broadly effective against most racs of leaf rust although several virulent races have been identified in Bulgaria, Israel and Turkey (Huerta-Espino, 1992) and more recently in South Africa (Pretorius and Bender, 2010). As with *Sr33*, *Sr45* and *Sr46*, *Lr32* has not been deployed in wheat breeding programs (McIntosh et al., 1995) perhaps due to its presence in an agronomically poor synthetic-derived genetic background.

The leaf rust resistance gene *Lr42* was transferred from the *Ae. tauschii* accession TA2450 by Cox et al. (1994) and maps to a distal region on chromosome 1DS (Sun et al., 2010). This gene has been widely utilized in leaf rust resistance breeding efforts in the United States (Martin et al., 2003) and internationally with *Lr42* present in the pedigrees of high yielding, rust resistant lines from CIMMYT, including the Kenyan cultivar Robin (Singh et al., 2011a).

Stripe Rust

A single gene conferring resistance to the wheat stripe rust pathogen *Puccinia striiformis* f.sp. *tritici* has been derived from the D genome of *Ae. tauschii*. The gene *Yr28* has been mapped to chromosome 4DS and is derived form the *Ae. tauschii* accession WX219 (TA2465) (Singh et al., 2000). The D genome of this *Ae. tauschii* accession is represented in the synthetic hexaploid wheat parent of the ITMI reference mapping population (http://wheat.pw.usda.gov/GG2/index.shtml) from the cross Synthetic W7984 (Altar84/*Aegilops tauschii* CIGM86.940) × Opata M85. *Yr28* has not been widely used in breeding for stripe rust resistance.

The Wheat Stem Rust Pathogen

Life Cycle

The wheat stem rust fungus *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. E. Henn. (*Pgt*) has a complex life cycle consisting of both sexual and asexual reproductive cycles, different sexual and asexual hosts, multiple spore stages and nuclear conditions. *Pgt* is a heteroecious fungus requiring two hosts to complete the entire life cycle. Wheat stem rust is a biotrophic fungus and

does not exist in nature apart from the primary host, wheat, or the secondary host, common barberry (*Berberis vulgaris* L.) (Leonard and Szabo, 2005).

Teliospores form on wheat stems infected with *Pgt* late in the growing season. These abiotic stress-tolerant spores are capable of overwintering on infected straw. Upon formation of the two cells of teliospores, two haploid nuclei (n+n) are present in each cell. These nuclei undergo karyogamy and form a single nucleus (2n). Meiosis takes place after karyogamy but is arrested until germination the following spring season (Boehm et al., 1992).

Germination of teliospores is synchronized with new growth of the alternate host, barberry (*Berberis vulgaris*). Each teliospore produces a basidium from which four basidiospores are produced. Each basidiospore is haploid (n) and contains one of the four products of meiosis from the fusion of the haploid nuclei. The meiotic products are of different mating types, two basidiospores will be + and two will be -. A mitotic division in the basidiospores produce two haploid nuclei in each basidiospore. The basidiospores are products of recombination and it is through meiosis in teliospores that novel variation in *Pgt* can be produced. The basidiospores ejected from the basidia infect adaxial surface of Barberry leaves (Roelfs, 1985).

The hyphae of basidiospores grow within the leaf mesophyll as haploid hyphae and produce a pycnium on the adaxial leaf surface. Haploid pycniospores (n) of + and – mating types are exuded from the top of the pycnium. Serving as male gametes, pycniospores are brought into contact with haploid female (n), flexuous hyphae of the opposite mating type that extrude from the top of the pycnium (Anikster et al., 1999). A dikaryon (n+n) consisting of two haploid nuclei is formed and the resutling hyphae grow throughout the leaf mesophyll to produce an aecium on the abaxial leaf surface. From the aecium, single celled, dikaryotic (n+n) aeciospores are produced which can then infect the wheat host.

The primary infection of wheat is by aeciospores that infect and produce hyphae within the host. These hyphae then produce uredinia that yield dikaryotic urediniospores (n+n) that represent the asexual stage of the life cycle. Urediniospores re-infect the host during the growing season and cause the principle damage to wheat plants resulting in yield losses. Upon maturity of the host,

teliospores (n+n) are produced which will overwinter and begin the cycle the following growing season.

Physiologic races and variation

Puccinia graminis f.sp. *triticii* is divided into physiologic races based on virulence and avirulence specificities of isolates based on a differential set of stem rust resistance gens (Roelfs and Martens, 1987). The differentials consist of five sets of genes comprised of four genes per set. To discriminate races, a letter code for each set is assigned. The specific pattern of high and low infection type on genes within each set determines the letter code with a higher letter indicating virulence to more genes within the set.

Host genotype and spacial scale can dramatically affect the population structure of *Pgt.* The cultivation of a wheat variety carrying a single major gene for resistance to stem rust places intense directional selection on existing avirulent *Pgt* genotypes (Van der Plank, 1968). In the case of major genes encoding for NB-LRR type receptors that function in the detection of specific *Pgt* effector activity, the selective pressure on *Pgt* populations is for the loss of detection of effector activity which can simply be achieved through allelic changes by mutation or recombination during the sexual stage (McDonald and Linde, 2002). As only *Pgt* genotypes carrying the loss of avirulence are virulent on the host carrying the major gene resistance, these genotypes increase in frequency. The result of this virulence shift is widespread disease on the newly-susceptible and widely cultivated variety. As the frequency of virulence increases in *Pgt* populations and epidemics worsen, the susceptible variety is planted on fewer acres, usually replaced by a new variety with a different source of major gene resistance, thereby perpetuating the boom and bust cycle (Sun and Yang, 1999).

Virulence shifts in *Pgt* populations take place during both sexual and asexual cycles. Sexual recombination allows the opportunity to bring virulence to multiple resistance genes together in a single genotype. The absence of the sexual cycle removes the possibility of sexual recombination and most common genotypes of *Pgt* have lost the ability to produce teliospores (Zambino et al., 2000). The barberry eradication program undertaken in the United States in the 20th century served to limit the diversity of *Pgt* populations by removing the impact of sexual reproduction

and limiting evolutionary potential. The greatest diversity in aeciospore and urediniospore collections from Minnesota are from prior to large scale Barberry eradication efforts (Peterson et al., 2005). However, barberry and Mahonia sp. continue to provide a source of sexual variation in *Pgt* races in regions west of the Rock Mountains (Yue Jin, personal communication).

Historical and contemporary significance

Wheat stem rust is the most destructive of all the wheat rust pathogens due to its ability to decimate a healthy wheat crop late in the season and is capable of causing up to 100% yield loss from lodging and disruption water and photosynthate movement (Roelfs et al., 1992). For as long as agrarian societies have cultivated wheat, stem rust has been a threat to food security. The Bible refers to rust epidemics as punishments on the Israelites from God for their sins (Chester, 1946). Around 700A.D., the Roman festival of Robigalia was celebrated annually to pacify the rust god Rubigus to ensure a healthy crop (Chester, 1946; Peterson, 2001).

During the last century, stem rust has caused major epidemics in all wheat producing countries. In the 1940's and 1950's, China experienced major stem rust epidemics due to higher than average temperatures and rainfall leading to ideal conditions for the pathogen that flourishes under high temperature and moisture (Roelfs, 1977). Severe epidemics took place in North America in the first half of the 20th century (Roelfs and Bushnell, 1985; Hodson, 2011). In North America, the spring wheat growing regions are most affected by stem rust epidemics causing yield losses up to 50% (Leonard, 2001).

Emergence of the Ug99 lineage of wheat stem rust races

Stem rust has again become a major threat to global wheat production and food security with the emergence of the Ug99 lineage stem rust races that are virulent to most of the stem rust resistance genes deployed in wheat cultivars worldwide (Singh et al., 2011a). The first race of the Ug99 lineage is designated race TTKSK based on the North American Nomenclature developed by Roelfs and Martens (1987) and has resulted in the addition of a fifth differential set to the nomenclature system (Jin et al., 2008). This race was first collected in Uganda in 1999 and became significant due to virulence on the gene *Sr31* that is present on a 1BL·1RS translocation derived from 'Petkus' rye (*Secale cereale* L.)(Pretorius et al., 2000). *Sr31* is a critical source of

stem rust resistance in spring wheat lines under cultivation in developing countries (Singh et al., 2008) and is present in US wheat breeding lines and cultivars (Olson et al., 2010a). The combination of *Sr31* virulence in combination to the majority of stem rust derived from cultivated wheat makes *Pgt* race TTKSK and its derivatives a major threat to wheat crops.

Stem rust races of the Ug99 lineage are a highly diverse group. Genotypically Ug99 races of stem rust form a distinct lineage apart from less virulent contemporary races of stem rust (Visser et al., 2009; Visser et al., 2011). Furthermore, race TTKSK has rapidly developed virulence to additional *Sr* genes that are widely deployed resistance sources. The genes *Sr24* and *Sr36* are both widely deployed in hard winter and soft winter wheat, respectively, in US wheat breeding lines and cultivars (Olson et al., 2010). TTKSK quickly combined virulence to *Sr24* with *Sr31* virulence to produce race TTKST (Jin et al., 2008) and spreading beyond East Africa to wheat growing regions of South Africa (Pretorius et al., 2010). TTKSK gained additional virulence combining *Sr36* and *Sr31* virulence to produce race TTTSK (Jin et al., 2009). The development of expanded virulence is likely accelerated by the presence of the sexual host, common barberry (*Berberis vulgaris* L.), in Eastern Africa (Yue Jin, personal communication).

The highlands of east Africa have historically been a hotspot for the development of highly virulent races of *Puccinia* sp. (Saari and Prescott, 1985). Factors contributing to the development of highly virulent races of wheat rust include the year-round cultivation of susceptible wheat genotypes creating ideal conditions for disease development. The *Yr9*-virulent race of wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) that has reached global dispersal and has been a major challenge to wheat production developed in the East African highlands and spread across the CWANA region to northern India (Singh et al., 2004b). Stem rust races of the Ug99 lineage were first identified in east Africa in 1999 and have since expanded south to South Africa, north to Sudan and to the northeast into Yemen with the farthest expansion of Ug99 into northern Iran (Nazari et al., 2009; Pretorius et al., 2010; Singh et al., 2011a; Mukoyi et al., 2011).

Approximately 1 billion people reside in the predicted path of Ug99. Many of the people present in this region are in countries that consume all the wheat produced within their borders.

International attention has been given to Ug99 and large-scale efforts are underway supporting the development of resistant cultivars, seed increase and delivery of resistant varieties to farmers and the identification and introgression of new sources of resistance (http://www.globalrust.org/traction; http://www.wheatrust.cornell.edu). From these efforts multiple new sources of Ug99 stem rust resistance have been made available (Liu et al., 2011a; Liu et al., 2011b; Qi et al., 2011).

Stem Rust Resistance

To date, 55 genes have been designated for resistance to wheat stem rust (Mcintosh et al. 1995, 2008, 2009, 2010, 2011). Over the last century, these genes have been identified within common wheat and wild relatives. Many *Sr* genes of common wheat origin have been deployed during major efforts to incorporate genetic resistance to stem rust in wheat cultivar development (Stakman, 1954; McIntosh et al., 1995). In concert with barberry eradication efforts (Peterson et al., 2005), deployment of genetic resistance along with earlier maturity wheat cultivars (Eversmeyer and Kramer, 2000) has minimized the impact of stem rust on wheat production in the United States and globally.

Mechanisms of resistance

Stem rust resistance genes generally operate under two mechanisms. Of the designated *Sr* genes 53 are single-locus major genes (McIntosh et al., 1995) conferring resistance at all stages of plant development, sometimes with varying effectiveness at the adult plant stage (Singh et al., 2011a). Resistance can also be quantitative, conferred by multiple minor genes that individually contribute small effects but together contribute significantly to the resistance phenotype (Poland et al., 2008). Stem rust resistance from two genes *Sr2* and *Sr55*, are unique in that they confer quantitative adult plant resistance to stem rust and are pleiotropic (McIntosh et al., 1995; Krattinger et al., 2009), conferring resistance to diseases including leaf rust, stipe rust and powdery mildew.

Seedling Resistance

Major gene resistance to rust pathogens of wheat generally operates in a gene-for-gene manner (Flor, 1956) where a single disease resistance gene corresponds to a single avirulence factor in

the pathogen. Most plant disease resistance genes that function in effector-triggered immunity (ETI) and encode NB-LRR proteins that operate in detection of pathogen activity (Jones and Dangl, 2006). Seedling resistance genes that have been cloned in wheat encode proteins with NB-LRR domains (Huang et al., 2003a; Feuillet et al., 2003; Yahiaoui et al., 2004; Cloutier et al., 2007). These disease resistance proteins generally function to detect perturbations of host cellular components by pathogen-derived molecules operating in mechanisms known as the guard hypothesis (van der Biezen and Jones, 1998; Dangl and Jones, 2001) and the decoy hypothesis (van der Hoorn and Kamoun, 2008). Upon detection of pathogen molecule activity, disease resistance proteins signal to downstream factors resulting in the induction of the defense response.

Most stem rust seedling resistance genes confer a strong defense response involving chlorosis or necrosis that limit the formation and spread of fungal hyphae and uredinia in host tissues. The type of defense response and the presence of either chlorosis or necrosis differs, sometimes greatly, between individual *Sr* genes and is a metric is used to classify the phenotypic expression of resistance (Stakman et al., 1962). *Sr5, 17, 27, 35* and *36* all confer low, hypersensitive infection types whereas *Sr22*, *Sr29* and *Sr33* confer low chlorotic infection types (McIntosh et al., 1995). The Stakman stem rust rating scale ranges from 1 to 4 with 1 and 2 usually indicating an incompatible resistance response infection types and 3 and 4 indicating a compatible susceptibility response. Infection types of 0, ; and 1 infer a necrotic infection type, and 2 infers chlorosis or necrosis. Infection types of 3 and 4 infer the presence or absence of chlorosis, respectively, in compatible interactions.

Adult Plant Resistance

In contrast to major gene resistance to stem rust that is race-specific and observed at the seedling stage, stem rust resistance conferred by adult plant resistance (APR) genes is non race-specific and is expressed in adult plants. Most APR genes are minor genes acting as quantitative trait loci (QTL). The accumulation of multiple minor genes contributing to resistance has the effect of generating high levels of resistance in adult plants (Singh et al., 2011b).

One of the most widely utilized adult plant resistance genes is *Sr2* (McFadden, 1930).The gene is most effective in concert with up to five other genes with small effects. Resistance from *Sr2* in the cultivar "Hope" and other emmer-derived resistance in the cultivar "Thatcher" provided a foundation for stem rust resistance in spring wheat germplasm of the United States and widely adapted lines developed by Dr. N. E. Borlaug (Hare and McIntosh, 1979). The "*Sr2*-Complex" comprised of *Sr2* in concert with up to five additional genes with small effects continues to be foundational to adult plant resistance to stem rust in international breeding efforts (Singh et al., 2011b). The pleiotropic adult plant resistance gene *Lr34* has demonstrated effects on the expression of stem rust resistance (Liu and Kolmer, 1998; Vanegas et al., 2008; Kolmer et al., 2011) and has been designated as *Sr57*. *Sr57* (*Lr34*) serves to enhance the effects of other QTL conferring stem rust resistance (Kolmer et al., 2011).

Durability of resistance

Both major gene and minor gene resistance drive evolution of *Pgt* populations. Major genes, when deployed singly, have the effect of generating directional selection towards virulence resulting in boom and bust cycles (Sun and Yang, 1999). The result is of continuous boom and bust cycles is a diminished gene pool of effective stem rust resistance genes. Minor genes also exert selection on *Pgt* populations. However, the result of selection is not a qualitative change as a single mutation from *Avr* to *avr* but as a quantitative increase in aggressiveness (Kolmer and Leonard, 1986). Presumably, resistance from minor genes does not involve the recognition of *Avr* gene products to trigger resistance and will not induce selection pressure in pathogen populations for mutations in major gene targets. Both mechanisms of resistance show the potential to break down but quantitative resistance has shown to be more durable over time and space.

Single genes deployed over large acreages have short life spans. Pyramids of stem rust resistance genes show promise in prolonging the durability of major gene resistance. Having multiple genes together, to which no virulence exists in the pathogen population, should in theory prolong the effectiveness of each individual gene. The effectiveness of resistance gene pyramids lies in the low probability of simultaneous mutation towards virulence to all pyramided genes in the pathogen population. Virulence may develop at random to one of the genes in the pyramid. The

virulence may not persist in the pathogen population as individuals with the acquired virulence cannot reproduce because of the presence of additional resistance genes to which they are avirulent. To cause disease on a cultivar carrying three major genes for resistance, a pathogen would have to acquire virulence to all three genes simultaneously which may be a very low probability event occurring at a frequency of the product of the probability of virulence to each gene individually.

An assumption made in pyramiding of major gene resistance is that different genes detect the activity of different pathogen molecules. Two genes recognizing the activity of the same effector could be made ineffective simultaneously. In order to assure that multiple modes of pathogen recognition are active, more than two major genes present together in a single genetic background should be sufficient to provide multiple modes of pathogen recognition and thereby prevent the simultaneous mutation to virulence. A mutation in a pathogen virulence component to overcome resistance could compromise pathogen fitness resulting in fitness cost (Bahri et al., 2009). This could potentially increase the durability of a resistance gene product that recognizes a virulence component that, if lost, confers a fitness penalty to the pathogen.

