MOLECULAR AND GENETIC CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a devastating fungal disease of both durum and common wheat. Multiple sources of FHB resistance have been found in common wheat, but not in durum wheat. Lack of effective FHB resistance sources and complex inheritance of FHB resistance genes in durum limit the development of FHB-resistant durum varieties. This research aimed to map FHB resistance genes and to understand the inheritance of the hexaploid wheat-derived FHB resistance genes in durum. Molecular mapping positioned the wild emmer wheat (*Triticum dicoccoides*)-derived FHB resistance QTL *Qfhs.ndsu-3AS* to a chromosomal interval of 5.2 cM flanked by the molecular marker *Xwgc501* and *Xwgc510* on the short arm of chromosome 3A (3AS). This study reduced the linkage drag associated with *Qfhs.ndsu-3AS* and developed new molecular markers to assist its selection breeding. The FHB resistance genes in common wheat 'Sumai 3' were normally expressed in the F_1 with hexaploids, but not in the F_1 with durum. The common wheat PI 277012-derived FHB resistance gene(s) exhibited complete dominance in the F_1 with durum. In addition, 'Langdon' (LDN) chromosomes 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, and 7A were found to contain genes that suppress expression of the Sumai 3-derived FHB resistance genes in the F_1, and 4A, 6A, and 6B contain genes required for expression of the PI 277012-derived FHB resistance genes in the F_1. Apparently, Sumai 3-derived FHB resistance genes exhibited a more complex inheritance pattern than PI 277012 in durum. Evaluation of LDN-'Chinese Spring' (CS) D-genome disomic substitution lines and LDN-*Aegilops tauschii* D-genome addition lines for FHB resistance indicated that LDN chromosome 5A and CS chromosome 6D may contain genes for FHB susceptibility and/or suppression of FHB resistance. LDN chromosome 2B and *Ae. tauschii* 5D may carry genes for FHB resistance. Addition of the entire D genome to LDN increased susceptibility to FHB. Multiple LDN durum and D-genome

chromosomes were identified to have either negative/suppression or positive/enhancement effects on FHB resistance. Thus, special genetic manipulation is needed to eliminate the genes with negative/suppression effects and to ensure normal expression of FHB resistance genes in durum.

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CHAPTER 1. GENERAL INTRODUCTION

Durum wheat is an important cultivated wheat species worldwide. Durum production has been greatly threatened by Fusarium head blight (FHB), a serious fungal disease in wheat and barley, due to the lack of effective FHB resistance. Outbreaks of FHB may result in significant economic losses because of reduced grain yield and quality caused by the contamination of deoxynivalenol (DON). Host resistance has been recognized as the most economic and efficient means to manage FHB.

Wild relatives of durum wheat as a potential gene pool for FHB resistance have been screened and accessions with acceptable FHB resistance have been identified (Oliver et al. 2007, 2008). Resistance QTL have been identified and mapped using molecular markers in some of the FHB-resistant accessions. A major FHB resistance QTL, designated *Qfhs.ndsu-3AS*, was identified and mapped on chromosome 3A of *Triticum dicoccoides* accession Israel-A (Otto et al. 2002; Stack et al. 2002). Saturation mapping positioned this QTL to an 11.5 cM chromosomal interval by Chen et al. (2007). However, molecular marker density within this QTL region remained low, making it difficult to precisely tag the QTL for marker-assisted selection in durum breeding. Thus, further saturation and fine mapping of *Qfhs.ndsu-3AS* will elucidate the exact location of the QTL and facilitate its use in wheat breeding. The comparative mapping strategy has proven to be an effective approach to saturate a genomic region with molecular markers in cereal crops (Faris et al. 2000; Liu and Anderson 2003; Liu et al. 2006; Qin et al. 2011)

Introgression of hexaploid wheat-derived FHB resistance genes into durum wheat could be an alternative approach to improve its FHB resistance. Hexaploid wheat 'Sumai 3' with a high level of FHB resistance has been widely used in common wheat breeding and many common wheat cultivars with comparable resistance as 'Sumai 3' have been developed from this

resistance source worldwide. However, Sumai 3-derived FHB resistance has not been successfully introgressed into durum. Multiple genetic factors in either Sumai 3 or durum wheat backgrounds or both could hamper this introgression (Rudd et al. 2001). Recently, another hexaploid wheat accession PI 277012 has been identified highly resistant to FHB. It seemed that FHB resistance genes in PI 277012 could be expressed in the durum wheat background (Chu et al. 2011). Genetic analysis of the expression of Sumai 3- and PI 277012-derived FHB resistance in durum and influence of hexaploid wheat-harbored genetic factors such as D-genome chromosomes on FHB resistance in the durum background should enhance utilization of hexaploid-derived FHB resistance sources in durum improvement.

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CHAPTER 2. LITERATURE REVIEW

Infection of Wheat by the FHB Pathogen

Fusarium head blight (FHB), also known as scab, is a destructive fungal disease in wheat and barley worldwide (Parry et al. 1995; McMullen et al.1997; Stack 1999). At least 17 *Fusarium* species could cause FHB with production of various mycotoxins (Parry et al. 1995; Bottalico et al. 1998; Bottalico and Perrone 2002). *F. graminearum* Schwabe [teleomorph:*Gibberella zeae* (Schw.) Petch] and *F. culmorum* (W.G. Smith) Sacc. (teleomorph unknown) are frequently encountered species associated with FHB infection in wheat (Parry et al. 1995; Wilcoxson et al. 1998; Siranidou et al. 2002). *F. graminearum* is a predominant species associated with FHB epidemics of wheat in the US (Parry et al. 1995; McMullen et al. 1997). Wilcoxson et al. (1998) collected 23,726 isolates from 24 counties in the state of Minnesota and 75% of them belonged to *F. graminearum*.

Outbreak of FHB is favored by high humidity and warm temperature during the flowering stage of host plants. Rossi et al. (2001) observed the development of four fungal species in wheat plants at temperatures ranging from 10 °C to 35 °C, and concluded that *F. graminearum* exhibited the highest infection frequency at 28-29 °C. Brennan et al. (2005) compared the influence of two different temperatures (i.e. 16 °C and 20 °C) on FHB severity in wheat, and observed that the temperature of 20 °C led to a greater yield loss than 16 °C. Siou et al. (2014) studied the infection of eight isolates from *F. graminearum*, *F. culmorum*, and *F. poae* in wheat, and found that wheat plants were most vulnerable to fungal infection at anthesis. Hence, a temperature from 15 °C to 30 °C and humidity equal or greater than 90% during anthesis would favor FHB fungal infection and disease development (De Wolf et al. 2003; Cowger et al. 2009).

Infection of the *Fusarium* fungus starts from the extruded anthers and subsequently the caryopsis, floral bracts, and rachis. Afterwards, symptoms can spread through the rachis to the close spikelets. The infected spikelets are prematurely bleached (Nakajima 2010). Infection of *F. graminearum* in wheat spikes reduces kernel number and weight, and causes contamination of grain by the pathogen-produced deoxynivalenol (DON). DON is harmful to human and livestock health when diseased grains are consumed (Snijders and Perkowski 1990; Brennan et al. 2005; Pestak 2007). In the USA, it is recommended that the DON content of human food should be less than 1ppm (http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx). This disease has caused tremendous economic losses to wheat growers and industries due to reduced yield and quality of wheat grain. For instance, wheat production was reduced up to about 25% (2.72 million metric tons) due to epidemics of FHB over 11 states in the USA in 1991 (McMullen et al. 1997). Serious outbreaks of FHB occurred during the 1990s in the Northern Great Plains and central region where durum wheat was mainly produced (McMullen et al. 1997).

Management of FHB Disease

Several strategies have been investigated and applied to control FHB. Rotation and tillage approaches can be used to reduce the infection from survived *F. graminearum* in crop residue (McMullen et al. 1997). However, the wide range of hosts for the *Fusarium* fungi makes these methods less effective (Bai and Shaner 1994; Parry et al. 1995). Fungicide application is another option to reduce FHB risk. However, this does not always provide effective control of FHB (Horsley et al. 2006; Nakajima 2010). Moreover, the usefulness of spraying is greatly influenced by the application time and cost. Also, there are environmental and food safety concerns associated with the extensive application of fungicide in crop production. Thus, FHB

management cannot rely only on fungicide application in wheat (Bai and Shaner 1994; Nakajima 2010).

Host resistance has been considered the most effective method to control FHB. Variation in the susceptibility of wheat to FHB was first observed in the late 1800s (Bai and Shaner 1994). Since then, great efforts have been made to identify FHB resistance sources. The existence of a gene for gene relationship between a resistance gene in host and an avirulence gene in the parasite was first proposed by Flor (1956, 1971), and was found to apply to many plant-pest interactions. This was followed by the formulation of the concepts of vertical and horizontal resistance in hosts. Mesterhazy et al. (1999) showed that no host specificity existed in *F. graminearum* and *F. culmorum* isolates through evaluation of the interaction between 45 wheat genotypes with different levels of resistance and 15 isolates of *F. graminearum* and *F. culmorum*. Toth et al. (2008) observed similar FHB reactions of 20 wheat genotypes to different isolates of *F. graminearum*. Thus, FHB resistance is neither race-specific nor species-specific in wheat. As a result, it was proposed that FHB resistance in wheat has a horizontal nature (Snijders and van Eeuwijk 1991; van Eeuwijk et al. 1995; Mesterhazy et al. 1999).

Types of FHB Resistance

Generally, resistance to FHB has been classified into two categories: physiological (active) or morphological (or passive) (Miedaner 1997; Rudd et al. 2001). Wheat morphological traits including height, presence of awns, peduncle length, opening of the flower, heading time, and spikelet compactness were investigated for their contribution to FHB resistance (Mesterhazy 1995; Paillard et al. 2004; Gilsinger et al. 2005; Schmolke et al. 2005; Chu et al. 2007; Klahr et al. 2007). However, no clear relationships between these morphological traits and FHB

resistance have been observed (Miedaner 1997; Holzapfel et al. 2008). Thus, selection of FHB resistance based on morphological traits is not preferable in wheat.

Two types of physiological resistance to FHB were proposed by Schroeder and Christensen (1963): (I) resistance that impedes the initial infection by the pathogen, and (II) resistance that delays the spread of the pathogen in the infected plant organ. Further studies revealed great variation among wheat accessions in their ability to degrade and tolerate the secondary metabolite DON during infection by *F. graminearum*. Miller et al. (1985) analyzed the ratio of ergosterol, a biomarker for fungal biomass, to DON. It was observed that the ratio was higher in FHB resistant wheat accessions than in susceptible ones, suggesting resistant wheat had greater ability to degrade DON. Miller and Arnison (1986) observed the suspension cultured wheat 'Frontana' degraded 18% of DON 72 hr post-inoculation, which partly accounted for FHB resistance identified in Frontana. These results indicated that the synthesis and metabolism of DON were related with FHB resistance in wheat. Analysis of coleoptile development under different concentrations of DON in 14 spring wheat accessions indicated that FHB-resistant wheat accessions tolerated higher concentrations of DON than susceptible accessions (Wang and Miller 1988). In addition, resistance to kernel infection and yield tolerance were also identified associated with genotypes in wheat (Mesterhazy 1995; Mesterhazy et al. 1999). As a result, five types of FHB resistance were proposed: (I) resistance to initial infection, (II) resistance against fungus spread within the spike, (III) resistance against kernel infection, (IV) yield tolerance, and (V) decomposition of mycotoxins in the infected spikes.

FHB Inoculation and Evaluation

Multiple inoculation methods have been developed and used to evaluate the five types of FHB resistance in wheat. Single-floret and spray inoculation methods are commonly used in the

greenhouse and field for FHB resistance evaluation (Buerstmayr et al. 2009). In the single-floret inoculation method, 5-10 µl of inoculum with a concentration typically ranging from 10,000 to 100,000 conidiospore mL^{-1} is injected into the central floret in a spike at anthesis (Rudd et al. 2001; Stack et al. 2002; Rosyara et al. 2009). The single-floret inoculation method provides the same amount of inoculum for each inoculated spike and reduces variation in FHB infection due to different amounts of inoculum. Afterwards, the inoculated spikes are kept under a high level of humidity for 72 hr with a plastic bag or misting system to favor infection. FHB severity is scored 14 days and 21 days post-inoculation. Time and labor requirements are the major shortcomings of this method.

The spray inoculation method is also commonly used for its convenience. In this method, the inoculum with a concentration of 50,000 conidiospore mL^{-1} is sprayed at the mid-anthesis stage, and the treatment is repeated two or three days later (Somers et al. 2003; Steiner et al. 2004; Yang et al. 2005; Chen et al. 2006). After inoculation, a mist-irrigation system is used to maintain a high humidity for three days to favor disease development. FHB severity is scored 21 days post-inoculation. This method is widely used for large-scale evaluations in the field due to its convenience, but it is greatly influenced by environmental conditions, especially the temperature.

Grain spawn is another large-scale evaluation method widely used in the field. In this method, the inoculum is carried by grain such as wheat or corn. The grain spawn is usually prepared in a laboratory and is evenly distributed in the field about three weeks prior to the flowering stage. Field plots are misted for disease development after spawn application (Chu et al. 2007). FHB is usually assessed 21 or more days post anthesis depending on disease development.

FHB resistance can be assessed as FHB spread, FHB severity, FHB incidence, disease index, Fusarium damaged kernels (FDK), and DON content (Buerstmayr et al. 2009). The percentage of infected spikelets in each spike can be visually scored as a value of FHB spread with a scale from 0 to 100% (Kumar et al. 2007). This value is usually used to evaluate resistance to pathogen spread (Type II) along the rachis of infected spikes. For convenience of scoring, a modified scale was proposed with the assumption of 13 to 14 spikelets in a spike (Stack and McMullen 1998). The scale was classified into ten infection types (0%, 7%, 14%, 21%, 33%, 50%, 66%, 79%, 90%, and 100%). The value 7%, 14%, and 21% represent one, two and three infected spikelets in a spike, respectively, and 33%, 50%, and 66% are for 1/3, 1/2, and 2/3 of spikelets being infected in a spike. FHB severity is a mean value of the percentage of diseased spikelets per unit and has been used for the evaluation of Type II resistance (Paul et al. 2005; Buerstmayr et al. 2009). The percentage of infected spikes per unit is scored as incidence. It is used to determine Type I resistance (Chen et al. 2006). The disease index is calculated by multiplying incidence by FHB severity (Stack and McMullen 1998). Positive correlation was observed among incidence, FHB severity, and disease index (Paul et al. 2005; Oliver et al. 2008; Kollers et al. 2013). However, negative correlation between incidence and FHB severity was also reported in some genotypes (Gilbert and Woods 2006). FDK is estimated by calculating the percentage of diseased kernels in a random sample (Chu et al. 2007; Cowger et al. 2009). DON content (mg kg^{-1}) of kernels is determined using gas chromatograph-mass spectrometry (GC-MS), enzyme-linked immunosorbent assay (ELISA) or other chromatographic methods (Hart et al. 1998; Zhou et al. 2002; Chu et al. 2007). Among the above evaluation methods, visual measurement of FHB spread and severity for Type II resistance has been widely adopted for FHB disease evaluation in wheat (Buerstmayr et al. 2009).

Sources of FHB Resistance

Extensive screening of FHB resistance has been performed in the primary, secondary, and tertiary gene pools of wheat since the discovery of variation in FHB resistance among different genotypes (Snijders 1990a; Cai et al. 2005; Oliver et al. 2007, 2008). The widely used sources of FHB resistance are categorized into three groups: (1) winter wheat from Europe, (2) spring wheat from China/Japan, (3) spring wheat from Brazil (Schroeder and Christensen 1963; Snijders 1990a). In addition, new sources of resistance to FHB have been identified from the germplasm pools derived from other parts of the world (Rudd et al. 2001; Zhang et al. 2008; Liu and Anderson 2013). Most of the resistance sources exhibit moderate resistance to FHB (Rudd et al. 2001; Liu et al. 2013).

'Sumai 3', a widely used FHB resistance source of Chinese origin, has played an important role in wheat breeding for FHB resistance worldwide. It was developed from the cross of two FHB susceptible accessions 'Funo' and 'Taiwan Xiaomai' and released in the early 1970's in China (He et al. 2001). As a cultivar derived from transgressive segregation, Sumai 3 exhibited a high level of FHB resistance. However, minor genetic variations have been observed among the Sumai 3 accession from different countries (Niwa et al. 2014). A number of Sumai 3-derived cultivars such as 'Ning 7840', 'Een 1', and 'Alsen' have been released in China and the USA and exhibited good levels of FHB resistance (http://www.ag.ndsu.nodak.edu/alsen.htm; He et al. 2001). Another Chinese landrace 'Wangshuibai' has also been identified possessing moderate Type II resistance to FHB. This resistance source has not been used as widely in wheat breeding as Sumai 3 (Zhou et al. 2004; Lin et al. 2004). PI 277012 is a newly reported spring wheat accession with a similar level of FHB resistance as Sumai 3 (Chu et al. 2011). Other FHB resistance sources such as Frontana from Brazil (Steiner et al. 2004), 'Arina' from Switzerland

(Paillard et al. 2004), and ‘Nobeokabozu’ from Japan (Steiner et al. 2004) have been reported.

Complete resistance to FHB has not been found in wheat and its relatives although tremendous efforts have been made to search for new sources of FHB resistance. In addition, FHB resistance gene introgression into adapted genotypes has been a challenge due to complex inheritance and poor understanding of FHB resistance genes in wheat. Sumai 3-derived common wheat cultivars exhibited moderate resistance and introgression of FHB resistance from Sumai 3 into durum wheat has been hampered by unknown genetic factors. Therefore, identification of novel genes for FHB resistance remains essential in wheat, especially in durum. Extensive attention has been placed on searching for FHB resistance genes from wild relatives of wheat. FHB resistance has been identified in several wheat-related genera including *Aegilops*, *Agropyron*, *Hystrix*, *Elymus*, *Kengyilla*, *Secale*, and *Thinopyrum* in the tribe *Triticeae*, and even higher levels of FHB resistance than that in Sumai 3 were observed in some wild species (Cai et al. 2005). These wild species represent a promising gene pool for wheat improvement with regard to FHB resistance. However, linkage drag associated with alien chromatin/genes and presence of the presence of *Ph* gene that inhibits homoeologous recombination in wheat makes the introgression of alien genes into wheat more difficult than to use native FHB resistance genes (Wang et al. 1977; Cai et al. 2008). Accessions from different species in the genus *Triticum* have been evaluated for FHB resistance, and some accessions of *T. timopheevii*, *T. karamyschevii*, *T. dicoccum*, and *T. dicoccoides* have been identified with FHB resistance. FHB resistance identified in *T. dicoccoides* has become an important source in durum breeding (Otto et al. 2002; Stack et al. 2002; Chen et al. 2007). Various methods have been developed to overcome the obstacles in the utilization of alien FHB resistance genes in wheat breeding. Embryo rescue makes it possible to recover hybrids between alien species and wheat. Meiotic pairing and

recombination between homoeologous chromosomes of wheat and related wild species have been enhanced using a mutant *Ph* gene, i.e. *ph1b* mutant (Wang et al. 1977; Sharma and Ohm 1990). In addition, a number of wheat-alien species derivatives with FHB resistance have been developed and identified. They are ideal ‘bridge’ materials for alien gene introgression from wild species into wheat.

