MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN TWO ADAPTED SPRING WHEAT CULTIVARS

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Ahmed ElFatih Amin ElDoliefy

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Title

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Ahmed ElFatih Amin ElDoliefy

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Mohamed Mergoum
Chair

Dr. Elias M. Elias

Dr. Phil McClean

Dr. Shaobin Zhong

Approved:

10/30/2014
Date

Dr. Richard D. Horsley
Department Chair

ABSTRACT

Releasing bread wheat (*Triticum aestivum*) cultivars with resistance to *Fusarium* head blight (FHB) disease can be endangered by narrowing the variation in genetic sources. However, resistance to FHB rely on five different types. FHB resistance types I, II, III, IV and V were assessed in field and greenhouse under multiple locations and years experiment's combination. In our study, the genetic of FHB resistance in two widely cultivated hard red spring wheat varieties ('Glenn' and 'Parshall') were dissected. The specific objectives of the study were to generate recombinant inbred lines (RIL) populations, phenotypic assessment for FHB resistance, different informative genotypic marker data, genetic map, and finally QTL analysis. For Glenn/MN00216-4 (GM) population, 112 RIL were developed; while for Parshall/Reeder (PR) population, 110 RIL were developed. The RIL, checks and the two parents were evaluated for five FHB-related and one agronomic-related traits over two to six environments in North Dakota, Minnesota, and South Dakota. Two genetic maps were developed covering 2,229 cM of length using 645 DArT markers for GM population, and 470.4 cM length using 154 DArT/SNP combined markers for PR population. Composite interval mapping identified 37 QTL for the GM population, and 10 QTL for the PR population. Results showed that Glenn lacks the major consistent (*Fhb1* and *Fhb5A*) QTL from the Chinese source Sumai3, while acquired (*Fhb2*). Parshall proved to be domestic with no exotic resistance background, though it acquired similar genomic regions to *Fhb2* of Sumai3. PR genome contains five major QTL including three novel QTL with multiple FHB resistance and two with stable effect (1AS and 4BL) across at least two environments. Along with these previously identified QTL for FHB resistance, in both populations, new QTL were also identified such as *Fhb-1B1L.c* and *7D1S.b* in Glenn, and *Fhb.5AL, 7AS* and *4BL* in Parshall. In conclusion, our study added to the wheat genome, two genetic maps, new QTL for FHB resistance and two germplasms with new recombination for QTL and/or resistance sources. Finally, Glenn and Parshall can be of great importance if implemented in wheat enhancement and molecular assisted breeding programs nationally and internationally.

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DEDICATION

Always one beloved family: Mom, Dad, Brothers, Wife, Children…

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

قُلْ بِفَضْلِ ٱللَّهِ وَبِرَحْمَتِهِۦ فَبِذَٰلِكَ فَلْيَفْرَحُوا۟ هُوَ خَيْرٌ مِّمَّا يَجْمَعُونَ ٥٨

“Say, ‘in the bounty of Allah and in his mercy - in that let them rejoice; it is worthiest than whatsoever they collect safely’."

PREFACE

This dissertation consists of three chapters and a fourth section for general conclusion. Chapter 1 includes a general introduction about the dissertation research, along with the objectives, and a literature review. Chapters 2 and 3 were written as two papers to be submitted for publication to the appropriate scientific journals. Therefore, each of these paper chapters includes an abstract, introduction, material and methods, results, discussion, conclusion/summary and references. Final section was the general conclusion of all chapters.

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LIST OF ABBREVIATIONS

INC .... Incidence

SEV .... Severity

DON .... Deoxynivalonyl

FDK .... Fusarium Damaged Kernel

HD .... heading dates

HGT .... Plant Height

KS .... Kernels per spike

KSk .... Kernels per spikelet

GPC .... Grain protein content

GY .... Grain Yield

RIL .... Recombinant Inbred Line

NSk .... Number of spikelets

TKW .... Thousand kernel weight

CHAPTER 1. INTRODUCTION

1.1. General Introduction

Fusarium head blight (FHB) is caused primarily by *Fusarium graminearum* Schwabe. In wheat, the disease is favored by warm humid environmental conditions during flowering and early stages of kernel development. FHB is considered a serious cereal grains fungal disease in the United States (US) (Cuthbert *et al.* 2007). Lightweight *Fusarium*-damaged kernels (FDK) may contain high concentrations of mycotoxins making the grain unsuitable for food or feed (Gilbert and Tekauz, 2000). The major criterion of mycotoxin be traced in the FDK is generally known as deoxynivalenol and denoted as "DON". The nature of inheritance in FHB resistance is complicated due to the huge influences of environmental factors on the disease expression. Therefore, FHB assessment is based on screening large numbers of matured host plants, which intricate breeding efforts (Yang *et al.* 2005). Although other managements were practiced to help control FHB (ex. crop rotation, appropriate use of fungicides and weed control), yet developing resistant cultivars through breeding procedures was the safest and most economical procedures (Pirgozliev et al. 2003). So far, five types for FHB disease resistance (Type I, II, III, IV, and Type V) based on rate of spike infection and FDK, and resistance to the mycotoxin accumulation (Mesterhazy, 1995).

The Chinese wheat cultivar 'Sumai3' (PI- 481542) is the most common source of FHB resistance type II (Bai and Shaner 2004). Robust DNA molecular-assisted marker is considered the fastest and most efficient tool to screen for the genes of resistance especially under greenhouse condition (Kolb *et al.* 2001). Several studies have identified molecular markers linked to quantitative trait loci (QTL) associated with Sumai3-derived FHB resistance type II on chromosomes 3BS (Waldron et al. 1999), 5A (Buesrstmayr *et al.* 2002) and 6B (Yang *et al.* 2003). However, other genomic regions such as 6DS have been associated with multi-FHB resistance types (I, and II) based on studies using wheat doubled haploid (DHL's) populations (Handa *et al.* 2008).

Globally, substantial breeding efforts, especially in the US, are addressed to study the threatening impact of the FHB as a disease on the cereal economics in the last two decades. These research efforts have flourished many released cultivars and germplasms encompassing various levels of resistance. In

North Dakota (ND), the hard red spring wheat (HRSW) breeding program at the North Dakota State University (NDSU), have effectively developed many FHB resistant wheat cultivars. Some of these cultivars include `Alsen` (PI- 615543-released in 2000 (Frohberg *et al.* 2006)), `Steele-ND` (PI- 634981- released in 2004 (Mergoum *et al.* 2005a)), `Parshall` (PI- 613587- released in 1999), `Glenn` (PI- 639273- released in 2005 (Mergoum *et al*. 2006a), `Howard` (PI- 642367- released in 2006 (Mergoum *et al.* 2006b)), `Faller` (PI- 648350- released in 2008 (Mergoum *et al.* 2008)), `Barlow` (PI- 658018- released in 2009 (Mergoum *et al,* 2011)). These cultivars dictate the spring climate regions and ND, for instance, Alsen was accounted for 29% and 23% of spring wheat planted acreage during 2004 to 2005 (USDA, 2007), while recently, Glenn dominates the grown acreage in ND since 2007 (USDA, 2007, 2008).

Thus, understanding the complex genetics of FHB resistance and mapping the location of QTL/genes will be necessary to facilitate the introgression and pyramiding of FHB resistance genes into adapted wheat cultivars. The proposed research was to study the genetics of FHB-resistance in the two cultivars Glenn and Parshall. Glenn is a cultivar that is planned to have its source of resistance from Sumai3 (based on its pedigree) and Parshall is a cultivar that is believed to have an indigenous FHB resistance. Yet genetics of resistance in both cultivars is questionable.

1.2. Objectives

1.2.1. General Objective

The overall objective of this study was to determine the genetics of FHB resistance in two major cultivars (Glenn, and Parshall) of HRSW in ND using the advanced methodologies of breeding procedures assisted by the DNA molecular marker biotechnology, and bioinformatics studies.

1.2.2. Specifics Objectives

- Deciphering the genetic source of resistance in the spring wheat cultivar `Glenn`.
- Mapping the FHB genes in both cultivars of the HRSW `Glenn` and `Parshall`
- Studying the QTL, which contribute to FHB resistance
- Identifying molecular markers related to FHB-resistance

1.2.3. Expected Outcomes

This study will help to clarify the genetic source of resistance of the cultivar Glenn plus its agronomic and quality characteristics related to this trait. Confirm the Sumai3 genetic background in Glenn. Identify the source of genetics for FHB resistance in Parshall. Meanwhile, the results from this study will allow us to map the QTL/genes controlling FHB resistance and eventually develop/identify molecular markers that can be used by breeders in future MAS schemes.

1.3. Literature Review

1.3.1. Economic Importance of FHB Disease

In temperate agricultural regions, *Fusarium graminearum* Schwabe, perfect state *Gibberella zeae* (Schwein), and *F. culmorum* predominate as the causal agents for FHB (Snijders, 1994). FHB reduces kernel set and weight, causing grain yield loss. Invasion of the kernel by *Fusarium* destroys the starch granules, cell walls, and affects endosperm storage proteins; which reduces grain quality. Generally, *Fusarium* species produce three major mycotoxins (trichothecenes deoxynivalenol (DON), 3-acetyldeoxynivalenol (3A-DON) and nivalenol (NIV)) (Gang et al. 1998). Quality loss due to mycotoxin contamination has led to additionally important economic losses (Bai and Shaner, 2004).

According to a report estimating the regional economic impacts of FHB in wheat and barley by Nganje et al. 2011; the FHB disease in the upper Midwest region of the US (ND and Minnesota (MN)) led to hundreds of millions of dollars in losses for farmers since 1993. Losses at production level were estimated as 27.6 out of 47.8 million bushels in the period (1998-2000). North Dakota accounted for 41% of the overall direct impact of FHB during the three years followed by Minnesota and Ohio. For HRSW, FHB price reduction ranged from 1.04 to 6.81 cents per bushel. This resulted in direct economic impact of $457 million losses during the same period (1998-2000). In 2000 only $160 million losses were recorded; reflecting the importance of the released resistant varieties; albeit the annually loss was on average $290 million.

1.3.2. Types of FHB Resistance

Disease pressure was influenced by several factors including initial natural inoculum supply, resistance level of the wheat variety, and climatic conditions. Breeding for FHB resistance was complicated by many factors, such as 1) screening methods of the host plants at maturity; 2) expression of resistance, which was highly, affected by the environmental conditions 3) the type of the resistance. The later factor was basically divided into many types according to the stage of disease development. Some other resistance types were less characterized such as the disproportionate reductions in the accumulation of DON, which also was described as Type IV resistance (Cuthbert et al. 2007).

The first type of resistance (Type I) is assessed as the incidence of infection (initial infection) under natural or artificially field inoculation. The first procedure was spraying the spore suspension at the time of anthesis and after 21 days of spore spraying, the number of infected spikes was counted on each plant and divides by the total number of spikes. The second procedure was using grain spawn to evaluate large number of plants in the field nurseries. Therefore, instead of spraying the spore suspension, this protocol uses colonized grains (wheat or maize) that were spread throughout the field. Three weeks after flowering, the disease symptoms are assessed. This protocol is simulating the natural epidemics of the disease (Rudd et al. 2001).

The second type of resistance (Type II) is defined as the spread of infection within the spike following single/dual floret injections (SFI/DFI). Type II is the most used and well characterized in Sumai3 and its derivatives. Type II is measured in greenhouse experiments by using artificially point inoculation with syringe or needle. Typically, a single central floret was inoculated at anthesis with 2-10 µl of macroconidial spore suspension. The range of spore suspension concentration could be 50,000-100,000 spores/ml. Three weeks after inoculation, number of infected spikelets is counted and the percentage of infection is estimated by dividing the number of infected over the total number of spikelets per single spike. Disease severity is measured as the percentage of infected spikelets per spike. Usually 10-25 spikes are scored per plot to estimate disease severity. FHB-index is measured by multiplying the disease severity by incidence (Burlakoti, 2008).

Resistance to the accumaulated levels of the FHB mycotoxin, especially DON, is assessed as the type III. Resistance type III is related to the mycotoxin (DON) content in the analyzed milled kernels (flour) from infected spikes. Resistance to kernel infection, known as Type IV, is measured by scoring the number/percentage of FDK (tombstones), other grain characters such as, kernel number reduction, kernel weight, and test weight can also be used. On the other hand the resistance type V is related to the yield losses of infected plot compared to checks (resistant and susceptible), and may include, test weight (TW) and 1000 kernels weight (TKW). A lower disease incidence in a field ensures lower mycotoxin content in the grain (Bai et al. 2001).

1.3.3. Role of Morphological Traits in FHB Resistance

In wheat, several morphological characteristics that help reduce natural infection had been reported (Gervais et al. 2003). Tall plants without awns and plants with lax ears tend to have lower rates of infection (Mesterhazy, 1987). Some studies showed that QTL for resistance could coincident with genes controlling morphological characteristics; such as resistance to kernel shattering (Zhang and Mergoum, 2007). Also, it was reported that selection to semi-dwarf types is possible to improved FHB resistance (Gervais et al. 2003). When resistance to FHB is based mainly on type I and II (Schroeder and Christensen, 1963); the resistant varieties showed, in addition to slow and late development of symptoms (incubation period), a delay in time of sporulation (latent period) (Ribichich et al. 2000). Other findings also showed that FHB spreads slowly in the genotypes with long peduncle, lax spikes and rapid grain fill period (Rudd et al. 2001).

1.3.4. Sources of Resistance

The main common sources, with a very high level of resistance, in spring wheat have been identified worldwide in the following cultivars: Sumai3, Ning8343, and Wuhan1 (China); Nobeokabozu komugi (Japan); and Frontana (Brazil) (Snijders, 1994). Classical inheritance studies of population segregating for FHB resistance have shown that the resistance to FHB in wheat has a quantitative nature, but relatively highly heritable and controlled by few genes with additive effects (Van Ginkel et al. 1996). Many studies suggested that FHB resistance in Sumai3 and its derivative Ning7840 was based on two major genes (Van Ginkel et al. 1996). Although, complete resistance has not been discovered and

transgressive segregation is observed in progenies for crosses involving resistant parents. This, plausibly, was showing that accumulation of resistance genes is possible (Buerstmayr et al. 1999).

1.3.5. Identified QTL Linked To Different FHB Resistance Types

Molecular evidences (simple sequence repeats (SSR) and the amplified fragment length polymorphism (AFLP)markers) for a major QTL of resistance type II in Sumai3 and Ning7840 has explained up to 60% of the variation for FHB resistance (Liu and Anderson, 2003). Finding a common QTL in different populations on chromosome 3BS was indicating that the identified markers have good potential to be used in marker assisted selection (MAS). Spring wheat varieties appear to have the same designated QTL as Sumai3, Ning7840 and Huapei57-2 (Bourdoncle and Ohm, 2003). However, other QTL of less magnitude have been identified on chromosomes 3BL, 3A, and 5B (Bourdoncle and Ohm, 2003). Successful conversion of one AFLP marker for the 3BS QTL yielded in sequence-tagged sites (STS) marker that explained about 50% of the variation in FHB resistance (Guo et al. 2003). Unlike AFLP, using STS marker-technology was more breeder-friendly and suitable tool for high-throughput screening especially at early seedling stage.

The FHB resistance type III was suggested to be controlled by the *Fhb1* genes, which cause low accumulation of DON due to encoding a DON-glucosyltransferase or regulating the expression of such enzyme (Lemmens et al. 2005). *Fhb1* locus was mapped on the distal segment of chromosome 3BS and linked to *Xgwm533* and *Xgwm493* markers (Cuthbert et al. 2006). Along with the QTL of *Fhb1* on chromosome 3BS, seven other genomic regions on chromosomes 2B, 2DL, 2DS, 5AL, 5BS, 5DS and 7AS were associated with type (I) not type (III) resistance (Xu et al. 2001). Additionally, six genomic regions on 1BS, 2DS, 3BS, 4AL, 5AS and 6DS were associated with type (II) (Xu et al. 2001). Moreover, one QTL controlling accumulation of DON was identified in the close region of 2DS (Yang et al. 2005). Surprisingly, two FHB-related QTL but functioning-contradictory to each other were closely located in the same chromosome region (2DS). This indicates the possibility that these two QTL might have pleiotropic effects of the same gene (Yang et al. 2005). Therefore, Sumai3 may not only contain resistance genes but also may have susceptible genes as well that halt building up a full resistance against FHB.

In addition to the resistance mechanisms for type II, there were other mechanisms of which may influence DON content in kernels such as DON degradation, conjugation and/or tolerance (Mesterhazy et al. 2002). Other reports have demonstrated that defense responsive genes were activated both in infected spikelets and un-colonized parts of infected heads (Makandar et al. 2006 and 2010). Genes such as *NPR1*, *PAD4*, *WRKY18*, and *LOXs* were shown to enhance resistance against FHB in *Arabidopsis* and wheat. In *Arabidopsis*, *NPR1* gene regulates the activation of systemic acquired resistance, while *PAD4* gene regulates multiple defense mechanisms, including salicylic acid synthesis and signaling. *WRKY18* encodes transcription factor that regulates defense gene expression. Unlike *NPR1*, *PAD4*, and *WRKY18*, the lipoxygenases (*LOXs*) activity contributes to host susceptibility to this fungus (Nalam et al. 2010).

1.3.6. Breeding for FHB Resistance

Since 1990's, FHB has been declared as a devastating disease. Few breeding programs in the US were engaged to breed for FHB resistance. One of the major objectives of a wheat breeding program for pathogen resistance was to use superior genotypes combining different sources and types of resistance suitable for selecting desired agronomic and quality traits. In average, to develop a new wheat variety, 10-12 years are needed for a single breeding program, starting with the crosses ending by the release. Fortunately, genetic resistance was available from eastern Asian origin (Sumai3). However; huge breeding effort to recover progenies that combine the desirable agronomic and quality traits with FHB resistance was required (Anderson, 2007). Although, multiple resistance types were detected in wheat, yet screening for the resistance type II was the applicable approach in greenhouse and field experiments (Dill-Macky, 2003). Field screening is time and resource consuming and results are often confounded by environmental factors, therefore, verification across multiple environments is necessary (Fuentes-Granados et al. 2005). Resistance genes from multiple sources can enhance the level of resistance (Miedaner et al. 2006). Marker repeatability, which reflects the identified markers using a collection of genotypes rather than a segregating population, was more likely to yield in markers applicable to other populations and breeding programs. Markers from traditional linkage studies need to be validated and tested for polymorphism with other populations to ensure their use in MAS (Sun et al. 2003).

1.3.7. Breeding Achievements for FHB Resistance

In Canadian soft winter wheat, the correlation is investigated among FHB index, DON accumulation, number of FDK, heading date (HD), plant height (HGT), quality traits and yield in a cross between `RCATL33` (FHB resistant and poor yielding) and `RC Strategy` (good yielding and FHB susceptible) genotypes (Tamburic-Ilincic 2011). The FHB resistance in 'RCATL33' was derived from two exotic sources, Sumai3 and Frontana, where the known QTL on chromosomes 3BS and A3 are identified (Tamburic-Ilincic 2006). Among the findings from this study were the highest correlation between DON content and FDK level (r = 0.82). Significant negative correlation between HGT and the three FHB-related traits (FHB index, DON accumulation, number of FDK), protein content and HD, while positive correlation with TKW was observed. On the other hand, protein content was highly and positively correlated with DON and FDK. In addition, HD was positively correlated with FDK, while negatively correlated with TKW.

Another study used Fronatana as an exotic source for FHB resistance and QTL for FDK to validate two populations (Szabó-Hevér *et al.* 2011). The two populations were the Austrian Frontana/Remus DH from Tulln (n = 210) and the Mini Manó/Frontana DH population from CRC, Szeged (n = 169). In the first population, QTL successfully identified on 2B, 2D, 3A, 3D, 4A, 4B, 5A, 6B and 7B chromosome regions. In the second population, significant association with the FHB and FDK on chromosome 5A and 6B was confirmed. Among the identified QTL, the 5A and 6B QTL were confirmed in Hungary in both populations.

At the level of QTL validation studies, the validation of four QTL on the chromosomal regions 2B, 3BSc, 4BL and 5AS is investigated and testing if these regions were truly associated with resistance type II and III in the soft red winter wheat line 'Ernie' (Abate et al. 2008). Of the six identified markers on the 5AS region, only (*Xbarc165*) locus was significant across combined data reflecting the lower level of marker saturation on the 5AS. Across many populations, results suggested the importance of *Xgwm319*, *Xgwm285*, and *Xgwm495* markers on 2B, 3BSc, and 4BL loci, respectively. All these markers together explained 67% of FHB susceptibility and 69% of DON levels as phenotypic variations.

Regionally, the HRSW breeding program at NDSU, ND, had successfully released, through the North Dakota Agricultural Experiment Station (NDAES), many varieties and germplasm with significant FHB

resistance. The range of identified resistance to FHB was good, medium to high. The varieties with good FHB resistance included Alsen, Steele-ND, Faller, and Howard. These cultivars combine high grain yield, excellent end-use quality for domestic and export wheat markets and leaf disease resistance. Other varieties/germplasm with medium FHB resistance includes ND 756 (PI-648034) and Barlow. Some other varieties/germplasm that had high level of FHB resistance were ND 2710 (PI-633976-released in 1998 (Frohberg et al. 2004)), ND 744 (PI-634936- released in 2004 (Mergoum et al. 2005b)), ND 751 (PI-642781- released in 2006 (Mergoum et al. 2007)), and Glenn.

1.3.8. Marker Assisted Selection for FHB Resistance

Autonomous differences in wheat DNA sequences were mostly linked to a gene/QTL that confers greater resistance to FHB. These DNA differences could be due to repetitive sequences (e.g. SSR; insertion/deletion of DNA segment(s); and/or single nucleotide polymorphism (SNP)). Most markers used today in wheat were SSRs and most of the QTL that were widely used in breeding programs were located on chromosomes 3BS and 5AS. The highest magnitude of FHB resistance gene/QTL identified to date was *Fhb1* in Sumai3. This gene can reduce the disease by 50%, but on average, depending on the genetic background, it can reduce FHB by 20–25% (Pumphrey et al. 2007). However, there was evidence that combining major QTL can increases FHB resistance level (Miedaner et al. 2006). One advantage of marker technology was that it can be used to rapidly select for combinations of multiple genes in a population. In contrast, field-based selection of same homozygous lines would be labor and time consuming. Therefore, MAS can increase chances of selection for desired traits, allowing breeders to concentrate their resources on materials that have a better chance of resulting in improved varieties (Anderson, 2007).

