Mapping quantitative trait loci for Fusarium Head Blight resistance in the U.S. winter wheat

by

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B. S., King Saud University, 2009
M.S., King Saud University, 2013

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Abstract

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Markers for the repeatable QTLs were converted into Kompetitive allele specific PCR (KASP) markers for marker-assisted breeding to pyramid these QTLs in U.S. winter wheat.

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Chapter 1 . Literature review

1.1 Wheat production in the United State

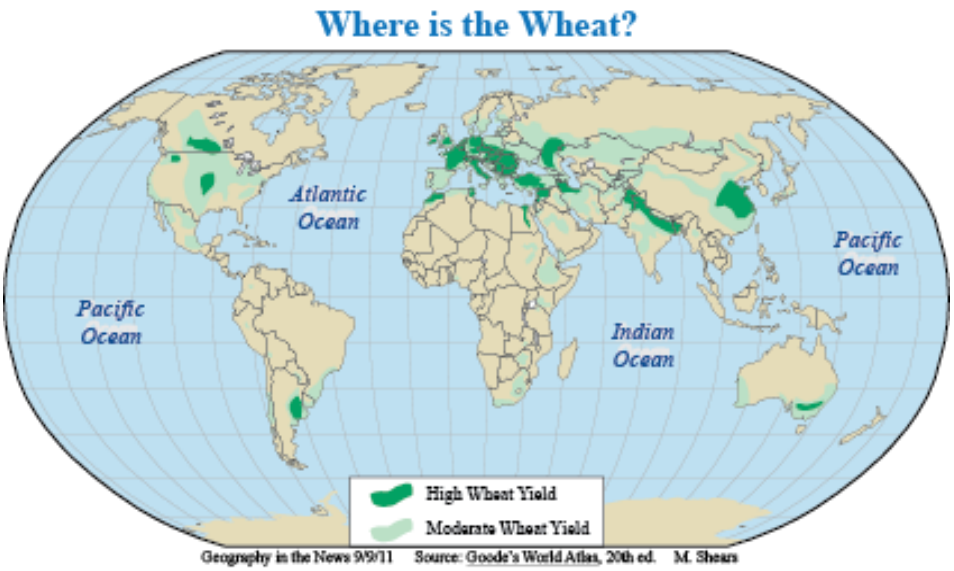
Wheat is one of the most important crops that provide human nutrition for more than 10,000 years (Ferdy, 2010). It ranks the third largest crop after the corn and soybean in the United States (U.S.). Although wheat production in the U.S. has been challenged in the past, the U.S. ranks the fifth in wheat production after China, European Union, India, and Russia, thus is still one of the major wheat-producing countries in the world (Curtis and Halford, 2014). The U.S. produces about 10% of the world wheat yearly on approximately 25 million hectares (McMullen *et al.*, 2012).

Wheat production mainly in five major regions of the world: North America, Europe, Asia, eastern Africa, Australia, and South America. In the U.S., many challenges have influenced the wheat production that has led to reduction of more than one-third in wheat harvest areas since 1981. The profitability of U.S. wheat has been dropped significantly compared to other crops, and foreign competition might be one of the major reasons for declining in the U.S. wheat production. In addition, the flexible policy in the U.S. that allows farmers to choose the crops they want to grow might be another reason for wheat production swinging in the last ten years (https://www.ers.usda.gov/topics/crops/wheat/background/).

1.2 Wheat classes in the U.S.

Durum (*Triticum durum* L. 2N = 4x = 28) and bread wheat (*Triticum aestivum L.* 2N=6X=42) are the two-major species of wheat in commercial production in the U.S.. Bread wheat has A, B and D genomes, with seven chromosomes per genome (Martínez-Pérez *et al.*, 1999). The A genome was derived from wild einkorn wheat (Shewry, 2009), the D genome was from *Aegilops*

tauschii, whereas the donor of B genome remains unknown.



Wheat in the U.S. can also be divided into six classes based on grain hardness, color, and the growing habits: hard red winter wheat (HRWW), hard red spring wheat (HRSW), soft red winter wheat (SRWW), durum, hard white wheat, and soft white wheat (Baenziger *et al.*, 2009). The soft wheat is usually used for cakes and flatbread, whereas the hard wheat is used for various types of bread. Hard wheat contains a higher level of gluten than soft wheat (Cai *et al.*, 2019). HRWW is usually grown in the Great Plains (Colorado, Kansas, Oklahoma, Nebraska, Texas, South Dakota, and Montana), whereas the HRSW is mainly grown in Northern Plains (South and North Dakota, Montana, Minnesota). Durum wheat is excellent for pasta and occupies ~3 - 4% of U.S. wheat hectarage mainly in the same area as HRSW. Based on the USDA report, 40% of the total wheat production is hard red winter wheat, SRWW accounted for 15 - 20% of the total production which primarily grown in the states along the Mississippi river and eastern states (Ohio, Missouri, Indiana, and Illinois). Kansas had the largest wheat hectarage in 2016 with about 3,400,000 hectares (8,500,000 acres) planted and is the leading state for wheat production in the

U.S. (https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/.)

1.3 *Fusarium* head blight in the wheat

Fusarium head blight (FHB) or scab is a fungal disease in Wheat, soybean, oat, barley. It not only reduces grain yield but also grain quality. Several *Fusarium* species can cause FHB, including *F. graminearum, F. avenaceum, F. culmorum* and *F. poae*. In the U.S., *F. graminearum* is the major pathogen for FHB. FHB usually occurs in warm and humid areas, and flowering spikelets can be infected easily (Aoki *et al.*, 2014; Bai and shaner 2004). *Fusarium* ascospores are usually transported by wind or water-splash to wheat spikes as initial inoculum (Fredy, 2010), which can be further spread to > 500 m through atmosphere (David, *et al*., 2015). The infected kernels are usually discolored and shriveled kernels, so also called *Fusarium*-damaged kernels (FDK) (Perlikowski D *et al.*, 2014).

Grain contaminated with deoxynivalenol (DON) can be a serious safety issue to human and animal health (Bai and Shaner, 1994; Parry *et al*., 1995; Perlikowski, 2014). About 1 ppm of DON may reduce animal feed intake and body weight, whereas at 10 ppm of DON can cause feed rejection of animal (De Wolf *et al.,* 2003). Therefore, many countries have set the maximum allowed DON content in food and feed. For example, the DON limits in Europe are 1.25 ppm for unprocessed bread wheat, 0.2 ppm for babies' food, and 0.5 ppm for bread and bakeries (Buerstmayr, 2009). In 1993, the FDA in the U.S. set the acceptable levels of DON as 1 ppm for final wheat products (http://www.cfsan.fda.gov/~dms/graingui.html).

Many methods including fungicide, biological control and cultural practice have been proposed for FHB control. However, the most effective methods to reduce the FHB damage is to use resistant cultivars (Bai and Shaner 1994, 2004). Moreover, sumai3 was reported to have the

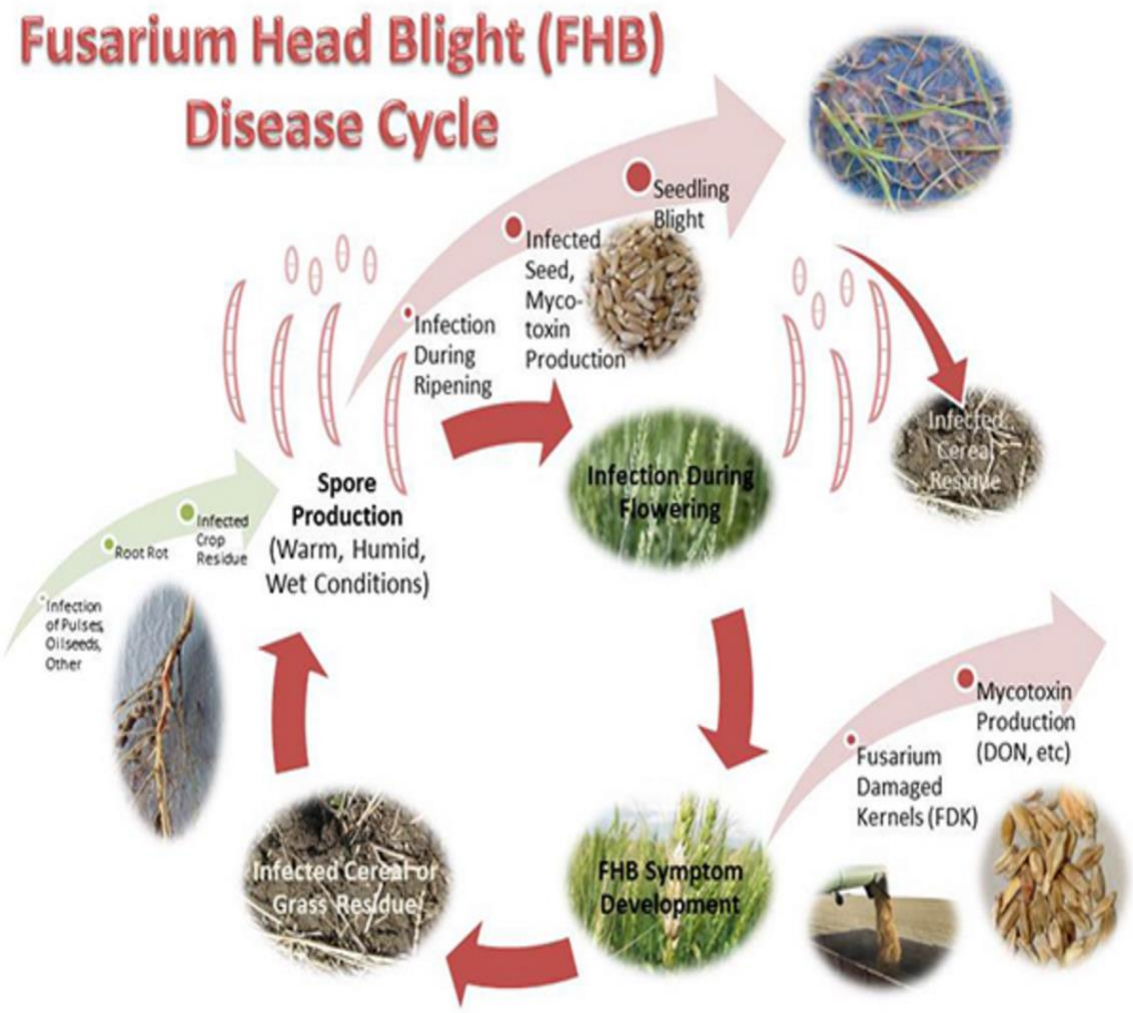
best resistance after thousands of germplasm and breeding lines were screened. It has been used as the main source of FHB resistance in breeding programs over the world. Quantitative trait loci (QTLs) have been identified for FHB resistance in all the 21 chromosomes (Bai and Shaner 2004; Yu *et al.*, 2008; Liu *et al.*, 2009; Buerstmayr *et al.*, 2009). *Fhb1* on the chromosome 3BS shows the best resistance to FHB spread within a spike among QTLs identified to date (Bai and Shaner, 2004; Buerstmayr *et al.*, 2009)

1.4 Life cycles of *F. graminearum*:

Mycelium of *F. graminearum* overwinters in crops residues including wheat, barley, corn and rice. In the spring, perithecia are developed on the residues, and ascospores are released from the matured perithecia and then attached to the spike's tissues during the anthesis. *F. graminearum* is transported to wheat spikes by wind and rain-splash (Fredy, 2010; Goswami, 2004). As soon as fungal spores land on the surface of florets and glums, it develops hyphae and grow to reach stomata during the inflorescence stage (Trail 2009; Xu and Nicholson 2009).

There are two mechanisms for fungus to spread within the tissues. The main way for the fungus to spread in wheat plant tissues is to start from a floret, then get inside a spikelet tissue, to other spikelets through vascular bundles. The xylem and phloem tissues are all infected. That may cause either partially damage or death of entire spikelet (Ribichich *et al.*, 2000; Jansene *et al.*, 2005) . There is an evidence that plant death is related to increased colonization in the host (Bushnell *et al.*, 2003). The fungus starts to produce DON as soon as the infection occurs, which helps *F. graminearum* enter wheat florets (Fredy, 2010). Three conditions are required for FHB infection: high humidity (> 90%) for at least 12 hours, warm temperature and enough inoculum available around wheat canopy during the flowering time.

(https://www.saskatchewan.ca/business/agriculture-natural-resources-and-industry/agribusiness-farmers-and-ranchers/crops-and-irrigation/crop-protection/disease/*fusarium*-head-blight).



https://www.saskatchewan.ca/business/agriculture-natural-resources-and-industry/agribusiness-farmers-and-ranchers/crops-and-irrigation/crop-protection/disease/*fusarium*-head-blight

1.5 Measures for FHB control

1.5.1 Control methods

To reduce FHB epidemics, first pathogen inoculum source and amount should be minimized (Parry *et al.,* 1995). There are many control methods have been used to achieve the goal, such as fungicide control, biological control.

1.5.1.1 Chemical controls

A. Metconazole (Caramba) is labeled for FHB suppression at 13.5 to17 fl oz./acre. For barley and wheat, it can be applied at the beginning of anthesis with good efficacy for FHB and DON content suppression. However, sometimes it is hard to be certain about the optimum time for the application due to the fields' environment (Yoshida *et al.,* 2008).

B. Propiconazole (Tilt) is also labeled for FHB control, but with poor efficacy. Suggested application rate is 4.0 fl. oz./acre. Barley and wheat should be sprayed at the flowering stage (50% plants flowering), but not after Feekes growth stage 10.5. Based on the USDA, farmers should not apply more than 8 fl. oz/acre per season. Also, the chemical treated plants should not be grazed by livestock or used as animal forage after treatment. However, after harvesting, the straw can be used for either animal bedding or feed. (Wilson and Lester, 1996).

Biological control has been explored as an additional strategy to manage FHB. Compared to fungicides, this method may cost lower, gives longer time protection and does not accumulate a hazard of toxins. (Bai and Shaner 2004; Homdork *et al.,* 2000; Parry *et al.,*1995). Biocontrol of pathogen can be accomplished by antibiosis, mycoparasitism, and competition (Legrand *et al.,* 2017). In vitro assays in both greenhouse and field demonstrated that some bacteria such as the genera *Bacillus* and *Pseudomonas* are able to reduce *F. gerimearum* growth, thus they may be used to reduce FHB infection (Da Luz *et al.,* 2003; Khan *et al.,* 2004; Palazzini, 2007). Some

filamentous fungi have been reported to reduce inoculum potential of *F. grminearum*, (Palazzini *et al.,* 2007). In Palazzini *et al.,* (2018) study, they evaluated *B. velezensis* RC 218 and *Streptomyces albidoflavus* RC 87B against FHB disease and DON content under the field condition. Both *B. velezensis* RC 218 and S. *albidoflavus* RC 87B reduced FHB incidence between 25% to 30% severity and up to 51% for DON accumulation.

C. Prothioconazole (Proline 480 SC) is labeled for FHB and DON suppression. On barley, the application rate for FHB is 5.0 - 5.7 fl oz./acre at Feekes 10.5, when barley heads on the main stem have fully emerged. On wheat, the rate is the same but should be sprayed at the early flowering (Mcmullen *et al.,* 2012). To provide the best protection for the wheat or other cereal against diseases such as FHB double fungicide applications are recommended. A single application for long season plant protection is not sufficient (Caldwell *et al.,* 2017).

1.5.1.2 Biological control

1.5.1.3 Other control methods

To control FHB, farmers have used multiple strategies to reduce FHB damage such as tillage practices to bury crop residues in order to reduce the initial source of inoculum (Dill-Macky 2008). Crop rotation is one of the classical methods that have been used to reduce initial inoculum (Bolley, 1913). Using tillage and crop rotation together, FHB severity and DON content in the gain can be significantly reduced. FHB symptom was the highest when wheat was planted after corn in Minnesota (Dill-Macky and Jones 2000), but the lowest when wheat was planted after soybeans (Wegulo *et al.*, 2015). However, cultural practice alone cannot reduce FHB to the desired level (Koehler *et al.,* 1924; Milus and Parson, 1994; Dill-Macky and Jones, 2000).

1.6 Wheat resistance to FHB

Two types of resistances are reported: morphological or physiological resistance. Morphological traits such as awn, peduncle, and compactness of spikes may play some roles in facilitating the disease initial infection and spread within spikes. However, the effects usually are smaller than physiological resistance (Meidaner, 1997; Mesterhazy, 1995; Rudd *et al.*, 2001). The physiological resistance mechanisms include the chemical pathways that produce obstacle chemicals to prohibit the pathogen from growing in wheat tissue (Rudd *et al.*, 2001). There are three main types of resistance to FHB: type I (resistance to initial infection), type II (resistance to fungal spread in the spike) (Schroeder and Christensen 1963), type III (refers to low DON accumulation) (Miller *et al.*, 1985). Mesterházy *et al.*, (1995) proposed two more types of resistance which are type IV (resistance to kernel infection), and type V (FHB tolerance). In wheat, type II resistance is extensively studied because it is the most stable and easy to be evaluated (Bai and Shaner 2004). Plants are usually rated for type II resistance using percentage of symptomatic spikelets in a spike (PSS) or using a 1-10 visual scale with 1 as highly resistant and 10 as highly susceptible (Stack, 2000). Type IV resistance can be measured using percentage of *Fusarium* damaged kernels (FDK) (Rudd *et al.*, 2001). Type I resistance, however, usually is more difficult to evaluate than type II or III resistance because it is hard to control the initial inoculum amount and most often it can be complicated by type II resistance.

Several hypotheses have been made for the mechanisms of FHB resistance although none of them have been clearly proved (Giancaspro *et al.*, 2016). One hypothesis is that resistance wheat may build thick cell walls to either delay mycelium spreading into other spikelets or accumulate the phenolic compounds that are toxic to the pathogen (Ribichich *et al.*, 2000). Another hypothesis is that plants may have a defense gene responding to early *F. graminearum* infection in wheat

spikes. The defense genes could be activated within 6 - 12 h after inoculation by producing plant resistance related (PR)-proteins including PR-1, PR-2 (- 1,3-glucanase), PR-3 (chitinase), PR-4, and PR5 (thaumatin). After 36 to 48 h of infection, expression of these genes could reach the highest levels. PR-4 and PR-5 proteins were accumulated higher and earlier in resistant wheat cultivars than susceptible cultivars. Correlation has been established between responding timing of defense genes and infection of *F. graminearum* in wheat cultivars (Pritsch *et al.,* 2000; Bai and Shaner, 2004). Another study showed that Jasmonate (JA) and ethylene (ET) might regulate the wheat resistance to FHB because of the increase of JA and ET in wheat plants after early infection (Jia e*t al.,* 2018). However, salicylic acid (SA)-regulated systemic resistance may not be important in this process (Ding *et al*., 2011; Makandar *et al.,* 2012). In Li and Yen (2008) study concluded that sprayed different amounts of SA content onto wheat spikes was not effective to enhance FHB resistance.

1.7 Genetics of FHB resistance

In the past several decades, breeders have started using resistant cultivar to ontrol the FHB, which is one of the most effective ways to control the disease (Bai and Shaner 2004). Many cultivars with various levels of resistance have been reported around the world especially in Japan, China, and Brazil. However, wheat cultivars with complete immunity have not found yet (Bai and Shaner, 2004). *Fusarium* head blight is a disease that usually controlled by a few major QTLs and several minor QTLs (Bai *et al.,* 2000). The expression of the QTLs can also be influenced by many environment factors. Classical genetic analysis suggested three resistance genes involved in FHB resistance of two Chinese's varieties 'Sumai-3' and 'Ning7840' (Bai, 1995). QTL mapping study indicated that 'Ning7840' carries a major QTL, *Fhb1,* and showed a high level of FHB resistance along with 'Sumai-3' (Bai and Shaner, 1996). *Fhb1* is stably detected in different environments.

1.8 Production of mycotoxins during FHB infection

Many species of *Fusarium* pathogens can produce mycotoxins during FHB infection, Mycotoxins are toxic to humans and animals (Steiner *et al.*, 2017) and can defend against other microorganisms (Wachowska *et al.*, 2017). Mycotoxicosis can be caused by using wheat flour from FHB-infected grain (Chen *et al.*, 2003). Deoxynivalenol (DON) is a major type of toxin that produces by *F. graminearum* in FHB infected wheat or barley in the U.S.. Different chemotypes of the fungus have been studied because the same species can produce different mycotoxins. DON contaminated flour can cause fever, nausea, headaches, and vomit. Therefore, reducing mycotoxin content in wheat kernels is important (Steiner *et al.*, 2017).

1.9 FHB resistance sources

Growing resistant wheat cultivars is the most economical approach to minimize the disease damage, and reduce food contamination by the mycotoxins (Zhang et al., 2008). Breeding FHB resistant wheat became an important breeding objective in many wheat breeding programs since it's new outbreak in the 1980s (Cai *et al.*, 2019). FHB resistant wheat germplasm has been reported worldwide (Bai and Shaner, 1996; Kolb *et al.*, 2001), yet wheat cultivars with a high level of resistance are rare (Bai and Shaner, 2004).

To date, wheat with immunity to FHB has not been reported (He *et al.*, 2013). Wheat resistance to FHB has been found in China and Japan (Yu *et al.*, 2008) and many other countries. In China, about 34,571 wheat lines have been screened in the 1980s, only 1,765 (5.1%) showed resistant or moderately resistant reactions to FHB (He et al., 2013). Due to the fact that resistance to FHB is controlled by several genes, with most showing minor effects (Bai and Shaner 2004) and the expression of the resistance influenced by environments and genotype x environments interaction, direct phenotypic selection for resistance to FHB is difficult (Wanguimwanki 2017). Presence of

several types of resistance to FHB (Schroeder and Christensen 1963; Mesterhezy 1995) also complicate FHB resistance selection results. Buestmayr (2009) summarized reported QTLs identified for FHB resistance and found that over 100 QTLs associated with FHB resistance have been reported in all the 21 wheat chromosomes from more than 50 resistant sources worldwide. For instance, several Chinese landraces have been used as sources of resistance, such as 'Wangshuibai', 'Sumai-3', and 'Ning7840' (Bai and Shaner1996). Among the Chinese FHB resistance germplasm, 'Sumai-3' and 'Ning 7840' showed a high level of FHB resistance due to the major QTL *Fhb1* on chromosome 3BS (Bai, 1996) and another QTL *Fhb2* on chromosome 6BS (Anderson *et al.,* 2001). These two QTLs show stable FHB resistance in different environments (Rudd *et al*., 2001). *Fhb1* has the largest effect on type II resistance among all reported loci and was mapped as a single gene using flanking sequence-tagged-site (STS) markers to a 1.2 cM interval (Cuthbert *et al.,* 2006). Later, *Fhb1* has been cloned using map-based cloning, which led to a report of the first *Fhb1* candidate as a pore-forming toxin-like (*PFT*) gene (Rawat *et al.,* 2016). However, Jia *et al.,* (2018) reported that (*PFT*) gene sequence of susceptible line 'PH691' was identical to that of 'WSB' and 'Sumai3', yet 'PH691' was highly susceptible to FHB. Indicating that there is an association between (*PFT*) with FHB resistance in some germplasms is due to its tight linkage to the resistance gene. On the other hand, Su *et al.,* (2019) pointed out that (*TaHRC*) a gene that encodes a putative histidine-rich calcium-binding protein conferring FHB susceptibility and by deleting the start codon of the gene that reflected on FHB resistances. As result, by manipulating the sequence of (*TaHRC*), it may help to improve FHB resistance. More recently, Su *et al.* (2018) developed two diagnostic markers based on the sequence deletion in the gene coding putative histidine calcium binding protein (*TaHRC*). These markers have been

validated in many breeding populations, thus are the best diagnostic markers for transferring *Fhb1* into new wheat cultivars.

Sources of FHB resistance has been reported from different regions of the world. FHB resistance sources from China and Japan usually have a high level of resistance to FHB, but they also have some unfavorable agronomic traits (Bai and Shaner, 2004). Some sources from Europe including Arina, Renan, and Dream are moderately resistant to FHB (Buerstmayr *et al.,* 2009). A spring wheat cultivar Frontana from South America shows a high-level resistance to type I and type II FHB resistance with the main QTL on chromosome 3A and 5A that explained collectively about 25.0% of the phenotypic variation (Steiner et al., 2004). Another wheat cultivar Encruzhilada is also from South American and shows a high level of resistance to FHB (Mesterhazy, 1995). *Fhb4* has been mapped at chromosome 4B from the RIL population Nada2419/Wangshuibai (Xue *et al.,* 2010) and may be associated with a *Rht* gene in the same chromosome, in which tall genotypes may escape infection when limited inoculum is available (McCarteny *et al.,* 2007).

In general, local cultivars with moderate resistance can be useful resistance sources for breeding because their good adaptation to local environments (Waldron et al.,1999). In the U.S., *Fhb1* has not been reported in any hard winter wheat cultivars. However, many of wheat cultivars have been reported to have moderate resistance to FHB and used as resistance sources in breeding, such as 'Everest', 'Overland', 'Lyman',' Heyne' and 'Hondo' (Bockus et al., 2009; Zhang *et al.,* 2012a; Fatima, 2016). Currently most HWW cultivars are highly susceptible to FHB. QTLs have been reported from some of those sources including these on 2DS, 3BS, 4BS and 4DS, 5AL (Fatima, 2016; Zhang *et al.,* 2012a; Bockus et al., 2009). QTLs for FHB resistance on 2D, 4B, 4D were detected from 'Art' (Clinesmith *et al.,* 2019). Another study on HWW concluded that QLTs on 4A, 4D, 5B, and 4DL for FHB resistance contributed by 'Overland' (Fatima, 2016). A QTL on

1B from Japanese hard red winter wheat 'Yumechikara' was mapped by Nishio *et al.*, 2016). Those QTLs may be easier to be incorporated into U.S. HWW cultivars due to good adaptation of these donor parents. Therefore, mapping the QTLs in U.S. HWW may be critical for deployment of these QTLs in U.S. HWW (Bai *et al.,* 2018).

1.10 Evaluation of FHB resistance

Phenotyping for FHB resistance is time-consuming and costly. Expression of FHB resistance is not only influenced by the environmental factors (G X E interaction) such as the humidity, temperature, and the plants' growth stage at the inoculation (Bai & Shaner 1994; Parry *et al.,* 1995; Klahr *et al.,* 2007), also morphological traits may play an important role in wheat resistance to FHB, especially in field conditions. Morphological traits, such as plant height (PH) and anther extrusion (AE) have been correlated with FHB resistance (He *et al.,* 2018). Narrow and short floral opening increases FHB resistance. Also, wheat verities with early flowering time show higher susceptibility than these with late flowering.

1.11 Relationship between plant height and FHB resistance

Plant height was reported to be negatively correlated with FHB severity. Short plants have been associated with increased FHB susceptibility in field conditions (Mesterhazy, 1995; Hilton *et al.,* 1999; Buerstmayr *et al.,* 2000, Miedaner & Voss, 2008; Ha¨berle *et al.,* 2009, and Yan *et al.,* 2011) In general, taller genotypes tend to have lower levels of FHB symptoms, because the shorter plants may be closer to the source of the inoculum in the crop residues on soil surface and may receive more ascospores than these of tall plants (Draeger *et al.,* 2007; Yan *et al.,* 2011). However, the differences in FHB resistance between tall and dwarf plants were also detected in the greenhouse experiments when point inoculation was used, thus the *Rht-B1* and *Rht-D1* loci coincident with FHB loci may be due to tightly linked QTLs or pleiotropic effect of the *Rht* genes

in these populations (Draeger *et al.,* 2007, Srinivaschary *et al.,* 2009). However, consistent associations were not found between *Rht* genes and FHB resistance (Liu *et al*., 2010 and Yan *et al.,* 2011). For an instance, Miedaner and Voss (2008) studied three winter wheat mapping populations (Apache/ Biscay, Romanus/Pirat and History/ Rubens) with Biscay, Pirat and Rubenen carrying *Rht-D1b* short allele and found that the lines carrying *Rht-D1b allele* were about 7-18% shorter than the lines with *Rht-D1a* alleles, but the taller plants (*Rht-D1a*) showed 22-53% higher FHB severity than short lines with *Rht-D1b*.

1.12 Genetic markers for FHB resistance QTLs

Quick development of molecular marker technologies has revolutionized the modern breeding and molecular markers have been used as an indispensable tool in modern breeding programs (Kelly *et al.,* 1999). Genetic markers can be defined as features to determine genetic loci or alleles of the genes that are transmitted from generations to generations, therefore, they can be used as experimental probes or tags to keep track of an individual gene or a chromosome (Jiang, 2013). Genetic markers have two types, classical markers including morphological, cytological and biochemical markers, and DNA markers (Jiang, 2013). Morphological marker is the oldest markers that have been used to select plants for desirable traits. Some of the morphological markers such as leaf- shape, flower color, pubescence color, pod color, seed color, and seed shape are mostly visible traits (Liu, 1999). The morphological markers directly reflect polymorphisms of traits. As a result, it is easy to be determined and used to select indirectly for linked traits. An example of the successful use of morphological markers was to select a semi-dwarf gene in rice and wheat for high yield (Jiang, 2013).

Cytological marker is another type of classical markers where the chromosomes are visualized based on bending patterns. The banding patterns differ in distribution of euchromatin

and heterochromatin (Jiang, 2013). Two typical examples of chromosomes bandings are Q bands that are produced by quinacrine hydrochloride and G bands that are produced by Giemsa stain. These chromosome landmarks can be used for physical mapping and linkage group identification. However, procedure for cytological marker assays are complicated thus are not suitable for routine plant breeding and genetic mapping (Nadeem, *et al.,* 2017).

Protein markers usually refer to isozyme markers in which the same proteins have different structures, molecular weights and electrophoretic mobility, thus they can be separated in a protein gel and used as genetic markers to map linked genes. However, the isozyme markers are limited in number in most crops so they are not sufficient in number for mapping of most traits (Tanksley, 1983).

DNA markers have been used to detect polymorphisms among different genotypes. The polymorphism occurs due to alternative DNA sequences (alleles) at a locus among individuals or populations (Qi *et al.,* 2014). If the sequence polymorphism between parents are tightly associated with a QTL, then they can be used as tools to tag the QTLs and tracking the movement of QTLs in the progeny during breeding selection (Mulualem and Bekeko 2016). To identify QTLs and linked markers to the QTLs, construction of genetic maps is the first step. Once markers are confirmed to be tightly linked to a QTL in a linkage map, the markers can be used to introgress the QTL into new breeding materials through marker-assisted selection, and conduct comparative mapping between different species and physical mapping to identify candidate gene as first step towards map-based cloning of the QTL (Semagn *et al.,* 2006).

There are two major methods for polymorphism detection: Southern blotting using a nuclear acid hybridization technique (Southern, 1975) and PCR using a polymerase chain reaction technique (Mullis,1990). Typical Southern blotting uses DNA restriction fragment length

polymorphism (RFLP) proposed by Botstein *et al.,* (1980) for human linkage mapping. This technique has been successfully used in human, animal and plant genetics researches. PCR-based methods include Random Amplified Polymorphic DNA (RAPD) (Williams J.G.K., 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.,*1995), Single-Strand Conformation Polymorphism (SSCP), SSR (microsatellite), Sequence-tagged Microsatellite Sites (STMS), Sequence Tag Sites (STSs), Expressed Sequence Tags (ESTs) and Single Nucleotide Polymorphisms (SNP) markers (Jordan and Humphries 1994; Gupta *et al.,* 1999). Among them, SSR markers have been widely used in QTL mapping and marker-assisted selection for a long time because of its reproducibility (Akkaya *et al.,* 1992). However, all those markers require prior known sequences for primer design except for RAPD and AFLP (Gupta *et al.,* 1999).

As quick development of next-generation-sequencing (NGS) techniques, whole-genome SNP chips and NGS-based marker platforms have emerged as popular marker systems for QTL mapping and breeding (Mommadov *et al.,* 2012). An SNP is a single nucleotide base difference between two DNA sequences or individuals. SNPs can be classified based on nucleotide substitutions as either transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). SNPs provide the simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance (Sobrino *et al.,* 2005). Therefore, they can offer unlimited markers for genetics and breeding applications. In plants, we can find one SNP in every 100-300 bp DNA sequence (Xue, 2010). It can be within coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes (Jiang, 2013).

NGS using high-throughput sequencing can generate gigabyte genomic data in hours with low costs (Gupta *et al.,* 2008), which makes it possible to discover genome-wide SNPs even in complex genomes (Khlestkina and Salina 2006). GBS method is an NGS-based high-throughput

marker platform that uses restriction digestion to reduce the complexity of genomes, and ligation of digested fragments to adaptors with barcodes to multiplex samples for NGS (Poland *et al.,* 2012). GBS-based SNPs is a high-throughput, multiplex marker system for QTL mapping and genome-wide marker screening. For marker-assisted selection for a specific gene/trait, GBS-SNPs can be converted into singleplex markers such as Kompetitive Allele Specific PCR (KASP) (Livak *et al.,* 1995; Mammadov *et al.,* 2012). Due to its low cost per datapoint, KASP assay has been widely used in many crops including wheat for marker-assisted selection (MAS) of specific QTL/gene in breeding (Poland *et al.,* 2012).

1.13 Mapping QTLs for FHB resistance

To identify QTLs for FHB resistance, constructing genetic linkage maps are important (Bai *et al.,* 2003; Collard *et al.,* 2005; Zhang *et al.,* 2004; Giancaspro, 2016; Cai *et al.,* 2019). Linkage maps which are represented as graphs or tables of the positions of the DNA markers have been utilized for identifying chromosomal regions that controls quantitative traits using QTL analysis (Collard *et al.,* 2005). The map positions are inferred by estimation of recombination frequencies (RF) between markers (Griffiths, 2005). DNA markers that are tightly linked to a QTL of interest can be used for MAS for the QTL in breeding programs (Collard *et al.,* 2005).

To build up a linkage map for QTL mapping, it requires a segregation population. The parents of the population must have contrasts in the trait (s) of interest. The earliest QTL mapping study was reported by Sax (1923). Since then this method has been developed using DNA markers (Cai *et al.,* 2019). Both the generation and size of the mapping population may affect the accuracy of QTL mapping (Somers et al. 2003). Several population types have been used and all of them have the pros and cons. For example, a F_2 population that derives from F1

hybrids is easy and quick to produce, but each plant is a single genotype and phenotype of each genotype cannot be repeated in different environments. Recombinant inbred lines (RILs) consist of homozygous lines derived from continuously selfing from F_2. Each of the lines contains a unique combination of chromosomal segments derives from the original parents. The disadvantage is that producing this type of population takes several growing seasons of selfing (Waldron *et al.,* 1999). Double haploid (DH) population can significantly reduce production time by regenerating plants from haploid tissue through chromosome doubling (Chen *et al.,* 2006). Both RI and DH populations are mainly homozygous lines and each genotype can be phenotyped multiple time to get repeatable data for QTL mapping (Kao, 2005). To construct of a linkage map, polymorphic DNA markers between parents were screened in the populations and recombination rates among markers were computed to calculate genetic linkage among markers. Usually, a large population of more than 150 RILs from F_5 or later generations are recommended for QTL mapping (Collard *et al.,* 2005).

