TRANSFER OF FHB RESISTANCE GENES INTO HARD RED WINTER WHEAT

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

November 2020

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Fusarium head blight (FHB) is a serious foliar disease of hard red winter wheat (HRWW) in North Dakota. Disease resistance was identified as major component in disease management. First objective of this study is to transfer resistance QTL, *Qfhb.rwg.5A.2* into HRWW germplasm. Second part of this study aims is to confirm FHB resistance of two-triticale-*Th. distichum* addition lines (W1450 and W1451) and attempt to bring acrocentric addition chromosome in W1450 into common wheat, and characterization of W1423X-3 progeny in search of true breed resistance genotype. *Qfhb.rwg-5A.2* is transferred into three different populations, and haplotype consisting of three SNP close to centromere was identified as suitable haplotype for the target gene. FHB severity of the addition lines was less than 20% (greenhouse trial). Backcrosses are being made to bring addition chromosome of W1450 into wheat. Small proportion of W1423X-3 progeny appears as homozygous resistant, it needs further confirmation with cytogenetic techniques.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor Dr Francois Marais, for sharing his knowledge and ideas on different plant breeding tools throughout my study. His expertise, enthusiasm, thoughtfulness and professionalism are always great guide for my current and future career. His kindness, patience and positiveness encouraged me to learn and progress, which I will never forget. I would like to thank my committee members, Dr. Nonoy Bandillo, Dr. Shaobin Zhong and Dr. Ted Helms for their valuable comments and suggestions for my research study. I would like to thank my past and current lab members Dylan Barry, Hongbin Tao and Sagar Adhikari.

I also express my sincere thanks to Dr Hongbin Tao for teaching me the hands-on lab techniques. I like to express my gratitude to Dr. Ajay Kumar for sharing knowledge, encouragement, and valuable advices throughout this study. I thank our research specialist Mr. Bradley Bisek (Plant Sciences, NDSU) for his help with greenhouse and field experiments throughout this study. I thank Dr. Jason Fielder and Mrs. Mary Osenga (Genotyping center, USDA-ARS, Fargo, ND) for genotyping samples used in this study and for their help in analysing the data. I thank Dr. Yueqiang Leng (Plant Pathology, NDSU) for preparing FHB inoculum for all of my research experiments. I thank Dr. Steven Xu (Cereal Crops Research of USDA-ARS, Fargo, ND) for providing germplasm used in this research. I thank Dr. Bernard Friebe and his lab (Plant Pathology, Kansas State University) for performing GISH on germplasm used in this study. I like to extend my sincere thanks to my friend Satya Dev Pasupuleti for his support and encouragement. Furthermore, my special thanks to all the faculty members, staff, and graduate students in the Department of Plant Sciences for their kind support.

Finally, my wholehearted gratitude and appreciation to my parents and my uncle for their continuous encouragement during my M.S. study.

This research was supported by the US Wheat and Barley Scab Initiative.

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CHAPTER I. GENERAL SUMMARY OF THE STUDY

Different species of *Fusarium* cause symptoms of *Fusarium* head blight (FHB) of which *Fusarium graminearum* (*Fg*)is the predominant causal agent in most areas of the world. The fungus infects both spring and winter wheat and causes serious grain test weight, yield and economic losses. Apart from this, fungal mycotoxins that remain in the affected grains make it unfit for animal and human consumption, thus compounding the losses. Breeding of resistant varieties will increase and stabilize wheat production, reduce health risks to humans and domestic animals, improve seed quality and thus provide economic, social and health benefits.

The NDSU (North Dakota State University) Hard Red Winter Wheat (HRWW) breeding program continuously seeks to acquire and introduce novel, effective FHB resistance Quantitative Trait Loci (QTL) in its breeding population. This study has two components to it. First, an attempt to complete the transfer to HRWW of two very effective, complementing FHB resistance QTL discovered in spring wheat accession PI277012. A previous project transferred one of the genes, *Qfhb.rwg-5A.1,* to the HRWW line Novus-4. This project aimed to transfer *Qfhb.rwg-5A.2* so that both genes can be used in the development of commercial varieties. To do this, a hard-red spring wheat (HRSW) donor line (GP80) was crossed with and backcrossed to winter wheat. In the absence of reliable markers, single nucleotide polymorphism (SNP) haplotyping was used to characterize the parents and select for the chromosome region containing *Qfhb.rwg-5A.2* during backcrossing. Progenies that retained the targeted region were recovered and evaluated for type II FHB resistance in a greenhouse trial to confirm the presence of *Qfhb.rwg-5A.2*.

The second study attempted to characterize FHB resistance that occurs in *Thinopyrum distichum*. It was previously found that two *Th. distichum* addition lines (W1450 and W1451)

from a set of 27 random, disomic alien addition lines in hexaploid triticale could carry FHB resistance genes. Evaluation of the two lines in a greenhouse and in a misted field nursery appeared to confirm the earlier study; however, their dwarf phenotype and late maturity complicated evaluation. An attempt was initiated to also transfer the addition chromosome through backcrosses to common wheat to better evaluate its potential usefulness in a wheat genetic background. In the absence of a diagnostic marker, the morphology of the addition chromosome (acrocentric) was used to identify critical plants during backcrossing. B_4F_1 seeds were produced; however, at the same time unpublished results from a different study suggested that the acrocentric addition chromosome derives from an unknown triticale R-chromosome and does not appear to have *Th. distichum* associated chromatin. No further backcrosses were made after that. Finally, related triticale lines that derived from triticale–*Th. distichum* secondary hybrids and appeared to segregate for resistance in greenhouse FHB tests were again tested in greenhouse and field trials. However, no consistent evidence of the possible presence of a *Th. distichum* translocation to triticale was found.

CHAPTER II. LITERATURE REVIEW

Introduction

Globally, the United States ranks fourth in total wheat production, with almost 53.3 million metric tons produced in the 2018-2019 growing season. China, India and Russia ranked 1st, 2nd and 3rd, respectively (USDA 2019). Wheat is the third most important field crop in the United States, however, the area planted to wheat shrunk drastically from about 28 million hectares in 1997/1998 to about 18.24 million hectares in 2018/2019. Accordingly, US wheat production decreased from 2.4 billion bu in 1997/1998 to 1.962 billion bu in 2018/2019 (USDA 2019). Among the five major classes of wheat, HRWW accounted for about 45% of the total production in the 1990s, but this number decreased since 2000 and became 36% in the 2014/2015 season. However, the acreage has since increased to about 50% in 2018/19 with 9.08 million hectares planted. The US exported approximately 40–50% of the HRWW produced during the past 2-3 decades. Due to reduced production, the export of wheat and HRWW decreased to 85 million and 27 million bu respectively in 2018/2019 as compared to 1.1 billion and 37 million bu respectively in 1990/1991(USDA 2019).

Among many factors that contributed to the decrease in wheat acreage and production, the International Maize and Wheat Improvement Centre identified FHB (or scab) as a major factor. A broad survey of American wheat producing areas showed that FHB resulted in billions of dollars of wheat yield and quality loss in the 1990s and early 2000s (McMullen et al., 2012), and resurfaced as a worldwide threat. As a result, FHB was given special status as an emerging disease in the 1995 and 1996 federal budgets. In 1997, the United States Wheat and Barley Scab Initiative (USWBSI) was established to coordinate collaboration of plant scientists, growers,

millers and food processors across the country to develop effective control measures to minimize losses due to FHB (https://scabusa.org/about).

Fusarium head blight

Fusarium head blight (FHB), is also known as Scab (Dubin et al., 1996), and losses to the disease were first described over a century ago (Dickson and Mains, 1929). Initially, it was a serious concern for spring wheat and spring barley producers, but later on it started to negatively affect all classes of wheat and other small grains. In 1919, losses caused by FHB amounted to 80 million bushels in wheat alone (Dickson, and Mains, 1929). Along with yield, FHB also reduces the quality of grains by producing mycotoxins such as deoxynivalenol (DON) during infection. Since 1990, United States wheat and barley farmers have lost more than 3 billion dollars due to FHB epidemics (Schmale III and Bergstrom 2003). From 1993 to 2001, losses to FHB epidemics were estimated at about $7.67 billion for nine major FHB affected states in the northern great plains and the central USA, and North Dakota alone lost $3.5 billion dollars accounting for 45% of the total losses (Nganje et al., 2004). Increased acreage planted to maize, increased use of minimum tillage and climate change appeared to be major drivers that aggravated FHB epidemics and spread to newer regions (Beyer et al., 2006; Gilbert and Haber, 2013).

Symptoms and favourable conditions

Fusarium head blight in wheat is caused by fungal species of the genus *Fusarium* such as *F. graminearum* (*Fg*) and *F. culmorum*, however the disease is mostly associated with *F. graminearum Schwabe* (sexual stage: *Gibberella zeae*). Two other species of *Fusarium, F. poae* and *F. avenaceum*, infects barley (McMullen et al. 1997). Wheat and barley are susceptible to head infection from the flowering (pollination) period through the soft dough stage of kernel development. First symptoms of FHB infection are the premature bleaching of one or two

spikelets on the bottom, middle or top of the spike as spores of the fungus land on the exposed anthers of the flower and then grow into the kernels, glumes, or other head parts through vascular bundles and eventually bleach the entire spike. As the fungus colonizes, the infected seed will get deformed and deposited with trichothecene mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), 3-acetyl DON, etc. (Abbas et al., 2013). Under favourable conditions, salmon or pink coloured spores will be produced on the rachis and glumes of the infected spikelets (Schmale III and Bergstrom 2003). The fungus overwinters on infested stubble and straw of cereals, weed grasses, and on stalks and rotted ears of corn (McMullen et al. 1997). Infection is favoured by continuous high moisture or relative humidity (>90%) and moderately warm temperatures (between 15 to 30°C); prevalence of such conditions during flowering results in rapid inoculum production, floret infection, and colonization of developing grains (Schmale III and Bergstrom 2003).

Disease management strategies

Yuen and Schoneweis (2007) described disease management strategies to reduce losses caused by FHB, they included cultural practices, fungicide spray, biological control, and utilization of resistant cultivars. Cultural practices for control of FHB aim to reduce the pathogen inoculum for disease development. Since *F. graminearum* survives saprophytically on residues of crops and produces both asexual (chlamydospores, macroconidia and microconidia) and sexual spores (ascospores) on these substrates, practices that reduce inoculum such as crop rotation with non-gramineaceous crops and tillage (burying of infested residue) and burning of crop residues may be effective for FHB management in individual cereal fields and over broader regions of cereal production. However, these agronomic practices are less attractive in terms of economic and environmental benefits. Fungicides with active ingredients of metconazole,

tebuconazole, and propiconazole have proved effective for FHB management (Paul et al. 2008). However, a very short window for fungicide application and high associated cost limit their usefulness (McMullen et al., 2012). Certain fungal, bacterial and yeast species were reported to be potential biological control agents of *F. graminearum* (Yuen and Schoneweis 2007; Gilbert et al., 2004; Jochum et al., 2006; Khan et al., 2004). However, biocontrol agents have not yet been applied successfully on a commercial scale. Cultural practices and fungicide application can reduce but not prevent yield loss caused by FHB. The use of resistant cultivars provides a much more efficient and environmentally friendly way to reduce the impact of this disease (Ruckenbauer et al., 2001; Bai and Shanner 2004; He et al., 2013). Due to the complex interaction between the host, pathogen, and environment, sole dependence on a single management strategy has little or no effect in reducing FHB damage (McMullen et al., 2012). An integrated disease management approach using a combination of host resistance, cultural practices and fungicide application is the most efficient and effective way to control FHB (McMullen et al., 1997). Disease resistance was shown to be a key component in FHB management (Nathen, 2019).

Basis of FHB resistance

FHB resistance is categorized into five different components: Type I (resistance to primary infection), Type II (resistance to disease spread within the spike), Type III (resistance to accumulation of DON), Type IV (resistance to kernel infection) and Type V (resistance to yield loss) (Mesterházy, 1995). Type II is the most widely explored and utilized form of resistance in wheat breeding, while Type I has gained subtle importance (McMullen et al., 2012). Type IV and Type V resistance are rarely utilized in breeding programs as it is poorly understood (Zhang et al., 2011a).

Resistance to FHB involves a series of physiological and molecular processes and was well described by Walter et al. (2010). FHB requires a suitable host and favourable environmental conditions for its establishment. Its infection involves a brief biotrophic phase before entering the necrotrophic stage, making *Fusarium graminearum* (*Fg*) a hemi-biotroph (Goswami and Kistler, 2004; Saville et al., 2012). Several receptor proteins like G protein-coupled receptor proteins, transduction beta sub-unit and tetraspanins encoded by the *Fg* genome facilitates the recognition process and appressorium formation (Walter et al., 2010). *Fg* spores land on a head of wheat, mostly on the middle portion of the spike which harbours spikelets with higher water content compared to spikelets on the top or bottom of the spike. Various signals are then transduced through the fungal membrane to their respective target sites in the fungal genome to facilitate pathogenicity and virulence. *Fg* then grows hyphae and secretes a diverse range of hydrolysing enzymes to enter through the cuticle. Hyphae gradually grow into the subcuticular, substomatal and intercellular regions of spikes and up to the middle lamella and cell wall. Pectin and other cell wall-degrading enzymes secreted by the pathogen helps in penetration through cell barriers. Eventually, hyphae extend into the cell apoplast and trigger a cytological turbulence which leads to cell death. This stage is characterized by the induction of DON, a trichothecene mycotoxin that is involved in the killing of cells by generating hydrogen peroxide, inhibiting the production of plant proteins and damaging cell components. The amount of DON produced by *Fg* is thus directly correlated to its virulence. *Fg* maintains its pathogenicity and colonization by neutralizing plant defence proteins or toxins as well as extracting nutrients from the plant to facilitate its own growth and spread (Walter et al., 2010).

A resistant plant will trigger a defence mechanism against these fungal actions based on the type of resistance in that plant. Morphological and physiological features will act as a first

wall of defence or Type I resistance against pathogen entrance and establishment (Walter et al., 2010). Once the fungal hyphae break physical barriers, plant chitin-binding proteins and chitinases sense and degrade fungal glucans. The integrity of the fungal membranes may also be destroyed by non-specific lipid transfer proteins (nsLTPs), thionins and puroindolines. Degeneration of the cuticle or cell membrane of resistant plants induces jasmonic acid (JA) and methyljasmonate (MeJA) through polyunsaturated fatty acid signalling. JA and MeJA are the two key components in signalling for activation of a defence response against necrotrophic pathogens. The two hormones also cascade a signal to alarm distant tissue about the invading pathogen, thereby preparing an entire region of plant cells for the defence response. Along with the disintegration of the fungus, resistant plants tend to repair and fortify their barriers by expanding the cell wall and establishing cell wall appositions to reduce incoming toxin and outgoing plant nutrients. Since *Fg* produces DON, resistant plants will either alter the toxin or its target by obstructing interaction of the toxin and target or by generating downstream signalling cascades. Wheat will also trigger a defence response against DON by opposing the oxidative stress induced by it. These concerted actions against FHB infection tends to provide both Type I and Type II forms of resistance. Type II resistance will mainly involve the mechanisms that tend to reduce the influx of FHB toxin as well as spread of the fungus through the plant. Reduced vessel size, solid sclerenchyma and cells walls, thicker vascular bundles and smaller internodes will aid in conferring Type II resistance (Walter et al., 2010).

Host resistance

FHB resistance in wheat can also be viewed as being of three types: passive (morphological), active (physiological), and due to tolerance (Mesterhazy 1995). The FHB infection process is complex and affected by morphological factors like presence of wax on the

cuticle, plant height, spikelet density, anther retention, awn characteristics, duration of flowering and grain filling (Rudd et al., 2001; Steiner et al., 2019). Furthermore, environmental conditions during pathogen infection and spread are of primary importance (Bai et al., 2018). Gibberellin-insensitive semi-dwarfing genes and anther retention make cultivars more prone to FHB infection (Lu et al., 2013; Steiner et al., 2019). Weak correlation was found between plant height, anther retention and FHB resistance (Chu et al., 2011). Physiological (active) resistance results when biochemical pathways produce chemicals that affect the initial infection and spread to other tissues and it is generally more useful. The ability of plants to withstand pathogen attack without loss of economic yield is called tolerance of the pathogen. So far, no immunity with respect to FHB has been found in wheat (Mesterhazy, 1995). However, the perennial grass *Elymus tsukushiensis*, a wild relative of wheat was reported to be immune (Cainong et al., 2015). Different genes imparting different types of resistance were identified in the wheat gene pool.

Gene pools

Harlan and de Wet (1971) grouped cultivated plants into three categories (gene pools). They are:

Primary gene pool

This includes all the cultivars, races or species that are easily crossable with one another. Chromosomes of each pair well with those of the other primary gene pool members during meiotic metaphase I. Hybrids produced are fertile, gene transfer and gene segregation within the gene pool are easy and normal. Hexaploid landraces, cultivated tetraploids, wild *Triticum dicoccoides* and diploid donors of the genomes of hexaploid wheat are included in this gene pool. Mostly, gene transfers from this pool is straightforward and requires only standard breeding methods like hybridization, backcrossing and selection (Harlan and de Wet, 1971).

Secondary gene pool

This includes species that are closely related to, and crossable with cultivated wheat. Meiotic chromosome pairing between the wild donor and wheat is limited or chromosomes may not pair thus producing weak and sterile hybrids. Embryo rescue may be needed to save the hybrids. Recovery of genotypes with desired traits is difficult. Thus, secondary gene pool species can be used as a source of genes for the improvement of cultivated wheat, but hybridization and gene transfer require special effort. This gene pool consists of polyploid *Triticum* and *Aegilops* species which share one genome among the three genomes of wheat (Harlan and de Wet, 1971).

Tertiary gene pool

Crosses employing these species can only be made with great difficulty. Meiotic pairing between chromosomes of these species and those of the crop species will not happen unless special techniques are employed. Hybrids produced are weak, embryo rescue and chromosome doubling are needed. Several generations of backcrosses to the desired crop species genetic background is needed to introgress desired chromatin. Transfer of genes from this gene pool presents unprecedented challenges. Polyploid species with Triticeae genomes other than A, B and D are included in this gene pool (Harlan and de Wet, 1971).

Many valuable resistance genes have been introduced into wheat from at least 52 species from 13 genera (Gill et al., 2008; Wulft and Moscou, 2014). Despite valuable resistance genes being present in the tertiary gene pool, plant breeders are reluctant to use them as a gene source because of a high likelihood of deleterious linkage drag (Mago et al., 2002). Linkage drag and the long time needed to transfer desired chromatin into a desirable genetic background limits the use of alien species as a source of resistance genes (Cainong et al., 2015); however, recent

advances in genome sequencing and bioinformatics will facilitate more rapid introgression of genes from the tertiary gene pool (Wang et al., 2020).

Transfer of Fusarium head blight resistance genes from related grasses

Although many FHB resistance QTL were discovered in wheat, their use in breeding programs is limited by small individual effects, environmental influence on their expression and segregation of unknown genetic background resistance QTL (Bai et al., 2018; Buerstmayr et al., 2019). Increasing efforts are being made to explore species in the secondary and tertiary gene pools in order to identify stronger-effect resistance QTL and to simultaneously increase genetic diversity for FHB resistance in cultivated wheat.

Hexaploid wheat has three distinct but genetically related sub-genomes (A, B and D). Homoeologous chromosomes in the three sub-genomes can compensate for each other, making it possible to incorporate alien chromatin segments containing target genes into the wheat genome through chromosome engineering (Qi et al., 2007; Gill et al., 2008; Marais et al., 2008). Following the identification of useful resistance in a wild relative, the first step is to produce a fertile hybrid with cultivated wheat which will then serve as the starting point for backcrosses to wheat to establish chromosome addition, substitution and translocation lines (Marais et al., 1998; Chen et al., 2001).

Chromosome pairing in wheat

Wheat belongs to the tribe Triticeae. It can form hybrids with many of its wild and cultivated relatives, providing a bridge by which valuable genes can be transferred to wheat. However, although the genomes of wheat and its gene pool member species are related, synapsis (pairing) between wheat and homoeologous alien chromosomes rarely occurs (Riley et al., 1959). This is the result of pairing control genes, located on wheat chromosomes, which suppress

recombination between homoeologous chromosomes. The most prominent of these genes is *Ph1* located on chromosome 5B (Riley and Chapman 1958). Reduced or no homology between different chromosomes along with many complex genetic factors leads to a-synapsis or de-synapsis (Sears 1972; Hamant et al., 2006). Under a-synaptic conditions, chromosomes never pair and the majority of the univalent chromosomes exhibit irregular distribution and dispersion in the cytoplasm. These unpaired chromosomes never congregate on the equatorial plate. With de-synapsis, initial pairing takes place between chromosomes, univalents and bivalents orient at the equatorial plate during cell division but pairing is disrupted before the exchange of chromatin (Perison et al., 1997). Univalents either get lost or are randomly transmitted to daughter cells giving rise to unbalanced gametes, which leads to aneuploidy in the offspring. With respect to a single unpaired chromosome, aneuploid (n-1 or n+1) or euploid (n) gametes will be produced through abnormal segregation to result in genotypes ranging from nullisomic (2n-2) to tetrasomic (2n+2). Univalents may also undergo mis-division to produce telocentric chromosomes (telosomes) or Robertsonian translocations (Friebe et al., 2005). Thus, all of the abnormal meiotic events associated with a-synapsis and de-synapsis cause variations in chromosome structure and number (Bhat and Wani, 2017). However, in the absence of the primary chromosome pairing control locus, Ph_1, homoeologous recombination between wheat and alien chromosomes can take place (King et al., 1993). This phenomenon has been used for the introgression of genes from wild relatives into wheat (Cainong et al., 2015). In addition to loci such as Ph_1 and Ph_2, that suppress homoeologous pairing in wheat, some genes (for example *Su1-Ph1, Su2-Ph1, Ph*^{1} in *Ae. speltoides*) can inhibit Ph_1 to allow for homoeologous pairing to occur even in its presence (Chen et al., 1994; Li et al., 2017).

