IMPROVING DISEASE RESISTANCE TO STEM RUST AND POWDERY MILDEW IN WHEAT USING D GENOME INTROGRESSIONS FROM AEGILOPS TAUSCHII

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ABSTRACT

IMPROVING DISEASE RESISTANCE TO STEM RUST AND POWDERY MILDEW IN WHEAT USING D GENOME INTROGRESSIONS FROM AEGILOPS TAUSCHII

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Stem rust (*Puccinia graminis*) and powdery mildew (*Blumeria graminis*) are persistent threats to hexaploid wheat (*Triticum aestivum* L., 2*n*=6*x*=42, AABBDD) production worldwide. Genetic variation for disease resistance has been limited in the D genome of wheat due to restricted gene flow between *T. aestivum* and the diploid D genome progenitor species, *Aegilops tauschii* Coss. (2*n*=2*x*=14, DD). One method to introgress disease resistance from *Ae. tauschii* to wheat is through direct hybridization and backcrossing with wheat. Using this method, the Ug99-effective stem rust resistance gene *SrTA10187* was previously introgressed from *Ae. tauschii* accession TA10187 and mapped to wheat chromosome 6DS. Development of a high-resolution genetic map surrounding *SrTA10187* assigned the resistance locus to a 1.1 cM interval on 6DS and enabled candidate gene identification. To introgress and map powdery mildew resistance in the D genome, introgression lines (ILs) were developed by direct hybridization of the resistant *Ae. tauschii* accession TA1662 with the susceptible wheat line KS05HW14. Following embryo rescue and recurrent backcrossing to KS05HW14, ILs were developed that only segregate for D genome alleles. Using a combination of genotyping-by-sequencing and KASP™ SNP markers, a novel powdery mildew resistance gene, designated *Pm58*, was mapped to 2DS and confirmed to be effective under field conditions. Powdery mildew resistant germplasm, fixed for *Pm58* were released for immediate use in disease resistance breeding.

To Kailyn, with love

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CHAPTER I

Introduction

Global importance of wheat

The historical, cultural, and economic significance of wheat is immense. The shift from nomadic- to agrarian-based human societies coincided with the domestication of wheat, and wheat has remained a cornerstone of ancient and modern civilizations (Shewry 2009). Presently, wheat accounts for approximately 20% of the calories consumed by humans worldwide (Brenchley et al. 2012). The success and wide-adoption of wheat can be attributed, in part, to grain storage proteins that form a gluten matrix. Gluten is an important source of protein and is responsible for the large diversity of wheat-based food products including bread, noodles, cake, pastries, and cereal (Shewry 2009). The genetic diversity and plasticity of wheat has also enabled rapid adaptation of wheat to various growing environments including Asia, Europe, the Americas, and Africa while maintaining high grain yield. Globally, annual production of wheat surpasses 700 million tonnes—95% of which is common wheat (*Triticum aestivum* L.) and the remaining 5% is durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) (FAOSTAT 2014, Dubcovsky and Dvorak 2007).

The origins of hexaploid wheat

The earliest forms of cultivated wheat include the diploid species *Triticum monococcum* L. ($2n = 2x =$ AA) and the tetraploid species *T. turgidum* subsp. *dicoccoides* L. ($2n = 4x =$ AABB) commonly known as einkorn and emmer wheat, respectively. These wheat species were domesticated from natural populations and likely maintained as landraces by early farmers (Feldman 2005). Approximately 9,000 to 10,000 years ago, the natural hybridization between *T. turgidum* and the diploid wild goat grass species, *Aegilops tauschii* ($2n = 2x =$ DD), led to the speciation of modern allohexaploid wheat, *T. aestivum* ($2n = 6x =$ AABBDD) (Kihara 1944,

McFadden and Sears 1946). Based on the limited divergence between the D genomes of *Ae. tauschii* and *T. aestivum*, most agree that the natural hybridization between *T. turgidum* and *Ae. tauschii* occurred very few times or only once in history (Cox 1997, Baidouri et al. 2017). Through the continued cultivation of hexaploid wheat, non-shattering and free-threshing variants were identified and preserved.

Aegilops tauschii

Extensive research has focused on the distribution, and genotypic and phenotypic diversity of *Ae. tauschii* because of its crucial role as the D genome progenitor of wheat. The native range of *Ae. tauschii* extends from Transcaucasia to central Asia between the 30°N and 45°N parallels (Pestsova et al. 2000). Using spike morphology, early studies classified *Ae. tauschii* into two subspecies—subsp. *tauschii* and subsp. *strangulata* (Dvorak 1998). More recent studies based on single nucleotide polymorphisms (SNPs) have classified *Ae. tauschii* into two distinct lineages, L1 and L2, with very few intermediate types (Wang et al. 2013). Generally, *Ae. tauschii* subpp. *tauschii* group within L1 and are acclimated to elevations higher than 400 m above sea level, while *Ae. tauschii* spp. *strangulate* group within L2 and are acclimated to elevations lower than 400 m above sea level (Mizuno et al. 2010, Wang 2013). Phylogenetic analysis, of *Ae. tauschii* collections indicated that accessions most closely related to the D genome of wheat belong to the L2E sublineage as described by Wang et al. (2013). Accessions belonging to this sublineage originate from the southern coast of the Caspian Sea, which supports the hypothesis that wheat was domesticated in that region (Wang et al. 2013).

Biotrophic wheat pathogens

Biotrophic pathogens cause many of the most destructive diseases affecting wheat. A few of the most notable biotrophic pathogens of wheat include *Puccinia triticina* f.sp. *tritici* (leaf rust), *P. striiformis* f.sp. *tritici* (stripe rust), *P. graminis* f.sp. *tritici* (stem rust), and *Blumeria graminis* f.sp. *tritici* (powdery mildew). As obligate biotrophs, they each rely on living plant tissue for growth and propagation. To remain undetected by the host, biotrophs use specialized structures called haustoria to infect and interface with the plant cell to suppress host-defense and obtain nutrients (Panstruga 2003). When conditions are favorable for disease development, each of these pathogens has the potential to cause large-scale epidemics that can reduce grain yield and cause economic and social hardship (McIntosh et al. 1995, Cowger et al. 2012).

Stem rust

Stem rust is a disease affecting wheat and other small grains that is caused by the fungal basidiomycete *Puccinia graminis* f.sp. *tritici*. Stem rust can be observed on wheat as red-brown (rust colored) oval lesions on leaves, leaf sheaths, and stems. When the infection is severe, tearing of the epidermal layer of plant tissues and brittle stems leads to reduced photosynthetic capacity and plant lodging (McIntosh et al. 1995). *Puccinia graminis* is a macrocyclic, heteroecious rust with a life cycle involving five types of spores and two plant hosts. Starting during the warm summer months, red-brown asexual urediniospores are produced on infected wheat which can then reinfect surrounding wheat. As the wheat begins to senesce and autumn approaches, black teliospores are produced that overwinter and undergo karyogamy and meiosis to produce basidiospores. In the spring, basidiospores infect the alternative host, common

Figure 1.1 Photographs of common barberry (*Berberis vulgaris*) identified in the Manistee National Forest of Michigan (Wiersma, 2016).

barberry (*Berberis vulgaris* L., Figure 1.1), where a dikaryotic mycelium is formed. Pycniospores and aeciospores are produced on the adaxial and abaxial surfaces of barberry leaves, respectively, and aeciospores are capable of infecting wheat—thereby completing the cycle (Petersen 1974).

In the early 20th century, after repeated stem rust epidemics in the central Great Plains of North America, and amidst rising fears about food security following World War I, a multi-state barberry eradication program began that ultimately saw the removal of approximately 500 million plants (Peterson et a. 2005). In theory, by disrupting the reproductive life cycle of *P. graminis*, the pathogen could no longer overwinter in northern states and eventually the disease might be eliminated. Unfortunately, the extent to which windborne urediniospores could travel had not yet been realized. The term "Puccinia Pathway" was coined to describe how asexual urediniospores were capable of overwintering in the Gulf States and Mexico, and of traveling on monsoon wind currents to northern states (Aylor 2003). While the barberry eradication did not eliminate stem rust in northern states, it was effective at delaying the arrival of the disease. Also, the number of stem rust races decreased and stabilized by reducing the sexual reproduction and genetic recombination of *P. graminis* (Jin 2011).

Powdery mildew

Wheat powdery mildew is caused by the fungal ascomycete *Blumeria graminis* f.sp. *tritici*, and appears as white-to-grey mycelium and conidia on wheat leaves, leaf sheaths, and stems. As *B. graminis* matures, dark reproductive structures called cleistothecia can be seen among the white mycelium. Characteristics making *B. graminis* particularly difficult to control are its short reproductive cycle and rapid production of secondary inoculum (conidia) that can

germinate in high relative humidity (rather than requiring free water) (Te Beest et al. 2008). Powdery mildew is most severe in intensively managed wheat production systems where the use of nitrogen fertilizers, irrigation, and semi-dwarf wheat varieties lead to dense and compact canopies, optimal for disease development. Powdery mildew is considered a cool season pathogen because it favors temperatures between 10 and 22°C (Cowger et al. 2012). Generally, powdery mildew has the greatest impact on wheat production in the northern hemisphere in coastal regions or areas with high humidity and annual rainfall—including the eastern and mid-west soft wheat growing regions of the United States (Niewoeher et al. 1998, Parks et al. 2008).

Disease management and resistance breeding in wheat

Integrated disease management

The most effective method to control biotrophic fungal diseases of wheat, including stem rust and powdery mildew, is one that integrates cultural methods with the use of foliar fungicides and genetic resistance. Common cultural methods used to minimize the risk of stem rust and powdery mildew are wider plant or row spacing, optimized irrigation timing, rotation to non-host crops, and removal of weeds, volunteer wheat, and alternative hosts (Schumann and D'Arcy 2012). Foliar fungicides such as strobilurin, triazole, and mixed mode of action fungicides can be applied at critical growth stages to protect grain yield (Dimmock and Gooding 2002). Despite the importance of cultural and chemical disease management, the most effective strategy also includes genetic resistance.