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Chapter 2 - Simultaneous transfer, genomic localization and introgression of genes for resistance to stem rust race Ug99 from the wheat D-genome progenitor species, *Aegilops tauschii* to cultivated wheat, *Triticum aestivum*

Introduction

Cultivated bread wheat, *Triticum aestivum* L. (AABBDD, 2n=6x=42), is a hexaploid species that arose approximately 8000 years ago from the hybridization of cultivated tetraploid (AABB) with the diploid D genome species, *Ae. tauschii* Coss. (Kihara, 1944b)(McFadden and Sears, 1946). A very limited number of *Ae. tauschii* accessions, perhaps as few as two, contributed to the original hybridization events (Talbert et al., 1998)(Dvorak et al., 1998). Hybridization of common wheat landraces with wild and cultivated teraploid wheat occurs naturally in its area of origin (Loureiro et al., 2007) and has introduced allelic variations in the A and B genomes of common wheat (Caldwell et al., 2004). However, in nature, ploidy level and incompatibility preclude direct hybridization and introgression of genes from *Ae. tauschii*. As a result, the D genome of common wheat is very limited in genetic diversity relative to the A and B genomes (Akhunov et al., 2010).

McFadden and Sears (1944) produced the first artificial hybrid between tetraploid wheat and *Ae. tauschii*. Doubling the chromosome number of F_1 hybrids produced synthetic wheat that is readily crossed with *T. aestivum* wheat making the allelic diversity of the *Ae. tauschii* transferrable to cultivated hexaploid wheat. Kihara and colleagues made large collections of *Ae. tauschii*, documented extensive genetic diversity including resistance to leaf and yellow rust and produced a large number of synthetic wheats incorporating rust resistance (Kihara et al. 1957 and Tanaka 1961). A direct hybrid between wheat and *Ae. tasuchii* was reported by The (1973) but backcrossing of F_1 hybrids to wheat was not attempted. Gill and Raupp (1987) developed a breeding strategy using the direct hybridization method for enriching the D genome of wheat. Direct hybridization of diploid *Ae. tauschii* accessions with hexaploid wheat (Gill and Raupp, 1987) provides a means to transfer D genome regions carrying target alleles of interest without

disrupting adaptive allelic combinations in the A and B genomes while providing D genome allelic diversity for agronomically important traits (Cox et al., 1995a).

In direct hybridization, the diploid *Ae. tauschii* (2n=2x=14, DD) accession is used as a male to directly pollinate *T. aestivum* (2n=6x=42, AABBDD) as a female (Gill and Raupp 1987). Alternatively, *Ae. tauschii* can be used as a female and *T. aestivum* as a male, resulting in a higher frequency of caryopsis formation (Sehgal et al. 2010). Imprinting and epigenetic phenomena are responsible for parent specific allele expression in seed endosperm development (Köhler and Makarevich 2006). Interploidy crosses between diploids and hexaploids display altered expression of paternal and maternal alleles leading to aberrant endosperm formation (Tiwari et al. 2010). The failure to develop functional endosperm after fertilization maintains a hybridization barrier between *Ae. tauschii* and *T. aestivum*. Hybrid embryos with the ABDD genomic constitution (2n=28) require embryo rescue and generation of seedlings on artificial growth media. To restore fertility and recover target alleles, hybrids are first backcrossed as females to hexaploid wheat. Partially fertile BC_1F_1 plants display aneuploidy (Gill and Raupp 1987) and are backcrossed again to hexaploid wheat as males generating a high frequency of euploid (n=21) gametes by certation. Target alleles can be recovered in BC_2 plants with restored chromosome number and fertility.

To date, three genes effective against TTKSK have been transferred from *Ae. tauschii* including *Sr33*, *Sr45* (Kerber and Dyck 1979)(Sambasivam et al. 2008) and *Sr46*. Kerber and Dyck (1979) developed synthetic hexaploids carrying *Sr33* and *Sr45* by hybridization of the extracted tetraploid of 'Canthatch'. The *Ae. tauschii* accessions carrying these genes originated from different geographies and subspecies of *Ae. tauschii*. The *Sr33* donor, *Ae. tauschii* ssp. *strangulata* accession RL5288 (syn. TA1600) originates from northern Iran, near Tehran and the *Sr45* source, *Ae. tauschii* ssp. *tauschii* RL5289 (syn. TA1599) originates from northwestern Iran near Gilan (Jon Raupp, personal communication). Sr33 is widespread throughout the *Ae. tauschii* center of diversity in countries bordering the South Caspian Sea. Innes and Kerber (1994) identified *Sr33*, by allelism test, in *Ae. tauschii* accessions from Armenia, Iran and Turkmenistan.

Genes transferred by earlier workers (Kerber and Dyck 1969; Kerber and Dyck 1979) and later Gill and Raupp (1987), Cox (Cox et al. 1992; Cox et al. 1995a; Cox et al. 1995b), and Murphy (Murphy et al., 1998, 1999) carried out the introgression of useful genes from *Ae tauschii* in a two-step approach. In the first step, they isolated true breeding lines homozygous for the target gene. In the second step, these lines were used for chromosome mapping of target using either monosomic analysis in earlier studies and molecular markers in later studies. In this study, we report the simultaneous transfer, genetic mapping and introgression into elite wheat germplasm, a new gene for resistance to the highly virulent stem rust race Ug99.

The *Ae. tauschii* accessions TA1662 (Rouse et al. 2011) and CDL4424 (M.Rouse unpublished) were identified to yield a low infection type of 2- to stem rust race TTKSK. The following work was conducted to transfer the stem rust resistance from TA1662 and CDL4424 by direct hybridization, to a hexaploid wheat genetic background suitable for hard winter wheat breeding. The genetic stocks developed in the process of introgression were used to map the resistance genes. The introgressed genes from both accessions were mapped to1DS.

Materials and Methods

Plant Materials and direct hybridization

The diploid *Ae. tauschii* accession TA1662 from Azerbaijan is resistant to *Pgt* races TTKSK (Ug99), TTTTF and QTHJC and is susceptible to *Pgt* races RKQQ and TPMK(Rouse et al. 2011). The *T. aestivum* recurrent parent is hard white winter wheat line KS05HW14 produced by the Kansas State University wheat breeding program in Hays, KS by Dr. Joe Martin. KS05HW14 is susceptible to *Pgt* races TTKSK, TTTTF, TPMKC, RKQQC and QTHJC. Emasculated florets of KS05WH14 were pollinated with TA1662 using the approach method (Rosenquist C.E, 1927). Between 14 and 17 days after pollination, caryopses containing fertilized embryos were removed from spikes for embryo rescue. Caryopses were surface sterilized for 20m in a 20% bleach solution containing 0.001% Tween20 and rinsed three times in 40mL ddH20 for 20m. All handling of sterilized caryopses and embryos was done under sterile conditions in a laminar flow hood. Embryos were removed from caryopses and transferred to embryo culture media containing a mixture of $4.1g^{-1}$L Murashige and Skoog salts (Murashige

and Skoog 1962) and Gamborg's B5 Vitamins (Gamborg et al. 1968) (Sigma Aldrich, M0404) with 3% sucrose, 2mg^{-1}L kinetin, 2g^{-1}L phytagel (Sigma Aldrich, P8169) at pH 5.7. Upon the development of shoot and root tissues, germinating embryos were transferred to 50mL culture tubes containing a mixture of 4.1g^{-1}L Murashige and Skoog salts and Gamborg's B5 Vitamins with 4% maltose, 1.9 g^{-1}L MES buffer, 0.1 g^{-1}L ascorbic acid and 2g^{-1}L phytagel at pH 5.7. When seedlings developed several roots greater than 10cm, they were placed in vernalization for 4 weeks at 4C°. Vernalized seedlings were transferred directly to 5" pots containing Metro Mix 200 growth media (Hummert, Earth City, MO) saturated with tap water. To prevent loss of turgor pressure, seedlings were immediately covered with a clear plastic dome maintaining a 100% humidity environment for 3 to 5 days. Plastic domes were removed when guttation water was observed on the secondary leaves.

F_1 plants were grown in the greenhouse for backcrossing under 10h of supplemental light at 21°C ± 4C°C six weeks and then transitioned to 16h of supplemental light at 23°C ± 3C°C. At female maturity, spikes of the hybrid plants were pollinated by KS05HW14 to generate BC_1F_1 seed. A single BC_1F_1 plant resistant to stem rust race QTHJC, called U6573-1R, was used as a male to generate a BC_2F_1 mapping population of 138 individuals called U6714.

After stem rust assay, BC_2F_1 plants from U6714 were vernalized for six weeks at 4°C, then placed in a growth chamber under 10h days at 20°C / 15°C for three weeks and then transitioned to a regimen of 16h days at 23°C / 20°C until maturity. BC_2F_2 seed from individual plants was used for progeny testing of stem rust resistance. Stem rust resistance from *Ae. tauschii* accession CDL4424 was transferred using a similar approach. Allelism test for *Sr33* was conducted using diploid *Ae. tauschii* accessions TA1600 (RL5288)(*Sr33*) and CDL4424. TA1600 (RL5288) is resistant to *Pgt* races TTKSK, TTTTF, TPMKC, RKQQC and QTHJC. TA1600 was crossed as a female to CDL4424. Progeny testing for stem rust resistance was done on 2,422 F_2 seedlings.

Stem rust assay

For inoculation of *Pgt* race QTHJC, urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42°C water bath for 5 minutes. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto

two to three leaf stage seedlings. Inoculated plants were incubated in a dew chamber at 20 ± 1°C and 100% relative humidity for 16 h and then placed in a growth chamber at 20 ± 1ºC with a 16 h light/ 8 h dark cycle. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings yielding a low infection type of 2- to 2 were considered resistant and seedlings yielding a high infection type of 3- to 4 were considered susceptible.

Inoculation of *Pgt* race TTKSK were done by USDA-ARS Cereal Disease Lab scientists in a BL3 facility at the University of Minnesota, in St. Paul, MN. TTKSK urediniospores were removed from storage at -80°C and heat shocked at 45°C for 15 min. Spores were rehydrated by placing spore capsules in an air-tight container at 80% humidity maintained by a KOH solution for 2 to 4 h. Urediniospores were then suspended in a light-weight mineral oil (Soltrol 70; Conoco- Phillips Inc., Houston) and sprayed onto seedlings. Plants were placed in dew chambers overnight as described previously (Jin et al., 2006). After dew chamber incubation, plants were kept in a greenhouse maintained at 25 ± 2°C. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings yielding a low infection type of 2- to 2+ to TTKSK were considered resistant and seedlings yielding a high infection type of 3- to 4 were considered susceptible.

BC_1F_1 plants derived from direct crossing with TA1662 were assayed with *P. graminis* f.sp. *tritici* (*Pgt*) race QTHJC that is avirulent to TA1662-derived resistance temporarily designated *Sr1662*. BC_1F_1 plants derived from direct crosses with CDL4424 were assayed with *Pgt* race RKQQC that is avirulent to *Sr4424*.

In the U6714 mapping population, 138 BC_2F_1 plants were assayed using QTHJC. Progeny testing of 15 individuals from 138 BC_2F_2 families of U6714 was done with QTHJC to confirm resistant and susceptible BC_2F_1 phenotypes. To demonstrate the unique identity of *Sr1662*, 10 individuals from 121 BC_2F_2 families were inoculated with *Pgt* race RKQQC that is virulent to *Sr1662*.

To confirm effectiveness of *Sr1662* against TTKSK, 10 QTHJC-resistant and 10 QTHJC-susceptible BC_2F_2 families from the U6714 mapping population, the *Ae. tauschii* donor accession

TA1662, and the recurrent parent KS05HW14 were assayed with stem rust race TTKSK. From theU6573-1R BC_1F_1 donor parent used to generate the BC_2F_1 mapping population U6714, 48 BC_1F_2 progenies were assayed with TTKSK to confirm segregation of resistance.

CDL4424 seedling stem rust resistance was assayed using *Pgt* race RKQQ that is avirulent to *Sr4424*. To evaluate the effectiveness of *Sr4424* resistance against Ug99 stem rust races in the field, evaluation was done in hill plots in the US Winter Wheat Nursery at the KARI research station in Njoro, Kenya. In the 2011 nursery, three $BC_1F_{2:4}$ lines homozygous for *Sr4424* were evaluated including, U6369R1-078, U6369R1-148 and U6369R1-200.

DNA isolation and PCR conditions

Genomic DNA from mapping populations and TA1662, CDL4424 and KS05HW14 parents was isolated using BioSprint 96 DNA Plant Kits (Cat. No. 941558) following the manufacturer's instructions (Qiagen, Valencia, CA).

Reaction conditions for SSR markers were as follows: 8.33 µL ddH_20, 2.4 µL 10X reaction buffer, 0.9 µL 50mM $MgCl_2$, 1.92 µL 2.5mM dNTPs, 1.9 µL 1pM of 19bp M-13 labeled forward primer (5'-ACGACGTTGTAAAACGAC), 2.2µL 10pM of reverse primer, 2.2µL 10pM of M-13 primer labeled with 6-FAM, VIC, PET, or NED, 0.1µL (0.5U) Taq polymerase. Cycling conditions for all markers included an initial denaturation of 95°C followed by 35 cycles of 95°C (60 s), primer-specific Tm (60 s), and 72°C (2 m), and a final extension at 72°C (10 m). Primer sequences and specific annealing temperatures for SSR markers used in the bulked segregant analysis and mapping were obtained from GrainGenes2.0 (http://wheat.pw.usda.gov/GG2/index.shtml).

Molecular marker analyses and genetic mapping

To identify the chromosomal location and simple sequence repeat (SSR) loci linked to *Sr1662* segregating in U6714, genomic DNA of 8 resistant and 8 susceptible BC_2F_1 plants were pooled to generate resistant and susceptible bulks. A set of 70 D genome-specific SSR markers comprised of 5 markers per chromosome arm were amplified from the resistant and susceptible bulks.

Six SSR loci on 1DS including *Xcfa2158*, *Xcfd15*, *Xgdm33*, *Xwmc147*, *Xwmc432* and *Xwmc222* and one EST-STS locus, *XBE424485*, were evaluated on U6714 following the PCR conditions described above (Table 2.1, Figure 2.1). All markers were scored as co-dominant.

Five SSR loci on 1DS were evaluated on the U6369R1 (BC_1F_2) mapping population. Two loci, *Xbarc152* and *Xwmc147,* were scored as dominant for the KS05HW14 parent allele. The *Xcfa2158, Xwmc222, Xwmc336*, and *Xwmc432* loci were scored as co-dominant. Two EST-STS loci were also evaluated, *XBE443103* and *XBE591682,* and scored as dominant for the CDL4424 allele (Table 2.1, Figure 2.1).

Sizing of PCR products was performed by capillary electrophoresis using a 3730*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). Genetic linkage analysis was performed using MAPMAKER v 3.0 (Lander et al. 1987). Marker orders were established using multipoint analysis and the Kosambi centimorgan function with a minimum LOD of 3.0 and validated using the ripple function. Segregation of marker loci and stem rust resistance was evaluated using a χ^2 goodness-of-fit test.

KS05HW14, TA1662, CDL4424, three stem rust resistant and three stem rust susceptible segregants from mapping populations were evaluated for the presence of *Sr33* using a marker designed from the genic sequence of *Sr33* (S.Periyannan and E. Lagudah, unpublished)(Table 2.3).

Results

Direct hybridization and population development

From direct crossing of KS05HW14 with TA1662, nine F_1 plants were recovered by embryo rescue. The aneuploid (n=28, ABDD) F_1 plants were male sterile and were backcrossed as females to KS05HW14 and nine BC_1F_1 seed were recovered. Two BC_1F_1 seedlings were screened with stem rust race QTHJC. The BC_1F_1 plant called U6573-1R, yielded an infection type of 2 to QTHJC that was lower than the recurrent parent, KS05HW14 with an infection type

of 4. The other BC_1F_1 plant, U6573-2S yielded a high infection type of 3 to QTHJC. The QTHJC-resistant BC_1F_1 plant U6573-1R was used as the male stem rust resistance donor parent to generate the BC_2F_1 mapping population, U6714.

Stem rust assay

In the U6714 mapping population, BC_2F_1 plants resistant to QTHJC yielded low infection types of 2- to 2 and susceptible individuals yielded high infection types of 3 to 4 (Figure 2.2). The stem rust susceptible recurrent parent KS05HW14 yielded a high infection type of 3+4 to QTHJC and the donor parent TA1662 yielded a low infection type of 2-. The segregation of 64 resistant: 74 susceptible in U6714 agrees with a 1:1 segregation ratio (χ^2= 0.72, p=0.39) consistent with a single stem rust resistance gene in a BC population.