Addition lines development in wheat

The development of wheat addition lines with single alien donor chromosomes aids identification of critical chromosomes harbouring desired genes and facilitates subsequent attempts to transfer such genes to homoeologous wheat chromosomes. In order to produce an addition line, a primary hybrid between wheat and the alien species is produced first. Following chromosome doubling of the F_1 with colchicine, a fertile amphiploid hybrid can be obtained. The amphiploid is backcrossed to wheat in order to select plants with fully restored wheat genomes plus an additional alien species chromosome (= monosomic addition line). Following self-pollination, disomic addition lines with stable transmission of the added chromosome pair can be selected. If during backcrossing, selection is done for the presence of a desirable alien trait, the addition line will carry the alien chromosome harbouring that trait (Riley and Chapman, 1951). Establishment of a complete set of alien chromosome addition lines in wheat allows study of the genetic effects of individual alien chromosomes in the background of hexaploid wheat (Liu et al., 2019). Over the past two decades, addition lines were widely used for the characterization of desirable alien resistance genes (Marais et al., 1998). Alien chromosome addition stocks have also been used in gene mapping (Chen et al., 2001), gene tagging (Marais et al., 2007) and gene transfer (Cainong et al., 2015).

Robertsonian translocation

Robertsonian translocations (whole-arm translocations) often occur in double-monosomic plants as a result of centric mis-division followed by the random fusion of the broken arms from different chromosomes (Qi et al., 2011; Robertson, 1916). The mechanism by which Robertsonian translocations arise was investigated in plants monosomic for chromosome 1A of wheat and 1H(t) of *Elymus trachycaulus* by Friebe et al. (2005). The two univalents stayed intact

(and were unaffected) while metaphase I meiotic recombination occurred. Robertsonian translocations appeared to arise from centric mis-division of univalents at ana-/telophase I, followed by segregation of the derived telocentric chromosomes to the same nucleus, and fusion of the broken ends during the ensuing interkinesis (Friebe et al., 2005). Complete fission-fusion cycles have been reported for wheat-rye translocations (Lukaszewski, 1993, 1994, 1997). Analysis of wheat-rye Robertsonian translocations derived from such mis-division events indicated that centric breakage-fusion can occur at different positions within the primary constriction without affecting centromere function. These studies showed that the centromere consist of functional subunits that can be divided and reshuffled even between chromosomes of species that belong to different genera without losing their function. Robertsonian translocations are widespread in plants (Jones, 1978). However, the frequency of Robertsonian translocation varies depending on the chromosome involved (Marais and Marais, 1994; Friebe et al., 2005). The production of compensating translocations involving homoeologous chromosomes of alien species and common wheat can result in rapid introgression of desired genes (Liu et al., 2013).

Chromosome engineering in wheat

Sears (1972) listed four basic methods for the transfer of genetic material from alien to wheat chromosomes. They are: 1) spontaneous translocation; 2) use of ionizing radiation to induce translocations; 3) induction of homoeologous pairing and recombination; and 4) Robertsonian translocation. Based on the likelihood of recovering a useful translocation and the ability to limit linkage drag, homoeologous chromosome pairing induction is the most effective. The homoeologues normally show little or no tendency to pair, however, they can be induced to pair in the absence of *Ph1* (Riley and Chapman, 1958). The *phlb* mutant line has a deletion at the *Ph1* locus on chromosome 5B. Thus, homoeologous pairing can occur in *ph1b* (recessive)

homozygotes. To allow for this to happen, a plant needs to be produced that is simultaneously *ph1bph1b* and a double monosomic for the alien donor chromosome and a homoeologous wheat chromosome. Such plants can be testcrossed with wheat and the testcross progeny analysed to recover the desired recombinants (Qi et al., 2007; Cainong et al., 2015). Alternatively, a Robertsonian translocated line with the desired chromosome fragment can be crossed and backcrossed to the wheat Chinese spring *ph1b* mutant. Backcross plants are phenotyped or genotyped to identify plants homozygous for *ph1b* and with the desirable gene fragment. These plants can be testcrossed to recover recombinants or can be selfed further to identify recombinant segregates with the target gene but reduced linkage drag. The proportion of desired, translocation-carrying genotypes recovered are usually very low (Guo et al., 2015). Once a suitable translocation is obtained it will be backcrossed into a normal, desirable wheat genetic background (Guo et al., 2015). C- banding or genomic in-situ hybridization (GISH) is often done on root tip cells to confirm the presence of introgressed alien chromatin (Somo et al., 2014). Use of *ph1b* may also induce unintended recombination between other wheat homoeologous chromosomes, which need to be remedied afterwards by backcrossing to wheat. Advances in molecular marker technology has greatly aided chromosome engineering and the selection of appropriately modified genotypes with the desirable gene.

Hybrid necrosis

Hybrid necrosis is a physiological disorder caused by complementary dominant gene action which results in premature death of leaf blades and leaf sheaths. It starts from the tip of the first leaf and gradually progresses to younger leaf tissues. After flowering, necrosis progresses to the whole plant causing senescence of leaves and leaf sheaths prior to maturity leading to the production of premature, shrivelled seeds (Hermsen, 1966; Chu et al., 2006).

Hybrid necrosis is a serious barrier to gene transfer from related alien species to commercial wheat (Tommar et al., 1991). Serious hybrid necrosis can complicate the genetic analysis of particular traits as it may prohibit the development of a representative mapping population. Hybrid necrosis is controlled by the complementary dominant genes *Ne1* and *Ne2* located on 5BL and 2BS of wheat, close to the centromeres (Tsunewaki, 1960; Zeven, 1972; Nishikawa et al., 1974; Chu et al., 2006). Marker locus *Xbarc74* was mapped 2cM from *Ne1* and *Xbarc55* was mapped 3.3cM from *Ne2* in F_2 and backcross populations obtained from CIMMYT (Chu et al., 2006). A large variation in the extent of necrosis was observed in different wheat crosses and is largely dependent on different combinations of the three alleles of *Ne1* and five alleles of *Ne2* (Singh et al., 1992; Hermsen, 1963). Along with these, two additional genes, *Ner1* and *Ner2* were identified in rye that are known to cause hybrid necrosis in triticale by interacting with necrosis genes of the B genome (Scoles, 1985; Ren and Lelley, 1989). The effect of some necrosis gene combinations can be minimized by exposure of the plants to higher temperature and can be eliminated by backcrossing F_1 hybrids with desired wheat cultivars lacking necrosis genes (Bizimungu et al., 1998).

Synthetic species

Gene transfer from homoeologous chromosomes in the secondary and tertiary gene pool is mostly difficult and often complicated by factors such as: the cross-ability of wheat parents, F_1 seed abortion, F_1 hybrid lethality, high sterility of F_1 hybrids, sterility caused by the abnormal chromosome numbers of segregates (Marais et al., 1998), and hybrid necrosis (Bizimungu et al., 1998).

The primary gene pool species are rich sources of genes for a broad range of resistance, tolerance and adaptational traits (Li et al., 2018). In order to increase diversity in common wheat

and to be able to utilize the diversity and valuable genes present in the primary gene pool species more effectively, synthetic wheat hexaploids were made. Rana et al., (2013) provided a review of the use of synthetic hexaploids in wheat breeding. Synthetic hexaploid wheat is developed by hybridizing *T. turgidum*, AABB (either *T. turgidum* ssp. *dicocccum* or *T. turgidum* ssp. *durum*) and diploid wild goat grass (*T. tauschii*, DD) and doubling the F_1 hybrid chromosome numbers. In recent years more emphasis is being placed on the durum wheat X *T. tauschii* crosses and numerous hybrids have been made to fully access the genetic diversity present in *T. tauschii*. Synthetic wheat is used as a bridge to transfer desirable genes from *Ae. tauschii* and durum wheat to hexaploid bread wheat. Synthetic hexaploid wheat has been a source of resistance to biotic stresses such as leaf blotch, glume blotch, yellow leaf spot, powdery mildew and abiotic stresses (Ginkel and Ogbonnaya, 2007). Rosyara et al. (2019A) estimated that 20% of a current total of 1600 advanced lines and their parents in CIMMYT's spring bread wheat breeding germplasm were derived from synthetic wheat. Apart from desirable genes, synthetic wheat possesses numerous agronomically undesirable characters such as non-free threshing grains of the wild species which makes it necessary to remove these undesired traits through cross breeding with elite common wheat germplasm and intensive selection.

A different way to utilize wild species germplasm is by making and establishing new, synthetic species. Triticale is such a man-made hybrid species developed by crossing wheat and rye. The first viable triticale was developed in 1888 by Rimpau (Mergoum et al., 2009). Triticale incorporates favourable alleles from both progenitor species. Triticale is better adapted to, and gives higher grain yield under marginal wheat growing conditions, but has poor bread making quality compared to wheat (Mergoum and Macpherson, 2004). Vital QTL for abiotic stress

tolerance such as root architecture under water stress (Ayalew et al., 2017) and resistance QTL for bacterial leaf blight were identified in triticale populations (Wen et al., 2018).

FHB resistance genes

FHB resistance is a quantitative trait controlled by many QTL, each of which provides only partial resistance and shows strong environmental interaction (Bai and Shanner, 2004). Since 1999, close to 500 resistance QTL have been reported in mapping studies (considering repeatedly reported QTL only) and 104 of them were reported as being major QTL by the authors (Buerstmayr et al., 2019). At least one QTL was reported on all of the hexaploid wheat chromosomes except 7D (Buerstmayr et al., 2009). Recently, a new resistance QTL was found on chromosome 7D (Ren et al. 2018), thus, all 21 chromosomes harbour one or more QTL controlling FHB resistance. After summarizing 52 publications, Buerstmayr et al. (2009) confirmed 22 reliable QTL regions in the A (6), B (11) and D (5) genomes. These are on chromosomes 1B (two regions), 1D, 2A (two regions), 2B (two regions), 2D (two regions), 3A, 3B (two regions), 3D, 4B, 4D, 5A, 5B, 6A, 6B, 7A and 7B (two regions). Among these, seven QTL have formally been assigned a gene name (*Fhb1*, *Fhb2*, *Fhb3*, *Fhb4*, *Fhb5*, *Fhb6* and *Fhb7*) (Catalogue of gene symbols, 2019). Despite the discovery of numerous resistance QTL, FHB resistant germplasm mostly have modest levels of resistance (Bai and Shanner, 2004). Only a few such as ‘Sumai3’ and its derivatives were found to have relatively strong FHB resistance and therefore received extensive attention from breeders and geneticists worldwide (Bai and Shaner, 2004).

Resistance genes identified in the primary gene pool

He et al. (2014) screened Chinese wheat germplasm at CIMMYT and found many of them to be resistant to FHB with lower levels of DON accumulation. Mapping experiments

identified many QTL for increased FHB resistance in CIMMYT cultivars (Buerstmayr et al., 2019). However, the most exploited resistance was from Sumai3 and its derivatives. Many small-effect QTL discovered in local cultivars were not repeatable across breeding programmes. Genome wide association analysis performed on Pacific North West and CIMMYT lines identified many previously mapped and novel QTL associated with FHB index and DON accumulation (Wang et al., 2017). Certain QTL, *Fhb1, Fhb2, Fhb4, Fhb5, Qfhs.ifa-5A, Qfhs.rwg.5A.2, Qfhs.ndsu.-3AS, Qfhb.nau-2B,* and *Qfhb.mgb-2A,* have been fine mapped (reviewed by Buerstmayr et al., 2019; Zhao, 2018) and *Fhb1* and *Qfhb.mgb-2A* were cloned (Gadaleta et al., 2019; Su et al., 2019).

Fhb1 on chromosome arm 3BS from 'Sumai3' was first named and is the most exploited QTL (Cuthbert et al., 2006). *Fhb1* provides resistance to fungal spread (type II resistance; Anderson et al., 2001) and it may be involved in the conversion of DON into the less phytotoxic DON-3-O-glycoside (Lemmens et al., 2005). In high resolution mapping populations segregating for *Fhb1*, this locus was mapped with high precision as a single Mendelian gene within a 1.2 cM interval bordered by wheat markers STS3B-80 and STS3BS-142 (Cuthbert et al., 2006). This region was later reduced to a 261 kb region containing seven putative genes (Liu et al., 2008). Map based cloning suggested that the *Fhb1* resistance is provided by a pore-forming toxin (PFT) like gene at the *Fhb1* locus (Rawat et al., 2016). A later study (Su et al., 2019) found that deletion in the *Fhb1* region encoding TaHRC, a putative histidine-rich calcium-binding protein, induces FHB resistance. Irrespective of its exact identity or the mechanism involved, *Fhb1* reportedly maintain strong and stable resistance across different genetic backgrounds (Anderson et al., 2001, Chen et al., 2006). *Fhb2* on chromosome 6B was mapped to a 2.2 cM region flanked by markers *Gwm133* and *Gwm644* (Cuthbert et al., 2007). *Fhb4*, *Fhb5* and *Qfhb.nau-2B* derived

from ‘Wangshuibai’ and were located on chromosomes 4B, 5A and 2B, respectively, providing type I resistance (Lin et al., 2006; Jia et al., 2018). *Fhb4* was mapped to the 4BL5-0.86-1.00 Chinese Spring deletion bin and fine mapped to a 1.7 cM chromatin interval flanked by *Xhbg226* and *Xgwm149*. *Qfhs.ifa-5A* is another QTL from Sumai3 that maps to 5AS (Buerstmayr et al., 2003). Xue et al. (2011) mapped *Qfhs.ifa-5A* to a 0.3 cM map distance which covered 75% of the 5AS physical length. *Qfhs.ifa-5A* showed stronger resistance to spray inoculation than single spikelet injection (Buerstmayr et al., 2003) and was fine mapped with radiation hybrids as it occurs in a recombination cold-spot close to the centromere (Buerstmayr et al., 2018). Recently, Steiner et al., (2019) fine mapped the region with a near-isogenic recombinant inbred population and concluded that two QTL, 0.3 cM apart, occur at this locus. One QTL (*Qfhb.ifa-5Ac*) mapped across the centromere and the other (*Qfhs.ifa-5AS*) mapped distal to the centromere on 5AS. Although the two QTL intervals were delimited to 0.1 and 0.2 cM respectively, the actual physical regions were large (44.1 and 49.2 Mbp, respectively). *Qfhs.rwg.5A.1* and *Qfhs.rwg.5A-2* were identified in PI277012 close to the *Q* gene and showed both type I and type II resistance (Chu et al., 2011; Zhao et al., 2018). Type I resistance is difficult to assess as it is confounded by type II resistance and the environment (Kolb et al., 2001). *Qfhs.mgb-2A* was identified in durum wheat and was mapped to a 5.3 cM region that included 27 SNP 2AS markers. A wall-associated receptor like kinase (WAK2) was suspected to be responsible for resistance based on expression analysis (Gadaleta et al., 2019). *Fhb3* and *Fhb7* were identified in alien germplasm, *Leymus racemosus* and *Thinopyrum ponticum*, respectively (Qi et al., 2007; Guo et al., 2015).

Of the resistance QTL described above, *Fhb1* shows high levels of stable type II resistance in different genetic backgrounds and is extensively studied and utilized by geneticists and breeders across the globe (Bai et al., 2018). Currently used FHB resistance sources in wheat

breeding programs can mostly be traced to very few parents, namely Sumai3 and its derivatives, Wangshuibai and Wuhan 1 (Bai and Shaner, 2004; Buerstmayr et al., 2009). *Fhb1* is often used in combination with *Qfhs-ifa-5A* in breeding programs (Buerstmayr et al., 2019). Another FHB resistance QTL combination that appears to be highly effective occurs in PI277012, a hexaploid wheat accession with two FHB resistance QTL on chromosome 5A, i.e. *Qfhb.rwg.5A.1* (5AS) and *Qfhb.rwg.5A.2* (5AL) explaining up to 20% and 32% of observed phenotypic variation, respectively (Chu et al., 2011). Along with type I and type II resistance, the two QTL also showed major effects in reducing the percentage of Fusarium damaged kernels and DON accumulation in seeds in both greenhouse and field experiments (Chu et al., 2011).

Many locally adapted cultivars also carry minor, native QTL for FHB resistance (Wang et al., 2017). The individual minor QTL provide much less resistance than that provided by *Fhb1,* but may show additive effects when combined (Cai, 2016). Examples of these cultivars include 'Chokwang' from Korea (Yang et al., 2005), 'Frontana' from Brazil (Mardi et al., 2006), 'Ernie', 'Freedom' and 'Roane' from the USA (Rudd et al., 2001) and 'Renan' and 'Arnia' from Europe (Gervais et al., 2003). FHB resistance has also been reported in many related grass species (Cai et al., 2008) including species of the genus *Thinopyrum* such as *elongatum, ponticum, intermedium,* and *distichum.*

Resistance QTL against DON accumulation

Strict restrictions are imposed on allowable DON levels in grains as they pose considerable health risks to both animals and humans (Wegulo et al., 2008). Some resistance genes lower both FHB symptoms and DON accumulation; however, resistance QTL exclusively for the degradation of DON were reported in multiple studies (Wang et al., 2017; He et al., 2019). Major effect QTL explaining more than 10% of the phenotypic variation for type III

resistance was identified on 2AS, 3BL (Semagn et al., 2007; He et al., 2019) and on 3DL. Minor effect QTL for type III resistance were reported on 3A, 4B, 7A and 7B (Ágnes et al., 2014). Lack of reliable molecular markers and the high cost and time associated with DON analysis impede the use of these QTL in breeding.

FHB resistance genes identified in the secondary and tertiary gene pools

Extensive attempts have been made to identify grass species with high levels of FHB resistance for their potential use in wheat breeding programmes. Resistance QTL *Fhb3, Fhb6* and *Fhb7* are examples of genes that have been identified and successfully transferred to wheat from the alien species *Leymus racemosus* (Wang et al., 1999), *Elymus tsukushiensis* (Cainong et al., 2015) and *Thinopyrum ponticum* (Wang et al., 2020), respectively. *Fhb3* was introgressed onto wheat chromosome 7AS, *Fhb6* onto chromosome 1A (Cainong et al., 2015) and *Fhb7* onto chromosome 7D (Guo et al., 2015). *Fhb3* imparts type II resistance and was transferred to wheat in the form of a compensating translocation T7AL.7Lr#1S (Qi et al., 2007). *Fhb3* was later transferred into winter wheat (Fatima, 2016). *Fhb6* was identified in the perennial, cross-pollinating, hexaploid species *Elymus tsukushiensis*, which is native to China, Korea, and Japan (Weng and Liu, 1989). Wang et al. (1999) produced chromosome addition lines in wheat and recovered a resistant, disomic addition with chromosome 1E of the wild relative. Chromosome engineering was used to replace a wheat chromosome 1AS homoeologous region with corresponding 1E chromatin that harbours *Fhb6*. The compensating translocation (T1AL.1AS-1E^{ts}#1S with *Fhb6*) was released as germplasm line KS14WGRC61 and does not affect agronomic performance (Friebe, 2013). A set of CAPS, SNP and KASP markers useful for marker assisted selection of *Fhb6* were developed (Cainong et al., 2015). Tao (2019) used the latter molecular markers to evaluate the *Fhb6* resistance and incorporate the gene into HRWW

germplasm. Shen et al. (2004) reported that the *Thinopyrum ponticum* - wheat substitution line, $7el_2(7D)$, has FHB resistance that equals that of Sumai3. The gene responsible for the FHB resistance was mapped to a 10.3 cM region towards the telomere of $7el_2$ (Shen and Ohm, 2007). A mapping population was developed from the parents, K11463 ($7el_1(7D)$) and K2620 ($7el_2(7D)$) and used to reduce the chromatin region to which the FHB resistance mapped to 3.7cM (Zhang et al., 2011b). The latter authors designated the gene as *FhbLop. FhbLop* was fine mapped to 1.7cM and renamed *Fhb7* (Guo et al., 2015). *Fhb7* was furthermore pyramided with *Fhb1* and shown to complement *Fhb1* (Guo et al., 2015). With the availability of the E genome sequence, *Fhb7* was cloned and it was concluded that *Thinopyrum elongatum* acquired the resistance through horizontal gene transfer from *Epichloe* species. The *Fhb7* translocation was shown to reduce FHB severity and DON accumulation without any yield penalty (Wang et al., 2020). Novel FHB resistance has also been reported in *Thinopyrum distichum* (Chen et al., 2001). This resistance has not yet been pursued in cereal breeding as a subsequent attempt to develop an addition line in durum wheat was not successful.

Genetics of resistance in wheat

FHB resistance in wheat is complex and quantitative in nature (Wang et al., 2017; Rudd et al., 2001). Different types of resistance are involved and apart from resistance with a physiological basis, morphological traits also have a role. Genotype X environment interaction is very important, and the expression of resistance reflected by FHB index and DON levels are greatly affected by genetic background (Buerstmayr et al., 2019). The complexity of FHB resistance was regarded to being similar to that for grain yield (Campbell and Lipps, 1998). Some resistance QTL are known to have additive effects and pyramiding was shown to be

effective in reducing disease symptoms (Jia et al., 2018); however, some other QTL combinations failed to increase resistance (Fatima, 2016; Tao, 2019).

Studies of the Sumai 3 resistance suggested the presence of two or three genes (including *Fhb1*) imparting two or more types of resistance (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2009). Fine mapping of *Fhb1* revealed its Mendelian nature and a candidate gene which encoded a protein with pore-forming toxin like domain (Rawat et al., 2016). Later, a different candidate gene (TaHRC) was suggested which had a deletion at the start codon that resulted in a defective protein product and was shown to be the reason for *Fhb1* resistance (Su et al., 2019). Uncertainty about the identity of *Fhb1* resulted in marker systems that are based on different putative loci. Heritability of FHB resistance varied greatly with populations and environments. Resistance showed higher heritability ($H > 0.75$) (Buerstmayr et al., 2000) in some studies while some other studies showed it to be a lowly heritable trait (Hall and Van Sanford, 2003). Direct selection for each resistance type is expensive and difficult. So, usually type I and type II resistance are measured as they are well correlated with FDK and DON accumulation (Paul et al., 2005). However, the latter authors concluded that this high correlation holds good only in the absence of type III resistance QTL and when late infection is prevented. Overall, the existence of five types of resistance mechanisms, large fluctuations in heritability and varying correlation between type I resistance, type II resistance and DON accumulation makes the identification, mapping and selection of FHB resistant genotypes extremely difficult.

Breeding for FHB resistance

The success of a resistance breeding program depends on the availability of resistant germplasm, variability created within the breeding population and accuracy in the selection of resistant genotypes to further improve the population (Buerstmayr et al., 2009). FHB resistance

from diverse sources are now available to breeding programmes and tools for the identification and selection of resistant plants are continuously being improved. Durable resistance is needed to ensure that changes in the pathogen population will not completely overcome the resistance established in cultivars and thus exacerbate FHB epidemics. Parents from diversified resistance sources that are without deleterious linkage drag are desirable for use in breeding programmes. So far, *Fhb1* resistance has not shown adverse effects on important agronomic traits and has been utilized in numerous breeding programmes (Li et al., 2019). Traditional breeding methods such as the pedigree and single seed descent methods are frequently used for improving resistance in wheat. The results from backcross breeding is strongly influenced by the genetic background of the recurrent parent. Recurrent selection has proven successful (Jiang et al., 1993) and can be useful to accumulate resistance genes conditioning different types and sources of resistance.