Genetic resistance to biotrophic plant pathogens takes advantage of the multilayered plant innate immunity. The primary line of defense against biotic threats is pattern-triggered immunity (PTI), which involves recognition of non-adapted microbes and phytopathogens at the cell

surface by pattern recognition receptors (PRRs) such as receptor-like kinases and receptor-like proteins (Nejat et al. 2016, Bautrot and Zipfel 2017). A secondary line of defense is effector-triggered immunity (ETI), which typically involves the indirect or direct recognition of pathogen virulence factors (effectors) by intracellular nucleotide-binding/leucine-rich-repeat (NLR) receptors (Cui et al. 2015). ETI leads to programmed cell death at the site of infection (termed the hypersensitive reaction), which restricts the invading pathogen to a tissue dead-zone and limits its growth and proliferation (Li et al. 2015).

Although many factors limit the effectiveness of genetic resistance, it remains the most economically affordable and environmentally safe approach to reduce the risk of stem rust and powdery mildew in wheat (Ellis et al. 2014, Acevedo-Garcia et al. 2017). Additionally, the harvest index (grain weight/above ground biomass weight) of modern wheat is nearly optimized, and additional yield gains will likely be made by protecting wheat photosynthetic tissue from biotic and abiotic stresses, including biotrophic pathogens (Curtis and Halford 2014).

Major gene and quantitative resistance

There are two primary types of genetic resistance in wheat disease resistance breeding—major gene (qualitative) and quantitative resistance. As the name suggests, major gene resistance is controlled by a single locus in the wheat genome, whereas quantitative resistance is controlled by many small effect loci (Poland et al. 2008). Major gene resistance is typically race-specific and involves detection of the pathogen or signatures of pathogen attack by large and genetically diverse families of NLR or PRR resistance genes (Petit-Houdenot and Fudal 2017). So far, all the stem rust and powdery mildew major genes that have been cloned in wheat, *Sr35*, *Sr33*, *Sr50*, *Sr22*, *Sr45*, *Pm3b*, and *Pm2*, belong to the NLR gene family (Saintenac et al. 2013, Periyannan et

al. 2013, Mago et al. 2015, Steuernagel et al. 2016, Yahiaoui et al. 2003, Sanchez-Martin et al. 2016). The immune response produced by major gene resistance create strong selection pressure on the pathogen which can lead to increased frequency of virulent races that breakdown host resistance. Alternatively, quantitative resistance is generally considered a more durable form of resistance because it is polygenic and it reduces disease rather than providing complete immunity (Brown 2015).

Adult plant resistance and Mlo genes

Other forms of resistance that do not fit neatly into the major gene or quantitative resistance categories include adult plant resistance and the recessive *mlo* alleles of barley and wheat (Li et al. 2014, Acevedo-Garcia et al. 2014). Adult plant resistance is described as a partial reduction in disease that is not correlated with a seedling resistance phenotype. Adult plant resistance is particularly useful to plant breeders because it is generally more durable than major gene resistance, it often enhances major gene resistance, and it can be effective against a broad range of races and pathogens (Ellis et al. 2014). One example of adult plant resistance in wheat is the pleiotropic locus *Lr34*/*Yr18*/ *Sr57*/*Pm38* which confers resistance to leaf rust, stripe rust (yellow rust), stem rust, and powdery mildew, respectively. Map-based cloning of *Lr34* revealed that a transmembrane ABC transporter was solely responsible for multi-pathogen resistance (Krattinger et al. 2009).

In barley, durable and broad-spectrum resistance to *B. graminis* f.sp. *hordei* is due to lost function of the *Mlo* gene which codes for a transmembrane protein that is necessary for pathogen penetration of the host-cell (Schulze-Lefert and Vogel 2000). While *mlo* genes could be a source of powdery mildew resistance in hexaploid wheat, all three homoeologous *Mlo* genes must be

mutated to provide resistance. This was first demonstrated by Wang et al. (2014) using a transcription activator-like effector nuclease (TALEN) to mutate a conserved region in each of the homoeologous *Mlo* alleles. Recently, the same result was reproduced without the use of transgenes. Acevedo-Garcia et al. (2017) screened a wheat TILLING population for missense mutations in *Mlo* homoeologues and pyramided three mutant alleles into a single wheat line to confer resistance.

The importance of continued disease resistance breeding

Breeding for genetic resistance to stem rust and powdery mildew remain key objectives in wheat due to the rapid evolution of *P. graminis* and *B. graminis*. In 1999, a new stem rust race with virulence to the widely used *Sr31* resistance gene posed a significant threat to global wheat production (Pretorius et al. 2000). What came to be known as the Ug99-race group spread from Uganda to surrounding eastern African countries and the Middle East, and gained additional virulence to *Sr24* and *Sr36* (Jin et al. 2008, Jin et al. 2009, Singh et al. 2011). More recently, in 2016, the highly virulent stem rust race TTTTF was discovered in Sicily and represents a major threat to European wheat production (Bhattacharya 2017). Likewise, powdery mildew isolates collected from the eastern soft wheat growing region of the United States have overcome most of the major gene resistance available in commercial varieties (Parks et al. 2008). Due to the rapid reproductive cycle of *B. graminis*, the Fungicide Resistance Action Committee has listed cereal powdery mildew as having high risk of developing resistance to fungicides (www.frac.info, Dec 2013). If the limited number of fungicides available to control powdery mildew become ineffective, wheat growers will be forced to rely even more heavily on genetic resistance.

Accessing D genome genetic variation for disease resistance

Limited D genome genetic variation

A great deal of effort has been focused on expanding the genetic variation in hexaploid wheat, especially within the D genome. As demonstrated by the wide global distribution of bread wheat, the acquisition of a D genome from *Ae. tauschii* enabled hexaploid wheat to be grown in more diverse environments compared to tetraploid wheat (Dubcovsky and Dvorak 2007). However, the genetic variation within the D genome of wheat is limited due to the domestication bottleneck, or founder effect, which involved very few natural hybridizations between tetraploid wheat and *Ae. tauschii* (Cox 1997). The comparatively large amount of genetic variation within the *Ae. tauschii* gene pool has not been fully incorporated into cultivated and landrace varieties of hexaploid wheat because gene flow is restricted between the species (Reif et al. 2005). Plant breeders rely on the genetic diversity available within wheat to select for improved wheat traits when confronted with diverse biotic and abiotic stresses. If the genetic variation within wheat is insufficient to make genetic gain towards a breeding target, additional genetic variation can be introduced from the wild relatives of wheat—especially *Ae. tauschii* (Feuillet et al. 2007). In fact, *Ae. tauschii* is one of the most accessible wheat relatives because it belongs to the primary gene pool of wheat and *Ae. tauschii* chromosomes can readily pair and recombine with the D genome of wheat (Ogbonnaya et al. 2013).

Plant breeders implement various methods of interspecific and backcross hybridization between wheat and its wild relatives to transfer desirable traits from *Ae. tauschii* to hexaploid wheat (Cox 1997). The two best defined methods are the development of synthetic hexaploid wheat and direct hybridization of hexaploid wheat with *Ae. tauschii*. To date, novel genetic variance derived from *Ae. tauschii* has been used to improve a wide variety of traits in wheat,

including disease and insect resistance, yield components, and tolerance to abiotic stresses such as precocious germination, drought, heat, boron, and aluminum (Borner et al. 2015). In 2011, Rouse et al. screened a diverse collection of *Ae. tauschii* accessions and discovered that approximately 22% of the accessions were resistant to Ug99 races of *P. graminis*. To date, six stem rust resistance genes have been introgressed from *Ae. tauschii* to wheat: *Sr33*, *Sr45*, *Sr46*, *SrTA1662*, *SrTA10171*, and *SrTA10187* (Periyannan et al. 2013, Periyannan et al. 2014, Yu et al. 2015, Olson et al. 2013a, Olson et al. 2013b). Likewise, *Ae. tauschii* has been used for introgression of powdery mildew resistance genes *Pm2*, *Pm19*, *Pm34*, *Pm35*, *PmY201*, *PmY212*, and *PmM53* (McIntosh and Baker 1970, Lutz et al 1995, Miranda et al. 2006, Miranda et al 2007, Sun et al. 2006, Li et al. 2011).

Synthetic hexaploid wheat

Synthetic hexaploid wheat (SHW) is developed by crossing tetraploid wheat with *Ae. tauschii* and followed by colchicine treatment or spontaneous meiotic restitution to obtain a full complement of chromosomes (McFadden and Sears 1947, Figure 1.2A). Typically, *T. turgidum* subsp. *durum* L. is used as the tetraploid parent because durum wheat is a domesticated, free-threshing wheat that is still widely grown. Alternatively, *T. turgidum* subsp. *dicoccoides* is used as a wild, hulled tetraploid parent to simulate the natural hybridization that may have occurred when hexaploid wheat was domesticated (Yang et al. 2009). In the last 30 years, the international breeding group, CIMMYT has developed more than 1,000 SHW populations that have been used extensively to improve wheat around the globe (Dreisigacker et al. 2008). One disadvantage of using SHW is that the entire D genome of *Ae. tauschii* is transferred—a digression to an

