META-ANALYSIS OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEAT LANDRACES USING GENOTYPING BY SEQUENCING

by

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B.S., Xuzhou Normal University, 2008
M.S., Kansas State University, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Abstract

Fusarium head blight (FHB) is a devastating fungal disease in wheat, reducing not only grain yield but also quality. The pathogen produces the mycotoxin deoxynivalenol (DON) that induces severe toxicological problems in human and animals. Using host resistance has been the most efficient way to control the disease. To identify quantitative trait loci (QTLs) for FHB resistance in Chinese landrace Haiyanzhong (HYZ), a recombinant inbred lines (RILs) population derived from a cross between HYZ and Wheaton was developed. The RILs were evaluated for percentage of symptomatic spikelets (PSS) in three greenhouse experiments, and genotyped using simple sequence repeats (SSRs) and single nucleotide polymorphism (SNPs) developed from genotyping-by-sequencing (GBS). Eight QTLs were identified for type II (PSS) resistance on chromosomes 5A, 6B, 7D, 2B (2), 3B, 4B, and 4D, with 5A as the major QTL. Ten SNPs closely linked to 5A, 6B, and 2B QTLs were successfully converted to Kompetitave allelic specific PCR (KASP) assays.

To identify common QTLs across different populations, we constructed high-density GBS-SNP maps in an additional four RIL populations derived from the Chinese landraces, Wangshuibai (WSB), Baishanyuehuang (BSYH), Huangfangzhu (HFZ), and Huangchandou (HCD) and conducted meta-analysis of the QTLs for FHB resistance using a consensus map developed from the five populations. We identified six MQTLs on chromosomes 3BS (2), 3A, 3D, 2D, and 4D and 23 tightly linked GBS-SNPs to the MQTLs. These GBS-SNPs were successfully converted to KASPs. The KASPs linked to MQTLs can be used for pyramiding these QTL in breeding programs.

To quickly reduce FHB damage in U.S. hard winter wheat (HWW), we transferred *Fhb1*, a major QTL with stable effects on FHB resistance, from Ning7840 into three adapted HWW

cultivars Overland, Jagger, and Overley, by marker-assisted backcross (MAB), and assessed the effect of *Fhb1* on FHB resistance in these different backgrounds. The results showed that *Fhb1* can significantly lower FHB severity, *Fusarium*-damaged kernel (FDK), and DON accumulation in the all the three HWW backgrounds. Some of the selected lines showed high levels of FHB resistance, but agronomically similar traits as recurrent parents, can be used as resistant parents to improve HWW FHB resistance.

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Acknowledgements

I would like to express my deepest appreciations to my major advisor, Dr. Guihua Bai for accepting me in his lab and giving me the opportunity to improve myself intellectually, academically, and spiritually. He always gave me advises, supports, and encouragements when I have troubles with my research projects. I am impressed by his passion and hard work as a scientist. Most importantly, I'm very grateful to him for providing me a warm and family-like environment while working. I am grateful to my co-advisor, Dr. Guorong Zhang, and the members of my advisory committee, Dr. Allan K Fritz, Dr. Jesse Poland, and Dr. Geoffrey Morris, who spent their precious time to serve on my supervisory committee and provided great guidance and assistance throughout my graduate program. I also would like to thank Dr. Michael Veeman for serving as my outside chair of my final examination committee.

I would like to give special thanks to Ms. Shuangye Wu for teaching me the methods of GBS library construction, Dr. Shan Wang and Mr. Hadi Ali Pour for helping me with GBS data analysis, and Ms. Yun Wu for the help given in QTL Meta-analysis statistical software. Many thanks to my lab members, Dr. Paul St. Amand, Dr. Amy Bernardo, Dr. Zhengqi Su, Dr. Jixin Zhao, Dr. Dongtao Liu, Ms. Nosheen Fatima, Ms. Meng Lin, Ms. Yue Lu, Mr. Mingqin Shao, Mr. Abdulrahman Hashimi, and Mr. John Curato for their help and friendship.

Last but not least, I would like to thank my family for all their support, love and encouragement. My parents gave me their unconditional love and care, without whom I would not make it this far. I would like to give thanks to my husband Jie Ruan for his love, care, and support, and my little son Derek Ruan for giving my happiness and sense of responsibility.

Chapter 1 - Literature Review

Wheat Crop

Origin and agronomic importance of wheat

The first cultivation of wheat can be traced back to southwest Asia about 10,500 years ago (Shewry 2009). The two most important commercial wheat types are durum wheat (*Triticum durum L.* 2n = 4x = 28) and bread wheat (*Triticum aestivum L.* 2n = 6x = 42). Bread wheat is an allohexaploid species with three genomes, A, B, and D. Each of the three genomes has seven chromosomes, which makes the total chromosome number of 42 (2n = 6x = 42). The three genomes in hexaploid wheat derived from three ancestral diploid progenitors (Martínez-Pérez et al. 1999). The A genome was clearly from the A genome of *Triticum urartu* (einkorn wheat) (Shewry 2009), while the D genome is clearly derived from *Aegilops tauschii*. There is not too much divergence between the D genomes present in the hexaploid and diploid species (Petersen et al. 2006). However, the origin of B genome in hexaploid wheat is not clearly defined, and it was probably derived from the S genome in the *Sitopsis* section of *Aegilops*, with *Ae. speltoides* being the closest species (Ceoloni and Feldman 1987). Hybridization between A and B genomes created the species *Triticum turgidum* about 580-820 thousand years ago. Hexaploid wheat arose from the hybridization between a domesticated form of tetraploid, wild emmer wheat (*Triticum turgidum* spp. *dicoccoides*) and the wild wheat species *Aegilops tauschii* about 7,000~12,000 years ago (Salse et al. 2008; Marcussen et al. 2014; Petersen et al. 2006). Wheat species are disomic in inheritance, because the chromosome pairing is genome specific. The specific chromosome pairing is controlled by paring suppressor genes *ph1*, *ph2* with other minor genes (Ceoloi et al. 1986; Martínez-Pérez et al. 1999).

This character allows hybridization fertility within and between species, providing the opportunity to achieve higher diversity (Wulff and Moscou 2014).

Wheat plays an important role in the world's food supply. It is grown in more than 70 countries and is the most widely grown crop worldwide (Dixon 2007). The production of wheat is the third most-produced cereal after maize and rice. In 2013, the worldwide wheat production was 713 million tons, and the U.S. production.during the same year was 58 million tons (FAOSTAT 2015.Verified August 2015 in http://faostat3.fao.org/browse/Q/*/E). Bread wheat is adapted to a wide range of temperate environments, due to its sufficient genetic diversity (Ceoloni and Feldman 1987). The optimum wheat growing temperature is about 25^{o}C, ranging from 3^{o}C to 32^{o}C (Briggle 1980). Wheat is also adapted to a broad range of moisture conditions, with precipitation ranging from 250 to 1750 mm (Leonard and Martin 1963). Its high productivity across diverse environments has permitted wheat to be the widely grown crops in the world (Shewry 2009). Wheat grain is a staple food used to make bread and a wide range of baked products including cakes, biscuits, pasta, noodles and so on (Shewry 2009).

Wheat growing regions and market classes in the United States

The two most important commercial wheat types are common wheat (*Triticum aestivum* L. 2*n* = 6*x* = 42), and durum wheat (*Triticum durum* L. 2*n* = 4*x* = 28). Based on its growth habits, wheat can be divided into three classes: winter wheat, facultative wheat, and spring wheat (Baenziger et al. 2009). Winter wheat is primarily sown in the fall, requiring vernalization to flower, and tolerant of freezing temperatures. Facultative wheat needs a shorter length of vernalization, can acting as either spring or winter wheat,

depending on the time of sowing. Spring wheat, mainly sown in spring and summer months, does not require vernalization to flower, and cannot withstand even a moderate period of freezing temperatures (Baenziger et al. 2009).

Wheat grown in the U.S. can be divided into six classes based on grain color and hardness, and their planting seasons. They are 1) hard red winter (HRW), 2) hard red spring (HRS), 3), soft red winter (SRW), 4) durum, 5) hard white, and 6) soft white wheat. The hard wheat, with the highest level of gluten among all wheat classes, is mainly used for making bread and rolls, while the soft wheat is mainly used for making flat bread, cakes, and muffins (Baenziger et al. 2009). Hard red winter (HRW) wheat is an extremely versatile class with excellent milling and baking characteristics for hard backed food, such as pan bread. HRW accounts for more than 40% of the U.S. wheat production, and is grown primarily in the Great Plains (Kansas, Oklahoma, Nebraska, Texas, Colorado, South Dakota and Montana); Hard red spring (HRS) wheat has the highest protein content, and accounts for about 20% of production primarily grown in Northern Plains (North Dakota, Montana, Minnesota, and South Dakota); Soft red winter (SRW) wheat is high yielding wheat with low protein and weak gluten content, which is excellent for cookies, crackers, and pie crust. SRW accounts for 15-20% of total production, and grown primarily in these states along the Mississippi River and the eastern state (Ohio, Missouri, Indiana, Illinois, and Pennsylvania); Soft white wheat with light-colored grain and low protein content accounts for 10-15% of total production, and is grown in Washington, Oregon, Idaho, Michigan, and New York states; Hard white wheat is the newest market wheat class in U.S, can be used in making pan bread, and especially noodles. The hard white wheat are mainly grown in Kansas and Colorado;

Durum wheat has very hard grain texture and high protein content (especially gluten protein) that is good for making pasta, and accounts for 3-5% of total production, primarily in North Dakota, Montana, and South Dakota, (http://www.ers.usda.gov/topics/crops/wheat/background.aspx#classes (Rohrich 2014)).

Factors affect wheat production yield

Wheat growing area, production and yield levels in the U.S. have remained stable during the past decades. In 2014, wheat acreages planted in Kansas were about 9,600,000 and harvested about 8,000,000 with a total production of 246.4 million bushels (6.7 million tons) and a yield of 28 bushels (762 kg) per acre (USDA 2014). The harvested acreages were lower than the previous years, mainly because of the extremely low temperature and precipitation. Environmental stresses such as drought, salinity, heat, and cold are common in the wheat growing regions that may cause a great reduction in wheat production and yield. According to "Kansas Wheat History", Kansas wheat suffered from extreme weather (low rainfall during germination, freezing temperature in mid-February, extremely dry in April) in 1989, which led to the lowest production of 213 million bushels (~5.8 million tons) since 1963 (USDA 2014). Other abiotic stresses such as aluminum toxicity and lengthy, wet harvesting seasons that cause sprouting in the wheat head also cause a great reduction in yield and yield. Diseases can also cause major crop losses. According to the Kansas Cooperative Plant Disease Survey, the cumulative wheat disease losses estimated for the 2013 wheat crop were 6.2% or 21.7 million bushels (~0.59 million tons) (Appel et al. 2013). Wheat diseases are mainly caused by fungi and viruses, with a few by bacteria. In Kansas, the important diseases to wheat production were *Septoria* leaf disease, wheat streak mosaic, tan spot, barley yellow dwarf, leaf rust,

Fusarium head blight (FHB), powdery mildew and bunt (Appel et al. 2013; Petersen et al. 2006).

Fusarium head blight in wheat

Impact of Fusarium head blight

Fusarium head blight (FHB), also called scab, is mainly caused by *Fusarium graminearum* Schw. It occurs mostly in cereal crops, such as wheat, barley (Bai and Shanner, 2004). FHB can cause reductions not only in grain yield but also in grain quality, especially when warm and humid weather from anthesis to early kernel filling stages (Bai and Shaner 1994). Kernels infected by FHB are mostly partially filled and are weighted much ligher than normal seeds (Bai et al. 2001). Thus, the infected kernels are very easy to be blown out during threshing, which can cause a severe reduction in grain yield. The *Fusarium*-damaged kernels (FDK) are also contaminated with mycotoxins, especially deoxynivalenol (DON), which is not suitable for human and animal consumption (De Wolf et al. 2003). For animal consumption, DON concentration of 1 ppm can cause a significant feed-intake reduction and weight losses, and 10 ppm can cause vomiting and feed refusal (Shephard 2008; Vincelli and Parker 2008). For human being, the allowable DON levels in wheat varied from 0.5 ppm to 2 ppm depending on different countries (Bai and Shanner, 2004). Exceeding the regulated minimum limit would cause the wheat grains rejection or value discount at grain intake point (Cowger et al. 2009). FHB has great impacts on grain value in feeding, processing, marketing, and exporting (McMullen et al. 1997).

FHB was firstly described in 1884 in England and was considered a major threat to wheat during the early 20th century (Goswami and Kistler 2004). Until now, the

epidemics of FHB have been reported from different regions worldwide including Asia, Europe, North America and South America (Bai and Shaner 1994; Goswami and Kistler 2004). In China, FHB has caused wheat yield losses more than 1 million tons (~35.7 million bushels) on more than 7 million hectares of the field in the 1990s (Bai and Shaner 2004). In the U.S., severe epidemics of FHB in Indiana and Ohio were recorded by J.C. Arthur in 1891 (Arthur 1891). In recent years, epidemics of the disease have occurred in many wheat-producing states such as North Dakota, Minnesota, South Dakota, Ohio, Indiana, Michigan, Missouri, Kansas, and Arkansas (De Wolf et al. 2003; McMullen et al. 1997). The disease has induced yield and quality losses to farmers in at least 18 states. Johnson et al. (1998) estimated that the direct losses in wheat and barley caused by FHB totaled about $1.3 billion during the period from 1991 to 1997 in the U.S., and the commulative economic losses during the peirod were about three times of the amount (Wegulo, 2012). In 1993, FHB struck the U.S., especially Minnesota, North Dakota, and South Dakota. The averaged wheat yields dropped 45% from an average of 49 bu/harvested acre in 1992 to 26.4 bu/acre in 1993 (McMullen et al. 1997). From 1993 to 2001 in the northern and central Great Plains, the direct economic losses attributable to FHB in wheat and barley were $2.5 billion with $1.07 billion from 1998 to 2001 (Nganje et al. 2004). In 2007 and 2008, serious FHB outbreaks occurred in parts of Nebraska and Kansas. In Kansas, FHB losses were estimated at 17.6, 15.8, and 8.75% for the northeast, east-central, and southeast districts, respectively, with the statewide losses estimated at 7.1 million bushels valued at $57 million in 2008 (McMullen et al. 2012).

Causal agents, infection pathways, and symptoms of FHB

F. graminearum is a homothallic fungus and is the most predominant FHB causal species in most of cereal growing area of the world (Bai and Shaner 1994; Xu and Nicholson 2009). At least 17 different *Fusarium* species including *F. culmorum*, *F. graminearum, Microdochium nivale, M. majus*, *F. avenaceum,* and *F. poae* have been associated with FHB in wheat or other small grains (Parry et al. 1995; Trail 2009; Xu and Nicholson 2009). *F. graminearum* has different isolates that may differ in pathogenicity (Bai and Shaner 1994). But, these isolates are not race-specific (Bai and Shaner 1994). Wheat cultivars resistant to *F. graminearum* are also strain or isolate non-specific. Therefore, FHB inoculation with a mixture of *F. graminearum* isolates is regularly used, that can be considered as a more efficient way compared to the inoculation with single isolates and were repeatable in different years and locations (Bai 1996; Zhou et al. 2002; ŠÍP et al. 2011).

At early anthesis stage, anther may be the first floral part to be infected (Ribichich et al. 2000b). After 6-12 h, conidia begin to germinate and then germ tubes produce hyphae that can grow and extend to the interior surface of florets and form dense mycelium networks (Xu and Nicholson 2009). The disease may then spread from anther to palea, lemma, and rachis (Schmale III and Bergstrom 2003 Updated 2010). The pathogen may also directly enter the host tissue through stomata, and then hyphae also grow through the interior surface of the lemma, glume, and palea (Xu and Nicholson 2009). The infection levels of *F. graminearum* have no significant differences throughout the whole floral parts (Argyris et al. 2005). Once the conidia reach rachilla and rachis, the disease may spread upward and downward the spike through vascula bundles and cortical

parenchyma tissues (Goswami and Kistler 2004; Xu and Nicholson 2009). The senescence premature spikes and shriveled seeds were produced due to that mycelium clog the vascular bundle tissue in the rachis and rachilla, and thus block the supply of water and nutrition (Xu and Nicholson 2009). Another reason might be that the pathogen secretes cell wall degradation enzyme that can degrade the host cells (Xu and Nicholson 2009). Overall, the pathogen hyphae may spread horizontally by invading anthers or bracts of adjacent florets within the infected spikelets, and then move to the neighbor spikelets through the rachis and rachilla; or spread vertically through vascular bundles and parenchyma to spikelets above or below the infected spikelets (Ribichich et al. 2000b). Besides, the pathogen can also produce mycotoxin, especially deoxynivalenol (DON) within 36 h after initial infection. Similar to the disease symptom, DON spreads upward and downward to neighbor spikelet through xylem vessels and phloem sieve tubes (Kang and Buchenauer 2002). Thus, DON contamination within a spike is unavoidable, especially when given favorable weather condition and enough time (Xu and Nicholson 2009). Secondary infection from spike to neighbor spike may also happen, however, it is very rare (Wise and Woloshuk 2010).

FHB symptoms are confined to wheat spikes. The most obvious symptoms are brown or dark brown necrotic lesions formed on the surface of glumes (Goswami and Kistler 2004), and bleaching of some of the spikelets, while the healthy spikelets are still green. The infected kernels that appear shriveled, discolored and light weighted are commonly called “tombstone” (Wise and Woloshuk 2010). FHB symptoms are different between resistant and susceptible germplasms. In highly resistant plants, dark brown discoloration limit to an inoculated or infected spikelets, sometimes, only a dark brown

spot showed on the lemma of the infected spikelet (Bai and Shaner 1994). In moderately resistant and moderately susceptible plants, the symptoms may also spread to neighboring spikelets about two weeks after initial infection, and many other spikelets in the spike remain uninfected. However, in highly susceptible plants, the whole spike can be blighted as bleach discoloration or dark brown on the spikelets, rachis and rachilla in 7-10 d after initial infection (Ribichich et al. 2000b). Therefore, susceptible plants show much higher disease severity than resistant plants.

Life cycle of *F. graminearum*

F. graminearum belongs to ascomycete with both sexual and asexual stages (Bai and Shaner 1994). The asexual stage of the fungus produces spores called macroconidia; while the sexual stage (*Gibberella zeae*) produces ascospores. Sexual stage is a critical part of the life cycle (Trail 2009). *F. graminearum* overwinters as binucleate hyphae on the infested residue of cereal crops such as corn, wheat and barley (Xu and Nicholson 2009). In spring, perithecia arise from the binucleate hyphae, and then forcibly discharge ascospores into the air to initiate initial infection when plants are ready. The ascospores travel through turbulent wind currents for long distances. Natural infection occurs when ascospores land on spikelets during flowering, germinate and enter through the anthers or other tissues such as glume, lemma, and palea (Trail 2009; Xu and Nicholson 2009). Asexual spores (conidia) may also be produced on the surface of infected crop residues during wet weather, and infect plant by rain-splash or the wind in short distances (Parry et al. 1995; Trail 2009). Host plants that get infected will later produce diseased kernels that are shriveled and wilted. Kernels that are colonized by the *F. graminearum* during

late kernel filling stage may not appear to be affected, but may still be contaminated with mycotoxin (Moretti et al. 2014; Schmale III and Bergstrom 2010).

FHB resistance mechanisms and assessment of disease resistance

FHB resistance in wheat can be classified into two types: morphological and physiological (Gilsinger et al. 2005). Morphological features include plant height, awn, the width of flower opening, and so on. Generally speaking, awned plants with a short peduncle and a compact spike have faster disease spread than plants that are awnless with a long peduncle, and a lax spike (Rudd et al. 2001); short genotypes with a long grain filling period have higher chances to get infected than tall genotypes with rapid grain filling (Rudd et al. 2001); plants with wider opening florets are more susceptible to FHB (Ban 2003). However, morphological characteristics are considered to be passive resistance to FHB, which is of minor significance compared with physiological resistance. The physiological mechanism involves biochemical pathways that produce chemicals barriers to prohibit pathogens growth after initial infection.

Mesterhazy (1995) proposed five types of FHB resistance: resistance to initial penetration of the pathogen (Type I) (Schroeder and Christensen 1963), resistance to spread within a spike (Type II) (Schroeder and Christensen 1963), kernel size and number retention (Type III), tolerance (Type IV), and decomposition or non-accumulation of mycotoxins (Type V) (Miller et al. 1985). Among them, type I, II and V are the three major types that are commonly accepted (Bai and Shaner 1994), thus type V is also reported as type III by (Miller et al. 1985). In wheat, FHB type II resistance is the most stable and easy to evaluate, thus is mostly studied and extensively used (Bai and Shaner 2004). Type I resistance has been reported in wheat, however, is not as common

as in barley, while type III resistance is commonly used in both wheat and barley. Type II can be evaluated by injecting inocula into central spikelet and rating the disease spread within a spike. Percentage of symptomatic spikelets within a spike is usually used to measure type II resistance (Bai et al. 1999). Plants with low PSS (<5%) are highly resistant, while plants with high PSS (>80%) are considered as highly susceptible. Plants with ratings in between can also be categorized into moderately resistant and moderately susceptible (Bai et al. 1999). Accurate assessment of type I resistance is more difficult than type II resistance, because type I measurement can be affected by many factors. It is usually measured by spraying inoculation, and a number of inocula that applied to a spike is difficult to be quantified. Also, the disease assessment can be confounded by the type II resistance (Rudd et al. 2001). Besides, assessment of all the other types of resistances relies on careful threshing. Type III resistance (kernel size and number retention) is measured by the percentage of *Fusarium*-damaged kernels (FDK) (Rudd et al. 2001). Type IV resistance is evaluated by measuring grain yield in FHB-infected plots compared with the plots with no disease. Type V resistance measures DON concentration in harvested grains. This resistance is important to grain end-use quality (Rudd et al. 2001).

The biochemical pathways that involve in physiological resistance are associated with FHB type II resistance. Although the disease spreading within a spikelet is non-selective, the biochemical responses to the infection varied between resistant and susceptible wheat germplasm (Ribichich et al. 2000a). Many studies proposed the biochemical mechanisms of FHB resistance, however, the mechanisms remain to be equivocal. One hypothesis is that resistant wheat plants may either produce physical barrier (such as thickened cell wall) to delay the mycilium rapid growth or accumulate

phenolic compounds and triticens that are toxic to the pathogen, thus, can prevent the spike from a sudden desiccation upon initial infection (Ribichich et al. 2000a). Another hypothesis is that *F. graminearum* may induce defense responsive genes during early infection in wheat spikes. The genes translated defense-related proteins PR-1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4, and PR-5 (thaumatin-like protein) can be detected as early as 6 to 12 h after inoculation, and can reach the peak after 36 to 48 h (Pritsch et al. 2000). Among the five proteins, a study found that expression of PR-4 and PR-5 was much earlier and greater in resistant wheat plants than in susceptible plants (Bai and Shaner 2004; Pritsch et al. 2000). However, another study showed that PR proteins might have nothing to do with FHB resistance, instead, Jasmonate (JA) and Ethylene (ET) mediated defense responses regulate wheat resistance to FHB based on the observation of more JA or ET biosynthesis after inoculation and JA or ET biosynthesis increased in resistant plants after initial infection (Ding et al. 2011; Li and Yen 2008). In JA pathway, two substances, lipoxygenase (LOX2) and chalcone synthase, are up-regulated in resistant wheat plants rather than in susceptible plants. While in ET pathway, ET can lead plant organs senescence, cell wall to dissolve and finally cell death (Li and Yen, 2008). Besides, many other biochemical compounds including choline, betaine, and amino acids glutamine, glutamate alanine, trans-aconitate, and sucrose are also associated with fungal hyphae growths, thus, affect FHB infection (Browne and Brindle 2007). However, other studies cannot find the significant associations. Therefore, the biochemical mechanisms of FHB resistance are still a debatable topic.

Mycotoxins and their relationships to FHB infection

Fusarium species are widely distributed plant pathogens that produce a great diversity of toxic secondary metabolites such as trichothecenes that are detrimental to human and animal health. Trichothecenes have been identified as an important class of the mycotoxins (Schollenberger et al. 2007). Nowadays, more than 170 trichothecenes have been isolated. They have been divided into A-, B-, C-, D-type trichothecenes according to their characteristic functional groups (Schollenberger et al. 2007). B-type trichothecenes include the mycotoxins fusarenon-X, nivalenol, and deoxynivalenol (DON) (Bennett and Klich 2003). The trichothecenes are an extremely potent inhibitor of eukaryotic protein synthesis, thus are harmful to both animals and plants. DON is one of the most common mycotoxins that found in grains, and delays seed germination and the subsequent development of plants (Ji et al. 2015). When agricultural animals ingest DON in high doses, they may experience nausea and vomit; while ingested at low doses, animals may exhibit food refusal and weight loss. Therefore, DON is also called 'vomitoxin' (Bennett and Klich 2003). DON produced by *F. graminearum* is the most prevalent and commonly found trichothecene in small grain and can cause significant economic and health consequences although it's less toxic than many other major trichothecenes (Bennett and Klich 2003; Foroud and Eudes 2009). DON causes tissue necrosis, and is the only trichothecene that has been considered as a virulence factor (Desjardins et al. 1996; Trail 2009).

DON accumulation may be involved in FHB infection (Bai et al. 2001; Hernandez Nopsa et al. 2012; Lemmens et al. 2004; Ma et al. 2006a; Paul et al. 2006; Wegulo 2012). Hernandez Nopsa et al. (2012) found significant correlation coefficients,

ranging from 0.57 to 0.77, between FHB severity and DON concentration in two winter wheat cultivars in all three years experiments. Paul et al. (2006) used meta-analysis to analyze 163 studies, and found the mean correlation coefficient between FHB severity and concentration was 0.53. Thus, FHB severity is a major factor influencing DON accumulation in wheat. Meanwhile, the significant correlations between FHB symptom ratings and DON content indicate the percentage of scabbed spikelets and *Fusarium*-damaged kernels (FDK) can be used to predict DON contents in harvested wheat grains (Bai et al. 2001). However, some studies showed the associations between FHB severity and DON content in harvested grains are not consistent. A field study conducted in China showed that DON content in infected grain didn't consistently correlate with FHB incidence (Ji et al. 2015). Many factors may affect FHB infection and DON content, including timing and methods of inoculation, environmental conditions, and DON measurement. DON content was greatest when a plant was inoculated at early to mid anthesis, but lowest when inoculated during ear emergence and after anthesis (Lacey et al. 1999). Lemmens et al. (2004) found that environmental conditions had important impacts on both FHB symptoms and DON levels, and indicated that the high correlation between FHB and DON was only obtained under moderate disease pressure, not at high disease pressure with only susceptible and moderately susceptible cultivars tested in one experiment (Bai et al. 2001; Lemmens et al. 2004). Another factor affecting FHB and DON correlation is the way DON content is measured. Disease kernels are often blown out by the air flow in the combine thresher, which will lead to under-estimation of DON in susceptible cultivars (Bai and Shaner 2004; Mesterhazy et al. 1999).

DON produced by *F. graminearum* during FHB infection has been proposed as a virulence factor. Disruption of the gene encoding a trichothecene synthase (*Tri5*) in *F. graminearum* reduced FHB severity, and restoration of the synthase gene resulted in the increased FHB severity and DON accumulation (Bai and Shaner 2004; Desjardins et al. 1996). However, trichothecenes may not be a virulence factor for FHB initial infection in wheat floret (Jansen et al. 2005). When green fluorescence protein (GFP) labeled wild type, and trichothecenes knocked out mutant of *F. graminearum* strain was used to inoculate wheat plants, *Fusarium* hyphae of both enter the cytosol of the epicarp cells in wheat, leading to a cell death in plants in both cases (Jansen et al. 2005).

Control methods

To reduce the risk of the FHB epidemics, we shall reduce the number of available inocula, prevent the dispersal of inocula, minimize susceptible wheat available, thus prevent FHB epidemics when inocula present (Parry et al. 1995). To achieve these goals, many control methods have been applied, including the use of the cultural practice, the application of fungicide or biological antagonists, and growing resistant cultivars (Bai and Shaner 2004; Parry et al. 1995). Control of FHB by crop rotation was proposed firstly by Bolley (1913). High FHB incidence was observed in the plot where wheat was continuously grown after maize (Koehler et al. 1924; Latta et al. 1891) because the maize debris is a good medium for pathogen production (Parry et al. 1995). Thus, avoiding maize-wheat rotation can reduce the incidence of FHB. In addition, tillage systems used have a great impact on FHB incidence. No-tillage or minimum tillage approaches would leave most of crop residues on the soil surface that take long time to decompose and increase the chances of FHB outbreaks (Dill-Macky and Jones 2000). However,

conventional deep tillage systems enable crop residued incorporated into the soil that make the crop residues easier to decompose, thus, reduce the chance of FHB outbreaks (McMullen et al. 1997). Besides, sowing date is another element that indirectly affects FHB infection. Growing early maturity cultivars and early sowing are also good practices for wheat to escape from favorable conditions for heavy FHB infection (Champeil et al. 2004).

Among the FHB control methods, the fungicide application is still a major method of commercial wheat production. Proper use of fungicides is critical to reducing both FHB severity and DON concentration, especially in FHB moderately resistant plants (Wegulo et al. 2011). Some effective chemicals have been reported, such as tebuconazole, prochloraz and Guazatine, however, none of them are consistently effective (Parry et al. 1995). Difficulties in the determination of an optimal time to apply fungicide, high cost, lacking fungicide with the specific active ingredient, the length of protection, accumulation of toxins, and environmental conditions are all problems involved with fungicide application (Bai and Shaner 2004; Homdork et al. 2000; Parry et al. 1995). Disease forecasting together with newly developed fungicides and application methods can improve fungicide application effectiveness (Mesterhazy 2002; Wegulo et al. 2011). Compared with the fungicide application, inhibition of FHB through biological control agents is environmentally friendly. For example, inoculating *Sporobolomyces spp.* at anthesis stage or *Cladosporium spp.* before anthesis would both significantly reduce disease severity and DON accumulation (Parry et al. 1995; Riungur et al. 2007). However, the most effective and efficient approach to control FHB and reduce FHB severity in wheat could be growing resistant cultivars. A combined approach of cultural

practices, fungicide application, and resistance sources can function together to control FHB.

FHB resistance sources

Among the different control methods, use of cultivars resistance is the most effective and economical approach for disease reduction (Bai and Shaner 2004). Since FHB were firstly described in the U.S. in late 19th century (Arthur 1891), many efforts have been made to find resistant sources. Although cultivars with various levels of resistance have been reported worldwide, wheat germplasm with a high level of FHB resistance is very rare (Bai and Shaner 2004). To date, no source of complete immunity has been identified (He et al. 2013). Resistant wheat sources with a high level of FHB resistance are mostly found from China and Japan (Yu et al. 2008a). In China, with the cooperation of multiple institutes in China, about 34,571 wheat lines were screened in 1980's, and only 1,765 (5.1%) showed resistant or moderately resistant reactions to FHB (He et al. 2013). Chinese wheat cultivar Sumai3 and its derivatives, especially 'Ning7840' were reported to carry the major QTL *Fhb1*, and show a high level of FHB resistance (Bai 1996). The resistance was quite stable across different environments, thus has been extensively used in the world's breeding programs (Rudd et al. 2001; Bai 1996). Other Chinese landraces such as Wangshuibai, Baishanyuehuang, Huangcandou, Huangfangzhu, and Haiyanzhong also show high levels of FHB high resistance (Jia et al. 2006; Lin et al. 2006; Yu et al. 2008b). In Japan, wheat cultivars such as Shinchunaga, Nobeokabouzu, and Nyu Bai are also highly resistant to FHB (Bai and Shaner 2004; Ban 2001). However, the use of either Chinese or Japanese landraces or in conventional breeding is not successful because of the linkage drag to their unfavorable agronomic traits. In addition

to FHB resistance sources from Asia, germplasm with FHB resistance are also reported in South America, North America, and Europe, such as Frontana and Encruzilhada from Brazil (Ban 2001; Mesterhazy 1995; Singh and Ginkel 1997); soft red winter wheat Ernie, Freedom and Roane from the U.S. (Jin et al. 2013; Rudd et al. 2001); and winter wheat Arina, Renan, and Fundulea 201R from Europe (Gervais et al. 2003; Somers et al. 2004; Steiner et al. 2004; Paillard et al. 2004) . These cultivars may carry different QTLs for FHB resistance from those in Asian sources (Jin et al. 2013).

Moderately resistant cultivars from local regions are also good sources of breeding parents (Waldron et al. 1999) because they have good adaptation to the region. Pyramiding FHB resistance QTLs from Asian sources to U.S. locally adapted cultivars with moderate resistance can enhance the level of FHB resistance. Also, a cross from moderately resistant and moderately susceptible parents may develop some highly resistant progenies, which is due to transgressive segregation. QTLs of some moderately resistant cultivars have been mapped, such as Chokwang (Yang et al. 2005a), Frontana (Mardi et al. 2006a) and Chinese Spring (Grausgruber et al. 1999). Many U.S. wheat sources have been reported to show moderate resistance to FHB, but do not carry *Fhb1*.In soft winter wheat (SWW) cultivars, ‘Truman’, ‘Massy’, ‘Roane’ show moderate resistance (Liu et al. 2013; Sneller et al. 2012). In hard spring wheat (HSW) growing region, more than 54% of the wheat acreages were grown with moderately resistant wheat cultivars (Anderson et al. 2012a). Several HSW cultivars with *Fhb1* were released, such as ‘Sabin’ from Minnesota, ‘Alsen’ and ‘Glenn’ from North Dakota (Anderson et al. 2012a; Anderson et al. 2012b; ElDoliefy et al. 2015). For hard winter wheat (HWW) cultivars in the Great Plains, only a few cultivars have moderate resistance, such as

‘Everest’, ‘Overland’, ‘Lyman’, ‘Heyne’, and ‘Hondo’ (Jin et al. 2013, Bockus et al. 2009; Zhang et al. 2012a), and none of them carry *Fhb1*.

In addition to cultivars and landraces, alien chromosome introgression is used to breed resistant cultivars. FHB resistance has been identified in tetraploid wheat species, such as wild emmer wheat (*T. turgidum ssp. dicoccoides*), and other alien species including *Aegilops tauschii, Ae. ventricosa, Ae. speltoides, Thinopyrum ponticum, Th. elongatum, Th. intermedium, Dasypyrum villosa, Secale cereale, Leymous racemosus,* oats (*Avena sativa*), and *Elymus tsukushiensis* (Cai et al. 2008; Oliver et al. 2005; Cainong et al. 2015; Qi et al. 2008). To transfer resistant genes from alien sources to adapted common wheat, resistant alien species need to be crossed with wheat to produce amphiploids. Then, amphiploids are backcrossed with common wheat to generate addition, substitution, translocation, or recombinant lines. Sometimes, *Ph1* and *Ph2* genes are used to regulate homologous chromosome pairing (Wulff and Moscou 2014). However, the main problems of the effective use of introgressed resistance genes are the genetically linked deleterious traits (linkage drag), and fast breakdown when single genes introduced (Cai et al. 2005; Wulff and Moscou 2014). These problems associated with sexually imcompatibility and linkage drags can be solved by transgenes in a single cassette (Wulff and Moscou 2014). Transgenic wheat exhibit improved level of FHB resistance has been reported in multiple studies. One example would be *Arabidopsis thaliana NPR1*-expression wheat induce defense response gene PR1 when challenged by fungus (Makandar et al 2006); Another example is a barley UDP-glucosyltransferase expressed wheat show significantly higher type II resistance than non-transformed controls (Li et al. 2015); The third example is a β-1.3-glucanase transgenic wheat line

enhanced FHB type II, type III and type IV resistance (Mackintosh et al. 2007). However, none of the transgenic wheat has been used as a source of resistance in breeding so far. .

Genetics of FHB resistance

FHB resistance is a quantitative trait that usually controlled by a few major QTLs and multiple minor QTLs, and also affected by environmental effect (Bai et al. 2000; Parry et al. 1995). Genetic variation of FHB resistance mainly consists of three components: Additive effect, dominant effect, and epistasis effect, among which additive effect accounts for the largest part of genetic variation (Bai et al. 2000). Some studies showed the FHB resistance is controlled by many minor QTLs (Chen 1983; Liao and Yu 1985) while other studies showed the disease resistance was controlled by a few major QTLs together with several minor QTLs (Bai et al. 1990). In most of the studies, only 1~3 QTLs control FHB resistance (Bai et al. 2000), however, a few studies show several minor QTLs together responsible for the disease (Cai et al. 2015, Chapter2). The additive effect of FHB resistance enables the pyramiding of several QTLs from different resistance source to achieve a better level of FHB resistance. One example is that progenies are possible to have superior FHB resistance than the parental lines they derived from, mainly due to transgressive segregation (Bai et al. 2000; Yang et al. 2005a).

