TRANSFER OF FUSARIUM HEAD BLIGHT RESISTANCE TO HARD RED WINTER WHEAT

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Fusarium head blight (FHB; caused by *Fusarium graminearum* Schwabe) is a serious disease of hard red winter wheat (HRWW) in North Dakota. Current varieties are lacking in resistance. Many resistance quantitative trait loci (QTL) were discovered in spring wheat that can be employed in HRWW. The North Dakota State University breeding program aims to acquire and pyramid useful FHB resistance QTL into its breeding population. Therefore, hybrid populations derived from CM82036 (*Fhb1* and *Qfhs.ifa-5A*), PI277012 (*Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*), Frontana (*Qfhs.ifa-3A*), and TA5660 (*Fhb6*) were tested for type II resistance to: derive new FHB resistant HRWW lines with pyramided QTL (*Fhb1*+) using molecular marker-assisted selection; evaluate the ability of the different QTL to complement *Fhb1*. A second project objective was to assess FHB resistance in *Thinopyrum distichum* (wheat wild relative), random *Thinopyrum* single chromosome additions to wheat and triticale, and a triticale-*Th. distichum* secondary hybrid population segregating for a small translocation from *Th. distichum*.

Useful winter wheat lines carrying *Fhb1* with 1-2 additional resistance QTL were developed; however, transfer of *Qfhb.rwg-5A.2* was not completed as it was not in the spring wheat intermediate, RWG21. Loci *Qfhs.ifa-5A* and *Qfhb.rwg-5A.1* are probably alleles of the same locus with similar additive effects relative to *Fhb1* on the overall resistance. Addition of *Qfhs.ifa-3A* to *Fhb1* plants resulted in no convincing improvement in type II resistance. Combining either *Qfhs.ifa-5A* or *Fhb6* with *Fhb1* improved type II resistance; however, pyramiding of three QTL did not give further symptom reduction. No associated, detrimental phenotypic and yield effects were detected in a greenhouse assessment of the alien-derived *Fhb6* resistance. The pyramids will be used in crosses to initiate full integration of the new QTL in the

breeding germplasm. A select group of pyramids will be evaluated in field trials to better assess the full resistance.

Strong FHB resistance was found in triticale-*Th. distichum* addition lines and secondary hybrid population (W1423). However, the W1423 lineage showed a high incidence of aneuploidy ascribable to aberrant segregation of chromosome 7A and an unknown chromosome. It was not possible to select a translocation homozygote or to identify the translocation chromosome.

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CHAPTER I. GENERAL INTRODUCTION

Rational and Significance

Several Fusarium species cause symptoms of *Fusarium* head blight (FHB); however, *Fusarium graminearum* is the predominant causal agent of FHB in most areas of the world. The fungus attacks developing heads of small grains directly and can result in serious yield loss and reduced grain quality. Losses are compounded by mycotoxins produced in the diseased grain. As hard red winter wheat (HRWW) (*Triticum aestivum* L.) accounts for about 40% of the total U.S. wheat production, breeding of FHB resistant varieties will increase and stabilize production, reduce health risks to humans and domestic animals, improve wheat seed quality, and thus, provide economical, ecological, and social benefits.

Currently available and suitably cold-hardy HRWW germplasm in North Dakota has a narrow genetic base and is seriously lacking in disease resistance, particularly with respect to FHB. Many FHB resistance quantitative trait loci (QTL) were discovered in spring wheat that can also be employed in HRWW. Recently, the HRWW breeding program at North Dakota State University (NDSU), transferred the FHB resistance QTL, *Fhb1*, *Qfhs.ifa-5A*, and *Qfhb.rwg-5A.1*, from spring wheat to winter wheat. Since these QTL confer only partial resistance to FHB it is necessary to continue to acquire and transfer additional restance genes.

This study had two main objectives: Firstly, the NDSU HRWW breeding program decided to introduce additional QTL for FHB resistance from spring wheat. The targeted loci included *Fhb1*, *Qfhs.ifa-5A* (ex CM82036), *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* (ex PI277012), *Qfhb.ifa-3A* (ex Frontana) and *Fhb6* (ex TA5660), which has an *Elymus tsukushiensis* translocation). One purpose of this project was to assemble subsets of the QTL into HRWW gene pyramids using molecular markers and to evaluate these pyramids (initially greenhouse and

eventually in the field) for their potential to increase resistance levels in new varieties. The multigenic nature of FHB resistance and low repeatability of biotest results necessitated strong reliance on the use of molecular markers to achieve QTL transfer and pyramiding. For the same reasons, validation of marker-pyramided gene combinations in greenhouse tests is only preliminary and actual presence of the targeted QTL needs to be confirmed in ongoing field experiments. A second project objective was to evaluate a FHB resistance QTL found in triticale - *Thinopyrum distichum* secondary hybrids and their derivatives with the ultimate aim to transfer the resistance to common wheat.

Literature Review

The United States ranks fourth in global wheat production, with almost 1,884 million bushels produced in the 2018/2019 growing season (USDA, 2019). The European Union, China and India ranked 1st, 2nd and 3rd, respectively. Wheat is the third most important field crop in the United States. However, the area planted to wheat decreased drastically from about 72.2 million acres in 1992/1993 to 47.8 million acres in 2018/2019. Accordingly, U.S. wheat production decreased from 2,447 million bushels in 1992/1993 to 1,884 million bushels in 2018/2019. 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 35% in the 2018/2019 season. Approximately 40 to 50% of the HRWW produced in the U.S. was exported during the past 2 to 3 decades. Due to reduced production, the export of HRWW decreased to 317 million bushels in 2017/2018 as compared to 559 million bushels in 1991/1992 (USDA, 2019).

Many factors contributed to the decrease in wheat acreage and production. According to a broad survey of American wheat producing areas, FHB resulted in billions of dollars of wheat yield and quality loss in the 1990s and early 2000s (McMullen et al., 1997; McMullen et al.,

2012) and resurfaced as a worldwide threat. In the United States, severe FHB epidemics from 1991–1997 resulted in about $1.3 billion of total direct economic losses to the wheat and barley industries and accumulated losses amounting to $4.8 billion (Johnson et al., 2003). In 1998–2000, FHB infestations led to direct economic losses of approximately $871 million and secondary losses of $1.8 billion in the upper Midwest region of the United States. North Dakota and Minnesota accounted for 55% of the total losses over the period (Nganje et al., 2004). In 2003, forty counties across Maryland, North Carolina, and Virginia experienced wheat yield losses of 28.3, 52.0, and 54.2%, respectively, with the total monetary loss estimated at $13.6 million (Cowger and Sutton, 2005). In 2008, FHB losses were about $57 million in Kansas (McMullen et al., 2012).

Fusarium head blight and plant resistance to FHB

FHB negatively affects all classes of wheat and other small grains. In North America, FHB is caused mainly by *Fusarium graminearum* Schwabe, with occasional involvement of other *Fusarium* species (McMullen et al., 1997). Wheat and barley are susceptible to head infection from the flowering (pollination) period up through the soft dough stage of kernel development. Spores of the causal fungus may land on the exposed anthers of the flower and then grow into the kernels, glumes, or other head parts. Infected plant debris is important for the overwintering of the fungus while infection is favored by continuous high moisture or relative humidity (>90%) and moderately warm temperatures (between 15 to 30 ℃). If such conditions prevail during flowering then inoculum production, floret infection, and colonization of developing grains can occur (Schmale III and Bergstrom, 2003).

Damages done by FHB include lower yield, reduced grain quality, and toxicity of infected grain to livestock and humans (Schröder and Christensen, 1963). Salgado et al. (2015)

reported a significant negative linear relationship between FHB index and grain yield, and thus predicted a yield reduction of 1MT ha^{-1} under 19% FHB infection (index based). In addition to yield, grain quality is also critical for marketing, processing and export of wheat (McMullen et al., 1997). FHB infection reduced test weight (Salgado et al., 2015), and very importantly, elevated mycotoxin concentration in the grain (Bai and Shaner, 2004). Many *Fusarium* species (including *F. graminearum*) produce mycotoxins that are harmful to animals. The major mycotoxin produced by *F. graminearum* in wheat and barley is deoxynivalenol (DON). DON is also called vomitoxin because of its deleterious effects on the digestive system of swine and other monogastric animals. DON concentrations higher than 4-5mg kg^{-1} in pig feed rations can cause rejection of feedstuff intake and fertility disorders (Rotter et al., 1996). DON disrupts normal cell and organ function by inhibiting protein synthesis and organ functioning (Pestka et al., 1989). Humans consuming wheat flour made from DON contaminated grain will often demonstrate symptoms of nausea, fever, headaches, and vomiting (Sobrova et al., 2010). Maximum allowable DON concentration in wheat grain and products for human consumption ranges from 0.2 to 2.0 mg/kg worldwide (Bai and Shaner, 2004; Shaner, 2003).

Plant resistance to FHB may involve passive and active mechanisms (Mesterházy, 1995). Passive resistance, i.e. avoidance or escape from infection at the most susceptible stage, is the result of morphological features (Wiese, 1985) like tall plant height, absence of awns, low spikelet density within a spike, and flowering in the booting stage (escape) (Mesterházy, 1995). Active resistance results from physiological processes (Crute et al., 1985). To date, five types of active FHB resistance are recognized: Type I resistance is to initial pathogen penetration (Schröder and Christensen, 1963); type II resistance is to infection symptom spread within a spike (Schröder and Christensen, 1963); type III resistance is to kernel damage (Mesterházy,

1995); type IV resistance is to DON accumulation (Miller et al., 1985); and type V resistance is tolerance (Mesterházy, 1995). Type I resistance is measured as incidence under natural infection and is the calculated percentage of initially infected spikes in the field. It is relatively difficult to discriminate type I and type II resistance under field conditions (Burt et al., 2015). Type II resistance is measured as disease infection severity, which can be calculated as the percentage of infected spikelets in a spike (Bai and Shaner, 1994). Single floret (spikelet) inoculation (inoculum is injected into a central spikelet of a spike at anthesis) under greenhouse conditions (Stack, 1989), or grain-spawn inoculation in the field (Bai and Shaner, 1994), are commonly used for the evaluation of type II resistance. Type III resistance is reflected by the amount of *Fusarium* damaged kernels (FDK), which can be visualized as shriveled, lightweight, and chalky white kernels (Cuthbert et al., 2006). Individual grain samples are rated for the percentage of FDK using a 1 to 9 scale (Miedaner et al., 2006). Type IV resistance can be directly evaluated by measuring DON concentration in the grain, using chromatographic methods (Schollenberger et al., 1998), immunoassays (Hart et al., 1998) or other indirect methods (Abramson et al., 1998). A very high correlation was obtained between infection severity and FDK, and between FDK and DON under greenhouse conditions (Jin et al., 2014; Miedaner et al., 2004). However, others reported poor or no correlation between infection severity and DON contents (Mesterházy et al., 1999). To date, type II resistance is the most commonly used criterion due to its better repeatability and easier assessment compared with other types (Bai and Shaner, 2004).

The use of specific cultivation practices has been proposed as a mean to lessen the impact of FHB. Crop rotation is an effective manner in which to reduce or eliminate sources of primary inoculum (McMullen and Luecke, 1996). Fungicide application to seed before seeding or during heading at anthesis (Mesterházy, 2003) can also serve to reduce the impact of FHB. Considerable

improvement in genetic resistance of varieties has been achieved through conventional breeding and selection. Compared to the impact of cultivation practices and fungicides, growing resistant cultivars has proved to be the most effective and environment friendly approach to minimizing the damage caused by the disease (Bai and Shaner, 2004; McMullen et al., 2012). However, selection for a trait such as FHB resistance is relatively difficult and cost intensive due to its quantitative inheritance. Being quantitatively inherited, FHB resistance shows continuous distribution among the progeny of parents that differ in resistance (Bai and Shaner, 1994; Snijders, 1994). Dominant resistance QTL and modifier genes that can alter the expression of resistance QTL have been noted. Additive effects of different resistance QTL occur and make it possible to accumulate (pyramid) resistance genes in a single genotype in order to further improve overall host resistance (Bai and Shaner, 1994; Snijders, 1994). Numerous QTL mapping studies have been completed, and many FHB resistance sources and QTL have been found in the past years (Bai et al., 2018; Buerstmayr et al., 2009), which facilitates resistance breeding.

Fusarium head blight resistant germplasm

Wheat gene pools

Harlan and de Wet (1971) proposed a logical germplasm classification with respect to cultivated crops in which the total available germplasm is assigned to primary, secondary and tertiary gene pools, based on their accessibility. Members of the primary gene pool (GP-1) correspond to the traditional concept of the biological species. Members of this gene pool are probably of the same species and can inter-mate freely. GP-1 includes both spontaneous races (wild and/or weedy) and cultivated races. Within GP-1, crossing is easy, hybrids are generally fertile with good chromosome pairing, and gene transmission is approximately normal. The transfer of genes from a member of the primary gene pool to the cultivated crop is generally easy.

The secondary gene pool (GP-2) is normally composed of different species than the crop species in GP-1. However, these species are relatively closely related to the cultivated crop. Gene transfer from GP-2 to the cultivated crop is possible, but difficult. Hybrids of GP-2 species and the crop species may be sterile, weak or inviable or show poor/no chromosome pairing during meiosis. Thus, recovery of desired traits in advanced generations of crosses may be difficult. GP-2 can be used as a source of genes for the improvement of the cultivated crop, but hybridization and transfer may be difficult, requiring great effort. The tertiary gene pool (GP-3) comprises species at the extreme outer limit of the potential gene pool of a crop, i.e. wild relatives distantly related to GP-1. Crosses can be made between GP-3 species and the crop, but the hybrids tend to be anomalous, inviable or completely sterile. Gene transfer is either not possible or require specific techniques, i.e. embryo culture or grafting to obtain hybrids, doubling of the chromosome number or using bridging species to obtain some fertility.

A large gene pool indicates extensive genetic diversity, while low genetic diversity can reduce biological fitness and increase the risk of extinction (Frankham, 2005). Although wheat is a genetically narrow species throughout its entire existence (Cox, 1997), it has a very large GP-2 and a substantial GP-3 (Harlan and de Wet, 1971). Wheat GP-1 includes the hexaploid landraces, cultivated tetraploids, wild *T. dicoccoides*, and diploid donors of the A and D genomes of hexaploid wheat (Chaudhary et al., 2014; Qi et al., 2007). Wheat GP-2 includes several hundred polyploid *Triticum* and *Aegilops* species, which share one genome among the three genomes of wheat (Chaudhary et al., 2014). Wheat GP-3 includes cultivated and wild relatives with non-homologous genomes to wheat, i.e. *Secale cereale* (RR), *Elymus* ssp., *Thinopyrum* ssp., etc. (Chaudhary et al., 2014).

Useful GP-1 germplasm for wheat FHB resistance breeding

The majority of the wheat FHB resistant germplasm is of Asian origin. Spring wheat Sumai3 and its derivatives (such as Ning7840 and CM82036) confer strong FHB resistance (Anderson et al., 2001; Buerstmayr et al., 2003) and have superior agronomic traits compared to other resistance sources (Bai and Shaner, 2004), and thus, became major materials for FHB resistance breeding worldwide. The Chinese landrace, Wangshuibai, has a high level of FHB resistance (Ma et al., 2006), but is associated with undesirable agronomic traits (Bai and Shaner, 2004). Other Chinese (Baishanyuehuang, Huangcandou and Huangfangzhu) and Japanese landraces (Nobeokabozu and NyuBai) have different levels of FHB resistance, but poor agronomic performance (Bai et al., 2018; Yu et al., 2008; Zhang et al., 2012). The associated poor agronomic performance of the latter sources limited their application in breeding programs.

Native FHB resistance was found in locally adapted wheat germplasm from South America, the United State and Europe. Brazilian spring wheat Frontana provides moderate native resistance to FHB and was successfully applied in many breeding programs (Mardi et al., 2006; Schröder and Christiansen, 1963). In the United States, cultivars Ernie (McKendry et al., 1995), Freedom (Gooding et al., 1997) and Roane (Griffey et al., 2001) exhibit different degrees of FHB resistance. In Europe, cultivars Arina (Ruckenbauer et al., 2001), Renan (Gervais et al., 2003) and Fundulea 201R (Ittu et al., 2001) were identified as native FHB resistance sources. These sources may carry resistance genes different from the Asian sources (Jin et al., 2013), and are good candidates to pyramid with FHB resistance QTL from Asian sources.

To date, more than 250 QTL for FHB resistance have been reported in wheat (Peterson et al., 2016), many of which have been detected in more than one mapping population. After summarizing 52 publications, Buerstmayr et al. (2009) confirmed 22 reliable QTL regions in the

A (6), B (11) and D (5) genomes. These occur on two regions in chromosome 1B, one region in 1D, two regions in each of 2A, 2B and 2D, one region in 3A, two regions in 3B, one region in each of 3D, 4B, 4D, 5A, 5B, 6A, 6B, and 7A and two regions in 7B.

FHB resistance from GP-2 and GP-3

Due to its recent origin, common wheat has a narrow genetic base, which prompted numerous attempts to enrich and broaden genetic diversity (Cai et al., 2005; Jiang et al., 1993). Both close (GP-2) and distant relatives (GP-3) of wheat were targeted for gene discovery and transfer. Many alien-derived genes were applied to wheat breeding programs worldwide. A study of the diploid wheat relative *Aegilops tauschii* identified many accessions that confer high levels of FHB resistance (Brisco et al., 2017). Japanese indigenous species of the genus *Elymus* were demonstrated to have strong resistance to FHB (Ban, 1997). The perennial grass *Elymus tsukushiensis* Honda exhibited strong FHB type I and type II resistance (Weng and Liu, 1989). It was also reported that *Elymus gigantus* L. has strong FHB resistance (Mujeeb-Kazi et al., 1983; Wang et al., 1986). Perennial wheatgrass species of the genus *Thinopyrum* are also rich sources that confer disease resistance (Turner et al., 2013). FHB resistance was found in *Th. ponticum* (Kim et al., 1993), *Th. elongatum* (Jauhar, 2008), *Th. intermedium* (Cai et al., 2005; Oliver et al., 2005), *Th. junceiforme* (Jauhar and Peterson, 2011), and *Th. distichum* (Chen et al., 2001). All these germplasms are potentially valuable source materials for wheat FHB resistance breeding.

Introgression of alien *Fusarium* head blight resistance to wheat

Common wheat has limited genetic variability due to its short evolutionary existence, and its genetic variation was further reduced by domestication (Cox, 1997). Worldwide, only limited FHB resistance was discovered in the common wheat gene pool, which restricted the improvement of wheat FHB resistance through breeding (Cai et al., 2005). Therefore, increasing

efforts are being made to explore species in GP-2 and GP-3 in order to enrich and broaden the genetic diversity of cultivated wheat (Cai et al., 2005; Jiang et al., 1993).

Hexaploid wheat has three distinct but genetically related sub-genomes (A, B and D). Homoeologous chromosomes in the three sub-genomes can genetically compensate for each other. Thus, it possible to incorporate alien chromatin containing a target gene into the wheat genome through chromosome manipulation or chromosome engineering (Cai et al., 2005; Sears, 1972). Chromosome engineering is a process that employs a range of cytogenetic and marker techniques to modify ploidy, chromosome number, or chromosome structure with the ultimate purpose to incorporate and tailor a small segment of targeted alien chromatin. The initial transfer of alien chromatin from wild species into wheat is achieved through the development of chromosome addition, substitution, and translocation lines (Zhang et al., 2017).

Chromosome pairing

Proper chromosome pairing (synapsis) and recombination are prerequisites for accurate segregation of homologous chromosomes during meiosis. Several genes are essential for encoding proteins required for synapsis (Hamant et al., 2006; Lew and Burke, 2003). Mutation of these genes cause a-synapsis and de-synapsis. A-synapsis leads to complete failure of homologous chromosome pairing during the first meiotic division. De-synapsis occurs after the onset of prophase and results in failure to maintain the association of homologous chromosomes in the subsequent stages of meiosis causing them to dissociate prematurely (Wani and Bhat, 2017). In a-synaptic mutants, the majority exhibit irregular distribution and random dispersion of univalents in the cytoplasm at prophase I and metaphase I. These univalents never congregate at the equatorial plate during metaphase I. In the case of de-synaptic mutants, bivalents and univalents were oriented at the equatorial plate during metaphase I (Peirson et al., 1997). Univalents either get lost or are randomly transmitted to daughter cells, resulting in

chromosomally unbalanced gametes and eventually aneuploids in the offspring. In addition, univalents may undergo misdivision (transverse division, produce telocentric, acrocentric, acentric chromosomes and/or isochromosomes) (Friebe et al., 2005; Sears, 1952). All these abnormal meiotic events associated with a-synapsis and de-synapsis induce variations in chromosome structure and number (Wani and Bhat, 2017).

Wheat monosomics

Aneuploids may arise spontaneously in wheat when the members of a bivalent fail either to pair (a-synapse) or separate prematurely (de-synapse) during meiosis (Morris and Sears, 1967; Sears, 1954). A low level of meiotic irregularity may occur in stable varieties; however, certain varieties/ chromosomes may behave less stably than normal, giving rise to a higher frequency of a- or de-synapsis. Aneuploid gametes (*n*-1 or *n*+1 chromosomes) or euploid gametes (*n*) may result, giving rise to monosomics (2*n*-1) (most frequently) and trisomics (2*n*+1). Monosomics are available for each of the wheat chromosomes and have been extensively used for gene mapping and transfer. In a monosomic common wheat plant, the unpaired chromosome gets lost in approximately 75% (average) of female gametes produced. This results in a 75:25 ratio of *n*-1 to *n* gametes. In the male germline, most of the *n*-1 gametes are eliminated after meiosis due to their reduced viability/ competitiveness. This changes the average ratio of *n*-1 to *n* male gametes that will participate in fertilization to about 4:96 (Morris and Sears, 1967). Consequently, following self-pollination, a monosomic plant is expected to produce (on average) 24% disomic: 73% monosomic: 3% nullisomic progeny.

Chromosome engineering

Gene transfer from a GP-3 species to a cultivated crop begins with making a cross. The allo-haploid hybrid is normally completely infertile and its chromosome number needs to be doubled to restore homologous chromosome pairing and thus, fertility. Addition lines can be

developed by backcrossing the allopolyploid hybrid to wheat followed by self-pollination. Rapid loss of unpaired alien chromosomes will result from successive backcrosses to produce aneuploids. Extensive screening of selfed progeny from backcrosses will allow for the identification of random plants with a normal diploid set of wheat chromosomes plus an unknown alien chromosome (O'Mara, 1940). Individual alien chromosome addition lines can be tested for expression of the targeted trait and the homoeologous relationship of the critical alien addition chromosome to wheat chromosomes established. Addition lines are normally not directly suited to commercial production due to the presence of a complete alien chromosome that is home to numerous deleterious genes. It is possible to substitute the alien addition chromosome for a homoeologous wheat chromosome, thus, establishing a substitution line and removing redundant gene copies. While substitution lines are better adapted than addition lines, they are normally not commercially viable either. Both addition and substitution lines are hampered by meiotic and chromosome instability and deleterious linkage drag (Cai et al., 2005).

Thus, physical and genetic methods were applied to induce random chromosome breaks and/or promote homoeologous pairing and recombination (Qi et al., 2007). Instead of transferring the entire chromosome, chromosome translocation integrates only a segment (with the target gene) from alien chromosome into the wheat genome, which is the most effective approach for alien gene introgression (Cai et al., 2005; Friebe et al., 1996; Jiang et al., 1993). A small size translocation in which the alien segment compensates for (is homoeologous to) the lost wheat chromosome segment will have a less/no linkage drag, and thus be more useful to breeders (Friebe et al., 1996; Qi et al., 2007). Compensating translocations are usually the result of meiotic recombination between wheat chromosomes and their wild species homoeologues (Zhang et al., 2017).

Homoeologous pairing is usually inhibited by *Ph1* gene in wheat (Cai et al., 2005). *Ph1* is located on wheat chromosome 5B, and imposes diploid-like chromosome behavior during meiosis, even though the constituent sub-genomes of this hexaploid species are known to be very closely related to one another (Rey et al., 2015). Presence of *Ph1* ensures the integrity of the wheat genome, but prevents the introduction of wild species chromatin into the wheat genome. In the absence of *Ph1*, homoeologous recombination will occur, and thus, homoeologous pairing becomes possible. Therefore, *Ph1* is used in chromosome engineering (Riley and Chapman, 1958). Different types of materials were developed to suppress *Ph1* activity, including *ph1b* deletion mutant (Sears, 1977), the *Ph1* inhibitor gene (Chen et al., 1994), the chromosome 5D(5B) substitution in durum wheat (Klindwirtg et al., 2012), and chromosome 5D(5B) nulli-tetrasomics in common wheat (Feldman, 1988).

Among these, the *ph1b* mutant (a large deletion on wheat chromosome 5BL; Sears, 1977) is widely used to induce meiotic homoeologous pairing and recombination between wheat chromosomes and alien homoeologues (Cai and Jones, 1997). The advantage of using the *ph1b* mutant is that the translocations are not random, but involve the exchange of genetically related material; however, it occurs at very low frequencies between chromosomes of more distantly related genomes (Miller et al., 1994; Rey et al., 2015).

Robertsonian translocations

If a double-monosomic plant is produced which is simultaneously monosomic for an alien addition chromosome and a homoeologous wheat chromosome, Robertsonian translocations can be invoked. Both monosomic chromosomes will remain unpaired during meiotic metaphase I. Since univalents have a tendency to break at the centromeres, random fusion of the broken arms may occur, giving rise to Robertsonian whole arm translocations

(Robertson, 1916). If a compensating Robertsonian translocation is derived it could have direct economic application. The formation of compensating translocations in a double-monosomic plant requires that the derived telocentric chromosomes from opposite arms of homoeologous chromosomes in ana-/telophase I migrate to the same spindle pole followed by the fusion of the broken ends during interkinesis (Qi et al., 2011).

The most successful Robertsonian translocations in wheat improvement are the T1BL·1RS and T1AL·1RS translocations (Mettin et al., 1973; Zeller, 1973). Translocation T1BL·1R#1S carrying *Sr31* (stem rust) and other disease resistant genes such as *Lr26* (leaf rust), *Yr9* (yellow rust) and *Pm8* (powdery mildew) (Friebe et al., 1996). Recently, alien genes such as *Sr44* (Liu et al., 2013) and *Sr52* (Qi et al., 2011) were introgressed into wheat using Robertsonian translocations

Use of irradiation to induce chromosome translocations

Irradiation induces random chromosome breakage and fusion of the broken segments can result in translocation chromosomes (Qi et al., 2007). Ionizing radiation can accomplish the transfer of genetic information from distantly related and hardly pairing species to wheat, even in the presence of the homoeologous-pairing suppressor *Ph1* (Sears, 1993). This technique has been widely applied to wheat for the production of interspecific translocations (Friebe et al., 1991; Knott, 1987; Sears, 1956). Theoretically, irradiation methods can introgress any alien chromosome segment into a wheat chromosome with a relatively higher efficiency (Jiang et al., 1993). However, the majority of translocations caused by irradiation is between non-homoeologous chromosomes, and involves duplications/deficiencies. Therefore, they are genetically non-compensating and most likely to produce undesirable agronomic effects (Qi et al., 2007).

Sears (1956) first transferred leaf-rust resistance (*Lr9*) from *Aegilops umbellulata* Zhuk. to wheat. In this research, plants with an added, derived iso-chromosome for the resistance-carrying arm were X-irradiated prior to meiosis and their pollen was used in crosses with normal untreated plants. Pollen was irradiated in order to improve the chances of obtaining a small translocation to a wheat chromosome (a wheat translocation chromosome was expected to be normally transmitted through pollen, whereas the complete alien chromosome had very low transmission and was virtually excluded from microspores). The frequency of recovery of a desirable translocation was low with only one potentially useful translocation occurring among 6,091 progeny (Sears, 1956). Despite the fact that the translocation carried very useful resistance it was not used commercially due to its non-compensatory nature.

Another leaf rust resistance gene, *Lr19*, was transferred to wheat by Sharma and Knott (1966). In this research, winter wheat Argus (with a complete *Th. ponticum* chromosome 7E substituted for wheat chromosome 7D) was backcrossed to Thatcher. The derivatives of this backcross were subjected to irradiation. This resulted in the translocation line Agatha (T7DS-7DL-7Ae#1L) which proved to be compensating and was associated with improved yield in certain environments.

Important *Fusarium* head blight resistance QTL in wheat breeding

Mapping, cloning and useful markers of Fhb1

Of the reported FHB resistance QTL, *Fhb1* showed the strongest and most stable resistance against FHB infection across multiple wheat genetic backgrounds (Bai et al., 2018), and is therefore being widely applied in breeding programs. *Fhb1* was derived from the Chinese cultivars Sumai3 and Ning7840 (Bai et al., 1999; Cuthbert et al., 2006; Waldron et al., 1999; Zhou et al., 2003) and was mapped to chromosome 3BS by Waldron et al. (1999). This QTL can

also be found in landraces that are not related to Sumai3, such as Wangshuibai (Ma et al., 2006; Yu et al., 2008). However, many reports showed that the *Fhb1* resistance allele is only found in landraces from southern China and Japan, suggesting that *Fhb1* likely originated from this region (Bai et al., 2018). It is widely accepted that *Fhb1* provides resistance to fungal spread (type II resistance) (Anderson et al., 2001; Buerstmayr et al., 2002). Additionally, 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 gene could be mapped with high precision as a single Mendelian gene (Liu et al., 2008).