Genetic and Molecular Analysis of FHB Resistance Genes

The quantitative nature of inheritance conferred by minor or major genes for FHB resistance has been reported in various studies (Snijders 1990b; Van Ginkel et al. 1996; Holzapfel et al. 2008). FHB resistance is mainly controlled by additive genetic effects, but non-additive genetic effects such as dominance or epistasis are also observed. Snijders (1990b) found that both additive and dominance genetic effects were associated with FHB resistance, but additive genetic effects were more important. Predominant additive effect in FHB resistance was also reported in other studies (Ottler et al. 2004; Yang et al. 2005). In addition, dominance effect was found to play a major role in FHB resistance in some crosses (Snijders 1990c).

Due to strong genotype by environment interaction, wide-range heritability of FHB resistance was observed for different genotypes. Snijders (1990b) analyzed broad-sense heritability of ten winter genotypes in F_2 populations and observed large variation from 0.05 to 0.89 for individual F_2 families derived from different genotypes. Fakhfakh et al. (2011) calculated heritability of 0.42 and 0.84 for FHB severity and FDK at 21 days post-inoculation, respectively. This result illustrated the effectiveness of scoring these two traits at three weeks after inoculation. Transgressive segregants for FHB resistance were observed in many segregating populations (Buerstmayr et al. 2002; Steiner et al. 2004). Sumai 3 was selected from the transgressive segregants of the cross between two FHB-susceptible cultivars (He et al. 2001).

Genomic resources and tools have been widely used to identify and characterize FHB resistance genes in wheat. Quantitative trait loci (QTL) mapping has been frequently performed to detect and locate the genes for FHB resistance. Several types of molecular markers including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), sequence tagged site (STS), target region amplification polymorphism (TRAP), diversity arrays technology (DArT), sequence tagged microsatellite (STM), and single nucleotide polymorphism (SNP) have been employed in the molecular mapping of FHB resistance QTL in wheat (Waldron et al. 1999; Zhou et al. 2002; Liu and Anderson 2003; Chen et al. 2007; Basnet et al. 2012; Bernardo et al. 2012; Buerstmaryr et al. 2013). Recombinant inbred lines (RILs) and doubled haploids (DHs) are commonly used populations for FHB resistance QTL mapping because of their nature of true breeding and utility in the replicated FHB evaluation experiments at multiple locations and seasons (Waldron et al. 1999; Chen et al. 2007; Buerstmaryr et al. 2009).

All 21 wheat chromosomes have been found to contain FHB resistance QTL. Many wheat accessions harbor multiple QTL on different chromosomes (Buerstmaryr et al. 2009; Cativelli et al. 2013). Sumai 3 has FHB resistance QTL on chromosome 2B, 3B, 6B, and 7A (Zhou et al. 2002). The major QTL on 3BS in Sumai 3, designated *Qfhs.ndsu*-3BS or *Fhb1*, has been verified in different studies (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002). A high-density linkage map of *Fhb1* was constructed using a comparative mapping strategy. Efforts toward map-based cloning of *Fhb1* have been made; however, no functional gene has been identified (Liu et al. 2006; Liu et al. 2008). Zhuang et al. (2012) proposed that the genes in Sumai 3 could possibly confer FHB resistance by reducing the susceptibility of Sumai 3 rather than expressing an active FHB resistance reaction. It was

identified that the Chinese landrace Wangshuibai harbors FHB resistance genes on chromosomes 2A, 3A, 5A, 7A, 1B, 3B, 4B, 5B, 6B, 2D, 3D, 5D, and 7D explaining 5.0% to 37.3% of the phenotypic variation (Lin et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Jia et al. 2005; Mardi et al. 2005; Lin et al. 2006; Ma et al. 2006; Yu et al. 2008; Zhang et al. 2010). Frontana was identified to contain FHB resistance QTL on chromosomes 3A, 5A, 7A, 2B, and 6B (Steiner et al. 2004; Mardi et al. 2006). Two FHB resistance QTL were identified on the short and long arms of chromosome 5A, respectively, in the common wheat accession PI 277012 (Chu et al. 2011). The nature of the polygenic inheritance makes the introgression and breeding for FHB resistance a challenge in wheat.

A number of FHB resistance genes have been identified from various sources. Some of them have been successfully utilized in the development of superior wheat cultivars. However, genes for FHB susceptibility should not be ignored. Manipulating those genes may be another approach to improve resistance of wheat to FHB. Zhou et al. (2002) developed a set of 'Chinese Spring' (CS)-Sumai 3 disomic substitution lines where a pair of CS chromosomes was substituted by a pair of homologous chromosomes from Sumai 3. FHB evaluation of these substitution lines revealed that Sumai 3 chromosomes 1B, 2D, and 4D contained genes for DON accumulation. Also, FHB-susceptible genotypes may contain FHB resistance genes that are not normally expressed due to genetic suppression. Basnet et al. (2012) identified three FHB resistance QTL on chromosomes 3B, 6B, and 2D in a population derived from the cross of Sumai 3 with a susceptible Tibetan landrace, Y1193-6. The QTL on chromosome 2D originated from the susceptible parent, Y1193-6. Shen et al. (2003) and Handa et al. (2008) also identified FHB resistance genes on chromosome 2D from susceptible wheat accessions.

FHB Resistance in Durum Wheat

Multiple sources of resistance to FHB have been identified in common wheat (*Triticum aestivum* L., 2n = 6x = 42, genome AABBDD). Some of them have been successfully utilized in cultivar development. Several tetraploid relatives of durum wheat (*T. durum* Desf., 2n = 4x = 28, genome AABB) and durum landraces exhibited improved FHB resistance compared to current durum cultivars (Otto et al. 2002; Kumar et al. 2007; Ghavami et al. 2011; Talas et al. 2011). However, a source of resistance to FHB comparable to the widely used hexaploid resistance source Sumai 3 has not been found in durum and its tetraploid relatives. Moreover, introgression of FHB resistance from hexaploid wheat including Sumai 3 into durum wheat has not been very successful. It has been postulated that, 1) the D-genome chromosomes possibly play a role in the expression of Sumai 3-derived FHB resistance; 2) the complete set of FHB resistance genes in Sumai 3 is difficult to recover; 3) suppressors of the Sumai 3-derived FHB resistance genes exist in the durum wheat background (Gilbert et al. 2002; Liu and Anderson 2003; Rudd et al. 2011; Basnet et al. 2012).

Both cultivated and wild tetraploid relatives of durum wheat were screened to identify novel FHB resistance genes for durum wheat improvement (Oliver et al. 2007, 2008). Evaluation of 376 accessions under five cultivated tetraploid wheat sub-species related to durum identified a few FHB resistant accessions in Persian wheat and Poulard wheat (Oliver et al. 2008). Oliver et al. (2007) evaluated 416 wild emmer wheat accessions in the greenhouse, observed tremendous variation in FHB resistance, and identified accessions with significant resistance to FHB. Several FHB resistance QTL have been detected and mapped to specific chromosomes in several tetraploid species including *T. dicoccoides*, *T. dicoccum*, *T. cathlicum*, and *T. durum* (Otto et al. 2002; Somers et al. 2006; Stack and Faris 2006; Kumar et al. 2007; Buerstmayr et al. 2012).

Among these QTL, *Qfhs.ndsu-3AS* conferring Type II resistance from *T. dicoccoides* accession Israel-A has been further characterized through molecular mapping (Otto et al. 2002; Stack et al. 2002; Chen et al. 2007; Zhu et al. 2013). It was positioned to a chromosomal interval of 11.5 cM flanked by the markers *Xfcp401* and *Xfcp397.2* on chromosome 3A by Chen et al. (2007). Further saturation and high-resolution mapping were performed in this study to better understand this QTL and develop effective molecular markers for marker-assisted selection in durum breeding.

Conclusions

Fusarium head blight disease in terms of pathogen infection, disease evaluation and control, inheritance pattern of host resistance, and identification and development of resistant germplasm have been extensively studied in wheat. Significant progress has been made in the management of FHB especially by improving host resistance in common wheat. The lack of an effective resistance source has limited improvement of FHB resistance in durum. In addition, complex inheritance of FHB resistance genes in durum makes it difficult to incorporate hexaploid wheat-derived FHB resistance genes into durum. It is essential to have a better understanding of the expression and inheritance of FHB resistance genes in durum. This will facilitate utilization of the FHB resistance sources currently available in hexaploid and tetraploid wheat to improve FHB resistance of durum.

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CHAPTER 3. TOWARD A BETTER UNDERSTANDING OF THE CHROMOSOMAL REGION HARBORING FUSARIUM HEAD BLIGHT RESISTANCE QTL *QFHS.NDSU-3AS* IN DURUM WHEAT

Abstract

Comparative mapping has been an efficient strategy for molecular mapping of the wheat genome, especially with respect to saturation and fine mapping, by taking advantage of the genomic collinearity of wheat with other species in the grass family. In this study, the genomic region harboring Fusarium head blight (FHB) resistance quantitative trait locus (QTL) *Qfhs.ndsu-3AS* on wheat chromosome 3A was saturated with 42 newly developed sequence tagged site (STS) and simple sequence repeat (SSR) markers. They spanned a genetic distance of 232 cM on the short arm of chromosome 3A. The STS and SSR primers were designed from the wheat ESTs identified based on the micro-collinearity of this QTL region with the corresponding rice and *Brachypodium* genomic regions. *Qfhs.ndsu-3AS* was mapped to a chromosomal interval of 7.4 cM flanked by *Xbcd1532/Xbarc45* and *Xwgc510*. Moreover, the map resolution of the QTL region was improved by genotyping 372 F_2 individuals derived from the cross of the recombinant line RICL#10 containing *Qfhs.ndsu-3AS* in the shortest *T. dicoccoides* chromosomal fragment with 'Langdon' (LDN) durum. As a result, five co-segregating markers in the QTL region mapped to three loci proximal to *Xgwm2*. The other four STS markers (*Xwgc1226*, *Xwgc510*, *Xwgc1296*, and *Xwgc1301*) mapped further proximal to the above markers in a higher resolution. Five homozygous recombinant lines with smaller *T. dicoccoides* chromosomal fragments have been selected for FHB evaluation using the molecular markers in the F_3, F_4, and F_5 generations. *Qfhs.ndsu-3AS* was further mapped to a 5.2-cM interval flanked by marker *Xwgc501* and *Xwgc510* in the advanced populations. This has reduced the *T. dicoccoides*

chromatin around *Qfhs.ndsu-3AS* and minimized the linkage drag associated with the alien chromatin, making this alien FHB resistance QTL more useful in durum breeding.

Introduction

Fusarium head blight (FHB), caused mostly by *Fusarium graminearum* Schwabe [telomorph:*Gibberella zeae* (Schw.) Petch], is a destructive fungal disease of wheat. Epidemic infection of FHB is accelerated by warm and humid conditions (Stack and McMullen 1985; Xu et al. 2008). Outbreak of FHB can cause severe losses in grain yield and quality in both common wheat (*Triticum. aestivum* L., 2n=6x=42, genome AABBDD) and durum wheat (*T. durum* Desf., 2n=4x=28, genome AABB) (McMullen et al. 1997). Multiple genes for FHB resistance have been identified and successfully deployed in common wheat cultivars (Liu and Anderson 2003; Liu et al. 2006, 2008; Buerstmayr et al. 2009; Chu et al. 2011). However, FHB remains a major threat to durum wheat production due to the lack of effective resistance sources in durum (Oliver et al. 2007). Incorporation of FHB resistance genes from common wheat into durum has not been very successful because of complex inheritance of hexaploid-derived FHB resistance in the durum background (Buerstmayr et al. 2012; Zhu et al. 2012).

Sources of FHB resistance have been found in durum wheat and its tetraploid relatives even though they are not as effective as those in common wheat (Stack et al. 2002; Buerstmayr et al. 2003; Cai et al. 2005; Oliver et al. 2007, 2008; Ghavami et al. 2011; Talas et al. 2011; Ruan et al. 2012). Molecular mapping has identified several wild emmer wheat (*T. dicoccoides*)-derived FHB resistance QTL, including *Qfhs-ndsu-3AS* on chromosome 3A (Otto et al. 2002), *Qfhs.fcu-7AL* on 7A (Kumar et al. 2007), and another one on 6B (Stack et al. 2006). Also, FHB resistance QTL has been detected in the durum-related tetraploids *T. cathlicum* (Somers et al. 2006) and *T. dicoccum* (Buerstmayr et al. 2012; Zhang et al. 2014). In addition, extensive screening of durum

accessions for FHB resistance has identified several durum landraces with detectable resistance to the disease over the last few years (Ghavami et al. 2011; Talas et al. 2011; Zhang et al. 2014). Some of these tetraploid-derived FHB resistance QTL have been mapped to the same genomic regions as those identified in hexaploids, suggesting collinearity of the resistance gene loci in tetraploids and hexaploids.

Qfhs.ndsu-3AS is a wild emmer-derived FHB resistance QTL located on the short arm of chromosome 3A (Otto et al. 2002; Stack et al. 2002). It is the first FHB resistance QTL identified in tetraploid wheat and confers moderate resistance in the durum background. Saturation mapping positioned *Qfhs.ndsu-3AS* to a chromosomal interval of 11.5 cM flanked by the molecular markers *Xfcp401* and *Xfcp397.2* on 3AS (Chen et al. 2007). Wheat chromosomes in homoeologous group 3 are collinear to rice chromosome 1 and *Brachypodium* chromosome 2 (Moore et al.1995; the International Brachypodium Initiative 2010; Sehgal et al. 2012; Luo et al. 2013). Wheat researchers have been taking advantage of the collinearity to characterize the large and complex polyploid genome of wheat using the genomic resources available in these two grass models, rice and *Brachypodium* (Liu and Anderson 2003; Liu et al. 2006; Foote et al. 2004; Kumar et al. 2009). This study aimed to utilize the micro-collinearity of the chromosomal region harboring *Qfhs.ndsu-3AS* with rice chromosome 1 and *Brachypodium* chromosome 2, and to precisely identify the position of *Qfhs.ndsu-3AS* within a smaller chromosomal region. This will facilitate understanding of the genomic region harboring the FHB resistance QTL *Qfhs.ndsu-3AS* and provide effective molecular markers to assist selection of this resistance QTL in wheat breeding.

Materials and Methods

Mapping Populations

Eighty-three recombinant inbred chromosome lines (RICLs) developed by Joppa (1993) from the cross between durum wheat cultivar 'Langdon' (LDN) and disomic LDN-*T. dicoccoides* Israel-A (ISA) substitution line 3A [LDN (DIC-3A)] were employed for saturation mapping in this study. RICL#10, having the shortest *T. dicoccoides* chromosomal fragment harboring *Qfhs.ndsu-3AS*, was crossed with LDN to generate additional meiotic recombinants within the chromosomal region for fine mapping of the QTL. A large F_2 population (n>1,800) segregating only in the QTL region was developed from that cross for fine mapping. The F_2 individuals with smaller *T. dicoccoides* chromosomal fragments than RICL#10 were screened to develop recombinants homozygous at the marker loci within the *Qfhs.ndsu-3AS* region. Homozygous recombinants were evaluated for FHB resistance to further map *Qfhs.ndsu-3AS* to a smaller chromosomal interval.

Comparative Analysis and Molecular Marker Development

An initial comparative analysis was performed to develop additional EST (expressed sequence tag)-derived STS (sequence tagged site) and SSR (simple sequence repeat) markers for saturation mapping of the *Qfhs.ndsu-3AS* QTL region based on the collinearity of the QTL region with the genomic regions on rice chromosome 1. The EST sequences of BE517736 and BF484475, from which the STS markers *Xfcp402* and *Xfcp399* flanking the QTL region were developed (Chen et al. 2007), were used as queries to perform BLASTn against rice and *Brachypodium* genomic sequences and to identify the collinear counterparts of the QTL region on rice chromosome 1 and *Brachypodium* chromosome 2 in the J. Craig Venter Institute (JCVI) wheat genome database (http://blast.jcvi.org/euk-blast/index.cgi?project=tae1) and

Brachypodium database (http://www.brachypodium.org/gmod/alignment/blast_finders/new), respectively. A threshold of an expected (E) value equal to or less than e^{-15} was adopted in the BLAST searching and comparative analysis. The rice and *Brachypodium* bacterial artificial chromosome (BAC)/P1-derived artificial chromosome(PAC) clones hit in the BLAST search were used as anchor points to identify the rice and *Brachypodium* genomic regions collinear to the QTL region (http://www.ebi.ac.uk/ena/data/view/GCA_000005425.2; http://ensembl.gramene.org/Brachypodium_distachyon/Info/Index?db=core;r=2:1-4999). Then the rice and *Brachypodium* genomic sequences collinear with the QTL region and the adjacent rice and *Brachypodium* sequences outside of the anchor points were used as queries to search for wheat ESTs through BLASTn in the JCVI wheat genome database. The corresponding tentative sequences (TCs) (http://compbio.dfci.harvard.edu/cgi-bin/tgi/est_ann.pl?gudb=wheat) or contigs in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml) for the identified ESTs were adopted to eliminate redundant EST sequences. The STS and SSR primers were designed from the EST sequences using Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

Molecular Marker Analysis and Genetic Mapping

All EST-derived STS and SSR primers were screened for polymorphisms between LDN and LDN (DIC-3A). The polymorphic markers were employed to genotype 83 RICLs. PCR amplification was carried out in a 20-µl mixture containing 40 ng genomic DNA, 0.5 µM each of forward and reverse primers, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP and 0.25 U of Taq DNA polymerase. PCR was performed according to the following protocol: 94 ºC for 3 min; 45 cycles of 94 ºC for 1 min, 52~60 ºC (varied with specific primer pairs) for 1 min and 72 ºC for 1.5 min; then with a final 72 ºC for 7 min. PCR products were separated on 8% polyacrylamide gel and visualized by ethidium bromide staining or separated on 5% denaturing polyacrylamide

gel and visualized by silver staining (Liu and Anderson 2003; Chen et al. 2007). Additional molecular marker techniques used in this study included cleaved amplified polymorphism (CAP) (Chen et al. 2003), single-strand conformation polymorphism (SSCP) (Kumar et al. 2006), and heteroduplex analysis (Mohamed et al. 2004). The genetic maps were constructed by using Mapmaker 2.0 for Macintosh as described by Lander et al. (1987). All the newly developed molecular markers in this project were designated *Xwgc* followed by a number.