History and current status of genetic markers

The genetic marker, acting as landmarks for genes or QTLs, is the most popular tool for tagging the genes or QTLs of interest in modern plant breeding. They can be divided into two major categories: classical markers and DNA-based markers (Jiang 2013). Classical markers include morphological, cytological and biochemical markers. Morphological markers are earliest markers that applied in breeding programs (Stadler

1929). The visible traits such as leaf shape, pigment differences, vernalization habit and plant height were used as indirect selection criteria. However, morphological markers were very limited, thus, cannot be extensively used in breeding (Worland et al. 1987). Cytological markers, shown by chromosome karyotypes and bands, are not only very limited in number, but also difficult to be used in genetic mapping and plant breeding due to highly technical demand (Jiang 2013). Protein isozymes have been used in the 1970s, this marker replaced morphological marker for a very short time but has not been widely used in breeding. In the 1980s, DNA markers became popular because the marker was abundant compared to the previous markers. DNA markers can be classified into three categories: 1) hybridization-based; 2) PCR-based; 3) sequence-based markers. After the 1970s, genetic markers based on DNA-DNA hybridization was developed, this type of markers include restriction fragment length polymorphism (RFLP) (Bostein et al. 1980), fluorescent in situ hybridization (FISH), and microarray. In the 1990s, PCR-based marker became popular because it needs a small amount of DNA, avoids radioisotopes, and generates a high level of polymorphisms. Many types of PCR-based markers were widely used for QTL mapping studies, such as RAPD (random amplified polymorphic DNA) (Williams J.G.K. 1990), AFLP (amplified fragment length polymorphism) (Vos et al. 1995), and SSR (simple sequence repeats) (Akkaya et al. 1992). SSR marker is also called microsatellite marker, which is 2-6 bp tandem repeats, highly abundant, polymorphic, and widely throughout the whole genome. SSR marker has relatively high throughput and reproducibility, thus has been used in QTL mapping and marker-assisted selection for a long time (Akkaya et al. 1992). The sequence-based markers including nucleotide polymorphism (SNP) (Jordan and Humphries 1994), sequence tag sites (STSs)

and expressed sequence tags (ESTs) (Gupta et al. 1999) were also developed in the 1990s. Among them, STS marker is a unique DNA fragment that designed from known sequences (Gupta et al. 1999). SNP is the newest type of markers that detect individual nucleotide polymorphism, have an unlimited number and are ready for high throughput genotyping, thus can be broadly used in genetic research and breeding programs. Before next-generation sequencing (NGS) technology, researchers used Sanger's method to resequence unigene or used in silico SNP discovery method to mine EST database (Mammadov, Aggarwal, et al., 2012, Wright, Bi, et al., 2005). But this method is expensive and unable to discover SNPs in intergenic spaces. With the emergence of NGS, transcriptome resequencing allows faster and less expensive SNP discovery technologies and can reduce genome complexity (Morozova and Marra 2008). The NimbleGen sequence capture technology (Roche Applied Science, IN), including exon sequence capture and NimbleGen microarray by NGS for target resequencing, can discover gene-based SNPs in plants in a higher throughput and coverage (Springer et al. 2009). However, those technologies focus on coding regions only (Mammadov et al. 2012). For genome-wide SNPs discovery, Complexity reduction of polymorphic sequences (CRoPS) (Keygene N.V., Wageningen, The Netherlands) (Orsouw et al. 2007) and restriction site associated DNA (RAD) (Floragenics, Eugene, OR, USA) (Baird et al. 2008) were successfully applied in crop research. These methods together with new computational technology can be used to filter out duplicated SNPs. Most recently, genotyping by sequencing (GBS) technique was developed, and enable discovery of a large number of SNPs in maize, sorghum, and wheat (Mammadov et al. 2012). GBS is developed as a simple but robust approach by genome complexity reduction and

multiplexing samples (Poland et al. 2012). Similar to RAD, GBS targets the genomic sequences flanked by restriction sites to produce a reduced representation of a genome, but GBS library construction is greatly simplified compared to that of RAD. The high throughput GBS approach is becoming a powerful tool for SNP discovery and genomic-assisted breeding in species that lack reference genomes (Poland et al. 2012). To convert the GBS-based SNPs into high-throughput or breeder-friendly markers for marker-assisted breeding, the assays need to be redesigned. Several genotyping platforms are available: Illumina's BeadArray technology-based GoldenGate (GG) (Fan et al. 2003) and Infinium assays (Mammadov et al. 2012) for high-throughput marker analysis, and Life Technologies' TaqMan assay (Livak et al. 1995) and KBiosciences' competitive allele specific PCR (KASPar) for breeder-friendly single SNP analysis. More recently, high-density SNP genotyping arrays with about 90,000 gene-associated SNPs were developed as a powerful tool to characterize genetic variations in allopolyploid wheat (Wang et al. 2014).

Genetic maps and QTL for FHB resistance

The molecular markers that discussed in the previous paragraph have been used to construct genetic linkage maps to locate QTLs for FHB resistance (Anderson et al. 2001; Bai et al. 2003; Bai et al. 1999; Ban 2000; Buerstmayr et al. 2002; Burt et al. 2015; Cuthbert et al. 2006; Guo et al. 2015; Liu et al. 2007; Liu and Anderson 2003; Ma et al. 2006a; Mardi et al. 2006a; Poland et al. 2012; Somers et al. 2003; Steiner et al. 2004; Sun et al. 2003; Waldron et al. 1999; Yu et al. 2008b; Zhang et al. 2004). Linkage maps are constructed based on recombination frequency (RF) among markers in a mapping population to determine relative positions of these markers. Marker positions and

intervals may be different between different populations. Thus, a consensus map that combining map information from different populations is a good tool to determine marker and QTL positions for further study. The first SSR map in wheat was constructed in the 1990s' with 279 SSR markers (Roder et al. 1998). In 2004, a wheat consensus map with 1,235 SSR markers was constructed using four different populations (Somers et al. 2004). This consensus map is a useful reference for future works on mapping QTL for traits of interest, as well as map-based cloning of QTLs for different traits (Somers et al. 2004) (http://wheat.pw.usda.gov). Recently, a new wheat SNP consensus map was constructed using high density 90,000 SNP arrays. A total of 46,977 SNPs were genetically mapped in a combination of eight double haploid populations (Wang et al. 2014). This map provides a valuable source for not only genetic diversity studies but also a high-resolution dissection of complex traits in wheat. Most recently, a haplotype map of allohexaploid wheat has been published (Jordan et al. 2015), which will be a useful genetic resource for SNP mapping projects.

QTL mapping method was firstly proposed in 1923 by Sax and later elaborated in 1961 by Thoday. In the 1990s', QTL mapping method has been used to dissect quantitative traits, and map QTLs that are underlining traits of interest in genetic maps and identify the QTL effects and interactions (Kearsey 1998). A quantitative trait is usually controlled by a few major QTLs and several minor QTLs. Each of the QTL may segregate under Mendelian law and also affected by environments. QTL mapping model fits phenotypic variation into the predicted genetic models to estimate QTL numbers, genotyping by environment interactions and heritability. Mapping population is the start point of QTL mapping. For mapping QTL for FHB resistance, the parents for a mapping

population could either show significant contrast (Collards et al. 2005; Liu 1998), or no big contrasting for FHB resistance as long as the constructed population has significant phenotypic variations (Mardi et al. 2006). Population sizes of 70 to 250 lines were reported in preliminary QTL mapping (Mohan et al. 1997), however, large populations are required for high-resolution QTL mapping (Collards et al. 2005). Different types of mapping populations has been reported in QTL mapping studies, such as F2, backcross (BC) (Buerstmayr et al. 1999), recombinant inbred lines (RILs) (Waldron et al. 1999; Yu et al. 2008b), double haploid (DH) (Chen et al. 2006; Jia et al. 2006; Yang et al. 2005b) and chromosome recombinant inbred lines (CRILs) (Garvin et al. 2009; Jayatilake et al. 2011; Ma et al. 2006a). RIL population have been the most studied type of mapping FHB resistance because the phenotyping conducted on RIL can be repeated in different years and locations (Collard et al. 2005).

Several different approaches for QTL mapping have been reported, including single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), and multiple interval mapping (MIM) (Tanksley 1993). SMA is the easiest method for QTL detection with individual markers. The statistical method such as t-test, analysis of variance (ANOVA) and linear regression can be used to identify the marker-trait associations (Collards et al. 2005; Young 1996). However, SMA can detect QTL only when a marker closely linked to the QTL is found. SIM uses linkage maps to calculate the association between the phenotypic scores and linked markers to identify QTLs intervals (Manly and Olson 1999). Thus, SIM is more powerful than SMA. However, when two QTLs located in close marker intervals, SIM cannot separate them (Manly and Olson 1999). CIM can detect a closely linked QTL by considering some

background markers as a window size, and control background noise by using the background markers as cofactors (Manly and Olson 1999; Zeng 1994). However, MIM method can detect epistasis between QTLs by considering multiple marker intervals simultaneously, thus is powerful in detecting QTL interactions (Wang et al. 2006). To determine the significance of QTLs, the logarithmic of odds (LOD) (Lander and Kruglyak 1995) and the likelihood ratio statistics (LRS) (Haley and Knott 1992) are commonly used. The significant threshold of LOD or LRS is calculated by 1000 permutations with 95% CI (Churchill and Doerge 1994). If the peak of a QTL exceeds the threshold, the QTL can be claimed as significant. An empirical threshold of LOD at 3.0 is usually used for claiming significant QTL (Collard et al. 2005). Empirically, major QTLs can usually explain a large percentage of phenotypic variations (R^2>10%), and are more stable across different environments and locations, especially those for disease resistance (Collard et al. 2005; Li et al. 2011), while minor QTLs accounts for only a relatively small percentage of phenotypic variation (R^2 < 10%) (Collard et al. 2005; Li et al. 2011). Many factors may affect the detection power of QTL mapping, such as population size, marker density, the accuracy of phenotypic and genotypic data, and environmental effects (Darvasi et al. 1993). A large population size, high-density genetic map, accurate and reproducible phenotyping are preferrable for QTL mapping (Collards et al. 2005; Cuthbert et al. 2006; Kolb et al. 2001). The same QTL may express different levels of effects on different environments, especially minor QTLs. Thus, QTL mapping experiments should be conducted with replications under multiple years and environments (Collard et al. 2005; Haley and Knott 1992; Kolb et al. 2001).

QTLs for the four types (type I, II, III, and FDK) of resistance have been mapped in more than 50 wheat cultivars on all 21 wheat chromosomes (Table 1.1) (Buerstmayr et al. 2009; Liu et al. 2009). Among them, the QTLs on chromosome 1B, 2B, 2D, 3A, 3B, 3D, 4B, 4D, 5A, 6B, 6D, and 7A have been mapped in at least two populations according to the previous reports (Buerstmayr et al. 2009; Liu et al. 2009). Seven of the mapped QTLs were formally designated with a gene name. They were (1) *Fhb1* on the short arm of chromosome 3B from Sumai3 (Cuthbert et al. 2006). This QTL shows the largest effect on FHB type II and III resistance (Bai and Shaner 2004; Waldron et al. 1999), and validated by several studies (Anderson et al. 2001; Yu et al. 2008b; Zhou et al. 2002). (2) *Fhb2* on chromosome 6B from Sumai3 (Anderson et al. 2001; Cuthbert et al. 2007); (3) *Fhb3* on chromosome 7AS derived from an alien species *Leymus racemosus* (Qi et al. 2008); *Fhb4* on chromosome 4B from Wangshuibai (Xue et al. 2010); *Fhb5* on chromosome 5A also derived from Wangshuibai (Xue et al. 2011), *Fhb6* on chromosome 1A derived from $1E^{ts}$#1S of *Elymus tsukushiensis* (Cainong et al. 2015); and *Fhb7* on 7D derived from *Thinopyrum ponticum* (Guo et al. 2015). However, *Fhb1*, originally derived from Sumai3, is the only one that has been reported to be stable in more than 30 studies. This QTL has also been reported in wheat germplasm that are not related to Sumai3, such as Chinese landraces Wangshuibai (Lin et al. 2006; Zhou et al. 2004), Huangcandou (Cai and Bai 2014), Huangfangzhu (Li et al. 2012), Baishanyuehuang (Zhang et al. 2012b), and Japanese wheat landrace Nyu Bai (Cuthbert et al. 2006; Somers et al. 2003). Due to its large and stable effects on FHB type II and type III resistance across different genetic backgrounds (Anderson et al. 2001; Bai et al. 1999; Bourdoncle and Ohm 2003; Buerstmayr et al. 2003; Chen et al. 2006; Cuthbert et al. 2006; Jayatilake et al. 2011;

Jiang et al. 2007a; Jiang et al. 2007b; Lemmens et al. 2005; Shen et al. 2003; Somers et al. 2003; Yang et al. 2005a; Yang et al. 2005b; Yu et al. 2008b), *Fhb1* has been extensively utilized in wheat breeding programs. Besides, *Fhb2* was also a major QTL that explained a wide range of phenotypic variations in FHB resistance (especially type II) from 4.4% to 23% (Anderson et al. 2001; Bonin and Kolb 2009; Cuthbert et al. 2007; Häberle et al. 2009; Li et al. 2011; Li et al. 2012; Semagn et al. 2007; Shen et al. 2003; Yang et al. 2005b; Zhang et al. 2010). *Fhb3* was transferred from *L. racemosus* to wheat chromosome 7A and are different from these QTLs mapped in Wangshuibai (Zhou et al. 2004), and CS-Sumai3-7ADSL (Jayatilake et al. 2011). *Fhb4* was identified in Wangshuibai with type I resistance (Jia et al. 2006; Lin et al. 2004), and in Ernie (Liu et al. 2007), Chokwang (Yang et al. 2005), and Wuhan1 (Somers et al. 2003) for type II resistance, explained 4.7% (Yang et al. 2005) to 17.5% (Lin et al. 2006) of phenotypic variation. *Fhb5* is mainly conferring FHB type I resistance was identified in Wangshuibai with type II and III resistance and explained from 4% (Li et al. 2011) to 30% (Buerstmayr et al. 2012; Buerstmayr et al. 2011) of the phenotypic variations. The QTL on 2DS was mapped close to *Xgwm261* for type I, II, and III resistance (Cai and Bai 2014; Handa et al. 2008; Somers et al. 2003). The 2D QTL is also linked to the same marker linked to a reduced height locus *Rht8*, however, the genetic relationship between *Rht8* and FHB resistance at this region remains to be investigated. The QTL for FHB type II resistance was also mapped on chromosome 7D in a Chinese landrace Haiyanzhong (Li et al. 2011) and explained a large percentage of disease symptom spread variation, thus, can be an interesting source for QTL validation.

Progress in breeding for FHB resistance

Breeding for improved FHB resistant cultivars requires moving high levels of FHB resistance to locally adapted backgrounds (Bai et al. 2000). Because the additive effect is a major component of FHB resistance, developing lines by pyramiding FHB resistance QTLs from diverse gene pools, such as Chinese landraces, to local cultivars can achieve a better level of resistance (Rudd et al. 2001). Major QTLs with stable effects on FHB resistance are preferable in gene pyramiding, thus transferring *Fhb1* into locally adapted moderately susceptible or resistant cultivars may significantly improve FHB resistance in commercial wheat cultivars (Kolb et al. 2011). However, most FHB highly resistant sources are Chinese or Japanese landraces, such as Wangshuibai, and Ning7840, that have many unadapted agronomic traits (Bai et al. 2000). To avoid or reduce linkage drag of these poorly adapted agronomic traits, we could use marker-assisted selection (MAS) to transfer the resistant QTLs into adapted backgrounds (Bai et al. 2000).

Many elite wheat lines and cultivars were reported to show moderate resistance these years due to the efforts made on improving FHB resistance. Some of these lines may carry QTLs from Asian resistant sources, such as *Fhb1*, others may contain native resistance QTLs only. In the U.S., several soft winter wheat (SWW) cultivars with FHB resistance have been released including ‘Truman’, ‘Massy’, ‘Ernie’, and ‘Freedom’ etc., however, they do not carry *Fhb1* (Liu et al. 2013; Rudd et al. 2001; Sneller et al. 2012). Some commercial soft wheat cultivars harbor *Fhb1* have been released, such as Pioneer Brands 25R18, 25R42, and 25R51, most of which are developed by marker-assisted backcross (Brown-Guedira et al. 2008). In the U.S. spring wheat growing regions, more than 54% of the total acreage was grown with moderate FHB-resistant cultivars (Strunk

2012). Some U.S. hard spring wheat (HSW) cultivars also have FHB resistance, including ‘Bacup’ and ‘Sabin’ developed in Minnesota, and ‘Alsen’, ‘Steele’, ‘ND2710’ and ‘Glenn’ developed by North Dakota State University (Mergoum et al. 2007). ‘Among them ‘Sabin’, ‘Alsen’, and ‘Glenn’ were reported to have *Fhb1* (Anderson et al. 2012a; Anderson et al. 2012b; ElDoliefy et al. 2015), thus can either be directly used in commercial production or as resistant parents in breeding programs. Besides, a few U.S. hard winter wheat (HWW) cultivars have been reported to have moderate FHB resistance including ‘Everest’, ‘Overland’, ‘Lyman’, ‘Heyne’ and ‘Hondo’, and none of them carry *Fhb1* (Bockus et al. 2009; Zhang et al. 2012a). To improve FHB resistance in these wheat cultivars, they can serve as recurrent parents to transfer QTLs from Asian sources. Combining QTLs from the Asian sources would not only improve resistance level but also broaden the genetic diversity (Bai et al. 2003). Besides, transgressive segregation has been successfully used in creating FHB resistant cultivars (Young 1996; Bai et al. 2000). Examples are some wheat cultivars developed from southern China including Sumai 3, Zhen7495, Xiangmai 2, Jingzhou 1 and Jingzhou 47 (Bechtel et al., 1985; Bai et al., 2000). Thus, elite resistant lines selected from transgressive segregation may have a higher level of FHB resistance than their breeding parents.

Prospective in future FHB resistance research

Functional markers are preferred for marker-assisted selection for FHB resistance. However, the functional markers are still not available because none of FHB resistance genes has been cloned to date (He et al. 2013). Flanking markers are available for some FHB resistance QTLs, but they may not be diagnostic for these QTLs in different populations. The *Fhb1* linked marker *Xumn10* is a tightly linked marker to *Fhb1*, and

easy to use in marker-assisted selection, thus, have been widely used in breeding. However, false positive has frequently been observed for *Xumn10* in soft winter wheat breeding program, thus, functional markers are desired for improving FHB resistance in breeding programs.

Use of wheat resistance to FHB is the most effective and sustainable method of defeating FHB. To date, Asian sources of FHB resistance have been widely used, and the other sources of resistance are still limited. The introduction of resistance from alien species has become quite popular in recent years (Bai and Shaner 2004). Sources with higher levels of resistance to FHB than Sumai3 were reported in hybrids *Triticum aestivum-Leymus racemosus*, T. *aestivum-Roegneria komoji,* and *T. aestivum-R. ciliaris* (Chen et al. 1993). More QTL mapping papers published recently have focused on the QTLs from alien species (Buerstmayr et al. 2013; Buerstmayr et al. 2012; Buerstmayr et al. 2011; Zhang et al. 2014). However, successful use of the QTL from alien species has not been reported. Besides, the local FHB resistance sources have usually been overlooked until recently (Cativelli et al. 2013; Chu et al. 2011; Jin et al. 2013; Liu et al. 2012; Liu et al. 2013; Zhang et al. 2012a). Pyramiding resistance QTL from different sources could yield durable and highly resistant genotypes.

Table 1.1 Summary of reported FHB resistance QTLs from different studies

Type of FHB resistance	Chromosome	Population name	Population type	References
Type II resistance	3BS, 6BS (Sumai3) 2AL, 4B (Stoa)	Sumai3 (R)/Stoa (MS)	RIL	(Waldron et al. 1999)
Type II resistance	3BS	Ning7840(R)/Clark (S)	RIL	(Bai et al. 1999)
Type II resistance	3AL,6AS, 3BS	ND2603(R)/Butte86(MS)	RIL	(Anderson et al. 2001)
Type II resistance	2AL, 3BS, 4BS, 6BS	Sumai3(R)/Stoa(MS)	RIL	(Anderson et al. 2001)
Type II resistance	2AS, 2BL and 3BS	Ning7840(R)/Clark(S)	RIL	(Zhou et al. 2002)
Type II resistance	5A, 1B and 3BS	CM-82036(R)/Remus(S)	DH	(Buerstmayr et al. 2002)
Type II resistance	3BS	Ning7840(R)/Wheaton(S)	$F_{2:3}$	(Zhou et al. 2003)
Type II resistance	3BS	Ning7840(R)/IL89-7978(S)	$F_{3:4}$	(Zhou et al. 2003)
Type II resistance	3BS, 5A	CM-82036(R)/Remus(S)	DH	(Buerstmayr et al. 2003)
Type II resistance	1B, 3A, 3D, 5A	F201R(R)/cv. Patterson (MS)	RIL	(Shen et al. 2003)
Type II resistance	3A, 3BS, 3BL and 5B	Huapei57-2(R)/Patterson (MS)	RIL	(Bourdoncle and Ohm 2003)
Type II resistance	2DL, 3BSc and 4B	Wuhan-1(R)/Maringa(MS)	DH	(Somers et al. 2003)
Type II resistance	1B and 3BS	Wangshuibai(R)/Alondra(S)	RIL	(Zhang et al. 2004)
Type II resistance	7AL, 3BSd, 1BL and 3BSc	Wangshuibai(R)/Wheaton(S)	RIL	(Zhou et al. 2004)
Type II resistance	3BS, 6BS, 2DS and 7BL	DH181(R)/AC Foremost(S)	DH	(Yang et al. 2005b)
Type II	3BS, 4BL and	Chokwang(R)/Clark(S)	RIL	(Yang et al. 2005a)

resistance	5DL			
Type II resistance	6AL, 1B, 2BL, and 7BS	Dream(R)/Lynx(S)	RIL	(Schmolke et al. 2005)
Type II resistance	3BS, 4BL and 5DL	Chokwang(R)/Clark(S)	RIL	(Yang et al. 2005a)
Type II resistance	7A, 3B, 5B, and 2D	Wangshuibai(R)/Alondra(S)	DH	(Jia et al. 2006)
Type II resistance	5AS and 3BS	W14(R)/Poin2684(S)	DH	(Chen et al. 2006)
Type II resistance	6A, 3B, 2D, and 4D	Chinese spring sumai3 disomic substitution line (R)/Annong 8455(S)	RIL	(Ma et al. 2006a)
Type II resistance	3B, 2A	Wangshuibai (R)/Annong 8455	RIL	(Ma et al. 2006b)
Type II resistance	3BS	Sumai3*5(R)/Thatcher(S) and HC374(R)/3*98B69-L47(S)	RIL	(Cuthbert et al. 2006)
Type II resistance	3AL, 7AS and 1BL	Frontana (MR)/Seri82(S)	$F_{3:5}$	(Mardi et al. 2006b)
Type II resistance	1AS, 3BS, 7BS, 2BL, 1BC	CJ9306(R)/Veery(S)	RIL	(Jiang et al. 2007b)
Type II resistance	3BSc, 5A, 6B	BW278(R)/AC Foremost(S)	RIL	(Cuthbert et al. 2007)
Type II resistance	1AL, 7AL 1BL and 6BS	Arina (MR)/NK93604(MR)	DH	(Semagn et al. 2007)
Type II resistance	5A, 2B, 3B, and 4BL	Ernie(MR)/MO 94-317(S)	RIL	(Liu et al. 2007)
Type II resistance	1A, 5AS, 7AL 3BS, 3DL and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	(Yu et al. 2008b)
Type II resistance	1A and 2BL	G16-92(R)/Hussar(S)	RIL	(Schmolke et al. 2008)
Type II resistance	2DS	Sumai3(R)/Gamenya(S)	DH	(Handa et al. 2008)
Type II resistance	2B, 3B, 4B, and 6B	IL94-1653/Patton	RIL	(Bonin and Kolb 2009)
Type II	7BS /5BL,	G93010 (R)/Pelikan	RIL	(Häberle et al.

resistance	6BS and			2009)
Type II resistance	7A, 1B, 3B, 6B and 2D	Wangshuibai(R)/Sy95-7(S)	$F_{2:3}$	(Zhang et al. 2010)
Type II resistance	2A, 5A, 2B, 5B	T. macha(R)/Furore(S)	RIL	(Buerstmayr et al. 2011)
Type II resistance	7AC and 3BS	CS-Sumai 3-7ADSL	CRIL	(Jayatilake et al. 2011)
Type II resistance	1AS, 5AS, 6BS(2) and 7DL	Haiyanzhong (R)/Wheaton	RIL	(Li et al. 2011)
Type II resistance	5AS, 5AL	PI 277012 (R)/Grandin	DH	(Chu et al. 2011)
Type II resistance	3A, 6A, and 4D	Frontana (R)/Chris	Reciprocal backcross monosomic (RBCM)	(Yabwalo et al. 2011)
Type II resistance	1AS, 5AS, 7AL, 1B and 3BS	Huangfangzhu(R)/Wheaton	RIL	(Li et al. 2012)
Type II resistance	3AS, 4AL and 4DL	Heyne(R)/ Trego	RIL	(Zhang et al. 2012a)
Type II	3BSd, 3BSc, 3A, 5A	Baishanyuehuang (R)/Jagger	RIL	(Zhang et al. 2012b)
Type II resistance	3B, 5A, 5B, 7A, 7B	BGRC3487/2*DT735 (Moderate R)	BCRIL	(Ruan et al. 2012)
Type II resistance	3B, 5A, 3A	RCATL33(R)/RC Strategy	RIL	(Tamburic-Ilincic and Miedaner 2012)
Type II resistance	1BL, 2A, 2DL, 5B, 6A, and 7A	VA00W-38 (Moderate R)/26R46	RIL	(Liu et al. 2012)
Type II resistance	1A, 2B, 2D, 3B, 6A, 7A, and 7B	Jamestown/LA97113UC-124	RIL	(Wright et al. 2012)
Type II resistance	1B, 2B, 3A, and 6A	Pioneer25R47/Jamestown	RIL	(Wright et al. 2012)
Type II resistance	3A, 6B	Mt. Gerizim #36 (R)/Helidur	BC	(Buerstmayr et al. 2013)

Type II resistance	1DS, 3BL	Becker/Massey	RIL	(Liu et al. 2013)
Type II resistance	2DS, 4BS, 4DS, 5AL, 3BL, 4BS	Ernie/MO 94-317	RIL	(Liu et al. 2013)
Type II	7DS, 3BS, 5DL	Catbird/Milan	DH	(Cativelli et al. 2013)
Type II resistance	3BSc, 3BSd, 3AS, 2D, and 6D	Huangcandou(R)/Jagger	RIL	(Cai and Bai 2014)
Type II resistance	2A (Ben) 3A, 5A from PI41025	Ben(Durum)/PI41025	RIL	(Zhang et al. 2014)
Type II resistance	2B, 2D, 3B, 5B, 6B, 7A, 7D	Glenn/MN00216-4 (GM)	RIL	(ElDoliefy 2015)
Type II resistance	3BL, 5AL, 4BL	Parshall/Reeder	RIL	(ElDoliefy 2015)
Type II resistance	1A, 2A, 6A	NC-Neuse (Moderately resistant)/AGS	RIL	(Petersen et al. 2015)
Type I resistance	3B, 5A, 1B	CM-82036(R)/Remus(S)	DH	(Buerstmayr et al. 2002)
Type I resistance	3A and 5A	Frontana(MR) and Remus(S)	DH	(Steiner et al. 2004)
Type I resistance	3AS, 5AS, 3BS, 3BSc, 6BS, 2DS and 4DL	DH181(R)/AC Foremost(S)	DH	(Yang et al. 2005b)
Type I resistance	5A, 4B, and 5B	Wangshuibai (R)/Nanda2419	RIL	(Lin et al. 2006)
Type I resistance	3AS, 5AS, 3BS, 4B, and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	(Yu et al. 2008b)
Type I resistance	1B, 2B, 3A, 6A, 6B, 7A and 7D	RL4137 (R)/Timgalen(MR)	RIL	(Srinrvasachary et al. 2008)
Type I resistance	3BS, 6BL, 2DS	Sumai3 (R)/Y1193-6	RIL	(Basnet et al. 2011)
Type I	3A, 6A, and	Frontana (R)/Chris	Reciprocal	(Yabwalo et al.

	4D		backcross monosomic (RBCM)	2011)
Type I resistance	4B, 6A, 6B	T. dicoccum-161 (R)/ DS-131621 (durum wheat)	BC_1F_4	(Buerstmayr et al. 2012)
Type I resistance	3B, 4B, 6B	T. dicoccum-161 (R)/ Floradur (durum wheat)	BC_1F_4	(Buerstmayr et al. 2012)
Type I resistance	4B, 7B	T. dicoccum-161 (R)/ Helidur (durum wheat)	BC_1F_4	(Buerstmayr et al. 2012)
Type I resistance	2A, 3B, 5B, 7A	BGRC3487/2*DT735 (Moderate R)	BCRIL	(Ruan et al. 2012)
Type I resistance	3A, 4A, 6B, 2B, 4B, 5A, 7B	Frontana (R)/Remus	DH	(Szabó-Hevér et al. 2012)
Type I resistance	1A, 2B, 2D, 3B, 6A, 7A, and 7B	Jamestown/LA97113UC-124	RIL	(Wright et al. 2012)
Type I resistance	1B, 2B, 3A, and 6A	Pioneer25R47/Jamestown	RIL	(Wright et al. 2012)
Type I resistance	2D and 4BS	Becker/Massey	RIL	(Liu et al. 2013)
Type I resistance	4BS, 4DS, 5AL	Ernie/MO 94-317	RIL	(Liu et al. 2013)
Type I resistance	1A, 1B, 2D, 3B, 4A, 5A, 5B, 6A, 7B	GKMini Mano ′/Frontana	DH	(Ágnes et al. 2014)
Type I resistance	1AS, 3B, 6A, 7A	Glenn/MN00216-4	RIL	(ElDoliefy 2015)
Type I resistance	1AS, 4BL	Parshall/Reeder	RIL	(ElDoliefy 2015)
Type I resistance	1A, 5B, 6A	NC-Neuse (Moderately resistant)/AGS	RIL	(Petersen et al. 2015)
Type III resistance	5AS, 2DS	Wuhan-1(R)/Maringa (MS)	DH	(Somers et al. 2003)

Type III resistance	3BS, 5A	CM-82036(R)/and Remus	DH	(Lemmens et al. 2005)
Type III resistance	5AS and 3BS	W14(R)/Poin2684(S)	DH	(Chen et al. 2006)
Type III resistance	2DL, 1AS, 3BS, 5AS	CJ9306(R)/Veery(S)	RIL	(Jiang et al. 2007a)
Type III resistance	1AL and 2AS	Arina (MR)/ NK93604(MR)	DH	(Semagn et al. 2007)
Type III resistance	1A, 5AS, 7AL, 1BL, 3BS and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	(Yu et al. 2008b)
type III resistance	7AC and 3BS	CS-Sumai 3-7ADSL	CRIL	(Jayatilake et al. 2011)
Type III resistance	5AS, 5AL	PI 277012 (R)/Grandin	DH	(Chu et al. 2011)
Type III resistance	3B, 5A, 3A	RCATL33(R)/RC Strategy	RIL	(Tamburic-Ilincic and Miedaner 2012)
Type III resistance	1BL, 2A, 2DL, 5B, 6A, and 7A	VA00W-38 (Moderate R)/26R46	RIL	(Liu et al. 2012)
Type III resistance	1A, 2B, 2D, 3B, 6A, 7A, and 7B	Jamestown/LA97113UC-124	RIL	(Wright et al. 2012)
Type III resistance	1B, 2B, 3A, and 6A	Pioneer25R47/Jamestown	RIL	(Wright et al. 2012)
Type III resistance	4DL	Becker/Massey	RIL	(Liu et al. 2013)
Type III resistance	1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D	GKMini Mano ⁄Frontana	DH	(Ágnes et al. 2014)
Type III resistance	5B	Glenn/MN00216-4 (GM)	RIL	(ElDoliefy 2015)
Type III resistance	1A, 1B, 1D, 2A, 4A, 5B	NC-Neuse (Moderately resistant)/AGS	RIL	(Petersen et al. 2015)
FDK resistance	5AS and 3BS	W14(R)/Poin2684(S)	DH	(Chen et al. 2006)

FDK resistance	2B, 4B, and 6B	IL94-1653/Patton	RIL	(Bonin and Kolb 2009)
FDK resistance	5AS, 5AL	PI 277012 (R)/Grandin	DH	(Chu et al. 2011)
FDK resistance	3A, 6A, and 4D	Frontana (R)/Chris	Reciprocal backcross monosomic (RBCM)	(Yabwalo et al. 2011)
FDK resistance	3B, 5A, 3A	RCATL33(R)/RC Strategy	RIL	(Tamburic-Ilincic and Miedaner 2012)
FDK resistance	1BL, 2A, 2DL, 5B, 6A, and 7A	VA00W-38 (Moderate R)/26R46	RIL	(Liu et al. 2012)
FDK resistance	3D, 2B, 4B, 5A, 7B	Frontana (R)/Remus	DH	(Szabó-Hevér et al. 2012)
FDK resistance	4BS	Becker/Massey	RIL	(Liu et al. 2013)
FDK resistance	4BS, 4DS, 3BL, 4BS	Ernie/MO 94-317	RIL	(Liu et al. 2013)
FDK resistance	1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D	GKMini Mano ′Frontana	DH	(Ágnes et al. 2014)
FDK resistance	1B, 2B, 3D, 5B, 7B, 7D	Glenn/MN00216-4 (GM)	RIL	(ElDoliefy 2015)
FDK resistance	1A, 1B, 1D, 4A,	NC-Neuse (Moderately resistant)/AGS	RIL	(Petersen et al. 2015)

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Chapter 2 - Mapping QTLs for Fusarium head blight resistance in Chinese wheat landrace Haiyanzhong

Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastative disease in wheat (*Triticum aestivum* L.). FHB epidemics reduce not only grain yield, but also grain quality. Use of host resistance is one of the most effective strategies to minimize the disease damage. Haiyanzhong (HYZ) is a Chinese wheat landrace that shows a high level of resistance to FHB type II resistance. To map the quantitative trait loci (QTLs) in HYZ and identify markers tightly linked to the QTLs for FHB resistance, we genotyped 186 recombinant inbred lines (RILs) derived from a cross between HYZ and Wheaton, a susceptible cultivar, using simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs) derived from genotyping-by-sequencing (GBS). The population was also phenotyped for the percentage of symptomatic spikelets (PSSs) per spike in three greenhouse experiments using single floret inoculation. Eight QTLs were identified for type II resistance with six from HYZ. The absence of *Fhb1* in HYZ suggests that an additive effect of multiple minor QTLs can also provide a high level of resistance in wheat. A major QTL for FHB resistance was mapped on chromosome 5AS with a 1.88-cM interval flanked by SNP *GBS3127* and SSR *Xbarc316*. The other seven minor QTLs were mapped on the chromosomes 6B, 7D, 2B (2), 3B, 4B, and 4D. Critical SNPs linked to the QTLs on chromosomes 5A, 6B, and 2B were converted into KBioscience competitive allelic-specific PCR (KASP) assays that could be used for marker-assisted selection (MAS) to pyramid these QTLs in wheat.

Introduction

Fusarium head blight (FHB) is mainly caused by *Fusarium graminearum* Schwabe [telomorph, *Gibberella zeae* (Schw.) Petch], and is one of the most destructive diseases of wheat (*Triticum aestivum*), especially in humid and semi-humid wheat-growing regions of the world (Bai and Shaner 2004; Goswami and Kistler 2004). It causes significant yield losses and grain quality reduction. Infected grain is also contaminated with mycotoxins, especially deoxynivalenol (DON), which is a major health concern for humans and animals (Cetin and Bullerman 2005). Although progress has been made in managing FHB during the last decade, FHB and DON continue to cause significant economic losses in many regions in the U.S. and many other countries (McMullen et al. 2012). No single strategy is completely effective in mitigating FHB damage. However, growing FHB-resistant cultivars coupling with appropriate cultural practices can minimize FHB damage.

FHB resistance in wheat is a quantitative trait controlled by multiple quantitative trait loci (QTLs) and affected by environmental factors (Bai and Shaner 1994; Buerstmayr et al. 1999). To date, more than 50 QTLs have been reported on all 21 chromosomes to be associated with FHB resistance (Buerstmayr et al. 2009). Several QTLs have repeatedly been mapped on chromosomes 3BS, 5AS, 6BS, 3A, 4B, 2D, 1B, 7A and 5B etc (Liu et al. 2009), and seven have been formally designated with a gene name including *Fhb1* on chromosome 3BS derived from Sumai3 (Cuthbert et al. 2006), *Fhb2* on 6BS derived from Sumai3 (Anderson et al. 2001; Cuthbert et al. 2007), *Fhb3* on 7AS derived from *Leymus racemosus* (Qi et al. 2008), *Fhb4* derived from 4B of Wangshuibai (Xue et al. 2010), *Fhb5* derived from 5A of Wangshuibai (Xue et al. 2011),

Fhb6 on 1A derived from *Elymus tsukushiensis* (Cainong et al. 2015), and *Fhb7* on 7DS derived from *Thinopyrum ponticum* (Guo et al. 2015). However, most of the QTLs were mapped using low-density maps. A high-density map is critical to the identification of tightly linked markers to these QTLs. Genotyping-by-sequencing (GBS) is a simple, but effective, approach for spontaneous discovering and mapping of SNP markers in diverse species (Poland et al. 2012), and is a useful marker system for fine mapping of QTLs for FHB resistance.

FHB resistance genes used in current wheat breeding programs can be traced back to very few sources with most of them derived from Sumai3 (Bai and Shaner 2004). Limited resistant sources used in breeding may pose vulnerability to resistance breakdown and severe disease epidemics. Exploring new sources of resistance will facilitate pyramiding of different QTLs to increase the resistance level and diversity of resistant sources. Several Chinese landraces showed a high level of FHB resistance (Yu et al. 2008a). One of them is Haiyanzhong (HYZ) that shows a similar level of FHB resistance as Sumai3 (Li et al. 2011). Using 136 recombinant inbred lines (RILs) derived from a cross between HYZ and Wheaton, a susceptible cultivar, Li et al. (2011) did not find *Fhb1*, the most common QTL for FHB resistance in Chinese sources, instead, they identified a major QTL on 7DL that explained 15.9-22.6% of the phenotypic variance in both greenhouse and field experiments and four other QTLs with minor effects. Thus, HYZ might be a different source of resistance from Sumai3.

The objectives of the present study were to (1) validate the previous mapped QTL on 7D in HYZ using a new and larger population; (2) identify new QTLs by using a high-density SNP map; (3) develop tightly linked markers to the QTLs and convert them to

breeder friendly Kompetitive allele specific PCR (KASP) assays for marker-assisted selection (MAS).

Materials and methods

Plant materials and FHB evaluation

A population of 186 F_7-derived RILs was developed from a cross between HYZ and the U.S. FHB-susceptible hard red spring wheat variety Wheaton by single-seed descent. The RILs were evaluated for FHB resistance in the greenhouses on spring and fall 2012 and spring 2013 at Kansas State University in Manhattan, Kansas. Seeds of the RILs and two parents were planted in plastic trays filled with Metro-mix 360 soil mix (Hummert International, Topeka, KS). After 50 d of vernalization at 6^{o}C in a cold room, about 12 seedlings per line were separated into two replications and transplanted into 4" x 4" Dura pots filled with Metro-mix 360 soil mix. The pots were arranged on greenhouse benches in a randomized complete block design (RCBD) with two replications (pots) per line. The greenhouse was maintained at 17 $\pm$2^{o}C at night and 22 $\pm$5^{o}C during the day with 12 h supplemental daylight.

A Kansas strain of *F. graminearum* (GZ3639) was used as inocula, and a conidial spore suspension was prepared following Bai et al. (1999). At early anthesis, wheat spikes were inoculated by injecting 10 µl of the conidial spore suspension (~1000 spores/spike) into a floret of a central spikelet in a spike using a syringe (Hamilton, Reno, NV). Five spikes per pot were inoculated and maintained in a moist chamber at 100% relative humidity and 20 to 22^{o}C for 48 h to initiate fungal infection. Then the plants were returned to the greenhouse benches for further FHB development. FHB symptom spread within a spike (type II resistance) was evaluated by counting the symptomatic

spikelets and total spikelets in an inoculated spike 15 d after inoculation. Percentage of symptomatic spikelets (PSS) from each RIL in each experiment and mean PSS across all three experiments were calculated and used for QTL analysis.