The first *Fhb1* mapping experiment utilized restriction fragment length polymorphism (RFLP) markers (Waldron et al., 1999) to assign the gene to 3BS. A population of 112 F_5-derived recombinant inbred wheat lines from the cross Sumai3 (resistant)/Stoa (moderately susceptible) was evaluated for Type II resistance. The nearest RFLP marker (*Xcdo981*) explained 15.4% of the phenotypic variation. However, the Cdo981 clone used in the experiment appeared to differ from the original Cdo981 clone; therefore, another closely linked marker (*Xbcd907*) was considered a more reliable marker for *Fhb1*.

Anderson et al. (2001) conducted mapping experiments with two Sumai3 derived populations using 512 molecular markers, including RFLP, amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) markers. The latter included all of the published SSR markers from chromosome 3BS (Röder et al., 1998). *Fhb1* was mapped between *Xgwm493* and *Xgwm533* on chromosome 3BS. Marker *Xgwm493* was significantly associated with FHB resistance with an R^2 value of 41.6%. Buerstmayr et al. (2002) used a doubled-haploid (DH) population derived from the cross CM-82036 (resistant, Sumai3 derivative)/Remus (susceptible) to map Type II resistance. The most-prominent effect was

detected on the short arm of chromosome 3B, explaining up to 60% of the phenotypic variance for Type II FHB resistance.

Based on replicated evaluations of homozygous recombinant inbred lines for Type II FHB resistance, the location of *Fhb1* was narrowed down to a 1.2cM marker interval flanked by STS3B-189 and STS3B-206 (Liu et al., 2006). Further study narrowed this interval to a 261kb region with seven putative genes, and also proposed a highly diagnostic co-dominant marker (Umn10) for the detection of *Fbh1* (Liu et al., 2008). Umn10 has been successfully applied in many breeding programs worldwide for about 10 years. However, more and more results showed that Umn10 provided false positive identifications in Chinese germplasm due to genetic background similarity of Sumai3 to many susceptible Chinese landraces and cultivars. Thus, Umn10 also became less diagnostic for *Fhb1* in instances where more Chinese germplasm sources are used in US wheat breeding programs (Bai et al., 2018). Several single nucleotide polymorphism (SNP) markers were also developed (Bernardo et al., 2012), but they did not prevent false positives either. Lack of a reliable diagnostic molecular marker of *Fhb1* decelerates breeding progress and can even steer it in the wrong direction.

As understanding of the *Fhb1* genetic region improved, it aided the development of more efficient molecular markers. Recent research provided reference sequences for the *Fhb1* region. Rawat et al. (2016) reported the positional cloning of *Fhb1* from Sumai3. They suggested that the candidate gene encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain (PFT). Genotypes with the *Fhb1* resistance allele produced the functional PFT, while genotypes with the susceptible allele had no functional PFT (Rawat et al., 2016). However, many susceptible genotypes within a large collection of wheat samples, especially Chinese landraces, have functional PFT domains, implying that the PFT domain may not be the

determinant of *Fhb1* resistance (Bai et al., 2018). Moreover, Schweiger et al. (2016) reported four clearly expressed genes in the *Fhb1* region that were absent in the susceptible reference. The latter genes encoded: (1) a protein with agglutinin and 'pore-forming toxin-like' domains; (2) a GDSL lipase; (3) an F-box protein; and (4) a hypothetical protein. Of these, the GDSL lipase gene is the only gene in the sequenced contig that shows a significant increase in expression in response to the pathogen. The gene that encodes the F-box protein is among the strongest constitutively expressed genes on the contig.

Very recently, results from map-based cloning and EcoTILLING provided an alternative candidate gene in the *Fhb1* region (Su et al., 2017), which was reported to encode a putative histidine-rich calcium-binding protein (*TaHRC*) (Schweiger et al., 2016). Two markers (*TaHRC-GSM* and *TaHRC-KASP*) were developed based on a deletion mutation in the *TaHRC* gene (Su et al., 2018). These markers were validated in large, diverse populations and demonstrated to be superior to all previously reported markers.

Molecular markers for chromosome 5A resistance QTL

In addition to 3BS, chromosome 5A proved to be valuable region with more than 14 FHB resistance QTL having been mapped here (Cai et al., 2016). Of these, *Qfhs.ifa-5A* (Buerstmayr et al., 2003) and *Qfhi.nau-5A* (*Fhb5*) (Lin et al., 2006; Xue et al., 2011) are comparatively stronger effect FHB resistance QTL. Both QTL were mapped to the peri-centromeric region of chromosome 5A (Buerstmayr et al., 2009). Recently, another two important QTL (*Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*, respectively) were identified on chromosome arms 5AS and 5AL of the germplasm line PI277012 (Chu et al., 2011).

Qfhs.ifa-5A contributes to type I resistance by reducing initial infection (Buerstmayr et al., 2003), and to a lesser extent confers type II resistance (Schweiger et al., 2013). *Qfhs.ifa-5A* was

found in Sumai3 and its derivatives and explained about 20% of the phenotypic variation observed by Buerstmayr et al. (2003). *Qfhs.ifa-5A* is flanked by markers *Xgwm293* and *Xgwm156*. Within this interval, marker loci *Xgwm293*, *Xgwm304a*, *Xgwm1057*, *Xbarc186*, *Xbarc117*, and *Xbarc56* appeared to be closely or completely linked with the centromere. To map this QTL more accurately, Buerstmayr et al. (2018) used DH and near-isogenic recombinant inbred lines (NI-RIL) populations and narrowed the critical region down to a 1.6cM interval flanked by *Xbarc186* (deletion bin 5AS3-0.75-0.97) and *Xwmc805* (deletion bin 5AL5-0.46-0.55). Among the markers tested, *Xcfa2250*, *Xjfio6* and *Xgpg503* mapped closest to the centromere with a genetic distance of 0.9cM between *Xcfa2250* and *Xbarc186* in the NI-RIL map, containing seven loci. A recombination-independent radiation hybrid mapping (RH mapping) technique was also employed to improve the resolution in the 5AS peri-centromeric region (Buerstmayr et al., 2018). Through RH mapping, map resolution increased 389-fold for the *Qfhs.ifa-5A* interval compared to the genetic map, and 66 loci were discovered in the same interval, covering a distance of 350.3cR (cR = unit on RH-map, 1cR corresponds to ~0.77Mb for 5AS; Hukriede et al., 1999). A lipid transfer protein was identified in this region, which is constitutively expressed and is at least 50-fold more abundant in plants with the *Qfhs.ifa-5A* resistance allele (Schweiger et al., 2013).

Qfhi.nau-5A (*Fhb5*) was discovered in Wangshuibai and its derivatives and explained up to 27.0% of the observed phenotypic variation in the study of Lin et al. (2006). *Qfhi.nau-5A* mapped to the 0.4cM interval between *Xwmc96* and *Xgwm304*, and 0.1cM from *Xwmc96* on chromosome 5A (Lin et al., 2006). Xue et al. (2011) confirmed that *Qfhi.nau-5A* inherited like a single dominant gene and narrowed its location down to a 0.3cM interval flanked by *Xgwm304* and *Xgwm415* (Xue et al., 2011).

Qfhb.rwg-5A.1 and *Qfhb.rwg-5A.2* occur in PI277012 (hexaploid spring wheat) and were first reported by Xu et al. (2010). The two genes give a similar level of type II resistance as Sumai3. *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* were mapped to 5AS and 5AL of PI277012, explaining up to 20 and 32% of the variation in FHB severity, respectively (Chu et al., 2011). The two QTL also had strong effects in reducing the percentage of *Fusarium* damaged kernels (FDK) and DON accumulation in seeds (Chu et al., 2011). *Qfhb.rwg-5A.1* was mapped within a 40.8cM interval flanked by markers *Xcfa2104* and *Xgwm617* with a peak at marker *Xbarc40* on 5AS. *Qfhb.rwg-5A.2* was mapped within a 40.4cM interval flanked by markers *Xwmc470* and *Xbarc48* with a peak at marker *Xcfd39* (Chu et al., 2011).

When considering the published genetic maps of Buerstmayr et al. (2003, 2009, and 2018), Chu et al. (2011), Somers et al. (2004), and Sourdille et al. (2004), there is a clear overlap of the chromosome regions that border *Qfhs.ifa-5A*, *Qfhb.rwg-5A.1* and *Fhb5*. Buerstmayr et al. (2003) concluded that *Qfhs.ifa-5A* and *Fhb5* seemed to occupy a similar position. Chu et al. (2011) also concluded that *Qfhs.ifa-5A* and *Qfhb.rwg-5A.1* might be the same locus or different alleles of the same locus. However, the exact relationship of the three 5AS QTL remain unclear and it is not known whether they constitute different loci or whether they are alleles of the same locus (Xu, personal communication, 2016, USDA-ARS).

Qfhb.rwg-5A.2 represents a novel FHB resistance QTL in wheat (Chu et al., 2011). PI277012 was deposited in the US National Plant Germplasm System (NPGS) in 1961 by Luis M. Villena, Zaragosa, Spain, who described it as a rust resistant *Triticum aestivum* L. variety. According to the NPGS database (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?1206987), PI277012 has the pedigree: Extremo Sur/Argelino//*T. timopheevii* and its spikes have tough glumes and semi-brittle rachis. Thus, it is likely that

PI277012 obtained the FHB resistance QTL from *T. timopheevii.* PI277012 is non-free threshing (has tough glumes) due to it having the recessive allele, *q*, of the domestication locus *Q*, which was first isolated by Simons et al. (2006). The *Q* gene is located on chromosome 5AL, and mapped about 5.4cM from *Xcfd39* (the marker locus closest to the *Qfhb.rwg-5A.2* peak; Chu et al., 2011). In a more recent study, the *Q* gene was mapped at 8.5cM from *Xcfa39*, using the same PI277012 derived DH population (Zhao, 2017). Thus, the *Q* locus and *Qfhb.rwg-5A.2* are closely linked, but not the same locus (Zhao et al., 2018). A relatively large segregating population was searched to find recombinants in which the linkage between *q* and *Qfhb.rwg-5A.2* had been broken. One of 130 DH lines (DH#80 or GP80) had both *Qfhb.rwg-5A.2* and *Q* (Chu et al., 2011).

Chu et al. (2011) provided closely linked molecular markers for *Qfhb.rwg-5A.2* that occurred within the *Xwmc470* - *Xbarc48* interval. Zhao (2017) narrowed the region containing *Qfhb.rwg-5A.2* to a 0.4cM genomic region, and developed three CAPS markers (M2375, M2620, and M2781) that are associated with *Qfhb.rwg-5A.2* and believed to be more accurate.

Molecular markers for Qfhb.ifa-3A

Brazilian spring wheat Frontana was demonstrated to have both type I and type II resistance to FHB (Schröder and Christiansen, 1963; Singh et al., 1995). Several FHB resistance QTL were detected in Frontana (Mardi et al., 2006; Srinivasachary et al., 2008; Steiner et al., 2004), including one large effect QTL on 3AL QTL (*Qfhb.ifa-3A*), and other relatively minor effect QTL on 5A, 1BL, 7AS and other chromosome regions. Of these, *Qfhb.ifa-3A* was consistently detected in different studies, and explained up to 16% of the observed phenotypic variation (Mardi et al., 2006; Steiner et al., 2004; Yabwalo et al., 2011).

Further resistance QTL were discovered near the 3AS centromere in non-Frontana derived germplasm. These were closely associated with *Xgwm2* (Otto et al., 2002), or *Xgwm5*

(Bourdoncle and Ohm, 2003); however, the latter markers did not show polymorphism in Frontana-derived populations. *Qfhb.ifa-3A* of Frontana mapped within a 6.5cM interval between *Xgwm720* and *Xdupw227* (Steiner et al., 2004) and could therefore be a different QTL (Steiner et al., 2004). Mardi et al. (2006) narrowed down the interval containing *Qfhb.ifa-3A* to 5.0cM between *Xgwm720* and *Xgwm1110*. In the gene pyramiding experiment of Tamburic-Ilincic (2012), markers Gwm5, Barc45 and Wmc264 were applied to select for *Qfhb.ifa-3A*. Chromosome 3A genetic maps differ with respect to the marker order in the *Qfhb.ifa-3A* region. Sourdille et al. (2004) placed *Xgwm5* and *Xwmc264* on the long arm of chromosome 3A whereas *Xbarc45* and *Xgwm5* were assigned to the short arm of chromosome 3A by Song et al. (2005). Inadequate markers could have been part of the reason why some studies failed to show a clear advantage to the inclusion of *Qfhb.ifa-3A* in resistance gene pyramids (Tamburic-Ilincic, 2012).

The transfer and mapping of Fhb6

The perennial genus *Elymus* is one of the largest and most widely distributed genera within the *Triticeae* (Dewey, 1984). Some *Elymus* species show notable levels of FHB resistance. In China, *Elymus tsukushiensis* Honda ($2n = 6x = 42$, $S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$, syn. *Roegneria kamoji* C. Koch) was investigated by Weng and Liu (1989). FHB resistance was found in this perennial, cross-pollinating, hexaploid species. It is a distant wild relative of hexaploid wheat ($2n = 6x = 42$, AABBDD), and exhibits strong FHB type I and type II resistance (Weng and Liu, 1989). In Japan, four species of indigenous *Elymus* were investigated, including *Elymus humidus* Osada, *E. tsukushiensis* Honda, *E. racemifer* Tsvel. and *E. mayebaranum* (Ban, 1997). All accessions showed strong resistance to invasion that was comparable to the Sumai3 resistance. Specifically, no spread of the fungus was detected in most of the *E. humidus* accessions.

Many attempts were made to develop hybrids of *Elymus* and wheat (Muramatsu et al., 1983; Weng and Liu, 1989). Also, many disomic and double monosomic addition lines containing FHB resistance were developed (Takata and Muramatsu, 1992; Weng et al., 1993). A wheat-*E. tsukushiensis* disomic addition line having an *E. tsukushiensis* group 1 chromosome was produced by Wang et al. (1999). This addition line conferred strong FHB type II resistance, similar to the resistance in Sumai3 (Cainong et al., 2015; Wang et al., 1999). These efforts facilitated the understanding of FHB resistance in *Elymus* and revealed a novel FHB resistance QTL - *Fhb6*. *Fhb6* was mapped to a sub-terminal region in the short arm of chromosome $1E^{ts}$#1S of *E. tsukushiensis* (Cainong et al., 2015). Chromosome engineering were then applied to replace a corresponding homoeologous region of chromosome 1AS of wheat with the *Fhb6* associated chromatin derived from $1E^{ts}$#1S of *E. tsukushiensis*. Two potentially useful recombinants were obtained in this research. One is a compensating wheat-*E. tsukushiensis* recombinant chromosome that consists of the long arm of wheat chromosome1A, a proximal part of the 1AS arm and a small distal segment derived from $1E^{ts}$#1S (described as T1AL.1AS-$1E^{ts}$#1S). The second recombinant is non-compensating and involves an interstitial translocation of an *E. tsukushiensis* segment to an unidentified wheat chromosome. Both recombinants provide resistance to infection and are believed to harbor *Fhb6* (Cainong et al., 2015). However, only T1AL.1AS-$1E^{ts}$#1S is useful for agronomic purposes because the chromosomes involved in this translocation belong to the same homoeologous group, and homoeologous chromosome regions were exchanged during translocation. This recombinant line was released as germplasm line KS14WGRC61 by Friebe et al. (2013). Plant progenies homozygous for *Fhb6* had a disease severity rating of 7% compared to 35% for the null progenies (Cainong et al., 2015). A set of perfect markers was developed for tagging the *E. tsukushiensis* segments in wheat backgrounds,

including CAPS markers (tplb0017E15, tplb0029J02, and AK357509), SNP markers and a KASP marker (Cainong et al., 2015). These markers enable the transfer of *Fhb6* to different wheat backgrounds using marker assisted breeding.

Transfer and mapping of QTL derived from Thinopyrum

Perennial *Thinopyrum* wheatgrass species were found to be a rich source of genes that confer resistance to biotic and abiotic stresses (Turner et al., 2013). Several species of *Thinopyrum* were demonstrated to have strong FHB resistance (Oliver et al., 2005). Several sets of common wheat - *Thinopyrum* substitution lines and translocation lines were developed in the past decades, including: wheat - *Th. elongatum* (EE) substitution lines in a Chinese Spring background (Dvořák, 1980; Tuleen and Hart, 1988); two substitution lines in the Thatcher background 7D-7el$_1$ (Knott, 1968) and 7D-7el$_2$ (Knott et al., 1977); translocation lines derived from the substitution line 7D-7el$_1$ (Sharma and Knott, 1966) and 7D-7el$_2$ (Kibirige-Sebunya and Knott, 1983); and wheat and triticale - *Thinopyrum* addition lines (Littlejohn and Pienaar, 1985; Marais and Marais, 1998).These lines are valuable for the introgression of resistance from *Thinopyrum* into wheat.

Thinopyrum ponticum (Podp.) Barkworth & D.R. Dewey (2*n* = 10*x* = 70) substitution and translocation lines consistently showed lower FHB disease severities than the wheat parents (Oliver et al., 2006; Shen et al., 2004). The best characterized QTL was found on *Th. ponticum* chromosome 7el$_2$ (Shen and Ohm, 2007). This QTL explained 15.1-32.5% of the phenotypic variation observed in the mapping experiment of Shen and Ohm (2007) and was designated as *Fhb7* by Guo et al. (2015). Following its transfer to wheat, molecular marker analysis and genomic in situ hybridization showed that the translocation line (KS24-2) carrying *Fhb7* was a 7DS.7el$_2$L Robertsonian translocation (Guo et al., 2015). *Fhb7* was mapped to the distal region

of the long arm of $7el_2$ within a 10.4cM interval between flanking markers *XBE445653* and *Xcfa2240* (Shen and Ohm, 2007). This interval was narrowed down to a 1.7cM interval between *XsdauK66* and *Xcfa2240* by Guo et al. (2015). Useful co-dominant SSR markers for this gene (*Xcfa2240*, *XsdauK352*, and *XsdauK66*) were identified or developed, which facilitated the continued introgression and marker-assisted selection of *Fhb7* (Guo et al., 2015).

Thinopyrum elongatum (Host) Á. Löve ($2n = 2x = 14$; EE) was reported to show only 3.8 % infection in comparison with 60% to 90% infection in susceptible durum cultivars (Jauhar and Peterson, 1998). Two FHB resistance QTL were found on *Th. elongatum* chromosomes 1E and 7EL respectively. FHB resistance on 1E was first reported by Jauhar et al. (2009) who observed that disomic alien addition lines developed less than 21% infection (6.5% infection on average). Shen et al. (2004) evaluated 19 Chinese Spring (CS) - *Th. elongatum* substitution lines, and significant resistance was identified in the substitution lines 7E(7A), 7E(7B), and 7E(7D). Also, Miller et al. (2011) found a striking difference between CS and addition line CS-7EL with respect to FHB spread within the spike. These consistent resistance responses to FHB infection clearly indicated that chromosome 7E of *Th. elongatum* confers excellent type II resistance (Miller et al., 2011; Shen et al., 2004). Importantly, Chinese Spring - *Th. elongatum* 7E substitution lines showed normal fertility (Shen et al., 2004), which suggests a high degree of homoeology with the wheat group 7 chromosomes. This translates into a strong likelihood that the resistance can be introgressed through homoeologous pairing induction, and that a compensating translocation carrying the resistance can be produced.

Chromosomes 1E to 7E of *Th. elongatum* are largely homoeologous to the group 1 to 7 chromosomes of common wheat (Dvořák, 1980; Liu et al., 1999), while the E genome is most closely related to the D genome (Liu et al., 2007). Genomic and molecular marker information

and genetic maps pertaining to *Thinopyrum* species are limited (Mahelka et al., 2011; Wang et al., 2010), which limits the accessibility of *Thinopyrum* germplasm for gene transfer. Recent studies provided some SNP markers, which may also be applicable for genetic studies within and among the *Thinopyrum* species carrying E and/or St genomes (Lou et al., 2017).

A recent study reported a newly discovered (common wheat) QTL (*QFhb.cau-7DL*) on chromosome arm 7DL, which is closely linked to the marker Gwm428 (Ren et al., 2019). This is a further indication that group 7 chromosomes (long arm) could harbor a homoeo-locus that affects FHB resistance.

FHB resistance was also observed in an accession of *Th. junceiforme* (L öve & L öve) L öve ($2n = 4x = 28$, $J_1 J_1 J_2 J_2$). Hybrid derivative lines ($2n = 4x = 28$; ABJ_1J_2) between durum wheat (*Triticum turgidum*; $2n = 4x = 28$; AABB) and *Th. junceiforme* showed significantly lower mean infection, of which, the best hybrid showed 10.93% infection in comparison with 70.34% to 89.46% infection of the parental durum wheat (Jauhar and Peterson, 2011). *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey ($2n = 6x = 42$) showed Type II FHB resistance equal to Sumai3 in greenhouse trials (Cai et al., 2005; Oliver et al., 2005). Strong FHB resistance was also reported in *Th. distichum* (Thunb.) Á L öve ($2n = 4x = 28$, $J_1^dJ_1^dJ_2^dJ_2^d$) by Chen et al. (2001). The genetic bases of these resistances are not known.

Marker-assisted selection to improve FHB resistance

Marker assisted selection (MAS) is an indirect selection method whereby a trait of interest is selected based on the presence of a marker linked to it (Ribaut and Hoisington, 1998), which can be morphological, biochemical, cytological, or molecular in nature. Good markers are highly polymorphic, exhibit simple inheritance, are abundant throughout the genome, are easy and fast to detect, exhibit minimum pleiotropic effects, and their detection is not dependent on

the developmental stage of the organism. Therefore, molecular MAS for major QTL has much higher selection efficiency than conventional phenotypic selection (Wilde et al., 2007).

Pyramiding of single resistance genes with the use of molecular markers is a well-established procedure (Sanchez et al., 2000). However, the overall FHB resistance phenotype is determined by many QTL that each makes a small or medium size contribution and occurs in different regions of the wheat genome. As a result, resistance is inherited quantitatively (Bai and Shaner, 1994). The expression of resistance QTL is modified by environmental factors and measurements are subject to experimental error, both of which complicate the accurate assessment of resistance (Miedaner et al., 2006). While molecular markers allow for accurate detection of specific, targeted QTL, it has to be kept in mind that anonymous or uncharacterized background QTL could occur which may affect the overall disease phenotype.

Objectives

This study aimed to:

(1) Develop new FHB resistant lines with pyramided QTL (*Fhb1* +) in the NDSU HRWW breeding population using molecular markers. The pyramided lines were phenotyped to evaluate the ability of different QTL to complement *Fhb1*. QTL combinations that proved to be advantageous and without obvious deleterious agronomic effects, will now be used to strengthen the baseline resistance of the NDSU HRWW breeding population. Such lines will continue to be evaluated in field trials and will also be used in new crossing blocks.

(2) Evaluate an FHB resistance QTL that segregates in triticale – *Th distichum* hybrid progenies. An unknown translocation that carries a gene for FHB resistance could be present in line W1423. The study aimed to select a stable homozygote for more accurate characterization.

An attempt was also made to identify the complete *Thinopyrum* addition chromosome on which the resistance occurs.

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CHAPTER II. COMBINING FUSARIUM HEAD BLIGHT RESISTANCE GENES ON WHEAT CHROMOSOME 5A WITH *FHB1*

Abstract

Fusarium head blight (FHB; caused by *Fusarium graminearum* Schwabe) is a serious disease of hard red winter wheat (HRWW) in North Dakota, and current varieties are generally lacking in resistance. This study aimed to transfer and pyramid FHB resistance QTL from the hard red spring wheat (HRSW) germplasm lines CM82036 (*Fhb1*, *Qfhs.ifa-5A*) and PI277012 (*Qfhb.rwg-5A.1*, *Qfhb.rwg-5A.2*) to HRWW. Previously, each source was crossed with HRWW followed by backcrosses, single seed descent inbreeding or doubled haploid production to develop FHB resistant, winter habit intermediates. Marker-assisted selection and FHB evaluation (field and greenhouse) were employed to select HRWW lines 14K456-K-1 (*Fhb1* and *Qfhs.ifa-5A*) and Novus-4 (*Qfhb.rwg-5A.1* and/or *Qfhb.rwg-5A.2*). In this study, the two lines were crossed and 18 F_2 segregates homozygous for *Fhb1* plus marker allele *Xbarc186-1* (associated with *Qfhs.ifa-5A*), and 16 F_2 homozygous for *Fhb1* plus the alternative *Xbarc186-2* allele were recovered. Five subfamilies derived from each of the 34 families were then analyzed for their chromosome 5A single nucleotide polymorphism (SNP) haplotypes and resistance (greenhouse). The results suggested that *Qfhb.rwg-5A.2* was absent whereas *Qfhs.ifa-5A* and *Qfhb.rwg-5A.1* are probably alleles of the same locus with similar effects on the overall resistance phenotype. The RWG21 source material was clearly heterogeneous and lacked *Qfhb.rwg-5A.2*, which appears to be the more useful of the PI277012 QTL.

Introduction

Fusarium head blight (FHB) is a devastating plant disease that harms wheat production worldwide; however, considerable improvement in genetic resistance of varieties has been

achieved through breeding and selection. Up to now, more than 250 Quantitative Trait Loci (QTL) for FHB resistance have been reported in wheat, of which, *Fhb1* (first designated as *Qfhs.ndsu-3BS*) has the largest individual effect, explaining 60% of the phenotypic variation for disease severity (Waldron et al., 1999). *Fhb1* originated from Chinese wheat, and provides resistance to fungal spread (type II resistance) (Anderson et al., 2001; Buerstmayr et al., 2002). The gene may be involved in the conversion of deoxynivalenol (DON) into the less phytotoxic DON-3-O-glycoside (Lemmens et al., 2005). Due to its prominence, *Fhb1* became widely introduced in wheat breeding programs, including HRWW at North Dakota State University (NDSU).

In addition to 3BS, 5A proved to be another valuable chromosome region with more than 14 FHB resistance QTL having been mapped here (Cai et al., 2016). *Qfhs.ifa-5A*, derived from Sumai3, showed stronger response to spray inoculation than single spikelet injection (Buerstmayr et al., 2003). *Qfhs.ifa-5A* was found to be flanked by marker loci *Xbarc186* and *Xcfa2250* within a 0.9cM genetic interval on the short arm of chromosome 5A (Buerstmayr et al., 2018). Another two important QTL, *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A*.2 (in PI277012) were also mapped to chromosome 5A, explaining up to 20% and 32% of the variation in FHB severity, respectively (Chu et al., 2011).

Qfhb.rwg-5A.1 occurs within the interval *Xcfa2104* and *Xgwm617* on the short arm of chromosome 5A, whereas *Qfhb.rwg-5A.2* peaked at marker *Xcfd39* that is located between *Xwmc470* and *Xbarc48* in an interval that includes the *Q* gene (Zhao, 2017). The two QTL had strong effects in reducing the percentage of *Fusarium* damaged kernels and DON accumulation in seeds (Chu et al., 2011). Markers *Xgwm304* and *Xgwm415* on 5AS flank resistance QTL *Fhb5* (first designated as *Qfhi.nau-5A*) that derives from Wangshuibai and segregates as a single

dominant gene (Xue et al., 2011). Genetic maps produced by Buerstmayr et al. (2003, 2009, and 2018), Chu et al. (2011), Somers et al. (2004), and Sourdille et al. (2004) suggest that the chromosome regions that border *Qfhs.ifa-5A*, *Qfhb.rwg-5A.1* and *Fhb5*, overlap. However, the exact relationship between these 5AS QTL is not clear and it is not known whether they constitute different loci or whether they are alleles of the same locus.

FHB resistance is controlled by multiple QTL, each conferring partial resistance and as a result, gene pyramiding is a logical way to improve overall FHB resistance (Eckard et al., 2015; Miedaner et al., 2006). Molecular markers closely linked to target QTL facilitate and accelerate gene pyramiding, and are particularly useful when dealing with a trait of low to moderate heritability (Buerstmayr et al., 2009; Somers et al., 2004; Sourdille et al., 2004). However, favorable marker polymorphisms are required between the parents whereas the recombination distance between the marker and target trait should be small. The use of closely linked (and suitably polymorphic) flanking markers greatly improves the accuracy of marker-predicted genotypes.

This study aimed to identify and cross the two most useful lines from the two introgression attempts and to select their hybrid progeny for recovery of genotypes with higher order pyramided resistance genes. The purpose was twofold: to determine whether the QTL have marked additive effects that can be pursued in breeding, and, whether winter-hardy genotypes with acceptable agrotype and superior FHB resistance can be developed for use as breeding parents.

Materials and Methods

General outline

The parental lines that were used to initiate the study and the winter habit inbred lines that were developed from it are listed in Table 2-1. The experimental material was first tested with appropriate markers to determine the polymorphisms and to identify suitable markers for selection. The material was then also evaluated for type II FHB resistance in a greenhouse trial to confirm the presence of resistance QTL.

