MOLECULAR MAPPING OF RUST RESISTANT QTLS IN A SYNTHETIC HEXAPLOID WHEAT POPULATION

By

Jeffrey David Kovach

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ABSTRACT

MOLECULAR MAPPING OF RUST RESISTANT QTLS IN A SYNTHETIC HEXAPLOID WHEAT POPULATION

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Triticum aestivum, common wheat, is a widely grown crop that is responsible for a large portion of worldwide consumed calories. Bread wheat is an allohexaploid derived from a series of hybridization and polyploidization events between three diploid progenitor species. Due to its origin, genetic diversity across the hexaploid wheat genome is lacking, especially in the D genome. *Ae. tauschii*, the D genome diploid progenitor, is an important source of D genome variation and has been used to identify novel genes for multiple traits including disease resistance. The evolution and spread of new pathogenic *Puccinia* races highlight the need to identify and utilize novel sources of disease resistance. A recombinant inbred line population was derived from a cross between the synthetic 9.131.15(tetraPrelude/TA2474) and KS05HW14, a hard white winter wheat. This population was phenotyped for resistance to stem, stripe and leaf rust. All-stage resistance to stem and stripe rust was mapped on 5BL and 4DS originating from 9.131.15 and TA2474, respectively. Leaf rust resistance was mapped to 3BL and adult plant resistance QTLs were mapped to the 6D and 7D chromosomes for leaf and stem rust resistance in Mason, MI in 2017. This study identifies resistance to all three *Puccinia* rust species in this RIL population. However, further work is needed to determine if the 4DS and 5BL loci are novel and the identified adult plant resistance QTLs can be repeatedly observed. A single RIL was phenotyped as resistant to all three *Puccinia* fungal pathogens in all tested environments. This line can be used as a useful source of disease resistance to these fungal pathogens.

This thesis is dedicated to my family, friends, and loving fiancée. Thank you for all your support over the years. This would not be possible without you.

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KEY TO ABBREVIATIONS

APR Adult plant resistance

CIM Composite interval mapping

GBS Genotyping-by-sequencing

GH Greenhouse

HTAPR High temperature adult plant resistance

LOD Logarithm of the odds

MM Mason, MI

MYA Million years ago

Pt Puccinia triticina

Pgt Puccinia graminis f. sp. *tritici*

Pst Puccinia striiformis f. sp. *tritici*

QTL Quantitative trait loci

RIL Recombinant inbred line

RH Relative humidity

SHW Synthetic hexaploid wheat

CHAPTER 1: Introduction

Wheat's Importance, Evolution and Origin

Wheat's Importance in Agriculture

Wheat is one of the most important row crops grown worldwide and a common sight when traveling through agricultural regions. However, a field full of amber wheat spikes can quickly transform into shriveled diseased heads attached to broken stems due to the presence of evolving virulent pathogens. In 2016, wheat was planted on over 50 million acres in the United States alone (USDA, 2016). Flour from wheat is used to produce a wide range of food products ranging from crackers to bread. Around nineteen percent of calories consumed by humans are from wheat (Braun et al. 2010). Since wheat is widely grown and an important food source there is a considerable world wide effort to continually improve yields. However, wheat yields are affected by both the environment they are grown in and the diseases present in their environment. The *Puccinia* fungal species are treacherous wheat pathogens because of their capability to evolve new aggressive and virulent races to overcome presently deployed resistance genes (Milus et al., 2009; Singh et al., 2011b; Hovmøller et al., 2016; Bhattacharya, 2017). In order to better understand methods to improve hexaploid wheat, it is important to understand the origin of hexaploid wheat to more effectively utilize available wild germplasm.

Evolution and Origin of Hexaploid Wheat

The evolution of hexaploid wheat began over 10,000 years ago with the domestication of cereals beginning where present day Turkey and Syria are located and is tied with the development of agriculture (Lev-Yadun et al., 2000; Tanno, 2006). Wheat is a member of the grass family *Poaceae* and the tribe *Tritceae* which include barley and rye. Divergence between

wheat and these other members of *Tritceae* occurred 11 to 7 MYA respectively (Huang et al., 2002). Common wheat, *Triticum aestivum* (AABBDD, 2n=6x=42), is the most widely cultivated wheat worldwide. Common wheat is an allohexaploid which consists of the independently segregating A, B, and D genomes. The grouping of these genomes during the evolution of common wheat involved a series of interspecific hybridizations, followed by subsequent polyplodization, between three different diploid progenitor species. The first in this series of hybridizations occurred between the A genome progenitor and the B genome progenitor species which, when followed by spontaneous chromosome doubling, generated the wild tetraploid emmer species *T. turgidum* ssp. *dicoccoides* (AABB, 2n=4x=28) (Feuillet et al., 2008). The A genome progenitor species that produced this wild tetraploid has been demonstrated to be, *T. uratru* (AA, 2n=2x=14) (Chapman et al., 1976; Dvorák et al., 1993). The identification of the B genome progenitor species has been difficult. It was originally speculated to be the wild goat grass species *Ae. speltoides* (SS, 2n=2x=14),(Sarkar and Stebbins, 1956). However, current research supports the theory that *Ae. speltoides* is instead a closely related relative of the B genome progenitor species (Salse et al., 2008). This wild emmer species was subsequently cultivated resulting in the formation of *T. turgidum* ssp. *dicoccum* (Peng et al., 2011).

The second interspecific hybridization and spontaneous chromosome doubling occurred between cultivated emmer *T. dicoccum* (AABB, 2n=4x=28) and the D genome progenitor species resulting in the formation of hexaploid wheat (McFadden and Sears, 1946). The D genome of hexaploid wheat originated from the wild goat grass species *Ae. tauschii* ssp. *strangulata* (DD, 2n=2x=14) (Kihara, 1944; McFadden and Sears, 1946). It is thought that cultivated emmer, and not wild emmer, hybridized with *Ae. tauschii* to generate common wheat due to the shared geographical distribution of *T. dicoccum* with *Ae. tauschii* (Zohary et al., 1969;

Cox, 1997). *Ae. tauschii* itself is hypothesized to have arisen from a homoploid hybridization between the A and B genome progenitor species (Marcussen et al., 2014). However, taking into account the presence of additional genomes in the Aegilops-Triticum complex and using both nuclear and chloroplast DNA, evidence supports a hybrid origin of *Ae. tauschii* but further work is needed to determine the correct model describing its origins (Li et al., 2015). The origin of common wheat helps to explain the amount of genetic diversity present in current breeding populations and identify potential sources to improve it.

Genetic Diversity in Hexaploid Wheat

Genetic Diversity within the Hexaploid Subgenomes

The recent polyploidization of common wheat has resulted in a narrow genetic base (Feuillet et al., 2008; Peng et al., 2011). However, this lack of genetic diversity is not consistent between the three subgenomes. The A and B genomes contain a higher amount of polymorphisms compared to the D genome (Poland et al., 2012; Cavanagh et al., 2013; Wang et al., 2014). An explanation for the increased diversity in the A and B genomes present in hexaploid wheat is the potential formation of female fertile hybrids by tetraploid and hexaploid wheat crossing (Cox, 1997; Dvorak et al., 2006). These fertile hybrids would enable hybridization between tetraploids and the hexaploid population increasing genetic diversity of the shared genomes (Figure 1.1). However, in the case of the D genome, the recent polyplodization event between *Ae. tauschii* and *T. turgidum* created a genetic bottleneck, reducing the genetic diversity present in the D genome of *T. aestivum* compared to the diploid progenitor species (Dvorak et al., 1998; Caldwell et al., 2004; Reif et al., 2005).

It is widely thought that the Green Revolution, while significant in increasing wheat production, has reduced the genetic diversity in breeding populations (Trethowan et al., 2007). There was an initial decrease in genetic diversity after the Green Revolution where intense selection for high yielding and disease resistant varieties was undertaken (Warburton et al., 2006). In efforts to introduce a greater amount of genetic diversity, specifically in the D genome, breeders can use available landraces or wild relatives in their programs. The use of wild species has increased the genetic diversity of both spring hexaploid and durum wheat varieties since the Green Revolution (~1980s to present) (Reif et al., 2005; Ren et al., 2013). However, the difference in genomewide genetic diversity between land races and modern cultivars is minor, which is speculated to be because selection from breeding acts on only a subset of the genome (Cavanagh et al., 2013). In contrast, *Ae. tauschii* accessions have greater D genome variation compared to both landraces and modern bred cultivars (Reif et al., 2005). The introduction of novel variation from wild species, such as *Ae. tauschii*, is paramount to continue the improvement of modern wheat varieties.

Identification and Utilization of Ae. tauschii Genetic Diversity

As modern breeding practices are used to develop new elite wheat varieties, there is an ever increasing emphasis to identify new sources of beneficial alleles to enhance current germplasm. Novel genetic variation that is available for use can be separated into different gene pools based on observed meiotic pairing and production of fertile hybrids with the primary gene pool allowing for recombination and fertile hybrids to be generated through hybridization (Figure 1.2) (Cox, 1997; Feuillet et al., 2008). Those species that are included in this primary gene pool include *T. turgidum*, and the progenitor diploid species (Harlan and de Wet, 1971; Cox, 1997). The low genetic diversity of the D genome can potentially be improved through the

introduction of novel alleles from *Ae. tauschii*. The lineage of *Ae. tauschii* accessions and the method of introgression can impact the successful introgression of beneficial alleles to common wheat. The *Ae. tauschii* gene pool itself can be split into two separate lineages based on DNA marker data (Dvorak et al., 1998; Wang et al., 2013). While it is unknown the specific number of hybridizations that occurred between *Ae. tauschii* and *T. dicoccum* to form hexaploid wheat, it has been demonstrated that a single *Ae. tauschii* sublineage is the most related to the original D genome progenitor ancestor (Wang et al., 2013). Therefore, *Ae. tauschii* accessions that are not genetically similar to this sublineage may be of additional interest to use to introduce novel genetic variation. The introduction of novel variation from wild species into cultivated wheat has several key problems that need to be addressed including: (1) the phenotyping of wild and alien species for desired traits, (2) the introduction of genetic information from donor species into an adapted variety.

Identification of *Ae. tauschii* accessions that contain desirable alleles can be performed in a straightforward manner for those qualitative traits that are controlled by a few major effect genes. One example is disease resistance, especially major gene resistance, which can be evaluated directly in wild species. Examples of this include *Septoria tritici* blotch (McKendry and Henke, 1994) and the rusts (Zaharieva et al., 2001; Assefa and Fehrmann, 2004; Liu et al., 2010; Rouse et al., 2011). Thus, the identification of novel genetic variation that contributes to highly heritable traits can be done before the introgression of wild relatives into a common wheat background. However, identifying wild germplasm with desirable alleles that affect quantitative traits controlled by many minor effect QTLs, such as grain yield, can be difficult due to the presence of several negative alleles in the background of these un-adapted varieties (Cox, 1997). In order to identify genomic regions that confer positive alleles from wild germplasm it is often

necessary to evaluate wild material in an adapted background and use backcross methods to reduce linkage drag to eliminate negative alleles (Tanksley and Nelson, 1996).

Introduction of genetic variation from *Ae. tauschii* can be done through either direct crossing or the production of synthetic hexaploids. Direct crossing between hexaploid and diploid *Ae. tauschii* creates an F_1 hybrid that has an AABBD genome. However, endosperm development of the F_1 hybrids is halted and requires embryo rescue to successfully produce a plant (Gill and Raupp, 1987). These F_1 hybrids are male sterile and are backcrossed twice to the recurrent parent to return to a normal ploidy level (Olson et al., 2013a). It has been previously demonstrated that the genotype of wild relatives other than *Ae. tauschii* has an impact on the number of F_1 hybrids recovered during direct crosses (Sharma and Ohm, 1990; Valkoun et al., 1990; Tixier et al., 1998; Fedak, 1999). Differences in the number of F_1 embryos per direct cross produced has been observed between white and red winter wheat and different *Ae. tauschii* accessions (E. Olson, Personal Communication). However, there are no known reasons for this genotypic effect in direct hexaploid wheat x *Ae. tauschii* crosses. Synthetic wheat is produced through the direct hybridization of tetraploid wheat (AABB) with *Ae. tauschii* (DD) to create a triploid (ABD), which, through chromosome doubling, produces a hexaploid wheat line (AABBDD) (McFadden and Sears, 1946). Production of direct hybrids can be technically challenging in comparison to the production of synthetic lines. However, it can allow for an accelerated return to an adapted background after the initial direct cross.

The tetraploid parent in a synthetic cross can be a *T. turgidum* line or an extracted tetraploid. Extracted tetraploids are made through the crossing of a hexaploid line to a tetraploid line to create a pentaploid F_1 hybrid which is then backcrossed for several generations to create an AABB extracted tetraploid (Kerber, 1964). These extracted tetraploids retain the A and B

genomes from their hexaploid form. The extracted tetraploids have less vigor than their hexaploid counterparts owing to the elimination of a third of their genome, though differences in vigor may be impacted by the tetraploid accession and the number of backcrosses used to produce the extracted tetraploid (Figure 1.3) (Kerber, 1964; Kaltsikes, P. J. , Evans, L.E. , Larter, 1969; Yang et al., 1999).

Overview of the *Puccinia* Genus

A major group of pathogens that affect wheat and other grass species are the fungi that are members of the *Puccinia* genus. The fungi in this genus can be delineated into multiple species dependent on the host plants they are capable of infecting. *Puccinia striiformis f.sp. tritici* (wheat stripe rust), *Puccinia graminis f. sp. tritici* (wheat stem rust) and *Puccinia triticina f. sp. tritici* (leaf rust) being among the rust fungi that infect wheat. These species can be further delineated into "races" which describes a specific virulence/avirulence to a specific set of resistance genes (Roelfs, 1984).

Life Cycle

The life cycle of the members of the *Puccinia* genus is well understood (Chen, 2005; Leonard and Szabo, 2005; Chen et al., 2014). Briefly, the asexual uredinospores (n+n) germinate and fungal hyphae enter through the stomata. After the fungus has developed, hyphae burst through the epidermis and produce new uredinospores asexually. As the wheat plant begins to go through senescence, diploid teliaspores form through the fusion of the haploid genomes of the uredinospores. These teliospores undergo meiosis to produce haploid basidospores which infect the alternative host. After successful germination and proliferation, basidospores develop pycinia which produce spermagonia that fuse with other spermagonia to produce dikaryotic mycelium.

These mycelium develop and produce aecia which release aceiospores that infect wheat. The asexual uredinospores that are subsequently produced are capable of spreading over thousands of miles and can cause multiple cycles of infection over a single growing season (Roelfs and Bushnell, 1984).

Stem, stripe, and leaf rust are examples of heterocious biotrophic fungal pathogens that complete their life cycle on two different hosts. Asexual propagation occurs on the primary host while an alternative host is required for the successful completion of sexual reproduction. The alternative host for stem rust has been known to be barberry but it has not been demonstrated until recently that stripe rust can develop on both barberry species and *Mahonia aquifolium,* Oregon grape (Jin, 2011; M.N, 2013; Zhao et al., 2013). Unlike stem and stripe rust, leaf rust's alternative host is *Thalictrum speciosissimum* (Bolton et al., 2008; Kolmer, 2013). The presence of this mixed reproduction in the rusts creates a situation where new virulent races are capable of being produced at a high rate (McDonald and Linde, 2002; Jin, 2011). Two factors that can influence the impact of these spores are the environment a wheat crop is grown in and the deployment of resistant varieties. Rust spores are sensitive to both humidity and temperature, which can affect spore germination and limits the initial presence of spores at the beginning of the growing season in more temperate growing zones (McGregor and Manners, 1985; Kramer and Eversmeyer, 1992; Chen, 2005). The evolution of new rust races that are more aggressive and are virulent to currently deployed resistance genes can have a potentially devastating effect on wheat yield. Thus, it is necessary for new novel sources of rust resistance to be identified.

Puccinia graminis as a Pathogen

Highly Virulent Races are Capable of Overcoming a Majority of Current Resistance

The overwintering of stem rust on the alternative host, *Berberis vulgaris L*, or what is known commonly as Common Barberry, allows for both sexual recombination and earlier infection to occur in these regions where it is grown (Jin, 2011). In the early 20th century, there was a concerted effort to eliminate the common barberry from the United States and this program was a success with only occasional sightings of common barberry in non-grain growing areas (Roelfs, 1982). This "elimination" of the sexual recombination step in the stem rust's life cycle can negatively impact its ability to adapt and overcome deployed resistance genes. However, the elimination of the alternative host does not completely eliminate the potential for new aggressive races. The proliferation of clonally propagated uredinospores and the accumulation of spontaneous mutations can lead to the formation of new virulent races (Jin, 2011). Several new stem rust races have been identified including *Pgt*-TTKSK, also known as Ug99, and *Pgt*-TTTTF that are virulent against a large number of stem rust resistance genes that are currently used, with Ug99 being capable of successfully infecting over 80% of wheat cultivars (Singh et al., 2011b; Bhattacharya, 2017). There is a need to identify new sources of genetic variation in current wheat breeding germplasm and wild relatives that can be used to identify sources of resistant to these constantly evolving pathogens.

Stem Rust Resistance Genes from Ae. tauschii

Stem rust resistance genes have been identified on all three genomes. The diploid progenitor for the D genome *Ae. tauschii* has served as a useful source of resistant genes. Efforts have been directed towards identifying these disease resistance genes with 42 stem rust genes

officially designated(McIntosh et al., 2014). Currently designated stem rust genes successfully introgressed from *Ae. tauschii* into common wheat include *Sr33, Sr45, Sr46, SrTA1662, SrTA10171*, and *SrTA10187* (Kerber and Dyck, 1979; Marais et al., 1998; McIntosh et al., 2013; Olson et al., 2013a; b). *Sr33* and *Sr45* are located on the 1D chromosome and originated from *Ae. tauschii* accessions RL5288 and RL5289, respectively (Marais et al., 1998). *Sr46* is located on the 2D chromosome and is from the *Ae. tauschii* accession TA1703 (Rouse et al., 2011). *SrTA1662*, from the *Ae. tauschii* accession TA1662, is located on the 1D chromosome (Olson et al., 2013a). *SrTA10171* and *SrTA10187*, from *Ae. tauschii* accessions TA10171 and TA10187, are located on the 7D and 6D chromosomes, respectively (Olson et al., 2013b). All six of these genes are all-stage resistance genes that are effective against Ug99 (Rouse et al., 2011; Olson et al., 2013a; b). However, *Sr33, Sr45,* and *Sr46* were susceptible to *Pgt*-TTTTF based on seedling tests (Rouse et al., 2011).

***Puccinia striiformis* as a Pathogen**

Aggressive Virulent Stripe Rust Races are becoming Widespread

Historically, the temperature sensitivity of stripe rust has limited its primary range to where local inoculum can survive until it is spread by wind into more temperate regions. The potential for stripe rust to develop adaptations to survive at a wider range of temperatures as well as the changing climate could be disastrous for wheat growing regions that had previously been mostly unaffected by stripe rust (Milus et al., 2009; Chakraborty et al., 2011; Hovmøller et al., 2011). There has been a massive shift in the worldwide stripe rust population in the 2000s with high temperature adapted strains, such as *PstS1, PstS2*, and *Pst*-Warrior currently widespread (Hovmøller et al., 2016; Walter et al., 2016; Ali et al., 2017). In addition to temperature adaptation, stripe rust populations have developed virulence to a significant number of resistance

QTLs. In a survey of the global yellow rust population, only *Yr5* and *Yr15* displayed resistance to all tested races out of all of the tested QTLs, *Yr1, Yr2, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr25, Yr27, Yr32* (Ali et al., 2017). Evidence has demonstrated that the *Pst*-Warrior rust races, that has replaced the pre-2011 population of stripe rust in the United Kingdom, is more genetically diverse than the previous population (Hovmøller et al., 2016). Both the adaptation to a wider temperature range and the presence of genetically diverse virulent strains of stripe rust increases the need for the identification of novel stripe rust genes.

Stripe Rust Resistance Genes from Ae. tauschii

Major efforts have focused on identifying stripe rust resistance with 61 resistance genes officially designated (McIntosh et al., 2014). The current officially identified stripe rust genes successfully introgressed from *Ae. tauschii* include *Yr28* and *YrAS2388* (Singh et al., 2000; Huang et al., 2011). Both of these genes were introgressed into hexaploid wheat through the use of synthetic lines. The diploid source of *YrAS2388* has a highly resistant infection type (0-1) based on inoculation with a mixture of stripe rust races native to China (Huang et al., 2011). However, the AS2388 accession displays a higher resistance in the diploid background compared to the synthetic indicating that there may be interactions suppressing resistance from *Ae. tauschii* (Huang et al., 2011). *YrAS2388* is present on the distal end of the 4DS chromosome and originates from AS2388 (Huang et al., 2011). *Yr28* has a resistant infection type (2-4) as a synthetic and screening was not performed on the diploid parent (Singh et al., 2000). *Yr28* originated from the W-219 *Ae. tauschii* accession and is located on the distal end of 4DS (Singh et al., 2000). These genes are both all-stage resistance genes and have similar infection types in the synthetic background. Presently, it is unknown if *Yr28* and *YrAS2388* are the same or

different loci though Huang et. al. 2011 claims a difference based on differing IT reactions of *Yr28* and *YrAS2388* to the same rust race in separate studies.