DNA extraction and analysis of simple sequence repeats

Leaf tissue was collected at the three-leaf stage in 96-deepwell plates, dried in a freeze dryer (ThermoSavant, Holbrook, NY) for 48 h, and ground using a Mixer Mill (MM 400, Retsch, Germany). Genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide protocol (Maguire et al. 1994).

A core set of 384 simple sequence repeat (SSR) primer pairs were used to screen the two parents, HYZ, and Wheaton. This core primer set was originally selected from 2000 primer pairs (http://wheat.pw.usda.gov) based on the result of previous studies conducted at the USDA Central Small Grain Genotyping Laboratory in Manhattan, KS. The markers are distributed on all the 21 wheat chromosomes (Somers et al. 2004). Primers that amplified at least one polymorphic band between the parents were used to screen the 186 RILs. Polymerase chain reaction (PCR) amplification was done in an MJ Research PTC-200 Thermal Cycler (Bio-Rad, Hercules, CA). For SSR detection, an M13 tail (5'-ACGACGTTGTAAAACGAC) was added to 5'-end of all forward primers. A 10-µl PCR master mix contained 1X ASB buffer, 2.5 mM of MgCl2, 200 µM of dNTP, 100 nM of fluorescent dye-labeled M13 primer, 100 nM of M13 tailed forward primer, 200 nM of a reverse primer, 0.6 U of *Taq* polymerase, and 40 ng of template genomic DNA. PCR amplification was done using a touchdown program. The PCR mixture was incubated initially at 95^{o}C for 5 min, followed by five cycles of 96^{o}C for 1 min, annealing at 68^{o}C for 3 min with a decrease of 2^{o}C in each subsequent cycle, and extension at 72^{o}C

for 1 min. For another five cycles, annealing temperature started from 58^{o}C for 2 min with a decrease of 2^{o}C in each subsequent cycle, and then PCR went through an additional 25 cycles of 96^{o}C for 1 min, 50^{o}C for 1 min, and 72^{o}C for 1 min, ending with a final extension at 72^{o}C for 5 min. Amplified PCR products from four PCRs labeled with different fluorescent dyes (FAM, VIC, NED, and PET) were pooled and analyzed in an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Data were scored using GeneMarker v1.75 (SoftGenetics LLC, State Collage, PA).

GBS library construction and SNP genotyping

A GBS library was generated from 186 RILs and three replicates of both parents using a previously described protocol (Poland et al. 2012). In brief, DNA concentration was quantified using the Quant-iT^{TM} PicoGreen® dsDNA Assay (Life Technologies Inc., Grand Island, NY) and normalized to 20ng/µl. Each DNA sample was digested with *HF-PstI* (High-Fidelity) and *MspI* (New England BioLabs Inc., Ipswich, MA), ligated with one of 192 barcoded adaptors and the Y common adaptor using T4 ligase (New England BioLabs Inc.). Ligated samples with different barcodes were pooled into a single tube, cleaned up using a QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA), and then amplified by PCR with 5µl *Taq* 5X Master Mix (New England BioLabs Inc.) and 10uM Ion primers. The PCR mixture was incubated initially at 95^{o}C for 30 sec, followed by 16 cycles of 95^{o}C for 30 sec, 62 ^{o}C for 20 sec, and 68 ^{o}C for 1min, and then PCR end with a final extension at 72^{o}C for 5 min. The PCR products were cleaned up again using the QIAquick PCR Purification Kit, size-selected for a range of 250-300 bp in an E-gel system (Life Technologies Inc.), and sequenced in an Ion Proton system (Life Technologies Inc.). GBS data analysis was performed using UNEAK, and independent

reference pipeline of TASSEL (Lu et al. 2013; Poland et al. 2012). For those reads with less than 64 bp, a poly-A tail was added to the reads to ensure all reads were 64 bp. The accuracy of GBS-SNP calls was validated using Kbioscience allele-specific PCR (KASP) assays (LGC Biosearch Technologies, Petaluma, CA). The KASP assays were designed from the corresponding GBS sequences harboring the SNPs that were mapped to the QTL regions. KASP assays consisted of three KASP primers: two allele-specific forward primers and one common reverse primer. The KASP master mix for each reaction comprised of 3 µl of 2x KASP reaction mix, 0.0825 µl of KASP primer mix (100 µM) and 3 µl of DNA (~40 ng). Samples were incubated at 94^{o}C for 15 min, followed by 10 cycles of 94^{o}C for 20 s and annealing at 65^{o}C for 1 min with a decrease of 0.8^{o}C in each subsequent cycle. Then the PCR went through an additional 40 cycles of 94^{o}C for 20 sec and 57^{o}C for 1 min. After PCR, plates were read in an Applied Biosystems 7900HT Fast Real-Time PCR System (Life Technologies Inc.,). The number of mismatches between GBS-SNP and KASP-SNP data was counted and compared. If any mismatch between KASP markers and corresponding GBS-SNPs, the KASP markers were remapped with other GBS-SNPs to validate the map locations.

Genetic map construction and QTL analysis

A linkage map with both SSR and GBS-SNP markers was constructed using Kosambi mapping function (Kosambi 1944) and 'regression' mapping algorithm in JoinMap version 4.0 (Van Ooijen 2006). QTLs for PSS were determined using Composite Interval Mapping (CIM) in WINQTL Cartographer version 2.5 with Model 6 (Wang et al. 2005). The permutation test was performed 1000 times to determine the LOD threshold for claiming significant QTLs at $P < 0.05$ (Churchill and Doerge 1994).

Results

FHB variation among RILs and between parents

The resistant parent HYZ showed a high level of FHB resistance in all three greenhouse experiments, with an average PSS of 11.20%, ranging from 7.62 to 14.81%, whereas Wheaton, the susceptible parent, had a mean PSS of 97.75%, ranging from 95.5 to 100% (Figure 2.1). The mean PSSs of RILs across all the three experiments ranged from 7.61% to 100%. PSS frequencies showed continuous distribution skewed toward HYZ in spring and fall 2012, but toward Wheaton in spring 2013 (Figure 2.1). Mean PSS over all RILs was 46.16%, ranging from 39.91% (spring 2012) to 55.32% (spring 2013), indicating the highest disease pressure in spring 2013 and the lowest in spring 2012. Transgressive segregation was not evident in spring 2012, but obvious in fall 2012 and spring 2013, suggesting there might be QTL contributed by the susceptible parent. The positive correlations were highly significant among the three greenhouse experiments, ranging from 0.58 to 0.64 ($P < 0.001$). Significant variation in genotypes, environments, and genotypes by environments was observed in the three experiments (Table 2.1). The broad sense heritability was very high ($H = 0.81$).

Construction of a linkage map

The GBS- SNPs were analyzed for 172 RILs after removing 14 RILs that had excessive missing data. After four Ion Proton runs of 192 samples, 21740 GBS-SNPs were called with 80% missing data. Among them, 6232 had 20% or less missing data and were used for mapping. For SSR, a core set of 384 SSR markers were screened between parents, and 132 were polymorphic, thus used to screen all the RILs. Of the 6364 markers (6232 SNPs and 132 SSRs) analyzed in the mapping population, 4624 (72.7%) were

mapped to 48 linkage groups with at least three markers in each group. The map covered all 21 chromosomes at a genetic distance of 4044.34 cM with an average marker density of 0.87 cM per marker. Among the three genomes of wheat, the B genome has the most markers (49.2%), followed by the A genome (40.8%) and the D genome (10.0%). Marker density was the greatest in a linkage group corresponding to chromosome 3A, with an average density of 0.50 cM per marker, while the least on a linkage group corresponding to chromosome 3D, with an average interval of 5.84 cM between markers.

QTLs for FHB resistance

CIM mapping detected eight significant QTLs for FHB resistance on 5AS, 6BS, 7DL, 2B (two QTLs), 4D, 3B and 4B (Figure 2.2 and 2.3). The QTL on chromosome 5A showed a major effect. The QTLs on 5AS, 6BS and 7DL were previously mapped in Li et al. (2011), whereas the other five were newly mapped QTLs in the current study. The 5A QTL showed a significant major effect in all three experiments and explained 6.10~15.98% of the phenotypic variation (Table 2.2, Figure 2.2). This QTL was delineated to a 1.88 cM interval between SNPs *GBS3127* and *Xbarc316*. *GBS3127* showed the largest effect on FHB type II resistance in all the three experiments among all markers tested, and two genotypic groups carrying the contrasting alleles at the QTL had a significant difference in the mean PSS (Table 2.4). Other SNPs that closely linked to the QTL including *GBS1852* and *GBS5669* on the one side of QTL, and *GBS2573* and *GBS1691* on the other side of the QTL all showed significant effects on Type II resistance.

The QTL on 6BS, flanked by SNPs *GBS4963* and *GBS3704*, was significant with spring 2012 and 2013 data, and mean PSS data. This QTL explained 6.91~11.11% of the

phenotypic variation (Table 2.2, Figure 2.3). Six SNPs were mapped to a 2.39 cM interval, with *GBS4305* and *GBS 4116* showing the largest effect on FHB resistance.

The QTL on 7DL was flanked by *Xcfd46* and *Xwmc702*, with *Xcfd46* showing the largest effect on FHB resistance. The QTL was significant in spring 2012 and 2013, and the mean PSS, which explained 5.59~7.53% of the phenotypic variation (Table 2.2, Figure 2.3).

Two QTLs for FHB resistance were mapped on the short arm of chromosome 2B. The susceptible parent "Wheaton" contributed positive alleles for both QTLs. The 2B_1 QTL was significant in spring 2013 only, and flanked by SNPs *GBS1340* and *GBS0835*, explained 5.80% of the phenotypic variation (Table 2.2, Figure 2.3), whereas 2B-2 QTL was 40 cM away from 2B-1 QTL that was delimited to a 3.3 cM interval and flanked by SNPs *GBS5561* and *GBS0848*. This QTL was significant in fall 2012 and mean PSS, and explained 5.10~7.77% of the phenotypic variation (Table 2.2, Figure 2.3).

One QTL on the short arm of chromosome 4D was mapped between SNPs *GBS3233* and *GBS4883*, and significant in fall 2012 only. This QTL explained 14.54% of the phenotypic variation (Table 2.2, Figure 2.3). One QTL on the long arm of chromosome 3B that flanked by SNPs *GBS1778* and *GBS3048* was significant in the fall 2012 experiment, and explained 8.21% of the phenotypic variation (Table 2.2, Figure 2.3). Another QTL on the long arm of chromosome 4B was flanked by SNPs *GBS2348* and *GBS3434*, which was significant in spring 2012 only and explained 5.61% of the phenotypic variation (Table 2.2, Figure 2.3).

Seven GBS-SNPs were mapped in the 5A QTL region, nine in the 6B QTL region, five in the 2B-1 QTL region, seven in the 2B-2 QTL region, four in the 3BL QTL

region, two in the 4BL QTL region, and three in 4DS QTL regions. Twenty markers were mapped in the *Fhb1* region between markers *Xumn10* and *Xgwm493* (~12 cM), however, no significant QTL was identified in this region. To verify the accuracy of GBS-SNP data, and fill the missing data from the GBS-SNPs in the QTL regions, 21 KASP assays were designed according to the corresponding GBS sequences harboring the SNPs that were mapped in the QTL regions on 5AS, 6BS, or 2B_2. Fourteen KASPs assays amplified very well and were polymorphic between parents and among the RILs (Figure 2.4 A). Ten of them were remapped to the three corresponding significant QTL regions (four each on 6B and 5A, and two on 2B-2) (Table 2.3), and had identical allele calls with the corresponding GBS-SNPs across the RILs. The other four KASP markers were mapped outside the QTL regions with five mismatches in *GBS5920* and *GBS2732*, six mismatches in *GBS2577*, and more than ten mismatches in *GBS3018*, thus, these markers were not pursued further.

The ten KASPs (Table 2.3) that were remapped to the three significant QTL regions (6B, 5A, and 2B_2) were then validated in an association mapping (AM) population of 96 U.S. elite wheat lines and cultivars as well as four Chinese FHB resistant landraces, Huangcandou, Baishanyuehuang, Huangfangzhu and Wangshuibai, as controls. All of the ten KASPs amplified well in the AM population. Two KASPs on 6B QTL (*GBS4963*, and *GBS4116*) (Figure 2.4 (5, 7)) and one KASP on 5A QTL (*GBS2573*) (Figure 2.4 (3)) separated into almost equal clusters. Another three KASPs on 5A QTL (*GBS3127*, *GBS5669*, and *GBS1852*) (Figure 2.4 (1, 2, 4)) and another two on 6B QTL (*GBS0158* and *GBS4305*) (Figure 2.4 (6)) showed unequal cluster with more lines in 'Wheaton' allele cluster. Among them, SNPs *GBS3127* on 5A (Figure 2.4 (1))

and *GBS4305* and *GBS0158* on 6B had all of 'Wheaton' alleles in the AM population, except one or two with heterozygous genotypes. Two KASPs for 2B-2 QTL (*GBS5855*, and *GBS1713*) (Figure 2.4 (8, 9)) showed unequal clusters with more lines in 'HYZ' allele cluster. All of the ten KASPs can be useful for MAS in U.S. winter wheat if the markers are polymorphic in breeding parents. However, the ones with extremely unequal clusters (*GBS3127*, *GBS4305*, and *GBS0158*) may be effective to be used as diagnostic markers.

Effects of QTLs on FHB type II resistance

To investigate the effect of individual QTLs on FHB resistance, RILs were grouped according to their allele combinations at three QTLs (5A, 6B, and 7D), and their allele substitution effects were compared among the groups. The three QTLs were selected because they were significant in at least two experiments and the mean PSS over the three experiments. Eight possible allelic combinations at the three QTLs are designated AABBDD, AABBdd, AAbbDD, aaBBDD, AAbbdd, aaBBdd, aabbDD and aabbdd, where AA, BB, and DD represent 'HYZ' alleles at QTLs on 5A, 6B, and 7D, respectively (Figure 2.5). The average PSSs for the eight genotypic groups of RILs ranged from 28.7% to 63.4%. The closet KASP markers to each of the three QTLs were *GBS3127* on 5A, *GBS4305* on 6B and *Xcfd46* on 7D, thus the three markers were used to represent the three QTLs to estimate the allelic effects of the three QTLs. The mean PSSs for the genotypic groups that had only one of the three resistance QTLs were 44.9% for 5A, 46.3% for 6B, and 55.1% for 7D (Figure 2.5), whereas the PSS for the group of RILs with none of the three resistance alleles ("null" group) was 63.4%, suggesting all the three QTLs reduced the FHB severity. The QTL on 5A showed the largest effect on FHB

resistance, and FHB severity of the 5A-containing group was significantly lower than that of "null" group ($P < 0.05$) (Figure 2.5). Meanwhile, the mean PSS of the RIL groups with 5A QTL plus an additional QTL (6B or 7D) were always lower than the mean PSS of the RIL group with 5A QTL only, but the difference was not significant.

Discussion

Fhb1 is absent in HYZ

Many Chinese wheat cultivars and landraces show a high level of FHB type II resistance (Cai and Bai 2014; Li et al. 2011; Li et al. 2012; Yu et al. 2008a; Yu et al. 2008b; Zhang et al. 2012), and most of them, especially landraces, carry *Fhb1* on the short arm of chromosome 3B. HYZ showed similar levels of resistance as WSB, HCD, BSYW and HFZ (Cai and Bai 2014; Jia et al. 2006; Li et al. 2012; Yu et al. 2008a; Yu et al. 2008b; Zhang et al. 2012), but it does not carry the *Fhb1* as in other landraces (Li et al. 2011). However, in a population of 136 RILs derived from the cross HYZ x 'Wheaton', Li et al. (2011) found a QTL near *Xwmc121* on the 7DL that showed a major effect on FHB resistance. In the current study, we developed a new population of 186 RILs using the same parents and constructed a high-density GBS-SNP map to validate the QTL mapping results by Li et al. (2011). The results of the current study confirmed that *Fhb1* is absent in HYZ although both diagnostic and flanking markers for *Fhb1* are polymorphic, suggesting that a high level of resistance in HYZ is not conditioned by *Fhb1* as in other Chinese landraces and is due to additive effects of multiple QTLs with minor effects.

QTLs for type II FHB resistance in HYZ

Among the eight QTLs identified in the current study, the QTL on 5AS explained the largest phenotypic variation (6.10~15.98%) across different experiments. To date, more than 14 QTLs for FHB resistance have been reported in 5A and explained 4.5~32% of the phenotypic variation across different experiments (Buerstmayr et al. 2002; Chu et al. 2011; Li et al. 2011; Lin et al. 2006; Steiner et al. 2004; Xue et al. 2011; Yang et al. 2005b; Yu et al. 2008b; Zhang et al. 2012). Some of them were associated with type I resistance, such as in 'DH181' (Yang et al. 2005b), 'W14' (Chen et al. 2006), 'CM-82036' (Buerstmayr et al. 2003) and 'Wangshuibai' (Lin et al. 2006; Yu et al. 2008b), whereas others with type II resistance, such as in 'Wangshuibai' (Yu et al. 2008b), 'CM-82036' (Buerstmayr et al. 2002), 'Frontana' (Steiner et al. 2004), 'F201R' (Shen et al. 2003), 'CM-82036' (Buerstmayr et al. 2003), 'Renan' (Gervais et al. 2003), 'Ernie' (Liu et al. 2007), 'Baishanyuehuang' (Zhang et al. 2012). Two studies reported the 5A QTLs for type III resistance (low DON content) in 'NyuBai' (Somers et al. 2003) and 'Wangshuibai' (Yu et al. 2008b). More recently, two major QTLs were reported on the both arms of chromosome 5A of a wheat accession 'PI 277012' (Chu et al. 2011). The QTL on 5AS explained up to 20%, 14%, and 16% and the one on 5AL explained 32%, 12% and 10% of the phenotypic variation for type II, III and type IV (FDK) resistance, respectively (Chu et al. 2011). Because different studies reported QTLs in different chromosome locations for different types of resistance, several QTLs may condition different types of FHB resistance on the chromosome 5A. Meta-analysis found at least three QTL clusters (Liu et al. 2009) with two on the chromosome 5AL as mapped in a French cultivar 'Renan' (Gervais et al. 2003) and one with a major effect on type I, II, III,

IV resistance mapped near the centromere of 5AS from various sources including Wangshuibai, W14, and Frontana (Chen et al. 2006; Steiner et al. 2004; Yu et al. 2008b). Six SSRs (*Xbarc56, Xbarc117, Xgwm415, Xgwm304, Xwmc705, Xbarc180*) in one haplotype located in this major cluster were reported to be responsible for this 5AS QTL. Recently, this 5AS QTL was fine mapped to a 0.3 cM region flanked by *Xgwm304* and *Xgwm415* (Xue et al. 2011). In Li et al. (2011), a minor QTL was mapped on chromosome 5A flanked by SSR markers *Xbarc141* and *Xgwm129,* which belonged to the major QTL cluster on 5AS (Liu et al. 2009), but the QTL effect is quite small that explained only 3.9% and 7.4% of phenotypic variation in the greenhouse and the field experiments. In the current study, the QTL on 5A was mapped into a 1.88 cM interval between SNP *GBS3127* and SSR marker *Xbarc316,* which was 5.91 cM away from the 5A QTL mapped by Li et al. (2011), thus suggesting they are the same QTL. The slightly larger effect of the QTL detected in this study compared to that reported by Li et al. (2011) might be due to the presence of markers close to the QTL in this study.

The QTL on 6BS in the current study was assigned to the interval between *GBS4963* and *GBS3704*, which explained 8.26~11.11% of the phenotypic variation in two of the three experiments and the mean PSS. This QTL was mapped very closely (about 2 cM) to *Xgwm88* and *Xwmc397*, the markers linked to *Fhb2*, thus may be the same QTL as *Fhb2* (Cuthbert et al. 2007). *Fhb2* has been reported with varied effects ranging from 4.4~24.0% on type II resistance (Cuthbert et al. 2007; Li et al. 2011; Yang et al. 2005b) in different cultivars including 'Nanda2419' (Lin et al. 2004), 'Sumai3' (Waldron et al. 1999), 'Wangshuibai' (Lin et al. 2004), 'Arina' (Semagn et al. 2007), and 'DH181' (Yang et al. 2005b). This QTL was mapped on HYZ previously in a 6.0 cM

interval (Li et al. 2011). In the current study, it was also mapped in the same location but with a smaller marker interval of 2.39 cM. Apparently, the GBS-SNPs applied in this study increased the marker density in the QTL region.

The QTL on the 7DL in the current population coincides with the major QTL reported by Li et al. (2011), but explained much smaller phenotypic variation (5.59~7.53%) than in previous report (Li et al. 2011). Two QTLs have been mapped to 7D showed minor effects for type III resistance in Arina/Riband (Draeger et al. 2007) and type IV resistance in Nanda2419/Wangshuibai (Li et al. 2007). These two QTLs are most likely the same QTL mapped in HYZ, because 7D QTL in HYZ shared a commonly linked marker *Xcfd46* with Wangshuibai and clustered in the same meta-QTL with 'Arina' (Liu et al. 2009). The discrepancy in QTL effect between the current study and Li et al. (2011) might be partially due to the differences in population size, marker density in the maps used for QTL mapping and environment conditions. The QTL effect can be overestimated in a small population, thus, the increase of population size may reduce the number of false-positive QTLs and improve the estimation accuracy of QTL effects.

Two minor QTLs were mapped on the short arm of chromosome 2B with 2B-2 explained 5.10 and 7.77% of the phenotypic variation in fall 2012 experiment and mean PSS, respectively, and 2B-1 explained 5.80% of the phenotypic variation in spring 2013 experiment. Several QTLs on 2B have been previously reported in different populations with one QTL close to *Xgwm120* mapped on 2BL in Ning7840 (Zhou et al. 2002) and 'Ernie' (Liu et al. 2007) for type II resistance, and a QTL close to *Xgwm210* on 2BS in 'Renan' for type II resistance (Gervais et al. 2003) and in 'Patterson' x 'Goldfield' population for type I resistance (Gilsinger et al. 2005). Both *Xgwm120* and *Xgwm210*

were mapped in the current study with *Xgwm120* on the long arm and *Xgwm210* on the short arm of chromosome 2B, but they were far from the QTLs 2B-1 and 2B-2. Therefore, the both QTLs 2B-1 and 2B-2 were novel QTLs for type II resistance. Interestingly, they are all from the susceptible parent 'Wheaton', suggesting that some highly susceptible cultivars may also harbor minor QTLs for resistance.

The 4D QTL was significant in fall 2012 only, explained 14.54% of the phenotypic variation. A few QTLs have been reported on chromosome 4D in DH181 for FHB type I and IV resistance (Yang et al. 2005b), in Chinese Spring x SM3-7ADS for FHB type II resistance (Ma et al. 2006), in 'Arina' for type II resistance (Draeger et al. 2007), and in 'Spark' for type II resistance (Srinivasachary et al. 2008). However, their allelic relationship between this QTL and previously reported ones remains to be determined because common markers are not available among these QTLs.

The QTL on 3BL was flanked by SNPs *GBS1778* and *GBS3048*, with *Xbarc164* as the closest marker. This QTL was significant in fall 2012 only, and explained 8.52% of the phenotypic variation. Many studies mapped *Fhb1*, a QTL for type II resistance (Cai and Bai 2014; Cuthbert et al. 2006; Li et al. 2012; Yang et al. 2005b; Yu et al. 2008b; Zhang et al. 2012) and QTL near centromere region of chromosome arm 3BS (Cai and Bai 2014; Yu et al. 2008b; Zhang et al. 2012). Only one QTL from Huapei 57-2 has been mapped on 3BL with *Xgwm247* as the closest marker (Bourdoncle and Ohm 2003). However, *Xbarc164* was far away (about 100 cM) from *Xgwm247* according to 3B reference physical map (http://wheat.pw.usda.gov/GG3/), therefore, the 3BL QTL mapped in this study is most likely a new QTL.

The QTL near *Xgwm6* on 4B was flanked by SNPs *GBS2348* and *GBS3434*, explained 5.61% of the phenotypic variation in the spring 2012 experiment. A formally named QTL *Fhb4* on chromosome 4B was previously mapped in 'Ernie' (Liu et al. 2007), 'Chokwang' (Yang et al. 2005a), 'Wangshuibai' (Jia et al. 2006; Lin et al. 2004), and 'Wuhan1' (Somers et al. 2003), and explained 4.7~17.5% of the phenotypic variation. This QTL likely coincides with the *Fhb4*, because *Xgwm6* is closely linked to *Fhb4*-linked marker *Xgwm149* and they were all mapped on 4BL5-0.86–1.00 bin (Xue et al. 2010).

Conversion of GBS-SNPs into KASP assays

GBS facilitates quick identification of SNPs for QTL mapping and many other applications at a low cost by multiplexing samples using barcodes (Li et al. 2015; Lin et al. 2015; Poland et al. 2012; Talukder et al. 2014). However, GBS also generates a large number of missing data across a mapping population due to the limited sequence depth (Poland et al. 2012; Sonah et al. 2013; Spindel et al. 2013). One way to solve this problem is to impute the data based on available reference genome sequences to predict missing data (Spindel et al. 2013). However, the wheat reference genome sequences are not complete, and imputed data may not be accurate for QTL mapping. Another way is to increase the number of runs for each library to reduce the number of missing data. In the current study, four Ion Proton runs of this population significantly increased numbers of SNPs when compared with a single run. We totally got 21740 GBS-SNPs with 80% missing data and 6232 GBS-SNPs with <20% missing data from the four runs. For a small set of GBS-SNPs that were mapped in the QTL regions, missing data were filled by KASP data that not only eliminated missing data, but also verified the accuracy of GBS-

SNP data by comparing the GBS-SNPs with KASP data in the segregating population. Among the 21 KASP assays designed, 14 were amplified well, and seven did not because SNP positions are too close to one end of the sequence reads that cause difficulties in primer design. Ten of the 14 amplified KASPs were remapped to the same positions corresponding to GBS-SNPs mapped, while the other four were not mapped to the expected positions due to either GBS sequencing errors or SNP calling errors. The ten KASPs were then validated in an association mapping (AM) population with 96 U.S. elite lines and cultivars. The seven KASP assays separated into two unequal clusters of HYZ and Wheaton alleles. Five KASP assays (*GBS3127*, *GBS5669* and *GBS1852* for 5A, *GBS0158* and *GBS4305* for 6B) amplified 'Wheaton' alleles in most of U.S. elite wheat lines, with only a few or none of the lines amplifying HYZ alleles in the AM population, indicating most of the elite lines/varieties may not have these two QTLs yet and thus, these KASPs can be effectively used to transfer them. KASPs *GBS1713* and *GBS5855* on 2B amplified more lines on the cluster of HYZ alleles than the 'Wheaton' alleles. Because 2B QTL-2 was contributed by susceptible parent 'Wheaton', the HYZ alleles on these two markers were prevalent in the AM population, therefore, these markers are good markers for transferring the QTL into U.S. winter wheat. However, lines amplified with three KASPs (*GBS4963*, *GBS2573*, and *GBS4116*) were separated almost equally into two clusters, indicating that the HYZ alleles in half of the U.S winter wheat accessions studied, and these KASP markers can still be useful for MAS in U.S. winter wheat if the breeding parents are polymorphic, however, they may not be effective to be used for diagnostic purpose.

Conclusion

Using a high-density GBS-SNP map developed from 186 RILs of 'HYZ' x 'Wheaton', we identified eight QTLs, without *Fhb1*, in HYZ controlling FHB type II resistance, suggesting an additive effect of multiple QTLs can provide a high level of resistance in wheat. Among them, five QTLs on the chromosomes 2B (2), 3B, 4B, and 4D are different from these mapped in Li et al. (2011) using the population developed from the same parents. The QTL on 4B was the same as the previously mapped *Fhb4*. The two QTLs on chromosome 2BS and the one on 3BL are novel QTLs that were not mapped before. The allelic relation between QTL on chromosome 4D and previously reported QTL in this chromosome cannot be determined due to lack of common markers. Ten GBS-SNPs linked to the QTLs on 5A, 6B and 2B-2 were successfully converted to KASP assays and validated using 96 U.S. elite winter wheat lines, they can be used in MAS to pyramid these QTLs in breeding.

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Table 2.1 Analysis of variance (ANOVA) of percentage of symptomatic spikelets (PSSs) data for the RILs based on three greenhouse experiments

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Experiment	2	4.33	2.17	100.34	<0.0001
Genotype	185	40.22	0.22	10.08	<0.0001
Replication(experiment)	3	0.046	0.15	0.72	0.5420
Genotype*experiment	364	15.37	0.0422	1.96	<0.0001
Error	506	10.92	0.022		
Total	1060	72.48			

Table 2.2 Flanking markers, Logarithm of odds (LOD), coefficients of determination (R2) of the significant QTL regions detected by inclusive composite interval mapping based on spring 2012, fall 2012, and spring 2013 greenhouse FHB type II resistance data.

Locus	Resistance allele from	Flanking markers	Spring 2012		Fall 2012		Spring 2013		Combined mean	
			LOD	R^2%	LOD	R^2%	LOD	R^2%	LOD	R^2%
5AS	HYZ	*GBS3127~Xbarc316*	3.31	6.10	4.85	10.26	5.82	12.15	6.63	15.98
6BS	HYZ	*GBS4963~GBS3704*	3.97	8.26	-	-	5.80	11.11	3.57	6.91
7DL	HYZ	*Xcfd46~Xwmc702*	3.47	6.32	-	-	3.59	7.53	2.86	5.59
2B-1	Wheaton	*GBS1340~GBS0835*	-	-	-	-	3.28	5.80	-	-
2B-2	Wheaton	*GBS5561~GBS0848*	-	-	2.81	5.10	-	-	3.67	7.77
4D	HYZ	*GBS3223~GBS4883*	-	-	6.06	14.54	-	-	-	-
3B	HYZ	*GBS1778~GBS3048*	-	-	3.83	8.21	-	-	-	-
4B	HYZ	*GBS2348~GBS3434*	3.03	5.61	-	-	-	-	-	-

Note: '-' represents not significant.

Table 2.3 List of KASP assays developed from GBS sequences

Primer name	Position	Primer sequence (5'-3')
GBS3127_H	5A	GAAGGTGACCAAGTTCATGCTTACCCGCATTCCAGTCTCTt
GBS3127_W	5A	GAAGGTCGGAGTCAACGGATTTACCCGCATTCCAGTCTCTc
GBS3127_R	5A	TCCCTAGCTGCGACCTTTCC
GBS5669_H	5A	GAAGGTGACCAAGTTCATGCTCAGTGCCAATCTGTTCGCAa
GBS5669_W	5A	GAAGGTCGGAGTCAACGGATTCAGTGCCAATCTGTTCGCAg
GBS5669_R	5A	GGTGTGATCGCACGGGACTC
GBS2573_H	5A	GAAGGTGACCAAGTTCATGCTCAGCGAGCAGGCACAGTAAAAc
GBS2573_W	5A	GAAGGTCGGAGTCAACGGATTCAGCGAGCAGGCACAGTAAAAt
GBS2573_R	5A	TGGCTAGAAACGCTCGCAGA
GBS1852_H	5A	GAAGGTGACCAAGTTCATGCTCAGCAGTTTCACCAACATTAATCATACt
GBS1852_W	5A	GAAGGTCGGAGTCAACGGATTCAGCAGTTTCACCAACATTAATCATACa
GBS1852_R	5A	TGGTCTTTGATGTAGTGTTCGACATTT

GBS0158_H	6B	GAAGGTGACCAAGTTCATGCTGCCTCAGCCCCCCTTGAt
GBS0158_W	6B	GAAGGTCGGAGTCAACGGATTGCCTCAGCCCCCCTTGAc
GBS0158_R	6B	CGTGGGTTTGGGGATCTAGG
GBS4963_H	6B	GAAGGTGACCAAGTTCATGCTGAATCCTATTTGACACTGCAGGTGTt
GBS4963_W	6B	GAAGGTCGGAGTCAACGGATTGAATCCTATTTGACACTGCAGGTGTc
GBS4963_R	6B	GACAGCGCCCGTTAGCAAAA
GBS4305_H	6B	GAAGGTGACCAAGTTCATGCTCCCGTTAGCAAAATGCCCTATAAt
GBS4305_W	6B	GAAGGTCGGAGTCAACGGATTCCCGTTAGCAAAATGCCCTATAAc
GBS4305_R	6B	ACGTTTAAGGCGCCGAACAT
GBS4116_H	6B	GAAGGTGACCAAGTTCATGCTGCCGATTGACAGCGCg
GBS4116_W	6B	GAAGGTCGGAGTCAACGGATTGCCGATTGACAGCGCt
GBS4116_R	6B	TAGTGACATGGCCCGCGTAG
GBS5855_H	2B-2	GAAGGTGACCAAGTTCATGCTGCCCCTAAATGTGAAGAACTGGTCg

GBS5855_W	2B-2	GAAGGTCGGAGTCAACGGATTGCCCCTAAATGTGAAGAACTGGTCa
GBS5855_R	2B-2	AAAACGGCCGCTCTCTCTCC
GBS1713_H	2B-2	GAAGGTGACCAAGTTCATGCTGTTGGGGCTATCAAATTTTTCg
GBS1713_W	2B-2	GAAGGTCGGAGTCAACGGATTGTTGGGGCTATCAAATTTTTCa
GBS1713_R	2B-2	GACGGGGTTGGAATGAA

Note: H forward primer with 'HYZ' allele, W forward primer with 'Wheaton' allele, R reverse primer

Table 2.4 Difference between FHB resistance (R) and susceptible (S) alleles at QTL on chromosome 5A as reflected by the closely linked markers, and coefficients of determination of the allele estimated from HYZ/Wheaton RILs on three greenhouse experiments.

Locus	genotype	Spring 2012	Fall 2012	Spring 2013	Mean PSS
	HYZ	34.31	37.18	48.33	39.99
GBS3127	Wheaton	47.18	51.81	63.96	54.23
	Diff.	12.87*	14.63*	15.63*	14.24*
	R^2	0.0867	0.0844	0.1090	0.1242
	HYZ	34.23	35.73	46.31	38.73
Xbarc316	Wheaton	44.45	49.68	62.65	52.22
	Diff.	10.22*	13.95*	16.35*	13.49*
	R^2	0.0559	0.0794	0.1143	0.1120

Note: * means the significant difference between PSSs of group ‘HYZ’ and ‘Wheaton’.

Figure 2.1 Frequency distribution of mean percentage of symptomatic spikelets in a spike (PSS) for the recombinant inbred line (RIL) population derived from 'Haiyanzhong' (HYZ) x 'Wheaton' evaluated in spring 2012, fall 2012 and spring 2013 greenhouse experiments.