Several methods can be used for QTL mapping: single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), and multiple interval mapping (MIM) (Liu, 1997; Cai *et al.,* 2019). CIM method can be used to detect the markers closely linked QTLs by considering some background markers (Tanksley, 1993). On the other hand, the MIM method can be used to detect epistasis between QTLs by considering multiple marker intervals simultaneously, therefore, it is used to detect QTL interactions (Wang *et al.,* 2006; Cai *et al.,* 2019). The statistical methods used for single-marker analysis include t-tests, analysis of variance (ANOVA) and linear regression. Moreover, linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL. The major disadvantage of this method is that the recombination occurs

between the markers and the QTL may underestimate the QTL effects (Collards *et al.*, 2005). Using a large number of genome-wide segregating DNA markers may help to solve the problem (Tanksley, 1993; Collard *et al.*, 2005).

Table 1.1 Summary of reported FHB QTLs from different studies.

Type I resistance			
Source	**QTL location**	**Population**	**References**
Frontana	3A, 5A	(Frontana (R) and Remus (S))- DH	(Steiner *et al.*, 2004)
DH181, AC Foremost (3A)	3AS, 5AS, 3BS, 3BSc, 6BS, 2DS, 4DL	(DH181 (R) x AC Foremos (S))- DH	(Yang *et al.*, 2005a)
Wangshuibai	5A, 4B, 5B	(Wangshuibai (R) x Nanda2419)- RIL	(Lin *et al.*, 2006)
Wangshuibai	3AS, 5AS, 3BS, 4B, 5DL	(Wangshuibai (R) x Wheaton (S))- RIL	(Yu *et al*., 2008)
RL4137	1B, 2B, 3A, 6A, 6B, 7A , 7D	(RL4137 (R) x Timgalen (MR))	(Srinrvasachary *et al*., 2008)
Sumai-3, Y1193-6 (2DS)	3BS, 6BL, 2DS	(Sumai-3 (R) x Y1193-)-RIL	(Basnet *et al*., 2012)
Frontana	3A, 6A, 4D	(Frontana (R) x Chris Reciprocal) backcross monosomic (RBCM)	(Yabwalo *et al.*, 2011)
T. dicoccum-161	4B, 6A, 6B	(*T. dicoccum*-161 (R) x DS-131621) (durum wheat) BC1F4	(Buerstmayr *et al.*, 2012)
Floradur (3B)	3B, 4B, 6B	(*T. dicoccum*-161 (R) x Floradur) (durum wheat) BC1F4	(Buerstmayr *et al.*, 2012)
T. dicoccum-161	4B, 7B	(*T. dicoccum*-161 (R) x Helidur) (durum wheat) BC1F4	(Buerstmayr *et al.*, 2012)
DT735, BGRC3487(3B)	2A, 3B, 5B, 7A	(BGRC3487 x 2*DT735 (MR)) BCRIL	(Ruan *et al.*, 2012)
Frontana	3A, 4A, 6B, 2B, 4B, 5A, 7B	(Frontana (R) x Remus)- DH	(Szabo-Hever *et al*., 2012)
Jamestown	1A, 2B, 2D, 3B, 6A, 7A, and 7B	(Jamestown x LA97113UC-124)- RIL	(Wright *et al*., 2012)
Jamestown	1B, 2B, 3A, and 6A	(Pioneer25R47 x Jamestown)- RIL	(Wright *et al*., 2012)
Massey (4BS), Becker (2D)	2D, 4BS	(Becker x Massey (MR))- RIL	(Liu *et al.*, 2013)
Ernie, MO 94-317 (4BS)	4BS, 4DS, 5AL	(Ernie (MR) x MO 94-317)- RIL	(Liu *et al.*, 2013)
Frontana	1A, 1B, 2D, 3B, 4A, 5A, 5B, 6A, 7B	(GKMini Mano x Frontana)	(Agnes *et al*. ,2014)
NC-Neuse, AGS (5B)	1A, 5B, 6A	(NC-Neuse (MR) x AGS RIL (MR)) x AGS)-RIL	(Petersen *et al.*, 2015)
CM-82036	3B, 5A, 1B	(CM-82036 (R) x Remus (S))- DH	(Buerstmayr *et al*., 2003)

Type II resistance			
Source	**QTL location**	**Population**	**References**
Sumai-3, Stoa	3BS, 6BS	((Sumai-3 (R) x Stoa (MS))- RIL	(Waldron *et al.*, 1999)
Ning7840	3BS	(Ning7840 (R) x Clark (S))- RIL	(Bai *et al.*, 1999)
ND2603	3AL,6AS, 3BS	(ND2603 (R) x Butte86(MS))- RIL	(Anderson *et al.*, 2001)
Sumai-3, Stoa	2AL, 3BS, 4BS, 6BS	(Sumai-3 (R) x Stoa (MS))- RIL	(Anderson *et al.*, 2001)
Ning7840	2AS, 2BL, 3BS	((Ning7840 (R) x Clark (S))- RIL	(Zhou *et al.*, 2002)
CM-82036	5A, 1B, 3BS	(CM-82036 (R) x Remus (S))-DH	(Buerstmayr *et al.*, 2002)
Ning7840	3BS	(Ning7840 (R) x Wheaton (S))- F2:3	(Zhou *et al.*, 2003)
Ning7840	3BS	(Ning7840 (R) x IL89-7978 (S))-F3:4	(Zhou *et al.*, 2003)
CM-82036	3BS, 5A	(CM-82036 (R) x Remus (S))- DH	(Buerstmayr *et al.*, 2003)
F201R, Patterson	1B, 3A, 3D, 5A	(F201R (R) x cv. Patterson (MS))- RIL	(Shen *et al.*,2003)
Huapei57-2, Patterson	3A, 3BS, 3BL, 5B	(Huapei57-2 (R) x Patterson (MS))- RIL	(Bourdoncle *and* Ohm 2003)
Wuhan-1, Maringa	2DL, 3BS, 4B	(Wuhan-1 (R) xMaringa (MS))	(Somers *et al.*, 2003)
Wangshuibai, Alondra	1B, 3BS	(Wangshuibai (R) x Alondra (S))-RIL –Alondra (S))-RIL	(Zhang *et al.*, 2004)
Wangshuibai	7AL, 3BSd, 1BL, 3BSc	(Wangshuibai (R) x Wheaton (S))-RIL	(Zhou *et al.*, 2004)
DH181	3BS, 6BS, 2DS, 7BL	(DH181 (R) x AC Foremost (S))- DH	(Yang *et al.*, 2005a)
Chokwang	3BS, 4BL, DL	(Chokwang (R) x Clark (S))- RIL	(Yang *et al.*, 2005b)
Dream, Lynx	6AL, 1B, 2BL, 7BS	(Dream (R) x Lynx (S))- RIL	(Schmolke *et al.*, 2005)
Wangshuibai	7A, 3B, 5B, 2D	(Wangshuibai (R) x Alondra (S))- DH	(Jia *et al.*, 2006)
W14	5AS, 3BS	(W14 (R) x Poin2684 (S))- DH	(Chen *et al.*, 2006)
CS-SM3-7ADS	6A, 3B, 2D, 4D	(CS-SM3-7ADS (R) x Annong 8455(S))- RIL	(Ma *et al.*, 2006a)
Wangshuibai	3B, 2A	(Wangshuibai (R) x Annong 8455)- RIL	(Ma *et al.*, 2006b)
Sumai-3	3BS	(Sumai-3*5(R) x Thatcher (S) and HC374 (R) x 3*98B69-L47 (S))- RIL	(*Cuthbert et al.*, 2006)
Frontana, Seri82	3AL, 7AS, 1BL	(Frontana (MR) x Seri82(S))- F3:5	(Mardi *et al.*, 2006)
CJ9306	1AS, 3BS, 7BS, 2DL, 5AS	(CJ9306 (R) x Veery (S))- RIL	(Jiang *et al.*, 2007)

Sumai-3	3BSc, 5A, 6B	(BW278 (R) x AC Foremost (S))- RIL(from 1440) Sumai-3	(Cuthbert *et al.*, 2007)
Arina, NK93604	1AL, 7AL, 1BL, 6BS	(Arina (MR) x NK93604 (MR))- DH	(Semagn *et al*., 2007)
Ernie	5A, 2B, 3B, 4BL	(Ernie (MR) x MO 94-317 (S))- RIL	(Liu *et al.*, 2007)
Wangshuibai	5AS, 7AL, 3BS, 3DL, 5DL	(Wangshuibai (R) x Wheaton (S))- RIL	(Yu *et al*., 2008)
G16-92, Hussar	1A, 2BL	(G16-92 (R) x Hussar (S))- RIL	(Schmolke *et al.*,2008)
Gamenya	2DS	(Sumai-3 (R) x Gamenya (S))- DH	(Handa *et al.*, 2008)
IL94-1653, Patton	2B, 3B, 4B, 6B	(IL94-1653 x Patton)- RIL	(Bonin and Kolb, 2009)
G93010	7BS /5BL, 6BS	(G93010 (R) x Pelikan)- RIL	(Häberle *et al.*, 2009)
Wangshuibai	7A, 1B, 3B, 6B, 2D	(Wangshuibai (R) x Sy95-7 (S))	(Zhang *et al*., 2010)
T. macha	2A, 5A, 2B, 5B	(T. macha (R) x Furore (S))- RIL	(Buerstmayr *et al.*, 2011)
Sumai-3	7A, 3BS	(CS-Sumai 3-7ADSLC)- RIL	(Jayatilake *et al.*, 2011)
Haiyanzhong	1AS, 5AS, 6BS (2), 7DL	Haiyanzhong (R) x Wheaton RIL	(Li *et al.*, 2011)
PI 277012	5AS, 5AL	(PI 277012 (R) x Grandin)- DH	(Chu *et al.*, 2011)
Frontana	3A, 6A, 4D	(Frontana (R) x Chris) Reciprocal backcross monosomic (RBCM)	(Yabwalo *et al.*, 2011)
Huangfangzhu	1AS, 5AS, 7AL, 1B, 3BS	(Huangfangzhu (R) x Wheaton)- RIL	(Li *et al.*, 2012)
Heyne	3AS, 4AL, 4DL	(Heyne (R) x Trego)- RIL	(Zhang *et al*., 2012a)
Baishanyuehuang	3BSd, 3BSc, 3A, 5A	(Baishanyuehuang (R) x Jagger)- RIL	(Zhang *et al*., 2012b)
BGRC3487 DT735	3B, 5A, 5B, 7A, 7B	(BGRC3487 x 2*DT735 (MR))- BCRIL	(Ruan *et al.*, 2012)
RCATL33	3B, 5A, 3A	(RCATL33 (R) x RC Strategy)- RIL	(Tamburic-Ilincic and Miedaner, 2012)
VA00W-38 26R46	1BL, 2A, 2DL, 5B, 6A, 7A	(VA00W-38 (MR) x 26R46)- RIL	(Liu *et al.*, 2012)
Jamestown	1A, 2B, 2D, 3B, 6A, 7A, 7B	(Jamestown x LA97113UC-124)- RIL	(Wright *et al*., 2012)
Jamestown,	1B, 2B, 3A, 6A	(Pioneer25R47 x Jamestown)	(Wright *et al*., 2012)
Mt. Gerizim #36	3A, 6B	(Mt. Gerizim #36 (R) x Helidur)- BC	(Buerstmayr *et al.*, 2013)
Becker	1DS, 3BL	(Becker x Massey)- RIL	(Liu *et al.*, 2013)
Ernie, MO 94-317	2DS, 4BS, 4DS, 5AL, 3BL, 4BS	(Ernie x MO 94-317)- RIL	(Liu *et al.*, 2013)
Catbird	7DS, 3BS, 5DL	(Catbird x Milan)- DH	(Cativelli *et al*., 2013)
Huangcandou, Jagger	3BSc, 3BSd, 3AS, 2D, 6D	(Huangcandou (R) x Jagger)-RIL	(Cai and Bai, 2014)
Ben, PI41025	2A, 3A, 5A	(Ben (Durum) x PI41025) RIL	(Zhang *et al*., 2014)
Neixiang 188	2D, 4B, 4D, 5A, 5D 7B	(Neixiang188 x Yanzhan 1)	(Chao lv *et al*., 2014)

Yumechikara	1BS	(Yumechikara x Kitahonami)- (DH)	(Nishio *et al.*, 2016.)
NC-Neuse	1A, 2A, 6A	(NC-Neuse (MR) x AG (S)-RIL	(Petersen *et al.*, 2015)
Haiyanzhong (HYZ)	6B, 7D, 3B, 4B, 4D	(HYZ x Wheaton)	(Cai *et al.*, 2019)
Overland	4DL,4AL, 5BL	(Oveland x overley)	(Fatima, 2016)
Cultivar art	2D,4B, 4D	(Cultiar art x Everest)	(Clinesmith *et al.*, 2019)

Type III resistance

Source	**QTL location**	**Population**	**References**
Wuhan-1, Maringa	5AS, 2D, 3BS	(Wuhan-1 (R) x Maringa (MS))- DH	(Somers *et al.*, 2003)
CM-82036	3BS	(CM-82036 (R) x Remus)- DH	(Lemmens *et al.*, 2005)
W14	5AS, 3BS	(W14 (R) x Poin2684 (S))- DH	(Chen *et al.*, 2006)
CJ9306	2DL, 1AS, 3BS, 5AS	(CJ9306 (R) x Veery (S))- RIL	(Jiang *et al.*, 2007)
NK93604	1AL, 2AS	(Arina (MR) x NK93604 (MR))- DH	(Semagn *et al.*, 2007)
Wangshuibai	1A, 5AS, 7AL, 1BL, 3BS, 5DL	(Wangshuibai (R) x Wheaton (S))- RIL	(Yu *et al.*, 2008)
Sumai-3	7A, 3BS	(CS-Sumai-3 - 7ADSLC)	(Jayatilake *et al.*, 2011)
PI 277012	5AS, 5AL	(PI 277012 (R) x Grandin)- DH	(Chu *et al.*, 2011)
RCATL33	3B, 5A, 3A	(RCATL33 (R) x RC Strategy)- RIL	(Tamburic-I. and Miedaner, 2012)
VA00W-38, 26R46	1BL, 2A, 2DL, 5B, 6A, 7A	(VA00W-38 (MR) x 26R46)- RIL	(Liu *et al.*, 2012)
Jamestown	1A, 2B, 2D, 3B, 6A, 7A, 7B	(Jamestown x LA97113UC-124)- RIL	(Wright *et al.*, 2012)
Jamestown	1B, 2B, 3A, 6A	(Pioneer25R47 x Jamestown)- RIL	(Wright *et al.*, 2012)
Becker	4DL	(Becker x Massey)- RIL	(Liu *et al.*, 2013)
Frontana	1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D	(GKMini Mano x Frontana)	(Agnes *et al.*, 2014)
NC-Neuse, AGS	1A, 1B, 1D, 2A, 4A, 5B	(NC-Neuse (MR) x AGS)- RIL	(Petersen *et al.*, 2015)

Type IV resistance

Source	**QTL location**	**Population**	**References**
W14	5AS, 3BS	(W14 (R) x Poin2684 (S))- DH	(Chen *et al.*, 2006)
IL94-1653, Patton	2B, 4B, 6B	(IL94-1653 x Patton)- RIL	(Bonin and Kolb, 2009)
PI 277012	5AS, 5AL	(PI 277012 (R) x Grandin)- DH	(Chu *et al.*, 2011)
Frontana	3A, 6A, 4D	(Frontana (R) x Chris -Reciprocal backcross monosomic (RBCM)	(Yabwalo *et al.*, 2011)
RCATL33	3B, 5A, 3A	(RCATL33 (R) x RC Strategy)- RIL	(Tamburic-Ilincic and Miedaner, 2012)
VA00W-38 26R46	1BL, 2A, 2DL, 5B, 6A, and 7A 2DL, 5B, 6A, 7A	(VA00W-38 (MR) x 26R46)- RIL	(Liu *et al.*, 2012)
Frontana	2B, 4B, 5A, 7B	(Frontana (R) x Remus)- DH	(Szabo-Hever *et al.*, 2012)
Massey	4BS	(Becker x Massey)- RIL	(Liu *et al.*, 2013)
Ernie, MO 94-317	4BS, 4DS, 3BL	(Ernie x MO 94-317)- RIL	(Liu *et al.*, 2013)

Frontana	1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D	(GKMini Mano x Frontana)- DH	(Agnes *et al*., 2014)
NC-Neuse, AGS	1A, 1B, 1D, 4A,	(NC-Neuse (MR) x AGS)- RIL	(Petersen *et al.*, 2015)

1.14 References

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Chapter 2 . Mapping QTLs for *Fusarium* head blight resistance in winter wheat 'CI13227'

2.1 Introduction

Fusarium head blight (FHB) is one of the most devastating fungal diseases in wheat (*Triticum aestivum* L.) worldwide. It mainly caused by *Fusarium graminearum Schwabe* [telomorph, *Gibberella zea* (Schw.) petch]. (Bai and Shaner, 2004; McMullen, 1997). FHB not only causes grain yield losses, but also reduces grain quality. FHB infection usually bleaches spikes and sometimes causes premature plant death (Bai and Shaner, 1994). Mycotoxins produced by the pathogen are also harmful to human and animal health (Bai and Shaner, 2004). The total

economic losses due to FHB epidemics from 1993 to 2001 were about $2.49 billion in the U.S. (Nganje *et al*., 2004).

Many approaches have been used to reduce the damage caused by FHB, including tillage practice, fungicide application and crop rotation, growing resistant cultivars, however, is the most effective strategy for the disease control (McMullen *et al.,* 1997). *Fusarium* head blight resistance in wheat can be active or passive. Active resistance may include genetic and physiological resistance (Buerstmayr *et al.,* 2009), whereas passive resistance refers to morphological factors that help to avoid the infection such as plant height, flowering time, and spike morphology, etc. (Buerstmayr and Buerstmayr, 2015; Lu *et al.,* 2012). For plant height, two semi-dwarf genes, *Rht-B1b* and *Rht-D1b* for reduced plant height (*Rht)* (Buerstmayr *et al.,* 2009; Hedden 2003) that are gibberellins (GA) insensitive, have been associated with FHB resistance (He *et al.,* 2016). However, it still remains unknown if the *Rht* genes have pleiotropic effect or link to the genes for FHB resistance.

FHB resistance can also be divided into five types. Type I refers to wheat resistance to initial penetration and infection; type II refers to resistance to FHB symptom spread within a spike after initial infection (Schroeder and Chistensen 1963); type III refers to low DON accumulation (Miller, 1985). Other two types including low *Fusarium* damaged kernel (FDK), and tolerance to FHB (Mesterhazy, 1995). Since 1980s, breeders screened thousands of Chinese germplasm accessions for FHB resistance and identified ‘Sumai 3’ and ‘Ning 7840’ to have a high level of type II resistance (Bai and Shaner, 1994), thus they have been used extensively as the sources of resistance in breeding programs worldwide. Late, several other resistant germplasms have been reported from Europe, North America, and South America (Ban, 2001; Mesterhazy, 1995; Singh and Ginkel, 1997).

FHB resistance in wheat is a quantitative trait that is controlled by multiple QTLs. These QTLs are easily influenced by environmental factors (Bai and Shaner, 1994). To date, more than 50 QTLs for FHB have been reported on all 21 wheat chromosomes (Buerstmayr *et al.,* 2009), but only seven QTLs have been formally named as *Fhb1* to *Fhb7*. Among them, *Fhb1* on chromosome arm 3BS of 'Sumai 3' (Cuthbert *et al.,* 2006) shows a major effect on type II resistance and high stability in different backgrounds and across different environments (Bai and Shaner 2004). *Fhb2* for Type II resistance derived from 'Sumai-3' was mapped on chromosome 6BS (Cuthbert *et al.,* 2007; Waldron *et al.,* 1999), *Fhb3* was derived from' *Leymus racemosus'* (Qi *et al.,* 2008), *Fhb4* on chromosome 4B and *Fhb5* on chromosome 5A were both derived from 'Wangshuibai' (Xue *et al.,* 2010; 2011), *Fhb6* on chromosome 1A was derived from '*ELymus tsukushiensis'* (Cainong *et al.,* 2015), and *Fhb7* on 7DS was derived from '*Thinopyrum ponticum'* (Guo *et al.,* 2015). In the United States, several soft winter cultivars have shown moderate resistance to FHB, including soft wheat cultivars 'Ernie', 'Roane' and 'Freedom', therefore they are used as sources of resistance in several U.S. wheat breeding programs (Liu *et al.,* 2007). In hard winter wheat (HWW), several cultivars have also been reported with moderate FHB resistance such as 'Heyne', 'Hondo' and 'Overland' (Bockus *et al.,* 2009; Zhang *et al.,* 2012; Jin *et al.,* 2013). Recently, screening HWW for FHB resistance in field and greenhouse experiments identified several moderately resistant cultivars including 'Lyman', 'SD05210', 'Evereset', and 'Harry', etc (Jin *et al.,* 2013). These cultivars showed moderate to high resistance to FHB, and most of them are adapted to the wheat growing environments in the Great Plains (Zhang *et al.,* 2012).

The quick development of high-throughput genotyping technologies makes it possible to map QTLs using high-density maps. Several wheat SNP arrays with varied SNP density have been developed in wheat (Akhunov *et al.,* 2009; Paux *et al.,* 2006) and used to map QTLs for many

traits of interest (You *et al.*, 2018), especially Illumina 90K wheat SNP arrays (Wang *et al.*, 2014). In this study, we used the 90K wheat SNP arrays to map the QTLs for FHB resistance in winter wheat 'CI13227', identify tightly linked markers to the QTLs and convert these SNP markers into Kompetitive allele specific PCR (KASP) assays for marker-assisted selection (MAS) in breeding programs.

2.2 Materials and methods

2.2.1 Plant materials and FHB resistance evaluation

A population of 179 double haploids (DH) lines was developed from 'CI 13227' x 'Lakin' is an FHB susceptible Kansas hard white winter wheat cultivar derived from Arlin/KS89H130, whereas 'CI13227' (Cltr13227) is a moderate FHB resistant soft red winter wheat with the pedigree of Wabash//American Banner/Aniversario. The parents and their DH lines were evaluated for FHB resistance in the greenhouses at Kansas State University in Manhattan, Kansas in spring and fall 2016, and spring 2017, respectively. Wheat seeds were planted in plastic growing trays filled with Metro-mix 360 soil mix (Hummert International, Topeka, KS). After vernalization in a cold room at 6 °C for 50 d, five seedlings per line were transplanted into each pot and the pots were arranged on the greenhouse benches using a randomized complete block design (RCBD) with two replications per line. The greenhouse temperature was set at 17 ± 5 °C during the night and 22 ± 5 °C during the day with 14 h of supplement light. Powdery mildew was controlled by burning sulfur for 3 h each night in a closed greenhouse environment. This treatment has no impact on FHB infection. *Fusarium* inoculum was prepared following Bai *et al.*, (1999). At the early anthesis, plants were inoculated by injecting 10 µl of conidial spores (1000 spores/ spike) into a central spikelet of a spike using point inoculation. Five spikes per pot were inoculated. Inoculated plants were moved to a moist chamber at 100% humidity and 20 - 25 °C for 48 h to initiate infection.

Plants were then returned to the original greenhouse benches for developing the FHB symptoms. FHB was evaluated 14 d after inoculation by counting the infected spikelets and total spikelets to calculate the percentage of symptomatic spikelets (PSS) in a spike using the formula

PSS= 100* (number of infected spikelets/ total number of spikelets in a spike).

2.2.2 FHB evaluation in field experiments

The parents and their DH lines were also evaluated in field for FHB resistance at Rocky Ford, Manhattan, KS, in 2017 and 2018 using corn grain-spawn inoculation (Tuite,1969). A RCBD was used with two replications per line. About 1 g of seeds per line was planted in a single row plot of 1 m. The field was misted by sprinklers for 3 min every h between 21:00 h and 6:00 h daily from early flowering to dough stages. FHB severity was scored after 20 - 25 d post anthesis based on symptoms developed on control cultivars. FHB severity was scored using the overall percentage of symptomatic spikelets in spikes in each row. Plant height and heading date were recorded. Plants from each plot were harvested by hand, threshed using a thresher (Almaco, Nevada, IA) to visually score *Fusarium* damaged kernels (FDK). Percentage of FDK was calculated by dividing the number of FDK by total number of kernels harvested from each row. From each plot, five grams of infected seeds were randomly selected for DON evaluation using gas chromatography-mass spectrometry (GC-MS) at the University of Minnesota, St. Paul, MN (Mirocha *et al.,* 1998). DON concentration was measured by part per million (ppm).

2.2.3 DNA extraction and genotyping

At the three-leaf stage, six pieces of one-inch-long tissue from each DH lines and the parents were collected into 96-deep-well plates. The plates with tissue were dried in a freeze dryer (ThermoSavant, Holbrook, NY) for 72 h and ground to fine powder using a Mixer Mill (MM400, Retsch, Germany). DNA was extracted using cetyltrimethylammonium bromide method (Murray

and Thompson, 1980). Wheat 90K-SNP chips developed by Illumina Inc. (San Diego, CA), and assembled by the International Wheat SNP Consortium (Cavanagh *et al.*, 2013). the genotyping was contacted at USDA Small Grains Genotyping Laboratory in Fargo, ND. The SNPs were called using GenomeStudio v2011.1 (Illumina, San Diego, CA).

2.2.4 Linkage map construction and QTLs identification

A linkage map was constructed using SNP data generated from Illumina wheat 90K SNP chips and the regression mapping algorithm in JoinMap version 4.1 (Van Ooijen, 2006). Recombination fractions were converted into centiMorgans (cM) using Kosambi functions (Kosambi, 1943). The QTLs were analyzed for FHB resistance by composite interval mapping (CIM) using Windows QTL Cartographer v2.5 (Wang *et al*., 2005). The thresholds of LOD scores for significant QTLs ($P < 0.05$) were from 2.65 to 2.87 calculated from 1,000 permutations for all traits (Nettleton and Doerge, 2000).

2.2.5 Conversion of SNPs to KASP markers

The SNPs that closely linked to QTLs were converted to KASP assays. KASP primer mix contains three primers designed using Primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/) with two forward primers and one common reverse primer. The allele-specific primers each harbor a unique tail sequence that corresponds with a universal fluorescence resonant energy transfer-cassette; one labeled with FAM™ dye and the other with HEX™ dye. KASP markers were used to evaluate the polymorphisms between the two parents first and the polymorphic SNPs were used to screen the DH population. For KASP analysis, a 6 µl reaction volume consisted of 3 µl 2X KASP Master Mix, 0.0825 µl KASP primer mix and 3 µl of DNA at 20 ng/µl and the data were analyzed using BMG FLUOstar Omega microplate reader (https://www.bmglabtech.com/fluostar-omega/) following the manufacturers' instructions for KASP analysis (http://www.lgcgroup.com).

2.2.6 Data Analysis

Analysis of variance (ANOVA) and phenotypic correlation were calculated using SAS v.9.2 software (SAS Institute Inc., Cary, NC, United States). Broad-sense heritability was estimated using the equation $H^2 = \sigma_g^2 / (\sigma^2_G + \sigma^2_{G\times E/e} + \sigma^2_{E/el})$, where σ^2_G = genotypic variance; σ^2_{GxE} = genotype-by-experiment interaction variance; σ_E^2 = error variance; e = number of experiments and l = number of replications (Nyquist and Barker, 1991).

2.3 Results

2.3.1 FHB variation in DH population

'CI13227' showed moderate resistance to FHB with the mean PSS from 40 to 55%, whereas 'Lakin' was highly susceptible with FHB severities from 95 to 100% in the three greenhouse experiments (Fig. 2.1). PSS frequencies showed continuous distribution. The most severe FHB was observed in spring 2016 with a mean PSS of 57% and the least severe FHB in fall 2016 with a mean PSS of 41%. The broad-sense heritability for the greenhouse experiments was high (0. 76) (Table 2.1). The positive correlation coefficients (0.33 to 0.67) were significant ($P <$ 0.001) among the three greenhouse experiments (Table 2.2). The susceptible parent 'Lakin' was head about 21 earlier than the moderate resistant parent 'CI13227' (Fig. 2.2). The average of the plant height for the 'Lakin' was (97 cm) while the 'CI13227' was about (139 cm) (Fig. 2.3). Transgressive segregation was obvious in all the three greenhouse experiments.

In the 2016 and 2017 field experiments, FHB severity also showed continuous distribution (Fig. 2.4). The mean PSS of the DH population in 2016 and 2017 field experiments were 44% and 49%, respectively. 'CI13227' showed mean PSS of 45% and highly susceptible 'Lakin' was 95%. Unexpectedly, the broad-sense heritability for the field experiments were higher than the greenhouse experiments (Table 2.3). The FDK was 30% for the DH population, 35% for 'CI13227'

and 79% for the 'Lakin'. The DON was 21% for the DH population, 14% for 'CI13227' and 62% for 'Lakin'. The phenotypic correlations were calculated among the plant height, heading date, PSS, FDK and DON (Table 2.4). The susceptible parent head about 24 days earlier than the moderate resistant parent 'CI13227' (Fig. 2.5). The average height for 'Lakin' was (90 cm) while 'CI13227' was (130 cm) (Fig. 2.6). There was a negative correlation between field PSS (FPSS), heading date and plant height (Figs. 2.7, 2.8). Also, FDK was significantly positively correlated with DON content ($r = 0.41$; $P < 0.001$) (Fig. 2.9).

2.3.2 Construction of linkage Map

Among 5,570 polymorphic SNPs analyzed in the DH population, 3,553 were mapped in the linkage map. The map consists of 35 groups representing all 21 chromosomes and covers genetic distance of 4,670 cM with an average interval of 0. 84 cM between markers. The B genome had the most mapped markers (45.8%), whereas the D genome had the lowest (14.8%) (Fig. 2.10).

2.3.3 QTLs for FHB resistance

Composite interval mapping detected four significant QTLs for type FHB resistance including one each on chromosomes 7A (*QFhb.hwwgru.7A*), 2DS (*QFhb.hwwgru.2DS*), 4BS (*QFhb.hwwgru.4BS*) and 5AL (*QFhb.hwwgru.5AL)* in the DH population using PSS data from the three greenhouses and two filed experiments (Table 2.5, Fig. 2.11). The QTLs on chromosomes 7A, 2DS and 4BS were from 'CI13227', while *QFhb.hwwgru.5AL* was from 'Lakin'.

Among the four QTLs, *QFhb.hwwgru.4BS* showed the largest effect on type II resistance and was detected in the three greenhouse experiments, one field experiment, and the mean greenhouse and field data. It was flanked between SNPs *GBS1041* and *GBS1633* and explained 8%, 16%, 17%, 15.5%, 19.3 and 9.6 % of the phenotypic variation (Table 2.5). Also, the QTL for

FDK was contributed by ‘CI13227’ (Fig. 2.11). Another QTL from ‘CI13227’ was *QFhb.hwwgru.7A*, which was significant on in fall 2016 greenhouse experiment, flanked by SNPs *GBS112* and *GBS3612* and explained 7.5% of the phenotypic variation.

QFhb.hwwgru.2DS for low PSS was significant in one greenhouse experiment, 2018 field data and mean greenhouse and field data, explained 12.6%, 6.3%, 5.1% and 5.4% of the phenotypic variation, respectively. This QTL was from ‘CI13227’ *QFhb.hwwgru.2DS* overlapped with the QTL region for both plant height and low DON (Fig. 2.11, Table 2.5).

QFhb.hwwgru.5AL was significant in two greenhouse experiments, two field experiments and overlapped with the mean values for both the greenhouse and field data, explained 8.3%, 10.9%, 9.5%, 12.05%, 10.9% and 11.1% of the phenotypic variations, respectively. This QTL was flanked by SNPs *GBS288* and *GBS1098*, and, also showed a significant effect on low DON, with 5.2% of the phenotypic variation explained by the QTL. ‘Lakin’ contributes the resistance allele.

2.3.4 KASP design and verification

To verify the genotypic data generated by 90K SNP chips and convert the SNPs into KASP SNPs for MAS and breeding. Among 14 chip-based SNPs within or around the QTL regions that were used to design primers, seven were successfully converted to KASP markers. Two SNPs on chromosome 5AL and three on 4BS, one on 2DS and one on 7A showed polymorphisms between the parents and segregation in the DH population. All of them were remapped into the QTLs region with one mapped slightly outside the QTL region. Comparison between array-based SNPs and KASP-based SNPs showed that five of KASP have identical genotypic data as 90k-SNP data in the DH population (Fig. 2.12; Table 2.6).

2.4 Discussion

2.4.1. PSS variation in the DH population

Previously, many studies reported FHB QTL mapping results based on phenotypic data either from greenhouse or field experiments, but not both (Bai *et. al.*, 1999; Buerstmayr *et al.*, 2000; Cai and Bai, 2014; Clinesmith *et. al.*, 2016). In the current study, a DH population and their parents, 'CI13227' and 'Lakin', were evaluated for type II resistance (Schroeder and Christensen1963) in both greenhouse and field experiments to conduct QTL analysis. We identified some common QTLs that were consistent under both environments in the DH population although point inoculation was conducted in the greenhouses and corn grain-spawn inoculation in the field experiments. Positive correlations (r = 0.33 - 0.67) were significant among the three greenhouse experiments ($P < 0.001$) (Table 2.2), indicating that QTLs for FHB resistance identified in this study are reliable.

The transgressive segregation was observed in all greenhouse experiments for FHB resistance, which indicated that both parents might contribute resistance alleles. The transgressive segregation has been frequently reported in previous studies and several FHB resistant cultivars have been created through transgressive segregation including well-known resistant cultivars 'Sumai 3', and several other Chinese resistant cultivars 'Zhen 7495', 'Xiangmai 2', 'Jingzhou 1' and 'Jingzhou 47' etc. Thus, breeders may be able to develop highly resistant cultivars by use of transgressive segregation of FHB resistance to pyramid different genes through crossing two moderately resistant parents that carry different QTLs (Bai and Shaner, 1994; Buserstmayr *et al.*, 2000; Somers *et al.*, 2003; Malihipour, 2017).