Resistant and susceptible reactions to QTHJC were confirmed by progeny testing of 15 BC_2F_2 progeny from each BC_2F_1 plant in U6714 (Table 2.2). BC_2F_2 progenies from QTHJC-resistant BC_2F_1 plants segregated for resistance with low infection types of 2- to 2 and high infection types of 3- to 4. All BC_2F_2 progenies from QTHJC-susceptible BC_2F_1 plants were susceptible to QTHJC with infection types of 3- to 4. Avirulence of QTHJC to *Sr1662* confirms that stem rust resistance from TA1662 is not due to *Sr45* as QTHJC is virulent to *Sr45* (Rouse et al. 2011).

Susceptibility to RKQQC was confirmed in 121 BC_2F_2 families from U6714 with all plants in all BC_2F_2 families yielding high infection types of 3 to 4 to RKQQC (Table 2.2). All individuals in BC_2F_2 families with resistance to QTHJC, yielded susceptible infection types to RKQQC confirming RKQQC virulence to *Sr1662*. This race-specific reaction of QTHJC resistance and RKQQC susceptibility demonstrates that *Sr1662* is different from *Sr33* which confers resistance to RKQQC.

The *Ae. tauschii* accession TA1662, recurrent hexaploid parent KS05HW14, and 24 individuals from 7 QTHJC-resistant and 7 QTHJC-susceptible BC_2F_2 families from U6714 were assayed with TTKSK to confirm resistance and susceptibility (Table 2.2). TA1662 yielded a low infection type of 2- and KS05HW14 yielded a high infection type of 4 to TTKSK. All BC_2F_2families yielding low infection types to QTHJC were confirmed to yield low infection

types of 2- to 2+ to TTKSK. All BC_2F_2 families yielding high infection types to QTHJC were confirmed to yield high infection types of 3+ to 4 to TTKSK (Figure 2.2). These results demonstrate that *Sr1662* mapped using QTHJC confers resistance to TTKSK.

The stem rust-resistant *Ae. tauschii* accession CDL4424 yields a low infection type of 2- to RKQQC and the stem rust-susceptible recurrent parent KS05HW14 yields a high infection type of 3+4 to RKQQC. CDL4424-derived stem rust resistance followed segregation for a single dominant gene in BC_2F_1 (χ^2=2.08, p= 0.15) and BC_1F_2 (χ^2 =0.79, p=0.67) populations in which RKQQC-resistant individuals yielded low infection types of 2- to 2 and susceptible individuals yielded high infection types of 3+ to 4. Three $BC_1F_{2:4}$ lines homozygous for *Sr4424* were evaluated in the field in Njoro, Kenya in 2011. U6369R1-078, U6369R1-148, U6369R1-200 yielded adult plant infection types of 20RMR, 25RMR and 25RMR, respectively.

Bulked segregant analysis and mapping of Ae. tauschii-derived stem rust resistance on 1DS

In the BSA, markers *Xwmc222* and *Xdgm33* differentiated the resistant and susceptible bulks from the BC_2F_1 population, U6714. The resistant bulk was heterozygous for the 182bp TA1662 allele and the 186bp KS05HW14 allele at *Xwmc222* whereas the susceptible bulk was homozygous for the 186bp allele from KS05HW14. The 173bp allele from TA1662 at *Xgdm33* differentiated the resistant and susceptible bulks. The resistant bulk was heterozygous for the 173bp allele and the 178bp KS05HW14 alleles at *Xwmc222* whereas the susceptible bulk was homozygous for the 178bp allele from KS05HW14.

The seven loci linked to *Sr1662* on 1DS cover a genetic distance of 35.8 cM in U6714 (Table 1)(Figure 2.1). Two SSR loci, *Xwmc432* and *Xwmc222* flank *Sr1662* at 4.4 cM distal and 4.4 cM proximal, respectively.

Two co-segregating SSR loci, *Xcfd15* and *Xwmc432*, map distal to *Sr1662* at 4.4cM (Figure 2.1). Both *Xcfd15* and *Xwmc432* have been previously mapped proximal to *Sr33* (Sambasivam and Bansal, 2008) at distances of 1.8 cM and 3.6 cM, respectively (Figure 2.1). Three recombinant genotypes are present in U6714 that are segregating for stem rust resistance and

fixed for KS05HW14 alleles at *Xwmc432*, *Xcfd15*, *Xwmc147*, and X*gdm33* suggesting *Sr1662* is located proximal to the *Sr33* locus. Map position places *Sr1662* distal to *Sr45* (Figure 2.1). The gene-specific marker for *Sr33* is absent from the TA1662 parent, BC_2F_1 individuals segregating for *Sr1662* stem rust resistance and stem rust-susceptible BC_2F_1 individuals from U6714 (Table 2.3).

The 179bp allele from CDL4424 at *Xwmc222* differentiated resistant and susceptible bulks. The resistant bulk was heterozygous for the 179bp CDL4424 allele and the 186bp KS05HW14 alleles at *Xwmc222* whereas the susceptible bulk was homozygous for the 186bp allele from KS05HW14. Polymorphism between resistant and susceptible bulks at *Xwmc222* identified 1DS as the chromosome location of *Sr4424*. Genetic mapping places *Sr4424* in the same interval on 1DS as *Sr1662* (Figure 1). Map data suggest the stem rust resistance gene from CDL4424 is located proximal to the *Sr33* locus (Figure 1). However, the gene-specific marker for *Sr33* is present in the CDL4424 parent and homozygous lines while the *Sr33* marker allele is absent from susceptible lines (Table 2.3). All 2,422 F_2 seedlings from the allelism cross TA1600 (Sr33)/ CDL4424 yielded a low infection type to stem rust race RKQQC confirming that *Sr4424* occupies the same locus as *Sr33*.

Marker allele sizes for TA1662, CDL4424, and KS05HW14 parents are given in Table 3.

Discussion

The diploid D genome progenitor species, *Ae. tauschii* is an accessible source of resistance to the Ug99 stem rust races that threaten global wheat production. The methods used in this study facilitate the transfer, introgression and simultaneous mapping of genes from a wild relative species to cultivated hexaploid wheat in one integrated process. Direct hybridization between *Ae. tauschii* and an elite winter wheat breeding line integrates gene transfer and introgression to elite wheat breeding germplasm. Generation of segregating populations in the process of transferring genes from *Ae. tauschii* to *T. aestivum* facilitates chromosome localization and genetic mapping of stem rust resistance genes. Phenotypic selection of lines for use in stem rust resistance breeding can be done on progenies derived from BC_2F_1 populations either from segregating lines

within families or lines derived from single seed descent. This study highlights the expedience with which genes from the D genome of *Ae. tauschii* and homologous genomes can be transferred, mapped and incorporated into wheat breeding germplasm.

Bulked segregant analysis using BC_2F_1 plants can identify linked markers and the chromosomal location of stem rust resistance genes for targeted mapping. Microsatellite markers are generally co-dominant and highly polymorphic between the wheat D genome and the *Ae. tauschii* D genome allowing bulked segregant analysis of heterozygous BC_2F_1 individuals. Using BC_2F_1 individuals for mapping of stem rust resistance genes accelerates the process of genetic mapping and identification of closely linked markers. This approach contrasts with phenotypic selection for stem rust resistance in multiple generations of backcrossing followed by subsequent inbreeding to develop a homozygous line for genetic studies. The approaches used in this study facilitated the transfer and characterization of stem rust resistance genes from *Ae. tauschii* within three generations.

As stem rust races of the Ug99 lineage are not present outside of Africa and the Middle East, mapping of TA1662 resistance on 1DS was done using race QTHJC that is endemic to North America. Effectiveness of *Sr1662* mapped on 1DS against TTKSK was confirmed in BC_2F_2 families from the U6714 mapping population with families having resistance to QTHJC also having resistance to TTKSK.

This study provides *Sr1662* from the *Ae. tauschii* accession TA1662 as a new gene for resistance to the widely virulent stem rust race TTKSK. Race specificity of resistance differentiates *Sr1662* from *Sr33* demonstrated by RKQQC virulence on *Sr1662*. Absence of the diagnostic *Sr33* allele provides further evidence that *Sr1662* is different from *Sr33*. The diploid *Ae. tauschii* accession TA1662 and stem rust resistant BC_2F_1 individuals carrying *Sr1662* from U6714 lack the diagnostic *Sr33* allele. Both *Sr1662* and *Sr33* from CDL4424 map to a region on 1DS flanked by the SSR loci *Xwmc432* and *Xwmc222*. However, presence-absence polymorphism and structural variation has been shown to generate haplotype variation for disease resistance genes and resistance gene analogs in *Ae. tauschii* (Brooks et al., 2006). Similar structural variation could exist among *Ae. tauschii* accessions carrying *Sr1662* and *Sr33*.

Genetic evidence from allelism test using diploid *Ae. tauschii* accessions indicated stem rust resistance transferred from CDL4424 (*Sr4424*) is conditioned by *Sr33*. This result is confirmed by CDL4424 having the diagnostic *Sr33* allele. However, results from previous *Sr33* mapping studies suggest that CDL4424 stem rust resistance occupies a unique locus (Figure 2.1)(Sambasivam and Bansal, 2008). The source of discrepancy between genetic maps of *Sr33* from CDL4424 and TA1600 (RL5288) is unknown.

The addition of *Sr1662* and the *Sr33* allele from CDL4424 provide depth to the pool of stem rust resistance genes effective against the Ug99 lineage of stem rust races. The effectiveness of *Sr1662* to TTKSK has been demonstrated at the seedling stage. The effectiveness of CDL4424-derived *Sr33* has been demonstrated in Kenya against both races TTKSK and TTKST yielding low infection types from 20-25RMR, indicating this allele of *Sr33* is effective under heavy disease pressure in the field. Germplasm is currently under development for distribution of *Sr1662* and CDL4424-derived *Sr33* to wheat breeding programs.

Three stem rust resistance genes effective against TTKSK transferred from *Ae. tauschii* have now been characterized on 1DS including *Sr33*, *Sr45* (Sambasivam et al. 2008), and *Sr1662*. Based on current mapping results these genes occupy different loci and the availability of markers for these genes should enable the phasing of all genes in coupling. Markers for *Sr1662*, *Sr45* and *Sr33* provide a means to generate a three-gene pyramid on 1DS. Utilizing rye-specific markers for *Sr50* (formerly *SrR*) (Anugrahwati et al. 2008), if *Sr50* occupies a different locus, would allow a four-gene pyramid to be generated on 1DS. With marker-assisted selection (MAS) for stem rust resistance genes targeted to a single chromosome arm, genome-wide constraints associated with selection of multiple unlinked genes are relaxed, thereby reducing constraints placed on breeding population sizes by early generation enrichment of target alleles by MAS.

The D genome of *Ae. tauschii* and other wild wheat relatives will remain a valuable genetic resource for future generations. This work underscores the need to preserve the genetic variation of wild wheat relatives. The variation present among in-situ germplasm collections for resistance to current pathogen species, races and biotypes have been generated over millennia of

selection in native populations of wild wheat relatives. The co-localization of wild relatives with cultivated species place genetically diverse wild relative populations under the same directional selection pressures that new pathogen populations place on cultivated monocultures, leading to fixation of effective resistance genes in wild relative populations (Huang et al., 2009). The ongoing evolutionary process of selection among wild species populations for resistance to current pathogens of cultivated species should be prolonged by ex-situ conservation efforts for the variation generated presently to be available for millennia in the future.

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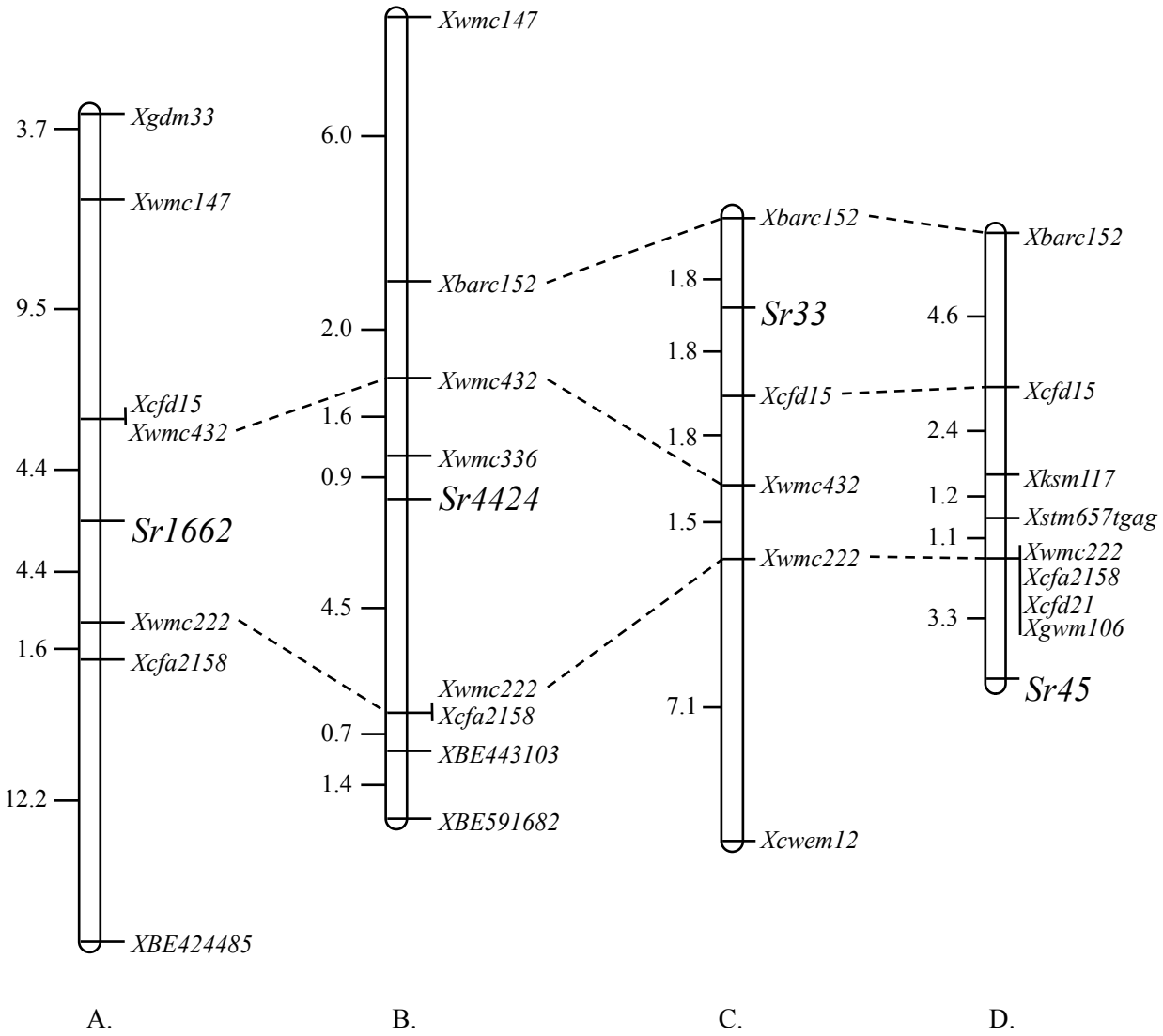
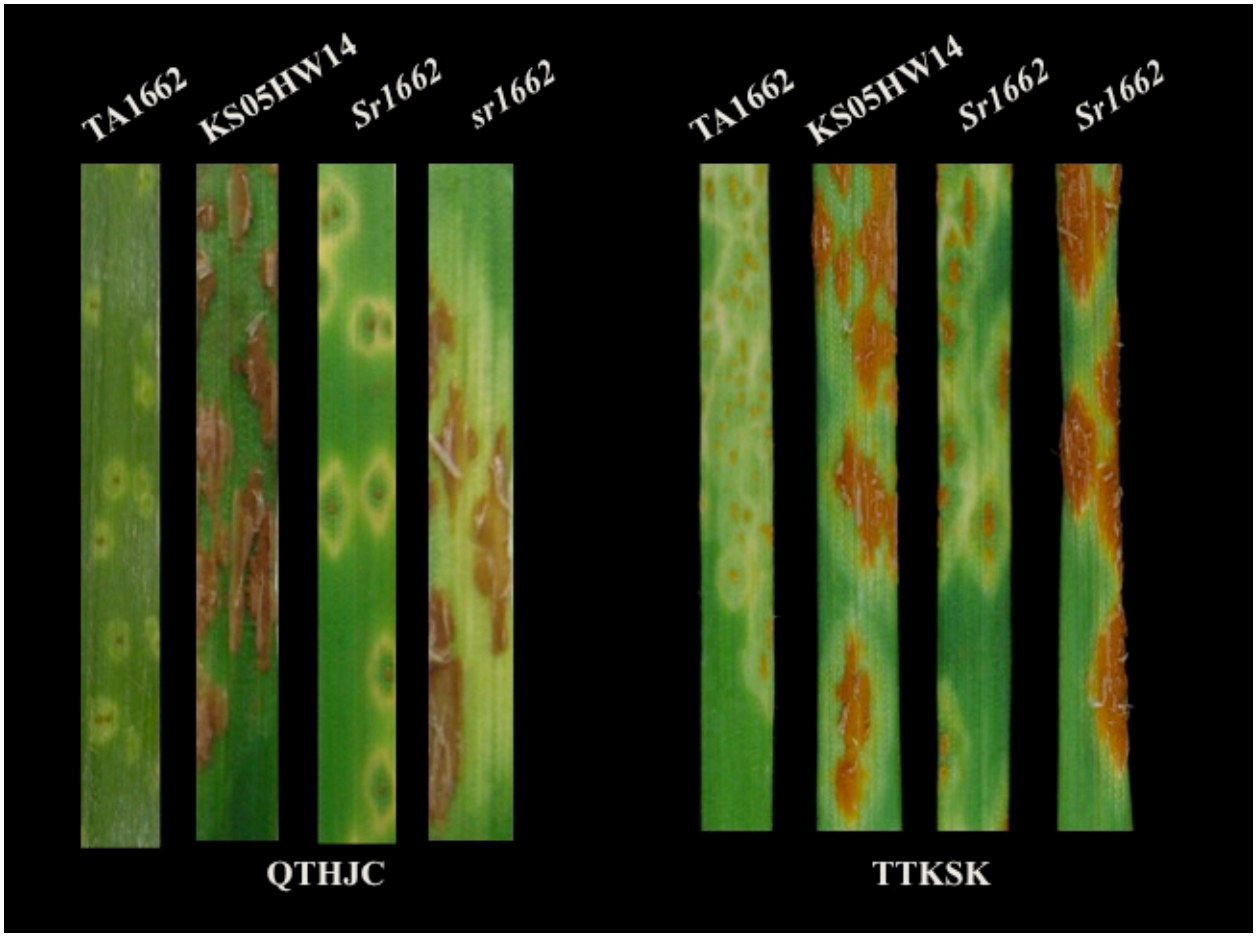
Figure 2.1 *Sr1662*, *Sr4424*, *Sr33* and *Sr45* on 1DS.