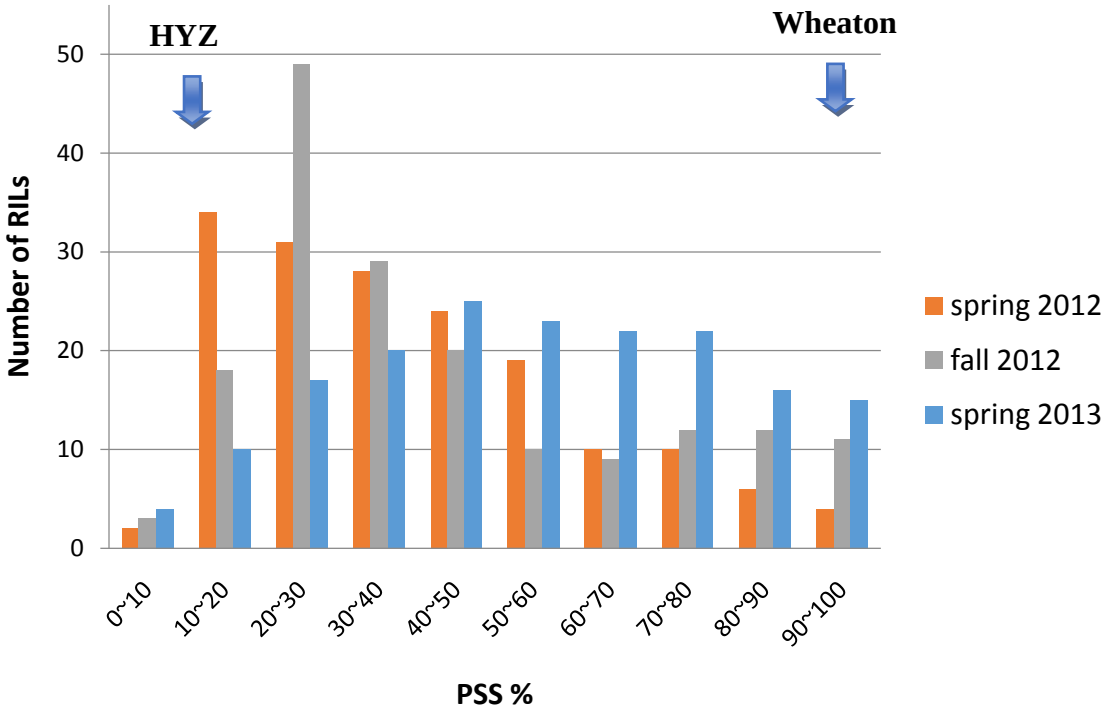


Figure 2.2 Maps of QTLs on 5A for FHB type II resistance constructed from the RIL population derived from the cross 'HYZ' x 'Wheaton' based on three greenhouse experiments

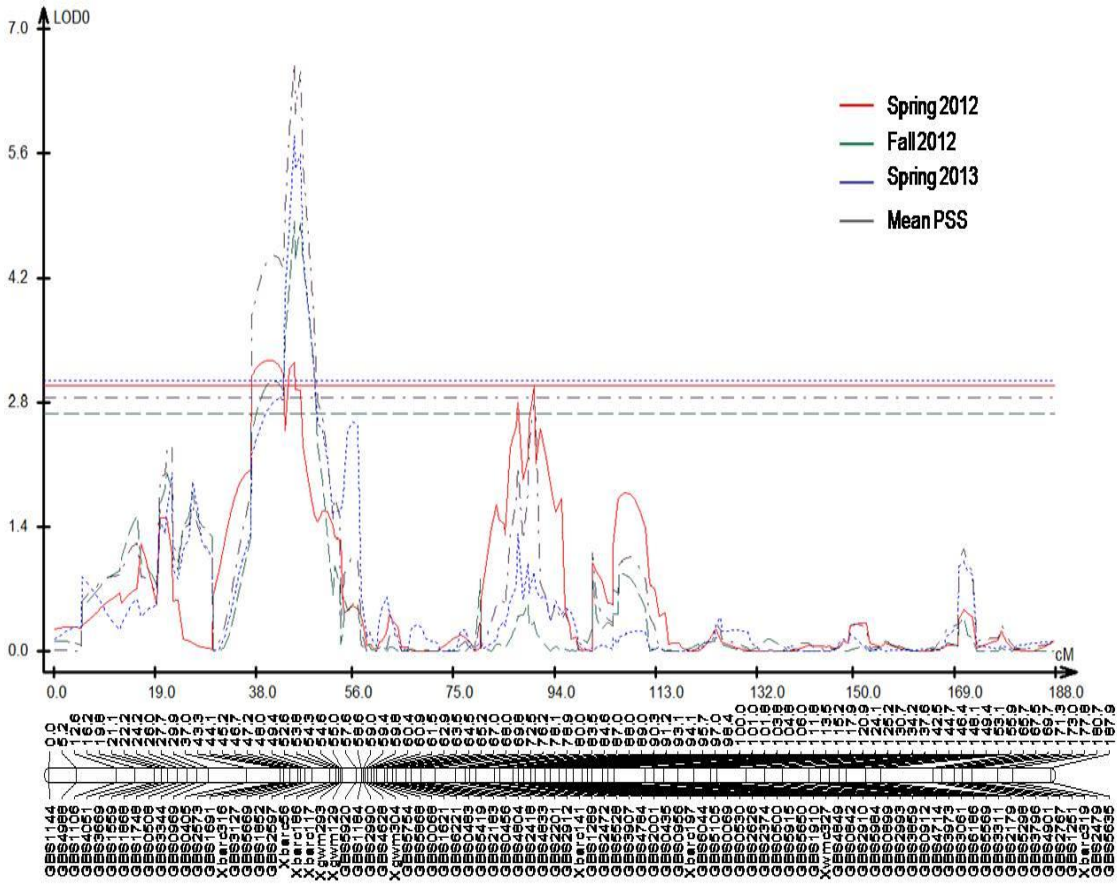
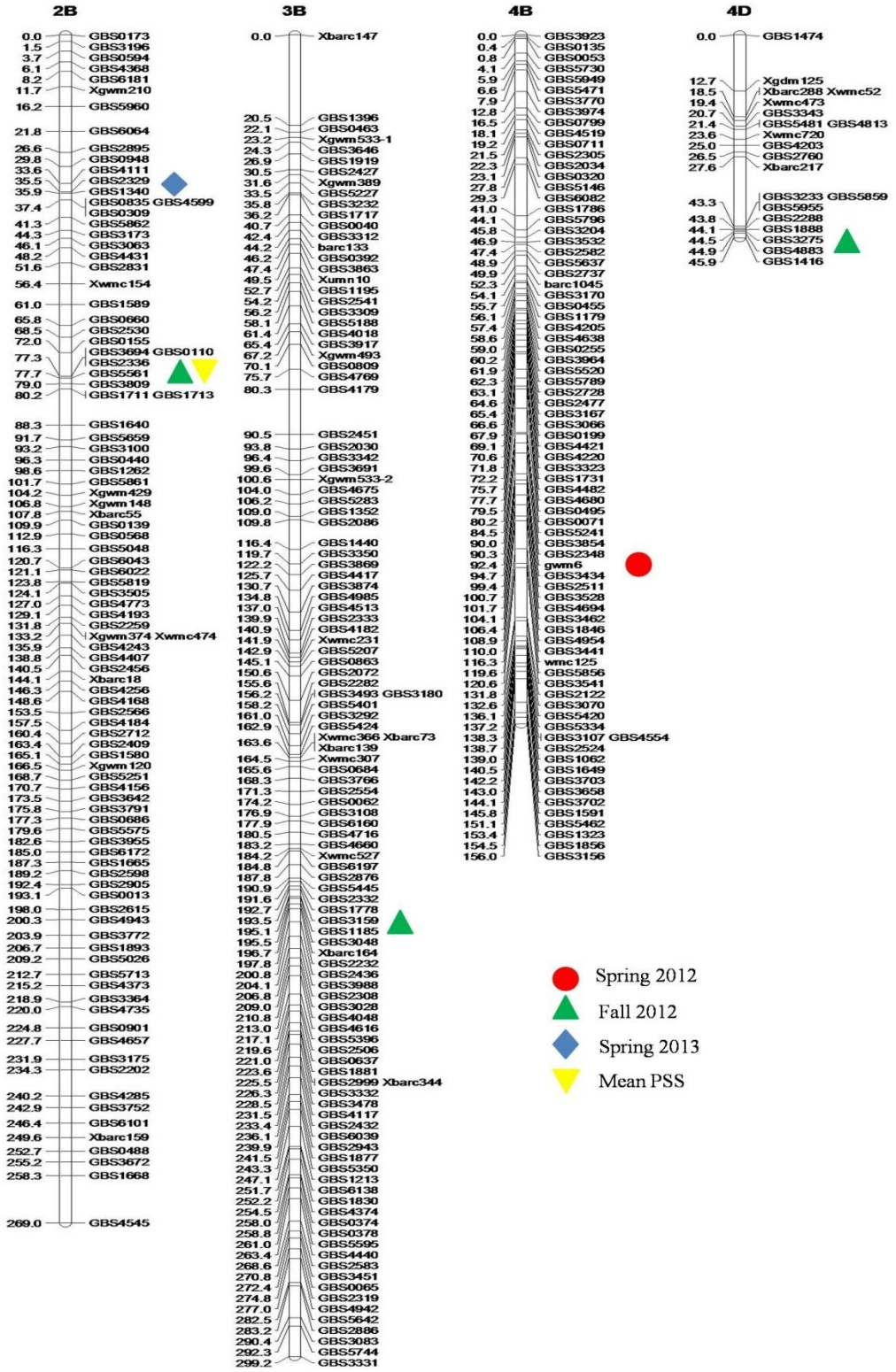


Figure 2.3 Maps of QTLs on 2B, 3B, 4B, 4D, 6B, and 7D for FHB type II resistance constructed from the RIL population derived from the cross 'HYZ' x 'Wheaton'



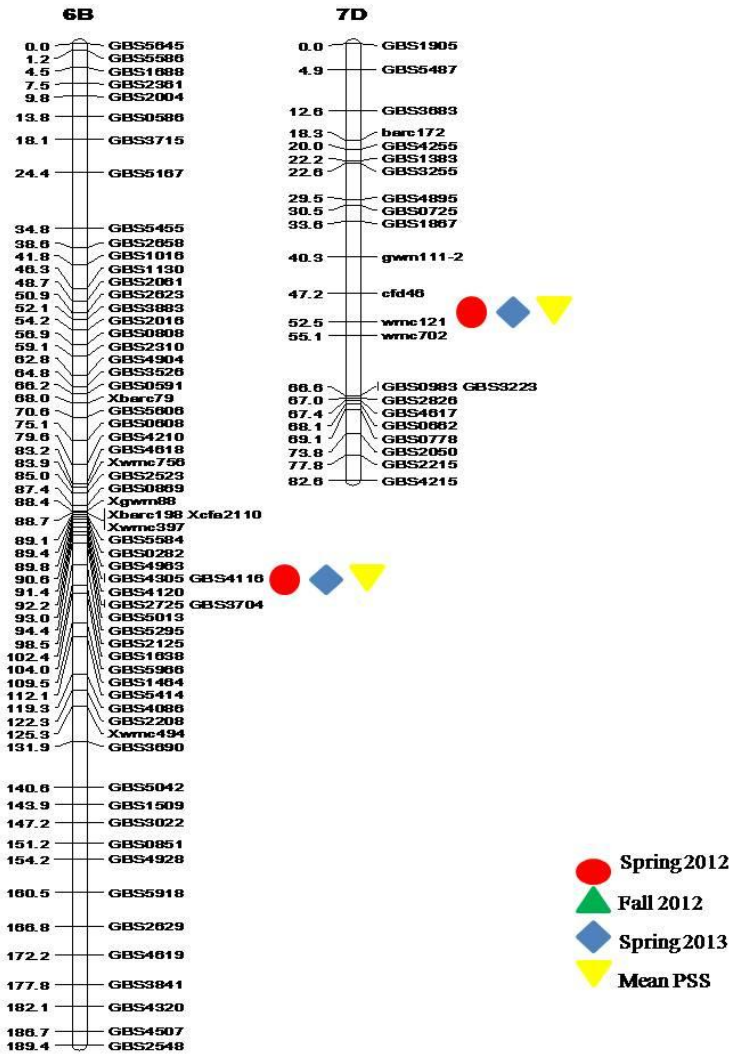
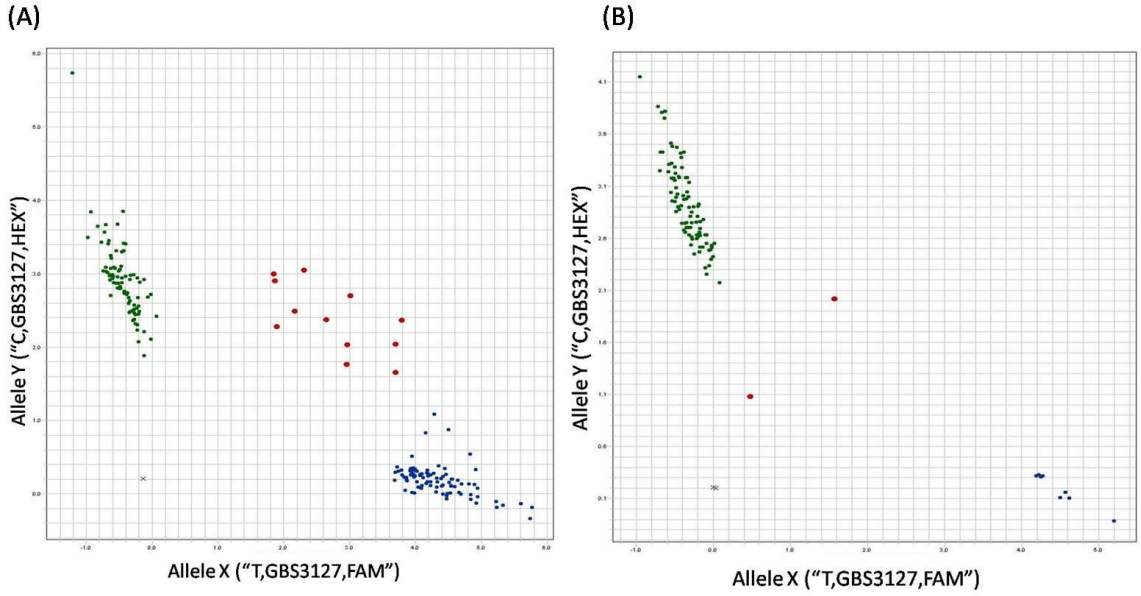
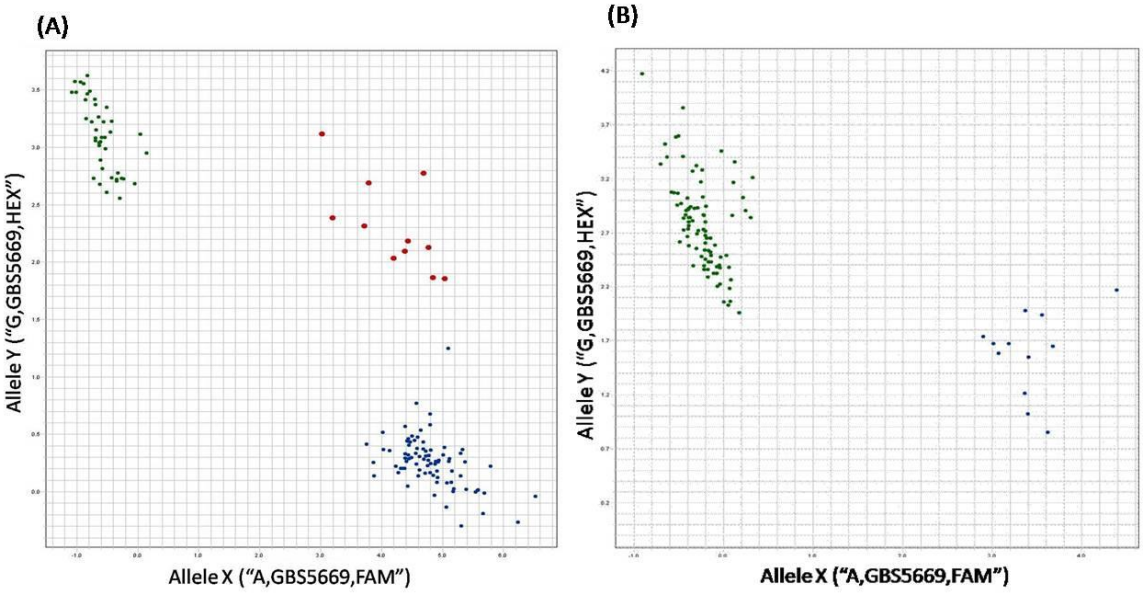

6B
0.0 GBS5645
1.2 GBS5586
4.5 GBS1688
7.5 GBS2361
9.8 GBS2004
13.8 GBS0586
18.1 GBS3715
24.4 GBS5167
34.8 GBS5455
38.6 GBS2658
41.8 GBS1016
46.3 GBS1130
48.7 GBS2061
50.9 GBS2623
52.1 GBS3883
54.2 GBS2016
56.9 GBS0808
59.1 GBS2310
62.8 GBS4904
64.8 GBS3526
66.2 GBS0691
68.0 Xbarc79
70.6 GBS5606
75.1 GBS0608
79.6 GBS4210
83.2 GBS4618
83.9 Xwmc756
85.0 GBS2523
87.4 GBS0869
88.4 Xgwm88
88.7 Xbarc198 Xcfa2110
Xwmc397
89.1 GBS5584
89.4 GBS0282
89.8 GBS4963
90.6 GBS4305 GBS4118
91.4 GBS4120
92.2 GBS2725 GBS3704
93.0 GBS5013
94.4 GBS5295
98.5 GBS2125
102.4 GBS1638
104.0 GBS5966
109.5 GBS1464
112.1 GBS5414
119.3 GBS4088
122.3 GBS2208
125.3 Xwmc494
131.9 GBS3690
140.6 GBS5042
143.9 GBS1509
147.2 GBS3022
151.2 GBS0851
154.2 GBS4928
160.5 GBS5918
166.8 GBS2629
172.2 GBS4619
177.8 GBS3841
182.1 GBS4320
186.7 GBS4507
189.4 GBS2548
7D
0.0 GBS1905
4.9 GBS5487
12.6 GBS3683
18.3 barc172
20.0 GBS4255
22.2 GBS1383
22.6 GBS3255
29.5 GBS4895
30.5 GBS0725
33.6 GBS1867
40.3 gwm111-2
47.2 cfd48
52.5 wmc121
55.1 wmc702
66.6 GBS0983 GBS3223
67.0 GBS2826
67.4 GBS4617
68.1 GBS0882
69.1 GBS0778
73.8 GBS2050
77.8 GBS2215
82.6 GBS4215
Spring 2012
Fall 2012
Spring 2013
Mean PSS

Figure 2.4 KASP assay profiles of SNP. (1) KASP *GBS3127* on 5A, (2) KASP *GBS5669* on 5A, (3) KASP *GBS2573* on 5A, (4) KASP *GBS1852* on 5A, (5) KASP *GBS4963* on 6B, (6) KASP *GBS4305* on 6B, (7) KASP *GBS4116* on 6B, (8) KASP *GBS5855* on 2B, (9) KASP *GBS1713* on 2B. Blue dots represent HYZ alleles, green dot represents U.S. wheat cultivar alleles, red dots refer to heterozygotes, and the black crosses or dots are ddH_2O.

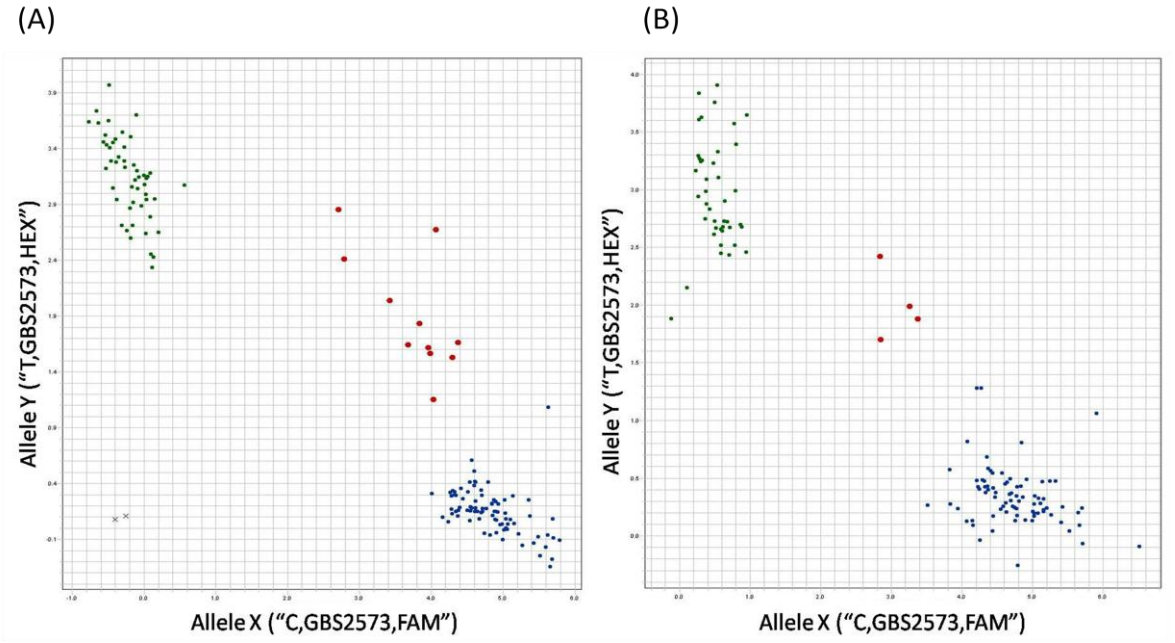
(1) KASP *GBS3127* on 5A



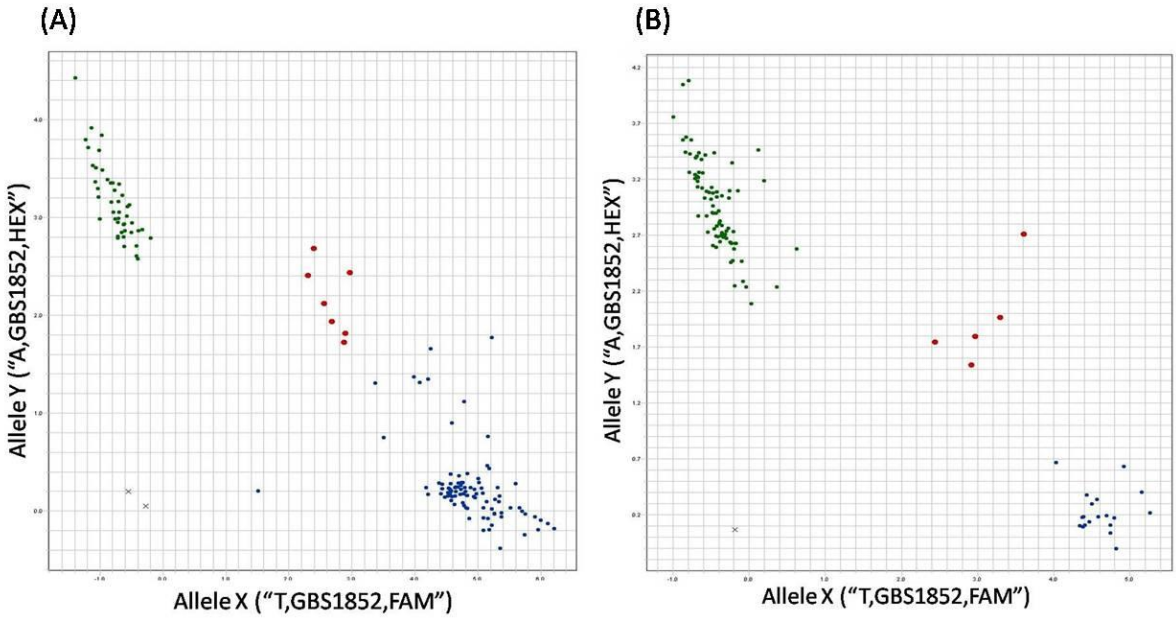
(2) KASP *GBS5669* on 5A



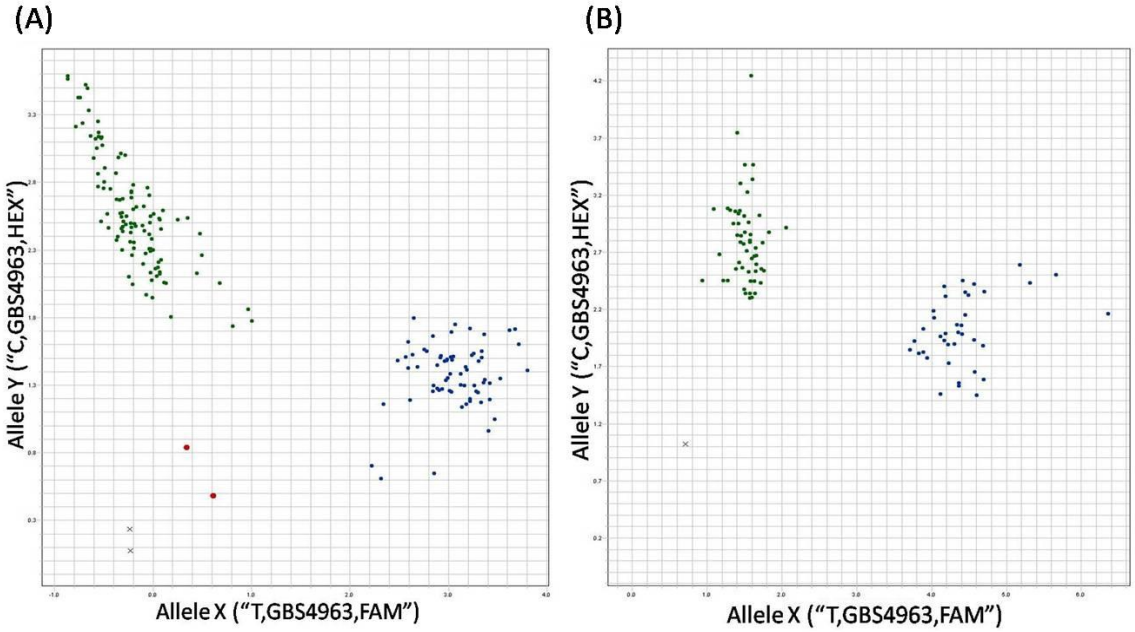
(3) KASP *GBS2573* on 5A



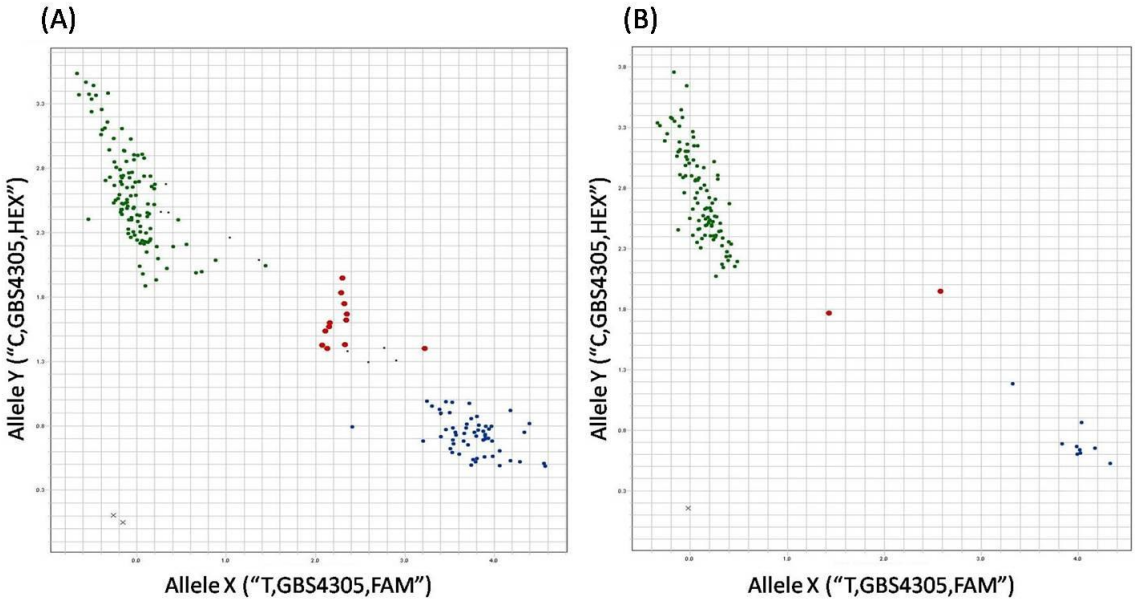
(4) KASP *GBS1852* on 5A



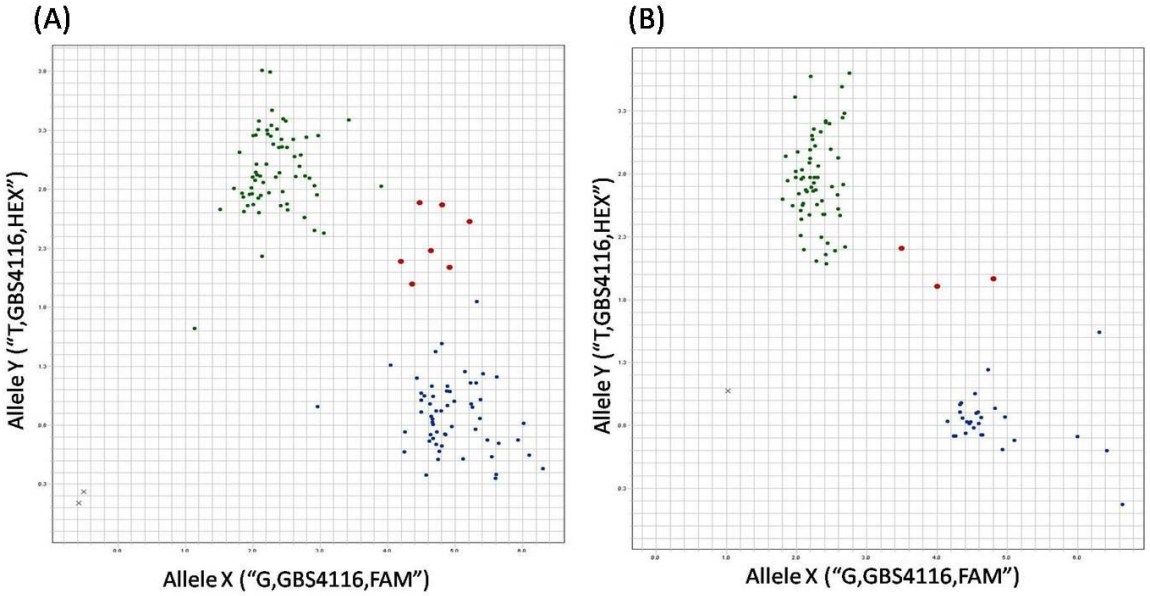
(5) KASP *GBS4963* on 6B



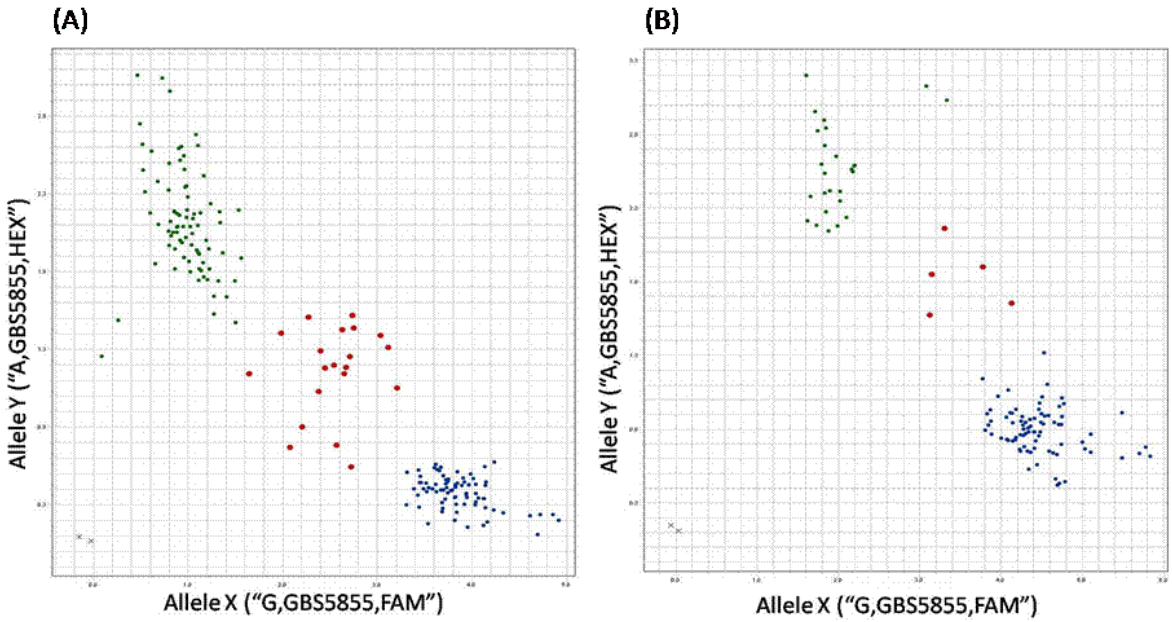
(6) KASP *GBS4305* on 6B



(7) KASP *GBS4116* on 6B



(8) KASP *GBS5855* on 2B



(9) KASP *GBS1713* on 2B

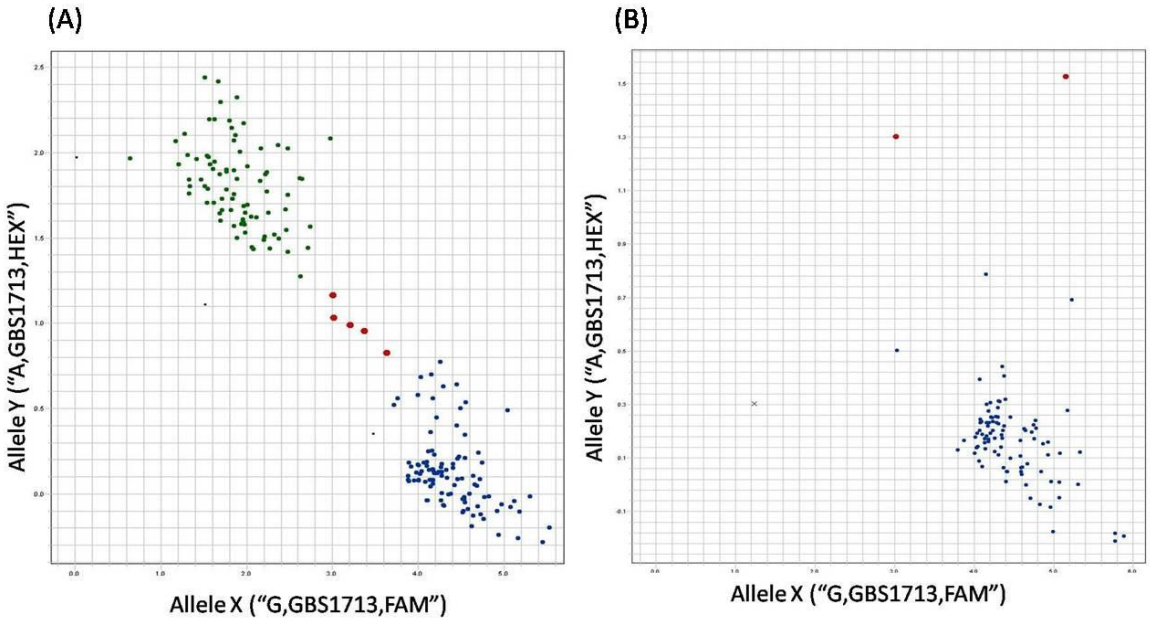
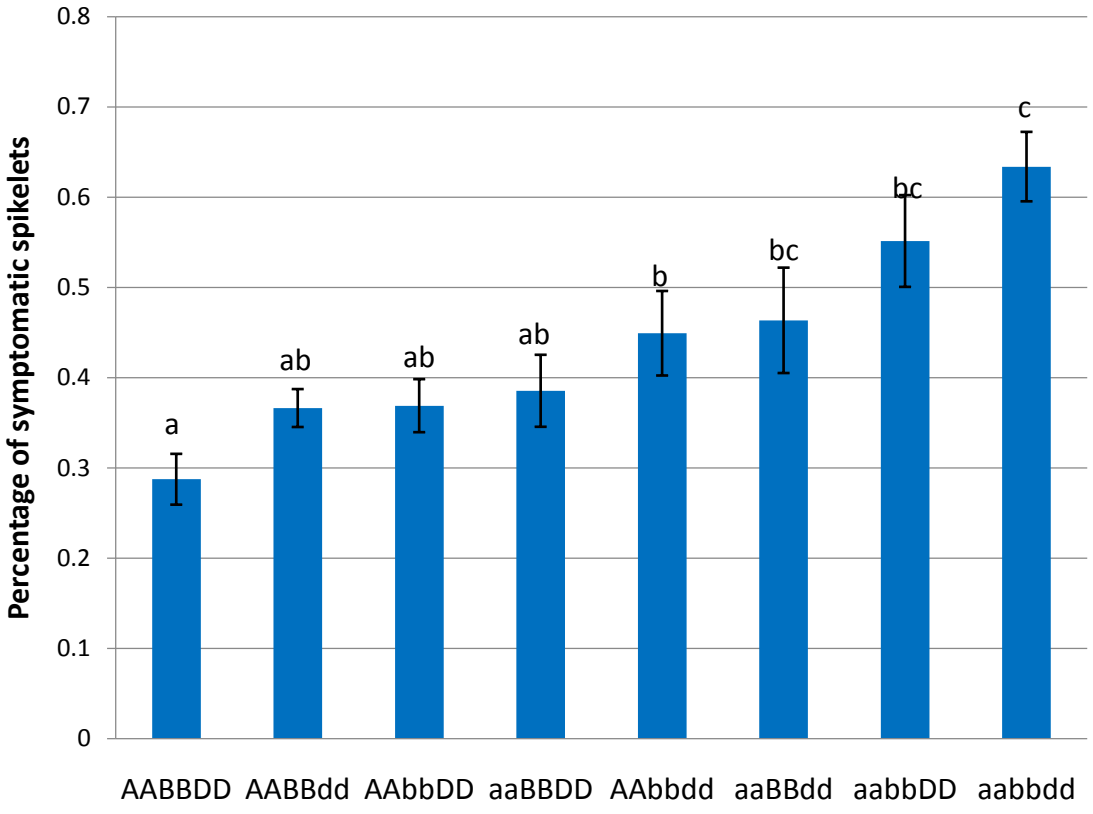


Figure 2.5 Effects of different combinations of three QTLs on 5A, 6B and 7D for percentage of symptomatic spikelets in a spike (PSS) analyzed in the RIL population. HYZ alleles were assigned as AA (5A), BB (6B) and DD (7D) and 'Wheaton' alleles aa (5A), bb (6B) and dd (7D). The solid bars stand for mean PSS of each group, and the length of each line refers to standard errors.



Chapter 3 - Meta-analysis of FHB resistance QTL in Chinese wheat landraces using GBS-SNPs

Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating disease in wheat (*Triticum aestivum* L.). FHB epidemics reduce both grain yield and quality. Many quantitative trait loci (QTLs) for FHB resistance have been reported from several sources, especially Chinese sources. *Fhb1*, a QTL from a Chinese cultivar Sumai 3, has been well characterized in many studies, however, other QTLs from Chinese sources are poorly characterized. In previous studies, QTLs for FHB resistance have been identified from five populations developed from five Chinese landraces, Haiyanzhong (HYZ), Wangshuibai (WSB), Baishanyuehuang (BSYH), Huangfangzhu (HFZ) and Huangcandou (HCD), using low-density maps constructed with simple sequence repeats (SSR). In the current study, we constructed high-density maps using genotyping-by-sequencing (GBS) and mapped 31 QTLs on 16 chromosomes in the five populations. Meta-analysis of the QTLs for FHB resistance using a consensus map developed from the five populations identified six meta-QTLs with two QTLs on the chromosome arm 3BS (3BSd and 3BSc), and one each on chromosomes 3A, 3D, 2D, and 4D. Closely linked markers were identified for all the QTLs. Twenty-three GBS-SNPs that tightly linked to the six meta-QTLs were successfully converted to breeder friendly Kompetitive allele specific PCR (KASP) assays. Those KASP markers tightly linked to QTLs mapped in multiple populations should be useful for marker-assisted selection of these QTLs in breeding programs.

Introduction

A substantial number of QTL studies on FHB resistance has been conducted during the past decades. Meta-analysis of QTLs based on statistical methods has been used to estimate the confidence intervals (CIs) of QTLs for soybean cyst nematode resistance (Guo et al. 2006), for maize flowering time (Chardon et al. 2004), for cotton fiber and other quality traits (Rong et al. 2007), for wheat earliness trait (Hanocq et al. 2007), and for rice blast resistance (Ballini et al. 2008). It is also used to summarize QTLs for FHB resistance in wheat (Löffler et al. 2009; Liu et al. 2009). Löffler et al. (2009) identified 19 meta-QTLs (MQTLs) with varying confidence intervals from 30 populations across 12 chromosomes, while Liu et al. (2009) summarized 45 studies and identified 19 repeatable QTLs. However, all of the meta-analyses were conducted based on previously reported low-density maps, and a high-density consensus map is critical to the identification of new QTLs and tightly linked markers to these QTLs. Genotyping-by-sequencing (GBS) is a simple, but effective, approach to spontaneous discovering and mapping of single nucleotide polymorphism (SNP) markers in diverse species (Poland et al. 2012), and thus is a useful marker platform for fine mapping of QTLs for FHB resistance. Meta-analysis using a new high-density consensus map from different populations will provide more precise positions for MQTLs, and better markers for marker-assisted selection (MAS) of these QTLs in breeding programs.

FHB resistance sources have been reported from many different regions in the world, including the U.S.A., Asia, Europe, and South America (Bai and Shaner 2004). Chinese sources, especially Chinese landraces, show the best resistance (Bai and Shaner 2004; Yu et al. 2008a). QTLs for FHB resistance have been mapped in some of these

landraces including Haiyanzhong (HYZ) (Li et al. 2011), Huangcandou (HCD) (Cai and Bai 2014), Baishanyuehuang (BSYH) (Zhang et al. 2012), Huangfangzhu (HFZ) (Li et al. 2012), and Wangshuibai (WSB) (Yu et al. 2008b).