In the field experiments, significantly positive correlations were observed for FPSS between the two experiments (r = 0.50), suggesting reasonable repeatability between two field

experiments, in agreement with previous studies (Wegulo 2012; Góral *et. al.*, 2018). Significant correlations were observed between FPSS and FDK (r = 0.23; P< 0.001) and between FDK and DON (r = 0.51; P< 0.001), which is consistent with previous studies (Mesterhazy *et al.*, 1999; Cai *et al.*, 2019; Malihipour, 2017). Paul *et al.*, (2006) summarized more than 163 wheat FHB related studies and found that almost 65% of them showed correlations between FHB severity and DON, suggesting that high PSS usually results in high DON content in infected grain. These results agree with Mesterhazy (2003) that susceptible genotypes usually had moderate to high DON accumulation whereas resistant genotypes usually showed low DON accumulation.

2.4.1 QTLs for type II FHB resistance

Four QTLs for FHB resistance, (*QFhb.hwwgru.4BS*, *QFhb.hwwgru.5AL*, *QFhb.hwwgru.2DS* and *QFhb.hwwgru.7A*) were significant in at least two experiments in the current study. *QFhb.hwwgru.4BS* showed the largest effect on type II resistance. It was located between the flanking markers *GBS1041* and *GBS1633* and explained 8.0 to 17.1% of the phenotypic variation across different experiments. The QTL overlapped with QTL for the plant height and FDK, indicating that the tall plants had better Type II resistance (low PSS and FDK) than shorter plants, in agreement with previous studies (Worland *et al.*, 1998; McCartney *et al.*, 2007; Yu *et al.*, 2008; Tian *et al.*, 2008; Malihipour 2017). To determine if the QTL for FHB resistance is overlapped with the *Rht-B1* gene, a diagnostic KASP marker for *Rht-B1* was analyzed in the population and significant correlations were observed between *Rht-B1* marker and the FHB severity generated from the greenhouses (r = 0.39) and field experiments (r = 0.43 - 0.49; P< 0.001) where plants were inoculated using different methods under different environments. These results indicated *RhtB1* may be either tightly linked to or has a pleiotropic effect on FHB resistance, in agreement with previous studies (Srinivasachary *et al.*, 2008; Buerstmayr, M., and Buerstmayr,

H, 2016). Further research to determine the genetic relationship between FHB resistance and semi-dwarf genes will provide useful information for wheat breeders to select right *Rht* gene to improve plant yield when FHB resistance is one of the major breeding objectives.

QFhb.hwwgru.5AL explained 8.3% and 11.5% of the phenotypic variation across different experiments. Moreover, *QFhb.hwwgru.5AL* overlapped with the QTLs for plant height and DON accumulation, but not FDK. QTLs on 5A have been reported to contribute type I and type II FHB resistance, and low FDK and DON in diverse wheat germplasm (Buerstmayr *et al.,* 2009; Malihipour 2017). Szabo-Hever *et al.* (2012) found more than one QTL for FHB resistance in the chromosome 5A. To date, a number of QTLs for FHB resistance have been reported in the intervals of 30 - 90 cM (Buerstmayr *et al.,* 2009), 146 -167 cM (Buerstayr *et al.,* 2011), and 103-142 cM intervals (Somers *et al.,* 2004) on chromosome 5A. Some of these QTLs overlapped with low DON (Buerstmayr *et al.,* 2009; Malihipour *et al*., 2017). However, the QTL reported in current study may be different from these previously reported QTLs in this chromosome because it does not overlap with any of the QTLs that have been reported so far.

Several QTLs have been reported on 2D previously (Ma *et al.,* 2006; Basnet and Glover 2011; Wangui, 2017). *QFhb.hwwgru.2DS* identified in this study was located in the interval between *GBS2829* and *GBS187* for FHB resistance. Semi-dwarfing gene *Rht8* is located in the same region with the QTL for FHB resistance in the current study, which was also reported in McCartney *et al., (*2016). This QTL was significant for low DON accumulation, plant height, and heading date. The marker for *Ppd-D1* gene, a locus controlling photoperiod insensitivity on chromosome 2DS (Niwa *et al.,* 2018) was also in this region and segregated in the DH population. Several traits segregated in the genomic region may be due to a pleiotropic effect or tightly linked genes at the QTL region.

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Figures and Tables

Figure 2.1 Frequency distribution of mean percentage symptomatic spikelets (PSS) data of DH lines derived from 'CI13227'x 'Lakin' evaluated in three greenhouse experiments conducted in spring 2016, fall 2016, and spring 2017

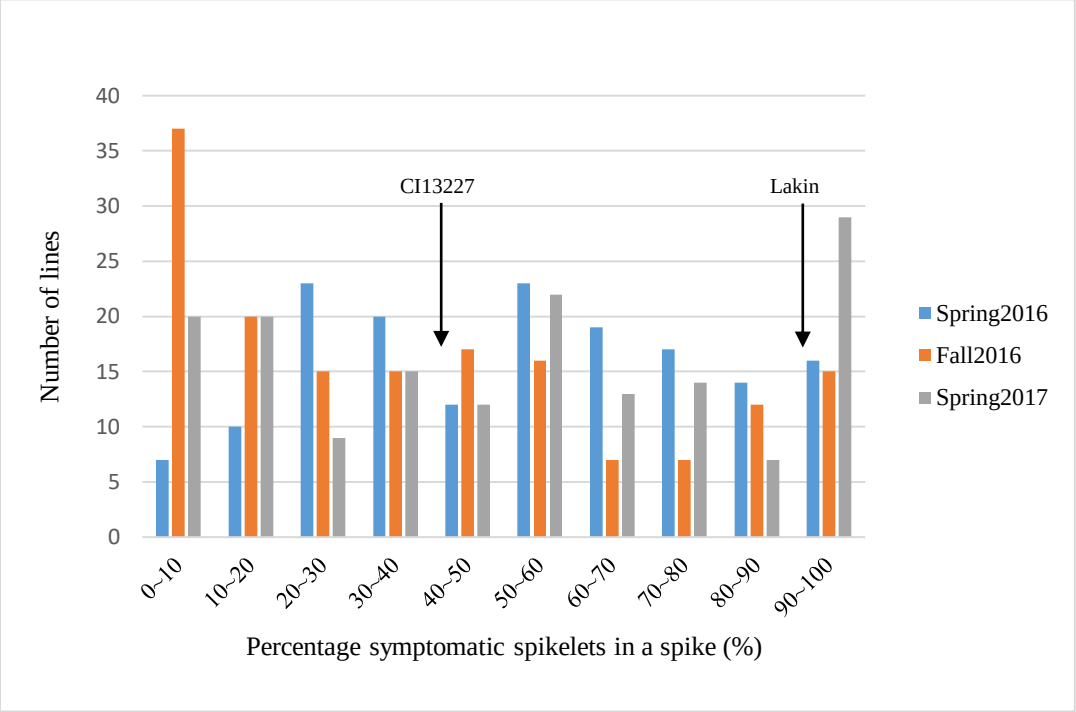


Figure 2.2 Frequency distribution of means of heading date (from the first day the genotype started heading) in the DH lines derived from 'CI13227'x 'Lakin' in three greenhouses experiments conducted in spring 2016, fall 2016, and spring 2017

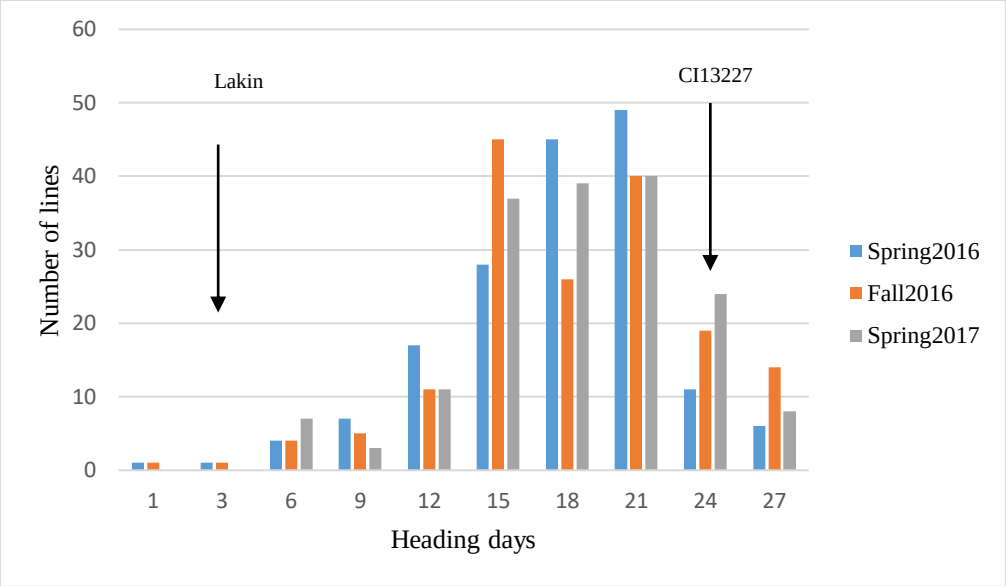


Figure 2.3 Frequency distribution of plant height in the DH lines derived from 'CI13227'x 'Lakin' in three greenhouses experiments conducted in spring 2016, fall 2016, and spring 2017

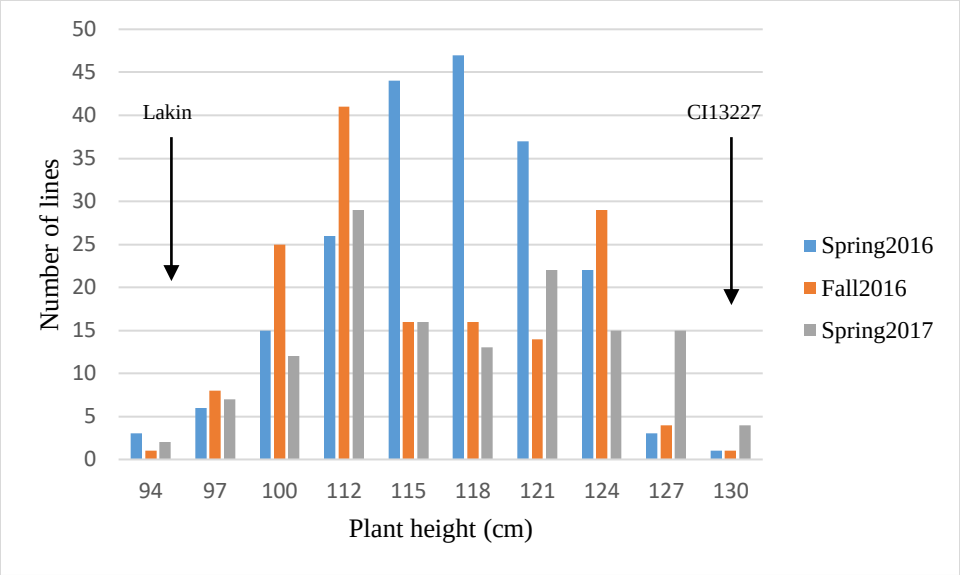


Figure 2.4 Frequency distribution of mean percentage symptomatic spikelets (PSS) data of DH lines derived from 'CI13227'x 'Lakin' in two field experiments conducted in 2017-2018

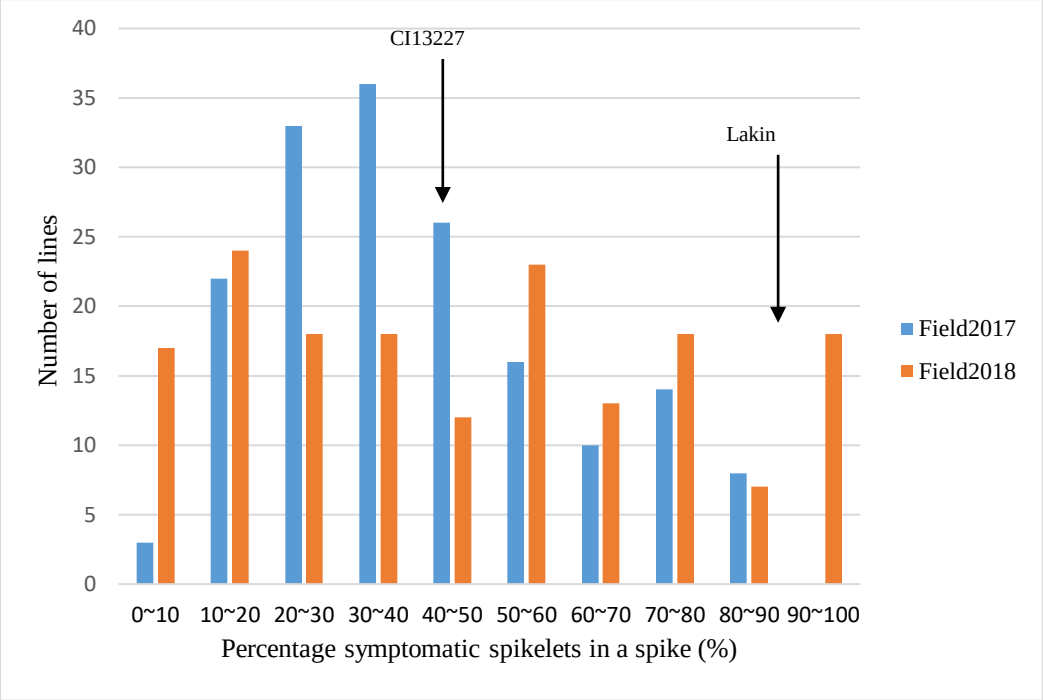


Figure 2.5 Frequency distribution of means of heading date (from the first day genotype started heading) in the DH lines derived from 'CI13227'x 'Lakin' evaluated in two field experiments conducted in 2017 and 2018

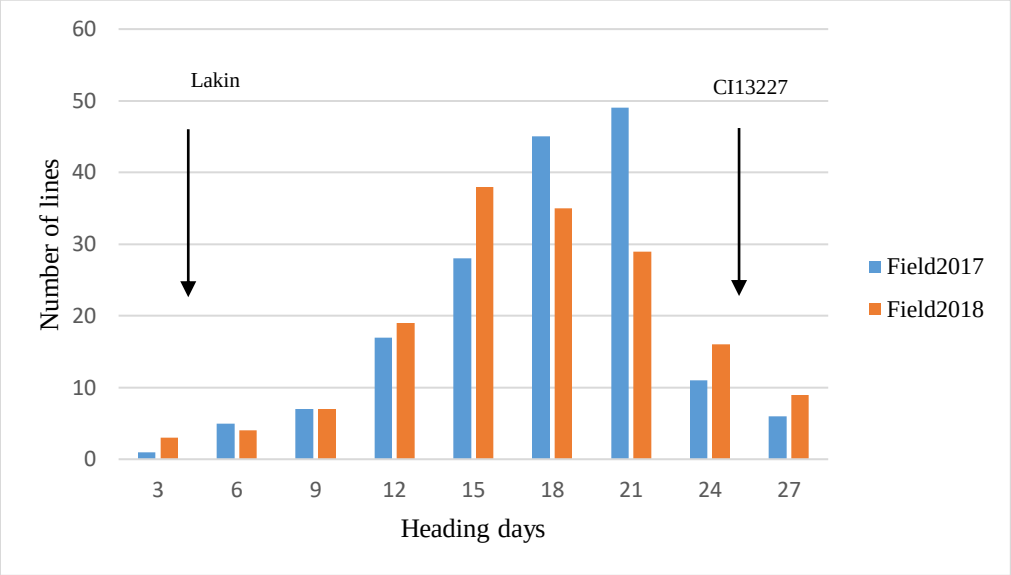


Figure 2.6 Frequency distribution of plant height in the DH lines derived from 'CI13227'x 'Lakin' evaluated in two field experiments conducted in 2017 and 2018

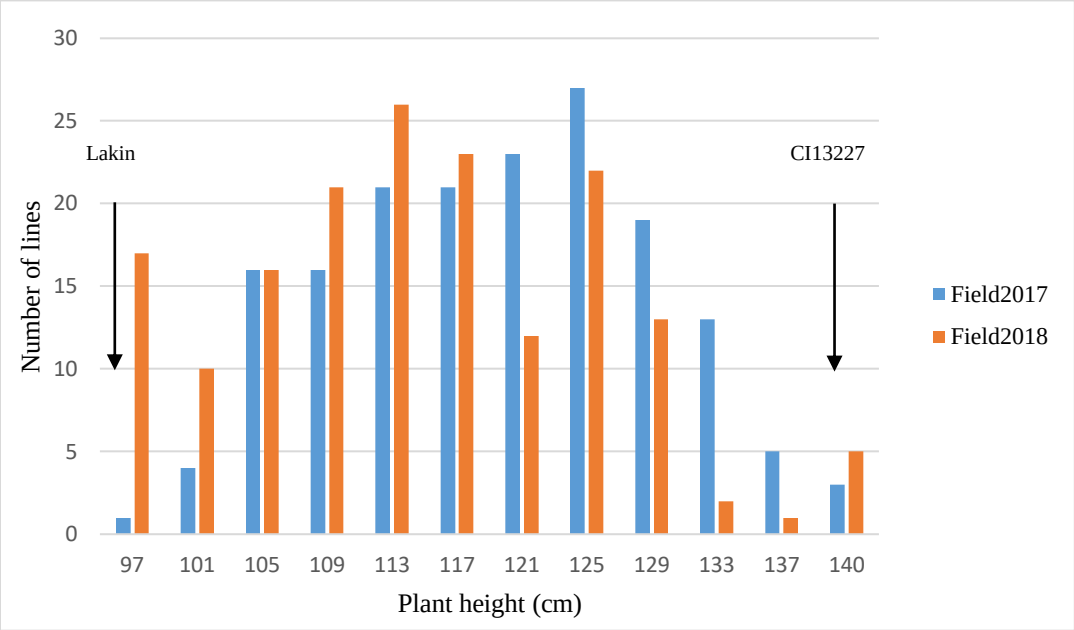


Figure 2.7 Correlation between FHB severity and plant height (ht) in the DH lines derived from 'CI13227'x 'Lakin' evaluated in the 2017 filed experiment

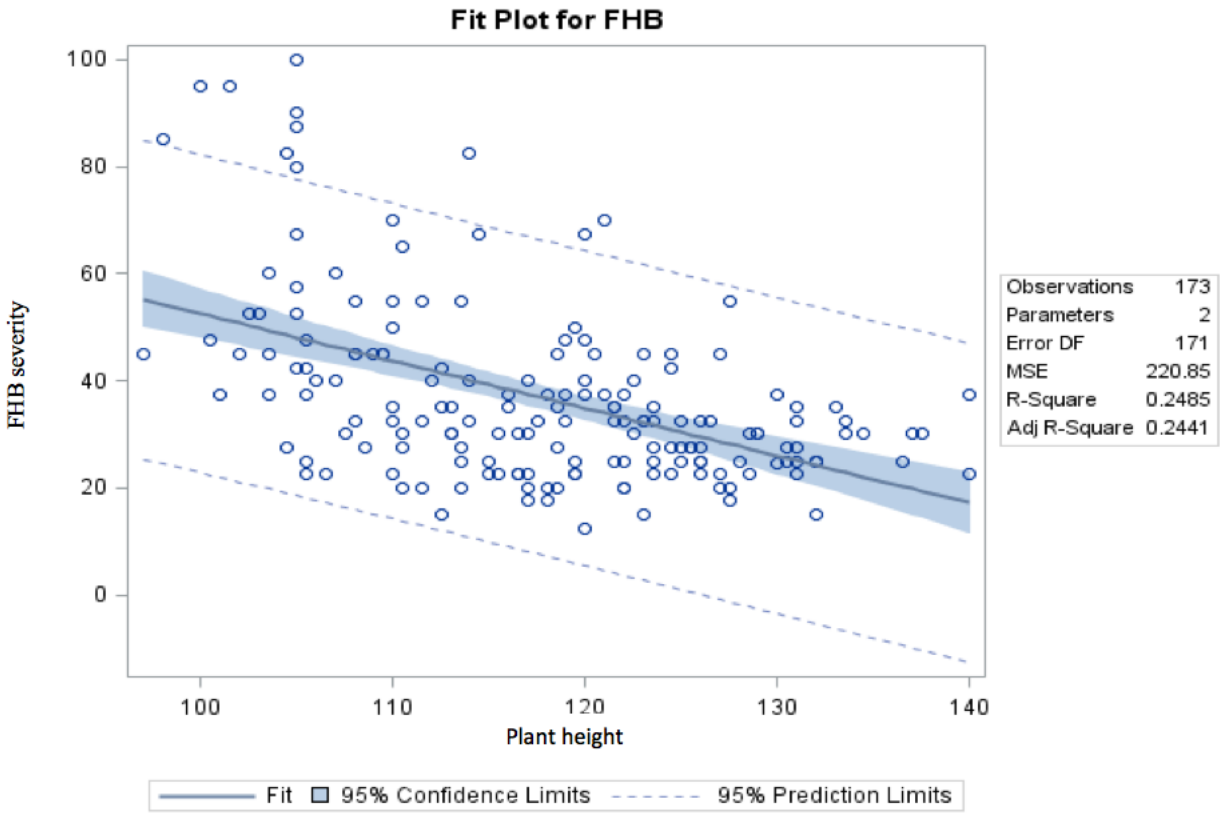


Figure 2.8 Correlation between FHB severity and heading date in the DH lines derived from 'CI13227'x 'Lakin' evaluated in the 2017 filed experiment

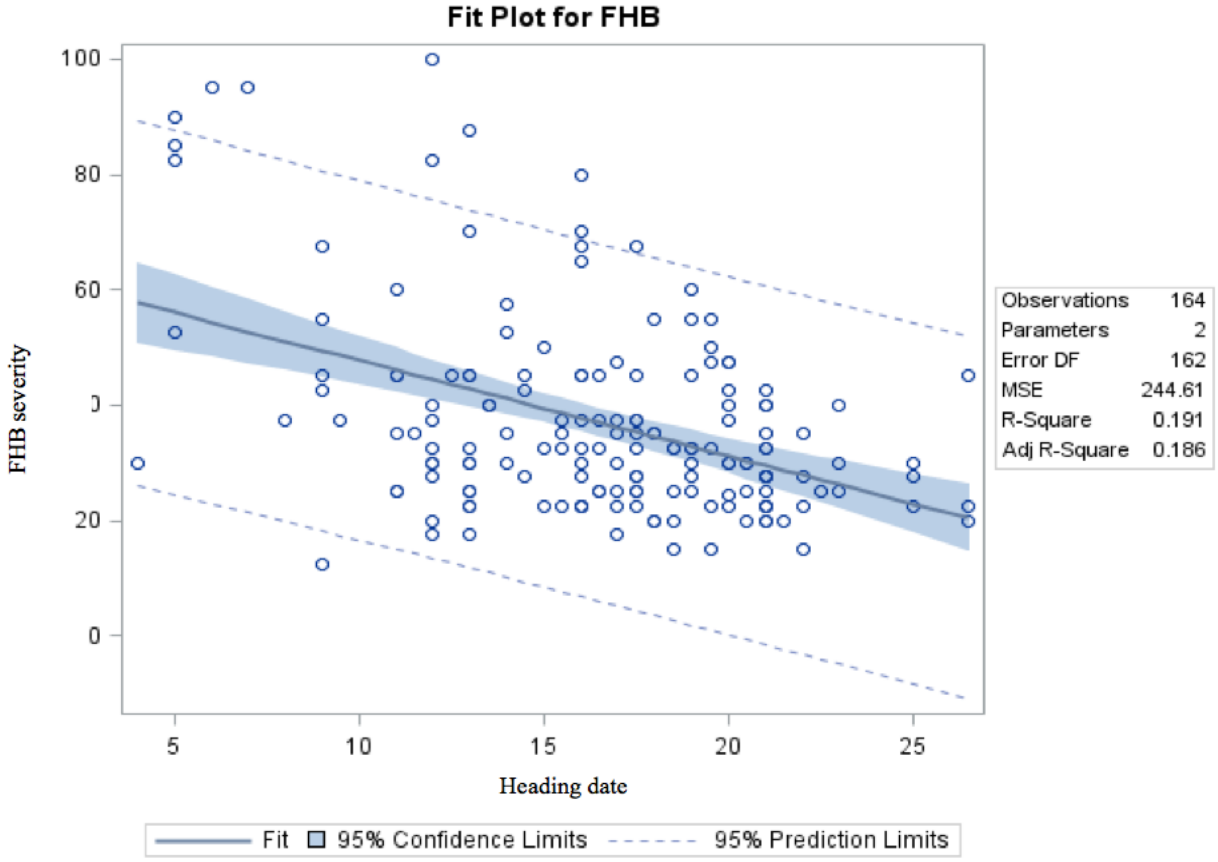


Figure 2.9 Correlation between of *Fusarium* damage kernel (FDK) and the content of deoxynivalenol (DON) in the double haploid (DH) lines derived from 'CI13227'x 'Lakin' evaluated in the 2017 filed experiment

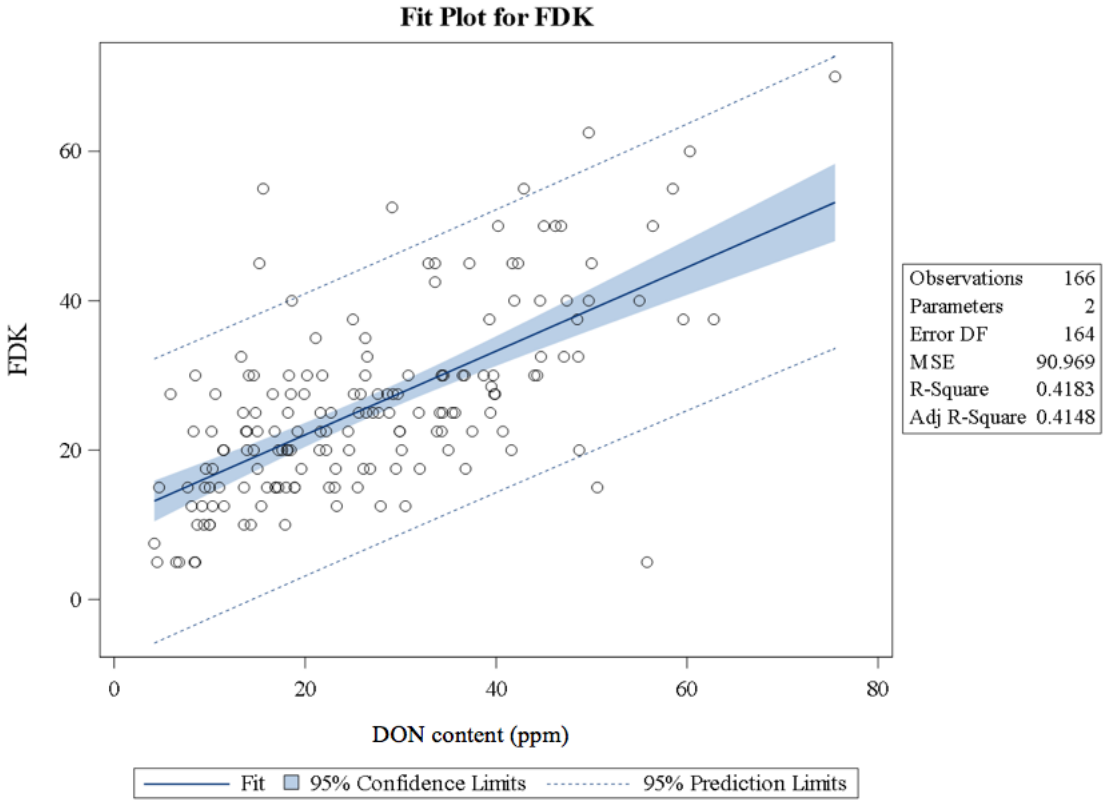


Figure 2.10 Distribution of 90K-SNPs on each chromosome in the doubled haploid (DH) population derived from 'CI13227'x 'Lakin' (under 50% missing data)

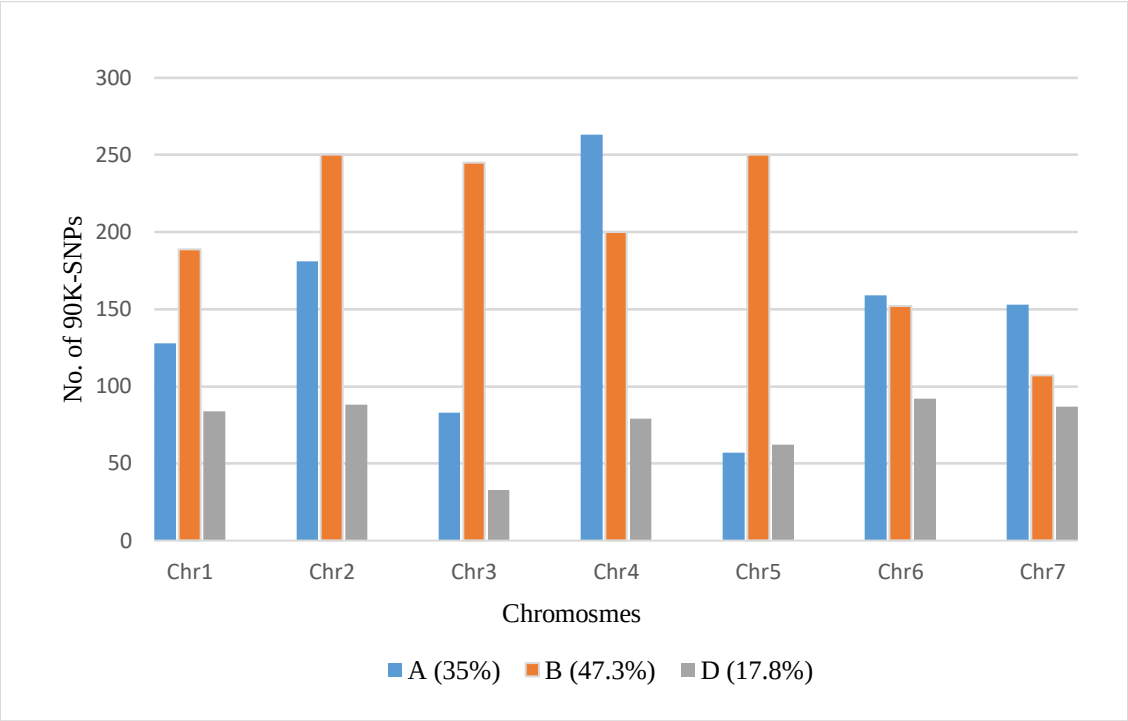
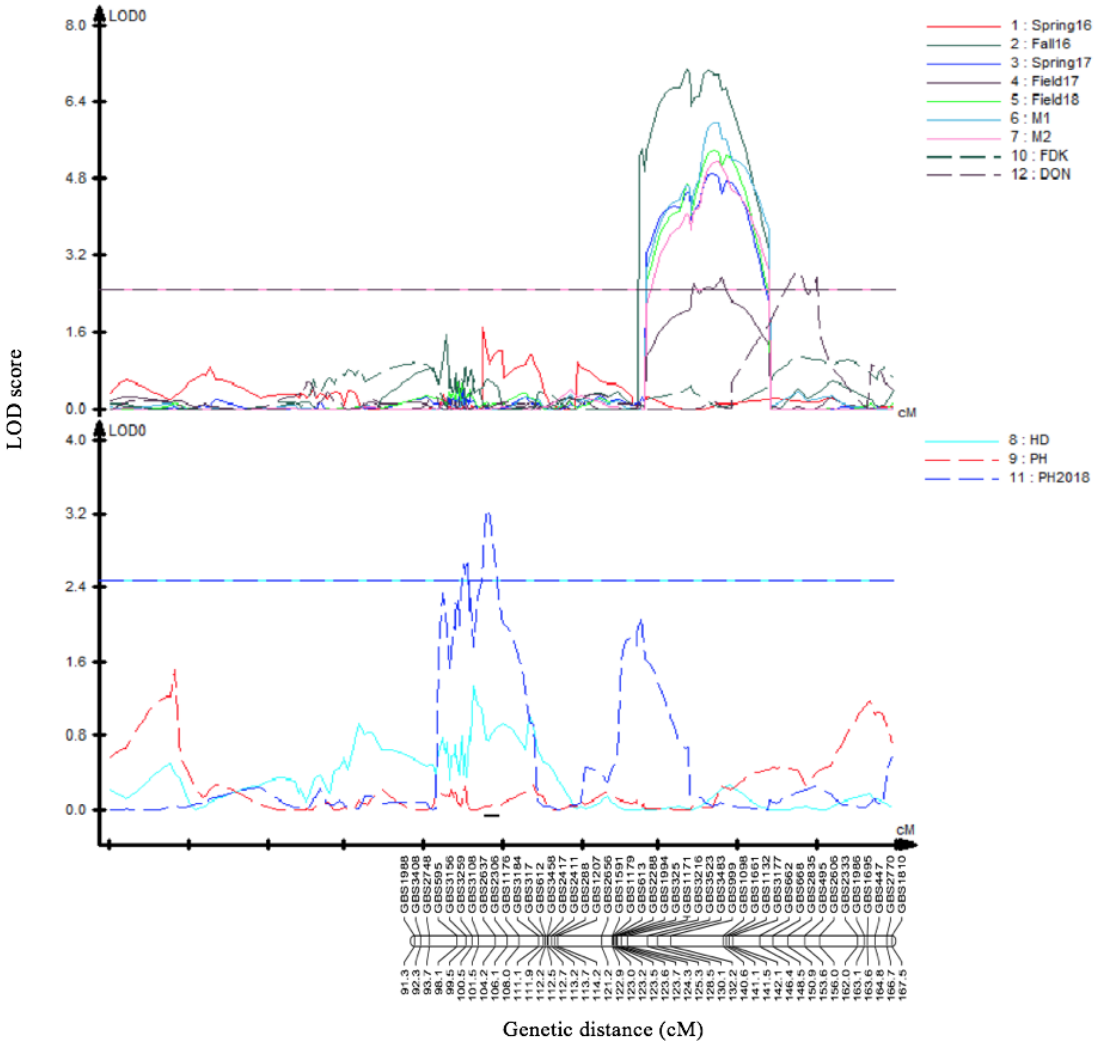
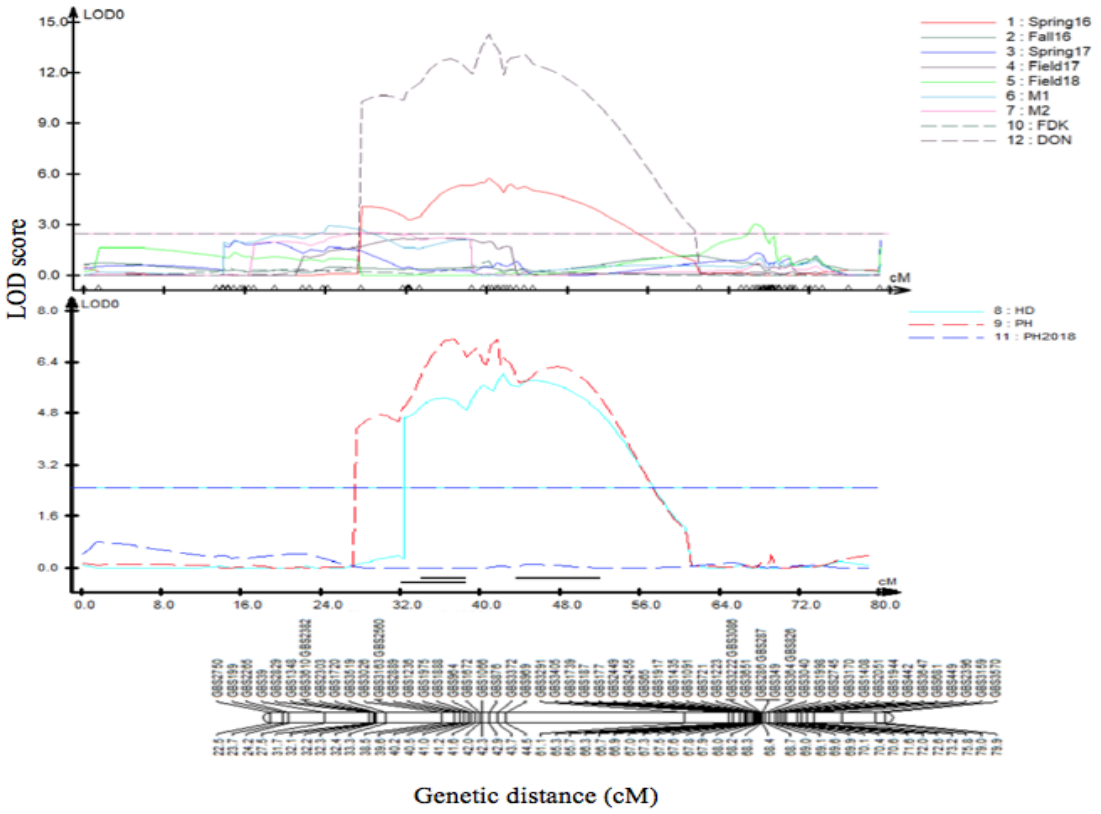