Comparative maps of *Sr1662*, *Sr4424*, *Sr33* and *Sr45* on 1DS. A) *Sr1662* from *Ae. tauschii* ssp. *tauschii* accession TA1662 in the BC_2F_1 mapping population U6714. B) *Sr4424* from *Ae. tauschii* ssp. *tauschii* accession CDL4424 in the BC_1F_2 mapping population U6369. C) *Sr33* from *Ae. tauschii* ssp. *strangulata* accession TA1600 (RL5288). D) *Sr45* from *Ae. tauschii* ssp. *tauschii* accession TA1599 (RL5289). *Sr33* and *Sr45* maps are from Sambasivam et al. (2008).

Figure 2.2 *Sr1662* infection types



Infection types to stem rust races QTHJC and TTKSK of *Aegilops tauschii* accession TA1662, recurrent parent hard winter wheat line KS05HW14 and backcross progenies with (*Sr1662*) and without (*sr1662*) TA1662-derived stem rust resistance.

Table 2.1 Parental allele sizes in base pairs of SSR loci on 1DS linked to *Sr1662* and *Sr4424*

Locus	TA1662	CDL4424	KS05HW14
Xgdm33	154	-[a]	-
Xwmc147	143	-	150
Xbarc152	-	-	134
Xwmc432	174	202	193
Xcfd15	196	-	159
Xwmc336	-	91	97
Xwmc222	161	160	150
Xcfa2158	214	214	204
XBE591682	-	921	930
XBE424485	339	-	342
XBE443103	-	781	783

[a] - indicates data not available or no parental allele is amplified at the marker locus

Table 2.2 Infection types (Stakman, 1962) of TA1662, KS05HW14, and BC_2F_2 families from U6714 to wheat stem rust races QTHJC, RKQQC and TTKSK.

Line	QTHJC[a]	RKQQC[a]	TTKSK[b]
TA1662	2-	3-	2-
KS05HW14	3	3+	4
U6714-004	3	3	4
U6714-022	3,3-	3	4
U6714-074	3	3	33-
U6714-100	3	3-,3	3,3-
U6714-125	3	3	3,4
U6714-133	3	3	3,4
U6714-153	3	3,3-	4
U6714-002	2-/3[c]	3	2,2+/3
U6714-016	2-/3	3,3-	2+/4
U6714-069	2-/3	3-,3	2/3
U6714-075	2-/32+,3	3,3-	2+/3
U6714-110	2/3-	3,3-	2+/4
U6714-141	2,2-/3	3	22+/4
U6714-155	2/3	3	22+/4

[a] QTHJ and RKQQ phenotypes at 20C°, taken 14 days after inoculation
[b] TTKSK phenotypes taken at 25C°+/- 2C°, 14 days after inoculation
[c] Segregation for resistant and susceptible phenotypes indicated by h

Table 2.3 *Sr33* status of *Ae. tauschii* donors, *T. aestivum* recurrent parent, and derived lines based on assay with a gene-specific marker.

Line	Source	Genotype	*Sr33* [a]
CDL4424	*Ae. tauschii*	*SrSr*	+
U6369R1-25	BC_1F_2	*SrSr*	+
U6369R1-75	BC_1F_2	*SrSr*	+
U6369R1-78	BC_1F_2	*SrSr*	+
U6369R1-24	BC_1F_2	*srsr*	-
U6369R1-42	BC_1F_2	*srsr*	-
U6369R1-60	BC_1F_2	*srsr*	-
TA1662	*Ae. tauschii*	*SrSr*	-
U6714-09	BC_2F_1	*Srsr*	-
U6714-43	BC_2F_1	*Srsr*	-
U6714-86	BC_2F_1	*Srsr*	-
U6714-04	BC_2F_1	*srsr*	-
U6714-28	BC_2F_1	*srsr*	-
U6714-85	BC_2F_1	*srsr*	-
KS05HW14	*T. aestivum*	*srsr*	-

[a] + indicates presence of *Sr33* allele
[a] - indicates absence of *Sr33* allele

Chapter 3 - Further enrichment of Ug99 wheat stem rust resistance diversity with *Sr10171* and *Sr10187* from the D genome species, *Aegilops tauschii*

Introduction

Deployment of resistance genes in wheat cultivars played a major role in controlling wheat stem rust caused by the basidiomycete fungus *Puccinia graminis* Pers.:Pers f. sp. *tritici* Eriks. & E. Henn during the latter half of the 20th century in North America and globally. Stem rust has great destructive potential that can transform a field of filling grain to a tangled mass of lodged stems causing yield losses up to 100%. The impact of stem rust on wheat production has been limited in recent decades due to effrorts to eradicate the alternate host, Common Barberry (*Berberis vulgaris* L.) and a focus on incorporating genetic resistance in cultivar development. An isolate of *Pgt* collected from Uganda, commonly referred to as 'Ug99' (Pretorius et al., 2000) designated TTKSK based on the North American nomenclature, (Roelfs and Martens, 1987) is virulent to the widely deployed stem rust resistance gene *Sr31* and most stem rust resistance genes derived from cultivated wheat (Singh et al., 2011a).

To increase the number of stem rust resistance genes effective against TTKSK available to wheat breeding programs it becomes necessary to utilize the allelic diversity in the wild relatives of wheat. Cultivated wheat, *Triticum aestivum* L., is an allohexaploid (AABBDD, 2n=6x=42) species derived from hybridization events between related Triticeae species (Sears, 1948). Species with genomes with perfect homology to the A, B and D genomes of *T. aestivum* can be considered part of the primary gene pool of hexaploid wheat. Species with homoeologous and non-homologous genomes represent the secondary gene pool of wheat. Stem rust resistance genes transferred from the secondary and tertiary gene pools of wheat are present among elite U.S. wheat cultivars and breeding lines including *Sr24* from *Agropyron elongatum*, *Sr31* and *Sr1RS^{Amigo}* from *Secale cereale*, and *Sr36* from *T. timopheevi* (Olson et al., 2010a), and *Sr38* from *Ae. ventricosa* (McIntosh et al., 1995). Stem rust resistance genes effective against TTKSK originally present on large chromosome translocations have been made available on smaller introgressions including *Sr22* from *T. boeoticum* (Olson et al., 2010b), *Sr26* from *Agropyron*

elongatum (Dundas et al., 2007), *Sr39* from *Aegilops speltoides* (Niu et al., 2011), *Sr44* from *A. intermedium* (Bernd Friebe, personal communication), *Sr50* (formerly *SrR*) from *S. cereale* (Anugrahwati et al., 2008) and *Sr53* from *Ae. geniculata* (Liu et al., 2011b). In response to the threat of TTKSK, new stem rust resistance genes *Sr51*, *Sr52* and *Sr53* transferred from the secondary gene pool of wheat have been identified in chromosome addition lines and Robertsonian translocations from *Aegilops searsii*, *Dasypryum villosum*, and *Aegilops geniculata*, respectfully (Liu et al. 2011a)(Qi et al. 2011)(Liu et al. 2011b).

The D genome species, *Ae. taushii* is a highly tractable source of genes for resistance to rust pathogens of wheat (Cox, 1998). Currently, four *Ae. tauschii*-derived stem rust resistance genes effective against Ug99 are available for resistance breeding efforts in including *Sr33*, *Sr45* (Kerber and Dyck, 1979), *Sr46* (Rouse et al., 2011) and *Sr1662* (Olson et al. 2012). A screening of *Ae. tauschii* accessions with stem rust race TTKSK (Ug99), identified 98 accessions with resistance that is readily transferrable to *T. aestivum* (Rouse et al., 2011). Direct hybridization of diploid *Ae. tauschii* accessions with hexaploid wheat (Gill and Raupp, 1987) provides a means to transfer D genome regions carrying target genes of interest without disrupting adaptive allelic combinations in the A and B genomes while providing D genome allelic diversity for agronomically important traits (Cox et al., 1995a; Cox et al.,1995b). Further, homologous recombination between *Ae. tauschii* and *T. aestivum* D-genome chromosomes can break undesirable linkages between target genes and alleles associated with linkage drag.

An integrated direct hybridization approach combining gene transfer, genomic localization and introgression (Olson et al., 2012) has been shown as an efficient method of expediting genes from *Ae. tauschii* into wheat breeding germplasm. This method of direct hybridization with an elite hard winter wheat, and development of backcross mapping populations has yielded two additional sources of TTKSK resistance from *Ae. tauschii* accessions TA10171 and TA10187. This work underscores the exceptional feasibility of transferring genes from *Ae. tauschii* and highlights the utility of D genome genetic resources in meeting future challenges to wheat production.

Materials and Methods

Plant materials and direct hybridization

The diploid *Ae. tauschii* ssp. *tauschii* accessions TA10171 and TA10187 are from Turkmenistan and resistant to an array of Pgt races including TTKSK (Ug99), TTKST (Ug99 + *Sr24* virulence), TTTTF, RKQQC, TPMKC. The *T. aestivum* recurrent parent is hard white winter wheat line KS05HW14 produced by the Kansas State University wheat breeding program in Hayes, KS by Dr. Joe Martin. KS05HW14 is susceptible to *Pgt* races TTKSK, TTKST (Ug99 + *Sr24* virulence), TTTTF, TPMKC, RKQQC and QTHJC.

Emasculated florets of KS05WH14 were directly pollinated by TA10171 and TA10187 using the approach method (Rosenquist C.E, 1927). Between 14 and 17 days after pollination, caryopses containing fertilized embryos were removed from spikes for embryo rescue. Caryopses were surface sterilized for 20m in a 20% bleach solution containing 0.001% Tween20 and rinsed three times in 40mL ddH20 for 20m. All handling of sterilized caryopses and embryos was done under sterile conditions in a laminar flow hood. Embryos were removed from caryopses and transferred to embryo culture media containing a mixture of $4.1g^{-1}L$ Murashige and Skoog salts (Murashige and Skoog 1962) and Gamborg's B5 Vitamins (Gamborg et al., 1968) (Sigma Aldrich, M0404) with 3% sucrose, $2mg^{-1}L$ kinetin, $2g^{-1}L$ phytagel (Sigma Aldrich, P8169) at pH 5.7. Upon the development of shoot and root tissues, germinating embryos were transferred to 50mL culture tubes containing a mixture of $4.1g^{-1}L$ Murashige and Skoog salts and Gamborg's B5 Vitamins with 4% maltose, 1.9 $g^{-1}L$ MES buffer, 0.1 $g^{-1}L$ ascorbic acid and $2g^{-1}L$ phytagel at pH 5.7. When seedlings developed several roots greater than 10cm, they were placed in vernalization for 4 weeks at 4C°. Vernalized seedlings were transferred directly to 5" pots containing Metro Mix 200 growth media (Hummert, Earth City, MO) saturated with tap water. To prevent loss of turgor pressure, seedlings were immediately covered with a clear plastic dome maintaining a 100% humidity environment for 3 to 5 days. Plastic domes were removed when guttation water was observed on the secondary leaves.

F_1 plants were grown in the greenhouse for backcrossing under a regimen of 10h of supplemental light at 21°C ± 4C°C six weeks and then transitioned to a regimen of 16h of supplemental light

at 23°C ± 3C°C. At female maturity, spikes of the hybrid plants were pollinated by KS05HW14 to generate BC_1F_1 seed.

A single BC_1F_1 plant derived from direct crossing to TA10171 resistant to stem rust race QTHJC, called U6584-2, was used as a male to generate a BC_2F_1 mapping population of 107 individuals called U6721. A single BC_1F_1 plant derived from direct crossing to TA10187 resistant to stem rust race QTHJC, called U6586-1, was used as a male to generate a BC_2F_1 mapping population of 105 individuals called U6731.

After stem rust assay, BC_2F_1 plants from U6721 and U6731 were vernalized for six weeks at 4°C and then placed in a growth chamber under a photoperiod and temperature regimen of 10h light at 20°C and 14h dark at 15°C for three weeks and then transitioned to a regimen of 16h light at 23°C and 8h dark 20°C until maturity. BC_2F_2 seed from individual plants was used for progeny testing of stem rust resistance.

Stem rust assay

BC_1F_1 seedlings were evaluated with *Pgt* race QTHJC. However, for reasons of inoculum availability and higher perceived levels of aggressiveness, U6721 and U6731 were evaluated with *Pgt* race RKQQC. For inoculation of QTHJC and RKQQC, urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42°C water bath for 5 minutes. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto two to three leaf stage seedlings. Inoculated plants were incubated in a dew chamber at 20 ± 1°C and 100% relative humidity for 16 h and then placed in a growth chamber at 20 ± 1ºC with a 16 h light/ 8 h dark cycle. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings yielding a low infection type of ; to ;1 were considered resistant and seedlings yielding a high infection type of 3- to 4 were considered susceptible.

For inoculation of *Pgt* race TTKSK, urediniospores were removed from storage at -80°C and heat shocked at 45°C for 15 min. Spores were rehydrated by placing spore capsules in an air-tight container at 80% humidity maintained by a KOH solution for 2 to 4 h. Urediniospores were

then suspended in a light-weight mineral oil (Soltrol 70; Conoco- Phillips Inc., Houston) and sprayed onto seedlings. Plants were placed in dew chambers overnight as described previously (Jin et al., 2006). After dew chamber incubation, plants were kept in a greenhouse maintained at 25 ± 2°C. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings yielding a low infection type of 1- to 2; to TTKSK were considered resistant and seedlings yielding a high infection type of 3- to 4 were considered susceptible.

BC_1F_1 plants derived from direct crossing with TA10171 and TA10187 were assayed with *P. graminis* f.sp. *tritici* (Pgt) race QTHJC that is avirulent to *Sr10171* and *Sr10187*.

In the U6721 mapping population, 107 BC_2F_1 plants were assayed using RKQQC. Progeny testing of 10 individuals from 107 BC_2F_2 families of U6721 was done with RKQQC to confirm resistant and susceptible BC_2F_1 phenotypes. In the U6731 mapping population, 105 BC_2F_1 plants were assayed using RKQQC. Progeny testing of 10 individuals from 105 BC_2F_2 families of U6721 was done with RKQQC to confirm resistant and susceptible BC_2F_1 phenotypes.

To confirm effectiveness of *Sr10171* against TTKSK, 24 individuals from 10 RKQQC-resistant and 10 RKQQC-susceptible BC_2F_2 families from the U6721 mapping population, the *Ae. tauschii* donor accession TA10171, and the recurrent parent KS05HW14 were assayed with stem rust race TTKSK. Likewise, to confirm *Sr10187* effectiveness against TTKSK, 24 individuals from 10 RKQQC-resistant and 10 RKQQC-susceptible BC_2F_2 families from the U6731 mapping population and the *Ae. tauschii* donor accession TA10187 were assayed with stem rust race TTKSK. Additionally, 48 BC_1F_2 progenies from BC_1F_1 stem rust-resistant donor plants U6584-2 and U6586-1 used to generate the BC_2F_1 mapping populations U6721 and U6731, respectively, were assayed with TTKSK.

DNA isolation and bulked segregant analysis

Genomic DNA from U6721, U6731 BC_2F_1 mapping populations and TA10171, TA10187 and KS05HW14 parents was isolated using BioSprint 96 DNA Plant Kits (Cat. No. 941558) and a BioSprint 96 robot following manufacturers instructions (Qiagen, Valencia, CA).

Bulked segregant analysis was done according to Olson et al. (2012). Briefly, genomic DNA of 8 resistant and 8 susceptible BC_2F_1 plants from U6721 and U6731 were pooled to generate resistant and susceptible bulks. A set of 70 D genome-specific SSR markers comprised of 5 markers per chromosome arm were amplified from the resistant and susceptible bulks.