There are limited sources of commercially useful resistance to FHB, inheritance of resistance is often quantitative and complex. Therefore, there is a constant need for evaluating and identifying new sources of resistance in alien germplasm as well as in wheat (Cainong et al., 2015).

Marker assisted selection

Fhb1 is a major QTL responsible for type II resistance; however it was of little use when introduced into certain genetic backgrounds (Buerstmayr et al., 2019). Combining different resistance QTL is of great importance for improving FHB resistance and can be achieved more efficiently with the help of molecular markers. The use of molecular markers as proxy for QTL alleles that improve resistance is referred to as marker assisted selection (MAS). The underlying principle of MAS is the use of a difference in a DNA sequences genetically closely linked to a gene that confers FHB resistance to predict the presence of the resistance in other genotypes

(Anderson, 2007). These differences could for example be due to variation in the number of simple sequence repeat units (SSR), or single base differences (Semagn et al., 2006). The biggest advantage of marker technology is that it can be used to rapidly select for combinations of multiple target genes in a population and this can be done at any stage in the breeding programme, mostly for initial selection. MAS resembles constructing an ideal genotype with the desired building blocks based on marker alleles. However, pyramiding of multiple favourable QTL alleles in a single genotype is difficult and its difficulty increases with increase in QTL number (Poland and Rutkoski, 2016). So, only well-validated, major resistance QTL are normally targeted. Diagnostic markers that have marker alleles that are very unique to the QTL are very useful for MAS (Liu and Anderson, 2003). MAS can reduce time and cost for resistance evaluation and results in higher genetic gain in FHB resistance improvement compared to phenotypic selection when reliable markers are employed (Miedaner et al., 2009). A critical consideration for the use of molecular markers is therefore that their use should be more effective than conventional screening (Anderson, 2007). Although many FHB resistance QTL have been discovered, only few QTL with large effect have reliable marker systems. Marker assisted selection has been used widely to select for *Fhb1* and *Qfhs.ifa-5A* in breeding programmes (Anderson, 2007). However, reliable KASP markers were developed only for *Fhb1* (Su et al., 2018; Singh et al., 2019). Time and cost in phenotyping for FHB resistance caused researchers to depend more on molecular markers and marker assisted selection. Pyramiding of resistance QTL with additive effect helps in achieving higher levels of quantitatively inherited resistance (Buerstmayr et al., 2019).

Marker assisted backcrossing

Pyramiding of resistance genes *Fhb1, Fhb2, Fhb4,* and *Fhb5* in marker-aided backcross schemes drastically reduced disease symptoms (Anderson, 2007; Jia et al., 2018). Marker assisted backcrossing was more efficient when QTL showing additive effects such as *Fhb4* and *Fhb5* were targeted (Jia et al., 2018). Pyramiding of *Fhb1* and *Qfhs.ifa.5A* with the help of molecular markers reduced DON accumulation by 78% and disease severity by 55% in European winter wheat backgrounds (Miedaner et al., 2006). *Fhb1* and *Qfhs.ifa-5A* that were stacked with the use of marker-assisted backcrosses, reduced disease symptoms more than either of the QTL individually in nine European winter wheats backgrounds (Salameh et al., 2011). The same study recovered lines exhibiting good FHB resistance with neither *Fhb1* nor *Qhs.ifa.5A* present. On the contrary, at times lines with both *Fhb1* and *Qfhs.ifa.5A* exhibited FHB resistance comparable to that of lines without either of the QTL implying the importance of genetic background. Recovery of lines with the donor parent level of FHB resistance is extremely difficult (Salameh et al., 2011; Brar et al., 2019). Marker assisted pyramiding of *Fhb3* with *Fhb1* resulted in poor resistance far lower than the resistance of *Fhb1* alone in two winter wheat backgrounds (Fatima, 2016). These results suggest that the interaction of novel resistance QTL in combination with established QTL needs to be evaluated before deployment in a breeding program. Advances in genomic technology and the establishment of regional genotyping centres allow for dense genotyping of breeding material, marker development and marker assisted selection. However, since FHB resistance is conditioned by many small effect QTL along with major QTL, genomic selection could be more efficient than conventional MAS for improving FHB resistance (Buerstmayr et al., 2009, 2019; Bai et al., 2018). Genomic selection is a more encompassing

breeding tool that captures all the markers affecting a phenotype and select the genotypes with all such markers (Bernardo, 2016).

Role of morphological traits in FHB resistance

FHB susceptibility is significantly affected by plant height. Pathogen inoculum that overwintered in crop residue can more easily reach the spikes of shorter plants than those of taller plants thus increasing inoculum pressure on the shorter plants. In addition, higher humidity around the spikes of short statured plants creates more favourable disease conditions (Jenkinson and Parry, 1994; Yan et al., 2011). Although linkage was detected between *Rht1* and some FHB resistance genes, an unfavourable microclimate around the tall spikes rather than physiological responses could have been the reason (Yan et al., 2011). Srinivasachary et al. (2009) concluded that the semi-dwarfing allele *RhtD1-b* increases susceptibility to FHB more than the semi-dwarfing allele *RhtB1-b*. Buerstmayr and Buerstmayr (2016) concluded that the difference in resistance of semi-dwarf genotypes with either *Rht-D1b* or *Rht-B1b* could be related to differences in anther retention. Anther retention was shown to be much less correlated with FHB disease traits despite its strong correlation with plant height (Tessmann and Van Sanford, 2019). Shorter flag leaves and sparse spikes create less favourable conditions and could also reduce disease severity on the spikes (Jones et al., 2018). An association between flowering time and FHB resistance was also shown; however, this could be due to prevailing microclimate conditions around anthesis (escape) and may not be genetically controlled (Buerstmayr et al., 2008). A detailed study of associations between plant morphology and FHB traits by Tessmann and Van Sanford (2019) revealed negative correlation between FHB disease traits and morphological traits like plant height, peduncle length, spike length, spikelet number and yield in soft red winter wheat population. A study done by Buerstmayr et al. (2011) found negative

associations of morphological traits such as plant height, days to anthesis, spike density, spike length and glaucousness with FHB resistance QTL derived from *Triticum macha* (Georgian spelt wheat) in an advanced backcross population.

Study objectives

The first objective of this study was to transfer the FHB resistance gene *Qfhb.rwg-5A.2* from spring wheat to winter wheat breeding material utilizing marker-assisted selection that was based on SNP haplotypes. This objective is addressed in Chapter III of the study.

The second study objective was to continue to characterize FHB resistance that was previously reported in certain triticale-*Th. distichum* hybrid progenies (single chromosome addition lines and secondary backcross hybrids). Chapter IV of this study describes and summarizes these results.

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CHAPTER III. TRANSFER OF FHB RESISTANCE QTL *QFHB.RWG.5A.2* FROM HARD RED SPRING WHEAT (GP80) TO HARD RED WINTER WHEAT

Abstract

Fusarium head blight (FHB) is a fungal disease of small grains that attacks spikes after flowering. The disease is of global importance and can cause substantial loss of grain yield and quality. The complexity of disease infection, host resistance and the plant-pathogen interaction compounds resistance breeding. Resistance is conditioned by numerous small effect QTL that are strongly affected by the environment and genetic background. However, there is general consensus that genetic resistance needs to be the cornerstone of an integrated disease management strategy. Resistance QTL *Fhb1* and *Qfhs.ifa.5A* from Sumai-3 spring wheat, and *Qfhb.rwg.5A.1* and *Qfhb.rwg.5A.2* from wheat accession PI277012, are among the most promising of the larger-effect QTL described in published mapping and breeding experiments. *Fhb1*, *Qfhs.ifa-5A* and *Qfhb.rwg.5A.1* have already been incorporated into hard-red winter wheat (HRWW) germplasm at NDSU. To also transfer *Qfhb.rwg.5A.2*, crosses were made with a hard HRSW donor line (GP80 = Pedigree: PI277012/Grandin) that has the gene, and backcrosses were conducted to HRWW. Three different B_2F_1 populations were developed and all parents and B_2F_1 were genotyped with the Illumina 90K SNP platform. Genotyping data were analysed to identify SNP haplotypes that corresponded to PI277012 chromosome 5A segments. B_2F_2 plants from each B_2F_1 family were then tested in a greenhouse for type II FHB resistance. The combined marker and FHB resistance data showed that the backcross which employed HRWW line ND Noreen as final backcross parent included the most resistant plants. Two F_2 plants from 19M13-67 of this group that appeared to be homozygous for both *Qfhb.rwg.5A.1* and *Qfhb.rwg.5A.2* were used in a further backcross to ND Noreen. The more resistant (disease

severity less than 40%) B_2F_2 of all three populations were identified and the B_2F_3 was field planted for continued evaluation and selection in the HRWW breeding program.

Introduction

FHB resistance is a complex, quantitative trait and its expression is strongly influenced by the environment and genetic background (Schroeder and Christensen, 1963; Buerstmayr et al., 2011). Approximately 500 FHB resistance QTL have been mapped and 104 of these were described as major QTL by the authors. The latter QTL were present on almost all of the wheat chromosomes except on 7D (Liu et al., 2009; Buerstmayr et al., 2019). Despite the many reports, *Fhb1* on chromosome arm 3BS, *Fhb2* on 6BS and *Qfhs.ifa.5AS* on 5A were the only QTL that were utilized in breeding programs around the world (Anderson, 2007; Bai et al., 2018; Brar et al., 2019; Buerstmayr et al., 2019). Mapping experiments conducted by Waldron et al. (1999) and Anderson et al. (2001) first identified the major resistance QTL, *Fhb1,* on chromosome 3BS of Sumai 3 and lines derived from it. *Fhb1* was shown to reduce FHB symptoms by 20-25% on average in different genetic backgrounds (Anderson, 2007). Consequently, Chinese cultivar 'Sumai 3' and its derivatives have most often been used as sources for FHB resistance breeding (Buerstmayr et al., 2009). A KASP marker (Bai et al., 2018) was developed for marker-aided selection and pyramiding of *Fhb1* in breeding applications.

PI277012 was identified as a promising new source of resistance that harboured resistance QTL on chromosome arms 5AS and 5AL (Chu et al., 2011). The PI277012 resistance genes were mapped utilizing SSR markers to characterize a doubled haploid population obtained from the cross PI277012/Grandin. Of the two QTL, *Qfhb.rwg.5A.2* on 5AL was the major QTL explaining 32% of phenotypic variation and it was mapped between the SSR markers *Xwmc 470* and *Xbarc 48*. This QTL was weakly correlated with plant height. *Qfhb.rwg-5A.1* was mapped

on 5AS between the markers *Xcfa 2104* and *Xgwm 617*. The two QTL showed strong type I and type II resistance along with resistance to DON accumulation (Chu et al., 2011). *Qfhb.rwg.5A.1* was mapped to the same position as *Fhb5* and explained up to 20% of phenotypic variation in some studies (Buerstmayr et al., 2009; Liu et al., 2009). A study conducted by Tao (2019) suggested that *Qfhs.ifa-5A* and *Qfhb.rwg.5A.1* could be alleles of the same locus with similar effects on the overall resistance phenotype. Resistance QTL were also discovered in the approximate location of *Qfhb.rwg-5A.2* in European cultivars but explained much less than 32% of the phenotypic variation (Gervais et al., 2003; Buerstmayr et al., 2009; Chu et al., 2011). *Qfhb.rwg-5A.2* in PI277012 is believed to be closely linked (in repulsion phase) with the *q* allele (non-free-threshing) at the domestication locus *Q*. Its location raised the possibility that the reported 5AL resistance could actually be a pleiotropic effect of the *q* allele. However, in the doubled haploid line, GP80, the *Q*-allele appears to occur in coupling phase with *Qfhb.rwg-5A.2*, which suggests that the 5AL FHB resistance is not a pseudo-resistance effect induced by the hard spikes of PI277012 (Chu et al., 2008). The chromosome 5A region with *Q* allele for easy thresh-ability is physically large, and reported to affect yield and yield attributes positively (Xie et al., 2018). Zhao (2018) fine mapped *Qfhb.rwg-5A.2* to a 1.09-Mbp genomic region in the PI277012/Grandin RIL population using SNP, SSR, CAPS and STRAP markers. However, markers suitable for marker-assisted selection (MAS) were not developed, which was likely due to low recombination and low polymorphism in the QTL region.

A hard-red spring wheat line, RWG21 (pedigree = Russ 2*/PI277012); believed to have both *Qfhb.rwg-5A.1* (5AS) and *Qfhb.rwg-5A.2* (5AL), was obtained (2014) by the NDSU HRWW breeding program from Dr S. Xu (USDA, ARS, Fargo). Following crosses to winter wheat, the line Novus-4 (= RWG21/Jerry) was selected from a large population of doubled

haploids and single seed descent progenies from this cross. Novus-4 has a winter growth habit and has intermediate to good winter-hardiness; however, it has been found to carry only *Qfhb.rwg-5A.1* but to lack *Qfhb.rwg-5A.2* (which was subsequently found to also be absent from RWG21).

The present study aimed to transfer *Qfhb.rwg-5A.2* to HRWW. To achieve this, a different source of the PI277012 genes, GP80 (= PI277012/Grandin), was used. GP80 is believed to have both of the PI277012 resistance QTL and was obtained from Dr Xu in 2017. It is also believed that GP80 has the *Q*-allele of the domestication locus that occurs in regular common wheat and which results in easy thresh-ability. In PI277012, *Qfhb.rwg-5A.2* is said to be closely linked to the *q*-allele that causes tough bracts, making the material difficult to thresh. Since the earlier introgression attempt was complicated by lack of suitable markers in the QTL region, it was decided to employ SNP haplotypes for MAS in the renewed attempt rather than to rely on individual SSR markers.

Material and methods

General outline of the study

The wheat genotypes used in the study are listed in Table 2.1. An outline of the crosses and backcrosses made to winter wheat and the research plan is given in Fig. 2.1 (B_1F_1: 18M6 seeds were available at the onset of the study). The B_1F_1: 18M6 (= GP80/Novus-4//Monument) and parental lines (GP80, Novus-4, and Monument) were initially tested with the simple sequence repeat (SSR) markers Barc186 and Gwm304 (closely linked to *Qfhb.rwg-5A.1*; Chu et al., 2011 and Tao 2019) and Gpw2136, Gpw2181, and Gpw2172 (linked to *Qfhb.rwg-5A.2*; Chu et al., 2011 and Tao 2019) to confirm the presence of useful marker polymorphisms and the likely presence of *Qfhb.rwg-5A.2*. Three B_1F_1 plants, believed to have *Qfhb.rwg-5A.1* and

Qfhb.rwg-5A.2, were selected with the help of SSR markers. Each selection was crossed with one or more of the winter wheats ND Noreen, Monument and 18Nord-114 to generate three B_2F_1 populations. B_2F_1:19M13 (= GP80/ Novus-4// Monument/3/ ND Noreen) consisted of 22 plants; B_2F_1:19M14 (= GP80/ Novus-4// *2 Monument) consisted of 21 plants, and B_2F_1:19M15 (= GP80/ Novus-4// Monument/3/ 18Nord-114) consisted of 26 plants. The 69 B_2F_1 plants together with the parents and controls were genotyped with the Illumina Infinium iSelect 90K SNP platform (Wang et al., 2014). For each of the 69 B_2F_1 families, B_2F_2 seeds were harvested. Twenty to thirty B_2F_2 seeds per family as well as the parents and two non-critical controls were evaluated for FHB type II resistance in a greenhouse experiment. Controls Jerry and CM82036 were not used in any crosses but their resistance phenotypes were used for comparison.

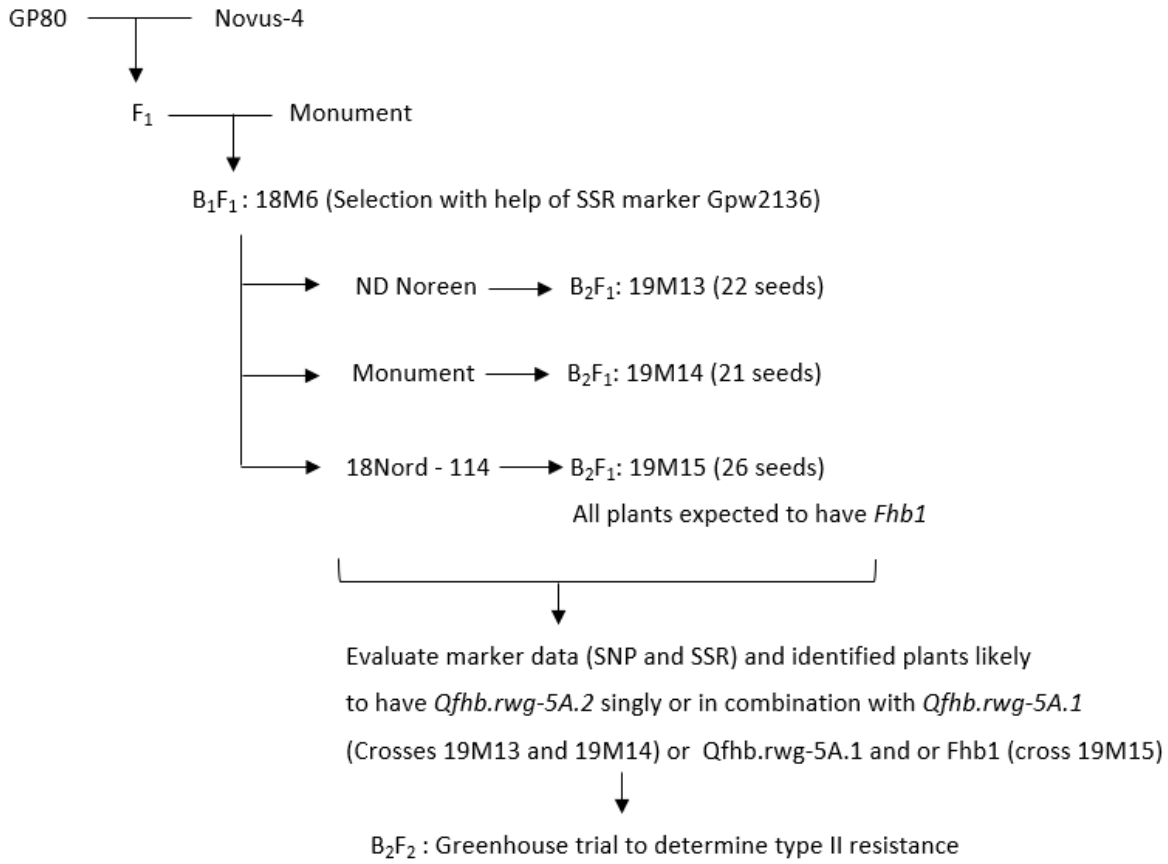

Figure 2.1. Outline of the experiment.

Table 2.1. Wheat cultivars and breeding lines employed in the study.

Genotype	Pedigree	Description
PI277012		HRSW source of *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*
RWG21	Russ 2*/PI277012	HRSW
Grandin		HRSW
GP80[1]	PI277012/Grandin	HRSW doubled haploid line with *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*. It also has the dominant allele *Q* (improved thresh-ability) at the domestication locus, *Q*.
Novus-4	RWG21/Jerry	A HRWW selection derived through single seed descent inbreeding and believed to have *Qfhb.rwg-5A.1*[2].
Monument		A HRWW cultivar released by Syngenta[3]
ND Noreen		A HRWW cultivar released by NDSU
18Nord-114		A HRWW inbred line (NDSU) with *Fhb1*
14K456-K-1		A HRWW inbred line (NDSU) with *Fhb1* and *Qfhs.ifa-5A* (control)
14K456-1-35F-9		A HRWW inbred line (NDSU) with *Fhb1* and *Qfhs.ifa-5A* (control)
Accipiter		A Canadian HRWW cultivar[4] (control)

[1] Developed and provided by Dr S Xu [Cereal Crops Research Center, USDA-ARS, Fargo, ND]
[2] Tao, H., 2019. Transfer of Fusarium head blight resistance to hard red winter wheat. North Dakota State University
[3] https://agriprowheat.com/variety/sy-monument
[4] https://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/whe/app00007416e.shtml

Molecular marker analyses

The SSR marker analyses were done in the HRWW laboratory in the Plant Sciences department, Loftsgard Hall, NDSU campus. Seedling leaves from B_1F_1:18M6 plants were cut and used for DNA extraction following a modification of the Triticarte Pty. Ltd (http://www.triticarte.com.au/) protocol. Quality and concentration of extracted DNA were checked using agarose gel electrophoresis and staining with ethidium bromide. DNA concentration was adjusted to 10 ng/µl before using it in polymerase chain reaction (PCR) reactions. The marker primer sequences and PCR conditions for the markers that were employed are available in the Graingenes website (http://www.wheat.pw.usda.gov).

The 69 B_2F_1 plants that resulted from crosses 19M13, 19M14 and 19M15, were again used for marker analyses and selection of marker-predicted resistant progeny. Leaves were cut on B_2F_1 plants and parental controls for DNA extraction and were genotyped with the Illumina iSelect 90K SNP array at the USDA-ARS Biosciences Research Lab in Fargo, North Dakota, USA (https://www.ars.usda.gov/plains-area/fargo-nd/etsarc). The same set of DNA samples was also tested for polymorphism with respect to additional loci that occur in the same chromosome region as *Qfhb.rwg-5A.1* (*Xbarc186*) and *Qfhb.rwg-5A.2* (*Xgpw2136, Xgwm179* and *Xgwm126*). The Wheat_2014_90K SNP consensus map served as reference to select chromosome 5A SNP markers. SNPs were clustered using manual option of GenomeStudio 2.0 with polyploid clustering module (https://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html). SNPs with a GenTrain score more than 90% were selected and exported to MS-EXCEL. PI277012 and Grandin polymorphic SNPs were used to identify PI277012 and Grandin derived chromatin on GP80 chromosome 5A based on the 90K

consensus map of Wang et al. (2014). Further, polymorphic SNPs were identified for each backcross population and used to characterize the recombinants.