Puccinia triticina **as a Pathogen**

Leaf Rust

Recently, attention has been focused on new highly virulent stem and stripe rust races. However, leaf rust is still a threat that, like stem and stripe rust, has the ability to quickly develop virulent races that can overcome widely deployed resistant genes. In 1977, the presence of virulent races to J73, a widely grown variety of wheat in northwest Mexico, resulted in yield losses of over 40% and required government backed fungicide applications to protect the affected wheat in the region (Dubin, 1981). Leaf rust is more amiable to a wider range of temperatures compared to stripe and stem rust allowing it to spread across a greater number of geographical regions (Bolton et al., 2008). Leaf rust can cause around 7-50% yield loss dependent on stage of growth infection occurs (Singh et al., 2011a). Leaf rust not only can affect the yield of a wheat line but also may have an impact on its quality (Everts et al., 2001). Leaf rust is a highly diverse pathogen with new races identified annually from multiple countries (Bolton et al., 2008). A survey of North American leaf rust races demonstrated races are geographically localized with virulence to wheat cultivars resistance genes (Kolmer and Hughes, 2015). Identifying novel sources of resistance to leaf rust will help protect cultivars from this diverse widespread rust pathogen.

Leaf Rust Resistance Genes from Ae. tauschii

Over 55 leaf rust resistance genes have been officially designated (McIntosh et al., 2014). Those official leaf rust genes that originate from *Ae. tauschii* include *Lr21*, *Lr22a*, *Lr32*, *Lr39*

Lr41, and *Lr42* (Kerber, 1987; Cox et al., 1994; Raupp et al., 2001; Hiebert et al., 2007; Sun et al., 2009). *Lr21* is seedling resistance gene present on the 1DS chromosome and originated from the RL5289 *Ae. tauschii* accession(Cox et al., 1994). *Lr22a* is the only adult plant resistance gene from *Ae. tauschii* that has been officially identified and is present on the 2DS chromosome (Hiebert et al., 2007). *Lr32* is a seedling resistance gene (IT 0;-1) that originated from RL5497-1 and is located on the 3D chromosome (Kerber, 1987). The location of *Lr43* is unknown (Cox et al., 1994). *Lr41*and *Lr42* are seedling resistance genes located on the 1D chromosome and originated from TA2460 and TA2450, respectively (Cox et al., 1994). Recently, *Lr39* was determined to be allelic with *Lr41,* which is actually located on 2DS (Singh et al., 2004). *Lr39* is located on 2DS and originated from multiple tested germplasm lines (Raupp et al., 2001; Singh et al., 2004).

Breeding for Disease Resistance

Types of Genetic Resistance

The original hypothesis concerning gene-for-gene interactions was first identified by Flor concerning resistance of flax to rust (Flor, 1955). This hypothesis states that an interaction between a substrate from a pathogen is recognized by the host organism resulting in a resistant phenotype. These gene-for-gene interactions in terms of rust resistance are exemplified by seedling resistance genes. Seedling resistance is characterized by a qualitative hypersensitive response to infection that is present throughout all growth stages of the plant and tends to be race specific (Leonard and Szabo, 2005; Chen et al., 2014). This hypersensitive response typically entails necrosis occurring around the point of infection which prevents the spread of this biotrophic pathogen past the point of infection (Stakman, 1915). Adult plant resistance (APR) is typically quantitative, is present in the adult growth stages and tends to be non-race specific.

APR does not prevent initial infection but lessens its proliferation (Singh et al., 2011c). While APR tends to be non-race specific, there are examples of stripe rust race specific APR genes (Milus et al., 2015). Rust resistance can be influenced by the environment with some environments decreasing the effectiveness and others increasing the effectiveness of certain APR genes (Chakraborty et al., 2011). An example of environment being a positive influence on rust resistance is high temperature adult plant resistance (HTAPR) (Qayoum and Line, 1985; Chen, 2005; Lin and Chen, 2007). HTAPR is characterized by the susceptibility of a variety at the seedling stage but as the plant develops and the temperature increases, host resistance increases as well.

In addition to the environment affecting the expression of resistance QTLs, epistatic interactions have been identified between these QTLs and genomic regions present in different genetic backgrounds. These regions are capable of reducing the effect of a resistance QTL and termed as suppressors. Examples of suppressors are present on all three wheat subgenomes and lessen the impact of introgressed resistance QTLs for stem (Kerber and Green, 1980; Dyck, 1987; Bai and Knott, 1992), stripe (Ma and Singh, 1995), as well as leaf rust (Bai and Knott, 1992). Thus, the background of the recurrent parent needs to be taken into account when determining the effectiveness of resistance QTL.

Due to its phenotype, all-stage resistance genes are relatively easy and inexpensive to screen for. However, these seedling resistance genes when deployed can be quickly overcome by increased selective pressure to produce virulent stripe rust races through random mutations (Poland et al., 2009; Hulbert and Pumphrey, 2014). Thus, a common method that can be used to increase the durability of newly identified seedling resistance genes, called gene pyramiding, is to combine multiple seedling resistance genes together in one cultivar (St.Clair, 2010; Mundt,

2014). This reduces the chances that races will become virulent when compared to deployment of a single seedling resistance gene (Mundt, 2014; Bourget et al., 2015). In addition to the use of seedling resistance genes in breeding program, there is an increased effort to identify and use quantitative APR resistance. APR is thought to be the more durable resistance compared to seedling resistance. However, even when pathogens develop virulence to major gene resistance, these "defeated" genes can still have a residual effect on resistance (Bodny et al., 1986; Li et al., 1999). Enough of these defeated QTLs together can potentially provide a durable resistance to disease (Li et al., 1999). Screening for APR typically requires more time, a greater amount of space, and a greater risk of environmental variation and contamination from other field diseases when compared to seedling assays, which occur in a controlled environment. However, work has been done that attempts to measure APR in artificially inoculated and controlled setting in the greenhouse for both stripe (Pretorius et al., 2007) and stem rust (Bender and Pretorius, 2016).

Apparent Association between the Origins of Ae. tauschii Accessions and Disease Resistance

Ae. tauschii is a useful source for disease resistance QTL for breeders but it's necessary to be able to identify potentially useful accessions before undergoing the process of creating direct crosses or synthetic lines for greater efficiency. As discussed earlier in the chapter, genetic analysis and screening for disease resistance has been performed previously on the *Ae. tauschii* accessions themselves before their introduction into a hexaploid genetic background. In order to identify those accessions useful for screening, a breeder may select lines that originate from certain geographical regions. The geographic origin of a line has been demonstrated to be associated with resistance to stem rust (Cox et al., 1992), stripe rust (Liu et al., 2010), and fusarium head blight (Brisco et al., 2017). Those environments that are favorable to disease tend to contain more resistant accessions as these environments would exhibit a higher disease

pressure to drive R gene evolution and selection (Cox et al., 1992). Breeders can use associations between environments conducive to disease development and resistance to more effectively identify *Ae. tauschii* accessions more likely to contain disease resistance QTLs in order to introgress them into their breeding germplasm.

Problem Statement

Common wheat is a widely grown row crop that first originated in the Middle East and evolved through a series of interspecific hybridizations between three diploid progenitor species. These recent hybridizations resulted in a diminished genetic diversity in common wheat, specifically in the D genome. *Ae. tauschii*, the D genome progenitor, has been used to increase the novel genic variation in hexaploid wheat. This progenitor species has been shown to harbor numerous QTLs for disease resistance to all three rust species. New races of rust have been observed that have increased aggressiveness and virulence to currently utilized resistance. In the study described in this thesis, a synthetic RIL population was used to identify novel genes conferring resistance to stem, stripe, and leaf rust. This RIL population was created by crossing 9.131.15x (tetraPrelude/TA2474) with KS05HW14 and tested for all-stage resistance and APR in the field and greenhouse. QTL analysis allowed for the identification of disease resistance in this population that can be used to improve current wheat varieties.

APPENDIX

APPENDIX

Figure 1.1. *Hybridization and polyploidization events that result in the formation of hexaploid wheat.* Dash lines indicate potential hybridizations that can occur between domesticated and cultivated tetraploid wheat and between tetraploid wheat and hexaploid wheat. Figure adapted from Feuillet et al., (2008).

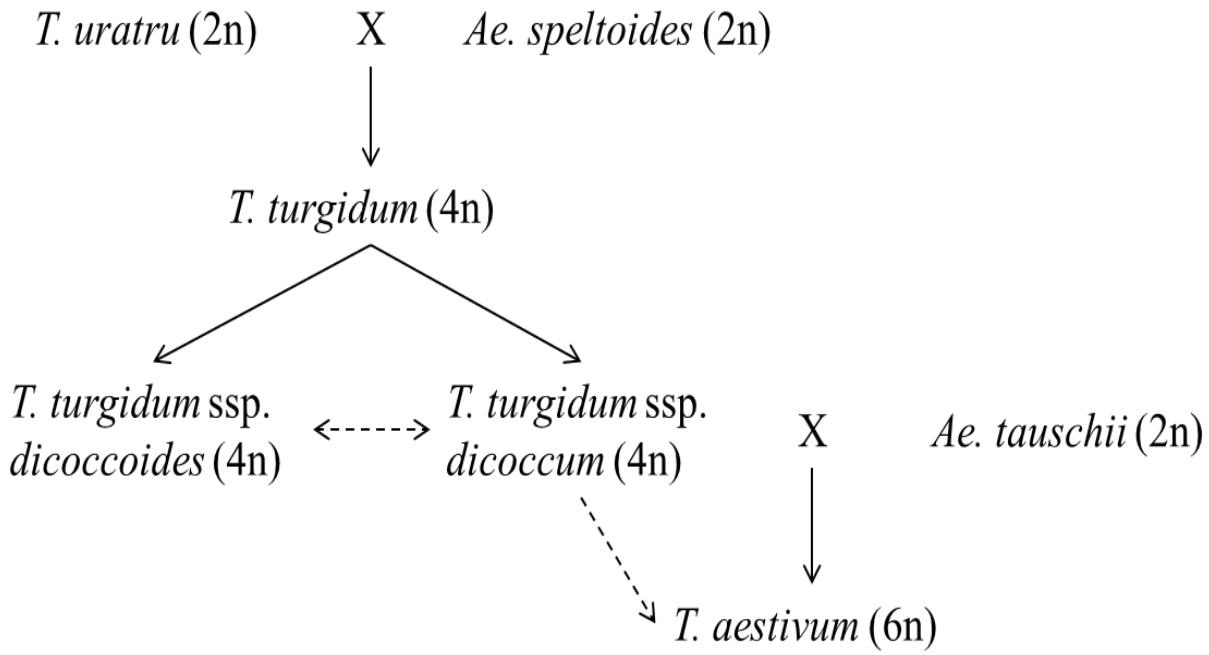


Figure 1.2. *The gene pools of T. aestivum.* Gene pools are determined by F_1 fertility and meiotic pairing. *Ae. tauschii* is a part of the primary gene pool due to its homology to the D genome regardless of its ploidy difference with bread wheat. Figure adapted from Cox, (1997) and Feuillet et al., (2008).

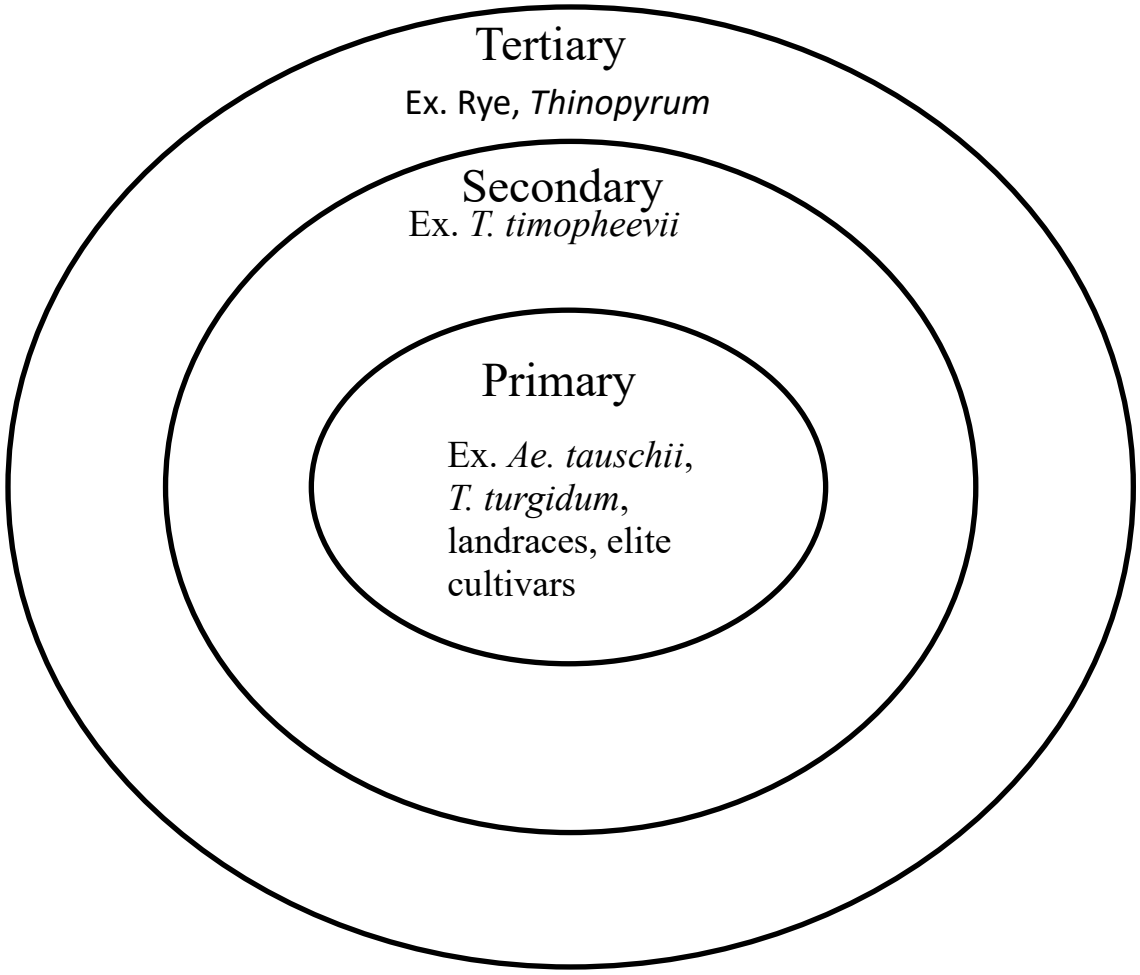
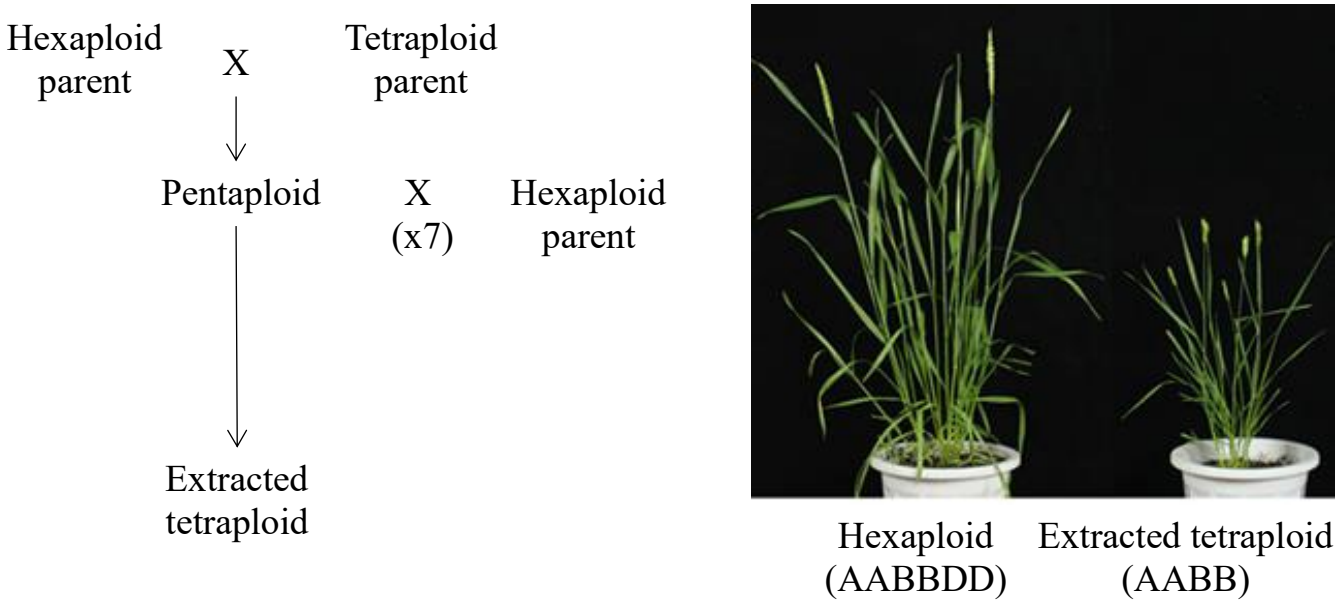


Figure 1.3. *Development of an extracted tetraploid from a hexaploid parent.* Briefly, the hexaploid parent is crossed to a tetraploid variety and pentaploid F_1 plants are backcrossed back to the hexaploid parent. Pentaploid progeny are selected in each generation and backcrossed multiple times. Pentaploid progeny from the last backcross were selfed and tetraploid progeny were retained. The size and vigor of the extracted tetraploid is stunted compared to the hexaploid. Flow through adapted from and photograph from Zhang et al., (2014). Extracted tetraploid procedure originates from Kerber, (1964.



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CHAPTER 2: Mapping Rust Resistance in a Synthetic (tetraPrelude/TA2474) Hexaploid Wheat Population

Abstract

New pathogenic *Puccinia* races highlight the need to identify new sources of disease resistance. Disease resistance from the D genome progenitor of common wheat, *Ae. tauschii,* can be introgressed into hexaploid wheat. Two $BC_1F_{5:6}$ RIL families (n = 70) were derived from a cross between synthetic hexaploid 9.131.15x (tetraPrelude/TA2474) and a hard white winter wheat line, KS05HW14. All-stage and adult plant resistance to stripe, stem, and leaf rust were evaluated in the growth chamber, greenhouse and field environments. Both families segregated for stem rust resistance in all environments tested and only one family segregated for stripe rust resistance. Using composite interval mapping, a region on 4DS was identified that confers all-stage resistance to stripe rust and a region on 5BL confers all-stage resistance to stem rust. The 4DS region is inherited from the *Ae. tauschii* donor TA2474 and the 5BL region is from 9.131.15x. Resistance to leaf rust, originating from KS05HW14, segregated in both families on 3BL. Adult plant resistance to leaf and stem rust, on 6D and 7D respectively, segregated in U6708-03 and originated from TA2474. Currently, there are no officially designated adult plant resistance genes on either 6D or 7D from *Ae. tauschii.* This study identifies potentially novel resistance to all three *Puccinia* rust species and can be used to develop rust resistant varieties.

Introduction

There is a constant need to identify new effective sources of resistance as new highly pathogenic diseases emerge throughout the world and threaten the yield of common wheat. The need to ensure crop productivity worldwide is becoming increasingly important as the climate

shifts and the world population continues to increase (Lobell et al., 2008; Godfray et al., 2010). Over 224 million bushels of wheat were produced in 2016 and provide about a fifth of human consumed calories worldwide (Shiferaw et al., 2013; FAO, 2015). In consequence, there has been a continued focus by plant breeders both to improve the yield of new varieties and to protect against wheat fungal pathogens.

Identifying new sources of genetic diversity and disease resistance allows breeders to respond to newly evolved pathogen races. The potential for a pathogen to evolve highly virulent races is exemplified by stripe rust (*Puccinia striiformis* f. sp. *tritici*, *Pst*), stem (*Puccinia graminis* f. sp. *tritici*, *Pgt*) and leaf rust (*Puccinia triticina*, *Pt*), all members of the *Puccinia* genus. These rusts are heteroecious biotrophic fungi which are capable of both asexual and sexual propagation. Infection of susceptible plants by these pathogens can affect yield by reducing quantity and quality of produced grain (Leonard and Szabo, 2005; Chen et al., 2014).

Despite the extensive efforts by breeders to find and utilize effective resistance genes, new *Pst* and *Pgt* races have developed new virulence patterns (Milus et al., 2009; Singh et al., 2011b). The Ug99 *Pgt* race, *Pgt*-TTKSK, is capable of overcoming a majority of known resistance genes that are currently deployed in cultivated wheat (Singh et al., 2011b). While Ug99 is currently contained in Africa and parts of the Middle East, the potential for its spread is concerning. The emergence of new *Pgt* races such as *Pgt*-TTTTF with similar virulence patterns to Ug99 raises the concern that currently grown cultivars are inadequate in their protection (Bhattacharya, 2017). *Pst* races, like *Pst*-Warrior and *PstS2*, are spreading quickly worldwide and are virulent to many resistance genes (Hovmøller et al., 2016; Ali et al., 2017). While there are currently no widespread hyper-virulent *Pt* races similar to *PstS2* and Ug99, this pathogen is capable of evolving new virulent races like *Pgt* and *Pst*. Mexico experienced a widespread leaf

rust epidemic in 1977 which resulted in yield losses of over 40% and required government backed fungicide applications to control the spread of an aggressive *Pt* race (Dubin, 1981). Also, *Pt* is capable of tolerating a wider temperature range compared to either *Pgt* or *Pst* and is a highly diverse pathogen with multiple new races identified annually in different countries (Bolton et al., 2008). A survey of North American leaf rust races demonstrated that races are geographically localized with virulence to wheat cultivars resistance genes (Kolmer and Hughes, 2015).Thus, the identification of new rust resistance genes and their introgression into hexaploid wheat is necessary to counter the proliferation of new rust races.