Figure 2.11 Composite interval maps of quantitative trait loci (QTLs) for FHB type Ⅱ resistance constructed from DH lines derived from 'CI13227'x 'Lakin' based on three greenhouses and two field experiments on (a) chromosome 5AL (b) chromosome 2D (c) chromosome 4BS (d) chromosome 7A, logarithm of the odds (LOD score), genetic distance (cM), mean from three greenhouse data (M1), and mean from two field data (M2)

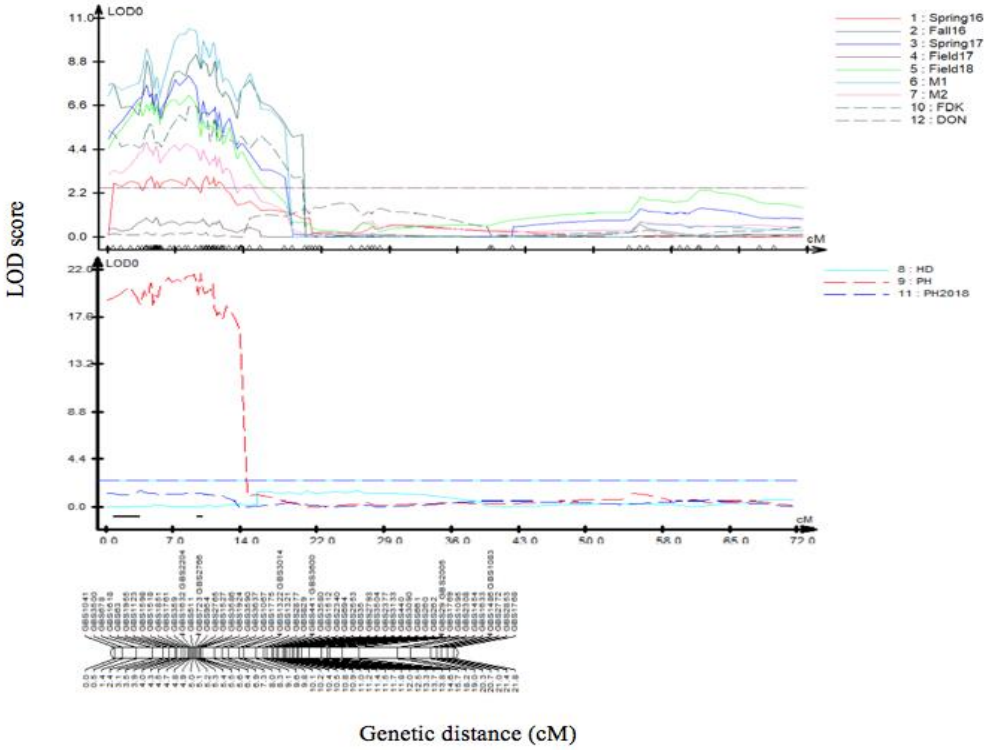
A. (5AL)



B. (2DS)


LOD0
LOD score
15.0
12.0
9.0
6.0
3.0
0.0
cM
1 : Spring16
2 : Fall16
3 : Spring17
4 : Field17
5 : Field18
6 : M1
7 : M2
10 : FDK
12 : DON
LOD0
8.0
6.4
4.8
3.2
1.6
0.0
cM
8 : HD
9 : PH
11 : PH2018
0.0
8.0
16.0
24.0
32.0
40.0
48.0
56.0
64.0
72.0
80.0
Genetic distance (cM)

C. (4BS)


LOD score
LOD0
11.0
8.8
6.6
4.4
2.2
0.0
cM
1 : Spring16
2 : Fall16
3 : Spring17
4 : Field17
5 : Field18
6 : M1
7 : M2
10 : FDK
12 : DON
LOD0
22.0
17.6
13.2
8.8
4.4
0.0
cM
8 : HD
9 : PH
11 : PH2018
0.0
7.0
14.0
22.0
29.0
36.0
43.0
50.0
58.0
65.0
72.0
Genetic distance (cM)

D. (7A)

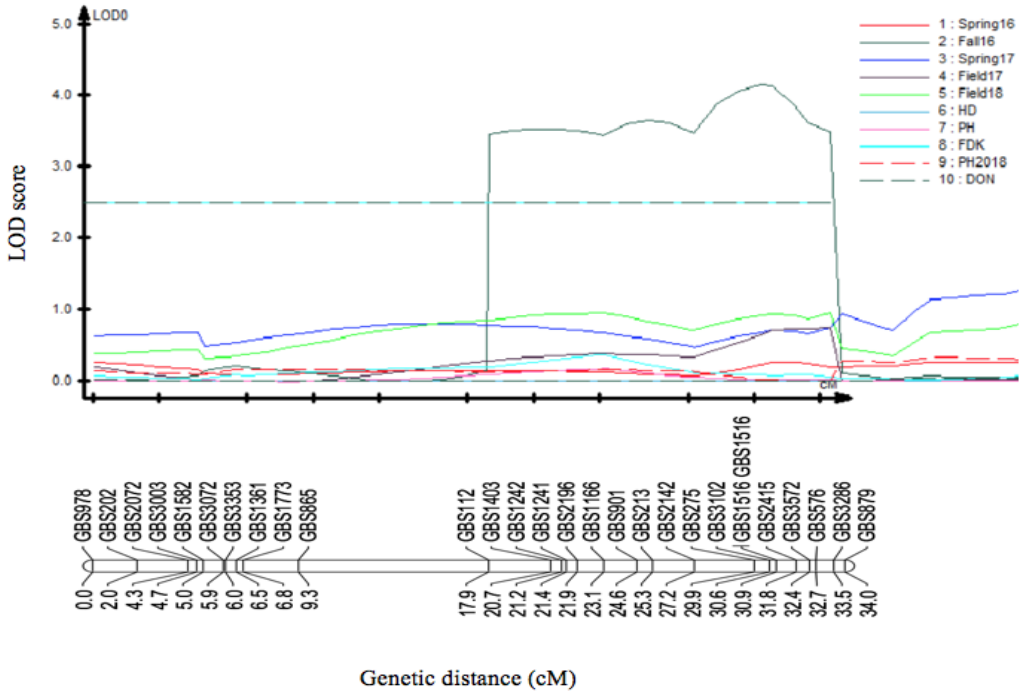
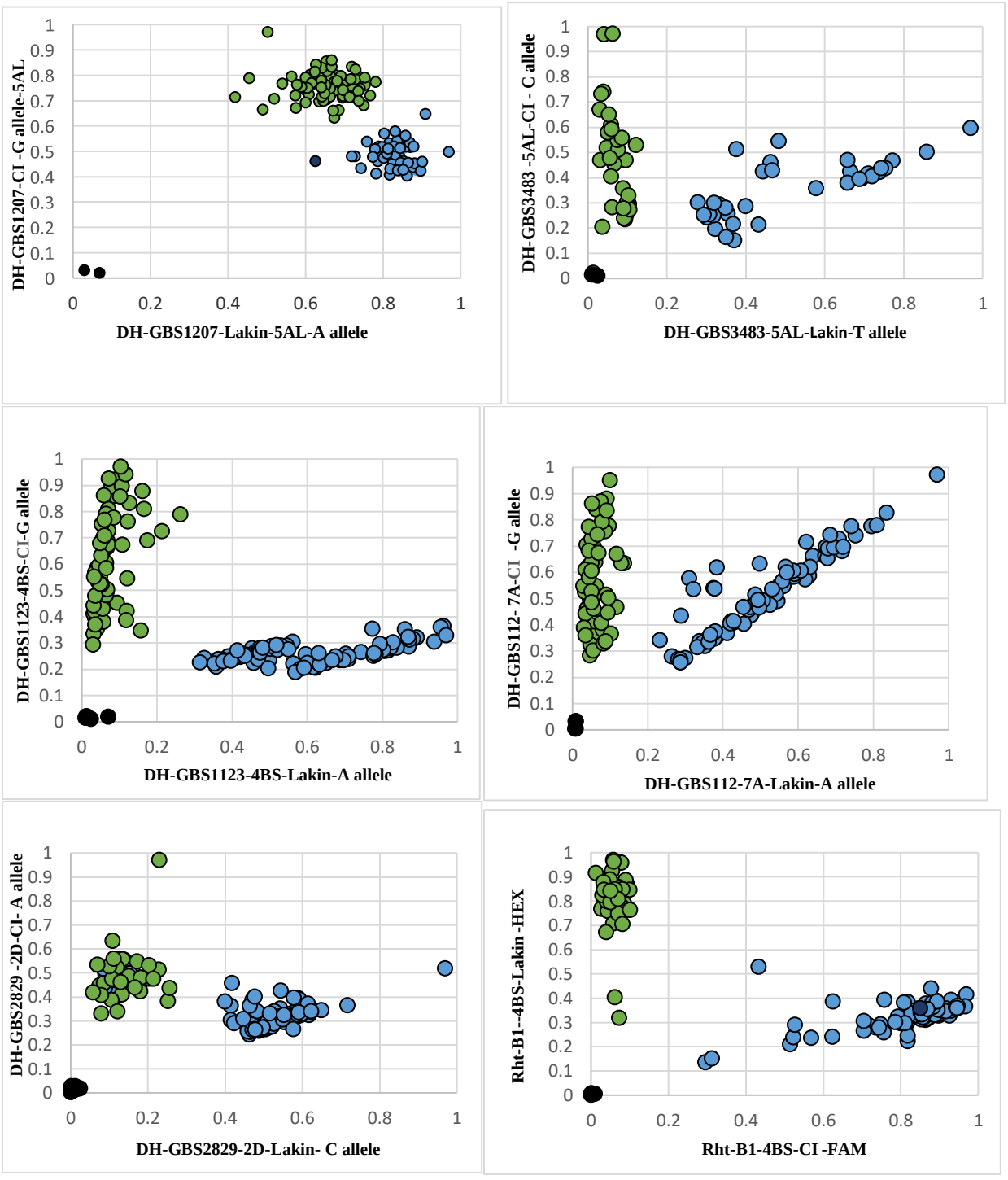
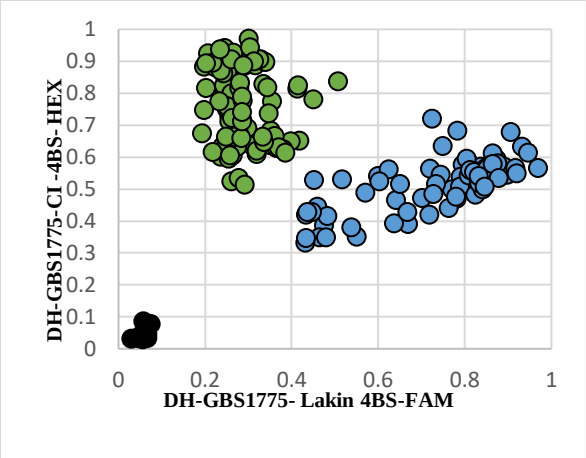
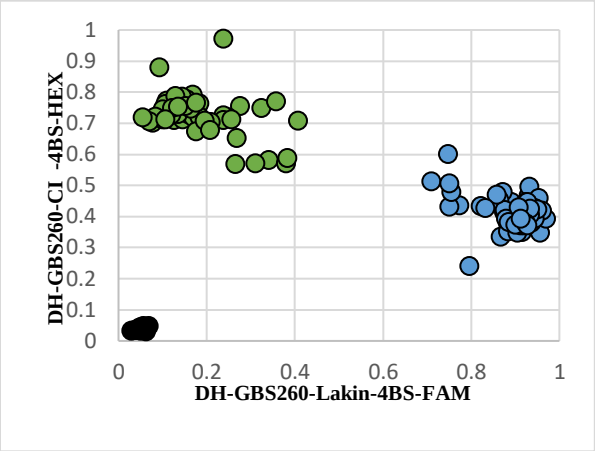

LOD0
LOD score
5.0
4.0
3.0
2.0
1.0
0.0
cM
1 : Spring16
2 : Fall16
3 : Spring17
4 : Field17
5 : Field18
6 : HD
7 : PH
8 : FDK
9 : PH2018
10 : DON
GBS978 0.0
GBS202 2.0
GBS2072 4.3
GBS3003 4.7
GBS1582 5.0
GBS3072 5.9
GBS3353 6.0
GBS1361 6.5
GBS1773 6.8
GBS865 9.3
GBS112 17.9
GBS1403 20.7
GBS1242 21.2
GBS1241 21.4
GBS2196 21.9
GBS1166 23.1
GBS901 24.6
GBS213 25.3
GBS2142 27.2
GBS275 29.9
GBS3102 30.6
GBS1516 30.9
GBS1516
GBS2415 31.8
GBS3572 32.4
GBS576 32.7
GBS3286 33.5
GBS879 34.0
Genetic distance (cM)

Figure 2.12 KASP assay profile to allelic segregation of SNPs in the double haploid (DH) lines derived from 'CI13227' x 'Lakin'. The blue and the green dots show different alleles and the black dots are water control or samples with failed PCR




DH-GBS1775-CI -4BS- HEX
DH-GBS1775- Lakin 4BS-FAM


DH-GBS260-CI -4BS-HEX
DH-GBS260-Lakin-4BS-FAM

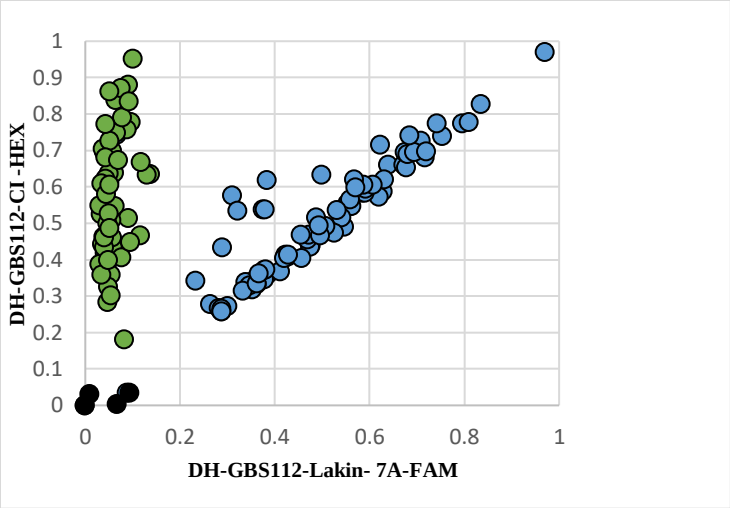

DH-GBS112-CI -HEX
DH-GBS112-Lakin- 7A-FAM

Table 2.1 Analysis of variance (ANOVA) and broad-sense heritability (H^2) for percentage of symptomatic spikelets in a spike (PSS) from DH population 'CI13227' x 'Lakin' in the greenhouse experiments conducted in spring 2016, fall 2016, and spring 2017

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	2	36975.04	18487.52	5402.68	<.0001
Replication (Experiment)	3	247.49	82.49	56. 91	<.0001
genotype	173	629728. 87	3640.05	1063.75	<.0001
Genotype *Experiment	346	275645.96	796.66	232. 81	<.0001
Error	519	1730.07	3.35		
Corrected Total	1043	944327.44			
H^2		0.78			

Table 2.2 Correlations among percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), plant height (PH), and deoxynivalenol (DON) in the DH lines derived from 'CI13227'x 'Lakin' evaluated in the three greenhouses experiments conducted in spring 2016, fall 2016, and spring 2017

Trait	HD	PH	GPSS-Spring2016	GPSS-Fall2016	GPSS-Spring2017	FDK
GPSS-Spring 2016	-0.19*	0.17				
GPSS-Fall 2016	- 0.13*	0.31	0.33***			
GPSS-Spring 2017	- 0.12*	0.3	0.52***	0.67***		
FDK2017	-0.1	-0.4	0.15**	0.21***	0.19***	
DON2017	0.35***	0.041	- 0.21**	-0.11**	- 0.17**	0.39***

PH= Plant height, HD=heading date, GPSS= Percentage of symptomatic spikelets in the greenhouse,

FDK= Fusarium damaged Kernels, DON= Deoxynivalenol

P≤ 0.05P≤ 0.01*** P≤0.001*

Table 2.3 Analysis of variance (ANOVA) and broad-sense heritability(H^2) for percentage of symptomatic spikelets (PSS) in a spike from double haploid (DH) population 'CI13227' x 'Lakin' evaluated in the two field experiments conducted in 2017 and 2018

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	1	166.02	166.02	43.66	<.0001
Replication (Experiment)	2	51.85	25.92	6.82	0.0013
genotype	169	316011.64	1869. 89	491.79	<.0001
Genotype *Experiment	169	491. 85	2.91	0. 77	0.97
Error	338	1285.15	3.80		
Corrected Total	679	318006.52			
H^2		0.82			

Table 2.4 Correlations among percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), plant height (PH), and deoxynivalenol (DON) in the DH lines derived from 'CI13227' x 'Lakin' evaluated in the three greenhouses experiments conducted in spring 2016, fall 2016, and spring 2017

Trait	HD	PH	GPSS-Spring2016	GPSS-Fall2016	GPSS-Spring2017	FDK
GPSS-Spring 2016	-0.09*	0.17				
GPSS-Fall 2016	- 0.13*	0.31	0.33***			
GPSS-Spring 2017	- 0.12*	0.3	0.52***	0.67***		
FDK2017	-0.1	-0.4	0.15**	0.21***	0.19***	
DON2017	0.35***	0.041	- 0.21**	-0.11**	- 0.17**	0.39***

PH= Plant height, HD=heading date, GPSS= Percentage of symptomatic spikelets in the greenhouse

, FDK= Fusarium damaged Kernels, DON= Deoxynivalenol

P≤ 0.05P≤ 0.01*** P≤0.001*

Table 2.5 Correlations among percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), plant height (PH), and deoxynivalenol (DON) in the DH lines derived from 'CI13227'x 'Lakin' evaluated in the two field experiments conduct in 2017 and 2018

Trait	HD	PH	FPSS-2017	FPSS-2018	FDK
PH	0.29***				
FPSS-2017	- 0.22**	-0.25**			
FPSS-2018	-0.12	-09*	0.50***		
FDK2017	-0.09	-0.40***	0.26**	0.23***	
DON2017	0.35***	0.04	-0.06*	- 0.19*	0.41***

PH= plant height, HD=heading date, FPSS= percentage of symptomatic spikelets in the field, FDK= *Fusarium* damaged kernels, DON= Deoxynivalenol

P≤ 0.05P≤ 0.01*** P≤ 0.001*

Table 2.6 Chromosomal locations, determination coefficients(R^2), additive effects and logarithm of the odds (LOD) values for significant quantitative trait loci (QTLs) in the DH lines derived from 'CI13227' x 'Lakin' for *Fusarium* head blight (type II) resistance

Traits	experiments	QTLs	Position (cM)	LOD	PVE(R^2)[a]	add. [b]	Flanking markers		Physical location		Contributed by
PSS	Spring 2016	*QFhb.hwwgru. 2DS.*	40.53	5.99	0.126	-0.103	*GBS2829*	*GBS187*	20691420	746431712	CI13227
DON	Field 2018	*Qdon.hwwgru. 2DS*	40.51	14.6	0.29	-0.582	*GBS2829*	*GBS187*	20691420	746431712	CI13227
Plant height	Field 2017	*QFhb.hwwgru. 2DS*	37.31	3.85	0.327	-0.113	*GBS2829*	*GBS187*	20691420	746431712	CI13227
PSS	Field 2018	*QFhb.hwwgru. 2DS*	66.7	3.1	0.064	-0.114	*GBS3291*	*GBS187*	73588901	746431712	CI13227
Mean	All greenhouses	*QFhb.hwwgru. 2DS*	24.23	3	0.051	-0.058	*GBS2429*	*GBS3163*	70886932	77495641	CI13227
Mean	All fields	*QFhb.hwwgru. 2D.*	27.53	2.61	0.054	-0.052	*GBS2429*	*GBS3163*	70886932	77495641	CI13227
PSS	Spring 2016	*QFhb.hwwgru.4BS*	4.04	3.73	0.08	-0.08	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
PSS	Fall2016	*QFhb.hwwgru.4BS*	0.91	9.06	0.161	-0.131	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
PSS	Spring 2017	*QFhb.hwwgru.4BS*	4.04	7.62	0.172	-0.126	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
PSS	Field 2018	*QFhb.hwwgru.4BS*	4.04	7.31	0.156	-0.1175	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
FDK	Field 2018	*QFDK.hwwgru.4BS*	4.09	6.54	0.127	-0.487	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
Plant height	Field 2017	*Qph.hwwgru.4BS*	6.81	21.7	0.389	-0.16	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
Mean	All greenhouses	*QFhb.hwwgru.4BS*	8.32	10.48	0.193	-0.109	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
Mean	All fields	*QFhb.hwwgru.4BS*	6.91	4.66	0.096	-0.07	*GBS1041*	*GBS1633*	30283858	77298194	CI13227
PSS	Fall2016	*QFhb.hwwgru.5AL*	122.91	4.34	0.083	-0.131	*GBS288*	*GBS1098*	552780515	568273645	CI13227
PSS	Spring 2017	*QFhb.hwwgru.5AL*	122.9	4.48	0.109	0.093	*GBS288*	*GBS1098*	552780515	568273645	Lakin
PSS	Field 2017	*QFhb.hwwgru.5AL*	130.01	2.67	0.095	0.059	*GBS288*	*GBS1098*	552780515	568273645	Lakin
PSS	Field 2018	*QFhb.hwwgru.5AL*	128.51	4.37	0.121	0.103	*GBS288*	*GBS1098*	552780515	568273645	Lakin
DON	Field 2018	*Qdon.hwwgru.5AL*	146.4	2.71	0.0523	-0.312	*GBS999*	*GBS2835*	595425884	597750881	CI13227
Plant height	Field 2018	*QPh.hwwgru.5AL*	113.31	2.79	0.065	-0.476	*GBS1176*	*GBS1179*	552780515	568273645	CI13227
Mean	All greenhouses	*QFhb.hwwgru.5AL*	128.34	6.01	0.109	0.084	*GBS288*	*GBS1098*	552780515	568273645	Lakin
Mean	All fields	*QFhb.hwwgru.5AL*	129.49	5.19	0.111	0.075	*GBS288*	*GBS1098*	552780515	568273645	Lakin
PSS	Fall 2016	*QFhb.hwwgru.7A*	30.2	3.36	0.076	-0.09	GBS112	*GBS3612*	726686022	735716943	CI13227

[a] PVE: phenotypic variation explained;
[b] Add.; additive effect

Table 2.7 List of sequence and primers for Kbioscience competitive allele-specific PCR (KASP) assays developed from 90K-SNPs arrays

SNP name	Sequence	Position	Dye	Tailed Primer
GBS1207-5AL	TGCAGCCACACACCGCC[A/G]CCGCAGACCGAGC CAACGGACGGGAATTTTACGAGAGAATCCAACC	18	FAM	GAAGGTGACCAAGTTCATGCTTGCAGCCACACACCGCCG
			HEX	GAAGGTCGGAGTCAACGGATTTGCAGCCACACACCGCCA
			R	GTAAAATTCCCGTCCGTTGG
GBS3483-5AL	TGCAGTGTCAAACTTGTCTTGACACGTCCACT[C/ T]ATGATATATCCAATGAAGGTGCCACATGAT	33	FAM	GAAGGTGACCAAGTTCATGCTCAAACTTGTCTTGACACGTCC ACTc
			HEX	GAAGGTCGGAGTCAACGGATTCAAACTTGTCTTGACACGTCC ACTt
			R	ATGTGGCACCTTCATTGGAT
GBS1123-4BS	TGCAGCATCTGGTGGCC[G/A]AATTCATAAGCTG GATCAGAAGTCGCAAAAGGCTGGTGAAGGCAGC	18	FAM	GAAGGTGACCAAGTTCATGCTTGCAGCATCTGGTGGCCg
			HEX	GAAGGTCGGAGTCAACGGATTTGCAGCATCTGGTGGCCa
			R	AGCCTTTTGCGACTTCTGAT
GBS260-4BS	TGCAGACGCTGAAGAGGTCCG[C/T]CGCTCTGGA TGGGTATGGCGGCGGCGGTGGCGGCGGGGAAGC	22	FAM	GAAGGTGACCAAGTTCATGCTGACGCTGAAGAGGTCCGC
			HEX	GAAGGTCGGAGTCAACGGATTGACGCTGAAGAGGTCCGT
			R	GCCATACCCATCCAGAGC
GBS1775-4BS	TGCAGCGTGCA[A/C]ACAAACAACCCTGCTTAGC ACAACAAGACAAAGACAAACAAGGAGGAAACA T	11	FAM	GAAGGTGACCAAGTTCATGCTGCTAAGCAGGGTTGTTTGTTT
			HEX	GAAGGTCGGAGTCAACGGATTGCTAAGCAGGGTTGTTTGTTC
			R	AGCCATACTGCATTTGGAAT
GBS2829-2D	TGCAGGGGTCCATGCCGCTGCAC[A/C]GCCGTCG TCTCTCTTGGGTCAGCCACTTCCGCATGTCGCC	24	FAM	GAAGGTGACCAAGTTCATGCTGGTCCATGCCGCTGCACA
			HEX	GAAGGTCGGAGTCAACGGATTGGTCCATGCCGCTGCACC
			R	GCTGACCCAAGAGAGACGAC
GBS112-7A	TGCAGAAGCTCACCATCAAGGCCGAC[A/G]AGGC TGAGGATGCGCTGGATGAGCTTCACTACTTCAT	27	FAM	GAAGGTGACCAAGTTCATGCTTCACCATCAAGGCCGACA
			HEX	GAAGGTCGGAGTCAACGGATTCACCATCAAGGCCGACG
			R	AAGCTCATCCAGCGCATC

Chapter 3 . Mapping the quantitative trait loci for *Fusarium* head blight resistance in a hard winter wheat Lyman

3.1 Introduction

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe is a worldwide concern for wheat production. It reduces not only yield, but also grain quality. Mycotoxins accumulated in infected grain are fungal secondary metabolites that are toxic to humans and animals (Bai and Shaner, 2004). In the U.S. Great Plains, most of cultivars are highly susceptible to FHB, thus, improving wheat FHB resistance is an urgent task for wheat breeders. Recently several cultivars have been released with moderate FHB resistance including 'Everest', 'Overland', 'Lyman', 'Heyne' and 'Hondo' (Bockus *et al.*, 2009; Zhang *et al.*, 2012b; Jin *et al.*, 2013). Those HWW may carry native resistance QTLs different from those in Chinese sources. (Cai and Bai, 2014) and can be good candidates for pyramiding with resistance QTLs from exotic sources such as *Fhb1* to enhance FHB resistance (Burlakoti *et al.*, 2009). However, identities of the QTLs in those native sources remain unknown. Objectives of this study are to map QTLs in 'Lyman' and identify closely linked markers to these QTLs for marker-assisted selection

3.2 Materials and methods

3.2.1 Plant materials and FHB evaluation

A population of 183 $F_{5:6}$ recombinant inbred lines (RILs) was developed by crossing a moderate FHB resistant hard winter wheat (HWW) 'Lyman' (KS93U134/Arapahoe) (Eckard *et al.*, 2015) to an FHB highly susceptible HWW 'Overley' (U1275-1-4-2-2 / Heyne'S' //Jagger) (http://kswheatalliance.org/varieties/overley/).

Pathogen inocula were prepared from a Kansas strain of *F. graminearum* (GZ3639) following Bai *et al.,* (1999). Methods for FHB disease inoculation and evaluation were described in the Chapter 2. The RIL population was evaluated for type II resistance in the greenhouse at Kansas State University, Manhattan KS. The materials were phenotyped for FHB resistance in fall 2016, spring and fall 2017, and spring 2018. Both the RIL lines and their parents were planted in plastic trays with Metro-mix 360 soil mix (Hummert International, Topeka, KS). The seedlings were transplanted to 4" x 4" plastic pots after vernalization in a cold room at ~ 6 °C for 50 d. The pots with the RILs and their parents were organized in a randomized complete block design (RCBD) in greenhouse's benches. The greenhouse temperature was set at 20 ~ 25 °C with 12 h light period.

The RIL population and their parents were also evaluated in field experiments at the KSU Plant Pathology FHB Nursery at Rocky Ford, Manhattan, KS in 2017 and 2018. The procedure has been described in Chapter 2.

3.2.2 DNA extraction and genotyping

Leaf tissue was collected from wheat seedlings at three-leaf stage into 96-deepwell plates, and dried in a freeze dryer (ThermoSavant, Holbrook, NY) for 48 h and ground into fine powder in a Mixer Mill (MM400, Retsch, Germany). DNA was extracted using the OKtopure™-(LGC Group) as described in Chapter 2.

3.2.3 GBS library construction

The DNA from RILs and two replications of each parent were used to construct a library following the protocol of (Poland *et al.*, 2012). DNA quantified using Quant-iT PicoGreen dsDNA Assay (Life Technologies Inc. NY), then a normalized DNA 17.5 ng/l was digested and ligated following the protocol that described in the Chapter 2.

3.2.4 Linkage map construction and QTL analysis

Linkage map construction and QTL analysis were described in Chapter 2. In brief, the linkage map was constructed using GBS-SNP data, Kosampbi mapping function (Kosambi1994) and regression algorithm in JoinMap V 4.0 software (Van Ooijen, 2006). The QTLs mapped consistently of 24 linkage group with at least 5 markers per group, representing all 21 chromosomes at length of average interval 0.7 cM between markers. QTLs for PSS, heading date, FDK were used for Composite Interval Mapping (CIM) via WINQTL Cartographer version 2.5 (Wang *et al.*, 2006). A 1000-time permutation was conducted to determine the LOD threshold for claiming significant QTL at $P < 0.05$ (Doerge and Churchill, 1994).

3.3 Results

3.3.1 FHB variation among RILs and between their parents

The resistant parent 'Lyman' showed moderate FHB resistance in all greenhouses experiments with a mean PSS of 21.4% ranging from 15.5% to 28.4%, whereas the susceptible parent 'Overley' was highly susceptible with a mean PSS of 98.2%, ranging from 97% to 100% (Fig. 3.1). The frequency distribution of mean PSS in the RIL population across all the greenhouse experiments ranged from 10.2% to 100%, with skewness to right toward the susceptible parent 'Overley' in all greenhouse experiments (Fig. 3.1). The broad sense heritability was medium to high (0.91) (Table 3.1). Positive correlations in PSS were highly significant ($P \leq 0.001$) among the four greenhouse experiments (Table 3.2). The transgressive segregation was not observed in any of the greenhouse experiments.

In the two field experiments, FHB scores showed continuous distribution (Fig. 3.2). The mean PSS for RILs in 2017 and 2018 field experiments were 42.7 % and 66.7%, respectively. The mean PSS for the resistant parent 'Lyman' was 25%, whereas the mean for the susceptible parent

‘Overley’ was 95%. The broad sense heritability for the field experiments were lower than the greenhouse experiments (Table 3.3). The average of height for susceptible parent ‘Overley’ was (97 cm) while the resistnt parent ‘ Lyman’ was (120 cm) (Fig. 3.3). ‘Overley’ head about 16 days earlier than ‘Lyman’ (Fig. 3.4)

The mean FDK was 45.3% for the RILs. A significantly positive correlation was obtained between FPSS and FDK in the 2017 field experiment (Fig. 3.5; Table 3.4). A negative correlation was significant between FPSS and heading date (Fig. 3.6), but not between FPSS and plant height in the RIL population.

3.3.2 Construction of a linkage map

The GBS-SNPs were analyzed for 170 RILs after removing seven RILs with a high number of missing data. Initially, 15,079 GBS-SNPs were called with 80% missing data. Among them, 1,674 had 20% less missing data and were used for QTL mapping. The markers were mapped to 27 linkage groups with at least seven markers in each group. The map covered all 21 chromosomes with an average marker density of 0.86 cM per marker. Among the three wheat genomes, B genome has the most markers (47.9%), whereas the D genome the least (14.4%) (Fig. 3.7).

3.3.3 QTLs for FHB resistance

Seven significant QTLs were detected for FHB resistance on 1A (2), 2A, 3A, 1B, 2B and 4B (Fig. 3.8 and Table 3.4). The QTL *QFhb.hwwgru.3A* showed the largest effect in two greenhouse experiments with the resistance allele from ‘Lyman’, and this QTL was delineated to 2.4 cM intervals between SNPs *LO4345* and *LO18017* (Fig 3.9, Table 3.5).

Two QTLs were significant on chromosome 1A. The first QTL (*QFhb.hwwgru.1A.1)* was significant in fall 2016 greenhouse, 2018 field and mean PSS data, explained 6.0%, 8.4%, and 7.7% of the phenotypic variation and flanked by SNPs *LO19826* and *LO20733*, respectively (Fig.

3.8 and Table 3.5). Another QTL on 1A, *QFhb.hwwgru.1A.2* was only marginally significant in one field experiment, flanked by markers *LO19826* and *LO20733* (Fig. 3.8, Table 3.5). This QTL explained 6.0% of the phenotypic variation and was not overlapped with *QFhb.hwwgru.1A.1*.