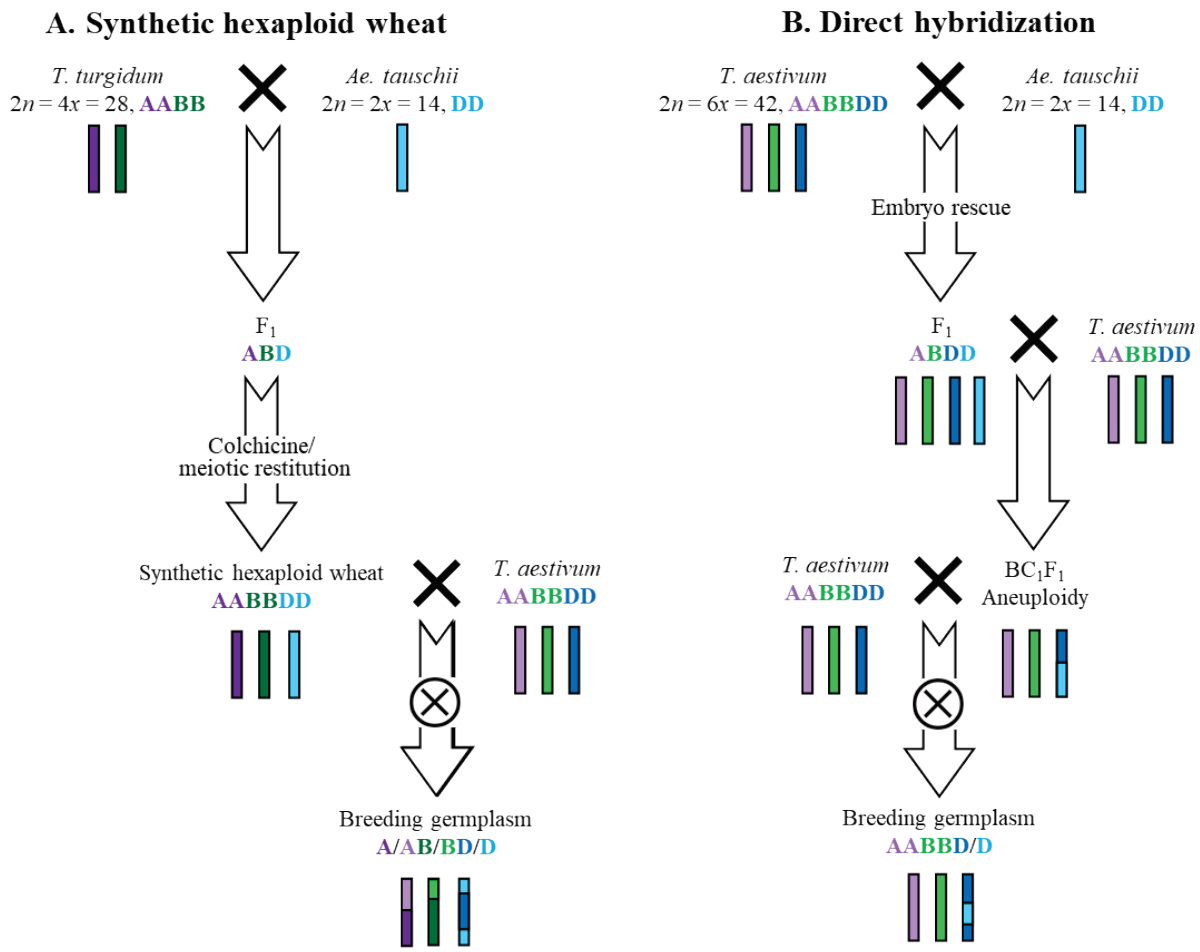

Figure 1.2 Diagrams of A) synthetic hexaploid wheat development and B) direct hybridization between *Ae. tauschii* and hexaploid wheat. Shades of purple, green, and blue represent homoeologous A, B, and D genomes, respectively. In steps involving hybridization, female parents are on the left and males are on the right. Encircled X = self-hybridization, forward slash = recombination between homologous chromosomes. This figure was adapted from Cox 1997 and Ogbonnaya et al. 2013.

unadapted D genome unsuitable for cultivation. Another disadvantage is that the A and B genomes of the tetraploid wheat parent do not necessarily have good combinability with the D genome of *Ae. tauschii*, whereas the A, B, and D genomes of hexaploid wheat have co-evolved for thousands of years (Ogbonnaya et al. 2013). To address these limitations, primary SHW is usually backcrossed to well-adapted hexaploid wheat before it is used for commercial wheat breeding objectives (Figure 1.2A).

Direct hybridization

An alternative approach to SHW is the use of direct hybridization between *Ae. tauschii* and hexaploid wheat followed by recurrent backcrossing to hexaploid wheat (Figure 1.2B). Using this method, Gill and Raupp (1987) first demonstrated that F_1 aneuploid embryos lacking typical endosperm could be rescued on culture media and backcrossed with hexaploid wheat to restore fertility. Then, by using BC_1F_1 individuals as pollen donors in a second backcross to hexaploid wheat, it was possible to select against aneuploid gametes and restore euploid chromosome segregation (Cox 1997). The primary advantage of this method is that stable, recombinant D genome chromosomes can be recovered without perturbation of the homoeologous A and B genomes of hexaploid wheat (Ogbonnaya et al. 2013). Additionally, if the same inbred hexaploid wheat line is used for recurrent backcrossing, the genetic background will be identical to that of the recurrent parent with the exception of introgressed D genome *Ae. tauschii* loci (Figure 2B). In this way, the allohexaploid wheat genome is effectively reduced to diploid segregation of D genome alleles, and the complex interactions between co-evolved homoeologous genomes are retained.

Advancements in genetic and genomic tools for wheat

Genome sequencing

Sequencing the genomes of wheat and its wild relatives represents the most significant advancements in the field of wheat breeding and genetics in recent years. Initially, due to the immense size (17-gigabases) and complex structure of the allohexaploid wheat genome, ditelosomic chromosome arms were isolated using flow-cytometry, sequenced, and assembled individually to produce the first partial reference genome of wheat (The International Wheat Genome Sequencing Consortium 2014). A year later, the first whole-genome shotgun assembly of wheat was released by Chapman et al. (2015). This assembly was anchored to a dense genetic map, but not annotated and only ~50% of the genome was represented. Finally, with longer reads and improved assembly algorithms designed for large complex genomes, a new whole-genome shotgun assembly of wheat was released that represents nearly 80% of the wheat genome (Clavijo et al. 2017). Due to the smaller size of the diploid *Ae. tauschii* and *T. urartu* genomes, whole-genome shotgun assemblies of each were released to the public in 2013 (Jia et al. 2013, Ling et al. 2013). Also, a 4-gigabase physical map of *Ae. tauschii* was developed by Luo et al. (2012) by finger printing bacterial artificial chromosomes and assembling/mapping contigs using a 10K Illumina® Infinium SNP array. More recently, using long-read single-molecule sequencing technology in conjunction with short reads, the *Ae. tauschii* genome was independently re-sequenced and -assembled to improve contig length (Zimin et al. 2017). Equipped with these genomic resources, substantial progress can now be made towards further understanding the origins of wheat, the genetic diversity present in wheat and its wild relatives, and the genetics underlying wheat response to abiotic and biotic stresses.

Wheat genotyping platforms

The primary genotyping tool used by wheat breeders and researchers in the early 2000's were microsatellite markers (namely simple sequence repeats). A gradual shift towards single nucleotide polymorphism (SNP) genotyping platforms has since occurred. The first wheat microsatellite map was produced in 1998 by Roder *et. al.* and was composed of 279 microsatellites. An improved wheat microsatellite map was released in 2004 with a total of 1,235 microsatellite loci mapped at an average interval distance of 2.2 cM (Somers *et. al.* 2004). At that time, microsatellite markers were the most affordable marker technology, and high-throughput capillary electrophoresis allowed researchers to map these polymorphic co-dominant markers in relatively large populations. Although microsatellite markers remain an important technology for applications in marker assisted selection (MAS) and trait mapping, recent advances in SNP genotyping have led to the large-scale adoption of SNP genotyping platforms including genotyping-by-sequencing (GBS), SNP arrays, and KASP™ markers. The primary advantage offered by these newer SNP genotyping platforms is higher throughput and increased genome-wide marker coverage (Mammadov et al. 2012). Furthermore, decreased sequencing cost, more user-friendly bioinformatics software, and increased genotyping platform flexibility are making SNP marker data more accessible for all crop species, including wheat (Poland and Rife 2012).

Genotyping-by-sequencing

The wheat breeding and research community has been very receptive to GBS technology because it offers a cost-effective method to genotype many plants at several thousand loci. Unlike other SNP genotyping platforms, no prior sequence data is needed for genotyping. This

allows GBS to be applied to novel germplasm and provides versatility. GBS was first demonstrated by Elshire *et. al.* in 2011. The basic method for GBS involves DNA digestion by restriction enzymes, adapter and barcode ligation, sample pooling and amplification, sequencing, and SNP calling. One important advancement in GBS was the application in wheat by Poland *et. al.* in 2012 using a novel two-enzyme approach. This approach included a rare-cutting restriction enzyme and a common-cutting enzyme which together resulted in higher specificity and reproducibility. While GBS has many advantages over alternative genotyping platforms, there are many disadvantages too: missing data, non-uniform distribution of sequence data, sequencing errors, SNP identification in duplicated loci, and the necessity for bioinformatics expertise when analyzing GBS data (Beissinger *et. al.* 2013, Kim et al. 2016). Development of more accessible GBS analysis software, SNP filtering, and a variety of data imputation methods have attempted to mitigate these issues with mixed success (Rutkoski *et. al.* 2013, Glaubitz *et. al.* 2014, Limborg et al. 2016).

KASP™ Markers

KASP™ markers are an important newer technology used to assay specific SNPs using a competitive allele-specific polymerase chain reaction (Smith and Maughan 2015). Two forward primers and a common reverse primer compete to amplify a specific segment of DNA. The two forward primers are identical except for the 3' nucleotide, which is the SNP that differentiates one allele from the other. Two fluorescent dyes are included in the mix, with one binding to the product from one allele and the other binding to the product from the alternative allele. Using a fluorimeter, the signals from a given reaction can be measured. Individuals that express a single fluorescence signal are homozygous for that allele, and individuals that express a combination of

both fluorescence signals are heterozygous. The substantial reduction in missing data points and its use as a targeted approach to saturate regions of interest with markers are the primary advantages of this technology (Rasheed et al. 2016). KASP™ markers were first used effectively in wheat in 2011 when Allen *et. al.* identified 1,114 SNPs and designed KASP™ markers to genotype 23 varieties. Many researchers have also found KASP™ markers to be complimentary to GBS; while GBS is effective at identifying important polymorphisms, KASP™ markers can be used to assay individuals with unknown genotypes (Gao et al. 2015, Lin et al. 2015). The primary disadvantages of KASP™ markers are the need for prior SNP identification, higher cost and lower throughput compared to other SNP platforms, and patents that limit the use of the technology (Ertiro et al. 2015, www.lgcgroup.com/products/kasp-genotyping-chemistry).