Screening for type II FHB resistance under greenhouse conditions

Twenty to thirty B_2F_2 from each B_2F_1 family along with the parents involved, and controls Jerry and CM82036 (Table 2.1) were used for a greenhouse FHB trial. Five to six plants of each entry were spaced in a 6" plastic pot. Five pots with 5-6 seeds were planted for each entry in a completely randomized trial layout. Three to five spikes per plant were inoculated with FHB spores.

The single spikelet injection method was used for inoculating a single central spikelet per spike at anthesis in a greenhouse (Stack, 1989). A mixture of *Fusarium graminearum* isolates (*Fg*08_13, *Fg*10_135_5, *Fg*10_124_1 and *Fg*13_79) was provided by Dr. Shaobin Zhong (Department of Plant Pathology, North Dakota State University). An approximately 10 µl-droplet containing the isolate mix (approximately 100,000 conidia per ml) was injected into a floret in the middle of the spike. Inoculated spikes were covered with a moist plastic bag immediately following inoculation and left for 48-72 hours. The temperature of the greenhouse was increased to 72 – 76 ^{o}F after the first inoculation. Infection severity was calculated by manually counting the total number of spikelets and number of infected spikelets per spike at 21-24 days after inoculation.

Statistical analyses

JMP Pro15 (https://www.jmp.com/en_us/software/new-release/new-in-jmp-and-jmp-pro.html) and RStudio 1.3.1073 (https://npackd.appspot.com/p/rstudio/1.3.1073) were used for performing statistical analysis and generating graphs.

Results and discussion

SSR marker results

SSR markers linked to *Qfhb.rwg-5A.1* (Barc186, Gwm304 and Gmw293) and *Qfhb.rwg-5A.2* (Gpw2136, Gpw2172 and Gpw2181) were tested on the genotype panel listed in Table 2.1 plus 14 F_1: 18M6 (= GP80/Novus-4//Monument) plants. Only markers Barc186 (Fig. 2.2) and Gpw2136 (Fig. 2.3) showed useful polymorphisms. With respect to Barc186; GP80, PI277012 and Novus-4, all of which are believed to have *Qfhb.rwg-5A.1*, had the smaller (± 200 bp) of two critical bands, named band 2 in Fig. 2.2. The remaining parents and controls had the slightly larger band (band 1; ± 210 bp). Since both GP80 and Novus-4 have *Qfhb.rwg-5A.1* whereas Monument does not, all of the F_1: 18M6 would have been heterozygous for this QTL. While the Barc186 bands in the F_1 heterozygotes (Fig. 2.2) were not sharply distinct, the F_1 plants were identifiable as heterozygotes. Marker Gpw2136, on the other hand produced very clear polymorphisms (Fig. 2.3). PI277012 and GP80 produced a characteristically smaller band (± 190 bp; band 2) than all the other genotypes. The winter wheat parents of cross 18M6 (Monument and Novus-4) produced an intermediate sized band (band 1 of ± 202 bp). Among the B_1F_1:18M6, five plants were heterozygous for *Gpw2136* bands 1 and 2 and were therefore likely *Qfhb.rwg-5A.2* heterozygotes. The same five plants were also clear heterozygotes for the *Barc186* marker (*Qfhb.rwg-5A.1*) loci. The five selected dihybrid plants were transferred to a greenhouse and three plants that had the better agrotypes were used for crosses with ND Noreen, Monument and 18Nord114 to produce respectively, 22, 21 and 26 hybrid seeds. The 69 F_1 seeds were then grown and samples were cut for 90K SNP genotyping.

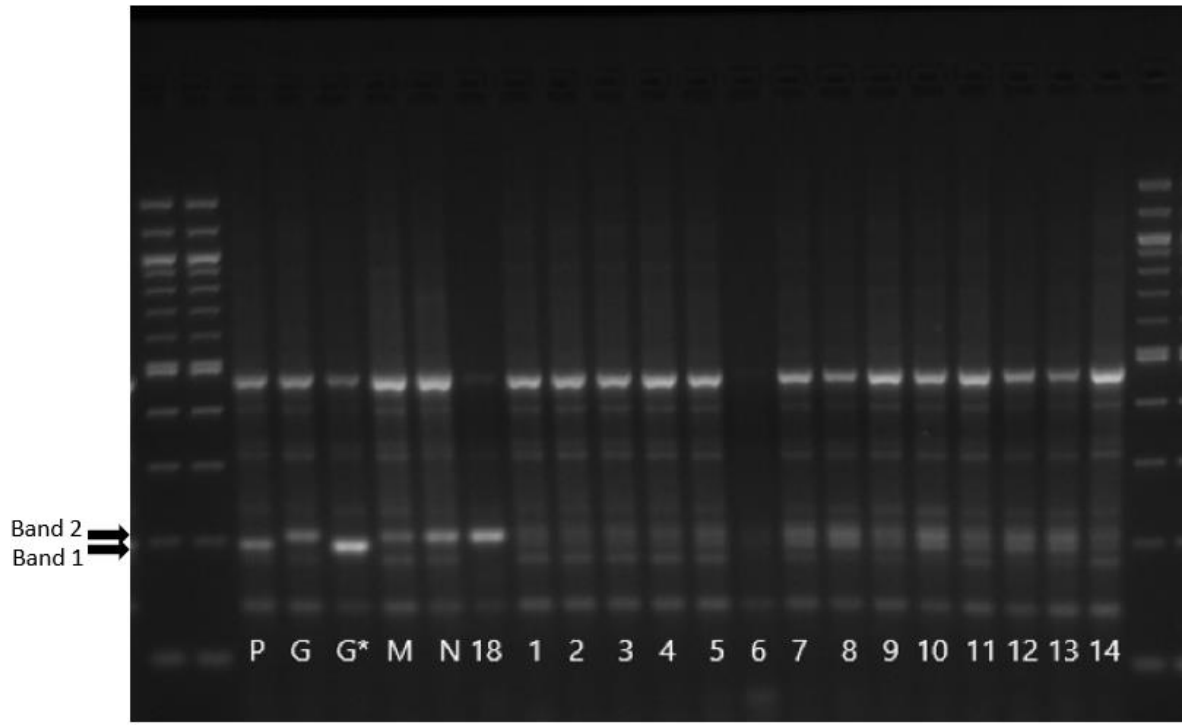

Figure 2.2. Agarose gel showing Barc186 marker polymorphisms used for the detection of FHB resistance QTL Qfhb.rwg.5A.1 among parents, controls and B1F1:18M6 plants. P = PI277012, G = Grandin, G* = GP80, M = Monument, N = ND Noreen, 18 = 18Nord-114, and 1-14 = Individual B1F1:18M6 plants and C = control. The critical polymorphic bands (approximate sizes 201bp and 198 bp) are indicated with arrows and the PI277012 polymorphism is designated as band 2. Genotypes G, P and N are homozygous for the presence of band 2. All of the F1 (1-14) are heterozygous band 1, band 2.

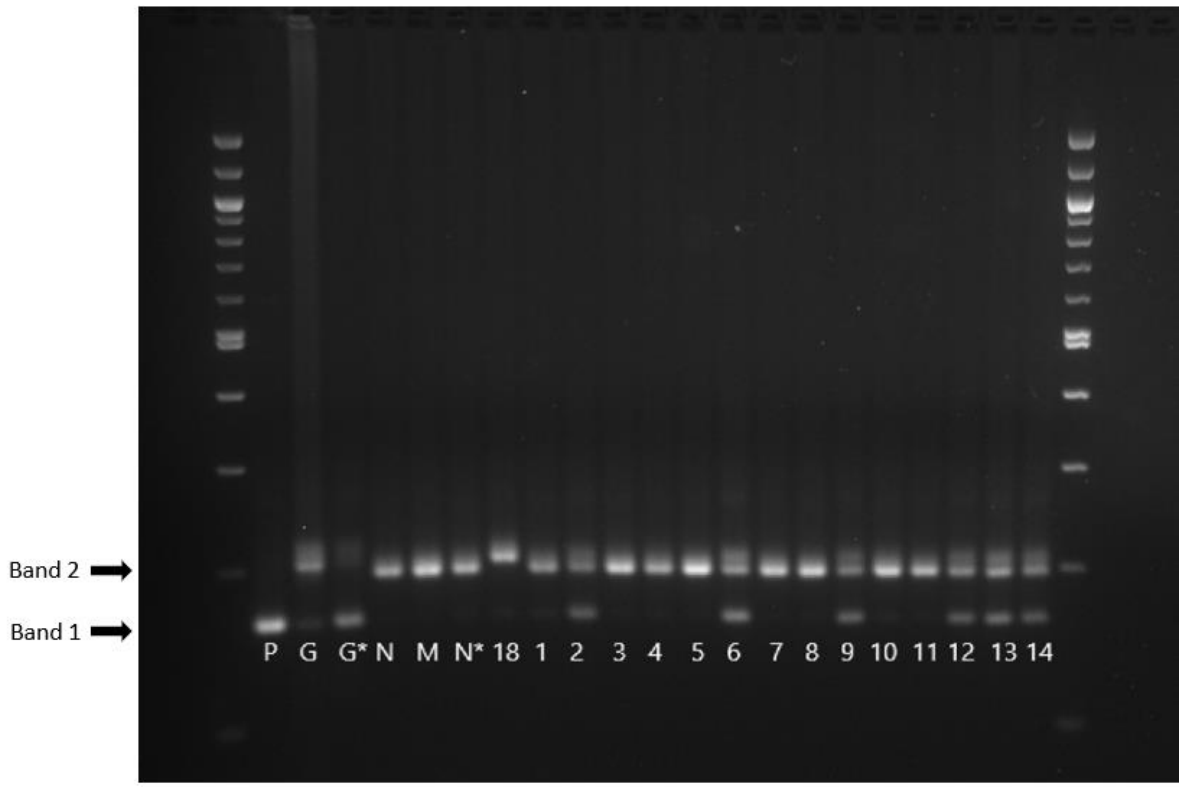

Figure 2.3. Agarose gel showing Gpw2136 marker polymorphisms used for the detection of FHB resistance QTL *Qfhb.rwg.5A.2* among the parents and progeny of B_1F_1:18M6. P = PI277012, G = Grandin, G* = GP80, N = Novus-4, M = Monument, N* = ND Noreen, 18 = 18Nord-114, and 1-14 = Individual B_1F_1:18M6 plants. The critical polymorphic bands (smallest190bp) are indicated with arrows and the PI277012 polymorphism was designated as band 1 B_1F_1 18M6 plants 2, 6, 9, 13 and 14 are heterozygous for the diagnostic bands.

SNP marker results

Only SNPs that have previously been mapped to chromosome 5A (Wang et al., 2014) were manually curated using GenomeStudio 2.0. Chromosome 5A markers that proved to be polymorphic were then exported to Excel. Two hundred and twenty-eight polymorphic SNPs were identified on chromosome 5A spanning the region between 8.12 and 148.3 cM (the length of the published map is 148.3 cM). However, only 118 markers were found to be polymorphic between PI277012 and Grandin, and were therefore used to construct a chromosome 5A map of the doubled haploid GP80. These 118 loci on this smaller map are presented in Fig. 2.4. It appeared that chromosome 5A of GP80 contains primarily PI277012 chromatin with a smaller

intercalary region of Grandin derived chromatin. This intercalary region (Region II, Fig. 2.4) was detected in the sequence of markers starting from 67424 (83 cM) through 76124 (101.2 cM). Two markers (75812, 9970 at 98.7 cM) within region II showed the PI277012 polymorphisms. Rather than being the result of two crossovers that occurred within very close proximity, the above two markers are probably incorrectly mapped. Another two markers (Grandin polymorphisms) occurred at 19.9 cM within region I that derives from PI277012 and are similarly believed to be incorrectly assigned. Since GP80 reportedly has the dominant allele for thresh-ability at the *Q* locus, the result suggests that the *Q* locus is contained within the region of Grandin-derived chromatin. Microsatellite marker results obtained by Tao (2019) similarly suggested that *Qfhb.rwg-5A.2* occurs in the PI277012-derived 5AL distal region (region III in Figure. 2.4.) which also harbours microsatellite markers Gpw2136, Gpw2172, Gwm179 and Gwm126. Novus-4 and RWG21 derive from a backcross of PI277012 to spring wheat cultivar Russ. Based on the present data, Novus-4 and RWG21 (both lack *Qfhb.rwg-5A.2*) retained the PI277012 SNP alleles at the very distal 5AL chromosome end (map positions 141.7 cM and 148.3 cM) which would suggest that *Qfhb.rwg-5A.2* is located proximally to this region.

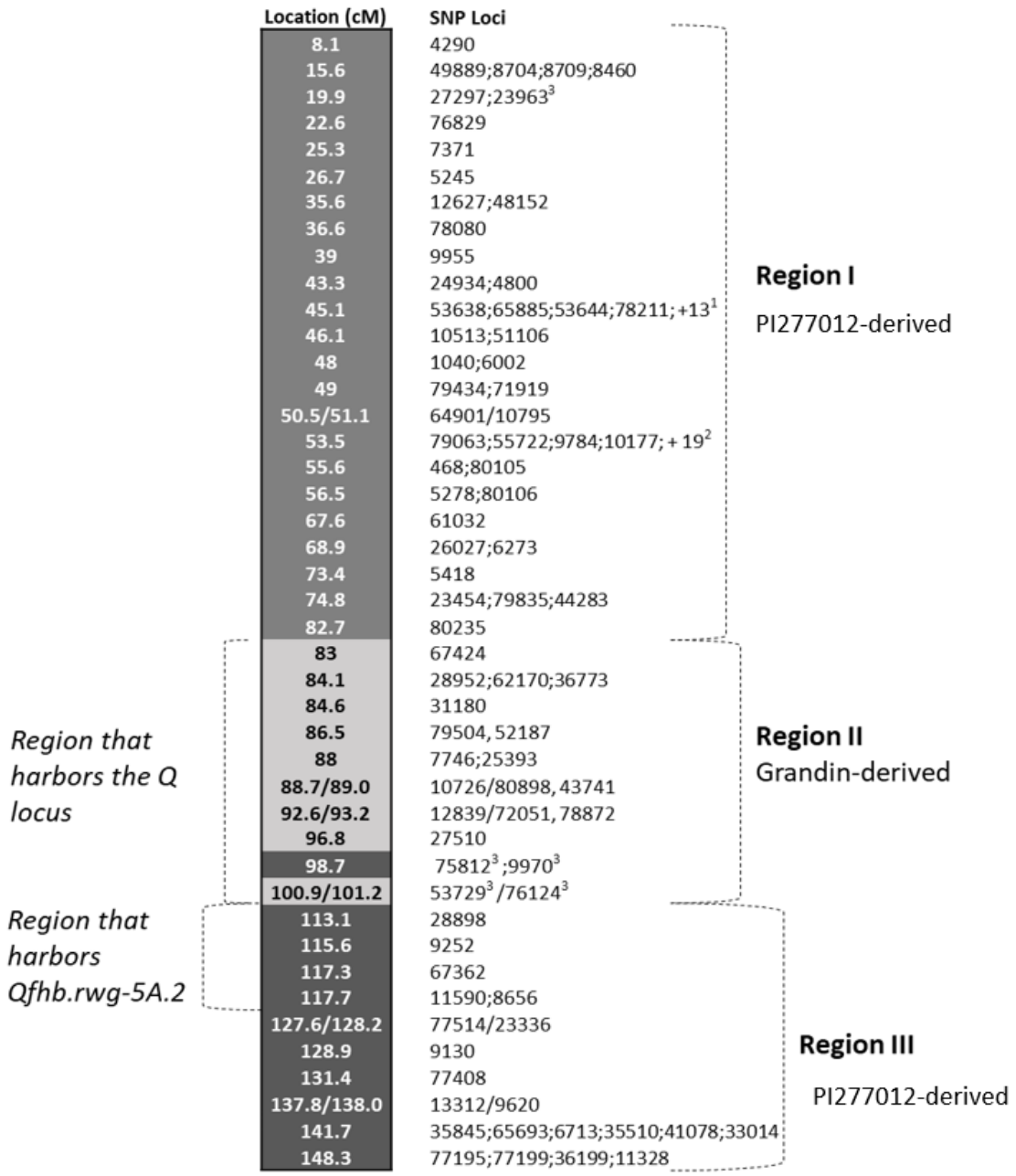

Figure 2.4. A haplotype map of GP80 chromosome 5A showing the genetic map positions. SNP polymorphisms that derived from PI277012 (indicated in dark grey) and positions where the SNP polymorphisms derived from Grandin (light grey) were detected. The genetic map positions of the SNP markers were obtained from wheat 90K consensus map (Wang et al., 2014). The locations of the *Q* and *Qfhb.rwg.5A.2* loci are based on the results of Chu et al. (2011) and Tao (2019).

[1] 13 more SNP loci mapped to the 45.1 cM position (53637; 65662; 9723; 9138; 9139; 53640; 79442; 74436; 72022; 8862; 98790; 71584; 51874)

[2]19SNP loci mapped at 53.5 cM (10055; 28986; 10427; 66880; 61929; 60075; 77049; 81033; 42774; 11967; 79916; 49769; 12016; 7125; 71630; 29241; 42775; 3647; 78593).
[3]Marker 23963 produced the Grandin polymorphism yet mapped to region I; whereas the positions of markers 75812 and 9970 (Region III) appears to be incorrect relative the positions of markers 53729 and 76124 (region III). These small inconsistencies did not affect the overall conclusions.

Since it appears that *Qfhb.rwg-5A.2* occurs within region III of GP80 chromosome 5A, and proximal to 141.7 cM, it is appropriate to use polymorphic SNP loci from this region for deriving haplotypes for MAS. In order to find SNP haplotypes for each of populations 19M13, 19M14 and 19M15; loci were identified that were polymorphic for the GP80-derived genotype versus the respective recipient winter wheat backgrounds (Novus-4, Monument and ND Noreen for F_1: 19M13; Novus-4 and Monument for F_1: 19M14 and Novus-4, Novus-4, Monument and 18Nord-114 for F_1: 19M15). Suitably polymorphic SNPs occurred at ten map locations within the 113.1 cM to 141.7 cM region. The SNP data obtained with regard to the parents and F_1 at the ten "haplotyping" loci are summarized in Table 2.2 and shows the haplotypes encountered in each population. The results obtained with the haplotyping loci were comparable to testcross results and each locus that was dihybrid for GP80 and winter wheat alleles, segregated into approximately 50% heterozygotes and 50% homozygotes in each B_2F_1 group. In the three respective B_2F_1 populations (19M13, 19M14 and 19M15), three, four and five distinct region III haplotypes were identified (Table 2.2). The 69 B_2F_1 were also tested with both Barc186 and Gpw2136 for determining which plants were likely to have *Qfhb.rwg-5A.1*, *Qfhb.rwg-5A.2*, or both. These results are included in Table 2.2. Two additional SSR markers, Gwm179 and Gwm126, located distally from *Xgpw2136* in region III (Tao, 2019), were also evaluated; however, only Gpw2136 proved to be useful in this study. Comparison of the absence/presence patterns of GP80 and winter wheat-derived chromatin across the three crosses suggested that *Xgpw2136* is likely located within a 20 cM region in between 117.7 – 137.9 cM.

Table 2.2. Summary of SNP and SSR marker results, and mean disease severities of B_2F_2 families in each population.