QFhb.hwwgru.1B on the short arm of chromosome 1B was significant in the fall 2016 greenhouse experiment, 2017 and 2018 field experiment, and overlapped with the mean of the greenhouse and the field data. The QTL was flanked by SNPs *LO31640* and *LO12252* and explained 7.5%, 9.1%, 8.5%, and 9.3% of the phenotypic variation, respectively (Fig. 3.8, Table 3.5).

QFhb.hwwgru.4B was mapped in 'Lyman' between SNPs *LO1866* and *LO14790*, and significant only in the 2017-2018 field experiment. This QTL explained 6.5% of the phenotypic variation (Fig. 3.8, Table 3.4). *QFhb.hwwgru.2A* was flanked by SNPs *LO18144* and *LO31050*, significant only in fall 2016 greenhouse experiment and explained 5.5% of the phenotypic variation (Fig. 3.8, Table 3.4). *QFhb.hwwgru.3BS* was detected for low FDK between flanking SNPs *LO2099* and *LO 17251* and explained 12.1% of phenotypic variation only in 2017 field experiment. However, QTL for low PSS was not significant in the region (Fig. 3.8, Table 3.5).

3.3.4 KASP design and verification

To verify the genotypic data generated from the GBS, we converted the SNP markers to KASP assays for MAS in breeding. Sequence reads harboring 22 GBS-SNPs in the four QTL regions *QFhb.hwwgru.3A, QFhb.hwwgru.1A.1, QFhb.hwwgru.1B,* and *QFhb.hwwgru.2B* were used to design KASP primers, and 14 of them showed polymorphisms between the parents and in the RIL population (Fig. 3.9, Table 3.6). Four SNPs on chromosome 2B, five SNPs on chromosome 1A, three on 3A, and two on 1B were associated with low PSS in the RIL population. Eleven of them were remapped into the same QTL region and three were located outside the QTLs.

Comparison of array-based SNPs with KASP SNP data, nine KASP data showed identical allele calls as array-based SNPs called in the RIL population.

3.4 Discussion

3.4.1 QTLs for type II FHB resistance in 'Lyman'

Among the seven QTLs detected in the current study, *QFhb.hwwgru.3A* showed the largest effect and explained 17.8 and 21.5 % of the phenotypic variation in the two greenhouse experiments. To date, more than eight QTLs for FHB have been reported in 3A chromosome and explained between 7.0 -17.5 % of phenotypic variation across different experiments (Tamburic-Ilincic, 2012; Buerstmayr *et al.*, 2013; Wright, 2014; Zhang *et al.*, 2014; Cai and Bai, 2014). Some of them were associated with type I resistance such as in 'Frontana' (Szabo-Hever *et al.*, 2012), and 'Jamestown' (Wright, 2014), whereas the others were associated with type II resistance such as in 'ND2603' (Anderson *et al.*, 2001), 'Huapei 57-2' (Bouroncle and Ohm, 2003), 'Frontana' (Steiner *et al.*, 2004; Yang *et al.*, 2005; Mardi *et al.*, 2006; Yabwalo *et al.*, 2011). The QTL at 3A identified in this study showed a larger effect on type II resistance than previously reported. Liu and Anderson (2003) reported a marker *Xgwm2* close to the highest peak for the QTL, and this marker is close to *QFhb.hwwgru.3A* found in this study, therefore, they are most likely the same QTL. However, *QFhb.hwwgru.3A* appeared to have a larger effect on type II resistance than the QTL reported previously.

QFhb.hwwgru.1B for FHB resistance was significant in the fall 2016 greenhouse and 2017-2018 field experiments. It explained 7.6 and 9.1% of the phenotypic variation, respectively. Among 55 previous studies that reported QTLs for FHB resistance, nine QTLs were on the chromosome 1B including one in the 1BL.1RS translocation derived from the rye chromosome (Nishio, 2016). Several studies mapped QTLs on the long arm of chromosome 1B, including 'CM-

82036' (Buerstmayr *et al.,* 2002), CIMMYT cultivar 'Seri 28' (Mardi *et al.,* 2006), Chinese landrace Wangshuibai (Zhou *et al.,* 2004), 'Arina' (Semagn *et al.,* 2007), and European winter wheat 'Cansas' (Häberle *et al.,* 2009). Liu *et al.,* (2013) reported a QTL on 1B that linked to Xgwm33 marker and was about 8 Mb from *QFhb.hwwgru.1B,* thus they are likely the same QTL.

Two QTLs on chromosome 1A, *QFhb.hwwgru.1A.1* and *QFhb.hwwgru.1A*.2, were detected in the current study. *QFhb.hwwgru.1A.1* explained 6.9 and 8.3% of the phenotypic variation in fall 2016 greenhouse and 2018 field experiments, respectively. *QFhb.hwwgru.1A*.2 was only significant in 2017 field experiment, which may not be a consistent QTL for FHB resistance. A few QTLs for FHB type II resistance have been reported on chromosome 1A including CJ9306 (Jiang *et al.,* 2007), G16-92 (Schmolke *et al*., 2005) and 'Arina' (Semagn *et al.,* 2007). *QFhb.hwwgru.1A.1* was likely close to the QTL reported by Jiang et al., (2007). Giancaspro *et al.,* (2016) reported a QTL for 1A at position 161. 8 cM and overlapped with the plant height QTL and indicated that the QTL may have pleiotropic effects on FHB resistance.

*QFhb.hwwgru.4B*S showed significance only in one field experiment. This QTL was flanked by *LO1866* and *LO14790* and explained 6.5% of the phenotypic variation. Based on flank marker indicated that QTL was located at the region outside *Rht-B1b* dwarf gene. Several QTLs have been reported in chromosome 4B including these in 'Erine' (Liu *et al.,* 2008), 'Chokwang' (Yang *et al.*, 2005a), 'Wangshuibai' (Jia *et al*., 2005), 'DBC-480' (Part *et al.,* 2015), and '02-5B-318' (Giancaspro *et al*., 2016). However, none of these reported QTLs were located at the same position with the QTL identified in this study based on the physical location of the flanking markers.

3.5 Conversion of GBS-SNPs into KASP assays

Genotyping-by-sequencing has been wildly used for identification of SNPs and mapping QTLs because of low cost per datapoint and high-throughput compared to other technologies (Cai *et al.*, 2019; Lin *et al.*, 2015; Poland *et al.*, 2012). However, one of the GBS limitations is that it generates many missing data due to limited sequence depth (Cai *et al.*, 2019; Poland *et al.*, 2012; Spindel, 2013). One way to solve the problem is to increase the number of the sequencing run for each library to reduce the missing data, however, it may significantly increase the assay cost. To improve quality of mapping data for these SNPs in the QTL region, KASP assays were conducted to eliminate the missing data and verify QTL locations. Among 19 KASP assays designed, 14 showed polymorphisms between parents and segregated in the population (Fig. 3.9; Table 3.5). These KASP markers can be used for MAS in U.S winter wheat if they are polymorphic between breeding parents.

3.6 References

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Figure and Tables

Figure 3.1 Frequency distribution of mean percentage symptomatic spikelets in a spike (PSS) data of RIL population lines derived from 'Lyman' x 'Overley' evaluated in four greenhouse experiments conducted in fall 2016, spring 2017, fall 2017, and spring 2018

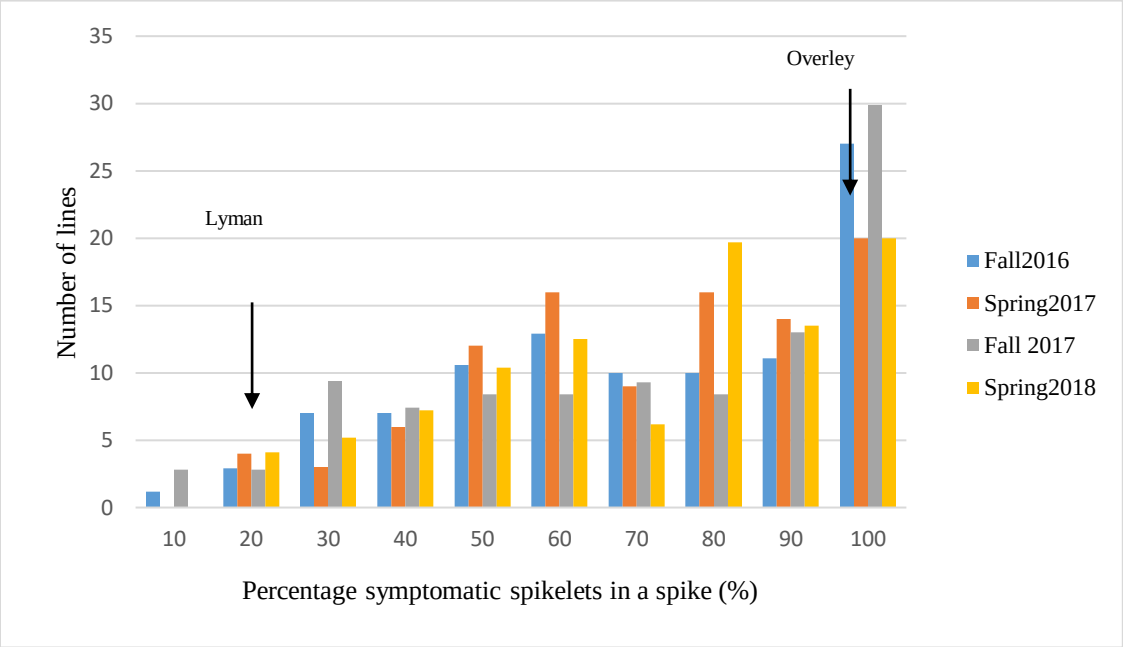


Figure 3.2 Frequency distribution of mean percentage of symptomatic spikelets in a spike (PSS) of RIL population lines derived from 'Lyman' x 'Overley' evaluated in the 2017 and 2018 field experiments

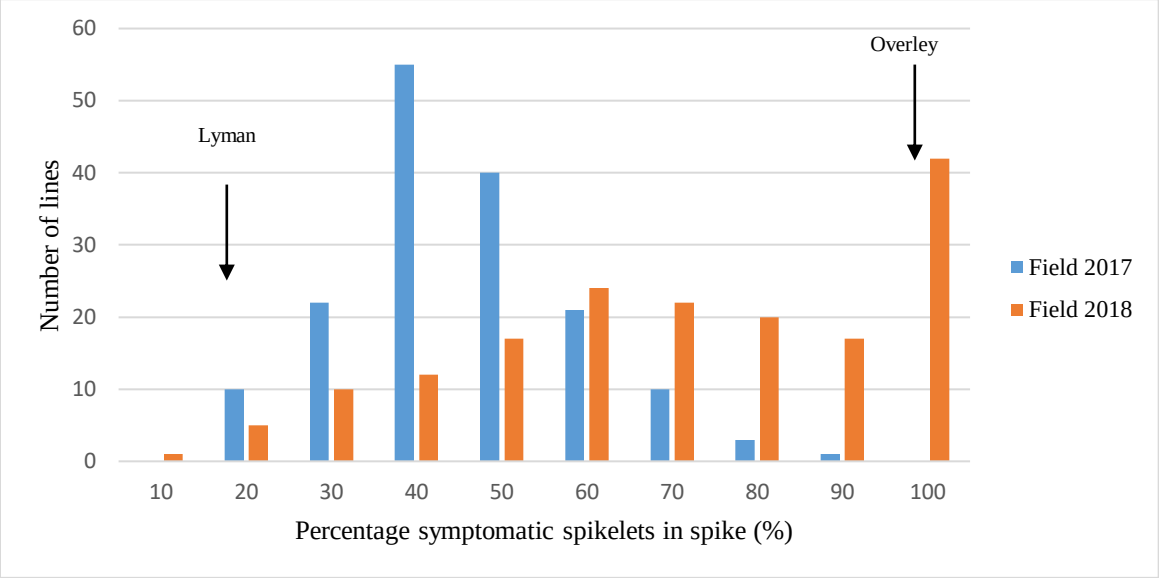


Figure 3.3 Frequency distribution of mean of Plant height (PH) data of the recombinant inbred line population derived from 'Lyman' x 'Overley' evaluated in the 2017 and 2018 field experiments

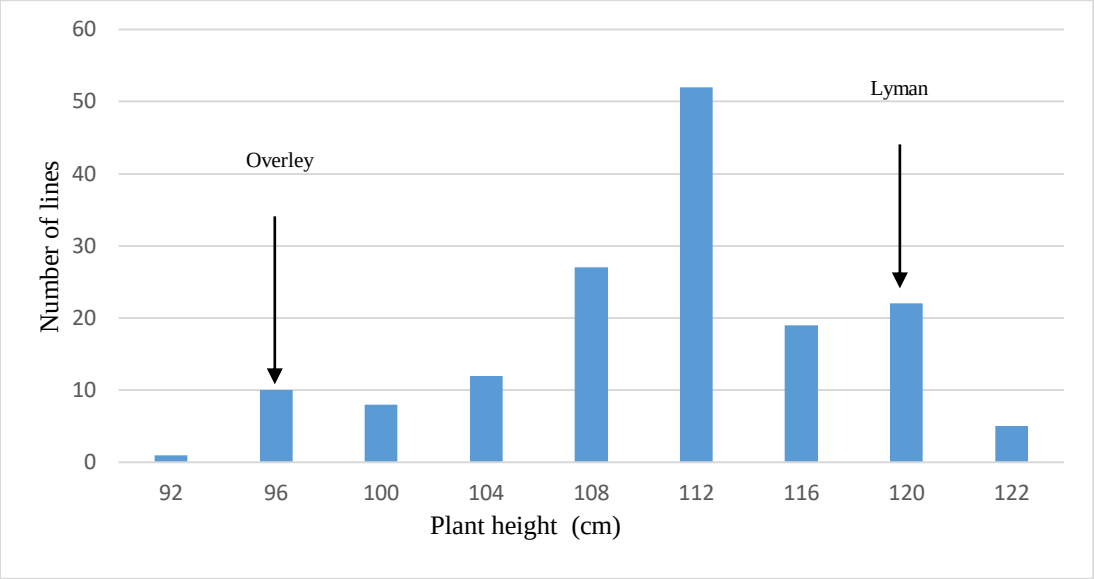


Figure 3.4 Frequency distribution of mean heading date (from the first day the genotype started heading) of the recombinant inbred line population derived from 'Lyman' x 'Overley' evaluated in the 2017- 2018 field experiments

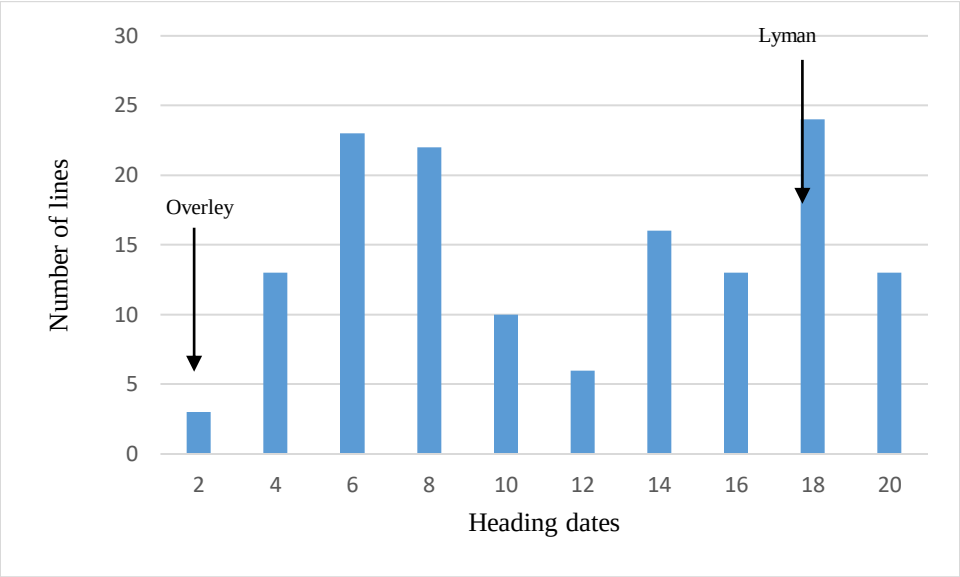


Figure 3.5 Correlations between percentage of symptomatic spikelets in a spike (PSS) and Fusarum damaged kernels (FDK) in the recombinant inbred line population derived from 'Lyman' x 'Overley' evaluated in the 2017 filed experiment

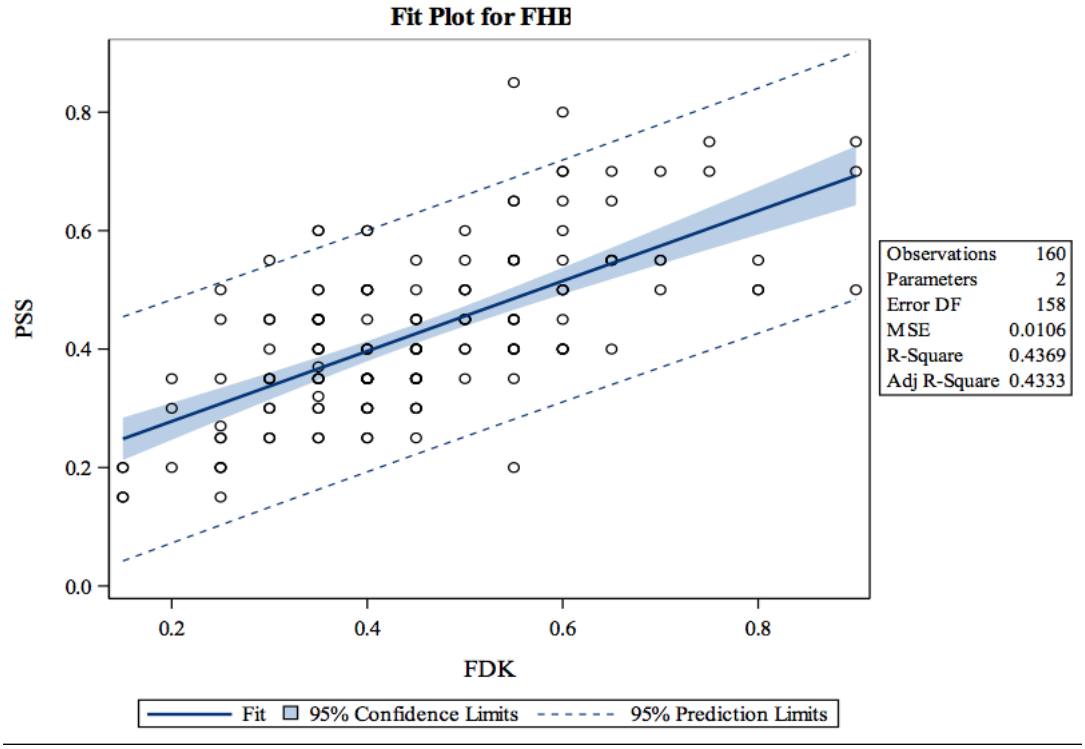


Figure 3.6 A correlation between percentage of symptomatic spikelets in a spike (PSS) and heading date (HD) in recombinant inbred line population derived from 'Lyman' x 'Overley' evaluated in the 2017 filed experiments

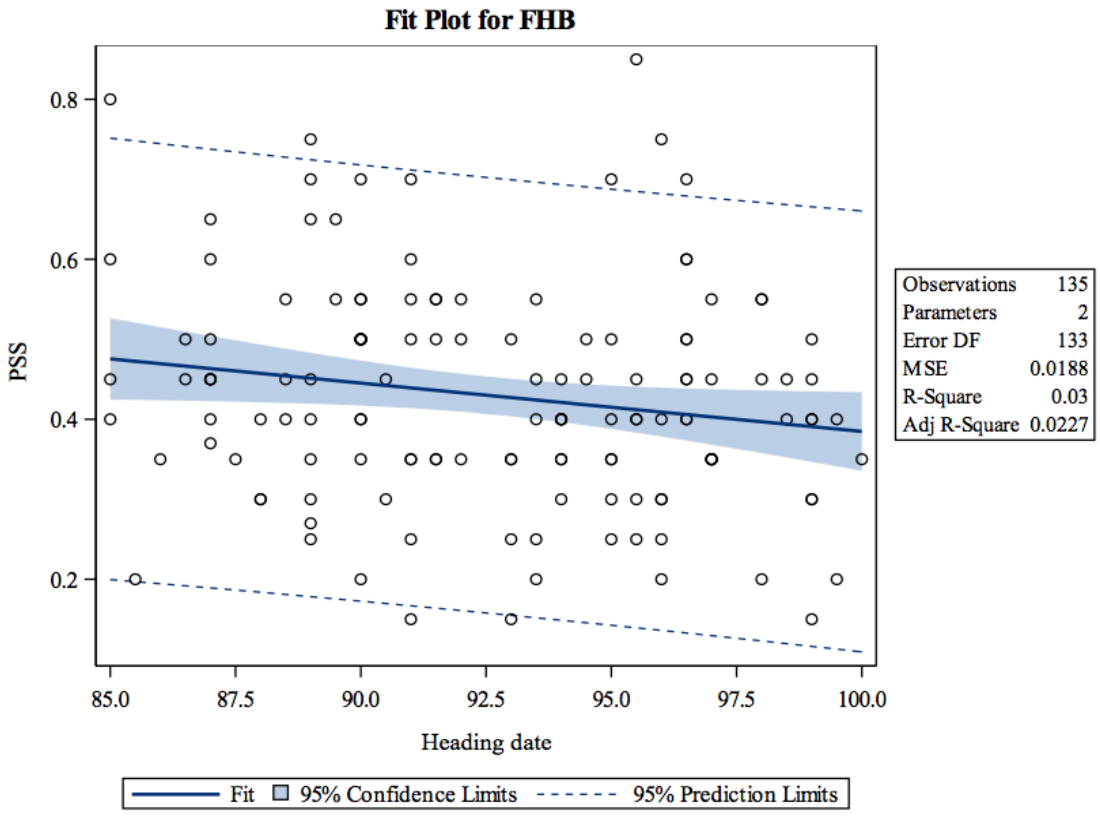


Figure 3.7 Distribution of genotype-by-sequencing-SNPs on each chromosome in the recombinant inbred line population derived from 'Lyman' x 'Overley'

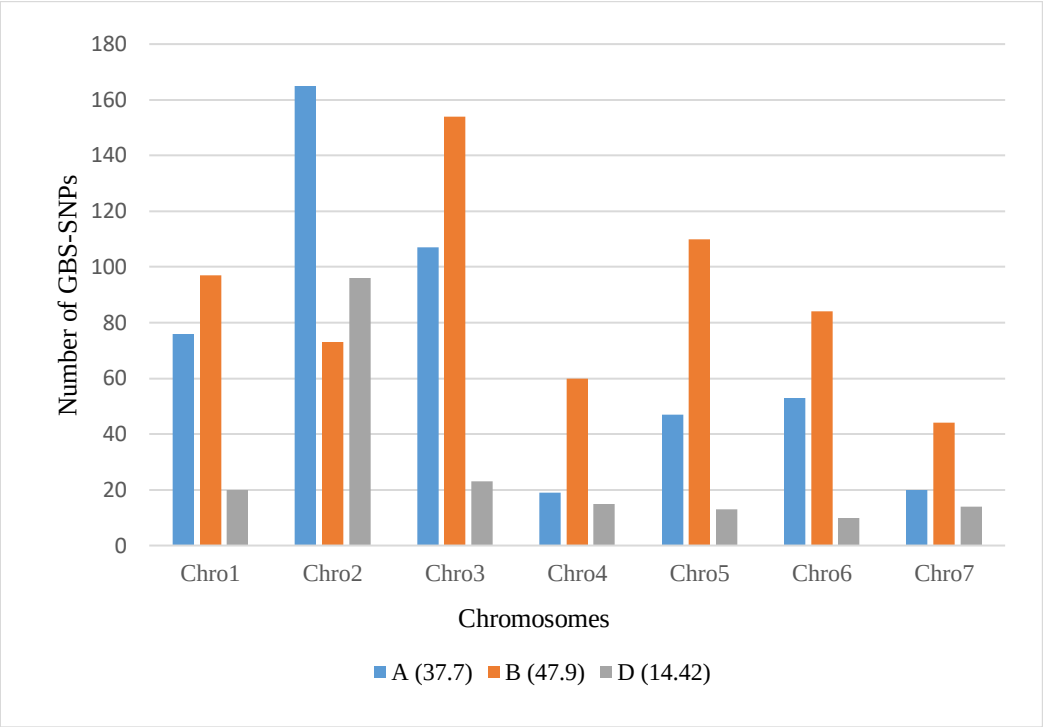
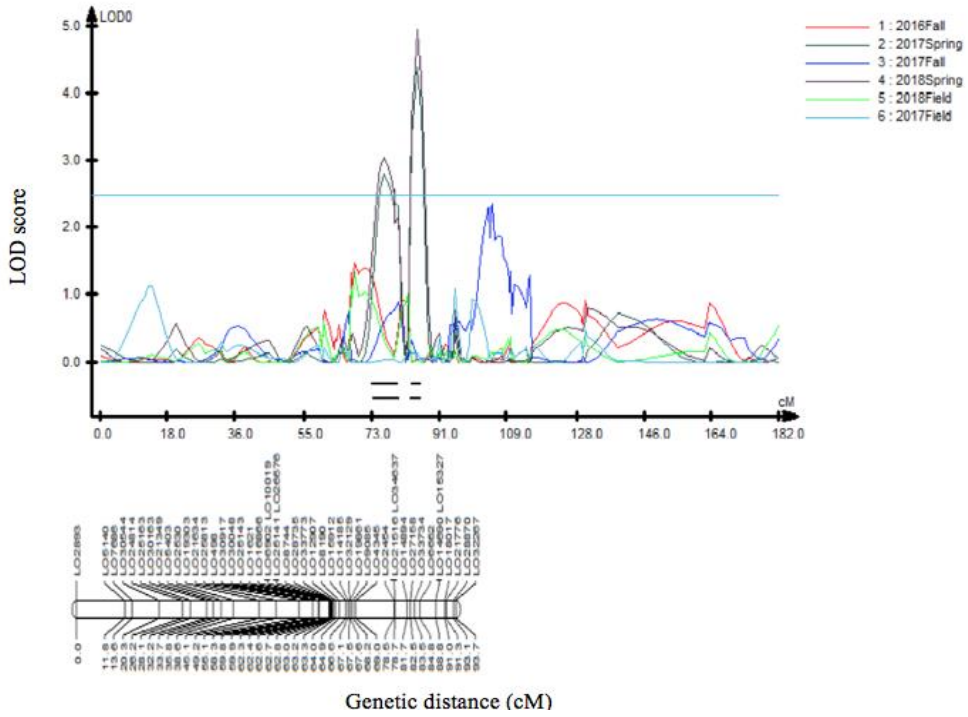
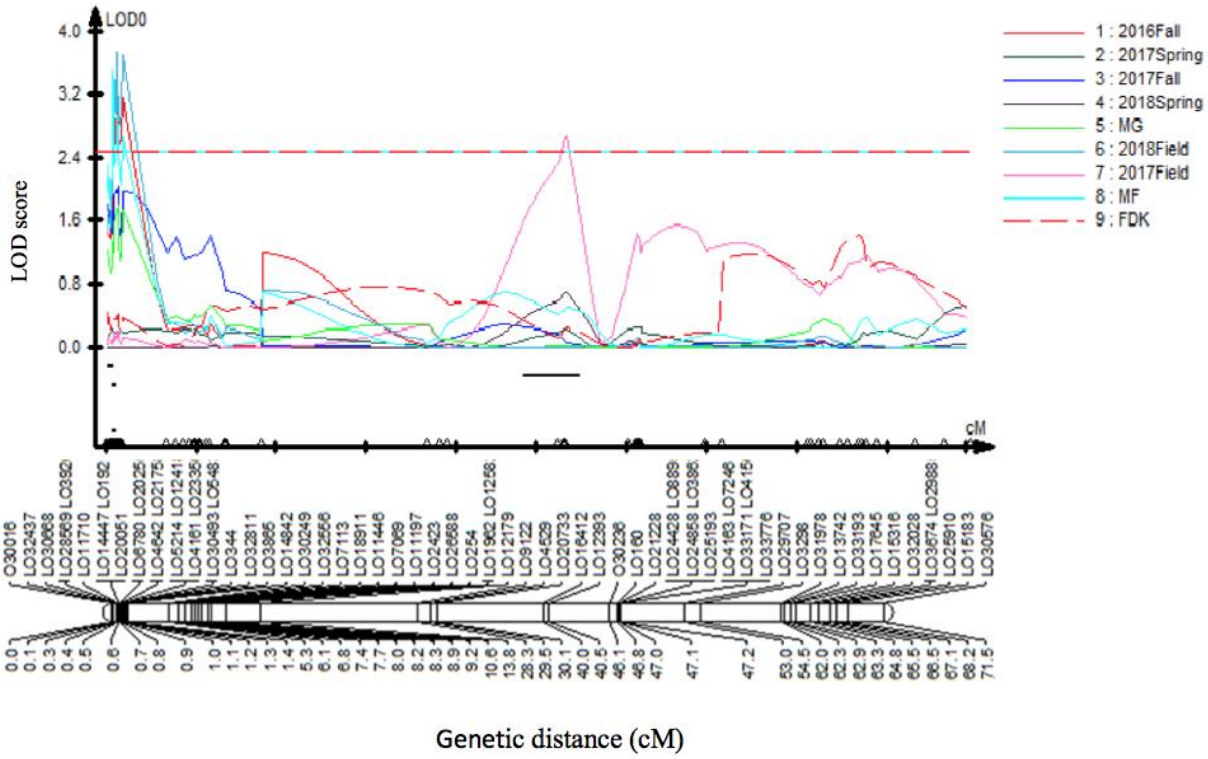


Figure 3.8 Quantitative trait loci (QTLs) for FHB type II resistance in a recombinant inbred line (RIL) population of 'Lyman' × 'Overley' phenotyped using percentage of symptomatic spikelets in a spike (PSS), and *Fusarium* damaged kernel (FDK) evaluated in 2016 fall, 2017 spring, 2017 fall, and 2018 spring greenhouse experiments, as well as mean of greenhouse (MG) and mean of field (MF)

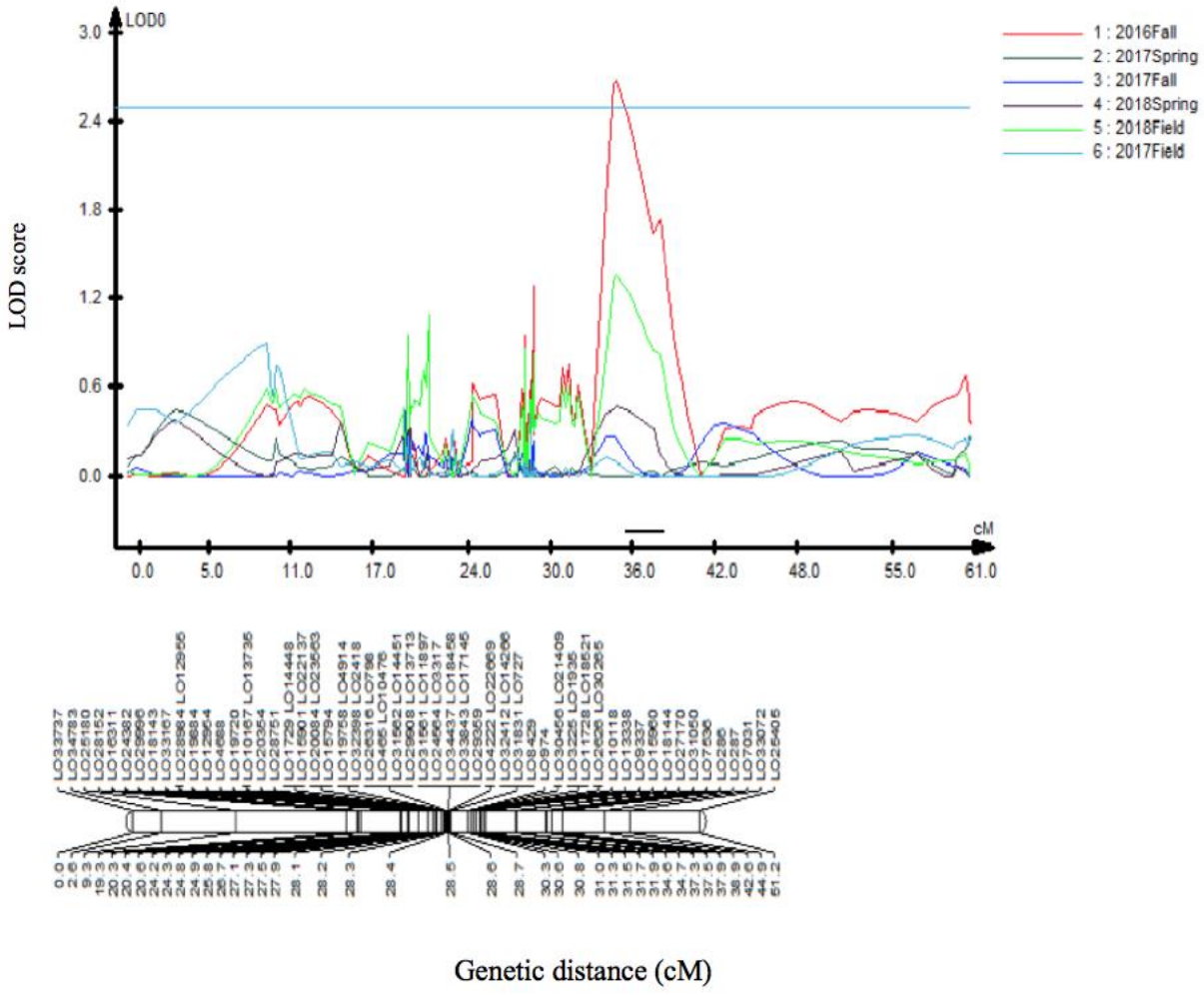
(a) 3A



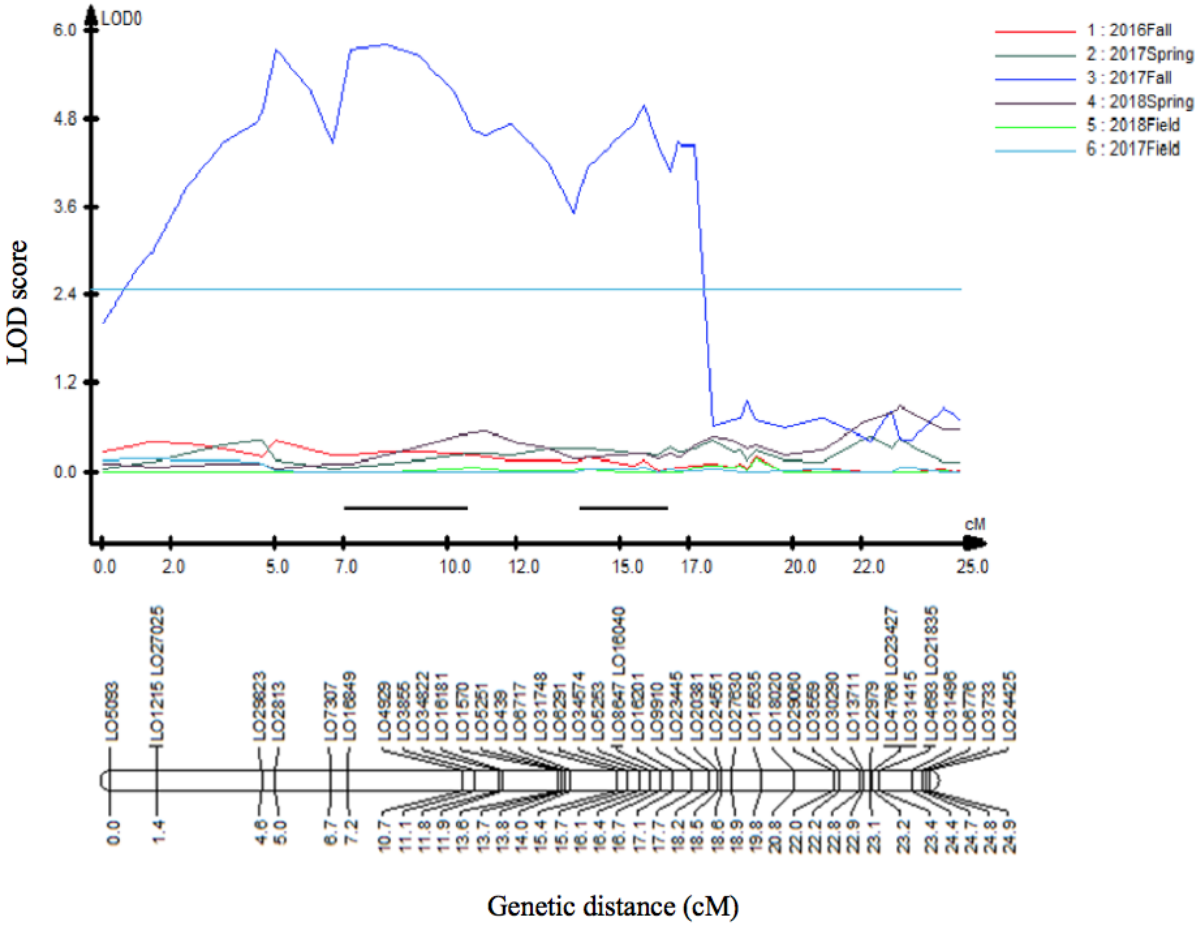
(b) 1A


LOD0
LOD score
4.0
3.2
2.4
1.6
0.8
0.0
cM
1 : 2016Fall
2 : 2017Spring
3 : 2017Fall
4 : 2018Spring
5 : MG
6 : 2018Field
7 : 2017Field
8 : MF
9 : FDK
Genetic distance (cM)