There is concern that intense selection by plant breeders has decreased the amount of genetic diversity present in modern cultivars (Warburton et al., 2006; Trethowan et al., 2007). This loss in genetic diversity is not consistent across the three subgenomes of wheat. The D genome has the least amount of genetic diversity compared to both the A and B genomes based on the presence of significantly fewer polymorphisms (Poland et al., 2012; Cavanagh et al., 2013; Wang et al., 2014). The lack of genetic diversity in hexaploid wheat, particularly in the D genome, is also a consequence of the evolutionary history of hexaploid wheat which resulted in severe genetic bottleneck (Dvorak et al., 1998; Caldwell et al., 2004; Reif et al., 2005). Hexaploid wheat is an allohexaploid composed of three subgenomes: A, B, and D. This allohexaploid is derived from the hybridization between *T. turgidum* (AABB, n=14) with the D genome progenitor *Ae. tauschii* (DD, n=7) followed by spontaneous chromosome doubling (Kihara, 1944; McFadden and Sears, 1946).

Wheat breeders use a wide range of gene pools composed of diploid and polypoid wheat progenitor species and wild relatives to improve genetic diversity and identify novel variation. The use of wild relatives introduces challenges to breeders based on differences in ploidy, lack of

homology, infertile offspring, and linkage drag (Cox, 1997; Feuillet et al., 2008). One relative that has been extensively used to improve hexaploid wheat is *Ae. tauschii*. *Ae. tauschii* contains a greater amount of genetic polymorphisms in its genome compared to the D genome of both elite and landrace hexaploid wheat (Reif et al., 2005). Thus, *Ae. tauschii* can be used as a source of novel alleles by plant breeders to increase D genome variation. One common method used to introgress novel variation from *Ae. tauschii* into common wheat is the production of synthetic hexaploid wheat (SHW) lines. SHW is produced by the same process that originally resulted in the formation of hexaploid wheat. A tetraploid parent is crossed to an *Ae. tauschii* accession resulting in an F_1 triploid. This F_1 triploid has its genome doubled by a doubling agent which results in the formation of a hexaploid plant (Ogbonnaya et al., 2013). *Ae. tauschii* has been used to improve multiple traits including quality (Hsam et al., 2001; Kunert et al., 2007) and disease resistance (Liu et al., 2010; Rouse et al., 2011; Brisco et al., 2017). Utilizing these genetic resources is critical when it comes to disease resistance as continued evolution of pathogens can render currently utilized resistance genes ineffective.

Resistance to rust can be categorized as seedling resistance or adult plant resistance. Seedling resistance tends to be race-specific and confers resistance throughout all growth stages while adult plant resistance (APR) is typically non race-specific and is only effective during the adult stages of development (Leonard and Szabo, 2005; Singh et al., 2011c). It has been demonstrated in *Pst* that infection type decreases and latency period increases as wheat continues to develop with resistance greatest during anthesis (Ma and Singh, 1996). Some APR genes have been identified that provide protection against multiple diseases either through a pleiotropic effect of a single gene or a haplotype of tightly linked genes. Two examples of pleiotropy include *Sr2* and *Lr67/Yr46*. *Sr2* is a *Pgt* APR gene that is either tightly linked with other

resistance genes or confers by itself, resistance to *Pt* and powdery mildew (Mago et al., 2011). *Lr67/Yr46*, located on chromosome 4DL, is associated with powdery mildew, *Pgt*, *Pst*, and *Pt* resistance (Herrera-Foessel et al., 2011, 2014). Although APR is commonly viewed as more durable than seedling resistance, proper utilization of seedling resistance can help mitigate the potential for virulent populations to evolve. Deployment of seedling resistance in gene pyramids or use of different resistance genes in back-to-back seasons can reduce the likelihood of pathogen races developing the necessary mutations to bypass deployed resistance genes.

Currently two *Pst*, eight *Pgt*, and five *Pt* resistance genes originating from *Ae. tauschii* have been officially designated (McIntosh et al., 2014). *Yr28* and *YrAS2388* on chromosome 4D, *Sr46* on chromosome 2D, and *SrTA10187* on chromosome 6D are four examples of known rust resistance genes derived from *Ae. tauschii* (Singh et al., 2000; Huang et al., 2011; Rouse et al., 2011; Olson et al., 2013b). Both *Yr28* and *YrAS2388* were derived from SHW lines developed from *Ae. tauschii* accessions, W-219 and AS2388 respectively and are seedling resistance genes (Singh et al., 2000; Huang et al., 2011). *Sr46* is an seedling resistance gene effective against several *Pgt* races including Ug99 but is susceptible to *Pgt*-TTTTF (Rouse et al., 2011). Sr*TA10187* is an seedling resistance gene that is resistant to multiple *Pgt* races such as *Pgt*-TTKSK, *Pgt*-QFCSC and *Pgt*-RKQQC (Olson et al., 2013b; Wiersma et al., 2016). *Pt* resistance genes that originate from *Ae. tauschii* include *Lr21*, *Lr22a*, *Lr32*, *Lr39, Lr41,* and *Lr42* (Cox et al., 1994; Raupp et al., 2001; Hiebert et al., 2007; Sun et al., 2009). Currently, *Lr22a* is the only identified *Pt* APR gene from *Ae. tauschii* (Hiebert et al., 2007). Wheat breeders have been able to use other wheat relatives, landraces, and elite varieties to identify novel disease resistance genes located on the A and B genomes. On chromosome 5BL there are two officially designated

Pgt resistance genes, *Sr49* and *Sr56*. Both of these genes originate from common wheat (Bansal et al., 2014, 2015). *Sr49* is a seedling resistance gene and *Sr56* is an APR gene.

Due to the evolution of more aggressive and virulent races of *Pst*, *Pgt*, and *Pt* there is a need to identify new sources of disease resistance. The SHW accession 9.131.15x (tetraPrelude/TA2474) is resistant to *Pst-37* and *Pgt*-QFCSC. The objective of this study was to identify and map sources of *Pst* and *Pgt* resistance in two RIL families derived from a cross between 9.131.15x (tetraPrelude/TA2474) and KS05HW14. Regions were identified that confirm seedling resistance to *Pst* on 4D and to *Pgt* on 5B. Additionally, *Pt* resistance was phenotyped in both families in response to an endemic population of leaf rust in Mason, MI, in 2017.

Materials and Methods

Plant Materials

The SHW, 9.131.15x, was derived from a direct cross between the *Ae. tauschii* accession TA2474 and an extracted tetraploid of the hexaploid wheat variety 'Prelude', tetraPrelude (Kerber, 1964), followed by colchicine treatment as described in Kalia (2015). TA2474, was confirmed to be resistant to *Pgt*-TTKSK (Rouse et al., 2011) and seedling susceptible to a number of leaf rust races (Kalia et al., 2017). 9.131.15x was crossed with KS05HW14 and the resulting F_1 progeny was self-pollinated to produce the F_2 population herein referred to as U6523 (Figure 2.1). A single F_2 plant from this population, U6523-1-156, was identified as resistant to *Pgt*-RKQQC and backcrossed with KS05HW14. Two BC_1F_1 individuals were self-pollinated and the resulting BC_1F_2 progeny from each population were advanced by single seed descent to generate the RIL families U6708-03 (n=71) and U6708-04 (n=73) (Figure 2.1).

Stem Rust Phenotyping

Stem rust phenotyping was performed on U6708-03 and U6708-04 at the seedling stage using *Pgt*-QFCSC. Ten to twenty seeds per pot were planted for each RIL, ten to fifteen seeds per pot for KS05HW14 and five seeds per pot for each inbred parent using a standard *Arabidopsis thaliana* potting soil media. Each inoculated tray contained one check of KS05HW14. Seeds were cold-imbibed (4ºC) to promote even germination. After cold-imbibing, plants were placed into a growth chamber (20 ±0.5ºC). Lines were inoculated with *Pgt*-QFCSC spores suspended in Solitrol using an atomizer after the emergence of the second leaf. After inoculation, the seedlings were incubated in a dew chamber for 16h (20ºC, 100% RH). Seedlings were then returned to the growth chamber. Disease measurements for infection type were based on a 0-4 scale (Stakman et al., 1962). The *Pgt* seedling phenotypic scores for the U6708 populations were subsequently converted to a quantitative 1-5 scale from the 0-4 scale (Dunckel et al., 2015).

Stem rust phenotyping was performed on U6708-03 and U6708-04 families at the adult plant stage under greenhouse conditions using *Pgt*-QFCSC. Lines from the U6708-03 and U6708-04 families were planted in SureMix plant media in 4" pots. Lines were unreplicated and 1-6 seeds were planted per line. Pots were assigned randomly to trays and placed into a greenhouse (70±2ºC) on a 16/8 h day/night cycle. Lines were inoculated at anthesis with an atomizer using *Pgt*-QFCSC spores suspended in Solitrol. These plants were incubated in a dew chamber for 16h (20ºC, 100% RH). Plants were returned to the greenhouse and individual inoculated plants were scored for infection type and severity. Infection type was scored resistant (R), moderately resistant (MR), intermediate (M), moderately susceptible (MS), or susceptible (S) (Roelfs et al., 1992). This APR infection type score was converted to a 1-5 quantitative scale

to allow QTL mapping. Rust severity on the flag leaf was measure on a scale of 0-100% based on the Cobb scale (Peterson et al., 1948). Scores within pots for the adult plant screening in the greenhouse were averaged together.

Stem rust phenotyping was performed on U6708-03 and U6708-04 at the adult plant stage under inoculated field conditions using *Pgt*-QFCSC. Lines from both U6708 families were planted in the field using 1.5m single row plots in April 2017. Lines were replicated twice. However, twenty-six lines out of a total of 132 lines for both families had only a single replicate due to seed availability and plant survival. 'Morocco' was planted around and within the trial to serve as spreader row as well as a susceptible check. The entire trial was inoculated with *Pgt*-QFCSC spores suspended in Solitrol using a commercial RoundUp® H-style sprayer. The trial was inoculated three times, with around 0.5 g of spores per inoculation, at growth stages 5, 9, and 12 based on the feekes scale. Lines were phenotyped for both infection type and severity in the same manner as performed in the greenhouse.

Stripe Rust Phenotyping

Stripe rust phenotyping was performed on U6708-03 and U6708-04 at the seedling stage using *Pst-37*. Ten to twenty seeds per pot were planted for each RIL, ten to fifteen seeds per pot for KS05HW14 and five seeds per pot for each inbred parent. Each inoculated tray contained one check of KS05HW14. Planting and imbibing was performed using the same previously described procedure for seedling screens. After cold-imbibing, these plants were placed into the growth chamber (14±0.5ºC). Lines were inoculated with *Pst-37* suspended in Solitrol using an atomizer after the emergence of the second leaf. After inoculation, the seedlings were incubated in a dew chamber for 16h (14ºC, 100% RH). Seedlings were then returned to the growth chamber. Disease

measurements for infection type were based on a 0-9 infection type (IT) scale (McNeal et al., 1971).

Stripe rust phenotyping was performed on U6708-03 at the adult plant stage under greenhouse conditions using *Pst-37*. Lines from the U6708-03 family were planted in two replicates in 4" square pots using SureMix plant media. Each pot consisted of 1-6 seeds. Pots were randomly assigned to a tray and were placed into a greenhouse (65±2ºC) on a 16/8 h day/night cycle. Plants were inoculated using an atomizer with *Pst-37* spores suspended in Solitrol at approximately Feekes 10. After inoculation, plants were incubated in a dew chamber for 16h (14ºC, 100% RH). Plants were then returned to the greenhouse. Individual flag leaves, or lower leaves if the flag leaf could not be scored, were scored for infection type using the 0-9 IT scale and severity based on the Cobb scale (Peterson et al., 1948; McNeal et al., 1971). Scores within pots for the adult plant screening in the greenhouse were averaged together.

Adult Plant Leaf Rust Phenotyping

Leaf rust phenotyping was performed on U6708-03 and U6708-04 at the adult plant stage under endemic leaf rust inoculation at Mason, MI, for the 2017 growing season. The U6708 families were planted in 1.5m single row plots in April 2017 in two separate trials with lines replicated twice per trial with U6708-03 lines replicated a total of four times. However, due to seed availability and plant survival, six lines out of a total of 143 lines for both families had only a single replicate. Morocco was planted on the borders of each trial and within each trial to serve as a susceptible check and spreader. Lines were naturally inoculated with leaf rust endemic to Mason, MI, during the summer of 2017. Plant flag leaves were scored for infection type using a categorical system (R: resistant, MR: moderately resistant, M: intermediate, MS: moderately susceptible, S: susceptible) (Roelfs et al., 1992). This APR infection type score was converted to

a quantitative 1-5 scale to allow QTL mapping. Severity was measured and was based on the Cobb scale (Peterson et al., 1948).

Phenotypic Data Analysis

Mean *Pst* and *Pgt* seedling scores were obtained for each genotype. Using Proc GLM in SAS® 9.4 (SAS Institute Inc, Cary, NC, USA), a mixed model was developed for *Pst* infection type. Genotype was considered a fixed factor while inoculation group and leaf scored were treated as random factors. An AUDPC was generated for each line from severity scores.

In order to account for field effect in Mason, MI, in 2017, both infection type and severity for *Pgt* and *Pt* ratings were adjusted using the susceptible check Morocco. For *Pgt* rating adjustments, the stem rust nursery was split into two separate replications with each replication consisting of two incomplete blocks. The grand mean of Morocco for infection type and severity was calculated for each replication and was compared to the mean of Morocco within the respective incomplete blocks. The phenotypes of the lines were adjusted based on these differences between incomplete blocks nested within replicates. SAS® 9.4 (SAS Institute Inc, Cary, NC, USA) was used to perform an ANOVA with genotype as a fixed factor and replication as a random factor. LSMeans for both severity and infection type were generated using this mixed model for *Pgt* APR. *Pt* rating adjustments were performed in a similar manner, however both the *Pgt*-inoculated nursery and a second nursery inoculated with *Pst* were included (note: *Pst* disease development was not observed). Effect of replication and nursery were ANOVA tested with SAS® 9.4 (SAS Institute Inc, Cary, NC, USA). A mixed model with replication as a random effect and line as a fixed effect was used to calculate LSMeans for *Pt* adult plant phenotypes.

Genotyping-by-sequencing

Leaf tissue of BC_1F_5 seedlings from each line (U6708-03, n=71; and U6708-04, n=73) were collected in a 96-well format and stored at -80ºC. DNA was extracted, quantified, and normalized according to Wiersma et al., (2016). Genotyping-by-sequencing (GBS) libraries were prepared using a two enzyme protocol as performed by Poland et al., (2012). Briefly, DNA was normalized to 20 ng/µL, samples were digested using PstI and MspI restriction enzymes, adapters and barcode sequences were ligated to digested genomic fragments, and pooled libraries were PCR amplified and confirmed using gel electrophoresis.

Pooled libraries were sequenced at the MSU sequencing facility on an Illumina HiSeq 2500 platform. Tags were called using the TASSEL 5.0-GBS pipeline (Bradbury et al., 2007; Glaubitz et al., 2014). Tags were then aligned to the IWGSC (2014) Chinese Spring v1.0 reference genome and filtered using TASSEL 5 for greater than 0.05 MAF and less than 70% missing data (Bradbury et al., 2007; Poland et al., 2012; Glaubitz et al., 2014). GBS tags were retained if they were polymorphic between the recurrent parent KS05HW14 and 9.131.15x resulting in 3,554 unique tags.

Linkage Map Construction

Using JoinMap 4.0 (Van Ooijen, 2006), separate linkage maps for the U6708-03 and the U6708-04 families with the 3554 retained GBS markers were constructed. Markers were grouped together based on independent LOD scores with a minimum of LOD score of 3.0. Markers were sorted in each group using the “fixed” and “start” order options. Map distances were calculated using the Kosambi’s mapping function with default parameters (Kosambi,

1943). Comparisons between these linkage maps to the IWGSC (2014) Chinese Spring v1.0 reference genome were visualized using MapChart (Supp. Figure 2.1-7A,B) (Voorrips, 2002).

QTL Analysis

QTL analysis was performed using Windows QTL cartographer version 2.5 (WinQTL) (Wang et al., 2012). Composite interval mapping (CIM) was performed for each trait based on the following parameters: walk speed=1.0 cM, window size=10.0 cM, control markers=5, and backwards regression. Genomewide threshold values were generated for each trait individually using 1000 permutations at α=0.05 (Sup. Table 1). Graphical representations of QTL positions were generated using MapChart (Voorrips, 2002).

Results

Linkage Map Construction

There were a total of 96,164 GBS raw tags generated using genotyping-by-sequencing. After filtering, for markers that had a maximum of 30% missing data and 0.05 MAF, and removing, indels, non bi-allelic, heterozygous and missing markers, a total of 5,831 markers that were polymorphic or monomorphic between 9.131.15x and KS05HW14 were identified. Out of those markers, around 39% were monomorphic and fail to segregate in the population. After selecting for polymorphic markers, a total of 3,554 GBS SNPs were retained for further analysis. A total of 34 and 32 linkage groups were assembled for U6708-03 and U6708-04 respectively (Sup. Figure 2.1-7). In total, 1,840 polymorphic markers segregate in the U6708-04 family and 1,967 polymorphic markers segregate in the U6708-03 family. There was an average of 57 markers per linkage group for each family (Sup. Table 2). However, some linkage groups are composed of a large amount of markers (Sup. Figure 2.3A, B) and others are composed of very

few markers (Sup. Figure 2.1A, B) indicating that there is a wide range in the number of markers per linkage group. No linkage groups were able to be constructed that aligned to chromosome 2B in U6708-04 and chromosome 3D from U6708-03 due to a lack of polymorphic markers present. Linkage groups typically aligned to the telomeric ends of the chromosomes with only a few, notably 6D in both families, capturing regions near the centromere.

Screening for Stripe Rust Seedling Resistance

Both U6708 families were screened for resistance to *Pst-37* at the seedling stage, however, only U6708-03 segregated for *Pst* resistance while U6708-04 was completely susceptible. Prelude was susceptible while the synthetic was resistant (Figure 2.5). Infection type measured at the seedling stage was mapped to two distinct 4D linkage groups. The first significant association between IT and GBS-SNP markers is located on the short arm of 4D and originated from TA2474 (Table 2.1) with an R^2 of 0.64 and the allelic effect (a) is -1.60 based on the 0-9 IT scale (Table 2.1). The second significant association between IT and GBS-SNP markers is located on the long arm of 4D and originates from the recurrent parent KS05HW14 (Table 2.1).

Screening for Stem Rust Seedling Resistance

The U6708-03 and U6708-04 families were screened for resistance to the *Pgt*-QFCSC race in a seedling assay and both families segregated for *Pgt* resistance. An intermediate IT to *Pgt*-QFCSC was observed in the synthetic while a highly resistant reaction was observed in Prelude (Figure 2.6). In the U6708-03 family, the 2D, 4D, and 5B linkage groups contained significant regions associated with IT measured in the three tested environments (Figure 2.2). In the U6708-04 family, regions on the 2D and 5B linkage groups were associated with IT

measured in the three environments (Figure 2.3). Both significant associations between the IT phenotype measured in the GC with GBS-SNP markers located on the short arm of chromosome 2D in the U6708-03 family (R^2 = 0.12 and a= -0.29) and the U6708-04 family (R^2 = 0.17 and a= -0.38) originated from TA2474 (Table 2.1). Significant associations between the IT phenotype measured in the GC with GBS-SNP markers located on the long arm of chromosome 5B in the U6708-03 family (R^2 = 0.29 and a= -0.45) and U6708-04 family (R^2 = 0.25 and a= -0.56) originated from 9.131.15x.

Greenhouse Screening of Adult Plants for Stem and Stripe Rust Resistance

The U6708-03 family segregated for *Pst* resistance at the adult plant stage for both infection type (IT) and severity in the greenhouse. Significant associations between markers and adult plant ITs were located on the short arms of chromosome 4D and 3B (Figure 2.2). An AUDPC score was generated from the *Pst* severity scores. Both the AUDPC and adult plant IT phenotype co-localize with the seedling IT phenotype on chromosome 4D (Figure 2.2).

Both U6708-03 and U6708-04 segregated for *Pgt* resistance at the adult plant stage for both infection type and severity in the greenhouse. Significant associations between markers on 4B and 5B for infection type in the U6708-03 family were identified (Table 2.1). However, no significant associations for severity in the U6708-03 family were identified. Infection type measured in the U6708-03 family in the greenhouse (R^2 = 0.29 and a = -0.84) co-localized with seedling IT (Figure 2.2). Significant markers located on 4B associated with IT in the U6708-03 family are derived from KS05HW14, the recurrent parent (Table 2.1). Adult plant infection type measured in the U6708-04 family had significant associations with markers on chromosome 5B and 4B. Additionally, severity measured in the U6708-04 was significantly associated with markers located on chromosome 5B. Both adult plant infection type (R^2 = 0.46 and a= -0.86) and

severity (R^2=0.16 and a= -7.47) measured in the greenhouse co-localize with the seedling IT measured in the growth chamber (Figure 2.3). Infection type associated with markers on chromosome 4B does not co-localize with any other phenotypes identified in the U6708-04 family.