The objectives of this study were to (1) remap QTLs for FHB resistance using newly developed GBS-SNP maps from the populations generated between the five Chinese resistant landraces (HYZ, HCD, BSYH, HFZ, and WSB) and one of two susceptible U.S. wheat cultivars (Jagger and Wheaton), (2) construct a consensus map using the genetic maps from the five populations, (3) conduct QTL meta-analysis to narrow down the confidence intervals (CIs) of the MQTLs, (4) identify closely linked GBS-SNPs to the MQTLs and convert them to Kompetitive allele specific PCR (KASP) assays for MAS in wheat breeding programs.

Materials and methods

Plant materials and FHB evaluation

The five mapping populations were recombinant inbred lines (RILs) developed by crossing five Chinese landraces (HYZ, HCD, BSYH, HFZ, and WSB) to one of FHB susceptible U.S. wheat cultivars, Jagger or Wheaton (Table 3. 2). FHB type II resistance (resistance to FHB spread within a spike) of the populations was evaluated using point inoculation in the greenhouses at Kansas State University (Manhattan, KS) as previously described (Cai and Bai 2014; Cai et al. 2015 Chapter 2; Li et al. 2011; Li et al. 2012; Yu et al. 2008b; Zhang et al. 2012).

Genotyping-by-sequencing library construction and SNP analysis

Genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide protocol (Maguire et al. 1994). Different numbers of SSR markers were screened for

polymorphisms in each population (Cai and Bai 2014; Cai et al. 2015 Chapter 2; Liu et al. 2012; Yu et al. 2008b; Zhang et al. 2012) (Table 3. 2). GBS libraries were generated for all the five RIL populations as described in Chapter 2 (Poland et al. 2012). GBS data were analyzed using UNEAK, a reference-independent pipeline of TASSEL (Lu et al. 2013; Poland et al. 2012). Poly-As were added to these read sequences that were shorter than 64 bp to ensure all reads had 64 bp.

The accuracy of GBS-SNP genotypes was validated using KASP assays that were designed based on their corresponding GBS read sequences harboring the SNPs that were mapped to QTL regions. KASP assay master mix used for each reaction and PCR cycles were described in Chapter 2. The genotypes of GBS-SNP and KASP-SNP data were compared, and the mismatches were counted.

Genetic maps and consensus map construction

Linkage maps with both SSRs and GBS-SNPs were constructed using Kosambi mapping function (Kosambi 1944) and 'regression' mapping algorithm in JoinMap version4.0 (Van Ooijen 2006). QTLs for low PSS were determined using Inclusive Composite Interval Mapping (ICIM) in QTL IciMapping V4.0 (Wang et al. 2011). Linkage groups with QTLs mapped in two or more populations were then used to construct a consensus map. For map integration, the 'regression' mapping algorithm was used. In general, a statistical approach 'weighted least square' was used to merge multiple individual genetic maps into a single consensus map by investigating heterogeneity of recombination rates between different studies (Van Ooijen 2006). The consensus map and comparative linkage maps with common markers from different studies were depicted using MapChart (Voorrips 2002).

QTL projection and meta-analysis of QTLs

QTLs from different populations were projected onto a consensus map by referring the initial QTL positions, confidence intervals (CIs), and r^2 values from the individual maps using QTLProj command in MetaQTL V1.0 (Veyrieras et al. 2007). The Gaussian mixture model was used to fit the distribution of the 'projected QTLs' on a chromosome, and cluster them to determine how many QTLs under the distribution of the observed QTLs using QTLClust command. The predicted MQTL positions and confidence intervals were extracted using QTLClustInfo command in Meta-QTL V1.0 (Veyrieras et al. 2007). The final consensus FHB-resistance QTL map with 95% CIs was drawn using MapChart software (Voorrips 2002).

Results

Molecular markers and linkage maps

For each of the five populations, the GBS library was analyzed in two Ion Proton runs. A total of 17,277 GBS-SNPs were called with less than 50% missing data, and 3429 GBS-SNPs had less than 20% missing data across all the five populations. Only the GBS-SNPs with less than 20% missing data were used in the meta-analysis. However, not all of the 3429 GBS-SNPs were polymorphic in all the five populations. An average of 1977 GBS-SNPs was combined with the original SSR markers to make a linkage map for each population, with 1950 SNPs in BSYH/Jagger, 1959 SNPs in HCD/Jagger, 1945 SNPs in HYZ/Wheaton population, 2060 SNPs in HFZ/Wheaton population, and 1972 SNPs in WSB/Wheaton population (Table 3.1). The number of markers mapped in each population varied from 1604 (81.3%) in WSB/Wheaton to 1776 (86.2%) in HFZ/Wheaton (Table 3.1). The marker density was the highest in HFZ/Wheaton

population (1.14 marker/cM), and the lowest in HCD/Jagger population (0.67 markers/cM) (Table 3.1). Among the five populations, HYZ/Wheaton population had the most markers in A genome, but all the other four populations had the most markers in B genome. On average, the B genome (45.4%) had the most markers, followed by the A genome (39.0%) and the D genome (15.5%) (Table 3.2).

QTLs for FHB resistance in individual populations

A total of 31 QTLs were mapped on 16 chromosomes (1A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5D, 6A, 6B, 6D, 7A, and 7D) in the five mapping populations (Table 3.2). QTLs on 3BSd and 3A were mapped in four populations with the 3BSd QTL mapped in the populations of BSYH/Wheaton, HCD/Jagger, HFZ/Wheaton and WSB/Wheaton, and the 3A QTL mapped in the populations of BSYH/Wheaton, HCD/Jagger, HYZ/Wheaton and HFZ/Wheaton (Figure 3.1, 3.2). QTLs on chromosomes 3BSc, 3D, 2D, 4D, 1A and 4B were each mapped in two populations, with 3BSc QTLs mapped in the populations of BSYH/Jagger and HFZ/Wheaton and 3D QTLs in the populations of HFZ/Wheaton and WSB/Wheaton, 2D QTLs in the populations of HCD/Jagger and HYZ/Wheaton; 4D QTLs in the populations of BSYH/Wheaton and HYZ/Wheaton, and 1A QTLs in the populations of HCD/Jagger and HFZ/Wheaton, and 4B QTLs in the populations of HYZ/Wheaton and HFZ/Wheaton (Figure 3.1, 3.2). These eight chromosomes had QTLs mapped in at least two populations, and thus were used for meta-QTL analysis. The other 11 QTLs were mapped in only one of the five populations and were not used for further meta-analysis.

Consensus map and MQTLs for FHB resistance

To conduct a meta-analysis of QTLs, a consensus map was constructed for the chromosomes 1A, 3A, 3BS, 3D, 2D, 4D, and 4B using the marker data from the relevant populations. The genetic distances of the seven consensus linkage groups ranged from 31.98 cM in 4D to 184.90 cM in 3A (Figure 3.1, 3.2). The marker density of the consensus linkage groups was the highest (1.85 markers/cM) in 3BS and the lowest (0.48 markers/cM) in 2D. Meta-analysis of FHB resistance QTLs resulted in six MQTLs from the 16 QTLs mapped in the five populations (Figure 3.1, 3.2), four remaining individual QTLs on chromosomes 1A and 4B were not clustered into MQTLs. The CIs of MQTLs ranged from 0.33 cM in the QTL 3BSc to 3.50 cM in the QTL 2D with an average of 1.90 cM (Table 3.4). QTL clustering often resulted in a reduction in CI of MQTLs compared to the mean individual initial CIs, and the reduction varied from 3.34 cM in QTL 3BSc to 8.88 cM in QTL 3A (Table 3.4).

On chromosome 3B, six individual QTLs were projected onto the consensus linkage group. The six initial QTLs were clustered in two MQTLs. The MQTL clustered on the distal end of 3BS (3BSd) was located at 21.60 cM and had a narrow CI of 3.25 cM (Table 3.4). All initial QTLs were distributed around the MQTL's position with the QTL position varied from 19.55 cM (HFZ) to 21.64 cM in (HCD), and the CIs varied between 4.43 cM (WSB) and 10.43 cM (HCD) (Table 3.4). Only slightly narrower CI of MQTL (3.25 cM) was observed compared to the narrowest individual initial CI (4.43 cM). Seven markers consisting of two SSRs/STSs (*Xbarc133* and *Xumn10*) and five GBS-SNPs (*GBS1663, GBS1100, GBS0800, GBS2377*, and *GBS1989*) were mapped within the CI of MQTL on 3BSd (Figure 3.2). The other MQTL clustered near the centromere region of

3BS was positioned at 80.41 cM, and had the narrowest CI (0.33 cM) among the MQTL investigated (Table 3.4). The two initial QTLs were located at 79.38 and 81.71 cM with CIs of 4.74 and 2.59 cM, respectively (Table 3.4). Five GBS-SNPs were mapped within the 3BSc MQTL region (*GBS2385*, *GBS0672*, *GBS0725*, *GBS2882*, and *GBS2285*) (Figure 3.2).

On chromosome 3A, four QTLs were clustered to one MQTL, positioned at 113.77 cM with a CI of 1.36 cM (Table 3.4). The initial four QTLs varied around this 3A MQTL in a range of 9 cM. Three of the four initial QTLs were distributed around the MQTL's position. The QTL from WSB was located 6.1 cM away from the 3A MQTL region, but was aggregated to the 3A MQTL because it has a relatively broad CI (11.76 cM). Seven markers including five SSR markers (*Xgwm2*, *Xwmc651*, *Xwmc527*, *Xgwm674*, *Xbarc306*) and only two GBS-SNPs (*GBS2002* and *GBS0782*) were mapped within the MQTL region, thus, the other five GBS-SNPs (*GBS2373*, *GBS3080*, *GBS2600*, *GBS0940*, and *GBS0340*) close to the MQTL 3A region (< 2 cM away) were also used to convert KASP assays (Figure 3.2).

On chromosome 3DL, two QTLs were clustered to one 3D MQTL, positioned at 31.17 cM with the narrow CI of 1.57 cM (Table 3.4). The initial QTLs of 3D MQTL, from WSB and HFZ, were also located at 31.17 cM with CIs of 7.57 cM and 6.47 cM, respectively (Table 3.4, Figure 3.2). One SSR marker (*Xgwm114*) and three GBS-SNPs (*GBS1480*, *GBS2389*, and *GBS1203*) were located within the MQTL region.

On chromosome 2D, two QTLs were clustered to one MQTL, positioned at 49.80 cM with the CI of 3.50 cM (Table 3.4). The initial QTLs of from HYZ and HCD, were located at 45.96 and 51.14 cM with CIs of 14.46 and 8.51 cM, respectively (Table 3.4,

Figure 3.2). The two initial QTLs were located ~5 cM away from each other, but both could be aggregated to the 2D MQTL because of the broad CI in HYZ (14.46 cM). Five GBS-SNPs (*GBS2554*, *GBS0096*, *GBS0097*, *GBS1487*, and *GBS1572*) were located within the MQTL region (Figure 3.2).

On chromosome 4D, two QTLs were clustered to one MQTL, positioned at 16.94 cM with the CI of 1.41 cM (Table 3.4). The initial QTLs of the 4D MQTL were from HYZ and BSYH, and located at 11.26 cM and 18.02 cM with CIs of 5.06 and 11.64 cM (Figure 3.2, Table 3.4). The two initial QTLs were ~6 cM away from each other, but a large CI (11.64 cM) of the QTL from BSYH aggregated the both QTLs to the 4D MQTL. Markers located within the 4D MQTL regions were four SSR markers (*Xcfd23*, *Xbarc288*, *Xwmc52*, *Xbarc98*) (Figure 3.2). GBS-SNPs were not mapped in the 4D MQTL region.

Conversion and validation of KASP assays

Five GBS-SNPs were mapped in the 3BSd MQTL region, six to the 3BSc MQTL region, seven within or close to the 3A MQTL region, seven in or close to the 3D MQTL region, five in the 2D MQTL regions, and three close to the 4D MQTL region. To verify the accuracy of GBS-SNP data, and fill the missing data of the GBS-SNPs in the QTL regions, 27 KASP assays were designed according to the corresponding GBS read sequences harboring the SNPs that were mapped in the MQTL regions on 3BSd, 3BSc, 3A, 3D, 4D, 2D. Twenty three KASP assays amplified successfully and were polymorphic in at least one of the five populations (Table 3.5). Twenty-two of them were remapped to the six corresponding significant MQTL regions (five on 3BSd, six each on 3BSc and 3A, one on 2D, three on 3D, two on 4D), and had identical allele calls with the

corresponding GBS-SNPs across the RILs. Only one KASP marker was mapped outside the QTL region with two mismatches in *GBS2882*.

Eleven of the 23 KASP assays (three on 3BSd, one each on 2D and 4D, and two each on 3BSc, 3A, 3DL) were then validated in an association mapping (AM) population with 96 U.S. elite breeding lines or cultivars. Eight KASPs (*GBS1989* (Figure 3.3 c), *GBS2882* (Figure 3.3 d), *GBS2285* (Figure 3.3 e), *GBS1487* (Figure 3.3 h), *GBS2373* (Figure 3.3 f), *GBS2600* (Figure 3.3 g), *GBS0781*(Figure 3.3 j), and *GBS2377*(Figure 3.3 b)), showed unequal clusters with more lines in 'Wheaton' or 'Jagger' allele cluster. However, three KASPs (*GBS1100* (Figure 3.3 a), *GBS2710* (Figure 3.3 i), and *GBS3012* (Figure 3.3 k) showed unequal clusters with fewer lines in 'Wheaton' or 'Jagger' allele cluster. Two of the KASP SNPs, *GBS2373* (Figure 3.3 f) on 3A and *GBS0781* (Figure 3.3 j) on 3DL, had most lines in either 'Wheaton' or 'Jagger' cluster in the AM population, with only five lines in the Chinese landrace's allele cluster.

Discussion

QTLs mapped in the newly constructed GBS-SNP maps

ICIM mapping detected significant QTLs for FHB resistance in each of the five populations. Previous QTL mapping studies using low-density SSR maps identified 27 QTLs for FHB type II resistance on 14 chromosomes in the five populations using Chinese landraces as resistant parents (1AS, 1BS, 2D, 3A, 3B, 3DL, 4B, 4D, 5AS, 5DL, 6BS, 6D, 7AL, and 7D) (Table 3.3). After adding GBS-SNPs into the new maps, most QTLs with relatively large and stable effects were remapped, but some minor ones disappeared including QTLs on chromosome1AS, 1BS, 5AS, and 7AL. However, several new QTLs were identified including those on chromosomes 1A, 2B, 2D, 3A, 3D, 4B, 4D,

6A, and 7A (Table 3.3). This is because newly developed GBS-SNPs maps in the current study added >1000 new SNP markers in each population and provided much better chromosome coverage than the old maps that had only ~200 markers mapped in each population.

MQTLs for FHB resistance in the five Chinese landraces

Meta-analysis of QTLs is used to integrate multiple QTLs from different studies to investigate the congruence of these QTLs between those studies (Veyrieras et al. 2007). In the current study, we remapped QTLs using newly constructed GBS-SNP maps, projected the QTLs to the consensus map, and clustered the projected QTLs to determine the number of the QTLs underlining the distribution of the mapped QTLs from the five populations. A total of six MQTLs were identified on the chromosomes 3BSd, 3BSc, 3A, 3D, 2D, and 4D using meta-analysis in this study. These MQTLs showed narrower CIs and improved accuracy of map locations than for originally mapped QTLs. For example, the 3BSd MQTL centered by STS marker *Xumn10* had a 95% CI of 3.25 cM, whereas the original CIs for the QTLs mapped in the four populations were at least 4.43 cM in WSB. The 3BSc MQTL was located near the centromere region of 3BS, centered by GBS-SNP *GBS2882* and flanked by *GBS2385* and *GBS2285*. The CI for 3BSc MQTL was 0.33 cM, but the CIs were 4.74 cM in BSYH and 2.59 cM in WSB.

However, the QTLs on chromosome 4B from HFZ and HYZ and the QTLs on 1A from HCD and HFZ could not be clustered together because the initial QTLs did not have common markers between different populations. The 4B QTL from HYZ corresponding to *Fhb4* which was centered on *Xgwm6* and flanked by *GBS2746* and *GBS1303*; while the 4B QTL from HFZ, centered on *GBS2125*, was about 25 cM from *Xgwm6* and 41 cM

from *GBS2746* according to both the HYZ 4B linkage maps and the 4B consensus linkage group maps. Thus, they were not the same QTL and cannot be mapped together. The 1A QTL from HCD was centered by *GBS2817*, and flanked by *GBS1035* and *GBS1427*; while the 1A QTL from HFZ was centered by *GBS1402* and *GBS1707*. The two QTLs were about 40 cM away from each other in the 1A consensus map, thus, they were different QTLs, and cannot be clustered together as a single MQTL.

Among these MQTLs, 3BSd MQTL was centered by *Xumn10*, a diagnostic marker for *Fhb1* (Liu et al. 2008), in HCD and BSYH populations, GBS SNP *GBS1663* in HFZ population, and *GBS0800* in WSB population. The three markers were mapped to a 2.47 cM interval. After meta-analysis, the 3BSd MQTL was still centered at *Xumn10*, and thus this MQTL corresponds to *Fhb1* (Cuthbert et al. 2006). The position of this MQTL coincided with these in original mapping studies because two GBS SNPs, *GBS0800* and *GBS1663*, are within the CI of *Fhb1*. However, the CI of *Fhb1* was shortened after meta-analysis, thus, the MQTL location is more accurate than that from individual populations. *Fhb1* from Sumai3 and its derivatives shows a large and stable effect on FHB type II resistance (Anderson et al. 2001; Bai et al. 1999; Waldron et al. 1999). Liu et al. (2008) fine mapped *Fhb1* to a 261 kb region harboring *Xumn10*. In the current study, *Fhb1* mapped in four of the five Chinese wheat landraces (Cai and Bai 2014; Li et al. 2012; Yu et al. 2008b; Zhang et al. 2012), but with varied effects on FHB type II resistance, ranging from 15.0% in BSYH to 30.3% in WSB. The large variation of the QTL effects may be due to the differences in FHB phenotyping, genetic backgrounds of populations, population sizes for map construction, and FHB inoculation methods used.

The second MQTL on 3BS identified in this study is 3BSc MQTL. This QTL was also reported in several other studies including Chinese and Japanese landraces (Somers et al. 2003; Yang et al. 2005; Yu et al. 2008b; Zhou et al. 2004), and US wheat (Liu et al. 2007; Perugini 2007). Although this MQTL shows much smaller effect than *Fhb1*, it may exist in both Chinese and US wheat, thus is an important QTL for improving FHB resistance in these countries. Liu et al. (2009) located this QTL to an interval between *Xgwm566* and *Xbarc344* using meta-analysis, which agrees with the current study. However, the current study identified a more tightly linked marker to this MQTL, GBS SNP *GBS2882* and narrowed the interval to < 1cM between *GBS2385* and *GBS2285*. The new interval for the MQTL is much smaller than the original ones (4.74 cM for BSYH and 2.59 cM for WSB). Sequence search in GenBank of National Center for Biotechnology Information (NCBI) using the SNPs (*GBS2385*, *GBS0672*, *GBS0725*, *GBS2882*, and *GBS2285*) in the MQTL region confirmed that all the SNPs are from wheat 3B chromosome. The MQTL can be physically located in an interval of 50,581,775 bp from 329,891,303 bp to 380,473,078 bp near the centromere. Among the five GBS SNPs in the MQTL region, *GBS2285* and *GBS2882* showed the best correlation with PSS ($r=0.26\sim0.42$, $P<0.05$), thus are good markers for marker-assisted selection of this QTL.

The MQTL on 3A is present in BSYH, HFZ, HYZ, and HCD. The original QTLs were mapped to a 9 cM interval from 110.78 to 119.85 cM in the consensus map. The MQTL was mapped to an interval of 1.36 cM, flanked by SSR markers *Xgwm2.1* and *Xbarc306*. Two GBS SNPs *GBS2002*, *GBS0782*, and five SSR markers *Xgwm2.1*, *Xwmc651*, *Xwmc527*, *Xgwm674*, and *Xbarc306* were mapped within the CI of 3A

MQTL. Two GBS SNPs *GBS2002* and *GBS0782* within the MQTL region showed a significant correlation of 0.28 and 0.27, but were lower than that of SSR marker *Xgwm674* (r=0.22~0.39). We then tried GBS SNPs *GBS2373*, *GSB3080*, *GBS2600*, and *GBS0340* that were only 0.2~ 2.0 cM away from the MQTL region, and found out they all had slightly lower correlations ranged from 0.21 to 0.35 ($P < 0.05$). None of the other markers outside of the MQTL regions showed a significant marker-trait association. Thus, the MQTL 3A region was accurate, but the CI calculated may be too short when the initial QTL regions vary a few cM away from each other. The QTL was also mapped in different sources besides Chinese landraces including in Huapei 57-2 (Bourdoncle and Ohm 2003), F201R (Shen et al. 2003b), and Frontana (Mardi et al. 2006; Steiner et al. 2004). Frontana is a cultivar from Latin America (Buerstmayr et al. 2009; Mardi et al. 2006; Steiner et al. 2004). Since this MQTL is not only present in Chinese landraces, but also in America germplasm, it should be a good candidate for QTL pyramiding.

The 3DL MQTL was from WSB and HFZ. The two original QTLs were projected to the same region at 31.17 cM with CIs of 6.47 and 7.57, respectively. The CI of the MQTL was narrowed down to 1.57 cM, centered at *GBS2389*, and flanked by *Xgwm114-1* and *GBS1203*. QTLs on 3D have been reported in Patterson (Shen et al. 2003b), Cansas (Klahr et al. 2006), and a Swiss susceptible winter wheat Forno (Paillard et al. 2004). However, the QTLs in Patterson and Cansas were mapped to the short arm of 3D, thus, they are different from the QTL identified in this study. The one mapped in Forno didn't have common markers with the current study. Therefore, the 3DL MQTL may be a unique QTL from Chinese landraces. Using meta-analysis and GBS-SNP maps, more tightly linked markers for the MQTL were identified, thus, they can be useful for MAS.

The 2D MQTL was from Jagger and Wheaton, centered at *GBS0097,* and flanked by *GBS2554* and *GBS1572*. This MQTL is near *Rht8,* a semi-dwarfing gene (Handa et al. 2008) and has been mapped in Alondra (Shen et al. 2003a), Wuhan 1 (Somers et al. 2003), Gamenya (Handa et al. 2008), WSB (Jia et al. 2005), and Jagger (Cai and Bai 2014) according to the common SSR markers *Xwmc25*, *Xgwm261,* and *Xgwm296*. Handa et al. (2008) identified a multidrug resistance-associated protein (MRP) gene as a candidate gene for the QTL. This MQTL is mainly contributed by susceptible or moderately susceptible parents from different countries, suggesting it may be a common QTL for FHB resistance in many commercial varieties. Further screening of locally adapted cultivars may identify more adapted parents as a donor of the QTL or as adapted parents for pyramiding of other QTLs in breeding programs. In the current study, we identified more closely linked markers, *GBS0096*, *GBS0097* and *GBS1487,* than *Xgwm261* on this chromosome. However, only *GBS1487* was successfully converted to KASP assay, because *GBS0096* and *GBS0097* have more than one SNP near the SNP positions. *GBS1487* can be used to select for the QTL if it is polymorphic in breeding populations.

The 4D MQTL was from HYZ and BSYH and originally mapped between 11.26 and 18.02 cM interval in the consensus map. On chromosome 4D, two QTLs have been reported with one near *Rht-D1*, a semi-dwarfing gene on 4DS and the other on the 4DL. This 4DS QTL was mapped in Arina (Draeger et al. 2007) and Spark (Srinivasachary et al. 2008), whereas the 4DL QTL has been reported in DH181 (Yang et al. 2005) and a ‘Chinese Spring’ ‘Sumai 3’chromosome 7A disomic substitution line (CS-SM3-7ADS) (Ma et al. 2006). The MQTL in the current study was the same as the one mapped on the

4DL because of the common marker *Xwmc457* was mapped 0.46 cM away from the MQTL. Four SSR markers *Xbarc288, Xwmc52, Xcfd23*, and *Xbarc98* were mapped within the MQTL region. No new SNPs were mapped within the MQTL region, and SSR marker *Xwmc52* was the best markers for MAS. Thus, KASP assays were designed using GBS SNPs *GBS1498, GBS3012*, and *GBS1836* that were a few cM away from the MQTL region. The derived KASP markers showed slightly lower but significant associations with FHB resistance ($r=0.21\sim0.30$, $P < 0.05$).

Unique QTLs in five populations

MQTLs showed QTLs that can be mapped in at least two of the five populations. However, many QTLs can only be mapped in a single population including QTLs on chromosomes1A, 2B, 3BL, 4B, 5AS, 5DL, 6A, 6BS, 6D, 7A and 7DL. Some of them including QTLs on 5AS, 6BS, 4B, 1A, and 7A were repeatedly mapped in previously studies (Buerstmayr et al. 2009; Liu et al. 2009). Others, however, were unique QTLs on chromosomes 3BL, 6A, and 6D, mapped in this study only. The QTL on 6BS mapped in HYZ/Wheaton population coincided with the previously mapped *Fhb2*, because of the common markers *Xgwm88* and *Xwmc397* (Cai et al. 2015 Chapter 2; Cuthbert et al. 2007). The 4B QTL, also mapped in HYZ only, was the same QTL as *Fhb4* (Cai et al. 2015 Chapter 2; Xue et al. 2010). The 5A QTL mapped as a major QTL in HYZ, was also the same QTL as *Fhb5* (Cai et al. 2015 Chapter 2; Xue et al. 2011).

Interestingly, the 5A QTLs were significant in four of the five populations when the SSR maps were used (Li et al. 2011; Li et al. 2012; Yu et al. 2008b; Zhang et al. 2012). However, it was only significant in HYZ when GBS-SNP maps were used for the QTL mapping. Further analysis found that in BSYH/Jagger population, the 5A QTL was

significant in only one of the three experiments with LOD score of 2.3, and explained only 4.5% of phenotypic variation (Zhang et al. 2012). In WSB/Wheaton population, the 5A QTL was significant in two of four experiments, with LOD of 1.8 and 2.6, and explained 3.5-5.5% of phenotypic variation (Yu et al. 2008b). In HFZ/Wheaton population, the 5A QTL was detected through single marker analysis (SMA) only (Li et al. 2012). The 5A QTL was a confirmed QTL for FHB type I resistance that had a large effect and was stable in both greenhouse and the field experiments (Buerstmayr et al. 2003; Chen et al. 2006; Lin et al. 2006; Yang et al. 2005; Yu et al. 2008b). However, it showed a much smaller and unstable effect for FHB type II resistance (Buerstmayr et al. 2009). Thus, the 5A QTL was not significant in meta-analysis due to its inconsistency among different environments.

Conversion of GBS-SNPs to KASP assays

The GBS SNPs are powerful for simultaneous SNP discovery and genotyping at a relatively low cost per sample (Poland et al. 2012; Sonah et al. 2013). However, GBS usually generates SNPs with a large number of missing data. This GBS generated 17277 GBS SNPs with less than 50% missing data in the five populations, but only 3429 GBS SNPs have less than 20% missing data. The high percentage of missing data was probably because of the limitation in sequence depth (Poland et al. 2012; Sonah et al. 2013; Spindel et al. 2013). One way to solve the problem is data imputation to predict missing data based on available reference genome sequences (Poland et al. 2012; Spindel et al. 2013). However, the wheat reference genome sequences are not complete so far, so that the imputed data may not be accurate enough for QTL mapping. To increase the number of Ion Proton runs for each library can reduce missing data, but the sequencing

cost will increase significantly. In the current study, two Ion Proton runs of each population significantly increased the number of SNPs called compared with a single run. To verify the accuracy of GBS-SNPs and eliminate the missing data, GBS-SNPs that were mapped in or close to the MQTL regions were converted to KASP assays. Among the 27 KASP assays designed, twenty three amplified very well, and four didn't amplify because SNP positions are too close to one end of the sequence reads, or more than one SNP is close to the SNP position. Twenty two of the twenty three KASP assays amplified can be remapped to the same positions corresponding to GBS-SNPs mapped, and only one cannot because of SNP calling errors. Twelve KASP assays were then selected from the 23 KASPs to validate in an AM population with 96 U.S. elite lines and cultivars. Nine KASPs (*GBS1100*, *GBS1989*, *GBS2882*, *GBS2285*, *GBS1487*, *GBS2373*, *GBS2600*, *GBS2710*, *GBS0781*, and *GBS3012*) showed unequal clusters, thus these markers can be good markers for transferring QTLs. Whereas, *GBS2377* and *GBS1836* showed almost equal clusters indicating that half of the U.S. winter accessions might have the resistant allele, but the KASP assays could be useful for MAS in U.S. winter wheat if the breeding parents were polymorphic.

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Table 3.1 Numbers of the original SSR or STS markers, newly added GBS-SNPs, markers mapped, and marker density in five populations derived from five Chinese landraces and the consensus map.

Population	Number of SSR/STS markers	Number of GBS-SNPs called (<20% missing)	Number of markers mapped	Genetic map length (cM)	Marker density (marker/cM)
BSYH/Jagger	91	1950	1662	2308.33	0.72
HCD/Jagger	182	1959	1613	2407.46	0.67
HYZ/Wheaton	118	1945	1713	2519.12	0.68
HFZ/Wheaton	98	2060	1776	1557.89	1.14
WSB/Wheaton	260	1972	1604	2269.21	0.71

Note: 'BSYH' is short for 'Baishanyuehuang'; 'HCD' is short for 'Huangcandou'; 'HYZ' is short for 'Haiyanzhong'; 'HFZ' is short for 'Huangfangzhu'; 'WSB' is short for 'Wangshuibai'.

Table 3.2 Number of markers distributed in three genomes.

Population	Genome A	Genome B	Genome D	Total
BSYH/Jagger	574	663	425	1662
HCD/Jagger	615	768	230	1613
HYZ/Wheaton	736	692	285	1713
HFZ/Wheaton	790	821	165	1776
WSB/Wheaton	548	865	191	1604
Average	652.6	761.8	259.2	-

Note: 'BSYH' is short for 'Baishanyuehuang'; 'HCD' is short for 'Huangcandou'; 'HYZ' is short for 'Haiyanzhong'; 'HFZ' is short for 'Huangfangzhu'; 'WSB' is short for 'Wangshuibai'.

Table 3.3 Previously published QTLs and newly mapped QTLs for FHB type II resistance in five mapping populations.

Population	Number of RILs	Previously mapped QTLs	References	QTL mapped in current study
BSYH/Jagger	188 F_6	3A, 5AS, 3BS (2)	(Zhang et al., 2012)	3A, 3BS (2), 4D*
HCD/Jagger	190 F_6	3A, 3BS (2), 2D, 6D	(Cai et al., 2014)	1A*, 3A, 3BS, 2D, 6D
HYZ/Wheaton	186 F_7	5A, 3BL, 4B, 6BS, 4D, 7DL	(Cai et al. unpublished)	5AS, 2B (2)*, 3BL, 4B, 6BS, 2D*, 4D, 7DL, 3A*
HFZ/Wheaton	102 F_6	1AS, 5AS, 7AL, 1BS, 3BS	(Li et al., 2011)	1AS, 3A*, 7AL, 3BS, , 4B*, 3DL*, 4A*
WSB/Wheaton	124 F_6	1AS, 5AS, 7AL, 3BS (2), 3DL, 5DL	(Yu et al., 2008)	3BS (2), 3DL, 6A*, 5DL

Note: 'BSYH' is short for 'Baishanyuehuang'; 'HCD' is short for 'Huangcandou'; 'HYZ' is short for 'Haiyanzhong'; 'HFZ' is short for 'Huangfangzhu'; 'WSB' is short for 'Wangshuibai'.

Table 3.4 Original and meta QTL positions, Confidence Intervals (CIs) of the QTL clusters constructed by MetaQTL V 1.0.

QTL cluster	Original QTL position	Original QTL CIs (95%)	Meta QTL position	Meta QTL CIs (95%)	Reduction of CIs
2D	45.96~51.14	8.51~14.46	49.80	3.50	7.98
3A	110.78~119.85	6.66~13.60	113.77	1.36	8.88
3BSd	19.55~22.19	4.43~10.43	21.60	3.25	4.33
3BSc	79.38~80.71	2.59~4.74	80.41	0.33	3.34
3DL	31.17~31,17	6.47~7.57	31.17	1.57	5.45
4D	11.26~18.02	5.06~11.64	16.94	1.41	7.21

Table 3.5 List of 23 KASP assays developed from GBS sequences.

Primer name	Position	Primer sequence (5'-3')	Polymorphic in
GBS1663_F	3BSd	GAAGGTGACCAAGTTCATGCTGGAACAAAACTGCAAAGTGGTGTc	HFZ/W WSB/W HYZ/W
GBS1663_H	3BSd	GAAGGTCGGAGTCAACGGATTGGAACAAAACTGCAAAGTGGTGTt	
GBS1663_R	3BSd	GGACCCTTGCTGATTCATTTCG	
GBS1100_F	3BSd	GAAGGTGACCAAGTTCATGCTTGGTTCCTACACACTGTTGCATTTa	HFZ/W WSB/W HYZ/W
GBS1100_H	3BSd	GAAGGTCGGAGTCAACGGATTTGGTTCCTACACACTGTTGCATTTg	
GBS1100_R	3BSd	GCATTCACCTGTGTCCAGAGAGA	
GBS0800_F	3BSd	GAAGGTGACCAAGTTCATGCTTGACCTCGGACACTGCAGCa	HFZ/W WSB/W HYZ/W
GBS0800_H	3BSd	GAAGGTCGGAGTCAACGGATTTGACCTCGGACACTGCAGCg	
GBS0800_R	3BSd	GTGACGGCAATCGAGCACAC	
GBS2377_F	3BSd	GAAGGTGACCAAGTTCATGCTGGCGCAACGTGATCACAc	HCD/J
GBS2377_H	3BSd	GAAGGTCGGAGTCAACGGATTGGCGCAACGTGATCACAt	
GBS2377_R	3BSd	TGTGAATCTCCATGCCTCCTT	
GBS1989_F	3BSd	GAAGGTGACCAAGTTCATGCTGATCGCCACCGTCCTTCCa	BSYH/J HYZ/J

GBS1989_H	3BSd	GAAGGTCGGAGTCAACGGATTGATCGCCACCGTCCTTCCg	HFZ/W WSB/W
GBS1989_R	3BSd	CGTGAACGGGCCTGATTGAA	
GBS2385_F	3BSc	GAAGGTGACCAAGTTCATGCTAGGCGCCCATCACGCAc	
GBS2385_H	3BSc	GAAGGTCGGAGTCAACGGATTAGGCGCCCATCACGCAt	BSYH/J HCD/J
GBS2385_R	3BSc	CGCGTCTCTTCAAGCTCGTC	
GBS0672_F	3BSc	GAAGGTGACCAAGTTCATGCTTGCAGATTAAACCTGTGCa	BSYH/J HCD/J
GBS0672_H	3BSc	GAAGGTCGGAGTCAACGGATTTGCAGATTAAACCTGTGCc	HYZ/W WSB/W
GBS0672_R	3BSc	TCTACAGCTGACGCATGGAG	HFZ/W
GBS0725_F	3BSc	GAAGGTGACCAAGTTCATGCTTGCAGCAAATCAACTGCTTTc	BSYH/J HCD/J
GBS0725_H	3BSc	GAAGGTCGGAGTCAACGGATTTGCAGCAAATCAACTGCTTTg	HYZ/W WSB/W
GBS0725_R	3BSc	TGCTCCTCTGTTTCTGATCTCC	HFZ/W
GBS2882_F	3BSc	GAAGGTGACCAAGTTCATGCTGTTTGGTTTGTATCTCAGTGGTa	BSYH/J HCD/J
GBS2882_H	3BSc	GAAGGTCGGAGTCAACGGATTGTTTGGTTTGTATCTCAGTGGTg	HYZ/W WSB/W
GBS2882_R	3BSc	CAGATCTGGTGAAATAGCAGTC	HFZ/W
GBS2285_F	3BSc	GAAGGTGACCAAGTTCATGCTCCCGCGTTGCGGGTCTc	BSYH/J HCD/J

GBS2285_H	3BSc	GAAGGTCGGAGTCAACGGATTCCCGCGTTGCGGGTCTt	HYZ/W WSB/W HFZ/W
GBS2285_R	3BSc	CCAGGCTCTCGTTTTCCTCGT	
GBS2312_F	3BSc	GAAGGTGACCAAGTTCATGCTGCTGTTGCTGCTCCTTGAACc	HYZ/W HFZ/W WSB/W
GBS2312_H	3BSc	GAAGGTCGGAGTCAACGGATTGCTGTTGCTGCTCCTTGAACt	
GBS2312_R	3BSc	CATAGGTCCGCCCTTTGTCT	
GBS1487_F	2D	GAAGGTGACCAAGTTCATGCTGCAGCGCCCCTATATATTTGc	HFZ/W HCD/J
GBS1487_H	2D	GAAGGTCGGAGTCAACGGATTGCAGCGCCCCTATATATTTGt	
GBS1487_R	2D	TTGCAGTCAAGGGAGTGAGTG	
GBS2373_F	3A	GAAGGTGACCAAGTTCATGCTGCAGGCGAGGGAAGAACa	BSYH/J HCD/J HYZ/W WSB/W HFZ/W
GBS2373_H	3A	GAAGGTCGGAGTCAACGGATTGCAGGCGAGGGAAGAACg	
GBS2373_R	3A	AGCCACTTCTCCATCGATCC	
GBS3080_F	3A	GAAGGTGACCAAGTTCATGCTGCTCAAAAAAGACAATGAGCAGTGAt	BSYH/J HCD/J HYZ/W WSB/W HFZ/W
GBS3080_H	3A	GAAGGTCGGAGTCAACGGATTGCTCAAAAAAGACAATGAGCAGTGAc	
GBS3080_R	3A	CACTGTCACCCCTCTCCCTGA	
GBS0340_F	3A	GAAGGTGACCAAGTTCATGCTCTGGCGAATATGTTCTGCTCc	HYZ/W HFZ/W