							Map position (cM) and locus[1]										
Parents/B_2F_2 family	**FHB Severity**						**113.1**	**115.6**	**117.7**		**137.9**	**138**	**141.7**	**141.7**	**141.7**	**141.7**	**141.7**
	Xbarc186[2]	**Plants**	**Lowest**	**Highest**	**Mean**	**Rec**[3]	**28898**	**9252**	**11590**	**Xgpw2136**[5]	**13312**	**9620**	**35845**	**65693**	**6713**	**35510**	**41078**
CM82036	2	24			0.50		AA	AA	AA	1	AA	BB	AA	BB	BB	BB	BB
PI277012	2	22	0.07	0.45	0.24		BB	BB	AA	2	BB	BB	AA	BB	BB	BB	BB
Grandin	1	29	0.82	1	0.99		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
RWG21	2	24	0.83	1	0.95		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
Jerry	1	22	0.7	1	0.86		AA	BB	AA	1	AA	AA	BB	AA	AA	AA	AA
GP-80	2	23	0.17	0.71	0.42		BB	BB	AA	2	BB	BB	AA	BB	BB	BB	BB
Novus-4	2	21	0.43	0.8	0.58		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
Monument	1	21	0.9	1	0.97		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
ND Noreen	1	20	0.6	1	0.76		AA	BB[6]	AA[6]	1	AA	AA	BB	AA	AA	AA	AA
18Nord-114	1	21	0.66	1	0.94		AA	BB[6]	BB	1	AA	AA	BB	AA	AA	AA	AA
19M13-55	1	24	0.43	1	0.92		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-65	1	21	0.31	1	0.83		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-66	1	24	0.46	1	0.87		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-70	1	20	0.23	0.84	0.53		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-71	**1**	26	0.28	1	0.83		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-73	1	27	0.25	1	0.74	0.79	AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-56	1,2	24	0.33	1	0.76		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-59	1,2	24	0.68	1	0.94		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-61	1,2	27	0.4	1	0.72		AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-69	1,2	27	0.44	1	0.81		AA	AB	AB	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-74	1,2	22	0.43	1	0.76	0.80	AA	AB	AB	1	AA	AA	BB	AA	AA	AA	AA
19M13-60	1	26	0.26	0.8	0.49		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-68	1	22	0.34	1	0.65		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-75	1	23	0.36	1	0.77		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-76	1	24	0.23	1	0.73	0.66	AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-57	1,2	26	0.3	1	0.74		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-58	1,2	26	0.17	1	0.59		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-62	1,2	30	0.37	1	0.78		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA

Table 2.2. Summary of SNP and SSR marker results, and mean disease severities of B_2F_2 families in each population. (continued)

							Map position (cM) and locus[1]										
Parents/B_2F_2 family	**FHB Severity**						**113.1**	**115.6**	**117.7**		**137.9**	**138**	**141.7**	**141.7**	**141.7**	**141.7**	**141.7**
	Xbarc186[2]	**Plants**	**Lowest**	**Highest**	**Mean**	**Rec**[3]	**28898**	**9252**	**11590**	**Xgpw2136**[5]	**13312**	**9620**	**35845**	**65693**	**6713**	**35510**	**41078**
19M13-63	1,2	26	0.32	1	0.77		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-64	1,2	24	0.29	1	0.76		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-67[7]	1,2	23	0.21	1	0.77		AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
19M13-72	1,2	22	0.26	1	0.69	0.73	AB	BB	AA	1,2	AA	AA	BB	AA	AA	AA	AA
Mean					**0.75**												
19M14-77	1	24	0.67	1	0.94		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-87	1	28	0.44	1	0.79		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-81	1	22	0.38	1	0.86	0.86	AA	AA	BB	1	AB	AB	BB	AA	AA	AA	AA
19M14-82	1,2	24	0.24	1	0.77		AA	AA	BB	1	AB	AB	BB	AA	AA	AA	AA
19M14-79	1,2	23	0.51	1	0.92		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-92	1,2	22	0.64	1	0.88		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-93	1,2	29	0.5	1	0.81		AA	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-86	1,2	25	0.35	1	0.79	0.83	AB	AA	BB	1	AA	AA	BB	AA	AA	AA	AA
19M14-78	1	28	0.26	1	0.87		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-84	1	21	0.7	1	0.91		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-89	1	24	0.27	1	0.87		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-94	**1**	21	0.39	1	0.90		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-95	1	26	0.71	1	0.91		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-98	1	24	0.65	1	0.90	0.89	AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-83	1,2	25	0.53	1	0.87		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-85	1,2	28	0.32	1	0.76		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-88	1,2	23	0.43	1	0.93		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-90	1,2	22	0.46	1	0.86		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-91	1,2	26	0.35	1	0.80		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-96	1,2	24	0.38	1	0.87		AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
19M14-97	1,2	25	0.2	1	0.80	0.84	AB	AB	AB	1,2	AB	AB	BB	AA	AA	AA	AA
Mean					**0.85**												

Table 2.2. Summary of SNP and SSR marker results, and mean disease severities of B_2F_2 families in each population. (continued)

							Map position (cM) and locus[1]										
Parents/B_2F_2 family	**FHB Severity**						**113.1**	**115.6**	**117.7**		**137.9**	**138**	**141.7**	**141.7**	**141.7**	**141.7**	**141.7**
	***Xbarc186*[2]**	**Plants**	**Lowest**	**Highest**	**Mean**	**Rec[3]**	**28898**	**9252**	**11590**	**Xgpw2136[5]**	**13312**	**9620**	**35845**	**65693**	**6713**	**35510**	**41078**
19M15-99	1	21	0.53	1	0.84		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-101	1	19	0.37	1	0.80		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-104	1	26	0.42	1	0.79		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-107	1	30	0.14	1	0.62		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-110	1	21	0.65	1	0.89		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-111	1	28	0.31	1	0.76		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-115	1	25	0.85	1	0.96		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-116	1	22	0.69	1	0.94		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-117	1	27	0.76	1	0.90		AA	AB	BB	1	AA	AA	BB	AA	AA	AA	AA
19M15-112	1,2	22	0.39	1	0.87	0.84	AA	AB	BB	1,2	AA	AA	BB	AA	AA	AA	AA
19M15-109	1	25	0.26	1	0.73		AA	AB	BB	1	AB	AB	AB	AB	AB	AB	AB
19M15-119	1	25	0.6	1	0.89		AA	AB	BB	1	AB	AB	AB	AB	AB	AB	AB
19M15-122	1	26	0.26	1	0.89		AA	AB	BB	1	AB	AB	AB	AB	AB	AB	AB
19M15-124	1	20	0.4	1	0.69	0.80	AA	AB	BB	1	AB	AB	AB	AB	AB	AB	AB
19M15-103	1,2	27	0.26	1	0.70		AB	BB	AB	1,2	AA	AA	BB	AA	AA	AA	AA
19M15-106	1,2	25	0.49	1	0.90		AB	BB	AB	1,2	AA	AA	BB	AA	AA	AA	AA
19M15-121	1,2	28	0.33	1	0.70	0.77	AB	BB	AB	1,2	AA	AA	BB	AA	AA	AA	AA
19M15-100	1,2	22	0.37	1	0.77		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-102	1,2	27	0.32	1	0.84		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-105	1,2	26	0.12	1	0.69		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-113	1,2	24	0.29	1	0.77		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-114	1,2	23	0.22	1	0.71		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-118	1,2	24	0.33	1	0.82		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-120	1,2	26	0.17	1	0.69		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-123	1,2	25	0.54	1	0.89		AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
19M15-52	1,2	27	0.37	1	0.71	0.77	AB	BB	AB	1,2	AB	AB	AB	AB	AB	AB	AB
Mean					**0.80**												

[1] Locus positions are as provided by Wang et al., (2014). Recombinants within the region containing *Qfhb.rwg-5A.2* were grouped for each cross with dark grey indicating the presence of GP80-derived chromatin and light grey indicating the presence of winter wheat parent derived chromatin.
[2] *Xbarc186* was used as marker for detecting the presence of *Qfhb.rwg-5A.1*
[3] Average of a subgroup of recombinants, ordered according to the likely presence of *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*
[4] *Qfhb.rwg-5A.2* was mapped to the region in between markers 53729 (100.9 cM) and 11590 (117.7 cM) by Zhao et al. (2018). Here, no suitably polymorphic markers were found in the 100.9 to 113.1 cM interval
[5] *Xgpw2136* was used as marker for the detection of *Qfhb.rwg-5A.2*
[6] Although these SNP alleles were the same as in GP80, the winter wheat parent was only used in the final cross which still allowed for detection of the GP80-derived SNP allele
[7] B_2F_3 Progeny from population 19M13-67 that were homozygous resistant for the *Xbarc186* and *Xgpw2136* markers were used for continued backcrosses to ND Noreen

Within population 19M13 (final backcross parent = ND Noreen), the major haplotypes were: (i) GP80 region 113.1_117.7_*Xgpw2136* (11 plants); (ii) haplotype winter wheat only (10 plants); and (iii) haplotype GP80 region *Xgpw2136* (1 plant) (Table 2.2). Haplotypes (i) and (ii) must already have been present in the B_1F_1 18M6 plant that was crossed with ND Noreen to produce the B_2F_1 19M13 plants. Haplotype (iii) had to be the result of a crossover between the latter two haplotypes in between locus position 117.7 cM and *Xgpw2136* in the F_1 18M6.

In the 19M14 population (final backcross parent = Monument), four haplotypes were found as is shown in the Table 2.2: (i) Haplotype GP80 region 113.1 to 138 (13 plants); (ii) a winter wheat only haplotype (5 plants); (iii) a GP80 region 137.9 haplotype (two plants) and (iv) a GP80 region 113.1 haplotype (1 plant). The two shorter haplotypes (iii) and (iv) must have resulted from a further crossover between a chromosome with haplotype (i) and a chromosome with normal winter wheat haplotype. Haplotype (iv) in line 19M14-86 is the shortest, potentially most useful recombinant as it encompasses the 113.1 GP80 chromosome interval within which *Qfhb.rwg-5A.2* is most likely located (Zhao et al., 2018). As such, it may be very useful for continued study and use in breeding. It could also be useful to investigate the possible conversion

of SNP 28898 into a new PCR-based marker as the linkage with *Xgpw2136* had been broken. GP80 region) fairly large region of GP80 chromatin.

Population 19M15 was developed using 18Nord-114 as the final backcross parent. In this population, five haplotypes were observed (Table 2.2): (i) Haplotype GP80 region 113.1_141.7 appears to contain the full GP80 region II and is present in nine B_2F_1 families. The remaining haplotypes are: (ii) a winter wheat only haplotype (nine plants); (iii) a haplotype G80 region 113.1_*Xgpw2136* (three plants); (iv) a GP80 region 137.9_141.7 haplotype (four plants); and (v) a GP80 *Xgpw2136* haplotype (one plant). Haplotypes (iii) and (iv) likely resulted from two different single crossovers between haplotypes (i) and (ii). Haplotype (v) probably resulted following a single crossover between haplotypes (ii) and (iii).

Characterization of parents and B_2F_2 families for type II FHB resistance

Strong disease development occurred on all the families, parents and controls. Infection severities recorded on parents in this experiment were higher than in other experiments (Chu et al., 2011; Tao 2019) confirming high disease pressure. A one-way analysis of variance (unequal numbers of measurements/plants) was done on the controls and parents only and the results are summarized in Table 2.3.

Table 2.3. Analysis of variance on parents and controls used in this study.

SOV	DF	SS	MSS	F Ratio
Name	9	15.03	1.67	163.87[****]
Error	217	2.21	0.01	
Total	226	17.24		

SOV=source of variance, DF=degree of freedom, SS=sum of squares, and MS=mean squares
[1]*** represents significance at 0.001 level.

Table 2.4. Mean disease severities and significant differences among the parents and controls used in this study.

Genotype	No. of plants screened	Mean[1]	Significant differences[2]
GRANDIN	29	0.99	A
MONUMENT	21	0.96	A
RWG-21	24	0.95	AB
18NORD-114	21	0.93	AB
JERRY	22	0.86	B
ND Noreen	20	0.75	C
NOVUS-4	21	0.58	D
CM82036	24	0.49	DE
GP-80	23	0.41	E
PI277012	22	0.23	F

[1] Means arranged from higher to lower order of disease severity
[2] Means connected with different letters were significantly different at $\alpha = 0.05$; $q = 3.196$

As expected, the parents showed significant differences in type II resistance (Table 2.3). PI277012 (0.24) and GP80 (0.42) were the most resistant (both have *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*) while CM82036 (0.50; *Fhb1, Qfhs.ifa-5A*) was the third best. Novus-4 (0.58; *Qfhb.rwg-5A.1*) showed intermediate resistance. Although, Novus-4 and RWG21 (0.95) both have *Qfhb.rwg.5A.1*, they differed significantly in resistance which is likely due to the very susceptible backcross parent Grandin (0.99) in the RWG21 genetic background. Excepting 18Nord-114 (which has the *Fhb1* marker allele), the remaining parents do not have any of the named FHB resistance QTL and showed lower to moderate disease resistance. Winter wheat varieties ND Noreen (0.76) and Jerry (0.86) performed better than the very susceptible parents 18Nord-114 (0.94), Monument (0.97), and Grandin (0.99).

Since FHB resistance levels were shown to be affected by the genetic back-ground (Bai et al., 2018; Brar et al., 2019), the disease phenotype data of the three hybrid populations were analysed separately. The number of individual plants tested, the range in disease severity among individual plants, and the mean disease severity of parents, controls and 69 individual B_2F_2 families are provided in Table 2.2. The distribution of individual plant data for the three hybrid populations are shown in Fig. 2.5. Since each population derive from backcrosses with the dihybrid F_1, 18M6, a broad range of infection severities are expected. From Table 2.2 and Fig. 2.5 it appears that population 19M13 (mean = 74.8) had the highest overall level of resistance followed by 19M15 (mean = 79.5) and 19M14 (mean = 85.4). These differences are probably related to genetic background as the respective backcross parents ND Noreen (mean disease severity = 0.76), 18Nord114 (94.0) and Monument (97.0) differed in susceptibility. The most promising families with lowest disease severities occurred in cross 19M13.

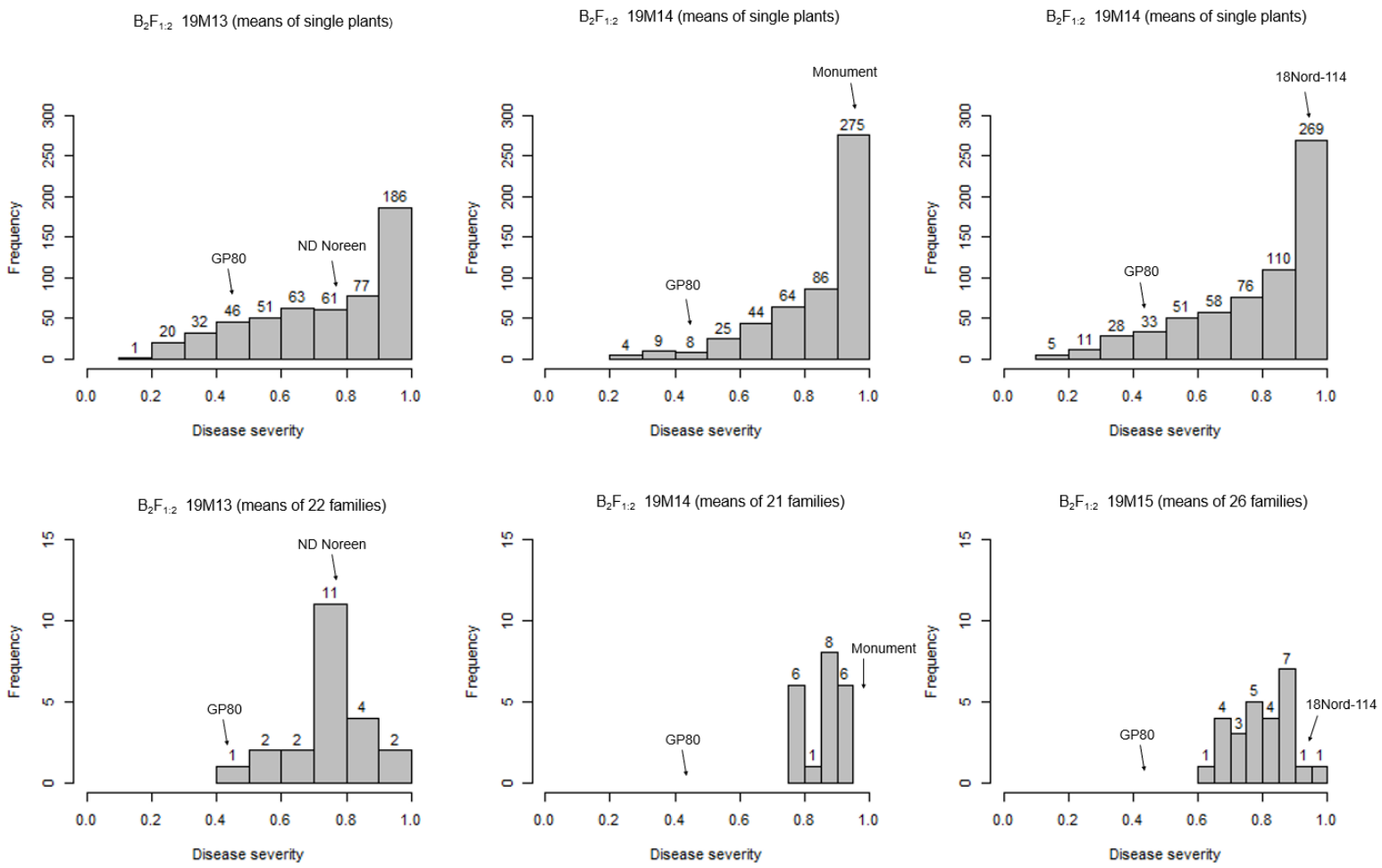

Figure 2.5. Distribution of Fusarium head blight infection severity in $B_2F_{1:2}$ families derived from three backcrosses. 19M13=GP80/Novus-4//Monument/3/ND Noreen; 19M14 = GP80/Novus-4//2* Monument; 19M15 = GP80/Novus-4/3/18Nord-114. The populations segregate for *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*.

With regard to the FHB results, an attempt was made to relate the mean disease severity of B_2F_2 families with the presence of marker Barc186 (*Qfhb.rwg-5A.1*), marker Gpw2136 (*Qfhb.rwg-5A.2*) and the GP80 region III haplotype (Table 2.2). For cross 19M13, the winter wheat only haplotype (*Qfhb.rwg-5A.2* likely absent) combined with the *Qfhb.rwg-5A.1* marker present (0.80) or absent (0.79) were very similar. Comparing the GP80 region 113.1_117.7_*Xgpw2136* haplotype in combination with the *Qfhb.rwg-5A.1* marker (0.73) or without (0.66) suggested that the presence of the latter haplotype improved resistance. The lower mean associated with the absence of Barc186 was largely due to one family (19M13-60) having a comparatively low score (0.49). The most likely cause of this low severity score is that it was due to the segregation of background QTL. If the family 19M13-60 result is excluded, the two groups (Barc186 + and -) are very similar. Since this cross produced better average results than crosses 19M14 and 19M15; the family with best agrotypes (19M13-67) that also has the GP80 region 113.1_117.7_*Xgpw2136* haplotype was chosen for continued backcrosses to ND Noreen. Two B_2F_3 plants that were homozygous for both this haplotype (marker Gpw2136) and the Barc186 marker were selected and used for the cross.

In population 19M14, there was no pattern when comparing the mean infection severities of the four haplotypes that were observed in this population with the absence or presence of the Barc186 marker (Table 2.2). The largest haplotype, GP80 region 113.1_138 with Barc186 marker present (0.84) or without (0.89) produced infection severities that were very similar to the winter wheat only and three small haplotypes in combination with (0.83) or without (0.86) the Barc186 marker. A most likely explanation of the result is that the very susceptible Monument genetic background lacks background QTL that advances resistance or contains QTL that interacts negatively with the chromosome 5A QTL. An alternative possibility is that *Qfhb.rwg-*

5A.2 is located within the 100.9-113.1 cM region for which no polymorphic SNP marker was obtained and was not present in this population.

With regard to population 19M15, five haplotypes were revealed (Table 2.2). Three of the haplotypes (winter wheat only, GP80 region *Xgpw2136* and haplotype GP80 region 137.9_141.7) occur outside the region suggested by Zhao et al. (2018) to harbor *Qfhb.rwg-5A.2*. Only one of the 14 families segregated for the presence of the Barc186 marker. This latter group had an average disease severity of 0.83. The disease severity of the remaining 12 families (all of which had the Barc186 marker) was 0.77. Since the latter group had the markers for both *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* present, it seems likely that the two genes had only small effects in reducing overall disease symptoms against a background of high susceptibility contributed by Monument (cross 18M6) and 18Nord-114 in the subsequent cross.

With reference to Table 2.2, single plants that had infection severities equal to, or lower than 0.25 were found in all three populations suggesting that it may be possible to derive more resistant lines than is suggested by the family means if pure line breeding is initiated from the three crosses. Family 19M13-60 produced the lowest disease severity (0.49) among the 69 families which seems to be comparable to the resistance in GP80 (0.42); especially since the *Qfhb.rwg-5A.1* marker could not be detected in this family. In general, the data suggest the successful introgression of *Qfhb.rwg-5A.2* from GP80 into winter wheat.

It is likely for *Qfhb.rwg-5A.1* to be located at the proximal end of GP80 region III. Haplotypes involving the three SNP loci closest to the *Q*-gene and spanning 113.14 to 117.67 cM are likely the smallest GP80 chromatin regions that include *Qfhb.rwg-5A.2*. Although a single recombinant with only GP80 113.15 cM fragment was recovered (VR-86), the phenotype

data do not confirm the presence of *Qfhb.rwg-5A.2*. Low availability of polymorphic SNP loci in region III limits any further inferences.

The FHB infection severities and marker results did not always follow a predictable pattern. The 19M14 and 19M15 crosses were generally more susceptible than the 19M13 cross despite the fact that the three crosses differed only with respect to the final backcross parent. Disease severity sometimes varied widely among those families of the same cross that had the same chromosome 5A region III haplotype with the Barc186 marker either present or absent. On the other hand, family 19M13-70 (ND Noreen final backcross parent) had neither of the chromosome 5A resistance QTL marker regions, yet exhibited strong resistance. Similar differences in FHB resistance was observed among spring wheat lines carrying same resistance genes (Miedaner et al., 2006). Resistance of the lines/populations obtained from a cross often depends on the level of resistance in the parents (Buerstmayr et al., 2008; Brar et al., 2019). This is the most likely reason for low disease severity of ND Noreen population and also for recovery of families with resistance comparable to the resistance donor parent within the ND Noreen population. The results suggest the possible existence of useful native resistance QTL in ND Noreen. Brar et al., (2019) suggested that epistatic interactions occur which could influence the expression and penetrance of FHB resistance genes. In this study, disease screening was performed on F_2 populations rather than on highly homozygous lines which not only limited the numbers of spikes evaluated per genotype but also maximized genetic effects such as dominance, over-dominance and epistasis. These factors may have contributed to differences in resistance among crosses and also among families with the same haplotype. Most families that expressed stronger FHB resistance had both of the *Qfhb.rwg-5A.1* and *Qhfb.rwg-5A.2* markers. In many FHB resistant germplasm enhancement programs, it is very difficult to recover lines with

resistance levels comparable to the donor parents because of the segregation of undetected background QTL and unknown epistatic interactions between discovered and undiscovered QTL (Salameh et al., 2011; Bai et al., 2018; Brar et al., 2019; Buerstmayr et al., 2019). Pyramiding of highly characterized QTL (*Fhb1, Fhb2* and *Fhb5*) in different genetic backgrounds failed to identify lines with resistance comparable to the donor parent (Brar et al., 2019).

Conclusion

The results suggest that gene *Qfhb.rwg-5A.2* was successfully introgressed into winter wheat. This locus has previously been mapped to a chromosome 5A region stretching from 100.9 cM to 117.67 cM on the 90K SNP consensus map. In this study, the parents and progeny of three backcross populations as well as control genotypes were subjected to 90K SNP genotyping. From the data, a GP80 chromosome 5A genetic map was constructed and it was determined that *Qfhb.rwg.5A.2* occurs in region III on this chromosome map. Polymorphic loci that were suited to SNP haplotyping were then identified and used to map 69 B_2F_1 progeny plants with regard to the presence/absence of GP80 region III-derived chromatin. Ten distinct GP80 haplotypes were found, including "parental" haplotypes containing winter wheat chromatin only or GP80-derived chromatin only. Following the evaluation of the same material for FHB type II resistance, the presence or absence of the *Xbarc186* marker locus (detects *Qfhb.rwg-5A.1*) was related to the FHB data and ten haplotypes on a per-cross basis. Due to the heterogeneity of the B_2F_2 populations and the high susceptibility of backcross parents Monument and 18Nord-114, the FHB data did not reveal clear differences among backcross families. There is strong indication that the segregation of background QTL and epistasis confounded the resistance expression of the targeted QTL. Population 19M13 (final backcross parent = ND Noreen) was the most resistant and the results were consistent with the location of *Qfhb.rwg-5A.2* as suggested by Zhao

et al. (2018). As the resistance appears to be well expressed in the ND Noreen genetic background, a recombinant (19M13-67) from this cross that had the best agrotype and both chromosome 5A QTL (markers) was used for making a further backcross to winter wheat. While marker *Xgpw2136* was useful in the initial transfer to winter wheat, there was ample evidence of recombination on either side of the marker locus indicating that it may not be located sufficiently close to *Qfhb.rwg-5A.2* to be a reliable marker. For continued introgression of *Qfhb.rwg-5A.2* it will be better to continue to use the SNP haplotype in conjunction with the marker for selection. The study evaluated only type II resistance, moreover in only one environment. More comprehensive screening involving both greenhouse and field trials in multiple environments will be necessary to confirm the resistance of families that showed good resistance in this study.