Based on these evaluations, the winter-hardy breeding line Novus-4 (homozygous for either or both *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*) was crossed with line 14K456-K-1 that is homozygous for resistance genes *Fhb1* and *Qfhs.ifa-5A*. The new cross was named 15K353. Jerry is a FHB susceptible HRWW with broad adaptation to North Dakota and excellent winter hardiness. The FHB resistance in Novus-4 derives from a cross between Jerry and the HRSW line RWG21. RWG21 derives from accession PI277012 that was crossed with and backcrossed (once) to Russ. The resistance genes *Fhb1* and *Qfhs.ifa-5A* in line14K456-K-1 were derived from the HRSW CM82036. Segregating progeny of cross 15K353 were analyzed employing both single nucleotide polymorphism (SNP) markers and greenhouse FHB resistance tests to identify progeny with combinations of resistance genes.

To confirm the results of the SNP analyses, a further genotype panel was tested for the presence of *Qfhb.rwg-5A.2* markers. The panel consisted of: PI277012, RWG21, GP80 (a new HRSW doubled haploid line with the PI277012 resistance that was obtained from Dr. Xu and which has the *q*-allele replaced with the *Q*-allele for better thresh-ability; Chu et al., 2011), Novus-4, Jerry, CM82036 and 14K456-K-1.

Table 2-1. Codes, pedigrees, known/inferred FHB resistance genes, and associated markers of the wheat germplasm that was used to initiate the study.

No.	Line/Variety	Description/Pedigree	Known/inferred resistance genes	Associated markers
1	CM82036	HRSW line	*Fhb1, Qfhs.ifa-5A*	Umn10, Barc186
2	12DH172	CM82036/Jerry	*Fhb1*	Umn10
3	11M221-24-1	CM82036/Jerry	*Fhb1, Qfhs.ifa-5A*	Umn10, Barc186
4	14K456-K-1	CM82036/Jerry/3/*Lr56*-157/Superb//4*Jerry	*Fhb1, Qfhs.ifa-5A*	Umn10, Barc186
5	14K456-L-5	CM82036/Jerry/3/*Lr56*-157/Superb//4*Jerry	*Qfhs.ifa-5A*	Barc186
6	PI277012	HRSW donor	*Qfhb.rwg-5A.1, Qfhb.rwg-5A.2*	Cfa2104, Cfd39
7	RWG21[1]	Russ 2*/PI277012 (HRSW)	Heterogeneous[2]	
8	Novus-4	RWG21/Jerry	*Qfhb.rwg-5A.1*	Cfa2104
9	11M228-19-1	RWG21/Jerry	*Qfhb.rwg-5A.1*	Cfa2104
10	RWG10[1]	BG282/3* Alsen (HRSW)	*Fhb1*	Umn10
11	RWG28[1]	(BG290/3* Alsen)/(BG282/3* Alsen) (HRSW)	*Fhb1*	Umn10
12	11M225-7-2	RWG10/Jerry		
13	11M225-97-1	RWG10/Jerry	*Fhb1*	Umn10
14	11M237-A-1-2	RWG28/Norstar	*Fhb1*	Umn10
15	Jerry	HRWW variety		
16	Norstar	HRWW variety		
17	Superb	HRSW variety		

[1] Provided by Dr. S. Xu (USDA/ARS, Cereal Crops Research, Fargo, ND 58102).
[2] Segregated for the presence of a single resistance QTL.

Molecular marker analyses

Marker information from several published chromosome maps was consulted when deciding on appropriate markers for the characterization of the resistant introgression lines and hybrid progenies. To simplify explanation and interpretation, we integrated the relevant chromosome 5A map information published by Buerstmayr et al. (2003, 2009, and 2018), Chu et al. (2011), Somers et al. (2004), and Sourdille et al. (2004) using a neighbor mapping approach (Barabaschi et al., 2015) in combination with MapChart 2.32 (Voorrips, 2002). This integrated map is given in Fig. 2-1.

Total genomic DNA was extracted from young leaves following the Triticarte Pty. Ltd protocol (http://www.triticarte.com.au/). The quality and concentration of extracted DNA was checked using agarose gel electrophoresis and staining with ethidium bromide. DNA concentration was adjusted to approximately 10 ng/µl before Polymerase Chain Reaction (PCR) amplification. The markers that were evaluated for the selection of the FHB resistance QTL were: (1) *Fhb1:* Fhb_USDA, designed based on the Umn10 marker (Liu et al., 2008) by the USDA-ARS Genotyping Center (1605 Albrecht Blvd N, Fargo, ND 58102); (2) *Qfhs.ifa-5A*: Gwm304, Gwm293, Barc186 (Buerstmayr et al., 2009; Somers et al., 2004); (3) *Qfhb.rwg-5A.1:* Wmc752, Barc40, Barc165, Wmc795 (Chu et al., 2011); (4) *Qfhb.rwg-5A.2*: Wmc470, Cfa2163, Cfa2185, Barc232, Wmc96, Cfd39, Gpw2273, Gpw2120, Gpw2172, Gpw2181, Gpw2136, Abg391, Abg366, Wg114, Gwm179, Gwm126, Gwm6, Gwm595, Barc48, Gwm291, Gwm410 (Chu et al., 2011; Sourdille et al., 2004). The microsatellite primer sequences are available in Röder et al. (1998) and the GrainGenes website (http://www.wheat.pw.usda.gov). PCR conditions were as described by Röder et al. (1998). PCR products were visualized by agarose

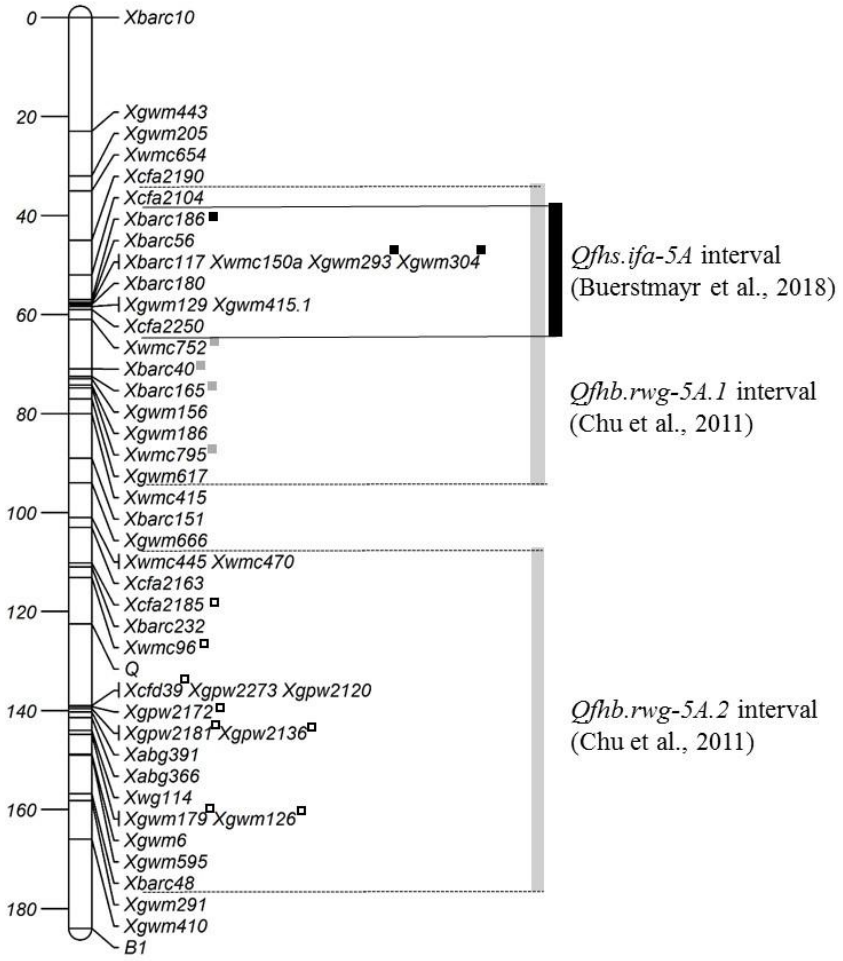

Figure 2-1. Relative locations of chromosome 5A markers used in this study. Map positions were obtained from genetic maps published by Buerstmayr et al. (2003, 2009, and 2018), Chu et al. (2011), Somers et al. (2004), and Sourdille et al. (2004). Markers that detect *Qfhs.ifa-5A*, *Qfhb.rwg-5A.1*, and *Qfhb.rwg-5A.2* are indicated with black, grey, and white squares, respectively.

(all except Umn10) or denaturing polyacrylamide (Umn10) gel based electrophoresis with ethidium bromide staining. The markers were tested on the parental genotypes to determine whether favorable allelic differences for marker-assisted selection could be detected using standard PCR. For confirmation, *Fhb1* and *Qfhs.ifa-5A* were also detected by utilizing the Kompetitive Allele Specific PCR genotyping system (KASP^{TM}). These analyses were conducted by the USDA-ARS Genotyping Center, Fargo, ND. A two-inch young leaf segment was sampled,

put directly into a 96-well plate, and then dried/stored at room temperature for KASP™ measurement. If it was necessary to confirm the position of a band, use was made of the genotype panel Chinese Spring (CS), CS nullisomic 5A tetrasomic 5B (CSN5AT5B), CS di-telosomic 5AL, CSN5BT5D and CSN5DT5A.

The genotypes listed in Table 2-1 were first analyzed with marker Umn10 to detect *Fhb1*. Second, seven microsatellite markers (Fig. 2-1) that are linked to the FHB resistance QTL on 5AS were analyzed. Of these, Barc186, Gwm304 and Gwm293 were used in the study of Buerstmayr et al. (2003) to determine the chromosome location of *Qfhs.ifa-5A.* Markers Wmc752, Barc40, Barc165 and Wmc795 were used in the study of Chu et al. (2011) to determine the chromosomal location of *Qfhb.rwg-5A.1*. Third, eight markers that were mapped by Chu et al. (2011) and occur in the *Qfhb.rwg-5A.2* region (Fig. 2-1) were tested for marker polymorphism.

Selection within cross 15K353

Genomic DNA of 406 15K353 F_2 plants were sampled and tested for the presence of *Fhb1* and *Qfhs.ifa-5A*, using the KASP™ method. Sixty-nine plants were homozygous for *Fhb1*. Of these, 20 were also homozygous for a 210 bp band amplified by the Barc186 primers in CM82036 (here referred to as allele *Xbarc186-1*, and used as marker to identify *Qfhs.ifa-5A);* 16 *Fhb1* homozygotes were also homozygous for the alternative allele *Xbarc186-2* (band size 201bp) that occurs in Novus-4 and therefore lacked *Qfhs.ifa-5A*. Thirty-three *Fhb1* homozygotes were heterozygous for *Xbarc186*. The two groups of homozygous plants (20 *Fhb1* and *Qfhs.ifa-5A* and 16 *Fhb1* only) were grown to obtain $F_{2:3}$ seeds. Five plants each from 34 $F_{2:3}$ families from both subgroups (18 *Fhb1* and *Qfhs.ifa-5A* and 16 *Fhb1* only) were then planted. Four of the five plants were used for genomic DNA extraction. This provided 144 samples in addition to the

parental lines RWG21, Jerry, PI277012, Novus-4, CM82036, and 14K456-K-1 (3 plants each). These samples were genotyped at the USDA-ARS Genotyping laboratory at Fargo, ND using the Illumina wheat 9K iSelect genotyping assay (Cavanagh et al., 2013). Polymorphic SNP markers were analyzed and selected using GenomeStudio Genotyping Module V 1.0 (Illumina Inc.).

Upon ripening, the five plants in each family were harvested separately to obtain F_4 seeds for FHB phenotyping (greenhouse) to determine type II resistance.

Greenhouse evaluation of FHB resistance

Trial 1: The starting material listed in Table 2-1 was evaluated for type II resistance in a greenhouse from October 2015 to March 2016. The greenhouse trial was set up as a completely randomized design within six replicates. Three plants were grown in each replicate (pot). Winter wheat genotypes were vernalized one day after planting for 56 days at 4 ℃ in a cold chamber, while spring wheat genotypes were planted seven days before the winter wheat vernalization ended.

Trial 2: A second greenhouse trial was conducted to evaluate the F_4 generation of cross 15K353 from November 2016 to June 2017. Five plants each of 36 $F_{2:4}$ families that originated from *Fhb1* homozygotes were planted, in total 180 lines. In addition, parental lines, 14K456-K-1 and Novus-4 were used as controls. The greenhouse trial was set up as a completely randomized design within four replicates. Five plants were grown in each replicate. All lines were vernalized one day after planting for 56 days at 4 ℃ in a cold chamber.

Field evaluation

Following the FHB resistance tests, 168 of the 180 lines produced enough F_5 seed to allow for their evaluation at two locations in 2017-18 growing season. A randomized complete block design (RCBD) experiment with two replicates was conducted at Fargo, ND; with each

entry planted in a single 0.6-m row for each replicate. An un-replicated 2-m row of each entry was also planted at Casselton, ND. The intention was to evaluate the individual rows for winter survival, FHB resistance, plant height and agrotype.

Fusarium inoculation and symptom evaluation

In the greenhouse trials, the single spikelet injection method was used for inoculating wheat spikelets at anthesis (Chu et al., 2011; Stack, 1989). A mixture of approximately equal amounts of spores from four *Fusarium graminearum* isolates (Fg_124_1, Fg10_135_5, Fg13_79 and Fg08_13) was provided by the Department of Plant Pathology at North Dakota State University. A 10 µl-droplet containing the mixture (approximate concentration = 100,000 conidia per ml) was injected directly into a floret in the center of the spike. Within each replicate, ten spikes were inoculated per entry, and thus approximately 60 spikes were inoculated for each line. Inoculated spikes were covered with a (wet) plastic bag for 72h immediately after inoculation.

Afterwards, the infection severity was assessed visually by counting the percentage of infected spikelets per spike at 21 days after anthesis (DAA). Inoculated spikes from each replicate were harvested in bulk and manually threshed. The seeds were used for determining *Fusarium* damaged kernels (FDK) and mycotoxin (DON, 3ADON, 15ADON, and nivalenol) accumulation. FDK were defined as those that were shriveled, lightweight, and chalky white (Cuthbert et al., 2006). Individual grain samples were rated for the percentage of FDK using the following scale: 1 = no visible FDK; 2 = <5%; 3 = 6–15%; 4 = 16–25%; 5 = 26–45%; 6 = 46–65%; 7 = 66–85%; 8 = 86–95%; and 9 = >95% (Miedaner et al., 2006). Mycotoxin accumulation was measured using the GC/MS method; analyses were conducted by the Veterinary Diagnostic Laboratory at NDSU. Two replicates were bulked and ground in order to obtain sufficient material for DON panel measurement, thus there were three replicates for this dataset.

Statistical analyses

Analyses of variance of phenotypic characters were conducted using SAS version 9.3 (SAS Institute, Cary, NC). The general linear model (GLM) was applied to compare the differences among lines tested in the greenhouse (completely randomized design), using model $Y = L_i + e$. In the field trial, a randomized complete block design was applied, using model $Y = R_i + L_i + e$.

Results and Discussion

Marker characterization of the CM82036 derivatives

Donor line CM82036 and its derivatives 12DH172, 11M221-24-1, and 14K456-K-1 each had the critical Umn10 marker allele whereas 14K456-L-5 lacked it (Table 2-1). The *Fhb1* controls which included RWG10 and RWG28 plus their derivatives 11M225-97-1 and 11M237A-1-2 also tested positive for Umn10 favorable allele, whereas derivative 11M225-7-2 lacked the positive allele. The remaining lines PI277012, RWG21, Novus-4 and 11M228-19-1 all lacked Umn10 favorable allele.

The *Qfhs.ifa-5A* markers Barc186, Gwm304 and Gwm293 from Buerstmayr et al. (2003) had favorable polymorphisms that could be used to detect this QTL among the *Fhb1* segregates. Results of the three markers were in agreement and showed that the marker allele associated with *Qfhs.ifa-5A* occurs in CM82036, 11M221-24-1, 14K456-K-1 and 14K456-L-5 but not in 12DH172. This QTL is not known to occur in any of the other genotypes in Table 2-1.

Markers that were found to be associated with *Qfhb.rwg-5A.1* by Chu et al. (2011), were also tested on CM82036, 14K456-K-1, Superb and Jerry. Primer sets Wmc752, Barc40, Barc165, and Wmc795 each amplified a common band in CM82036 and 14K456-K-1 which did not occur

in the recipient genetic backgrounds Superb and Jerry. Thus, these markers confirmed that the target segment had been transferred from CM82036 to 14K456-K-1.

Marker characterization of the PI277012 derivatives

Four of the seven 5AS markers that produced useful polymorphisms for *Qfhs.ifa-5A* were also polymorphic for *Qfhb.rwg-5A.1*. As shown in Table 2-1, RWG21 is a backcross-derived (recurrent parent = Russ) line, while Novus-4 was produced from the cross RWG21/Jerry. The PI277012 alleles of *Xwmc752* and *Xbarc40* only, were retained in RWG21. In the production of Novus-4, the RWG21 marker polymorphisms for *Xbarc165* and *Xwmc795* only, were transferred. In no instance was the original PI277012 marker polymorphisms retained in Novus-4. This suggested one of the following: (a) crossover occurred between the markers and *Qfhb.rwg-5A.1*; or (b) the *Qfhb.rwg-5A.1* QTL was not transferred to Novus-4.

Eight markers (Cfa2185, Wmc96, Cfd39, Gwm179, Gwm126, Gpw2136, Gpw2172, Gpw2181), that were found to be associated with *Qfhb.rwg-5A.2* by Chu et al. (2011), were tested on the Table 2-1 panel. None of these proved to be useful for the selection of *Qfhb.rwg-5A.2*. Six of the markers were polymorphic; however, in no instance had the PI277012 polymorphism been transferred to RWG21 or its derivatives, including Novus-4. Therefore, in the absence of useful markers, the disease phenotypes were of primary importance in the selection of the PI277012 derivatives.

Characterization of parental material and derivatives for FHB type II resistance

FHB disease development data were collected in a greenhouse trial using the genotypes of Table 2-1. Analysis of variance showed significant differences in infection severity, FDK and DON concentration among the parental materials and derivatives (Table 2-2). Based on the marker-predicted genotypes (Table 2-1) and the resistance data, the lines were ordered into five

groups: a susceptible group plus four possible resistant groups, i.e. *Fhb1* only, *Qfhs.ifa-5A* only; *Fhb1* and *Qfhs.ifa-5A*; and PI277012 derived material (containing *Qfhb.rwg-5A.1* and/or *Qfhb.rwg-5A.2*).

Inoculation with *Fusarium graminearum* caused disease development for all groups that in time became more severe. In group I (Fig. 2-2) the three susceptible control lines plus 11M225-97-1 and RWG21 showed severe infection at 21-days after anthesis (DAA). While the infection severity of both Superb and Norstar was significantly higher than in Jerry, RWG21 had the worst infection (87.1%). It seemed that the presence of Umn10 in 11M225-97-1 could have been the result of crossover within the *Fhb1-Xumn10* interval. Line 11M225-7-2 was included with group II (the *Fhb1* group, Fig. 2-2) even though it did not have the Umn10 marker (Table 2-1), as the level of resistance in this line suggested that it might have lost the marker. Lines 11M237A-1-2 and 12DH172 were also placed in group II. 12DH172 showed significantly lower infection severity than the other two lines and lower FDK than 11M237A-1-2. Group III (*Qfhs.ifa-5A*) contained line 14K456-L-5 only and its diseased phenotype was very comparable to the group II lines that had *Fhb1* singly. This appeared to contradict previous reports who found *Qfhs.ifa-5A* to be less effective than *Fhb1* (Buerstmayr et al., 2003); however, as was pointed out by the latter authors and Salameh et al. (2011) these two large effect QTL explained only about half of the FHB resistance in CM82036, implying the presence additional, undetected

Table 2-2. Analysis of variance (completely randomized design) to compare greenhouse type II FHB resistance of parental lines and derivatives. Measurements included infection severity, *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) concentration.

SOV[1]	Infection severity				FDK				DON			
	DF	SS	MS	*F* value[2]	DF	SS	MS	*F* value	DF	SS	MS	*F* value
Lines	14	66528.85	4752.06	58.17***	14	514.49	36.75	64.60***	14	7985.64	570.40	20.05***
Error	75	6127.29	81.70		75	42.67	0.57		30	853.29	28.44	
Total	89	72656.1			89	557.16			44	8838.93		

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

small effect QTL in this line. Un-monitored background QTL could therefore account for differences in overall resistance of lines carrying the same large effect QTL, and even among susceptible lines, the relative level of susceptibility varies, again pointing at the influence of lesser background QTL for FHB resistance (Salameh et al., 2011). The group IV lines 11M221-24-1 (13.4% infected at 21 DAA) and 14K456-K-1 (14.1% infected at 21 DAA) showed the strongest and similar resistance as their donor parent, CM82036. Thus, considering the likelihood of background effects, the results are in broad agreement with the conclusion of Salameh et al. (2011) that the CM82036–derived QTL appear to improve FHB resistance in the order *Qfhs.ifa-5A* < *Fhb1* ≤ *Qfhs.ifa-5A* plus *Fhb1*.

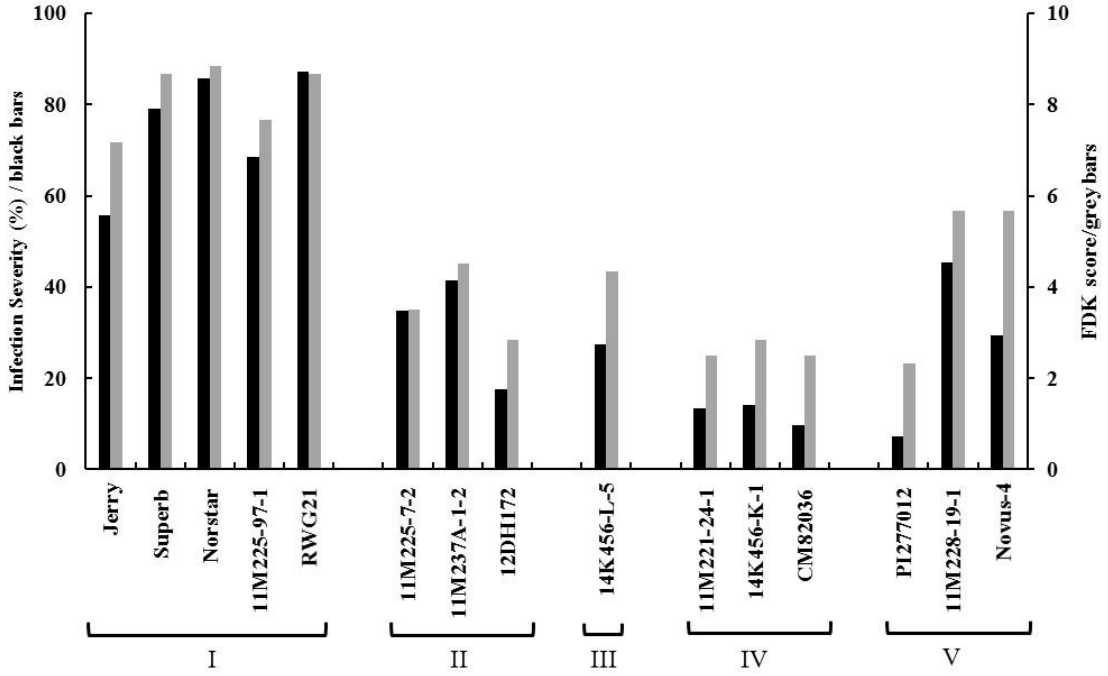

Figure 2-2. *Fusarium* head blight infection severity (black bars) 21 days after inoculation with *Fusarium graminearum* isolates (LSD=10.4%, α=0.05), and *Fusarium* damaged kernels (FDK, grey bars) determined at harvest (LSD=1.0, α=0.05). Entry groups are: I = without known resistance QTL; II = *Fhb1*; III = *Qfhs.ifa-5A*; IV = *Fhb1* and *Qfhs.ifa-5A*; V = PI277012 derived material (with *Qfhb.rwg-5A.1* and/or *Qfhb.rwg-5A.2*).

Within the *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* group, donor parent PI277012 showed the strongest resistance, which is similar to CM82036. Line RWG21 (Fig. 2-2) clearly does not have any of the PI277012 resistance QTL. It is believed that the original RWG21 source must have been heterogeneous for the resistance. Several RWG21 plants had been planted at the time it was used for making crosses. These plants were subsequently harvested separately. A single QTL had likely been transferred from a resistant RWG21 plant to each of Novus-4 and 11M228-19-1 while the seeds used in the FHB screening test must have derived from a different, susceptible plant. The two RWG21 progeny lines showed intermediate resistance, with Novus-4 (29.5% infected at 21 DAA) having significantly better resistance than 11M228-19-1 (45.5% infected at 21 DAA).

The FDK determinations mirrored the infection severity data (Fig. 2-2) with 12DH172, 11M221-24-1 and 14K456-K-1 having low scores that were similar to the donor parent CM82036, and reflective of strong resistance to *Fusarium* infection. PI277012 had the lowest FDK score (FDK=2) of the 15-line panel, although not significantly different from the *Fhb1* and *Qfhs.ifa-5A* group. RWG21 had the highest FDK score of 9 (>95 % damaged kernels), whereas Novus-4 and 11M228-19-1 had FDK scores of nearly 6 (approximately 45% damaged kernels).

Mycotoxin accumulation in seeds of the parental lines after *F. graminearum* inoculation

The 3-A DON and nivalenol contents of all lines were negligible (< 0.5 ppm). The 15-A DON contents exceeded 0.5 ppm only with respect to four of the five susceptible lines in Fig. 2-2 (Jerry being the exception) with RWG21 showing the highest level, i.e. 2.8 ppm. However, DON accumulation varied considerably and was high in the susceptible lines (Table 2-3). RWG 21 had the highest DON content (> 40 ppm) whereas the Jerry samples contained significantly (5% level) lower levels than the other four susceptible lines. Jerry samples did not differ significantly from

the group II and III entries, as well as 11M221-24-1 (group IV), 11M228-19 and Novus-4 (group V). Lines 14K456-K-1 (0.9 ppm), CM82036 (1.0 ppm) and PI277012 (< 0.5 ppm) contained the least DON. Of the three PI277012 derivatives, Novus-4 (6.3 ppm) had the lowest DON content.

In summary, line 14K456-K-1 (*Fhb1* and *Qfhs.ifa-5A*) showed very low infection severity, FDK score and DON content, comparable to that of CM82036 and PI277012, whereas Novus-4 showed intermediate values for the three measurements and is homozygous for either of *Qfhb.rwg-5A.1* or *Qfhb.rwg-5A.2*.

Table 2-3. Vomit toxin (DON, 3ADON, 15ADON, and nivalenol) accumulation in winter wheat seeds following inoculation with *Fusarium graminearum* isolates at anthesis.

		DON[1]	3-A DON	15-A DON	Nivalenol
	Lines	ppm	ppm	ppm	ppm
Susceptible	Jerry	10.5 $_{D}$	<0.5	<0.5	<0.5
	Superb	29.0 $_{BC}$	0.5	1.0	<0.5
	Norstar	26.9 $_{C}$	<0.5	1.7	<0.5
Fhb1	11M225-7-2	9.9 $_{D}$	<0.5	0.5	<0.5
	11M225-97-1	37.8 $_{AB}$	<0.5	1.7	<0.5
	11M237A-1-2	9.2 $_{DE}$	<0.5	<0.5	<0.5
Qfhs.ifa-5A	14K456-L-5	1.8 $_{DE}$	<0.5	<0.5	<0.5
Fhb1 & *Qfhs.ifa-5A*	11M221-24-1	3.7 $_{DE}$	<0.5	<0.5	<0.5
	14K456-K-1	0.9 $_{E}$	<0.5	<0.5	<0.5
	12DH172	2.8 $_{DE}$	<0.5	<0.5	<0.5
	CM82036	1.0 $_{E}$	<0.5	<0.5	<0.5
Qfhb.rwg-5A.1 & *Qfhb.rwg-5A.2*	RWG21	>40 $_{A}$	0.5	2.8	<0.5
	PI277012	<0.5 $_{E}$	<0.5	<0.5	<0.5
	11M228-19-1	8.1 $_{DE}$	<0.5	<0.5	<0.5
	Novus-4	6.3 $_{DE}$	<0.5	0.5	<0.5

[1] Means followed by the same letter do not differ statistically (LSD=8.0, α=0.05).
>40 ppm means that the value exceeds the detection limit of the GC/ECD method.
<0.5 ppm means that the value is smaller than the detection limit of the GC/ECD method.