Problem definition

As the demand for wheat increases and virulent plant pathogens threaten global wheat production, breeders must accelerate genetic gain by increasing genetic diversity and rapidly integrating novel resistance loci into elite varieties. While this is a challenging task, elegant population development, improved genomic resources, and higher-throughput SNP genotyping will facilitate ongoing efforts. By hybridizing *Ae. tauschii* directly with hexaploid wheat and using the same elite wheat variety for recurrent backcrossing, forward breeding with *Ae. tauschii* is possible. The effect of *Ae. tauschii* alleles can be characterized and mapped in hexaploid wheat without the confounding effects of homoeologous chromosome segregation, the exact genomic context can be determined using new reference genomes, and more accurate marker assisted selection is enabled by contemporary SNP markers. Together, these advancements will

streamline the development of disease resistant wheat germplasm using foreign introgressions from *Ae. tauschii*.

Objectives

To characterize and map genetic variation for stem rust and powdery mildew disease resistance from *Ae. tauschii* in hexaploid wheat and to develop disease resistant germplasm and genetic markers for ongoing breeding efforts.

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CHAPTER II

Fine mapping of the stem rust resistance gene *SrTA10187*

The Ug99-effective stem rust resistance gene *SrTA10187* was fine-mapped to a 1.1 cM interval on 6DS, candidate disease resistance genes were identified, and molecular markers were developed for ongoing wheat breeding efforts. For a full text of this work go to: Theoretical and Applied Genetics, 2016, 129(12):2369-2378, doi: 10.1007/s00122-016-2776-1

CHAPTER III

Identification of *Pm58* from *Aegilops tauschii*

Using a population of wheat-*Aegilops tauschii* introgression lines, the novel powdery mildew resistance gene *Pm58* was mapped to chromosome 2DS and confirmed to be effectie under field conditions. Additionally, molecular markers were developed for ongoing wheat breeding efforts. For a full text of this work go to: Theoretical and Applied Genetics, 2017, 130:1123-1133, doi: 10.1007/s00122-017-2874-8

CHAPTER IV

Registration of Two Wheat Germplasm Lines Fixed for *Pm58*

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Abstract

Powdery mildew, caused by *Blumeria graminis* (D.C.) f. sp. *tritici*, is a persistent threat to global wheat (*Triticum aestivum* L.) production. To broaden the genetic base for resistance to powdery mildew in wheat, germplasm lines U6714-A-011 and U6714-B-056 were developed at Michigan State University and are fixed for the novel powdery mildew resistance gene *Pm58*. This gene was identified in *Aegilops tauschii* Coss. accession TA1662, introgressed, and mapped to wheat chromosome 2DS. The two germplasm lines described are BC_2F_4-derived inbred backcrossed lines from a direct cross between TA1662 and the recurrent wheat parent KS05HW14, a hard white winter wheat line adapted to western Kansas. In addition to exhibiting resistant reactions to multiple *Bgt* isolates with broad virulence profiles, both lines have moderate yield potential and good agronomic characteristics, making them suitable as breeding germplasm. The availability of these lines will enable the incorporation of *Pm58* into wheat breeding programs, providing additional genetic variation for resistance to powdery mildew.

Introduction

As the causal agent of powdery mildew, the biotrophic ascomycete *Blumeria graminis* f. sp. *tritici* (*Bgt*) remains among the most important fungal pathogens affecting wheat (*Triticum aestivum* L.). The potential for large-scale grain yield loss due to powdery mildew and the breakdown of host resistance necessitate ongoing discovery of additional sources of genetic resistance to *Bgt* (Cowger et al. 2012).

The wild wheat relative *Aegilops tauschii* Coss. is an important source of genes conferring resistance to biotrophic pathogens, including powdery mildew. Due to D genome homology between *Ae. tauschii* and *T. aestivum*, chromosomes from interspecific crosses

recombine normally, allowing for migration of alleles by meiotic recombination. One of the most widely deployed powdery mildew resistance genes in wheat, *Pm2*, was derived from *Ae. tauschii* (McIntosh and Baker 1970, Bennett 1984), and multiple *Ae. tauschii* accessions have been used as powdery mildew resistance donors to improve germplasm (Murphy et al. 1998, Murphy et al. 1999).

In the ongoing effort to improve powdery mildew resistance in wheat, Wiersma et al. (2017) identified and mapped the novel powdery mildew resistance gene *Pm58*. The resistance donor, *Ae. tauschii* accession TA1662 from Azerbaijan, was crossed directly with the susceptible hard white wheat line KS05HW14 and lines fixed for *Pm58* were identified. The objectives of this study are to characterize powdery mildew resistance and grain yield in two germplasm lines, U6714-A-011 and U6714-B-056, fixed for *Pm58*. Availability of these well-adapted germplasm lines to the breeding community will facilitate the broadening of the genetic base for powdery mildew resistance in wheat.

Methods

Germplasm line development

The susceptible wheat line KS05HW14 (KS98HW452/CO960293//KS920709B-5-2), was used as the wheat parent in a direct interspecific cross with the powdery mildew-resistant *Ae. tauschii* accession TA1662 (Gill and Raupp 1987, Wiersma et al. 2017). The hard white winter wheat line KS05HW14 was developed by Dr. Joe Martin of Kansas State University in Hays, KS. The powdery mildew resistance donor, TA1662 is an *Ae. tauschii* accession collected from Azerbaijan and maintained by the Wheat Genetics Resource Center at Kansas State University. Following embryo rescue, F_1 hybrids were backcrossed to KS05HW14 as females,

and a single BC_1F_1 plant with powdery mildew resistance was backcrossed again to KS05HW14. A population of BC_2F_1-derived plants were maintained by single-seed-descent to the BC_2F_4 generation when seed of BC_2F_4-derived head rows was produced in 2014. Yield trials and powdery mildew tests were conducted in subsequent generations. The two genotypes U6714-A-011 and U6714-B-056 were identified as being fixed for *Pm58* based on powdery mildew disease scores from field studies and detached-leaf assays (Wiersma et al. 2017). U6714-A-011 and U6714-B-056 are also homozygous for the TA1662 haplotype at the KASP™ marker loci *K-TP127986* to *K-TP69304*.

Powdery mildew evaluation

Isolate-specific reactions of U6714-A-011, U6714-B-056, KS05HW14, and the susceptible check cultivar Jagalene to 20 *Bgt* isolates were tested using detached-leaf assays by the USDA-ARS Plant Science Research Unit in Raleigh, North Carolina. The 20 *Bgt* isolates were selected based on broad geographic distribution and virulence profiles (Cowger et al. 2017). Detached leaf segments (10-12 days, 1.5 cm long) were floated on 0.5% (w/v) water agar amended with benzimidazole (50 mg L^{-1}) (Parks et al. 2008). Two replicate leaf segments of U6714-A-011, U6714-B-056, and KS05HW14, and four replicate leaf segments of Jagalene were rated on each plate. Each plate was inoculated with an individual *Bgt* isolate, and four plates total were rated for each isolate. Leaf segments were rated 10 to 11 days post inoculation using a 0-9 rating system described previously by Parks et al. 2008, where ratings 0-3, 4-6, and 7-9 are classified as resistant, intermediate, or susceptible, respectively.

Agronomic evaluation

Yield trials were grown in three locations in 2015: Hays, KS; Ashland, KS; and Richville, MI. In 2016, four additional yield trial locations were included: Brookings, SD; Champaign, IL; Marianna, AR; and Pullman, WA. Yield trial fertilization varied by location and fungicides were applied at several locations to control infection by stripe rust (*Puccinia striiformis* f.sp. *tritici*). U6714-A-011 and U6714-B-056 were each planted in single replicate plots in an augmented design containing six incomplete blocks. Replicated plots of KS05HW14, and a locally adapted check were included in each block to control for variation. LS-means of grain yield and 95% confidence intervals were calculated using a mixed linear model in RStudio® (RStudio, Boston, MA, USA) using R version 3.2.1 and the packages lme4 (v.1.1-12) and lsmeans (v.2.25-5). Genotype was treated as a fixed effect and block was treated as a random effect.

Characteristics

Powdery mildew resistance

U6714-A-011 and U6714-B-056 were tested for their reaction to 20 *Bgt* isolates collected from widely separated locations in hard and soft wheat growing regions of the central and eastern United States (Table 4.1). Based on means of replicate leaf segments, U6714-A-011 expressed resistant to intermediate reactions to 15 *Bgt* isolates and susceptible reactions to 5 isolates; U6714-B-056 expressed resistant to intermediate reactions to 12 *Bgt* isolates and susceptible reactions to 8 isolates. U6714-A-011 and U6714-B-056 had consistently lower scores than both the recurrent wheat parent, KS05HW14, and the susceptible check, Jagalene (Table 4.1). In general, U6714-A-011 and U6714-B-056 were more resistant to powdery mildew

Table 4.1 Isolate-specific powdery mildew reactions identified in wheat lines U6714-A-011, U6714-B-056, KS05HW14, and the *Bgt* susceptible check Jagalene. Powdery mildew disease severity was rated on a 0-9 scale, and the mean score of replicate leaf segments is reported with the standard deviation in parenthesis.