Molecular marker analyses and genetic mapping

Four SSR loci on 7DS including *Xcfd30*, *Xgdm88*, *Xwmc463*, and *Xwmc827* were evaluated on the U6721 mapping population. Marker loci were amplified following the PCR conditions described in Olson et al. (2012) (3.3.1, Figure 3.3). SSR loci *Xbarc70* and *Xgwm130*, distally located on 7DS, were evaluated on U6721 to confirm a recombination breakpoint distal to *Sr10171*. All markers were scored as co-dominant.

Three SSR loci on 6DS were evaluated on the U6731 mapping population including *Xbarc173*, *cfd49* and *psp3200*. Marker loci were amplified following the PCR conditions described in Olson et al. (2012) (Table 3.1, Figure 3.4) and scored as co-dominant.

Sizing of PCR products was performed by capillary electrophoresis using a 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). Genetic linkage analysis was performed using MAPMAKER v 3.0 (Lander et al. 1987). Marker orders were established using multipoint analysis and the Kosambi centimorgan function with a minimum LOD of 3.0 and validated using the ripple function. Segregation of marker loci and stem rust resistance was evaluated using a $\chi 2$ goodness-of-fit test.

Results

Stem rust resistance from TA10171

Direct hybridization and population development

One F_1 plant was recovered by embryo rescue from direct crossing of KS05HW14 with TA10171. The male sterile F_1 plant was backcrossed as a female to KS05HW14 and 13 BC_1F_1

seed were generated. Six BC_1F_1 seedlings were screened with stem rust race QTHJC. Two BC_1F1 seedlings yielded resistant infection types of ;1= and four yielded susceptible infection types of 3 to 34. A single QTHJC-resistant BC_1F_1 plant called U6584-2 was used as the male stem rust resistance donor parent to generate the BC_2F_1 mapping population, U6721.

Stem rust phenotypic assay

The U6721 mapping population, BC_2F_1 plants resistant to RKQQC yielded low infection types of ; to ;1 and susceptible individuals yielded high infection types of 3 to 4. The stem rust susceptible recurrent parent KS05HW14 yielded a high infection type of 3+4 to RKQQC and the donor parent TA10171 yielded a low infection type of ; (Figure 3.1). The segregation of 42 resistant: 65 susceptible in BC_2F_1 individuals of U6721 shows distorted segregation from 1:1 segregation for stem rust resistance (χ^2=4.94, p=0.026), however, expression of TA10171-derived resistance in heterozygous individuals indicates resistance is completely dominant.

Resistant and susceptible reactions to RKQQC were confirmed by progeny testing of 10 BC_2F_2 progeny from each BC_2F_1 plant in U6721. BC_2F_2 progenies from RKQQC-resistant BC_2F_1 plants segregated for resistance with low infection types of ; to ;1 and high infection types of 3- to 4. All BC_2F_2 progenies from RKQQC-susceptible BC_2F_1 plants were susceptible to RKKQC with infection types of 3- to 4 (Table 3.2).

The *Ae. tauschii* accession TA10171, recurrent hexaploid parent KS05HW14, and 24 individuals from 10 RKQQC-resistant and 10 RKQQC-susceptible BC_2F_2 families from U6721 were assayed with TTKSK to confirm resistance and susceptibility (Table 3.2). TA10171 yielded a low infection type of ; and KS05HW14 yielded a high infection type of 4 to TTKSK. All BC_2F_2families yielding low infection types to RKQQC were confirmed to yield low infection types of ;2- to 2 to TTKSK (Figure 3.1). All BC_2F_2 families yielding high infection types to RKQQC were confirmed to yield high infection types of 3+ to 4 to TTKSK (Table 3.2). These results demonstrate that *Sr10171* mapped using RKQQC confers resistance to TTKSK.

Bulked segregant analysis and mapping of Sr10171

The 151bp allele from TA10171 at *Xwmc827* was polymorphic between resistant and susceptible bulks. The resistant bulk was heterozygous for the 151bp allele and the 146bp KS05HW14 alleles at *Xwmc827* whereas the susceptible bulk was homozygous for the 146bp allele from KS05HW14. Polymorphism between resistant and susceptible bulks at *Xwmc827* identified 7DS as the chromosome location of *Sr10171*.

The four loci linked to *Sr10171* on 1DS cover a genetic distance of 5.8 cM in U6721 (Table 3.1)(Figure 3.3). Two co-segregating SSR loci, *Xgdm88* and *Xwmc827* map 0.9 cM proximal to *Sr10171*. The SSR loci *Xbarc70* and *Xgwm130* were fixed for the KS05HW14 allele in U6721 indicating a recombination event took place in between these loci and *Sr10171* in the female gamete forming the BC_1F_1 plant U6584-2 that was used to develop the BC_1F_1 mapping population U6721.

Stem rust resistance from TA10187

Direct hybridization and population development

From direct crossing of KS05HW14 with TA10187, four F_1 plants were recovered by embryo rescue and 11 BC_1F_1 seed were generated by backcrossing the male sterile F_1 plants to KS05HW14. Four BC_1F_1 seedlings were screened with stem rust race QTHJC. Three BC_1F_1 seedlings yielded resistant infection types of ;1 to ;2- and one seedling yielded a susceptible infection type of 33+. A single QTHJC-resistant BC_1F_1 plant called U6586-1 was used as the male stem rust resistance donor parent to generate the BC_2F_1 mapping population, U6731.

Stem rust phenotypic assay

In the U6731 mapping population, BC_2F_1 plants resistant to RKQQC yielded low infection types of ; to ;1+ and susceptible individuals yielded high infection types of 3 to 4. The stem rust susceptible recurrent parent KS05HW14 yielded a high infection type of 3+4 to RKQQC and the donor parent TA10187 yielded a low infection type of ;1= (Figure 3.2). The segregation of 39 resistant: 66 susceptible in BC_2F_1 individuals of U6731 shows distorted segregation for stem rust resistance ($p=0.008$), but as with *Sr10171*, stem rust resistance is completely dominant and low infection types are observed in heterozygous BC_2F_1 plants.

Resistant and susceptible reactions to RKQQC were confirmed by progeny testing of 10 BC_2F_2 progeny from each BC_2F_1 plant in U6731. BC_2F_2 progenies from RKQQC-resistant BC_2F_1 plants segregated for resistance with low infection types of ; to ;1 and high infection types of 3- to 4. All BC_2F_2 progenies from RKQQC-susceptible BC_2F_1 plants were susceptible to RKKQC with infection types of 3- to 4 (Table 3.3).

The *Ae. tauschii* accession TA10187, recurrent hexaploid parent KS05HW14, and 24 individuals from 10 RKQQC-resistant and 10 RKQQC-susceptible BC_2F_2 families from U6731 were assayed with TTKSK to confirm resistance and susceptibility . TA10187 yielded a low infection type of ; and KS05HW14 yielded a high infection type of 4 to TTKSK. All BC_2F_2 families yielding low infection types to RKQQC were confirmed to yield low infection types of ;1 to ;2= to TTKSK (Figure 3.2). All BC_2F_2 families yielding high infection types to RKQQC were confirmed to yield high infection types of 3+ to 4 to TTKSK (Table 3.3). These results demonstrate that *Sr10187* mapped on 6DS using RKQQC confers resistance to TTKSK.

Bulked segregant analysis and mapping of Sr10187

The 148bp allele from TA10187 at *Xcfd135* was polymorphic between resistant and susceptible bulks suggesting 6DS as the chromosomal location of *Sr10187*. The resistant bulk was heterozygous for the 148bp CDL4424 allele and the 124bp KS05HW14 alleles at *Xcfd135* whereas the susceptible bulk was homozygous for the 124bp allele from KS05HW14. Clear polymorphism between resistant and susceptible bulks at *Xcfd135* identified 6DS as the chromosome location of *Sr10187*, however, this marker was not evaluated in the U6731 mapping population.

The three loci linked to *Sr10187* on 6DS covered a genetic distance of 20.9 cM in U6731 (Table 3.1)(Figure 2). The SSR locus, *Xcfd49* maps 2.9 cM distal, and *Xbarc173* maps 13.9 cM proximal to *Sr10187*.

Discussion

Through this work we present two additional genes for resistance to stem rust race race TTKSK from diploid *Ae. tauschii*-accessions TA10171 and TA10187 on chromosomes 7DS and 6DS, respectively. The integrated direct hybridization approach used by Olson et al. (2012) delivers novel stem rust resistance genes in a hexaploid wheat breeding background with good agronomic adaptation and closely linked genetic markers for marker-assisted selection and gene pyramiding. The timeline from initial direct crosses with the diploid to mapping of *Sr* genes in a hexaploid background is three generations. Additionally, BC_2 derived populations used for mapping *Sr* genes serve a dual purpose in their use for phenotypic selection and development of a superior breeding germplasm. Novel stem rust resistance genes on 6DS and 7DS add depth to the available pool of genes for stem rust resistance breeding efforts.

As *Pgt* race TTKSK is localized to East Africa and the Middle East, identification and mapping of *Sr10171* and *Sr10187* was done with *Pgt* races QTHJC and RKQQC that are endemic to North America. All derived lines resistant to QTHJC and RKQQC were confirmed to be resistant to TTKSK, thereby validating the effectiveness of genes mapped on 6DS and 7DS.

In the BC_2F_1 mapping population, U6721, we have mapped *Sr10171* on chromosome 7DS using closely linked SSR loci. The *Xwmc827* locus, which was identified as polymorphic between resistant and susceptible bulks in the BSA, maps 0.9 cM proximal to *Sr10171* and will be informative in marker-assisted selection. Further high-resolution mapping and SNP marker development will allow for MAS of *Sr10171* in more high throughput genotyping format.

Distal markers were fixed for the recurrent parent alleles and did not segregate in the BC_2F_1 mapping population, U6721, allowing only markers located proximal to *Sr10171* to be mapped. This suggests a recombination breakpoint located very closely distal to *Sr10171* in the meiotic event leading to the female gamete that generated the BC_1F_1 plant U6584-2 used as the donor parent in the U6721 mapping population. The distal recombination breakpoint would be fixed in the BC_2F_1 mapping population, U6721.

Segregation distortion was observed for *Sr10171* stem rust resistance was observed in U6721. A possible explanation for this observation is aneuploidy for 7D the BC_1F_1 plant. The female F_1

gamete forming the BC_1F_1 plant U6584-2 may have been aneuploid for 7D. U6584-2 would then have one 7D chromosome from the recurrent parent male gamete and multiple 7D chromosomes from the female gamete. Somatic chromosome counts were not made on the BC_1F_1 plant U6584-2 to address the effects of aneuploidy on the deviation of stem rust resistance from the expected 1:1 ratio, as the deviation became apparent only after phenotypic evaluation of the U6721 mapping population. However, aneuploidy is a likely explanation as the F_1 hybrid plant (KS05HW14/TA10171) pollinated as a female also produced viable F_2 seed with 2n= 40 to 44 chromosomes (data not shown). Self-pollination is very unlikely in a 28-chromosome ABDD plant, suggesting a spontaneous doubling event during embryo culture generating a 56-chromosome AABBDDDD F_1 plant. Another possibility is that gametes carrying *Sr10171* or some linked factor, suffer a fitness penalty thereby decreasing the frequency of *Sr10171* in backcross generations.

TA10187-derived stem rust resistance maps distally on chromosome 6DS. Stem rust resistance in the BC_2F_1 population U6731 followed segregation of a single dominant resistant gene. The *Xcfd49* locus maps 1.9 cM distal to *Sr10187* and should be useful in MAS. The most closely linked proximal locus is *Xbarc173* at 13.6 cM. Marker development in the region proximal to *Sr10187* and high-resolution mapping will yield a more closely linked proximal marker for use in SNP genotyping assays.

Both *Sr10171* and *Sr10187* can be pyramided on the same chromosome with other Ug99-effective *Sr* genes. *Sr10171* is linked very closely in repulsion to *Sr55* (*Lr34*) on 7DS (Krattinger et al., 2009). A recombinant carrying both genes would be highly desirable to combine high levels of stem rust resistance with pleiotropic effects on leaf rust, stripe rust and powdery mildew resistance. Further, the combination of seedling resistance genes and adult plant resistance genes for stem rust resistance has been shown to confer higher levels of resistance than each gene independently (Hiebert et al., 2011). *Sr10187* is linked in repulsion approximately 5cM distal to *Sr42* (Ghazvini et al., 2012) and *SrCad* (Hiebert et al., 2011). If *Sr42* and *SrCad* occupy different loci, all three genes could be phased in coupling to generate a three-gene pyramid on 6DS to enhance the durability of each resistance gene while targeting marker-assisted selection to a single chromosome. Both *Sr10171* and *Sr10187* will serve to complement existing stem rust

resistance genes in chromosome-specific gene pyramids that will enhance their utility in breeding and long term durability.

The D genome of *Ae. tauschii* is a highly tractable source of allelic variation for *T. aestivum* as linkage drag associated with chromosomal regions from *Ae. tauschii* can be eliminated through normal meiosis and phenotypic selection in segregating progenies. In direct hybridization with diploid *Ae. tauschii* accessions, adapted allelic complexes of the A and B genomes are undisrupted allowing for agronomic type to be recovered within two backcrosses. In contrast, crossing to primary synthetics composed of the A and B genomes of a durum or wild *T. dicoccoides* genotype and the D genome of an *Ae. tauschii* accession requires multiple backcrosses or topcrosses to restore adaptive alleles across all three genomes and recover segregants with desirable agronomic potential.

The diversity of stem rust resistance genes from *Ae. tauschii* and the feasibility of gene transfer underscores the value of this genetic resource in meeting challenges to wheat production. This study further highlights that genes from exotic D genome sources are highly accessible and readily transferrable to hexaploid wheat. Phenotyping accessions of exotic and wild relatives for disease resistance and abiotic stress tolerance serve as a starting point for it their use in wheat improvement. The utility in providing agronomic improvements are fully realized upon the transfer of the underlying genes to cultivated wheat backgrounds that can be used in breeding. In the case of *Ae. tauschii*, the shift from identification of phenotypes in wild accessions to mapping the genes underlying target phenotypes and developing useful breeding germplasm is three to four generations by direct hybridization. *Ae. tauschii* and wild wheat relatives will remain a valuable genetic resource for future generations to meet the continuously evolving challenges to wheat production.