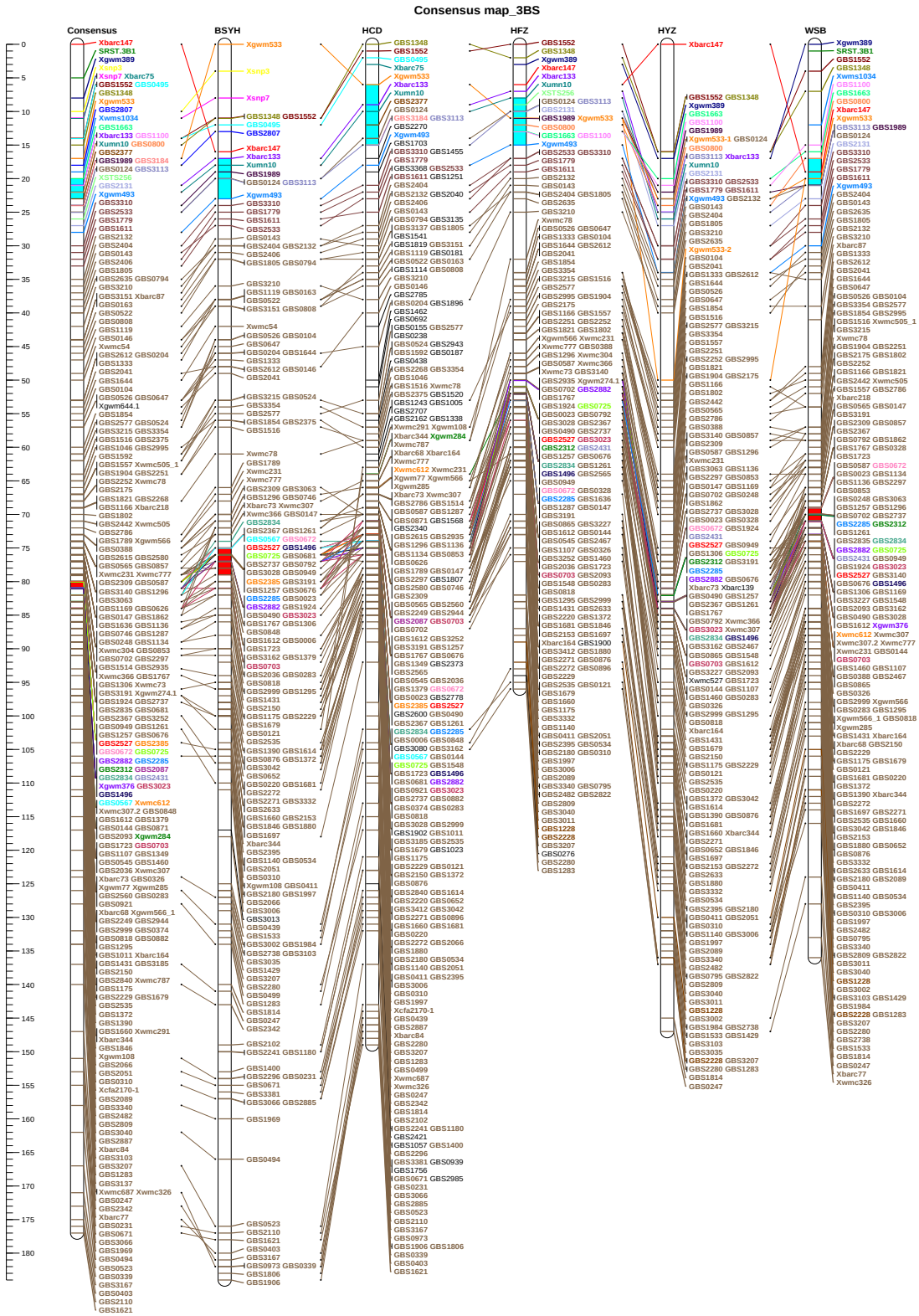
GBS0340_H	3A	GAAGGTCGGAGTCAACGGATTCTGGCGAATATGTTCTGCTCg	WSB/W
GBS0340_R	3A	TGTCCGGACGCTGTCAGTCT	
GBS2600_F	3A	GAAGGTGACCAAGTTCATGCTGCTTGACCATACTCCCGCa	BSYH/J HCD/J HYZ/W WSB/W HFZ/W
GBS2600_H	3A	GAAGGTCGGAGTCAACGGATTGCTTGACCATACTCCCGCt	
GBS2600_R	3A	TTGGCGAGCATCTGCTGGTA	
GBS2002_F	3A	GAAGGTGACCAAGTTCATGCTGTGGCCTGCAGCTTGCAc	HYZ/W WSB/W HFZ/W
GBS2002_H	3A	GAAGGTCGGAGTCAACGGATTGTGGCCTGCAGCTTGCAt	
GBS2002_R	3A	CATGGGAGGCACCAGAACAA	
GBS3320_F	3A	GAAGGTGACCAAGTTCATGCTGGGGTGACCTCGGGGa	HYZ/W WSB/W HFZ/W
GBS3320_H	3A	GAAGGTCGGAGTCAACGGATTGGGGTGACCTCGGGGg	
GBS3320_R	3A	AAGGGTGGGCAGCAAAAC	
GBS2710_F	3DL	GAAGGTGACCAAGTTCATGCTAGGTGCAGGGCCGTGGc	HYZ/W HFZ/W WSB/W
GBS2710_H	3DL	GAAGGTCGGAGTCAACGGATTAGGTGCAGGGCCGTGGt	
GBS2710_R	3DL	ACCTGGACGCGGAGGCTAC	
GBS1529_F	3DL	GAAGGTGACCAAGTTCATGCTTGCAGCGCTAAATAGGATTTg	BSYH/J HCD/J

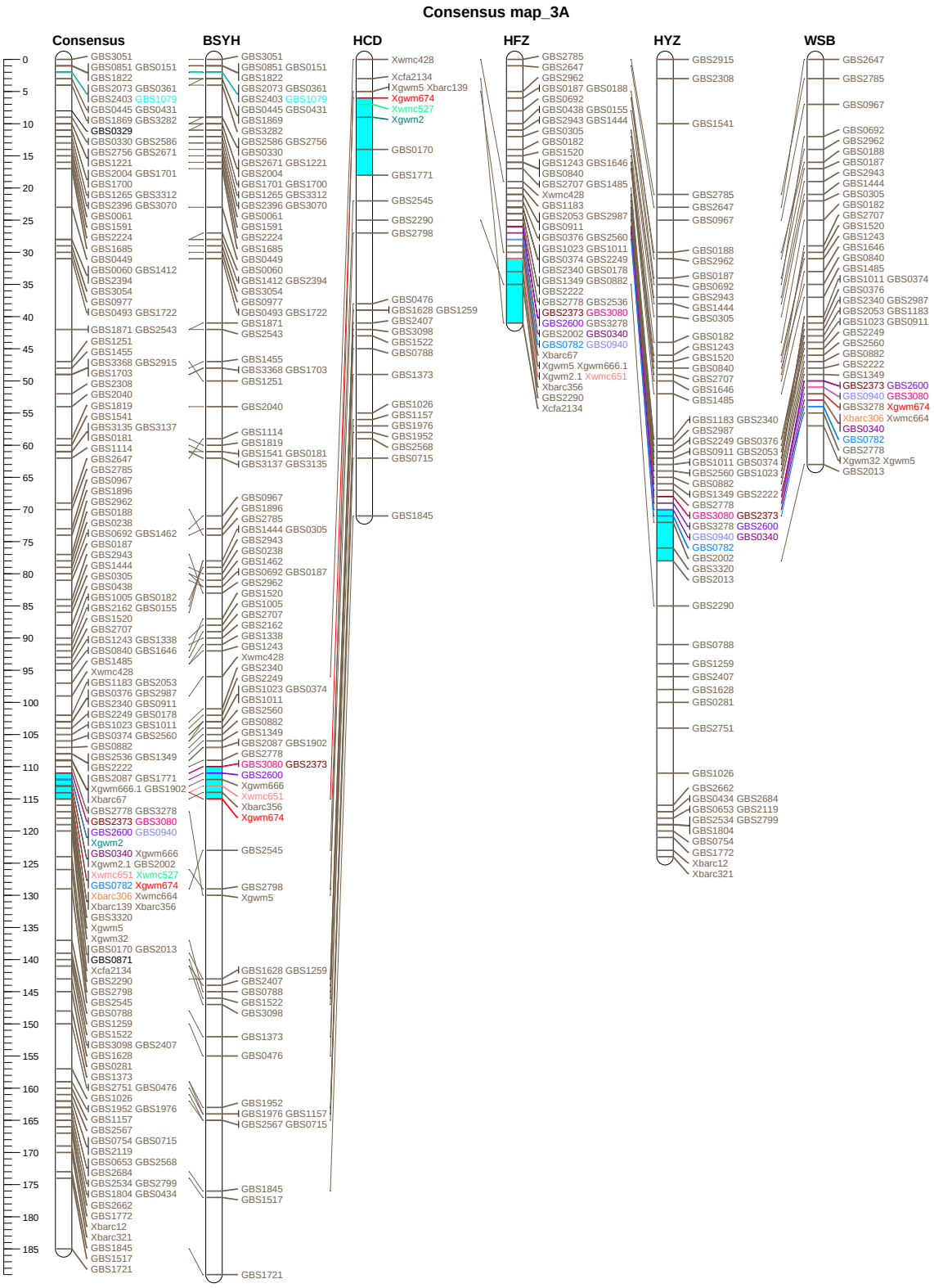
GBS1529_H	3DL	GAAGGTCGGAGTCAACGGATTTGCAGCGCTAAATAGGATTTt	HYZ/W HFZ/W WSB/W
GBS1529_R	3DL	TGTGATTACGTGCGTGGAGTC	
GBS0781_F	3DL	GAAGGTGACCAAGTTCATGCTGTGCCTCATAGCACTTAGCAGc	BSYH/J HCD/J HYZ/W HFZ/W WSB/W
GBS0781_H	3DL	GAAGGTCGGAGTCAACGGATTGTGCCTCATAGCACTTAGCAGt	
GBS0781_R	3DL	TCCCATCCACTCTGTTCACAT	
GBS1498_F	4D	GAAGGTGACCAAGTTCATGCTCTAGTCCTGCAGCGCCGTc	BSYH/J
GBS1498_H	4D	GAAGGTCGGAGTCAACGGATTCTAGTCCTGCAGCGCCGTt	
GBS1498_R	4D	GGTTGCAGACGTCCTCGTGA	
GBS3012_F	4D	GAAGGTGACCAAGTTCATGCTTGCAGTCGTCCATCTTCa	HCD/J BSYH/J
GBS3012_H	4D	GAAGGTCGGAGTCAACGGATTTGCAGTCGTCCATCTTCg	
GBS3012_R	4D	GACTTCCAAACAATCAGACACG	

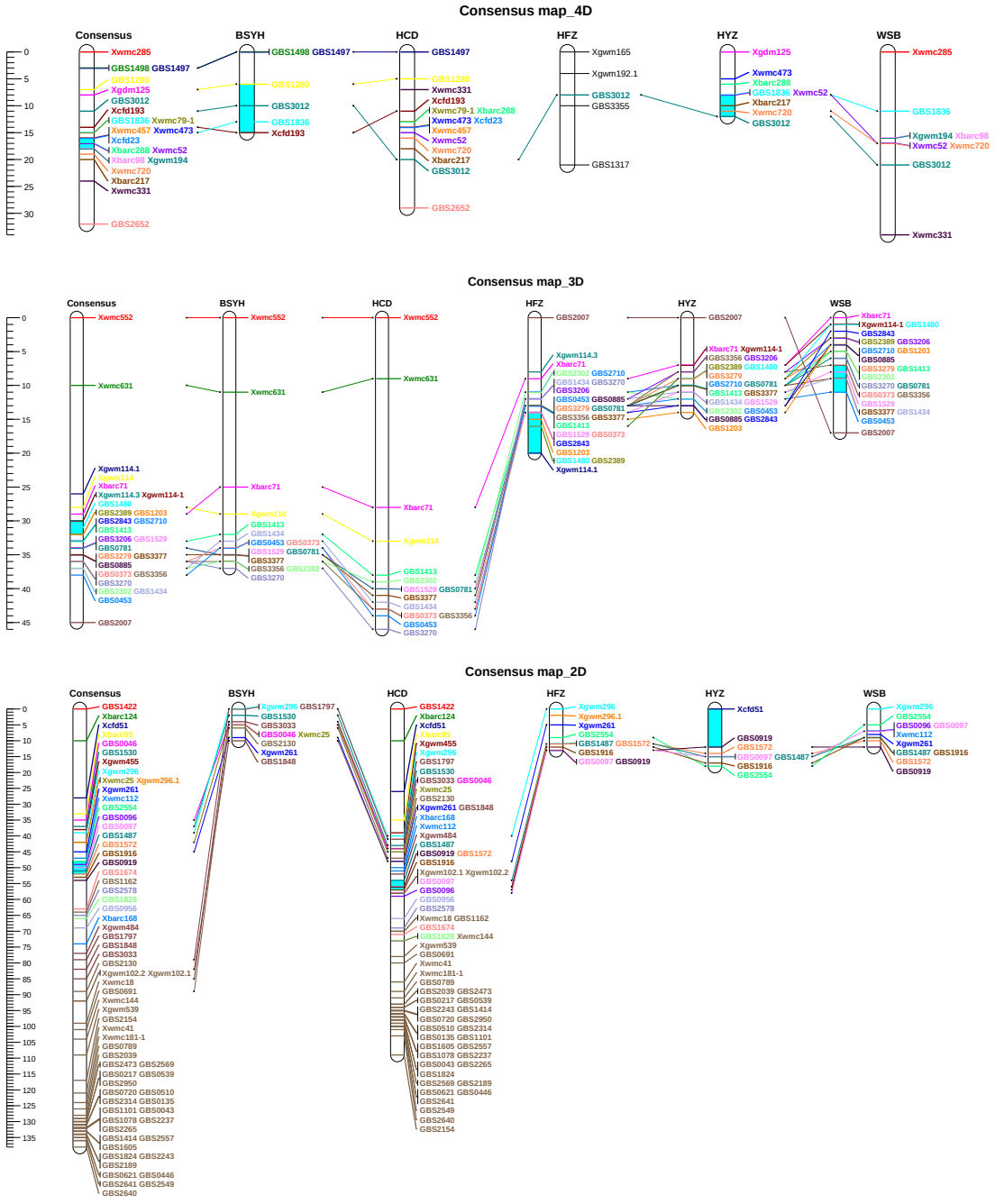
Note: 1. For KASPs *GBS2285*, *GBS2600*, *GBS3012*: H forward primer with alleles from Chinese landraces, F forward primer with alleles from Wheaton or Jagger; All the other KASPs: F forward primer with alleles from Chinese landraces, H forward primer with alleles from Wheaton or Jagger, R reverse primer.

2. BSYH/J represents Baishanyuehuang/Jagger; HCD/J represents Huangcandou/Jagger; HYZ/W denotes Haiyanzhong/Wheaton; HFZ/W denotes Huangfangzhu/Wheaton; WSB/W represents Wangshuibai/Wheaton.

Figure 3.1 Consensus map constructed from 3BS, 3A, 2D, 3D, and 4D linkage maps derived from the five mapping populations




Consensus map_3A
Consensus
BSYH
HCD
HFZ
HYZ
WSB



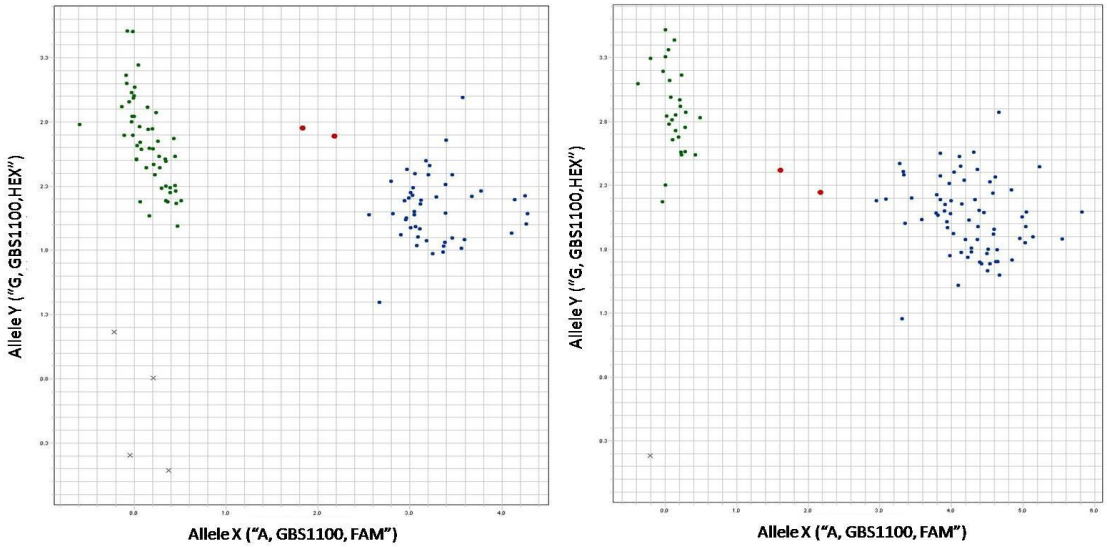
Note: ‘BSYH’ is short for ‘Baishanyuehuang’; ‘HCD’ is short for ‘Huangcandou’; ‘HYZ’ is short for ‘Haiyanzhong’; ‘HFZ’ is short for ‘Huangfangzhu’; ‘WSB’ is short for ‘Wangshuibai’.

Figure 3.2 Meta- map of QTLs associated with FHB type II resistance mapped to chromosomes 3BS (2), 3A, 2D, 3D and 4D of the consensus map (95% confidence intervals) developed from five populations with Chinese wheat landraces as the sources of FHB resistance

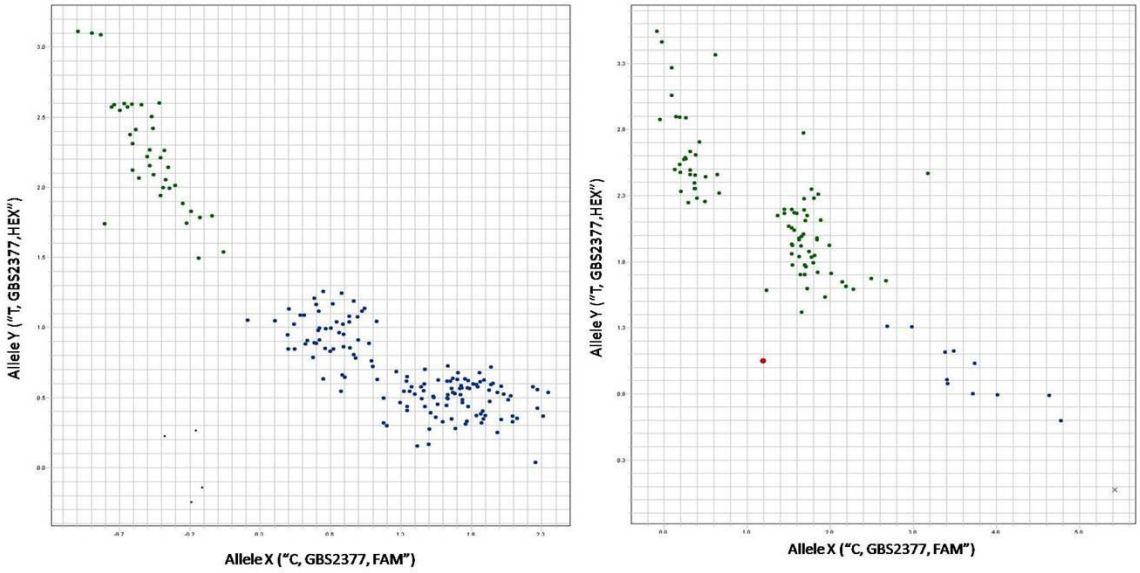
Note: ‘BSYH’ is short for ‘Baishanyuehuang’; ‘HCD’ is short for ‘Huangcandou’; ‘HYZ’ is short for ‘Haiyanzhong’; ‘HFZ’ is short for ‘Huangfangzhu’; ‘WSB’ is short for ‘Wangshuibai’.

Figure 3.3 KASP assay profiles of SNP.(a) KASP *GBS1100* on 3BSd, (b) KASP *GBS2377* on 3BSd, (c) KASP *GBS1989* on 3BSd, (d) KASP *GBS2882* on 3BSc, (e) KASP *GBS2285* on 3BSc, (f) KASP *GBS2373* on 3A, (g) KASP *GBS2600* on 3A, (h) KASP *GBS1487* on 2D, (i) KASP *GBS2710* on 3DL, (j) KASP *GBS0781*on 3DL, (k) KASP *GBS3012* on 4D in RIL populations and AM population. Blue dots represent Chinese landraces alleles, green dot represents U.S. wheat cultivar alleles, red dots refer to heterozygotes, and the black crosses or dots are ddH2O.

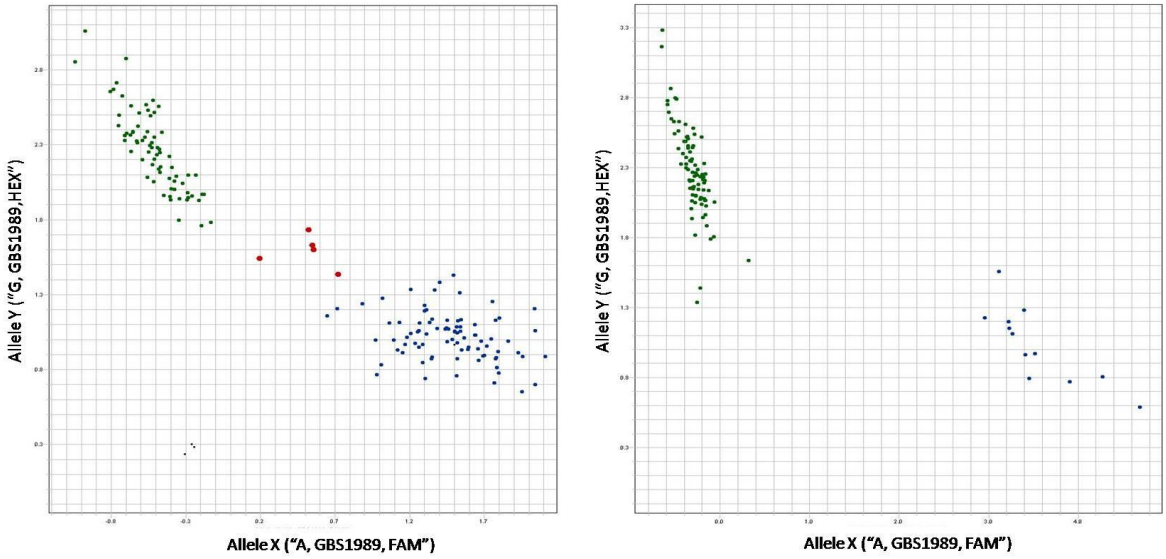
(a) KASP *GBS1100* on 3BSd in HFZ/Wheaton and AM population



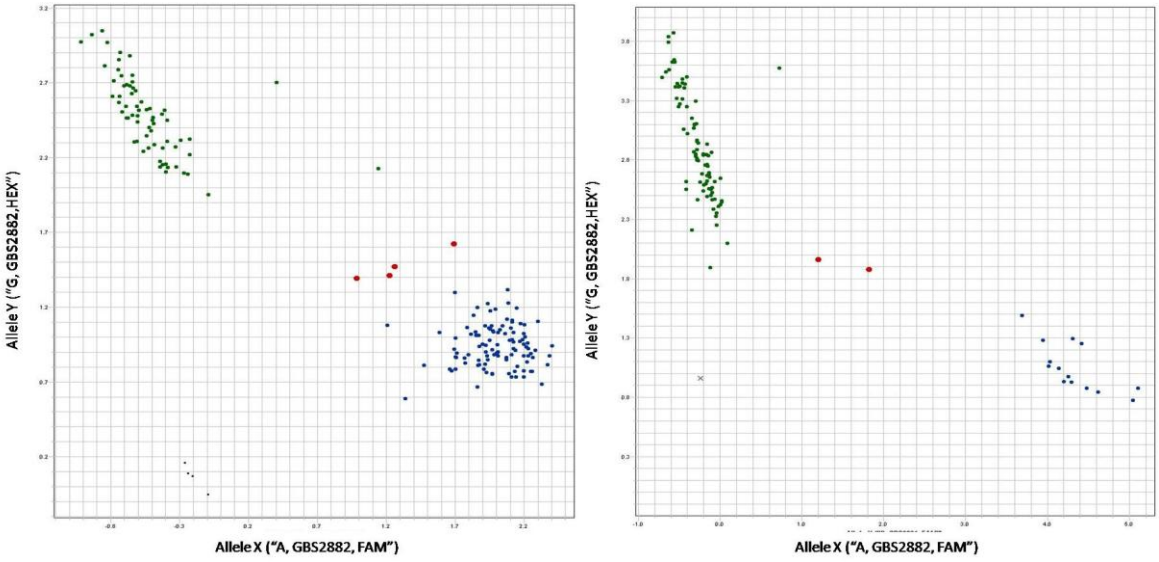
(b) KASP *GBS2377* on 3BSd in HFZ/Wheaton and AM population



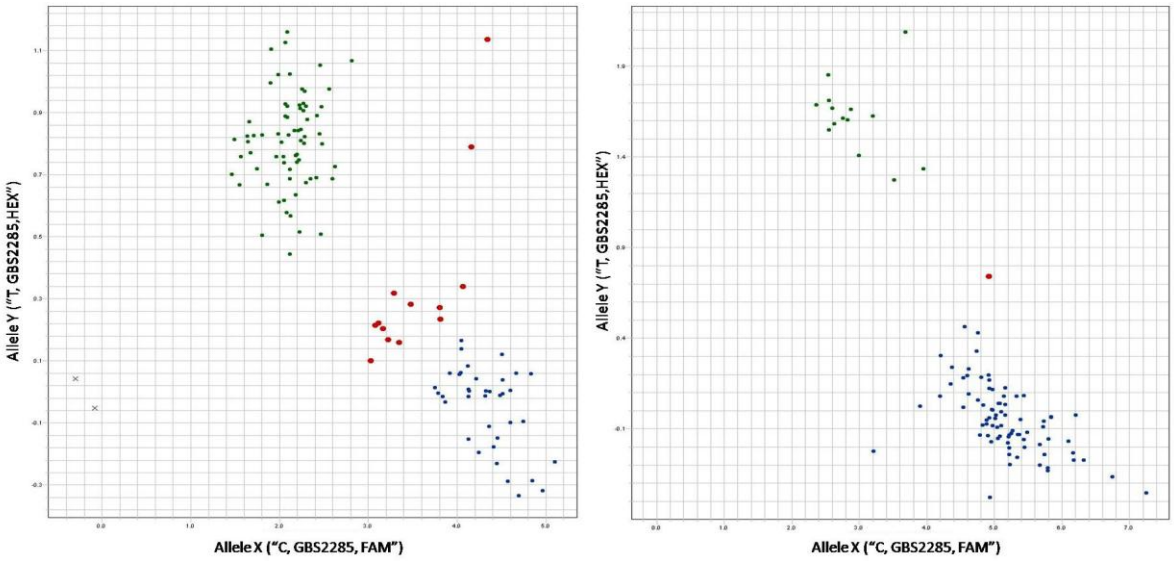
(c) KASP *GBS1989* on 3BSd in BSYH/Jagger and AM population



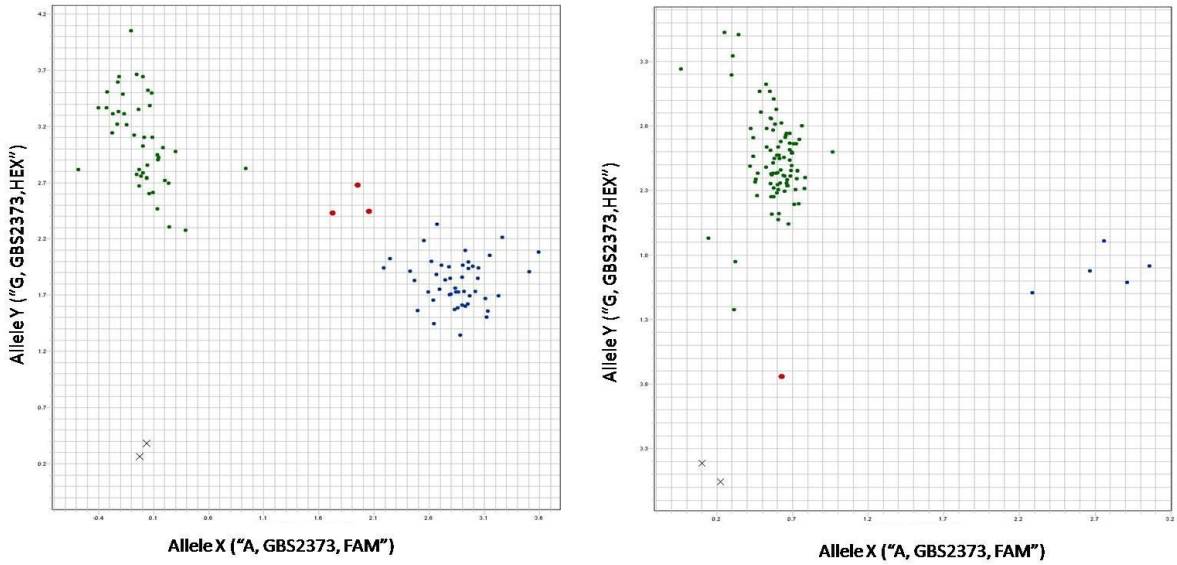
(d) KASP *GBS2882* on 3BSc in BSYH/Jagger and AM population



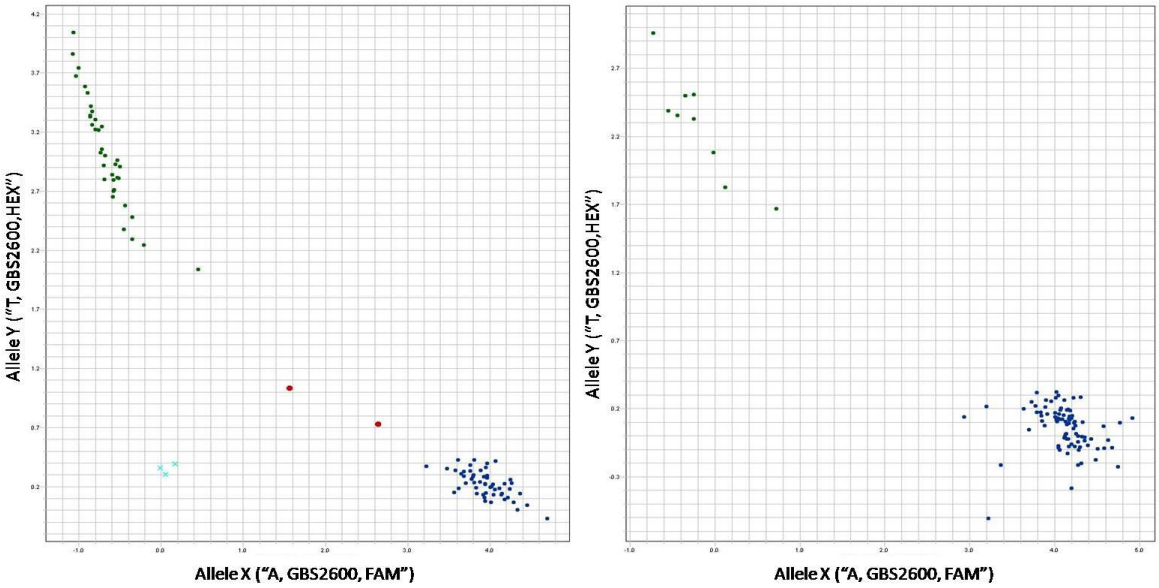
(e) KASP *GBS2285* on 3BSc in WSB/Wheaton and AM population (Chinese landrace allele on 'Allele Y', Wheaton or Jagger allele on 'Allele X')



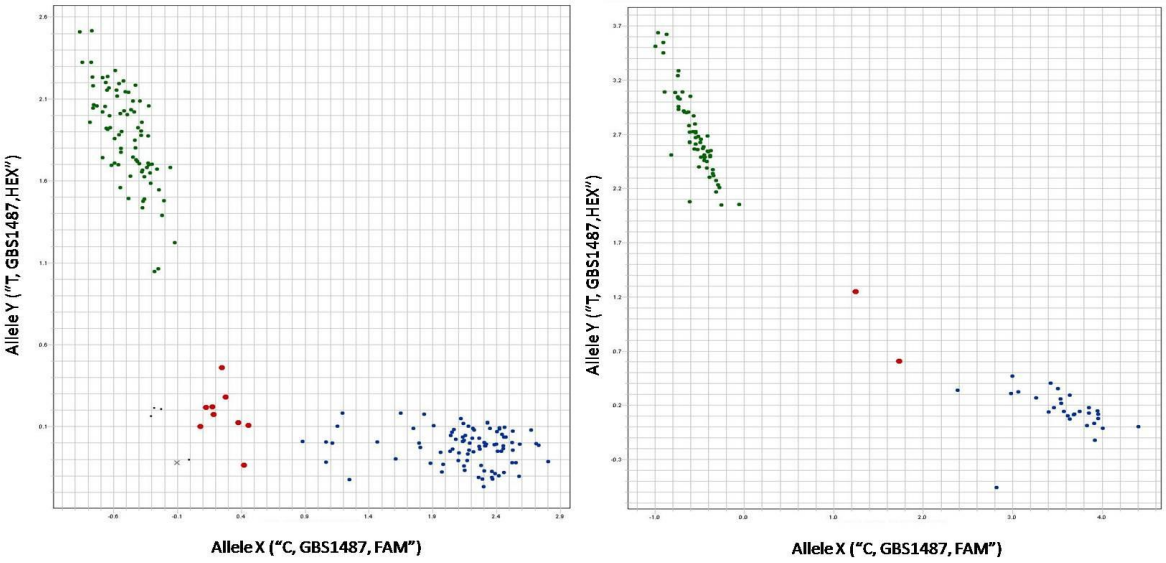
(f) KASP *GBS2373* on 3A in HYZ/Wheaton and AM population



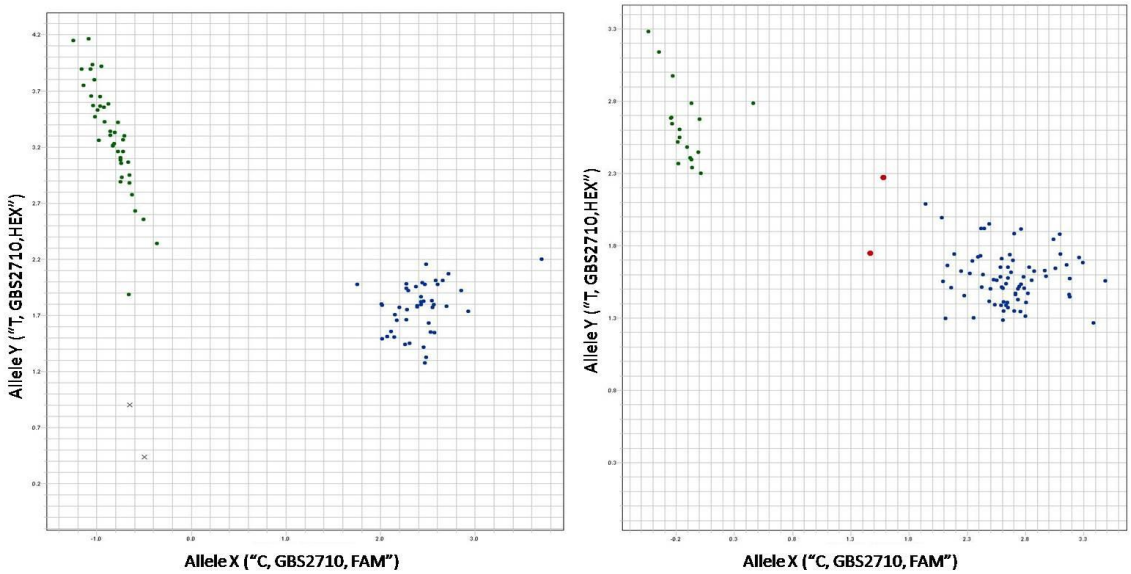
(g) KASP *GBS2600* on 3A in HYZ/Wheaton and AM population (Chinese landrace allele on 'Allele Y', Wheaton or Jagger allele on 'Allele X')



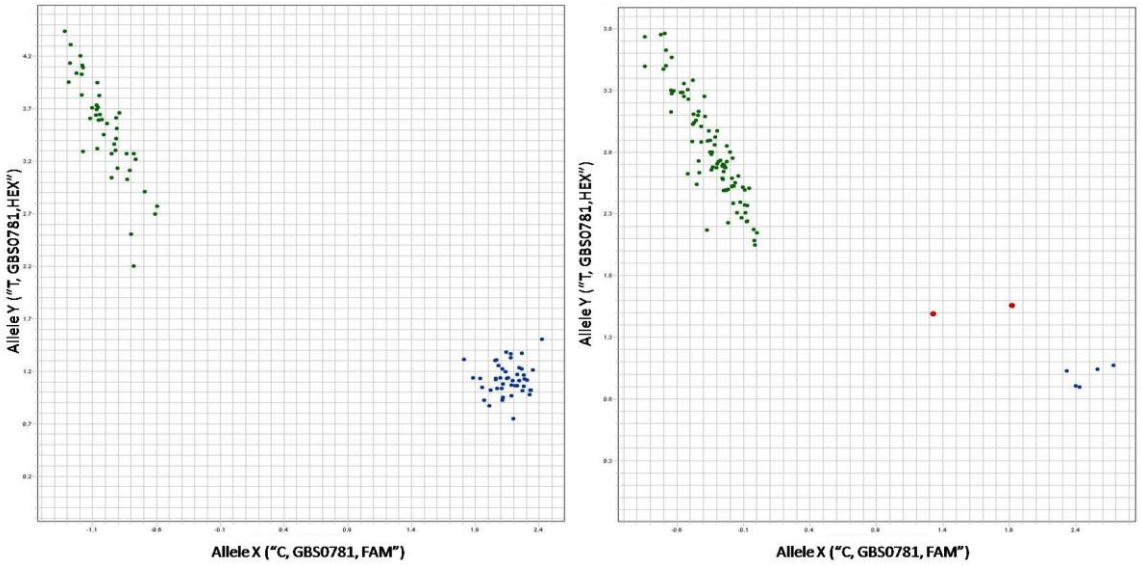
(h) KASP *GBS1487* on 2D in HCD/Jagger and AM population



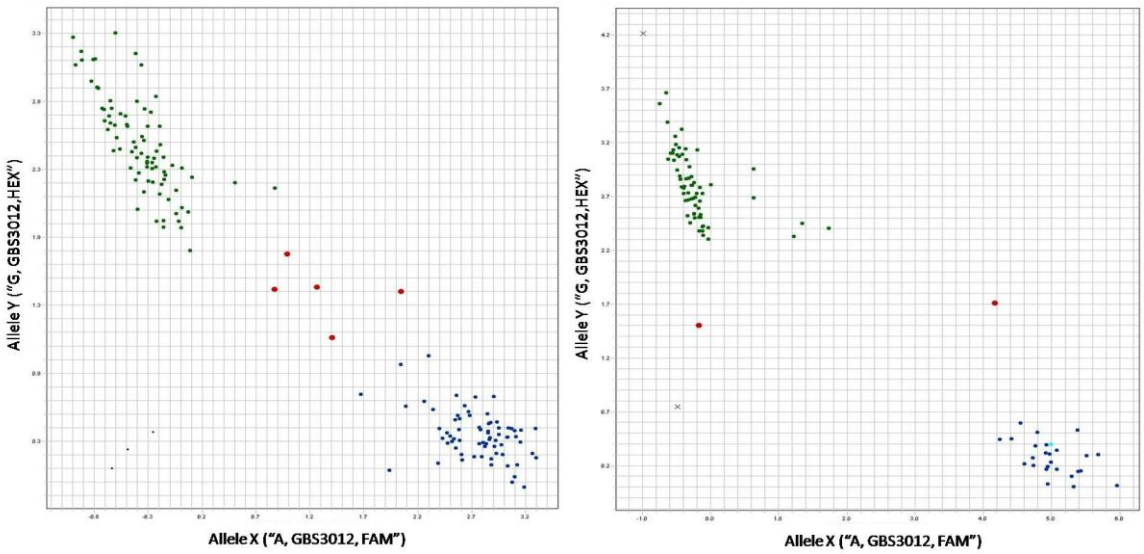
(i) KASP *GBS2710* on 3DL in WSB/Wheaton and AM population



(j) KASP *GBS0781*on 3DL in WSB/Wheaton and AM population



(k) KASP *GBS3012* on 4D in BSYH/Jagger and AM population (Chinese landrace allele on ‘Allele Y’, Wheaton or Jagger allele on ‘Allele X’)



Note: ‘BSYH’ is short for ‘Baishanyuehuang’; ‘HCD’ is short for ‘Huangcandou’; ‘HYZ’ is short for ‘Haiyanzhong’; ‘HFZ’ is short for ‘Huangfangzhu’; ‘WSB’ is short for ‘Wangshuibai’.