FHB Disease Evaluation

All homozygous recombinant plants were grown in 6-inch plastic pots randomly arranged on the bench in the greenhouse with one plant in each pot. The control RICL #10 was planted in three pots with two plants in each pot in each greenhouse environment. FHB resistance was evaluated with the point inoculation method following the procedure described by Stack et al. (2002). A single floret from a central spikelet was inoculated at first anthesis using 10 μl of conidiospore suspension. The inoculum was prepared from four spcies of *F. graminearum* and concentrated to 1×10^5 conidiospores per mL. The temperature in the greenhouse was kept at approximately 25 ^oC with 16 hr photoperiod during the disease development stage. To facilitate disease development, high humidity was maintained for 72 hr with the inoculated spikes covered by a plastic bag. At 21 days post inoculation, the percentage of infected spikelets in a spike was scored. For each pot, the average percentage of all spikes (5 to 10) was calculated as FHB severity for each individual homozygous recombinant. With regard to the control, the mean value of three pots was recorded as FHB severity.

Results

Identification and Analysis of Micro-Collinearity

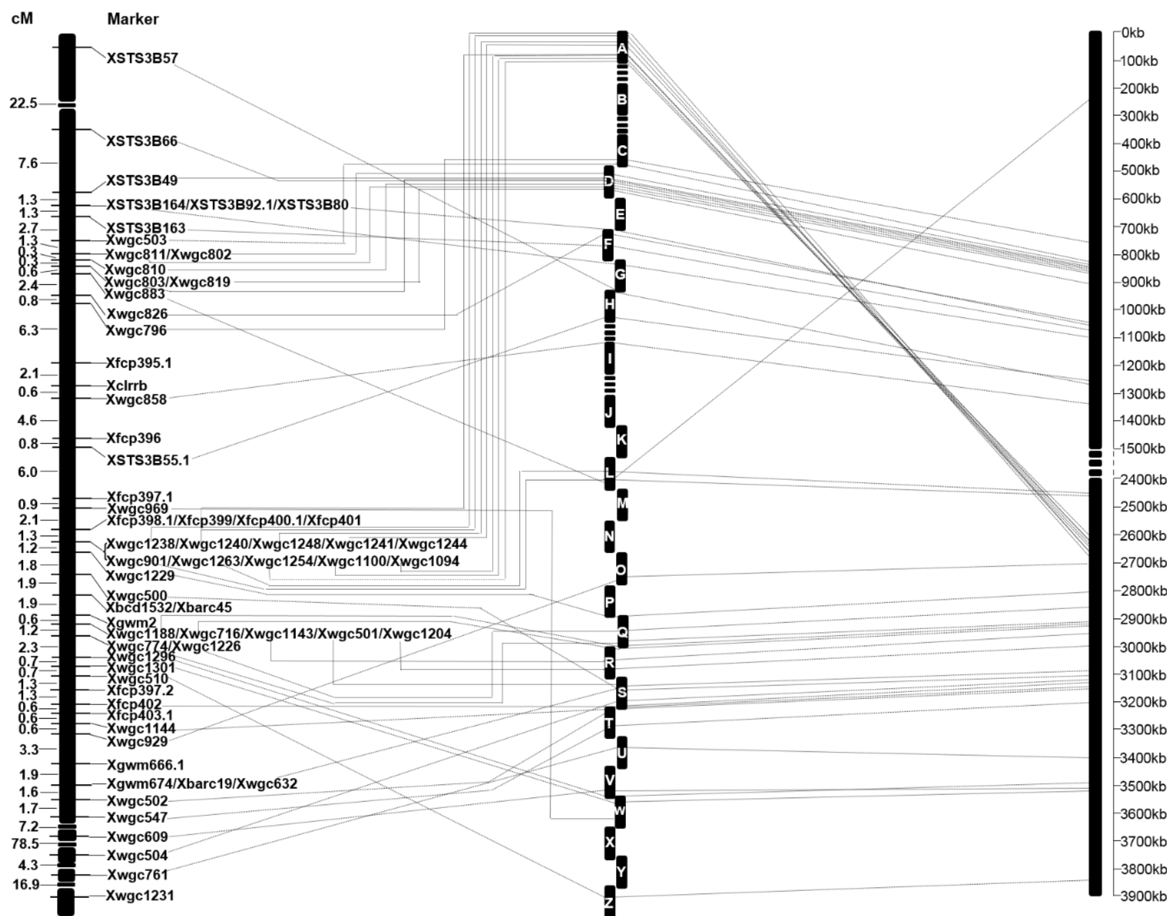
The wheat ESTs BE517736 (*Xfcp402*) and BF484475 (*Xfcp399*) mapped proximally and distally to the FHB resistance QTL *Qfhs.ndsu-3AS* on 3AS, respectively (Chen et al. 2007; Figure 3.1). The contig sequences of BE517736 (Ta.9622.1.S1_x_at) and BF484475 (Ta.1999.3.S1_a_at) were used as queries to BLAST rice genomic sequences. One rice PAC (AP003282) and one rice BAC (AP004225) were hit by the contig 'Ta.9622.1.S1_x_at' with lowest E values of $3.2e^{-144}$ and $6.8e^{-90}$, respectively. AP003282 is located on the short arm of rice chromosome 1, while AP004225 occurs on the long arm. Since *Qfhs.ndsu-3AS* mapped to the short arm of wheat chromosome 3A (3AS), AP003282 was selected as the anchoring point of BE517736 on rice chromosome 1. BLASTing with the contig 'Ta.1999.3.S1_a_at' identified three PACs (AP003610, AP002969, and AP003727) on rice chromosome 1 with similar E values, i.e. $5.4e^{-40}$, $5.8e^{-40}$, and $8.0e^{-40}$, respectively. Sequence alignment of the three PACs in NCBI indicated that AP003610 was part of AP003727 and 84% of AP002969 was included in AP003727. Thus, AP003727 was considered the anchoring point of BF484475 on rice chromosome 1. Therefore, the rice genomic region from AP003727 to AP003282 was considered collinear with the wheat genomic region spanning *Qfhs.ndsu-3AS* on 3AS. BLASTing the *Brachypodium* genome with BE517736 identified a genomic region from 3,139,341 bp to 3,141,007 bp on chromosome 2 with an E value of $1.4e^{-131}$, while BF484475 identified a genomic region from 2,644,263 bp to 2,644,440 bp with an E value of $4.7e^{-53}$ on the same chromosome. So, the *Brachypodium* genomic region from 2,644,263 bp to 3,141,007 bp on chromosome 2 was considered collinear with the wheat genomic region spanning *Qfhs.ndsu-3AS* on 3AS.

Saturation Mapping

A total of 793 pairs of STS primers and 42 pairs of SSR primers were designed from the wheat EST singletons, TCs, and contigs identified according to the micro-collinearity of the chromosomal interval harboring *Qfhs.ndsu-3AS* with corresponding genomic regions of rice and *Brachypodium*. Forty-eight pairs of STS primers and three pairs of SSR primers amplified polymorphisms between the two parents of the mapping population. Forty-two STS/SSR markers mapped to a genomic region of 232 cM on chromosome 3A (Figure 3.1; Table 3.1). Twenty-two of the 42 markers mapped within or near the QTL region. Five co-segregating STS markers, *Xwgc501*, *Xwgc716*, *Xwgc1143*, *Xwgc1188*, and *Xwgc1204*, mapped 0.6 cM proximal to *Xgwm2*. A new marker locus has not been detected between *Xgwm2* and *Xbcd1532*/*Xbarc45* (Figure 3.1). Twelve new EST-derived markers mapped closely distal to the *Xbcd1532*/*Xbarc45* locus. Ten of them, *Xwgc1238*, *Xwgc1240*, *Xwgc1248*, *Xwgc1241*, *Xwgc1244*, *Xwgc901*, *Xwgc1263*, *Xwgc1254*, *Xwgc1100*, and *Xwgc1094*, co-segregated in the mapping population. Another cluster of 14 markers, which spanned a chromosomal interval of 11.0 cM, mapped distally to the QTL region. This chromosomal region is collinear with the region harboring the major FHB resistance gene locus *Fhb1* on the short arm of wheat chromosome 3B (Chen et al. 2007; Figure 3.1).

Fine Mapping

A large F_2 population (n>1,800) was developed from the cross between LDN and one of the 83 RICLs, RICL#10 which retained the shortest chromosomal fragment from *T. dicoccoides* harboring *Qfhs.ndsu-3AS*. A total of 372 random individuals from the population were used in fine mapping of the QTL. Nine co-dominant STS markers (*Xwgc1188, Xwgc716, Xwgc1143, Xwgc501, Xwgc1204, Xwgc1226, Xwgc1296, Xwgc1301 and Xwgc510*) and two SSR markers (*Xbarc45* and *Xgwm2*) previously mapped to the QTL region (Figure 3.1) were used to genotype



Genetic map of the distal region of wheat 3AS

Physical map of the distal region (4,360 kb) of rice 1S

Distal region of *Brachypodium* 2S

Figure 3.1. Comparative analysis of the genomic region harboring *Qfhs.ndsu-3AS* on wheat 3AS, the distal region of rice 1S, and the distal region of *Brachypodium* 2S

Rice PACs/BACs: A-AP003727; B-AP002882; C-AP002747; D-AP002541; E-AP002868; F-AP002487; G-AP003046; H-AP003233; I-AP002538; J-AP002872; K-AP002540; L-AP002522; M-AP003045; N-AP003225; O-AP002521; P-AP003209; Q-AP003301; R-AP003339; S-AP003282; T-AP003215; U-AP002523; V-AP002903; W-AP002524; X-AP003118; Y-AP003047;Z-AP002484

Table 3.1. Primer sequences of STS/SSR markers mapped on chromosome 3A

Marker	Forward primer sequence(5'-3')	Reverse primer sequence(5'-3')	Source*
Xwgc500	ATATTGGAGTTGCTGTGGAC	TACGCCTACATGTTCTCCTT	TC253371
Xwgc501	CTGGGGCAACTACTTCTACT	AGAAACAAGCCTCAACCAC	TaAffx.516.1.S1_at
Xwgc502	TAGGAAGAGAAACGCGATAG	GTACTGGGTGACGTTGGTAT	TC237031
Xwgc503	CGCCTCCACCATTCTTCTT	GTAGAGCGTGATCACCGTGG	BF293133
Xwgc504	CTGGGTCTACTCCTGTATGG	CGTGGAGGCAAAGATATAAG	TC252511
Xwgc510	GCATACCTCCCTCTCATGTA	TTACAAACATCGTCTGTCCA	TC242060
Xwgc547	CTTTATTTCCGCCACCAC	TCCAGACCCAGCTTAGTAGA	TC254174
Xwgc609	GCAAGTTCTGCTCCCACTTC	ACGACCCAAGAGCATCAAGT	TC267460
Xwgc632	GACCTCAACACCATCGAAGC	GAGCAGGCTGATGTCGAACT	TaAffx.93289.1.S1_at
Xwgc716	CATCTGCTGCAATCCTTGAA	GCTCGGATATCAAACCTCCA	TC255907
Xwgc761	AGGCAGAACCTCAGACACTA	AGGAGAAAGTGGAAAGGAAG	CJ573891
Xwgc774	TGAAGATGGTGAGGATGATG	TGGAGTGCTCTTCTGACAAA	TC254977
Xwgc796	ATGACAGGCCGACAATGAG	TCAACCAATTAAGCAGTTGGAG	TC257223
Xwgc802	AAGAGCGAGGAGAAGAAGAC	ACACACACAACTCGAAGACA	TC238553
Xwgc803	GGCACGTGTCCTTCTCCTAC	CGCTGCCAATACAGCTAACA	TC263447
Xwgc810	CAGGAACTCTGTGAAGAAGG	GAATCGGAGGAGAAAAGAAT	TC259639
Xwgc811	AGAGCTCGCTCAAGGACAAG	GTAGTCGGCCGTCAACATTT	TC248298
Xwgc819	GGTACCTCGAGTACAGCATC	CTCCACCAAGAAAGAAAATG	TC242796
Xwgc826	GGCTGTCGTGGAAGAAGAAG	AAGGCGATGAACACCAAAAC	TC254539
Xwgc858	GTTGGGGGAGAGCATGAAG	CCTGTTGTGATGCGAAAATG	CJ795235
Xwgc883	TGCAAGAAGACGACACAAGG	GGATGAAGCCAATCTTCCAA	DR734177
Xwgc901	CTTTCCCTCCGGCCTACTAC	CAACATTCGGCACGAGACTA	CJ807010
Xwgc929	CGGACGAGAAGAAGCTCAAG	GCTACAATCCAGGGACCAAA	TC268280
Xwgc969	ATCCGTGTTACCCAAATGGA	GCAACAGCTGCAAATCGTAA	TC265006

Table 3.1. Primer sequences of STS/SSR markers mapped on chromosome 3A (continued)

Marker	Forward primer sequence(5'-3')	Reverse primer sequence(5'-3')	Source*
Xwgc1094	CTGGTGTCCGTTGTTTCCTT	CACATTGGTCCTCCACACAG	TC264939
Xwgc1100	GGGCAACCAAAAAGACAAGA	TTTCAACCCGCTTCTCAAAG	BE412385
Xwgc1143	TTCCAGATCACTCCTCTCC	TGCATGAAACAAAACAACAT	TC273545
Xwgc1144	TATGCAACCATTGATCTTGA	AACACCAGGAAACTTGACAC	TC247145
Xwgc1188	TCTCGCAACTTGTGATGAAA	GGGTACCAGTAGCTGAAGCA	TC326279
Xwgc1204	TCCTTTCTCTCCAGCAGCAT	CTCCGGTTATCTTCCACCAA	TC260079
Xwgc1226	CACTTGGCAGAGCTCAACAG	AGGAGCAGGAGGAGGATCAC	TC258122
Xwgc1229	GCAGGCTGTAAACTCCTTGA	TAACCGGATCAGGATACGAA	TC354664
Xwgc1231	ATGCCAACAGGAAGGTCTTG	CAGCATGCAAATCTCTGGAC	TC288309
Xwgc1238	CGTCAAGACCATTGCTGAGT	CGACACATTCAACATCTCCA	TC278865
Xwgc1240	GTGATTGGGAAGCCAGACAT	ATATTGGAGGCCTTGTGTGC	TC294980
Xwgc1241	GTTCATCACCCGAGCTCATT	TGTGTGGCTTTCTGCACTTC	TC246390
Xwgc1244	GTAGTGCAAGACCCCAAGGT	GACAGCTCCCTCGTCTGAG	CJ670128
Xwgc1248	CTACCACCAGGAGCAGGAAG	AGATTCAAGGAGGAGCGACA	TC247595
Xwgc1254	AGATGTACCCGGAGACGAAG	GACAGCGTAGCAGCATGTTT	TC344003
Xwgc1263	TTCATCAAGAGGCACGACAG	GCTTTGCCATGTTTCACAGA	TC307328
Xwgc1296	TGGTGACTGATGGGATGGTA	GCTGGGGGAAAGGGTAAAT	TC278201
Xwgc1301	AACATCGCCCAGCAGAAC	CGTCGCAGTGTATTTCATTTG	TC252087

*The GenBank wheat EST accession number, the TC number of the TIGR *Triticum aestivum* gene index and the GrainGenes 2.0 accession contig number

the population. *Xwgc774*, co-segregating with *Xwgc1226*, was not included because it is a dominant marker and could affect mapping accuracy in an F_2 population (Jiang and Zeng 1997). *Xbarc45*, a dominant SSR marker, was used to identify homozygous recombinants rather than for fine mapping. *Xwgc716* and *Xwgc1188* still co-segregated and mapped 0.8 cM proximal to *Xgwm2* in the F_2 population. *Xwgc1143* and *Xwgc1204* also co-segregated and mapped 0.1 cM proximal to *Xwgc716*/*Xwgc1188* (Figure 3.2). The other five STS markers, including *Xwgc501*, *Xwgc1226*, *Xwgc510*, *Xwgc1296*, and *Xwgc1301*, mapped further proximal to the above markers in a higher resolution (Figures 3.1-2).

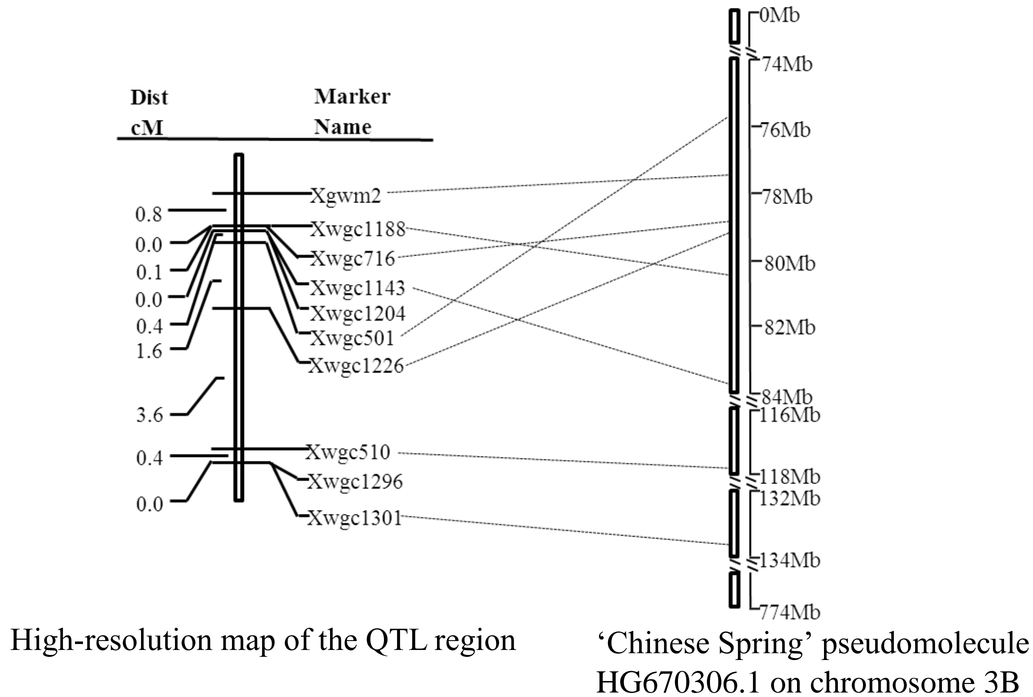


Figure 3.2. Comparative analysis of the high-resolution map of the QTL region with common wheat 'Chinese Spring' pseudomolecule HG670306.1 on chromosome 3B

Identification and Evaluation of Recombinants for FHB Resistance

Three F_2 individuals (3AS07-39-17, 3AS07-42-26, and 3AS07-53-16) were identified to have recombination in the QTL region and contained a shortened *T. dicoccoides* 3AS fragment harboring the QTL (Chen et al. 2007; Figure 3.1; Table 3.2). 3AS07-39-17 was a homozygous recombinant designated Type I recombinant, whereas 3AS07-42-26 and 3AS07-53-16 were heterozygous for a recombination in the QTL region (Table 3.2, 3.3). Two homozygous recombinants with the same genotype at the marker loci were identified from the 10 F_3 progenies of 3AS07-42-26, and designated Type II recombinants (Table 3.3). One of the two Type II recombinants was used to develop an F_4 family for further FHB evaluation (Table 3.4). Three heterozygous recombinants with different sized fragments from *T. dicoccoides* were developed from 3AS07-53-16 in the F_3 generation. Forty F_4 individuals derived from each of these three heterozygous recombinants were screened to recover homozygous recombination in the QTL region. Eleven individuals were identified as homozygous recombinants in each of two F_4 families and designated Type III and Type IV recombinants (Table 3.2, 3.3), respectively. Nine homozygous recombinants derived from the third F_4 family were designated Type V recombinants (Table 3.2, 3.3). In total, five types of homozygous recombinants harboring shortened *T. dicoccoides* fragments were identified within the QTL region (Table 3.3).