***Bgt* isolate**	**Origin of isolate (City, State, Year)**	**Avirulent/virulent on *Pm* genes†**	**U6714-A-011** (*n* = 8)	**U6714-B-056** (*n* = 8)	**KS05HW14** (*n* = 8)	**Jagalene** (*n* = 16)
GAP-A-2-3	Plains, GA, 2013	*Pm1a,1b,4b,16,17,36 / 2,3a,3b,4a,6,8*	**6.4** (2.1)	7.5 (0.8)	8.0 (0.0)	8.0 (0.0)
GAP-B-2-2	Plains, GA, 2013	*Pm1a,1b,4b,16,17,36 / 2,3a,3b,4a,6,8*	**6.1** (2.1)	7.5 (0.5)	7.9 (0.4)	7.8 (0.4)
MIR(14)-D-3-3	Rogers City, MI, 2014	*Pm1a,1b,3b,4a,4b,17,34,37 / 2,3a,6,8,25,35*	7.5 (0.5)	7.4 (0.7)	7.9 (0.4)	7.9 (0.3)
MIR(14)-E-1-3	Rogers City, MI, 2014	*Pm1a,1b,2,3b,4a,4b,17,25,34,35,37 / 3a,6,8*	7.9 (0.4)	7.4 (0.7)	8.0 (0.0)	7.9 (0.3)
MSG-A-3-1	Greenwood, MS, 2013	*Pm1a,1b,2,4a,4b,8,16,17,36 / 3a,3b,6*	7.5 (0.8)	7.0 (0.8)	8.0 (0.0)	8.0 (0.0)
MSG-C-3-4	Greenwood, MS, 2013	*Pm1a,1b,4b,16,17,36 / 2,3a,3b,4a,6,8*	**5.1** (2.5)	**5.0** (3.7)	7.6 (0.7)	7.6 (0.8)
MTG1-1a	Geraldine, MT, 2016	*Pm1a,1b,2,3a,3b,4a,4b,6,8,17,25,34,37 / 35*	**4.4** (3.7)	**5.1** (2.7)	7.0 (2.8)	7.6 (0.6)
MTG1-3a	Geraldine, MT, 2016	*Pm1a,1b,3a,3b,4b,8,17,25,34,35,37 / 2,4a,6*	**3.0** (3.4)	**5.5** (2.6)	8.0 (0.0)	7.9 (0.3)
NCC-B-1-3	Chocowinity, NC, 2013	*Pm1a,1b,2,4b,8,16,36 / 3a,3b,4a,6,17*	**4.1** (3.2)	**5.3** (2.4)	8.0 (0.0)	7.8 (0.4)
NCF-D-1-1	Four Oaks, NC, 2013	*Pm1a,1b,2,4a,4b,16,17,36 / 3a,3b,6,8*	8.0 (0.0)	7.8 (0.5)	8.0 (0.0)	8.0 (0.0)
NEI-1-3	Ithaca, NE, 2016	*Pm1a,1b,2,3a,4a,4b,25,34,35,37 / 3b,6,8,17*	**3.8** (4.1)	**4.3** (3.1)	7.0 (2.8)	8.0 (0.0)
NEI-3-1	Ithaca, NE, 2016	*Pm1a,1b,2,3a,3b,4a,4b,17,25,34,35,37 / 6,8*	**3.1** (2.6)	**6.3** (1.8)	8.0 (0.0)	8.0 (0.0)
NEI-5-5	Ithaca, NE, 2016	*Pm1a,1b,3a,3b,4b,8,25,34,37 / 2,4a,6,17,35*	**6.4** (2.7)	**5.4** (2.5)	8.0 (0.0)	8.0 (0.0)
NYA-E-3-3	Aurora, NY, 2013	*Pm1a,1b,4b,6,8,16,17,36 / 2,3a,3b,4a*	**6.9** (1.6)	**6.8** (2.1)	8.0 (0.0)	7.6 (0,5)
NYB-E-1-2	Brockport, NY, 2013	*Pm1a,1b,2,4b,16,17,36 / 3a,3b,4a,6,8*	**6.8** (0.9)	7.1 (1.0)	7.4 (0.7)	7.8 (0.4)
OKH-A-2-3	Hinton, OK, 2013	*Pm1a,1b,2,3a,3b,4a,4b,8,16,17,36 / 6*	**3.1** (2.4)	**1.9** (2.7)	7.9 (0.4)	7.6 (0.5)
OKS-A-2-2	Stillwater, OK, 2013	*Pm1a,1b,2,3a,3b,4b,16,36 / 4a,6,8,17*	**2.4** (2.6)	**3.0** (3.4)	8.0 (0.0)	7.6 (0.5)
OKS-B-2-2	Stillwater, OK, 2013	*Pm1a,1b,2,3a,3b,4a,4b,16,17,36 / 6,8*	**5.8** (1.5)	**6.1** (2.8)	**6.9** (2.8)	7.8 (0.4)
PAF(14)-D-1-2	Pennsylvania Furnace, PA, 2014	*Pm1a,1b,3b,4b,17,25,37 / 2,3a,4a,6,8,34,35*	7.9 (0.4)	8.0 (0.0)	8.0 (0.0)	7.9 (0.3)
PAF-E-2-2	Pennsylvania Furnace, PA, 2013	*Pm1a,1b,4a,4b,8,16,17,36 / 2,3a,3b,6*	**4.3** (3.7)	**5.9** (2.0)	8.0 (0.0)	7.9 (0.3)

†*Bgt* isolate was considered avirulent if the single-gene differential line expressed a reaction <7.0. Genotypes with isolate-specific reactions less than 7 are indicated in bold. *n* indicates the number of leaf segments rated.

isolates collected from the central states (Oklahoma, Nebraska, and Montana), compared to those collected from eastern states. Higher standard deviations in disease scores were observed in resistant and intermediate reactions compared to susceptible reactions (Table 4.1).

Agronomic evaluations

U6714-A-011 and U6714-B-056 are both free-threshing hard white winter wheat lines that exhibit good agronomic characteristics. In Michigan during the 2015 and 2016 growing seasons, the average plant height of both lines was 84 cm and anthesis occurred within one day of the recurrent parent KS05HW14 (late May to early June). Plant type and early maturity were stable and uniform across years and locations, indicating seed purity with very low levels of off types or heterozygosity.

When yield was tested under multiple environments including locations in Kansas, South Dakota, Illinois, Arkansas, Washington, and Michigan, U6714-A-011 and U6714-B-056 had moderate grain yield potential (Table 4.2). With the exception of Hays, KS in 2015, the two germplasm lines did not yield higher than the locally adapted check variety. On average, U6714-B-056 had higher yield potential than U6714-A-011, but both lines yielded lower than the recurrent parent KS05HW14 in all locations except Pullman, WA. In Pullman, WA, U6714-A-011 yielded within the 95% confidence interval of KS05HW14, and U6714-B-056 had grain yield higher than KS05HW14 (Table 4.2).

Discussion

The germplasm lines described here are the first publicly available wheat lines fixed for the novel powdery mildew resistance gene *Pm58* derived from *Ae. tauschii*. Based on its

Table 4.2 Grain yield LS-means of locally adapted check varieties, KS05HW14, U6714-A-011, and U6714-B-056 in 10 diverse environments throughout the United States.

		t ha^{-1}						
		Locally adapted check varieties			KS05HW14		U6714-A-011	U6714-B-056
Location	Year	Name	Grain yield	95% CI	Grain yield	95% CI	Grain yield (% KS05HW14)	Grain yield (% KS05HW14)
Ashland, KS	2015	Everest	4.64	4.45 - 4.82	4.66	4.41 - 4.89	3.54 (76%)	3.23 (69%)
Ashland, KS	2016	Everest	4.85	4.65 – 5.05	4.20	4.11 – 4.30	3.06 (73%)	3.84 (91%)
Brookings, SD	2016	Lyman	3.60	3.30 - 3.90	3.30	3.17 - 3.44	0.36† (11%)	1.64 (50%)
Champaign, IL	2016	IL07-19334	7.85	7.47 - 8.22	5.67	5.50 - 5.85	3.28 (58%)	4.96 (87%)
Hays, KS	2015	Ernie	2.73	2.34 - 3.12	3.22	3.05 - 3.40	1.51 (47%)	3.36 (104%)
Hays, KS	2016	Joe	6.76	6.65 - 6.87	3.61	3.56 - 3.66	2.88 (80%)	2.85 (79%)
Marianna, AR	2016	AR11LE24	4.12	3.86 - 4.39	2.73	2.60 - 2.85	2.52 (92%)	2.27 (83%)
Pullman, WA	2016	Jasper	9.06	8.75 - 9.37	4.45	4.31 - 4.59	4.43 (100%)	5.34‡ (120%)
Richville, MI	2015	AC Mountain	5.18	5.09 - 5.26	4.95	4.76 - 5.15	4.32 (87%)	4.12 (83%)
Richville, MI	2016	Ambassador	5.63	5.31 - 5.95	5.31	5.15 - 5.46	2.40 (45%)	4.32 (81%)

†Exceptionally low yield was the result of a severe stripe rust and some difficulty threshing in Brookings, SD in 2016. ‡ Grain yield is significantly higher than the recurrent parent, KS05HW14. CI = confidence interval.

performance against the present, geographically representative set of *Bgt* isolates, *Pm58* is likely to be particularly effective in the hard wheat region (Kansas, Nebraska, Oklahoma), where the *Bgt* population has lower virulence complexity than in soft wheat growing areas (Cowger et al, 2017). However, our results indicate *Pm58* also confers partial resistance to some isolates from predominantly soft wheat-growing areas, and thus can be useful in those regions in combination with other sources of resistance.

U6714-A-011 and U6714-B-056 have acceptable agronomic adaptation, perform well under conventional wheat management, and can be used in the development of elite wheat lines with improved powdery mildew resistance. Additionally, by using KASP™ markers linked to *Pm58* (Wiersma et al. 2017), plants can be selected in the absence of disease pressure and multiple powdery mildew resistance genes can be pyramided simultaneously.

Availability

Small quantities of unrestricted seed are available immediately for distribution. Written requests should be submitted to Dr. Eric L. Olson at Michigan State University, East Lansing, MI. Breeder seed was produced in individual yield trial plots at the Michigan State University, Saginaw Valley Research and Extension Center in Frankenmuth, MI (43.395, -83.676). Five years from the publication date, the National Small Grains Collection (USDA-ARS) in Aberdeen, Idaho is responsible for continued organization and maintenance of seed stocks. Seed was also deposited in the USDA-ARS National Center for Genetic Resources Preservation (NCGRP) in Ft. Collins, Colorado. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new breeding line or cultivar.