To be effective and applicable to breed for FHB-resistance, MAS must guarantee some criteria: 1) efficiency/gain compared to phenotypic selection; 2) usefulness of markers in relevant breeding populations; and repeatability across populations 3) the cost, throughput, and essential expertise; and 4) the type of molecular markers used, and intensity of genome coverage (Anderson, 2007). Diagnostic markers, for breeding populations, must acquire polymorphic alleles to differentiate between the two parents and their descendent progenies (Liu and Anderson, 2003). Several major QTL from Sumai3 for FHB resistance on chromosomes 3BS, 5A, 6BS were identified with major concern given to the QTL

Qfhs.ndsu-3BS on chromosomes 3BS (Yang et al. 2003). Recently, another effective marker (*Umn10*) is identified with close linkage to the 3BS *Fhb1* locus (Liu et al. 2008). This new marker was widely used in many breeding programs including HRSW breeding program.

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CHAPTER 2. 'GLENN': A NEW SOURCE OF FHB RESISTANCE IN USA HARD RED SPRING WHEAT

2.1. Abstract

Fusarium head blight (FHB; *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schw. (Petch)]) is a major disease of wheat (*Triticum aestivum* L.) that affects kernel yield and quality in many wheat-growing regions, including the US Northern Plains. In recent years, the hard red spring wheat (HRSW) breeding program at the North Dakota State University has released several FHB resistant cultivars, including the high-quality 'Glenn'. Pedigree in Glenn presumes 'Sumai3' as the resistance source. However, molecular analysis showed no marker evidence of the major FHB resistance QTL (*Fhb1*) from Sumai3. Therefore, to identify the genetic nature of FHB resistance in Glenn, a 112 RIL population (GM) was developed from a cross between Glenn and a moderate resistant line MN00261-4. GM population was evaluated for four FHB resistance types, one agronomic trait and one FHB index at multiple locations in two states (ND and MN) over three years (2010-2012). FHB resistance types and index are [types I (incidence, INC); types II (severity, SEV); types III (toxin deoxynivalenol level, DON); types IV (*Fusarium* damaged kernels, FDK); disease index (NDX)] and the agronomic trait is heading date (HD). A framework linkage map was developed using 645 out of 2500 polymorphic diversity array technology (DArt) gene-based markers for GM population. Composite interval mapping (CIM) was used to identify a total of five QTL for resistance type I, 11 for type II; six each for type IV and NDX; one for DON and nine for HD. These included two major QTL with stable effects (detected in ~50% of tested environments) out of total 37 major QTL (R^2>10%) for FHB resistance. Our study did not find the major consistent QTL of Sumai3 (*Fhb1*, *Fhb4,* and *Fhb5A*), though found the *Fhb2* QTL, suggesting achievement of high FHB resistance in GM population without these major QTL. Additionally, GM population elucidates some new FHB resistant QTL (Q*NDX.1BL1.b, QFHB.5Bl1.c, QFDK.5BL2,* and QSEV.*7DS1*). The high FHB resistance pyramided in Glenn along with its high yield and end-use quality can be an excellent source to be implemented in national/international genome wide selection and/or molecular assisted breeding programs in wheat.

2.2. Introduction

Fusarium head blight (FHB) is a devastating disease in spring wheat (*Triticum aestivum* L.) production regions worldwide. FHB is mainly caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schw. (Petch)] in warm, humid growing regions. In the upper Midwest region of the US, FHB has led to farmers losses of hundreds of millions of dollars since 1993. For example, in the year 2000, $160 million losses were recorded (Nganje et al. 2011). *F. graminearum* is spread through asexual conidia or sexual ascospores in the field by wind or rain (Gilbert and Fernando 2004). FHB pathogenesis depends on two phases: the biotrophic phase, [<72 hours after injection (hai)] (factors type I resistance), followed by the necrotrophic phase [>72 hai (factors type II resistance)]. In resistant cultivars, transcriptomic studies suggested the release of biotrophic resistance metabolites during the first 48 hours of infection (Basnet et al. 2012). After depositing on wheat spikes, macroconidia germinate within 5-6 hours, elongate a germ tube over the glume and floret surface, and enter the host cells through stomata or direct penetration (Bushnell et al. 2003; Seong et al. 2008). Fungi spread within the apoplast, causing cytological changes and cell death (Zhuang et al. 2013). Additionally, the fungus produces a mycotoxin deoxynivalenol (DON) that spreads within the spike via subcuticular growth (Gunnaiah et al. 2012). DON kills plant cells and accumulates in infected kernels. Heavily infected grains are not suitable for feed and food consumption, and result in lower seed quality and commodity value (Shin et al. 2012).

FHB resistance is a complex quantitative trait and consists of five main discrete types: i) type I (resistance to initial infection measured by incidence); ii) type II (resistance to pathogen spread measured by severity); iii) type III (resistance to toxin -DON- accumulation); iv) type IV (resistance to kernel infection or FDK); and v) type V (resistance to yield loss) (Mesterhazy, 1995). Resistance type II is the most common and is less affected by environmental changes than other resistance types (Bai and Shaner 1996). Consistent screening for resistance type II depends upon minimizing escapes and is usually performed under controlled environments involving direct spore placement (Buerstmayr et al. 2013). Morpho-physiological traits such as plant height, spike trait, heading dates, etc. are also associated with FHB type I and II resistance (Buerstmayr et al. 2009). Plant height is negatively associated with type II resistance (Lu et al. 2012). The major QTL for resistance type I and II are associated previously with plant height *Norin10*

genes (*Rht-D1b* and *Rht-B1b*) (Srinivasachary et al. 2009; Buerstmayr et al. 2012). Other studies report anther extrusion is associated with avoidance mechanism of both resistance type I and II, where more pronounced extrusion characteristics confer less risk of infection (Lu et al. 2012). Other traits associated with FHB resistance type I and II include awnless spikes and low spikelet density, respectively. However, QTL regions of both resistance types are also associated with QTL of low yield (Lu et al. 2012), which propose a linkage drag for these specific regions. Numerous QTL for different FHB resistance types have been found throughout the wheat genome in various cultivars using different populations (Liu et al. 2009). Most notably are the major QTL on wheat chromosomes 2D (Lin et al. 2006), 3A (Yu et al. 2008), 3BS (*Fhb1*; Anderson et al. 2001), 4B (*Fhb4*; Randhawa et al. 2013), 5A (*Fhb5*; Xue et al. 2011), and 6B (*Fhb2*; Cathbert et al. 2007). These QTL are associated with type I and II resistance originating from Sumai3 (PI-481542) or its derivatives such as DH181 (Yang et al. 2005), CJ9306 (Jiang et al. 2007), Ning7840 (Zhou et al. 2002), CM82036 (Buerstmayr et al. 2002), and Line685 (Lu et al. 2011), making Sumai3 the most popular and common source for FHB resistance especially type II.

Breeding FHB-resistant cultivars is the most efficient method to reduce the economic impact of FHB (Lu et al. 2013). Breeding programs can accelerate the development of such cultivars by utilizing molecular tools for selection. However, the success of molecular-assisted breeding (MAB) generally depends on: i) close linkage between a marker and gene/QTL of interest; ii) marker validation under the specific environments in different genetic backgrounds; and iii) assessment methods must be highly consistent, repeatable, cost- and time-efficient (Randhawa et al. 2013). The most widely used QTL for FHB resistance in wheat breeding programs is *Fhb1* located on 3BS of Sumai3 (Waldron et al. 1999). The closest effective marker (*umn10*) is less than 1 cM from *Fhb1* (Liu et al. 2008) and is being used, frequently, in many breeding programs. Usually, *Fhb1* can increase type II resistance by 20-25% on average, depending on the genetic background (Pumphrey et al. 2007). Evidence suggests that combining *Fhb1* with other major QTL (eg. *6BL* QTL) can further increase the FHB resistance by delaying the necrotrophic pathogenicity phase (Zhuang et al. 2013). Glenn (PI- 639273; Mergoum et al. 2006a), an FHB resistant cultivar, is developed by the hard red spring wheat breeding program (HRSW) at North Dakota State University (NDSU), Fargo, ND for the US Upper Midwest region. Released in 2005, Glenn has become the leading cultivar in ND from 2007 to 2012 and is characterized by very high end-use quality, excellent

agronomic performance, and high FHB resistance (Mergoum et al. 2006a). Based on its pedigree, the source of FHB resistance in Glenn was expected to originate from Sumai3. However, molecular analysis (data not shown) showed that Glenn does not possess molecular markers (including *umn10*) associated with the widely reported major FHB QTL *Fhb1* from Sumai3. Therefore, the objectives of this study were to investigate i) the genetic factors controlling FHB resistance in Glenn, ii) whether *Fhb1* has any contribution to FHB resistance in Glenn, iii) if it is possible to achieve high levels of FHB resistance by combining several loci other than *Fhb1*, and iv) if there are any associations between heading date (HD) and FHB resistance in GM population. To accomplish these objectives, a RIL population is developed from the cross Glenn/MN00261-4 (GM). GM was phenotyped for FHB traits and HD under several field and greenhouse environments for three years. Further, GM was genotyped using DArT markers, genetically mapped and analyzed molecularly for potent QTL.

2.3. Material and Methods

2.3.1. Plant Material and Population

The present study used a RIL population (called GM) developed by single seed descent from the cross Glenn/MN00261-4 at NDSU. Glenn is a HRSW cultivar developed from the cross between ND2831 (PI- 665931; Mergoum et al. 2012) and 'Steele-ND' (PI- 634981; Mergoum et al. 2005). The ND2831 is an experimental line with Sumai3 in pedigree (SUMAI-3/STOA; SUMAI-3/WHEATON//GRANDIN/3/ND-688; ND-2709/ND-688) (http://wheatpedigree.net/sort/show/49574). Glenn combines high level of FHB resistance, high yield and grain volume, as well as excellent end-use quality for domestic and export wheat markets (Mergoum et al. 2006a). MN00261-4 is an experimental line developed from the cross MN95286/MN94155//VERDE by the University of Minnesota wheat breeding program (Anderson et al. 2010). MN00261-4 has high yield, good leaf rust (*Puccinia tritici* Eriks.) resistance and strong gluten strength. Comparatively, Glenn has earlier HD, higher FHB resistance and better end-use quality than MN00261-4 (Anderson et al. 2010). A total of 112 RILs were evaluated in field and greenhouse trials. Seven checks were evaluated, including a high FHB resistant line 'ND2710' (PI- 633976; Frohberg et al. 2004), two moderate resistant cultivars 'Faller' (PI- 648350; Mergoum et al. 2008) and 'Alsen' (PI- 615543;

Frohberg et al. 2006), two moderate susceptible cultivars Steele-ND and Barlow (PI- 658018; Mergoum et al. 2011), a susceptible line 'Vida' (PI- 642366; called MT0245; Lanning et al. 2006), and a highly susceptible line (ND2398).

2.3.2. Field Experiment

Field evaluations were conducted for three years (2010-2012) at one location in Minnesota (MN) (Minneapolis, 44°59′N 93°16′W) and three locations in North Dakota (ND) [Carrington (47.45000N, 99.12390 W), Prosper (46.96300N, 97.01980 W) and Langdon (48°45′42″N 98°22′18″W)]. Field experiments were conducted in FHB nurseries using the methods of FHB artificial inoculation (Stack et al. 1997) and overhead mist irrigation (Rudd et al. 2001), where scoring occurs 21 days after flowering time. All experiments were conducted in a randomized complete block design (RCBD) with four replicates in ND and two replicates in MN. Experimental unit consisted of 10-15 plants in 0.3 m hill/plot.

2.3.3. Greenhouse (GH) Experiment

Evaluations conducted during 2011 and 2012 in an RCBD layout with four replicates in 8-inch diameter pots contained five plants. The planting soil was Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA USA), augmented with 20 g Osmocote® slow release fertilizer (Scott's Company LLC, Marysville, OH USA). At flowering, 5-10 spikes per pot were artificially inoculated using the FHB spore-suspension injection method (Bekele, 1995). Briefly, one middle floret of a single spikelet of each spike was manually injected, using a needle containing about ~4 µl (100 k/ml) of spore suspension. Inoculated spikes were covered individually by misted plastic bags for 72 hours. Severity scores were collected about three weeks after injection.

2.3.4. Phenotypic and Genotypic Data Recording

Each genotype in all replicates was assessed for all FHB resistance traits. In field experiments, FHB incidence (INC/resistance type I) was assessed as the proportion of infected spikes of total spikes in a plot/hill. INC was assessed in five environments (combinations of year by location) in field only, including MN 2010 and 2012 (M10 and M12), Carrington 2011 (C11) and Prosper 2011and 2012 (P11 and P12). No

INC recorded in GH. FHB severity (SEV/resistance type II) was assessed as the proportion of infected spikelets per spike averaged across randomly chosen 10 spikes per plot in field and 5-10 spikes per pot in GH experiments. Phenotypic data used for QTL analysis included the means of FHB responses. Records of HD were assigned to determine flowering dates and inoculum applications in the field and GH. SEV was recorded for seven environments including M10, M12, Prosper 2010 (P10), P11, P12, C11, and GH 2011 (G11). DON (resistance type III) concentration was determined in 10 gm of milled kernels for each genotype using gas chromatography and/or mass spectroscopy (Schwarz et al. 1995). DON was assessed in three environments including P10, P11, and G11. *Fusarium* damaged kernels (FDK/resistance type IV) was determined as the proportion of infected kernels found in 200 kernels randomly sampled out of each individual genotype per replicate. FDK was scored in five environments including M10, MN 2011 (M11), M12, G11, and GH 2012 (G12). Disease index (NDX) was calculated by multiplying INC and SEV percentages at different environments for each genotype.

Fig 2.1. Percentage of FHB severity developed on wheat spikes. Courtesy G. Bergstrom (http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx).

Fig 2.2. Percentage of the FHB severity scale used to score the disease on wheat spikes. Stack, R. and McMullen, M. 1995. A visual scale to estimate severity of *Fusarium* head blight of wheat. (http://www.ext.nodak.edu/extpub/plantsci/smgrains/pp1095w.htm).

Fig 2.3. The different shapes of shapes of FDK seeds. (http://www.grainscanada.gc.ca/str-rst/fusarium/fhbwc-foc-eng.htm).

One agronomic traits, HD, was evaluated as the number of days from planting to Feekes growth stage 10.5, where 50% of spikes per plot were fully emerged from boot leaves. Early, medium, and late HD was assigned at seven days intervals (seven, 14, and 21 days) in seven environments including M10, M12, P10, P11, P12, C11, and Langdon 2011 (L11).

Genomic DNA was extracted from lyophilized tissue of young leaves for each individual RIL, parent and checks using Qiagen DNeasy Plant mini kit (Cat# 69106) with minor modifications. For each genotype, 30 µl of DNA (80ng/µl) were sent to Triticarte Pvt. Ltd (Canberra, Australia; http://www.triticate.com.au) for Diversity Array Technology (DArT) analysis (Akbari et al. 2006). Out of 2500 DArT markers, 659 polymorphic markers were revealed; where 645 markers were used to generate the genetic map.

2.3.5. Map Construction and QTL Analysis

Scores of all polymorphic DArT markers were converted into genotype codes according to the parental scores. Segregation deviation of individual markers from expected ratios (1:1) was determined by χ^2 tests. Genetic maps were constructed using a combination of MapMaker 3.0 (Lander et al. 1987) and CarthaGene V.1.2.3R (De Givry et al. 2005) software with a minimum LOD score of 3.0 and maximum recombination frequency of 40%. MapMaker 3.0 was used to construct the groups based on few anchor markers from each chromosome, while CarthaGene was used to construct final map orders using build10, greedy search, genetic algorithm, annealing, flips, and polish functions. The genetic distances were obtained using the Kosambi mapping function (Kosambi, 1944). Final maps were compared with the DArT consensus maps (Huang et al. 2012) using the program Autograph (Derrien et al. 2007; http://autograph.genouest.org/) to check the accuracy of the markers order. QTL mapping was carried out by CIM (Zeng 1994) using the QGene4 (Joehanes and Nelson 2008). A scanning interval of 1 cM between markers and putative QTL with a window size of 10 cM to detect QTL. Five controlling markers were set to control background by forward and revers regression. Significant QTL was considered at 3 LOD value. Additive QTL effects, positive and negative signs indicate, respectively, the contribution of Glenn and MN00261-4 toward higher trait values. Proportion of phenotypic variance (PV) explained by single QTL was determined by the squared partial correlation coefficient (R^2). Confidence intervals (CI) were obtained using positions ±2 LOD away from QTL peak. Graphical representation of linkage groups and QTL was carried out using MapChart 2.2 software (Voorrips 2002).

2.3.6. Statistical Analysis of Phenotypic Data

Data from all traits were subjected to analysis of variance (ANOVA) for RCBD design using the Mixed procedure of the statistical analysis system 9.3® (SAS Institute, Cary, NC 2004). The RIL, parental, and checks genotypes were considered fixed effects, while environments and replicates were considered random. ANOVA was calculated for each individual environment (field and GH) separately and for combined data across all environments to estimate genotype X environment interaction. The statistical model of *F*max ratio (<10-fold) for homogeneity (Tabachnick and Fidell 2001) was tested to combine

ANOVA. If the *Fmax* ratio between the major and minor experimental error of combined environments was less than 10, combining error terms was considered valid at $p \leq 0.05$. Correlation coefficients (r) was carried out for each trait location individually, and for FHB combined trait means using Proc Corr of the SAS 9.3® and considered significant at $p \leq 0.05$. Means separation for genotype was determined using *F*-protected least significant differences (LSD, $p \leq 0.05$) generated by the Proc Mixed of SAS analysis. Broad sense heritability (h^2) was calculated based on family mean basis (Holland et al. 2003) using the output ANOVA random model of SAS Proc Mixed analysis excluding the parents and checks means. To calculate h^2 we used the combined trait means with insignificant *F*-max homogeneity (Otto et al. 2002) where $h^2 = \frac{\delta^2 G}{\delta^2 G + \frac{\delta^2 GE}{e} + \frac{\delta^2 E}{re}}$ and $\delta^2 G$=genotypic variance, $\delta^2 GE$=genotype X environment interaction, $\delta^2 E$=experimental error variance, e =total number of environments, and r =total number of replications within an environment.

2.4. Results

2.4.1. Phenotypic Analyses

Phenotypic data is generally showed segregation for FHB resistance traits. Except for DON and FDK in P10 and MN locations, Glenn showed lower mean values at the HD and other FHB resistance types than MN00261-4 (Table 2.1). Generally, this reflects the better performance of Glenn compared to MN00261-4. The mean of RIL (94.90%) for INC trait was lower than both parents and all checks (Table 2.1). For SEV and NDX, RIL's mean (41.50% and 38.10%, respectively) was lower than the parent MN00261-4, the medium and the high susceptible checks (Bacup and ND2398), while higher than the parent Glenn, the high and the good resistant checks (Alsen and Parshall). This may suggest that alleles co-contributed from the MN00261-4 parent to FHB resistance type II and NDX ceased the overall RIL's mean to reflect better resistance means than the parent Glenn. It is also may suggest that MN00261-4 parent is negatively reduced resistance type II and NDX in GM population. Which means, alleles with high resistant effects from Glenn could be less contributed for the resistance type II and NDX due to the deleterious alleles contributed by the MN00261-4. The mean of RIL in GM population under ND environments for DON and FDK (GH) and for HD trait was always higher than both parents and checks. This reflects the high effect of HD and photoperiod trait on these resistance types in GM population.

Besides, it reflects the preference of lateness trait contributed by the MN00261-4 parent under ND condition and the less earliness trait contributed by Glenn under ND environment. Meanwhile, RIL produced lower mean values of DON (P10) than the high resistant parent (Glenn) and the good resistant check (Parshall), and higher values than the medium resistant parent (MN00261-4). Meaning that, some GM RIL has better resistance type III than Glenn but still lower than MN00261-4. Besides, it may reflect that resistance type III in MN00261-4 is more effective than that exist in Glenn. Since both Glenn and Parshall are ND released cultivars, while MN00261-4 is an MN line, this may reflect the environmental preference contributed for resistance type III and IV in the GM population. This may mean, that GM population acquired resistance types III and IV in addition to resistance types I and II especially under the ND environments. Given that, under the MN conditions, the mean of RIL was almost equal to the mean of the parent MN00261-4 for resistance type IV and HD trait (Table 2.1). This may mean that MN00261-4 contributed to lower mean values of FDK and with lateiness effect on HD trait than the ND cultivar (Glenn). Further, cultivars released under MN conditions may contain alleles of resistance types III and IV, while ND cultivars may contain alleles for resistance types I and II, which needs further comparative molecular analysis.

Table 2.1. Means, minimum and maximum values of RIL, parents, checks in GM population for FHB severity (SEV), incidence (INC), index (NDX), DON, damaged kernels (FDK) and heading dates (HD).

Trait	INC%	SEV%	NDX%	DON (ppm)		FDK%		HD (days)	
Env	A†	A††	A‡	P10	G11	MN	G	ND	MN
Parents									
Glenn	95.60	38.20	37.10	00.71	00.50	18.20	68.40	53.60	30.80
MN00261-4	98.10	46.50	42.20	00.04	02.00	17.20	82.50	56.70	33.30
LSD (a)	10.06	12.14	12.88	-	-	06.68	15.10	05.20	03.24
Checks									
ND2398	100.00	55.40	53.30	-	-	-	-	58.50	-
Alsen	98.10	36.80	37.20	-	-	10.70	69.10	56.10	32.80
Parshall	100.10	39.10	35.50	00.30	01.60	-	-	54.20	-
Bacup	98.80	51.10	50.70	-	-	21.50	-	-	28.00
RIL									
Mean	94.90	41.50	38.10	00.22	04.73	17.30	84.50	58.00	33.90
Min	66.30	22.30	14.40	00.04	00.05	07.30	59.40	53.20	28.00
Max	103.30	88.10	86.50	01.60	28.40	68.20	99.40	61.90	39.50
LSD (b)	08.24	10.56	10.61	-	-	06.80	21.10	05.20	03.24

Trait, FHB traits and HD. Env, environments for each dataset as location x year. INC%, percentage of Field FHB incidence. A†, combined disease incidence in P11 (Prosper 2011); C11, (Carrington 2011); M10 (MN 2010) and M12 (MN 2012). SEV%, percentage of FHB severity scores. A††, combined SEV in P10 (Prosper 2010); P 11 (Prosper 2011); P12 (Prosper 2012); C11 (Carrington 2011); M10 (MN 2010) and M12 (MN 2012); and G11 (GH 2011). NDX%, product of multiplied SEV% x INC% scores. A‡, combined NDX% in P11, P12, C11, M10 and M12; DON, toxin deoxynivalenol concentration in ppm. FDK%, percentage of damaged kernels in 200 random seeds. G, combined FDK% scored in G11 and G12. HD, means measured based on number of days from planting dates in ND and at 1st of June in MN. ND, combined HD data from ND state only (P10, P11, P12, C11, Langdon 2011 (L11)). MN, combined HD data from MN state only (M10, M11, and M12). Parents: means of the resistant Glenn and the moderate resistant MN00261-4; Check, means of high resistance cultivars 'Alsen'; the high susceptible 'ND2398'; the medium susceptible 'Bacup' and the good resistance 'Parshall. RIL, Recombinant inbred lines. Mean, means of RIL only excluding the means of parents and checks; Min, minimum mean scored in RIL; Max, the maximum mean scored in RIL; least significant differences (*LSD*) at *P*=0.05 for individual entry. (a), LSD for parents and checks. (b), LSD for mean of RIL vs individual RIL, parents, and checks.