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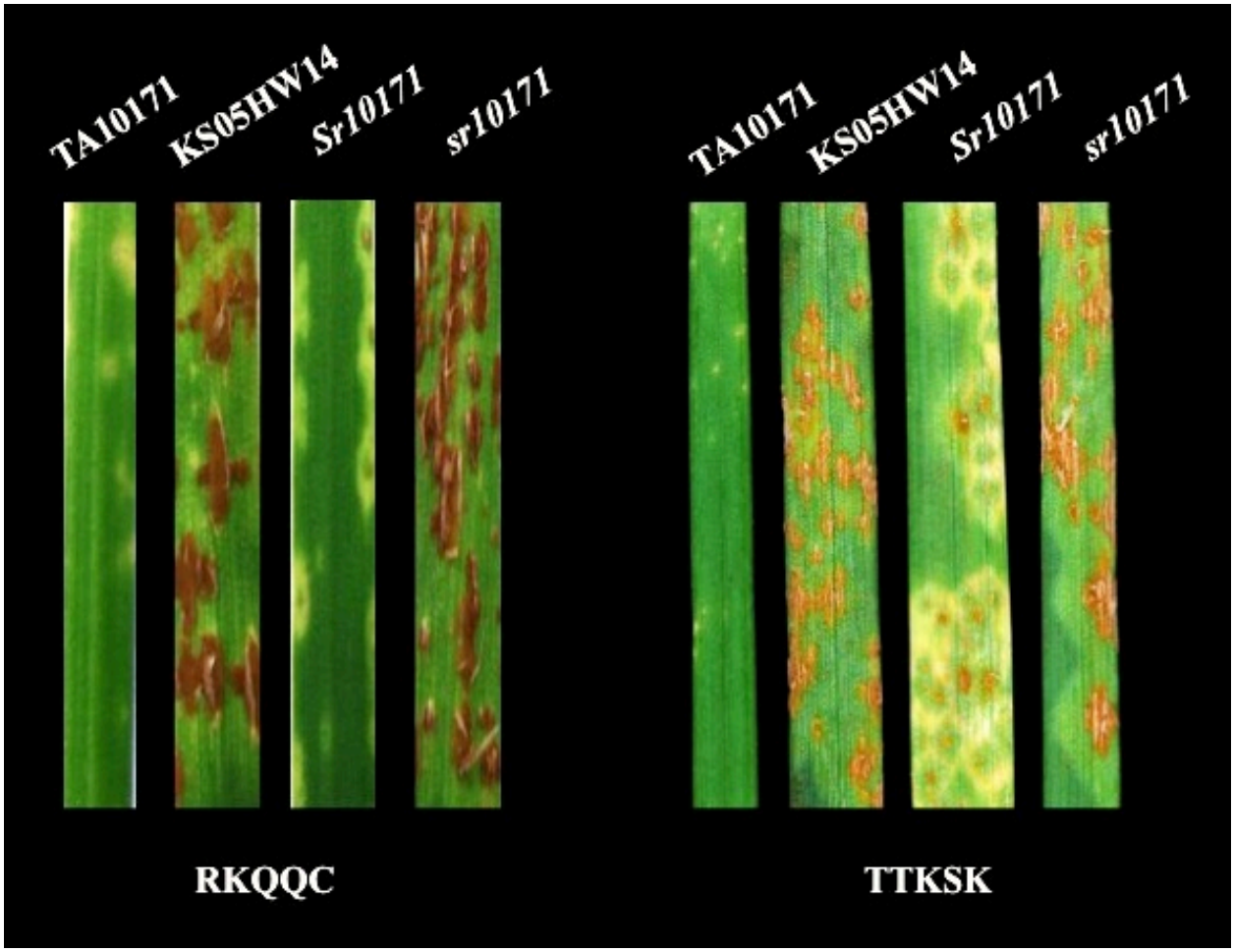
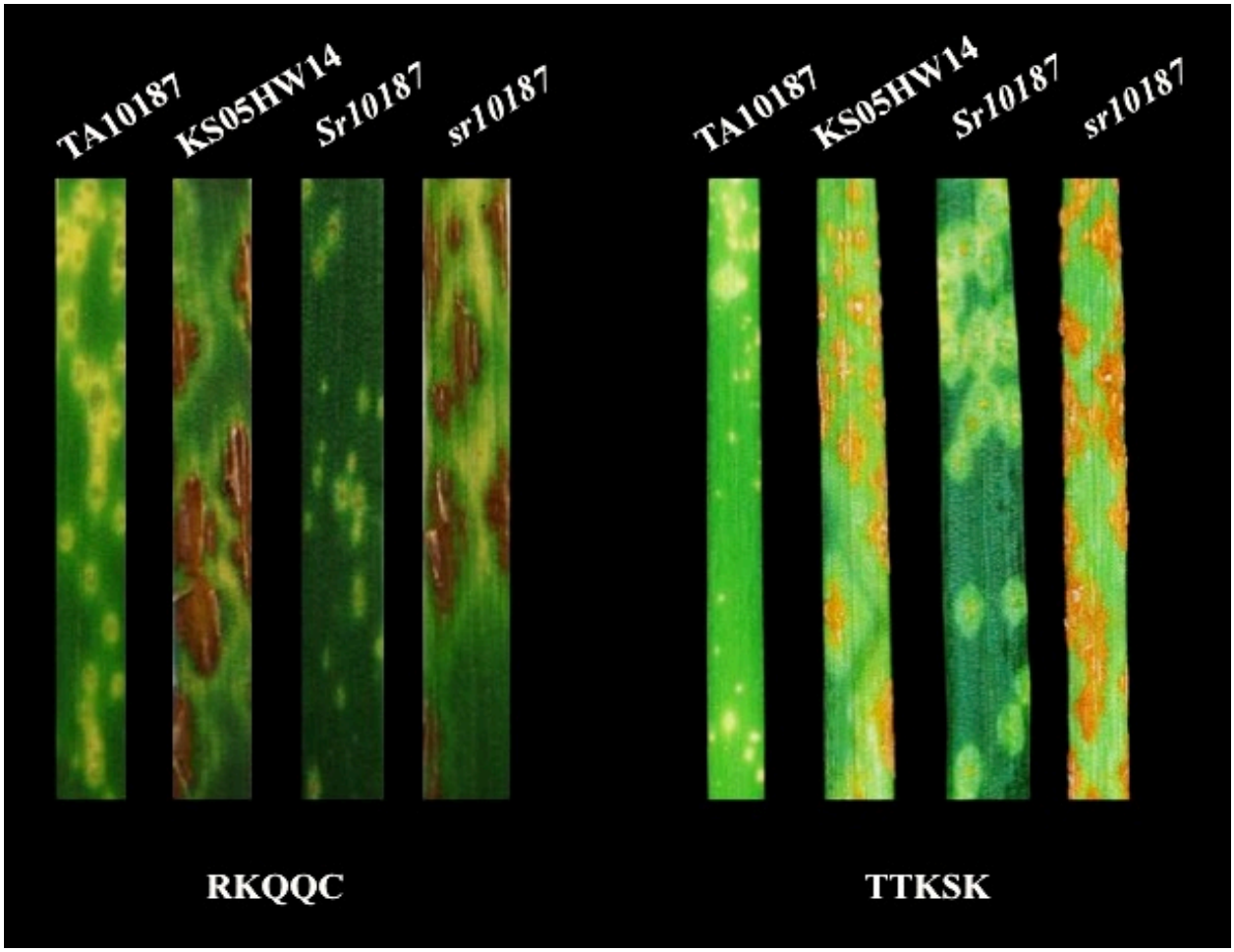
Figure 3.1 *Sr10171* infection types



Infection types to stem rust races RKQQC and TTKSK of *Aegilops tauschii* accession TA10171, recurrent parent hard winter wheat line KS05HW14 and backcross progenies with (*Sr10171*) and without (*sr10171*) TA10171-derived stem rust resistance.

Figure 3.2 *Sr10187* infection types



Infection types to stem rust races RKQQC and TTKSK of *Aegilops tauschii* accession TA10187, recurrent parent hard winter wheat line KS05HW14 and backcross progenies with (*Sr10187*) and without (*sr10187*) TA10187-derived stem rust resistance.

Figure 3.3 Genetic map of *Sr10171* on 7DS

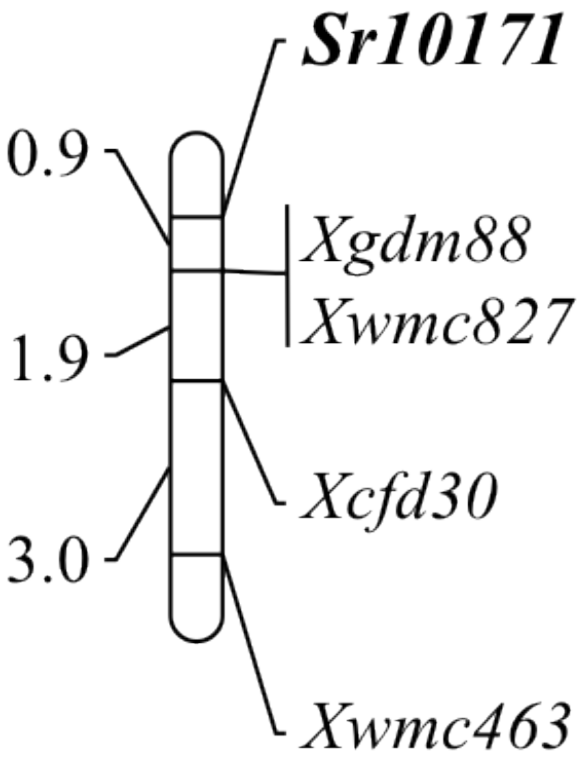


Figure 3.4 Genetic map of *Sr10187* on 6D

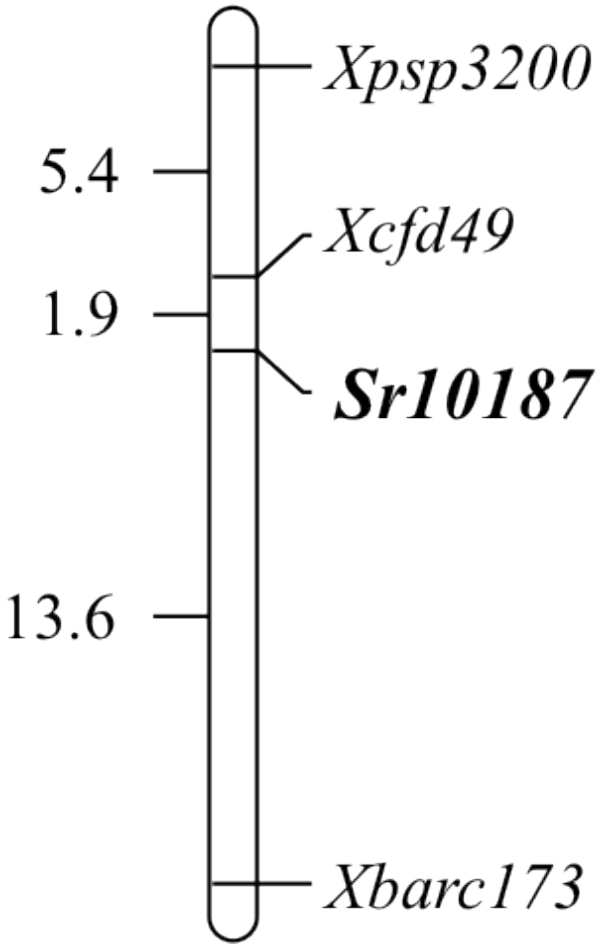


Table 3.1 Marker allele sizes for TA10171, TA10187 and KS05HW14

Locus	TA10171	TA10187	KS05HW14
Xwmc827	132		171
Xgdm88	118		116
Xcfd30	177		174
Xwmc463	156		152
Xpsp3200		182	165
Xcfd49		196	219
Xbarc173		275	237

Marker allele sizes are given in base pairs, from the *Sr10171* and *Sr10187 Aegilops tauschii* donor accessions TA10171 and TA10187, respectively, and the hard winter wheat recurrent parent, KS05HW14.

Table 3.2 *Sr10171* infection types of segregating BC_2F_2 from U6721

Line	TTKSK[a]	RKQQC[b]
KS05HW14	4	3+
TA10171	;0	;
U6721-002	3,3+	3
U6721-062	3hif,4	3
U6721-063	3,4	3
U6721-086	3,3-	3
U6721-094	3,3-	3
U6721-102	3	3
U6721-157	3	33-
U6721-212	3,3+	3
U6721-215	3-	33-
U6721-238	3	3-
U6721-026	;1+h3[c]	;1
U6721-056	1+2h3	;1
U6721-061	1h4	;1
U6721-093	12+h3	;1
U6721-100	2h3	;1-
U6721-187	;2,2h3	;1
U6721-191	;2,2h3	;1
U6721-222	2h3	;1
U6721-223	2h3	;1=
U6721-241	2h3	;1-

[a] indicates phenotype in segregating BC_2F_2 plants
[b] indicates phenotypes of BC_2F_1 plants
[c] segregation for resistant and susceptible phenotypes indicated by h

Table 3.3 *Sr10187* infection types of segregating BC_2F_2 from U6731

Line	TTKSK[a]	RKQQC[b]
KS05HW14	4	;
TA10187	;	;1=
U6731-033	3	3
U6731-064	3,3+	3
U6731-121	3+4	3
U6731-124	3+	3
U6731-126	4	33-
U6731-135	4	3
U6731-147	4	3-
U6731-148	4	3
U6731-159	3,4	3
U6731-160	34	3
U6731-004	;1h3[c]	;
U6731-037	1-h3	;
U6731-039	;1h3	;
U6731-060	;1h3+	;
U6731-062	1;h4	;
U6731-114	;1-h3	;1
U6731-146	;2--h4	;1
U6731-157	;2--h4	;1
U6731-014	;1-h4	;1=
U6731-058	1h3	;1=

[a] indicates phenotype in segregating BC_2F_2 plants
[b] indicates phenotypes of BC_2F_1 plants
[c] segregation for resistant and susceptible phenotypes indicated by h

Chapter 4 – Transfer and recovery of additional Ug99 stem rust resistance from the D genome species, *Aegilops tauschii*

Introduction

Wheat stem rust caused by the basidiomycete fungus, *Puccinia graminis* Pers.:Pers f. sp. *tritici* Eriks. & E. Henn (*Pgt*) has been the most destructive pathogen of wheat. Genetic resistance has been a successful means of controlling this disease, however differences exist in the durability of individual resistance genes. The stem rust resistance gene *Sr31* is widely deployed across global wheat germplasm and has provided resistance to stem rust for decades. The identification of *Pgt* race TTKSK with virulence to *Sr31* in combination with virulence to most stem rust resistance genes in cultivated wheat presents a serious threat to wheat production (Singh et al., 2011a).

The wild relatives of wheat have been a valuable resource for resistance to an array of pathogens and pests of wheat (Friebe et al., 1996). Many stem rust resistance genes effective against TTKSK come from wild relative species transferred by cytogenetic methods and chromosome engineering (Qi et al., 2007). The D genome species of *Aegilops tauschii* Coss. represents a highly accessible source of resistance genes that are readily transferrable to wheat by direct hybridization (Gill and Raupp, 1987). In the following work, an integrated approach was attempted to simultaneously transfer, genetically map and introgress stem rust resistance genes from multiple *Ae. tauschii* accessions in parallel. Crosses with all accessions were initiated during the same greenhouse season. However, many complications prevented the movement of genes from all accessions in parallel including fertility and tissue culture problems, recessive gene action, race specificity of resistance, and the presence of multiple race-specific genes. Resistance to *Pgt* race TTKSK was transferred, mapped and introgressed to a wheat breeding genotype from three *Ae. tauschii* accessions CDL4424, TA1662 (Chapter 2), TA10171 and TA10187 (Chapter 3). TTKSK resistance from *Ae. tauschii* accessions CDL4366, TA1615, TA1642, TA1693 has been recovered in segregating backcross and topcross derived populations is reported in this chapter.

Materials and Methods

Plant Materials

Five *Ae. tauschii* accessions including CLD4366, TA1615, TA1642, TA1693 and TA1718 were targeted to transfer resistance to stem rust race TTKSK to hexaploid wheat by direct crossing. Race specificity of resistance is for each accession is given in Table 4.1. The *T. aestivum* recurrent parent is hard white winter wheat line KS05HW14 produced by the Kansas State University wheat breeding program by Dr. Joe Martin. KS05HW14 is susceptible to *Pgt* races TTKSK, TTKST (Ug99 + *Sr24* virulence), TTTTF, TPMKC, RKQQC and QTHJC. Additional hard winter wheat lines used as male and female parents include Duster, a hard red winter wheat developed at Oklahoma State University, Fuller, a hard red winter wheat developed at Kanas State University, Lakin, a hard white winter wheat developed at Kansas State University, Overley, a hard red winter wheat developed at Kansas State University, Postrock, a hard red winter wheat released by AgriPro-Sygenta, TAM304, hard red winter wheat cultivar developed at Texas A&M University, or WL711, a widely adapted hard red spring wheat from India.

Population development began with pollination of emasculated florets of KS05WH14 with *Ae. tauschii* accessions using the approach method (Rosenquist, 1927). Embryo rescue and generation of F_1 hybrids was done according to Olson et al. (2012). F_1 plants were grown in the greenhouse for backcrossing under 10h of supplemental light at 21°C ± 4C° six weeks and then transitioned to16h of supplemental light at 23°C ± 3C°C. At female maturity, spikes of F_1 hybrids were pollinated by KS05HW14, Duster, Fuller, Lakin, Overley, Postrock, TAM304 or WL711 to generate BC_1F_1 and topcross F_1 (TCF_1) seed. Seed was germinated by placing on moist filter paper in germination boxes at 4°C for one week and then at ambient room temperature, 21°C ± 2C° for 24h. BC_1F_1 and TCF_1 plants were self pollinated to produce BC_1F_2 and F_2 families or used as males or females in a second backcross or topcross to KS05HW14 or a hard red winter wheat parent. $BC_1F_{2:3}$ and $F_{2:3}$ families were developed from CDL4366-derived lines. After stem rust assay, all plants were vernalized for 6 weeks at 4°C and grown in the greenhouse at 24 ± 3°C with a 16 h light/ 8 h dark cycle for crossing and self-pollination. The direct crossing strategy is outlined in Figure 4.1

Stem Rust Assay

Inoculation of *Pgt* race QTHJC, RKQQC and TPMKC was done at Kansas State University. Urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42°C water bath for 5 minutes. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto two to three leaf stage seedlings. Inoculated plants were incubated in a dew chamber at 20 ± 1°C and 100% relative humidity for 16 h. Inoculated seedlings were placed either in a growth chamber at 20 ± 1ºC with a 16 h light/ 8 h dark cycle or in a greenhouse at 24 ± 3°C with a 16 h light/ 8 h dark cycle. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962).

Inoculation with *Pgt* race TTKSK were done by USDA-ARS Cereal Disease Lab scientists in a BL3 facility at the University of Minnesota, in St. Paul, MN. TTKSK urediniospores were removed from storage at -80°C and heat shocked at 45°C for 15 min. Spores were rehydrated by placing spore capsules in an air-tight container at 80% humidity maintained by a KOH solution for 2 to 4 h. Urediniospores were then suspended in a light-weight mineral oil (Soltrol 70; Conoco- Phillips Inc., Houston) and sprayed onto seedlings. Plants were placed in dew chambers overnight as described previously (Jin et al., 2006). After dew chamber incubation, plants were kept in a greenhouse maintained at 25 ± 2°C. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings yielding a low infection type of 2- to 2+ to TTKSK were considered resistant and seedlings yielding a high infection type of 3- to 4 were considered susceptible.