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Chapter V

Conclusions and future perspectives

Overview of dissertation research

High resolution mapping of the Ug99-effective stem rust resistance gene *SrTA10187* was accomplished using more than one thousand BC_3F_2 individuals and a combination of SNP, SSR, and STS markers. A total of fifteen KAPS™ markers were designed based on SNPs available in public databases and identified internally using genotyping-by-sequencing (GBS). A genetic map with increased marker density was developed, and *SrTA10187* was mapped to a 1.1 cM genetic interval. To identify candidate resistance genes and to determine the genomic context surrounding *SrTA10187* two approaches were used. In the first approach, genetic markers were aligned to the reference *Aegilops tauschii* genome developed by Jia et al. (2013). Due to large reference genome recombination bins and conflicting marker orders, the genomic context surrounding *SrTA10187* could not be resolved. Alternatively, the use of a higher resolution *Ae. tauschii* genetic map and pooled BAC library sequences developed by Luo et al. (2013) proved to be more successful. After aligning common markers and annotating BAC library sequence in the region surrounding *SrTA10187*, at least one NLR was identified in the interval of interest (of the reference *Aegilops tauschii* accession). The marker resources and high resolution genetic map developed in this study will facilitate continued efforts to improve stem rust resistance in wheat using marker-assisted selection, and will enable gene pyramiding of *SrTA10187* to improve the durability of major-gene resistance.

Identification of the novel powdery mildew resistance gene *Pm58* from *Ae. tauschii* began by screening a small collection of *Ae. tauschii* accessions for seedling resistance using detached-leaf assays. Of the nine accessions screened, TA1662 stood out with resistance to 18 of the 20 isolates tested. After confirming that powdery mildew seedling resistance was segregating as a single-locus trait in a population of wheat-*Ae. tauschii* introgression lines, the resistance

locus was mapped to wheat chromosome 2DS and formally designated *Pm58*. Genome-wide genetic maps (including the resistance locus) were developed from GBS data, and tag sequences were aligned to the reference *Ae. tauschii* genome to determine the chromosome identities of linkage groups. To confirm the effectiveness of *Pm58* in adult plant tissues, the same introgression lines were rated for powdery mildew resistance in two naturally infected field trials. In both environments, single major-effect QTLs were identified at the same locus where *Pm58* seedling resistance mapped. To improve the genetic map in the region immediately surrounding *Pm58*, nine GBS-SNP markers were converted to KASP™ markers. Four KASP™ markers colocalized with *Pm58* and will be useful for marker-assisted selection.

For either *SrTA10187* or *Pm58* to have an impact on wheat disease resistance breeding, lines with acceptable agronomics that are fixed for the resistance locus must be accessible to the public. To accomplish this for *Pm58*, two homozygous lines, U6714-A-011 and U6714-B-056, were yield tested in multiple locations across the United States. Generally, U6714-B-056 had higher yield potential than U6714-A-011, but both lines underperformed relative to locally adapted check varieties and the recurrent wheat parent KS05HW14. To further characterize isolate-specificity of *Pm58*, the two lines were inoculated with 20 powdery mildew isolates collected throughout central- and eastern-United States. Isolate-specific interactions differed slightly between U6714-A-011 and U6714-B-056, but both lines were more powdery mildew resistant than the recurrent wheat parent KS05HW14. Seed of these germplasm lines has been archived in the USDA-ARS National Center for Genetic Resources Preservation storage facility in Fort Collins, CO, and active seed stocks will be maintained by the National Small Grains Collection in Aberdeen, Idaho. Similar efforts to release *SrTA10187* germplasm are underway

through a collaboration with the Hard Winter Wheat Genetics Research Unit, USDA-ARS, in Manhattan, KS.

Overcoming past limitations in disease resistance breeding

Since the earliest attempts to map agronomically important traits in wheat, recombination rate and marker availability have remained the primary limitations. Matters are further complicated by introgression of alien loci that may not recombine readily with wheat and can be linked to loci that reduce grain quality and yield (Wulff and Moscou 2014). This linkage drag often hampers genetic gain for important breeding targets and discourages disease resistance breeding. As demonstrated in the studies described here, development of wheat-*Ae. tauschii* introgression lines with high background wheat isogeneity and using current SNP genotyping platforms progress has been made towards overcoming some of these hurdles. By using the D genome progenitor species *Ae. tauschii*, homologous D genome loci could recombine normally and mapping efforts were simplified to diploid segregation of only D genome loci. Higher throughput GBS and KASP™ marker platforms increased the speed and resolution of mapping. With improved marker resolution and genetic maps, the location of disease resistance genes could be more accurately defined and tightly linked genetic markers developed. Now efforts can be refocused on reducing the genetic load of deleterious loci from *Ae. tauschii*, identifying well-adapted germplasm, and deploying novel resistance genes in combination other major-gene or adult-plant resistance to increase durability.

Future perspectives

In the past, well-funded plant breeding programs have engaged in positional cloning of disease resistance genes. Now, the argument can be made that resistance gene cloning should be practiced in specialized labs using advanced techniques that rely on variations of bulked segregant analysis, chemical mutagenesis, or genomic reduction by resistance gene enrichment (RenSeq) or chromosome flow sorting (Bent 2016, Sanchez-Martin et al. 2016). Two recent studies relied on a RenSeq approach to preferentially capture and sequence DNA fragments belonging to the NLR gene family (Jupe et al. 2013). In the first study, multiple lines were chemically mutagenized to independently knock-out resistance gene function. Then, by searching for a single resistance gene that was mutated in all the knock-out lines, the resistance gene was cloned (Steuernagel et al. 2016). In the second study, after fine mapping the resistance locus, RenSeq was used in combination with single-molecule real-time sequencing to *de novo* assemble and clone six NLR genes in the region (Witek et al. 2016). Alternatively, genome reduction has also been done using flow cytometry to isolate and sequence single chromosomes linked to resistance. Again, by identifying contigs that were mutated in multiple knock-out lines, the resistance gene was identified and cloned (Sanchez-Martin et al. 2016). Although none of these techniques are the "silver bullet" for disease resistance gene cloning, they each represent progress towards more cost-effective and less time-consuming methods. One caveat, however, is that labs with established protocols, specialized equipment, and bioinformatics expertise will be much better suited for the task.

APPENDIX

Characterizing *YrTA1718* from *Aegilops tauschii* in hexaploid wheat and germplasm development

Introduction

Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), has become one of the most problematic diseases affecting wheat (*Triticum aestivum* L.; Schwessinger 2017). In the last 50 years, *Pst* virulence and race diversity has gradually increased with the introduction of major gene resistance in wheat (Liu et al. 2017). Starting around the year 2000, an unprecedented rise in *Pst* virulence and disease severity was observed in the United States, Australia, and Europe (Hovmoller et al. 2010, Hubbard et al. 2015). This recent trend is especially concerning in the eastern United States soft wheat region where stripe rust was not problematic in the past and most commercial wheat varieties are *Pst*-susceptible (Cereal Rust Bulletins, www.ars.usda.gov). Due to recent stripe rust epidemics, wheat breeders are rapidly searching for effective sources of resistance.

There is a long tradition of using wild wheat relatives, including the diploid D genome progenitor species *Aegilops tauschii* Coss., as reservoirs for novel disease resistance genes. Although numerous attempts have been made to transfer stripe rust resistance from *Ae. tauschii* to hexaploid wheat, only one formally designated *Ae. tauschii* resistance gene, *Yr28*, has been mapped in wheat (Singh et al. 2000). Another temporarily designated resistance gene, *YrAS2388* (also derived from *Ae. tauschii*), was mapped to a 4DS locus near *Yr28* and expressed different *Pst* race-specificity (Huang et al. 2011). Many other attempts to transfer resistance from *Ae. tauschii* to wheat were less successful due to suppression of stripe rust resistance in hexaploid genomic backgrounds (Ma et al. 1995, Yang et al. 2003, Chen et al 2013).

A small population of BC_2F_4-derived wheat introgression lines (U6719) was developed by direct hybridization and backcrossing of the stripe rust resistant *Ae. tauschii* accession TA1718 and the susceptible wheat line KS05HW14. The objectives of this study were to characterize seedling and adult plant resistance segregating in U6719, and to develop improved germplasm and F_2 mapping populations. Additionally, the resistance from TA1718 will be integrated into the soft white winter wheat variety Ambassador using backcross-breeding to improve stripe rust resistance resources in the eastern US soft wheat growing region.

Methods and Materials

Plant materials

The stripe rust resistant *Ae. tauschii* accession TA1718 was originally collected in Iran and is currently maintained by the Wheat Genetic and Genomic Resource Center in Manhattan, KS (www.genesys-pgr.org). TA1718 was hybridized directly with the hard white winter wheat line KS05HW14 (Figure 5.1). Interspecific F_1 embryos were rescued on growth media following the method described by Olson et al. (2013). Recovered F_1 plants were used as females in an initial backcross to the recurrent wheat parent KS05HW14. A single BC_1F_1 plant was recovered and backcrossed to KS05HW14 as the male parent. A total of 15 BC_2F_1 plants belonging to the U6719 family were advanced by single-seed-descent to the BC_2F_4 generation when the seed from a single plant was harvested and increased in subsequent generations to produce BC_2F_4-derived lines.