Chapter 4 - Effects of *Fhb1* on FHB Resistance in Hard Winter Wheats

Abstract

Fhb1, a quantitative trait locus (QTL) on 3BS from Sumai3 and its derivative Ning7840, has shown the largest effect on FHB resistance. In this study, we transferred *Fhb1* from Ning7840 into three adapted hard winter wheat (HWW) cultivars, Overland, Jagger, and Overley, by marker-assisted backcross (MAB), and assessed the effect of *Fhb1* on FHB resistance in these U.S. HWW backgrounds. High correlations were found between the percentage of symptomatic spikelet (PSS) and *Fusarium*-damaged kernel (FDK), PSS and deoxynivalenol (DON), and FDK and DON in the field experiments, suggesting visual scoring of PSS is useful to estimate FHB resistance. The *Fhb1* carrying lines selected from each population showed significantly lower mean PSS, FDK, and DON accumulation than the recurrent parents in both field and greenhouse experiments, although the levels of reduction varied among the populations from different recurrent parents and the environments these populations were tested in. Haplotype analysis using GBS-SNPs indicated the presence and the sizes of the *Fhb1* segment, and enabled us to check the proportion of recurrent parent genome recovery. A total of thirty two lines were selected from the three *Fhb1*-populations showed the relatively high level of FHB resistance and recurrent parents-like agronomic traits, thus can be used as resistant parents in U.S. HWW breeding programs.

Introduction

Fhb1 is a major QTL originally mapped on the short arm of chromosome 3B of a Chinese variety Sumai3 and shows a major effect on FHB resistance (Anderson et al. 2001; Cuthbert et al. 2006; Waldron et al. 1999). Chinese wheat line Ning7840 is a derivative of Sumai3 and *Fhb1* in Ning7840 explained up to 60% of the phenotypic variation for FHB resistance (Bai et al. 1999; Bai 1996; Cuthbert et al. 2006). However, both Sumai3 and Ning7840 carry many unadapted agronomic traits, thus, *Fhb1* has not been integrated into US hard winter wheat (HWW) after a decade of breeding effort. In the U.S., FHB epidemics originally occurred mainly in hard spring wheat and soft winter wheat (SWW) regions, thus breeding for wheat resistance to FHB started earlier than HWW. Several SWW cultivars with FHB resistance have been released for production including 'Truman', 'Massy', 'Roane', 'Ernie', and 'Freedom' etc. (Liu et al. 2013; Rudd et al. 2001; Sneller et al. 2012). These cultivars do not carry *Fhb1* (Liu et al. 2005), but several minor genes for FHB resistance with most of them from native sources. Several hard spring wheat with *Fhb1* have been released for production including Sabin from Minnesota and Alsen from North Dakota (Anderson et al. 2012a; Anderson et al. 2012b). Spring wheat cultivar 'Glenn' was also reported to have *Fhb1*, however, it cannot be detected using the closely linked marker *Xumn10* (ElDoliefy et al. 2015). In the U.S., most HWW cultivars used in production in the Great Plains are highly susceptible. Only a few HWW cultivars have been reported to have some levels of FHB resistance including 'Everest', 'Overland', 'Lyman' 'Heyne' and 'Hondo' (Bockus et al. 2009; Zhang et al. 2012a), but none of them carry *Fhb1*. Since *Fhb1*has a large effect, pyramiding *Fhb1* with native resistance genes from the U.S. locally adapted cultivars

may enhance the level of FHB resistance, and enrich the genetic diversity of FHB resistance in U.S. wheat cultivars. The current study used marker-assisted backcross (MAB) to transfer *Fhb1* into locally adapted U.S. HWW backgrounds without bringing in undesired agronomic traits from the Asian sources to develop locally adapted HWW germplasms with FHB resistance and to determine the *Fhb1* performance in different backgrounds.

Materials and methods

Plant materials

Ning7840 is a highly FHB resistant Chinese wheat line derived from Sumai3, (Bai et al. 1999), and used as the *Fhb1* donor for backcrosses; Chokwang is a moderately FHB resistant wheat cultivar from Korea (Yang et al. 2005), and used as a donor for QTLs on 5D and 4B (Yang et al. 2005). Three locally adapted U.S. HWW cultivars Overland from Nebraska, Overley, and Jagger from Kansas were used as recurrent parents. Among the three recurrent parents, Overland is moderately resistant to moderately susceptible, Jagger is moderately susceptible, and Overley is highly susceptible to FHB.

The backcross procedure is depicted in Figure 4.1. In brief, Ning7840 and Chokwang were crossed to Jagger, respectively, to obtain Ning7840/JaggerF_1 and Chokwang/JaggerF_1. The F_1 plants from the two crosses were then crossed to each other to generate (Ning7840/JaggerF_1 x Chokwang/JaggerF_1)F_1. The derived F_1 plants were genotyped with three markers (*Xumn10, SNP8,* and *Xgwm533)* linked to *Fhb1* to select *Fhb1* heterozygous plants for backcrosses or selfing. Selected F_1 plants were backcrossed to Overland, Overley, and Jagger, respectively, for two or three times to develop BC_2F_1 or BC_3F_1. At least 20 heterozygous plants were identified in each cross for backcrossing.

The selected *Fhb1* heterozygous BC_2F_1 and BC_3F_1 were selfed and homozygous BC_2F_2 or BC_3F_2 were selected with markers (*Xumn10*, *SNP8*, and *Xgwm533*) and advanced (Figure 4. 1). The selected homozygous BC_2F_2 or BC_3F_2 lines and later generations were used to evaluate the percentage of symptomatic spikelets (PSS) in the greenhouse experiments in spring and fall 2011. Based on the greenhouse PSS, lines with good resistance were further selected and evaluated for PSS in spring and fall 2012 greenhouse experiments, and for PSS, fusarium damaged kernel (FDK), and Deoxynivalenol (DON) in 2013 and 2014 field experiments.

Evaluation of FHB resistance

FHB inoculation and evaluation for type II resistance (PSS) in the greenhouse were described in Chapter 2. Field experiments were conducted in the Rocky Ford FHB Nursery in Kansas State University (Manhattan, KS). About 40 seeds per line were planted in a 1.3 m long plot. Each experiment used a randomized complete block design (RCBD) with two replications. The FHB nursery was inoculated using spawn inoculation by scattering *Fusarium graminearum*-infected corn (*Zea mays L.*) kernels on the soil surface twice at booting and heading stages. The FHB nursery was misted by sprinklers for 3 min /h from 2100 to 0600 h daily from flowering to early dough stage. PSS was estimated based on the overall performance of a plot at 19~21 d after heading dates, and was rechecked one more time after 3 d.

Plants from each plot were harvested by hand, threshed using a plant thresher (Almaco, Nevada, IA) and then cleaned by hand to keep as many tombstones as possible. FDK was visually evaluated using all kernels harvested from each plot based on checks with 5, 25, 50, 75, and 100% FDK.

Infected seed samples were hand cleaned to remove all trash and 5 grams randomly sampled grain were weighed for DON determination using gas chromatography-mass spectrometry (GC-MS) (Mirocha et al. 1998) at the University of Minnesota, St. Paul. DON concentration was measured in part per million (ppm).

Genotyping-by-sequencing

A genotyping-by-sequencing (GBS) library was constructed for the selected 128 *Fhb1* lines (49 Overland-*Fhb1* lines; 59 Jagger-*Fhb1* lines; 20 Overley-*Fhb1* lines) and parents with three replications each (Ning7840, Chokwang, Overland, Overley, and Jagger). The GBS library construction and data analysis were described in Chapter 2. The data were imputed using 'W7984' and 'Chinese spring reference sequences (IWGSC) 2014; Chapman et al. 2015). The imputed data were then blasted using 'Popseq' and 'W7984' wheat genome references for a 'gbs_loc' (http://129.130.90.211/wpdb/gbsloc) (Chapman et al. 2015). The SNPs were then arranged according to the positions in 'W7984' SNP map. The population genotypes were organized as 'A' for the donor parent genotype and 'B' for the recurrent parent genotypes.

Results

Selected FHB resistant lines

After genotyping with *Xumn10*, *SNP8* and *Xgwm533*, a total of 834 BC_2F_2 or BC_3F_2 plants with homozygous *Fhb1* marker alleles (207 Overland-*Fhb1* plants, 252 Jagger-*Fhb1* plants, and 375 Overley-*Fhb1* plants) were selected from 1000 plants of the each population. The selected lines were evaluated for FHB in the spring and fall 2011 greenhouse. Based on the data, 150 Overland-*Fhb1* lines, 131 Jagger-*Fhb1* lines, and 35 Overley-*Fhb1* lines that showed at least moderate resistance (PSS<60%) were selected

for further greenhouse and field FHB evaluations. A total of 128 lines (49 Overland-*Fhb1,* 59 Jagger-*Fhb1* and 20 Overley-*Fhb1* lines) were selected for GBS analysis based on the 2013 field PSS and FDK data. For Overland-*Fhb1* lines, 28 were BC_2 lines, and 21 were BC_3 lines (Figure 4.1 a). For Overley-*Fhb1* lines, 5 were BC_2, and 15 were BC_3 lines (Figure 4.1 b). All Jagger-*Fhb1* lines were BC_3 lines (Figure 4.1 c).

PSS of *Fhb1* lines in the greenhouse and field experiments

In the greenhouse experiments, *Fhb1* lines had lower mean PSS than their recurrent parents, and the PSS reduction ranged from 8.63 to 77.43% (Table 4.1). The mean PSS varied among the three *Fhb1* populations. Overland- *Fhb1* lines had the lowest mean PSS of 20.54%, and ranged from 7.14 to 65.93% (Table 4.1, Figure 4.2). Overley-*Fhb1* lines had the highest mean PSS of 46.31%, ranged from 27.04 to 73.08% (Table 4.1, Figure 4.2). And Jagger-*Fhb1* lines were in between with a mean PSS of 40.39%, ranged from 21.03 to 58.61% (Table 4.1, Figure 4.2). The recurrent parent Overland (40.35%) had the lowest mean PSS and Overley (95.08%) had the highest (Table 4.1). However, the mean PSS reduction was significant ($P < 0.01$) in Jagger and Overley *Fhb1* populations, but not significant in Overland *Fhb1* lines (Table 4.1).

In the field experiments, the PSS of the recurrent parents was the lowest in Jagger (51.67%), and the highest in Overley (80.05%) (Table 4.1, Figure 4.2). The mean PSS for the progenies was lower than those in the greenhouse experiments with the lowest (19.44%) in Jagger-*Fhb1* lines, and the highest in Overland-*Fhb1* lines (33.08%) (Table 4.1, Figure 4.2). A slightly different trend of PSS was observed between two years with the highest PSS for Overley-*Fhb1* (33.13%) in 2013 experiment and for Overland-*Fhb1* lines (38.96%) among three populations in 2014 field experiment (Table 4.1). The

reduction of PSS was significant ($P < 0.01$) for all the three *Fhb1*-populations compared to their recurrent parents (Table 4.1).

FDK and DON concentrations in *Fhb1*-carrying lines

In the field experiments, FDK and DON concentrations were also evaluated in the 2013 and 2014 experiments. All of the *Fhb1*-populations had significantly lower FDK than their recurrent parents (Table 4.2). Similar to PSS data, the mean FDK was the lowest in Jagger-*Fhb1* lines (11.94%), and the highest in Overland-*Fhb1* lines (20.61%) (Table 4.2). The recurrent parent Jagger had the lowest mean FDK (31.34%), and Overley had the highest FDK (67.50%) (Table 4.2). The DON concentrations were different among recurrent parents with the lowest mean DON concentration (9.51 ppm) in Jagger and the highest (20.13 ppm) in Overley (Table 4.2). The mean DON concentrations were similar among three populations with 6.61 ppm for Jagger-*Fhb1* lines, 7.65 ppm for Overley-*Fhb1* and 9.23 ppm for Overland-*Fhb1* lines and they were all lower than their recurrent parents (Table 4.2). Both FDK and DON concentrations in 2014 were much higher than those in the 2013 experiment.

The three *Fhb1*-carrying populations had lower PSS, FDK and DON accumulation than their recurrent parents, with the greatest reduction in Overley-*Fhb1* population (Table 4.1, Table 4.2). Therefore, transferring *Fhb1* into U.S. HWW can significantly reduce FHB severity in U.S HWW cultivars.

Relationship between PSS, FDK and DON in the field experiments

In the field experiment, the correlation coefficients were the highest between field PSS and FDK ($r = 0.82$, $P < 0.01$) (Figure 4.2 a), the second between field PSS and DON concentration ($r = 0.68$, $P < 0.01$) (Figure 4.2 b), and lowest between FDK and DON

concentration (r = 0.60, P < 0.01) (Figure 4.2 c), suggesting that the *Fhb1*-lines with low PSS usually have a low FDK and DON concentration in field.

Haplotype analysis

The GBS analysis of 128 *Fhb1* lines (49 Overland-*Fhb1*, 59 Jagger-*Fhb1*, and 20 Overley-*Fhb1* lines) obtained 18,376 GBS-SNPs with 80% missing data. Among them, 1,253 had 20% or less missing data. After imputation, the GBS-SNPs with 20% missing increased to 15,677 GBS-SNPs. These GBS-SNPs were used for mapping using 'Popseq', and 14,113 GBS-SNPs were mapped to specific chromosome locations for haplotype analysis.

For each *Fhb1*-line, Ning7840 or Chokwang's alleles were designated as genotype 'A' and recurrent parent allele as 'B' to show parental allele distribution in the wheat genome. Haplotype analysis revealed a large block of 15 GBS-SNPs in Overland, 23 in Jagger and 26 in Overley from the donor on the short arm of 3B chromosome that cover genetic distances of 12.9 cM in Overland-*Fhb1*, 13.67 cM in each of Jagger and Overley-*Fhb1* populations (Figure 4. 4). GBS-SNPs within the SNP blocks were then blasted in National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). The physical distance of the SNP blocks on 3BSd was 12,417,272 bp in Jagger-*Fhb1* lines, 12,526,290 bp in Overley-*Fhb1* lines, and 9,900,850 bp in Overland-*Fhb1* lines. In the Overland-*Fhb1* population, 42 of the 49 lines have all 'A'genotype within the *Fhb1* block, line 4 has all 'B' genotype, and lines 18, 28, 29, 39, 41 and 47 have four to thirteen SNPs with 'A' genotype in the *Fhb1* block (Figure 4. 4 a). For Jagger-*Fhb1* population, 54 of the 59 lines have all 'A' genotypes within the *Fhb1* block, line 40, 48 have five SNPs with 'A'genotype each, and line 5, 18, and 50 have 10,

13, and 14 SNPs with 'A' genotype, respectively (Figure 4. 4 b). For the Overley-*Fhb1* population, 16 out of 20 lines have all 'A' genotypes within the *Fhb1* block, line 11 and 20 have 25 SNPs with 'A' genotype, and line 3 and 16 have 10 SNPs with 'A' genotype (Figure 4. 4 c). We then ran two *Fhb1* diagnostic markers (*cg8* and *Xumn10*) to figure out if all the lines from the three populations have *Fhb1* or not. The genotyping results showed that all of the lines of Jagger and Overley populations have *Fhb1*. However, Lines #4 and #18 of the Overland population showed no *Fhb1* fragment in haplotype analysis was also genotyped as 'b' using the *Fhb1* diagnostic markers (Appendix A table 1).

For Overland and Overley-*Fhb1* populations, we developed both BC_2 and BC_3 progenies and haplotyping analysis indicated that BC_2 progenies usually have better FHB resistance, but lower genome recovery than BC_3 progenies. In Overland-*Fhb1* lines, the BC_2 lines have lower mean greenhouse and field PSS, and mean DON accumulation, however, slightly higher mean FDK than BC_3 lines (Table 4.3). The proportions of Overland genome recovery ranged from 85.59 to 98.56%, with an averaged genome recovery of 93.42% in BC_2 plants, and 95.46% in BC_3 plants (Table 4.3). For Overley-*Fhb1* progenies, the BC_2 lines showed significantly lower mean field PSS, FDK, and DON concentration, and slightly lower greenhouse PSS than BC_3 plants (Table 4.3). The proportion of genome recovery ranged from 64.37 to 97.71% with an averaged genome 87.23% in BC_2 and 94.87% in BC_3 lines (Table 4.3). Therefore, BC_2 plants showed better FHB resistance for both *Fhb1* populations, but less recurrent parent's genome recovery than the BC_3 plants.

Fhb1-lines with outstanding FHB resistance, as well as the reasonable proportion of recurrent parent genome recovery were selected from each of the Overland, Overley, and Jagger-*Fhb1* populations. The twenty five percent of lines with top FHB resistance and recurrent parent genome recovery were selected from each *Fhb1*-population (Appendix A). Twelve out of the forty nine Overland-*Fhb1* lines (GH PSS < 17%, field PSS < 23%, FDK < = 20%, and DON concentration < 11 ppm) (Appendix A Table 1), fifteen of the fifty nine Jagger-*Fhb1* lines (GH PSS < 40%, field PSS < = 20%, FDK < 18%, and DON concentration < 10 ppm) (Appendix A Table 2), and five of the twenty Overley-*Fhb1* lines (GH PSS < 46%, field PSS < 30%, field FDK < 17%, and DON concentration < 10 ppm) (Appendix A Table 3) were selected from the three *Fhb1*-populations. The selected lines were listed in Appendix A labeled with asterisks.

Discussion

FHB resistance of Fhb1 in different genetic backgrounds

FHB epidemics in U.S. HWW in the Great Plains have become more severe and frequent in the recent years, and transferring *Fhb1* from Sumai3 and its derivatives can be an effective way to quickly improve the resistance in U.S HWW (McMullen et al. 2012; Rudd et al. 2001). In the current study, we transferred *Fhb1* into three U.S. HWW using marker-assisted backcrossing, and the selected *Fhb1* lines showed significantly lower PSS, FDK, and DON than their recurrent parents (Table 4.1, 4.2), indicating that *Fhb1* can significantly improve FHB resistance and reduce DON in HWW genetic backgrounds.

Among the three recurrent parents, Overland is moderately resistant to moderately susceptible and Jagger is moderately susceptible, thus, they may carry some indigenous

minor QTLs for FHB resistance, while Overley is highly susceptible and may not have any FHB resistance QTL. Mean PSS were different among the three *Fhb1*-populations. In the greenhouse experiments, Overland-*Fhb1* lines had the lowest mean PSS (20.54%), and Overley-*Fhb1* lines had the highest (46.31%), which agreed with the ranks of the recurrent parents. However, Jagger-*Fhb1* lines performed better than Overland-*Fhb1* lines in both field experiments. The discrepancy between greenhouse and field experiments was probably due to earlier heading date of Jagger -*Fhb1* than Overland-*Fhb1* that escaped from a long period of warm and wet FHB inductive conditions in the Manhattan field experiments.

Effects of *Fhb1* on the reduction of PSS are similar among the three populations in the greenhouse experiments, but different in the field experiments. The Overland-*Fhb*1 lines had a lower reduction in the three types of resistance than other two populations. This is probably due to later flowering time of Overland population than other two populations that increased FHB severity in the field.

Correlation among PSS, FDK, and DON

Fhb1 was previously identified as a major QTL for FHB resistance, especially, type II resistance as measured by PSS (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2003; Cai and Bai 2014; Cuthbert et al. 2006; Jia et al. 2006; Shen et al. 2003; Waldron et al. 1999; Yu et al. 2008; Zhang et al. 2012b). Significant high correlations were found between PSS and FDK ($r = 0.82$, $P < 0.01$) (Figure 4. 2 a), PSS and DON accumulation ($r = 0.68$, $P < 0.01$) (Figure 4. 2 b), FDK and DON accumulation ($r = 0.59$, $P < 0.01$) (Figure 4. 2 c) in the field. These agree with several previous reports (Bai et al. 2001; Lemmens et al. 2004; Paul et al. 2005; Wegulo et al. 2011). However, opposite

results were also reported between visual FHB rating and DON accumulation (Liu et al. 1997; Wisniewska et al. 2004). This discrepancy might be due to the way of threshing or cleaning seeds. The light weigh tombstones can be easily blown away if special care was not taken during threshing, which can underestimate both FDK and DON accumulation. Also, field PSS rating was to visually estimate FHB severity for each plot on the 20th day after the heading, rather than counting scabbed spikelets in each spike as in greenhouse experiments, thus, the personal experience might significantly affect PSS ratings in the field. Nevertheless, if the seeds are carefully threshed and cleaned, an experienced scientist could get consistent FHB scores that result in high correlations among PSS, FDK, and DON concentration.

Haplotype analysis

By using haplotype analysis *Fhb1* region is clearly identified as a block of GBS-SNPs in all the three *Fhb1*- derived populations. The block with *Fhb1* transferred from the donor consist of 15 SNPs spanning 12.9 cM in Overland-*Fhb1* population, 23 SNPs covering 13.67 cM in Jagger-*Fhb1* population, and 26 SNPs covering 13.67 cM in Overley-*Fhb1* population. The donor fragment size was not too long compared to most *Fhb1* QTLs reported previously (Cai and Bai 2014; Yu et al. 2008; Zhang et al. 2012b). The physical lengths of the transferred fragments were 9.9 Mb in Overland-*Fhb1* lines, 12.5 Mb in Overley-*Fhb1* lines, and 12.4 Mb in Jagger-*Fhb1* lines, ranged from 304,411 bp to 12,721,683 bp on 3B chromosome. Analysis of the *Fhb1*-populations showed that lines 4 and 18 of Overland-*Fhb1* lines showed higher field PSS, FDK, and GH PSS than the averaged value of lines with all 'A' genotype in the *Fhb1* SNP block (Figure 4.4 a). *Fhb1* diagnostic markers (*Xumn10* and *cg8*) screening over the three populations showed

that *Fhb1* was absent in lines 4 and 18 of the Overland population. The line 4 showed no *Fhb1* fragment in haplotype analysis actually had no *Fhb1*, while the line 18 showed the donor fragment, however, was negative with diagnostic markers *Xumn10* and *cg8*, thus, can be excluded from further selection. The other lines with single "B" alleles were seen in *Fhb1* fragments of all the three populations, this occurs probably because the lines are actually heterozygous on *Fhb1*. Besides, the *Fhb1* region, donor alleles were randomly present throughout the genome, indicating that MAB without background selection might bring in some unwanted donor fragments into the genome of *Fhb1*-carrying populations. However, GBS for background screening can be helpful to identify lines with fewer and shorter donor fragments that may be associated with unadapted traits. Besides, some other common SNP haplotype blocks from donor genotypes were identified across all the three *Fhb1*-populations. Because the selected lines in this study have been through several cycles of FHB resistance evaluation, those blocks may carry some other QTLs for FHB resistance from the donor by chance. SNP haplotypes blocks on 4B of Overland, Overley, and Jagger-*Fhb1* populations and on 5D of Overland and Overley-*Fhb1* population may have been transferred from Chokwang. Therefore, haplotype analysis using GBS-SNPs on the three *Fhb1*-populations help us to determine the presence and the sizes of the target fragments transferred from the donor genome, and enable us to check how much recurrent parents' genomes were recovered after two or three rounds of backcrossing.

For Overland and Overley, we found that BC_2 plants had better FHB resistance for both *Fhb1*-populations, but less recurrent parent genome recovery than BC_3 plants. The BC_2 plants had a larger proportion of donor genome than BC_3 plants, thus, may have

a bigger chance to carry resistant alleles from donor plants. The averaged genome recovery in Overley-*Fhb1* lines were 87.23% in BC_2, and 94.87 in BC_3 plants, which agreed with the theoretical proportions of 87.5% in BC_2, and 93.75% in BC_3. However, Overland-*Fhb1* lines showed much higher genome recovery than expected (93.42% in BC_2 plants, and 95.46% in BC_3 plants). This is probably because we used Jagger to cross with Ning7840 and Chokwang in the initial steps, so that some 'B' genotypes may be contributed by Jagger rather than Overland. Therefore, one more cycle of backcrossing significantly improved the genome recovery, thus enable us to select *Fhb1*-lines with good FHB resistance as well as better recurrent parent genome recovery.

A total of 32 *Fhb1*-lines (12 of Overland, 15 of Jagger, and 5of Overley-*Fhb1* populations) were selected from the three populations. Lines selected were the top 25% of FHB resistance from each population, however, with different criteria of selection on PSS, FDK, and DON accumulation. For example, the selection criteria in greenhouse PSS was high in Overley (< 46%) and Jagger-*Fhb1* (< 40%) population, however, relatively low in Overland-*Fhb1* lines (< 17%). This is because most of the Overley and Jagger-*Fhb1* lines showed a moderate level of resistance in the greenhouse, however, half of Overland-*Fhb1* lines show PSS less than 20%. Therefore, we cannot set one selection criteria for all three populations. The selected *Fhb1*-lines showed relatively good FHB resistance in both greenhouse and field experiments, with large proportions of recurrent parent genome recovery. The selected *Fhb1*-lines with recurrent parents-like agronomic traits can be used as resistant parents in U.S. HWW breeding programs.

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Table 4.1 Comparison of PSS of selected lines from Overland-Fhb1, Jagger-Fhb1 and Overley-Fhb1 populations with their corresponding recurrent parents.

	Spring 2011 GH %	Fall 2011 GH %	Spring 2012 GH %	Fall 2012 GH %	Mean GH %	GH PSS Range %	2013 field %	2014 field %	Mean field %
Overland-*Fhb1* lines (49)	24.67	19.22	19.93	18.34	**20.54**	7.14~65.93	27.19	38.96	**33.08**
Overland	27.00	34.19	66.22	34.00	**40.35**		51.00	66.67	**58.84**
PSS reduction due to *Fhb1* (%)	8.63	43.78	69.90**	46.06	**49.10**		46.69**	41.56**	**43.78****
Jagger-*Fhb1* lines (59)	20.20	49.62	45.05	44.15	**40.39**	21.03~58.61	16.86	22.01	**19.44**
Jagger	89.51	77.42	82.72	85.00	**83.66**		55.00	48.33	**51.67**
PSS reduction due to *Fhb1*(%)	77.43**	35.91	45.54*	48.06*	**51.14****		69.35**	54.46**	**62.38****
Overley-*Fhb1* lines (20)	26.89	49.67	46.76	64.67	**46.31**	27.04~73.08	33.13	24.75	**28.94**
Overley	87.63	100.00	97.14	95.54	**95.08**		81.00	80.00	**80.50**
PSS reduction due to *Fhb1*(%)	69.31*	50.33	51.86*	32.30	**49.14****		59.10**	69.06**	**64.05****

Note: * indicates statistically significant with $P < 0.05$, ** indicates statistically significant with $P < 0.01$.

Table 4.2 Comparison of field FDK and DON of selected lines from Overland-Fhb1, Jagger-Fhb1 and Overley-Fhb1 populations with their corresponding recurrent parents.

	2013 FDK %	2014 FDK %	Mean FDK %	2013 DON (ppm)	2014 DON (ppm)	Mean DON (ppm)
Overland-*Fhb1* lines (49)	8.85	32.37	**20.61**	4.02	14.44	**9.23**
Overland	25.00	56.67	**40.84**	4.46	20.60	**12.53**
FHB reduction due to *Fhb1*(%)	64.60*	42.88*	**49.53****	9.87	29.90	**26.34**
Jagger-*Fhb1* lines (59)	7.37	16.21	**11.94**	2.44	10.48	**6.61**
Jagger	26.00	36.67	**31.34**	3.91	15.10	**9.51**
FHB reduction due to *Fhb1*(%)	71.65**	56.75*	**62.92***	37.60	30.60	**30.49**
Overley-*Fhb1* lines (20)	16.23	18.18	**17.21**	4.36	10.94	**7.65**
Overley	65.00	70.00	**67.50**	12.65	27.60	**20.13**
FHB reduction due to *Fhb1*(%)	75.03**	74.03**	**74.50****	65.53*	60.36*	**62.00****

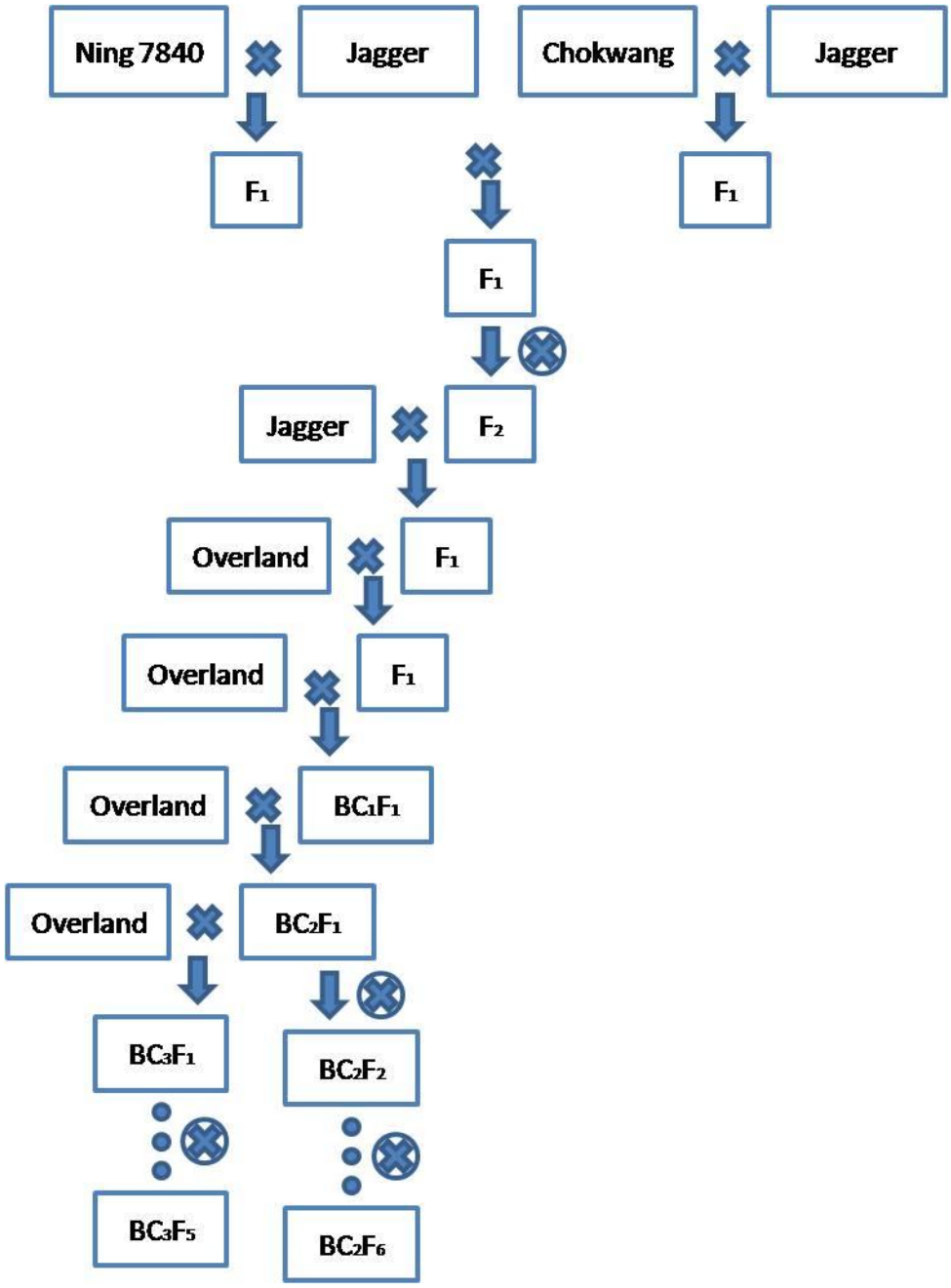
Note: * indicates statistically significant with $P < 0.05$, ** indicates statistically significant with $P < 0.01$

Table 4.3 Comparison of the mean greenhouse PSS, field PSS, FDK and DON of BC2 and BC3 lines from Overland-Fhb1 and Overley-Fhb1 populations.