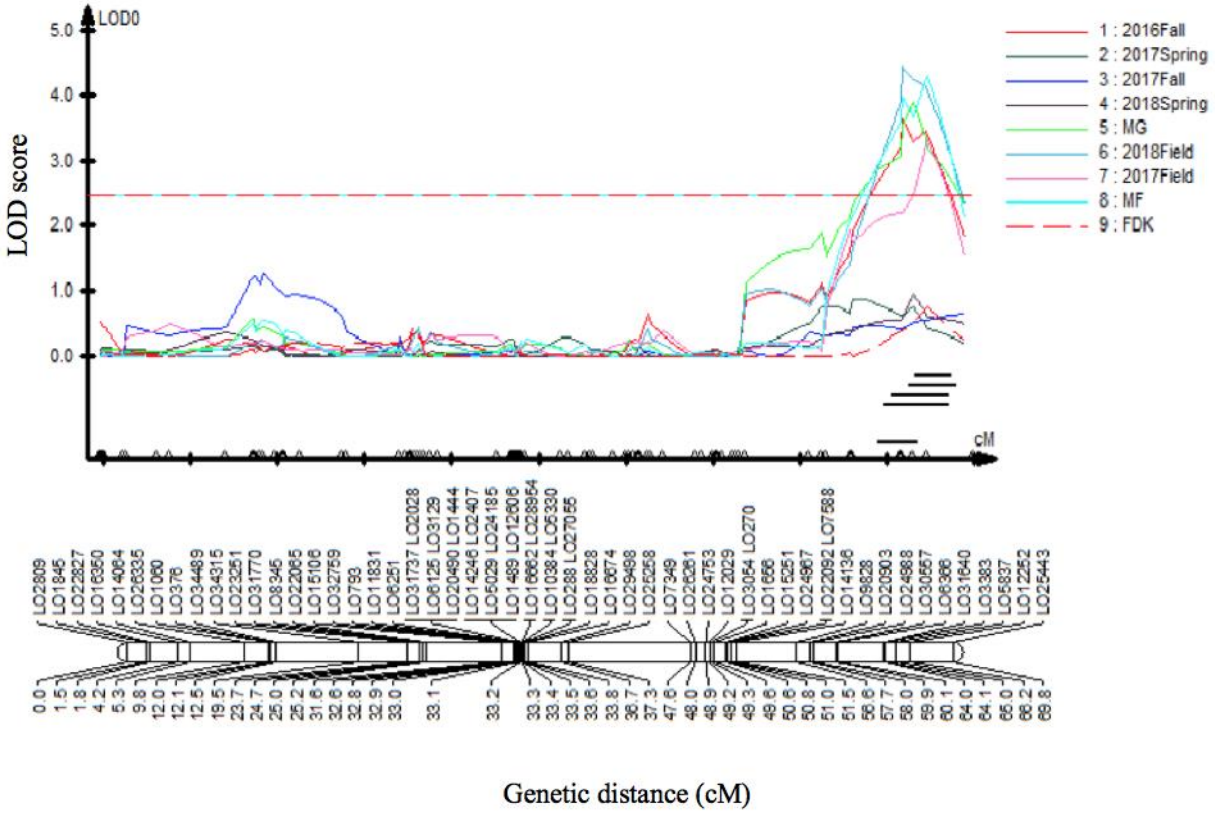
(C) 2A


LOD0
LOD score
3.0
2.4
1.8
1.2
0.6
0.0
cM
0.0
5.0
11.0
17.0
24.0
30.0
36.0
42.0
48.0
55.0
61.0
1 : 2016Fall
2 : 2017Spring
3 : 2017Fall
4 : 2018Spring
5 : 2018Field
6 : 2017Field
Genetic distance (cM)

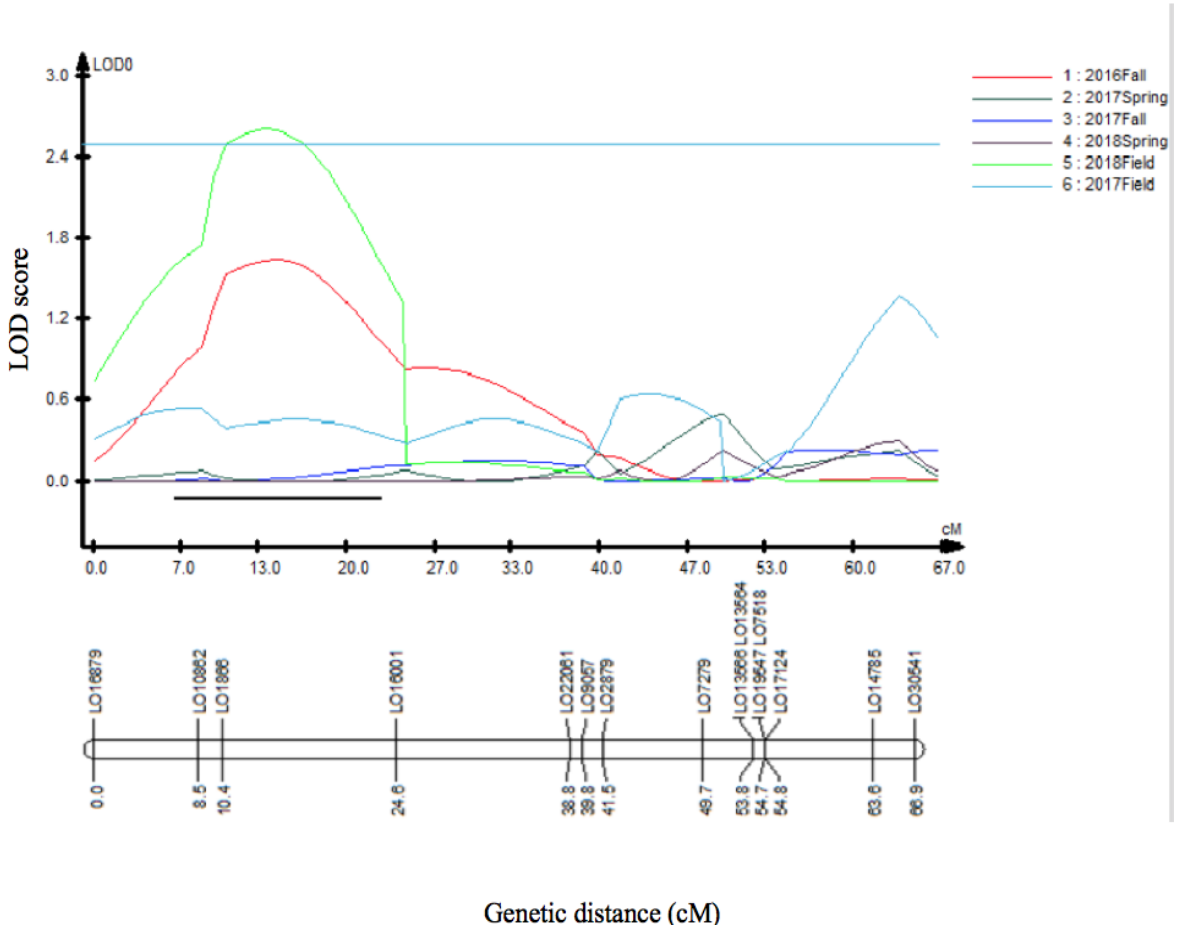
(D) 2B


LOD0
LOD score
6.0
4.8
3.6
2.4
1.2
0.0
cM
0.0
2.0
5.0
7.0
10.0
12.0
15.0
17.0
20.0
22.0
25.0
1 : 2016Fall
2 : 2017Spring
3 : 2017Fall
4 : 2018Spring
5 : 2018Field
6 : 2017Field
LO5093
LO1215 LO27025
LO29823
LO2813
LO7307
LO16849
LO4929
LO3855
LO34822
LO16181
LO1570
LO5251
LO439
LO6717
LO31748
LO6291
LO34574
LO5253
LO8647 LO16040
LO16201
LO9910
LO23445
LO20381
LO24551
LO27030
LO15535
LO18020
LO29060
LO3559
LO30290
LO13711
LO2979
LO4766 LO23427
LO31415
LO4668 LO21835
LO31496
LO6776
LO3733
LO24425
0.0
1.4
4.6
5.0
6.7
7.2
10.7
11.1
11.8
11.9
13.6
13.7
13.8
14.0
15.4
15.7
16.1
16.4
16.7
17.1
17.7
18.2
18.5
18.6
18.9
19.8
20.8
22.0
22.2
22.8
22.9
23.1
23.2
23.4
24.4
24.7
24.8
24.9
Genetic distance (cM)

(E)1B


LOD0
LOD score
5.0
4.0
3.0
2.0
1.0
0.0
cM
1 : 2016Fall
2 : 2017Spring
3 : 2017Fall
4 : 2018Spring
5 : MG
6 : 2018Field
7 : 2017Field
8 : MF
9 : FDK
LO2809 LO1845 LO22827 LO16350 LO14064 LO26335 LO1060 LO376 LO34489 LO34315 LO23251 LO31770 LO8345 LO22065 LO15106 LO32759 LO793 LO11831 LO6251 LO31737 LO2028 LO6125 LO3129 LO20490 LO1444 LO14246 LO2407 LO5029 LO24185 LO1489 LO12806 LO16662 LO28954 LO10384 LO5330 LO288 LO27055 LO18828 LO16674 LO29498 LO25258 LO7349 LO26261 LO24753 LO12029 LO3054 LO270 LO1660 LO15251 LO24967 LO22092 LO7588 LO14136 LO9828 LO20503 LO24988 LO30557 LO6386 LO31640 LO3383 LO5837 LO12252 LO25443
0.0 1.5 1.8 4.2 5.3 9.8 12.0 12.1 12.5 19.5 23.7 24.7 25.0 25.2 31.6 32.6 32.8 32.9 33.0 33.1 33.2 33.3 33.4 33.5 33.6 33.8 36.7 37.3 47.6 48.0 48.9 49.2 49.3 49.6 50.6 50.8 51.0 51.5 56.6 57.7 58.0 59.9 60.1 64.0 64.1 65.0 66.2 69.8
Genetic distance (cM)

(F) 4B



Genetic distance (cM)

(G) 3BS

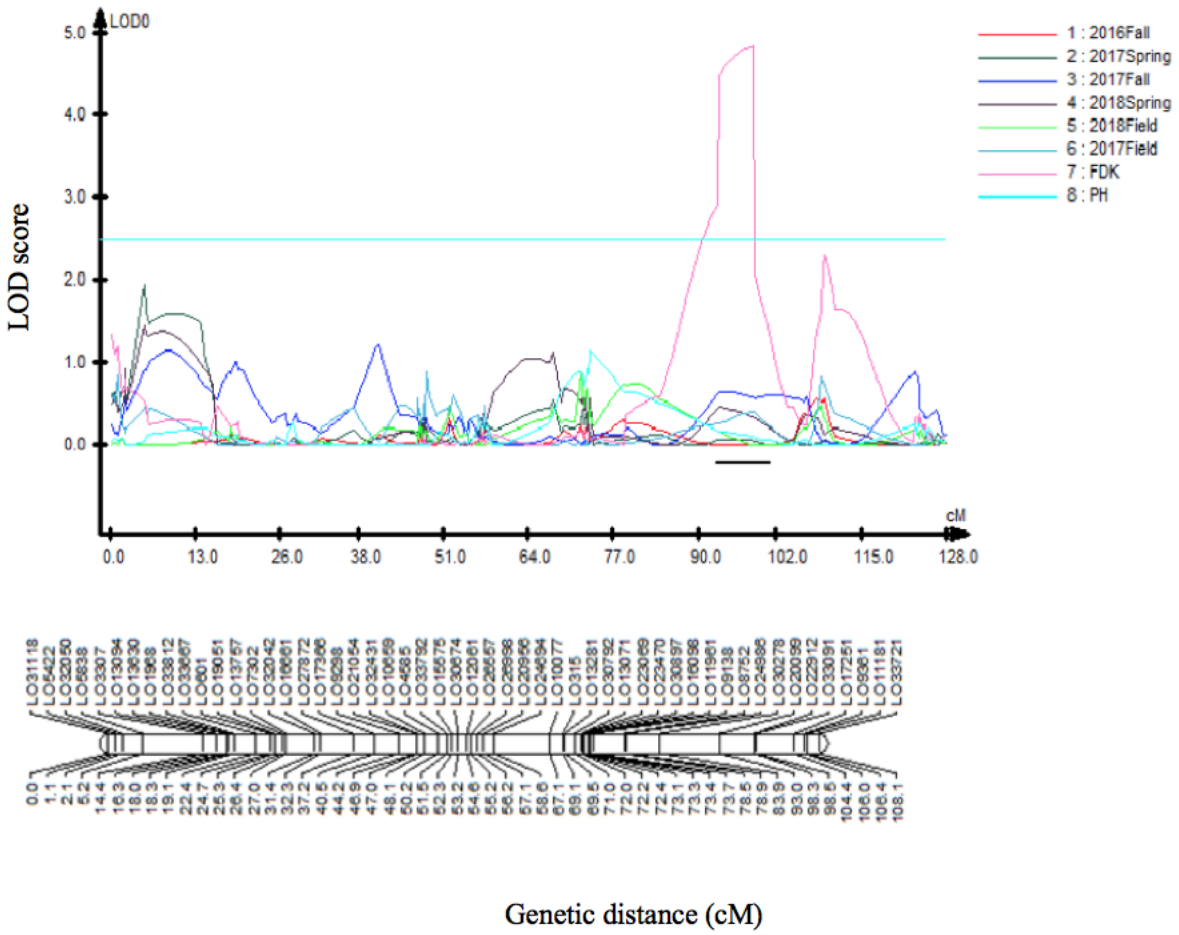
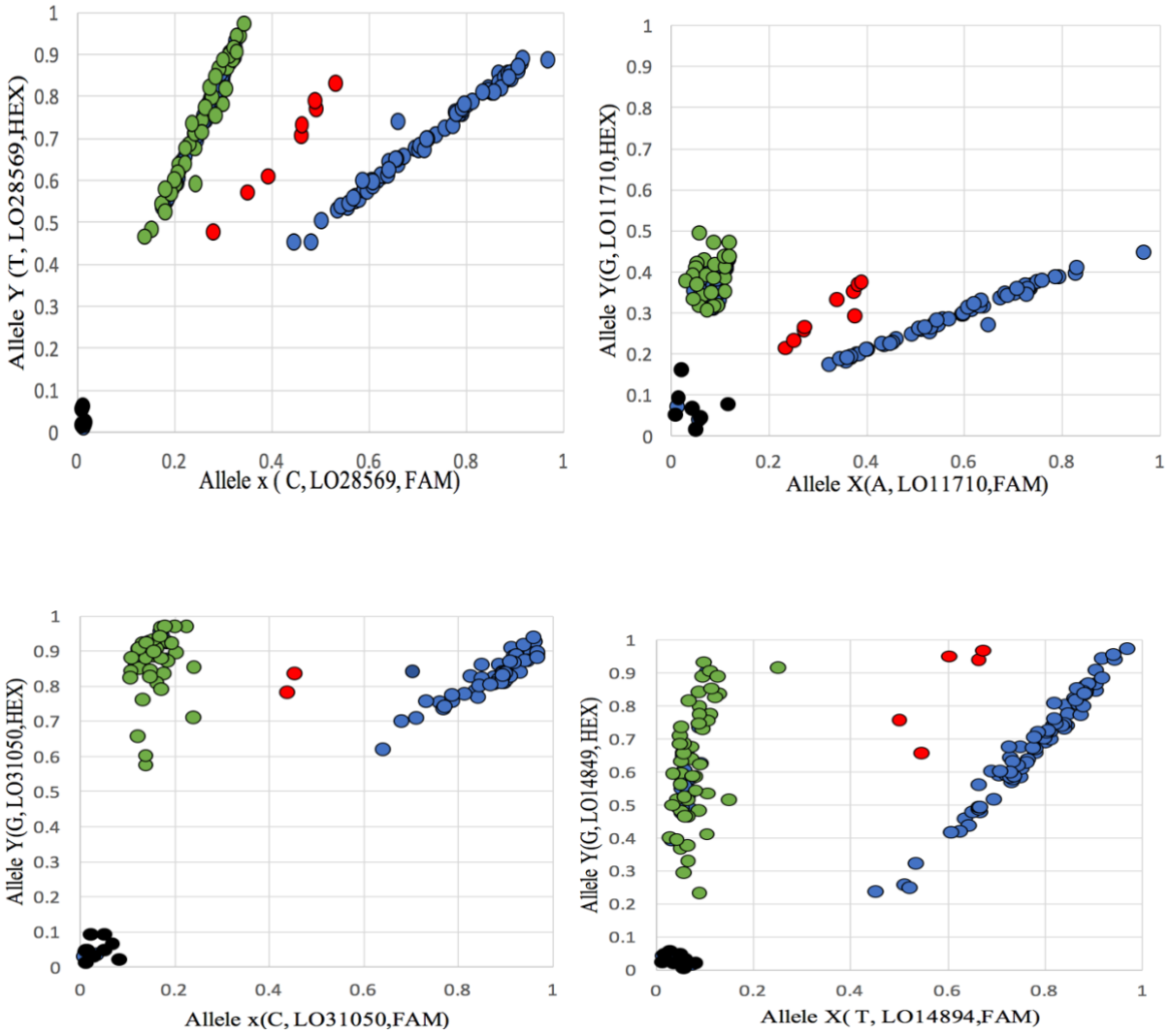
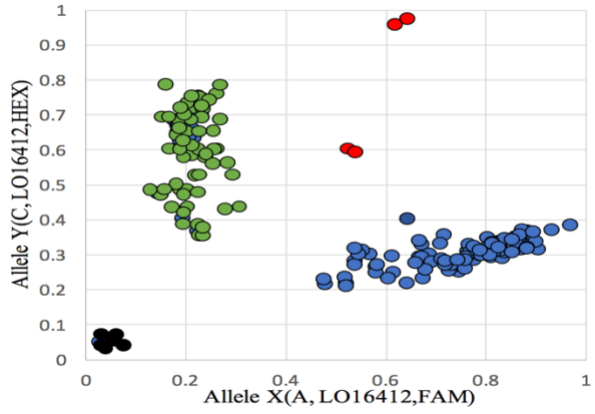
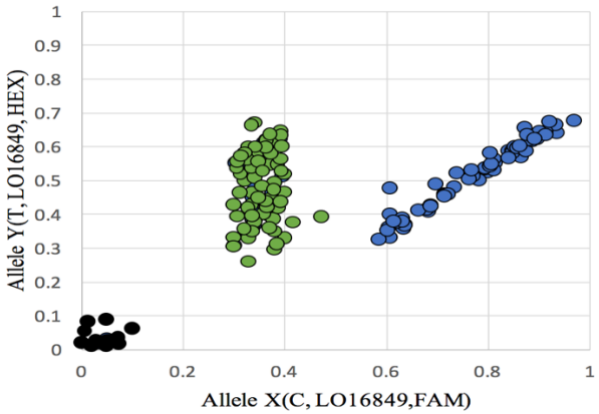
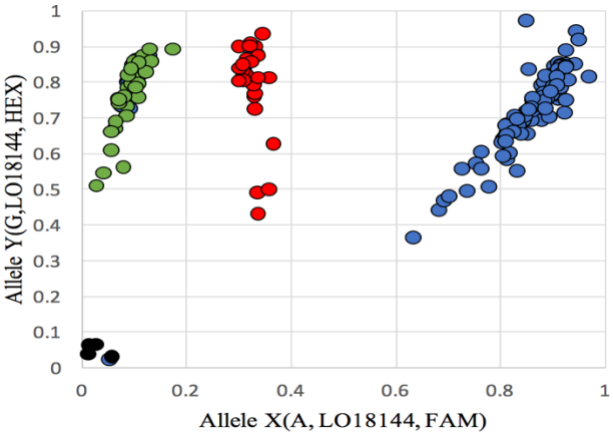

LOD0
LOD score
5.0
4.0
3.0
2.0
1.0
0.0
cM
0.0
13.0
26.0
38.0
51.0
64.0
77.0
90.0
102.0
115.0
128.0
1 : 2016Fall
2 : 2017Spring
3 : 2017Fall
4 : 2018Spring
5 : 2018Field
6 : 2017Field
7 : FDK
8 : PH
Genetic distance (cM)

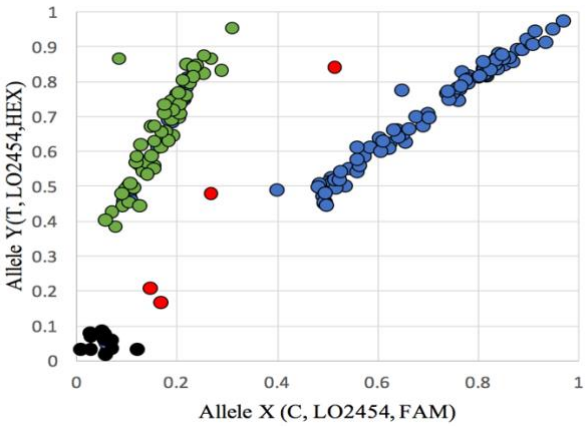
Figure 3.9 Kompetitive allele specific polymorphism (KASP) assays to show SNP allele segregation in the recombinant inbred line (RIL) population of 'Lyman' x 'Overley'. The blue and green dots are contrasting homozygous alleles, and red and black dots are heterozygotes and water control or samples with failed PCR.




Allele Y(C, LO16412,HEX)
Allele X(A, LO16412,FAM)


Allele Y(T, LO16849, HEX)
Allele X(C, LO16849,FAM)


Allele Y(G,LO18144, HEX)
Allele X(A, LO18144, FAM)


Allele Y(T, LO2454,HEX)
Allele X (C, LO2454, FAM)

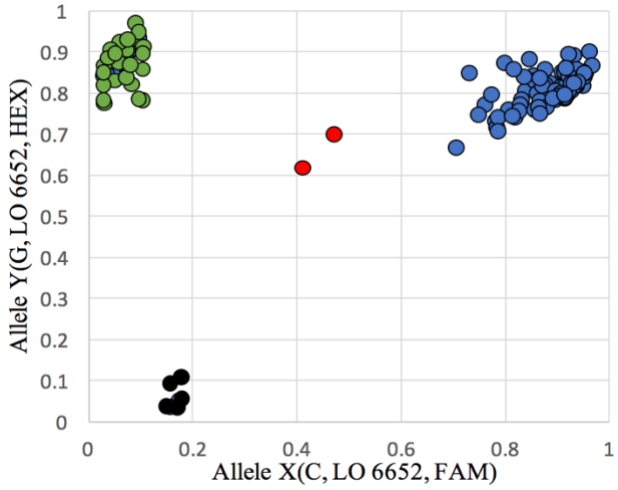

Allele Y(G, LO 6652, HEX)
Allele X(C, LO 6652, FAM)

Table 3.1 Analysis of variance (ANOVA) and broad-sense heritability (H^2) for percentage of symptomatic spikelets in a spike (PSS) from recombinant inbred line (RIL) population 'Lyman' x 'Overley' evaluated in the greenhouse experiments conducted in fall 2016, spring 2017, fall 2017, and spring 2018

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	3	1028.00	342.66	59.96	<.0001
Replication (Experiment)	4	17.15	4.28	0.75	0.55
genotype	173	583157.72	3371.04	589. 84	<.0001
Genotype *Experiment	519	313314.69	603.62	105. 63	<.0001
Error	694	3949.20	5.71		
Corrected Total	1390	901497.04			
H^2		0.91			

Table 3.2 Correlations among percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), and plant height (PH) in the recombinant inbred line population (RIL) 'Lyman' x 'Overley' evaluated in the four greenhouses experiments conducted in fall 2016, spring 2017, fall 2017, and spring 2018

Trait	PSS-Fall2016	PSS-Spring2017	PSS-Fall2018	PSS-Spring2018	HD
PSS-Fall2016	-				
PSS-Spring2016	0.13	-			
PSS-Fall2018	0.18 ***	0.35***	-		
PSS-Spring2018	0.92***	0.37***	0.38***	-	
HD	-0.04	-0.15 *	- 0.14*	- 0.09*	-
PH	0.013	0.03	0.15	0.12	0.07

PH= Plant height, HD=heading date, PSS= Percentage of symptomatic spikelets in a spike.

* refers to $P \leq 0.05$; ** refers to $P \leq 0.01$; *** refers to $P \leq 0.001$

Table 3.3 Analysis of variance (ANOVA) and broad-sense heritability (H^2) for percentage of symptomatic spikelets in a spike (PSS) from recombinant inbred line population (RIL) 'Lyman' x 'Overley' evaluated in the two field experiments conducted in 2017- 2018

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	1	50892.93	50892.93	37884.30	<.0001
Replication (Experiment)	2	50.92	25.13	23.02	<.0001
genotype	172	228012. 89	1325.65	986. 81	<.0001
Genotype *Experiment	172	104012.22	604.72	468.40	<.0001
Error	345	463.46	1.29		
Corrected Total	691	383412.44			
H^2		0.54			

Table 3.4 Correlations among percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), and plant height (PH) in the recombinant inbred line population (RIL) 'Lyman' x 'Overley' evaluated in the four greenhouses experiments conducted in fall 2016, spring 2017, fall 2017, and spring 2018

Trait	PSS-Fall2016	PSS-Spring2017	PSS-Fall2018	PSS-Spring2018	HD
PSS-Fall2016	-				
PSS-Spring2016	0.13	-			
PSS-Fall2018	0.18 ***	0.35***	-		
PSS-Spring2018	0.92***	0.37***	0.38***	-	
HD	-0.04	-0.15 *	- 0.14*	- 0.09*	-
PH	0.013	0.03	0.15	0.12	0.07

PH= Plant height, HD=heading date, PSS= Percentage of symptomatic spikelets in a spike.

* refers to $P \leq 0.05$; ** refers to $P \leq 0.01$; *** refers to $P \leq 0.001$

Table 3.5 Correlations among percentage of symptomatic spikelets in a spike (PSS), *Fusarium* damaged kernels (FDK), heading date (HD) and plant height (PH) in the recombinant inbred line population of 'Lyman 'x 'Overley' evaluated in the two field experiments conducted in 2017- 2018

Trait	PSS-2017	PSS-2018	FDK	HD
FPSS-2018	0.14**	-	-	-
FDK	0.44***	-	-	-
HD	- 0.018*	- 0.03	-0.14*	-
PH	0.018	-0.04	0.06	0.07

PH= Plant height, HD=heading date, PSS= Percentage of symptomatic spikelets in the field.

FDK= *Fusarium* damaged kernels.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 3.6 Chromosomal locations, marker intervals, determination coefficients (R^2), additive effect, logarithm of the odds (LOD) values and possible physical locations for significant quantitative trait loci (QTLs) identified in this study

Traits	Experiments	QTLs	Position (CM)	PVE (R2)[a]	Add. [b]	Marker interval		Physical location		Contributed by
PSS	Fall 2016	*QFhb.hwwgru.1A.1*	0.406	0.069	-0.073	*LO30016*	*LO5214*	105965471	319774804	Lyman
PSS	Field 2018	*QFhb.hwwgru.1A.1*	0.406	0.083	-0.078	*LO30016*	*LO5214*	105965471	319774804	Lyman
Mean	All the fields	*QFhb.hwwgru.1A.1*	0.53	0.077	-0.067	*LO30016*	*LO5214*	105965471	319774804	Lyman
PSS	Field 2017	*QFhb.hwwgru.1A.2*	40.61	0.06	-0.045	*LO19826*	*LO20733*	533901553	589247643	Lyman
PSS	Fall 2016	*QFhb.hwwgru.1B*	64.51	0.076	-0.0732	*LO31640*	*LO12252*	639941296	642547719	Lyman
PSS	Field 2018	*QFhb.hwwgru.1B*	64.51	0.091	-0.079	*LO31640*	*LO12252*	639941296	642547719	Lyman
PSS	Field 2017	*QFhb.hwwgru.1B*	66.61	0.079	-0.051	*LO31640*	*LO12252*	639941296	642547719	Lyman
Mean	All greenhouses	*QFhb.hwwgru.1B*	65.39	0.085	-0.061	*LO31640*	*LO12252*	639941296	642547719	Lyman
Mean	All the fields	*QFhb.hwwgru.1B*	66.58	0.093	-0.059	*LO31640*	*LO12252*	639941296	642547719	Lyman
PSS	Fall 2016	*QFhb.hwwgru.2A*	35.81	0.056	0.061	*LO18144*	*LO31050*	24259092	26494882	Overley
PSS	Fall 2017	*QFhb.hwwgru.2B*	8.21	0.179	-0.127	*LO16849*	*LO3855*	59220191	72714192	Lyman
PSS	Spring2017	*QFhb.hwwgru.3A*	76.01	0.215	-0.121	*LO4345*	*LO18017*	18448755	30867409	Lyman
PSS	Spring 2018	*QFhb.hwwgru.3A*	84.81	0.178	-0.104	*LO4345*	*LO18017*	18448755	30867409	Lyman
FDK	Field 2017	*QFhb.hwwgru.3B*	98.31	0.121	0.083	*LO20099*	*LO17251*	783042700	794765681	Lyman
PSS	Field 2018	*QFhb.hwwgru.4B*	13.41	0.066	-0.067	*LO1866*	*LO14790*	4038721	9070001	Lyman

[a] PVE: phenotypic variation explained;
[b] Add.: Additive affect.