FHB evaluation of the five types of homozygous recombinants was carried out in three greenhouse seasons (Table 3.4). Homozygous recombinant plants for FHB evaluation in each season were either selected from a segregating population at early generations by markers (PSM), or derived from a homozygous recombinant with unknown reaction to FHB (PDU) or with resistance to FHB (PDR) at later generations. Ten plants in the PDU family and 20 plants in PDR were employed for FHB evaluation. The observed number of the plants for FHB evaluation less

Table 3.2. Genotypes of LDN, RICL#10, and three F_2 individuals at the marker loci within the QTL region

	Xbarc 45	*Xgwm* 2	*Xwgc* 716	*Xwgc* 1188	*Xwgc* 1204	*Xwgc* 1143	*Xwgc* 501	*Xwgc* 1226	*Xwgc* 510	*Xwgc* 1296	*Xwgc* 1301
LDN	A*	A	A	A	A	A	A	A	A	A	A
RICL#10	B	B	B	B	B	B	B	B	B	B	B
3AS07-39-17	A	A	A	A	A	A	A	B	A	A	A
3AS07-42-26	B	H	A	A	H	H	H	A	A	A	A
3AS07-53-16	A	H	H	H	H	H	A	H	A	A	A

* 'A' refers to homozygous LDN genotype, 'B' refers to homozygous *T. dicoccoides* genotype, and 'H' refers to heterozygous genotype

Table 3.3. Genotypes of five homozygous recombinants selected in the *Qfhs.ndsu-3AS* region

Recombinant types	F_2 sources	*Xbarc45*	*Xgwm* 2	*Xwgc* 716	*Xwgc* 1188	*Xwgc* 1143	*Xwgc* 1204	*Xwgc* 501	*Xwgc* 1226	*Xwgc* 510	*Xwgc* 1296	*Xwgc* 1301
I	3AS07-39-17	A*	A	A	A	A	A	A	B	A	A	A
II	3AS07-42-26	B	B	A	A	B	B	B	A	A	A	A
III	3AS07-53-16	A	A	A	B	B	A	A	B	A	A	A
IV	3AS07-53-16	A	A	B	B	B	A	A	B	A	A	A
V	3AS07-53-16	A	B	B	B	B	B	A	B	A	A	A

* 'A' refers to homozygous LDN genotype, and 'B' refers to homozygous *T. dicoccoides* genotype

Table 3.4. FHB evaluation of homozygous recombinants in three seasons

Recombinant type	Fall 2013					Spring 2014					Summer 2014				
	No. plants	Generation	Plant type	FHB severity (%)	PPLC (%)	No. plants	Generation	Plant type	FHB severity (%)	PPLC (%)	No. plants	Generation	Plant type	FHB severity (%)	PPLC (%)
I	9	F_3	PDU	11.6-76.9	44.4	10	F_4	PDU	58.3-71.9	0	20	F_4	PDR (28.1%)	14.6-48.6	90.0
II	9	F_4	PDU	36.6-63.9	0	9	F_4	PDU	49.4-72.2	0		-	-	-	-
III	9	F_4	PIM	14.6-50.3	33.3	8	F_5	PDU	21.7-51.6	37.5	20	F_5	PDR (14.6%)	19.0-54.7	80.0
IV	8	F_4	PIM	30.5-72.9	37.5	10	F_5	PDU	19.6-59.4	70.0	-	-	-	-	-
V	9	F_4	PIM	9.9-54.2	66.7	-	-	-	-	-	20	F_5	PDR (9.9%)	13.6-48.7	80.0
RICL#10				34.4					31.39					36.0	

PPLC: Percentage of plants with FHB severity lower than the control (RICL#10)
PDU: A family in which plants derived from a homozygous recombinant with unknown FHB resistance level
PIM: A family in which homozygous recombinant plants were identified based on marker genotype
PDR: A family in which plants derived from a homozygous recombinant with known FHB resistance level (bracketed scores are FHB severity for the recombinants)

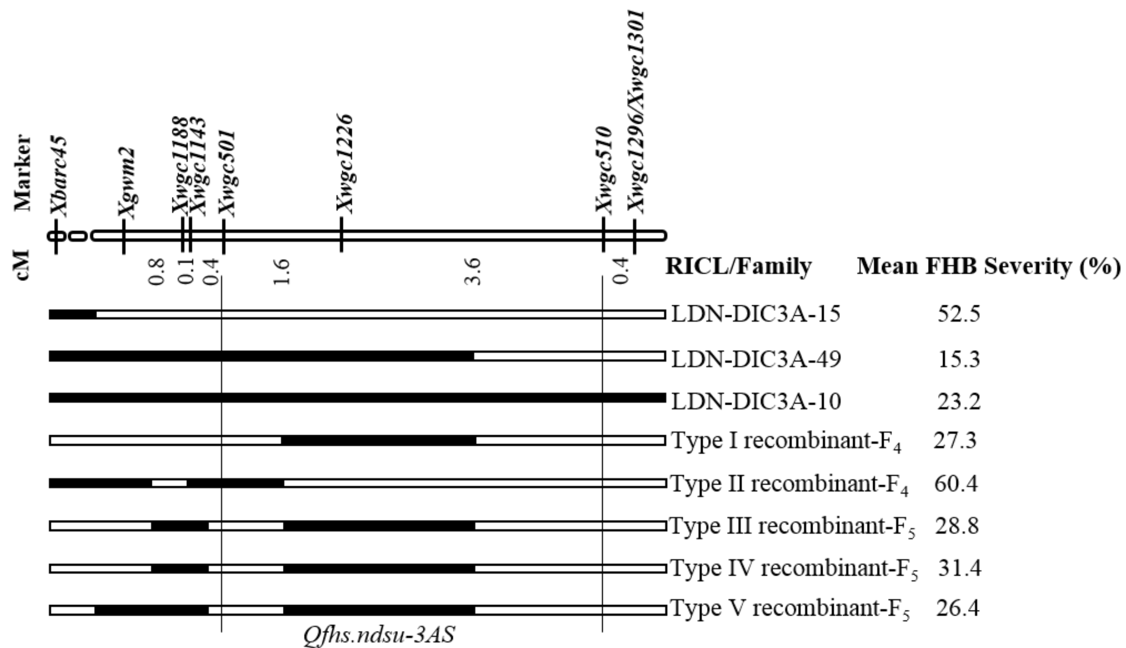


Figure 3.3. Graphical genotypes of three RICLs and five homozygous recombinants. Alleles from *T. dicoccoides* and LDN were represented by the black and open boxes, respectively. The recombination breakpoints were assumed to be in the middle of the interval flanked by two markers. *Qfhs.ndsu-3AS* was placed within a 5.2cM interval flanked by markers *Xwgc501* and *Xwgc510*

than 10 in PDU family or that of initially identified recombinants in PIM family resulted from the death of plants or the plants saved for the development of advanced seeds in some cases (Table 3.4). FHB evaluation results indicated that 33.3% to 70% homozygous recombinants in either PIM or PDU families exhibited a resistance level higher than or similar to the control, RICL#10, for the four types of recombinants (I, III, IV, V). However, no homozygous recombinants with lower FHB severity than RICL#10 was observed in one PDU family of Type I recombinant in the spring of 2014 (Table 3.4). High percentage of resistant plants (80% to 90%) was identified in three PDR families of Type I, III and V recombinants. No resistant plants were identified in two PDU families derived from the same homozygous F_3 individual of Type II recombinants.

Comparative Analysis

Complex collinearity has been revealed among wheat chromosome 3A, rice chromosome 1, and *Brachypodium* chromosome 2 through comparative analysis. The chromosomal interval proximal to *Xgwm2* harbors two groups of co-segregating markers and three other closely linked markers within the QTL region. The co-segregating markers, including *Xwgc501*, *Xwgc716*, *Xwgc1143*, *Xwgc1188*, and *Xwgc1204*, are 0.6 cM away from *Xgwm2*. These five EST-derived STS markers identified a collinear region of ~154 kb on the short arm of rice chromosome 1 (1S) and ~100 kb on the short arm of *Brachypodium* chromosome 2 (2S) (Figure 3.1). The other two co-segregating markers, *Xwgc774* and *Xwgc1226*, is 1.2 cM proximal to the five co-segregating markers. These two markers identified a collinear region of ~78 kb and ~50 kb on rice 1S and *Brachypodium* 2S, respectively. The orientation of the chromosomal interval harboring these two groups of co-segregating markers on 3AS are inverted to the corresponding collinear regions on rice 1S and *Brachypodium* 2S. The three markers proximal to the two co-segregating markers, *Xwgc1296, Xwgc1301,* and *Xwgc510*, detected a collinear region of ~339 kb on rice 1S and ~330 kb on *Brachypodium* 2S. Both are located proximally to the collinear regions identified by the five co-segregating markers (Figure 3.1). The chromosomal interval distal to the QTL region, defined by *Xwgc1229* and *Xgwc500*, shows good collinearity with a genomic region of ~340 kb on rice 1S and ~300 kb on *Brachypodium* 2S. A group of ten co-segregating markers distal to *Xwgc1229* detected two collinear regions on rice 1S and *Brachypodium* 2S, respectively. Two of the ten markers *Xwgc901* and *Xwgc1263*, identified a collinear region of ~13 kb on rice 1S and ~10 kb on *Brachypodium* 2S. This collinear region on rice 1S is about 2,658 kb away from the terminal end of this chromosome arm. The other eight co-segregating markers detected a collinear region of ~141 kb near the terminal end of rice 1S. However, the collinear region

identified by these eight markers on *Brachypodium* 2S (~90 kb) is located 2,600 kb away from the terminal end of the chromosomal arm and ~150 kb proximal to the collinear region detected by *Xwgc901* and *Xwgc1263* on *Brachypodium* 2S. The collinear regions on rice 1S and *Brachypodium* 2S are in inverted orientations (Figure 3.1). Fifteen marker loci were identified within a chromosomal region of 18.6 cM farther distal to the QTL region. Some of them co-segregated in the mapping population. The fifteen markers residing within this chromosomal region detected a collinear region of 244 kb on rice 1S, which is about 910 kb away from the terminal end of the chromosomal arm. The collinear region identified by these 15 markers is about 320 kb long on *Brachypodium* 2S. Greater collinearity has been found within these genomic regions, in terms of order and chromosomal locations of the marker loci in the respective genomes (Figure 3.1). Apart from the inversion of marker loci, deletions of marker loci on the *Brachypodium* chromosome were also observed. For example, the TC sequence of *Xwgc501* and *Xwgc1301* has an orthologous sequence on rice chromosome 1, but not on *Brachypodium* chromosome 2 (Figure 3.1).

To predict the possible physical size of the QTL region, comparative analysis between the refined QTL region and the released 'Chinese Spring' chromosome 3B pseudomolecule was performed. In this process, EST singletons, TCs or contig sequences corresponding to the markers in the refined QTL region were used as queries to perform BLASTn against the Chinese Spring chromosome 3AS-specific survey sequence database to identify the corresponding contigs (https://urgi.versailles.inra.fr/blast/blast.php) with a threshold E value equal to or less than e^{-15} (Sehgal et al. 2012). The 40-bp primer sequence of *Xgwm2*, an SSR marker closely linked to the *Qfhs.ndsu-3AS* QTL peak (Chen et al. 2007), was used as query to search for the potential representative sequence in the wheat database through BLASTn in NCBI. Two

identical *T. durum* microsatellite sequence clones, *Dwm213* and *Dwm2*, were identified. The microsatellite sequence of *Xgwm2* was used as a query of BLASTn against the 3AS-specific survey sequence database to identify the corresponding contigs (https://urgi.versailles.inra.fr/blast/blast.php) with a threshold E value equal to or less than e^{-15}. Then the identified contig sequences with the lowest E value were used as queries to BLASTn against Chinese Spring chromosome 3B pseudomolecule HG670306.1 in NCBI. Six markers (*Xgwm2*, *Xwc1188*, *Xwgc716*, *Xwgc1143*, *Xwgc501*, and *Xwgc1226*) around the QTL region were identified to have anchor points in the 3BS pseudomolecule. Comparative analysis identified a ~3.4Mb region (75.7 Mb to 79.1 Mb) in the pseudomolecule collinear with the chromosome interval flanked by *Xwgc501* and *Xwgc1226* (Figure 3.2). A ~38.8Mb genomic region in the pseudomolecule was found to be collinear with the chromosome interval flanked by *Xwgc1226* and *Xwgc510*. Moreover, a ~8.0Mb region (75.8 Mb to 83.8 Mb) in the pseudomolecule was collinear with the region harboring the QTL on 3AS (Figure 3.2). However, there was discrepancy in the orders of the markers on 3AS and their collinear regions in the 3BS pseudomolecule, indicating complex collinearity between these two homoeologous regions on chromosome 3A and 3B.

Discussion

The *T. dicoccoides*-derived FHB resistance QTL *Qfhs.ndsu-3AS* was positioned within the chromosomal interval flanked by two targeted region amplification polymorphism (TRAP) markers *Xfcp401* and *Xcfp397.2*, but only three markers, *Xbcd1532*, *Xbarc45* and *Xgwm2*, mapped within this region in a previous study by Chen et al. (2007). In the present study, this chromosomal interval was saturated by adding 22 newly developed markers, which further narrowed the location of *Qfhs.ndsu-3AS* to a smaller chromosomal region. Based on the

graphical genotypes of two RICLs, LDN-DIC3A-15 (susceptible to FHB) and LDN-DIC3A-49 (resistant to FHB), *Qfhs.ndsu-3AS* was placed within a 7.4 cM chromosome interval flanked by markers *Xbcd1532*/*Xbarc45* and *Xwgc510* (Figure 3.3).

Some of the 22 newly developed markers within the QTL region co-segregated in the RICL population. Five of these co-segregating markers were near *Xgwm2*, the most significant marker for *Qfhs.ndsu-3AS* (Figure 3.1). Thus, a larger mapping population was needed to resolve the co-segregating markers within the QTL region. Here, an F_2 population consisting of 372 individuals derived from the cross of LDN with RICL#10 (with the shortest *T. dicoccoides* fragment harboring *Qfhs.ndsu-3AS*) was developed for fine mapping of the chromosome region around *Xgwm2*. As a result, the map resolution was greatly improved and five co-segregating markers 0.6 cM proximal to *Xgwm2* were resolved into three marker loci. Moreover, five types of homozygous recombinants with shortened *T. dicoccoides* fragments were identified through the selection of marker alleles. Various levels of FHB resistance ranging from resistant to susceptible among the plants in the family of Type I, III, IV, and V recombinants suggested that *Qfhs.ndsu-3AS* was not co-segregating with these markers, and was possibly harbored in the genomic region flanked by two of these markers. The percentage of plants with high levels of FHB resistance in the later generations was higher than in the early generations in Type I, III, IV, and V homozygous recombinants, which could be caused by the segregation of heterozygous alleles at the *Qfhs.ndsu-3AS* locus over generations. No resistant plants were identified in Type II homozygous recombinants indicating the possible absence of the QTL in this type of recombinant. The *T. dicoccoides* fragment flanked by *Xwgc501* and *Xwgc510* was shared by Type I, III, IV, and V recombinants but was absent in Type II recombinants, which suggested that the QTL resides within this 5.2 cM genomic region flanked by *Xwgc501* and *Xwgc510*

together with the analysis of the graphical genotypes of three RICLs reported in Chen et al. (2007) (Figure 3.1, 3.3). No co-segregation of *Xwgc1226* with *Qfhs.ndsu-3AS* suggests that *Qfhs.ndsu-3AS* is possibly flanked either by *Xwgc501* and *Xwgc1226* or by *Xwgc1226* and *Xwgc510*.

The chromosomal interval harboring *Qfhs.ndsu-3AS* was found to be collinear to a large physical distance (~38.8Mb) on the 3B pseudomolecule. This shows why more precise mapping of *Qfhs.ndsu-3AS* would be necessary for a better understanding of the sequence information of the QTL. Although there is complex micro-collinearity between chromosomes 3A and 3B, the chromosome interval flanked by *Xgwm2* and *Xwgc510* exhibited good collinearity to the 3B pseudomolecule with regard to the content of marker loci. This suggests that the CS 3B pseudomolecule sequence could be a better reference sequence for further fine mapping of *Qfhs.ndsu-3AS*.