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CHAPTER IV. CHARACTERIZATION OF FHB RESISTANCE IN *THINOPYRUM DISTICHUM* DERIVATIVES

Abstract

FHB is a serious fungal disease that causes significant economic losses in wheat and barley production in the Northern Great Plains and Central United States. The pathogen is particularly harmful when optimal infection and disease development conditions prevail. Past research showed that genetic disease resistance is the most effective disease management strategy. Numerous FHB resistance QTL were identified and mapped to all of the wheat chromosomes. However, only *Fhb1* has been widely exploited in breeding programs around the world as it expresses resistance in most genetic backgrounds and could reduce FHB symptoms by up to 20-25%. To further reduce disease symptoms and damage caused by FHB, it is critical to identify and introduce additional diverse resistance genes. FHB resistance has previously been reported in *Thinopyrum distichum* Thunb. Löve (2n = 4x = 28; $J_1^dJ_1^dJ_2^dJ_2^d$). However, this resistance has not been transferred to wheat and remains poorly characterized. This study evaluated addition lines of *Th. distichum* in hexaploid triticale; secondary hybrids derived from backcrosses of a primary triticale/*Th. distichum* allodecaploid (2n = 10x = 70; $AABBRRJ_1^dJ_1^dJ_2^dJ_2^d$) to triticale; and from *Th. distichum* based tritipyrum lines (durum wheat X *Th. distichum* partial amphiploids). First, di-telosomic triticale addition lines W1450 and W1451 exhibited strong FHB resistance in a greenhouse trial but results from a field trial could not confirm that they were more resistant than the triticale parent Rex. Based on greenhouse results, both addition lines were crossed with spring wheat in an attempt to also transfer the addition telosome to hexaploid wheat cultivar Inia 66. As the B_1F_1 exhibited strong hybrid necrosis and poor seed set, two additional spring wheats were used for continued backcrosses. The final

backcross populations were found to segregate for the presence of the telosome, which was thought to be of *Th. distichum* origin. However, GISH results showed that the addition telosome in both addition lines is actually an unknown rye telosome. Since no *Th. distichum* chromatin could be detected on the addition telosome, the transfer attempt was not continued. Secondary hybrids from *Th. distichum* X hexaploid triticale backcrosses were studied to determine the possible presence of a *Thinopyrum* derived translocation in the material. Individual plants that showed better FHB resistance were selected from lines W1423X, W1423X-3 and its sister lines and were evaluated for somatic chromosome number and FHB resistance in several FHB resistance trials. Progeny of two plants, one each from W1423X-3 and W1423X-21 showed good FHB resistance in a final replicated greenhouse trial and will be pursued further. Plants that seemed to be resistant among the progeny of W1423X-3 (B_2F_7 resistant plant with 2n = 42 chromosomes) were also crossed with triticale cultivar Tobie and then either crossed with spring wheat Inia 66 or were backcrossed Tobie. The Tobie backcross progeny did not show any significant differences in resistance as compared to Tobie. A small proportion of cross progeny with Inia 66 showed good resistance suggesting the presence of FHB resistance. However, the latter progeny exhibited strong hybrid necrosis and seed set was very poor. Also, the seeds that were recovered were shriveled and did not germinate. 15 Individual plants from W143X lineage with better FHB resistance and agrotype were selected from field trial and were screened in 2019 fall greenhouse trial. Based on the phenotype data, W1423X-3-2 and W1423X-21-2 appears as homozygote resistants. Further cytogenetic studies are needed to confirm their breeding nature. Finally, two tritipyrum lines, one of which showed good FHB resistance in prior trials, were evaluated in greenhouse and field trials. The results were inconclusive due to the very short stature of the plants that were overgrown by weed grass.

Introduction

Numerous, valuable disease resistance genes have been transferred to wheat from its wild relatives to protect the crop against destructive diseases such as leaf rust, stem rust, and *Fusarium* head blight. Among these are the alien resistance genes *Lr19, LrAp, Lr53, Lr56, Lr59, Lr62, Sr-1644-1Sh, Sr-1644-5Sh* and *Fhb7* (Sharma and Knott, 1966; Marais et al., 2018; Narang et al., 2020; Sharma and Knott 1995; Guo et al., 2015). Linkage drag may sometimes result in the co-introgression of deleterious genes that are closely associated with the introduced alien resistance; however, these can be removed by applying chromosome-engineering methodology (Marais et al., 2018). In some instances useful genes are transferred from wild relatives without apparent deleterious linkage drag (Gill et al., 2008; Zeng et al., 2013; Wang et al. 2020).

FHB resistance is a complex, polygenic trait. FHB resistance QTL have small individual effects on the overall level of resistance and their resistance expression is strongly influenced by the environment (Bai et al., 2018; Buerstmayr et al., 2019). The degree of resistance obtained from the use of an FHB resistance QTL in a cross is affected by the genetic background of the recipient parent. Some resistance QTL (for example *Fhb1* and *Qfhs.ifa-5A*) may interact in a complementary manner to provide increased resistance (Salameh et al., 2011). Most wheat breeding programs depend solely on Sumai 3 derived resistance for cultivar development (Buerstmayr et al., 2019). Therefore, there is a need for more, diverse, and novel FHB resistance sources, which has led to attempts to identify and transfer resistance from wild relatives to wheat (Cai et al., 2008; Cainong et al., 2015). *Thinopyrum* species are a rich source of biotic and abiotic stress tolerance and resistance genes (Marais et al., 1998; Li et al., 2008; Li and Wang, 2009). *Thinopyrum ponticum* 7E substitution lines in wheat and 7el$_2$-7D wheat-*Th. ponticum* translocations exhibited higher levels of type II FHB resistance. QTL on chromosome 7el2 was

mapped to the distal region of its long arm and markers were designed for its tracking (Shen et al., 2004; Shen and Ohm, 2007). Chromosome engineering was done to characterize and introgress a short chromosome segment from *Th. ponticum* by employing the *ph1b* mutant. This paved the way for identification of a 1.7cM chromatin interval that harbours the resistance gene (named *Fhb7*) with the help of SSRs, diversity array technology and EST-derived STS markers (Guo et al., 2015). *Fhb7* was first identified on chromosome $7el_2$ of the decaploid species *Th. ponticum* by Kim et al., (1993) and was recently cloned. The candidate gene was determined to be a glutathione S-transferase gene based on the assembled genome sequence of *Thinopyrum elongatum* (Wang et al., 2020). Apart from a reduction in disease severity, *Fhb7* detoxifies DON without any yield penalty. The gene also confers resistance to crown rot in wheat. Greenhouse trials employing chromosome 7E di-telosomics and GISH suggested that a gene for resistance to the spread of infection occurs on 7ES (Fu et al., 2012). Two *Triticum–Secale–Thinopyrum* trigeneric hybrids (both 2n = 42) that were derived from a cross between hexaploid triticale and hexaploid *Triticum trititrigia* (2n = 6x = 42, AABBEE), exhibited higher levels of resistance to Fusarium head blight, leaf rust and stem rust race Ug99 (Dai et al., 2017).

Chromosome 1E of *Thinopyrum elongatum* exhibited strong type II resistance in a durum wheat genetic background in greenhouse trials (Jauhar, 2008). *Thinopyrum intermedium* amphidiploids with FHB resistance were reported by Zeng et al. (2013). Fifteen QTL for FHB resistance were discovered in *Th. intermedium* (intermediate wheatgrass), a crop that is currently being domesticated (Bajgain et al., 2019). FHB resistance was first reported in *T. durum –Th. distichum* hybrids by Chen et al. (2001) but its transfer to wheat was not attempted.

Thinopyrum distichum (2n = 28) is a maritime grass, native to the eastern, southern and south-western coastline of the Cape Province, South Africa. It is a hardy, salt-tolerant and slow-

growing perennial (Pienaar 1990). Cytological studies revealed that it is a segmental allotetraploid (Pienaar 1983, Pienaar et al. 1988) with genomes, J_1^d and J_2^d. Hybrids of *Th. distichum* with durum and hexaploid wheat (Pienaar 1981, 1983, 1990), rye and triticale (Marais et al. 1998, Marais and Marais 2003) have been produced. Littlejohn and Pienaar (1995) produced 11 of 14 possible disomic single chromosome addition lines in common wheat cultivar Inia 66. Marais and Marais (1998) hybridized *Thinopyrum distichum* with hexaploid triticale and pollinated the C_1 hybrid (2n = 70) with triticale. F_1 plants (2n = 54-56) were backcrossed to the triticale cultivar Rex to derive B_2F_1 (2n = 45-49). B_2F_2 plants with 43 or 44 chromosomes were selected and selfed. B_2F_3 with 2n = 44 were selected from the latter progenies to produce a set of 30 random triticale-*Th. distichum* addition lines (Marais et al., 2007). A preliminary study by Tao (2019) found that two of the latter triticale addition lines (W1450 and W1451) had seemingly strong FHB resistance. The two lines appeared to have the same (acrocentric) addition chromosome.

During the search for triticale addition lines (Marais et al., 2007), progenies that appeared to carry triticale-*Th. distichum* translocations occurred. W1423 was one such stock that seemed to segregate for a translocation. A preliminary screen of F_4 W1423 plants (unpublished data; Pirseyedi and Marais, 2016) suggested that a low frequency of plants from this stock showed FHB resistance. Tao (2019) continued to select resistant single plants from the W1423 lineage in an attempt to recover a homozygote through continuous selfing and screening for FHB resistance. However, that was not possible even after four selection cycles due to abnormal segregation of chromosome 7A and a second unknown chromosome thought to carry a *Th. distichum* translocation. One resistant F_7 plant (W1423X-3) had 42 chromosomes, appeared to be

disomic 7A and produced a low frequency of aneuploids which were believed to result from de-synapsis of a translocated chromosome and its normal homologue.

Marais et al. (2014) described the development of *Th. distichum* based tritipyrum plants. These, so-called d-tritipyrums, have 2n = 42 chromosomes and the genomic formula $AABBJ^dJ^d$. Each individual J^d chromosome derives primarily from either of the J_1^d or J_2^d genomes and might have been altered through recombination with its homoeologue. Due to the hybrid origin of their J^d genome, d-tritipyrum lines are characterized by high genotypic and phenotypic variability and some lines/hybrids show infertility. When grown in the field, a tritipyrum line, 16M1001, showed strong FHB resistance.

The first study objective was to confirm FHB resistance that was previously reported in two triticale-*Th distichum* addition lines (W1450, W1451) and to initiate crosses to transfer the acrocentric addition chromosome in W1450 to common wheat. Secondly, an attempt was made to confirm FHB resistance in tritipyrum line 16M1001. Lastly, F_8 progenies of selection W1423X-3 were characterized in greenhouse and field evaluations of FHB type II resistance in an attempt to select a line that would breed true for the resistance.

Materials and methods

Three sets of unconfirmed material that could harbour FHB resistance from *Th. distichum* were evaluated with the purpose to continue to validate the resistance and derive true breeding, resistant stocks. Four FHB resistance evaluation trials were done. The first trial was a greenhouse trial that was done in the fall of 2018. A second greenhouse experiment was done in the spring of 2019. The third trial was a misted field trial that was done in the spring/summer of 2019. A fourth trial was done in a greenhouse in the fall of 2019. Each trial included lines or segregating material that derived from the three sources that were investigated.

Hexaploid triticale di-telosomic addition lines W1450 and W1451

Addition lines W1450 and W1451 were produced by Marais et al. (2007). As is summarized in Fig. 3.1, the two lines were included for further evaluation in both greenhouse trial 1 and the field evaluation trial. An attempt was also made to transfer the addition chromosome in W1450 to a hexaploid wheat background. For this purpose, W1450 was crossed with the spring wheat cultivar Inia 66. F_1 progeny from this cross was backcrossed to Inia 66. B_1F_1 seeds were germinated and roots were cut from the seedlings to do root tip somatic chromosome counts. Seedlings with 2n = 42-43 chromosomes (that included the telosomic addition chromosome) were grown for continued backcrosses. Since the hybrids exhibited strong hybrid necrosis and seed development was very poor, two additional spring wheat lines (SST66 and W84-17) were used for making the second backcross. Three sets of B_2F_1 were produced that segregated for the telosomic addition.

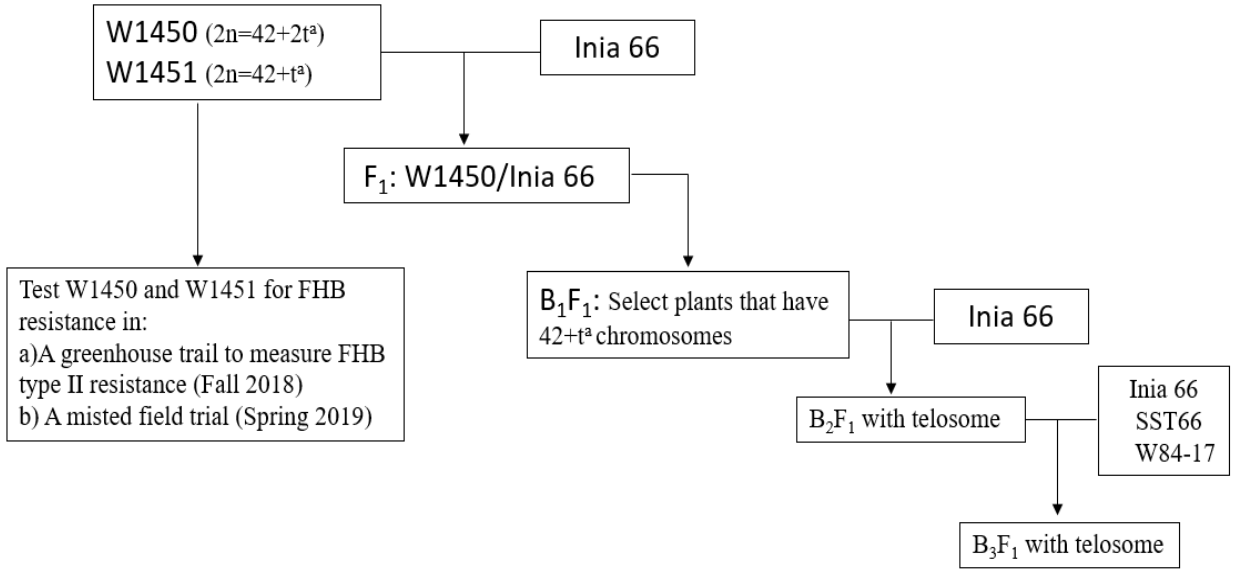

Figure 3.1. Evaluation of triticale addition lines W1450 and W1451 for FHB resistance and initiation of backcrosses to transfer the addition chromosome to spring wheat.
t^a = Acrocentric addition chromosome.

Table 3.1. Genotypes that were evaluated in a misted field nursery at Fargo (spring/summer 2019).

Entry	**Description**
GP80	HRSW control with *Qfhb.rwg-5A.1, Qfhb.rwg.5A.2*
CM82036	HRSW control with *Fhb1, Qfhs,ifa-5A*
Rex	Hexaploid spring triticale cultivar
Tobie	Hexaploid spring triticale cultivar
W1450	Triticale di-telosomic addition line
W1451	Triticale di-telosomic addition line
W1423X (Selfed progeny)	
1423X-1 (Selfed progeny)	W1423X-3 sister line
1423X-2 (Selfed progeny)	W1423X-3 sister line
W1423X-3 (Selfed progeny)	
1423X-12 (Selfed progeny)	W1423X-3 sister line
1423X-18 (Selfed progeny)	W1423X-3 sister line
1423X-21 (Selfed progeny)	W1423X-3 sister line
F_2: W1423X-3/Tobie	
16M1001	Tritipyrum
16M1005	Tritipyrum
F_2 :16M1001/16M1005	

Evaluation and selection of W1423X-3 progenies

Plant W1423X-3 (B_2F_7: *Th. distichum*/3* hexaploid triticale) occurred among segregating populations that were evaluated for FHB type II resistance in a greenhouse experiment done by Tao (2019). Since plant W1423X-3 appeared to be strongly resistant to FHB, it was pollinated with hexaploid triticale cultivar, Tobie (Marais 2018, Personal communication). The W1423X-3/Tobie F_1 plants were subjected to somatic chromosome counts and were evaluated in greenhouse trial 1. The two most resistant F_1 plants were then backcrossed to Tobie (34 viable seeds were produced) and was also pollinated with Inia 66 pollen (10 viable seeds were produced). The W1423X-3/2* Tobie backcross was named 18M11 whereas the W1423X-3/Tobie//Inia-66 cross was named 18M13. Both Tobie and Inia 66 are highly susceptible to FHB. The 18M11 and 18M13 F_1 seeds were germinated for root tip chromosome counts and were then evaluated together with the parents in the second greenhouse FHB resistance experiment.

F_2 populations derived from W1423X-3 (six populations) and the W1423X-3/Tobie F_1 (two populations) were included in the 2019 field experiment. Five plants each from W1423X-3/Tobie and W1423X-3, one from W1423X/Tobie, and four from W1423X-21 were selected from the field trial and were evaluated in a 2019 fall greenhouse trial.

Tritipyrum line 16M1001

Two tritipyrum lines were derived among single rows that were naturally infected with FHB in the field. Line 16M1001 appeared to have strong resistance whereas line 16M1005 was highly susceptible (Marais 2018, Personal communication). The two lines were included for resistance evaluation in the first greenhouse trial and in the field trial.

Greenhouse FHB resistance trials

The pure line genotypes that were evaluated in greenhouse trial 1 included: addition lines W1450 and W1451; tritipyrum lines 16M1001 and 16M1005; hexaploid spring wheat control Inia 66; hexaploid spring triticales Rex and Tobie. Five replicates (pots) were planted of each pure line with five seeds planted per pot. A total of 25 spikes were inoculated per line. The F_1 18M11 and 18M13 populations were evaluated in a second greenhouse trial; each seedling differed genotypically from every other individual and only 1 to 8 spikes could be inoculated per plant. Inia 66, Rex and Tobie plants were included as controls.

The third greenhouse trial: Five plants each from W1423X-3 and Tobie/ W1423X-3, four plants from W1423X-21 and one from Tobie/W1423X were selected from the field trial. Four seeds from each selected single plant (one head) were planted in a single pot (three pots per entry) and used for progeny testing. Thus, 9-12 plants were screened per line. For each plant, one to seven spikes were inoculated with FHB.

The single spikelet injection method was used for inoculating spikelets at anthesis in a greenhouse (Stack, 1989). A mixture of *Fusarium graminearum* isolates (*Fg*08_13, *Fg*10_135_5, *Fg*10_124_1 and *Fg*13_79) provided by Dr Shaobin Zhong (Department of Plant Pathology, North Dakota State University) was used for inoculation. A 10µl-droplet containing the isolate mix (approximately 100,000 conidia per ml) was injected directly into a floret in the middle of the spike. After the first inoculation, the greenhouse temperature was raised to 72-76 ^{o}F. Inoculated spikes were covered with a wet plastic bag for 72 h immediately after inoculation. When disease symptoms developed, the infection severity was visually assessed by determining the percentage of infected spikelets per spike at 21 days after inoculation.

Field FHB nursery

Genotypes analysed in the field nursery are shown in the Table 3.1. Each genotype/line was planted in a single 2m row with five replications. At the booting stage, FHB infected corn kernel spawn that was obtained from Dr Shaobin Zhong (Department of Plant pathology, North Dakota State University) was spread on the soil surface as described by Xue et al. (2006). Corn kernels were kept moist with a misting system to stimulate *F. graminearum* sporulation and maintain high humidity for *F.graminearum* infection and FHB symptom development. The date when 50% of the spikes in a plot were in anthesis was recorded for each line. The nursery was overhead misted for 5 min at 15-min intervals over a 12h period extending from 4:00 p.m. to 4:00 a.m. each day. Misting continued until 14 days after anthesis of the last line. For each line, 20-25 spikes were visually scored for both disease incidence and severity. The disease incidence and severity scores were multiplied for each spike to calculate the FHB index.

Root tip chromosome counts

Chromosome counts were done based the methodology described by Darlington and La Cour (1976). Seeds were germinated at room temperature on Whatman's No. 1 filter paper in a petri dish. 2-3 healthy roots were cut into clean glass vials; placed on crushed ice and kept in a refrigerator for 30 hrs then water was replaced with fixative (3:1 mixture of methanol and propionic acid). The fixative was replaced with distilled water for 30 min. and the roots transferred to preheated (60^0C) 1N HCL for 7½ minutes. The roots were returned to distilled water for 1-2 minutes to stop hydrolysis and then placed in 1ml leuco-basic fuchsin (prepared according Darlington and La Cour 1960) in a refrigerator for 2 hrs. The leuco-basic fuchsin was drained off and the roots rinsed twice with distilled water for about 2-5 minutes. Following a final rinse with pH 4.5 sodium acetate buffer (3.16 g sodium acetate and 3.47 ml glacial acetic

acid in 1 L distilled water; adjusted to pH 4.5) for 1 minute the roots were transferred to 2.5% pectinase (in pH 4.5 sodium acetate buffer) for 30 min at room temperature. The pectinase was replaced with distilled water. Roots were cut on a clean glass slide and mounted in a small drop of 1% Rosner aceto-carmine (dissolve 2g carmine in 110 ml boiling water, add 90 ml acetic acid, reflux-condense for 4 hours). The chromosomes were counted under 100x magnification.

Statistical analyses

Analyses of variance were conducted using JMP (version 15.0)

Results and discussion

FHB resistance in W1450 and W1451 and transfer of the addition chromosome to wheat background

The two addition lines and controls Rex, Tobie and Inia-66 were tested in a completely randomized design with five replicates in greenhouse trial 1. The resultant analysis of variance is shown in Tables 3.2 and 3.3. The two addition lines showed the lowest mean disease severities and were significantly different from the two triticale cultivars used in the development of triticale-*Th. distichum* addition lines and also from Inia 66. The resistance of the two addition lines and the two triticale controls were also evaluated in the field trial (Fig. 3.2 and 3.3). The disease severities of W1450 and W1451 were lower and significantly different from that of Tobie but not from the other triticale parent, Rex. The disease index of W1450 was very high and equalled the susceptible triticale cultivar, Tobie, whereas the disease indices of W1451 and Rex were similar.