GM population displayed normal distribution for almost all FHB– except for DON- as well as for HD traits (Figure 1). Moreover, it produced transgressive RIL segregation based on parental performance. However, distribution of RIL means showed negative skewedness for DON (P10 and G11) and HD (MN) trait. Meanwhile, some RIL showed less values for INC, SEV, FDK, and NDX than both parents specially the high resistant parent Glenn. Similarly, some other RIL showed earliness and lateness photoperiod relative to both parents, with preference allocations to MN and ND, separately.

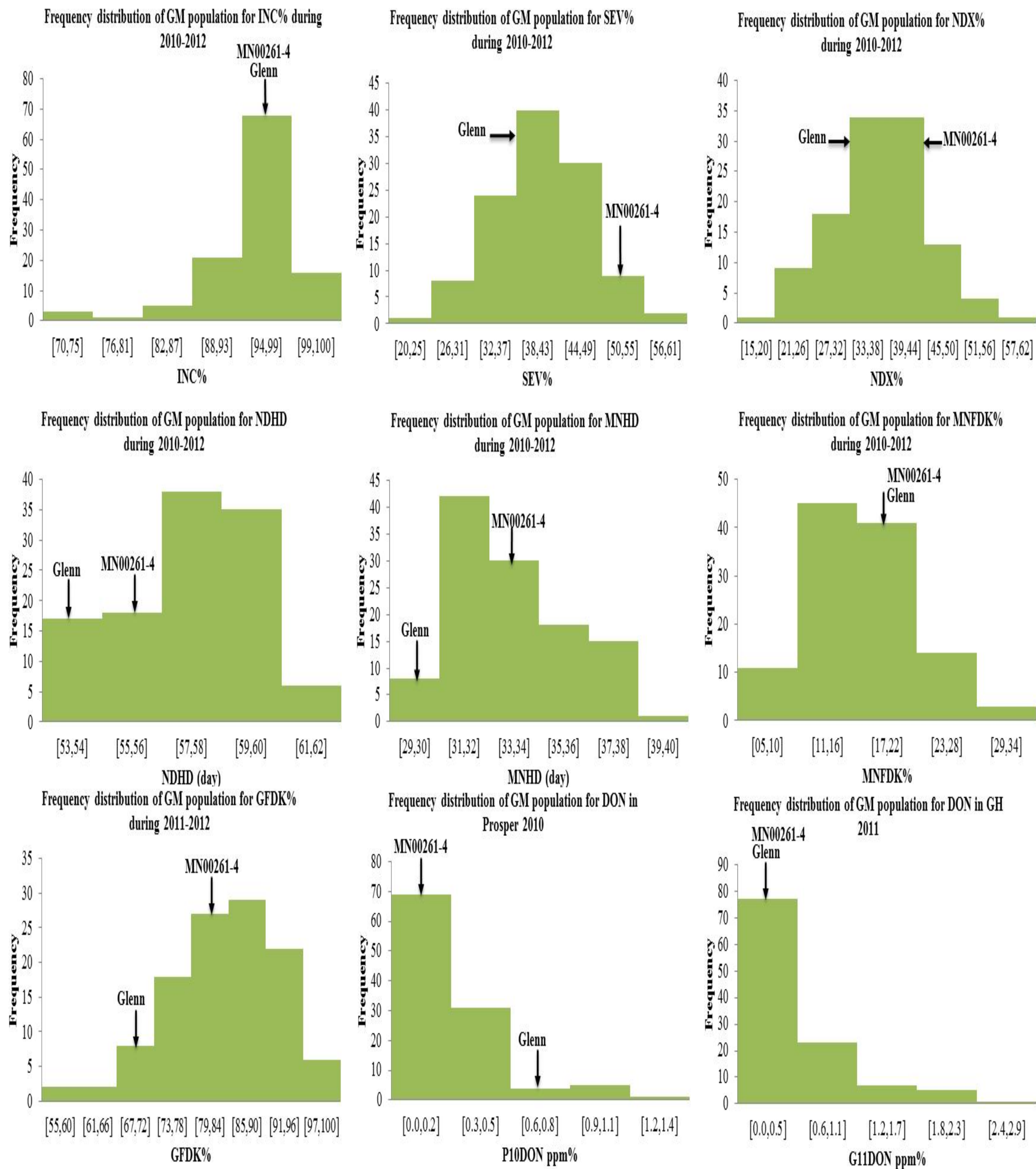

Fig 2.4. Frequency means distribution of GM population for FHB variables (SEV, INC, NDX, DON and FDK) and agronomic traits (HD). Arrows indicate the range of means of the two parents (Glenn and MN00261-4).

Genotypes in GM population displayed significant variation in all FHB and agronomic traits. However, E and G×E effects were significant (Table 2.2), except for FDK trait in MN and GH environments,

it was insignificant. This may be due to the low number of combined environments compared to other FHB traits and the variable influence of environment on FDK trait. Even with the significant *F*-values for E and GXE variances, that confirms the variation due to environments; the sum of squares calculated from the combined ANOVA showed that genotypes (RIL, parents, and checks) are the main sources of variation (data not shown). Given that, the mean squares of genotypes in the combined ANOVA was high to produce high h^2 values (0.69 - 0.93) for SEV, FDK (MN), NDX, and HD (Table 2.2). Also, moderate h^2 (0.48–0.53) for INC and FDK (GH) were produced. ANOVA, h^2, and *LSD* values could not be estimated for DON, because it was a single replicate in ND. Coefficient of correlation values for each trait in different pair of environments were positive, except for SEV in P10, P12, and G11 was insignificantly negative (data not shown). The range of the coefficient of correlations for the combined trait data was between $r = 0.02$ and $r = 0.94$ (Table 2.3). INC trait was consistently and positively correlated with all other FHB resistance types, while negatively correlated with DON (P10), FDK, and HD traits. SEV trait was consistent and positively correlated with all other FHB resistance types, while negatively correlated with FDK (GH) and HD. Resistance to FHB NDX was consistent and positively correlated with other FHB resistance types, while negatively correlated with DON (P10) and FDK (GH). This reflects that resistance types I, II and NDX may have different allele contribution and effects than those for resistance types III and IV. DON trait (G11) was consistently and positively correlated with all other FHB resistance types, while negatively correlated with HD. Meanwhile, DON trait (P10) showed negative correlation with INC and FHB NDX traits. This may reflect the difference between assessment methods of FHB traits under field and GH environments. Likewise, the FDK trait (MN) showed the same fluctuate correlation observed in the DON trait. FDK trait (GH) was negatively correlated with INC, SEV, NDX and HD traits, while positively correlated under field environment (MN). This variation between filed and GH environment is logic due to the controlled conditions in GH. Generally, performance of FHB resistance types I (INC), II (SEV) and NDX is negatively correlated with HD and positively correlated with type III (DON) and type IV (FDK). This means, the earliness the cultivar, the faster it escapes from the early infection of the fungi, the less INC, SEV, and NDX. However, if late infections occurs especially under field conditions, the more time the cultivar is exposed to the disease pressure, and the more effect on kernel developmental stages the higher FDK% and DON levels.

Table 2.2. Heritability, standard error of heritability and analysis of variance for FHB traits and heading dates in GM population across combined environments.

Trait (h^2)	SEV% (h^2=0.69[a]; SE=0.13)				INC% (h^2=0.48[a]; SE=0.13)			
Source	*df*	MS	*F* value	*P*	*df*	MS	*F* value	*P*
G	124	1081.20	3.43***	0.0001	124	307.46	1.99***	0.0001
E	6	40260.00	17.07***	0.0001	3	5261.80	8.90***	0.0027
Rep/E	17	2254.80	11.94***	0.0001	8	517.40	7.50***	0.0001
G x E	710	325.23	1.72***	0.0001	351	159.03	2.30***	0.0001
Error	1980	188.86	.		972	68.99	.	
	NDHD (h^2=0.87[b]; SE=0.13)				MNHD (h^2=0.93[c];SE=0.13)			
	df	MS	*F* value	*P*	*df*	MS	*F* value	*P*
G	121	101.97	7.54***	0.0001	118	24.99	15.31***	0.0001
E	4	15669.00	560.37***	0.0001	1	1095.30	466.02***	0.0001
Rep/E	15	21.13	3.24***	0.0001	2	1.43	2.01	0.1358
G x E	484	13.52	2.07***	0.0001	117	1.63	2.30***	0.0001
Error	1852	6.52	.		235	0.71	.	
	MNFDK (h^{22}=0.83[c]; SE=0.13)				GFDK (h^2=0.53[c]; SE=0.14)			
	df	MS	*F* value	*P*	*df*	MS	*F* value	*P*
G	118	347.28	5.94***	0.0001	122	619.25	2.14***	0.0001
E	2	1242.20	1.74	0.3105	1	21756.00	5.89	0.1331
Rep/E	3	702.81	15.61***	0.0001	2	4362.50	14.86***	0.0001
G x E	230	58.60	1.30**	0.0142	118	289.38	0.99	0.5275
Error	332	45.03	.		360	293.51	.	
	NDX% (h^2=0.72[a]; SE=0.13)							
	df	MS	*F* value	*P*				
G	125	1094.40	3.64***	0.0001				
E	4	17286.00	11.21***	0.0003				
Rep/E	11	1431.10	8.50***	0.0001				
G x E	473	311.11	1.85***	0.0001				
Error	1333	168.37	.					

Trait, FHB traits; SEV%, FHB severity of combined locations P, Prosper; 10, the year 2010; 11, the year 2011; 12, the year 2012; C11, Carrington 2011; M10, data from locations in MN;M12; G11, Greenhouse year 2011. h^2, broad sense heritability based on family basis calculated from random analysis of variance (ANOVA) model output; SE, standard error of h^2; INC%, the FHB incidence for combined locations P11; C11; M10 , M10; and M12; NDX%, the FHB index for combined locations P11, P12, C11, M10 and M12; ND-HD combined data of heading days (HD) from ND locations only P10, P11, P12, L11 (Langdon 2011), and C11; MN-HD, the data for HD combined from M10 and M12; MN-FDK, the combined data of *Fusarium* damaged kernel (FDK) percentage in M10, M11, and M12; G-FDK, combined FDK% data from the GH experiment for the years 2011 and 2012; G, variance due to genotypic factor; E, variance due to environmental factor; Rep/E, variance due to number of replicates per environment; G x E, variance due to genotype by environment interaction; Error, variance due to experimental error. MS, means squares; *df*, degrees of freedom; *F*-value; [a] h^2 was estimated based on harmonic means; [b] h^2, was estimated based on means 4 replications; [c] h^2,was estimated based on means 2 replications; *P*, probability of *F*-value at α=0.05, 0.01, 0.001.

Table 2.3. Pearson correlation coefficient (*r*) calculated in GM population between mean values of FHB traits and phenotypic trait tested in combined ND, MN and GH environments through 2010-2012.

Trait	SEV	INC	NDX	NDHD	MNHD	P10DON	G11DON	MNFDK
INC	+0.64***							
NDX	+0.94***	+0.71***						
NDHD	-0.48***	-0.34***	-0.52***					
MNHD	-0.62***	-0.54***	-0.66***	+0.89***				
P10DON	+0.02	-0.11	-0.01	+0.09	+0.07			
G11DON	+0.32***	+0.23*	+0.35***	-0.40***	-0.43***	+0.06		
MNFDK	+0.64***	+0.50***	+0.66***	-0.35***	-0.42***	+0.04	+0.29***	
GFDK	-0.16	-0.12	-0.21*	+0.39***	+0.35***	+0.18*	+0.02	-0.08

Trait; FHB traits; SEV%, FHB severity combined across environments P, Prosper; 10, the year 2010; 11, the year 2011; 12, the year 2012; C11, Carrington 2011; M; MN location; INC%, FHB incidence combined across environments P11; C11; M10 , M10; and M12; NDX%, FHB index combined across environments P11, P12, C11, M10 and M12; ND-HD combined data of heading days (HD) from ND environments P10, P11, P12, L11 (Langdon 2011), and C11; MN-HD, HD data combined across M10 and M12; P10-DON, toxin deoxynivalonel (DON) levels data from single location P10; G11DON, DON data from single location greenhouse in 2011; MN-FDK, combined data of *Fusarium* damaged kernel (FDK) percentage in MN locations M10, M11, and M12; GFDK, combined FDK% data from the G11 and G12; *r*, *Pearson* coefficient of correlation using significant level at $P < 0.05, 0.01,$ and 0.001. (-), reflects negative correlations. (+), reflects positive correlation. (*), reflects significance at P=0.05. (***), reflects significance at P=0.001.

2.4.2. Map Construction

Out of total 2,289 of DArT markers, 659 were polymorphic and used to generate a framework linkage map. A total of 645 markers representing 458 unique loci were successfully mapped to 37 linkage groups belonging to 19 different chromosomes (Table 2.4). Five chromosomes (2B, 4B, 6B, 7A and 7D) had three linkage groups each; eight chromosomes (1B, 2A, 2D, 4A, 5B, 6A, 6D and 7B) had two linkage groups each, while six chromosomes (1A, 1D, 3A, 3B, 3D and 5A) had a single linkage group each. No markers were mapped to 4D or 5D chromosomes. The 645 markers covered a total map distance of 2229 cM, with an average distance of 3.46 cM between two markers. Total map lengths of genome A, B, and D were 879.4, 1106, and 243.6 cM, respectively (Table 2.4).

Table 2.4. Number of chromosomes, groups, markers, unique loci, cumulative kusambi distance and the average distance between two loci in the mapped genome of GM population.

Chromosome	Groups	Markers	Unique loci	K-Distance (cM)	Average Distance (cM)
1A	1	58	39	166.1	4.3
2A	2	26	17	80.1	4.7
3A	1	30	26	153.5	5.9
4A	2	14	11	71.5	6.5
5A	1	17	11	88.7	8.1
6A	2	58	45	237.1	5.3
7A	3	37	25	82.4	3.3
Total A	12	240	174	879.4	38.0
1B	2	50	42	196.7	4.7
2B	3	49	38	151.2	4.0
3B	1	30	21	241.6	11.5
4B	3	21	16	113.2	7.1
5B	2	40	33	215.3	6.5
6B	3	37	30	88.7	3.0
7B	2	38	23	99.3	4.3
Total B	16	265	203	1106.0	41.0
1D	1	10	8	67.3	8.4
2D	2	9	7	27.3	3.9
3D	1	40	22	70.3	3.2
4D	0	0	0	0.0	0.0
5D	0	0	0	0.0	0.0
6D	2	13	10	12.5	1.3
7D	3	68	34	66.2	2.0
Total D	9	140	81	243.6	18.7
Genome	37	645	458	2229.0	4.9

Genome/Chromosomes: numbers refer to the specified genome number, while letters refer to the chromosome. Groups: the number of chromosomal groups generated in each specified chromosome. Markers: the number of markers mapped in each chromosome. Unique loci: the number of unique (unrepeatable) markers after removing the co-segregating markers in each specified chromosome. Distance: the total commutative Kosambi mapping distance identified in each chromosome. Average distance: the means of distance calculated by dividing the Distance/ the number of unique loci in each chromosome.

2.4.3. QTL Analysis

2.4.3.1. QTL Identified for FHB Resistance Types I, II, and NDX

CIM for resistance type I revealed a total of five major QTL on four different chromosomes (Table 2.5; Fig 2.5). QTL contributing to ≥10% of PV were considered major QTL. *QINC.1AS* QTL was detected in two environments including the combined means across environments. All other remaining QTL were detected in single environment in MN, except the *QINC.3B* QTL was detected in ND environment. The range of PV explained by the QTL for resistance type I ranged between 12.00 and 26.10%. The range of additive effect was between -03.70 and -06.80%.

Eleven major QTL were identified for resistance type II on seven different chromosomes (Table 2.5; Fig 2.5). One major and stable QTL (*QSEV.5B1L.d*) was identified in 50% of environments. This QTL was identified preferentially expressed in ND environments only. Another QTL (QSEV-*6B2*) was detected in two environments and confirmed by the combined means across environments. The *QSEV.5B1.c* QTL was detected in only two environments, while the remaining eight QTL were detected in only one environment. The range of PV explained by the QTL for resistance type II was between 11.90 to 21.40%. The range of additive effect was between +03.20 and ±09.50%.

For FHB NDX, six major QTL were identified on five different chromosomes (Table 2.5, Fig 2.5). Two QTL (*QNDX.5BL1.a* and *QNDX.5BL1.b*) were detected exclusively on chromosome 5B. One major QTL (*QNDX-5BL1.b*) was detected in two environments and confirmed by the combined means across environments and therefore, considered stable. The range of PV for NDX was between 12.10 to 22.70%. The range of additive effect was between -03.20 and -06.20%.

Glenn contributed alleles at two QTL (*QINC.3B* and *6A2*), while MN00261-4 contributed alleles at the remaining three QTL for resistance type I. Meaning that, alleles provided for resistance type I by MN00261-4 has lowered INC values (increased resistance) especially at the major and stable *QINC.1AS* QTL. Both parents Glenn and MN00261-4 contributed alleles at two QTL (*QSEV.5BL1.c* and *5BL1.d*) for resistance type II. Meanwhile, Glenn only increased SEV values (lowered resistance) at three QTL (*QSEV.2B2*, *5BL1.b* and *5BL1.e*) and MN00261-4 reduced the SEV values (increased resistance) at the remaining six QTL for resistance type II. Glenn increased NDX values (lowered resistance) at three QTL including the major and stable QTL, while MN00261-4 reduced the NDX values (increased resistance) at the remaining three QTL for FHB NDX. This means, GM population acquired new improvement alleles form MN00261-4, while negatively affected by alleles from Glenn at these QTL for resistance types I, II and NDX. Given that, all QTL were expressed under ND environments, this may propose MN00261-4 as the major source of improved alleles for resistance types I, II and NDX in GM population. This theory is supported based on the susceptible allelic effect existed in Glenn from the Sumai3 background. Further, allelic forms at these loci in Glenn has reached its homologous saturation effect by recombination, while in MN00261-4 the non-homologous effects are to be added. This means that recombination frequencies for resistance

types I, II and NDX at these alleles in Glenn are depleted, meanwhile, crossing over events generated by crossing Glenn to Mn00261-4 in the GM population allowed for new recombination at these QTL positions. This theory may explain the transgressive segregation produced in GM population and the RIL with higher resistance phenotype than both parents.

Table 2.5. QTL identified for FHB resistance type I (INC), II (SEV) and FHB-index (NDX), III (DON), IV (FDK) and HD in GM population.

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QINC.1AS	INC	wPt-6269-wPt-7872	M10, A	22	18-38	-04.60	03.50***	13.30	2.86-3.74
QINC.3B	INC	wPt-8140-wPt-6047	P12	56	52-68	+06.80	04.30***	16.10	2.86-3.36
QINC.6A1	INC	wPt-6904-wPt-733976	M12	90	88-90	-03.70	03.50**	13.30	2.85-3.52
QINC.6A2	INC	tPt-6661-wPt-667430	M12	32	28-32	+04.80	03.10**	12.00	2.85-3.52
QINC.7A1	INC	wPt-740561-wPt-0002	M12	56	46-56	-06.80	07.40***	26.10	2.85-3.52
QSEV.2B2	SEV	wPt-798970-tPt-6487	P11	32	28-34	+03.20	04.50***	16.90	2.90-3.55
QSEV.2D1	SEV	wPt-0330-wPt-671665	P12	06	04-08	-05.30	03.10**	11.90	3.00-3.75
QSEV.3B	SEV	wPt-798970-tPt-6487	C11	00	00-04	-03.60	03.90***	14.70	3.00-3.68
QSEV.5BL1a	SEV	wPt-5737-wPt-3012	P12	88	86-94	-05.30	04.00***	15.20	3.00-3.75
QSEV.5BL1b	SEV	wPt-4246-wPt-3049	G11	132	126-132	+09.50	04.60***	17.10	3.00-3.98
QSEV.5BL1c	SEV	wPt-4246-wPt-3049	P10, G11	138	134-144	±09.50	05.10***	19.10	3.00-3.98
QSEV.5BL1d	SEV	wPt-3049-wPt-5604	P10, P12, C11, G11	154	146-156	±06.80	05.30***	19.40	3.00-3.98
QSEV.5BL1e	SEV	wPt-5604-wPt-2707	C11	158	158-160	+03.70	05.20***	19.30	3.00-3.68

Table 2.5. QTL identified for FHB resistance type I (INC), II (SEV) and FHB-index (NDX), III (DON), IV (FDK) and HD in GM population (continued).