Field Analysis of Stem Rust

Both Prelude and 9.131.15x showed an intermediate infection type in Mason, MI, in 2017. *Pgt* resistance segregated in both the U6708-03 and U6708-04 families (Figure 2.2, 2.3). Associated regions for severity and infection type on chromosome 5B co-localized with IT mapped in the seedling and greenhouse adult plant screen for the presumed major effect *Pgt* seedling resistance QTL (Figure 2.2, 2.3). Multiple genomic regions associated with severity, inherited from KS05HW14, were identified in U6708-04 located on 7D and 3D and, in U6708-03, on 1B (Table 2.1). Infection type and severity in the U6708-03 family co-localized on the short arm of chromosome 7D with marker alleles which originated from TA2474 (Figure 2.2).These putative QTLs did not align with any of the previously mapped *Pgt* phenotypes in either the seedling or greenhouse experiments. In the U6708-04 family a genomic region associated with severity, with alleles inherited from 9.131.15x, was mapped to 3BS with infection type slightly under the genomewide threshold level on the same linkage group (Figure 2.3). Both infection types with significant associations on 4B identified in the greenhouse, and infection types with significant associations on 2D identified in the growth chamber did not co-localize with any phenotypes that were deemed significant in the 2017 field analysis.

Adult Plant Reaction to Leaf Rust in Mason, Michigan 2017

Both the U6708-03 and U6708-04 families segregated for resistance to *Pt* during the 2017 field season in Mason, MI. Prelude and 9.131.15x were scored as susceptible in the field with moderately resistant phenotypes occurring in the RILs (Figure 2.5). In the U6708-03 family, there were two putative resistance QTLs, one identified on 3BL and one on 6DL (Table 2.1). Both the severity and infection type phenotypes co-localized on the 3B linkage group and originated from the recurrent parent KS05HW14 (Figure 2.2). The 6DL QTL, associated with the infection type phenotype, originated from the *Ae. tauschii* accession TA2474. Both the severity and infection type phenotypes co-localized to the 3BL linkage group present in the U6708-04 population and originated from KS05HW14 (Figure 2.3). KS05HW14 was not included in the study because it is a winter wheat. In 2006, the Regional Germplasm Observational Nursery (RGON) included KS05HW14 in their field trials. It garnered a 50% severity and a moderately susceptible IT rating in St. Paul, MN.

Discussion

Stripe Rust Resistance from TA2474

A seedling resistance QTL, *QYr.msu-4DS,* has been identified in the SHW 9.131.15x on 4D for resistance to *Pst*. Infection type observed in the seedling stage and co-localized with adult plant infection type and AUDPC phenotypes measured in the greenhouse environment (Figure 2.2). *QYr.msu-4DS* is located on the short arm of chromosome 4D and originated from the *Ae. tauschii* accession TA2474. Currently, two officially designated *Pst* seedling genes have been identified on 4D, *Yr28* (Singh et al., 2000)and *YrAS2388* (Huang et al., 2011), both at the distal end of the short arm of chromosome 4D. *Yr28* and *YrAS2388* are distinct based on their

differential resistance and physical position (Huang et al., 2011). These genes originate from two different *Ae. tauschii* accessions, W-219 and AS2388, respectively, while *QYr.msu-4DS* is from TA2474 (Singh et al., 2000; Huang et al., 2011). Owing to its own distal nature on 4DS, *QYr.msu-4DS* is likely either a novel resistance QTL or a potential variant of *YrAS2388*. Further tests, like an allelism test, need to be performed to confirm the novelty of *QYr.msu-4DS*.

Although numerous *Ae. tauschii* accessions and other wild wheat relatives have been screened for stripe rust resistance, either in their diploid form or in a synthetic, the designated stripe rust genes derived from these accessions have only been observed at the proximal end of 4DS (Singh et al., 2000; Huang et al., 2011). A recent survey of *Ae. tauschii* germplasm has postulated that *YrAS2388* is a common gene present in multiple *Ae. tauschii* accessions and is most likely a variant of *Yr28* (Liu et al., 2013). This indicates the possibility that common rust resistance genes are shared among *Ae. tauschii* germplasm. Further investigation into the distribution of *YrAS2388* in the *Ae. tauschii* germplasm can provide insight into the evolution of major gene resistance to *Puccinia* in natural populations.

Stem Rust Resistance from the Synthetic 9.131.15x

The putative stem rust seedling resistance QTL *QSr.msu-5BL* was identified on chromosome 5B in both families and with infection type and severity taken in multiple environments co-localizing. Both of these QTLs were linked to similar markers. Therefore, it is likely that the 5B *Pgt* seedling resistance QTL is the same locus in both families (Sup Fig 2.5A,B). The origin of resistance for this QTL is the synthetic parent. There are currently two officially designated stem rust genes present on 5BL, *Sr56* and *Sr49*. Both of these genes originated from hexaploid wheat. However, *Sr49* is a seedling resistance gene and *Sr56* is an APR gene (Bansal et al., 2014, 2015). *Sr49* originated from the land race Mahmoudi (Bansal et

al., 2015). The very distal nature of *QSr.msu-5BL* indicates the potential for it to be a novel *Pgt* seedling resistance QTL or a variant of *Sr49*. *Sr49* confers an intermediate resistant phenotype in Mahmoudi similar to *QSr.msu-5BL* in 9.131.15x (Bansal et al., 2015). However, the putative originator of the 5B *Pgt* seedling resistance QTL, hexaploid Prelude, exhibits a highly resistant fleck phenotype to both *Pgt*-QFCSC (Figure 2.4) as well as the *Pgt*-98-1,2,(3),(5),6 (Hiebert et al., 2016), a *Pgt* race from Australia used to map *Sr49* (Bansal et al., 2015). This supports the possibility that *QSr.msu-5BL* and *Sr49* confer different ITs, although only if *QSr.msu-5BL* is present in different hexaploid backgrounds. A potential 7D APR QTL originating from TA2474 was identified in the 2017 field trial at Mason, MI. This QTL is a small effect QTL and was not observed in the greenhouse study. There are currently no officially designated *Pgt* APR QTLs located on 7D that originate from *Ae. tauschii* making this a potential, novel QTL (McIntosh et al., 2013). However, this will require further testing to confirm that this 7D QTL is repeatable.

The origin of resistance for the *QSr.msu-5BL* QTL is the synthetic parent and rationally from tetraPrelude (due to its location in the B genome). This is supported additionally by the highly resistant phenotype of the hexaploid Prelude. However, sequencing of hexaploid Prelude revealed significant marker differences present throughout the A and B genome from the synthetic. It has been observed that the creation of the tetraPrelude may have resulted in translocation events between the 1D chromosome and the A and B genome (Dronzek et al., 1970). In order to test this, an LD analysis was performed between SNP markers aligned to the 1D chromosome and SNPs aligned to the A and B genome (data not shown). Our results indicate that it is unlikely that the tetraploid Prelude used to create this SHW suffered from the same translocation events that have previously been observed (Dronzek et al., 1970). Further investigation will be needed to pinpoint the exact origin of the *QSr.msu-5BL* QTL.

Alignment of YrAS2388 and Sr49 Markers to the Reference Genome

In an effort to discover additional evidence on the potential novelty of these identified QTLs, the recent release of the IWGSC (IWGSC, 2014b) Chinese Spring v1.0 reference genome was used to align markers linked to *Sr49* and *YrAS2388* using BLAST (Huang et al., 2011; Bansal et al., 2015). Both the *Sr49* and *YrAS2388* markers that were aligned to the reference were situated in overlapping molecular positions with markers associated with *QSr.msu-5BL* and *QYr.msu-4DS* respectively (data not shown). This additional data supports the hypothesis that these identified seedling resistance QTLs may be variants of *YrAs2388* and *Sr49*. However, both the length of DNA sequence associated between flanked markers as well as the propensity for R genes to be clustered together in the genome, indicates the needs for additional tests. Therefore, allelism tests should be performed to better determine the novelty of QTLs that may be located very close together (Bergelson et al., 2001; Leister, 2004).

Adult Plant Reaction to Leaf Rust Segregating in the U6708 Population

The 9.131.15x synthetic (tetraPrelude/TA2474) was originally created at Kansas State University by Dr. Bhanu Kalia for the purpose of identifying adult plant resistance to leaf rust isolates present in Kansas (Kalia, 2015). TA2474 and 9.131.15x were phenotyped as susceptible to a number of leaf rust isolates at the seedling stage (Kalia, 2015; Kalia et al., 2017). A mapping population was created by crossing 9.131.15x to WL711 which was phenotyped at several locations, and APR QTLs were identified on 1B, 1A, 2D, 5D, 5A and 6B (Kalia, 2015). All regions from the D genome were contributions from TA2474.

None of the previously identified leaf rust APR QTLs from 9.131.15x were identified in the U6708 population. There are a multitude of reasons why this may be the case. First, the

populations could have had a differential inheritance of genetic information from the synthetic parent. This is supported by the extremely small 1A and 1B linkage groups that are present in the U6708 population, indicating the probability that these previously identified QTLs were not captured during the creation of this population. Second, the genetic background of this population is different. The recurrent parent in the U6708 population is KS05HW14, a hard white winter wheat, and a parent in the other mapping population was W7111, a spring wheat variety. The genetic background of wheat has been shown to be significant in influencing QTLs in the case of rust where suppressors can alter the effectiveness of rust resistance QTLs (Bai and Knott, 1992; Assefa and Fehrmann, 2004). Third, the endemic leaf rust isolates likely differ between Kansas/CIMMYT disease nurseries compared to Mason, MI. Thus, previously identified QTLs that are shared between these two populations may be either environmental or race specific.

The susceptible reaction of Prelude and 9.131.15x compared to the moderately resistant phenotype that is segregating through the population indicates that either KS05HW14 is the contributor of *Pt* resistance or suppression is occurring in the SHW. There were three putative QTLs identified in these two families, two occurring on 3B and one occurring on 6D. The major effect QTLs that have been identified in both populations occur on the 3B chromosome with both originating from KS05HW14, appear to be located near one another, and are likely shared between the populations (Figure 2.2, 2.3). The 6D QTL originates from TA2474 and was only observed in a single family (Table 2.1). KS05HW14, a winter wheat, was not rated for leaf rust during the 2017 field season as the U6708 population is spring type, but it was rated in 2006 in the RGON and displayed a moderately susceptible infection type and a 50% severity. A follow

up study testing KS05HW14 for APR to leaf rust in Mason, MI, will need to be performed to test if the resistance observed may originate from a race-specific resistance QTL.

The substitution at this genomic region for KS05HW14 alleles providing enhanced leaf rust resistance is an indication of two possibilities: an unidentified resistance QTL is present in KS05HW14 on 3B, or this substitution eliminates a suppressor. Phenotyping KS05HW14 for *Pt* resistance and testing 9.131.15x for *Pt* resistance in different hexaploid background could test these two hypotheses. Only one *Pt* resistance QTL originating from *Ae. tauschii* was observed and it was only present in one family (Table 2.1). The large effect of the 3B QTLs in both families may make it difficult to identify minor effect QTLs present, especially with the small number of individuals in this population (Beavis, 1998; Xu, 2003). Thus, resistance may be originating from multiple TA2474 derived QTLs, but their effect is masked by the presence of a highly significant suppressor. No QTL that have been identified for stem or stripe rust in the U6708 population co-localized with the QTL on 3B or on 6D. Thus, the *Pt* resistance QTLs identified in this experiment is not currently thought to be either pleiotropic or linked to previously identified QTLs. Further testing of the population with a single-spore *Pt* isolate can help further delineate whether or not the segregating resistance in U6708 is a seedling resistance gene or an APR loci.

Phenotyping of Adult Plant Resistance

Recent efforts have demonstrated the feasibility of using the greenhouse to both screen and identify new sources of genetic resistance to stripe and stem rust at the adult plant stage (Pretorius et al., 2007; Bender and Pretorius, 2016). In this study, greenhouse screening of adult plants was successful in identifying both major and minor effect QTLs (Table 2.1). However, greenhouse screening of adult plants failed to identify minor effect seedling resistance QTLs

from the seedling assay as well as APR QTLs identified in Mason, MI, for *Pgt* resistance. Minor effect QTL for *Pst* and *Pgt* resistance originating from the KS05HW14 were identified, which was rated as susceptible at the seedling stage to both stripe and stem rust. There can be a multitude of reasons for this lack of repeatability of these minor effect QTLs between environments. The lack of replication due to space and time constraints in the greenhouse and loss of replication for some lines in the field may impact the ability to detect QTLs with a low R^2 value, especially in the presence of other major QTLs. Also, due to the population size, it is likely the Beavis effect influenced the ability to detect authentic minor effect QTL and caused spurious minor effect QTLs to appear (Beavis, 1998; Xu, 2003). While minor seedling QTLs are more readily identified in controlled assays, the question remains for the breeder if these QTLs will be effective in the field environment when deployed. Minor seedling QTLs for stem rust that were detected in the seedling stage and not in the greenhouse were also not detected in the field environment (Table 2.1). However, the major seedling QTL present on 5BL could be identified in all three environments and the major seedling QTL present on 4DS could be detected in all *Pst* experiments. Those minor effect QTLs that failed to display significance in multiple environments are thus likely aberrations due to population size limitations and, if they are real seedling resistance genes, likely not effective in the adult plant stage, making their identification difficult regardless of the method.

Summary

The SHW 9.131.15x was analyzed using seedling, greenhouse, and field experiments to identify sources of resistance to both *Pst-37* and *Pgt*-QFCSC. U6708-03 and U6708-04 both segregated for *Pgt* resistance while only U6708-03 segregated for *Pst* resistance. Two seedling resistance loci, *QYr.msu-4DS* and *QSr.msu-5BL*, were identified in the seedling, greenhouse, and

field environments. *QYr.msu-4DS* is located on 4DS and originated from the *Ae. tauschii* parent TA2474. It is either a novel stripe rust QTL or a variant of the most distal *Pst* gene on 4DS, *YrAS2388*. *QSr.msu-5BL* is located on 5BL and originated from 9.131.15x. Due to its distal nature on 5BL, this is likely a novel *Pgt* QTL or a variant of *Sr49*. In addition to *Pgt* and *Pst* resistance, QTLs for *Pt* resistance are also present in the population. Thus, in addition to harboring potentially new putative disease resistance QTLs for stem, stripe, and leaf rust, lines extracted from this population can be used to increase resistance to multiple Puccinia species when crossed into a breeding program.

APPENDIX

APPENDIX

Table 2.1. *Quantitative trait loci for resistance to stem, stripe, and leaf rust.*

a: *Sr*= Stem rust, *Lr*=Leaf rust, *Yr*= Stripe rust, IT was rated based on the 0–9 McNeal scale for *Pst* resistance at both the seedling and adult stage. IT for *Pgt* and *Pt* resistance were measured using the 0–4 Stakman scale for seedling resistance and were rated as susceptible (S), moderately susceptible (MS), moderate (M), moderately resistant (MR) or resistant (R) as adult plants. Severity was rated from 0–100% based on the Cobb scale, AUDPC = area under the disease progression curve. GC = growth chamber phenotyping environment; GH = greenhouse phenotyping environment; MM = Mason, Michigan, 2017 phenotyping environment.
b: Most significant marker based on LOD.
c: Additive effect of the alleles.

Disease	QTL	Family	Stage	Trait	Location	Chr	Position	LOD	R^2	Effect[c]	Origin
Stem Rust	*QSr.msu-1BS*	U6708-03	Adult	IT	Field	1BS	665,403,509	3.96	0.12	-7.36	KS05HW14
	QSr.msu-3BL	U6708-04	Adult	IT	Field	3BL	573,497,145	6.66	0.08	-0.35	Synthetic
	QSr.msu-4BL	U6708-03	Adult	IT	GH	4BL	619,589,061	4.72	0.13	-0.56	KS05HW14
		U6708-04	Adult	IT	GH	4BL	626,604,202	3.78	0.08	-0.37	KS05HW14
	QSr.msu-5BL	U6708-03	Seedling	IT	GC	5BL	699,455,437	10.77	0.29	-0.45	Synthetic
		U6708-04	Seedling	IT	GC	5BL	699,728,464	7.07	0.25	-0.56	Synthetic
		U6708-03	Adult	IT	GH	5BL	693,686,064	8.23	0.29	-0.84	Synthetic
		U6708-04	Adult	IT	GH	5BL	699,728,464	14.03	0.46	-0.86	Synthetic
		U6708-04	Adult	Severity	GH	5BL	697,611,190	4.37	0.16	-7.47	Synthetic
		U6708-03	Adult	IT	Field	5BL	699,455,437	15.76	0.52	-0.84	Synthetic
		U6708-04	Adult	IT	Field	5BL	699,407,657	25.21	0.69	-1.01	Synthetic
		U6708-03	Adult	Severity	Field	5BL	699,422,973	8.83	0.32	-11.81	Synthetic
		U6708-04	Adult	Severity	Field	5BL	697,865,267	7.69	0.29	-12.83	Synthetic
	QSr.msu-2DS	U6708-03	Seedling	IT	GC	2DS	7,527,954	6.34	0.12	-0.29	TA2474
		U6708-04	Seedling	IT	GC	2DS	13,242,604	5.61	0.17	-0.38	TA2474
	QSr.msu-3DL	U6708-04	Adult	IT	Field	3DL	45,015,647	3.87	0.05	-0.26	KS05HW14
	QSr.msu-7DS.1	U6708-04	Adult	Severity	Field	7DS	1,570,012	5.63	0.16	-9.69	KS05HW14
	QSr.msu-7DS.2	U6708-03	Adult	IT	Field	7DS	62,120,102	3.81	0.074	-0.33	TA2474
		U6708-03	Adult	Severity	Field	7DS	72,699,333	5.25	0.17	-8.90	TA2474
Stripe Rust	*QYr.msu-3BS*	U6708-03	Adult	IT	GH	3BS	14,482,106	4.21	0.12	-0.63	KS05HW14
	QYr.msu-4DS	U6708-03	Adult	IT	GH	4DS	2,828,876	4.78	0.15	-0.70	TA2474
		U6708-03	Adult	AUDPC	GH	4DS	1,704,666	6.72	0.22	-17.17	TA2474
		U6708-03	Seedling	IT	GC	4DS	1,242,429	19.43	0.64	-1.60	TA2474
	QYr.msu-4DL	U6708-03	Seedling	IT	GC	4DL	345,415,047	5.85	0.09	-0.62	KS05HW14
Leaf Rust	*QLr.msu-3BL*	U6708-03	Adult	IT	Field	3BL	748,448,161	22.83	0.76	-1.06	KS05HW14
		U6708-04	Adult	IT	Field	3BL	748,448,161	14.62	0.47	-0.81	KS05HW14
		U6708-03	Adult	Severity	Field	3BL	748,448,161	16.63	0.68	-13.75	KS05HW14
		U6708-04	Adult	Severity	Field	3BL	748,448,161	10.11	0.38	-11.48	KS05HW14
	QLr.msu-6DL	U6708-03	Adult	IT	Field	6DL	347,713,271	6.73	0.08	-0.37	TA2474

Sup Table 2.1. *Genomewide threshold values generated for each trait individually using 1000 permutations at α=0.05.*

	U6708-04	U6708-03
Trait	LOD	LOD
Yr IT GC	N/A	3.6
Yr IT GH	N/A	3.51
Yr AUDPC GH	N/A	3.58
Sr IT GC	3.58	3.59
Sr IT GH	3.58	3.7
Sr Severity GH	3.47	6.16
Sr IT Mason, Michigan 2017	3.67	3.73
Sr Severity Mason, Michigan 2017	3.66	3.65
Lr IT Mason, Michigan 2017	3.62	3.78
Lr Severity Mason, Michigan 2017	3.62	3.62

Figure 2.1. *Flow through of the population development of the U6708-03 and U6708-04 families.* A single F2 individual from the U6523 population, U6523-1-156, was backcrossed to KS05HW14 to create the two individual U6708 families.

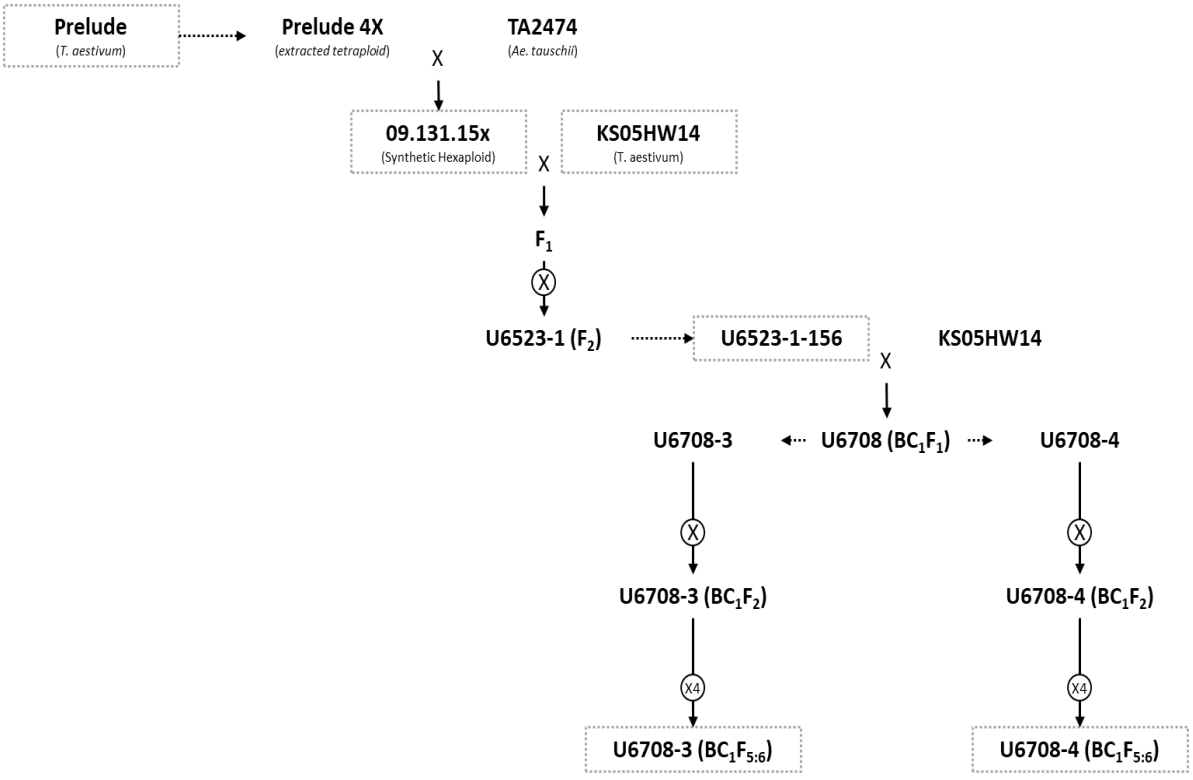


Figure 2.2. *QTL analysis of the U6708-03 family using composite interval mapping for stem, stripe, and leaf rust resistance QTL*. Eight linkage groups, of chromosomes 1B, 2D, 3B, 4B, 4D, 5B, 6D, and 7D, were identified that carry rust resistance QTL. Leaf, stripe, and stem rust resistance QTL are shown in blue, green and black, respectively.