Table 3.2. One-way analysis of variance of FHB type II infection severity data obtained with respect to two *Th distichum* – triticale disomic addition lines, two tritipyrum lines and three controls that were compared in greenhouse experiment, Fall 2018.

SOV	DF	SS	MS	F Ratio
Genotype	6	2.81	0.46	30.36***
Error	24	0.43	0.01	
Total	34	3.24		

SOV = source of variance; DF = degrees of freedom; SS = sum of squares, and MS = mean squares
***indicates significance at the 0.0001 level.

Table 3.3. Average infection severities of triticale-*Th. distichum* addition lines and tritipyrum lines in comparison with control plants (grown in a greenhouse trial).

Genotype	Mean[1]	Significance[2]
W1451	0.14	C
W1450	0.19	C
16M1001	0.38	BC
16M1005	0.46	B
Rex	0.58	B
Tobie	0.83	A
Inia 66	0.94	A

[1] Means are ordered from the lowest to the highest.
[2] Averages not connected by same letter are significantly different based on Tuckey HSD test.

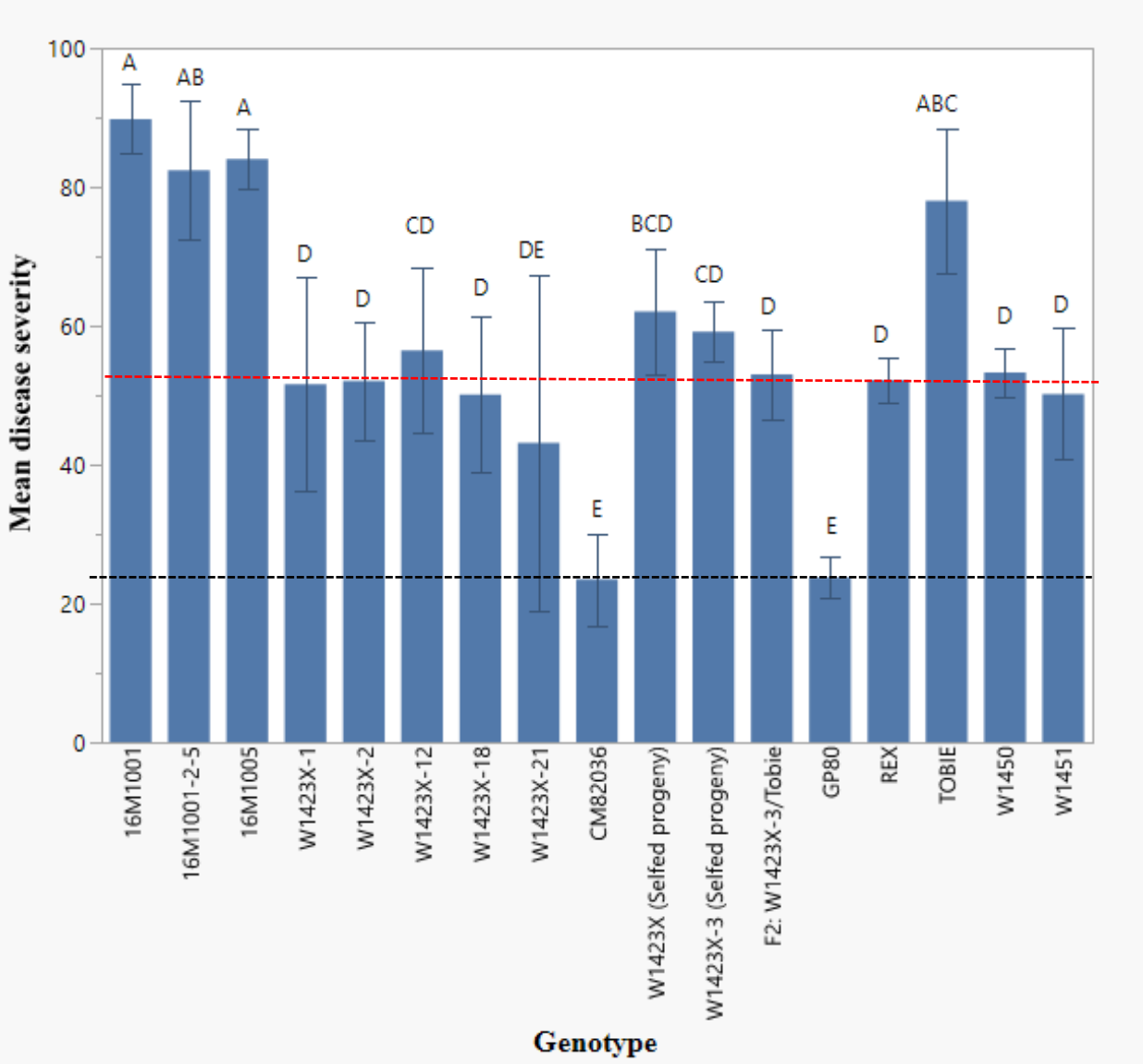

Figure 3.2. *Fusarium* head blight infection severity (%) of genotypes listed in the table 3.1. The bar on each column indicates the size of the standard deviation; Same letters on the bars suggests no significant differences at $P<0.05$. Disease severity of the resistant control is indicated with a black broken line, while disease severity of the resistant triticale parent Rex is indicated with a red broken line.

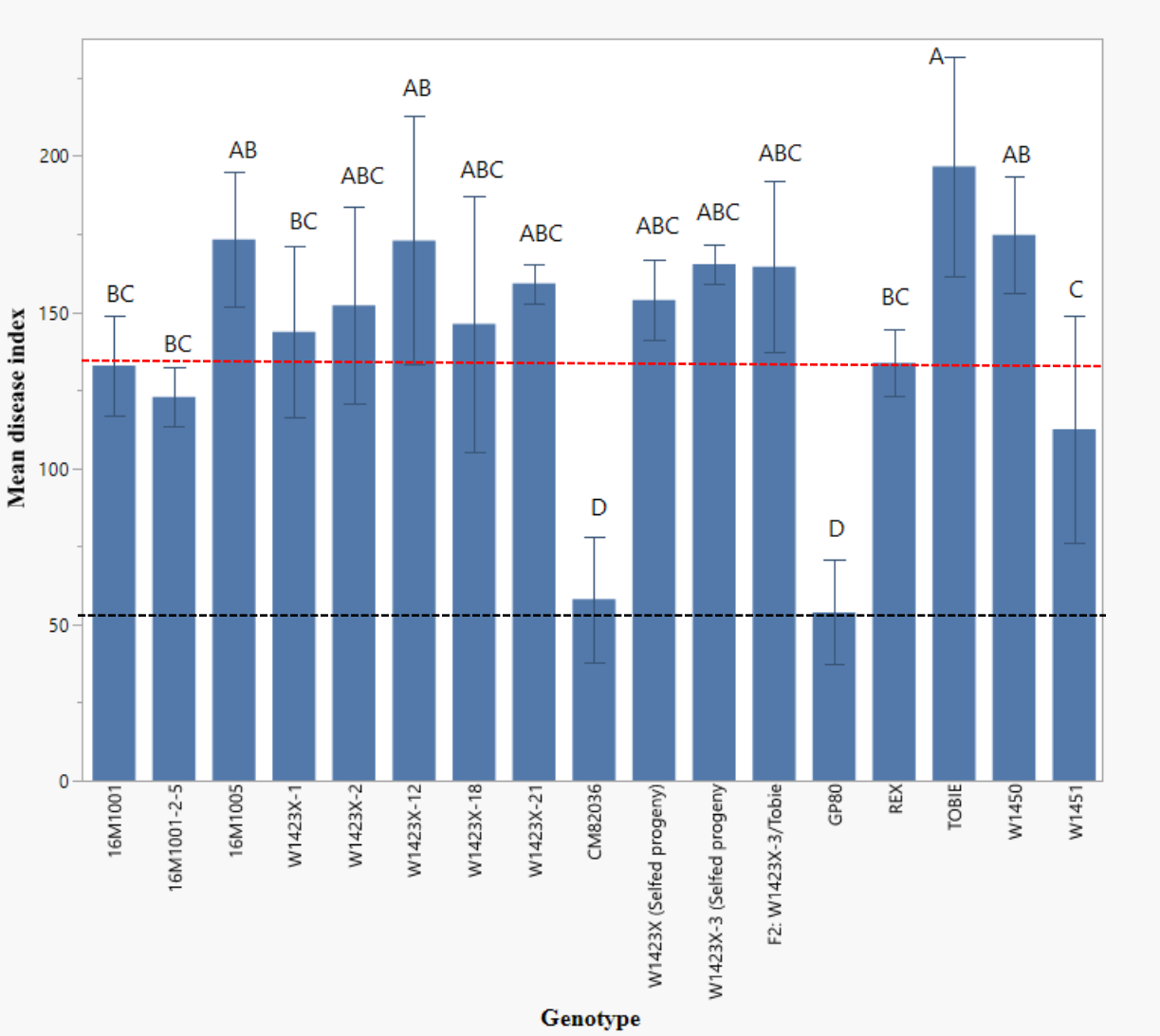

Figure 3.3. *Fusarium* head blight disease index (infection severity x disease incidence) of genotypes listed in the table 3.1. The bar on each column indicates the size of the standard deviation; Same letters on the bars suggests no significant differences at $P<0.05$. Disease index of the resistant control is indicated with a black broken line, while disease index of the resistant triticale parent Rex is indicated with a red broken line.

Based on strong FHB resistance in greenhouse trials, both W1450 (42+2t) and W1451 (42+2t) plants were initially used in backcrosses to attempt the transfer of the addition chromosome to hexaploid wheat as outlined in Fig. 3.4.

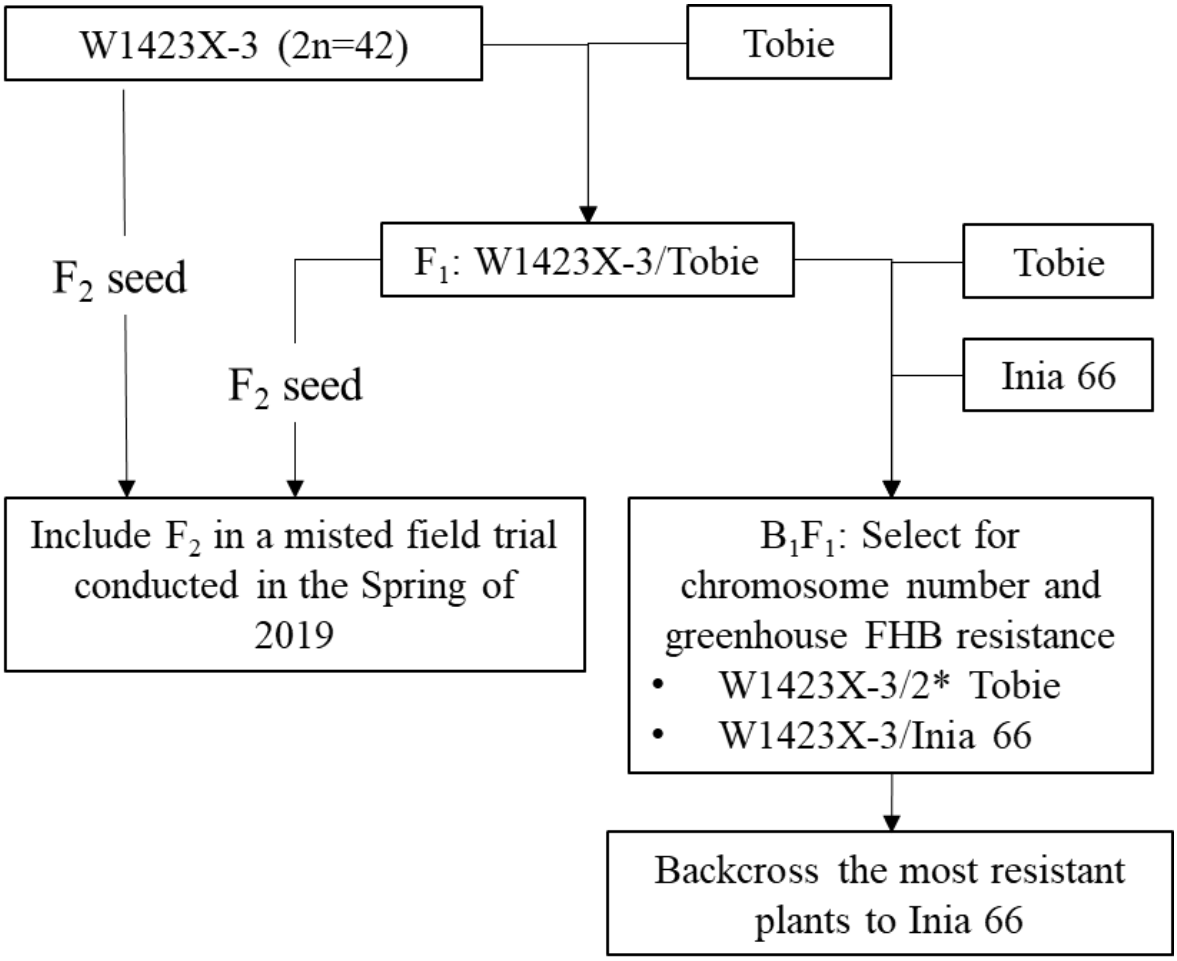

Figure 3.4. Attempt to select a resistant homozygote from the W1423X-3 lineage and to transfer the resistance to common wheat.

The backcross results obtained can be summarized as follows:

a. The W1450 (2n = 42+2t)/Inia-66 cross produced 14 F_1 hybrid seeds, five of which were viable. The somatic chromosome numbers of the five viable seedlings were: 2n = 41+t; 42; 42+t; 42+t and 42+2t, respectively which suggested that a level of meiotic metaphase I pairing irregularity occurred which probably involved the telocentric chromosome and a

normal triticale chromosome. The W1451 (2n = 44)/Inia-66 cross produced nine F_1 seeds. F_1 plants with 2n=42+t chromosomes from both crosses were backcrossed to Inia-66 to give B_1F_1 seeds; however, due to the similarity of W1450 and W1451, only the W1450 backcrosses were continued. In the absence of a marker to select for the addition chromosome, somatic chromosome counts were used to identify backcross F_1 for continuing the backcrosses.

b. Eight B_1F_1 W1450/2*Inia 66 seeds and four F_2 W1450/Inia-66 seeds were germinated of which five B_1F_1 (chromosome numbers: 39; 39+t; 41+t; 42 and 44) and two F_2 seedlings (both 40+t chromosomes) were viable. The two F_2 plants were completely sterile. Since strong hybrid necrosis and infertility occurred in the earlier crosses, the next backcross involved Inia 66, W84-17 and SST66 as recurrent wheat parents.

c. Three sets of B_2F_1 seeds were obtained and the chromosome numbers of viable seedlings were determined to be as follows: Cross 20M52 (W1450/3* Inia-66), 2n = 39, 40, 41; cross 20M53 (W1450/2* Inia-66/3/SST66), 2n = 38, 39, 39+t, 40, 40, 41, 41, 41, 42, 42; and cross 20M54 (W1450/2* Inia-66/3/W84-17), 2n = 38, 38+t,39, 39, 39+t, 40+t, 41, 42.

d. B_3F_1 seeds were produced on the telosome-carrying B_2F_1 with better agrotype and fertility and F_2 seeds were collected. It is believed that monosomic additions and subsequently, disomic additions can be derived from this material. Pictures of the Fuelgen-stained somatic chromosomes of some of the B_2F_1 are given in Fig. 3.3 (a, b). These clearly shows the targeted telosome and also shows its acrocentric morphology. Seeds of W1450 and W1451 addition lines were provided to Dal Hoe Koo and Bernd Friebe, Wheat Genetics Resources Center, Kansas State University (personal

communication, 10 June 2020) who characterized them using genomic in situ hybridization. Their results are given in Fig.3.5 and clearly showed that the extra telocentric chromosomes in W1450 and W1451 are of similar size and both are of rye origin. Using GISH, Koo and Friebe (2020) could not detect *Th. distichum* chromatin on the addition telosome. This suggests that it is highly unlikely that a *Th. distichum* translocation is present; however, the GISH technique is not sensitive enough to detect very small DNA inserts, which does not totally rule out the possibility that such a small translocation could be present. Even if a *Th. distichum* translocation is present, it will still be difficult to transfer it to a wheat chromosome seeing that the addition chromosome is of rye origin.

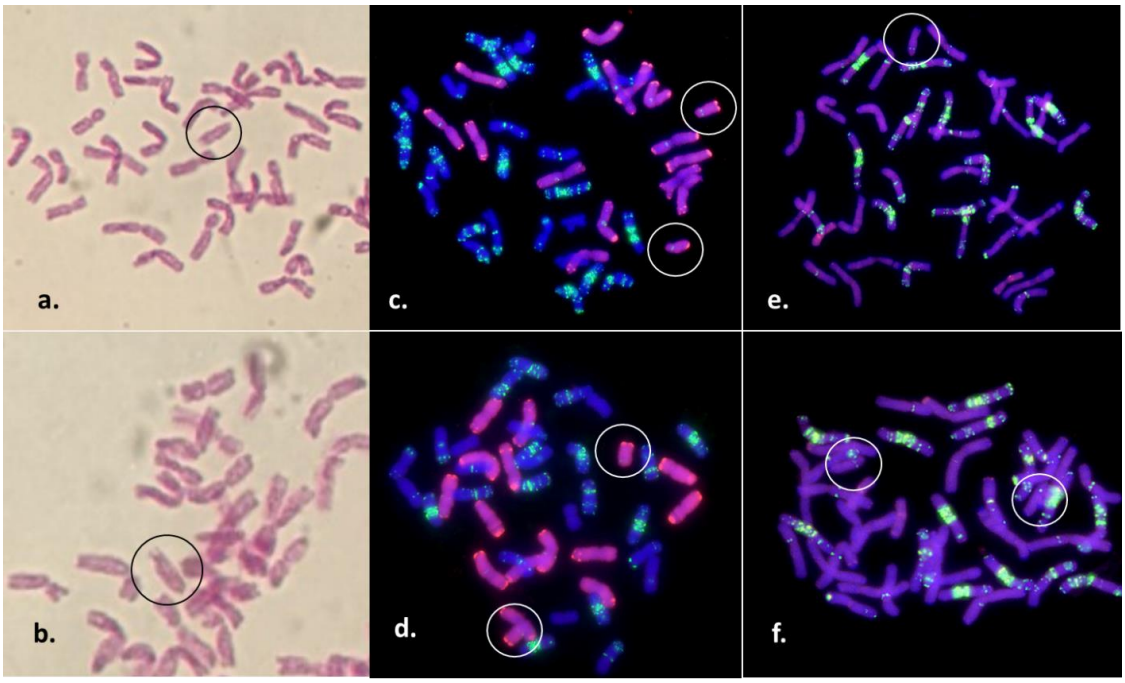

Figure 3.5. Pictures showing the telosome that occurs in disomic addition lines W1450 and W1451. Images a and b show Fuelgen-stained somatic metaphase chromosomes of B_2F_1 (W1450/3* common wheat) plants. Black circles show the telosomic chromosome with the acrocentric segment clearly visible in plate GISH images c and d show the telocentric chromosome in W1450 (white circles). The acrocentric nature of the telosome is clearly visible in plate c. Blue regions represent A and B genome chromatin whereas red indicate R genome chromatin, suggesting that the telosome is of rye origin. In GISH images e and f, blue is associated with triticale blocker DNA whereas the chromosomes were probed with *Thinopyrum distichum* genomic DNA (red). No evidence was found of the presence of a *Th. distichum* translocation. The GISH results and pictures were produced and provided by Dal Hoe Koo and Bernd Friebe, Wheat Genetics Resources Center, Kansas State University (personal communication, 10 June 2020).

The GISH results (Koo and Friebe, 2020) showed that the addition chromosome in W1450 and W1451 probably derive from a rye chromosome. While the data do not provide a reliable indication of which rye chromosome arm it might be, this would explain the low level of recurring aneuploidy that was seen in the disomic addition line. Also, it is very likely that the FHB resistance associated with W1450 and W1451 derives from rye rather than *Th. distichum*. Another, less likely explanation of the origin of the telosome is that it could be a rye B-

chromosome (Müntzing, 1948). B-chromosomes are often preferentially inherited and do not show regular Mendelian segregation (Houben, 2017).

Evaluation of W1423X-3 progeny

Plant W1423X-3 (2n = 42) showed good FHB resistance and fertility, and was therefore used in crosses with Tobie. The root tip chromosome numbers of the F_1 were determined to identify those plants that had 2n=42 or 43 chromosomes. The selected plants were then evaluated for FHB resistance in greenhouse trial 1. Tobie, W1423X-3 and Rex plants were included as controls. The ANOVA results and mean infection severities of this initial trial are summarized in Tables 3.4 and 3.5, respectively.

Table 3.4. Results of a one-way ANOVA to compare FHB type II infection severity of 29 W1423X-3/Tobie F_1 plants plus three controls that were evaluated in a greenhouse experiment that was conducted in the Fall (2018).

SOV	**DF**	**SS**	**MS**	**F Ratio**
Genotypes	30	6.72	0.22	1.91*
Error	127	14.87	0.11	
Corrected Total	157	21.60		

SOV = source of variance; DF = degrees of freedom; SS = sum of squares, and MS = mean squares
*indicates significance at the 0.05 level.

Table 3.5. Average infection severities of F_1: W1423X-3/Tobie plants in comparison with control plants (grown in a greenhouse trial).