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QSEV.6B2	SEV	wPt-6522-wPt-2564	P11, M12, A	54	52-60	-06.00	03.80***	14.30	2.90-3.75
QSEV.7A1	SEV	wPt-740561-wPt-0002	M12	56	48-56	-08.20	05.90***	21.40	3.00-3.75
QSEV.7D1	SEV	wPt-744917-wPt-666162	A	28	26-40	-04.10	04.80***	17.90	3.12-3.74
QNDX.1B1	NDX	wPt-1248-wPt-3566	M10	114	112-114	+04.10	03.10**	12.10	3.04-3.73
QNDX.2B2	NDX	wPt-798970-tPt-6487	P11	32	28-34	+03.30	04.60***	17.30	3.13-3.84
QNDX.5BL1a	NDX	wPt-5185-wPt-3012	C11	90	90	-03.20	03.10**	12.10	3.12-3.77
QNDX.5BL1b	NDX	wPt-3049-wPt-2707	C11, P12, A	156	146-160	+05.90	05.70***	21.00	3.08-3.84
QNDX.6B2	NDX	wPt-6522-wPt-3168	M12	56	54-58	-06.20	03.40**	13.00	2.96-3.71
QNDX.7A1	NDX	wPt-740561-wPt-0002	M12, A	56	48-56	-09.40	06.30***	22.70	2.96-3.84
QDON.5B1	DON	wPt-3049-wPt-5604	G11	150	146-156	+02.40	03.50***	13.60	2.80-3.39
QFDK.1B1	FDK	wPt-8168-wPt-3451	G12	102	100-102	-03.00	03.50**	13.20	3.10-3.72
QFDK.2B2	FDK	wPt-6477-wPt-1920	G11	36	36	-05.50	04.30***	16.10	3.15-3.79
QFDK.3D	FDK	wPt-740613-wPt-740653	G11	30	30	+04.90	03.90***	14.80	3.15-3.79
QFDK.5B2	FDK	wPt-6971-wPt-2804	M10	00	00	+01.80	03.20**	12.30	3.15-4.03
QFDK.7B2	FDK	wPt-669693-wPt-9813	G12	06	06	-02.90	03.30**	12.60	3.10-3.72
QFDK.7D1	FDK	wPt-663992-wPt-743384	M11, MN	12	12	-02.10	04.60***	17.30	3.10-3.94
QHD.1D	HD	wPt-666986-wPt-4971	M10	00	00-02	+00.80	03.70**	14.00	3.05-3.99
QHD.5BL1a	HD	wPt-9006-wPt-3055	MN	16	16	-00.70	03.20**	12.40	3.20-3.76
QHD.5BL1b	HD	wPt-4246-wPt-1733	P12, L11	146	136-152	-01.30	06.60***	23.70	3.02-3.76
QHD.5BL1c	HD	wPt-1733-wPt-2707	P10, C11, P11, M12, MN, ND, M10	158	154-160	-02.10	14.80***	45.50	3.05-4.10
QHD.6AS1a	HD	wPt-743282-wPt-9687	ND	58	50-62	+00.90	03.80***	14.50	3.10-3.80

Table 2.5. QTL identified for FHB resistance type I (INC), II (SEV) and FHB-index (NDX), III (DON), IV (FDK) and HD in GM population (continued).

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QHD.6AS1b	HD	wPt-741170-wPt-729877	P10, MN, M12	80	80-82	+01.20	04.30***	16.30	3.05-3.95
QHD.6B2	HD	wPt-9971-wPt-0052	P10	16	14-16	-01.20	03.20**	12.10	3.08-3.95
QHD.6DS1	HD	wPt-1695-wPt-732847	M12, MN	02	00-06	+00.90	04.30***	16.10	3.05-3.76
QHD.7A3	HD	wPt-1976-wPt-1706	P11	14	10-16	+01.30	03.70**	14.30	3.20-4.10

QTL, name of QTL assigned to its chromosomal groups. TRT, FHB trait/variable. MRK, flanking markers. LOC, environment locations. Pos, Position of QTL peaks. CI, Confidence intervals of QTL. Add, additive effect. LOD, Logarithmic likelihood of odds. R^2%, percentage of phenotypic value. Perm LOD, LOD value based on permutation test for each individual environment using 1000 replications and 30 threads. SEV, percentage of FHB severity. INC, percentage of FHB incidence. NDX, percentage of FHB-index. HD, days to heading. DON, concentration of deoxynivalenol. FDK, percentage of *Fusarium* damaged kernel. LOC, environment locations (P, Prosper. C, Carrington. L, Langdon. G, greenhouse. M, MN state. ND, combined means across ND state locations. MN, combined means across MN state locations. A, All combined data of the specified FHB traits (INC, SEV and NDX). 10, the year 2010. 11, the year 2011. 12, the year 2012. [a], significance thresholds were estimated by permutation test (1000 iterations) for α=0.05 and 0.01.

2.4.3.2. QTL Identified for FHB Resistance Types III, and IV

One major QTL was detected for resistance type III in single environment and located on 5BL1 chromosome (Table 2.5; Fig 2.5). The PV explained by this QTL was 13.60%. The additive effect was +02.40%. Six major QTL were detected for resistance type IV on six different chromosomes (Table 2.5; Fig 2.5). The range of PV was between 12.30 and 17.30%. The *QFDK.7DS1* QTL was all detected with preference to MN environments and the highest PV (17.30%). The range of additive effect was between +01.80 and -05.50%. Glenn increased values for DON (lowered resistance) at the only identified *QDON.5BL1* QTL for resistance type III. Glenn also increased FDK trait values (lowered resistance) at two QTL, while MN00261-4 lowered the FDK values (increased resistance) to the remaining four QTL for resistance type IV. This means that MN00261-4 parent was the major contributor of alleles to enhance resistance types III and IV in GM population, while Glenn still contains susceptible alleles from the Sumai3 parental background. Also it may suggest that alleles bred for FHB resistance in MN environments (MN00261-4) may have preferred expression effect in ND environments

2.4.3.3. QTL Identified for Agronomic Trait HD

Nine QTL were identified for HD on six different chromosomes (Table 2.5; Fig 2.5). The most stable *QHD.5BL1.c* QTL was detected in seven environments with 45.50% of PV. Two important (*QHD.5BL1.b* and *6AS1.b*) QTL were identified in two environments, where the *QHD.6AS1.b* QTL was additionally confirmed by the combined means across MN environments and explained 23.70 and 16.30% of PV, respectively. Another stable *QHD.6DS1* QTL was detected in one environment and confirmed by the combined means across MN with 16.10% of PV. The range of overall PV for HD was between 12.10 and 45.50%. The range of additive effect was between -00.70 and -02.10%. Glenn alleles increased the HD trait values (lateness) at five QTL, while the MN00261-4 alleles decreased HD values (earliness) at the remaining four QTL. This may suggest that earliness alleles affecting photoperiod in MN00261-4 have more effect to be added for earliness trait to GM population than effects of alleles from Glenn at the same specified QTL.

2.4.3.4. Linked/Pleiotropic QTL Identified for FHB Variables and Agronomic Traits

Seven QTL (*QFHB.1B1*, *2B2*, *5BL1.a*, *5BL1.b*, *6AS1*, *6B2* and *7A1*) were identified for multiple FHB resistance types and agronomic trait with overlapped CI (Table 2.6). This could be due to pleiotropic effect of gene/QTL affecting different FHB variables; tight linkage between different genes/QTL; or these CI regions were belonging to the same QTL. One important (*QFHB.5BL1.b*) QTL was detected in seven environments and confirmed by four combined means across environments with 45.50% of PV for resistance type II, III, NDX and HD and considered stable. Three other (*QFHB.1B1*, *2B2*, and *5BL1.a*) QTL out of the seven were detected in two environments with 13.20 to 17.30% of PV and considered stable for resistance types II, IV and NDX. One (*QFHB.6AS1*) QTL was detected in two environments and confirmed by means across MN environments with 16.30% of PV and considered stable for resistance type I and HD. One (*QFHB.6B2*) QTL was detected in two environments and confirmed by the combined means across environments for resistance types II and NDX with 14.30% of PV. One (*QFHB.7A1*) QTL was detected in one environment and additionally in combined means across environments for resistance types I, II and NDX with 26.10% of PV. Overall, the range of PV explained by the seven QTL with multiple FHB resistance

types was between 13.20 and 45.50%. The range of additive effect was between ±03.72 and ±09.46%. Both parents contributed alleles at four QTL, while the MN00261-4 contributed at the remaining three QTL.

Table 2.6. QTL identified in multiple datasets and/or multiple FHB-variables and agronomic traits in GM population.

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QINC.1A	INC	wPt-6269-wPt-7872	M10,AINC	22	18-38	-04.64	3.47***	13.30	2.86-3.74
QFHB.1B1	NDX,FDK	wPt-8168-wPt-3566	G12,M10	102	100-114	±04.05	3.45**	13.20	3.04-3.73
QHD.1D	HD	wPt-666986-wPt-4971	M10	00	00-02	+00.82	3.67**	14.00	3.05-3.99
QFHB.2B2	SEV,NDX, FDK	wPt-8492-wPt-1920	P11,G11	32	28-36	±05.51	4.62***	17.30	2.90-3.84
QSEV.2D1	SEV	wPt-0330-wPt-671665	P12	06	04-08	-05.30	3.08**	11.90	3.00-3.75
QSEV.3B	SEV	wPt-798970-tPt-6487	C11	00	00-04	-03.61	3.85***	14.70	3.00-3.68
QINC.3B	INC	wPt-8140-wPt-6047	P12	56	52-68	+06.77	4.27***	16.10	2.86-3.36
QFDK.3D	FDK	wPt-740613-wPt-740653	G11	30	30	+04.85	3.89***	14.80	3.15-3.79
QHD.5B1	HD	wPt-9006-wPt-3055	MN-HD	16	16	-00.70	3.21**	12.40	3.20-3.76
QFHB.5B1a	SEV,NDX	wPt-5737-wPt-3012	C11,P12	88	86-94	-05.30	4.00***	15.20	3.00-3.77
QFHB.5B1b	SEV,NDX, DON,HD	wPt-4246-wPt-2707	P10,G11,P12,L11HD,C11,ANDX,P11,M12HD,MNHD,NDHD,M10HD	158	126-160	±09.46	14.8	45.50	2.80-4.10
QFDK.5B2	FDK	wPt-6971-wPt-2804	M10	00	00	+01.82	3.18**	12.30	3.15-4.03
QHD.6A1	HD	wPt-730591-wPt-9687	NDHD	58	50-62	+00.85	3.80***	14.50	3.10-3.80
QFHB.6A1	INC,HD	wPt-7754-wPt-733976	P10,MNHD,M12	80	80-90	±03.72	4.33***	16.30	2.85-3.95
QINC.6A2	INC	tPt-6661-wPt-667430	M12	32	28-32	+04.79	3.11**	12.00	2.85-3.52
QHD.6B2	HD	rPt-1806-wPt-0052	P10	16	14-16	-01.16	3.15**	12.10	3.08-3.95
QFHB.6B2	SEV,NDX	wPt-6522-wPt-3168	P11,M12,ASEV	56	52-60	-06.16	3.40***	14.30	2.90-3.75
QHD.6D1	HD	wPt-1695-wPt-741955	M12,MNHD	02	00-06	+00.88	4.27***	16.10	3.05-3.76
QFHB.7A1	INC,SEV, NDX	wPt-740561-wPt-0002	M12,ANDX	56	48-56	-09.39	7.35***	26.10	2.85-3.84
QHD.7A3	HD	wPt-1976-wPt-1706	P11	14	10-16	+01.27	3.74**	14.30	3.20-4.10
QFDK.7B2	FDK	wPt-669693-wPt-9813	G12	06	06	-02.86	3.28**	12.60	3.10-3.72
QFDK.7D1	FDK	wPt-663992-wPt-743384	M11,MN	12	12	-02.07	4.55***	17.30	3.10-3.94
QSEV.7D1	SEV	wPt-744917-wPt-666162	ASEV	28	26-40	-04.10	4.78***	17.90	3.12-3.74

QTL, name of QTL assigned to its chromosomal groups. TRT, FHB trait/variable. MRK, flanking markers. LOC, environment locations. Pos, Position of QTL peaks. CI, Confidence intervals of QTL. Add, additive effect. LOD, Logarithmic likelihood of odds. R^2%, percentage of phenotypic value. Perm LOD, LOD value based on permutation test for each individual environment using 1000 replications and 30 threads. SEV, percentage of FHB severity. INC, percentage of FHB incidence. NDX, percentage of FHB-index. HD, days to heading. DON, concentration of deoxynivalenol. FDK, percentage of *Fusarium* damaged kernel. LOC, Locations. P, Prosper. C, Carrington. L, Langdon. G, greenhouse. M, MN state. ND, combined means across ND state locations. MN, combined means across MN state locations. A, All combined data of the specified FHB traits (INC, SEV and NDX). 10, the year 2010. 11, the year 2011. 12, the year 2012. [a], significance thresholds were estimated by permutation test (1000 iterations) for α=0.05 and 0.01.

Table 2.7. QTL identified for FHB resistance types based on genome type.

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QINC.1AS	INC	wPt-6269-wPt-7872	M10,AINC	22	18-38	-4.60	3.50***	13.30	2.86-3.74
QINC.6AL2	INC	tPt-6661-wPt-667430	M12	32	28-32	+4.80	3.10**	12.00	2.85-3.52
QHD.6AS1a	HD	wPt-743282-wPt-9687	ND	58	50-62	+0.90	3.80***	14.50	3.10-3.80
QHD.6AS1b	HD	wPt-741170-wPt-729877	P10,MN,M12	80	80-82	+1.20	4.30***	16.30	3.05-3.95
QINC.6AS1	INC	wPt-6904-wPt-733976	M12	90	88-90	-3.70	3.50**	13.30	2.85-3.52
QHD.7AL3	HD	wPt-1976-wPt-1706	P11	14	10-16	+1.30	3.70**	14.30	3.20-4.10
QFHB.7AS1	SEV,NDX,INC	wPt-740561-wPt-0002	M12,ANDX	56	48-56	-9.40	7.40***	26.10	2.85-3.84
QFDK.1BL1a	FDK	wPt-8168-wPt-3451	G12	102	100-102	-3.00	3.50**	13.20	3.10-3.72
QNDX.1BL1b	NDX	wPt-1248-wPt-3566	M10	114	112-114	+4.10	3.10**	12.10	3.04-3.73
QFHB.2BL2	SEV,NDX,FDK	wPt-798970-wPt-1920	P11,G11	32-36	28-36	±5.50	4.60***	17.30	2.90-3.84
QSEV.3BS	SEV	wPt-798970-tPt-6487	C11	0	00-04	-3.60	3.90***	14.70	3.00-3.68
QINC.3BL	INC	wPt-8140-wPt-6047	P12	56	52-68	+6.80	4.30***	16.10	2.86-3.36
QFDK.5BL2	FDK	wPt-6971-wPt-2804	M10	0	0	+1.80	3.20**	12.30	3.15-4.03
QHD.5BS1	HD	wPt-9006-wPt-3055	MN	16	16	-0.70	3.20**	12.40	3.20-3.76
QFHB.5BL1a	SEV,NDX	wPt-5737-wPt-3012	P12,C11	88-90	86-94	-5.30	4.00***	15.20	3.00-3.77
QFHB.5BL1b	SEV,NDX,DON ,HD	wPt-4246-wPt-2707	P10,P11,P12,L11 ,C11,ANDX,M10, M12,G11,MNHD, NDHD	132-158	126-160	±09.5 0	14.80***	45.50	2.80-4.10
QHD.6BS2a	HD	wPt-9971-wPt-0052	P10	16	14-16	-1.20	3.20**	12.10	3.08-3.95
QFHB.6BS2b	SEV,NDX	wPt-6522-wPt-3168	P11,M12,ASEV	54-56	52-60	-6.20	3.80***	14.30	2.90-3.75
QFDK.7BL2	FDK	wPt-669693-wPt-9813	G12	6	6	-2.90	3.30**	12.60	3.10-3.72
QHD.1DS	HD	wPt-666986-wPt-4971	M10	0	00-02	+0.80	3.70**	14.00	3.05-3.99
QSEV.2DS1	SEV	wPt-0330-wPt-671665	P12	6	04-08	-5.30	3.10**	11.90	3.00-3.75
QFDK.3DS	FDK	wPt-740613-wPt-740653	G11	30	30	+4.90	3.90***	14.80	3.15-3.79
QHD.6DS1	HD	wPt-1695-wPt-732847	M12,MN	2	00-06	+0.90	4.30***	16.10	3.05-3.76
QFDK.7DS1	FDK	wPt-663992-wPt-743384	M11,MN	12	12	-2.10	4.60***	17.30	3.10-3.94
QSEV.7DS1	SEV	wPt-744917-wPt-666162	ASEV	28	26-40	-4.10	4.80***	17.90	3.12-3.74

QTL, name of QTL assigned to its chromosomal groups. TRT, FHB trait/variable. MRK, flanking markers. LOC, environment locations. Pos, Position of QTL peaks. CI, Confidence intervals of QTL. Add, additive effect. LOD, Logarithmic likelihood of odds. R^2%, percentage of phenotypic value. Perm LOD, LOD value based on permutation test for each individual environment using 1000 replications and 30 threads. SEV, percentage of FHB severity. INC, percentage of FHB incidence. NDX, percentage of FHB-index. HD, days to heading. DON, concentration of deoxynivalenol. FDK, percentage of *Fusarium* damaged kernel. LOC, environment locations (P, Prosper. C, Carrington. L, Langdon. G, greenhouse. M, MN state. ND, combined means across ND state locations. MN, combined means across MN state locations. A, All combined data of the specified FHB traits (INC, SEV and NDX). 10, the year 2010. 11, the year 2011. 12, the year 2012. [a], significance thresholds were estimated by permutation test (1000 iterations) for α=0.05 and 0.01.

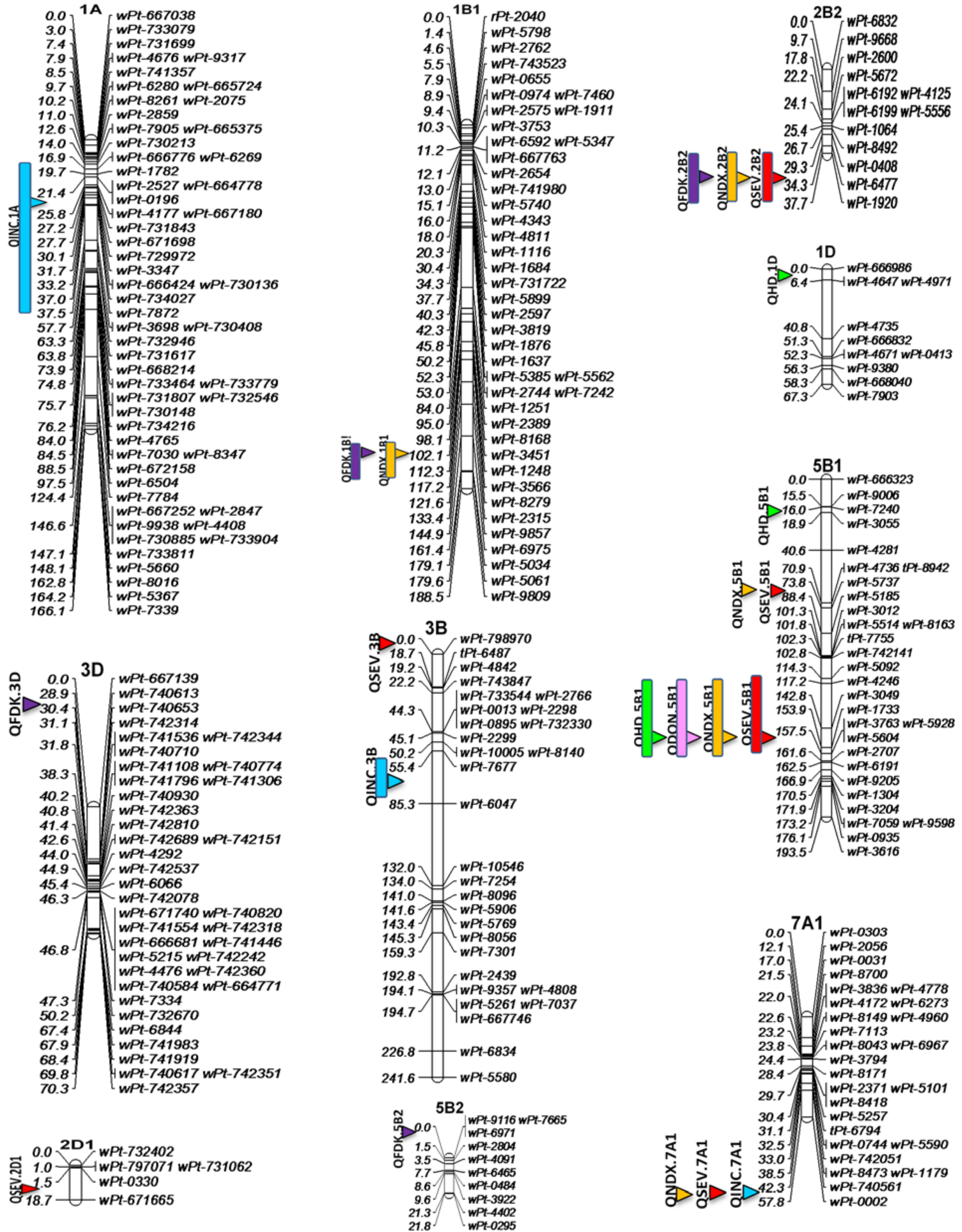

Fig 2.5. Chromosomal map of QTL identified in GM population. QTL intervals shown as (lines); QTL positions shown as (Triangles); QTL for INC, SEV, NDX, DON, FDK, and HD were shown in (Blue, Red, Yellow, Pink, Purple and Green) respectively).