Table 3.7 List of sequences of single nucleotide polymorphism (SNPs) markers derived from genotyping-by-sequencing (GBS) and primers for Kompetitive allele-specific PCR (KASP) assays developed from GBS sequences

SNP name	Sequence	Position	Dye	Tailed Primer
GBS1207-5AL	TGCAGCCACACACCGCC[A/G]CCGCAGACCG AGCCAACGGACGGGAATTTTACGAGAGAAT CCAACC	18	FAM	GAAGGTGACCAAGTTCATGCTTGCAGCCACACACCGCCG
			HEX	GAAGGTCGGAGTCAACGGATTTGCAGCCACACACCGCCA
			R	GTAAAATTCCCGTCCGTTGG
GBS3483-5AL	TGCAGTGTCAAACTTGTCTTGACACGTCCAC T[C/T]ATGATATATCCAATGAAGGTGCCACA TGAT	33	FAM	GAAGGTGACCAAGTTCATGCTCAAACTTGTCTTGACACGTCCAC Tc
			HEX	GAAGGTCGGAGTCAACGGATTCAAACTTGTCTTGACACGTCCAC Tt
			R	ATGTGGCACCTTCATTGGAT
GBS1123-4BS	TGCAGCATCTGGTGGCC[G/A]AATTCATAAG CTGGATCAGAAGTCGCAAAAGGCTGGTGAA GGCAGC	18	FAM	GAAGGTGACCAAGTTCATGCTTGCAGCATCTGGTGGCCg
			HEX	GAAGGTCGGAGTCAACGGATTTGCAGCATCTGGTGGCCa
			R	AGCCTTTTGCGACTTCTGAT
GBS260-4BS	TGCAGACGCTGAAGAGGTCCG[C/T]CGCTCT GGATGGGTATGGCGGCGGCGGTGGCGGCGG GGAAGC	22	FAM	GAAGGTGACCAAGTTCATGCTGACGCTGAAGAGGTCCGC
			HEX	GAAGGTCGGAGTCAACGGATTGACGCTGAAGAGGTCCGT
			R	GCCATACCCATCCAGAGC
GBS1775-4BS	TGCAGCGTGCA[A/C]ACAAACAACCCTGCTT AGCACAACAAGACAAAGACAAACAAGGAG GAAACAT	11	FAM	GAAGGTGACCAAGTTCATGCTGCTAAGCAGGGTTGTTTGTTT
			HEX	GAAGGTCGGAGTCAACGGATTGCTAAGCAGGGTTGTTTGTTC
			R	AGCCATACTGCATTTGGAAT
GBS2829-2D	TGCAGGGGTCCATGCCGCTGCAC[A/C]GCCG TCGTCTCTCTTGGGTCAGCCACTTCCGCATG TCGCC	24	FAM	GAAGGTGACCAAGTTCATGCTGGTCCATGCCGCTGCACA
			HEX	GAAGGTCGGAGTCAACGGATTGGTCCATGCCGCTGCACC
			R	GCTGACCCAAGAGAGACGAC
GBS112-7A	TGCAGAAGCTCACCATCAAGGCCGAC[A/G] AGGCTGAGGATGCGCTGGATGAGCTTCACT ACTTCAT	27	FAM	GAAGGTGACCAAGTTCATGCTTCACCATCAAGGCCGACA
			HEX	GAAGGTCGGAGTCAACGGATTCACCATCAAGGCCGACG
			R	AAGCTCATCCAGCGCATC

SNP name	Sequence	Position	Dye	Tailed Primer
LO18144-2B	CTGCAGCGGTGTGCAAGCAAGGTGAGATGC[A/G]GGAGCCGACAGACAATGACGAAGTTTTGAAGCAA	31	FAM	GAAGGTGACCAAGTTCATGCTCAAGCAAGGTGAGATGCA
			HEX	GAAGGTCGGAGTCAACGGATTCAAGCAAGGTGAGATGCGA
			R	AACTTCGTCATTGTCTGTCG
LO31050-2B	CTGCAGATAGCTATCTTCTGGTACATTCGCATCTACATGAAAAACAAC[C/G]ACAACCATCATGATTC	49	FAM	GAAGGTGACCAAGTTCATGCTGCATCTACATGAAAAACAACC
			HEX	GAAGGTCGGAGTCAACGGATTGCATCTACATGAAAAACAACG
			R	TCTCCTGATTAGGGGCTTC
LO16849-2B	CTGCAGCTATGGTGCATAGTAGTACAAGTCGAGGTAGTACAAGAGGTACAGG[C/T]GGAGGCAAGCGA	53	FAM	GAAGGTGACCAAGTTCATGCTGGTAGTACAAGAGGTACAGGC
			HEX	GAAGGTCGGAGTCAACGGATTGGTAGTACAAGAGGTACAGGT
			R	AGGCTTGGACCATAATGAAA
LO6291-2B	CTGCAGGTGGAGCTTATGCCGTGGAGGCCCTGAGGGC[A/G]GAACTTGCATTAGCCAATTAACAAGTC	38	FAM	GAAGGTGACCAAGTTCATGCTAATTGGCTAATGCAAGTTCT
			HEX	GAAGGTCGGAGTCAACGGATTAATTGGCTAATGCAAGTTCC
			R	CTGCAGGTGGAGCTTATG
LO31640-1B	CTGCAGAGGCTGCAAGATGGGACGCAAATTCGTCTCCAAGGT[A/G]CAAGATTTGTTTGATCGAGCTG	43	FAM	GAAGGTGACCAAGTTCATGCTGCAAATTCGTCTCCAAGGTA
			HEX	GAAGGTCGGAGTCAACGGATTGCAAATTCGTCTCCAAGGTC
			R	CAGCTCGATCAAACAAATCTT
LO5837-1B	CTGCAGGTTCTGAGCCTGAA[G/T]TCCTCCTCCAGGAAGCTGGATATCTCTGCTATGGCCGTCTACGA	21	FAM	GAAGGTGACCAAGTTCATGCTCAGGTTCTGAGCCTGAAG
			HEX	GAAGGTCGGAGTCAACGGATTCAGGTTCTGAGCCTGAAT
			R	AGACGGCCATAGCAGAGATA
LO14894-3A	CTGCAGCTGCGGTGGTGT[G/T]CGGTAGGCTCCCGCGGTGGCAGACAGCTAGATCTGGGCCCAACCAG	19	FAM	GAAGGTGACCAAGTTCATGCTGCGGTGGCAGACAGCTAG
			HEX	GAAGGTCGGAGTCAACGGATTGCGGTGGCAGACAGCTAT
			R	ACCAGTTGCGGCTACG

Chapter 4 . Mapping quantitative trait loci for *Fusarium* head blight resistance in the RIL population of 'Lyman' X 'CI13227'

4.1 Introduction

Wheat is one of the most consumed crops worldwide and is one of the important crops for future food security. *Fusarium* head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe is one of the most destructive diseases in wheat. FHB epidemics can result in significant losses in wheat grain yield and quality.

In the U.S., FHB used to occur mainly in hard spring wheat (HSW) regions in the northern states and soft winter wheat regions. By incorporating resistance genes into breeding materials, several U.S. FHB resistant cultivars including 'ND VitPro' have been officially released by North Dakota State University in 2017 (https://www.agupdate.com/theprairiestar/news/crop/). Recently more frequent occurrences of FHB epidemics have been reported in the hard winter wheat (HWW) region in the U.S. Great Plains, which has made breeding for FHB resistance one of the major breeding objectives in this region. After extensive screening HWW germplasm for FHB resistance, several cultivars with moderate resistance to FHB were identified, including 'Heyne', 'Hondo', 'Everest', 'Overland', and 'Lyman' and they may carry native resistance QTLs for FHB resistance (Bockus *et al.,* 2009; Zhang *et al.,* 2012a; Jin *et al.,* 2013). The haplotype analysis of markers for *Fhb1* indicated that these cultivars might not carry *Fhb1*, a major QTL from Chinese sources. Therefore, combining U.S. native resistance QTLs with major QTLs from Asian sources such as *Fhb1* may diversify the FHB resistance gene pool and enhance FHB resistance levels in U.S. hard winter wheat.

In the previous chapters, QTLs for FHB resistance in 'Lyman' (KS93U134/Arapahoe) from South Dakota (Eckard *et al.,* 2015) and 'CI13227' (Wabash//American Banner/Aniversario)

from Indiana (Shaner *et al.,* 1997) have been mapped using different recombinant inbred populations. The objectives of the current study were to validate the previous mapped QTLs in 'CI13227' and 'Lyman', respectively, and the SNP markers that tightly linked to these QTLs.

4.2 Materials and methods

4.2.1 Plant materials and FHB resistance evaluation in greenhouse experiments

A mapping population of 164 $F_{5:6}$ recombinant inbred lines (RILs) was developed by single seed descent from the cross Lyman x CI13227. 'Lyman' (KS93U134/Arapahoe) is a hard-red winter wheat with moderate resistance to FHB (Eckard *et al.,* 2015), while 'CI13227' (Cltr13227) is a soft red winter wheat line with moderate FHB resistance and a pedigree of Wabash//American Banner/Aniversario (Shaner *et al.,* 1997) from Indiana. This RIL population was evaluated for type II FHB resistance in the greenhouses at Kansas State University, Manhattan KS. Seeds of the RILs were planted in plastic trays filled with Metro-mix 360 soil mix (Hummert International, Topeka, KS). After 50 d of vernalization in a cold room at 6 °C, about 5 seedlings per line were transplanted into a 4 x 4'' Dura pot filled with Metro mix 360 soil mix. The pots were arranged on the greenhouse benches in a randomized complete block design (RCBD) with two replications. The greenhouse temperature was maintained 17 °C at night - 22 °C during day with 14 h of supplement light. For powdery mildew control, the sulfur powder was burned in the greenhouses for three hours each night.

F. graminearum (GZ3639) inoculum is a Kansas strain (Bai *et al.,* 1999*).* About 1000 conidial spores were injected into the central spikelet of a spike using a syringe (Hamilton, Reno, NV). Five spikes per pot were inoculated and inoculated plants moved into a moist chamber at 100% relative humidity at 24 °C for 48 h. The FHB symptoms for type II resistance was evaluated at 14 d after inoculation and FHB severity was calculated using the following formula:

PSS = 100* (number of infected spikelets / total spikelets in a spike)

4.2.2 FHB evaluation in field experiments

FHB for the RIL population and their parents were evaluated in the KSU Pathology FHB Nursery, Rocky Ford, Manhattan, KS in the 2015 and 2016 using the corn grain-spawn inoculation (Tuite, 1969). The experiments were arranged in a randomized complete block design (RCBD) design with two replications. One gram of seed per line was planted in a 1-m single row plot. *F. graminearum*-infected corn kernels were scattered twice at a two weeks' interval on the soil surface prior anthesis. The field nursery was misted for 3 min per h between 6:00 h and 21:00 h daily from heading to late grain filling using sprinklers. Type II resistance was evaluated after 21 d post anthesis using a scale of 1% (most resistant) to 100% (most susceptible) based on overall performance in each row. Heading date and plant height were measured for each entry. All plants were harvested and threshed manually. *Fusarium*-damaged kernel (FDK) was visually evaluated and the percentage of FDK was calculated by dividing the number of FDK by a total number of kernels that were harvested from each plot. DON concentration was determined from the harvested seeds from each plot using gas chromatography (Tacke and Casper, 1996). About 5 g per RIL was randomly selected for DON evaluation.

4.2.3 DNA extraction and genotyping

Six pieces of leaf tissue were collected in 96 deep-well plates and dried in freeze dryers (ThermoSavant, Holbrook,NY) for 72 h, ground using a Mix Mill (MM 400, Retsch, Germany) for DNA isolation. DNA was extracted using OKtopure™, an automated DNA isolation platform from LGC as described in Chapter 2. GBS library construction, sequencing, SNP calling, and quality control followed (Poland *et al.*, 2012) as described in Chapter 2. Linage map construction and QTL mapping were described in Chapter 2.

4.3 4.3. Results

4.3.1 FHB in RIL population

In the three greenhouse experiments, ‘Lyman’ showed a higher level of FHB resistance (with mean PSS of 20%, ranging from 15.0 to 25%) than ‘CI 13227’ (mean PSS of 50.0%, ranging from 45.4 to 55.0%) (Fig. 4.1). The frequency distribution of mean PSS of RIL population from three greenhouse experiments was skewed to left to Lyman. The mean PSS of the RIL population from the three greenhouse experiments was the lowest (23.4%) in the fall 2017 and the highest (40.9%) in spring 2018. Transgressive segregation was observed in both fall and spring 2017, suggesting both parents contributed QTLs to susceptibility. A broad sense heritability was high (0.80) (Table 4.1). A significantly negative phenotypic correlation was observed between PSS and heading date (Table 4.2), but not between PSS and plant height.

In the 2015 field experiment, the frequency distribution for PSS showed a continuous distribution (Fig. 4.2). The parents ‘Lyman’ and ‘CI13227’ had mean PSS of 18.0% and 55.0%, respectively and the RIL population was 46.2%. The PSS for both parents and RIL population were slightly lower in 2016 field experiment with 15% for ‘Lyman’, 50% for ‘CI 13226’ and 36.5% for RIL population. The broad sense heritability was high for the field experiments (0.72) (Table 4.3). The resistant parent ‘Lyman’ head about 8 days earlier than ‘CI 13226’ in average of both field experiments (Fig. 4.3). The mean plant height was 118 cm for the resistant parent ‘Lyman’ and 135 cm for ‘CI13227’ (Fig. 4.4). The mean FDK in the 2015 field experiment were 5% for ‘Lyman’ and 16% ‘CI13227’. DON content was lower in the resistant parent ‘Lyman’ (15 ppm) than in the susceptible parent ‘CI13227’ (18.5 ppm). However, 2016 field experiment had slightly lower disease scores with the FDK 5% for ‘Lyman’ and 9% for ‘CI13227’, and DON content of 12.9 ppm for ‘Lyman’ and 17.3 ppm for ‘CI13227’.

A correlation was positively significant ($P < 0.001$) for RILs between the 2015 and 2016 field experiments. Among the traits evaluated, the PSS was positively correlated with FDK (Table 4.2), negatively correlated with head date, but not correlated with plant height in the both field experiments. As expected, FDK was significantly ($P < 0.001$) correlated with DON content in the field experiments, indicating that wheat kernels with high FDK contained high DON (Fig. 4.5; Fig.4.6).

4.3.2 Construction of linkage map

The GBS-SNPs from two Ion Proton sequencing runs were called for 149 RILs using the TASSEL-GBS pipeline after removing 20 RILs that had too many missing data. A total of 2,256 GBS-SNPs with 30% or less missing data were used to construct a linkage map of 27 linkage groups, ranging from 7 to 230 markers per group. The map represents all 21 chromosomes with 2,007.8 cM long and an average marker distance of 0. 89 cM per marker. As in the other two maps in Chapters 2 and 3, B genome had the most markers (44.3 %), and the D genome least (20.9%) (Fig. 4.7)

4.3.3 QTLs for FHB resistance

Inclusive composite interval (CIM) mapping detected four significant QTLs for type II FHB resistance, *QFhb.hwwgru-1A*, *QFhb.hwwgru.3A*, *QFhb.hwwgru-7A*, and *QFhb.hwwgru-2B* in the 'Lyman' x 'CI13227' RIL population (Fig. 4.8, Table 4.5). *QFhb.hwwgru.3A* flanked by SNPs *GBS19781* and *GBS280* was significant in 2017 spring greenhouse experiment, 2016 field experiment, greenhouse mean PSS and field mean PSS data, and explained 10.18%, 9.43%, 13.79% and 11.94% of the phenotypic variation, respectively. 'Lyman' contributed the resistance allele. A QTL for heading date was significant in the same chromosome, but it was not overlapped with the FHB resistance QTL (Fig. 4. 8 and Table 4.5).

QFhb.hwwgru-7A was significant for FHB resistance and explained 12.3 % of the phenotypic variation (Fig. 4.8, Table 4.5). It was delineated to a 1.9 cM interval between SNPs *GBS11994* and *GBS4990*. It showed a large effect on type II resistance in spring 2018 greenhouse experiment and overlapped with the QTL for low DON accumulation in 2015 field experiment. The resistance allele of *QFhb.hwwgru-7A* was contributed by 'CI13227'.

QFhb.hwwgru-1A was flanked by SNPs *GBS17061* and *GBS12112*, significant in the 2016 field experiment and 2017 spring greenhouse experiments, and explained 6.4 % and 7.2% of the phenotypic variation, respectively. This QTL was also significant for DON content and FDK, and explained 3.8% and 5.2% of the phenotypic variation, respectively (Fig. 4.8, Table 4.5).

QFhb.hwwgru.2B on chromosome 2B was flanked by *GBS11644* and *GBS18945* markers and was significant in the 2015 field experiment and 2017 fall greenhouse experiment. This QTL explained 8.4% and 10.0% of the phenotypic variation, respectively, and the resistance allele was from the resistant parent 'Lyman' (Fig. 4.8, Table 4.5).

4.4 Discussion

4.4.1 QTLs for type II FHB resistance in 'Lyman' and 'CI13227'

Four significant QTLs for FHB resistance were detected on chromosomes 1A, 3A, 7A, and 2B in this population using the phenotypic data from the two greenhouses and two field experiments. Among them, 'CI13227' confers resistance alleles at the QTLs on chromosomes 1A and 7A while 'Lyman' confers resistance at the QTLs on chromosomes 2B and 3A.

QFhb.hwwgru.1A was significant in spring 2017 greenhouse and 2016 field experiment. The QTL was contributed by 'CI13227'. A number of studies reported QTLs for type II FHB resistance on chromosome 1A such as in 'Arina' (MR) (Semagn *et al.*, 2007), 'Wangshuibai' (Yu

et al., 2008b), 'Jamestown' (Wright *et al.*, 2012), 'NC-Neuse' (Petersen *et al.,* 2015), and 'C615' (Yi *et al.*, 2018). Based on the previous studies, the QTL that determined in current study is likely close to the QTL found by Yu *et al.,* (2008), because *QFhb.hwwgru.1A* is about 32 Mb from the marker *Xwmc120* reported by (Yu *et al.,* 2008 and Cai, 2012). Comparing to the 1A QTL identified in 'Lyman' x 'Overley' RIL population, the two QTLs were from different chromosome regions and contributed by different parents, by 'Lyman' in 'Lyman' x 'Overley' and by 'CI13227' in 'Lyman' x 'CI 13227'.

QTL *QFhb.hwwgru.3A* was significant in multiple experiments 'Lyman' x 'CI 13227' population including the field experiment 2016 and greenhouse experiment in spring 2017, and mean PSS from both the greenhouse and field experiments. The resistance allele was contributed by 'Lyman' and explained 9.4 ~ 13.8% of the phenotypic variation. Several QTLs on 3A chromosome have been reported, including these in 'F201R' from Europe (Shen *et al.,* 2003), 'Huapei57-2' (Bourdoncle & Ohm, 2003), 'Wangshuibai' (Yu *et al.,* 2008b) and several other landraces from China (Zhang *et al.*, 2012b; Cai & Bai, 2014; Cai *et al.*, 2019), 'Heyne' from the U.S. (Zhang *et al.*, 2012a), 'DH181R' (Yang *et al.*, 2005b) and 'Frontana' from Brazil (Steiner *et al.*, 2004). In this study, the QTL peak from 2018 spring experiment appeared in the right side of the QTL peak derived from the mean, but they were most likely the same QTL. The discrepancy might be due to FHB scoring error in different environments. The *QFhb.hwwgru.3A* is likely the same as that detected from 'Lyman' x 'Overley' and 'Lyman' contributed resistance allele in both populations.

The QTL on 2B was significant in the field experiment 2016, fall 2017 greenhouse experiment, and the mean of the greenhouse, and explained 8.4 ~ 19.0% of the phenotypic variation. Several QTLs on chromosome 2B have been reported in different populations such as

in 'Ning7840' (Zhou *et al.,* 2002b), 'Ernie' (Liu *et al.,* 2007), 'IL94-1653' (Carolyn *et al.,*2009) and 'HYZ' (Cai *et al.,* 2019). The current QTL was mapped close to the type II resistance QTL in 'Renan' because it is close to the marker *Xgwm120* in 'Renan' (Gervais *et al.,* 2003). Based on the physical location for the flanking marker, the *QFhb.hwwgru.2B* is located at the same region as the the 2B QTL detected from 'Lyman' x 'Overley' and 'Lyman' contributed resistant allele in the both populations

4.5 Conversion of GBS-SNPs into KASP assays

In the current study, among 11 KASP assays designed, six were successfully amplified PCR and five did not due to the SNP positions were too close to the end of the sequence reads that cause difficulties in primer design. All six markers were remapped to the four QTL regions. For each primer set, the KASP assays separated the RIL population into two clusters as "Lyman' and 'CI13227' alleles. *GBS6006* and *GBS5424* were linked to *QFhb.hwwgru.3A*, *GBS1965* and *GBS11994* to 7A and *GBS6720* to 1A and *GBS6507* to 2B (Fig. 4.9; Table 4.6). Sex KASP markers that have designed for the *QFhb.hwwgru.7A* from DH population 'CI13227' x 'Lakin' and *QFhb.hwwgru.3A* and *QFhb.hwwgru.2B* from the RIL population 'Lyman' x 'Overley' were tested in the RIL population 'Lyman' x 'CI13227'. Three KASP markers (*GBS112-7A, LO6652-3A,* and *LO6291-2B*) could be mapped on the corresponding QTL regions on chromosome 7A, 3A and 2B in 'Lyman' x 'CI13227' population. For the KASP assays *LO6652-3A* and *LO6291-2B*, they amplified 'Lyman' alleles correctly, but failed amplification of 'CI13227' alleles in the 'Lyman' x 'CI13227' population. In contrast, *GBS112-7A* amplified the 'CI13227' allele only, but not the 'Lyman' allele. One marker (*LO6291-2B*) was mapped at the QTLs regions in the RIL population 'Lyman' x 'CI13227' and (*LO6652-3A, GBS112-7A*) were not mapped in the QTL region, but they mapped with a 7 and 2 cM away respectively. As results, they may be same QTLs.

Therefore, these KASPs may be useful markers for selecting the QTLs in breeding programs when they are polymorphic.

4.6 Conclusion

To identify QTLs in U.S. winter wheat ‘Lyman’ and ‘CI13227’, One DH population of ‘CI13227’ x ‘Lakin’ and one RIL population of ‘Lyman’ x ‘Overley’ were developed, genotyped with SNP markers generated by either 90K Wheat SNP chips or GBS, and phenotyped in both field and greenhouse experiments for QTL analysis. Four QTLs were identified on chromosomes 4BS, 5AL, 2DS and 7A in the ‘CI13227’ x ‘Lakin’ population and Lyman contributed resistance alleles for QTLs on 7A, 2DS and 4BS. Six QTLs on the chromosomes 1A, 2A, 3A, 1B, 2B and 4B in RIL population ‘Lyman’ x ‘Overley’ and Lyman contributed all resistance alleles except one QTL on 2A. Most of the QTLs showed minor effects on type II resistance. QTL on 2DS and 4BS from CI 13227 overlapped with two plant height genes (*Rht8* and *Rht1*), suggesting they may be tightly linked genes or have pleiotropic effects. Both susceptible parents Lakin and Overley contributed resistance alleles at one or two QTLs, suggesting that using susceptible parents with some minor resistance genes may pyramid the minor genes from those parents to increase the resistance level of progeny. To confirm the QTLs identified from the two populations, a RIL population from ‘Lyman’ x ‘CI13227’ was evaluated in both field and greenhouse experiments. Four QTLs including these on chromosomes 1A, 3A, 7A and 2B were mapped for FHB type II resistance in the population. Among them, the QTLs on chromosomes 2B and 3A were contributed by ‘Lyman’ whereas the QTL on chromosome 7A were contributed by ‘CI13227’, which were mapped to the positions close to these mapped in ‘CI13227’ x ‘Lakin’ and ‘Lyman’ x ‘Overley’ populations and confirmed these QTLs. The QTL on 4BS contributed by both ‘CI13227’ and ‘Lyman’ was not significant in ‘Lyman’ x ‘CI13227’ population, which may be due to the same

QTLs in the both ‘Lyman’ and ‘CI13227’. Breeder-friendly KASP markers were developed for most of QTLs identified from the two resistance sources. They can be useful markers for transferring these QTLs into U.S. winter wheat.

4.7 References

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Figure and Tables

Figure 4.1 Frequency distribution of mean percentage symptomatic spikelets (PSS) in a spike of recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' in greenhouse experiments conducted in fall 2017, Spring2017, and Spring2018

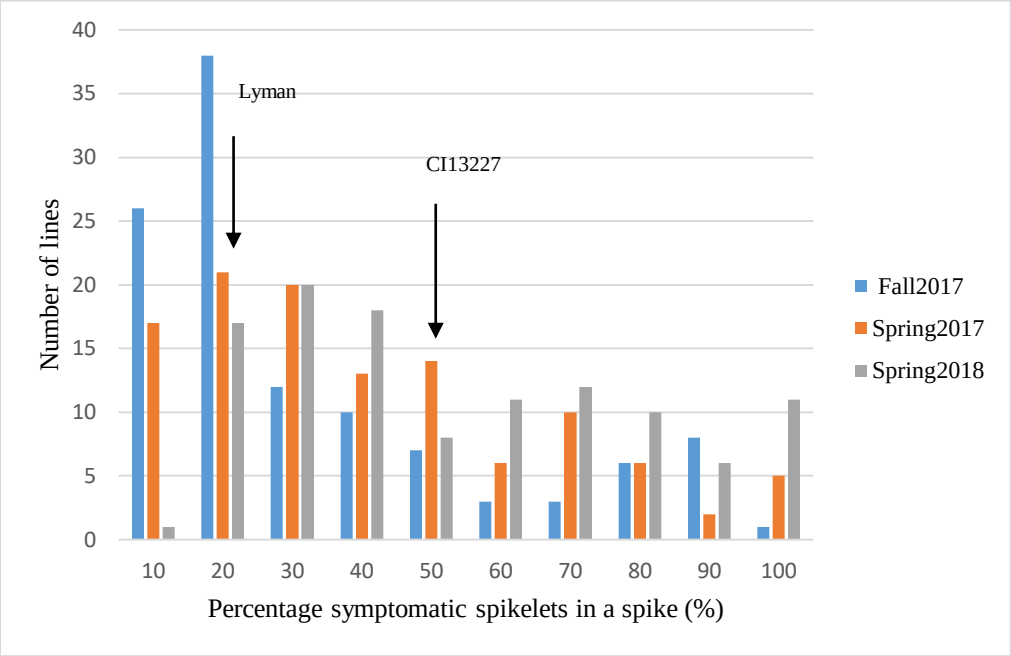


Figure 4.2 Frequency distribution of mean percentage symptomatic spikelets (PSS) in a spike of recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' in two field experiments conducted in 2015 and 2016

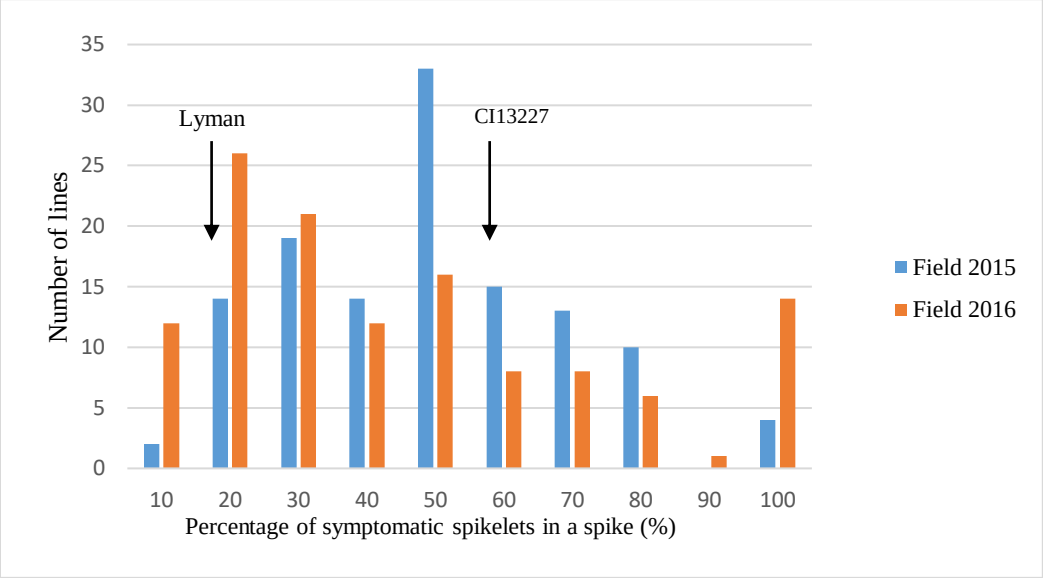


Figure 4.3 Frequency distribution of mean heading date of recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' in two field experiments conducted in 2015 and 2016

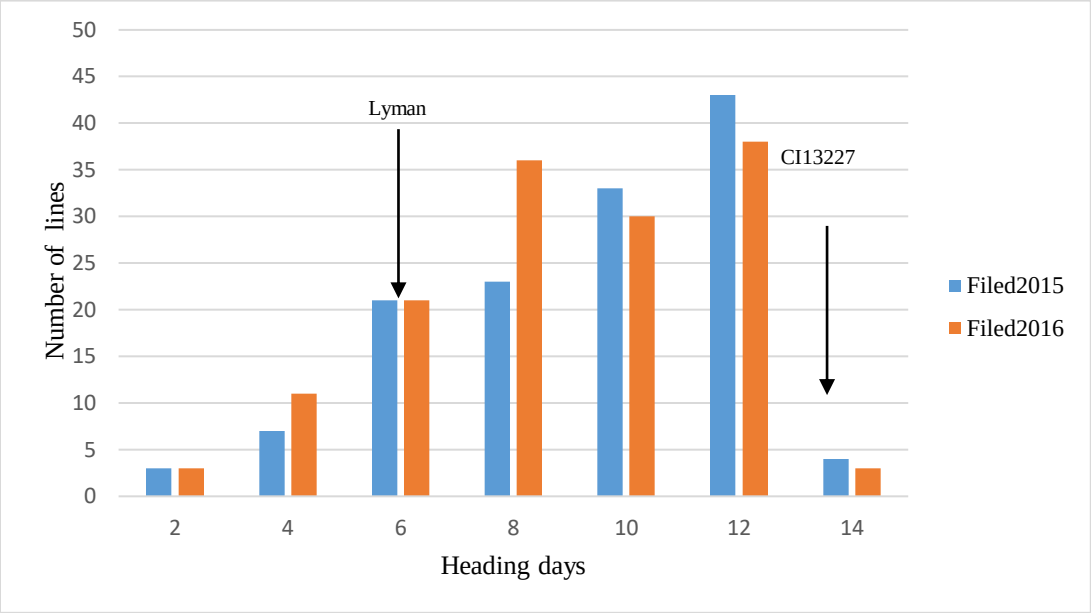


Figure 4.4 Frequency distribution of mean of plant height (PH) of recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' evaluated in 2015 (Field15) and 2016 (Field16) field experiments

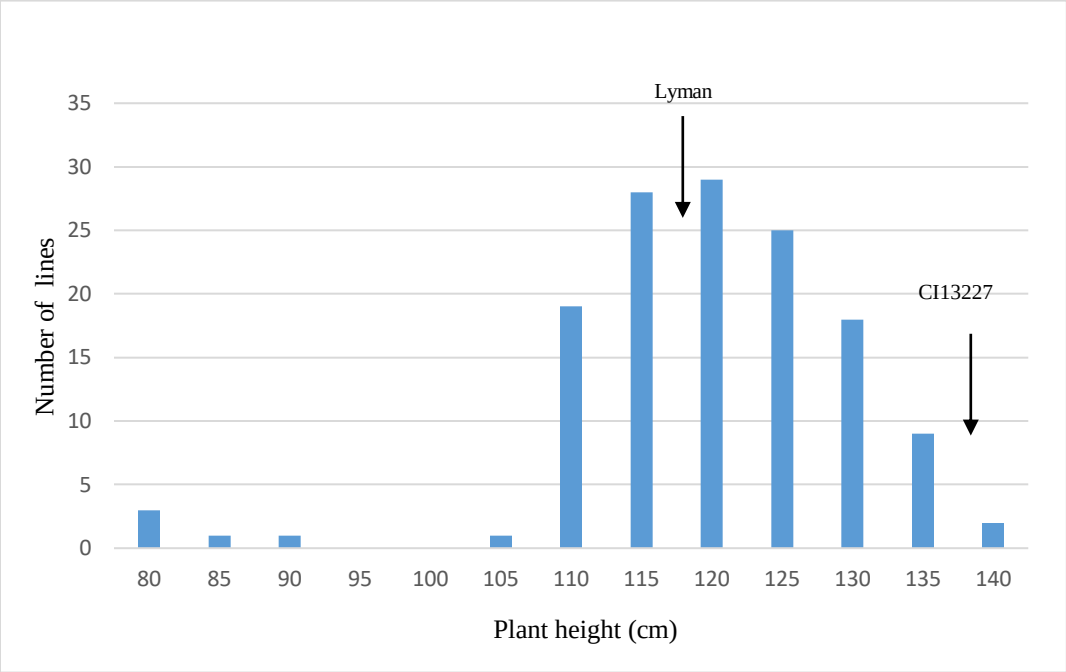


Figure 4.5 Correlation between percentage of symptomatic spikelets (PSS) in a spike and DON in the recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' evaluated in the 2016 field experiment

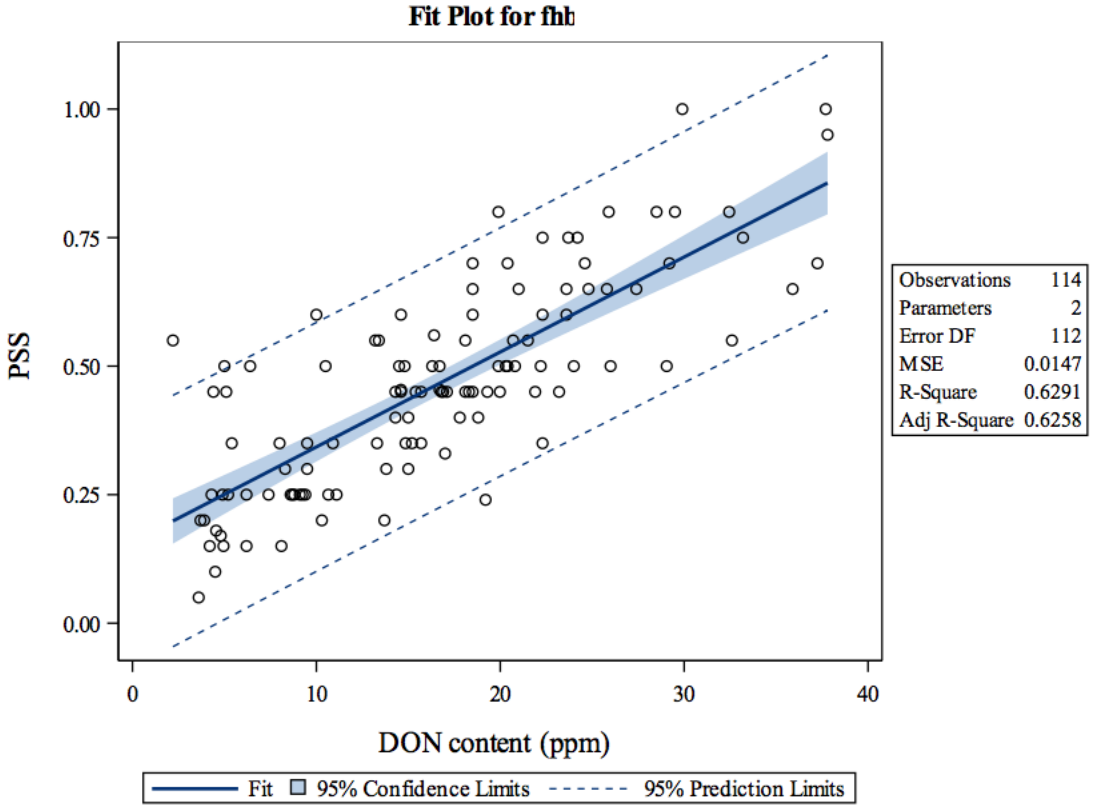


Figure 4.6 Correlation between percentage of symptomatic spikelets (PSS) in a spike and *Fusarium* damaged kernels (FDK) in the recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' evaluated in the 2017 filed experiment