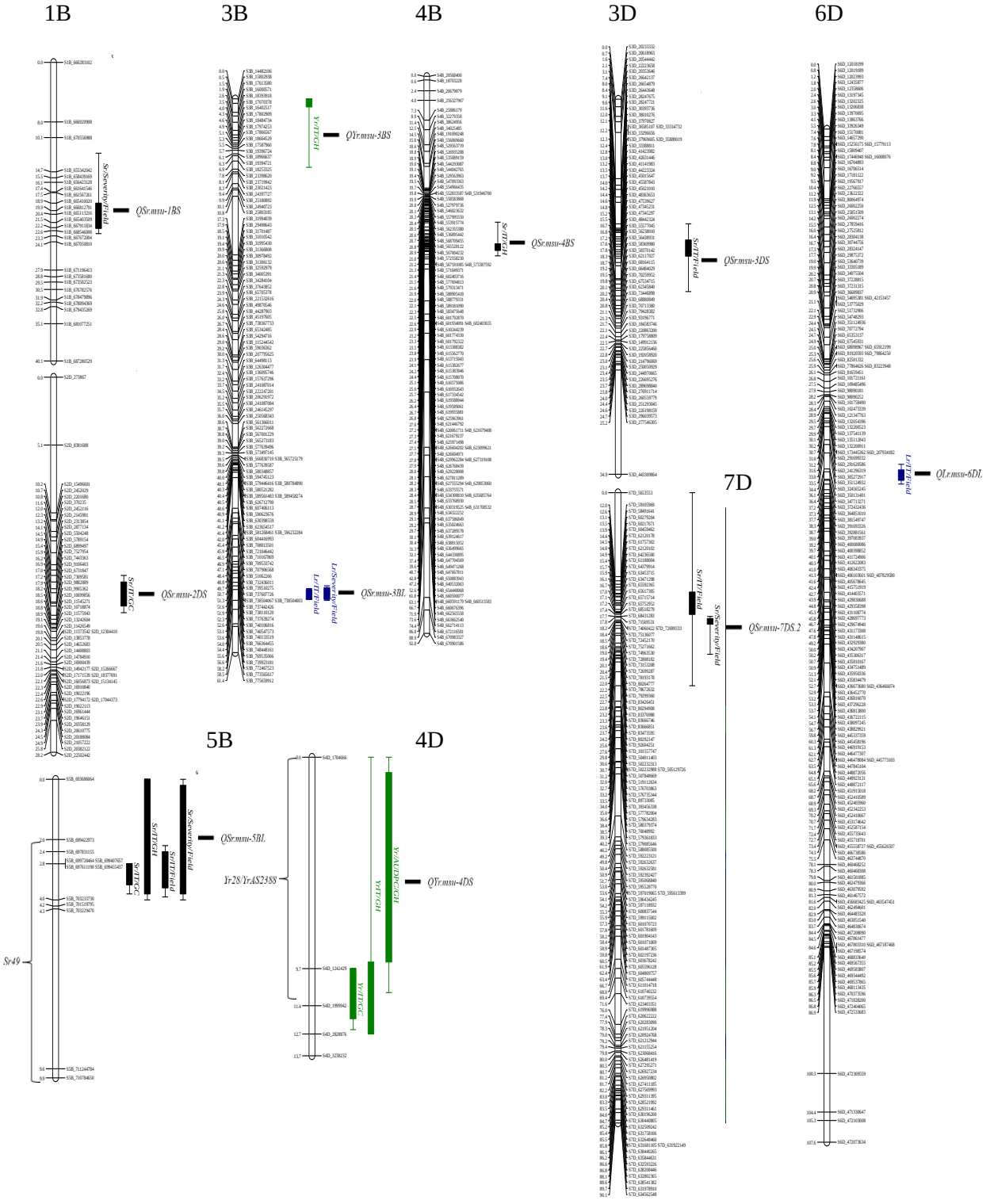


Figure 2.3. *QTL analysis of the U6708-04 family using composite interval mapping for stem and leaf rust resistance QTL*. Seven linkage groups, of chromosomes 2D, 3B, 3D, 4B, 5B, and 7D were identified that carry rust resistance QTL. Leaf and stem rust resistance QTL are shown in blue and black, respectively.

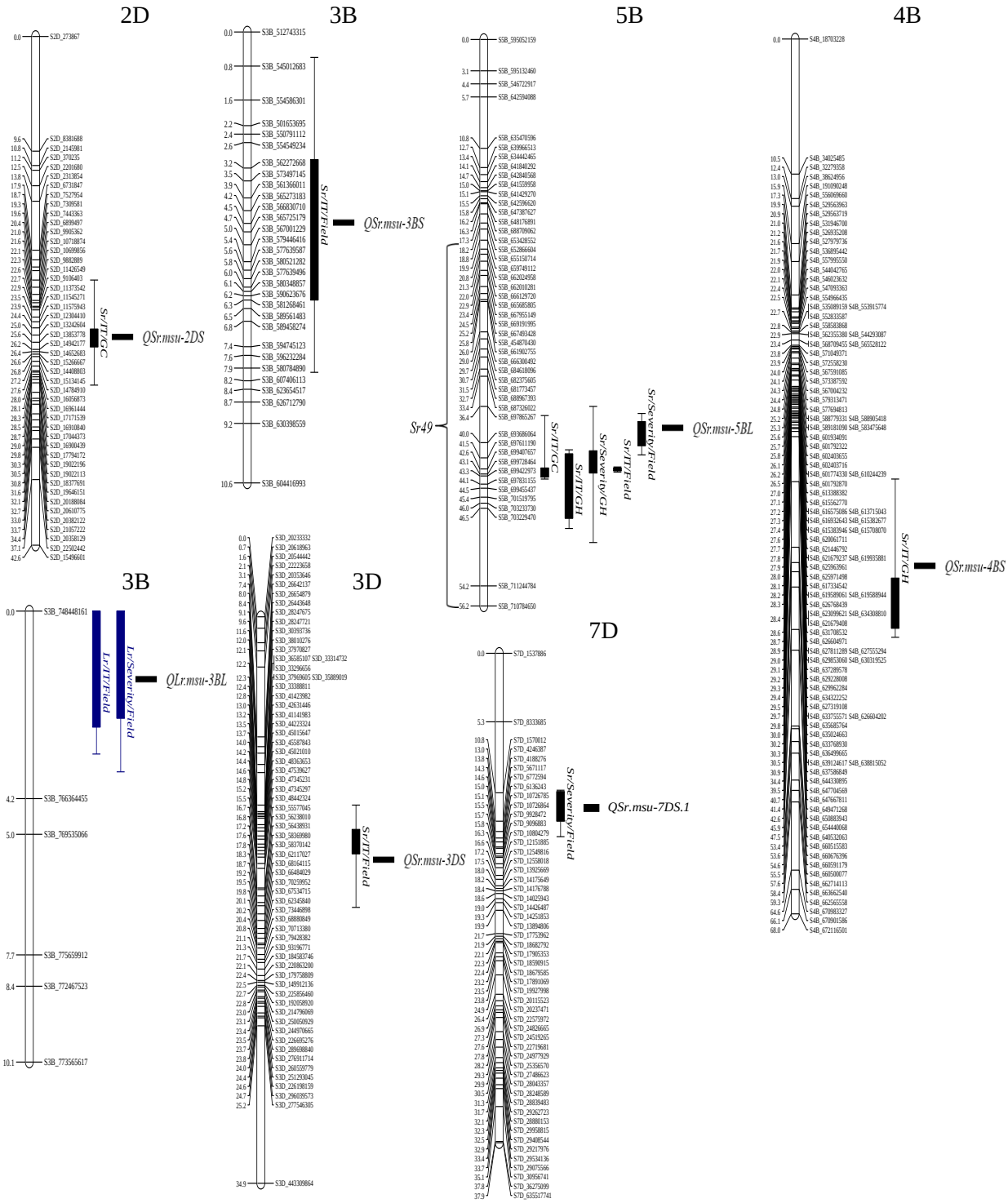


Figure 2.4. *Examples of Pst seedling reactions to Pst-37 in the U6523 F2:3 population*: **A.** Prelude displays a susceptible reaction. **B.** 9.131.15x exhibits a resistant reaction with a hypersensitive response seen by necrosis and low sporulation. **C.** U6523-1-87 is a susceptible line in the population with abundant sporulation. **D.** U6523-1-90 is resistant, displaying an infection type similar to 9.131.15x. The U6708 population has the same infection types segregating as the U6523 population.

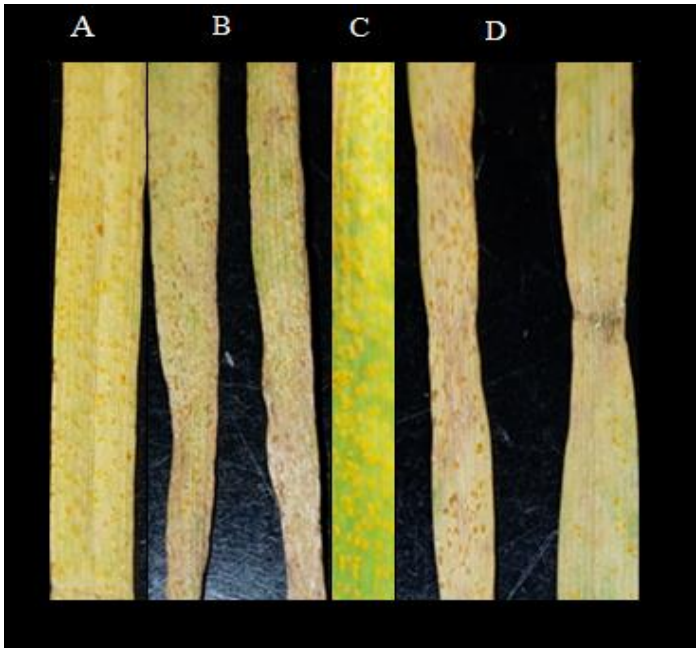


Figure 2.5. *Examples of adult plant reactions to leaf rust in the U6708 population in Mason, Michigan 2017*: **A.** Prelude displays a susceptible reaction with abundant sporulation and no chlorosis. **B.** 9.131.15x displays a susceptible to moderately susceptible reaction with abundant sporulation and slight chlorosis. **C.** A moderately resistant line with necrosis and small uredinia. KS05HW14, the recurrent parent, was not measured in the field as it was a winter wheat and not available to rate.

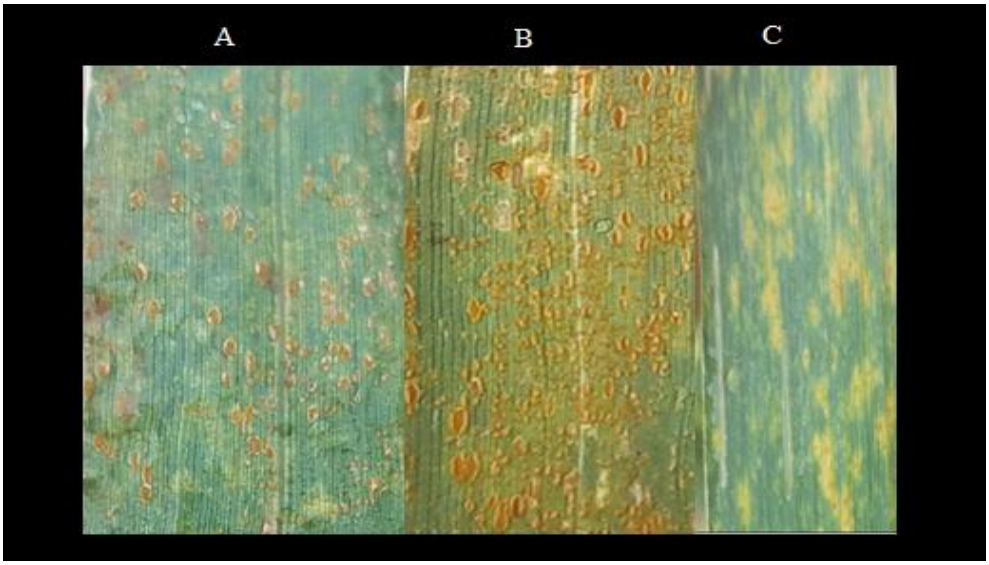
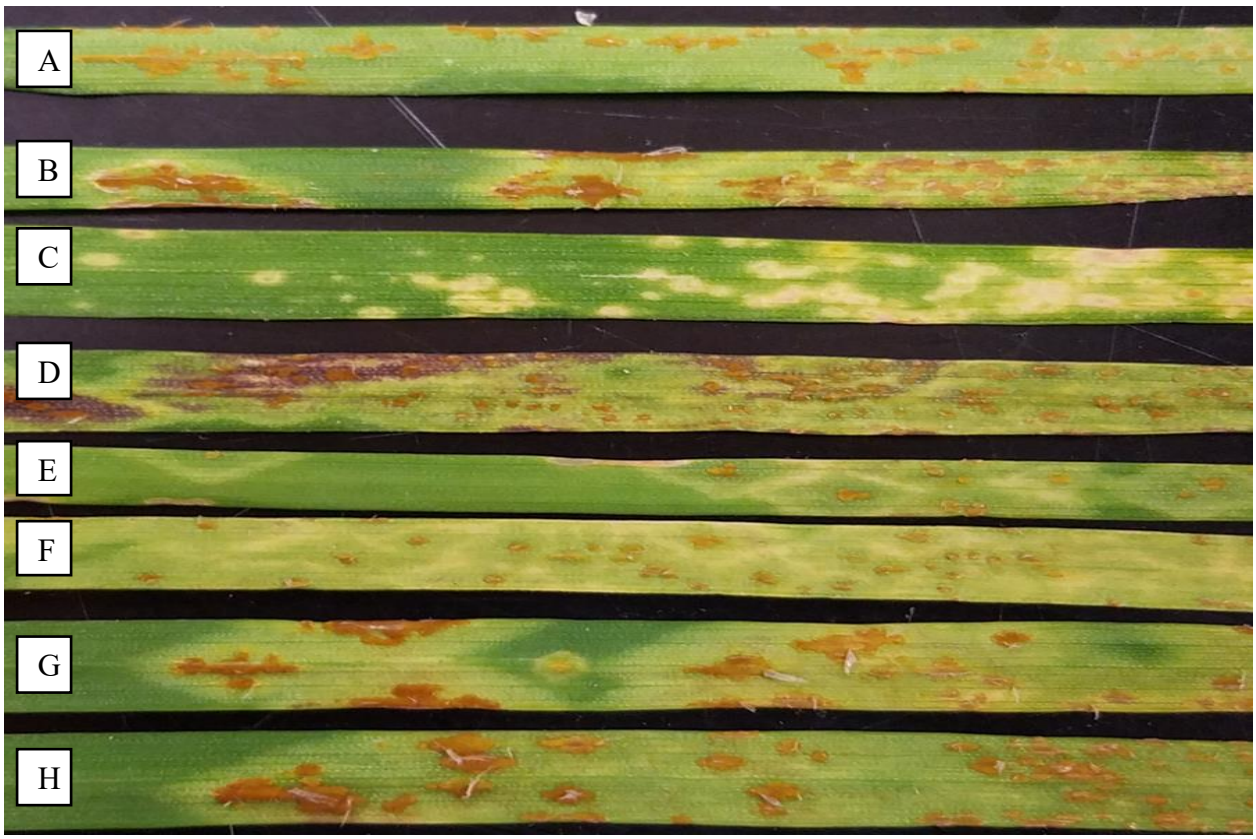


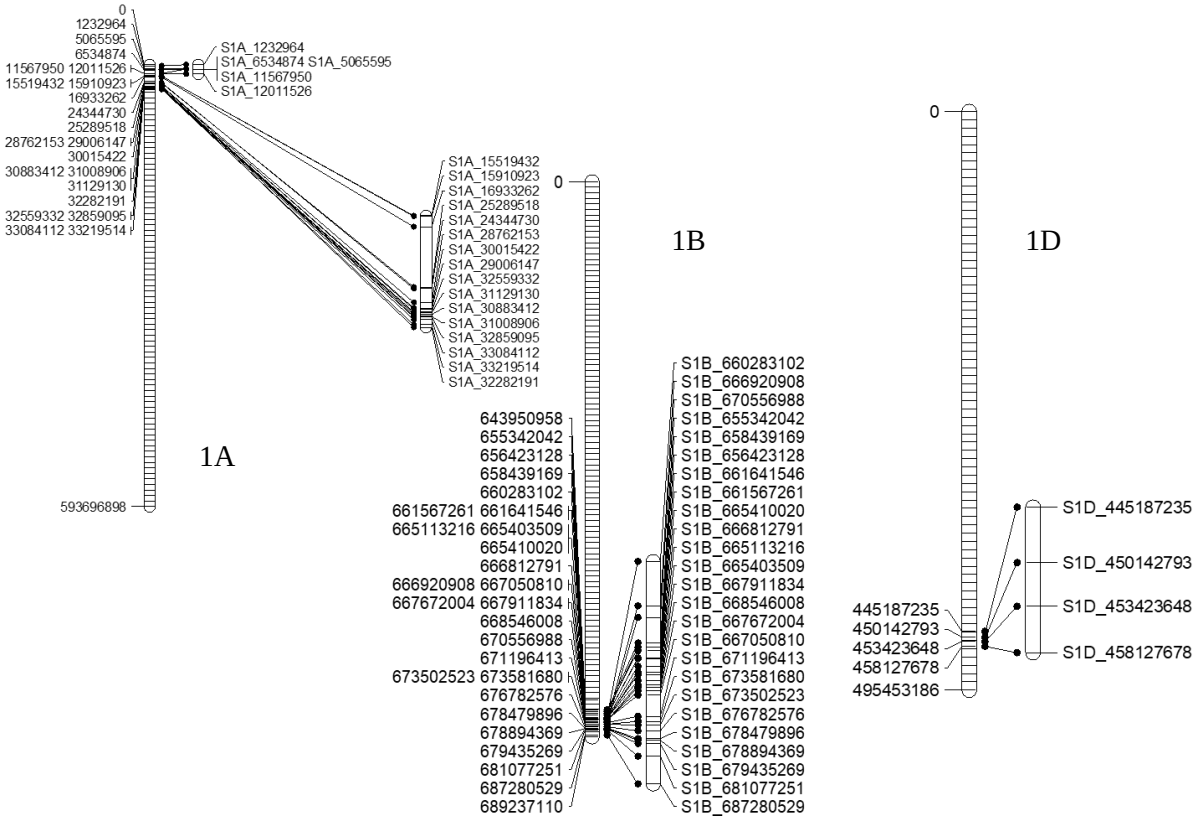
Figure 2.6. *Examples of Pgt seedling reactions to Pgt-QFCSC in the U6708 population*: **A.** Morocco serves as a susceptible check. **B.** KS05HW14 is the susceptible recurrent parent. **C.** Prelude displays a highly resistant fleck phenotype with no uredinia present and necrosis is evident. **D.** 9.131.15x displays an intermediate resistant phenoytpe. **E.** U6708-03-025 **D.** U6708-03-044 **G.** U6708-03-073 **H.** U6708-03-08. **E** and **D** are examples of the intermediate resistance segregating in the U6708 population. **G** and **H** are examples of susceptible reactions in the population. The same infection types for resistant and susceptible RILs were observed in both families.