Extensive collinearity among the genomes of cereal crops has been revealed (Moore et al. 1995). Wheat homoeologous group 3 chromosomes are collinear to rice chromosome 1 and *Brachypodium* chromosome 2 (Moore et al. 1995; the International Brachypodium Initiative 2010; Sehgal et al. 2012; Luo et al. 2013). In this study, almost all mapped STS/SSR marker sequences have orthologous sequences on both rice chromosome 1 and *Brachypodium* chromosome 2 (Figure 3.1). Rice chromosome 1 exhibited more conserved collinearity with *Brachypodium* chromosome 2 than with wheat chromosome 3 in terms of the orientation of marker loci. However, the similarity between the genomic sequences of wheat and *Brachypodium* is higher than between *Brachypodium* and rice based on the higher E value obtained following BLASTn of the wheat sequence against the *Brachypodium* genomic sequence than against the rice genomic sequence (data not shown). Thus, *Brachypodium* is more closely

related to wheat than rice, which agrees with previous studies (Catalan and Olmstead 2000; Draper et al. 2001; Foote et al. 2004; the International Brachypodium Initiative 2010; Zhang et al. 2013). Although in the present study the association wheat-*Brachypodium* was more productive, there are instances where the wheat-rice comparison is more useful. In this study, the deletions of marker loci on wheat chromosome 3A were detected on *Brachypodium* chromosome 2 instead of on rice chromosome 1. Faris et al. (2008) found that the collinearity was more conserved between wheat and rice than between wheat and *Brachypodium* with regard to the *Q* gene region. To date, many genes in wheat have been well characterized through comparative analysis with rice and *Brachypodium* genomic sequences (Liu et al. 2003, 2006; Bossolini et al. 2007; Faris et al. 2008; Somyong et al. 2011; Zhang et al. 2013). In these studies, complex micro-collinearity has been revealed among wheat, rice, and *Brachypodium*. Thus, utilization of multiple genomic sequences from different species to characterize wheat genes could be more efficient than the use of only one genomic sequence.

In summary, this study determined the location of *Qfhs.ndsu-3AS* within a smaller chromosomal interval. Germplasm with shortened *T. dicoccoides* introgressed regions harboring *Qfhs.ndsu-3AS* was developed, and thus the linkage drag from *T. dicoccoides* was further reduced in LDN background. In addition, the genomic region on the CS 3B pseudomolecule collinear to *Qfhs.ndsu-3AS* was identified. These results will facilitate continued detail analysis of the *Qfhs.ndsu-3AS* regions and its utilization in wheat breeding.

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CHAPTER 4. EFFECTS OF DURUM WHEAT BACKGROUND ON THE EXPRESSION OF HEXAPLOID WHEAT-DERIVED FUSARIUM HEAD BLIGHT RESISTANCE GENES

Abstract

Hexaploid wheat-derived Fusarium head blight (FHB) resistance exhibits a complex inheritance pattern in durum wheat background. This study aimed to characterize how the durum genetic background modifies the expression of hexaploid wheat-derived FHB resistance genes. Evaluation the FHB infection of the F_1 of 'Sumai 3' with durum and hexaploid wheat indicated that FHB resistance genes from Sumai 3 were normally expressed in the F_1 hybrids with other hexaploids, but not in the F_1 with durum wheat. The hexaploid wheat PI 277012-derived FHB resistance gene(s) exhibited complete dominance in the F_1 of PI 277012 with durum. FHB evaluation of the F_1 of Sumai 3 and PI 277012 with LDN D-genome substitution lines suggested that chromosomes 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, and 7A may contain genes that suppress expression of the Sumai 3-derived FHB resistance genes in the F_1, and 4A, 6A, and 6B may contain genes required for the expression of the PI 277012-derived FHB resistance genes in the F_1. A wide range of segregation for FHB severity (10-90%) was observed in the F_2 generation of crosses of Sumai 3 with durum varieties 'Langdon' (LDN) and 'Divide'. The distribution of FHB severity in F_3 families derived from the most resistant F_2 segregants was skewed towards the more susceptible end. A similar segregation trend was observed in the F_4 generation. In the crosses of PI 277012 with durum, resistance also seemed to be slightly diluted over successive generations, but multiple resistant segregants were recovered in each generation of these crosses. Thereby, durum wheat may contain multiple genes on different chromosomes that positively

and/or negatively regulate the expression of hexaploid wheat-derived FHB resistance genes. This has made FHB resistance introgression from hexaploids into durum a challenging task.

Introduction

Fusarium head blight (FHB), also called scab, is one of the major devastating diseases in wheat worldwide. It is mainly caused by the fungus *Fusarium graminearum*. The major epidemic regions of FHB in the USA are in the mid-western and eastern states including North Dakota (Stack 1999). Host resistance is considered the most efficient approach to reduce yield and quality losses caused by FHB compared to other management tactics, such as rotation and chemical control (McMullen et al. 1997; Rudd et al. 2001). However, an effective source of resistance to FHB has not been found in durum wheat (Oliver et al. 2007). Therefore, there is an urgent need to identify and implement effective FHB resistance in durum wheat.

To date, the commonly used FHB resistance sources are all derived from hexaploid wheat. ‘Sumai 3’, a Chinese common wheat cultivar, has been a widely used source of resistance to FHB in wheat breeding worldwide. Chromosomes 2B, 3B, 6B, and 7A have been identified harboring FHB resistance genes in Sumai 3 (Yao et al. 1997; Waldron et al. 1999; Zhou et al. 2002; Liu and Anderson 2003). Among these genes, the major gene on 3B, designated *Qfhs.ndsu-3BS* (*Fhb1*), explains 15.4-60.0% of the phenotypic variation (Buerstmayr et al. 2002, 2009; Jayatilake et al. 2011), and many other genes contribute minor resistance to FHB (Bai et al. 1989; Van Ginkel et al. 1996). Moreover, both Type II (resistance to the spread of the pathogen) and Type V (low accumulation of mycotoxins) resistance were identified in Sumai 3 (Jayatilake et al. 2011). Preliminary studies of the candidate functional gene for *Fhb1* were carried out and a diagnostic molecular marker *UMN10* was developed by Liu et al. (2008). FHB resistance of Sumai 3 has been successfully introgressed into many hexaploid wheat germplasm and cultivars

worldwide (Waldron et al. 1999; He et al. 2001; Mergoum et al. 2006, 2008; Teresa et al. 2013; http://www.extension.umn.edu/agriculture/small-grains/cultivar-selection-and-genetics/docs/freyr-wheat.pdf). In the 1980s, two hexaploid cultivars 'Een 1' and 'Yangmai 4' with moderate resistance to FHB were first developed from Sumai 3 and released in China (He et al. 2001). In the USA, the first FHB resistant cultivar, 'Alsen', derived from Sumai 3 was released in the late 1990s (Frohberg et al. 2006). After that, many more FHB-resistant cultivars, including 'Faller', 'Glenn', and 'Freyr' were developed from Sumai 3 (Mergoum et al. 2006, 2008; http://www.extension.umn.edu/agriculture/small-grains/cultivar-selection-and-genetics/docs/freyr-wheat.pdf).

Recently, another hexaploid spring wheat accession PI 277012 with high level of FHB resistance was identified (Chu et al. 2011). It has a level of FHB resistance similar to Sumai 3. Molecular mapping identified two FHB resistance QTL located on chromosome arms 5AS and 5AL, respectively, in PI 277012 (Chu et al. 2011). Moreover, it appeared that the PI 277012-derived FHB resistance genes could normally express in durum wheat and could be very useful in durum wheat breeding (Chu et al. 2011).

To identify novel FHB resistance sources in tetraploid wheat, extensive screening of wild relatives of durum wheat has been carried out (Cai et al. 2005; Oliver et al. 2007, 2008; Buerstmayr et al. 2012; Ruan et al. 2012). Several FHB resistance QTL have been identified in wild emmer wheat (*T. dicoccoides*) accessions (Otto et al. 2002; Stack and Faris 2006; Kumar et al. 2007). However, most of the identified QTL confer moderate to low levels of resistance to FHB (Fakhfakh et al. 2011). Of these QTL, *Qfhs-ndsu-3AS* located on the short arm of chromosome 3A has been more extensively characterized than others (Otto et al. 2002; Chen et al. 2007). The poor understanding of the chromosome region involved and associated linkage

drag of undesirable genes in the wild relatives makes it difficult to utilize these FHB resistance QTL directly in durum wheat breeding.

One of the strategies to improve FHB resistance of durum wheat is to transfer hexaploid wheat–derived FHB resistance genes into durum wheat. However, progress in the introgression of FHB resistance from Sumai 3 into durum wheat has been very limited. The possible mechanisms of hindering the progress may include: 1) the single configuration of the gene combination in Sumai 3 is difficult to be recovered during the introgression (Liu and Anderson 2003; Basnet et al. 2012); 2) the wheat D genome, which is absent in durum wheat, possibly harbors genes that affect expression of FHB resistance genes (Rudd et al. 2001; Fakhfakh et al. 2011); 3) durum may contain genes that suppress expression of Sumai 3-derived FHB resistance (Rudd et al. 2001; Gilbert et al. 2002). It has been found that there are suppressor genes to tetraploid wheat-derived rust resistance on D-genome chromosomes in bread wheat. This has become an obstacle in the introgression of rust resistance genes from durum wheat to bread wheat (Kerber and Green 1980; Bai and Kbitt 1992). Therefore, dissection of the genetic factors influencing expression of FHB resistance genes in durum wheat may facilitate utilization of hexaploid wheat-derived resistance genes in durum breeding. The objective of this study is to investigate the effects of durum background on the expression and inheritance of FHB resistance genes from Sumai 3 and PI 277012.

Materials and Methods

Wheat Germplasm and Crosses

Four durum wheat cultivars ‘Langdon’(LDN), ‘Grenora’, ‘Alkabo’, ‘Divide’ were used as female parents to cross with Sumai 3 and PI 277012, two highly FHB-resistant common wheat accessions. The F_1 hybrids along with their parents were evaluated for FHB severity in four

greenhouse seasons (Fall 2011, Fall 2012, Spring 2013, and Spring 2014). Four highly FHB-susceptible hexaploid wheat accessions ('2398', 'Choteau', 'AC Vista', and 'AC Lillian') were used as female parents to develop F_1 with Sumai 3, and these F_1 were evaluated for FHB resistance in two greenhouse seasons (Spring 2013 and Fall 2013).

A complete set of LDN-'Chinese Spring' (CS) D-genome substitution lines, developed by the USDA-ARS Cereal Crops Research Unit, Fargo, ND, were crossed with Sumai 3 and PI 277012. In each substitution line, a pair of homologous A- or B-genome chromosomes in LDN was substituted by a pair of its corresponding homoeologous D-genome chromosomes from CS. The F_1 of these crosses were evaluated for FHB resistance in either three (Spring 2013, Fall 2013 and Spring 2014) or two greenhouse seasons (Spring 2013 and Fall 2013).

F_1 hybrids in all crosses were confirmed by visual inspection of seed morphology and molecular marker analysis. Some of the F_1 were further verified by spike morphology comparison. True F_1 were employed for the evaluation of FHB resistance and advanced to the subsequent generations for inheritance studies.

Inheritance Analysis

FHB evaluation was performed from the F_2 to F_4 generations for the crosses of Sumai 3 with LDN and Divide, and PI 277012 with LDN. One spike of each plant was self-pollinated to derive the next generation. The advanced families were formed by the bulked seeds from resistant segregants in the previous generation.

Experimental Design and FHB Evaluation

All plants were grown in 6-inch plastic pots with one plant per pot for F_1, and two plants per pot for parental controls and LDN-CS D-genome substitution lines. Five to ten spikes in each pot were inoculated. The pots were randomly arranged on the greenhouse benches. Each pot was

regarded as one replicate. The number of replicates for each entry in each experiment ranged from two to five depending on the germination of the F_1. A completely randomized design (CRD) with unbalanced data was used for statistical analysis using SAS version 9.3 (SAS Institute 2011). The data obtained in different environments were pooled for combined analysis if the Bartlett's homogeneity test of error variance was not indicative of significant difference (P=0.05).

The greenhouse temperature was kept around 16 °C and 18 °C at night and daytime, respectively, with a 16 hr photoperiod in the greenhouse before anthesis. During the inoculation period, the temperature was increased to around 25 °C. The single-floret inoculation method was used to infect plants with the inoculum as described by Stack et al. (2002). An inoculum suspension was prepared from four species of *F. graminearum* with a concentration of 1×10^5 conidiospores per milliliter. For inoculation, 10 µl of inoculum was injected into a central floret of each spike during anthesis. The inoculated spikes were covered with plastic bags that were water-misted on the inside to maintain a relatively high humidity for disease development for 72 hr. At 21-day post inoculation, the percentage of diseased spikelets was recorded for each spike to evaluate Type II resistance, and the mean percentage of FHB severity over all spikes in each pot was calculated as the value of one replicate.

Results

Production of F_1 Hybrids

Initially, the morphology of F_1 hybrid seeds from all crosses was visually studied. True hybrid seeds derived from the crosses of 2398, Choteau, AC Vista, and AC Lillian with Sumai 3 and PI 277012 were significantly smaller than the normal self-pollinated seeds of their female parents (Table 4.1; Figure 4.1). The true F_1 seeds from the crosses of Sumai 3 and PI 277012 with durum wheat and LDN D-genome substitution lines were shriveled compared to the plump

seeds of their female durum parents (Figure 4.2). Molecular marker analysis is a more reliable method to verify F_1 hybrids (Ballester and de Vicente 1998). In this study, all the F_1 of Sumai 3 with durum wheat and LDN D-genome substitution lines were verified at the seedling stage with the molecular marker *UMN10*. The durum wheat and LDN D-genome substitution lines did not have any amplicons at the *UMN10* locus, and PCR amplification of true F_1 produced the same amplicon as Sumai 3 at the *UMN10* locus (Figure 4.3). In addition, spike morphology was used to verify F_1 derived from the crosses involving PI 277012. Spikes of true F_1 exhibited speltoid (spear-shaped) morphology, since PI 277012 has the *q* gene on chromosome 5A (Chu et al. 2011; Figure 4.4).

Figure 4.1. Seed morphology of Choteau (**a**) and its F_1 hybrid with Sumai 3 (**b**)

Table 4.1. Seed morphology of three hexaploid wheat and their F_1 hybrids with Sumai 3

Entry	Sample size	Means of seed width (mm)	Means of seed length (mm)
2398	39	3.04±0.27*	5.97±0.39
(2398×Sumai 3) F_1	50	2.76±0.28	4.34±0.41
Choteau	50	2.88±0.31	5.42±0.43
(Choteau×Sumai 3) F_1	50	2.39±0.22	4.14±0.35
AC Vista	27	2.8±0.31	6.5±0.52
(AC Vista×Sumai 3) F_1	34	2.43±0.26	4.8±0.40

*Mean± Standard deviation

Figure 4.2. Seed morphology of a) LDN, b) (LDN×Sumai 3) F_1, c) (LDN×PI 277012) F_1, d) Divide, e) (Divide×Sumai 3) F_1, and f) (Divide×PI 277012) F_1

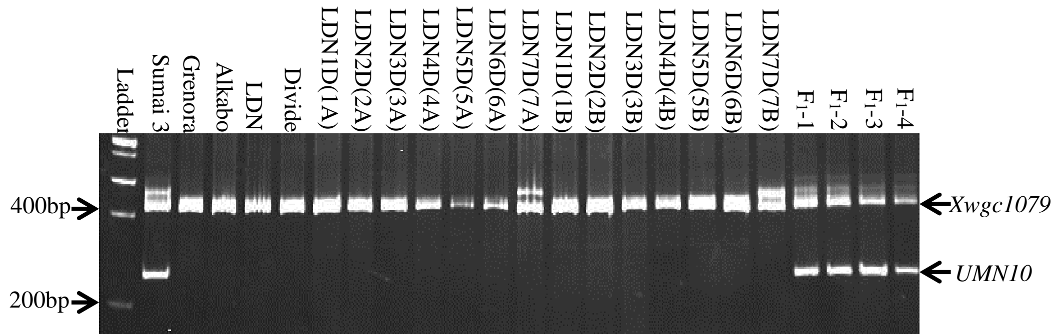

Figure 4.3. PCR amplification products (*UMN10* and *Xwgc1079* marker loci) produced in durum wheat, LDN-CS D-genome substitution lines, and the F_1 of LDN×Sumai 3 (F_1-1), Divide×Sumai 3 (F_1-2), LDN1D(1A)×Sumai 3 (F_1-3), and LDN2D(2A)×Sumai 3 (F_1-4)

Figure 4.4. Spike morphology of a) PI 277012, b) (Divide×PI 277012) F_1, c) Divide, d) (LDN×PI 277012) F_1, and e) LDN

FHB Resistance in the F_1 Hybrids of Sumai 3 with Durum and Hexaploid Wheat

All F_1 of Sumai 3 with durum wheat LDN, Divide, Grenora and Alkabo exhibited a resistance level similar to or lower than their durum parents in 1-3 greenhouse seasons (Table 4.2). The F_1 of Divide with Sumai 3 exhibited a FHB severity of 38.7% that was significantly higher than 28.0% for Divide (α=0.05). Statistical analysis indicated that the FHB severity of LDN, Grenora, and Alkabo were not significantly different from their respective F_1 with Sumai 3.

The F_1 of Sumai 3 with four highly FHB-susceptible hexaploid wheat accessions exhibited a resistance level intermediate to their parents (Table 4.3). FHB severity of the four hexaploids ranged from 73.6 % for AC Vista to 91.7% for 2398. All four F_1 exhibited around 40% FHB severity while the FHB severity for Sumai 3 was less than 20% in all greenhouse environments. A combined data analysis was not performed with the spring wheat line 2398 due to hoterogeneity of the error variance over the two greenhouse environments for this line. 2398 exhibited a high FHB severity (91.7%) in one greenhouse environment, which was consistent with the report by Mergoum et al. (2008). However, this line had a significantly lower FHB severity (40.2%) in another greenhouse environment.

Table 4.2. FHB severity of F_1 hybrids between Sumai 3 and durum wheat

Entry	FHB severity (%)	Evaluation environment
Sumai 3	18.4a*	Fall 2011; Spring 2013; Spring 2014
F_1	59.1b	
LDN	60.7b	
Sumai 3	17.1a	Fall 2011; Fall 2012
F_1	38.7c	
Divide	28.0b	
Sumai 3	11.7a	Fall 2011
F_1	31.4b	
Grenora	22.3ab	
Sumai 3	11.7a	Fall 2011
F_1	32.9b	
Alkabo	29.7b	

*Means followed by different letters in this column are significantly different at α=0.05 level

Table 4.3. FHB severity of F_1 hybrids between Sumai 3 and hexaploid wheat

Entry	FHB severity (%)	Evaluation environment
Sumai 3	10.3a*	Fall 2013
F_1	36.5b	
2398	91.7c	
Sumai 3	14.3a	Spring 2013; Fall 2013
F_1	39.9b	
Choteau	77.0c	
Sumai 3	14.3a	Spring 2013; Fall 2013
F_1	39.3b	
AC Lillian	74.3c	
Sumai 3	14.3a	Spring 2013; Fall 2013
F_1	38.1b	
AC Vista	73.6c	

*Means followed by different letters in this column are significantly different at α=0.05 level

Most of the LDN-CS D-genome substitution lines and their F_1 with Sumai 3 were evaluated for FHB resistance in 2-3 greenhouse seasons. Three of the substitution lines, including LDN2D(2A), LDN2D(2B), and LDN7D(7B), and their F_1 with Sumai 3 were

evaluated in only one greenhouse season due to seed shortage (Table 4.4). Substitutions of 2D for 2B, 3D for 3A and 3B, 4D for 4A and 4B, 5D for 5B, 6D for 6A and 6B, and 7D for 7A all augmented resistance in the F_1 of these substitution lines with Sumai 3. Statistical analysis indicated that FHB severity of all these F_1 was significantly lower than that of their corresponding LDN D-genome substitution lines. However, the F_1 of LDN1D(1A), LDN1D(1B), LDN2D(2A), LDN5D(5A), and LDN7D(7B) with Sumai 3 showed a resistance level similar to or higher than their corresponding substitution lines (Table 4.4).