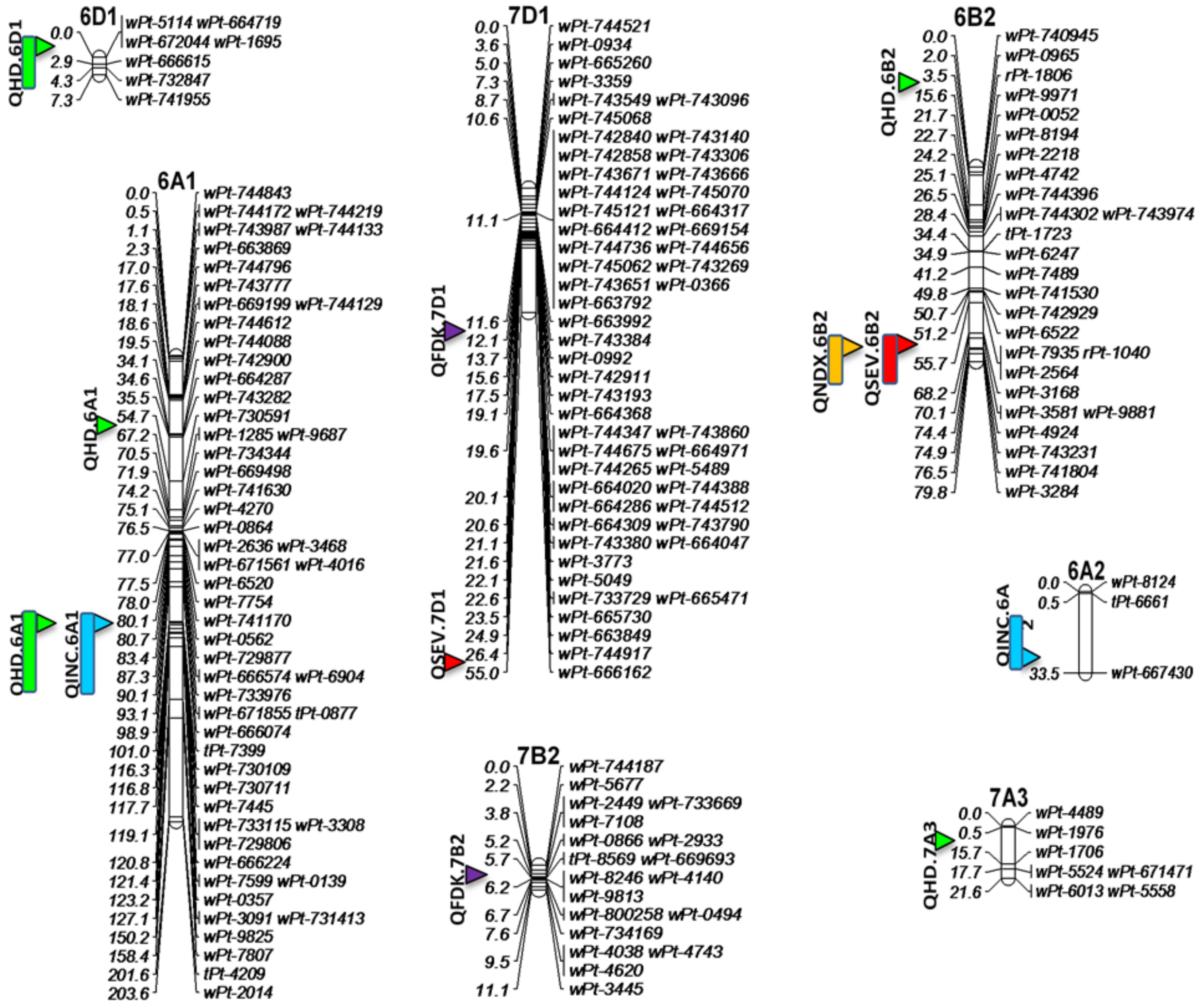

Fig 2.5. Chromosomal map of QTL identified in GM population (continued).

2.5. Discussion

2.5.1. Phenotypic Data

As expected, resistance type II was positively correlated with all other resistance types I, III, and IV and NDX. This reflects that the higher the resistance type II, the higher the resistance types I, III, IV and FHB NDX. Unexpectedly, a negative - though low - and insignificant correlation was observed between resistance type II and IV in GH environment; though found significantly positive in the MN field environment (Table 2.3). It was reported that single-floret injection (as applied in GH) conferred a lower correlation between type II resistance and some agronomic traits rather than the correlation conferred by grain-spawn

inoculation method (as applied in field) (Buerstmayer et al. 2013). Therefore, this observed fluctuation (GH/MN) in the correlation of resistance type II/IV may be influenced by the inoculation method. Further, deviation in correlation coefficient was previously reported (Paul et al. 2005) when two different resistance mechanisms (type II and IV) were assayed. Nevertheless, some RIL, such as RIL-102, showed high-significant transgressive resistance than both parents for all FHB resistance types (data not shown). Other RIL were highly and consistently resistant to FHB resistance type(s) I and/or II, while fluctuating in consistency for resistance type(s) III and/or IV. This may reflect the basic genetic differences in the resistance mechanisms and/or the genetic factors that modulate resistance types I/II and III/IV. Differences are also reflecting the lower liability of resistance type III and IV vs. the most common resistance types I and II to evaluate cultivars under FHB disease pressure.

Previous studies have reported negative correlation between HD and the different FHB resistance types under field conditions (Emrich et al. 2008). Knowing that Glenn heads earlier than MN00261-4 (Anderson, et al. 2010); only one RIL (GM-19) under ND and three RIL (GM-9, 42 and 86) under MN environments headed earlier than Glenn. RIL headed later than Glenn, have exposed to disease conducive conditions for shorter time. Controlled inoculation and assessment methods used in GH mitigated the interaction of HD and disease development. In field studies, the potential interaction between HD and disease onset could not be mitigated. The influence of HD on FHB traits in both ND and MN environments was negatively correlated with resistance type III and IV in MN. Exceptionally, HD was positively correlated with resistance type IV scored in GH environment; which could be due to the same reasons previously stated for the fluctuate correlations noticed between resistance type II/IV for field and GH environments.

Resistance type III was highly correlated with infection at early grain development (Randhawa et al. 2013). Therefore, the low positive correlation between type III and IV in Prosper may be due to early planting in 2010 that exposed the seeds to the disease pressure for a longer time. Given that, the inoculum level has a factor on disease development. Generally, this fluctuation in correlation data was evidence that resistance types III and IV were genetically independent of the other types I and II and was critically affected by G x E interactions and assessment methods. These observations support the generally held opinion that

FHB evaluation in wheat is most reliable and preferred if applied by using resistance type I, II and NDX (Bai and Shaner 1996; Buerstmayr et al. 2013).

2.5.2. Interactive QTL for FHB Resistance Identified in the A Genome

Seven total QTL were identified in the A genome. Four QTL were identified for resistance type I, one QTL each for resistance type II and FHB NDX and three QTL for HD (Tables 2.7). Among these seven QTL, the major QTL *QINC.1AS* for resistance type I was detected with preference to MN environments. This QTL was in the same region (18 - 38 cM), where a QTL for FHB resistance was previously reported in Chinese landrace 'Haiyanzhong' (Gilbert and Haber 2013). In a meta-QTL analysis, the same region was also reported in 'Frontana' and 'Arina' (Löffler et al. 2009) and in 'Pirate', 'Wheaton', 'CJ9306', and 'Pelikan' (Liu et al. 2009).

Another important *QINC.6AL2* QTL was associated with resistance type I in GM population was also reported for resistance type I, II and III (Zwart et al. 2008; Lu et al. 2013; Liu et al. 2013). The *6AL2* QTL region (28 32 cM) in GM was previously identified for conferring resistance type II in a Sumai3-derived hexaploid wheat population (Ma et al. 2006) and in a Tunisian-derived durum wheat population (Ghavami et al. 2011). In the winter wheat (Arina), the *6AL2* QTL region was associated with plant height (HGT) (Draeger et al. 2007), while associated with HD and wilted tips in European wheat (Zwart et al. 2008). The region (80 – 82 cM) of *QHD.6AS1.b* QTL was consistently associated with HD across three environments in GM population (Table 2.7). However, the *6AS1.b* QTL was previously associated with harvest index in wheat (Kumar et al. 2007). The region (10 - 16 cM) of *QHD.7AL3* QTL in GM population for HD was previously reported for reduced HGT (Liu et al. 2013) and grain yield in wheat (Kumar et al. 2007). Among these GM QTL on genome A, the most important QTL for FHB resistance was identified on 7AS1. This *QFHB.7AS1* QTL (48 – 56 cM) had major and stable effect on resistance type I, II and NDX with preference expression under MN environments. The alleles for increased resistance in this QTL were contributed by MN00261-4. The same locus was previously reported for resistance type II, IV and yield loss in winter wheat Arina (Draeger et al. 2007) and for number of grains per spike in wheat (Kumar et al. 2007). This may

suggest that, QTL provided by the A genome have pyramided advances of FHB resistance, agronomic and yield component traits in GM population.

2.5.3. Interactive QTL for FHB Resistance Identified in the B Genome

In the GM population, the B genome harbored total of 14 QTL; one QTL for resistance type I; six for resistance type II; five for NDX; one for resistance type III; four each for resistance type IV and HD (Tables 2.7). Among these 14 QTL, the two QTL on 1BL for resistance types IV and NDX. The most important region (100 - 102 cM) in GM population was associated to the major *QFDK.1BL1.a* QTL for resistance type IV. The same region was previously reported for resistance type II and III in a double haploid (DH) population derived from the cross Arina/'NK93604' (Semagn et al. 2007). Likewise, the same region was reported for resistance type II in Arina, 'Pirat', 'Biscay' and 'History' (Liu et al. 2009). Another region (112 - 114 cM) that was considered new on *QNDX.1BL1.b* QTL had a major effect for FHB NDX resistance in GM population with preference to the MN environments and was not reported in previous studies. In this study, the genomic region (28 - 36 cM) of *QFHB.2BL2* QTL was associated with resistance type II, IV and NDX (Table 2.7). This region was previously reported for resistance type II in cultivar 'Goldfield' (Buerstmayr et al. 2009) and for resistance type II and III in Shanghai-3/Catbird cross (Lu et al. 2013). The same region was also found closely linked to a HGT QTL in winter wheat (Liu et al. 2013) and earliness trait of photoperiod sensitivity *Ppd-B1* locus (Chu et al. 2011). The *2B2L* region was also reported for number of tillers per plant, spikelets per spike and grains per spike in wheat (Kumar et al. 2007). All this enlarging the importance of the 2B2L region as multiple trait effector on GM population for FHB resistance as well as for other important yield-related traits.

The 3B chromosome harbored the major and most famous QTL 3BS/*Fhb1* for FHB resistance. However, the GM population harbored one QTL each for resistance types I and II (Table 2.7) on the long and short arms of the 3B chromosome. The region of the *QSEV.3BS* QTL that was identified in the GM population for resistance type II was previously associated with type I, II, III, and IV resistance in the Chinese line 'W14' (Chen et al. 2006). The region of the *QINC.3BL* QTL that was identified in the GM population for resistance type I was previously associated with resistance type II and III in 'Apache', Wangshuibai, Arina,

‘Ernie’ and ‘Massey’ (Basnet et. al. 2012) and also for salinity tolerance in wheat (Sardouie-Nasab et al. 2013). This QTL has the only region in GM population with a potential drought/salinity tolerance effect.

On chromosome 5B, three QTL for resistance types II; two QTL for NDX, one QTL each for type III and IV and three QTL for HD (Table 2.7) were identified. The short arm on chromosome 5B harbored the *QHD.5BS1* QTL for HD with MN environment specificity, where MN00261-4 contributed alleles for earliness. This QTL at 16 cM position was previously reported for resistance type II in a Tunisian durum wheat population (Ghavami et al. 2011) and for resistance type I and NDX in the BGRC3487/2*DT735 tetraploid population (Ruan et al. 2012). Within the 74 cM region on 5BL1, two stable and major QTL (*FHB.5BL1.a* and *5BL1.b*) were identified across at least two environments. The most stable and major region (126 - 160 cM) was identified across 10 environments for resistance type II, III, NDX and HD (Table 7). This QTL has no previous identification and, therefore, was considered new. Additionally, the other stable and major (86 - 94 cM) region was lso identified across two environments for resistance type II and NDX in GM population. This region had a favorable allele contribution from MN00261-4 that reduced values of severity and FHB NDX under ND environments. Previously, the same region was associated with type II resistance in tetraploid and hexaploid wheat (Ruan et al. 2012; Buerstmayr et al. 2009). The *QFDK.5BL2* QTL that was associated in GM population to resistance type IV (Table 2.7), had allele contribution from Glenn with environmental preference to MN. This region was not previously detected and therefore was considered new. In this population, a stable and major *QFHB.6BS2.b* QTL (52 – 60 cM) was identified for resistance type II and NDX in GM population across three environments. This region was located in overlapping CI where MN00261-4 contributed for increased resistance type II and considered new with no previous records. The 7BL arm in the GM population harbored a major *QFDK.7BL2* QTL for resistance type IV, which was previously reported for resistance type II in ‘CJ9306’ and ‘Rubens’, and for resistance type I in ‘Cansas’ (Liu et al. 2009). This region, in other studies was additionally associated with flowering dates (Buerstmayr et al. 2012) and resistance type II in ‘Dream’ (Buerstmayr et al. 2009 and 2012).

2.5.4. Does the Parental QTL of Sumai3 (*Fhb1*) on 3BS Exist in Glenn?

The *3BS* QTL (*Fhb1*) from Sumai3 was extensively used for FHB resistance in wheat (Waldron et al. 1999). Many FHB mapping studies have reported *Fhb1* as a major source for resistance type I, II, III, and IV (Buerstmayr et al. 2009). Based on pedigree, it was believed that the source of FHB resistance in Glenn could be Sumai3 (and, plausibly, the *Fhb1* locus). However, the *Fhb1* locus acquired some major considerations in its deployment, such as: 1) tight linkage with unfavorable agronomic traits and susceptibility to other diseases (Cuthbert et al. 2007); 2) QTL × genetic background interaction effect (Pumphery et al. 2007); 3) multiple genes (~seven genes) localized in *Fhb1* QTL region (Liu et al. 2008); 4) multiple functions of *Fhb1* genes (Zhuang et al. 2013); 5) both parents of Sumai3 were susceptible (Bai and Shaner 1994); and 6) existence of a null gene close to *Fhb1* suggested a deletion event in this region (Liu et al. 2008). Besides, it was believed that the main gene *CA460991* in *Fhb1* (which explains 24-39% of the PV) was negatively correlated with resistance type II (Zhuang et al. 2013). Our results proved that Glenn depends mainly on resistance type I and II, likewise, Sumai3 depends on resistance type II generated by *Fhb1*. Given that, the gene *CA460991* may lose its regulatory site by binding to a suppressor molecule that may either be induced by the pathogen or produced by the host (Zhuang et al. 2013). This makes us suggest that Glenn may generated its resistance type I/II from sources other than *Fhb1* through losing *Fhb1* activity due to some suppressor activity or recombination events. Additionally, the QTL analysis in this study suggested that all allelic contribution lead by Glenn were all negatively affected the FHB resistance in the GM population. This fact may be reasoned to one or all of the above mentioned considerations that were taken against Sumai3 genetic heritage.

Our previous molecular analysis assured the absence of the closest SSR (*umn10*) marker for *Fhb1* in Glenn (data not shown). However, absence of markers close to *Fhb1* was common in breeding populations. For example, *Fhb1* was reported as missing for the first time in tetraploid durum wheat; however, the SSR marker associated with *Fhb1* in the donor ‘Floradur’ was absent in its population with ‘Helidur’ (Buerstmayr et al. 2012). Resistance gene analogs (*RGAs*) that were associated with the *Fhb1* region were absent in Ning7840/Clark cross (Guo et al. 2006). Specifically, *umn10* marker was absent in several accessions of hard winter and soft red wheat. Despite the absence of the markers, those lines

showed high levels of resistance in the GH and field (Jin et al. 2013). Allelic segregation at the *Fhb1* locus was examined in $F_{3:6}$ lines from the cross Alsen/NE00403//NE02584. The results showed some lines lacked the *Fhb1* QTL, while containing the *umn10* marker; meanwhile, other lines possessed the *Fhb1* QTL allele but lacked the *umn10* marker (Bakhsh et al. 2013). Moreover, genotypic ratio was determined as 3/16:2/16/:11/16 for (*Fhb1*/*Fhb1*) :(*Fhb1*/*fhb1*) :(*fhb1*/*fhb1*) (Bakhsh et al. 2013); which confirms crossover/recombination events within *Fhb1* region.

Above mentioned data suggested that, *umn10* marker was not the reliable marker for *Fhb1* in some wheat populations; deletion events were actively rich in *Fhb1* region, crossover events were possible in *Fhb1* region, and Glenn was not the first cultivar missing *Fhb1* QTL though showing high FHB resistance. Previous studies also showed that "the microcolinearity among wheat, rice, and barley at the *Fhb1* region was interrupted by rearrangements such as inversion, and insertion/deletions" (Liu et al. 2006). Therefore, the assumption that the *Fhb1* (~250.8 kb) region (having seven genes and *umn10* marker) was stable and allowed for no recombination events has become uncertain. Stability assumption of *Fhb1* region/marker (Liu et al. 2008) was evidently contrasting all the above-mentioned examples of suggested deletion (Guo et al. 2006; Jin et al. 2013) or recombination events (Bakhsh et al. 2013) that enabled missing of *umn10* marker and/or allelic segregation of *Fhb1*. Even though, a single major yet unstable QTL was identified in single environment on Glenn's 3BS region for resistance type II; that confirms the genetic background traced back to Sumai3. A fine map with comparative marker locations in the *Fhb1* region was analyzed. At *Fhb1* location, our major QTL (*QSEV.3BS*) was detected between 0.0 and 04 cM flanked by *wPt-798970 and tPt-6487* markers. In earlier studies, *Fhb1* region was placed to the CI (11.1 - 21.6 cM), (0.5 - 15.00 cM) (Song et al. 2005) and (24.9 - 46.0 cM) (Shen et al. 2006). This means, The GM *3BS* QTL is about 0.88 cM or 8.2 cM within the upstream region of *Fhb1* or about 10 cM distant away (Figure 6). Further, GM QTL might have the upstream part of the *Fhb1* genes due to a deletion or crossover event. This deletion/crossover in Glenn may cause a removal of most of the seven genes in the *Fhb1* region, while the remaining one or two genes were contributed to the GM 3BS unstable effect. Thus, the circumstance of having this GM 3BS with a high FHB resistance phenotype and lacking the *unm10* marker, was plausibly not due to *Fhb1*. Therefore, we can say that *Fhb1* did not play an important role in providing resistance in

Glenn. However, cytological and molecular fine mapping/cloning studies are needed to confirm inversion/conversion events occurred and genes entity/order placed in this region.

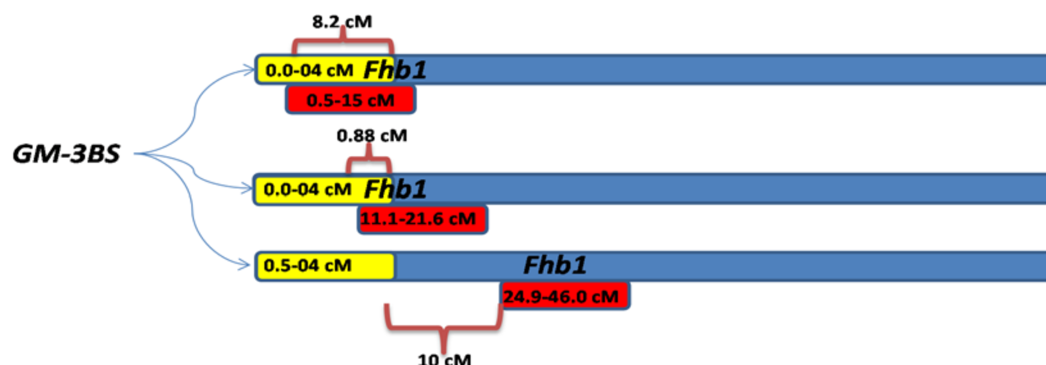

Fig 2.6. A Comparative position placement of *Fhb1* region on 3BS. The 3BS chromosome is shown in blue. The red squares are shown the position of *Fhb1* QTL on previous maps. The yellow squares are shown the GM *Fhb1* QTL region. Brackets are showing the over lapped CI of the *Fhb1* QTL region.

2.5.5. Interactive QTL for FHB Resistance Identified in the D Genome

In GM population, the D genome harbored total six QTL. Two QTL each for FHB resistance type II, IV and HD (Tables 2.7). The region (00 – 02 cM) on *QHD.1DS* QTL was associated with HD in our study (Table 2.7), while was previously associated with resistance type II in Sincorn/Ritmo (Buerstmayr and Anderson 2009) and Shanghai3/Catbird populations (Lu et al. 2013). An important region (04 – 08 cM) in GM population for the *QSEV.2DS1* QTL was associated with resistance type II (Table 2.7). This region was previously reported for resistance type I in a DH wheat population derived from Sumai3 (Handa et al. 2008), resistance type II in a backcross population of *Triticum mach/T. aestivum* (Buerstmayr et al. 2011), and resistance type III in Shanghai3/Catbird (Lu et al. 2013). Meta-QTL analysis showed the same 2DS1 region associated with resistance type II in Romanus, 'Biscay', and Wangshuibai; resistance type I, II and III in Sumai3 (Liu et al. 2009); and as stable multi-QTL (MQTL) in Wangshuibai (Löffler et al. 2009). This means that this region may contains allelic factors for FHB resistance types I, II, and III or a single allelic factor with multiple resistance effects that is regulatory modulated based on the population genetic background controlling it. This region was also harbored the photoperiod sensitive gene (*Ppd-D1*) and dwarfism in plant (*Rht-8*). In soft red winter wheat, a QTL for resistance type I was located in this 2DS1 region and associated with the photoperiod (*Ppd-D1*) and plant height (*Rht-8*) genes (Liu et al. 2013). Some other studies have also reported the association of *Ppd-D1*, *Rht-8* and FHB resistance in European wheat (Srinivasachary et

al. 2008) and in the US wheat (Liu et al. 2013). This actually add to the importance of this 2DS1 region in GM population. In addition to the 2DS1 region, the *QFDK.3DS* QTL region was associated with resistance type IV in GM population (Table 2.7). Previously, it was reported for resistance type II in a Wangshuibai/Wheaton population (Yu et al. 2008), in 'Arina' (Liu et al. 2009), in 'Patterson' (Buerstmayr et al. 2009), and for resistance type III in 'Tokai66' (Malla et al. 2010). A stable *QHD.6DS1* QTL region was detected in GM population and associated with HD (Table 2.7), while previously was reported for resistance type II in a CS-SM-7ADS/Annong8455 population (Ma et al. 2006).

The most important region for FHB resistance on the D genome was located on the 7DS chromosome. This short arm harbored two major and stable (*QSEV and QFDK.7DS1*) QTL expressed particularly in MN environments (Table 2.7). The *QSEV.7D1S* QTL was associated in GM population with resistance type II; as a novel QTL, with no previous identification, while the *QFDK.7D1S* QTL was associated with resistance type IV. The *QFDK.7D1S* region was previously reported for resistance type II and III in the DH population of Arina/Riband and for the trait of relative spikelets in 'Arina' (Draeger et al. 2007). The resistance at all these QTL was contributed by MN00261-4, which assures the importance of this parent in the enhanced FHB resistance phenotyped in GM population.