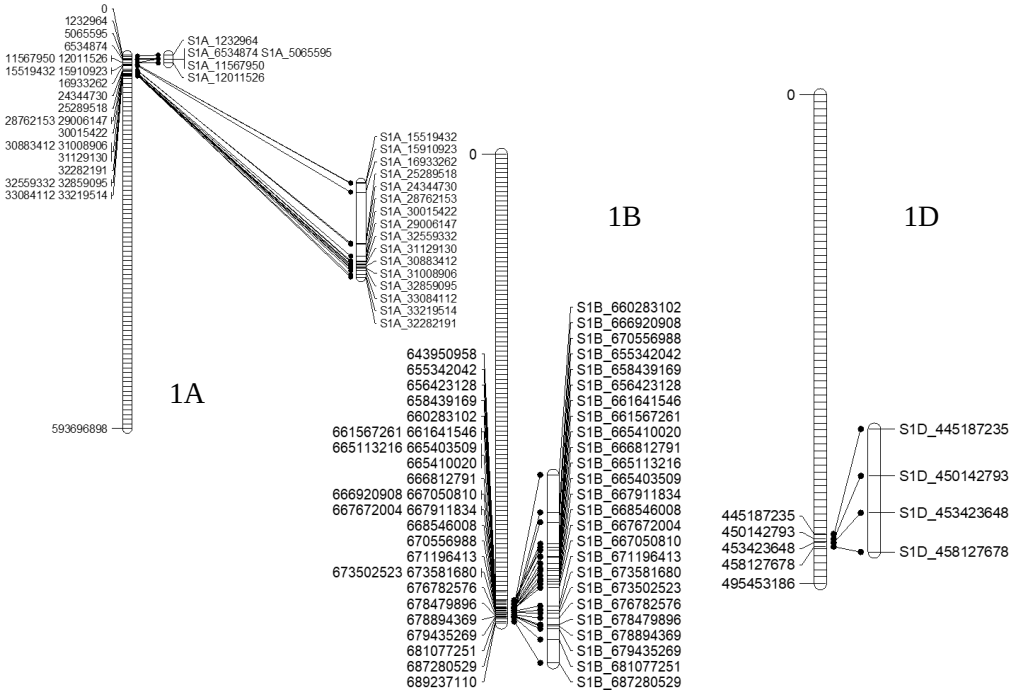
Sup Table 2.2. *Polymorphic segregating on each chromosome in the U6708-03 and U6708-04 families.*

Chromosome	U6708-04 Number of markers	U6708-03 Number of markers
1A	21	26
1B	26	25
1D	9	3
2A	140	113
2B	N/A	100
2D	132	191
3A	133	134
3B	92	105
3D	65	N/A
4A	59	50
4B	103	105
4D	49	98
5A	141	113
5B	48	86
5D	76	75
6A	5	76
6B	92	5
6D	173	172
7A	200	163
7B	215	170
7D	63	157

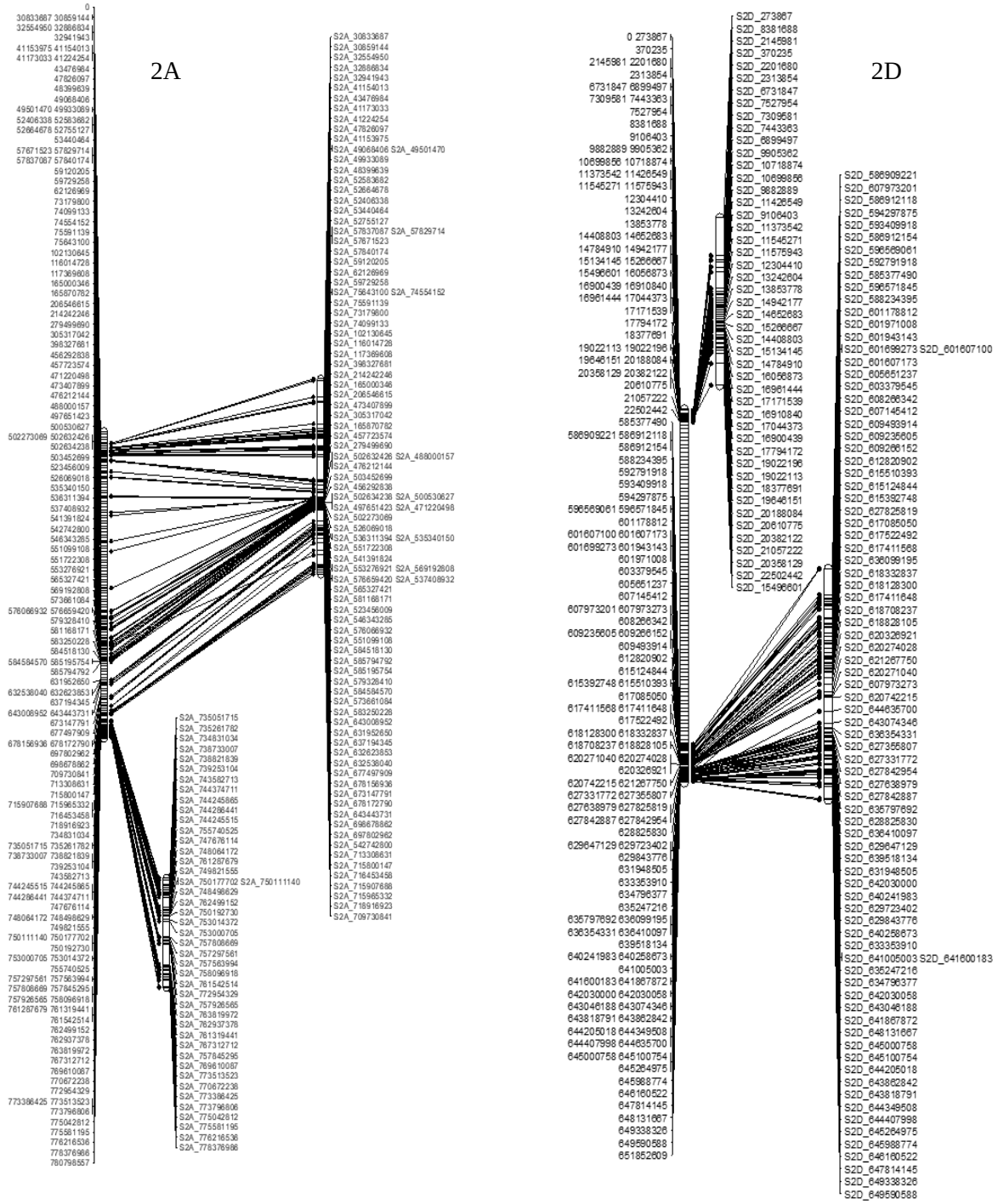
Sup Fig. 2.1A. *U6708-04 linkage groups for group 1 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



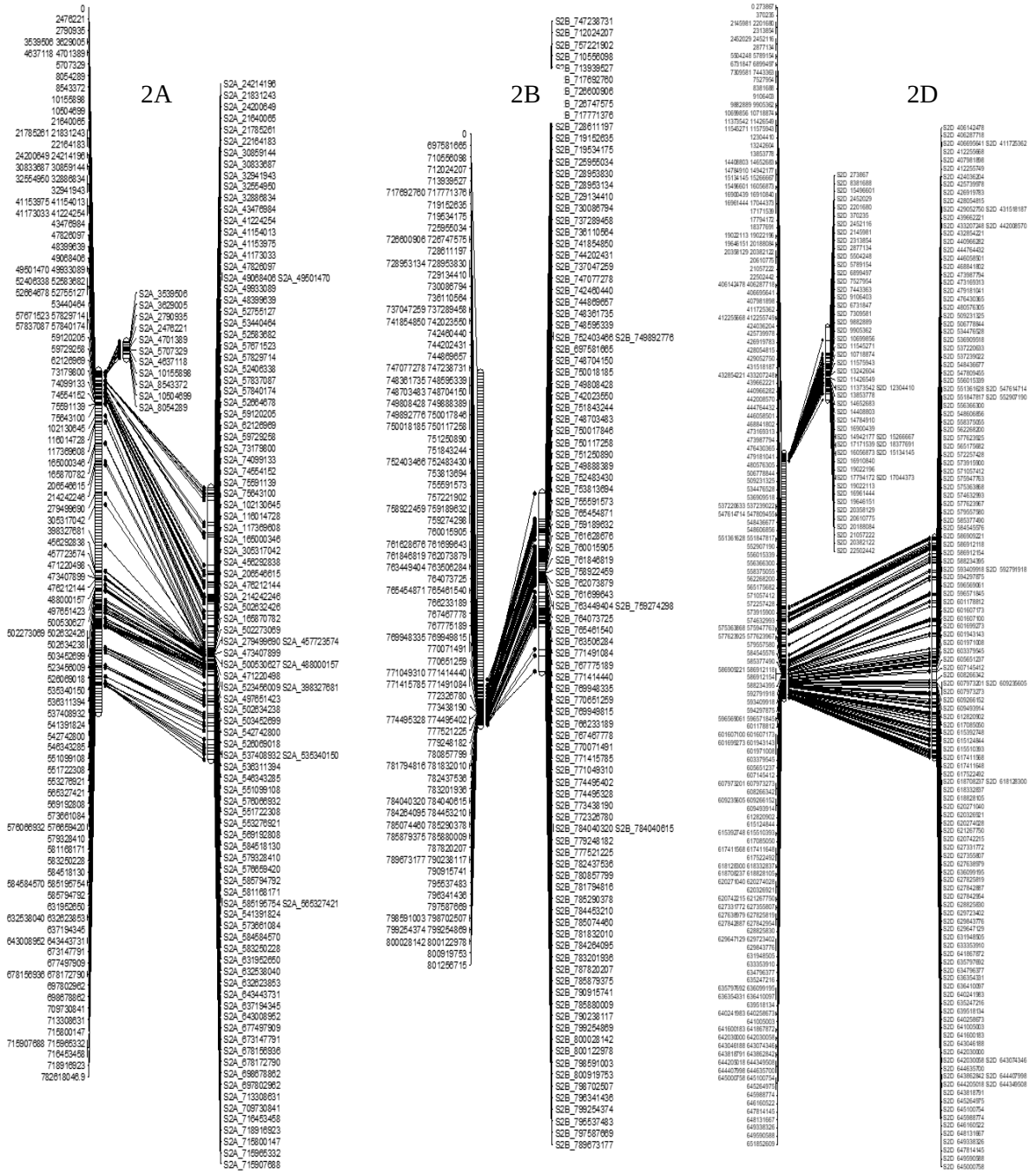
Sup Fig. 2.1B. *U6708-03 linkage groups for group 1 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



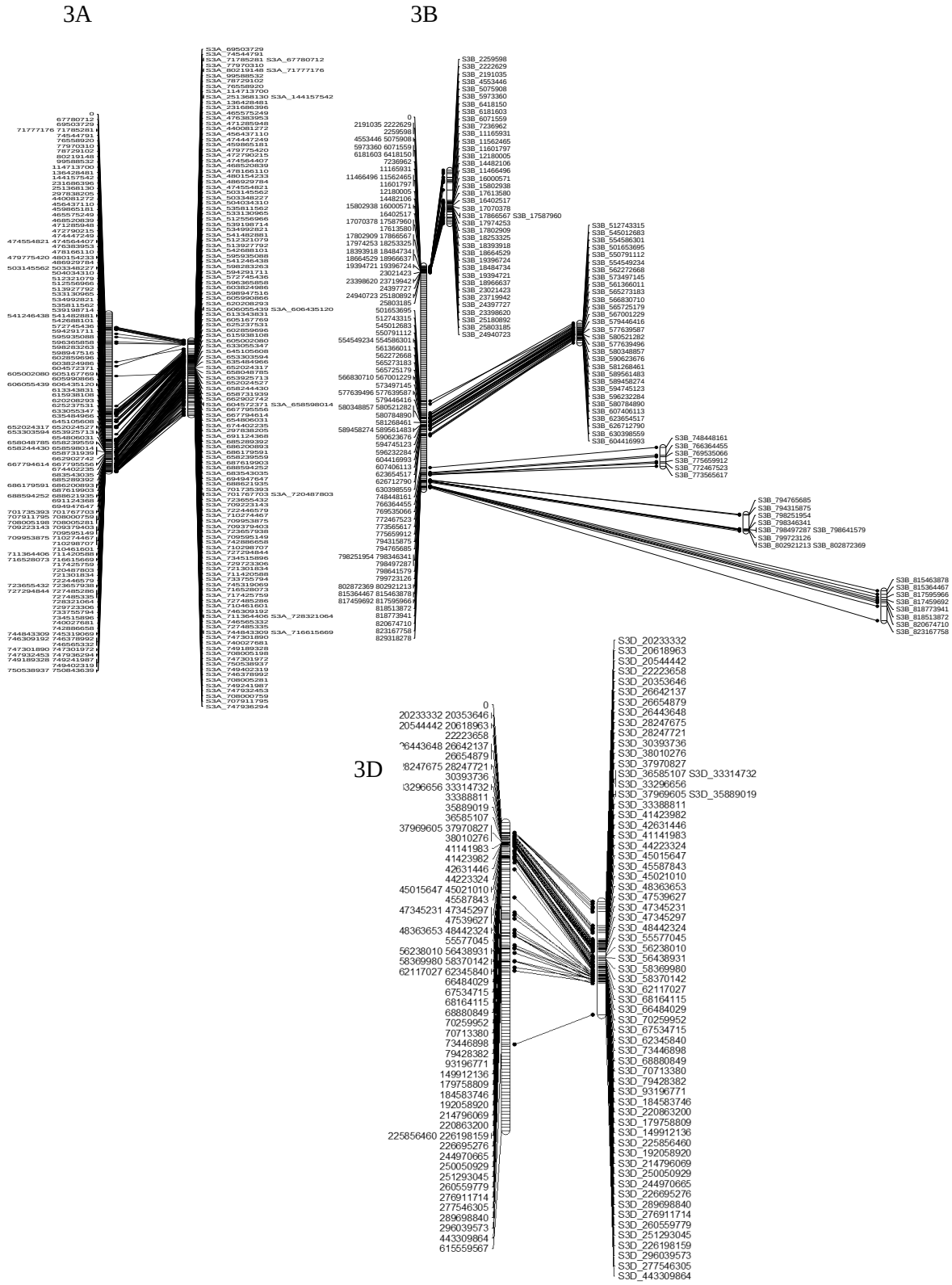
Sup Fig. 2.2A. *U6708-04 linkage groups for group 2 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



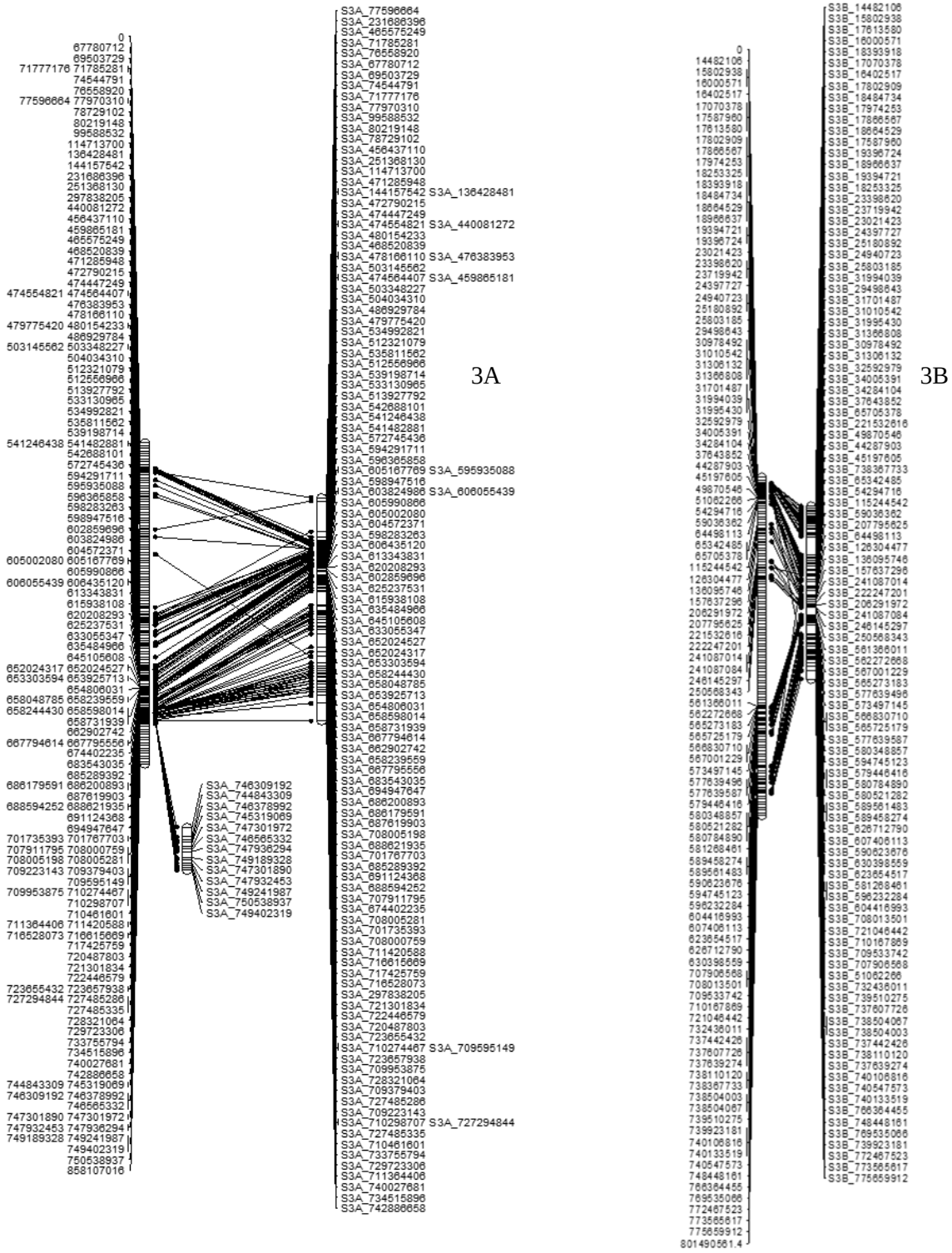
Sup Fig. 2.2B. *U6708-03 linkage groups for group 2 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



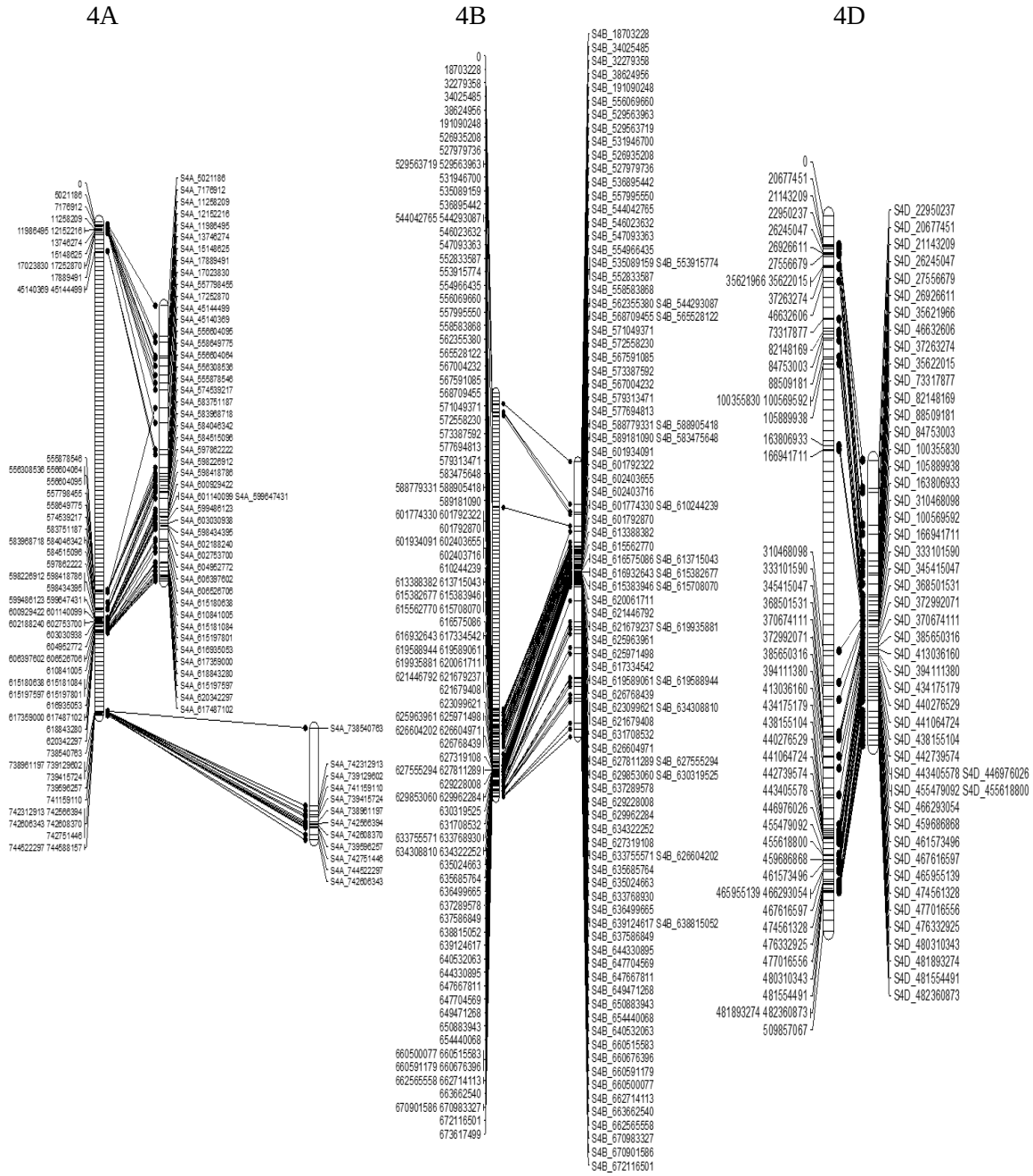
Sup Fig. 2.3A. *U6708-04 linkage groups for group 3 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