FHB resistance in cross 15K353

The average infection severity (Fig. 2-3) of the *Fhb1* plus *Qfhs.ifa-5A* homozygotes was 23.5 % (averaged over 18 families with 5 sub-families each), which was similar to that of *Fhb1* only homozygotes (24.3%; averaged over 16 families with 5 sub-families each). The distribution of resistance phenotypes within the two populations was also very similar, with the most resistant phenotypes being comparable to the 14K456-K-1 resistance in each case (Fig. 2-3). There was no instance of resistance that exceeded 14K456-K-1, as could be expected if a third locus was involved. This suggests that the resistance gene contributed by Novus-4 is most likely similar to, occurs in the same chromosome region as, and substituted for *Qfhs.ifa-5A*. The occurrence of genotypes with less resistance similar to Novus-4 could be due to variability in the precision of the FHB screening test, unknown genetic background interaction, or mis-identification of *Fhb1* due to recombination with *Xumn10*.

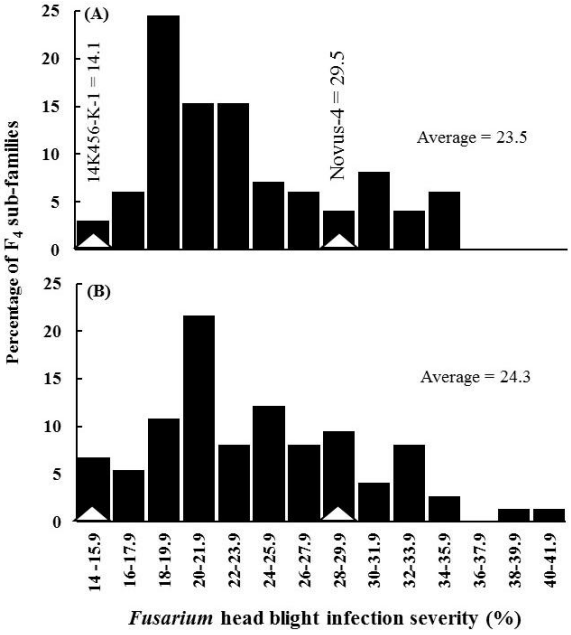

Figure 2-3. Average *Fusarium* head blight infection severity over 4 replications of 5 plants each per F_4 sub-family. (A) 18 families (each with 5 sub-families) were derived from F_2 plants that were homozygous for the *Fhb1* marker plus *Xbarc186* allele 1; (B) 16 families (each with 5 sub-families) were derived from F_2 plants that were homozygous for the *Fhb1* marker plus *Xbarc186* allele 2.

SNP haplotype comparisons within the 15K353 population

Chromosome 5A SNP loci were identified making use of the 9K consensus wheat map (Cavanagh et al., 2013). Those chromosome 5A markers that were polymorphic within the 15K353 $F_{2:3}$ population were then used for haplotype mapping. Haplotypes were assigned based on the parental (14K456-K-1 or Novus-4) genotypes at each locus. The complete haplotype map encompassed 55 SNP loci bordered by locus 14 (at 17.11cM) and locus 2646 (at 297.37cM) (Fig. 2-4). Since *Xbarc186* is closely linked with *Qfhs.ifa-5A*, a first logical step was to determine its position on the haplotype map. Families 1-17&36 derived from 18 F_2 plants that were homozygous for the 14K456-K-1 allele *Xbarc186-1*, while families 20-35 (16 in total) were homozygous for the Novus-4 allele of *Xbarc186-2*. Since the two groups of progeny were selected based on the presence of *Xbarc186* alleles, there were no heterozygotes at the map position of *Xbarc186*. For loci that segregate independently from *Xbarc186*, however, the ratio of heterozygotes: homozygotes should be 1:1. Thus, with respect to each locus, the data of sub-families were used to determine whether the family arose from an F_2 homo- or heterozygote. Chi-square values and probabilities for conformance to 1:1 segregation were calculated at each locus over the 34 families (Fig. 2-4). From the latter graph (Fig. 2-5) it was clear that *Xbarc186* must occur within the region bordered by markers 331 and 7220 (approximately 63.9cM) where the probabilities that these deviations were due to chance, ranged from 0.001-0.01.

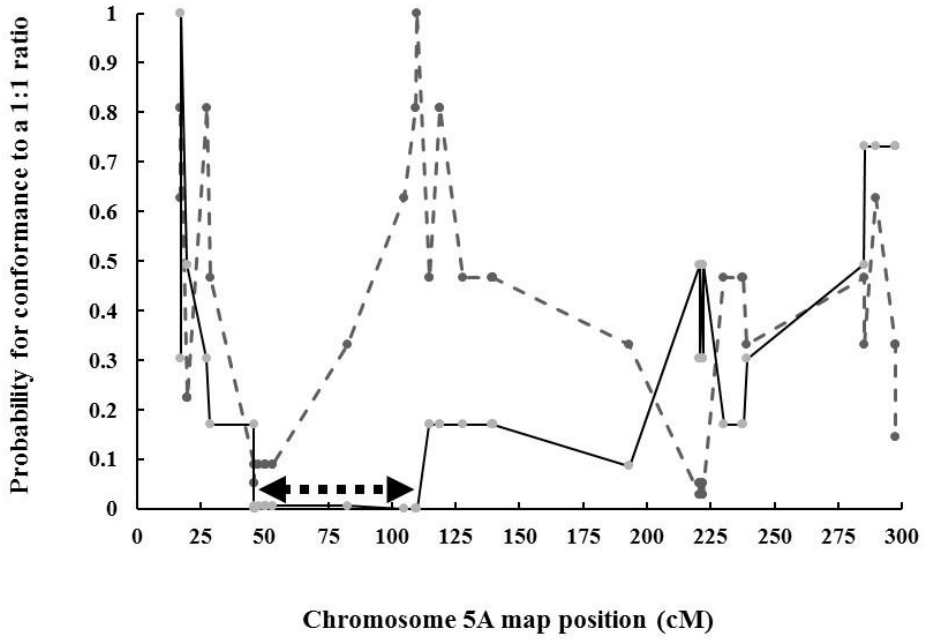

Figure 2-4. Segregation of 14K456-K-1 and Novus-4 alleles at 55 previously mapped chromosome 5A SNP loci (Cavanaugh et al., 2013) in 34 $F_{2:4}$ lines that were selected in the F_2 for being homozygous for either of two *Xbarc186* alleles. Based on marker allele patterns in the data, the most likely location of *Xbarc186* (arrowed) is within the marker 5728 (53.2cM) to 5380 (105cM) interval where the lowest frequency of heterozygotes were observed. The solid black line gives the probability that the observed ratio of heterozygotes to homozygotes = 1:1; whereas the broken grey line gives the probability that the maternal and paternal alleles at a locus segregated in a 1:1 ratio.

In order to determine the location of *Xbarc186* more closely, the haplotypes of all plants within this shorter interval (SNPs 331-7220) were compared (Fig. 2-5). Unfortunately, this only slightly narrowed the location of *Xbarc186* down to the region between marker 5728 (53.22cM) and marker 5380 (105.02cM). Within this region, the haplotype of each line is consistent with the presence of the specific *Xbarc186* allele that was detected. This result is in broad agreement with Somers et al. (2004), who mapped *Xbarc186* at approximately 57cM of chromosome 5AS, and Buerstmayr et al. (2009) mapped *Xbarc186* at approximately 58cM.

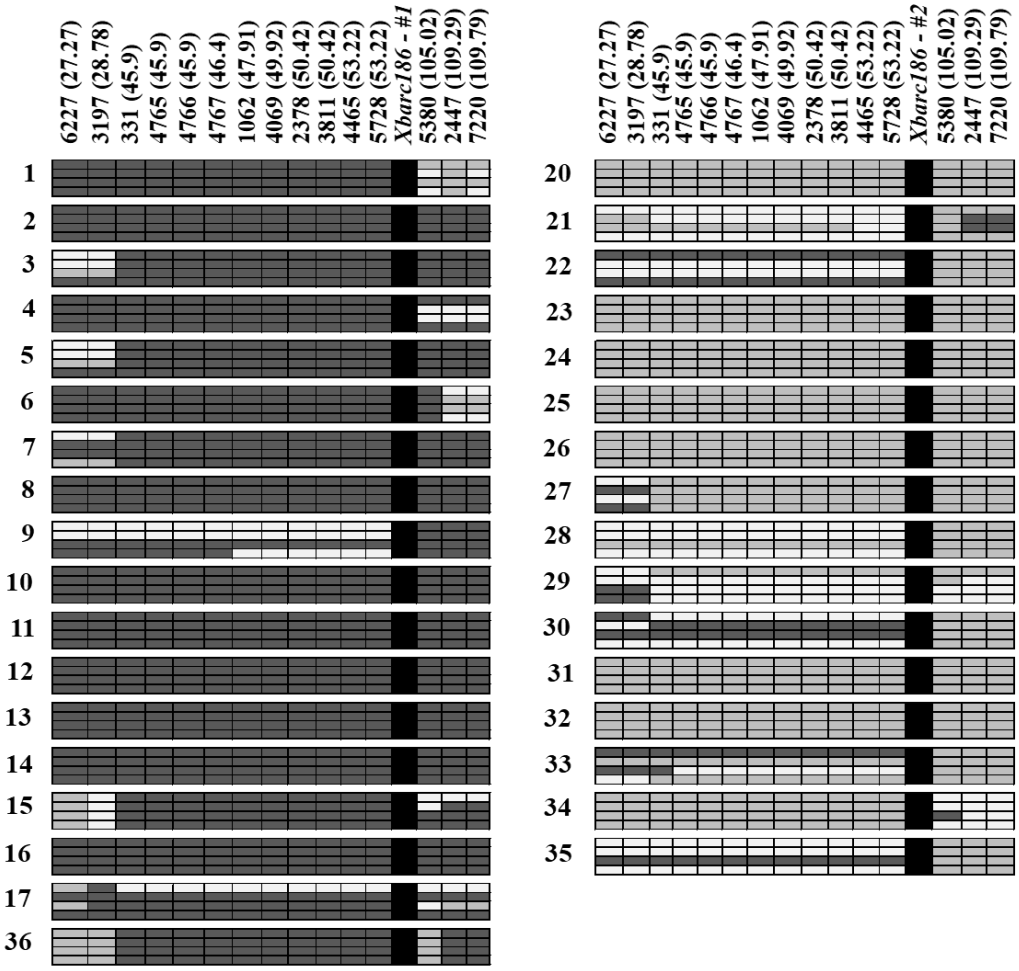

Figure 2-5. Haplotypes for the chromosome 5A region bordered by markers 6227 (27.27cM) and 7220 (109.79cM). Dark grey boxes indicate the 14K456-K-1 allele of a locus; light grey boxes indicate the Novus-4 allele, whereas white boxes indicate the presence of both alleles. Two sets of F_2-derived F_3 families (four F_3 plants per family) were used for SNP analyses: **A.** Family 1-17 &36 derive from F_2 that were homozygous for both *Umn10* (*Fhb1* marker) and the *Xbarc186* allele 1 that is associated with *Qfhs.ifa-5A* in 14K456-K-1; **B.** Family 20-35 derive from F_2 that were homozygous for the *Fhb1* marker as well as the alternative *Xbarc186* allele 2 from Novus-4.

In order to determine whether a chromosome 5A, Novus-4 derived allele outside the *Xbarc186* region affected the FHB scores, fifteen sub-families (each from a different family) with the best average infection values (14.2-18.8%) were compared for their polymorphism at all loci. Within this group, the frequency of the Novus-allele was ≤ 0.53 at all loci, except for a region (220.6 to 221.9cM) where it equaled 0.67. However, when considering the data of all families for this region (Fig. 2-4) it appeared that the ratio of Novus-4 and 14K456-K-1 alleles

was skewed in favor of the Novus-4 allele (0.62-0.63; P = 0.029 to 0.052). Since the material had not been pre-selected for anything other than the *Xbarc186* alleles, the observed segregation distortion at this position was not the result of the presence of an FHB resistance locus, but could instead originate from either small sample size (2n = 34) or an inherent genetic difference that affects gamete viability.

In summary, the 15K353 selections showed resistance that for the most resistant lines (infection severity = 14.2%) was comparable to, but not better than, the value of 14.1% of the 14K456-K-1 parent (donor of *Fhb1* and *Qfhs.ifa-5A*) while the majority of lines performed better than Novus-4 (donor of *Qfhb.rwg-5A.1* and/or *Qfhb.rwg-5A.2*) and had intermediate resistance levels. These observations are consistent with the possible segregation of additional minor resistance QTL, particularly from the 14K456-K-1 genetic background. No indication was found that a (Novus-4 derived) resistance QTL located outside the general *Qfhs.ifa-5A* region contributed to the resistance of the best performing lines. It seems highly likely that the Novus-4 derived resistance QTL is *Qfhb.rwg-5A.1* and that it fully substitutes for *Qfhs.ifa-5A* in cross 15K353.

Confirmation of the absence of *Qfhb.rwg-5A.2* in Novus-4

In a further attempt to test if *Qfhb.rwg-5A.2* is present in Novus-4 and its progenies, 21 chromosome 5A markers (loci *Xwmc470 – Xgwm410*; Fig. 2-1), were tested on a panel consisting of PI277012, RWG21, GP80, Novus-4, Jerry, CM82036 and 14K456-K-1. Twelve of the markers produced useful polymorphic bands within the latter interval. Four proximal marker loci (*Xcfa2163, Xcfa2185, Xbarc232* and *Xgpw2273;* Fig. 2-1) each produced a unique band in PI277012 while none of GP80, RWG21, and Novus-4, produced that same polymorphism, which indicates that these chromosome 5AL SSR loci have not been transferred from the PI277012

source. Another five marker loci (*Xcfd39, Xgpw2172, Xgpw2181, Xgpw2136* and *Xgwm410*) showed the same amplification products (null alleles for *Xcfd39, Xgpw2181 and Xgwm410*; unique alleles for *Xgpw2172* and *Xgpw2136*) with respect to both PI277012 and GP80 whereas RWG21 and Novus-4 had distinctly different bands. The three remaining markers (*Xgwm179, Xgwm126,* and *Xwms595*) produced the same polymorphism (null alleles for *Xwms595*; unique alleles for *Xgwm179 and Xgwm126*), in PI277012, GP80 and RWG21, which would suggest that PI277012 chromatin distally from *Xgpw2136* had been transferred to RWG21. Recombination events that could account for the observed results with respect to the PI277012 derivatives are summarized in Fig. 2-6 and suggest that *Qfhb.rwg-5A.2* had not been present in RWG21, and hence, not in Novus-4 and cross 15K353 either. This would suggest that *Qfhb.rwg-5A.1* co-segregated with *Qfhs.ifa-5A* in cross 15K353. Thus, both genes appear to strengthen the *Fhb1* resistance and their effect in reducing the spread of FHB is similar. While they are likely to be homoeo-alleles, the current data do not provide sufficient proof of that.

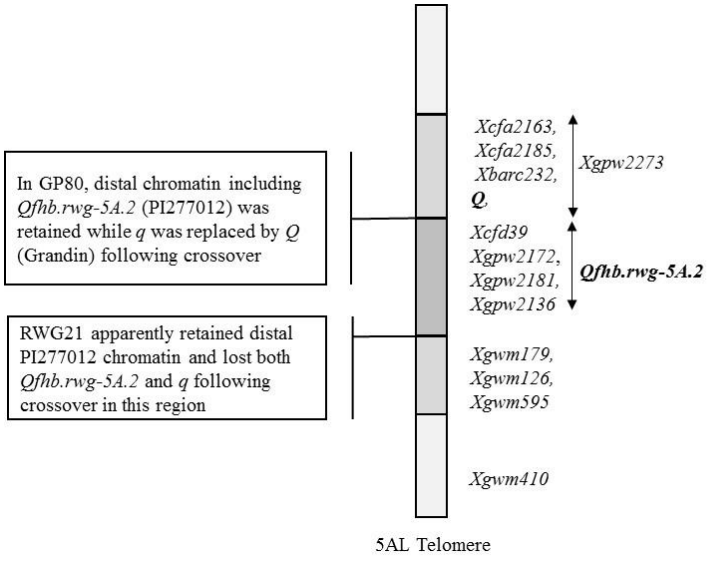

Figure 2-6. Possible recombination events that can explain the genetic make-up of RWG21, Novus-4 and GP80 with respect to the chromosome 5A region that contains the *Qfhb.rwg-5A.2* locus.

Winter survival (field)

Among the 15K353 F_4 sub-families, greenhouse infection severity ranged from 14.2 to 40.1; FDK scores ranged from 2 to 9; whereas DON contents ranged from 0 to 7.4. The field trials were conducted at two locations. However, the replicated trial in Fargo suffered severe winter kill and the survival rate was very poor. Only 25 lines in replicate 1 and 27 lines in replicate 2 had some surviving plants. Of these, only seven lines were common to both replicates. The numbers of surviving plants per row were too low to merit artificial inoculation. At Casselton, 16 sub-families within the *Fhb1* plus *Qfhs.ifa-5A* group and 26 within the *Fhb1* plus *Qfhb.rwg-5A.1* group showed more than 70% winter survival. Although a severe natural FHB epidemic developed in the hard red spring wheat fields at Casselton, the earlier maturing winter wheat escaped the epidemic and only sporadic infection was observed that could not be rated reliably. Among the latter 42 sub-families, those with greenhouse infection severity ≤ 24.1 %, FDK ≤ 4 and DON concentration ≤ 1.5 PPM were identified for continued evaluation (summarized in Table 2-4).

Table 2-4. Winter survival, infection severity, *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) concentration of the best lines from the 15K353 F_4 population based on a greenhouse evaluation and field trials.

	Sub-family	Winter Survival[1] (%)	Infection severity[2] (%)	FDK[2]	DON[2] (PPM)
Fhb1 & *Qfhs.ifa-5A*	3-1	80	19.2	4	1.43
	3-3	80	23.2	4	0.68
	3-4	100	18.2	4	1.06
	5-1	70	23.9	4	1.40
	6-2	70	19.7	3	0.97
	14-3	70	19.0	3	1.09
Fhb1 & *Qfhb.rwg-5A.1*	28-3	90	21.6	3	1.12
	31-4	70	16.5	3	1.27
	31-5	70	15.4	3	1.10

[1] Field data
[2] Greenhouse data

Conclusion

The study showed that line 14K456-K-1 has both *Fhb1* and *Qfhs.ifa-5A* from CM82036 whereas Novus-4 has *Qfhb.rwg-5A.1* from PI277012 only. It appears that additional, unnamed minor resistance QTL from CM82036 could have been transferred to 14K456-K-1; however, this needs corroboration. Absence of *Qfhb.rwg-5A.2* in Novus-4 resulted from its absence in the spring wheat donor line, RWG21. *Qfhs.ifa-5A* and *Qfhb.rwg-5A.1* are probably alleles of the same locus as they have similar effects on the overall resistance phenotype and occur in the same chromosome region; however, the present data are not conclusive in this regard. The introgression of *Qfhb.rwg-5A.1* will not provide additional benefit to breeders, and unfortunately, it has not been possible to evaluate the usefulness of *Qfhb.rwg-5A.2*, which appears to be the more promising of the PI277012 QTL.

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CHAPTER III. TRANSFER OF FUSARIUM HEAD BLIGHT RESISTANCE GENE *QFHS.IFA-3A* FROM SPRING WHEAT AND PYRAMIDING IT WITH *FHB1* IN HARD RED WINTER WHEAT

Abstract

Brazilian spring wheat cultivar Frontana is well known for its moderate resistance to *Fusarium* head blight (FHB). *Qfhs.ifa-3A* is one of several quantitative trait loci (QTL) identified in Frontana and reportedly contribute both type I and type II resistance. Pyramiding of *Qfhs.ifa-3A* with more regularly used resistance QTL such as *Fhb1*, could therefore result in better resistance. This study aimed to use markers to transfer *Qfhs.ifa-3A* from spring wheat to winter-hardy winter wheat and to evaluate its ability to complement *Fhb1*. The F_1: Frontana/Norstar was crossed with the near-isogenic line, Norstar-*Fhb1* and the segregating generations selected for the presence of *Fhb1* (marker Umn10), *Qfhs.ifa-3A* (markers Gwm5, Barc45, Dupw227, and Wmc264), plant height and winter habit. Pyramided genotypes homozygous for *Fhb1* only or for *Fhb1* plus *Qfhs.ifa-3A* were evaluated in a greenhouse test of FHB type II resistance. Norstar proved to be highly susceptible and Norstar-*Fhb1* plants were only slightly more resistant, whereas the added presence of *Qfhs.ifa-3A* did not convincingly improve type II resistance. Segregate #102 was the most resistant of the *Fhb1* plus *Qfhs.ifa-3A* pyramids yet was not as resistant as Frontana. Since *Qfhs.ifa-3A* may have a stronger effect on type I resistance, it will be necessary to continue to evaluate the selections in an artificially inoculated, irrigated field trial to obtain a more realistic evaluation of the combined, overall effect of the two genes. The various pyramids can be a valuable source for the continued introgression of *Qfhs.ifa-3A* into winter wheat germplasm.

Introduction

Fusarium head blight (FHB) is a significant threat to wheat production and use worldwide. The cultivation of genetically resistant cultivars is the most cost-effective method to control the disease (Buerstmayr et al., 2002); however, breeding for resistance is complicated by quantitative inheritance that requires the manipulation of multiple minor effect genes (Bai and Shaner, 1994).

Useful levels of resistance to FHB have been found in wheat germplasm from Asia, Europe, and South America. The majority of the resistance is of Asian origin, including the spring wheats Sumai3 and its derivatives (Anderson et al., 2001; Buerstmayr et al., 2003), and Wangshuibai (Ma et al., 2006). Many of the Asian sources contains the major effect QTL, *Fhb1* (Liu et al., 2008), which explained about 60% of phenotypic variation in the study of Waldron et al. (1999). Brazilian spring wheat Frontana is another important source that provides moderate resistance to FHB (Schröder and Christiansen, 1963). Multiple resistance QTL were identified in Frontana by Steiner et al. (2004). The most significant of these was a 3A QTL (*Qfhs.ifa-3A*) that explained about 16% of the observed phenotypic variation. Additional loci on 5A, 2B and 6B, respectively explained a further 8.8%, 6.1% and 6.7% variation. Mardi et al. (2006) estimated that 3AL and 7AS loci from Frontana explained about 7.7 and 7.6% of the variation in FHB resistance that was observed in their study.

Five FHB resistance types have been described: type I - resistance to initial infection; type II - resistance to spread within a spike (Schröder and Christensen, 1963); type III - resistance to kernel damage; type IV- resistance to DON accumulation (Lemmens et al., 2005); and type V - tolerance (Mesterházy, 1995). The outcomes of earlier studies (Anderson et al., 2001; Buerstmayr et al., 2003) suggested that *Fhb1* provides type II resistance. However,

Frontana was reported to have both type II resistance (Yabwalo et al., 2011) and type I resistance (Steiner et al., 2004). Combining these two types of resistance in a single genotype could therefore provide potentially more effective and durable resistance against FHB.

To date, several attempts were made to pyramid Frontana-derived resistance with *Fhb1* in spring wheat (Burrlakoti et al., 2010; Miedaner et al., 2006; Wilde et al., 2007) or soft red winter wheat (Tamburic-Ilincic et al., 2006). However, pyramids that added *Qfhs.ifa-3A* to the major QTL *Fhb1* and *Qfhs.ifa-5A* gave very minor further reduction in FHB incidence (type I resistance) or spread within the spike (type II resistance) in comparison to the donor parents; either Frontana or Sumai3 derivatives (Miedaner et al., 2006; Tamburic-Ilincic, 2012; Wilde et al., 2007). Although *Qfhs.ifa-3A* had the largest effect in the QTL mapping experiment of Steiner et al. (2004), it was not as prominent as *Fhb1* in the new pyramids, and its additive effect was relatively smaller (Miedaner et al., 2006). The latter authors estimated that, on average, *Fhb1*, *Qfhs.ifa-5A* and *Qfhs.ifa-3A* reduced the FHB rating by 10%, 10% and 5%, respectively. Adapted germplasm from different production regions can be expected to possess different background genes, which could interact differently with the Frontana-derived resistance genes (mainly *Qfhs.ifa-3A*). Introgression and effective use of the minor effect resistance QTL from Frontana may therefore be difficult to achieve in routine breeding programs.

North Dakota State University (NDSU) released the FHB-resistant hard red spring wheat (HRSW) cultivar ‘Alsen’ (Frohberg et al., 2006), which was developed from Sumai3. Alsen is well adapted and has been widely grown in the upper Midwest of the United States (Mergoum et al., 2007). Many new genotypes were successfully developed from Alsen, including HRSW Brick (Glover et al., 2010), Norden (Anderson et al., 2018), and other lines. NDSU hard red winter wheat (HRWW) breeders have also integrated the FHB resistance of Alsen into HRWW

and initiated an attempt to pyramid the resistance (mainly *Fhb1*) with Frontana-derived resistance (mainly *Qfhs.ifa-3A*). This study describes the use of conventional breeding and molecular marker assisted selection in an attempt to combine *Fhb1* and *Qfhs.ifa-3A* in winter-hardy winter wheat backgrounds and to evaluate the usefulness of *Qfhs.ifa-3A* for the improvement of overall FHB resistance.

Materials and Methods

Germplasm

Parental lines that were used to initiate the study are listed in Table 3-1. Cross 15M16 was produced by crossing the F_1: Frontana/Norstar with Norstar-*Fhb1*. Norstar-*Fhb1* is a near-isogenic line with pedigree RWG28/3*Norstar, while RWG28 is a hard red spring wheat (HRSW), carrying the resistance gene *Fhb1* from Alsen (which is a Sumai3 derived HRSW). RWG28 was kindly supplied by Dr. S. Xu (USDA/ARS, Cereal Crops Research Unit, Fargo, ND 58102). Norstar is an FHB susceptible HRWW with very good winter hardiness. Frontana (= Fronteira/Mentana) is a Brazilian spring wheat cultivar with moderate FHB resistance which is based in large part on *Qfhs.ifa-3A* (Mardi et al., 2006; Steiner et al., 2004). Additional control genotypes were included to facilitate the evaluation of the cross and progenies (also listed in Table 3-1).

Greenhouse trial 1

With the exception of Frontana and cross 15M16, all of the genotypes listed in Table 3-1 were evaluated for type II resistance in a greenhouse from October 2015 to March 2016. This was done to assess the level of resistance in Norstar and its near-isogenic line, Norstar-*Fhb1*. Unfortunately, HRSW cultivar Frontana could not be included in trial 1 due to unavailability of seeds (it was, however, evaluated at a later stage). The trial consisted of 12 entries, including six

Table 3-1. Codes, pedigrees, known resistance genes, and associated markers in the wheat germplasm that was used in the study.

Line/Cultivar/Cross	Description/Pedigree	Known FHB resistance genes	Associated markers
Calvin	Durum wheat cultivar		
Norstar	HRWW cultivar		
Superb	HRSW cultivar		
Jerry	HRWW cultivar		
Inia 66	HRSW cultivar		
Rex	Hexaploid triticale cultivar		
Norstar-*Fhb1*	RWG28/3*Norstar	*Fhb1*	Umn10
RWG28[1]	HRSW line (BG290/3* Alsen)/(BG282/3* Alsen)	*Fhb1*	Umn10
11M237-A-1-2	RWG28/Norstar	*Fhb1*	Umn10
RWG10[1]	BG282/3* Alsen	*Fhb1*	Umn10
12DH172	CM82036/Jerry	*Fhb1*	Umn10
Wesley-*Fhb1*	Near-isogenic line of HRWW cultivar Wesley	*Fhb1*	Umn10
Frontana	HRSW cultivar	*Qfhs.ifa-3A*	Gwm5
15M16	Frontana/Norstar//Norstar-*Fhb1*	Segregate *Fhb1, Qfhs.ifa-3A*	

[1]Provided by Dr. S. Xu (USDA/ARS, Cereal Crops Research, Fargo, ND 58102).

susceptible controls, donor parent Norstar-*Fhb1* and five genotypes previously confirmed to have *Fhb1* (Bai, USDA Kansas Genotyping Lab; Chapter II). The greenhouse trial was set up as a completely randomized design within six replicates. Three plants were grown in each replicate (pot). Winter wheat genotypes were vernalized one day after planting for 56 days at 4 ℃ in a cold chamber, while spring wheat genotypes were planted seven days before the winter wheat vernalization ended.

Greenhouse trial 2

Cross 15M16 (Frontana/Norstar//Norstar-*Fhb1*) F_1 were screened for *Fhb1* and *Qfhs.ifa-3A* dihybrids using molecular markers. F_2 seeds harvested from three dihybrid plants were planted and finally 201 plants were sampled and tested for the presence of *Fhb1*. Some of the F_2 were extremely tall and unsuited for breeding; in addition, there was segregation for spring versus winter habit, necessitating selection for these traits. Seeds from F_2 plants that were homozygous for *Fhb1* and with plant height 80-105 cm were harvested for further evaluation. A second greenhouse trial was then conducted to evaluate the F_3 generation of cross 15M16 for FHB type II resistance from September 2017 to March 2018. The parental lines, Frontana, Norstar-*Fhb1*, and Norstar, were included as controls (eight replicates each). Norstar-*Fhb1* and Norstar were vernalized with the selected lines for 56 days at 4 ℃ in a cold chamber and HRSW Frontana was planted seven days before the vernalization ended. The trial was set up as a completely randomized design within five replicates with four plants in each replicate. Four replicates of all selected lines were vernalized one day after planting, the fifth replicate was grown for three months without vernalization in order to identify and remove spring type lines. Only the lines that were pre-selected for height and winter habit were inoculated and evaluated

for FHB resistance. These included: (1) six lines that were homozygous for both *Fhb1* and *Qfhs.ifa-3A*, and (2) four lines homozygous for *Fhb1* only (based on molecular markers).