Genotype	Chromosome number	Number of spikes	Mean infection severity[1]	Std Error
Tobie/W1423X-3-26	42	3	0.15	0.20
Tobie/W1423X-3-21	42	3	0.17	0.20
Tobie/W1423X-3-19	43	1	0.27	0.34
Tobie/W1423X-3-7	42	3	0.36	0.20
W1423X-3[2]	42	41	0.49	0.05
Tobie/W1423X-3-23	42	2	0.52	0.24
Tobie/W1423X-3-12	42	2	0.54	0.24
Tobie/W1423X-3-13	42	2	0.56	0.24
Rex		7	0.58	0.13
Tobie/W1423X-3-14	42	6	0.58	0.14
Tobie/W1423X-3-5	42	1	0.62	0.34
Tobie/W1423X-3-9	42	1	0.63	0.34
Tobie/W1423X-3-6	42	2	0.63	0.24
Tobie/W1423X-3-2	42	7	0.69	0.13
Tobie/W1423X-3-17	43	5	0.72	0.15
Tobie/W1423X-3-4	43	6	0.72	0.14
Tobie/W1423X-3-1	42	7	0.75	0.13
Tobie/W1423X-3-16	43	4	0.76	0.17
Tobie		16	0.84	0.09
Tobie/W1423X-3-15	42	6	0.86	0.14
Tobie/W1423X-3-22	42	5	0.89	0.15
Tobie/W1423X-3-20	42	8	0.90	0.12
Tobie/W1423X-3-10	42	3	0.92	0.20
Tobie/W1423X-3-28	42	3	0.92	0.20
Tobie/W1423X-3-8	42	3	0.94	0.20
Tobie/W1423X-3-11	42	1	1.00	0.34
Tobie/W1423X-3-18	43	2	1.00	0.24
Tobie/W1423X-3-24	42	4	1.00	0.17
Tobie/W1423X-3-25	42	2	1.00	0.24
Tobie/W1423X-3-3	42	1	1.00	0.34
Tobie/W1423X-3-27	42	1	1.00	0.34

[1] Means are ordered from the lowest to the highest.
[2] Segregating progeny derived from plant W1423X-3

F_1 plants 26 and 21 (Table 3.4) were used for a further backcross to Tobie and for making a cross with Inia 66. Tao (2019) reported that W1423X progeny had high levels of aneuploidy that resulted from aberrant segregation of chromosome 7A and another unknown chromosome that was believed to carry the resistance. The transmission of resistance to progeny was generally low. Plants 26 and 21 were chosen for crosses based on their low infection severities; however, with the small number of spikes that could be inoculated per F_1 plant, it was not clear whether a resistance QTL did in fact segregate among the F_1. Since FHB resistance has polygenic inheritance and it is strongly influenced by the environment, single plant evaluation without the aid of markers is unreliable. Furthermore, the expression of resistance in the F_1 could have been reduced in the presence of a Tobie (highly susceptible) genome.

Secondary spikes on the two most resistant F_1: W1423X-3/Tobie plants were emasculated and fertilized with either Tobie or Inia 66 pollen. The hybrid seeds thus produced were again evaluated for somatic chromosome number and FHB resistance in greenhouse trial 2 that was conducted in the spring of 2019. In the latter trial, 30 B_1F_1: Tobie*2/W1423X-3 plants with 2n = 42 were grown with Inia-66 and Tobie control plants and inoculated with FHB. A minimum of five spikes were inoculated per plant. An analysis of variance and mean infection severity data relevant to the latter trial are given in Tables 3.6 and 3.7. The ANOVA F-test showed that there were no significant differences in mean disease severity among Tobie, Inia and the Tobie backcrosses. This would suggest that either the F_1: Tobie/W1423X plant that was pollinated in order to produce the backcross seeds lacked the resistance gene, or, the highly susceptible Tobie background did not allow for proper discrimination of the resistance QTL. Alternatively, it is possible that the resistance assessments based on single plants were simply not repeatable enough to discriminate among plants. In a final attempt to identify resistant,

preferably homozygous triticale segregates in the material, either F_2 or selfed seeds of (a) W1423X-3/Tobie; (b) W1423X/Tobie and (c) W1423X along with other translocation genotypes were included in a misted field trial to test for FHB resistance. At maturity spikes were selected that showed reduced infection. Seeds from such plants were again tested for FHB resistance in a December 2019 greenhouse trial.

Table 3.6. Results of a one-way ANOVA to compare FHB type II infection severity of 30 B_1F_1: Tobie*2/W1423X-3 plants plus two controls that were evaluated in a greenhouse experiment conducted in the spring of 2019.

SOV	**DF**	**SS**	**MS**	**F Ratio**
Entries	31	1.82	0.05	0.98
Error	154	9.17	0.05	
Total	185	11.00		

SOV = source of variance; DF = degrees of freedom; SS = sum of squares, and MS = mean squares

Table 3.7. Average FHB type II infection severity of 30 B_1F_1 plants plus two controls that were evaluated in a greenhouse experiment conducted in the spring of 2019.

Entry	Pedigree	Chromosome No.	No. of Spikes inoculated	Mean infection severity[1]	Std Error
18M11-5	B1F1: Tobie*2/W1423X-3	42	6	0.58	0.1
18M11-57	B1F1: Tobie*2/W1423X-3	42	5	0.72	0.11
18M11-43	B1F1: Tobie*2/W1423X-3	42	7	0.74	0.09
18M11-49	B1F1: Tobie*2/W1423X-3	42	6	0.75	0.1
18M11-42	B1F1: Tobie*2/W1423X-3	42	5	0.76	0.11
18M11-61	B1F1: Tobie*2/W1423X-3	42	5	0.77	0.11
18M11-6	B1F1: Tobie*2/W1423X-3	42	6	0.8	0.1
18M11-44	B1F1: Tobie*2/W1423X-3	42	5	0.84	0.11
18M11-63	B1F1: Tobie*2/W1423X-3	42	5	0.85	0.11
18M11-46	B1F1: Tobie*2/W1423X-3	42	6	0.86	0.1
18M11-48	B1F1: Tobie*2/W1423X-3	42	7	0.86	0.09
18M11-2	B1F1: Tobie*2/W1423X-3	42	6	0.87	0.1
18M11-38	B1F1: Tobie*2/W1423X-3	42	5	0.88	0.11
18M11-52	B1F1: Tobie*2/W1423X-3	42	6	0.9	0.1
18M11-64	B1F1: Tobie*2/W1423X-3	42	5	0.9	0.11
18M11-3	B1F1: Tobie*2/W1423X-3	42	7	0.91	0.09
Inia 66			5	0.92	0.11
18M11-51	B1F1: Tobie*2/W1423X-3	42	6	0.92	0.1
18M11-54	B1F1: Tobie*2/W1423X-3	42	5	0.92	0.11
18M11-65	B1F1: Tobie*2/W1423X-3	42	6	0.92	0.1
18M11-47	B1F1: Tobie*2/W1423X-3	42	5	0.93	0.11
18M11-55	B1F1: Tobie*2/W1423X-3	42	6	0.93	0.1
Tobie			6	0.94	0.1
18M11-41	B1F1: Tobie*2/W1423X-3	42	8	0.94	0.09
18M11-7	B1F1: Tobie*2/W1423X-3	42	6	0.97	0.1
18M11-50	B1F1: Tobie*2/W1423X-3	42	7	0.98	0.09
18M11-4	B1F1: Tobie*2/W1423X-3	42	6	1	0.1
18M11-39	B1F1: Tobie*2/W1423X-3	42	5	1	0.11
18M11-40	B1F1: Tobie*2/W1423X-3	42	5	1	0.11
18M11-53	B1F1: Tobie*2/W1423X-3	42	5	1	0.11
18M11-56	B1F1: Tobie*2/W1423X-3	42	6	1	0.1
18M11-62	B1F1: Tobie*2/W1423X-3	42	7	1	0.09

[1] Means are ordered from the lowest to the highest.

Transfer of the W1423X FHB resistance to Inia-66

The somatic chromosome numbers of 22 F_1: Tobie/W1423X-3//Inia hybrid seeds were determined. Of these, 17 seedlings were 2n = 42; four were 2n = 41 and one had 2n = 43 chromosomes. Thirteen of the 2n = 42 chromosome seedlings and one 2n = 41 seedling were planted and tested for FHB resistance in the second greenhouse trial. The results are summarized in Tables 3.8 and 3.9

Table 3.8. Analysis of variance of FHB type II infection severity data with respect to 14 F_1 (Tobie/W1423X-3//Inia) plants plus two controls that were evaluated in a greenhouse experiment.

SOV	**DF**	**SS**	**MS**	**F-ratio**
Entries	15	6.92	0.46	4.80*
Error	84	8.07	0.09	
Total	99	15.00		

SOV = source of variance; DF = degrees of freedom; SS = sum of squares, and MS = mean squares
*indicates significance at the 0.05 level

Table 3.9. Average FHB type II infection severity of 14 F_1 (Tobie/W1423X-3//Inia) plants plus two controls that were evaluated in a greenhouse experiment.

Entry	Pedigree	Chromosome no.	No. of Spikes inoculated	Average severity [1]	Std Error	Significance[2]
18M13-72	F1: Tobie/W1423X-3//Inia	42	5	0.13	0.14	D
18M13-82	F1: Tobie/W1423X-3//Inia	42	7	0.21	0.12	D
18M13-75	F1: Tobie/W1423X-3//Inia	42	7	0.25	0.12	CD
18M13-71	F1: Tobie/W1423X-3//Inia	42	6	0.28	0.13	BCD
18M13-74	F1: Tobie/W1423X-3//Inia	41	5	0.43	0.14	ABCD
18M13-67	F1: Tobie/W1423X-3//Inia	42	6	0.54	0.13	ABCD
18M13-83	F1: Tobie/W1423X-3//Inia	42	8	0.62	0.11	ABCD
18M13-84	F1: Tobie/W1423X-3//Inia	42	8	0.70	0.11	ABCD
18M13-68	F1: Tobie/W1423X-3//Inia	42	5	0.70	0.14	ABCD
18M13-73	F1: Tobie/W1423X-3//Inia	42	9	0.70	0.10	ABCD
18M13-70	F1: Tobie/W1423X-3//Inia	41	5	0.81	0.14	ABCD
18M13-66	F1: Tobie/W1423X-3//Inia	42	7	0.81	0.12	ABC
18M13-69	F1: Tobie/W1423X-3//Inia	42	6	0.84	0.13	ABC
Inia			5	0.92	0.14	ABC
Tobie			6	0.94	0.13	AB
18M13-58	F1: Tobie/W1423X-3//Inia	42	5	1.00	0.14	A

[1] Means are ordered from the lowest to the highest.
[2] Averages not connected by same letter are significantly different based on Tuckey HSD test.

The one-way ANOVA and Student's t-tests showed significant differences among the trial entries. Both Tobie and Inia 66 proved to be highly susceptible; however, some of the F_1, notably 18M13-72, 18M13-82, 18M13-75 and 18M13-71 were significantly more resistant than Inia 66, Tobie, and eight of the F_1 plants. Assuming that these four plants were the only resistant heterozygotes, a chi-square test was done. The results suggested that such a deviation from 1:1 segregation among 14 plants had a probability of 0.108. Although this cut-off point was chosen arbitrarily, it appears likely that the resistance could have been present in the population.

Unfortunately, the plants of this cross were highly sterile and also showed hybrid necrosis. With respect to the fourteen plants, only 1-2 seeds were collected from each plant and a maximum of four seeds were collected from one plant. The hybrid necrosis resulted in dark discoloration on the stems and the plants typically had hard/tough spikes. It is not clear if, and to what extent, the hybrid necrosis affected the FHB symptoms.

All of the F_1 were pollinated with Inia 66 and similarly very few hybrid seeds were obtained. Among the four likely resistant plants, one B_1F_1 seed was produced by each of plants 71, 72 and 75, whereas plant 82 produced three hybrid seeds. In addition to these, plant 71 produced one F_2 seed and 82 produced three. Thus, six B_1F_1 and five F_2 seeds were available for continued testing and backcrossing. Three of the viable F_2 seeds had respectively 39+t, 43 and 43 somatic chromosomes. Both of the two backcross F_1 seeds that were viable had 40 somatic chromosomes. When transplanted to a greenhouse the plants turned out to have poor vigour and showed strong hybrid necrosis. No viable selfed or hybrid seed could be produced.

The results obtained with the W1423X-3 derivatives that were evaluated in the field trial, are summarized in Figures 3.4 and 3.5, and Table 3.10 and 3.11 . From the data it is evident that there are no obvious, significant differences among the various W1423X-derived lines and cross progenies. It is known from earlier results (Tao, 2019) that the transmission of the resistance is low and irregular and that the W1423X lineage is characterized by high levels of aneuploidy. Combined with the fact that FHB resistance QTL are known to give only partial resistance and are strongly affected by the environment and genetic background (Bai et al., 2018; Brar et al., 2019) it can be expected that it will be hard to derive resistance-carrying lines. In the absence of a pure-line source of the resistance, the study relied on the identification of single, apparently

resistant plants from segregating populations that were selected in field FHB resistance evaluations.

Table 3.10. Analysis of variance on mean disease index of FHB field nursery, 2019.

SOV	DF	SS	MS	F Ratio[1]
Genotype	16	118831.37	7426.96	13.83***
Replication	4	7660.49	1915.12	3.56**
Error	64	34354.92	536.80	
Total	84	160846.78		

SOV=source of variance, DF=degree of freedom, SS=sum of squares, and MS=mean squares
[1]*** represents significance at 0.001 level, ** represents significance at 0.01 level.

Table 3.11. Analysis of variance on mean disease severity of FHB field nursery, 2019.

SOV	DF	SS	MS	F Ratio[1]
Genotype	16	27722.61	1732.66	18.99***
Replication	4	1036.86	259.21	2.84*
Error	65	5836.80	91.20	
Total	85	34595.70		

SOV=source of variance, DF=degree of freedom, SS=sum of squares, and MS=mean squares
[1]*** represents significance at 0.001 level, * represents significance at 0.05 level.

Five plants each from W1423X-3 and Tobie/ W1423X-3, four plants from W1423X-21 and one from Tobie/W1423X were selected from the field trial and tested in the final greenhouse trial. Nine to twelve plants were screened per line. Analysis of variance and mean disease severities are shown in Tables 3.12 and 3.13. There were significant differences in the disease severity of the individual plants (progeny) selected in the field trial (Fig. 3.6). The mean disease severity of the sub set of selections from line W1423X-3 varied from 0.43 to 0.74 with the W1423X-3-2 and W1423X-21-2 progenies appearing to be less susceptible. Mean disease severity of the progeny selected from the Tobie/W1423X-3 and Tobie/W1423X crosses were very high, which could be due to the FHB susceptible Tobie genetic background. Mean disease

severities of the W1423X-21 selections varied from 0.44 to 0.88. These results suggest that the best selections from the W1423X lineage should be tested further.

Table 3.12. Analysis of variance on mean disease severity of FHB greenhouse trial, 2019.

SOV	DF	SS	MSS	F Ratio[1]
Genotype	14	4.04	0.28	4.70*
Error	144	8.84	0.06	
Total	158	12.89		

SOV=source of variance, DF=degree of freedom, SS=sum of squares, and MS=mean squares
[1*] represents significance at 0.05 level of confidence.

Table 3.13. Average FHB type II infection severity of 15 field-selected progeny retested in a greenhouse trial, 2019.

Genotype	Number of inoculated plants	Mean	Std Dev	Std Err Mean
Tobie/W1423X-1	11	0.89	0.15	0.04
Tobie/W1423X-3-1	11	0.84	0.15	0.05
Tobie/W1423X-3-2	12	0.66	0.18	0.05
Tobie/W1423X-3-3	11	0.82	0.20	0.06
Tobie/W1423X-3-4	9	0.66	0.28	0.09
Tobie/W1423X-3-5	11	0.89	0.10	0.03
W1423X-3-1	9	0.48	0.37	0.12
W1423X-3-2	11	0.41	0.32	0.10
W1423X-3-3	9	0.64	0.24	0.08
W1423X-3-4	11	0.71	0.29	0.08
W1423X-3-5	10	0.74	0.23	0.07
W1423X-21-1	12	0.64	0.30	0.08
W1423X-21-2	11	0.44	0.23	0.07
W1423X-21-3	11	0.50	0.27	0.08
W1423X-21-4	10	0.87	0.30	0.09

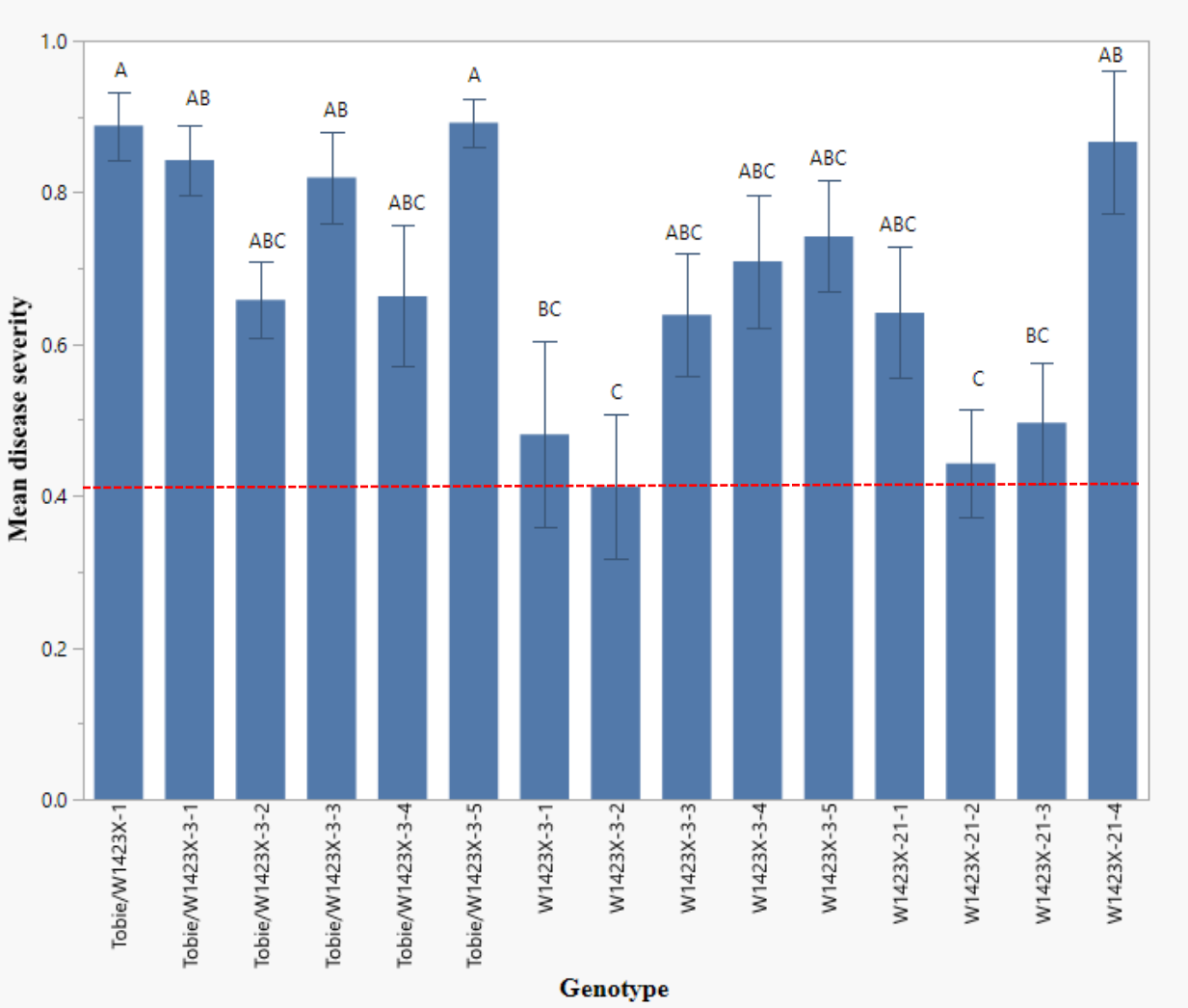

Figure 3.6. Fusarium head blight infection severity (%) of 15 field selected progeny. The bar on each column indicates the size of the standard deviation; Same letters on the bars suggests no significant differences at $P<0.05$. Lowest mean disease severity of field selected plant progeny is indicated with a red broken line.

Tritipyrum line 16M1001

The resistance results obtained with respect to 16M1001 in the greenhouse trial are given in Tables 3.2 and 3.3 and reflected moderate resistance. Contrary to the greenhouse results, the addition lines were highly susceptible in the field trial. The least mean square disease severities and disease indices for the genotypes in the FHB field nursery are shown in Figs. 3.4 and 3.5, respectively. The low levels of resistance were probably largely due to the short stature of the

tritipyrum lines which provided a more conducive micro-climate for infection as well pathogen development (Lu et al., 2013). Also, the tritipyrum plants have a much longer grain-filling period than wheat and triticale which might have prolonged the opportunity for infection and disease development.

Conclusion

Previous results suggested the presence of FHB resistance in certain triticale X *Thinopyrum distichum* wide hybrid progenies. These sources were of three types: (i) A *Th. distichum* disomic addition chromosome set in triticale included two addition lines with telosomic chromosome additions that appeared to derive from *Th. distichum*. These additions showed elevated FHB resistance in greenhouse trials, yet were poorly characterized. (ii) A tritipyrum line was selected from a naturally infected field plot that appeared to be more FHB resistant than the other lines included in the trial. (iii) A secondary triticale X *Th. distichum* hybrid lineage (W1423X) had approximately 42 chromosomes and showed meiotic irregularity that stemmed from abnormal segregation of two unknown triticale chromosomes. The lineage included a low frequency of plants with apparently strong FHB resistance that was suspected to be from a *Th. distichum* translocation. In parallel with the FHB evaluation trials (three greenhouse trials and one field trial) attempts were initiated to also transfer the addition chromosome in line W1450 and the FHB resistance in lineage W1423X to hexaploid wheat. The study failed to provide conclusive evidence of FHB resistance in the material and was complicated by the fact that genetically dissimilar single plants needed to be evaluated and selected for FHB resistance. Such single plant evaluations proved unreliable and the very susceptible genetic backgrounds (Inia 66 and Tobie) that were chosen for backcrosses further complicated selection. The extra chromosome in addition lines W1450 and W1451 appeared to

provide FHB resistance but turned out to be of rye origin. Marais et al. (2020) associated a small number of seemingly *Th. distichum*-specific DNA sequences with both W1450 and W1451. This suggests that a small translocation could occur on the addition chromosome, which GISH might have failed to identify. While the telosome was successfully transferred to wheat, it is unlikely to find further application. The tritipyrum line 16M1001 showed only intermediate to low resistance in follow-up trials. To evaluate it properly, further interbreeding to obtain earlier maturing plants of taller stature will be necessary. Study of the W1423X lineage proved inconclusive as well. While the possibility of strong FHB resistance in some plants cannot be ruled out, stable 2n = 42 progeny that breed true for the resistance has not yet been obtained. Two promising selections from the field experiment were re-tested in the final greenhouse experiment. Nine field-selected lines derived from plant W1423X had FHB disease severity values that ranged from 0.41 to 0.87. Since plant W1423X was highly inbred, the broad range in FHB resistance that was observed in its progeny is unexpected and could reflect the continued irregular segregation of a major FHB resistance factor. Progeny of plants W1423X-3-2 and W1423X-21-2 could be evaluated further in an attempt to derive a cytological stable line that breeds true for the resistance.

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