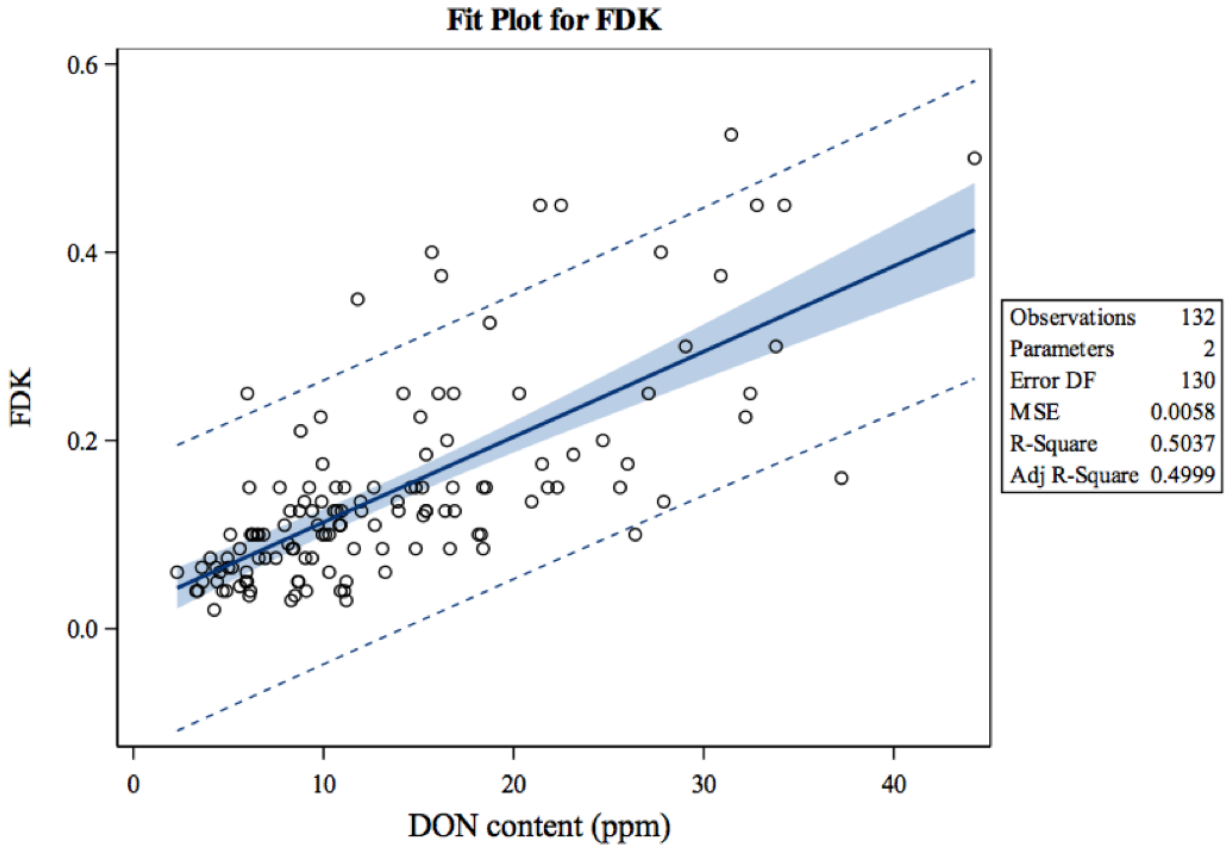


Figure 4.7 Genome-wide distribution of genotype- by- sequencing GBS-SNPs identified in the RIL population derived from recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227'

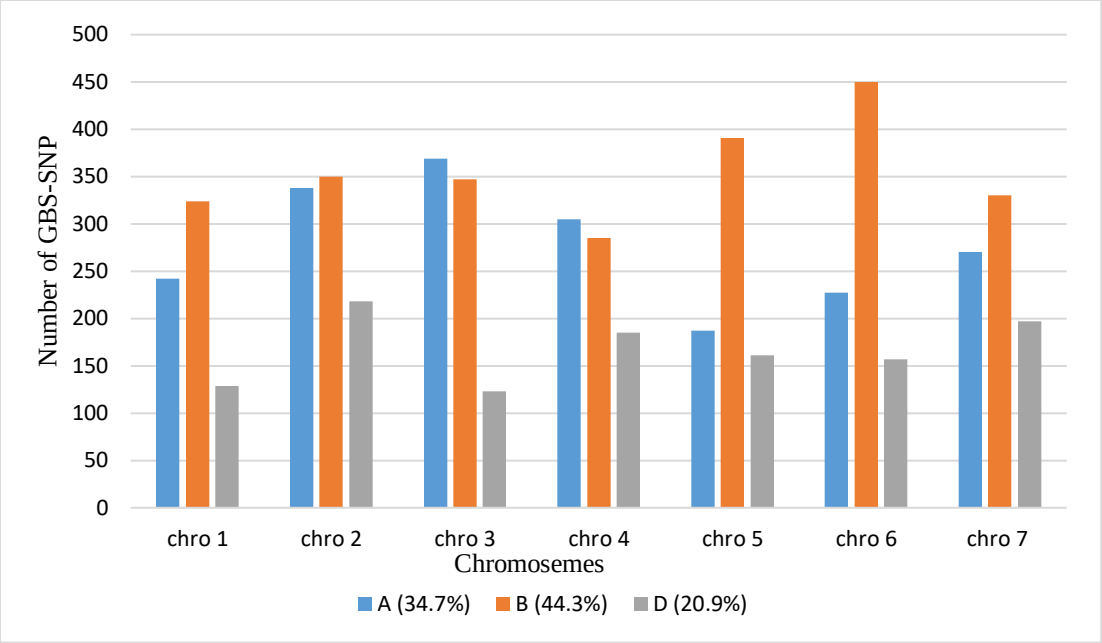
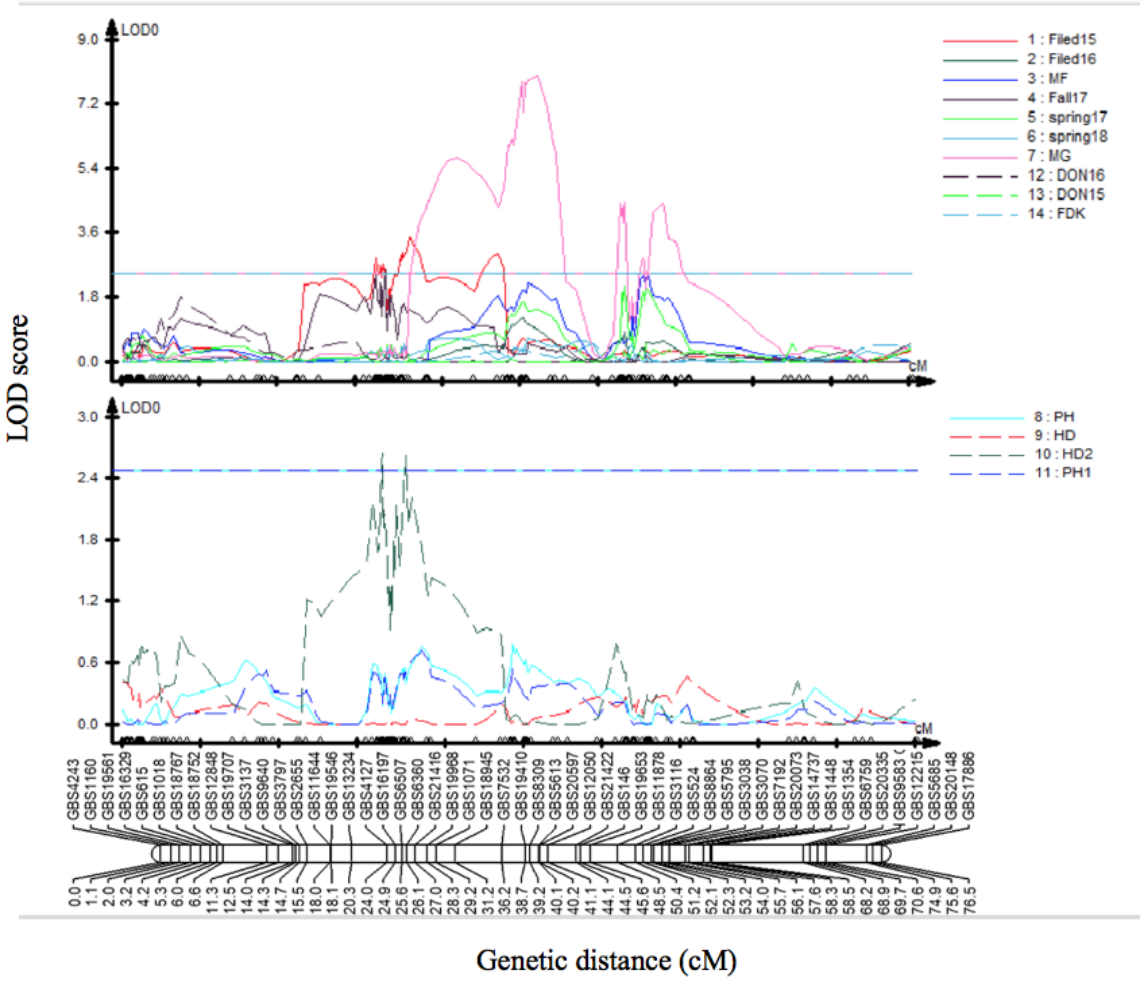
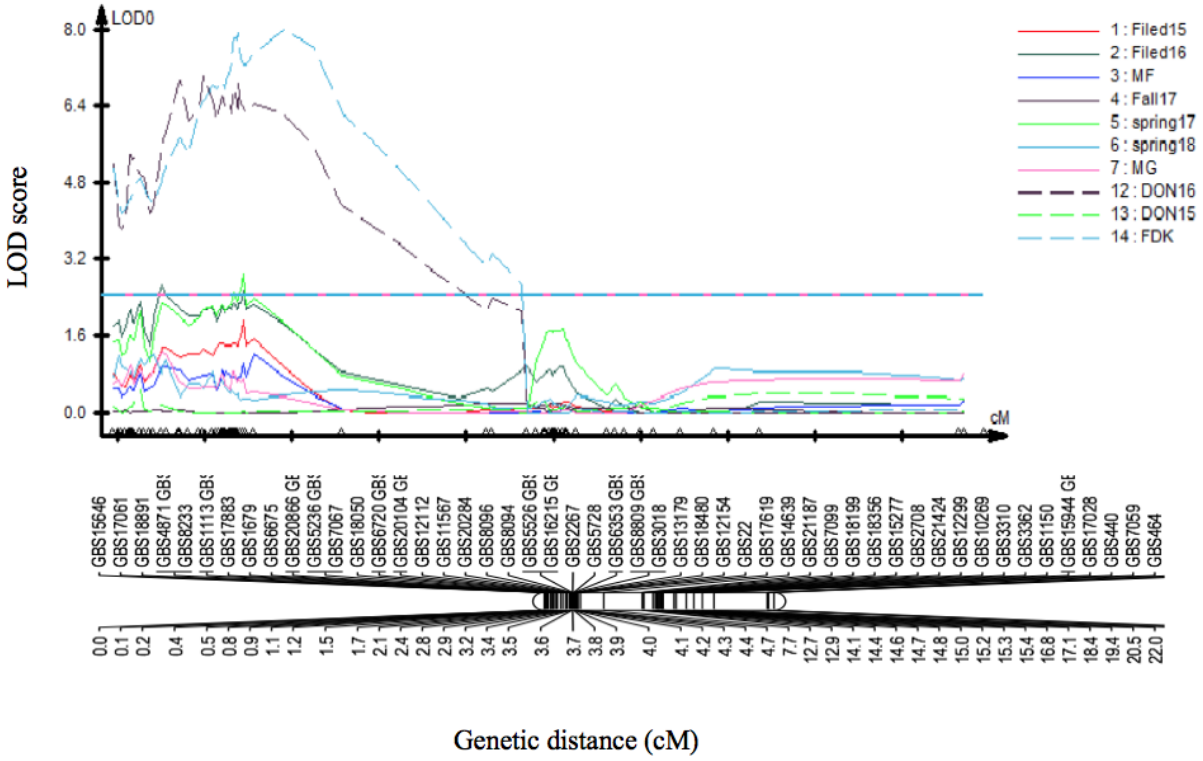


Figure 4.8 Composite interval mapping of quantitative trait loci (QTLs) for FHB type II resistance in a recombinant inbred line (RIL) population derived from 'Lyman' × 'CI13227' phenotyped in four greenhouse experiments (2016 fall, 2017 Spring, 2017 Fall, and 2018 Spring) using percentage of symptomatic spikelets (PSS), and *Fusarium* damaged kernel (FDK)

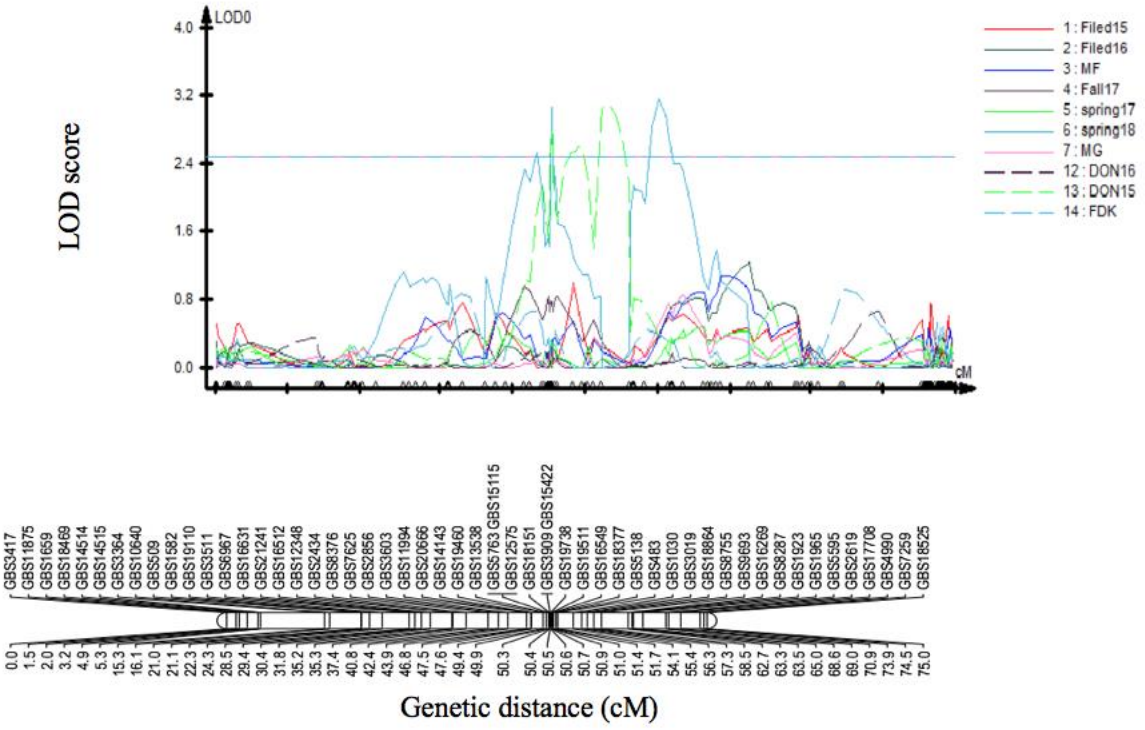
A. 2B



B. 1A


LOD0
LOD score
8.0
6.4
4.8
3.2
1.6
0.0
cM
1 : Filed15
2 : Filed16
3 : MF
4 : Fall17
5 : spring17
6 : spring18
7 : MG
12 : DON16
13 : DON15
14 : FDK
Genetic distance (cM)

C. 7A


LOD score
LOD0
4.0
3.2
2.4
1.6
0.8
0.0
cM
1 : Filed15
2 : Filed16
3 : MF
4 : Fall17
5 : spring17
6 : spring18
7 : MG
12 : DON16
13 : DON15
14 : FDK
GBS3417 GBS11875 GBS1659 GBS18469 GBS14514 GBS14515 GBS3364 GBS10640 GBS509 GBS1582 GBS19110 GBS3511 GBS8967 GBS16631 GBS21241 GBS16512 GBS12348 GBS2434 GBS8376 GBS7625 GBS2856 GBS3603 GBS11994 GBS20666 GBS14143 GBS19460 GBS13538 GBS5763 GBS15115 GBS12575 GBS18151 GBS3909 GBS15422 GBS19738 GBS19511 GBS16549 GBS18377 GBS5138 GBS483 GBS1030 GBS3019 GBS18864 GBS8755 GBS9693 GBS16269 GBS8287 GBS1923 GBS1965 GBS5595 GBS2619 GBS17708 GBS4990 GBS7259 GBS18525
0.0 1.5 2.0 3.2 4.9 5.3 15.3 16.1 21.0 21.1 22.3 24.3 28.5 29.4 30.4 31.8 35.2 35.3 37.4 40.8 42.4 43.9 46.8 47.5 47.6 49.4 49.9 50.3 50.4 50.5 50.6 50.7 50.9 51.0 51.4 51.7 54.1 55.4 56.3 57.3 58.5 62.7 63.3 63.5 65.0 68.6 69.0 70.9 73.9 74.5 75.0
Genetic distance (cM)

D. 3A

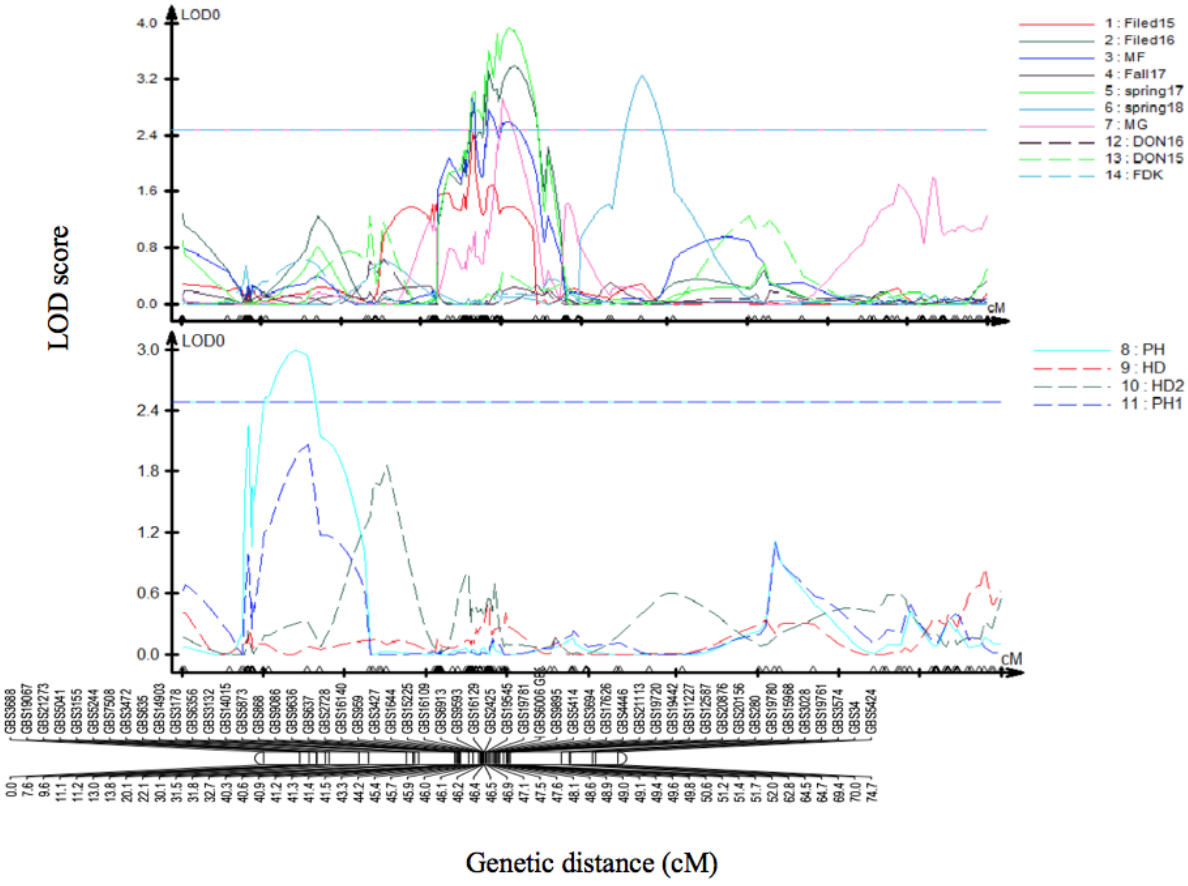
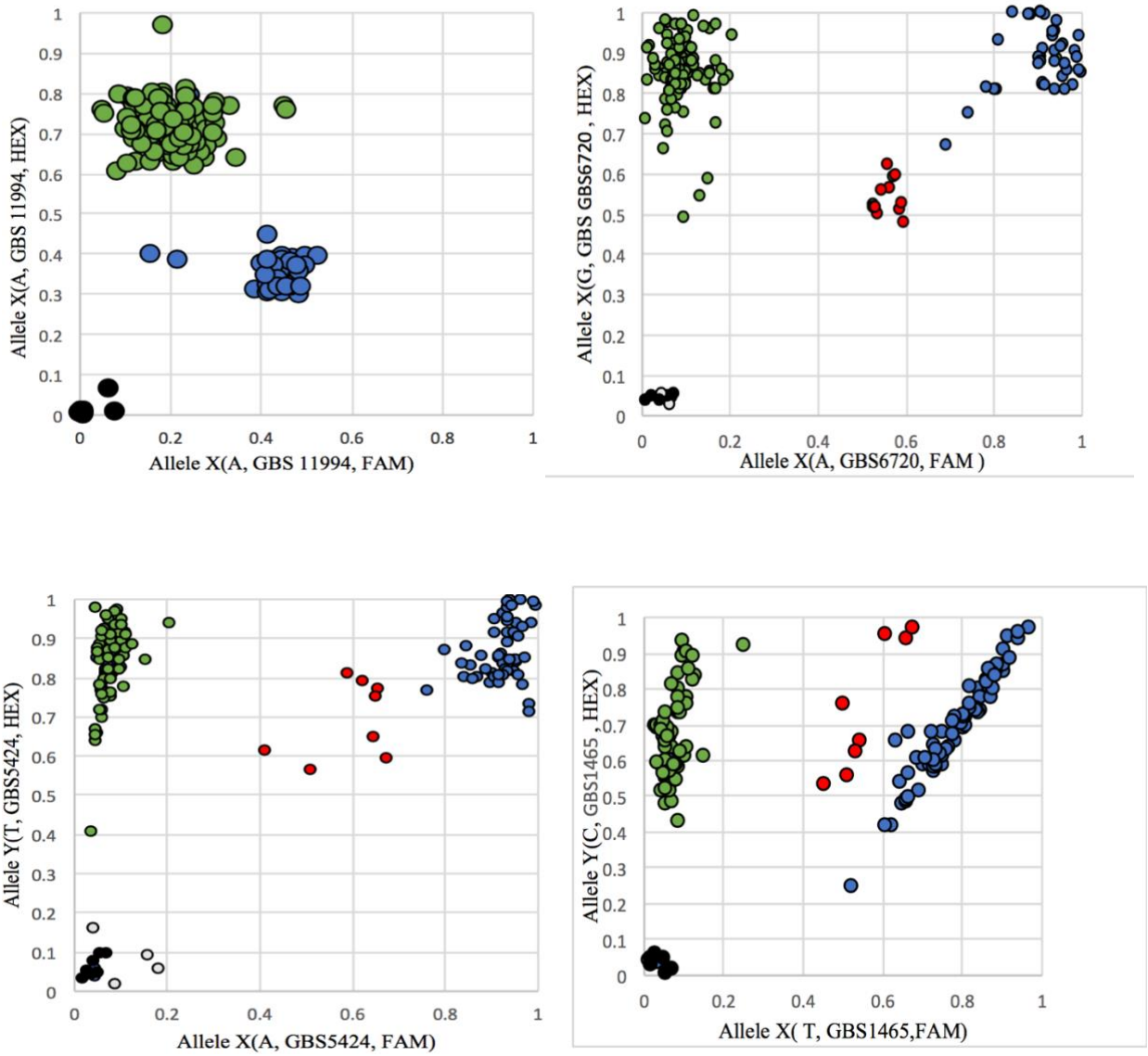
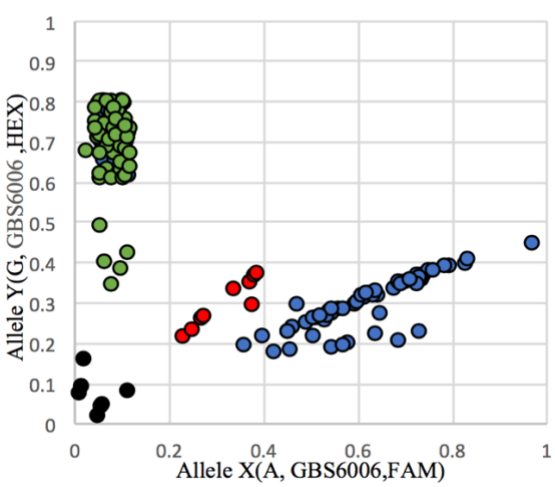

LOD0
4.0
3.2
2.4
1.6
0.8
0.0
cM
1 : Filed15
2 : Filed16
3 : MF
4 : Fall17
5 : spring17
6 : spring18
7 : MG
12 : DON16
13 : DON15
14 : FDK
LOD0
3.0
2.4
1.8
1.2
0.6
0.0
cM
8 : PH
9 : HD
10 : HD2
11 : PH1
LOD score
Genetic distance (cM)

Figure 4.9 KASP assay profile to show allele segregation of SNPs in the recombinant inbred line (RIL) population of 'Lyman' x 'CI13227'. The blue and green dots show different alleles; the red and black dots show the heterozygous and water control or samples with failed PCR




Allele Y(G, GBS6006 ,HEX)
Allele X(A, GBS6006,FAM)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

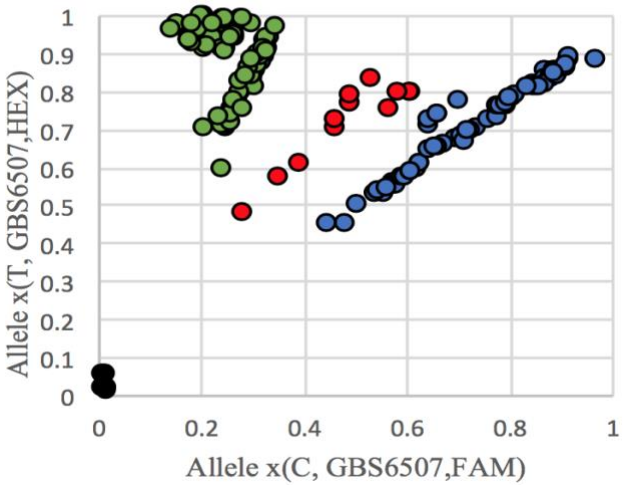

Allele x(T, GBS6507,HEX)
Allele x(C, GBS6507,FAM)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

Table 4.1 Analysis of variance (ANOVA) and broad-sense heritability (H^2) for percentage of symptomatic spikelets (PSS) in a spike from recombinant inbred line (RIL) population 'Lyman' x 'CI13227' in the three greenhouse experiments conducted in fall 2017, spring 2017, and spring 2018

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	2	13802.20	6901.10	3625. 38	<.0001
Replication (Experiment)	3	17.76	5.92	3.11	0.026
genotype	136	329626. 22	2423.72	1273.26	<.0001
Genotype *Experiment	272	134798.02	495.58	260.35	<.0001
Error	409	774.74	1.90		
Corrected Total	820	480024.28			
H^2		0. 80			

Table 4.2 Correlations among percentage of symptomatic spikelets in a spike (PSS), heading date (HD), and plant height (PH) in the recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' in the three greenhouses experiments conducted in fall 2017, spring 2017, and spring 2018

Trait	GPSS-Fall2017	GPSS- spring2017	GPSS- spring2018	HD
GPSS-Fall2017	-			
GPSS- Spring2017	0.289***	-		
GPSS- Spring2018	0.1917	0.53611***	-	
HD	-0.0531	-0.2041**	-0.1505	-
PH	-0.1995	-0.0497**	0.0203	-0.7169

PH= Plant height, HD=heading date, GPSS= percentage of symptomatic spikelets in a spike from the greenhouses

*$P \leq 0.05$**$P \leq 0.01$*** $P \leq 0.001$

Table 4.3 Analysis of variance (ANOVA) and broad-sense heritability (H^2) for percentage of symptomatic spikelets (PSS) in a spike from recombinant inbred line (RIL) population 'Lyman' x 'CI13227' in two field experiments conducted in 2015-2016

Source	DF	Type III SS	Mean Square	*F- Value*	*Pr > F*
Experiment	1	8651.528	8651.28	3518. 97	<.0001
Replication (Experiment)	2	1.03	0.51	0. 21	0. 81
genotype	135	222716.98	1649.75	672.03	<.0001
Genotype *Experiment	135	41297.60	305.90	124.43	<.0001
Error	271	663. 80	2.45		
Corrected Total	543	273330.95			
H^2		0.79			

Table 4.4 Correlations among percentage of symptomatic spikelets in a spike (PSS), *Fusarium* damaged kernels (FDK), heading date (HD), plant height (PH) in (cm) and deoxynivalenol (DON) content (ppm) in the recombinant inbred line (RIL) population derived from 'Lyman' x 'CI13227' from the two field experiments conducted in 2015- 2016

Trait	FPSS-2015	FPSS-2016	FDK	HD	DON
FPSS-2015	-				
FPSS-2016	0.72***	-			
FDK	0.49***	0.50159***	-		
HD	-0.16***	-0.172**	-0.116		
PH	-0.097	-0.0577	-0.1383	-	
DON	0.79***	0.72***	0.60***	-0.017	-

PH= Plant height, HD=heading date, FPSS= percentage of symptomatic spikelets in the fields

FDK= *Fusarium* damaged kernels, DON=deoxynivalenol

*$P \leq 0.05$**$P \leq 0.01$*** $P \leq 0.001$

Table 4.5 Chromosomal locations, marker intervals, determination coefficients (R^2), additive effect, logarithm of the odds (LOD) values and possible physical locations for significant quantitative trait loci (QTLs) identified in this study

Trait	Experiment	QTLs	Position(CM)	LOD	PVE (R2) [a]	Add. [b]	Marker interval		Physical location		Contributed by
PSS	Field 2016	*QFhb.hwwgru.1A*	1.71	3.19	6.4	6.05	*GBS17061*	*GBS12112*	311808357	400956976	CI13227
PSS	Spring 2017	*QFhb.hwwgru.1A*	1.67	2.98	7.2	6.15	*GBS17061*	*GBS12112*	311808357	400956976	CI13227
DON	Field 2016	*QDON.hwwgru.1A*	2.31	2.6	18.54	3.77	*GBS17061*	*GBS12112*	311808357	400956976	CI13227
FDK	Field 2016	*Q.FDK.hwwgru.1A*	5.91	8.4	22.21	5.225	*GBS17061*	*GBS12112*	311808357	400956976	CI13227
PSS	Field 2015	*QFhb.hwwgru.2B*	29.61	3.6	8.42	-6.53	*GBS11644*	*GBS18945*	62146196	70941951	Lyman
Head date	Field 2015	*QHD.hwwgru.2B*	29.01	2.49	12.13	6.66	*GBS11644*	*GBS18945*	62146196	70941951	CI13227
	Fall 2017	*QFhb.hwwgru.2B*	27.11	2.72	9.96	-6.66	*GBS11644*	*GBS6312*	62146196	70941951	Lyman
Mean	All greenhouses	*QFhb.hwwgru.2B*	42.76	8.03	18.99	-9.93	*GBS6507*	*GBS15959*	67796407	71776095	Lyman
PSS	Field 2016	*QFhb.hwwgru.3A.1*	54.01	3.4	9.43	-7.42	*GBS19781*	*GBS280*	47721720	57161118	Lyman
PSS	Spring 2017	*QFhb.hwwgru.3A.1*	53.01	4.83	10.18	-7.96	*GBS19781*	*GBS280*	47721720	57161118	Lyman
Mean	All greenhouses	*QFhb.hwwgru.3A.*	49.85	2.79	13.79	-5.22	*GBS19781*	*GBS280*	47721720	57161118	Lyman
PSS	Field mean	*QFhb.hwwgru.3A.*	52.01	2.94	11.94	-5.14	*GBS19781*	*GBS280*	47721720	57161118	Lyman
PSS	Spring 2018	*QFhb.hwwgru.3A.2*	74.71	3.27	10.72	9.33	*GBS3574*	*GBS5424*	68351925	68690425	Lyman
PSS	Spring 2018	*QFhb.hwwgru.7A.*	50.91	3.2	12.31	9.25	*GBS11994*	*GBS4990*	602098512	842473363	CI13227
DON	Field 2015	*QDON.hwwgru.7A.*	60.2	3.2	19.44	9.84	*GBS11994*	*GBS4990*	602098512	842473363	CI13227

[a] PVE: phenotypic variation explained
[b] Add: additive effect.

Table 4.6 Primer and sequence list of Kompetitive allele specific PCR (KASP) assays developed from GBS-SNPs

SNP name	Sequence	Position	Dye	Primers with a tail
GBS6507-2B	TGCAGGCAAACATGAATGCATGA[C/T]CCAGATTC TAGATGCACCCTTTAGTACAATAATCTCAGGC	24	FAM	GAAGGTGACCAAGTTCATGCTGATCCAGATTCTAGATGCACC
			HEX	GAAGGTCGGAGTCAACGGATTGATCCAGATTCTA
			R	CACTTTTCAGTATAGCCTGAGATT
GBS6720-1A	TGCAGGATCTCAGCGACGAATGTGTGACGATGGA CACACGTAGGGTA[A/G]TGACATTGTCTCGCGC	48	FAM	GAAGGTGACCAAGTTCATGCTGATGGACACACGTAGGGTAA
			HEX	GAAGGTCGGAGTCAACGGATTGATGGACACACGTAGGGTAG
			R	ACCAACATATCGACCTTGC
GBS5424-3A	TGCAGGCTTTTCTCGCATCCTCAGCTAGATGATGC AACTTTCTATAAAAA[A/T]TTGTTTTGTACA	51	FAM	GAAGGTGACCAAGTTCATGCTGATGATGCAACTTTCTATAAA AAA
			HEX	GAAGGTCGGAGTCAACGGATTGATGATGCAACTTTCTATAAA AAT
			R	TTAACACAGGGAAACATTCG
GBS6006-3A	TGCAGGCCTCCCTCTTCATCTGGTCGGGCCAG[G/A] CCCAAAGTGAGCAGCTCCCACCAGCCCAGTT	34	FAM	GAAGGTGACCAAGTTCATGCTGGGAGCTGCTCACTTTGGG
			HEX	GAAGGTCGGAGTCAACGGATTGGGAGCTGCTCACTTTGGA
			R	CCTCCCTCTTCATCTGGTC
GBS1965-7A	TGCAGTCGCTGCCC[C/T]GATGCAGCCGTAAGGAG ACGCCAGACTCGCTGATCGGAAGGAGCGGCGA	15	FAM	GAAGGTGACCAAGTTCATGCTCTCCTTACGGCTGCATCG
			HEX	GAAGGTCGGAGTCAACGGATTCTCCTTACGGCTGCATCA
			R	CACCCCACACACAGTACCT
GBS11994-7A	TGCAGCGATGGAGACTTGTGCGGCGGGAGGGCGA CGACA[A/G]CAAGAATTCCAGCCGTATCTGATG	40	FAM	GAAGGTGACCAAGTTCATGCTTACGGCTGGAATTCTTGT
			HEX	GAAGGTCGGAGTCAACGGATTTACGGCTGGAATTCTTGC
			R	CAGCGATGGAGACTTGTG