Molecular marker analyses

Total genomic DNA was extracted from young leaves following the Triticarte Pty. Ltd protocol (http://www.triticarte.com.au/). The quality and concentration of extracted DNA was checked using agarose gel electrophoresis and staining with ethidium bromide. DNA concentration was adjusted to approximately10 ng/µl before polymerase chain reaction (PCR). The detection of *Fhb1* utilized Kompetitive Allele Specific PCR (KASP) marker Fhb_USDA that were designed based on the Umn10 marker (Liu et al., 2008) by the USDA-ARS Genotyping Center (1605 Albrecht Blvd N, Fargo, ND 58102). For *Qfhs.ifa3A*, markers Dupw227 (Steiner et al., 2004), Gwm5, Barc45, and Wmc264 (Tamburic-Ilincic, 2012) were tested. The relative positions of the latter markers are explained in Fig. 3-1.

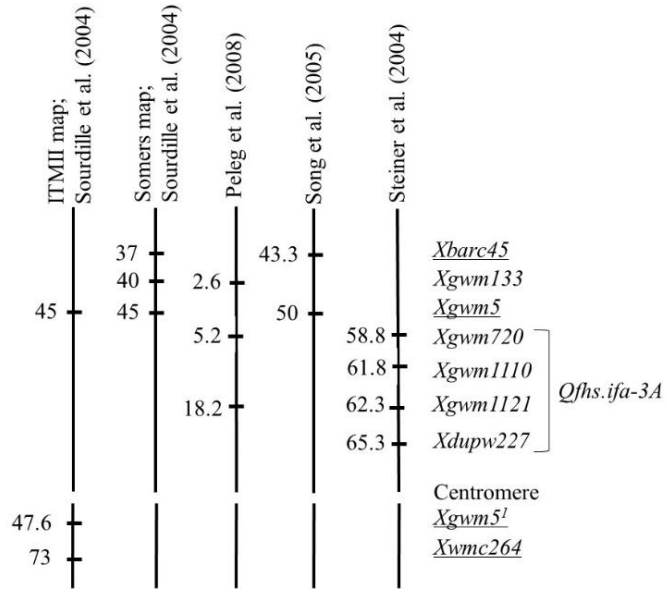

Figure 3-1. The location of *Qfhs.ifa-3A* relative to wheat chromosome 3A proximal markers was deduced from previous studies. *Qfhs.ifa-3A* has been mapped to the *Xgwm720 - Xdupw227* interval by Steiner et al. (2004). The underlined marker loci were utilized for the detection of *Qfhs.ifa-3A* in the study of Tamburic-Ilincic (2012). Integrated map information provided by Sourdille et al. (2004) showed that locus *Xgwm5* had been mapped to 3AS on the Somers consensus map and to 3AL on the ITMI map. Note: [1]Sourdille et al. (2004).

The microsatellite primer sequences are available in the GrainGenes website (http://www.wheat.pw.usda.gov). PCR conditions were as described by Röder et al. (1998). PCR products were visualized by non-denaturing polyacrylamide gel-based electrophoresis with ethidium bromide staining (Umn10), denaturing polyacrylamide gel-based electrophoresis with silver staining (Gwm5), and agarose gel-based electrophoresis with ethidium bromide staining (Dupw227, Barc45, and Wmc264). The markers were tested on the parental genotypes Norstar, RWG28, Norstar-*Fhb1* and Frontana to determine whether favorable allelic differences for marker-assisted selection could be detected using standard PCR. Testing for the presence of *Fhb1* utilized the Kompetitive Allele Specific PCR genotyping system (KASP^{TM}). These analyses were conducted by the USDA-ARS Genotyping Center, Fargo, ND. A two-inch long young leaf was sampled, put directly into a 96-well plate, and then dried/stored at room temperature for KASP^{TM} measurement.

FHB resistance tests

The single spikelet injection method was used for inoculating wheat spikelets at anthesis in a greenhouse (Stack, 1989). An approximately equal mixture of spores from four *Fusarium graminearum* isolates (Fg_124_1, Fg10_135_5, Fg13_79 and Fg08_13) was provided by the Department of Plant Pathology at North Dakota State University. A 10 µl-droplet containing the mix (approximately 100,000 conidia per ml) was injected directly into a floret in the middle of the spike. Within each replicate, ten spikes were inoculated per entry, and thus approximately 40 spikes were inoculated for each line. Inoculated spikes were covered with a (wet) plastic bag for 72 h immediately after inoculation. Afterwards, the infection severity was visually assessed by determining the percentage of infected spikelets per spike at 21 days after anthesis. Inoculated spikes from each replicate were harvested in bulk and manually threshed. The seeds were used

for determining *Fusarium* damaged kernels (FDK). FDK were defined as those that were shriveled, lightweight, and chalky white (Cuthbert et al., 2006). Individual grain samples were rated for the percentage of FDK using the scale of Miedaner et al. (2006).

Statistical analyses

Analyses of variance of phenotypic characters were conducted using SAS (version 9.3) (SAS Institute, Cary, NC). The general linear model (GLM) ($Y = L_i + e$) was applied to compare the differences among lines in a greenhouse experiment that was layed out as a completely randomized design. Program GLIMMIX was applied to compare the differences among F_3 families, groups, and F_3 families within groups.

Results and Discussion

Molecular marker evaluation of parental lines and derivatives

Genotypes listed in Table 3-1 were used either as parents or were included as controls. They were first analyzed with marker Umn10 using both polyacrylamide gel-based electrophoresis and KASP™ technology to confirm the presence/absence of *Fhb1*. These results were found to be in accordance with earlier reports and are summarized in Table 3-2.

Secondly, four microsatellite markers were evaluated for their suitability to predict the presence of *Qfhs.ifa-3A* in cross 15M16 derivatives (Table 3-3). Markers Dupw227, Barc45, and Wmc264 were monomorphic with respect to the parental lines Norstar-*Fhb1* and Frontana; however, marker Gwm5 produced a clear polymorphism and was used for marker assisted selection in the 15M16 population. As is explained in Fig. 3-1, it appears that *Xgwm5* is located in close proximity of *Qfhs.ifa-3A*; however, it is difficult to judge the actual cM distance between the two loci and hence the accuracy with which *Xgwm5* can be used to predict the presence of *Qfhs.ifa-3A*.

Table 3-2. Detection of *Fhb1* associated marker polymorphisms in the genotypes that were evaluated for FHB resistance and used in crosses to pyramid *Fhb1* with the Frontana-derived *Qfhs.ifa-3A*. For marker detection based on the KASP™ system, "+" indicates presence of the marker polymorphism associated with *Fhb1*. For marker detection based on acrylamide gel electrophoresis, presence of the larger fragment (upper band) is indicated with a "1", presence of the smaller fragment (lower band) is indicated with a "2".

Variety/Line	*Xumn10* (KASP™)	*Xumn10* (Acrylamide)	Likely genotype
Jerry	-	2	Absent
Norstar	-	2	Absent
Superb	-	2	Absent
Frontana	No data	2	Absent
12DH172	+	1	Present
RWG10	+	1	Present
RWG28	+	1	Present
11M237-A-1-2	+	1	Present
Wesley-*Fhb1*	+	1	Present
Norstar-*Fhb1*	+	1	Present

Table 3-3. Detection of *Qfhs.ifa-3A* associated marker polymorphisms in the genotypes that were evaluated for FHB resistance and used in crosses for gene pyramids that combine *Fhb1* with Frontana-derived *Qfhs.ifa-3A*. Presence of a larger fragment (upper band) is indicated with a "1", presence of a smaller fragment (lower band) is indicated with a "2".

Variety/Line	*Xdupw227*	*Xgwm5*	*Xbarc45*	*Xwmc264*	Likely genotype
RWG28	2	2	2	2	Absent
Norstar-*Fhb1*	2	2	2	2	Absent
Norstar	1	2	2	2	Absent
Frontana	2	1	2	2	Present

Greenhouse trial 1

Analysis of variance (ANOVA) revealed significant differences in infection severity among 12 parental and control genotypes that were evaluated at the onset of the study and differed with regard to the presence/absence of *Fhb1* (Table 3-4; Fig. 3-2).

Table 3-4. Analysis of variance (completely randomized design) of infection severity of parental material and derivatives.

SOV[1]	DF	SS	MS	*F* value[2]
Lines	11	40203.50	3655.14	30.07***
Error	60	7293.86	121.56	
Total	71	47500.36		

[1]SOV, source of variance; DF, degrees of freedom; SS, sum of squares; and MS, mean square.
[2] *** represents significance at 0.001 level.

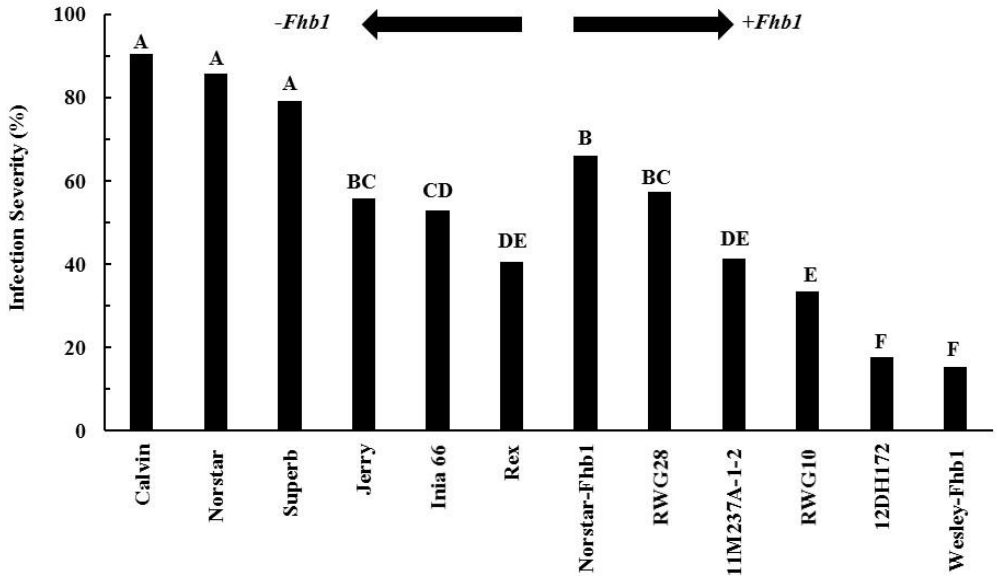

Figure 3-2. Effect of genetic background on *Fusarium* head blight (FHB) infection severity (%) at 21 days after inoculation with *Fusarium graminearum* isolates in a greenhouse experiment. Same letters on columns (entries) suggest that they are not significantly different (α=0.05).

Infection severity 21-days after anthesis ranged from 40.5% for Rex (triticale) to 90.5% for the durum wheat Calvin (Fig. 3-2). Variety Norstar (85.8%) was the most susceptible common wheat genotype whereas Inia 66 (53.0%) was the least susceptible. The *Fhb1*-carrying genotypes showed better resistance, however, also ranged widely with respect to infection severity. Norstar-*Fhb1* was the least resistant (infection severity = 66.2%) whereas Wesley-*Fhb1*

(15.4%) was the most resistant. Thus, Nostar-*Fhb1* appeared to be less resistant than Inia 66 and Jerry, both of which lack *Fhb1*. These differences clearly relate to genetic background, i.e. the presence in other chromosome regions of minor QTL that are unaccounted for and affect FHB resistance. Wesley is known to be only moderately susceptible to FHB, i.e. it showed 52-56% infection in greenhouse and field trials (Jin et al., 2006), implying the presence of minor resistance QTL. In the backcross-derivative Wesley-*Fhb1*, the combination of several QTL including *Fhb1* can account for its more pronounced resistance. Similarly, line 12DH172 has CM82036 as a parent. CM82036 has *Fhb1*, *Qfhs.ifa-5A* and a QTL on 1B (Buerstmayr et al., 2002), all of which confer a high level of FHB resistance similar to that of Sumai3 (Buerstmayr et al., 1996). Thus, the present data suggest that Norstar has few/no effective minor background QTL for FHB resistance which makes it highly susceptible to the disease. While the addition of *Fhb1* through backcrosses slightly improved its overall resistance, Norstar-*Fhb1* remains highly vulnerable to FHB.

Molecular marker selection within cross 15M16

All of the cross 15M16 F_1 plants were heterozygous for *Fhb1* and ten plants were therefore screened with marker Gwm5 to identify *Qfhs.ifa-3A* heterozygotes. Three dihybrid F_1 plants were identified and grown to obtain F_2 seeds (Table 3-5).

Seventy F_2 seeds of each selected line were planted from which 201 seedlings were obtained and screened for the presence of marker Umn10 (using the KASP^{TM} technique). Fourteen of the 201 plants could not be classified whereas 46, 92 and 49 lines were resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. Chi- square analysis showed that the *Fhb1* marker segregated in a 1:2:1 F_2 ratio [$\chi^2 = 0.144$, $\chi^2_{table}=5.99$ (df = 2, α = 0.05)]. With respect to the 46 *Fhb1* marker homozygous F_2 individuals, marker Gwm5 was

applied to select for *Qfhs.ifa-3A*. Of these, four plants died, twelve plants showed the same band pattern as Frontana, nineteen plants were heterozygotes, and eleven plants were recessive homozygotes (lacking *Qfhs.ifa-3A* marker locus). Chi- square analysis showed that the Gwm5 marker segregated in a 1:2:1 F_2 ratio [$\chi^2 = 0.429$, $\chi^2_{table}=5.99$ (df = 2, $\alpha = 0.05$)]. Upon maturity, the F_2 plants of the two homozygous resistant classes were measured to determine plant height and only those within the height range 80 to 105cm were kept and planted in greenhouse FHB trial 2.

Table 3-5. Detection of *Qfhs.ifa-3A* alleles using marker GWM5 within the 15M16 F_1 population. Presence of the larger fragment (upper band) is indicated with a "1", presence of the smaller fragment (lower band) is indicated with a "2", whereas "-" indicates failed amplification.

Variety/Line	Bands
Frontana	1
Norstar	2
Norstar-*Fhb1*	2
Norstar-*Fhb1-Sr26*	2
15M16-1	1,2
15M16-2	1,2
15M16-3	1,2
15M16-4	2
15M16-5	-
15M16-6	2
15M16-7	-
15M16-8	2
15M16-9	2
15M16-10	2

Greenhouse trial 2

All F_3 families with appropriate plant height were also evaluated for winter growth habit and the spring type and segregating families were excluded from FHB phenotyping. This left four homozygous for *Fhb1* marker locus only and six homozygous for *Fhb1* marker locus plus

Qfhs.ifa-3A marker locus resistant F_3 families which were then evaluated for FHB type II resistance (Fig. 3-3). Significant differences were observed among F_3 families, groups, and F_3 families within groups (Table 3-6). Both Norstar-*Fhb1* and Frontana (infection severities of 40.8% and 33.6%, respectively), were significantly more resistant than Norstar (53.9%). Moreover, Frontana provided slightly stronger resistance than Norstar-*Fhb1*. Both Norstar-*Fhb1* and Frontana confer moderate but different resistance, and their 15M16 progeny can be expected to include plants with a wide range of resistance, including improved, transgressive resistance. If in addition to *Qfhs.ifa-3A*, Frontana contributed another three resistance QTL (on chromosomes 5A, 2B and 6B; Steiner et al., 2004) their expected frequencies in F_2 families would have been: all additional QTL present = 0.08; two additional QTL present = 0.32; one additional QTL present = 0.42 and no additional QTL present = 0.18.

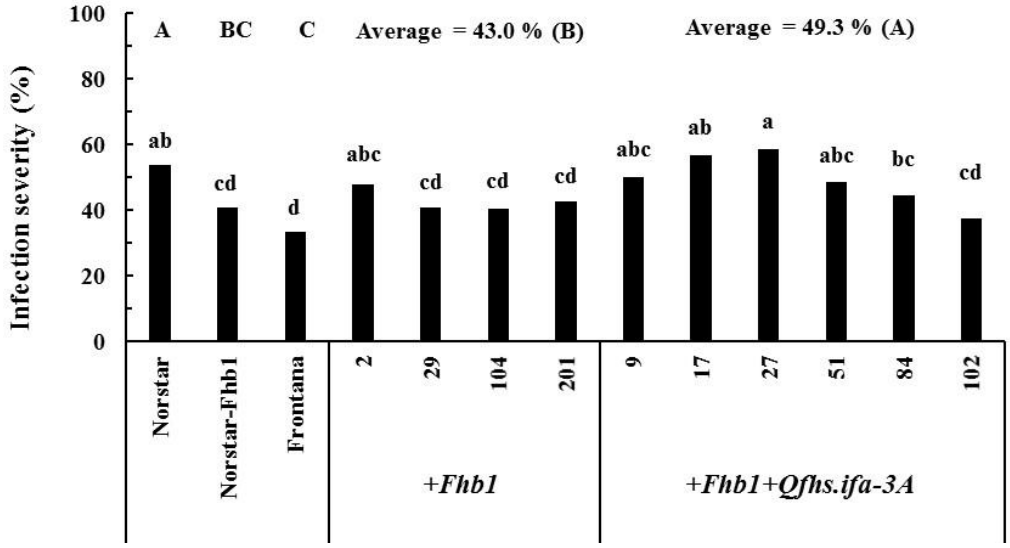

Figure 3-3. *Fusarium* head blight infection severity (%) of F_3 families 21 days after inoculation with *Fusarium graminearum* isolates. Same lowercase letters on columns suggest that those families are not significantly different (α=0.05); same capital letters with respect to family groups suggest that those groups are not significantly different (α=0.05).

Table 3-6. Statistical analysis of infection severity to compare performance among F_3 families, groups, and F_3 families within each group using program GLIMMIX of SAS. The partitioning of the 13 families into groups and families within groups is given in Fig. 3-3.

Effect	Num DF[1]	Den DF[2]	*F* Value[3]	Pr > *F*
F_3 families (F)	62	256	4.01***	<.0001
Group (G)	12	256	10.49***	<.0001
F(G)	50	256	2.60***	<.0001

[1] Num DF: the number of degrees of freedom in the model.
[2] Den DF: the number of degrees of freedom associated with the model errors.
[3] *** represents significance at 0.001 level.

Among the homozygotes for *Fhb1* marker locus, #29, #104 and #201 were similar to Norstar-*Fhb1* and significantly more resistant than Norstar (Fig. 3-3). Family #2 had intermediate resistance that was not significantly different from either Norstar or Norstar-*Fhb1*. On average, the *Fhb1* only families showed 43.0% infection, which was not statistically different from Norstar-*Fhb1*, but significantly more susceptible than Frontana. The *Fhb1* plus *Qfhs.ifa-3A* families showed more variability and an average of 49.3% infection, which was comparable to the susceptible parent Norstar and, on average, more than in Frontana and the *Fhb1* only families. Lines #17 and #27 were the most susceptible and did not differ significantly from Norstar. This result could suggest either the absence of *Fhb1* marker locus despite the presence of the Umn10 marker, or, alternatively Norstar may possess QTL that actually increase susceptibility. Lines #9, #51, #84 and #102 reacted similar to Norstar-*Fhb1* with #84 and #102 being significantly more resistant than Norstar. Thus, if *Qfhs.ifa-3A* was present as suggested by the marker data, then this QTL did not significantly improve resistance in these lines. Among the ten families, #102 showed the highest level of resistance which was not significantly different from Frontana. While the results were generally disappointing, it can probably be explained in part by the absence of resistance QTL in the Norstar genetic background (which constituted 75% of the

hybrid background of the cross). The high level of susceptibility of Norstar may be partly due to the presence of QTL that renders this genotype more sensitive to FHB attack (Oliver et al., 2007; Stack et al., 2002). Steiner et al. (2004) identified up to four small effect QTL in Frontana. Not only is it difficult to measure such small, individual effects with consistency, it is not certain that the resistance QTL will always interact additively either, which could make it difficult to predict the overall level of resistance. Lastly, it is also not clear whether the marker locus *Xgwm5* is actually linked closely enough to *Qfhs.ifa-3A* and whether there might have been false-positive identifications. Different map positions were assigned to *Xgwm5* in the Somers and ITMI maps (Sourdille et al., 2004), which could have implications for its reliability as a predictor of the presence of *Qfhs.ifa-3A*.

Qfhs.ifa-3A is believed to contribute mostly to type I resistance (Steiner et al., 2004), which was not measured in the testing methodology that was applied here. In the current protocol a single floret is injected with inoculum and disease spread from that infection point is monitored. A type I greenhouse resistance evaluation test or field testing based on an irrigated/misted nursery will expose all florets to random infection from sprayed inoculum or corn spawn and will provide a more exact and realistic evaluation of the *Qfhs.ifa-3A* effect.

With the possible exception of family #102, no evidence could be found that *Qfhs.ifa-3A* in combination with *Fhb1* will achieve a consistent and utilizable reduction in the level of type II FHB resistance that can be pursued in routine breeding. Family #102 will be studied further to confirm that it has retained *Qfhs.ifa-3A* and if confirmed, it will be used as a cross parent. Failure of the remaining five *Fhb1* plus *Qfhs.ifa-3A* families suggested either that the markers were not reliable and the respective resistance gene(s) were not present; or, that background QTL segregated which reduced rather than improved overall resistance.

The microsatellite markers that were available for marker-assisted selection of *Qfhs.ifa-3A* were problematic. These markers showed limited polymorphism and were mostly not useful for selection. The chromosome region containing the QTL is not well mapped either which raised concerns about the reliability of the existing markers. In order to achieve accurate and complete transfer of the Frontana resistance QTL and to develop a well-adapted, resistant HRWW introgression line, a genome-wide genotyping approach should be followed. This will allow for more accurate haplotype-based selection and targeted transfer of the critical genomic regions.

Conclusion

Norstar was found to be highly susceptible to FHB and its near-isogenic line, Norstar-*Fhb1*, remained susceptible to FHB albeit less so than Norstar. An attempt to transfer *Qfhs.ifa-3A* from the HRSW Frontana into HRWW and to evaluate its ability to complement the *Fhb1* resistance yielded disappointing results. Only one family (#102) that was homozygous for the marker polymorphisms of both resistance QTL had resistance stronger (but not significantly so) than Norstar-*Fhb1*. The overall resistance of family #102 was less, but not significantly different from the Frontana resistance. Family #102 can be a potentially useful HRWW cross parent and source of *Qfhs.ifa-3A*; however, it will be necessary to confirm that this resistance QTL had in fact been transferred. Although no deliberate attempt was made to select for the presence of another three Frontana background QTL with smaller individual effects than *Qfhs.ifa-3A*, there is a significant probability that some of these QTL may have been retained through chance in family #102.

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CHAPTER IV. INTROGRESSION OF *FHB6* INTO HARD RED WINTER WHEAT CONTAINING *FHB1* AND *QFHS.IFA-5A*

Abstract

Fusarium head blight (FHB; caused by *Fusarium graminearum* Schwabe) is a serious disease of hard red winter wheat (HRWW) in North Dakota. The North Dakota State University (NDSU) breeding program acquires and introgresses useful FHB resistance QTL (quantitative trait loci) into its breeding population, in particular genes that can complement *Fhb1*. Resistance gene *Fhb6* derives from *Elymus tsukushiensis* Honda and occurs on a chromosome 1AS translocation in the spring wheat germplasm line, TA5660. TA5660 was used to produce the winter wheat cross: TA5660/ Jerry// Radiant/3/ 14K456-K-1. Final F_1 plants that were trihybrid for *Fhb1*, *Qfhs.ifa-5A* and *Fhb6* provided an F_2 population. Marker-assisted selection and plant height screening were used to identify F_2-derived families that were homozygous for *Fhb1* only (1 family), *Fhb1* and *Qfhs.ifa-5A* (2 families), *Fhb1* and *Fhb6* (4 families), and *Fhb1*, *Fhb6*, and *Qfhs.ifa-5A* (6 families). The 13 families plus parental controls were evaluated for FHB type II resistance and agronomic traits in two separate, concurrent greenhouse trials. Although segregation of background genes with effect on FHB resistance confounded interactions among the three QTL, the results suggested that combining either *Qfhs.ifa-5A* or *Fhb6* with *Fhb1* can improve FHB type II resistance; however, pyramiding of all three QTL together did not give further symptom reduction. However, such conclusions can only be preliminary due to the small numbers of lines within each of the categories of pyramided QTL. Assessment in a greenhouse did not reveal associated, detrimental yield effects that could hinder the commercial use of *Fhb6*. Pyramided genotypes 198 and 225 (*Fhb1* and *Qfhs.ifa-5A*), 207 (*Fhb1* and *Fhb6*), and 217 (*Fhb1*,

Qfhs.ifa-5A and *Fhb6*) showed the most promising FHB resistance and plant type and will be used for continued field evaluation and breeding.

Introduction

Many breeding programs strive to improve *Fusarium* head blight resistance of wheat. Although more than 100 FHB resistance QTL have been reported (Bai et al., 2018), comparatively few were frequently used in breeding programs. *Fhb1* (first designated *Qfhs.ndsu-3BS*) and *Qfhs.ifa-5A* are two widely used resistance QTL that were estimated to explain 29-60% (Buerstmayr et al., 2003; Waldron et al., 1999) and 20% (Buerstmayr et al., 2003) of the phenotypic variation for disease severity, respectively. These two QTL have also been incorporated into HRWW lines of the NDSU breeding program. Selection 14K456-K-1 is one such derivative that has been confirmed to have both *Fhb1* and *Qfhs.ifa-5A* and conferred strong FHB resistance, which was not significantly different from the CM82036 resistance (Chapter II).

To date, three alien translocations that harbor FHB resistance QTL designated as *Fhb3*, *Fhb6* and *Fhb7*, have been introgressed from the alien species *Leymus racemosus* (Qi et al., 2008), *Elymus tsukushiensis* (Cainong et al., 2015), and *Thinopyrum ponticum* (Guo et al., 2015), respectively. *Elymus tsukushiensis* Honda ($2n = 6x = 42$, $S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$, syn. *Roegneria kamoji* C. Koch) is a perennial, cross-pollinating hexaploid species native to eastern Asia. This distant wild relative of hexaploid wheat ($2n = 6x = 42$, AABBDD), exhibits strong FHB type I and type II resistance (Ban, 1997; Weng and Liu, 1989). A wheat-*E. tsukushiensis* disomic addition line having an *E. tsukushiensis* group 1 chromosome ($1E^{ts}$#1S), was produced by Wang et al. (1999). This addition line conferred strong FHB type II resistance, similar to the resistance in Sumai3 (Cainong et al., 2015; Wang et al., 1999). Utilizing the addition line, a novel FHB-resistant wheat-*E. tsukushiensis* recombinant was developed and released as germplasm line

KS14WGRC61 (TA5660; Chinese Spring background) by Friebe et al. (2013). The translocated chromosome in line TA5660 consists of the complete 1AL arm, a proximal part of the 1AS arm and a small distal segment derived from $1E^{ts}$#1S. Importantly, the wheat and alien chromosome regions that were exchanged appears to be homoeologous and the translocation therefore seems to be compensating (Cainong et al., 2015). The $1E^{ts}$#1S translocated segment contains *Fhb6* that confers type II resistance to FHB. Molecular markers to detect the *E. tsukushiensis* alien segment in wheat backgrounds were developed (Cainong et al., 2015).

The introgression of alien chromatin through translocation inevitably results in linkage drag which could have beneficial, detrimental or no significant effect on the recipient species. Wheat-rye 1BL.1RS translocation lines (Zeller et al., 1973) improved both disease resistance (*Lr26*, *Sr31*, *Pm8*, etc.) and yield (Moreno-Sevilla et al., 1995), however, had negative effect on bread quality, causing dough stickiness, lack of mixing tolerance (Martin and Stewart, 1986), reduced SDS-sedimentation volume, and dough development time (Dhaliwal et al., 1987). The T4 translocation (Sharma and Knott, 1966) introduced the leaf and stem rust resistance genes *Lr19* and *Sr25* to wheat, yet was also associated with an undesirable gene that caused yellow endosperm pigmentation, whereas linkage drag from *Lr45* (McIntosh et al., 1995a, b) resulted in poor agrotype. As bread wheat is a hexaploid with three sets of chromosomes, it should generally be more tolerant and more likely to benefit from alien introgression (Gill et al., 2011). Apart from the FHB resistance, there are no previously reported positive or negative effects associated with the use of the TA5660 *Fhb6* translocation. This study aimed to produce and evaluate two and three QTL genotypic combinations of *Fhb1, Qfhs.ifa-5A* and *Fhb6* to determine whether additive genetic effects are evident and to evaluate the utility of *Fhb6* for breeding purposes. A second objective was to do a preliminary test for associated agronomic effects to the use of the

Fhb6 translocation and to determine whether winter-hardy genotypes with acceptable agrotype and superior FHB resistance can be developed for use as breeding parents.