2.6. Conclusions

Breeding for FHB resistance in wheat is a complex trait that is highly affected by environmental and genotypic by environmental interaction. Determining the genetically sources of FHB resistance depends on reliable genotypic and phenotypic data that are analyzed across multiple environments. The purpose of the current study was mainly to identify the genetic sources of FHB resistance in Glenn and if it was originated from Sumai3 or not. The widely used QTL in wheat breeding programs from Sumai3 included: 3BS-*Fhb1*, 6BS-Fhb2, 4B-Fhb4 and Fhb5A (Cathbert et al. 2007; Randhawa et al. 2013). Our DArT results augmented the worldwide wheat genetic maps and proved that the GM population is lacked the Sumai3 QTL (*Fhb1*, *Fhb4*, and *Fhb5A*), while acquired (*Fhb2*), and generated new QTL (*1BL1*, *5BL1*, *5BL2*, and *7DS1*). This means, even if Sumai3 was one of the parents in Glenn pedigree; however, three major Sumai3 QTL were suggested to be missed in Glenn, while the (*Fhb2*) could be existed. Significant linkage drag with reduced

protein content, due to association of resistance alleles from the *Fhb5A* QTL in Sumai3, was reported (Randhawa et al. 2013). However, high protein content in Glenn (Mergoum, et al. 2006) could be due to the molecular absence of *Fhb5A* QTL.

Astoundingly, the QTL identified in Glenn were reported with/close to important disease resistance and defense response (DR) genes and/or agronomic yield and quality traits. Most regions on the A genome were reported with association to multiple RGAs, while some others were pleiotropically affecting FHB resistance on other chromosomes. Noticeably in Glenn, the *FHB-6A2L* QTL showed syntenic relationship between spring, winter and durum wheat for FHB resistance. Besides, the DR genes of polyphenol oxidase (*Ppo*) was mapped to the same region of (*QINC.6AL2*) in GM population (Li et al 1999). Likewise, the DR gene of peroxidase for hypersensitive responses was previously mapped to the same region of the *QHD.6AS1.b* QTL in GM population. Additionally, the DR of Thaumatin (*Tha3*) and lipoxygenae (*Lpx*) genes were previously mapped to the same positions of *QHD.5BS1* and *QFHB.5BL1.a* QTL, respectively in the GM population. On the D genome two important genes (*Waxy*-2D and *Rip*) were previously mapped to the same regions of *QSEV.2DS1* and *QHD.6DS1* QTL, respectively, in the GM population. The importance of the *Rip* gene that codes for the ribosomal inactivating protein was shown in a multiple analysis between wheat relatives. In this study, *Rip* exists only in D genome species but not in A and B genome species (Li et al 1999). This indicate that this QTL region (as well as in Gm population) is underwent extensive exclusion in A and B genomes. The studies of host-pathogen interaction showed that the expression of DR genes is highly coordinated among synergistic or sequential families (Jabs et al 1997). Co-expressing of multiple DR genes may enhance fungus resistance quantitatively in transgenic plants (Jack et al 1995). Thus, the theoretical coexistence of FHB resistance QTL and DR genes in GM population may emphasize the unique FHB resistance phenotype existed in Glenn and produced transgressive segregants. A QTL on 1BL in GM population was reported with association to the photoperiod sensitivity *Ppd-B1* gene and the spike length trait, while another QTL on 2BL2 was reported for grain number per spike. However, on the D genome, the photoperiod sensitivity *Ppd-D1* gene and the HGT *Rht-8* gene were reported to disease avoidance mechanism.

A suppressor gene (*FA-15-3*) on a *2AS* QTL of Sumai3 is masking the QTL on *Qfhs.ndsu-5BL* especially if the *Fhb1*/3BS QTL was missing. Likewise, in GM population where the *Fhb1*/3BS is proved to be missing this may emphasize the inability to detect the major consistent QTL on 5BL region (243-247 cM). Controversially, the Sumai3 *Fhb1* QTL was believed to be the most major and stable source of FHB resistance in many wheat cultivars including Glenn. Herein and previous data showed that *Fhb1* may exhibit deleterious effects and population preferences including GM population. The *Fhb1* QTL was found absent in many other cultivars, rather than Glenn, and/or populations; though the closest (*umn10*) marker was found existed. Before, it was believed that the distance between *umn10* and *Fhb1* does not allow for crossing-over/recombination; however, some studies showed segregation between *Fhb1* and *umn10* marker. Albeit, our results suggest that the *FHB.3BS* QTL in GM population represents the upper portion of *Fhb1* region, and still miss the major *Fhb1* and its *umn10* marker. Therefore, our data supports previous foundations that *Fhb1* region may acquire recombination/deletion events and probably causing the loss of the closest SSR *umn10* marker.

In conclusion, our study has evidenced that FHB resistance in GM population was most likely not originated from *Fhb1*, *Fhb2*, *Fhb4* QTL of Sumai3. Additionally, we identified nine major and consistent QTL out of 37 total major QTL across at least two environments for FHB resistance in GM population. Three major and stable QTL (*QINC.1AS*, *QHD.6AS1.b* and *QFHB.7AS1*) were all identified in the A genome and with preference to MN environments. The B genome has more QTL than the A genome with four newly identified QTL (*QNDX.1BL1.b*, *QFDK.5BL2*, *QFHB.5BL1.c,* and *QFHB.6BS2.b*) and Three major and stable QTL across at least two environments (*FHB.5bL1.a*, *b*, *c and 6BS2.b*). The D genome has only one novel QTL (*QSEV.7DS1*) and two major and stable QTL (*QHD.6DS1* and *FDK.7DS1*) across two MN preferred environments. Additionally, GM population pyramids QTL (*FHB-2DS1* and *5BL1*), which were reported to high-yield components; and agronomic and quality end-use traits. Finally, GM population has proved to acquire five new FHB resistance sources; which could be implemented in genome wide selection (GWS) and molecular assisted breeding (MAB) programs to improve national and international FHB resistance in wheat.

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CHAPTER 3. 'PARSHALL': AN INDIGENOUS NEW FHB RESISTANCE SOURCE IN NORTH AMERICAN SPRING WHEAT

3.1. Abstract

Fusarium head blight is a devastating disease affecting wheat-growing regions worldwide. Economically, FHB epidemics had caused hundreds of millions in losses in the US alone since the 1990s. Producing wheat cultivars with FHB resistance that is satisfactory to producers and processors is critical. Parshall is a released cultivar with good FHB resistance. However, the genetics, underlying Parshall's FHB resistance, have yet to be characterized. A recombinant inbred lines (RIL) population was generated from the cross 'Parshall' × 'Reeder' (PR) and tested in three states (ND, MN, and SD) in the US. Three FHB-related (incidence/INC, severity/SEV, disease index/NDX) and one agronomic traits (heading date/HD) were evaluated in field and greenhouse experiments over three years (2010-2012). PR population was genotyped using combination of DArT and SNP markers. A genetic map of 154 markers was generated from 430 polymorphic markers, and analyzed by composite interval mapping to identify corresponding QTL. In total, ten (genome A=4; B=6 and D=0) QTL were identified on five different chromosomes, across locations and years. Three new QTL were identified in PR population with association to FHB resistance. FHB QTL regions in PR were verified by similar QTL genomic intervals in previous reports. Other FHB resistance regions in PR were previously reported for high yield, and quality traits. Most importantly, the newly identified *FHB.4BL* QTL in PR population for multiple FHB resistance types and HD trait; that was stable and adapted to the Northern American Central Plains region. Therefore, Parshall could be having an especial usefulness in wheat improvement and marker-based wheat breeding under stressed environments.

3.2. Introduction

Worldwide, wheat breeders exerted a considerable effort to counter the destructive effects of *Fusarium* head blight (FHB) in wheat production regions. FHB is caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schw. (Petch)]. The disease epidemic reached its zenith in the 1990's, when the economic impact exceeded hundreds of millions of dollars per year in the US Great Plains

wheat growing region (McMullen et al. 1997; Nganje et al. 2011). *Fusarium* spreads asexually through conidia or sexually through ascospores (Gilbert and Fernando 2004). After landing on wheat spikes, spores germinate and elongate the germ tube to enter cells through stomata or direct penetration (Seong et al. 2008). Fungi spread within the apoplast leads to cytological changes and cell death (Gunnaiah et al. 2012; Zhuang et al. 2013). Molecular studies suggest that the pathogen reacts as a biotrophic fungus in early infection, where responsive resistance metabolites were released in tolerant cultivars (Basnet et al. 2012).

Breeding for resistant cultivars was considered the most efficient method to mitigate FHB disease damage (Lu et al. 2013). Many wheat cultivars have been released with prominent FHB resistance from sources such as Sumai3 (PI481542) and its derivatives such as DH181 (Yang et al. 2005), CJ9306 (Jiang et al. 2007), Ning7840 (Zhou et al. 2002), CM82036 (Buerstmayr et al. 2002), and Line685 (Lu et al. 2011). FHB resistance is considered a complex quantitative trait. FHB resistance is generally categorized by five discrete types (Mesterhazy et al. 1999): 1) type I (resistance to initial infection), 2) type II (resistance to pathogen spread), 3) type III (resistance to toxin accumulation), 4) type IV (resistance to kernel infection) and 5) type V (resistance to yield tolerance) (Terzi et al. 2014). Type II resistance was generally more reliable and less affected by environmental factors (Bai and Shaner 1996) than other resistance indicies. Type II resistance is based on a response from the direct injection of *Fusarium* conidia, generally under controlled greenhouse environments (Buerstmayr et al. 2013). Cultivars demonstrating Type II resistance mainly depend on one or more of the previously identified QTL such as *Fhb5* (Xue et al. 2011) on chromosome 5A, chromosome 3A (Yu et al. 2008), *Fhb1* (Anderson et al. 2001) on chromosome 3BS, *Fhb2* (Cathbert et al. 2007) on chromosome 3BS and/or chromosome 2D (Lin et al. 2006). Agronomic traits such as plant height, spike trait, heading dates (HD) often were associated with type I and II resistance (Buerstmayr et al. 2009). Plant height was reported as negatively associated with type II resistance (Lu et al. 2012).

The 3BS QTL *Fhb1*, originating from the Chinese source Sumai3, was the most widely utilized QTL for FHB resistance in wheat breeding programs (Waldron et al. 1999). The effective FHB QTL marker (*umn10*) was confirmed with close linkage to the *Fhb1* (Liu et al., 2008). *Umn10* was widely used in many breeding programs, including the hard red spring wheat (HRSW) program, North Dakota State University

(NDSU), ND, US. The *Fhb1* reduces FHB damage, depending upon the genetic background, by an average of 20–25% (Pumphrey et al. 2007). Together, *Fhb1* and *Fhb2* QTL can explain > 60% of phenotypic variation in resistance derived from Sumai3. In this resistance the biotrophic phase depends upon delays in the necrotrophic phase of FHB pathogenicity (Zhuang et al. 2013). Other studies also conclude that combining major QTL in a single cultivar can pyramid FHB resistance (Miedaner et al. 2006). Therefore, integration of major QTL with multiple resistances using molecular-assisted breeding (MAB), for wheat improvement, was a strategy employed by many wheat breeders. However, MAB depends on: 1) close linkage of marker/gene, 2) validation under multiple environments, and 3) high consistency, repeatability, and cost- and time-efficient assessment methods (Randhawa et al. 2013).

'Parshall' was released in 1999, NDSU and developed using classical breeding methods with very high grain quality, excellent agronomic performance and moderate resistance to FHB (Mergoum et al. 2006). Based on its pedigree, the FHB resistance source in Parshall was unknown, though confirmed to be domestic, with no exotic background. Therefore, the objective of our study was to develop a RIL population and to identify the genetic factors of FHB resistance in Parshall using molecular markers.

3.3. Material and Methods

3.3.1. Plant Material and Population Development

A RIL population was developed from the cross Parshall × Reeder (PR) using single seed decent method. Parshall (PI-613587) is a HRSW cultivar developed from the cross Keene (PI-598224)/ND674, where Keene was developed from the cross (Stoa(Sib)/3/Ias-20*4/H-567.71//Amidon), and the 'ND674' (PI-592759) was developed from the cross (Grandin (PI-531005)*2/ND'Glupro' (PI-592759)) (http://wheatpedigree.net/sort/show/55032). Parshall has moderate earliness and good resistance to FHB. Also, it has good adaptation to ND and surrounding areas, test weight, resistance to lodging and shattering, and end-use quality for domestic and export wheat markets (Underdahl et al. 2008). Reeder (PI-613586) was an experimental line developed from the cross (Ias-20*4/H-567.71//Stoa/3/ND-674) at NDSU in 2000 (http://wheatpedigree.net/sort/show/60730). A total of 110 PR RIL were evaluated in field and GH experiments. Seven checks were evaluated, including the high FHB resistant line [ND2710 (PI-633976)],

two moderately resistant cultivars [Faller (PI-648350) and Alsen (PI-615543)], two moderate susceptible cultivars [Steele-ND (PI-634981) and Barlow (PI-658018)], a susceptible line ['Vida' (PI-642366)], and the highly susceptible check (ND2398).

3.3.2. Field Experiment

Multiple phenotypic evaluations were performed for FHB resistance types/traits and HD. Field experiments were conducted for three years (2010-2012) at one location in Minnesota (MN) (Minneapolis, 44°59′N, 93°16′W), in South Dakota (SD) (44°19′05″N, 96°47′00″W), and three locations in North Dakota (ND) [Carrington (47°45′00″N, 99°12′39″W), Prosper (46°96′30″N, 97°01′98″W) and Langdon (48°45′42″N, 98°22′18″W)]. Field experiments were conducted in FHB nurseries using artificial FHB inoculation (Stack et al. 1997). All experiments were conducted in randomized complete block design (RCBD) with four replicates in ND and two replicates in MN and SD. Field experiments were irrigated using overhead misted method (Rudd et al. 2001). Experimental unit consisted of hill/plot 0.3 M long. Each hill/plot contained at least 10 plants. FHB inoculum sprouted in field at flowering time and FHB traits were scored about three weeks after flowering time.

3.3.3. Greenhouse (GH) Experiment

Evaluations in 2011 and 2012 were conducted in RCBD layout with four replicates in eight-inch sized pots. Planting soil was Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA USA), augmented with 20 g Osmocote® slow release fertilizer (Scott's Company LLC, Marysville, OH USA). Each pot contained five plants. At least 5 spikes for each pot were artificially injected using FHB spore-suspension method (Bekele 1995). Briefly, one middle floret, of a single spikelet per spike, was manually injected, using a needle containing about ~4 µl of (100 k/ml) FHB spore suspension. Inoculated spikes were individually covered using plastic misted bags for 72 hours. Severity data was collected about three weeks after injection.

3.3.4. Phenotypic and Genotypic Data Recording

PR population and checks were assessed for all FHB resistance types/traits. Resistance type I, was conferring FHB incidence (INC) and assessed as the percentage of infected spikes per total number of spikes in a plot/hill. INC was assessed only in the field experiments for five environments (combinations of year by location) including MN 2010 (M10), SD 2010 (S10), Carrington 2011 (C11) and Prosper 2011 and 2012 (P11 and 12). Resistance type II, was conferring FHB severity (SEV) and assessed as the percentage of infected spikelets per spike averaged across 10 total randomly chosen spikes per plot/hill in the field, and across five to ten spikes in GH experiments. SEV was recorded for 12 environments, including M10; P10, P11, P12; C11; S10, S11, S12; GH 2011 and 2012 (G11 and G12); and the combined means across field data (FieldSEV); and GH data (GHSEV). Resistance type III, was conferring resistance to the accumulated levels of the toxin deoxynivalenol (DON) and was detected in 10 gm of milled kernels for each genotype using gas chromatography and/or mass spectroscopy (Schwarz et al. 1995). DON was assessed in six total environments; three for ND (P10, P11 and G11), and three for SD (S10, S11, and the combined SDDON environment). DON data in SD was assessed from two replicates, while in ND as a single replicate. Resistance type IV, was conferring the resistance to the high percentage of *Fusarium* damaged kernel (FDK) and assessed, as the percentage of infected kernels found in 200 kernels randomly chosen from each individual genotype per replicate. FDK was scored in four environments including S10, S11, G11, and G12. Disease index (NDX) was a calculated FHB variable and was the product of multiplying the INC and SEV for each genotype. NDX was assessed in six total environments including the combined data across all environments.

One agronomic trait was evaluated for HD. HD was measured as the number of days from planting until ~50% of the spikes were fully emerged from the boot leaves in each genotype at the growth stage Feekes 10.5. HD was recorded in seven total environments including P10, P11, P12, C11, Langdon 2011 (L11), M10, and the combined data across ND environments.

Genomic DNA was extracted from lyophilized young leaf tissue for each individual RIL, parent and check using Qiagen DNeasy Plant mini kit (Cat# 69106) with minor modifications. For each genotype, 30

µl of DNA (80ng/µl) were sent to Triticarte Pvt. Ltd (Canberra, Australia; http://www.triticate.com.au) for (DArT) analysis (Akbari et al. 2006). Additionally, DNA samples were also for SNP analysis (Wang et al. 2014) at the small grains genotyping lab, USDA, ARS, Plant Science Research Unit, Fargo, ND, US, using the 90 K iSelect wheat SNP chip (Illumina).

3.3.5. Map Construction and QTL Analysis

The scores of all polymorphic DArT and SNP markers were converted according to the parental codes. Linkage maps for each chromosome were constructed using Carthagene Software http://www.inra.fr/mia/T/Carthagene/.1.2-LKH (De Givry et al. 2005) specifying a RIL genetic model. A maximum distance of 30 centimorgans (cM) and a minimum logarithm of odds (LOD) threshold of 4 were used to partition markers into linkage groups. Cosegregating markers were merged into single markers. The most likely positions of the markers along the linkage groups were determined using the commands [mrkdouble, mrkmerges, group 0.3 4, mrkselset, build, flips, polish, detail]. The Kosambi mapping function (Kosambi, 1944) was applied to convert recombination rates into map distances in cM. Linkage groups were assigned to chromosomes according to SSR markers and their map information from GrainGenes (http://wheat.pw.usda.gov/ggpages/maps.shtml) and consensus map of DArT markers. Maps were compared to the high-density wheat consensus SSR and AFLP genetic map (Somers et al. 2004) available in GrainGenes. Final maps were compared with the DArT (Huang et al. 2012) and SNP consensus maps using the program Autograph (Derrien et al., 2007; http://autograph.genouest.org/) to confirm accuracy of the marker order. Combined DArT/SNP genotyping revealed 214 polymorphic markers and used to generate the genetic map that plotted 154 markers to 14 different wheat chromosomes.

QTL mapping was carried out by the composite interval mapping (CIM) method (Zeng 1994) using the software QGene 4.0 (Joehanes and Nelson 2008). A scanning interval of 1 cM between markers and putative QTL with a window size of 10 cM was used to detect QTL. The number of marker cofactors for background control was set by forward and revers regression with a maximum of five controlling markers. A QTL was considered significant when one markers was associated at 3 LOD. Permutation test was used at 1000 replications with 30 threads to confirm LOD score threshold for identified QTL in each trait.

Confidence intervals (CI) were obtained using ±2 LOD positions distant from each QTL peak. The proportion of phenotypic variance (PV) explained by a single QTL was determined by the square of the partial correlation coefficient (R^2). QTL with overlapping CIs were considered as one QTL. QTL with ≥10% of PV was considered major, and if repeated across ≥50% of total tested environments, was considered stable. For additive QTL effects, positive and negative signs of the estimates indicate, respectively, the contribution of Parshall and Reeder toward higher trait values. Graphical representation of linkage groups and QTL was carried out using MapChart 2.2 software (Voorrips 2002).

3.3.6. Statistical Analysis

Analysis of variance (ANOVA) were carried out using SAS 9.3® (SAS Institute, Cary, NC), where genotypes (G) were considered with fixed effect and the other sources of variation with random effects. ANOVA was conducted using Proc Mixed model for individual and combined environments across Field and GH. For individual environment analysis, data was analyzed as RCBD so that the model described genotypic effects only, while differences among replications within each environment were not considered. The statistical model *F*max ratio for homogeneity (Tabachnik and Fidell 2001) was performed prior to combine environments and to test significance of G, E and G×E interactions. If the *F*max ratio between the major and minor experimental error of combined environments was ≤10 fold, combining data was considered appropriate. Coefficient of correlations (*r*) was estimated using Proc Corr of the SAS 9.3® between the means of each trait locations and between combined means. Significance in Pearsons's correlations, *F*-test, and ANOVA was set at $p \leq 0.05$ value. Means separation for genotypes means was determined using *F*-protected least significant differences (*LSD*, $p \leq 0.05$) as a byproduct from the Proc Mixed model. Broad sense heritability (h^2) was calculated based on the family means basis as described by (Holland et al. 2003) using the random ANOVA Proc Mixed model output, excluding the parents and checks means. The equation used to calculate heritability was: $\boldsymbol{h^2}\ H^2 = \frac{\delta^2 G}{\delta^2 G + \frac{\delta^2 GE}{e} + \frac{\delta^2 E}{re}}$ where $\delta^2 G$=genotypic variance, $\delta^2 GE$= genotype × environment interaction, $\delta^2 E$= experimental error variance, *e*= total number of environments, and *r*= total number of replications within an environment.

3.4. Results

3.4.1. Phenotypic Traits Variation and Correlation

Genotypes of PR population have showed differential performance for the different phenotypic data (Table 3.1). At the level of parental performance, Parshall showed insignificant difference with mean values of Reeder, ND2710, ND2398 and RIL, while showed significant difference with mean value of Alsen for INC. the Reeder parent showed insignificant mean differences with Parshall, ND2398, and RIL, while significant differences with the high resistant checks (Alsen and ND2710). At the level of SEV trait, Parshall and Reeder were showing almost equal performance, where their means were insignificant different with Alsen, ND2710, and RIL, while significantly different from the mean of the highly susceptible check (ND2398). At the GH environment for SEV trait the performance of Parshall was insignificantly different from the means of Reeder, all checks and RIL. However the performance of Reeder, at the same environment, was significantly difference with the high resistant checks only. At the FHB NDX performance, Parshall and Reeder were equally performed where both insignificantly different with the means of all checks and RIL, except the mean of ND2398 was significantly different. This equal performance between Parshall and Reeder could be emphasized by the close parental genetic background between the two parents' pedigree. It may also suggest that Parshall could be more than moderate resistant for FHB.

Agronomically, the means of PR genotypes of RIL, checks and parents were insignificantly different for HD under ND and MN environments (Table 3.1). This may reflect the high level of homogeneity and specificity in the genetics controlling the photoperiod sensitivity traits in PR population. The PR population displayed transgressive segregation in all FHB and HD traits, where some RIL were recognized in both directions of distributed means (Fig 3.1). This reflects that both parents contributed to the traits of study. The distribution of means for INC trait showed positive skewness. Some PR lines showed lower values for FHB resistance traits than both parents, which reflect better performance and higher resistance. Similarly, other PR lines showed earliness and lateness relative to parents at HD in MN and ND environments.