FHB Resistance in the F_1 Hybrids of PI 277012 with Durum

The F_1 of PI 277012 with durum wheat exhibited a resistance level comparable to PI 277012 (Table 4.5). FHB severity of all these F_1 was significantly lower than their durum parents and not significantly different from PI 277011 (α=0.05), indicating complete dominance of the resistance genes in PI 277012 over the susceptible alleles in durum. The F_1 of PI 277012 with the LDN D-genome substitution lines LDN4D(4A), LDN6D(6A), and LDN6D(6B) had significantly higher FHB severity than LDN×PI 277012, whereas the F_1 of all other LDN D-genome substitution lines exhibited similar levels of resistance as LDN×PI 277012 (Tables 4.5 and 4.6).

Inheritance Analysis

A total of 57 F_2 plants from the cross of LDN with Sumai 3 were evaluated for FHB resistance. (1) The seed harvested from two F_2 plants with an FHB severity of 14.8% and 15.8%, respectively, were bulked to form an F_3 family consisting of 36 plants (Table 4.7). (2) Seed set on two F_3 plants with an FHB severity of 13.8% and 17.9%, respectively, were bulked to form an F_4 family consisting of 38 plants. Similarly, an F_2 population of 37 plants from the Divide×Sumai 3 cross were evaluated for FHB resistance. (1) An F_3 family consisting of 52 plants was derived from four F_2 plants with FHB severity ranging from 7.4% to 16.3%.

Table 4.4. FHB severity of F_1 hybrids between Sumai 3 and LDN-CS D-genome substitution lines

Entry	FHB severity (%)	Evaluation environment	Entry	FHB severity (%)	Evaluation environment
Sumai 3	15.4a*	Spring 2013; Fall 2013; Spring 2014	Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014
F_1	42.0b		F_1	42.6b	
LDN1D(1A)	40.1b		LDN4D(4B)	55.5c	
LDN	58.6c		LDN	58.6c	
Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014	Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014
F_1	55.4b		F_1	52.8c	
LDN1D(1B)	52.9b		LDN5D(5A)	30.2b	
LDN	58.6b		LDN	58.6c	
Sumai 3	21.8a	Spring 2014	Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014
F_1	87.6c		F_1	38.0b	
LDN2D(2A)	91.2c		LDN5D(5B)	67.7c	
LDN	67.2b		LDN	58.6c	
Sumai 3	19.3a	Spring 2013	Sumai 3	20.4a	Spring 2013; Spring 2014
F_1	52.2b		F_1	65.9b	
LDN2D(2B)	85.0c		LDN6D(6A)	84.4c	
LDN	52.4b		LDN	56.9b	
Sumai 3	20.4a	Spring 2013; Spring 2014	Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014
F_1	57.6b		F_1	62.2b	
LDN3D(3A)	69.0c		LDN6D(6B)	82.4c	
LDN	56.9b		LDN	58.6b	
Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014	Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014
F_1	40.0b		F_1	41.2b	
LDN3D(3B)	55.3c		LDN7D(7A)	59.9c	
LDN	58.6c		LDN	58.6c	
Sumai 3	15.4a	Spring 2013; Fall 2013; Spring 2014	Sumai 3	21.8a	Spring 2014
F_1	45.7b		F_1	66.0b	
LDN4D(4A)	62.6c		LDN7D(7B)	60.0b	
LDN	58.6c		LDN	67.2b	

*Means followed by different letters in this column are significantly different at α=0.05 level

Table 4.5. FHB severity of F_1 hybrids between PI 277012 and durum wheat

Entry	FHB severity (%)	Evaluation environment
PI 277012	12.2a*	
F_1	15.1a	Fall 2011; Spring 2013
LDN	57.9b	
PI 277012	12.2a	
F_1	15.3a	Fall 2011; Spring 2013
Divide	41.0b	
PI 277012	9.4a	
F_1	12.2a	Fall 2011
Grenora	22.3b	
PI 277012	9.4a	
F_1	14.4a	Fall 2011
Alkabo	29.7b	

*Means followed by different letters in this column are significantly different at α=0.05 level

(2) Twenty-two seeds from one F_3 plant with an FHB severity of 12.9% were used to establish an F_4 family of the Divide×Sumai 3 cross. Forty-one F_2 plants form the cross of LDN with PI 277012 were employed for FHB evaluation. (1) Seeds set on two F_2 plants that had FHB severity of 6.8% and 8.4%, respectively, were bulked to form an F_3 family of 59 plants. (2) Seeds from two plants with an FHB severity of 12.9% and 15.0% in F_3 family were combined to form an F_4 family of the LDN×PI 277012 cross.

A wide variation in FHB severity was observed in the F_2 population of the LDN×Sumai 3 cross. About 53% of the individual plants exhibited FHB severity less than 30% and 3.5% of the individuals were identified with FHB severity lower than 10% (Figure 4.5). All other segregants had an FHB severity ranging from 30% to 90%. However, only 22.2% and 2.6% of plants in the F_3 and F_4 families, respectively, were identified with an FHB severity of less than 30% in the cross of LDN×Sumai 3 (Figure 4.5). Individuals with an FHB severity of less than 20% were not observed in the F_4 family. Over 50% and 80% individuals in the F_3 and F_4 families, respectively,

Table 4.6. FHB severity of F_1 hybrids between PI 277012 and LDN-CS D-genome substitution lines

Entry	FHB severity (%)	Evaluation environment	Entry	FHB severity (%)	Evaluation environment
PI 277012	11.1a*	Spring 2013; Fall 2013	PI 277012	11.1a	Spring 2013; Fall 2013
F_1	15.9a		F_1	15.1a	
LDN1D(1A)	45.1b		LDN4D(4B)	62.8b	
LDN	56.4c		LDN	56.4b	
PI 277012	11.1a	Spring 2013; Fall 2013	PI 277012	12.2a	Spring 2013
F_1	15.5a		F_1	23.2a	
LDN1D(1B)	57.7b		LDN5D(5A)	21.4a	
LDN	56.4b		LDN	52.4b	
PI 277012	12.2a	Spring 2013	PI 277012	11.1a	Spring 2013; Fall 2013
F_1	16.9a		F_1	17.1a	
LDN2D(2A)	53.9b		LDN5D(5B)	62.7b	
LDN	52.4b		LDN	56.4b	
PI 277012	12.2a	Spring 2013	PI 277012	11.1a	Spring 2013; Fall 2013
F_1	22.0a		F_1	19.9b	
LDN2D(2B)	85.0c		LDN6D(6A)	96.0d	
LDN	52.4b		LDN	56.4c	
PI 277012	12.2a	Spring 2013	PI 277012	11.1a	Spring 2013; Fall 2013
F_1	16.7a		F_1	24.8b	
LDN3D(3A)	69.3c		LDN6D(6B)	78.9d	
LDN	52.4b		LDN	56.4c	
PI 277012	11.1a	Spring 2013; Fall 2013	PI 277012	10.4a	Fall 2013
F_1	15.4a		F_1	10.9a	
LDN3D(3B)	54.6b		LDN7D(7A)	50.4b	
LDN	56.4b		LDN	62.3c	
PI 277012	12.2a	Spring 2013	PI 277012		Missing
F_1	27.7b		F_1		
LDN4D(4A)	68.8d		LDN7D(7B)		
LDN	52.4c		LDN		

*Means followed by different letters in this column are significantly different at α=0.05 level

Table 4.7. Number of plants evaluated in F_2 populations, and F_3 and F_4 families of three crosses and selected plants for seed combination in the next generation

Population/Family	No. plants	No. selected plants (FHB severity %)
(LDN×Sumai 3) F_2	57	2 (14.8% and 15.8%)
(LDN×Sumai 3) F_3	36	2 (13.8% and 17.9%)
(LDN×Sumai 3) F_4	38	
(Divide×Sumai 3) F_2	37	4 (7.4%, 8.2%, 14.5%, and 16.3%)
(Divide×Sumai 3) F_3	52	1 (12.9%)
(Divide×Sumai 3) F_4	22	
(LDN×PI 277012) F_2	41	2 (6.8% and 8.4%)
(LDN×PI 277012) F_3	59	2 (12.9% and 15.0%)
(LDN×PI 277012) F_4	60	

were identified with an FHB severity higher than 50% (Figure 4.5). It was apparent that the F_3 and F_4 families segregated toward higher susceptibility compared with the F_2 population.

Similar segregation patterns to the LDN×Sumai 3 cross were observed in the F_2, F_3, and F_4 generations of the cross of Divide×Sumai 3. A little over 25% of the individuals were observed with an FHB severity of less than 20% in the F_2 population. However, over 80% of the individuals exhibited an FHB severity higher than 50% in the F_3 and F_4 families (Figure 4.6).

The cross of LDN×PI 277012 showed a segregation pattern of FHB severity similar to the LDN/Divide×Sumai 3 crosses in the F_2 generation with about 40% individuals having an FHB severity less than 20%. However, FHB severity in the F_3 and F_4 of LDN x PI 277012 segregated differently from the LDN/Divide×Sumai 3 crosses. The F_3 and F_4 families of LDN×PI 277012 maintained a high frequency (~55% in F_3 and ~85% in F_4) of individuals with an FHB severity less than 30% (Figure 4.7).

Discussion

Utilization of hexaploid-derived FHB resistance in durum wheat breeding has achieved limited success. It has been proposed that expression of hexaploid-derived FHB resistance genes

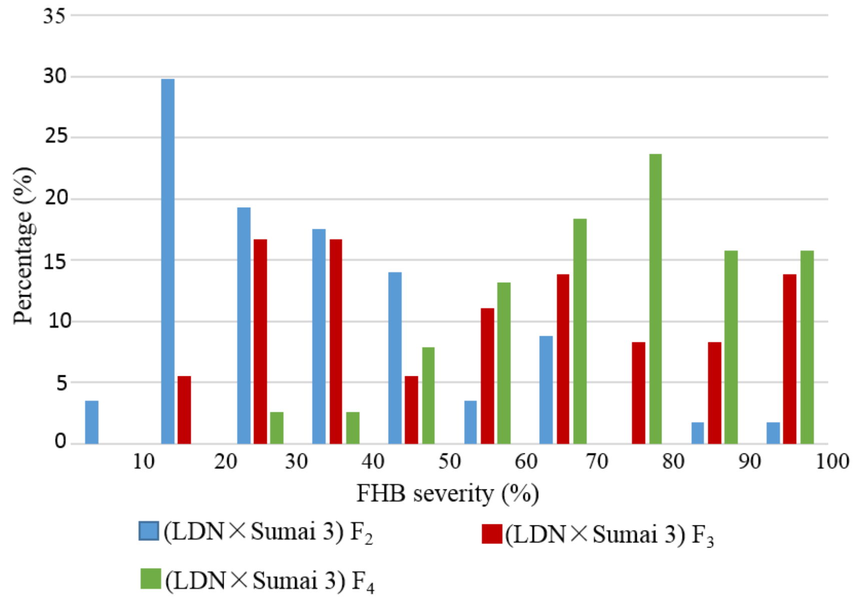

Figure 4.5. FHB severity in the F_2, F_3, and F_4 of the LDN×Sumai 3 cross

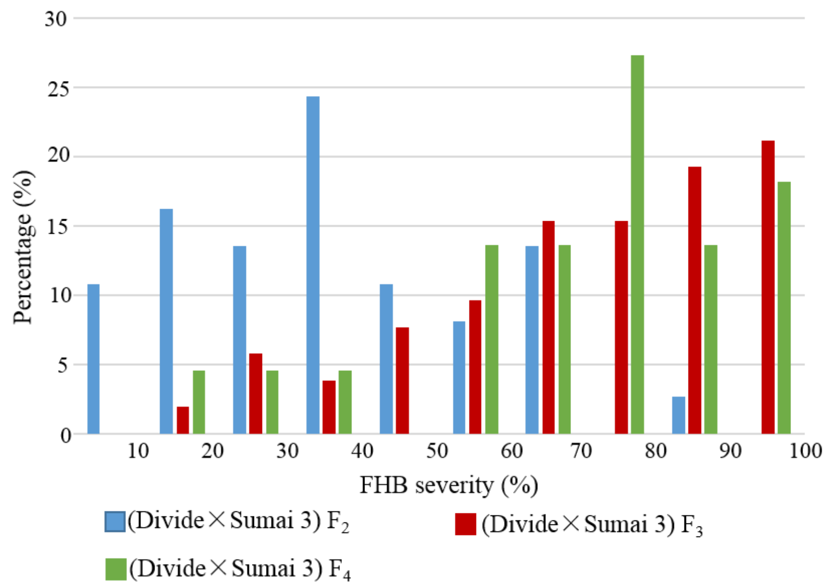

Figure 4.6. FHB severity in F_2, F_3, and F_4 of the Divide×Sumai 3 cross

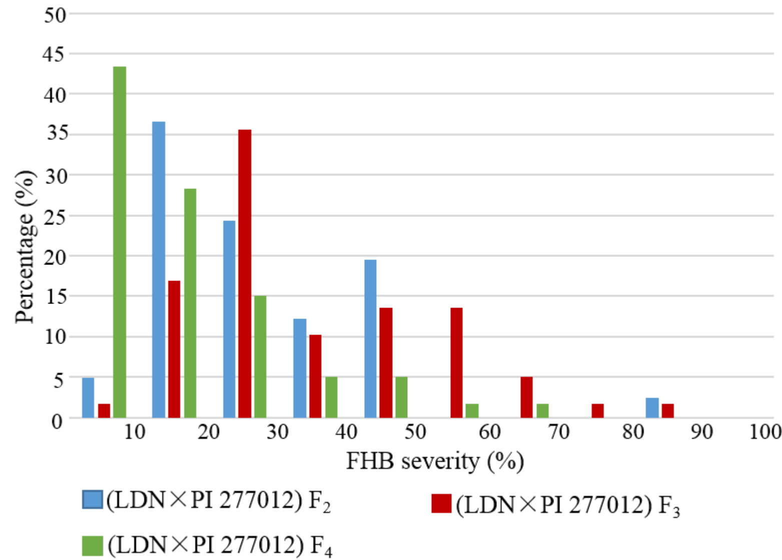

Figure 4.7. FHB severity in the F_2, F_3, and F_4 of the LDN×PI 277012 cross

may be influenced by genetic factors present in durum (Rudd et al. 2001; Fakhfakh et al. 2011). In this study, all F_1 of Sumai 3 with FHB susceptible hexaploid wheat exhibited a level of FHB resistance intermediate to the two parents. However, the F_1 of Sumai 3 with durum exhibited levels of FHB severity similar to or higher than durum. Similar results were also reported in the F_1 hybrids of other tetraploid wheat with FHB-resistant hexaploids including Sumai 3 (Gilbert et al. 2002). Obviously, Sumai 3-derived FHB resistance genes were normally expressed in the F_1 with hexaploids, but not in the F_1 with durum. The intermediate FHB resistance level in the F_1 of Sumai 3 with hexaploid wheat is consistent with polygenic inheritance in the hexaploid background. It was proposed that non-expression of Sumai 3-derived resistance genes in tetraploid wheat was caused by suppressor genes in the tetraploid wheat background (Gilbert et al. 2002). However, PI 277012-derived FHB resistance genes are normally expressed in the F_1 of durum wheat with PI 277012 (Table 4.5). Apparently, FHB resistance genes in PI 277012 are completely dominant over the susceptible alleles in durum wheat.

The F_1 of Sumai 3 with LDN2D(2B), LDN3D(3A), LDN4D(4A), LDN4D(4B), LDN5D(5B), LDN6D(6A), LDN6D(6B) and LDN7D(7A) had significantly lower FHB severity than their substitution line parents, suggesting that LDN chromosomes 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, and 7A may contain genes that suppress expression of the Sumai 3-derived FHB resistance genes. No significant increase of FHB resistance was observed in the F_1 of LDN1D(1A), LDN1D(1B), LDN2D(2A), LDN5D(5A), and LDN7D(7B) with Sumai 3, indicating that LDN chromosomes 1A, 1B, 2A, 5A, and 7B may not influence expression of Sumai 3-derived FHB resistance genes. The F_1 of LDN4D(4A), LDN6D(6A) and LDN6D(6B) with PI 277012 exhibited a resistance level lower than PI 277012, whereas the F_1 of PI 277012 with other substitution lines had a similar resistance level as PI 277012. These results suggested that LDN chromosomes 4A, 6A, and 6B likely contain genes required for the expression of the PI 277012-derived FHB resistance. Also, these expression results demonstrated that there might be different mechanisms underlying the Sumai 3- and PI 277012-derived FHB resistance genes. Zhuang et al. (2012) suggested that FHB resistance in Sumai 3 could be conferred by reducing the susceptibility rather than producing an active resistance reaction.

Wide variation of FHB severity was observed in the F_2 populations, F_3 and F_4 families of the crosses of Sumai 3 with LDN and Divide. Also, the frequencies of individuals with high levels of FHB resistance decreased from the F_2 to the F_4 generation. However, a high frequency of plants with high levels of FHB resistance was retained over the F_2 to F_4 generations in the cross of PI 277012 with LDN. The difference in inheritance pattern of Sumai 3- and PI 277012-derived FHB resistance could be caused by several factors. Firstly, FHB resistances QTL have been identified on several chromosomes including 7A, 2B, 3B and 6B in Sumai 3 (Yao et al. 1997; Waldron et al. 1999; Zhou et al. 2002; Liu and Anderson 2003). However, two FHB

resistance QTL were mapped on the same chromosome 5A in PI 277012 (Chu et al. 2011). Thus, Sumai 3-derived FHB resistance QTL segregate more frequently than those in PI 277012. Secondly, it was identified that introgression of a single FHB resistance gene from Sumai 3 could not provide a FHB resistance level comparable to Sumai 3 (Mergoum et al. 2006, 2008; http://www.extension.umn.edu/agriculture/small-grains/cultivar-selection-and-genetics/docs/freyr-wheat.pdf). However, one of the two QTL in PI 277012 could provide a level of FHB resistance comparable to PI 277012 in other FHB susceptible wheat genetic backgrounds (http://www.uky.edu/Ag/Wheat/wheat_breeding/New%20Folder/Steve%20Xu.pdf). Thirdly, the present study found that expression of Sumai 3-derived resistance genes was possibly suppressed by multiple genes on different durum chromosomes. However, PI 277012-derived FHB resistance can be completely expressed and the expression is possibly influenced by few genes in durum.