Materials and Methods

Crosses and outline

The common wheat lines used, the crosses that were made and the selection strategy that was followed to develop the various gene pyramids are outlined in Fig. 4-1. To initiate the transfer of *Fhb6* from HRSW to HRWW, the germplasm line TA5660 was first crossed with Jerry and the F_1 was then crossed with Radiant to produce F_1: 14M7 (TA5660/Jerry//Radiant). Accession TA5660 was kindly provided by Dr. B. Friebe (Department of Plant Pathology, Kansas State University, Manhattan, KS 66506) and has a small translocation from *E. tsukushiensis* containing the *Fhb6* gene at the distal end of chromosome 1AS. Jerry and Radiant are both susceptible to FHB. Jerry is a HRWW with broad adaptation to North Dakota and excellent winter hardiness. Radiant is a well-adapted Canadian HRWW with good winter survival and yield performance (Thomas et al., 2012). The 14M7 F_1 was then crossed with a winter-hardy breeding line, 14K456-K-1 (= CM82036/Jerry/3/*Lr56*-157/Superb//4*Jerry), which derived its FHB resistance from CM82036 and is homozygous for resistance genes *Fhb1* and *Qfhs.ifa-5A* (Chapter II). The final F_1 hybrid plants were screened with appropriate markers to identify trihybrids with respect to the three QTL. Progeny of the selected plants were grown and genomic DNA of 378 F_2 plants were sampled and tested for the presence of *Fhb1* and *Qfhs.ifa-5A*. First, the *Fhb1* homozygotes were identified. These plants were then screened to identify plants that were also homozygous for the presence or absence of *Qfhs.ifa-5A*. This yielded two homozygous groups, i.e. *Fhb1* only and *Fhb1* plus *Qfhs.ifa-5A* that were subsequently tested for the presence of *Fhb6* homozygotes. Finally, four categories of plants were obtained, including

four homozygotes having *Fhb1* only; two homozygotes having *Fhb1* and *Qfhs.ifa-5A*; six homozygotes having *Fhb1* and *Fhb6*; and six homozygotes having *Fhb1*, *Fhb6*, and *Qfhs.ifa-5A*.

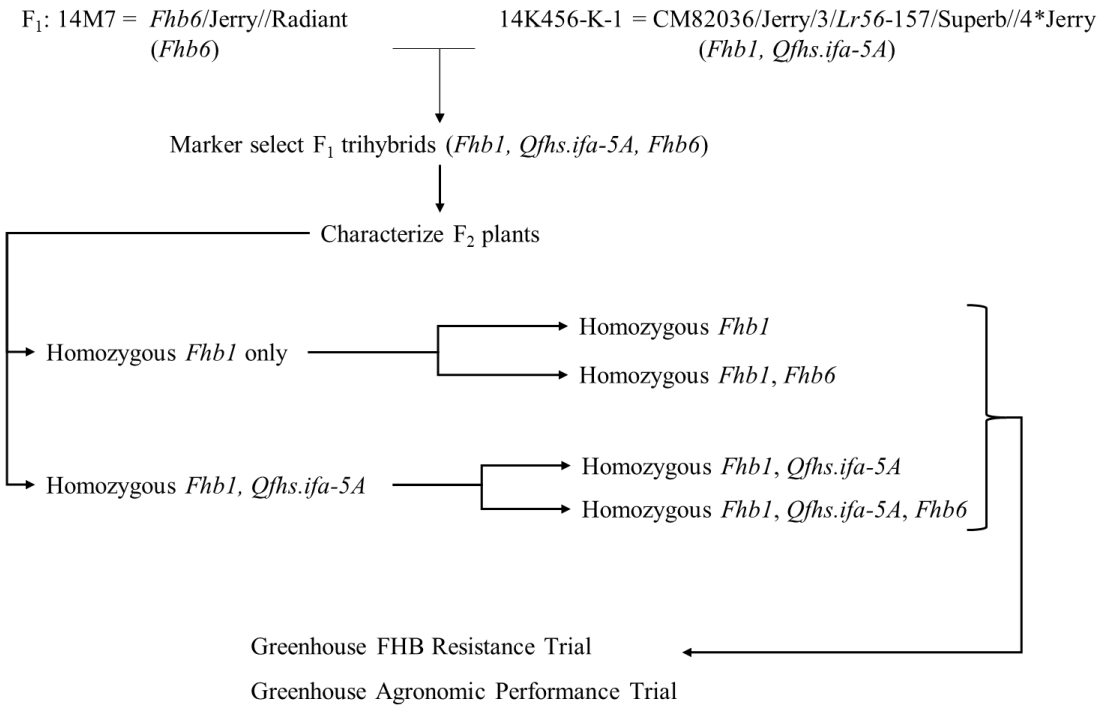

Figure 4-1. Crosses and selection strategy that were used to combine Fusarium head blight resistance QTL *Fhb1*, *Qfhs.ifa-5A* and *Fhb6* in hard red winter wheat genetic backgrounds.

Molecular marker analyses

The detection of *Fhb1* utilized two Kompetitive Allele Specific PCR (KASP) markers that were designed by the USDA-ARS Genotyping Center (1605 Albrecht Blvd N, Fargo, ND 58102). Fhb_USDA was based on the Umn10 marker (Liu et al., 2008) and Fhb_FM227 was based on the published sequence of *Fhb1* (Rawat et al., 2016). *Qfhs.ifa-5A* was detected with the simple sequence repeat (SSR) marker Barc186 (Buerstmayr et al., 2009; Somers et al., 2004); and *Fhb6* was tested for using markers tplb0017E15 and AK3575096 (Cainong et al., 2015). DNA extraction and genotyping (KASP^{TM}) to detect *Fhb1* were done by the USDA-ARS Genotyping Center, Fargo, ND. A two-inch, young leaf segment was sampled, put directly into a

96-well plate, and then dried/stored at room temperature for DNA extraction. DNA concentration was adjusted to approximately10 ng/µl before Polymerase Chain Reaction (PCR). The primer sequences and PCR conditions of marker Barc186 are available in the GrainGenes website (http://www.wheat.pw.usda.gov). The primer sequences and PCR conditions of the *Fhb6* markers were provided by Cainong et al. (2015). PCR products were digested with restriction enzyme *HaeIII* and incubated at 37 ℃ for 3h, then visualized by agarose gel-based electrophoresis with ethidium bromide staining. The respective markers were tested on the parental genotypes TA5660, Jerry, Radiant, CM82036, and 14K456-K-1 (Fig. 4-1) to determine whether favorable allelic differences for marker-assisted selection existed. Suitably polymorphic markers were used to evaluate the F_1 and F_2 progenies.

Phenotypic evaluation of F_3 sub-families

Greenhouse trials were conducted from May to December 2018 to evaluate the F_2-derived F_3 families of cross 14M7/14K456-K-1. Within the four categories of resistant homozygotes identified, F_2 plants that were too tall (> 100cm) were also excluded. This left one *Fhb1* only family, two *Fhb1* & *Qfhs.ifa-5A* families, four *Fhb1* & *Fhb6* families, and six *Fhb1*, *Fhb6*, & *Qfhs.ifa-5A* families. The thirteen resistant F_3 families (five replicates of each) and the parental controls (TA5660, 14K456-K-1, CM82036, Radiant, and Jerry; ten replicates of each) were planted in two identical trials. Each trial was set up as a completely randomized design. The experimental unit was a single pot containing four plants of that genotype. The first trial evaluated FHB Type II resistance and the second trial measured agronomic differences among genotypes.

In the first trial the single spikelet injection method was used for inoculating wheat spikelets at anthesis (Stack, 1989). A mixture of approximately equal quantities of spores from

four *Fusarium graminearum* isolates (Fg_124_1, Fg10_135_5, Fg13_79 and Fg08_13) was provided by the Department of Plant Pathology at North Dakota State University. A 10 µl-droplet containing the mixture (approximate concentration = 100,000 conidia per ml) was injected directly into a floret in the center of the spike. Within each pot, ten spikes were inoculated. Thus, 50 spikes were inoculated for each F_3 family and 100 spikes were inoculated for each control. Inoculated spikes were covered with a (wet) plastic bag for 72 h immediately after inoculation. Afterwards, the severity of infection was assessed visually by determining the percentage of infected spikelets per spike at 21 days after anthesis. Inoculated spikes from each replicate were harvested in bulk and manually threshed. The seeds were used for determining *Fusarium* damaged kernels (FDK). Individual grain samples were rated for the percentage of FDK using the scale described by Miedaner et al. (2006).

The second trial measured agronomic traits, including plant height (measured in cm from the base of the plant to the tip of the tallest tiller, ignoring the awns), number of productive spikes per plant, number of spikelets per main spike, thousand-kernel weight (g), and grain yield per plant (g/plant).

Statistical analyses

Analyses of variance of phenotypic characters were conducted using SAS (version 9.3) (SAS Institute, Cary, NC). A general linear model (GLM) ($Y = L_i + e$) was applied to compare the differences among greenhouse tested lines (completely randomized design). Program GLIMMIX was applied to compare the differences among F_3 families, groups, and F_3 families within groups.

Results and Discussion

Testing of the parental lines with KASP marker Fhb_USDA (based on Umn10) confirmed the presence of *Fhb1* in CM82036 and 14K456-K-1 (Table 4-1). Marker Barc186 amplified the same larger sized band (referred to as allele *Xbarc186-1*; approximately 210bp) in 14K456-K-1 that is associated with *Qfhs.ifa-5A* in CM82036. This is in accordance with the results in Chapter II, which reported the presence of both *Fhb1* and *Qfhs.ifa-5A* in 14K456-K-1. However, genotypes Radiant and TA5660 (without *Qfhs.ifa-5A*) also produced the *Xbarc186-1* allele whereas Jerry had allele *Xbarc186-2* (approximately 201bp).

Table 4-1. Marker polymorphisms in the parental genotypes that were crossed to develop gene pyramids with different combinations of *Fhb1*, *Qfhs.ifa-5A* and *Fhb6*. With respect to each marker, the marker allele associated with the favorable QTL allele was named allele-1 and the alternative allele was named allele-2.

Parent	*Xumn10* (*Fhb1*)	*Xbarc186 (Qfhs.ifa-5A)* Allele	*Xak357509 (Fhb6)* Allele
CM82036	1	1	2
14K456-K-1	1	1	2
Jerry	2	2	2
Radiant	2	1	2
TA5660	2	1	1

This meant that in order to be able to use *Xbarc186* for the selection of *Qfhs.ifa-5A*, it was necessary to select F_1:14M7 plants that were *Xbarc186* heterozygotes and to use these plants for making the 14M7/14K456-K-1 cross (Fig. 4-2A). Two CAPS markers (tplb0017E15 and AK357509; Cainong et al., 2015) were evaluated for the detection of *Fhb6* in TA5660. PCR products of both markers were visualized in 3.5% agarose gel and each showed a prominent, monomorphic band among the parental lines (the approximately 300-350bp product produced by AK357509 is shown in Fig. 4-2B). Following *HaeIII* digestion at 37 ℃ for 3h, the tplb0017E15 digestion products occurred in both resistant and susceptible parents (Table 4-1) and it could

therefore not be used for selection. However, the marker AK357509 digest retained a pronounced band of approximately 330bp (here referred to as allele *Xak357509-1*) in TA5660 (Fig. 4-2C). In the non-*Fhb6* parents, restriction digestion resulted in the appearance of a smaller, prominent band (approximately 260-270bp) which is here referred to as allele *Xak357509-2*.

Thirteen 14M7 F_1 plants (Fig. 4-1) were screened for the presence of *Fhb6* and seven heterozygotes (#1, #4, #7, #9, #10, #12 and #13) were found (Fig. 4-2C). The latter seven plants were then also tested for their Barc186 polymorphisms. Three plants (#1, #7, and #12) were homozygous for *Xbarc186-1* and were therefore discarded. The four remaining plants (#4, #9, #10, and #13) were heterozygotes and were kept for crosses with 14K456-K-1.

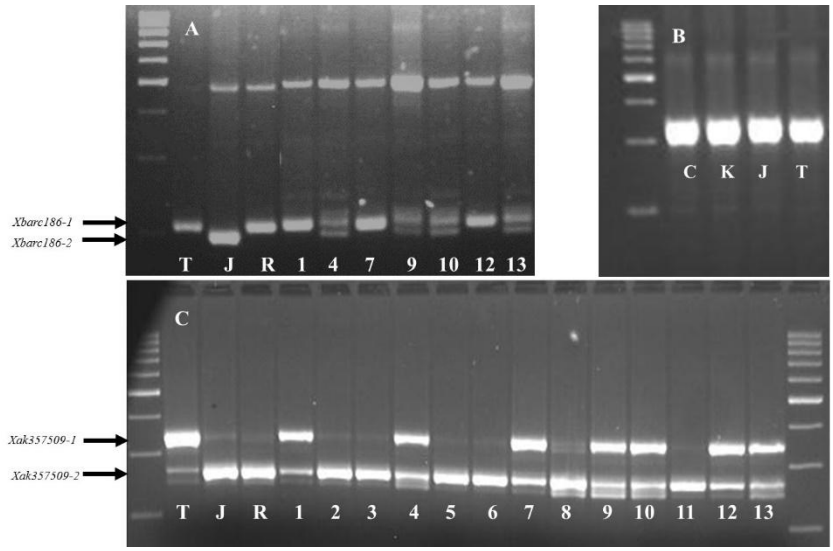

Figure 4-2. Agarose gel pictures showing marker polymorphisms used for the detection of FHB resistance QTL among the parents and progeny of cross 14M7. Where: T = TA5660, J = Jerry, R = Radiant, C=CM82036, K=14K456-K-1, and 1–13 = individual F_1 14M7 plants. (A) *Xbarc186* marker polymorphisms that were used for the detection of *Qfhs.ifa-5A*. (B) *Xak357509* (*Fhb6* marker) monomorphism before restriction enzyme digestion. (C) *Xak357509* marker polymorphisms after restriction enzyme digestion.

Twenty F_1 plants from cross 14M7/14K456-K-1 plus the parental lines (CM82036, 14K456-K-1, Jerry, Radiant, and TA5660) were tested for heterozygosity of *Fhb1*, *Qfhs.ifa-5A*, and *Fhb6*. First, KASP marker (Fhb_USDA) data were obtained (USDA-ARS Genotyping

Center, Fargo, ND) which unambiguously showed that 14 of the plants were heterozygous at this marker locus (Table 4-2). Next, marker Barc186 was applied and it appeared that six of the latter 14 plants were probably *Qfhs.ifa-5A* heterozygotes. Lastly, marker AK357509 was used to test the six selected dihybrid plants plus TA5660 and 14K-456-K-1 following which two plants (#3 and #13) were found to be *Fhb6* heterozygotes and were grown to maturity to obtain F_2 seed.

Table 4-2. Selection of *Fhb1*, *Qfhs.ifa-5A* and *Fhb6* trihybrids among cross14M7/14K456-K-1 F_1 plants using markers Fhb_USDA (KASP^{TM} system), Barc186 and AK357509. With respect to each marker, the marker allele associated with the favorable resistance QTL allele was named allele-1 and the alternative allele was named allele-2.

Lines/Segregates	*Fhb1* (*Xumn10* allele)	*Qfhs.ifa-5A* (*Xbarc186* allele)	*Fhb6* (*Xak357509* allele)
CM82036	1	1	-
14K456-K-1	1	1	2
Jerry	2	2	-
Radiant	Unknown	1	-
TA5660	2	1	1,2
1	1/2	1,2	2
2	1/2	1	-
3	1/2	1,2	1,2
4	1/2	1	-
5	1/2	1,2	2
6	1/2	1	-
7	1/2	1	-
8	Unknown	1	-
9	Unknown	1,2	-
10	Unknown	1,2	-
11	1/2	1	-
12	1/2	1,2	2
13	1/2	1,2	1,2
14	1/2	1	-
15	Unknown	1	-
16	Unknown	1	-
17	Unknown	1,2	-
18	1/2	1	-
19	1/2	1,2	2
20	1/2	1	-

Genomic DNA was obtained of 378 F_2 plants and tested for the presence of *Fhb1* (KASP marker Fhb_FM227) and *Qfhs.ifa-5A* (marker Barc186), using KASP^{TM} methodology. In total, there were 92 *Fhb1* homozygotes, 191 heterozygotes, and 94 recessive homozygotes [χ^2 = 0.0899, χ^2_{table}=5.99 (df = 2, α = 0.05)]. Moreover, there were 91 *Qfhs.ifa-5A* homozygotes, 191 heterozygotes, and 96 recessive homozygotes [χ^2 = 0.1745, χ^2_{table}=5.99 (df = 2, α = 0.05)]. The 92 *Fhb1* homozygotes were also tested with marker AK357509 to identify the *Fhb6* homozygotes. Within the latter group, the numbers of plants within the nine possible *Qfhs.ifa-5A* and *Fhb6* genotypic classes were determined and subjected to Chi-square analysis [χ^2 = 6.55, χ^2_{table}=15.51 (df = 8, α = 0.05)], which suggested normal Mendelian segregation. From this F_2 population, four categories of homozygotes were kept for continued evaluation according to their height (<100cm), including one homozygote having *Fhb1* only; two homozygotes having *Fhb1* and *Qfhs.ifa-5A*; four homozygotes having *Fhb1* and *Fhb6*, and six homozygotes having *Fhb1*, *Fhb6*, and *Qfhs.ifa-5A*.

FHB resistance of F_3 families

The FHB type II resistance data of F_3 families evaluated in the greenhouse revealed significant differences among F_3 families, groups, and F_3 families within groups (Table 4-3). The trial means and tests of significance outcomes are summarized in Table 4-4. Cultivar Radiant (pedigree = Norstar*6/PGR16635// Norwin/UT125512; Thomas et al., 2012) was the most susceptible genotype in the trial (IS = 90.4%). Since Norstar is also highly susceptible to FHB (Chapter II), Radiant's sensitivity could in part derive from Norstar which contributed about 25% of its genetic background. Jerry showed intermediate susceptibility (51.5%). Of the three resistant controls, CM82036 (13.5%; *Fhb1*, *Qfhs.ifa-5A* +) was the best and significantly more resistant than all other entries. 14K456-K-1 (33.3%; *Fhb1*, *Qfhs.ifa-5A*) was second best and

significantly better than TA5660 (*Fhb6*). TA5660 (40.7%) was better, but not significantly so, than Jerry (51.5%) and the *Fhb1* only homozygote (family I; 54.3%). TA5660 is derived from cross TA5655/*2 TA3809//*2 Everest (TA9121) (Friebe et al., 2013) where TA5655 is a disomic wheat-*Elymus tsukushiensis* Robertsonian translocation line reported to exhibit strong FHB resistance (infection severity = 6.2%; Cainong et al., 2015). However, TA5660 did not show such strong resistance in this experiment.

Unfortunately, only one *Fhb1* homozygous family was available. This family showed relatively little resistance which could be the result of sizeable background segregation within this cross. As could be expected, the two group II families (average = 35.7%) were not significantly different from 14K456-K-1. However, the group III families on average (= 44.5%), were significantly more susceptible than 14K456-K-1 and the group II families. While this may suggest that *Fhb6* does not complement *Fhb1* as well as *Qfhs.ifa-5A* does; it is also possible that the cross background is simply too complex/heterogeneous for making strict comparisons with regard to interactions of the three QTL. The apparent lack of resistance QTL (or the presence of susceptibility QTL) in Radiant, the more resistant Jerry background, and the anonymity of the background QTL involved, make it impossible to accurately evaluate the associated interactions. The four group III families showed a broad range of IS values (31.6% to 61.7%) and the most resistant family was very similar in its effect to 14K456-K-1 and the two group II families. The average infection severity of group IV did not differ significantly from that of 14K456-K-1, TA5660, group II and group III. Similar to group III, the IS values of the individual group IV families ranged widely, from 30.4% to 48.5% (average = 41.2%). In general, sizeable variation was seen within the family groups in terms of the levels of protection afforded by the resistance QTL. With respect to all of the pyramids, at least one family showed a reduced level of infection

that was very similar to that of 14K456-K-1. Thus, *Fhb1* in combination with either *Qfhs.ifa-5A* or *Fhb6* appears to result in significantly reduced infection depending on the background genes present. However, combining all three QTL did not result in significant, further reduction of Type II resistance, at least not in this experiment.

In the progeny groups evaluated here, segregation of unknown background QTL very likely affected the overall disease phenotype. Such effects were inseparable and clearly confounded interactions among the three known QTL. While it was possible to select progeny with significantly reduced resistance, it was not possible to quantify the real contributions of both the known and unknown QTL. To be able to do this more accurately, it will be necessary to incorporate the pyramids through backcrosses into identical genetic backgrounds. Such an endeavor will be time-consuming and of little additional benefit to the breeding program. Knowing that *Fhb6* is a novel resistance source, is able to interact with *Fhb1* and native background QTL to increase overall resistance and will diversify the pool of quantitative resistance that is accessible in the breeding program, is of great value.

Data on *Fusarium* damaged kernels (FDK, Table 4-4) were less informative, yet correlated broadly with the infection severity data. CM82036 showed the least (= 2) and Radiant (= 8.8) showed the most kernel damage. 14K456-K-1 (= 4.2), TA5660 (= 4.8) and Jerry suffered intermediate damage. Among the pyramids, group I (= 6.5) had the most damage. Groups II and IV (both = 4.9) and III (= 5.9) were in the same intermediate range as TA5660- and Jerry.

Table 4-3. *F*-values obtained following statistical analysis of infection severity (IS), *Fusarium* damaged kernels (FDK), plant height (PH), number of spikelets per main spike (SPS), number of spikes per plant (SPP), thousand-kernel weight (TKW), and yield per plant (YPP) of selected cross 14M7/14K-456-K-1 F_3 families.

Effect	Num DF[1]	Den DF[2]	IS[3]	FDK	PH	SPS	SPP	TKW	YPP
F_3 families (F)	17	95	11.05^{***}	6.51^{***}	6.74^{***}	1.40^{ns}	2.68^{**}	4.35^{***}	2.09^{*}
Group (G)	8	95	17.95^{***}	13.49^{***}	5.63^{***}	0.76^{ns}	4.06^{***}	7.03^{***}	3.09^{**}
F(G)	9	95	5.02^{***}	0.45^{ns}	7.36^{***}	1.98^{ns}	1.28^{ns}	1.99^{*}	1.21^{ns}

[1] Num DF: the number of degrees of freedom in the model.
[2] Den DF: the number of degrees of freedom associated with the model errors.
[3] ns, *, **, and *** represent not significant, significant at the 0.05, 0.01, and 0.001 levels, respectively.

Table 4-4. *Fusarium* head blight infection severity (IS, %) 21 days after inoculation with *Fusarium* graminearum isolates, *Fusarium* damaged kernels (FDK) determined at harvest; plant height (PH, cm); number of spikelets per main spike (SPS); number of spikes per plant (SPP); thousand-kernel weight (TKW, g), and yield per plant (YPP, g/plant) of selected cross 14M7/14K-456-K-1 F_3 families in comparison with the parental lines. Family group I-IV were homozygous for the following resistance QTL: I = *Fhb1*; II = *Fhb1* and *Qfhs.ifa-5A*; III = *Fhb1* and *Fhb6*; and IV = *Fhb1*, *Qfhs.ifa-5A* and *Fhb6*. Values within each column followed by the same letters were not significantly different at $P < 0.05$.

		IS (%)	FDK	PH (cm)	SPS (No.)	SPP No.)	TKW (g)	YPP (g/plant)
14K-456-K-1		33.3 D	4.2 D	106.5 A	44.8 AB	11.2 BCD	34.1 C	14.0 AB
CM82036		13.5 E	2.0 E	103.6 A	45.4 AB	6.2 D	32.4 C	6.8 C
TA5660		40.7 BC	4.8 CD	87.6 C	42.0 AB	10.4 BCD	27.6 D	8.0 BC
Jerry		51.5 BC	5.8 BC	100.2 AB	42.6 AB	11.4 BC	39.0 A	14.7 AB
Radiant		90.4 A	8.8 A	94.2 BC	49.8 A	8.8 CD	34.4 BC	13.7 AB
Group I	Family 222	54.3 B	6.5 B	109.4 A	45.4 AB	12.6 BC	35.4 ABC	13.3 ABC
Group II	Average	35.7 D	4.9 CD	104.4 A	40.8 B	16.5 A	37.5 AB	18.6 A
	Family 198	36.6	4.8	110.6	37.0	15.6	36.8	16.3
	Family 225	34.8	5.0	98.2	44.6	17.4	38.3	21.0
Group III	Average	44.5 BC	5.9 BC	101.6 AB	41.7 B	11.6 BC	35.0 BC	12.5 BC
	Family 73	61.7	6.0	91.8	45.0	13.2	34.3	16.1
	Family 93	41.7	5.8	102.0	45.8	11.0	37.0	12.4
	Family 207	31.6	5.4	106.6	41.6	11.8	33.9	11.8
	Family 283	43.8	6.7	106.0	33.5	10.3	35.0	9.6
Group IV	Average	41.2 CD	4.9 CD	106.6 A	42.8 AB	13.8 AB	34.5 BC	13.8 AB
	Family 125	48.5	5.2	97.1	38.5	14.2	33.8	12.1
	Family 217	30.4	4.6	102.9	45.9	11.6	36.4	15.3
	Family 238	39.3	5.0	116.1	47.9	13.6	35.8	16.5
	Family 241	47.4	5.0	109.7	43.2	14.1	35.7	12.1
	Family 260	39.9	4.8	114.2	39.6	17.1	34.0	14.0
	Family 322	42.8	4.8	99.8	44.6	12.1	31.4	12.5

Agronomic characteristics of the F_3 families

Agronomic performance data of F_3 families evaluated in the greenhouse trial indicated significant differences among F_3 families, groups, and F_3 families within each group in some cases (Table 4-3). Due to the exclusion of the very tall F_2 plants, the average plant heights of groups I to IV ranged from 102cm to 109cm and did not differ significantly (Table 4-4). The average numbers of spikelets per spike were also similar except for groups II and III that were slightly lower. The number of spikes per plant was highest for group II. The thousand-kernel weight and yield per plant averages of the four groups did not differ significantly either; however, there were sizeable variation among families within groups III and IV (Fig. 4-3). Unfortunately, group performance or trends could not be compared as too few families were available within groups I and II. Nonetheless, there was no evidence to suggest that the *Fhb6* translocation had a negative effect on yield or seed plumpness under greenhouse conditions. Within both groups III and IV, families occurred that performed very similar to the best families in the other groups and the controls. Family II-225 (*Fhb1* and *Qfhs.ifa-5A*) was clearly the best among the 13 families based on overall agronomic traits and had the highest TKW and yield values. The next best families in terms of FHB resistance, yield and TKW were 198 (*Fhb1* and *Qfhs.ifa-5A*); 207 (*Fhb1, Qfhs.ifa-5A* and *Fhb6*), and 217 (*Fhb1*, *Qfhs.ifa-5A* and *Fhb6*). The four lines will be tested further under field conditions in a misted nursery to get a more comprehensive indication of their FHB resistance, agronomic performance and utility as breeding parents.

Conclusion

There were strong indications that the segregation of unknown background QTL confounded the interactions of the three QTL studied here. The results suggested that pyramiding either *Qfhs.ifa-5A* or *Fhb6* with *Fhb1* increased FHB type II resistance to a level comparable

with that in the hard red winter wheat 14K456-K-1. However, it appeared that combining all three QTL did not achieve a further reduction in disease severity. It is realized, however, that the limited number of lines within each pyramid type detracts from the general validity of the latter observations. The study furthermore evaluated type II resistance only. Continued testing of the best lines under field conditions involving artificial or natural infection should allow for evaluation that is more comprehensive. The most promising families for continued breeding included 198, 225 (*Fhb1* and *Qfhs.ifa-5A*), 207 (*Fhb1* and *Fhb6*), and 217 (*Fhb1*, *Qfhs.ifa-5A* and *Fhb6*) which showed good FHB resistance and agronomic performance.

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CHAPTER V. A STUDY OF FUSARIUM HEAD BLIGHT RESISTANCE IN TRITICALE X *THINOPYRUM DISTICHUM* SECONDARY HYBRID DERIVATIVES

Abstract

Fusarium head blight (FHB) causes severe yield and quality losses in wheat and resistance breeding is an important component of crop health management. FHB resistance has quantitative inheritance and only a limited number of quantitative trait loci (QTL) are being employed by breeding programs, making it necessary to expand and diversify the available resistance. Effective resistance occurs in *Thinopyrum distichum*, however, has not been transferred to common wheat. To characterize this resistance, genetic stocks previously derived from common wheat – *Th. distichum* and triticale – *Th. distichum* secondary hybrids were utilized. First, four cycles of single plant selection for FHB resistance were done starting with an F_4 line (W1423) that segregated for an uncharacterized translocation as well as FHB resistance. After three cycles of single plant selection the progeny of the selected plants still varied for viability, fertility and FHB resistance. When the progeny of a single F_6 plant (W1423X) were analyzed, high levels of aneuploidy occurred that was ascribable to aberrant segregation of chromosome 7A and another unknown chromosome. The second, unknown chromosome might have been heterozygous for the resistance as the W1423X progeny included highly susceptible plants. However, it was not possible to confirm the presence of an alien translocation. A resistant F_7 plant (W1423X-3) had 42 chromosomes and appeared to be disomic 7A. Plant W1423X-3 produced a low frequency of aneuploids, believed to be the result of a low frequency of de-synapsis of a translocated chromosome and its normal homologue. Further studies are needed to select a homozygous, FHB resistant F_8 plant from line W1423X-3 to better assess the resistance, confirm the presence of an alien translocation, and transfer it to wheat. A second part of this

study involved the screening of 41 addition lines for FHB resistance. The data suggested that two triticale – *Th. distichum* addition lines, W1450 (a telosomic addition) and W1451 (a complete chromosome addition), have FHB resistance. However, the homoeology of the two addition chromosomes to wheat chromosomes is still unknown. The resistance in addition lines W1450 and W1451 must now be confirmed by also testing them in a misted FHB screening nursery. Furthermore, the target chromosomes must be transferred to common wheat and their homoeology to wheat chromosomes determined.