Table 3.1. Means, minimum and maximum values of RIL, parents and checks in PR population for FHB severity (SEV), incidence (INC), index (NDX) and heading dates (HD).

Trait	INC%	SEV%		NDX%			HD (day)	
Env.	A†	Field	G	A††	S12	P12	ND	M10
Parents								
Parshall	91.30	26.50	53.70	25.10	31.30	40.10	54.00	33.50
Reeder	95.10	34.40	65.80	25.90	30.50	48.60	54.20	34.00
LSD(a)	08.70	10.96	19.57	12.80	.	15.72	05.20	04.25
Checks								
Alsen	81.70	26.40	37.40	20.00	.	35.00	53.90	33.50
ND2710	85.50	26.70	44.10	26.80	.	40.70	53.20	.
ND2398	93.60	50.00	68.30	60.50	.	23.00	58.20	.
RIL								
Mean	94.00	29.70	51.00	27.40	27.20	37.30	56.00	34.30
Min	30.20	21.20	24.70	16.90	17.00	14.80	52.80	29.00
Max	100.20	37.80	75.80	36.90	41.30	64.10	59.80	38.50
LSD(b)	07.44	06.94	17.74	09.25	06.80	11.07	05.20	01.30

Trait, FHB traits and HD. Env, environment for each dataset as location x year. SEV, percentage of FHB severity scores. Field§, percentage of severity score combined in field experiments only in P10 (Prosper 2010); P11 (Prosper 2011); P12 (Prosper 2012); C11 (Carrington 2011); M10 (MN 2010); S10 (SD 2010); S11 (SD 2011) and S12 (SD 2012). G, percentage of FHB SEV combined in GH only in G11, GH 2011 and G12; GH 2012. INC, percentage of FHB incidence score; A†, percentage of FHB INC score in combined field data for P11, P12, C11, M10 and S10. NDX, percentage of FHB index calculated by multiplying INC x SEV for each genotype; A††, combined NDX score in field data for P11, C11, M10, S10 and S11. S12, percentage of NDX score in SD 2012 only. P12, NDX score in Prosper 2012 only. HD, heading dates measured by number of days from planting to heading in ND, and from 1st of June in MN. ND, combined data of HD in P10, P11, P12, C11, Langdon 2010 (L10). M10, HD records in MN 2010 only. Parents, means of Parshall the good FHB resistant and Reeder the FHB susceptible. Checks; high FHB resistant cultivars ‘ND2710’ and ‘Alsen’, and high susceptible line ‘ND2398’. RIL, recombinant inbred lines. Means, means of RIL only excluding the means of parents and checks. Min, the minimum means value in the RIL. Max, the maximum means value in the RIL. .*LSD*, least significant differences at P=0.05 for each entry. (a), LSD for checks and parents. (b), LSD for means of RIL vs individual RIL, Parents and Checks.

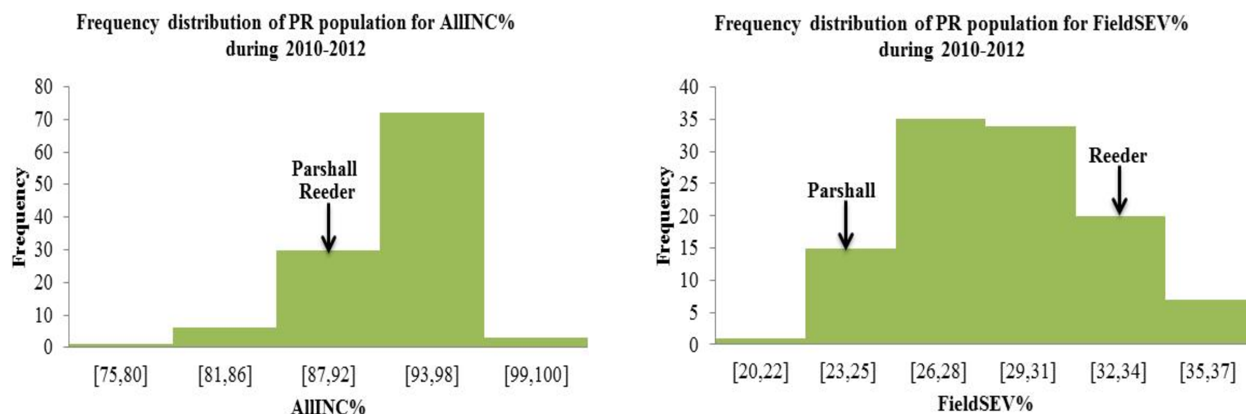
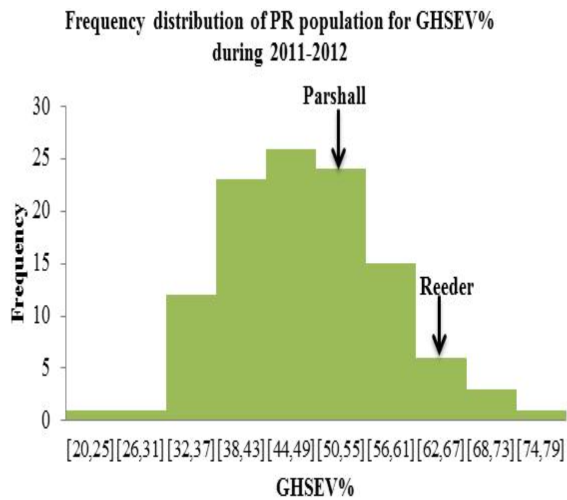
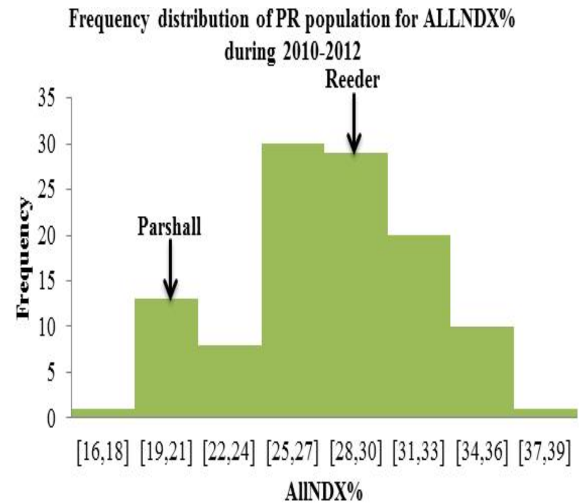
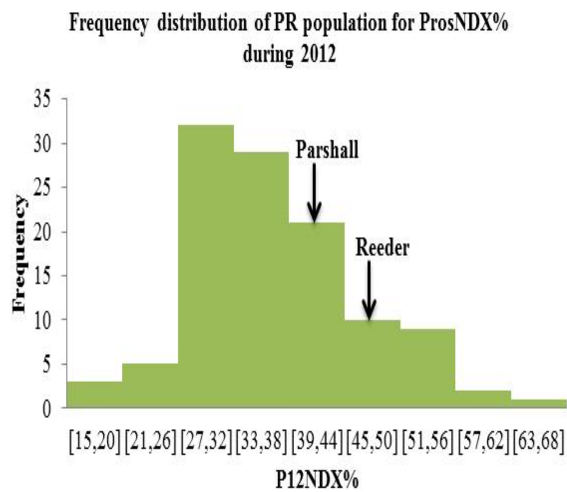
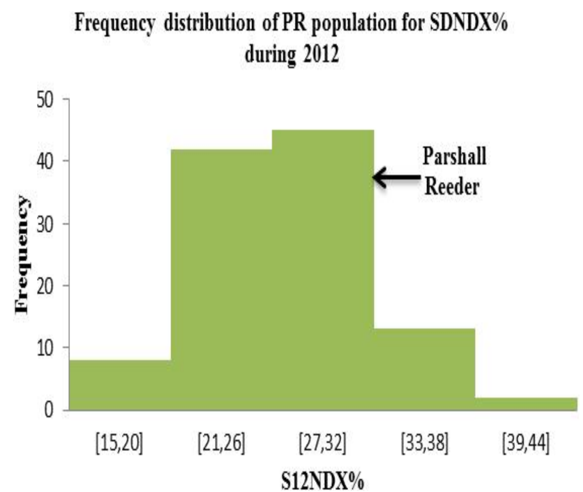


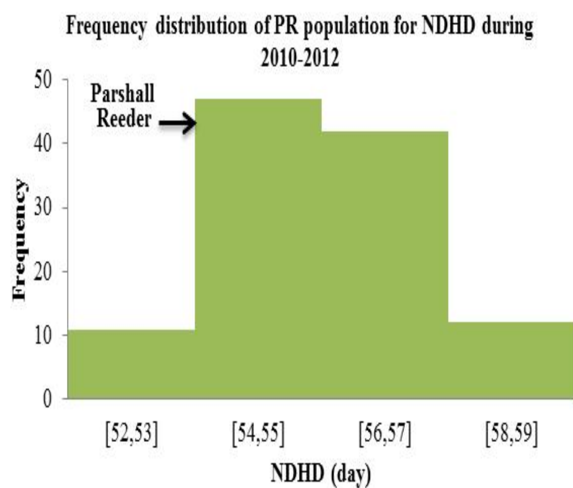
Fig 3.1. Frequency means distribution of PR population for FHB variables (AllINC, FieldSEV, GHSEV, AllNDX, Pros12NDX and SD12NDX) and agronomic traits (NDHD and MN10HD). Arrows indicate the range of means of the tow parents (Parshall and Reeder).











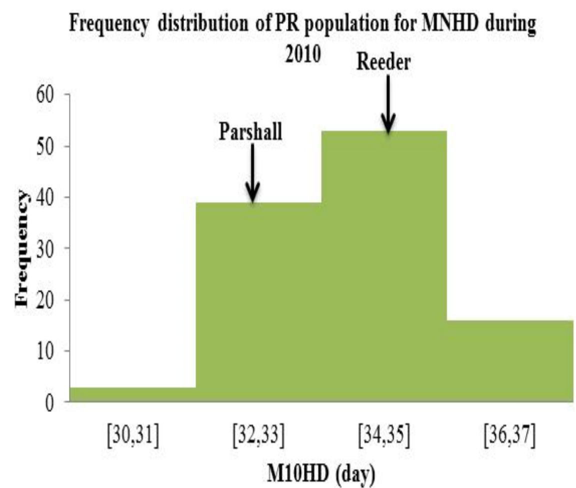


Fig 3.1. Frequency means distribution of PR population for FHB variables (continued).

ANOVA analysis showed high insignificance *F*-values describing PR means variation ($P<0.0001$) for all FHB resistance traits (Table 3.2) except for FHB NDX trait where it was significant. This means that variation between the means of PR population due to genotypes (G), environments (E), or GXE interaction in all FHB traits was insignificant, except for FHB NDX where the G and E were the source of significant variation. Likewise, the variation due to G, E, and GXE interaction between the means of PR population for HD trait was highly insignificant; based on *F*-values (Table 3.2). The sum of squares (data not shown) calculated from the combined ANOVA showed that genotypes (RIL, parents, and checks) are the main sources of variation for all FHB and HD traits. Despite the high means squares of E and G×E, the means squares of G was high to produce moderate to high broad sense heritability (0.45 - 0.79) for FieldSEV, AllNDX, and NDHD trait environments. Markedly, AllNDX trait environment showed high significant (P= 0.8 - 1.00) *F*-values for G and G×E variances, which reflects more dominant effect rather than additive effect in the PR population (Table 3.2). This dominance effect may support the lower breeding/additive values/effects showed in PR population, due to its domesticated attributes found in the parents. This domininance effect may was hidden and caused the moderate h^2 in the FHB traits (FieldSEV and GHSEV) and the low h^2 (0.16) in AllINC trait environments.

Pearson coefficient of correlation (Table 3) was generally positive in each trait in different pair of environments. Except for GHSEV trait environments, the correlation with INC and HD traits was negative. This was logic because HD was mitigated and no INC trait was estimated in GH environment. The range of coefficient of correlation was between $r = 0.03$ and $r = 0.86$. AllFHB traits were positively correlated with each other and also with HD trait, especially in field experiments. This reflects the high influence of photoperiod sensitivity genes on the FHB resistance types I and II in PR population under both ND and MN environments. It also may support that resistance types I and II in PR population is highly affected by environmental factors.

Table 3.2. Heritability, standard error of heritability and analysis of variance for FHB traits and heading dates in PR population across combined environments.

Trait	FieldSEV (h^2=0.52[a]; SE=0.13)				GHSEV (h^2=0.32[b]; SE=0.15)				AllINC (h^2=0.16[c]; SE=0.14)			
Source	*df*	MS	*F* value	*P*	*df*	MS	*F* value	*P*	*df*	MS	*F* value	*P*
G	122	393.3	2.1****	<.0001	120	479.4	1.5**	0.02	122	295.3	1.9****	<.0001
E	7	13140	9.6****	<.0001	1	8015.6	0.7	0.45	4	3355.6	7. 6***	0.003
Rep/E	16	1344.4	11****	<.0001	5	14696	40.6****	<.0001	11	428.4	3.2****	0.000
GxE	797	190.7	1.6****	<.0001	116	324.6	0.9	0.76	456	161.6	1.2***	0.005
Error	1926	122.4	.	.	420	361.9	.	.	1325	133.3	.	.
	AllNDX (h^2=0.45c; SE=0.13)				NDHD (h^2=0.79d;SE=0.13)							
	df	MS	*F* value	*P*	*df*	MS	*F* value	*P*				
G	124	68.6	0.2	1.00	118	57.4	4.8****	<.0001				
E	4	1255.1	0.4	0.81	4	15703	285.6****	<.0001				
Rep/E	9	2077.1	23.7****	<.0001	15	50.1	7.9****	<.0001				
GxE	449	3370.4	38.5****	<.0001	472	12.1	1.9****	<.0001				
Error	1075	87.6	.	.	1850	6.4	.	.				

Trait, FHB traits. Sources, the sourcses of variance. FieldSEV, the FHB severity variable for the locations (Prosper (2010-2012), Carrington (2011), MN (2010) and SD (2010-2012)); All-INC, the FHB incidence variable for the locations (Prosper (2011-2012), Carrington (2011), SD (2010) and MN (2010)); All-NDX, the FHB index variable for the locations (Prosper (2011), Carrington (2011) and MN (2010), and SD (2010-2011)); ND-HD, the days to heading in the ND locations (Prosper (2010-2012), Langdon (2011) and Carrington (2011)). MS, means squares; G, genotypes; E, environment; Rep/E, number of replicates per environment; G x E, genotype by environment interaction; Error, Residual experimental error; SE, standard error for heritability; h^2, broad sense heritability calculated based on family basis from a random model covariance output ANOVA; [a] h^2estimated based on harmonic mean (2.7) of replications; [b] h^2estimated based on harmonic mean (3.43) of replications; [c] h^2estimated based on harmonic mean (2.5) of replications; [d] $h^2$was estimated based on harmonic mean of (4) replications; *F*-value, *df*, degrees of freedom; *P*(0.05), probability of *F*-value.

Table 3.3. Pearson coefficient of correlation (*r*) calculated in PR population between mean values of FHB variables and phenotypic traits tested in ND, MN and GH environments through 2010-2012.

Trait.	FieldSEV	GHSEV	AllINC	AllNDX	NDHD
GHSEV	+0.10				
AllINC	+0.37***	-0.10			
AllNDX	+0.86***	+0.03	+0.53***		
NDHD	+0.34***	-0.19*	+0.37***	+0.46***	
MN10HD	+0.39***	-0.25**	+0.39***	+0.52***	+0.78***

Trait, FHB traits and HD. FieldFEV, the FHB severity variable for the locations (Prosper (2010-2012), Carrington (2011), MN (2010) and SD (2010-2012)); AllINC, the FHB incidence variable for the locations (Prosper (2011-2012), Carrington (2011), SD (2010) and MN (2010)); AllNDX, the FHB index variable for the locations (Prosper (2011), Carrington (2011) and MN (2010), and SD (2010-2011)); NDHD, the days to heading in the ND locations (Prosper (2010-2012), Langdon (2011) and Carrington (2011)). MN10HD, HD score in MN 2010 only. (*r*), *Pearson* coefficient of correlation using significant level at $P < 0.05$, 0.01, and 0.001. (-), reflects negative correlations. (+), reflects positive correlation. (*), reflects significance at P=0.05. (**), reflects significance at P=0.01. (***), reflects significance at P=0.001.

3.4.2. Mapping PR Population

Out of total 2,500 of combined DArT and SNP markers, 430 were polymorphic and used to generate a framework linkage map. A total of 154 markers representing 81 unique loci were successfully mapped to 17 linkage groups on 17 different chromosomes (Table 3.4). All chromosomes were consisted of one linkage group. The mapped distances of genome A, B, D were 105.6, 339.4, and 25.4 cM, respectively; and the total genomic mapped distance was 470.4 cM where the average distance between any two markers was 5.81 cM.

Table 3.4. Number of chromosomes, groups, markers, unique loci, cumulative kusambi distance and the average distance between two loci in the mapped genome of PR population.

Chromosome/Genome	Groups	Markers	Unique loci	Distance (cM)	Average distance (cM)
1AS	1	5	3	3.9	1.3
2AL	1	6	4	23	5.75
4AL	1	6	3	1.9	0.63
5AL	1	12	3	22.2	7.4
7AL	1	13	5	17.6	3.52
7AS	1	4	3	37	12.3
Genome A	6	46	21	105.6	5.03
1BS	1	4	3	8.8	2.93
3BS	1	18	10	48	4.8
3BL	1	8	5	36.3	7.26
4BL	1	21	8	46.6	5.83
5BL	1	6	5	13.4	2.68
6BL	1	3	3	44.5	14.83
6BS	1	4	3	1.8	0.6
7B	1	20	11	140	12.73
Genome B	8	84	48	339.4	7.1
2DL	1	8	5	17.6	3.52
6DL	1	6	3	5	1.67
7DS	1	10	4	2.8	0.7
Genome D	3	24	12	25.4	2.12
Genome	17	154	81	470.4	5.81

Chromosome/genome, numbers refer to the specified genome number, while letters refer to the chromosome. Groups, the number of chromosomal groups generated in each specified chromosome. Markers, the number of markers mapped in each chromosome. Unique loci, the number of unique (unrepeatable) markers after removing the co-segregating markers in each specified chromosome. Distance, the total commutative Kosambi mapping distance identified in each chromosome. Average distance, the means of distance calculated by dividing the distance/the number of unique loci in each chromosome.

3.4.3. QTL Identified for FHN Resistance and HD

3.4.3.1. QTL Identified for FHB Resistance Types I, II and NDX

CIM for resistance type I revealed two QTL (*QINC. 1AS*, and *4BL*) on two different chromosomes (Table 3.5; Fig 3.2). Both QTL were exhibited major effects, where the *QINC.1AS* QTL was repeated in two environments. The range of PV% explained by the QTL for resistance type I was between 11 and 17 %. The range of additive effect was between +03.42 and ±11.16%. Both parents contributed QTL for resistance to type I in 1AS QTL, while Parshall only contributed to the effects of the *4BL* QTL.

Table 3.5. QTL identified for FHB resistance type I (INC), II (SEV) and FHB index (NDX) and HD in PR population.

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm-LOD
QINC.1AS	INC	wPt-729972-wPt-4666	S10,A	02	00-02	±11.16	4.41***	17.0	2.49-3.25
QINC.4BL	INC	wPt-6149-tPt-7156	P12	28	26-32	+03.42	2.79**	11.0	2.35-2.92
QSEV.3BL	SEV	wPt-5295-wPt-4808	C11	28	26-28	-01.17	3.05**	12.0	2.43-3.26
QSEV.5AL	SEV	Ex_c7383_12655992-Ex_rep_c66900_65314083	S12	10	06-10	-01.61	3.33***	13.0	2.39-3.09
QSEV.4BL	SEV	Ku_c5210_9290700-wPt-6149	P10,S12, S11,Field	22	00-26	-06.15	8.27***	22.5	2.40-3.80
QNDX.3BL	NDX	wPt-0367-wPt-4808	C11	28	24-28	-01.30	3.41***	13.3	2.41-3.05
QNDX.5AL	NDX	Ex_c7383_12655992-Ex_rep_c66900_65314083	S12	10	04-10	-01.72	3.33***	13.0	2.35-3.04
QNDX.4BL	NDX	Ku_c5210_9290700-wPt-6149	S11,S12, A	22	18-26	-06.15	8.27***	29.3	2.35-3.29
QHD.7AS	HD	wPt-1928	P10	00	00	-00.93	3.56***	13.8	2.44-3.04
QHD.4BL	HD	Ku_c5210_9290700-wPt-744434	P10,C11, L11,M10, ND	10	00-20	-01.48	6.73***	24.8	2.44-3.42

QTL, name of QTL assigned to its chromosomal group. TRT, FHB trait/variable. MRK, flanking markers. LOC, environment locations. Pos, Position of QTL peak. CI, Confidence intervals of QTL. Add, additive effect. LOD, Logarithmic likelihood of odds. R^2%, percentage of phenotypic value ratio. [a], QTL significance based on permuted LOD values at 0.05 and 0.01 probabilities. Perm LOD, LOD value based on permutation test for each individual environment using 1000 replications and 30 threads. SEV, percentage of FHB severity. INC, percentage of FHB incidence. NDX, percentage of FHB index. HD, days to heading. LOC, environment locations. P, Prosper. C, Carrington. L, Langdon. M, MN state. ND, means across ND state combined locations. A, All combined data of the specified FHB trait. Field, all combined field SEV data. 10, the year 2010. 11, the year 2011. 12, the year 2012.

Three major QTL (*QSEV.3BL*, *5AL*, and *4BL*) were identified for resistance type II using six environments including the combined means across field environments. These QTL were located on Three different chromosomes (Table 3.5; Fig 3.2), and included one stable QTL across four environment. The range of PV% for resistance type II was between 12 and 22.5 %. The range of additive effect was between -1.17 and -6.15%. Parshall had no contribution to resistance type II, where Reeder had all the contribution

For FHB NDX resistance, three major QTL (*QNDX.3BL*, *5AL*, and *4BL*) were identified using five environments including the combined means across environments. These QTL were localized on three different chromosomes where the *4BL* QTL was stable across three environments (Table 3.5; Fig 3.2). The range of PV% for NDX was between 13 and 29.3%. The range of additive effect was between -1.30 and -6.15%. Parshall had no contribution to resistance type II, where Reeder had all the contribution. This means that FHB resistance type I that was expressed in the PR population was only affected by alleles from both parents. Meanwhile, the FHB resistance type II that was expressed in the PR population was coming from the alleles contributed by the parent Reeder only. This may suggest that Parshall had no alleles for resistance type II, where its sources of resistance depends mainly on alleles for resistance type I only

3.4.3.2. QTL Identified for HD

Two major QTL (*QHD.7AS*, and *4BL*) were detected for HD using six environments including the combined means across ND environments. These QTL were located on two different chromosomes (Table 3.5, Fig 3.2), where the 4BL QTL was stable across five environments. The range of PV% for HD was between 13.8 and 24.8 %. The range of additive effect was between -0.93 and -1.48%. Though the difference in HD between the two parents was ~1 day, the alleles for earliness were contributed by the parent Reeder only. Parshall did not contribute any alleles for HD in these QTL for HD.