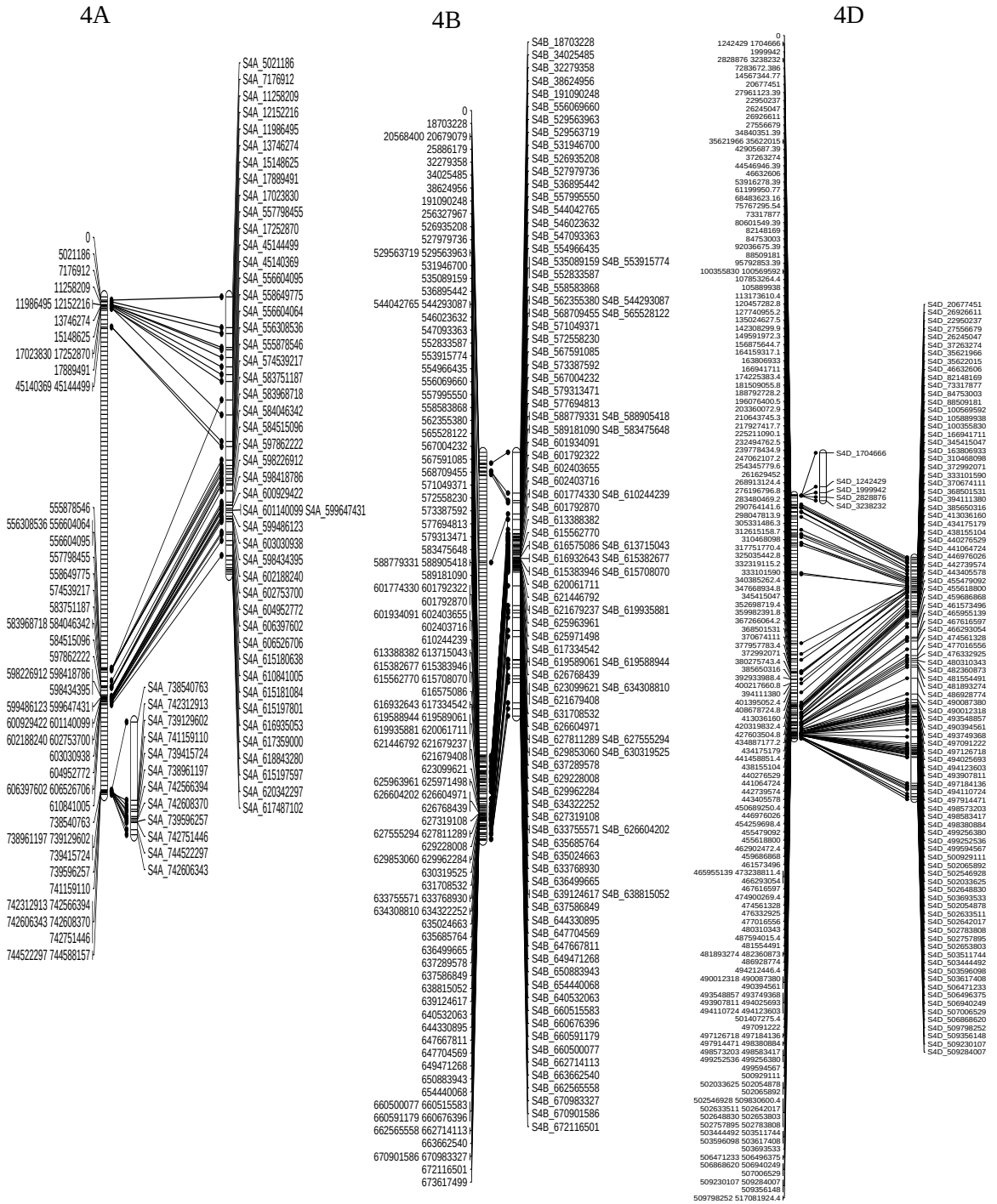
Sup Fig. 2.3B. *U6708-03 linkage groups for group 3 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



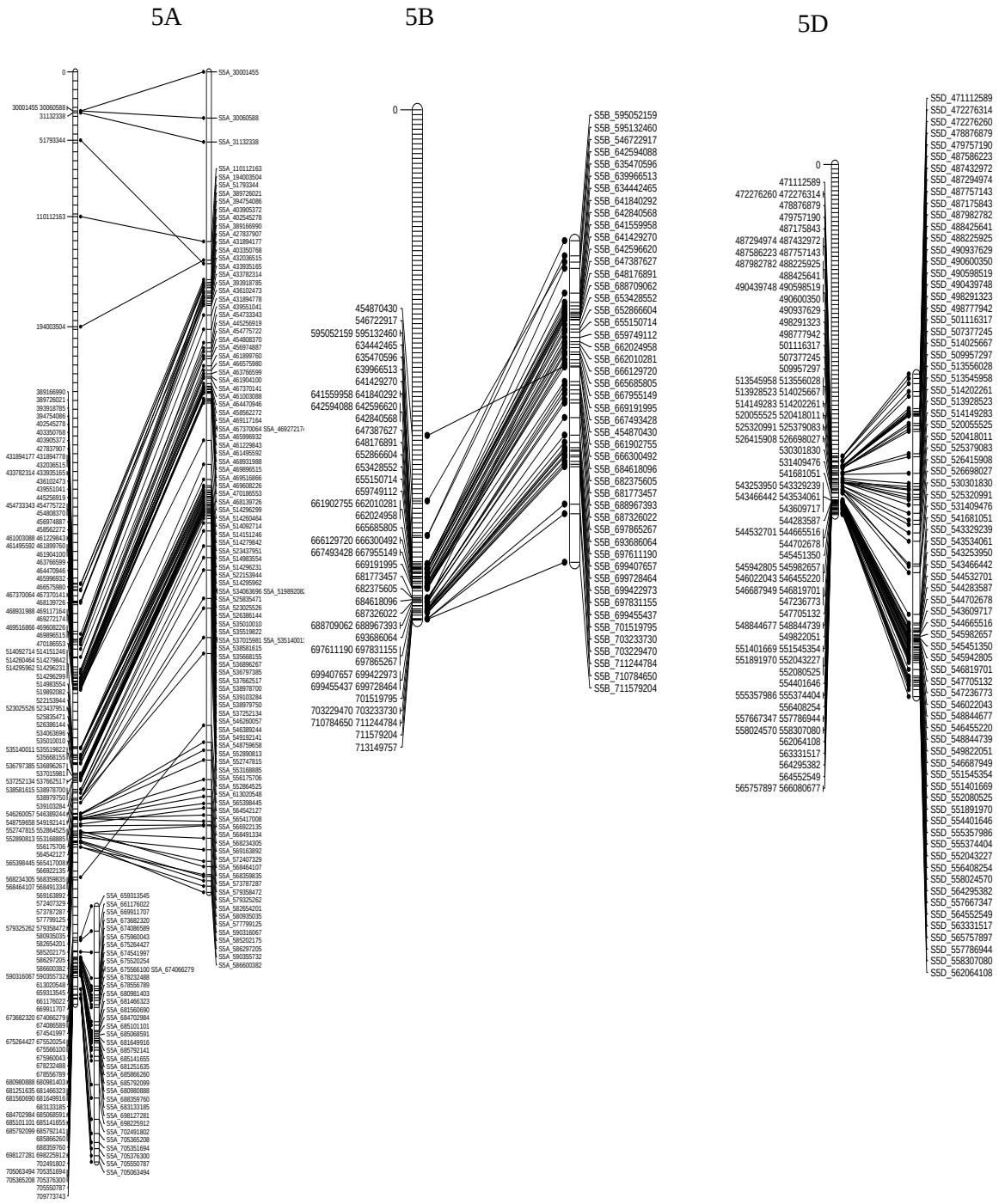
Sup Fig. 2.4A. *U6708-04 linkage groups for group 4 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



Sup Fig. 2.4B. *U6708-03 linkage groups for group 4 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



Sup Fig. 2.5A. *U6708-04 linkage groups for group 5 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



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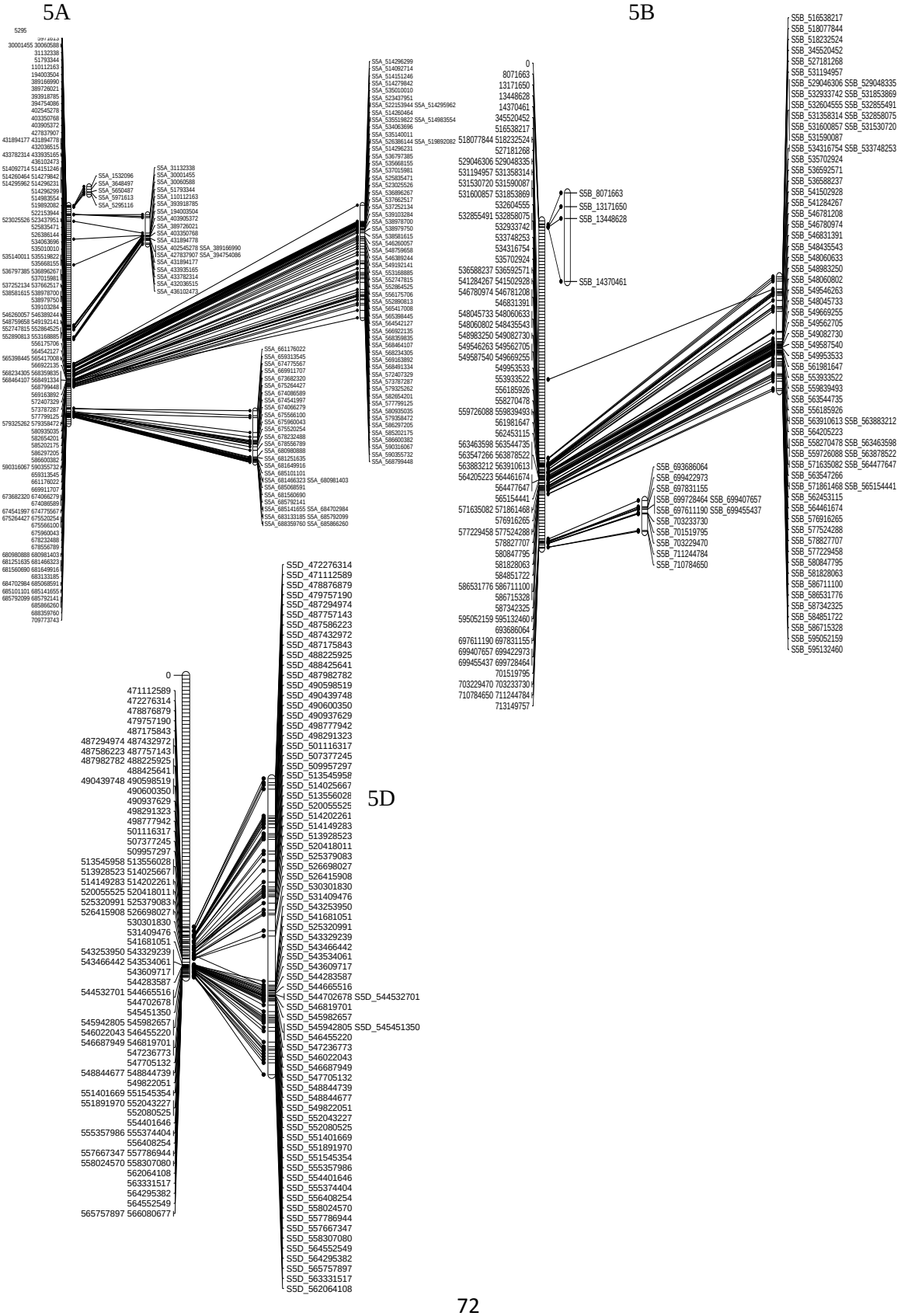
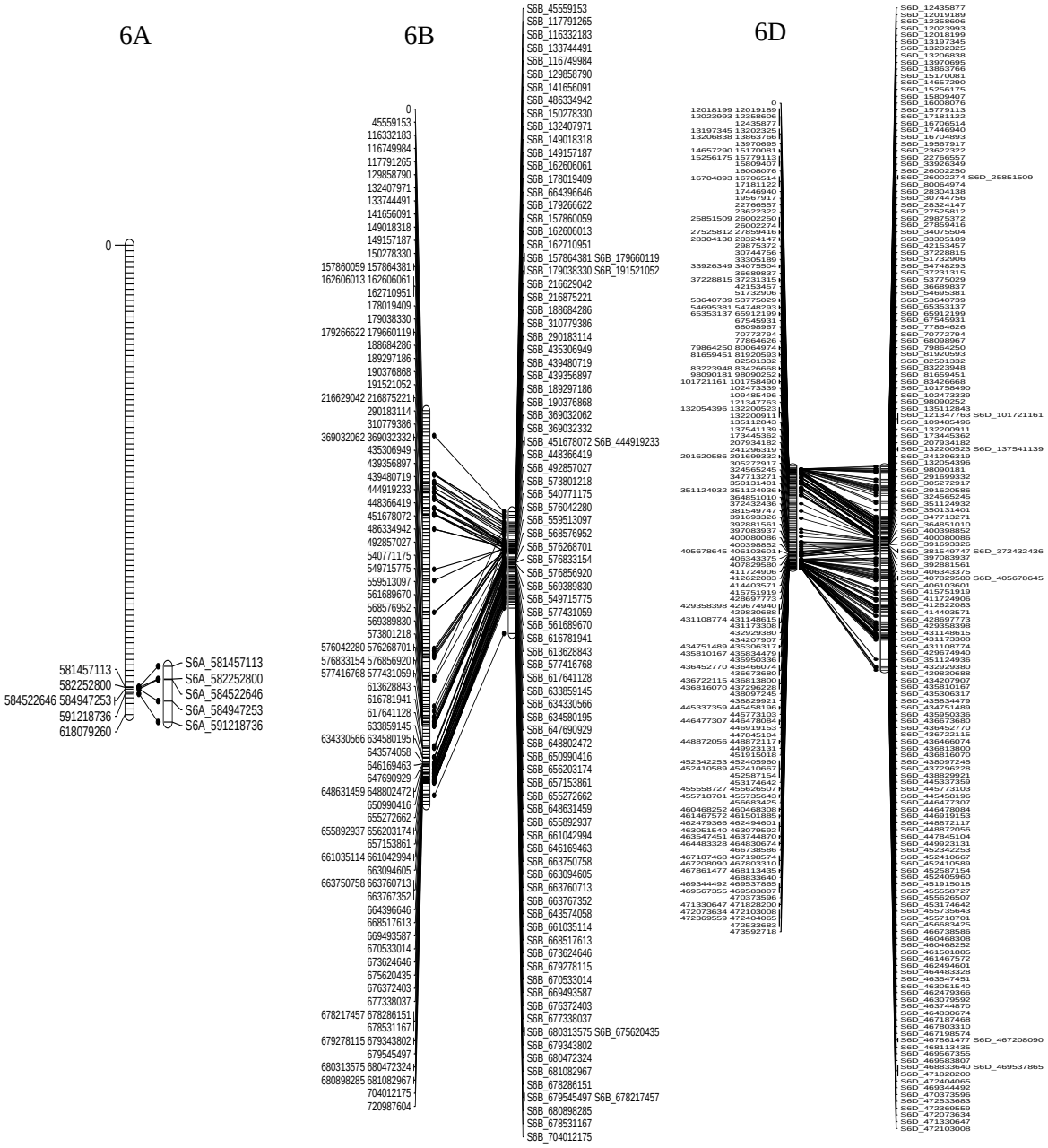
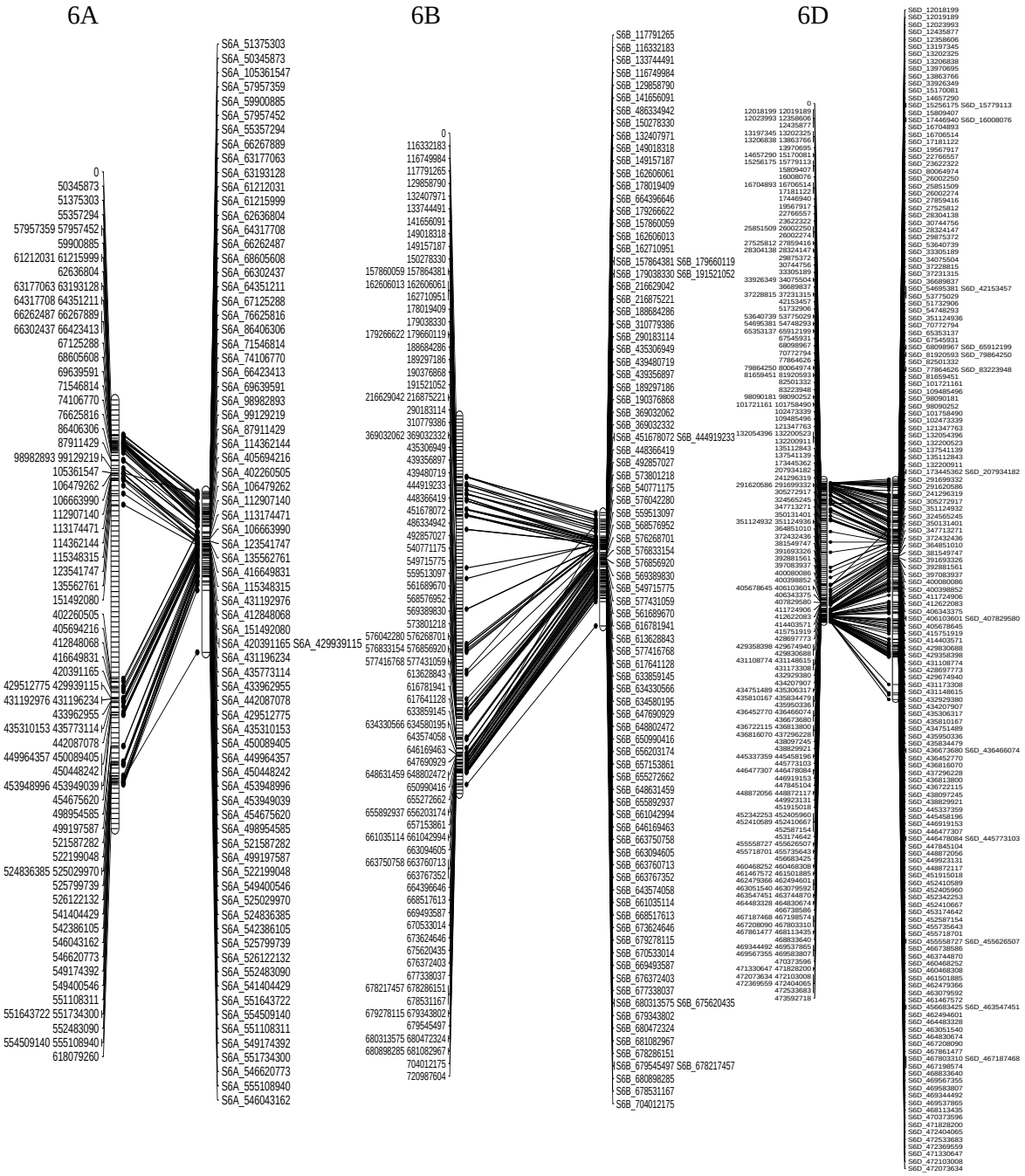


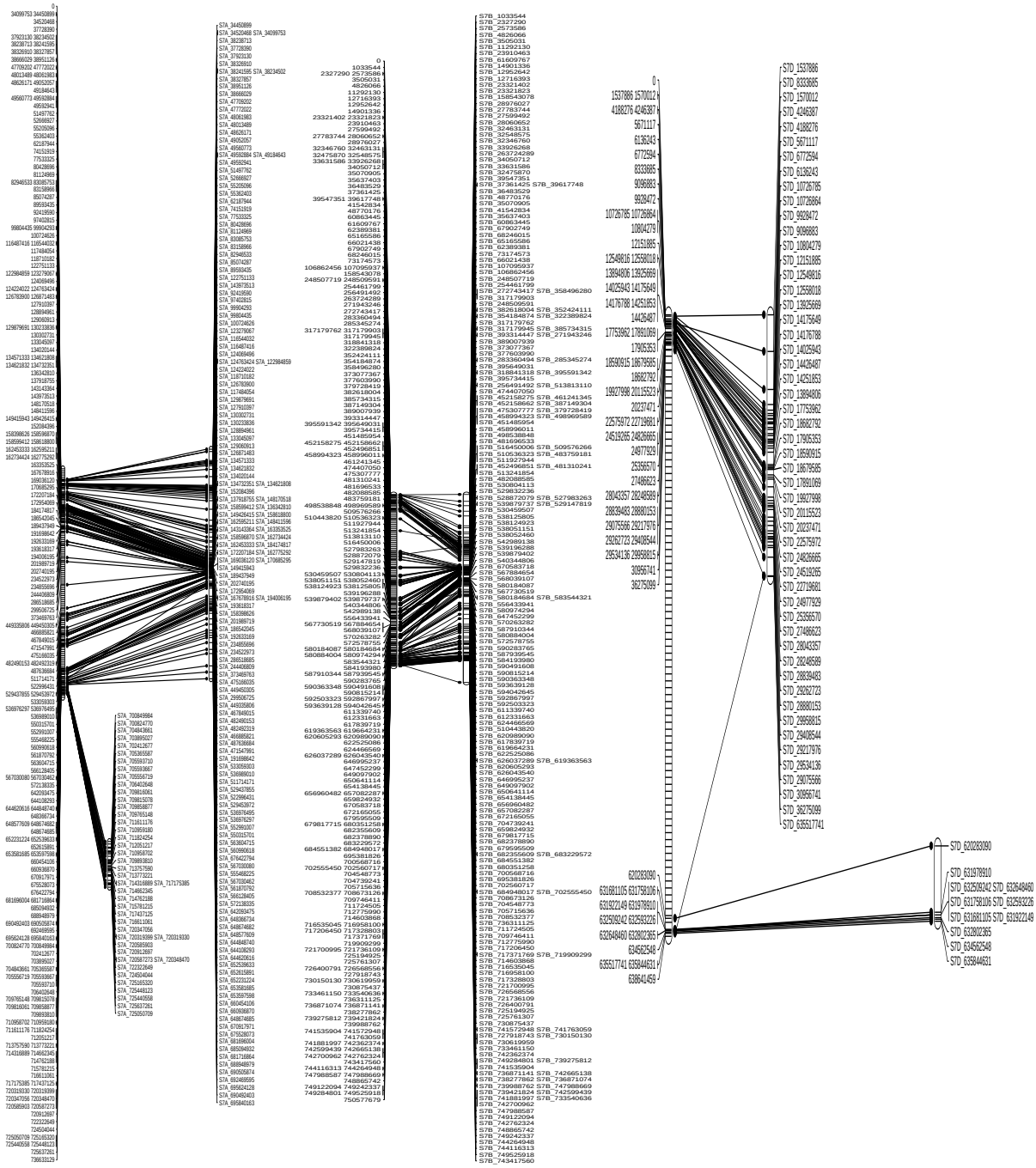
Sup Fig. 2.6A. *U6708-04 linkage groups for group 6 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