Introduction

Fusarium head blight (FHB) infection can drastically reduce wheat yield and quality (Parry et al., 1995). Resistant cultivars are a major tool for the control of FHB worldwide (Buerstmayr et al., 2009) and to date, more than 100 quantitative trait loci (QTL) were reported to confer FHB resistance (Bai et al., 2018). However, only a few large effect resistance QTL are employed in wheat breeding programs, including *Fhb1* (first designated *Qfhs.ndsu-3BS*) (Waldron et al., 1999), *Qfhs.ifa-5A* (Buerstmayr et al., 2003), and *Qfhs.ifa-3A* (Steiner et al., 2004). In addition to the use of FHB resistance genes from the cultivated wheat germplasm pool, the wild relatives can also serve to broaden the genetic diversity of wheat (Ceoloni et al., 2014). The tribe Triticeae comprises a vast array of annual and perennial, cultivated and wild grass species, which form a rich reservoir of genetic diversity (Mujeeb-Kazi et al., 2013).

Perennial wheatgrass species in the genus *Thinopyrum* are a rich source of genes that confer resistance to biotic and abiotic stresses (Turner et al., 2013). With respect to FHB, three significant QTL were found in *Thinopyrum* spp. A well-characterized QTL discovered on chromosome $7el_2$ of *Th. ponticum* (Podp.) Barkworth & D.R. Dewey ($2n = 10x = 70$) was transferred to wheat by first developing $7el_2$ substitution lines for wheat chromosome 7D (Kim et

al., 1993). This resistance gene was designated *Fhb7* by Guo et al. (2015) and found to explain 15.1-32.5% of the observed phenotypic variation (Shen and Ohm, 2007). *Fhb7* was mapped to the distal region of the long arm of $7el_2$ between flanking markers *XBE445653* and *Xcfa2240* (Shen and Ohm, 2007). Useful co-dominant markers for this gene (*Xcfa2240*, *XsdauK352*, and *XsdauK66*) facilitated the continued introgression and marker-assisted selection of *Fhb7*. The second and third QTL were found in *Th. elongatum* (Host) Á. Löve ($2n = 2x = 14$; EE) on chromosomes 1E and 7EL, respectively. The chromosome 1E QTL was demonstrated to have excellent FHB resistance in greenhouse evaluations, i.e. only 6.5% infection in the addition line as compared to 80% infection of the durum parent (Jauhar, 2008), but was less effective in the field when subjected to natural infection (Jauhar, 2014). The QTL on chromosome 7EL provided significant type II resistance to substitution lines (Shen et al., 2004).

FHB resistance was also found in other *Thinopyrum* species, including an accession of *Th. junceiforme* (Löve & Löve) Löve ($2n = 4x = 28$, $J_1 J_1 J_2 J_2$), with only 10.93% infection in comparison with 70.34 to 89.46% infection of the parental durum wheat (Jauhar and Peterson, 2011). *Th. intermedium* (Host) Barkworth & D. R. Dewey ($2n = 6x = 42$) showed Type II FHB resistance equal to Sumai 3 in greenhouse tests (Cai et al., 2005; Oliver et al., 2005). Strong FHB resistance was also reported in *Th. distichum* (Thunb.) Á Löve ($2n = 4x = 28$, $J_1^d J_1^d J_2^d J_2^d$) by Chen et al. (2001). *Th. distichum* is a highly salt-tolerant, perennial grass that is indigenous to the shoreline of southern Africa (Marais et al., 2014). Hybrids were made with durum wheat (*Triticum turgidum* L.), common wheat (*Triticum aestivum* L.) (Pienaar, 1983, 1990), and triticale (×*Triticosecale* Wittm) (Marais and Marais, 1998). Addition lines (Littlejohn and Pienaar, 1985; Marais and Marais, 1998) and useful molecular markers for some of the *Thinopyrum* chromosomes were developed (Marais et al., 2007) in wheat and triticale. During

the development of the triticale additions (unpublished data) several lines with apparent translocations to triticale chromosomes were noted and kept. One such translocation lineage was W1423, believed to segregate for the presence of an unconfirmed $7J_2^d$ translocation. Plants from population W1423 were included with diverse breeding lines that were evaluated in greenhouse tests of FHB type II resistance in 2015. When resistant segregates occurred, a more comprehensive evaluation of the material was initiated. The first aim was to identify a resistant homozygote in order to do a more comprehensive evaluation of the resistance.

Materials and Methods

Germplasm

Marais and Marais (1998) hybridized *Thinopyrum distichum* (female parent) with hexaploid triticale (*X Triticosecale* Wittmack) and pollinated the C_1 hybrid ($2n = 70$) with triticale. Backcross F_1 plants ($2n = 54$-56 chromosomes) were again backcrossed (reciprocally) to the triticale cultivar Rex to derive B_2F_1 **(**$2n = 45$-49**)**. B_2F_2 plants with 43 or 44 chromosomes were identified 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). During the search for addition lines, progenies that appeared to carry triticale-*Th. distichum* translocations occurred. W1423 was one such stock that seemed to segregate for a group 7 translocation based on marker polymorphisms observed at the time. Somo and Marais (2016, unpublished results) did genomic in situ hybridization (GISH) with a number of F_4 seeds from the W1423 lineage and observed a small translocation (Fig. 5-1) in only one of the seedlings. Unfortunately, this plant was lost. Thus, while it appeared that a small translocation from *Th. distichum* does in fact segregate in W1423, its exact nature remains unknown. A preliminary screen of wheat – *Th. distichum* hybrid derivatives that included F_4 W1423 plants (unpublished data; Pirseyedi and Marais, 2016)

suggested that a low frequency of plants from this stock showed FHB resistance. Seeds of two seemingly resistant plants (W1423-1 and W1423-2) served as starting material for the present study.

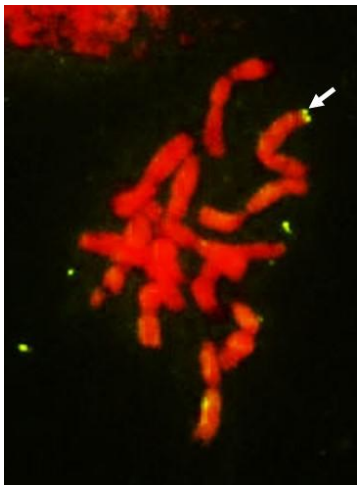

Figure 5-1. Genomic in situ hybridization (GISH) picture showing a small translocation from *Thinopyrum distichum* to triticale (Somo and Marais, unpublished data).

General outline of the study

The immediate study aim was to select a resistant homozygote from the W1423 progenies for further characterization. Accordingly, single, resistant plants were selected through four generations during 2015-2018 following greenhouse evaluation of Type II resistance. The most resistant individual of each generation provided seeds for evaluation and selection in the next generation. In the third generation, it became evident that the populations were also segregating for chromosome number and that it was necessary to introduce root tip chromosome counts and analyses for simple sequence repeat marker polymorphisms.

In a further attempt to identify the source of the resistance, a set of 11 wheat – *Th. distichum* addition lines and 30 triticale – *Th. distichum* addition lines were screened for FHB resistance.

Single plant selection for FHB resistance

For the first greenhouse-based selection cycle, six pots of each of the populations W1423-1 and W1423-2 were grown. Three plants were planted per pot, thus 36 plants total. Approximately 10 spikes in each pot were inoculated with mixed spores of *Fusarium graminearum* isolates. Infection severity and FDK were determined for each pot and used to identify the best plant. In selection cycle 2, 20 seeds from this most resistant individual were planted for resistance evaluation. Following identification of the best plant from the second cycle, its seeds were used for establishing and testing a further 20 progeny. The most resistant plant from cycle 3 was identified and named W1423X. When it appeared that three generations of progeny testing and single plant selection had failed to produce a pure line, it was decided to evaluate cycle 4 plants for somatic chromosome number and marker polymorphisms in addition to FHB resistance.

In cycle 4, progeny of plant W1423X were evaluated. Root tips were cut on 34 W1423X progeny in order to do somatic chromosome counts. Somatic chromosome numbers were determined using leuco-basic fuchsin staining (Darlington and La Cour, 1976). Nineteen of the plants were raised and evaluated for FHB resistance. The controls, Langdon (durum wheat) and Tobie (triticale) were included for comparison. Approximately 10 spikes in each individual were inoculated with *Fusarium graminearum* isolates. DNA extracts were made with respect to each.

Evaluation of addition lines for FHB resistance

Forty-one *thinopyrum* disomic addition lines were evaluated for type II resistance in a greenhouse in 2018, including eleven additions to Inia 66 (developed by Littlejohn and Pienaar, 1995) and thirty additions to triticale (developed by Marais et al., 2007). Parental lines Calvin (durum wheat), Inia 66 (common wheat), Rex (triticale) and Tobie (triticale), were used as

controls. The trial was set up as a completely randomized design within four replicates. Across replicates approximately twenty spikes were inoculated for each line.

FHB inoculation

The single spikelet injection method was used for inoculating wheat spikelets at anthesis in a greenhouse (Stack, 1989). A mixture of spore representative of four *Fusarium graminearum* isolates (Fg_124_1, Fg10_135_5, Fg13_79 and Fg08_13) was provided by the Department of Plant Pathology at North Dakota State University. A 10 µl-droplet containing the mix (approximately 100,000 conidia per ml) was injected directly into a floret in the middle of the spike. Inoculated spikes were covered with a (wet) plastic bag for 72 h immediately after inoculation. Afterwards, the infection severity was visually assessed by determining the percentage of infected spikelets per spike at 21 days after anthesis.

Molecular marker analyses

Marker analyses were done on 19 progeny of plant W1423X that had also been subjected to root tip chromosome counts and were evaluated for FHB resistance. Appropriate controls were added to these. Total genomic DNA was extracted from young leaves following the Triticarte Pty. Ltd protocol (http://www.triticarte.com.au/). The quality and concentration of extracted DNA were checked using agarose gel electrophoresis and staining with ethidium bromide. DNA concentration was adjusted to approximately10 ng/µl before polymerase chain reaction (PCR). Since W1423 was selected from material within which *Thinopyrum* chromosome $7J_2^d$ segregated, it was decided to start testing with group 7 markers first. Marker Cfa2240 was tested on a small panel of controls and an absence polymorphism was detected on chromosome 7A. Since the absence of wheat loci suggests that it could have been replaced with alien chromatin, it was decided to attempt comprehensive physical mapping of W1423 chromosome 7A, and more

limited physical mapping of chromosomes 7B and 7R employing a large group of homoeologous group 7 markers. Forty-seven molecular markers that map to the long arms of the group 7 chromosomes were chosen based on previous reports. (1) Wheat chromosome 7AL and 7BL markers were selected based on the studies of Song et al. (2005) and Sourdille et al. (2004). Primer 72 (F: TCCCTTCCTCGAACCAAGCA; R: GGATGTACTGGTAGAGGGCG) is an un-published marker that was designed from a group 7 EST sequence published by Hossain et al. (2004) and detects 7AS, 7BS and 7DS fragments (Pirseyedi and Marais, Personal Communication). (2) Markers that map to the long arm of *Th. ponticum* chromosome 7el were obtained from Guo et al. (2015). (3) *Thinopyrum disticum* chromosome 7JL markers (UST 11 and UST 12) were described by Marais et al. (2007). (4) Rye chromosome 7R markers, SCM86, SCM40, and cMWG682 were based on Saal and Wricke (1999) and Gustafson et al. (2009). (5) Barley chromosome 7H marker, MWG808, traces to Wenzl et al. (2006). (6) Finally, markers that are specific for homoeo-alleles of the phytoene synthase gene (*Psy 1*) on group 7L chromosome arms (Psy-A1, Psy-B1, Psy D1 and Psy-E1) were described in the study of Zhang and Dubcovsky (2008). Marker primer sequences are available in the above-mentioned publications and/or GrainGenes website (http://www.wheat.pw.usda.gov). PCR conditions were as described by Röder et al. (1998). PCR products were visualized by agarose gel-based electrophoresis with ethidium bromide staining.

Three sets of marker analyses were done: (1) Twenty-six microsatellite markers on wheat chromosome 7AL were first tested within the genotype panel Chinese Spring (CS), CS nullisomic 7A tetrasomic 7D (CSN7AT7D), CS di-telosomic 7AS (CSDT7AS), CSN7BT7A, CSDT7BL, CSN7DT7A, CSDT7DS, *Th. distichum*, Henoch (rye), Calvin (durum wheat), Tobie and Rex (triticale), W1417 (disomic triticale addition line $7J_2^d$, 2*n*=44), and W1423. (2) Forty-

one markers (all markers except Gpw2333, Gpw4100, Gpw2252, Gwm63, Gpw4410 and Gpw573) were tested within the genotype panel Chinese Spring (CS), *Th. disticum*, Henoch (rye), Calvin (durum), Rex (triticale), Tobie (triticale), W1417 (triticale addition line with $7J_2^d$), W1423X, and 21 W1423X progeny containing different numbers of chromosomes. (3) Markers Gmw63, Gpw2092, Psy-E1 and UST12 were applied to test the polymorphism among the 41 addition lines.

Statistical analyses

Analyses of variance of phenotypic characters were conducted using SAS (version 9.3) (SAS Institute, Cary, NC). The general linear model (GLM) ($Y = L_i + e$) was applied to compare the differences among lines tested in a completely randomized greenhouse experiment.

Results and Discussion

Selection within the W1423 lineage

The outcome of the selection experiment is summarized in Fig. 5-2. This shows the frequency distributions of infection severities for individual spikes in each cycle. The most resistant plant within each cycle was chosen for continued selection after comparison of the overall infection severity of all of the spikes on the individual plants and this was done prior to harvesting. The data suggested that following the first selection, there was a visible improvement in the overall resistance of population W1423; however, in subsequent cycles there was no obvious further improvement while the final population seems to have reverted and was again similar to the starting populations. The strong initial response is expected. With regard to a locus that was heterozygous in the F_1, only 3% (F_5), 1.6% (F_6) and 0.8% (F_7) heterozygosity remains (on average) in the advanced generations. Single plant selection in any of these generations should therefore readily yield a true breeding, resistant line. On the contrary, despite selection of

the most resistant and most fertile plant in each cycle, the next generation always included highly susceptible plants, plants with low viability (some of which died) and plants with low fertility. Thus, it appeared as though selection for strong FHB resistance and good plant type led to the perpetuation of a frequency of poor, weak plants among the progeny. This suggested that the resistance is associated with a chromosome structural change(s) that affects the regularity of meiotic pairing and causes genetic imbalances among the progeny. As a result, it was decided to analyze the W1423X progeny for their somatic chromosome numbers.

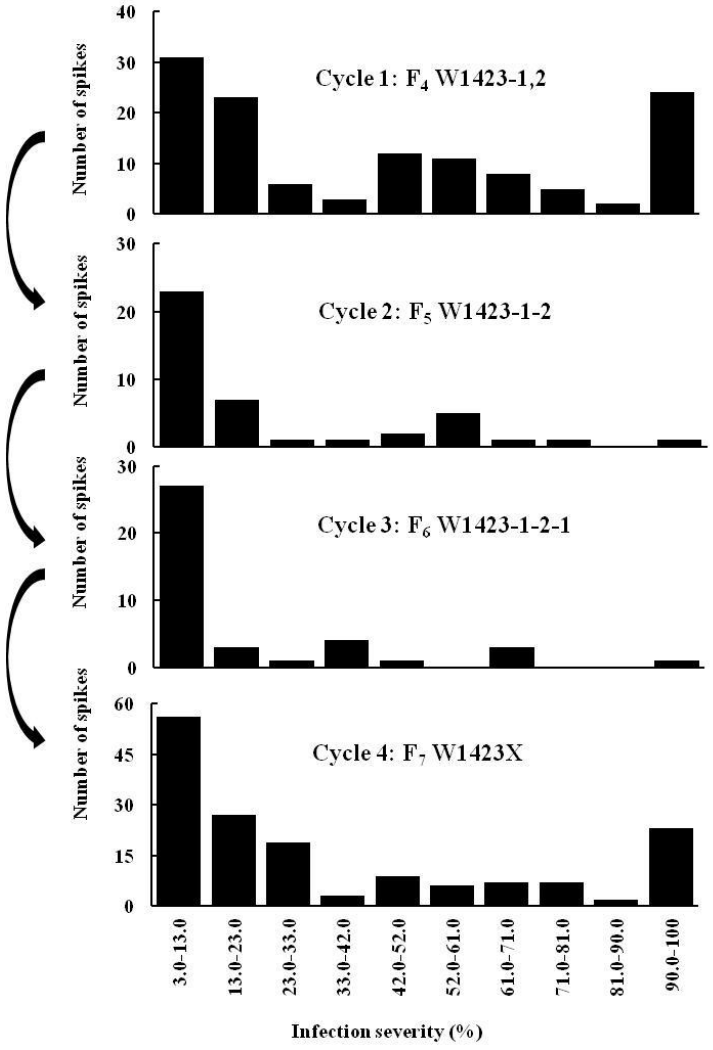

Figure 5-2. Distribution of infection severity of individual spikes during three single plant selection cycles that resulted in population W1423X.

Evaluation of the W1423X population

A wide range of chromosome numbers were encountered. There were two plants with 2*n* = 39, three plants with 2*n* = 40, eight plants with 2*n* = 41, three plants with 2*n* = 40 + telosome, three plants with 2*n* = 42, one plant with 2*n* = 42 + telosome, and one plant with 2*n* = 43 chromosomes. Two of the 2*n* = 39 plants had very low viability and eventually died. Nineteen of the remaining W1423X progeny plants were then tested for FHB type II resistance. The results of these tests are summarized in Fig. 5-3. FHB infection severity ranged from 10.1 to 95.9%. While there clearly was wide variation in FHB resistance among the plants, it is difficult to judge to what extent the variation in chromosome irregularities and genic imbalances affected the expression of resistance. The three 2*n* = 40 plants were the most susceptible and comparable to Langdon durum wheat. To the contrary, seven of the eight monosomic plants (plant #35 being the exception), one of three 2*n* = 40 + t plants, and one of the three 2*n* = 42 plants (# 3) showed relatively strong FHB resistance. Plant #3 was the best within the resistant group in terms of fertility, general phenotype and FHB resistance. Compared to #3, the two remaining disomics were inferior in phenotype and had intermediate resistance. Plants #4 (2*n* = 40 + t) and # 16 (2*n* = 42 + t) also showed intermediate resistance. The three remaining plants, #35 (2*n* = 41), #17 (2*n* = 40 + t) and #19 (2*n* = 43) showed more susceptibility.

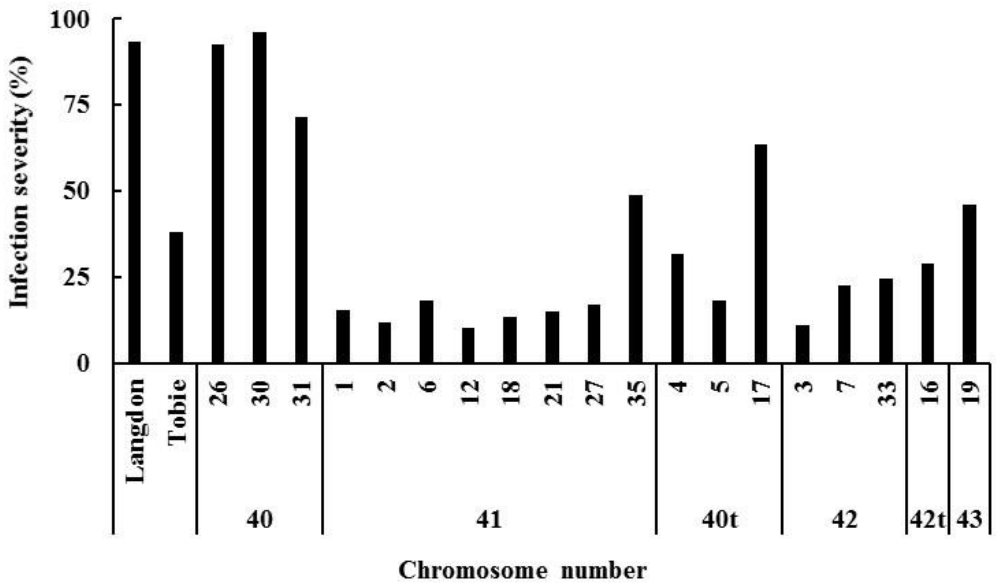

Figure 5-3. *Fusarium* head blight infection severity (%) of nineteen F_8 plants that derive from W1423X, and which differ in somatic chromosome number.

Since the chromosome numbers of the 34 plants ranged from 2*n* = 39 to 2*n* = 43, the aneuploidy must have been the result of at least two chromosome structural differences. The relatively high incidence of telosomes furthermore suggested frequent occurrence of unpaired chromosomes with accompanying centromeric breaks. Most commonly, normal segregation of two homologous chromosomes can be disrupted by monosomy (gives progeny with 2*n* = 40, 41 or 42), a-synapsis or de-synapsis (gives 2*n* = 40, 41, 42, and a low frequency of 2*n* = 43 or 44 progeny), or trisomy (gives 2*n* = 42, 43 or 44). Mechanisms that result in fewer that 2*n* = 40 chromosomes require the involvement of a further, non-homologous chromosome pair. A feasible explanation of the present results is that W1423X (2*n* = 41) was not only a monosomic with respect to one chromosome (7A as will be explained later), but that it was also heterozygous for a structural difference in a second chromosome. The latter difference caused a degree of a-synapsis or de-synapsis in meiosis I. Combined, the two mechanisms can generate a wide range

of aneuploids as is explained in Table 5-1. Not only will some of the predicted aneuploids have poor or no survivability, it will not be possible to calculate their expected frequencies or deduce viability without more specific knowledge of the aberration involved.

Table 5-1. Expected progeny chromosome numbers of a plant that is postulated to be monosomic with respect to a first chromosome and simultaneously harbors a structural difference between the two homologues of a second chromosome. It is assumed that the two homologues of the second chromosome will sometimes manage to form a bivalent and sometimes fail to form a bivalent due to a-synapsis or de-synapsis during meiotic metaphase I.

Euploidy/aneuploidy of chromosome: 7A[1]	Unknown[2]	2n	Somatic chromosomes[3]	Number of plants observed[4]
Nullisomic	Nullisomic	2n-4	38	0
Nullisomic	Monosomic	2n-3	39	2
Monosomic	Nullisomic	2n-3	39	
Nullisomic	Disomic	2n-2	40	3
Monosomic	Monosomic	2n-2	40	
Disomic	Nullisomic	2n-2	40	
Nullisomic	Trisomic	2n-1	41	23
Disomic	Monosomic	2n-1	41	
Monosomic	Disomic	2n-1	41	
Nullisomic	Tetrasomic	2n	42	3
Monosomic	Trisomic	2n	42	
Disomic	Disomic	2n	42	
Monosomic	Tetrasomic	2n+1	43	2
Disomic	Trisomic	2n+1	43	
Disomic	Tetrasomic	2n+2	44	0

[1] Marker data suggested that chromosome 7A occurred in a monosomic state in plant W1423X.
[2] A second, unknown pair of homologous chromosomes in plant W1423X apparently frequently failed to form a stable bivalent in meiosis.
[3] Telosomes probably arose through centric breaks in unpaired chromosomes and are therefore not distinguished from complete chromosomes here.
[4] The actual number of W1423X progeny that had this somatic chromosome number.

The 19 plants that were evaluated for FHB resistance plus 2 dead plants ($2n = 39$) and controls were also tested with chromosome 7A markers (Table 5-2). Before doing this, twenty-six chromosome 7A markers were tested on the genotype panel CS, CSN7AT7D, CSDT7AS,

CSN7BT7A, CSDT7BL, CSN7DT7A, CSDT7DS, *Th. distichum*, Henoch, Calvin, Tobie and Rex, W1417 and W1423 to confirm the presence of useful polymorphisms. The results obtained with the telosomic stocks suggested that marker loci *Xgpw2264*, *Xgpw7386*, and *Xgpw2119* actually maps to chromosome arm 7AS rather than 7AL (Sourdille et al., 2004) (Fig. 5-4).

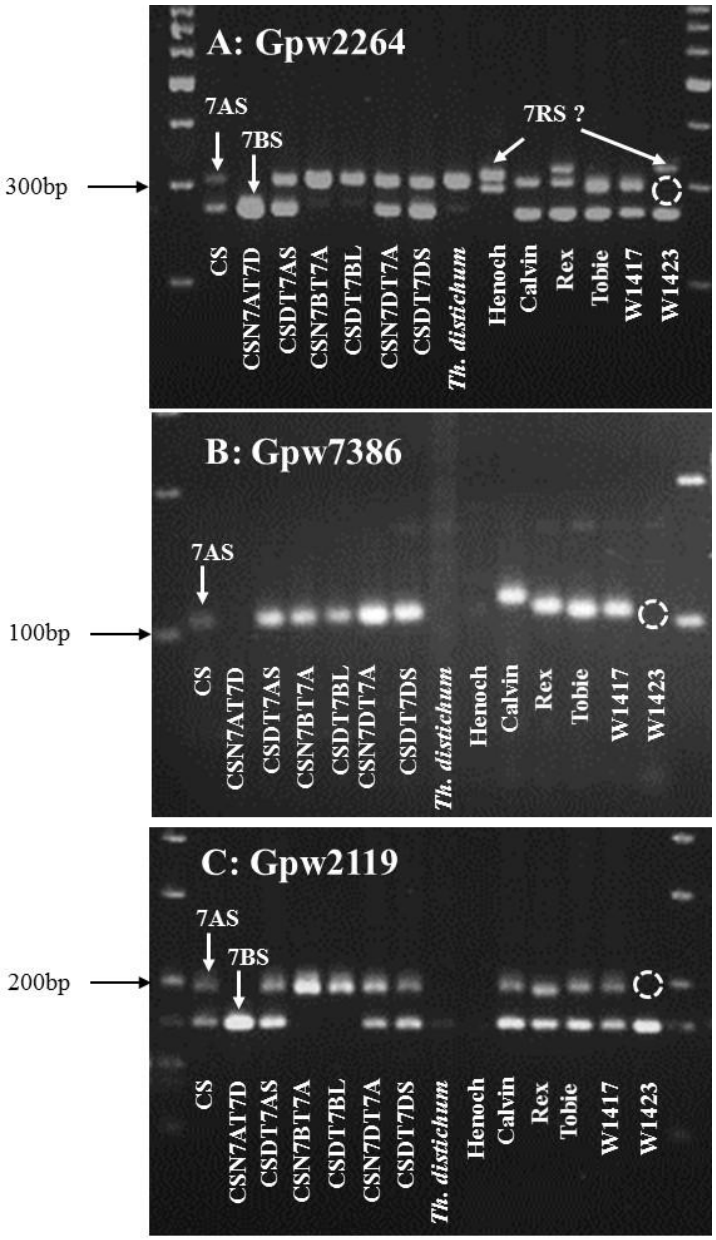

Figure 5-4. Chromosome 7A polymorphisms produced by markers Gpw2264 (A), Gpw7386 (B), and Gpw2119 (C), showed that the corresponding loci occur on chromosome arm 7AS rather than 7AL. Also shown (white circles), is nullisomy for these loci in a W1423 plant.

Therefore, 17 polymorphic 7AL markers and four polymorphic 7AS markers were scored on the W1423X panel. From the results (Table 5-2), the following became apparent: (1) Seven plants were nullisomic for chromosome 7A (Fig. 5-5 A, B). These included two plants with 2*n* = 39 (#10, #28; these died after planting), three plants with 2*n* = 40 (#26, #30, #31), one plant with 2n = 41 (#6) and one of the 2*n* = 40+t plants (#5). The observed incidence of nullisomy 7A was comparatively high (33.3%) as wheat monosomics are expected to produce (on average) only about 3% nullisomic progeny (Morris and Sears, 1967). This result suggests that there could have been a disadvantage to the presence of 7A, or that a structural change(s) was present that resulted in reduced transmission of 7A. (2) Among the 7A nullisomics, the 2*n* = 40 plants had very poor FHB resistance; however, their reduced viability might have contributed to their susceptibility. The two remaining 7A nullisomics (#5, #6) had comparatively good resistance, which would suggest that the resistance is not associated with the presence of chromosome 7A. (3) With respect to the four plants that had telosomes: The results suggest that the telosome in #4 is 7AL; the telosome in #5 does not derive from 7A, while the telosome in #16 and #17 could be from any chromosome in the genome. (4) In lines #4 (appears to lack 7AS) and #5 (appears to lack 7A), the *Xgpw7386* polymorphism is present which could suggest that one of the other chromosomes could have a small 7AS translocation. Such a (terminal) translocation would have competed for pairing with the normal chromosome 7AS and could have caused the 7A monosomic condition to arise. (5) The 7AL *Xpsy-A1* locus was detected in the controls CS, Calvin, Rex, Tobie and addition line $7J_2^d$; however, it was absent in W1423X and all its derivatives. This suggest several possibilities: the W1423X *Xpsy-A1* locus is intact, but allelic variation prevented adequate primer annealing; the locus had been lost through deletion, or, was replaced by a translocated segment.