The wheat introgression line U6719-004 was selected for mapping population development following stripe rust resistance screening and yield testing (Figure 5.1). U6719-004 was backcrossed as a male with KS05HW14, and BC_3F_1 plants were self-fertilized to establish a

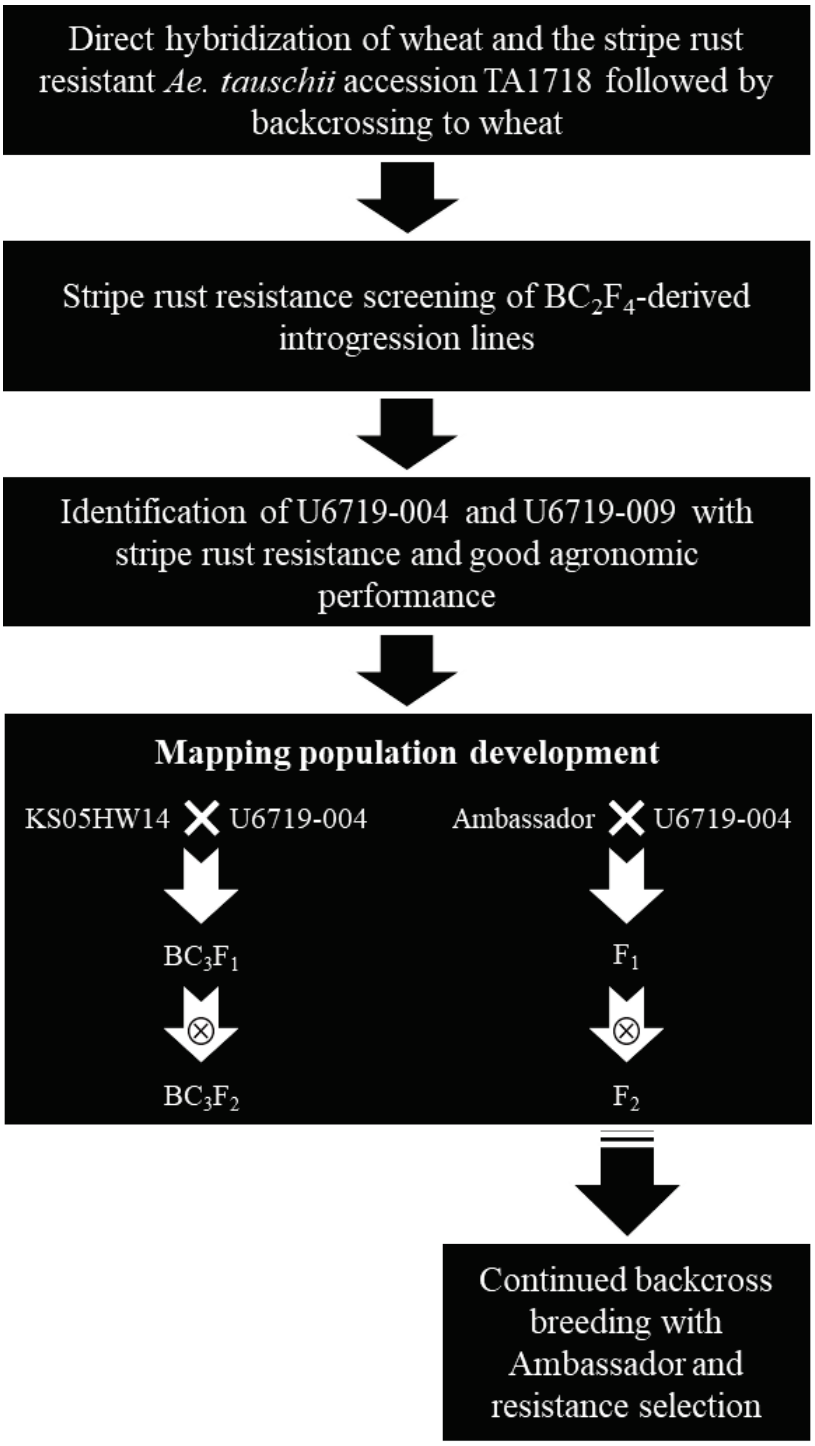

Figure 5.1 Introgression of stripe rust resistance from *Ae. tauschii* accession TA1718 and mapping population development. In hybridization diagrams, female parents are listed on the left-side. Encircled X indicates self-fertilization.

BC_3F_2 mapping population. Likewise, U6719-004 was crossed as a male with the soft white winter wheat variety Ambassador, and F_1 plants were self-fertilized to establish an additional F_2 mapping population. Ambassador is a high yielding, stripe rust susceptible wheat line that is well-adapted to Michigan growing environments.

Seedling stripe rust evaluation

Seedling stripe rust phenotyping of BC_2F_4-derived wheat lines and F_2 mapping populations was done in the growth chamber facility at Michigan State University. Stripe rust urediniospores used in this study were isolated from naturally occurring infections in Michigan and confirmed to be race *Pstv-37* based on reactions to a set of single-gene differential wheat lines (Wan and Chen 2014). Following a 5 min heat-shock at 45°C, urediniospores were suspended in Soltrol 170 isoparaffin oil (Chevron Philips Chemical Company LP, The Woodlands, TX) and sprayed onto two-leaf seedlings using an airbrush. Plants were then incubated in a dew chamber at 14°C and 100% relative humidity for 18 h. After incubation, plants were transferred back to a growth chamber held at 14°C. Seedling infection types (IT) were recorded at 18 days post inoculation using a 0 to 9 scale described previously (Wan and Chen 2014).

Adult plant stripe rust evaluation and yield testing

Adult plant stripe rust phenotyping of U6719-004, U6719-009, and the recurrent parent KS05HW14 was done at six locations across the United States in the year 2016. All the locations included in this study experienced near-epidemic levels of stripe rust in 2016 due to a mild winter followed by a cool spring which lead to early and severe onset of disease. Each location

was naturally infected by *Pst* races present in the region at that time. Four of the six locations were planted as yield trials: Brookings, SD; Hays, KS; Pullman, WA; and Richville, MI. The remaining two locations, Central Ferry, WA and Fayetteville, AR, were planted as head rows. Flag leaf severity was rated at all locations as the percentage (0-100%) of leaf area covered with stripe rust urediniospores (Peterson et al. 1948). At four locations, the *Pst* IT (0-9) was also rated on flag leaves (Wan and Chen 2014).

Yield testing of U6719-004, U6719-009, KS05HW14, and locally adapted check varieties was conducted at ten year-by-location environments. Lines were tested in two consecutive years (2015 and 2016) at Ashland, KS; Hays, KS; and Richville, MI. The remaining locations, Brookings, SD; Champaign, IL; Marianna, AR; and Pullman, WA, were only tested once in 2016. The yield trial locations are the same as those where adult plant stripe rust was rated. At each trial location, U6719-004 and U6719-009 were planted in single replicate plots in an augmented design containing six incomplete blocks. Locally adapted check varieties and the recurrent parent KS05HW14 were replicated in each block and used for yield comparisons and block corrections. Using a mixed linear model where genotype was treated as a fixed effect and block was treated as a random effect, grain yield least squares means (LS-means) and 95% confidence intervals were calculated in Rstudio® (RStudio, Boston, MA, USA, R version 3.2.1) using the packages lme4 (v.1.1-12) and lsmeans (v.2.25-5).

Preliminary Results

Identification of stripe rust resistance from TA1718

A small population of BC_2F_4-derived wheat introgression lines, U6719 (n = 15), was developed from the direct hybridization of the stripe rust resistant *Ae. tauschii* accession TA1718 and

susceptible wheat line KS05HW14. To ascertain if resistance from TA1718 was successfully transferred and expressed in a hexaploid wheat background, U6719 wheat introgression lines were screened for seedling stripe rust resistance. A total of 4 plants expressed seedling resistance to *Pstv-37* and had an IT ranging from 3-4 (Table 5.1 and 5.2). The segregation ratio of resistant to susceptible plants indicates that stripe rust resistance segregated as a single-locus trait (Table 5.1, $\chi^2 = 0.02$, *P*-value = 0.88) and was temporarily designated *YrTA1718*.

Characterizing adult plant resistance and yield in U6719-004 and U6719-009

Two BC_2F_4-derived wheat introgression lines, U6719-004 and U6719-009, confirmed to be stripe rust resistant by seedling tests were selected for adult plant stripe rust resistance characterization. In 2016, both lines were evaluated in six diverse United States environments including: Brookings, SD; Central Ferry, WA; Fayetteville, AR; Hays, KS; Pullman, WA; and Richville, MI. Naturally occurring stripe rust incidence and severity was high across all locations. U6719-004 and U6719-009 expressed adult plant resistance in every environment tested and had consistently lower IT and flag leaf severity compared to the recurrent parent KS05HW14 (Table 5.2). Variation in IT and severity scores between locations may be accounted for by differences in *Pst* race profiles.

The same two lines were also yield tested in 10 year-by-location environments between 2015 and 2016. Yield trial locations included: Ashland, KS; Brookings, SD; Champaign, IL; Hays, KS; Marianna, AR; Pullman, WA; and Richville, MI. U6719-004 and U6719-009 both exhibited exceptional yield performance relative to the recurrent wheat parent KS05HW14 (Table 5.3). In six out of ten environments, U6719-004 had higher yield than mean KS05HW14 yield. In five out of ten environments, U6719-009 had higher yield than mean KS05HW14 yield.

Table 5.1 Segregation of *Pst* resistance in BC_2F_4-derived introgression lines and BC_3F_2 and F_2 mapping populations

Family	Pedigree	Generation	Number of plants			Expected ratio (R:S)[b]	χ^2	*P*-value
			Resistant[a]	Susceptible	Total			
U6719	KS05HW14///KS05HW14/TA1718//KS05HW14	$BC_2F_{4:6}$	4	11	15	1:3	0.02	0.88
MSU16000953	KS05HW14/U6719-004	BC_3F_2	34	72	106	3:1	104.16	<0.001
MSU16000955	Ambassador/U6719-004	F_2	38	61	99	3:1	70.79	<0.001

[a] Plants with an infecgtion type < 7 were classified resistant

[b] Assuming *YrTA1718* dominance

Table 5.2 Seedling and adult *Pst* infection types and severity on wheat lines KS05HW14, U6719-004, and U6719-009.