Seedlings derived from direct crosses with CDL4366 were screened with either RKQQC or TPMKC. BC_1F_1 and TC_1F_1 seedlings derived from CDL4366 were assayed with *Pgt* race RKQQC to select for resistance. Segregating BC_1F_2, TC_1F_2, and TC_2F_1 families derived from CDL4366 were assayed with TPMKC including three BC_1F_2 families were screened with TPMKC (KS05HW14/CDL4366//KS05HW14), four F_2 families from Duster topcrosses (KS05HW14/CDL4366//Duster), six F_2 families from Lakin topcrosses (KS05HW14/CDL4366//Lakin), one Postrock topcross F_2 family (KS05HW14/CDL4366//Postrock) and 18 topcross F_1 seedlings with mixed pedigrees. Infection types to TPMK for CDL4366-derived lines are given in Table 4.5.

BC_1F_1 and TC_1F_1seedlings derived from direct crosses with TA1615, TA1642, TA1693 and TA1718 were assayed with QTHJC, TPMKC or RKQQC. Three BC_1F_1 seedlings derived from TA1615 (U6562), one BC1F1 seedling from TA1642 (U6567), two TC_1F_1 seedlings from TA1693 (U6573) and two BC_1F_1 seedlings from TA1718 (U6580) were assayed with QTHJC. Five TC_1F_1 plants derived from TA1615 (U6564), seven BC_1F_1 (U6567) and 10 TC_1F_1 (U6569, U6570, U6571, U6572) plants derived from TA1642 were assayed with TPMKC. One TA1642-derived BC_1F_1 (U6567) seedling, one TC_1F_1 seedling from a TA1693 (U6573), and two TA1718 BC_1F_1 (U6580) and TC_1F_1 (U6583) seedlings were screened with RKQQC.

Backcross and topcross families derived from direct crosses with *Ae. tauschii* accessions were assayed with *Pgt* race TTKSK. From CDL4366, 27 $BC_1F_{2:3}$ or $TC_1F_{2:3}$ families were selected for TTKSK assay based on resistance or susceptibility to TPMKC. All TA1615-derived BC_1F_2 and TC_1F_2 families, 17 in total, were assayed with TTKSK. From TA1642, all 19 BC_1F_2 families were assayed. From TA1693, two TC_1F_2 families were assayed. From TA1718, three BC_1F_2 and one T_1CF_2 families were assayed. Infection types of all families screened with TTKSK are given in Tables 4 through 8.

Results

CDL4366

From direct crosses between the diploid *Ae. tauschii* CDL4366 with the hexaploid wheat KS05HW14, 16 F_1 hybrid plants were recovered. An average of 2.2 BC_1 and TC_1 seed were recovered per spike pollinated with either Duster, KS05HW14, Lakin or Postrock yielding 65 backcross and topcross seed (Table 4.2). From these only 14 plants produced F_2 progenies. Due to fertility problems, very few F_2 seed were recovered from most plants. As plants were found to be sterile as males, seven TC_1 plants were pollinated as females to generate TC_2F_1 seed. Taken together, this suggested high levels of sterility possibly due to aneuploidy or genomic incompatibility.

Assay of BC_1F_1 and TC_1F_1 seedlings derived from CDL4366 with RKQQC yielded intermediate phenotypes from 2+ to 3 that were inconclusive. All RKQQC-assayed seedlings were then self pollinated in order to score stem rust resistance in segregating BC_1F_2 and TC_1F_2 families. Clear

segregation of resistance to *Pgt* race TPMKC was observed in BC_1F_2 and TCF_2 progenies of CDL4366-derived lines (Table 4.5). Infection types ranged from ;2+, 2-, 2++ in resistant individuals to 2+3, ;3, 3 LIF in intermediate types and 3 to 4 in susceptible genotypes.
The two $F_{2:3}$ families from TPMKC-susceptible F_2 plants were confirmed susceptible to TTKSK. Of the 25 $BC_1F_{2:3}$, $TC_1F_{2:3}$, or TC_2F_2 families derived from BC_1F_2, TC_1F_2 or TC_2F_1 plants with low infection types of ;2 to 2++ to TPMKC, all 25 segregated for infection types from 2- to 2+ to TTKSK (Table 4.5).

TA1615

In direct crosses with TA1615, 18 fertilized karyopses were collected from three spikes leading to the recovery of three F_1 hybrid plants (Table 4.3). Backcrossing the sterile F_1 hybrids to KS05HW14 resulted in 7 BC_1F_1 seed. TC_1F_1 seed was obtained by pollinating F_1 plants with Postrock, Duster, Lakin and TAM304 (Table 4.4). Not all BC_1F_1 and TC_1F_1 seed were viable upon germination.

Three BC_1F_1 seedlings, U6562-01, U6562-02 and U6562-03 were screened with QTHJC. All seedlings yielded low infection types of ;12-, ;1+ and 2C (Table 4.6). One BC_1F_1 plant was used as a male to generate a BC_2F_1 population of 270 individuals that was assayed with QTHJC. In the BC_2F_1, stem rust resistance : susceptibility followed a 2:1 ratio (p=0.60) which suggests two genes, possibly linked. Resistant plants showed low infection types of 0; and 2 while susceptible plants showed infection types of 3 to 4.

BC_1F_2 families from U6562 demonstrated race-specific resistance. The BC_1F_2 family from U6562-01 segregated for resistance to TTKSK with resistant plants showing infection types of 2 to 2+ and susceptible plants showing infection types of 4 (Table 4.6). The infection types for resistance to TTKSK in the BC_1F_2 were more similar to the 2 infection type to QTHJC observed a BC_2F_1 population derived from the BC_1F_1 plant U6562-01. The 0; infection type observed in the BC_2F_1 against QTHJC was not observed BC_1F_2 against TTKSK suggesting multiple race-specific genes transferred from TA1615 in the BC_1F_1 plant, U6562-01. BC_1F_1 plants U6562-02 and U6562-03 showed a low infection types of ;1+ and 2C to QTHJC, respectively, however,

BC_1F_2 progeny from these plants were all susceptible to TTKSK showing high infection types of 32+, 3 and 4. This clearly demonstrates that resistance to QTHJC from TA1615 is race-specific.

Five F_1 seedlings from topcrosses to Postrock (KS05HW14/TA1615//Postrock) were assayed with TPMKC (Table 4.6). All five F_1 seedling yielded intermediate to high infection types of 23C, 3 and 4. F_2 families from all six Postrock topcrosses were assayed with TTKSK. Four of the six families that were all susceptible to TPMKC, segregated for resistance to TTKSK showing low infection types of 2 to 2+ and high infection types of 3 to 4. Two families showed only high infection types to TTKSK. This further demonstrates that TTKSK resistance from TA1615 is race-specific.

Eight F_1 seedlings from topcrosses to Lakin (KS05HW14/TA1615//Lakin) were assayed with QTHJC (Table 4.6). Five seedlings showed low infection types of 2= to QTHJC, one showed an intermediate phenotype of 1+3X, one showed a high infection type of 3 and one seedling germinated slowly resulting in an escape. Eight F_2 families from Lakin topcrosses were screened with TTKSK. Two families from TPMKC resistant plants segregated for resistance to TTKSK showing low infection types of 2 and high infection types of 3 to 4. Three families from TPMKC resistant plants were all susceptible to TTKSK showing high infection types of 3 to 4. The F_2 family from the F_1 plant showing the intermediate 1+3X infection type segregated for resistance to TTKSK showing a low infection type of 2 and a high infection type of 3-. The F_2 family from the TPMK-susceptible F_1 plant was susceptible to TTKSK along with the remaining F_2 family with no data on TPMK. These families from Lakin topcrosses suggest multiple genes for resistance to TTKSK. One gene for TTKSK resistance also confers resistance to TPMKC and based on the Postrock topcross families, another gene confers resistance to TTKSK and not to TPMKC.

TA1642

Direct crosses between KS05HW14 and TA1642 yielded 33 fertilized karyopses from 7 pollinated spikes. Ten F_1 hybrid plants were recovered (Table 4.3). Backcrossing of F_1 hybrids to KS05HW14 yielded 17 BC_1F_1 seed. Topcrossing F_1 hybrids to Duster, Fuller, Lakin, Overley,

Postrock TAM304 and WL711 generated a total of 136 TC_1F_1 seeds (Table 4.4). Not all BC_1F_1 and TC_1F_1 seed were viable upon germination, however, 19 viable F_1 seedlings were germinated.

A single seedling germinated for assay with QTHJC that yielded a high infection type of 34. Seven BC_1F_1 seedlings were assayed with TPMKC and one seedling was assayed with RKQQC, all of which yielded high infection types of 3, 3+ and 4. Of the eight BC_1F_2 families derived from TPMKC and RKQQC susceptible BC_1F_1 plants, seven segregated for resistance to TTKSK with low infection types of 2 to 2+ and high infection types of 3, 3+ and 4. A single BC_1F_2 family from a TPMKC-susceptible BC_1F_1 plant was susceptible to TTKSK with infection types of 32+ and 3 (Table 4.7).

Seven TC_1F_1 seedlings from topcrosses to Postrock were assayed with TPMKC. Four F_1 seedlings yielded high infection types of 3 to TPMKC. Two F_1s yielded intermediate infection types of 2-3 and 23C. One F_1 yielded an infection type of ;2+. Three F_2 families derived from topcrosses to Postrock segregated for resistance to TTKSK with low infection types of 2 to 2+ and high infection types of 3- to3+ (Table 4.7).

Additional F_1 seedlings were recovered from topcrosses to Lakin, TAM304 and WL711. The Lakin F_1 yield a high infection type of 3 to TPMKC. The TAM304 F_1 yielded a low ;2+Y infection type. The WL711 F_1 yielded a low infection type of ;1. Segregation for TTKSK resistance was observed in the F_2 familiy derived from the Lakin TC_1F_1 with a low infection type of 2 to 2+. The TAM304-derived F_2 family was susceptible to TTKSK with a high infection type of 3 to 4. The WL711-derived F_2 family yielded intermediate types of 2++ to 32+ and high infection types of 4 (Table 4.7).

TA1693

From direct crosses with TA1693, 74 fertilized karyopses were recovered from 4 pollinated spikes. From embryo rescue, eight F_1 plants were recovered (Table 4.3). No BC_1F_1 seed were recovered and most TCF_1 plants did not germinate (Table 4.4). Extensive leaf variegation was observed in F_1 hybrids and F_1 seedlings derived from topcrossess to Postrock.

Only two viable seedlings derived from topcrosses to Postrock were recovered for screening with QTHJC and RKQQC yielding intermediate infection types of ;3 and 3;, respectively. One F_2 family yielded resistant infection types to TTKSK with low infection types of 2 and high infection types of 3. The second F_2 family yielded clearly susceptible infection types of 3 and 4 to TTKSK (Table 4.8).

TA1718

Direct crosses with TA1718 yielded 26 karyopses from two pollinated spikes. Three F_1 hybrid plants were recovered by embryo rescue (Table 4.3). Backcrossing F_1 hybrids yielded three BC_1F_1 seed. Additional topcrosses were made to Lakin, Overley and Postrock (Table 4.4).

Two BC_1F_1 seedlings derived from TA1718 were assayed with QTHJC yielding intermediate infection types of ;3Z and 2+3. An additional BC_1F_1 seedling and one F_1 seedling derived from an Overley topcross were assayed with RKQQC. The BC_1F_1 yielded a high infection type of 33+ and the TCF_1 yielded a low infection type that may have been due to *Sr38* from Overley. BC_1F_2 and TCF_2 families from each F_1 were assayed with TTKSK. Two BC_1F_2 families segregated for low infection types of 2+ and 2+LIF and high infection types of 3. One BC_1F_2 family yielded only high infection types of 3 and 4. The F_2 family derived from a topcross to Overley segregated for resistant infection types of ;2 and 2 and intermediate infection types of 3;2, 13Z and 23X (Table 4.9).

A BC_1F_2 and the Overly topcross F_2 segregated for a hybrid necrosis gene. The mechanism of the gene for hybrid necrosis is dominant as the BC_1F_1 and TCF_1 plants exhibited the hybrid necrosis phenotype. The hybrid necrosis gene segregated independently as a high frequency of stem rust-susceptible plants exhibited the necrosis phenotype. Hybrid necrosis began early in development, at the two-leaf stage and continued in adult plants extending to all organs. Hybrid necrosis had no observable effect on fertility.

Discussion

Despite confounding effects of fertility problems, multiple race-specific resistance genes and recessive gene action resistance to *Pgt* race TTKSK was recovered from *Ae. tauschii* accessions

CDL4366, TA1615, TA1642, TA1693 and TA1718. In the case of CDL4366 backcross and topcross progenies were recovered but few derived lines could be generated with high levels of fertility. Backcross and topcross seed derived fromTA1693 and TA1718 had low viability. Multiple race-specific resistance genes were transferred from TA1615. BC_1F_1 and TCF_1 plants derived from TA1642 as well as TA1693 and TA1718, showed susceptibility to stem rust, but segregated for resistance in F_2 populations suggesting recessive resistance.

Fertility problems were encountered in backcross and topcross progenies derived from direct crosses with CDL4366 as demonstrated by the high levels of sterility among BC_1F_1 and TCF_1 plants, and low number of F_2 progenies. Many BC_1F_1 and TCF_1 plants were male sterile and were pollinated as females in order to produce enough progenies to recover CDL4366-derived stem rust resistance.

By selecting for resistance to *Pgt* race TPMKC in CDL4366-derived progeny, TTKSK resistance was recovered in segregating families. All 25 families derived from TPMKC-resistant plants segregated for resistance to TTKSK. CDL4366-derived resistance to TTKSK will be recovered through backcrossing and selection using *Pgt* race TPMKC. Genetic analysis and mapping of stem rust resistance from CDL4366 can be done in BC_1F_1 or F_2 populations.

Multiple genes for race-specific resistance to stem rust were transferred from *Ae. tauschii* accession TA1615. Two BC_1F_2 families from BC_1F_1 plants resistance to QTHJC show susceptibility to TTKSK demonstrating that resistance to QTHJC is conferred by a different gene than resistance to TTKSK. Segregation of race-specific resistance was confirmed in the Lakin topcross F_2 families. Again, F_2 families derived from QTHJC-resistant F_1 plants show susceptibility to TTKSK. However, in both cases where F_1 plants were resistant QTHJC and F_2 families segregate for resistance to TTKSK. The opposite case was demonstrated in F_2 families derived form topcrosses to Postrock. F_1 plants show to be susceptible to TPMKC segregate for resistance to TTKSK in F_2 families. The mechanism of resistance to QTHJC and TPMKC in backcrosses and Lakin topcrosses can be assumed to be dominant as F_1 plants showed resistant infection types. The mechanism of TTKSK resistance is yet to be determined, and can be elucidated by a screening of sufficient number of BC_1F_2 plants with QTHJC or TPMKC. Also,

developing BC_2F_1 populations in KS05HW14 or Lakin backgrounds using different stem rust-resistant F_2 individuals from different BC and TC families as donor parents, and finding all BC_2F_1 plants from resistant F_2 plants to be susceptible will confirm that resistance is recessive. Backcrossing different F_2 plants will help to ensure TTKSK resistance is recovered.

Seven of eight BC_1F_2 families derived from BC_1F_1 plants susceptible to TPMKC or RKQQC segregated for TA1642-derived resistance to TTKSK. This suggests resistance derived from TA1642 to TTKSK, TPMKC and RKQQC from TA1642 is recessive as BC_1F_1 plants are susceptible and resistance was only identified in segregating BC_1F_2 populations. As we are not able to progeny test TTKSK-resistant plants, we may confirm TPMKC or RKQQC resistance in BC_1F_2 progenies using TPMKC or RKQQC. Recessive TA1642-derived stem rust resistance can be confirmed if TPMKC or RKQQC resistant plants are fixed for resistance in progeny tests and BC_2 progenies from these plants are susceptible to TPMKC or RKQQC. Resistant plants identified from screening BC_1F_2 and TC_1F_2 populations with TPMKC or RKQQC will be used as donor parents to backcross TTKSK resistance from TA1642 into the susceptible KS05HW14 background.

During the course of experiments it was discovered that the hard winter wheat cultivar Postrock carries *Sr38*. Therefore all F_1 plants from topcrosses to Postrock are heterozygous for *Sr38*. However, the penetrance of *Sr38* resistance appears quite variable depending on genetic background. This could explain the range of infection types in F_1 seedlings from Postrock topcrosses from ;2+ to 2-3 to 23Z. Resistance to TTKSK identified in segregating F_2 populations should be from derived from TA1642 as TTKSK is virulent to *Sr38*.

The recessive nature of TA1642-derived resistance was further confirmed in an F_2 family derived from a topcross to Lakin. The TC_1F_1 plant was susceptible to TPMKC, however segregation for TTKSK resistance was observed in the F_2. Both TAM304 and WL711 topcrosses were resistant to TPMKC, however F_2 families from both crosses yielded susceptible or intermediate infection types to TTKSK. TPMKC resistance in the topcross to TAM304 could be due to the presence of *Sr23* in TAM304 (Jackie Rudd, personal communication), however, the source of WL711 resistance to TPMKC is unknown.

A low number of plants were recovered from direct crosses with TA1693 relative to the number of fertilized karyopses. Fertilization events occur readily with this *Ae. tauschii* genotype, however incompatibilities limit the generation of F_1 hybrids in culture. Additionally, topcross seed were recovered at very low frequency from backcrossing TA1693 hybrids. Most TC_1F_1 seed did not germinate and only two F_1 seedlings were recovered.