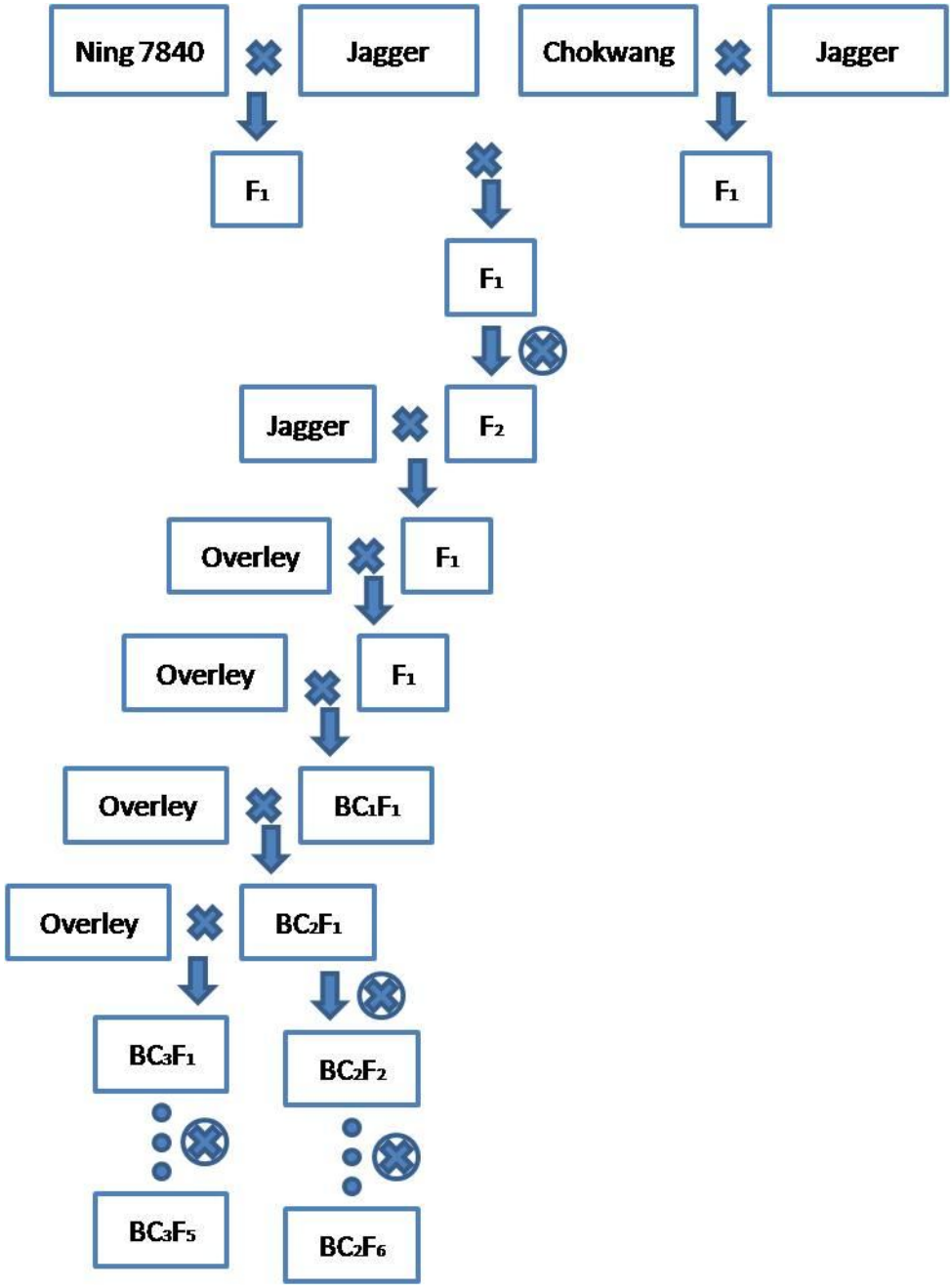
		Genome background recovered %	Mean GH PSS %	Mean field PSS %	Mean field FDK %	Mean field DON %
Overland-*Fhb1* lines	BC_2	93.42	18.06	32.55	21.29	9.15
	BC_3	95.46	21.94	34.10	20.48	9.68
	Diff.	2.04*	3.87	1.55	-0.81	0.53
Overley-*Fhb1* lines	BC_2	87.23	46.01	26.19	14.93	6.47
	BC_3	94.87	52.27	37.29	23.13	11.01
	Diff.	7.64	6.26	11.10*	8.20*	4.54**

Note: * indicates statistically significant with $P < 0.05$, ** indicates statistically significant with $P < 0.01$

Figure 4.1 Processes of backcrossing to (a) Overland, (b) Overley, and (c) Jagger



(b) Overley


Ning 7840
Jagger
Chokwang
Jagger
F1
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F1
Jagger
F2
Overley
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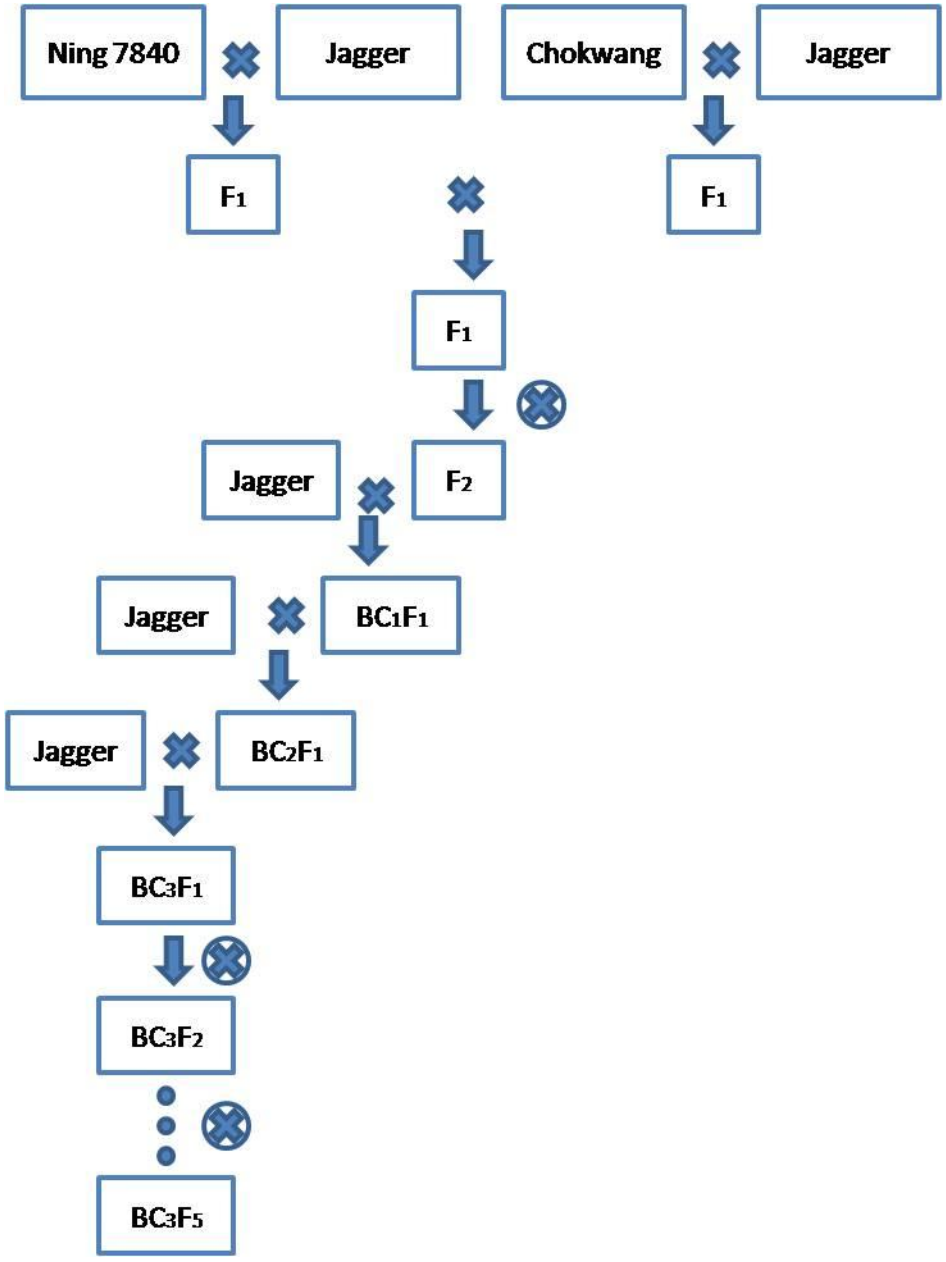
Overley
BC1F1
Overley
BC2F1
BC3F1
BC2F2
BC3F5
BC2F6

(c) Jagger


Ning 7840
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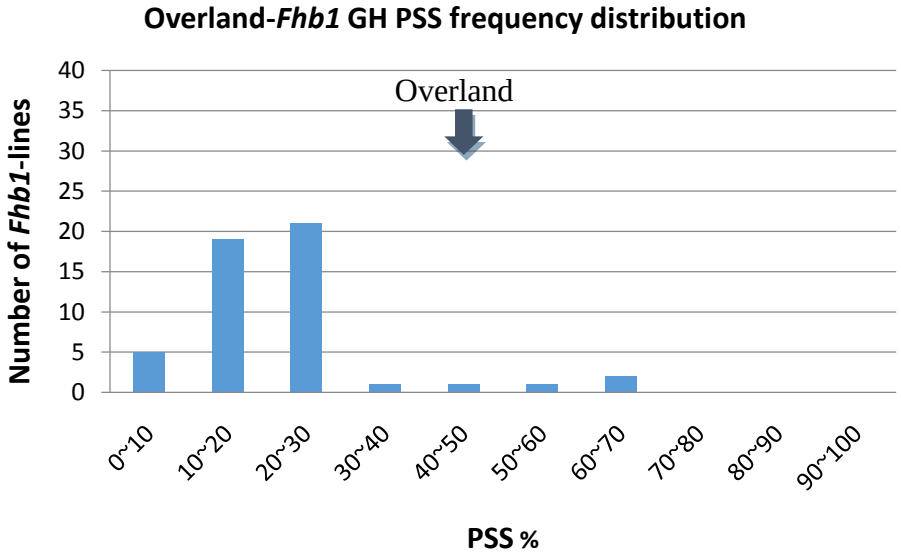
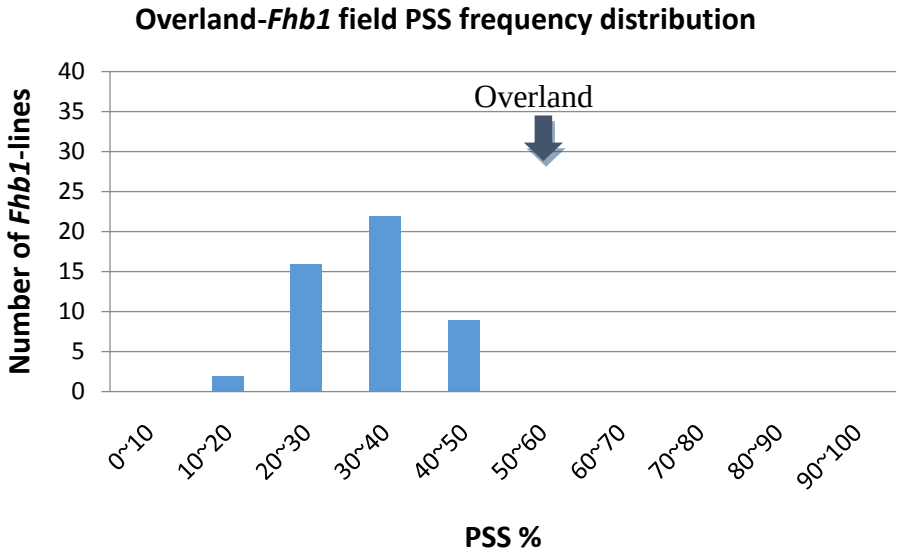
BC1F1
Jagger
BC2F1
BC3F1
BC3F2
BC3F5

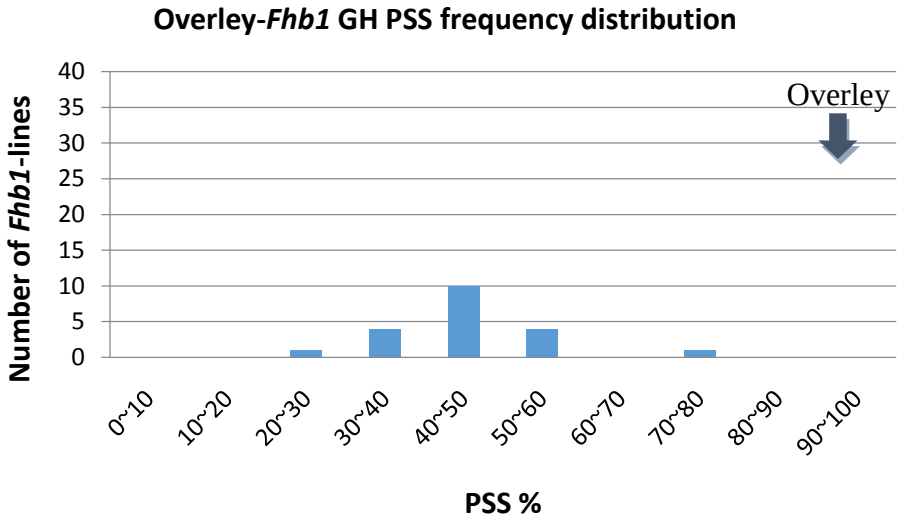
Figure 4.2 Distrubution of mean percentage of symptomatic spikelets for (a) Overland, (b) Overley, and (c) Jagger-Fhb1 lines in greehouse and field experiments.

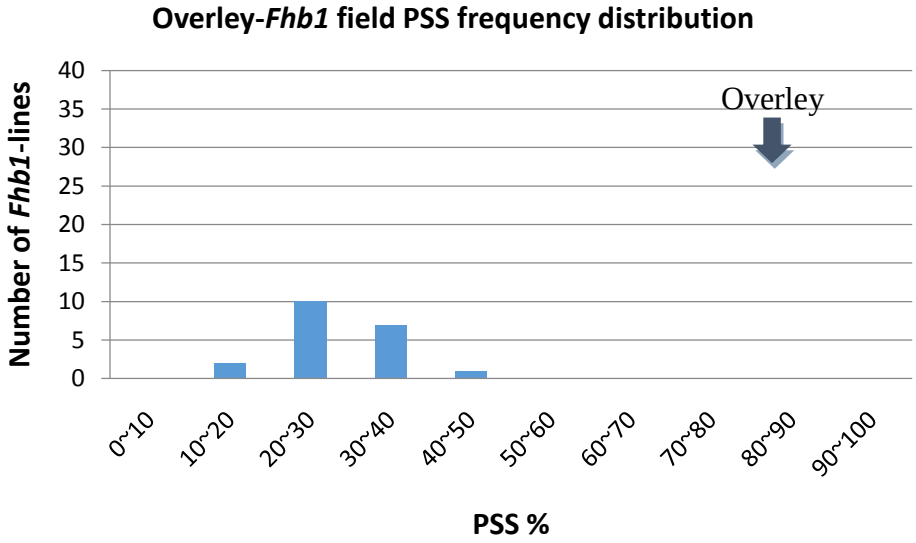
(a)



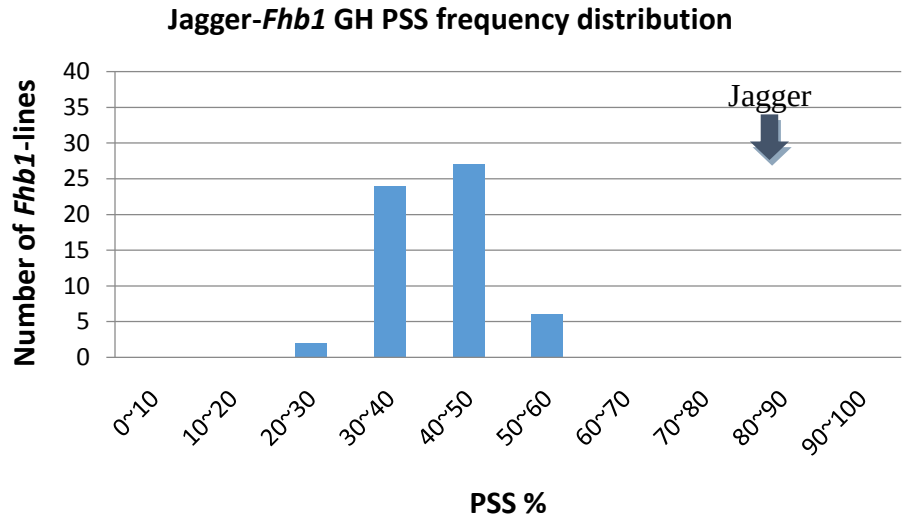


(b)


Overley-*Fhb1* GH PSS frequency distribution
Number of *Fhb1*-lines
40
35
30
25
20
15
10
5
0
Overley
0~10
10~20
20~30
30~40
40~50
50~60
60~70
70~80
80~90
90~100
PSS %


Overley-*Fhb1* field PSS frequency distribution
Number of *Fhb1*-lines
40
35
30
25
20
15
10
5
0
Overley
0~10
10~20
20~30
30~40
40~50
50~60
60~70
70~80
80~90
90~100
PSS %

(c)


Jagger-*Fhb1* GH PSS frequency distribution
Number of *Fhb1*-lines
40
35
30
25
20
15
10
5
0
Jagger
0~10
10~20
20~30
30~40
40~50
50~60
60~70
70~80
80~90
90~100
PSS %

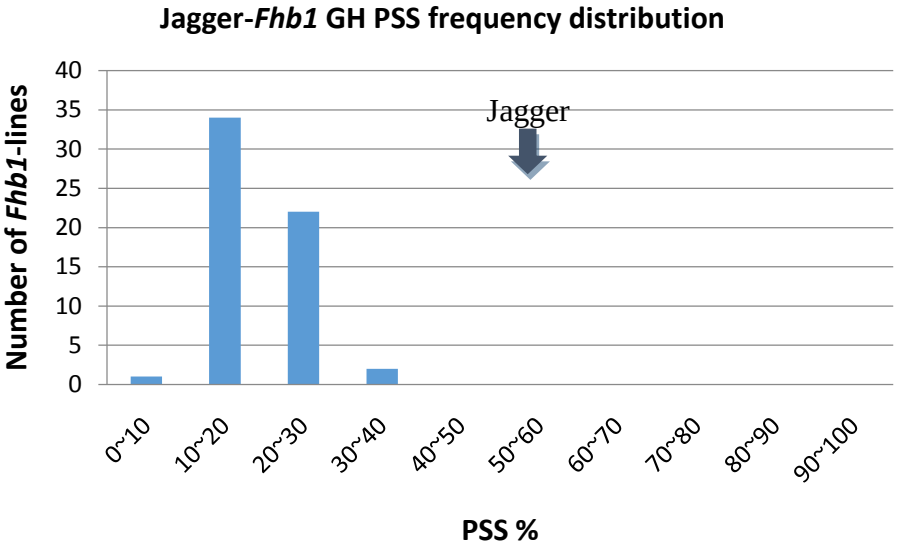
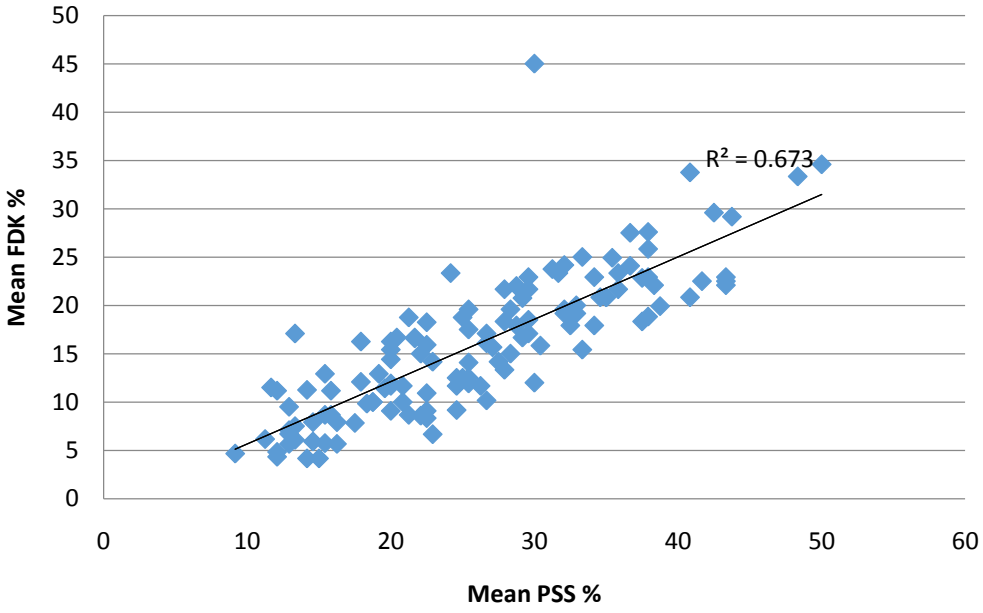
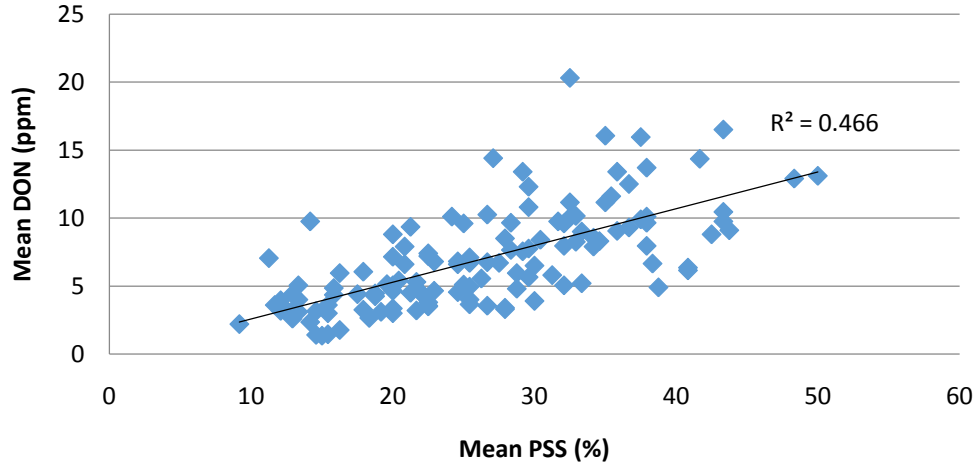

Jagger-*Fhb1* GH PSS frequency distribution
Number of *Fhb1*-lines
40
35
30
25
20
15
10
5
0
Jagger
0~10
10~20
20~30
30~40
40~50
50~60
60~70
70~80
80~90
90~100
PSS %

Figure 4.3 Correlations between field PSS, FDK, and DON concentration.

(a) Correlation of mean field PSS and FDK



(b) Correlation of mean field PSS and DON



(c) Correlation of mean field FDK and DON

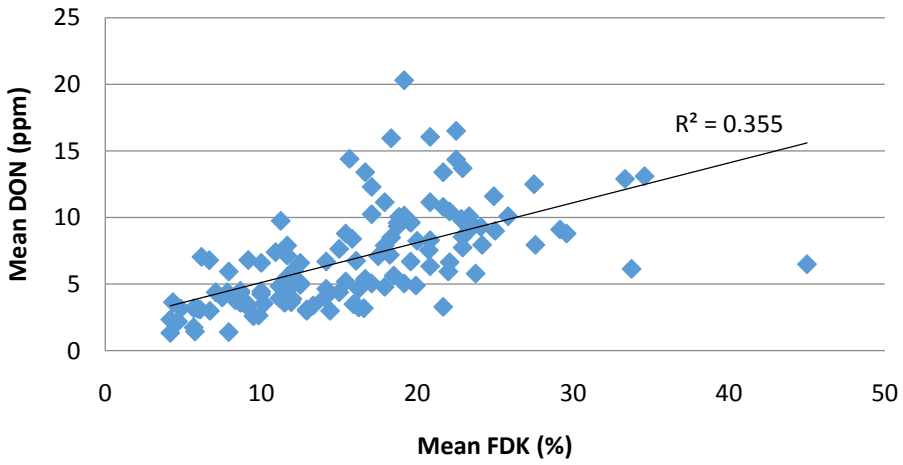


Figure 4.4 SNP haplotype blocks with donor genotypes in (a) Overland, (b) Jagger and (c) Overley-Fhb1 populations. 'A' in red represents the donor genotypes, 'B' in blue represents the recurent parents' genotypes, and 'H' in purple represens heterozygotes. The columns showed GBS-SNPs ordered in genetic distance of wheat genome, and the lines were *Fhb1*-lines in each population.

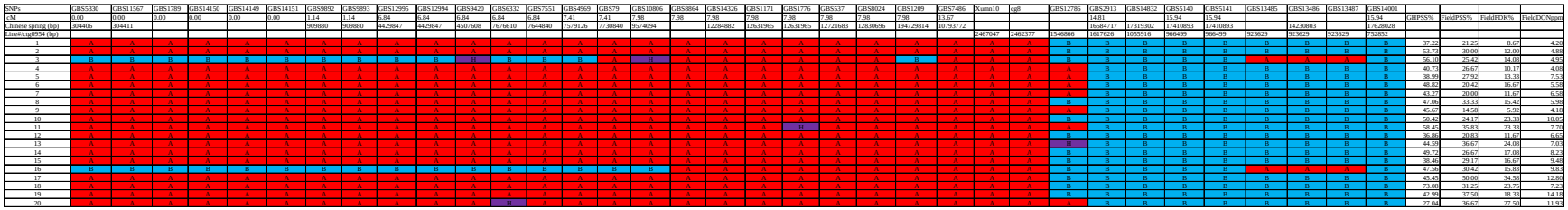
(a) SNP haplotype block in Overland-*Fhb1* population

SNPs	GBS7200	GBS9892	GBS9893	GBS9001	GBS5721	GBS4968	GBS10806	GBS14326	GBS1211	GBS1210	GBS1823	GBS7486	Xumn10	cg8	GBS13485	GBS13486	GBS13487	GBS14832	GBS5140	GBS5141	GBS14001	GBS13198	GBS15083	GBS5633	GBS957				
cM	1.1370	1.1370	1.1370	6.8430	6.8430	7.4115	7.9800	7.9800	7.9800	7.9800	11.3940	13.6680							15.943	15.943	15.943	17.08	17.08	18.217	18.217	GH PSS%	Field PSS%	Field FDK%	Field DONppm
Chinese spring (bp)	892922	909880	909880	7801088	7579126		9574094	12284882	194729814	194729814		10793772				14230803		17319302	16881671	16881671	17628028	18031327	18043966	19945225	21020177				
Line#/ctg0954 (bp)													2467047	2462377	923629	923629	923629	1055916	966499	966499	752852	350333	337689						
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	H	B	B	B	B	B	B	B	B	B	31	15	6	3
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	17	32	19	6
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	24	28	22	6
4	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	57	41	34	6
5	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	10	30	19	6
6	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	22	22	17	4
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	15	39	20	10
8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	14	33	25	9
9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	13	29	22	7
10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	20	30	23	9
11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	9	38	23	11
12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	12	38	28	11
13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	10	43	30	16
14	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	23	29	21	7
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	11	25	13	5
16	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	12	28	18	9
17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	11	38	26	15
18	A	B	B	A	A	A	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	66	41	21	6
19	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	12	29	18	10
20	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	26	27	16	10
21	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	13	44	29	11
22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	12	33	20	9
23	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	14	28	20	11
24	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	20	32	20	13
25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	19	43	22	13
26	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	28	35	25	12
27	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	16	38	22	10
28	B	B	B	B	A	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	7	18	8	7
29	A	H	H	A	A	A	B	A	A	A	A	H	A	A	A	A	A	H	B	B	B	B	H	H	B	60	34	18	7
30	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	24	35	21	10
31	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	15	38	19	7
32	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	23	38	23	12
33	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	27	34	23	7
34	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	24	30	17	9
35	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	27	23	8	5
36	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	13	21	19	10
37	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	23	32	24	9
38	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	21	35	21	13
39	B	B	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	25	35	21	7
40	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	22	33	19	7
41	A	H	H	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	25	25	19	11
42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	29	36	22	14
43	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	25	43	23	7
44	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	24	48	33	12
45	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	9	27	16	7
46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	11	43	23	11
47	B	B	B	B	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	24	33	18	11
48	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	23	42	23	10
49	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	26	33	19	15

(b) SNP haplotype block in Jagger-*Fhb1* population

SNPs	GBS1789	GBS11567	GBS14150	GBS14149	GBS14151	GBS9892	GBS9893	GBS12995	GBS12994	GBS6332	GBS4969	GBS79	GBS10806	GBS537	GBS1209	GBS8864	GBS7486	GBS11955	Xumn10	cg8	GBS2913	GBS5140	GBS5141	GBS13485	GBS13486	GBS13487	GBS14832	GBS14001	GBS13198	GBS15083	GBS957				
cM	0	0	0	0	0	1.137	1.137	6.843	6.843	6.843	7.4115	7.4115	7.98	7.98	7.98	7.98	13.668				14.8055	15.943	15.943					15.943	17.08	17.08	18.217	GHPSS%	FieldPSS%	FieldFDI%	FieldDONppr
Chinese spring (bp)		304411				909880	909880	4429847	4429847	7676610	7579126	7730840	9574094	12721683	1.95E+08		10793772	7.22E+08				14230803		16584717	16881671	16881671	17319302	17628028	18031327	18043966	21020177				
Line#/ctg0954 (bp)																		2924870	2467047	2462377	923629	923629	923629	1617626	966499	966499	1055916	752852	350333	337689					
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	32	21	10	9
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	30	25	12	5
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	43	12	11	6
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	37	16	9	4
5	B	A	H	H	H	B	B	B	B	H	H	B	H	A	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	B	B	30	9	5	3
6	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	37	13	7	4
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	48	25	20	11
8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	51	20	12	5
9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	32	13	6	4
10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	49	22	17	10
11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	33	12	4	3
12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	40	25	18	11
13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	45	20	9	5
14	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	51	14	11	10
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	31	19	13	7
16	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	40	23	11	8
17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	38	12	5	6
18	A	B	H	H	H	A	A	H	H	A	A	A	A	H	A	H	A	A	A	A	B	B	B	A	H	H	B	H	B	B	B	48	25	9	6
19	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	39	20	15	10
20	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	48	32	23	8
21	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	52	22	9	5
22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	37	28	15	9
23	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	48	25	13	9
24	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	41	19	10	5
25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	48	13	6	3
26	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	56	30	22	12
27	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	34	25	13	5
28	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	59	23	7	7
29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	48	33	19	9
30	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	40	19	10	4
31	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	43	25	12	5
32	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	40	16	8	5
33	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	31	11	6	5
34	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	41	20	11	6
35	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	32	13	7	3
36	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	44	23	18	14
37	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	44	28	14	8
38	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	21	18	12	6
39	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	34	13	10	4
40	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	42	18	16	6
41	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	34	14	4	4
42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	45	12	12	6
43	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	38	13	17	10
44	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	41	18	10	6
45	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	41	20	16	8
46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	54	16	11	5
47	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	34	23	14	6
48	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	B	42	30	45	20
49	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	40	23	16	5
50	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	31	13	8	7
51	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	32	20	14	3
52	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	31	22	15	6
53	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	49	15	8	3
54	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	34	26	12	8
55	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	28	15	9	3
56	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	38	23	9	5
57	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	44	15	13	11
58	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	47	16	6	4
59	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	44	15	4	3

(c) SNP haplotype block in Overley-*Fhb1* population



SNPs	GBS3330	GBS11567	GBS1789	GBS14150	GBS14149	GBS14151	GBS9892	GBS9893	GBS12995	GBS12994	GBS9420	GBS6332	GBS7551	GBS4969	GBS79	GBS10806	GBS8864	GBS14326	GBS1171	GBS1776	GBS537	GBS8024	GBS1209	GBS7486	Xumn10	cg8	GBS12786	GBS2913	GBS14832	GBS5140	GBS5141	GBS13485	GBS13486	GBS13487	GBS14001					
cM	0.00	0.00	0.00	0.00	0.00	0.00	1.14	1.14	6.84	6.84	6.84	6.84	6.84	7.41	7.41	7.98	7.98	7.98	7.98	7.98	7.98	7.98	7.98	13.67				14.81			15.94	15.94				15.94	GHPSS%	FieldPSS%	FieldFDK%	FieldDONppm
Chinese spring (bp)	364406	384411					909880	909880	4429847	4429847	4507608	7676610	7644840	7579126	7730840	9574094		12284882	12631965	12631965	12721683	12830696	19472981	10793772				16584717	17319302	17410893	17410893		14230803		17628028					
Line#/cnj0954 (bp)																									2467047	2462377	1546866	1617626	1055916	966499	966499	923629	923629	923629	752852					
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	37.22	21.25	8.67	4.20
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	53.73	30.00	12.00	4.80
3	B	B	B	B	B	B	B	B	B	B	H	B	B	B	A	H	A	A	A	A	A	A	B	A	A	A	B	B	B	B	B	A	A	A	B	56.10	25.42	14.08	4.99
4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	40.73	26.62	10.17	4.08
5	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	38.99	27.92	13.33	7.53
6	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	48.82	20.42	16.67	5.58
7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	43.27	20.00	11.67	6.58
8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	47.08	33.33	15.42	5.99
9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	45.67	14.58	5.92	4.18
10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	50.42	24.17	23.33	10.05
11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	58.45	35.83	23.33	7.79
12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	36.86	20.83	11.67	6.65
13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	B	B	B	B	B	B	B	B	44.59	36.67	24.08	7.03
14	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	49.72	26.67	17.08	8.23
15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	38.46	29.17	16.67	9.48
16	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	A	A	A	B	47.36	30.42	15.83	9.83
17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	45.45	50.00	34.58	12.80
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	73.08	31.25	23.75	7.23
19	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	42.99	37.50	18.33	14.18
20	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	27.04	36.67	27.50	11.93

Appendix A- Lists of Percentage of genome recovery, greenhouse and field mean PSS, field FDK and DON concentration in Overland, Overley, and Jagger-Fhb1 populations

Table 0.1. Overland-*Fhb1* lines

Line #	Percentage of genome recovery (%)	GH_mean PSS (%)	Field_mean PSS (%)	Field FDK (%)	Field DON (ppm)	*Cg8* genotype	*Xumn10* genotype
1	95.28	30.88	24.69	5.75	2.98	a	a
2*	98.20	16.86	21.94	19.17	5.60	a	a
3	94.51	24.37	25.56	21.67	6.48	a	a
4	94.22	56.88	50.46	33.75	6.40	b	b
5*	95.25	9.60	16.26	18.50	5.93	a	a
6	91.99	22.07	21.94	16.58	4.40	a	a
7	92.26	15.22	23.06	19.92	9.63	a	a
8*	93.84	13.72	20.26	25.00	9.40	a	a
9*	95.80	12.94	18.21	22.00	7.33	a	a
10	88.84	19.86	23.10	22.92	9.03	a	a
11	94.97	8.78	18.35	22.83	10.68	a	a
12	97.37	12.01	20.65	27.58	11.28	a	a
13	94.63	9.51	22.71	29.58	15.80	a	a
14	98.13	23.21	25.59	20.75	6.95	a	a

15*	89.69	10.88	15.72	12.50	4.53	a	a
16*	87.61	12.04	18.39	18.33	8.50	a	a
17	91.19	11.31	20.18	25.83	14.63	a	a
18	-	65.93	53.38	20.83	6.38	b	b
19*	95.75	11.72	17.40	17.92	10.00	a	a
20	93.78	25.61	26.10	15.67	10.13	a	a
21	96.11	13.09	23.31	29.17	10.88	a	a
22*	85.59	11.77	18.82	20.00	9.43	a	a
23*	85.89	14.13	18.86	19.58	10.65	a	a
24	90.69	19.56	23.73	19.58	12.60	a	a
25	97.94	18.98	27.10	22.08	13.33	a	a
26	96.59	28.25	30.64	24.92	11.73	a	a
27	92.89	16.44	23.74	22.08	9.70	a	a
28	-	7.14	14.05	7.83	7.10	a	a
29	-	60.46	49.94	17.92	7.38	a	a
30	96.17	23.53	27.36	20.83	9.75	a	a
31*	96.50	14.88	22.56	18.83	7.05	a	a
32	98.22	22.68	27.76	22.92	11.70	a	a
33	96.85	27.38	29.65	22.92	7.48	a	a
34	95.16	24.18	25.98	17.08	9.28	a	a
35	90.61	27.43	25.79	8.33	5.20	a	a
36*	88.08	13.06	15.79	18.75	9.90	a	a
37	93.41	22.98	26.01	24.17	8.78	a	a

38	96.07	21.31	25.87	20.83	12.83	a	a
39	97.66	24.50	27.86	20.83	6.93	a	a
40	96.23	22.13	25.73	19.17	7.38	a	a
41	92.57	24.71	24.83	18.75	10.58	a	a
42	98.56	29.27	31.46	21.67	13.73	a	a
43	97.49	24.89	31.04	22.92	7.05	a	a
44	94.38	23.73	31.93	33.33	12.05	a	a
45*	96.53	8.81	14.76	16.08	7.45	a	a
46	97.71	10.80	21.64	22.50	11.30	a	a
47	97.71	23.52	26.52	17.92	10.53	a	a
48	95.41	23.34	29.45	22.50	9.93	a	a
49	93.76	25.57	27.88	19.17	14.65	a	a
Overland	100	40.35	58.84	40.84	12.53	b	b

Table 0.2. Jagger-*Fhb1* lines

Line #	Percentage of genome recovery (%)	GH_mean PSS (%)	Field_mean PSS (%)	Field FDK (%)	Field DON (ppm)	*Cg8* genotype	*Xumn10* genotype
1	95.83	32.19	20.83	10.00	9.40	a	a
2	88.12	30.32	24.58	11.67	5.45	a	a
3	93.73	43.23	12.08	11.17	5.55	a	a
4*	94.38	37.17	15.83	8.67	4.03	a	a
5*	92.00	30.17	9.17	4.67	3.40	a	a
6*	95.02	36.73	12.92	6.71	4.30	a	a
7	91.83	47.88	25.42	19.58	10.55	a	a
8	89.39	50.62	20.00	11.92	5.35	a	a
9*	91.32	31.81	12.92	5.67	3.60	a	a
10	93.86	48.92	21.67	16.67	10.00	a	a
11	87.33	33.31	12.08	4.33	3.15	a	a
12	93.34	40.32	25.42	17.50	10.78	a	a
13	92.28	44.60	20.00	9.08	4.50	a	a
14	93.97	50.85	14.17	11.25	9.65	a	a
15*	93.88	30.98	19.17	12.92	7.28	a	a
16	91.85	40.01	22.50	10.92	7.85	a	a
17*	91.03	38.03	12.08	4.83	6.40	a	a
18	85.43	47.67	24.58	9.17	6.06	a	a
19	93.28	38.70	20.00	15.42	10.13	a	a

20	92.72	47.70	31.67	23.33	8.30	a	a
21	92.44	52.39	22.08	8.58	5.30	a	a
22	92.60	36.50	28.33	15.00	8.68	a	a
23	93.33	48.36	24.58	12.50	9.35	a	a
24	95.70	41.08	18.75	10.00	4.78	a	a
25	93.03	48.21	13.33	6.08	3.20	a	a
26	88.01	55.79	29.58	21.67	11.70	a	a
27	95.20	34.32	25.00	12.50	5.43	a	a
28	90.43	58.61	22.92	6.67	6.68	a	a
29	91.64	48.11	32.50	18.83	8.85	a	a
30	92.82	40.10	18.75	10.00	4.40	a	a
31	87.64	42.72	25.42	11.92	4.98	a	a
32*	94.95	39.85	16.25	7.92	5.43	a	a
33*	87.32	30.81	11.25	6.17	4.78	a	a
34	92.90	41.07	19.58	11.42	5.99	a	a
35*	96.05	31.88	12.92	7.08	3.23	a	a
36	81.78	44.25	22.50	18.25	13.95	a	a
37	96.68	44.24	27.50	14.17	7.93	a	a
38*	94.98	21.03	17.92	12.08	6.38	a	a
39*	93.18	34.07	12.92	9.50	4.13	a	a
40	89.30	42.37	17.92	16.25	5.53	a	a
41*	93.48	34.26	14.17	4.17	3.85	a	a
42	98.11	44.51	11.67	11.50	5.78	a	a

43*	89.42	37.65	13.33	17.08	9.55	a	a
44	88.92	41.16	18.33	9.83	5.93	a	a
45	93.86	40.52	20.00	16.25	7.93	a	a
46	88.53	54.46	15.83	11.17	5.23	a	a
47	90.75	34.36	22.92	14.17	5.60	a	a
48	89.27	42.25	30.00	45.00	19.65	a	a
49	90.15	40.03	22.50	15.92	5.40	a	a
50	87.79	30.51	13.33	7.50	7.05	a	a
51*	93.14	31.54	20.00	14.42	3.20	a	a
52	89.80	30.60	22.08	15.00	5.98	a	a
53	94.55	49.06	14.58	7.92	3.30	a	a
54	95.30	33.56	26.25	11.67	8.40	a	a
55*	95.82	27.70	15.42	8.67	3.38	a	a
56	88.25	38.48	22.50	9.08	5.30	a	a
57	89.02	43.73	15.42	12.92	10.68	a	a
58	85.20	47.43	16.25	5.67	3.91	a	a
59	94.16	44.44	15.00	4.17	3.35	a	a
Jagger	100	83.66	51.67	31.34	9.51	b	b

Table 0.3. Overley-*Fhb1* lines

Line #	Percentage of genome recovery (%)	GH_mean PSS (%)	Field_mean PSS (%)	Field FDK (%)	Field DON (ppm)	*Cg8* genotype	*Xumn10* genotype
1*	86.85	37.22	21.25	8.67	4.20	a	a
2	90.07	53.73	30.00	12.00	4.88	a	a
3	97.71	56.10	25.42	14.08	4.95	a	a
4	68.44	40.73	26.67	10.17	4.08	a	a
5	66.90	38.99	27.92	13.33	7.53	a	a
6	64.37	48.82	20.42	16.67	5.58	a	a
7*	90.58	43.27	20.00	11.67	6.58	a	a
8	94.92	47.06	33.33	15.42	5.98	a	a
9*	89.89	45.67	14.58	5.92	4.18	a	a
10	86.51	50.42	24.17	23.33	10.05	a	a
11	93.41	58.45	35.83	23.33	7.70	a	a
12*	94.40	36.86	20.83	11.67	6.65	a	a
13	95.51	44.59	36.67	24.08	7.03	a	a
14	92.83	49.72	26.67	17.08	8.23	a	a
15*	96.10	38.46	29.17	16.67	9.48	a	a
16	96.78	47.56	30.42	15.83	9.83	a	a
17	95.14	45.45	50.00	34.58	12.80	a	a
18	94.84	73.08	31.25	23.75	7.23	a	a
19	92.70	42.99	37.50	18.33	14.18	a	a

20	-	27.04	36.67	27.50	11.93	a	a
Overley	100	95.08	80.50	67.50	20.13	b	b

Note: Lines selected were labeled with astericks (*). ‘a’ represents genotype of Ning7840, and ‘b’ represents genotype of Overland, Overley, and Jagger.