In conclusion, the expression of hexaploid-derived FHB resistance genes was influenced by multiple genes on different chromosomes in the durum genetic background, which make it difficult to utilize FHB resistance genes in durum improvement. The expression difference of Sumai 3- and PI 277012-derived FHB resistance illustrated that proper selection of the hexaploid FHB resistance donor source could provide an opportunity to improve FHB resistance in durum.

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CHAPTER 5. INFLUENCE OF D-GENOME CHROMOSOMES ON FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

Abstract

Durum wheat (*Triticum durum*, 2n=4x=28, genome AABB) lacks the D sub-genome that common wheat (*T. aestivum*, 2n=6x=42, genome AABBDD) has. It has been anticipated that D genome may play a role in conditioning FHB resistance in wheat. The objective of this study was to elucidate the effect that the absence/presence of D-genome chromosomes might have on the expression of FHB resistance in durum backgrounds. Evaluation of a complete set of 'Langdon' (LDN) durum-'Chinese Spring' (CS) D-genome disomic substitution lines (DSLs) and a series of LDN-*Ae. tauschii* D-genome addition lines for FHB resistance indicated that LDN chromosome 5A and CS chromosome 6D possibly contain genes for FHB susceptibility and/or suppression of FHB resistance. LDN chromosome 2B and *Ae. tauschii* 5D could possibly carry genes for FHB resistance. Chromosome 1D may harbor a genetic factor(s) augmenting the 5D-derived FHB resistance. The high FHB severity of synthetic wheat illustrated that expression of D genome genes for FHB resistance could be suppressed by the presence of other D genome chromosomes in the durum backgrounds. It was also revealed that chromosome 2D could carry a gene(s) for FHB susceptibility and that LDN chromosome 6A and 6B may carry genes for FHB resistance.

Introduction

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe [teleomorph *Gibbberella zeae* (Schwein) Petch], has been a serious disease in wheat and barley worldwide. It is a severe threat to wheat production, especially in durum wheat due to the lack of effective FHB resistance in durum cultivars (McMullen et al 1997; Stack et al. 2002). In hexaploid wheat, multiple sources of FHB resistance, such as 'Sumai 3', 'Wangshuibai', and PI

277012, have been identified and successfully used in variety development (He et al. 2001; Chu et al. 2011). It has been observed that some Sumai 3-derived hexaploid wheat cultivars exhibited good levels of FHB resistance (He et al. 2001; Buerstmayr et al. 2009). To date, a durum accession with a high level of FHB resistance comparable to Sumai 3 has not been found (Oliver et al.2007, 2008), but a FHB resistance genes have been identified in wild emmer wheat (*T. dicoccoides*) (Otto et al. 2002; Kumar et al. 2007; Oliver et al. 2007). A major FHB resistance QTL, *Qfhs-ndsu-3AS*, identified from the wild emmer wheat accession Israel-A has been further characterized and used in durum breeding (Otto et al. 2002; Stack et al. 2002; Chen et al. 2007). Lack of effective resistance to FHB in durum wheat has prompted durum geneticists and breeders to utilize hexaploid wheat-derived resistance sources in durum breeding. However, little progress has been made with the introgression of FHB resistance from hexaploids to durum (Rudd et al. 2001; Oliver et al. 2008).

It has been anticipated that either the hexaploid resistance source, such as Sumai 3, or durum may contain genetic factors that account for the difficulties of deploying hexaploid-derived resistance genes in durum wheat (Rudd et al. 2001). The lack of a D genome in durum has been considered a major factor that limits the success of FHB resistance gene introgression from hexaploids to durum. Gilbert et al. (2000) studied the influence of the D genome from three FHB-resistant hexaploid wheat genotypes, Sumai 3, Ning8331, and 93FHB21 on FHB resistance in crosses of the hexaploids with tetraploid wheat. They observed variation of FHB severity in the pentaploid-derived F_2 plants harboring diverse combinations of D-genome chromosomes. However, a clear relationship could not be revealed between D-genome chromosomes and FHB resistance in that study.

Wheat has a variety of aneuploid stocks with various chromosome constitutions, such as amphiploids, chromosome addition, substitution, and translocation lines (Sears 1969). These aneuploids are very useful in the characterization and identification of genomes, individual chromosomes, and genes in wheat and its relatives. Both alien chromosome addition and substitution lines dissect an alien genome into individual chromosomes in the wheat genetic background. They have been commonly used to investigate the effect of specific alien chromosomes on various agronomic traits in wheat (Bai and Kbitt 1992; Watanabe et al. 1994; Law and Worland 1996; Stack et al. 2002; Zhou et al. 2002a; Faris et al. 2008; Chumanovaa et al. 2014; Du et al. 2014). For instance, 'Langdon' (LDN)-'Chinese Spring' (CS) D-genome substitution lines (LDN-CS DSLs) where one pair of LDN A- or B-genome chromosomes were substituted by one pair of homoeologous D-genome chromosomes have played an important role in the characterization of durum A and B genomes (Watanabe et al. 1994; Joppa LR and Williams 1988; Li et al. 2006; Klindworth and Xu 2008; http://wheat.pw.usda.gov/ggpages/GeneticStocks/Fargo_ARS_genetic_stocks.html). The advantage of LDN-CS DSLs lies in their isogenic background of LDN except of the substituted chromosomes. Wantanabe et al. (1994) investigated photosynthesis rates of LDN-CS DSLs and concluded that chromosome 1B and 3B contained the genes functionally decreasing photosynthesis. That conclusion was made base on the differences of photosynthesis rate between LDN1D(1A) and LDN1D(1B), and between LDN3D(3A) and LDN3D(3B), and their differences from LDN. In another study involving LDN-CS DSLs, Bai and Kbitt (1992) observed that LDN chromosomes 1B, 2B, and 7B carried the genes conferring stem rust resistance and CS chromosome 1D and 3D harbored suppressor genes for leaf rust resistance in LDN. Several other tetraploid substitution lines were developed and employed in durum genetic

studies in addition to LDN-CS DSLs. Stack et al. (2002) evaluated a complete set of LDN durum-*T. dicoccoides* chromosome disomic substitution lines [LDN (DIC)] where a pair of chromosomes from *T. dicoccoides* accession Israel-A substituted a pair of the corresponding homologous chromosomes in LDN for FHB resistance. Chromosome 3A of Israel-A was found to contain FHB resistance genes that are normally expressed in LDN background, but not in Israel-A. Based on this result, the FHB resistance QTL, *Qfhs-ndsu.3AS*, was identified and mapped to the short arm of chromosome 3A (Otto et al. 2002; Chen et al. 2007). Zhou et al. (2002a) evaluated a set of CS-Sumai 3 substitution lines and identified several chromosomes of Sumai 3 conferring accumulation or decomposition of mycotoxin deoxynivalenol (DON), a major metabolic product of *F. graminearum*, in CS background.

LDN-CS DSLs, LDN D-genome chromosome addition lines, and LDN durum-*Aegilops tauschii* (2n=2x=14, genome DD) amphiploids (i.e. synthetic hexaploid wheat) are ideal materials to characterize the effect of the D genome and individual D-genome chromosomes on various agronomic traits in LDN durum background. In this study, a complete set of LDN-CS DSLs were employed to investigate the effect of individual durum (A and B genome) and D-genome chromosomes on the expression of FHB resistance genes. Also, several durum-*Ae. tauschii* amphiploids (2n=6x=42, genome AABBDD) and a series of LDN-*Ae. tauschii* D-genome chromosome addition lines were developed and used to determine whether additions of the complete D genome and individual D-genome chromosomes to LDN background affect expression of FHB resistance genes.

Materials and Methods

LDN-CS DSLs, Durum, and *Ae. tauschii* Accessions

Fourteen LDN-CS DSLs were employed for FHB evaluation over seven greenhouse seasons [Fall 2011-Greenhouse1 (G1), Spring 2012-G1, Spring 2012-G2, Spring 2013-G1, Spring 2013 -G3, Fall 2013-G3, and Spring 2014-G3]. This set of substitution lines were developed through crossing the CS nullisomic-tetrasomic series to durum wheat LDN and backcrossing 12 times to LDN (http://wheat.pw.usda.gov/ggpages/GeneticStocks/Fargo_ARS_genetic_stocks.html). As a result, one pair of A or B genome chromosomes in LDN were substituted by one pair of homoeologous D genome chromosomes from CS. Due to the 12 cycles of backcrossing, each substitution line is isogenic with LDN except for the pair of substituted D genome chromosomes.

Three durum cultivars ('Lebsock', 'Divide', and LDN) with moderate susceptibility to FHB (Elias et al. 2001; Stack et al. 2002; Elias and Manthey 2007; Royo et al. 2009), an FHB-resistant Tunisian durum line Tunisian 7 (Tun 7) (Huhn 2008), and a FHB-resistant LDN-ISA substitution line 3A [LDN(3A)] where a pair of 3A chromosomes from *T. dicoccoides* accession Israel-A was substituted for the corresponding LDN durum chromosome (Joppa 1993) were included in this study. The *Ae. tauschii* accession RL5286 (2n=2x=14, DD) was used to develop synthetic hexaploid wheat in this study. CS nulli-tetrasomic lines where a pair of homologous chromosomes were substituted by a pair of homoeologous chromosomes (Sears 1966), and LDN-CS DSLs were used to identify D-genome chromosome-specific molecular markers.

Crosses, Chromosome Doubling, and Embryo Rescue

Crosses were made between tetraploid wheat accessions [Lebsock, Divide, LDN, Tun 7, and LDN (3A)] and RL5286 to develop triploid F_1 plants. The chromosomes of the triploid F_1

plants were doubled at the 3- to 4-tiller stage with colchicine. Plants were removed from the soil with roots rinsed with tap water, and put into a beaker containing colchicine solution with the knots of tillers submerged in the solution for 6-8 hr at room temperature in the dark. Mild aeration was applied to increase the oxygen concentration in the solution. The colchicine solution contained 0.5 g L^{-1} colchicine, 20 mg L^{-1} dimethyl sulfoxide (DMSO), 100mg L^{-1} gibberellic acid (GA_3) and 0.3mL L^{-1} Tween 80. Subsequently, the plants were rinsed under running tap water for 3 hours and kept in water overnight. Then, the plants were transplanted into soil and kept in a growth chamber at 16 ^{o}C with 16-hour photoperiod. After that, the plants were moved into the greenhouse for crosses and seed production.

Molecular Marker Analysis

PCR amplification was carried out in a 20-µl mixture containing 40 ng genomic DNA, 0.5 µM each of forward and reverse primers, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP and 0.25 U of Taq DNA polymerase. PCR was performed as follows: 94 ^{o}C for 3 min; 45 cycles of 94 ^{o}C for 1 min, 55 ^{o}C for 1 min and 72 ^{o}C for 1.5 min; with a final 72 ^{o}C for 7 min. PCR products were separated on 8% polyacrylamide gel and visualized by ethidium bromide staining (Chen et al. 2007).

FHB Evaluation

The FHB evaluation experiments were set up in a completely randomized design (CRD) with 3-5 replications. All materials were planted in 6-inch plastic pots with 1-2 plants in each pot in a greenhouse. Each pot of a line was considered a replicate. The pots were randomly arranged on the bench in the greenhouse where the temperature was set at 16 °C at night and 18 °C during daytime with a 16 hr photoperiod. The temperature was increased to 25 °C during inoculation. Four species of *F. graminearum* were used to prepare the inoculum suspension with a

concentration of 1×10^5 conidiospores per milliliter. The single-floret inoculation method described by Stack et al. (2002) was used for inoculation during anthesis. After inoculation, high humidity was kept for 72 hr by covering spikes with a plastic bag water-misted on the inside. FHB severity was evaluated at three weeks post-inoculation. The percentage of infected spikelets per spike was calculated as FHB severity. Statistical analysis was carried out with SAS version 9.3 (SAS Institute 2011). Fisher's protected least significant difference (LSD) was used to determine the mean separation at α=0.05 level. Bartlett's homogeneity test of error variance (P=0.05) was performed before doing a combined analysis of variance over greenhouse seasons.

Results

Production of Durum-*Ae. tauschii* Amphiploids and LDN D-Genome Addition Lines

Four durum wheat accessions (Lebsock, Tun 7, LDN, and Divide) and the LDN-ISA substitution line 3A [LDN(3A)], were crossed with *Ae. tauschii* accession RL5286 to develop durum-*Ae. tauschii* amphiploids (i.e. synthetic hexaploid wheat; 2n=6x=42, genome AABBDD). Five amphiploids involving these four durum accessions and substitution line and *Ae. tauschii* RL5286 were developed. The LDN-*Ae. tauschii* amphiploid was used as male parent to backcross to LDN. Consequently, a pentaploid (2n=5x=35, genome AABBD) in LDN background was produced. The pentaploid was self-pollinated to produce LDN D-genome addition lines with various D-genome chromosomes in each line (Figure 5.1).

Identification of D-Genome Chromosome-Specific Molecular Markers

Common wheat has three homoeologous sub-genomes A, B, and D. One molecular marker usually could detect three homoeoalleles from homoeologs in the three sub-genomes. A total of 93 simple sequence repeat (SSR) or sequence tagged site (STS) markers previously mapped on the D genome (~10-20 markers for each D-genome chromosome) were screened to identify D-

genome chromosome-specific markers (Roder et al. 1998; Pestsova et al. 2000; Guyomarch et al. 2002; Liu and Anderson 2003; Paillard et al. 2003; Sourdille et al. 2004; Somers et al. 2004; Song et al. 2005; Dobrovolskaya et al. 2011). Initial screening was carried out with CS and the seven CS nulli-tetrasomic lines each with a D-genome chromosome under nulli condition. For example, CS nulli1D-tetra1B (CSN1DT1B) contains four copies of chromosome 1B, but completely lacks chromosome 1D. The marker alleles amplified in CS, but absent in the specific D-genome nulli-tetrasomic lines were pre-selected as potentially D genome-specific. After that, the pre-selected D genome-specific markers were validated using CS, RL5286 (source of D genome for producing D-genome chromosome addition lines), durum parent LDN and other durum accessions (Divide, Tun 7, and Lebsock), CS D-genome nulli-tetra lines, and LDN-CS DSLs.

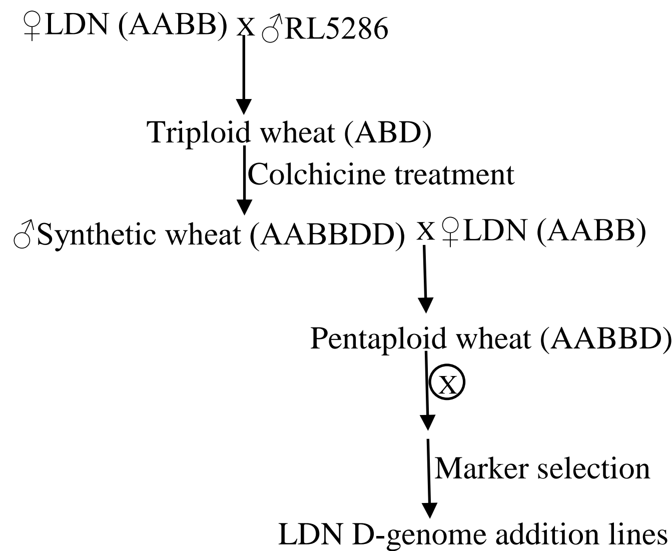

Figure 5.1. Scheme of development of LDN D-genome addition lines

One chromosome-specific SSR/STS marker was identified for each of the seven D-genome chromosomes (Figure 5.2; Table 5.1). Of the seven D-genome chromosome-specific

markers, *Xcfd76* for chromosome 6D amplified different sizes of PCR fragments for CS and RL5286, indicating a difference between the two alleles at this marker locus (Figure 5.2). All seven markers amplified D-genome chromosome-specific PCR bands in CS, RL5286, and LDN D-genome addition lines, but not in durum and the critical CS nulli-tetrasomic lines (i.e. the nulli-tetrasomics without the critical D-genome chromosome). For example, chromosome 1D-specific SSR marker *Xcfd63* amplified one clear PCR band from CS, as well as RL5286, LDN1D(1A), and LDN1D(1B) (Figure 5.2). However, that band was absent in CS nulli1D-tetra1B and durum wheat accessions, indicating *Xcfd63* is chromosome 1D-specific (Figure 5.2).

Table 5.1. Markers used to identify specific D genome chromosomes

Chromosome	Marker	Reference
1D	*Xcfd63*	Somers DJ et al. (2004); Paillard S et al. (2003)
2D	*Xgwm257*	Somers DJ et al. (2004); Paillard S et al. (2003); Roder MS et al. (1998)
3D	*STS3B-54*	Liu S et al.(2003)
4D	*Xgdm129*	Pestsova E et al. (2000)
5D	*Xgpw5238*	Dobrovolskaya O et al.(2011)
6D	*Xcfd76*	Guyomarch H et al. (2002)
7D	*Xgdm86*	Pestsova E et al. (2000)

Identification of LDN D-Genome Addition Lines (LDALs)

Wheat D genome-specific molecular markers were used to identify D-genome chromosomes in the progeny of the pentaploids (Figure 5.1). A total of 385 individuals from the progeny were genotyped with D genome-specific markers. Of the 385 individuals, 313 were found to contain D-genome chromosomes. Seventy-two individuals were identified to contain a single D-genome chromosome of 1D through 7D. Some of them contained the same D-genome chromosome, which were identified from the progeny of the pentaploid in the different greenhouse environments (Table 5.2). In addition, 54 addition lines were identified to contain double D-genome chromosomes in different combinations, designated LDN D-genome double

addition lines. Thus, a total of 126 LDN D-genome addition lines with 1-2 (non-homologous) D-genome chromosomes were identified with markers (Table 5.2).

FHB Severity of LDN-CS D-Genome Disomic Substitution Lines

LDN-CS D-genome disomic substitution lines (LDN-CS DSLs), where a pair of homologous A-genome or B-genome chromosomes were substituted by a pair of homoeologous D-genome chromosomes, dissect the CS D genome into individual chromosomes in LDN durum background. A complete set of DSLs (n=14) were employed to determine the effect of individual D-genome chromosomes on FHB resistance in LDN.