3.4.3.3. QTL Potential Linked/Pleiotropic QTL for FHB Resistance Types and Agronomic Traits

Overall, three QTL (*QFHB.3BL*, *5AL*, and *4BL*) were having overlapped CI for multiple FHB resistance and agronomic traits, including the most important and major 4BL QTL that was also consistent across 10 environments (Table 3.6 and Fig 3.2). This can be emphasized by pleiotropic gene/QTL affecting

different FHB variables, tight linkage between different genes/QTL, or the same QTL were coexisted in common CI. The other QTL (*QFHB.3BL* and *5AL*) were identified each in single environment with overlapped CI for resistance types II and NDX. Additional important QTL (QINC.1AS) was detected in two environments for resistance type I only. Both parents contributed alleles to the two major and consistent QTL, while Reeder contributed to the remaining three major QTL. (Table 3.6 and Fig 3.2).

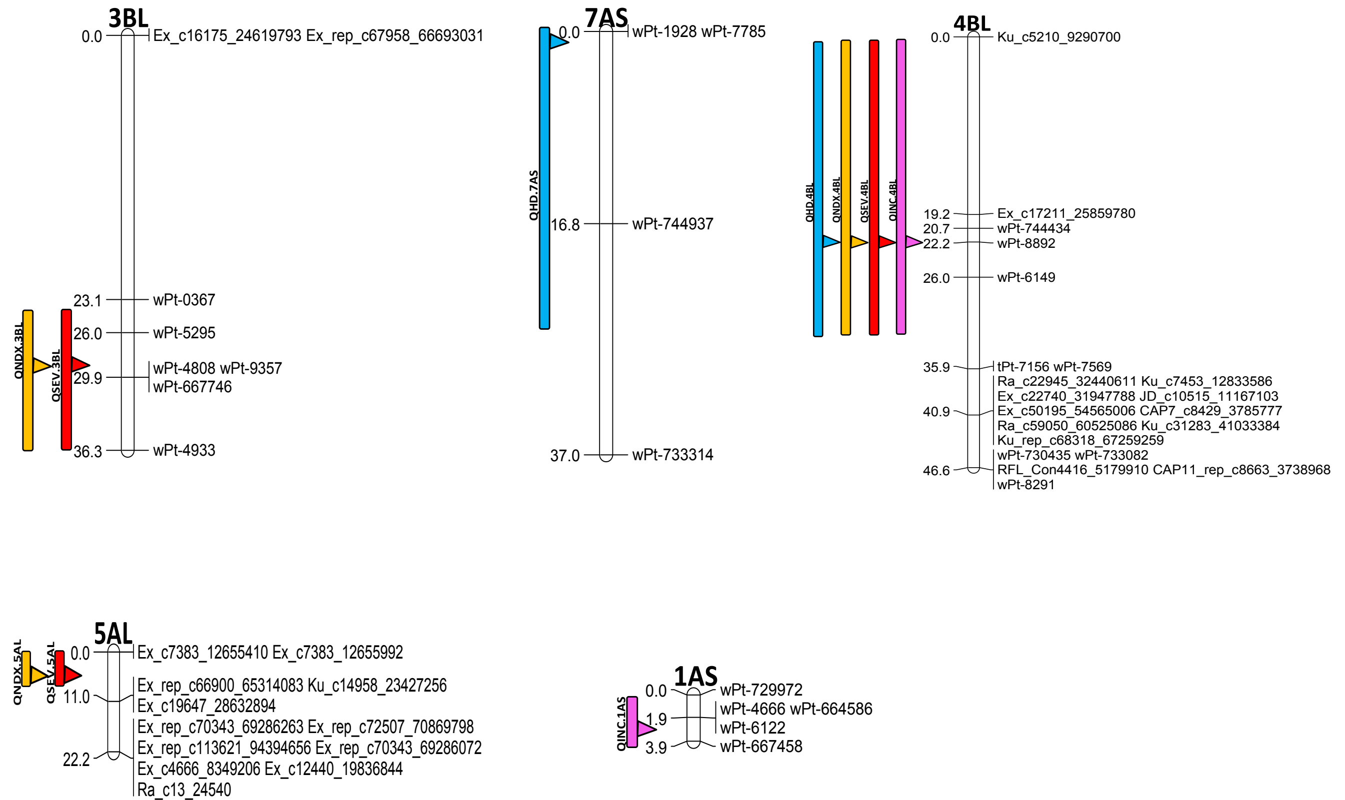


Fig 3.2. Chromosomal map of QTL identified in PR population. QTL intervals shown as (lines). QTL positions shown as (Triangles). QTL for INC, SEV, NDX and HD were shown in (Pink, Red, Yellow and Blue) respectively.

Table 3.6. QTL identified in multiple environments and/or multiple FHB and agronomic traits in PR population.

QTL	TRT	MRK	LOC	Pos	CI	Add	LOD[a]	R^2%	Perm LOD
QINC.1AS	INC	wPt-729972-wPt-4666	S10,AINC	02	00-02	±11.16	4.41***	17.00	2.49-3.25
QFHB.5AL	SEV, NDX	Ex_c7383_12655992-Ex_rep_c66900_65314083	S12	10	04-10	-1.72	3.33***	13.00	2.39-3.09
QHD.7AS	HD	wPt-1928	P10	00	00	-0.93	3.56***	13.80	2.44-3.04
QFHB.3BL	SEV, NDX	wPt-0367-wPt-4808	C11	28	24-28	-1.17	3.41***	13.30	2.43-3.26
QFHB.4BL	INC, SEV, NDX, HD	Ku_c5210_9290700-tPt-7156	P10,P12,C11,L11,M10,NDHD, S11,S12,ANDX, Field	10-28	00-32	±6.15	8.27***	29.30	2.44-3.8

QTL, name of QTL assigned to its chromosomal groups. TRT, FHB trait/variable. MRK, flanking markers. LOC, environment locations. Pos, Position of QTL peak. CI, Confidence intervals of QTL. Add, additive effect. LOD, Logarithmic likelihood of odds. R^2%, percentage of phenotypic value ratio. [a], QTL significance based on permuted LOD values at 0.05 and 0.01 probabilities. Perm LOD, LOD value based on permutation test for each individual environment for 1000 replications and 30 threads. SEV, percentage of FHB severity. INC, percentage of FHB incidence. NDX, percentage of FHB-index. HD, days to heading. LOC, locations. P, Prosper. C, Carrington. L, Langdon. Filed, combined SEV data in field environments only. M, MN state. ND, means across ND state combined locations. A, All combined data of the specified FHB trait (INC and NDX). 10, the year 2010. 11, the year 2011. 12, the year 2012.

3.5. Discussion

3.5.1. Impact of Phenotypic Assessment on FHB Resistance

Not surprisingly, resistance type II in PR population was found positively correlated with other resistance types I and NDX in field environments. However, a negative correlation, though low and insignificant, was detected between resistance type II and HD in the GH environment. This was logically possible, because in GH environments has mitigated photoperiod. Other reports also agree with this negative correlation between resistance type II and agronomic traits (HD) under FHB pressure (Buerstmayer et al. 2013; Paul et al. 2005). On one side, the normal frequency distribution of RIL means (Fig 3.1) showed some RIL (e.g., PR-10 and 101) with high and transgressive resistance for all FHB resistance types and agronomic traits except in GHSEV environment (data not shown). Other RIL (e.g., PR-50 and 98) showed consistently high resistance types I and II, but not to earliness (HD), while another RIL (e.g., PR-6, 52, and 96) showed high FHB resistance types I, II and NDX but also not to earliness (HD). Additionally, some RIL, such as PR-102, showed good performance in all FHB resistance types and agronomic traits, but not under GH or SD environments. Such observation establishes the reliability of the regular assessment for types I and II to evaluate resistant cultivars.

Many studies described a negative correlation between HD and the different FHB resistance types I, II, III and IV under field conditions (Emrich et al. 2008). However, HD in PR population showed a positive correlation with all FHB resistance types, except with resistance type II in GH environment. The reason can be the controlled environment in GH that may affect HD, or parents were having insignificant difference (~ 0.2 - 0.5 day) in HD that halt the phenotypic variation of RIL to contribute to the QTL analysis. Parshall was earlier in HD than Reeder (Table 3.1), and four RIL (PR-11, 33, 94 and 102) were transgressive earlier than both parents in ND and MN environments. Though RIL with early-heading were exposed to FHB pressure for longer time, lines such as PR-33 showed better resistance to type I, and II; while the line PR-11 showed better resistance to type I, and NDX; and the line PR-94 showed better resistance to type II (data not shown). Other RIL with latency in HD relative to parents were exposed to disease for shorter time; thus plausibly, performed better. These results strongly suggest that earliness (HD) in PR population

coordinately shows better impact on FHB resistance. This gave us impression that FHB resistance in PR population, and plausibly in Parshall, depends mainly on type I

In the PR population, the controlled conditions and time of assessment in GH, after inoculation at flowering stage, mitigated the effect of HD variation on the different FHB resistance types. Consistency in our data reflects the critical influence of environmental factors, specifically, the photoperiod length, factored by HD, on FHB disease development, factored by FHB-related traits. Means that, the more earliness (HD), the more avoidance, the higher resistance type I, the more uninfected florets (type II), the more healthy developed kernels (type IV), and the healthier milled grains with less DON (type III). This suggests that resistance in Parshall primarily confers type I. Earliness urge seed development to start earlier in Parshall than Reeder, which endangers seeds to FHB pressure for longer time and consequently lowers resistance types III and IV; fortunately PR population acquired lateness alleles from Reeder, which enhance FHB resistance types III/IV. Generally, the resistance types III and IV were genetically independent of other FHB resistance types, and were critically affected by G×E interactions and assessment methods. Most importantly, our results did not contrast the general opinion that the most reliable evaluation methods for FHB resistance were those reflected to types I, II and NDX.

3.5.2. QTL Identified for FHB Resistance

3.5.2.1. Effective QTL Identified on the A Genome

On the A genome, three QTL for FHB resistance and agronomic traits were identified in the PR population. Among these, one QTL for resistance type I, and one combined allelic effects for type II and NDX (Table 3.6). The 1AS QTL was consistently major across two environments, where both the 1AS and the 5AL QTL were showing preference expression under SD environments. Only the PR 7AS QTL for HD was expressed under the ND environment. The region (0.0-02 cM) on *1A1S* QTL was associated with resistance type I in PR population, while in previous meta-QTL analysis it was reported in association with resistance type II and III in ‘Wheaton’ and ‘Pirate’ (Liu et al 2009, Yu et al. 2008). This region was also in a close approximate to a previously reported region (08-09 cM) for resistance type II in Wheaton (Liu et al. 2009). This gives an implication of the potential multiple effects for FHB resistance that this region on PR

population can express. Besides, it may imply that this region in Parshall may have effects on resistance type I, II and III, as proved in other wheat cultivars.

The long arm on chromosome 5A contained a major QTL region (04-10 cM) that was eight cM away from the region (18.9-74.5 cM) that was previously reported for resistance type I (Anderson 2007; He et al. 2013), type II and III (He et al. 2013; Chu et al. 2011), *Qfhb.ifa.5A* QTL (Chen et al. 2006); *Qfhb.umc.5A QTL* (Buerstmayr et al. 2002), *Qfhi.nau.5A* (Xue et al. 2011). The same region was also reported for grain number per spike, plant height (HGT), spike length, spikelets number per spike and spike compactness traits (Yu et al. 2008; Rutkoski et al. 2012; Lu et al. 2014; Kumar et al. 2007). Moreover, it was previously reported for resistance type I, II, III and IV in 'W14', 'Spark', Wangshuibai, Fundulae201R, 'Riband', 'CJ9306', 'DH181', Frontana, 'CM82036', 'Ernie', 'Nyuba'i and 'Renan' (Loffler et al. 2009; Liu et al. 2009). This makes the PR 5AL QTL was 10 cM away from the 50 cM extended region on chromosome 5A that harbored a wide diverse of important genes/traits. The importance came due to the physical approximate of the PR 5AL region from the major 5A QTL originated from Sumai3 and proved in three different breeding populations (IFA, UMN, and NAU). However, this identified region was considered new to Parshall and PR population (NDSU), because the exact region was not previously reported. The genes controlling the major 5AL QTL that was previously identified in the three different populations, could be sharing the same origin in Parshall and PR population, even if Sumai3 has no traces in the parental background of PR pedigree. Moreover, it adds to the advents of Parshall to possibly acquire many agronomic traits for yield components. Interestingly, the regions on the 5AL were mainly attributed to resistance type III and HD. This may support the argument regarding the relationship between earliness (HD) in Parshall and its preference to factor the resistance types I/III. It may extend the hypothesis to include a high correlation between resistance types I/III in PR population, which needs more investigations. Finally, this region could be the most important region identified in PR population and Parshall that has the potential alleles to control multiple resistance types I, II, III, IV and agronomic traits (HGT and HD).

Chromosome 7A contained additional new and important region (00-22 cM) that was in approximate as 1.5, 12.5, and 15.1 cM away from three important regions on the short arm. All of the three region were previously detected for resistance type I, II and III (Ruan et al. 2012), for type II in Frontana

(Liu et al. 2009), and resistance to kernel shattering in Sumai3/Stoa population (Zhang and Mergoum 2007). This PR region have expression preference to ND environment. Moreover, it supports our claim that PR population may contain a single regions with multiple FHB resistance mechanisms I, II and III. It is also considered the second new region in PR population in the A genome that may have potential allelic effect on important agronomic traits (resistance to shattering). Overall, the A genome in PR population provided two novel genes with multiple potential to enhance FHB resistance types I, II, III, IV and important agronomic traits (HGT, HD, and resistance to grain shattering). This gives PR population a high performance and resistance against the FHB disease and high yield potentially as well.

3.5.2.2. Effective QTL Identified on the B Genome

In the B genome, the PR population, harbored two QTL regions; the 3BL region that combined the effects toward resistance type II and NDX in PR population, and the 4BL region that had major and consistent effects toward resistance types I, II, NDX and HD. (Table 3.6). This gives emphasis of how the QTL were organized on B genome with preference to resistance type II, NDX, and HD. The region on short arm of chromosome 3B (24-28 cM) in the PR population was previously reported for resistance types II, III and IV in Wangshuibai, Apache, Ernie, Arina and 'Massey' (Liu et al. 2009 and 2013). This region also shows environmental preference to ND. This region is the second region in PR population that has previous reports for FHB resistance in other studies along with the 1AS region. This region add to the PR population a potential allelic effect on combining multiple FHB resistance in single region. Specially, if we noticed that this region was consistent between spring and winter wheat cultivars. This gives implications about the importance of this region to the wheat genome for pathogen defense, which needs more investigation.

The PR region (00-32 cM) on the long arm of chromosome 4B was also considered new because it has no previous detection in earlier studies. However, it was ~30 cM away from a very important region (64.9-80.0 cM) that was previously reported for multiple FHB resistance, number of grains per spike, HGT and TKW traits (Lu et al. 2014). This 4BL region has especial importance to FHB resistance, since it enhanced the resistance types I, II and NDX in PR population. Besides it is the only new region in PR population that originally correlated tow FHB resistance types I and II to the agronomic trait HD in the same

intervals. This region is also considered the third novel region in PR population along with the 5AL and 7AS regions.

3.6. Summary

The economic impacts of FHB disease on wheat production were severe, and the genetic sources for FHB resistant were getting exhaled. Under such circumstances, it becomes sensible how the intensive usage of FHB resistance sources such as Sumai3, can narrow the resistance alleles in the wheat genetic pool. Hence, wheat researchers were pursuing new FHB resistant sources to be implemented in cultivar development. This study presents 'Parshall' as a new domestic and indigenous source of resistance to FHB in ND and neighboring states. Parshall was particularly well suited for breeding tasks, as a released cultivar with excellent adaption to the Northern Midwest (The Great Plains) wheat growing region of the US. Our study dissected the genetic factors in Parshall using a combined DArT/SNP genetic map and QTL analysis for three FHB resistance traits. Among three novel QTL regions identified in PR population, the (Q*FHB.4BL*) was the major and consistent QTL. Our results showed that resistance in PR population may not solely depend on resistance type II as the case with Sumai3. However, the new *QFHB.4BL* QTL combines the most reliable resistance types I, II and NDX and additionally associated with earliness HD. This earliness add to PR population an advent to escape from the early FHB infection if occurred. It also add to PR population a role in avoidance mechanisms that is not exist in most of Sumai3- dependent cultivars.

Therefore, we may imply that resistance in Parshall could rely on a delay in the necrotrophic pathogenicity phase. This suggestion was supported in the present study by QTL regions identified for resistance types I. The mechanism behind the resistance type I has more relation to the plant avoidance compartments. Comprehensively, if early incidence of FHB infection occurs, more spikes, spikelets and kernel development stages are going to be affected. As a result, elevated severity, DON and FDK values would be expected. If later incidence of FHB infection occurs, less spikes, spiklets, and kernels are exposed to disease. As a result, resistance types assessed based on kernel infection (DON and FDK) would be reduced. Therefore, we conclude that delayed pathogenicity contributes to the resistance observed in Parshall. In conclusion, PR genome contains five major QTL including three novel QTL with multiple FHB

resistance and two with stable effect across at least two environments. Environmental adaptation to SD region was revealed in PR genome for some QTL (*1AS, 5AL* and *4BL*), and to ND region (*7AS, 3BL,* and *4BL*) as well. The two major and stable QTL (*1AS* and *4BL)* explained between 17-29.30 % of the PV in PR population. No QTL identified on the D genome. Further, we may think that some pleiotropic interaction between QTL on genome A and B is possible, which needs further investigation. Also, a comparative molecular study of QTL identified on the two populations GM and PR would be very enriching to the wheat breeding and genome wide selection. Given that, a third population was developed as a direct cross (Glenn/Parshall). Finally, Parshall was proved as a well-adapted cultivar with excellent yield and quality traits. Parshall is also proved to acquire additional and valuable novel FHB resistance source that can enrich and widen the variation of alleles to combat FHB disease. Finally, Parshal can be considered a new source for breeders to be implemented in cultivars with improved FHB, agronomic, and quality traits in wheat.

3.7. References

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CHAPTER 4. GENERAL CONCLUSION

Disease resistance is a major pillar to many of the wheat breeding programs. *Fusarium* head blight is the most important major disease in humid wheat growing regions including the Great Plains area in the USA. The disease caused tremendous economic losses in the 1990's epidemics. However, breeders since that epidemics start a major awareness to the FHB attributes and scavenged wheat germaplsms in their programs for resistant lines. Meanwhile, they interrogate exotic national and international material to generate new populations with FHB resistant background. Many cultivars been released since 2002 till present with good to high FHB resistance. Among these cultivars were Glenn and Parshall the most dominated cultivars in ND, MN, MT, and SD regions.

In current study two populations (GM and PR) were generated to identify resistance to FHB. The aim of the 1st population (GM) was to find out the genetic factors controlling the FHB resistance in Glenn. Given that, Glenn has a Chinese genetic source (Sumai3); our molecular examination showed that Glenn could be missing the closest molecular marker (*umn10*) assigned to the *Fhb1* major QTL of Sumai3. Depending on different genetic backgrounds and populations, the *Fhb1* QTL on 3BS can explain ~25% of FHB resistance, mainly, type II. Our objectives were set to discover if Glenn has novel genetic sources for FHB resistance, or pyramids new recombination of pre-existed QTL, or has lost only this *umn10* marker of *Fhb1* on the 3BS region. Our results showed that Glenn has novel genetic regions (FHB-*1B1Lc* and *7D1Sb*) that were associated with FHB resistance. Moreover, we found that Glenn has missed the *umn10* marker more likely due to recombination. Our assumption was based on the discovery of a minor QTL in the same genomic region of *Fhb1*, yet still has missing the *umn10* locus. Knowing the *Fhb1* region contains seven genes, which hypothesizes a possible recombination insertion/deletion between the *Cis*-seven genes including the *umn10* marker locus; leaving behind other *trans*-genes to generate a minor QTL effect on Glenn. If we assumed a cultivar as a new derivative to the Sumai3, it needs at least three major consistent QTL that explains at least 40-50 % of its resistance to FHB. None of the major and consistently stable QTL of Sumai3 were identified in Glenn, which prove that genetic background in Glenn is new for FHB resistance. Glenn has also missed a major Sumai3 QTL (*Fhb5A*), that pleiotropically express low protein

contents this may explain the high protein quality in Glenn. More detailed fine mapping to the genes existed in these important QTL regions in Glenn would be highly beneficial.

The aim of the 2nd population (PR) was to investigate the genetic factors controlling FHB resistance in Parshall. Parshall was proofed to have no exotic resistance background specially the Chinese Sumai3. However, the exact source of resistance was unknown. Our results proved that Parshall has acquired three novel genomic regions. Only one QTL on 5AL was sharing the same CI of three major and stable QTL of sumai3 in three different populations. This QTL region could be inherited originally from wheat ancestor similar/close to Sumai3. We also proved that resistance in PR population is different from that of Sumai3 that depends on resistance types II. However, Parshall is strongly suggested to depend on resistance types I, and III/IV. Meaning that, due to some genetic differences in Parshall, these QTL with similar chromosomal allocations to Sumai3 may presented resistance types III/IV rather than I/II. Therefore, we may present Parshall as a new domestic source of FHB resistance in wheat, a well-adapted cultivar with other disease resistance genes, and excellent yield and quality traits. Parshall also proved to acquire many regions that previously identified with defense response genes. This gives advantage to Parshall to resist wide diverse of stresses especially salt, drought, and cold environments. In conclusion, Glenn acquired five new major and stable QTL for multiple FHB resistance, while the Parshall acquired three QTL. This study is presenting two cultivars as an addition and valuable wheat sources for breeders to have at their convenience to enhance FHB resistance, and integrate in MAB in wheat.