Sup Fig. 2.6B. *U6708-03 linkage groups for group 6 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*

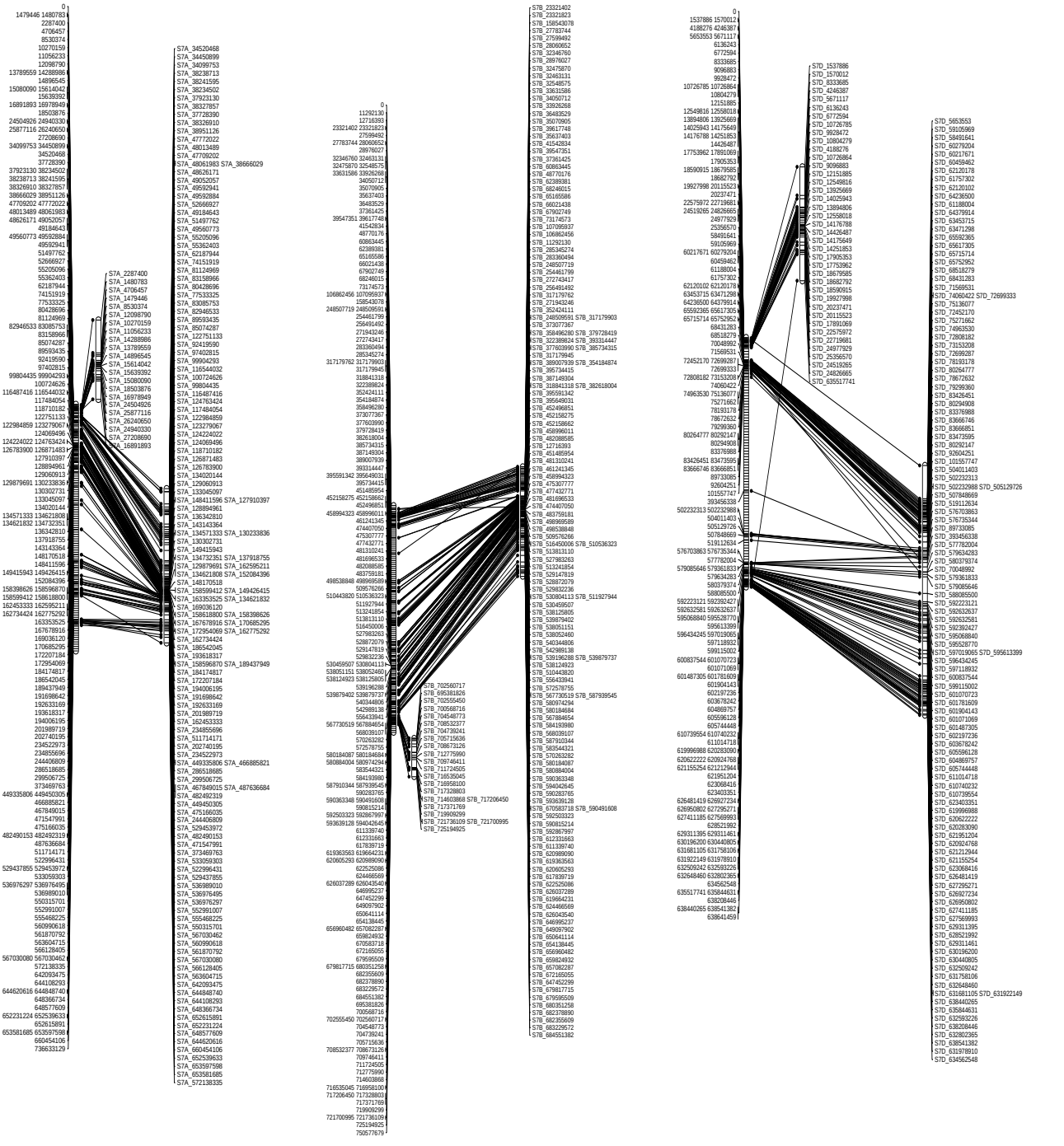


Sup Fig. 2.7A. *U6708-04 linkage groups for group 7 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



7A 7B 7D

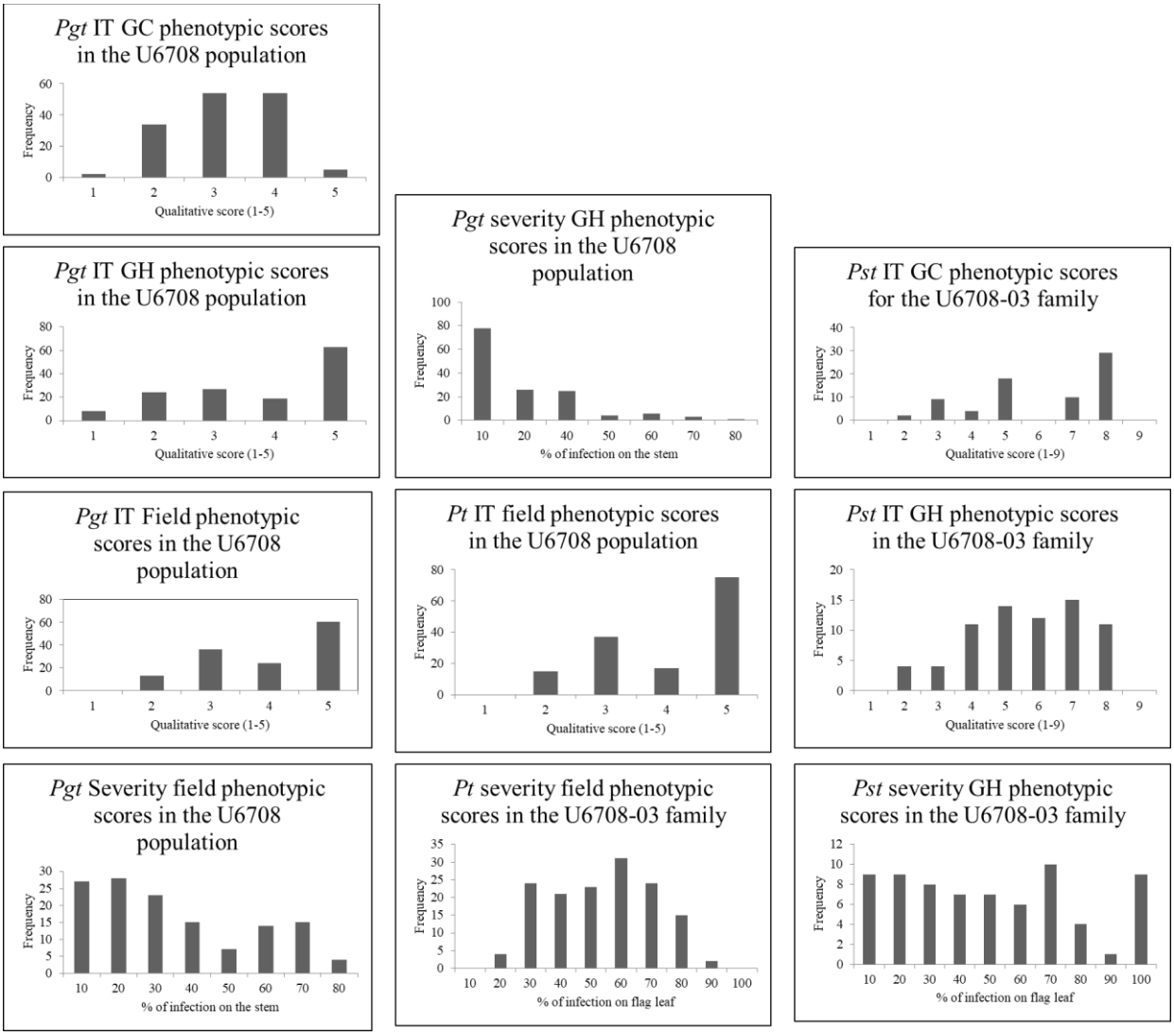
Sup Fig. 2.7B. *U6708-03 linkage groups for group 7 aligned to the respective representative chromosomes of the reference genome based on aligned marker positions.*



7A 7B 7D

Sup Fig. 2.8. *Histograms of both IT and severity scores recorded for the U6708 population in response to Puccinia inoculation.*

Severity was rated from 0–100% based on the Cobb scale, AUDPC = area under the disease progression curve. GC = growth chamber phenotyping environment; GH = greenhouse phenotyping environment; Field = Mason, Michigan, 2017 phenotyping environment. IT scores were converted to a 1-5 qualitative scale as described in materials and methods.



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CHAPTER 3: Conclusions

Conclusions of Current Research

A SHW RIL population, consisting of two families (U6708-03 and U6708-04), was developed from a cross between the synthetic line 9.131.15x (tetraPrelude/TA2474) and KS05HW14, a hard white winter wheat. The RIL population was genotyped using genotyping-by-sequence and GBS-SNP markers were identified using the TASSEL 5.0-GBS pipeline (Bradbury et al., 2007; Glaubitz et al., 2014). GBS-SNP markers were aligned to the recently released IWGSC (2014) Chinese spring v1.0 reference genome and linkage groups were created for each family. Twenty of twenty-one chromosomes segregated in each family with a majority of linkage groups aligning near the telomeres of each respective chromosome. The RIL population was phenotyped for disease resistance to three different *Puccinia* species, stripe (*P. striiformis* f. sp. *tritici, Pst*), stem (*P. graminis* f. sp. *tritici, Pgt*), and leaf rust (*P. triticina, Pt*). Phenotyping was performed at the seedling and adult plant stages with seedling evaluations performed in the growth chamber and adult plant evaluations performed in both the greenhouse and field. Greenhouse adult plant evaluations were implemented for additional phenotyping under a more controlled environment compared to the field. This controlled greenhouse screening was effective in mapping seedling resistance loci.

Seedling resistance to *Pst*, conferred by *QYr.msu-4DS* was mapped to the 4DS chromosome and originated from TA2474. *QYr.msu-4DS* was first mapped in a seedling test conducted in the growth chamber and segregated in only the U6708-03 family. Severity and infection type phenotypes, scored in a subsequent adult plant greenhouse evaluation, co-localized. Both growth chamber and greenhouse-grown plants were artificially inoculated with

Pst-37 uredinospores. Two other previously identified *Pst* seedling resistance genes have been mapped to chromosome 4DS, *Yr28* and *YrAS2338* which originated from two different *Ae. tauschii* accessions,W-219 and AS2338, respectively (Singh et al., 2000; Huang et al., 2011).

Seedling resistance to *Pgt,* conferred by *QSr.msu-5BL,* was mapped to the 5BL chromosome and segregated in both U6708 families. This seedling resistance locus originated from 9.131.15x and was associated with the same markers in both families indicating that the same QTL is present in both families. Phenotyping for *Pgt* resistance was performed in the growth chamber, greenhouse, and field environments, which were all artificially inoculated with *Pgt*-QFCSC uredinospores. Infection type and severity phenotypes, scored in both the greenhouse and field environments, co-localized with seedling resistance measured at the seedling stage. Only one *Pgt* seedling resistance gene has been previously mapped to 5BL, *Sr49*, which originated from the *T. aestivum* landrace Mahmoudi (Bansal et al., 2015). Further work will need to be carried out to determine the novelty of *QSr.msu-5BL*. Potential *Pgt* APR QTLs were identified in the greenhouse and field, but these were not repeated between environments. A putative APR QTL, originating from TA2474, was identified on the 7D chromosome in Mason, MI. Additional field trials could be performed to determine if the 7D QTL observed in the field can be observed in a repeatable manner.

Endemic *Pt* was present in Mason, MI, in 2017 and both families were phenotyped for *Pt* disease resistance against local *Pt* races. In both families, a major effect QTL that originated from KS05HW14 was mapped to 3BL at GBS-SNP markers shared between families. An additional *Pt* resistance QTL was identified on 6D segregating in the U6078-03 family and originated from TA2474. Previous work on 9.131.15x failed to identify leaf rust resistance on 6D and there is no currently known *Pt* QTLs on 6D that have originated from *Ae. tauschii* (Kalia,

2015). A synthetic RIL population consisting of two families U6708-03 and U6708-04 segregated for resistance to three different rust species with all three parents used in the construction of this U6708 mapping population conferring disease resistance QTL.

Future Directions

The study described previously was successful in mapping seedling resistance to both *Pgt* and *Pst* from 9.131.15x and identifying a *Pt* resistance QTL from KS05HW14. There are currently two officially identified *Pst* seedling resistance genes on 4DS (Huang et al., 2011) and one *Pgt* seedling resistance gene on 5BL (Bansal et al., 2015) that are at similar locations as *QYr.msu-4DS* and *QSr.msu-5BL* according to molecular positions based on the IWGSC v1.0 reference genome (2014). Additional testing is required to determine if *QYr.msu-4DS* and *QSr.msu-5BL* are novel resistance loci or variants of previously identified resistance genes. Future work will involve using an allelism test to better determine the novelty of these QTLs. Several lines from the U6708 population that are fixed for *QSr.msu-5BL* will be crossed to the landrace Mahmoudi, the hexaploid which is the origin of *Sr49* (Bansal et al., 2015), and lines from U6708-03 fixed for *QYr.msu-4DS* will be crossed with the synthetic harboring the *YrAS2388* gene (Huang et al., 2011). The resulting F_1 seeds will be taken to the F_2 generation and subsequently screened at the seedling stage for resistance with either *Pst-37* or *Pgt*-QFCSC. Segregation for resistance will be analyzed to better characterize the novelty of *QYr.msu-4DS* and *QSr.msu-5BL* with respect to previously identified resistance genes.

The U6708 population was screened against *Pst-37* and *Pgt*-QFCSC. However, with the emergence of new virulent rust races, it is important to determine if these loci are effective against the new highly virulent races like *Pgt*-TTTTF, *Pgt*-TTKSK, *Pst*-Warrior, and *PstS2* races. RILs will be selected from the population to be sent to St. Paul, MN to be screened for

seedling resistance to these rust races in a biocontainment safety level 3 (BSL-3) facility (Jin et al., 2007). If resistance is observed within the population to *Pgt*-TTKSK, lines may be sent to Njoro, Kenya for additional phenotyping for APR in a disease screening nursery.

Additional screening of the U6708 experiment will be required to decipher the segregating leaf rust resistance in this population. A seedling screen against the previously identified leaf rust race in Mason, MI, *Pt*-MCTNB, will be performed to identify if the major effect 3BL QTL is seedling resistance gene or an APR gene. Screening of KS05HW14 will need to be performed to confirm that it displays a resistant phenotype to local leaf rust races.

The U6708 population segregated for resistance to three rust species: stem, stripe, and leaf rust. Resistant lines from this population can be used in the Michigan State University Wheat breeding program and eventually released as useful germplasm lines for the wheat breeding community. There is a single RIL in the U6708-03 family that was phenotyped as resistant to stem, stripe, and leaf rust in all environments (Table 3.1). This RIL contains the *QYr.msu-4DS, QSr.msu-5BL, QSr.msu-7DS.2, QLr.msu-3BL,* and *QLr.msu-6DL*. This line would be useful to incorporate into wheat breeding programs as a multiple disease resistance donor.

APPENDIX

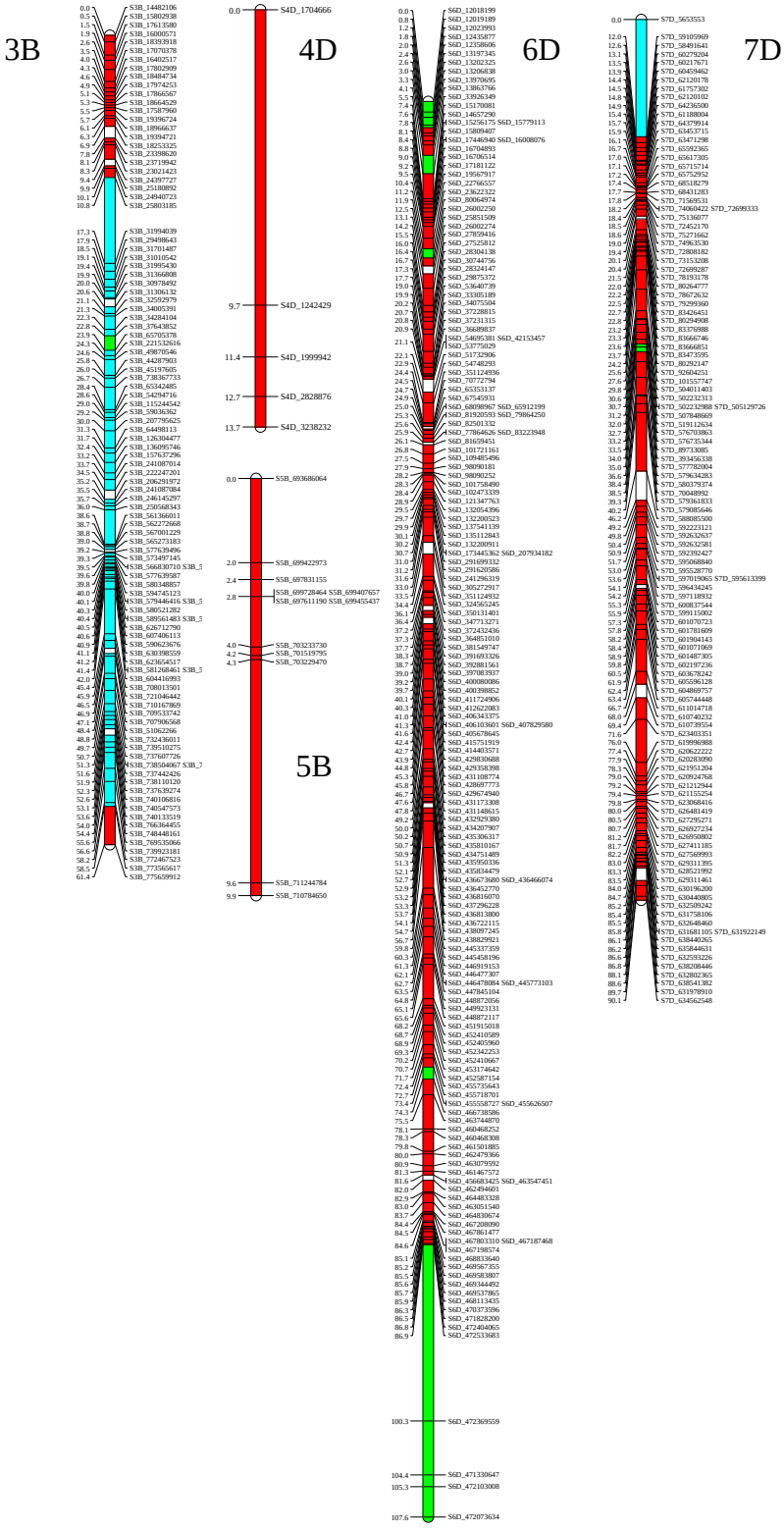
APPENDIX

Table 3.1. *Disease scores for a U6708-03 line that displays disease resistance to all three Puccinia species tested in all environments.*

Line	*Pst*			*Pgt*			*Pt*
	Seedling	Adult IT	Adult AUDPC	Seedling	Adult GH	Adult Field	Adult Field
U6708-03-028	2,3	3	25	22+	trR	trMR	25MR

Pst = stripe rust, *Pgt* = stem rust, *Pt* = leaf rust. Infection type (IT) on a 0–9 McNeal scale for *Pst* and 0–4 Stakman scale for *Pgt*. AUDPC = area under the disease progression curve.FR = field response rated as susceptible (S), moderately susceptible (MS), moderate (M), moderately resistant (MR) or resistant (R); severity, rated from 0–100% based on the Cobb scale, note: "tr" stands for trace levels of rust on the plant (Severity at, or around, 0).

Sup Fig. 3.1. *Graphical genotypes of linkage groups containing resistance QTLs in U6708-03-028.* Blue colored intervals contain KS05HW14 alleles, red colored intervals contain 9131.15x alleles, green intervals are heterozygous calls and black intervals are missing marker data.



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