Table 5-2. Polymorphisms of chromosome 7A markers produced by the 21 W1423X progenies plus controls, Chinese Spring (CS), *Th. distichum*, Henoch (rye), Calvin (durum), Rex (triticale), Tobie (triticale), W1417 (triticale addition $7J_2^d$), and W1423X and detected on agarose gels. Numbers in the table reflect the presence of different size polymorphisms: Zero indicates the absence of a band, numbers 1, 2, 3, etc each indicate a different size amplification product, while "?" means that the polymorphism is undetermined.

						C-7AL1-0.39	7A17-0.71-0.74	7A17-0.71-0.74	7A17-0.71-0.74	7AL21-0.74-0.86	7AL21-0.74-0.86	7AL21-0.74-0.86	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL18-0.90-1.00	7AL18-0.90-1.00	7AL18-0.90-1.00	7AL18-0.90-1.00		7AL18-0.90-1.00[4]
2n	Entry	*Xgpw7386*[1]	*Xprimer 72*[2]	*Xgpw2119*[1]	*Xgpw2264*[1]	*Xgpw2269*	*Xgpw1100*	*Xgpw3276*	*Xgpw7339*	*Xgpw2103*	*Xgpw4066*	*Xgwm2083*	*Xcfa2123*	*Xgpw3127*	*Xgwm282*	*Xpsp3094*	*Xcfa2019*	*Xcfa2257*	*Xcfa2240*	*Xgpw4050*	*Xsdauk71*[3]	*Xpsy-A1*
	CS	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
	Th. distichum	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	4	3	0	3	2	0
	Rye	0	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4	0	0	3	0
	Calvin	1	1	1	1	1	1	1	1	1	2	1	2	1	2	1	2	1	1	2	1	1
	Rex	1	1	1	1	1	1	1	1	2	3	1	3	1	2	1	2	1	1	2	1	1
	Tobie	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	3	1	1	2	1	1
	W1417	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	3	?	1	2	1	1
	W1423X	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
39	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
41	2	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0

Table 5-2. Polymorphisms of chromosome 7A markers produced by the 21 W1423X progenies plus controls, Chinese Spring (CS), Th. distichum, Henoch (rye), Calvin (durum), Rex (triticale), Tobie (triticale), W1417 (triticale addition $7J_2^d$), and W1423X and detected on agarose gels (continued).

						C-7AL1-0.39	7A17-0.71-0.74	7A17-0.71-0.74	7A17-0.71-0.74	7AL21-0.74-0.86	7AL21-0.74-0.86	7AL21-0.74-0.86	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL16-0.86-0.90	7AL18-0.90-1.00	7AL18-0.90-1.00	7AL18-0.90-1.00	7AL18-0.90-1.00		7AL18-0.90-1.00[4]
2n	Entry	*Xgpw7386*[1]	*Xprimer 72*[2]	*Xgpw2119*[1]	*Xgpw2264*[1]	*Xgpw2269*	*Xgpw1100*	*Xgpw3276*	*Xgpw7339*	*Xgpw2103*	*Xgpw4066*	*Xgwm2083*	*Xcfa2123*	*Xgpw3127*	*Xgwm282*	*Xpsp3094*	*Xcfa2019*	*Xcfa2257*	*Xcfa2240*	*Xgpw4050*	*Xsdauk71*[3]	*Xpsy-A1*
41	12	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
41	18	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
41	21	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
41	27	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
41	35	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
40+t	4	1	0	0	0	1	1	1	?	1	1	1	1	1	3	2	2	2	1	1	1	0
40+t	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40+t	17	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
42	3	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
42	7	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
42	33	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
42+t	16	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0
43	19	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	2	1	1	1	0

[1] These loci have previously been mapped on 7AL (Sourdille et al., 2004); however, as they actually occur on 7AS (See Fig. 5-4)
[2] Markers were assigned to deletion bins on the basis of their presence in the controls and based on the map positions of their homoeoloci in wheat and *Thinopyrum ponticum*.
[3] According to Guo et al. (2015) *Xsdauk71* is located 2.7cM from *Xcfa2240* near the long arm telomere of *Thinopyrum ponticum* chromosome $7el_2$.
[4] Zhang and Dubcovsky, 2008.

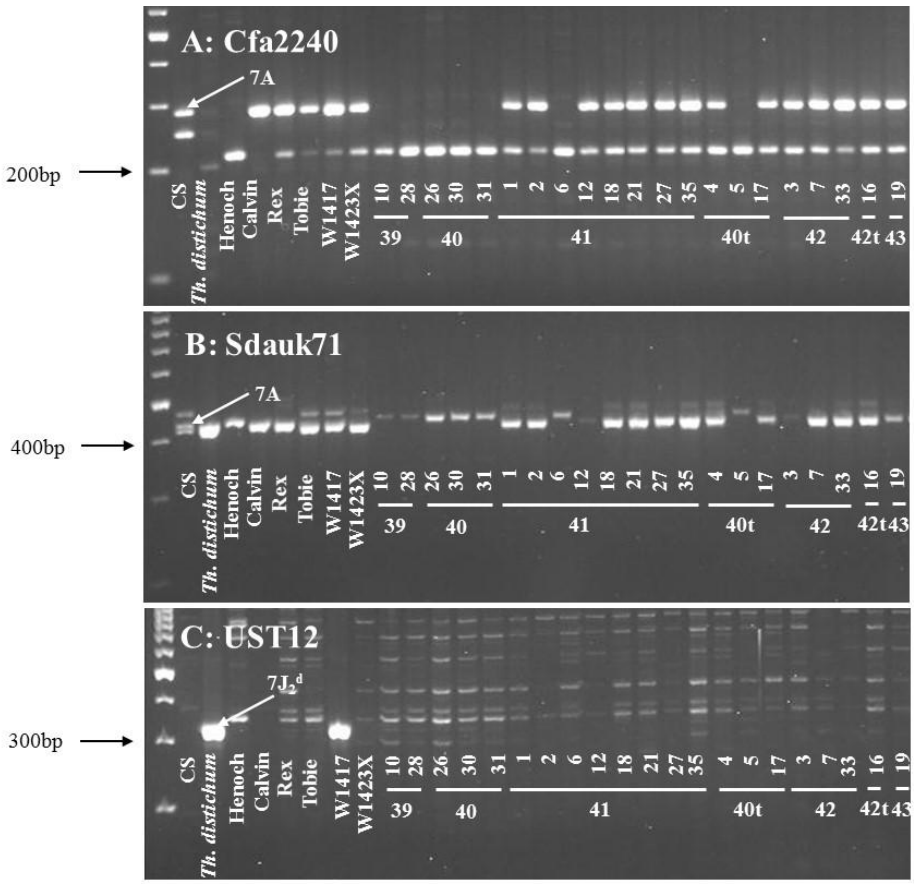

Figure 5-5. (A and B) Chromosome 7A polymorphisms produced by markers Cfa2240 and Sdauk71 showed that seven of 21 W1423X progeny (#10, #28, #26, #30, #31, #6, and #5) were nullisomic for this chromosome. (C) Marker UST12 detected a band diagnostic for *Th. distichum* chromosome $7J_2^d$.

The W1423X progeny were also tested with markers for the presence of chromosomes 7B and 7R (Table 5-3). The results suggested that there was no incidence of nullisomy of either of the two chromosomes among the lines. The chromosome 7BL markers *Xpsy-B1* and *Xbarc1073* did not amplify a product in either W1423X or its progeny, suggesting that a distal region of 7BL in this lineage could be deleted or substituted. However, the triticale cultivar Rex showed the same absence polymorphisms, suggesting that they are not unique to the W1423X lineage. Rex was one of the parents used in the production of the secondary triticale-*Th. distichum* hybrids and the polymorphisms may even derive from this cultivar.

Table 5-3. Polymorphisms of chromosome 7B, 7R, and $7J_2^dL$ markers detected on the 21 W1423X progenies plus controls, Chinese Spring (CS), *Th. distichum*, Henoch (rye), Calvin (durum), Rex (triticale), Tobie (triticale), W1417 (triticale addition $7J_2^d$), and W1423X. Numbers in the table reflect the presence of different size polymorphisms. Zero indicates the absence of a band, numbers 1, 2, 3, etc each indicate a different size amplification product, while "?" means that the polymorphism is undetermined.

		7BS	7BL					7RS		7RL							$7J_2^dL$	
2n	Entry	*Xgpw2264*	*Xgpw2269*	*Xgpw1100*	*Xcfa2019*	*Xpsy-B1*	*Xbarc1073*	*Xgpw2264*[1]	*Xscm40*	*Xgpw2269*[1]	*Xcfa2123*[1]	*Xcfa2293*[1]	*Xsdauk60*[2]	*Xcfa2240*[1]	*Xgpw4050*[1]	*Xscm86*	*Ust12*	*Xpsy-E1*
	CS	1	1	1	1	1	1	0	0	2	0	2	1	0	0	0	0	0
	Th distichum	2	0	0	2	0	0	0	0	3	2	0	0	0	0	0	1	1
	Henoch	0	0	0	0	0	0	2	1	1	1	0	1	1	1	2	0	0
	Calvin	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0
	Rex	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0
	Tobie	1	1	1	1	1	1	2	1	1	1	1	1	1	1	0	0	1
	W1417	1	1	1	1	1	1	2	1	1	1	1	1	1	1	0	1	1
	W1423X	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
39	10	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
39	28	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40	26	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40	30	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40	31	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	2	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	6	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	12	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	18	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	21	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
41	27	1	1	1	1	0	0	1	1	1	?	1	1	1	1	1	0	0

Table 5-3. Polymorphisms of chromosome 7B, 7R, and $7J_2^dL$ markers detected on the 21 W1423X progenies plus controls, Chinese Spring (CS), *Th. distichum*, Henoch (rye), Calvin (durum), Rex (triticale), Tobie (triticale), W1417 (triticale addition $7J_2^d$), and W1423X (continued).

		7BS	7BL					7RS		7RL							$7J_2^dL$	
2n	Entry	*Xgpw2264*	*Xgpw2269*	*Xgpw1100*	*Xcfa2019*	*Xpsy-B1*	*Xbarc1073*	*Xgpw2264*[1]	*Xscm40*	*Xgpw2269*[1]	*Xcfa2123*[1]	*Xcfa2293*[1]	*Xsdauk60*[2]	*Xcfa2240*[1]	*Xgpw4050*[1]	*Xscm86*	*Ust12*	*Xpsy-E1*
41	35	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40+t	4	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40+t	5	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
40+t	17	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
42	3	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
42	7	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
42	33	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
42+t	16	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0
43	19	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0

[1,2] Markers were assigned to chromosome arms on the basis of their presence in the controls and based on the map positions of their homoeoloci in wheat and *Thinopyrum ponticum*.

Two marker loci (*Xust12* and *Xpsy-E1*) were detected on *Th. distichum* chromosome $7J_2^dL$ (Table 5-3; the *Xust12* polymorphism is shown in Fig. 5-5C). However, the markers amplified the target sequence in *Th. distichum* and the $7J_2^d$ addition line only and not in W1423X and its progeny. A Psy-E1 amplification product was also seen in Tobie. The three Psy-markers were designed by Zhang and Dubcovsky (2008) for specific detection of the A-, B- and E-homoeo-alleles of the *Psy1* locus in their study and do not appear to show the same discriminatory ability with respect to the triticale variants of this locus. Thus, the two markers did not detect any *Thinopyrum* chromatin in W1423X or its progeny.

Evaluation of addition lines

Forty-one disomic addition lines were evaluated for type II FHB resistance (Fig. 5-6). These included eleven additions to Inia 66 (W627-W637) and thirty additions to triticale (W1411-W1417 and W1441-W1463). Of these, addition lines W1450 and W1451 showed the strongest resistance (17.8% and 13.0%, respectively); however, their resistance was not significantly better than that of Rex (24%). The addition in W1451 is a complete *Thinopyrum* chromosome whereas the addition in W1450 is a *Thinopyrum* telosome (long arm of an unknown chromosome). It is therefore possible that W1450 and W1451 are variations of the same chromosome. No additional information is available on these chromosomes and their homoeology to wheat chromosomes are unknown.

The marker data showed frequent aneuploidy of chromosome 7A which suggested that it is highly likely that the group 7 chromosomes were involved in structural exchanges during the development of addition stock W1423. Addition line W1417 was therefore included as a control in the marker analyses because the alien chromosome in this addition line has previously been identified as $7J_2^d$ (Marais et al., 2007). In the present study, markers Gmw63, Gpw2092, Psy-E1,

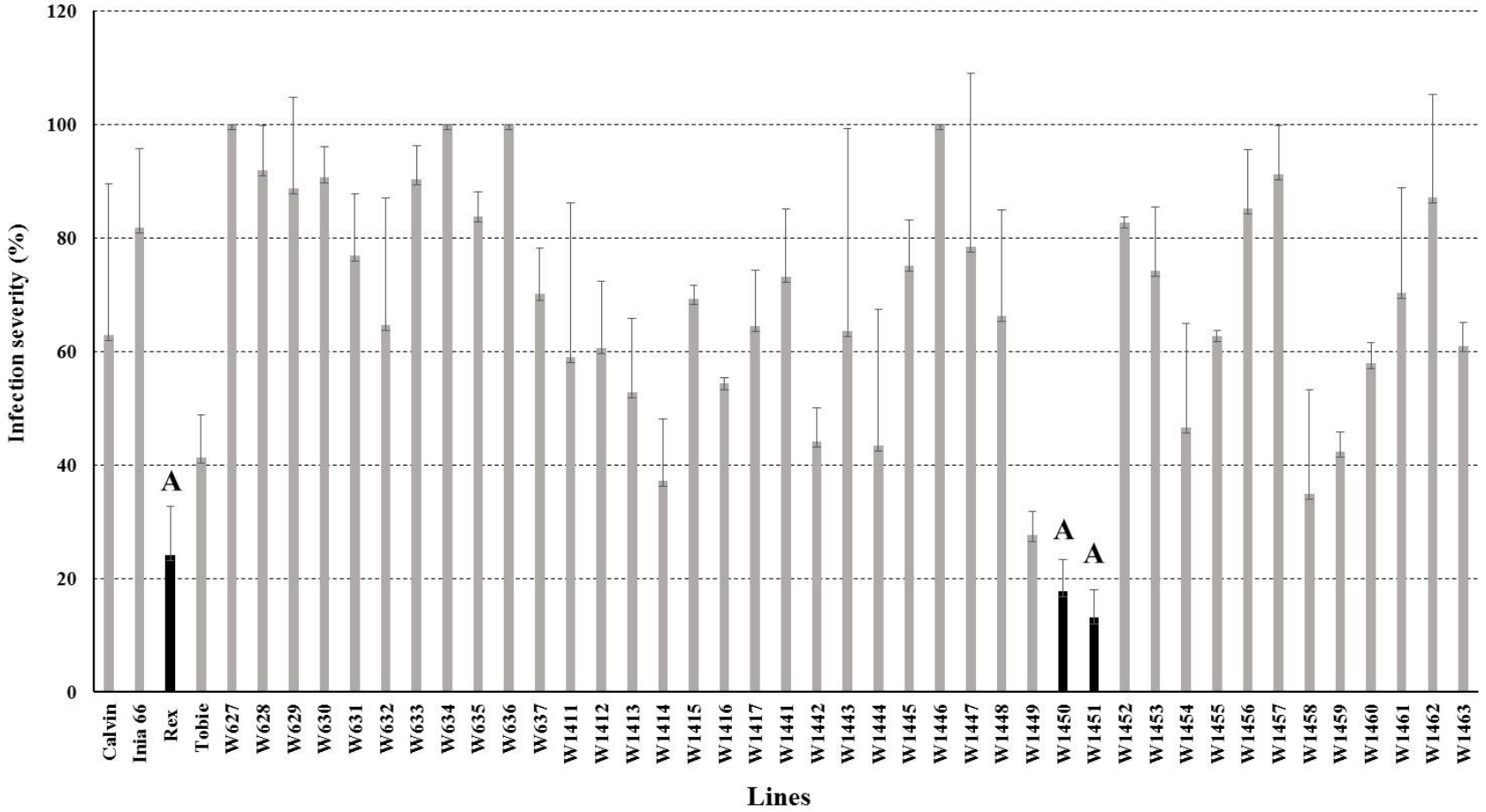

Figure 5-6. *Fusarium* head blight infection severity (%) of eleven Inia 66 - *Th distichum* addition lines (W627-W637) and thirty triticale - *Th. distichum* addition lines (W1411-W1417 and W1441-W1463). The bar on each column indicates the size of the standard deviation; same letters on the bars suggests no significant difference at $P<0.05$.

and UST 12 showed clear, *Thinopyrum*-specific polymorphisms. Psy-E1 and UST 12 were developed to specifically detect *Thinopyrum* group 7 loci, while Gmw63 and Gpw2092 detect wheat group 7 loci and likely amplified *Thinopyrum* group 7 homoeo-loci in the current marker tests. When the four markers were tested on the full set of addition lines, only markers Psy-E1 and UST12 detected the *Thinopyrum* polymorphisms in an addition line (W1417). However, addition line W1417 appears to be highly FHB susceptible and unlikely to be the source of the resistance in W1423. The second *Th. distichum* group 7 chromosome ($7J_1^d$) could not be detected among the addition lines using the four markers. Thus, if W1450 and/or W1451 are the source of the W1423 resistance, it is possible that they are not group 7 addition chromosomes.

Conclusion

FHB resistance was found in triticale × *Thinopyrum distichum* secondary hybrids as well as triticale-*Th. distichum* disomic addition lines W1450, and W1451. A lineage (W1423) derived from secondary triticale-*Th. distichum* hybrids appears to segregate for the presence of strong FHB resistance which could be due to a small alien translocation. In an attempt to select a resistant homozygote for further study, single, resistant plants were selected through four generations. However, the populations kept segregating for resistance and plant type despite being advanced generations. Chromosome count data of F_7 progeny (derived from an F_6 plant named W1423X) revealed a wide range of chromosome numbers, suggesting structural changes and abnormal segregation of at least two pairs of homologous chromosomes. Monosomy was then detected for chromosome 7A, implying the involvement of group 7 chromosomes in these genomic changes. Analysis with chromosome 7A, 7B, 7R and $7el_1$ markers did not reveal the presence of an alien chromosome region. Future attempts to characterize the W1423 resistance should therefore focus on finding a resistant homozygote with stable, euploid chromosome

number. Also, crosses and backcrosses should be made to both triticale and common wheat while simultaneously selecting for resistance and a euploid chromosome number. At least some of the chromosome abnormality in line W1423 appears to be due to chromosome modifications that do not involve the resistance. Substituting non-critical, structurally altered chromosomes in line W1423 with normal chromosomes through backcrosses should improve fertility and plant type and eventually allow for better assessment of the resistance. Once a stable, resistant, disomic plant is recovered it can be subjected to GISH analysis and an attempt to map the resistance and identify appropriate markers. However, it is possible that the W1423 resistance occurs on a structurally compromised chromosome or on a rye chromosome of the triticale genome. Thus, it may turn out not to be suited for application in wheat breeding in its present form. If this is the case, it will be necessary to aim to produce a new translocation to wheat through chromosome engineering. The resistance in the addition lines similarly needs confirmation in both greenhouse and field trials. It will then be necessary to transfer the addition chromosome into a wheat background through backcrosses. The telosomic addition (W1450) will be the best for this purpose as the telosome can be easily identified in somatic cells which will facilitate its selection for genetic engineering. Lastly, it will be necessary to identify the homoeology relationship of the addition chromosome with wheat chromosomes. Once this is known it will greatly facilitate the search for additional markers and the planning of its transfer to wheat through genetic engineering.

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CHAPTER VI. CONCLUSIONS

To date, numerous useful FHB resistance QTL were reported and applied to breeding programs in different regions of the world (Bai et al., 2018). At North Dakota State University the new HRWW breeding program is similarly trying to broaden its pool of FHB resistant germplasm. Therefore, diverse, validated FHB resistance QTL from spring wheat are being transferred to winter-hardy winter wheat backgrounds. This project attempted to transfer and establish QTL derived from the spring wheats PI277012 (*Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*), Frontana (*Qfhs.ifa-3A*), and TA5660 (*Fhb6* carrying translocation from *Elymus tsukushiensis*) in winter wheat. Marker-assisted selection was used in an attempt to select lines having *Fhb1* plus one or more additional QTL. Such genotypes were then evaluated for the level of resistance produced and those that showed the most promise for use as cross parents were identified (Table 6-1).

Table 6-1. Superior lines obtained from this research with different combinations of FHB resistance QTL

QTL combination	Cross number and pedigree	Line numbers
Fhb1 and *Qfhs.ifa-5A*	Cross 15K353 = 14K456-K-1/Novus-4	3-1; 3-3; 3-4; 5-1; 6-2 and 14-3
Fhb1 and *Qfhb.rwg-5A.1*	Cross 15K353 = 14K456-K-1/Novus-4	28-3; 31-4 and 31-5
Fhb1 and *Qfhs.ifa-5A*	Cross 15M26 = TA5660/ Jerry// Radiant/3/ 14K456-K-1	198 and 225
Fhb1 and *Qfhs.ifa-3A*	15M16 = Frontana/Norstar//Norstar-*Fhb1*	102
Fhb1 and *Fhb6*	Cross 15M26 = TA5660/ Jerry// Radiant/3/ 14K456-K-1	207
Fhb1, *Qfhs.ifa-5A* and *Fhb6*	Cross 15M26 = TA5660/ Jerry// Radiant/3/ 14K456-K-1	217

The primary objective with the attempt to transfer known resistance QTL from HRSW was to establish QTL combinations in HRWW that will provide significant FHB resistance and that can be employed directly in crosses with breeding lines to improve the overall level of

resistance in the program. Utilizing published markers, it was possible to incorporate all but one of the targeted QTL (*Qfhb.rwg-5A.2*) in winter wheat. Failure to incorporate *Qfhb.rwg-5A.2* was the result of the gene not being present in the original spring wheat donor source (RWG21). It was hoped that the resistance data gathered with respect to gene combinations would give an indication of the ability of the remaining QTL to complement the *Fhb1* effect. With the exception of *Qfhs.ifa-3A*, all of the QTL that were transferred appeared to strengthen the effect of *Fhb1*. However, the importance of genetic background effects in determining the overall level of resistance of a genotype was clearly evident. The lines developed from Jerry (HRWW) and CM82036 (spring wheat derived from Sumai3) showed strong type II resistance in the greenhouse (Chapter II), whereas the lines developed from Norstar-*Fhb1* on average showed very poor type II resistance (Chapter III). The Chapter III population was derived from cross Frontana/Norstar//Norstar-*Fhb1*, of which, both Frontana and Norstar-*Fhb1* are moderately resistant, but Norstar is highly susceptible. Both the Chapter II and Chapter III populations contained *Fhb1* plus a second QTL [*Qfhs.ifa-5A* (Chapter II) or *Qfhs. ifa-3A* (Chapter III)], each of which explained about 16-20% of the observed phenotypic variation and believed to also confer type I resistance. A big difference between these two populations is in their genetic background. The data implies absence of small effect resistance QTL/ epistatic genes in the Norstar genetic background (which constituted 75% of the hybrid background of the cross), and it is even possible that Norstar may possess QTL that makes it more sensitive to FHB attack.

It was necessary to confirm that *Fhb6* is free from deleterious genes that derive from *E. tsukushiensis.* While preliminary, the results of a greenhouse trial suggested that this is the case.

Numerous resistance QTL have been reported in literature, many of which appear to have comparatively small effects that are hard to detect with bio-testing, in particular when the bio-

test is aimed at Type II resistance only, as is the case with single spikelet injection. The better-characterized QTL such as *Fhb1* and *Qfhs.ifa-5A* appear to interact in an additive manner, however, it is possible that some of the background QTL act as modifiers or simply increase sensitivity to infection. These uncharacterized background QTL will of course add or detract from the overall resistance phenotype achieved when a major QTL such as *Fhb1* is introduced into a specific genotype.

Strong-effect resistance QTL have been detected in non-homologous chromosome regions of common wheat or its GP2 and GP3 relatives. This raises the possibility that at least some of the genes could be non-orthologous and employ different resistance mechanisms. These include: *Fhb6* (sub-telomeric region of chromosome $1E^{ts}$#1S of *Elymus tsukushiensis*); *Fhb1* (on 3BS of Sumai3); *Qfhs.ifa-5A/Qfhb.rwg.5A.1* (on 5AS of Sumai3/ *T. timopheevii*-derived chromatin in PI277012); *Qfhb.rwg-5A.2* (on *T. timopheevii*-derived chromatin in 5AL of PI277012); *Fhb7* (distal long arm of *Th. ponticum* chromosome $7el_2$), *Qfhb.cau-7DL* (on 7DL of winter wheat line AQ24788-83). Complementation appears to occur between *Fhb1* and *Qfhs.ifa-5A/Qfhb.rwg-5A.1*; *Qfhb.rwg-5A.1* (as well as *Qfhs.ifa-5A?*) and *Qfhb.rwg-5A.2*; and possibly between *Fhb1* and *Fhb6*. To accurately characterize all of the possible interactions it will be advisable to first develop near-isogenic lines with single or multiple pyramided QTL. In this study, the main purpose was to enrich the breeding germplasm pool and maintain diversity, therefore the QTL were introduced into diverse, winter-hardy backgrounds. Apart from the pursuit of favorable interactions among the QTL, diversification of the resistance germplasm pool in terms of the nature and origin of the FHB resistance QTL will help to deal with future shifts in the virulence of the pathogen population. The project currently lacks, and should strive to obtain, homoeologous group 7L resistance.

The study highlighted the necessity to regard the greenhouse FHB evaluations as being preliminary in nature, and to also evaluate the resistant lines under field-inoculated conditions to gain a better evaluation on the overall resistance. Due to time constraints, field testing could be done only with respect to (a) combinations involving *Fhb1* plus *Qfhs.ifa-5A/Qfhb.rwg-5A.1* (2016/17), and (b) *Fhb1* plus *Qfhs.ifa-5A/Fhb6* combinations (2017/18). In both seasons the irrigated FHB nursery at Fargo suffered extreme winter kill that even led to the complete loss of the most winter-hardy control, Jerry, in >90% of the plots. The 2017/18 nursery was duplicated at Casselton where the survival was still low but considerably better. Heavy FHB infections developed in HRSW late in the summer; however, the HRWW trials that flowered earlier escaped the epidemic.

Dependable molecular markers and genomic information of target QTL are extremely useful when introgressing promising resistance QTL. Very limited linkage map information was available with respect to the *Qfhs.ifa-3A* markers and it was not certain that this gene had actually been transferred with the marker. Attempts to confirm the presence of the QTL in recombinants by doing greenhouse testing was complicated by sizeable genetic background variation. Transfer of *Qfhb.rwg-5A.2* failed because the HRSW donor line, RWG21, lacked this QTL. Initially, RWG21 was said to have *Qfhb.rwg-5A.2* in conjunction with the *Q* allele of the *Q* locus. When the published markers were not polymorphic with respect to RWG21, it was decided to also apply chromosome 5A SNP markers in haplotype analyses in order to track the RWG21 5AL chromatin. Combined with greenhouse tests the marker data indicated that RWG21 lacked a strong effect FHB resistance QTL on 5AL. The introgression of *Qfhb.rwg-5A.2* is now being repeated with a different source germplasm, i.e. GP80.

In addition, a pilot study was conducted to test the FHB resistance in *Thinopyrum distchum*. First, a triticale - *Th. distichum* secondary hybrid population ($F_{4:}$ W1423) segregated for an uncharacterized translocation and a high level of FHB resistance and was therefore screened to obtain a translocation homozygote. However, the progeny of the selected plants still varied for viability, fertility and FHB resistance, even after three cycles of single plant selection. In the fourth cycle, a high level of aneuploidy was observed which was ascribable to aberrant segregation of chromosome 7A and another unknown chromosome. A resistant F_7 plant (W1423X-3) had 42 chromosomes, yet showed a low frequency of aneuploid progeny which was believed to be the result of a low frequency of de-synapsis of a translocated chromosome and its normal homologue. Preliminary indications are that the resistance is associated with homoeologous group 7; however, this needs to be confirmed. Two triticale – *Th distichum* addition chromosomes were found to produce strong resistance too. These addition chromosomes should be incorporated in common wheat through crosses and backcrosses so that it will be possible to use it in future chromosome engineering for transfer to wheat.