Both F_1 seedlings from topcrosses to Postrock exhibited an infection type similar to *Sr38*. However, one F_2 family segregated for resistance to TTKSK indicating that stem rust resistance has in fact been transferred from TA1693. The infection type of 2 to TTKSK in the F_2 is lower than the F_1 infection type to TPMK, suggesting a recessive mechanism of TA1693-derived resistance.

Clear differences in resistance and susceptibility to TTKSK were observed in BC_1F_1 and TC_1F_2 families derived from direct crosses to TA1718. Two BC_1F_1 seedlings yielded intermediate infection types to QTHJC but only one segregated for low infection type to TTKSK. An additional BC_1F_1 plant showed susceptibility to RKQQC but segregated for resistance to TTKSK. The F_1 plant derived from a topcross to Overley was resistant to RKQQC suggesting *Sr38* may be the source of resistance, however the ;2 infection type was much lower than the normal infection type conferred by *Sr38* which may indicate resistance is derived from TA1718. Further evidence for TA1718-derived resistance in the Overley topcross F_2 population is the low infection type of ;2 and 2 to TTKSK. Further, fully susceptible infection types to TTKSK were absent from the Overley TC_1F_2. Instead, a high number of intermediate infection types of 3;2, 13Z and 23X were observed.

One BC_1F_2 family and the Overley topcross F_2 family segregated for hybrid necrosis. Both families also segregated for resistance to TTKSK, however the relationship between the necrosis phenotype remains to be determined and can be studied in RIL populations derived from the F_2 families.

Generation of F_1 plants and BC_1F_1 seed viability are the two primary bottlenecks in the recovery of fertile plants carrying *Ae. tauschii*-derived stem rust resistance. The *Ae. tauschii* accessions TA1693 and TA1718 yielded the highest number of fertilizations and karyopses but led to the fewest number of F_1 hybrid plants and backcross or topcross seed. This suggests mechanisms controlling fertilization are separate from mechanisms controlling regeneration of hybrid plants in culture and viability of backcross and topcross progeny. Further, these mechanisms differ among *Ae. tauschii* genotypes significantly affect the feasibility of allele transfers hexaploid wheat.

Backcrossing recessive stem rust resistance genes to generate near isogenic lines (NILs) or breeding germplasm using phenotypic selection will require twice as many generations as backcrossing a dominant gene. All BCF_1 progenies must be assayed with stem rust in order to confirm the absence of dominant resistance and then all BCF_1 progenies must be self-pollinated to produce a BCF_2 population. Resistant plants will be identified in the segregating F_2 population, and can then be used as donor parents to generate a subsequent backcross generation.

Backcrossing will be necessary to isolate individual genes segregating, particularly from TA1615 where multiple genes are present. Future efforts on genetic characterization and mapping of recessive *Ae. tauschii*-derived stem rust resistance genes will yield closely linked molecular markers that will facilitate their use in wheat breeding. Markers can be used to select recessive genes in the heterozygous condition thereby allowing early generation enrichment of stem rust resistance genes. Linked markers will also aide in the pyramiding of recessive genes with other dominant resistance genes in pyramids in order to prolong their durability.

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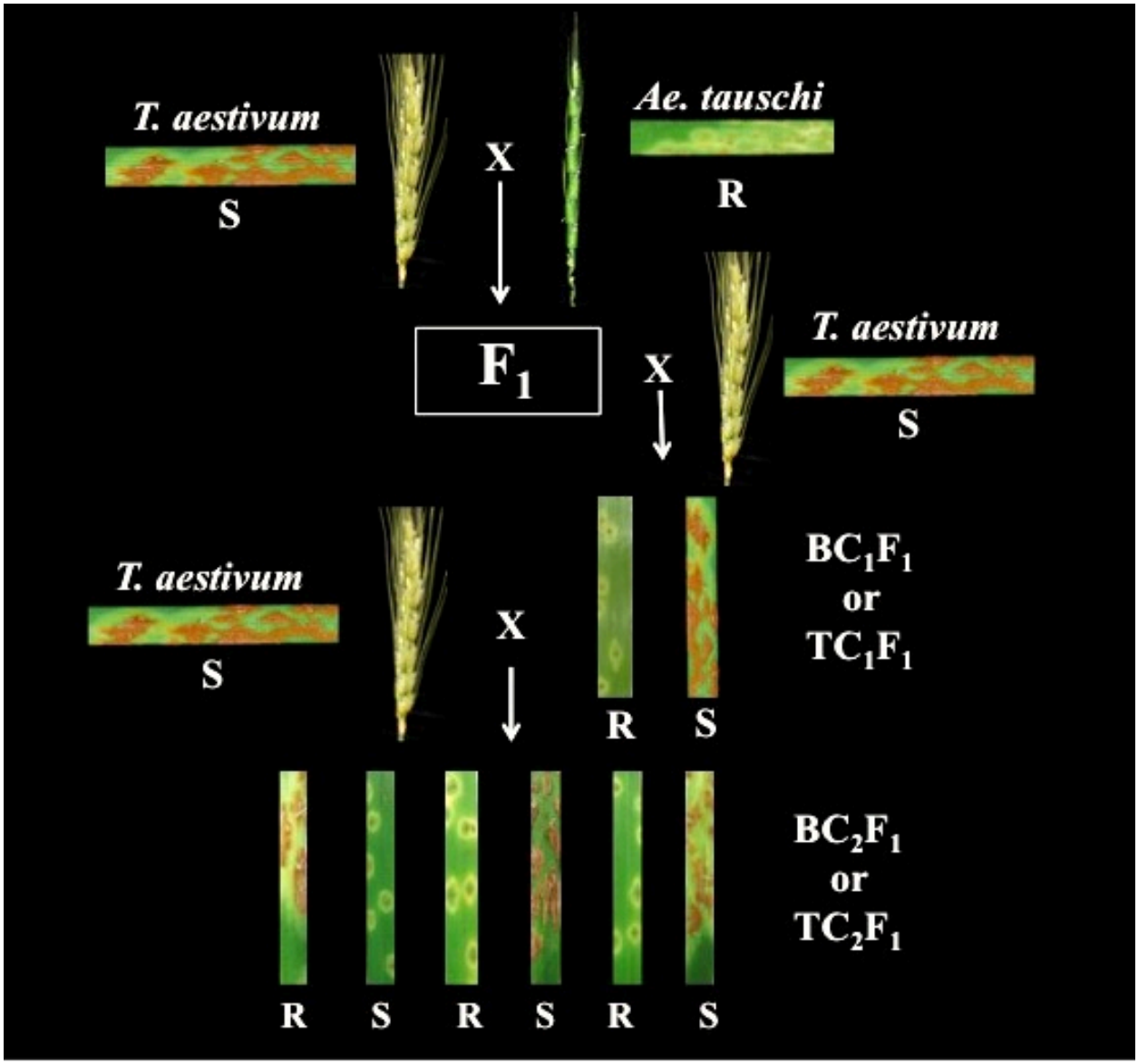
Figure 4.1 Crossing scheme to transfer *Sr* genes from *Aegilops tauschii*



Transfer of stem rust resistance genes was done using a direct hybridization protocol. A) The stem rust-susceptible hexpaploid wheat recurrent parent is pollinated by the stem rust-resistant diploid *Ae. tauschii.* B) Embryo rescue is done to recover hybrid 2n=28 (ABDD) plants that are backcrossed as females to the hexaploid recurrent parent. C) Screening of BC_1F_1 or topcross F_1 plants (2n=35 to 49) with the stem rust pathogen identifies stem rust-resistant plants that are backcrossed again, as males, to the hexaploid recurrent parent. D) BC_2 or TC_2 plants are screened with stem rust to identify resistant plants with the restored chromosome number (2n=42).

Table 4.1 Race-specific infection types of *Aegilops tauschii* accessions

Accession	TTKSK	QTHJC	RKQQC	TPMKC
CDL4366	1	;2	12-	12-
TA1615	2-	;1-	3,3--	1+
TA1642	;1	1-	11+LIF	;1-
TA1693	;1	1--1	1	22-
TA1718	;12-/;	1-,2	;1-	2+

Table 4.2 Frequency of BC and TC seeds derived from *Aegilops tauschii* accession CDL4366

Female	Male	Spikes	Seeds
KS05HW14-1/CDL4366	Duster	10	26
KS05HW14-1/CDL4366	KS05HW14	8	14
KS05HW14-1/CDL4366	Lakin	8	18
KS05HW14-1/CDL4366	Postrock	3	7

Table 4.3 Frequency of karyopsis formation and recovery of F1 hybrid plants from direct hybridization with *Aegilops tauschii* accessions TA1615, TA1642, TA1693 and TA1718

Cross	Karyopses	Spikes	Seed Set per Spike	F_1 Plants
KS05HW14 / TA1615	18	3	6	3
KS05HW14 / TA1642	33	7	4.7	10
KS05HW14 / TA1693	74	4	18.5	8
KS05HW14 / TA1718	26	2	13	3

Table 4.4 Backcross and topcross seed derived form direct hybridization with *Aegilops tauschii* accessions TA1615, TA1642, TA1693 and TA1718

F_1 Female	KS05HW14	Postrock	Duster	Lakin	Fuller	TAM304	WL711	Overley	Total
KS05HW14/TA1615	7	18	7	34	-	1	-	-	67
KS05HW14 / TA1642	17	7	23	14	4	5	1	-	71
KS05HW14 / TA1693	0	9	-	-	3	-	-	-	12
KS05HW14 / TA1718	3	1	-	8	-	-	-	1	13
Total	27	35	30	56	7	6	1	1	163

Table 4.5 Infection types of BC and TC lines carrying CDL4366-derived stem rust resistance to stem rust races TTKSK and TPMKC

Line	Pedigree	TTKSK[a]	TPMKC[b]
U6526-02-03	KS05HW14/CDL4366//KS05HW14	2+h3	;2+
U6526-03-06	KS05HW14/CDL4366//KS05HW14	2+h3	;2+
U6527-02-04	KS05HW14/CDL4366//Duster	2h3	2+2C
U6527-02-05	KS05HW14/CDL4366//Duster	3,3- h2	2C
U6527-02-10	KS05HW14/CDL4366//Duster	2h3	2C
U6527-02-16	KS05HW14/CDL4366//Duster	2,2--h3	2+
U6527-04-01	KS05HW14/CDL4366//Duster	2+3-h3	2C
U6527-04-03	KS05HW14/CDL4366//Duster	2+h3-,3	2C
U6527-04-08	KS05HW14/CDL4366//Duster	3,3+	2-C
U6527-04-11	KS05HW14/CDL4366//Duster	2h3-	2-C
U6528-01-07	KS05HW14/CDL4366//Lakin	2+h3	2+C
U6528-01-15	KS05HW14/CDL4366//Lakin	2,2+h3	22+C
U6528-02-09	KS05HW14/CDL4366//Lakin	2,2+ h3-hif	2++C
U6528-02-10	KS05HW14/CDL4366//Lakin	2h3	22+C
U6530-01-01b	KS05HW14/CDL4366//Postrock	2h3	2-C
U6530-01-02b	KS05HW14/CDL4366//Postrock	2h3-	2-C
U6530-01-100	KS05HW14/CDL4366//Postrock	2,2+h3	;2+
U6530-01-11b	KS05HW14/CDL4366//Postrock	2h3	2C
U6530-01-14b	KS05HW14/CDL4366//Postrock	22+h3	2C
U6530-01-15	KS05HW14/CDL4366//Postrock	2++,3	3
U6627-01-01	KS05HW14-1/CDL4366//Duster-2/3/Overley	2,2+h4	22-C
U6627-01-02	KS05HW14-1/CDL4366//Duster-2/3/Overley	2,2+h4	2++C
U6627-01-05	KS05HW14-1/CDL4366//Duster-2/3/Overley	2,2+h3	-
U6627-03-07	KS05HW14-1/CDL4366//Duster-2/3/Overley	2h3	2+
U6627-04-02	KS05HW14-1/CDL4366//Duster-2/3/Overley	2,2+h3	2+C
U6628-01-05	KS05HW14-1/CDL4366//Duster-2/3/Lakin	32+,3	2+
U6630-01	KS05HW14-1/CDL4366//KS05HW14-3/3/Lakin	4	4

a indicates phenotype in segregating F_2 plants
b indicates phenotypes of BC_1F_1 and Topcross F_1 plants

Table 4.6 Race-specific infection types of BC and TC lines carrying TA1615-derived stem rust resistance to stem rust races TTKSK, TPMKC and QTHJC

Line	Pedigree	TTKSK[a]	TPMKC[b]	QTHJC[b]
U6562-01	KS05HW14/TA1615//KS05HW14	2,2+ h4		;12-
U6562-02	KS05HW14/TA1615//KS05HW14	32+,3,4		;1+
U6562-03	KS05HW14/TA1615//KS05HW14	4		2C
U6564-01	KS05HW14/TA1615//Postrock	3,3hif	4	
U6564-02	KS05HW14/TA1615//Postrock	2+,2h3	4	
U6564-03	KS05HW14/TA1615//Postrock	2,2+h4	3+	
U6564-04	KS05HW14/TA1615//Postrock	3	-	
U6564-08	KS05HW14/TA1615//Postrock	2+3h4	23C	
U6564-09	KS05HW14/TA1615//Postrock	2+,2h3	3+	
U6565-01	KS05HW14-3/TA1615//Lakin	4		-
U6565-02	KS05HW14-3/TA1615//Lakin	4		2--
U6565-04	KS05HW14-3/TA1615//Lakin	2h3,3+		2--
U6565-05	KS05HW14-3/TA1615//Lakin	2+3,4		2-
U6565-06	KS05HW14-3/TA1615//Lakin	2h4		2--C
U6565-08	KS05HW14-3/TA1615//Lakin	3,4		2--
U6565-09	KS05HW14-3/TA1615//Lakin	2h3-		1+3X
U6565-10	KS05HW14-3/TA1615//Lakin	3,4		3

a indicates phenotype in segregating F_2 plants
b indicates phenotypes of BC_1F_1 and Topcross F_1 plants

Table 4.7 Infection types of BC and TC lines carrying TA1642-derived stem rust resistance to stem rust races TTKSK, RKQQC, TPMKC and QTHJC

Line	Pedigree	TTKSK[a]	RKQQC[b]	TPMKC[b]	QTHJC[b]
U6567-01	KS05HW14/TA1642//KS05HW14	2+h3			34
U6567-02	KS05HW14/TA1642//KS05HW14	2,2+h3		4	
U6567-03	KS05HW14/TA1642//KS05HW14	2,2+h3,4		3+	
U6567-04	KS05HW14/TA1642//KS05HW14	2h4		3	
U6567-05	KS05HW14/TA1642//KS05HW14	32+,3		3	
U6567-06	KS05HW14/TA1642//KS05HW14	2,2+h3+		3+	
U6567-07	KS05HW14/TA1642//KS05HW14	2,2+h4		3	
U6567-08	KS05HW14/TA1642//KS05HW14	2+h3		3	
U6567-09	KS05HW14/TA1642//KS05HW14	2h3+	4		
U6569-01	KS05HW14/TA1642//Postrock	2,2+h3		3	
U6569-02	KS05HW14/TA1642//Postrock	3,3+, 3hif, 3-		3	
U6569-03	KS05HW14/TA1642//Postrock	3,3-hif,3-		2-3	
U6569-04	KS05HW14/TA1642//Postrock	3h2		;2+	
U6569-06	KS05HW14/TA1642//Postrock	3,3-		23C	
U6569-07	KS05HW14/TA1642//Postrock	2h23?		3	
U6569-08	KS05HW14/TA1642//Postrock	3,3-		3	
U6570-03	KS05HW14/TA1642//Lakin	2,2+h3		3	
U6571-01	KS05HW14/TA1642//TAM304	3,4		;2+Y	
U6572-01	KS05HW14/TA1642//WL711	2++,32+,4		;1	

a indicates phenotype in segregating F_2 plants
b indicates phenotypes of BC_1F_1 and Topcross F_1 plants

Table 4.8 Infection types of BC and TC lines carrying TA1693-derived stem rust resistance to stem rust races TTKSK and TPMKC

Linea	Pedigree	TTKSK[a]	RKQQC[b]	QTHJC[b]
U6578-01	KS05HW14/TA1693//Postrock	2h3		;3
U6578-02	KS05HW14/TA1693//Postrock	3,4	3;	

a indicates phenotype in segregating F_2 plants
b indicates phenotypes of BC_1F_1 and Topcross F_1 plants

Table 4.9 Infection types of BC and TC lines carrying TA1718-derived stem rust resistance to stem rust races TTKSK and TPMKC

Line	Pedigree	TTKSK[a]	RKQQC[b]	QTHJC[b]
U6580-01	KS05HW14/TA1718//KS05HW14	2+,32h3		;3Z
U6580-02	KS05HW14/TA1718//KS05HW14	3,4		2+3
U6580-04	KS05HW14/TA1718//KS05HW14	2+lif h 3	33+	
U6583-01	KS05HW14/TA1718//Overley	;2,2h3;23,13Z,23X	;2	

a indicates phenotype in segregating F_2 plants
b indicates phenotypes of BC_1F_1 and Topcross F_1 plants