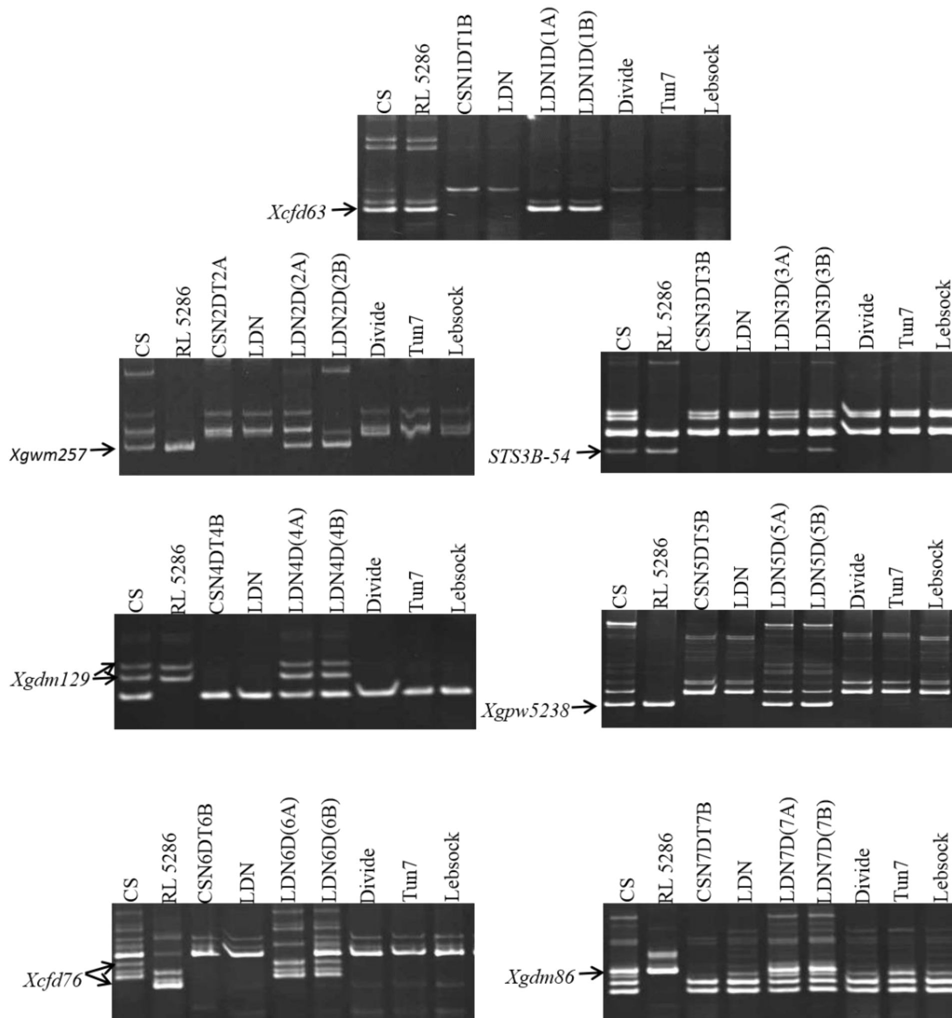

Figure 5.2. Gel images of D genome specific markers

Table 5.2. Number of plants with D-genome chromosomes identified with markers in three greenhouse environments

Seasons	No. plants	No. plants with D-genome chromosome(s)	No. plants with single D-genome chromosome	No. plants with double D-genome chromosomes
Fall 2013	162	110	32	24
Spring 2014	183	169	30	21
Summer 2014	40	34	10	9
Total	385	313	72	54

Fourteen LDN-CS DSLs were evaluated for Type II resistance at three greenhouse locations (G1, G2, G3) in five seasons (Fall 2011, Spring 2012, Spring 2013, Fall 2013, Spring 2014). Homogeneity test indicated there was no significant difference in the error variance among these seven greenhouse environments (P=0.2958>0.05), thus combined statistical analyses were performed. LDN exhibited a mean FHB severity of 55.9%. The mean FHB severity for each of the 14 DSLs over the seven greenhouse environments ranged from 29.3% for the DSL LDN5D(5A) to 80.4% for LDN6D(6A) (Table 5.3). FHB severity of LDN5D(5A) was significantly lower than LDN and other DSLs, including LDN5D(5B). On the other hand, LDN6D(6A), LDN6D(6B), and LDN2D(2B) exhibited significantly higher FHB severity than LDN. Also, FHB severity of LDN2D(2B) was significantly higher than LDN2D(2A) (Table 5.3).

FHB Severity of Durum-*Ae. tauschii* Amphiploids (Synthetic Hexaploid Wheat)

Five durum-*Ae. tauschii* amphiploids (i.e. synthetic hexaploid wheat) were evaluated at two greenhouse locations (G1 and G3) in three seasons (Fall 2012, Spring 2013, and Fall 2013). The test of homogeneity did not detect significant differences among the environments (P=0.0797>0.05). Therefore, combined statistical analyses were carried out with FHB data of the amphiploids and their durum parents. FHB severity of five amphiploids, ranging from 59.1% to 73.4%, was significantly higher than that of their corresponding durum parents (Table 5.4). Thus,

presence of the D genome from *Ae. tauschii* accession RL5286 in the durum background increased FHB susceptibility.

FHB Severity of LDN D-Genome Addition Lines (LDALs)

A total of 126 LDALs with 1-2 diverse D-genome chromosomes were employed for FHB evaluation in three greenhouse seasons (Fall 2013, Spring 2014, and Summer 2014). However, FHB evaluation data were obtained only from 98 plants with the missing data being due to 28 weak plants. There was less disease pressure in the greenhouse season of Spring 2014 than in the Fall 2013 and Summer 2014 (Table 5.5). The single D-genome chromosome addition lines LDAL-1D and LDAL-5D consistently exhibited lower FHB severity than LDN and other single chromosome LDALs consistently in the three greenhouse seasons. In addition, the double chromosome addition lines LDAL13F-1D+5D and LDAL14S-1D+5D that contained chromosome 1D and 5D had the lowest FHB severity among LDN and other LDALs in the greenhouse seasons of Fall 2013 and Spring 2014 (Table 5.5).

Apparently, both concurrent and separate addition of chromosome 1D and 5D to LDN enhanced FHB resistance. Also, some of the other double addition lines involving chromosome 1D or 5D, such as LDAL13F-1D+2D and LDAL13F-1D+7D, exhibited lower FHB severity than LDN, but this was not the case for other double addition lines involving chromosome 1D or 5D. Single addition of chromosomes 2D, 4D, 7D to LDN consistently increased FHB severity in all three greenhouse seasons. Chromosomes 3D and 6D showed an inconsistent effect on FHB resistance in LDN background in the three greenhouse seasons. It seemed that most of the double addition lines with other combinations of D-genome chromosomes exhibited a lower FHB severity than LDN even though a clear pattern for those double addition lines on FHB resistance could not be revealed (Table 5.5)

Table 5.3. Mean FHB severity of LDN and LDN-CS D-genome substitution lines in seven greenhouse environments

Line	FHB severity (%)
LDN5D(5A)	29.3a*
LDN1D(1A)	46.6b
LDN4D(4B)	50.9bc
LDN1D(1B)	54.0bcd
LDN	55.9bcd
LDN7D(7B)	57.0bcd
LDN7D(7A)	58.5cd
LDN3D(3B)	59.3cd
LDN4D(4A)	61.0cd
LDN3D(3A)	61.5cd
LDN5D(5B)	61.7d
LDN2D(2A)	64.8de
LDN6D(6B)	73.8ef
LDN2D(2B)	78.4f
LDN6D(6A)	80.4f

*Means in this column followed by different letters are significantly different at α=0.05 level

Table 5.4. Mean FHB severity of five durum-*Ae. tauschii* amphiploids with their durum parents in five environments

Entry	FHB severity (%)	Pedigree
LDN(3A)	31.7a*	
Tun7	36.5ab	
Lebsock	42.4bc	
Divide	42.6bc	
LDN	49.4c	
Syn3	59.1d	Lebsock/*Ae. tauschii* RL5286
Syn4	63.0de	Tun 7/ *Ae. tauschii* RL5286
Syn5	64.4def	LDN(3A)/ *Ae. tauschii* RL5286
Syn1	72.8ef	LDN/ *Ae. tauschii* RL5286
Syn2	73.4f	Divide/ *Ae. tauschii* RL5286

*Means in this column followed by different letters are significantly different at α=0.05 level

Table 5.5. FHB severity of LDN D-genome addition lines

Fall 2013-G3			Spring 2014-G3			Summer 2014-G3		
LDALs	No. plants	Mean FHB severity (%)	LDALs	No. plants	Mean FHB severity (%)	LDALs	No. plants	Mean FHB severity (%)
LDAL13F-1D*	6	47.1	LDAL14S-2D	12	34.3	LDAL14Su-1D	1	42.1
LDAL13F-2D	4	74.6	LDAL14S-5D	2	23.0	LDAL14Su-2D	1	84.8
LDAL13F-3D	3	55.0	LDAL14S-6D	3	50.5	LDAL14Su-3D	4	70.2
LDAL13F-4D	6	74.5	LDAL14S-7D	2	76.3	LDAL14Su-4D	1	76.9
LDAL13F-6D	2	62.7	LDAL14S-1D+5D	1	12.1	LDAL14Su-5D	1	46.4
LDAL13F-7D	5	74.6	LDAL14S-2D+3D	2	24.4	LDAL14Su-7D	2	83.1
LDAL13F-1D+2D	1	33.2	LDAL14S-2D+4D	2	71.6	LDAL14Su-2D+3D	1	84.3
LDAL13F-1D+4D	2	69.5	LDAL14S-2D+5D	2	27.3	LDAL14Su-2D+4D	1	73.3
LDAL13F-1D+5D	1	24.9	LDAL14S-2D+6D	4	59.8	LDAL14Su-3D+4D	1	70.5
LDAL13F-1D+7D	1	53.9	LDAL14S-2D+7D	2	57.0	LDAL14Su-3D+5D	2	70.7
LDAL13F-2D+3D	2	85.9	LDAL14S-3D+5D	1	24.7	LDAL14Su-5D+6D	1	80.3
LDAL13F-2D+4D	2	85.5	LDAL14S-3D+6D	1	51.4	LDAL14Su-5D+7D	1	98.4
LDAL13F-2D+5D	2	76.2	LDAL14S-4D+6D	1	65.2	LDAL14Su-6D+7D	1	83.7
LDAL13F-2D+6D	1	61.8	LDAL14S-all D	3	89.8	LDAL14Su-all D	1	83.9
LDAL13F-2D+7D	2	49.0	LDN	6	28.5	LDN	6	66.2
LDAL13F-3D+7D	1	100.0						
LDAL13F-4D+5D	2	86.7						
LDAL13F-5D+6D	1	86.1						
LDAL13F-5D+7D	1	34.2						
LDAL13F-all D[Φ]	4	74.5						
LDN	5	70.2						

*1D chromosome was identified in this LDAL

[Φ] All seven D-genome chromosomes were identified in this LDAL

Discussion

Few studies have reported on the effect of D-genome chromosomes on FHB resistance in durum (Gilbert et al. 2000). Characterization of such effect will lead to a better understanding of the expression of hexaploid wheat-derived FHB resistance genes and facilitate utilization of such resistance source in durum wheat. In the current study, substitutions of CS chromosome 5D for LDN chromosome 5A and 5B exhibited different levels of FHB resistance. FHB severity of LDN 5D(5A) and LDN5D(5B) is significantly lower than and similar to LDN, respectively. These results indicated that the augmented resistance level in LDN5D(5A) could be caused by the absence of 5A instead of the presence of 5D. Due to the loss of chromosome 5A, LDN5D(5A) exhibited a lax spike type, however, the spike morphology of LDN5D(5B) was similar to LDN due to the good compensation of 5D for the loss of 5B (Joppa and Williams 1988). The increased rachis length of LDN5D(5A) possibly increased the spread time of the fungus from the infected spikelet to other spikelets along the rachis, which resulted in a lower FHB severity. Thus, the gene(s) on 5A might be associated with FHB susceptibility in LDN. The LDN-*Ae. tauschii* 5D-addition line also exhibited lower FHB severity, which was apparently caused by the expression of genes on chromosome 5D. Thereby, chromosome 5D from *Ae. tauschii* RL5286 likely harbored FHB resistance gene(s) that were expressed in the LDN background. It seemed that chromosomes 5D from CS and *Ae. tauschii* RL5286 contained different alleles at the FHB resistance gene loci. The genomic difference of chromosome 6D from CS and *Ae. tauschii* RL5286 was observed at the marker *Xcfd76* locus (Figure 5.2). The role of chromosome 5D from CS in LDN needs to be evaluated further due to the smaller number of LDAL-5D plants evaluated. Chromosome 1D from both CS and *Ae. tauschii* RL5286 conferred positive effects on FHB resistance in LDN. The concurrent addition of 1D and 5D in LDALs resulted in the lowest

FHB severity and suggested that gene(s) on chromosome 1D might complement the *Ae. tauschii* 5D-derived FHB resistance in LDN background. Susceptibility of synthetic wheat to FHB also illustrated that expression of 5D-derived FHB resistance could be suppressed by other D-genome chromosomes.

Susceptibility to FHB conferred by D-genome chromosomes in LDN background was observed in some LDN-CS DSLs. Both substitutions of 6D for 6A and 6B exhibited a FHB resistance level significantly lower than LDN, suggesting that chromosome 6D possibly harbors a gene(s) for FHB susceptibility and/or suppression of FHB resistance in LDN or that 6A and 6B contain a gene(s) for FHB resistance in LDN. LDN2D(2B) exhibited significantly higher FHB severity than LDN, whereas LDN2D(2A) had FHB severity similar to LDN. This indicated that chromosome 2B might contain genes for FHB resistance in LDN. High FHB susceptibility contributed by chromosome 2D was observed in the LDN D-genome addition lines. Although FHB severity of LDN2D(2A) was not significantly higher than that of LDN, it was the fourth hightest value among the 14 LDN-CS DSLs (after LDN6D(6A), LDN6D(6B), and LDN2D(2B)). Thus, chromosome 2D might also harbor a gene(s) for FHB susceptibility.

In summary, LDN durum chromosomes 2B, 5A, 6A and 6B, and D-genome chromosomes 1D, 2D, 5D, and 6D possibly contain genes associated with FHB resistance in the LDN background. In previous studies, QTL associated with FHB resistance on these chromosomes were mainly identified in hexaploid wheat accessions, and few in tetraploid wheat. FHB resistance QTL on chromosome 2B have been widely identified in wheat accessions from Asia, North America, and Europe (Zhou et al. 2002b; Gervais et al. 2003; Paillard et al. 2004; Schmolke et al. 2005; Somers et al. 2006; Liu et al. 2007). However, FHB resistance QTL on chromosomes 5D and 6A were reported only in a few studies (Anderson et al. 2001; Schmolke et

al. 2005; Yang et al. 2005). Most of the FHB resistance QTL identified on chromosome 6B originated from hexaploid wheat accessions from China (Waldron et al. 1999; Shen et al.2003; Lin et al. 2004). Basent et al. (2012) concluded that chromosome 2D in Sumai 3 harbors a QTL for FHB susceptibility. Susceptibility genes on chromosome 5A and 6D have not been reported. In conclusion, FHB severity of LDN could be influenced positively or negatively by multiple chromosomes, and proper manipulation of these chromosomes is needed to improve FHB resistance in durum wheat.

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CHAPTER 6. GENERAL CONCLUSIONS

To date, durum wheat with acceptable FHB resistance has not been identified. FHB resistance QTL *Qfhs.ndsu-3AS* identified from *Triticum dicoccoides* has been regarded as a promising source to improve FHB resistance in durum wheat. In this study, complex micro-collinearity was observed among wheat chromosome 3A, rice chromosome 1, and *Brachypodium* chromosome 2 within the QTL *Qfhs.ndsu-3AS* region and the information was utilized to develop a new set of molecular markers. Employing these markers, the chromosomal interval spanning *Qfhs.ndsu-3AS* could be narrowed down from 11.5 cM in a previous study to 7.4 cM in the current study. Moreover, the map resolution was improved with a large F_2 population developed from the cross of RICL#10 harboring the shortest fragment from *T. dicoccoides* spanning *Qfhs.ndsu-3AS* with LDN. Evaluation of homozygous recombinants developed from the F_2 population further narrowed the location of the QTL to a 5.2 cM genomic interval flanked by *Xwgc50*1 and *Xwgc510*. In addition, recombinants with shortened *T. dicoccoides* chromosomal fragments surrounding *Qfhs*.ndsu-3AS were identified. Comparative analysis identified a ~42.2Mb genomic region on the common wheat 'Chinese Spring' (CS) chromosome 3B pseudomolecule that is collinear to the 5.2cM genetic region on 3AS spanning the QTL. These results will benefit continued fine mapping of *Qfhs.ndsu-3AS* and its utilization in durum improvement.

Problems associated with the introgression of FHB resistance from hexaploid to durum wheat were explored in this study. The F_1 of two hexaploid resistance sources ('Sumai 3' and PI 277012) with durum, four susceptible hexaploid wheat, and 14 LDN-CS D-genome substitution lines (LDN-DSLs) were evaluated for FHB resistance. The results indicated that expression of FHB resistance genes from Sumai 3 was possibly suppressed by genes on durum chromosomes

2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, and 7A. Chromosomes 4A, 6A, and 6B may contain genetic factors required for the expression of the PI 277012-derived FHB resistance genes in durum wheat. Suppression of Sumai 3-derived resistance and complete dominance of PI 277012-derived FHB resistance over susceptible alleles in durum wheat background was observed. Segregation within resistant-individual-derived families from the F_2 to F_4 generations in the cross of Sumai 3 with durum was skewed towards susceptibility, but a high frequency of resistant plants was retained over generations in the cross of PI 277012 with durum wheat. These results suggest that proper selection of the resistance source will be important for the introgression of hexaploid-derived FHB resistance into durum.

Evaluation of a complete set of LDN-DSLs and a series of LDN-*Ae. tauschii* D-genome addition lines (LDALs) for FHB resistance indicated that multiple chromosomes from LDN, CS, and *Ae. tauschii* had either negative or positive effects on FHB resistance. LDN chromosome 5A and CS chromosome 6D possibly harbor genes for FHB susceptibility. LDN chromosome 2B and *Ae. tauschii* chromosome 5D may carry genes for FHB resistance. Chromosome 1D possibly harbors a genetic factor(s) that augments the chromosome 5D-derived FHB resistance. It appeared that chromosome 2D may carry genes for FHB susceptibility whereas LDN chromosomes 6A and 6B may carry genes for FHB resistance. Thus, proper manipulation of these chromosomes is needed to ensure normal expression of FHB resistance genes in durum wheat.