Location	Year	Growth stage	KS05HW14		U6719-004		U6719-009	
			IT (0-9)	Severity	IT (0-9)	Severity	IT (0-9)	Severity
Growth Chamber (race *Pstv-37*)	2015	Seedling	8[a]	-	**3**	-	**4**	-
Brookings, SD (yield trial)	2016	Adult	-	41%[a]	-	16%	-	7%
Central Ferry, WA (head rows)	2016	Adult	7[a]	73%[a]	**3**	25%	**3**	20%
Fayetteville, AR (head rows)	2016	Adult	-	59%[a]	-	7%[a]	-	5%[a]
Hays, KS (yield trial)	2016	Adult	9[a]	74%[a]	**3**	10%	**1**	5%
Pullman, WA (yield trial)	2016	Adult	8[a]	48%[a]	**5**	30%	**6**	40%
Richville, MI (yield trial)	2016	Adult	7[a]	32%[a]	**3**	10%	**3**	10%

[a] Mean IT and severity reported for replicate growth chamber pots or field plots. Resistant infection types are indicated in bold.

Table 5.3 Grain yield LS-means of locally adapted check varieties, KS05HW14, U6719-004, and U6719-009 in 10 diverse environments throughout the United States.

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		Locally adapted check varieties			KS05HW14		U6719-004	U6719-009
Location	**Year**	**Name**	**Grain yield**	**95% CI**	**Grain yield**	**95% CI**	**Grain yield (% KS05HW14)**	**Grain yield (% KS05HW14)**
Ashland, KS	2015	Everest	4.64	4.45 - 4.82	4.66	4.41 - 4.89	4.99 (107%)	5.04 (108%)
Ashland, KS	2016	Everest	4.85	4.65 – 5.05	4.2	4.11 – 4.30	4.01 (95%)	3.37 (80%)
Brookings, SD	2016	Lyman	3.6	3.30 - 3.90	3.3	3.17 - 3.44	5.39 (163%)	5.32 (161%)
Champaign, IL	2016	IL07-19334	7.85	7.47 - 8.22	5.67	5.50 - 5.85	4.57 (81%)	4.26 (75%)
Hays, KS	2015	Ernie	2.73	2.34 - 3.12	3.22	3.05 - 3.40	4.02 (125%)	3.62 (112%)
Hays, KS	2016	Joe	6.76	6.65 - 6.87	3.61	3.56 - 3.66	4.69 (130%)	5.25 (145%)
Marianna, AR	2016	AR11LE24	4.12	3.86 - 4.39	2.73	2.60 - 2.85	2.86 (105%)	3.01 (110%)
Pullman, WA	2016	Jasper	9.06	8.75 - 9.37	4.45	4.31 - 4.59	4.08 (92%)	3.63 (82%)
Richville, MI	2015	AC Mountain	5.18	5.09 - 5.26	4.95	4.76 - 5.15	4.48 (91%)	4.43 (89%)
Richville, MI	2016	Ambassador	5.63	5.31 - 5.95	5.31	5.15 - 5.46	6.13 (115%)	5.03 (95%)

CI = confidence interval.

In a few locations, U6719-004 and U6719-009 even outperformed locally adapted check varieties. It is likely that stripe rust resistance fixed in U6719-004 and U6719-009 contributed to yield performance, especially when compared to susceptible check varieties including KS05HW14.

Mapping population seedling resistance

With the intent to eventually map *YrTA1718*, U6719-004 was crossed with KS05HW14 and the *Pst*-susceptible soft white wheat variety Ambassador to develop BC_3F_2 and F_2 mapping populations, respectively (Figure 5.1). Using the race *Pstv-37*, 106 BC_3F_2 and 99 F_2 plants were screened for seedling stripe rust resistance. In both populations, resistance was expressed in approximately 35% of individuals which did not conform to the expected segregation ratio of a dominant resistance gene (Table 5.1, BC_3F_2 $\chi^2 = 104.16$, *P*-value <0.001 and F_2 $\chi^2 = 70.79$, *P*-value <0.001). Although the resistance response was not as complete as that of the resistance donor *Ae. tauschii* accession TA1718, the resistant phenotype was evident from extensive leaf chlorosis and necrosis and reduced *Pst* sporulation. Susceptible BC_3F_2 and F_2 lines resembled the susceptible wheat parents KS05HW14 and Ambassador (Figure 5.2).

Discussion

The successful introgression of *YrTA1718* from *Ae. tauschii* into wheat was demonstrated by seedling resistance screening of BC_2F_4-derived wheat introgression lines. Two lines fixed for *YrTA1718* seedling resistance also expressed adult plant resistance at field sites throughout the United States—which may indicate that *YrTA1718* is effective against a broad range of *Pst* races

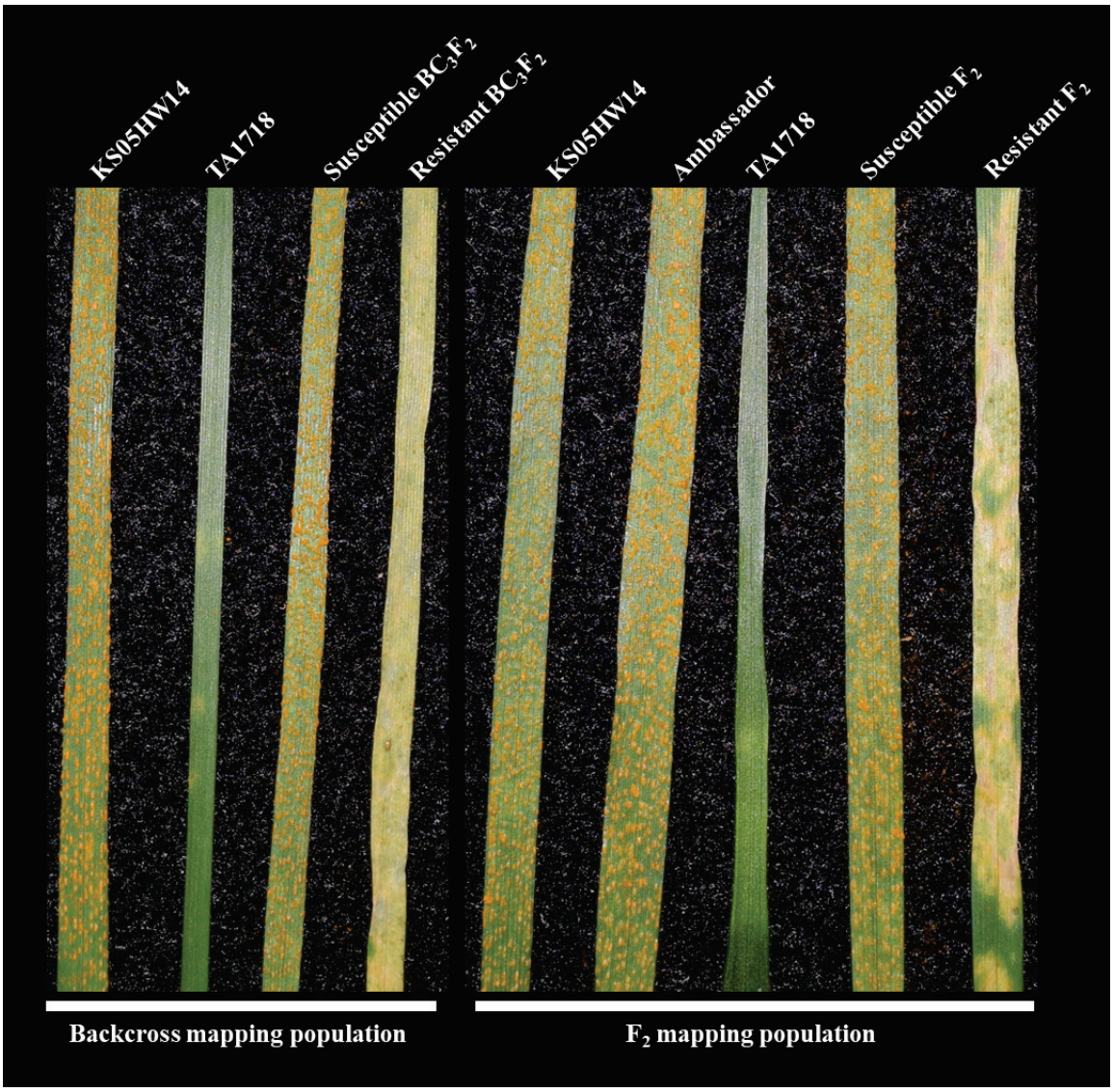

Figure 5.2 *Pstv-37* seedling leaf infection types of KS05HW14, TA1718, Ambassador, and resistant and susceptible BC_3F_2 and F_2 individuals.

(Annual Stripe Rust Race Reports, http://striperust.wsu.edu/races/data/). The importance of stripe rust disease resistance was also highlighted by higher grain yield of resistant lines U6719-004 and U6719-009 compared to the susceptible parent KS05HW14 at locations with high disease pressure. In fact, either line may be suitable for immediate germplasm release.

Ongoing efforts to map *YrTA1718* and backcross-breed resistance into a soft white winter wheat background were initiated by the development of BC_3F_2 and F_2 mapping populations. It is still unknown if *YrTA1718* is a novel disease resistance gene, or if it is one of the previously identified *Ae. tauschii* resistance genes *Yr28* or *YrAS2388*. Mapping of *YrTA1718* will help elucidate its relationship to other known resistance genes and inform breeding decisions. Resistant F_2 progeny were recovered from a cross between U6719-004 and Ambassador which indicates that *YrTA1718* was not suppressed. Additional backcrossing with Ambassador and resistance selection should be continued to develop a stripe rust resistant soft white winter wheat line that is well adapted to the eastern US soft wheat growing region (Figure 5.1).

Finally, based on the segregation ratio of resistant and susceptible plants in BC_3F_2 and F_2 mapping populations it appears that *YrTA1718* is not a dominant resistance gene. An alternative hypothesis that may explain the unexpected segregation ratio could be that *YrTA1718* is a recessive resistance gene, or that deleterious alleles from *Ae. tauschii* caused segregation distortion. It is also possible that *YrTA1718* behaves in a dosage-dependent manner, where heterozygous individuals express a more susceptible response that is hard to distinguish from homozygous susceptible individuals. Genetic markers linked to *YrTA1718* and progeny tests of $BC_3F_{2:3}$ and $F_{2:3}$ plants will likely clarify this observation.

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