MAPPING QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEAT LANDRACES

by

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Abstract

Fusarium head blight (FHB) is one of the most devastative diseases in wheat. Growing resistant cultivars is one of the most effective strategies to minimize the disease damage. Huangcandou (HCD) is a Chinese wheat landrace showing a high level of resistance to FHB spread within a spike (type II). To identify quantitative traits loci (QTL) for resistance in HCD, a population of 190 recombinant inbred lines (RILs) were developed from a cross between HCD and Jagger, a susceptible hard winter wheat (HWW) released in Kansas. The population was evaluated for type II resistance at the greenhouses of Kansas State University. After initial marker screening, 261 polymorphic simple-sequence repeats (SSR) between parents were used for analysis of the RIL population. Among three QTL identified, two from HCD were mapped on the short arms of chromosomes 3B (3BS) and 3A (3AS). The QTL on the distal end of 3BS showed a major effect on type II resistance in all three experiments. This QTL coincides with a previously reported *Fhb1,* and explained 28.3% of phenotypic variation. The QTL on 3AS explained 9.7% of phenotypic variation for mean PSS over three experiments. The third QTL from chromosome 2D of Jagger explained 6.5% of phenotypic variation. Allelic substitution using the closest marker to each QTL revealed that substitution of Jagger alleles of two QTL on 3AS and 3BS with those from HCD significantly reduced the PSS. HCD containing both QTL on 3AS and 3BS with a large effect on type II resistance can be an alternative source of FHB resistance for improving FHB type II resistance in wheat. Besides, meta-analyses were used to estimate 95% confidence intervals (CIs) of 24 mapped QTL in five previously mapped populations derived from Chinese landraces: Wangshuibai (WSB), Haiyanzhong (HYZ), Huangfangzhu (HFZ), Baishanyuehuang (BSYH) and Huangcandou (HCD). Nineteen QTL for FHB type II resistance were projected to 10 QTL clusters. Five QTL on chromosomes 1A, 5A, 7A, and 3BS (2) were identified as confirmed QTL that have stable and consistent effects on FHB resistance and markers in these meta-QTL regions should be useful for marker-assisted breeding.

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Chapter 1 - LITERATURE REVIEW

Impacts of *Fusarium* head blight

Fusarium head blight (FHB) is a devastative disease that can cause severe reduction in grain yield and quality in humid and semi-humid wheat growing regions worldwide (Bai and Shaner, 1994). When warm and wet weather coincides with anthesis and early kernel filling period, fungus can easily infect wheat plants and develop FHB. *Fusarium* infected florets often fail to produce kernels if infection occurs early or produce partially filled kernels that weight much less than normal ones. The infected kernels are light-weighted and very likely removed during threshing process, which significantly reduces harvested grain yield. FHB infection also lowers grain quality by reducing test weights and contaminating grain with mycotoxins such as Deoxynivalenol (DON) and zearalenone (De Wolf, 2003). Thus, FHB infection also causes severe impacts on the quality of cereals due to undeveloped kernels and mycotoxin accumulation. A significant positive relationship was observed between aggressiveness of the isolates and DON produced in the infected grain (Parry et al. 1995). This suggests DON content might be a virulence component (Burlakoti et al., 2010; Bai et al., 2001; Desjardins et al., 1996). In addition, high DON content is also a food safety concern. Consumption of grain products contaminated with mycotoxins is detrimental to human and animal health. As low as 1 ppm of DON can cause significant reduction in feed intake and lower weight gain in animals, and 10 ppm DON can cause vomiting and feed refusal (De Wolf et al. 2003). For human consumption, the acceptable DON levels in wheat have been set from 0.5 ppm to 2 ppm varied with countries. Thus, FHB not only reduces grain yield but also significantly lowers grain value in marketing, exporting, processing and feeding (Mcmullen, 1997).

FHB epidemics have been reported from many countries in Asia, Europe, North America and South America (Bai and shaner, 1994; Goswami and Kisler, 2004). In China, FHB has affected more than 7 million hectares of wheat and has caused more than 1 million tons of yield losses in 1990's (Bai and Shaner, 2004). In the U.S.A., direct value losses due to FHB from 1991 to 1997 in FHB-affected regions were estimated at $1.3 billion with the cumulative economy losses as high as $4.8 billion (Johnson, 1998). North and South Dakota and Minnesota suffered most from FHB outbreaks, accounted for about two-thirds of the total dollar losses due to all

diseases (Nganje, 2004). In 1996, FHB has expanded to more than ten states in the central Great Plains areas of U.S.A. The disease continue to spread in Europe and South America, thus, FHB in wheat has become one of the most important crop diseases around the world.

Causal organism, inoculums sources and dispersion

About 19 *Fusarium* species can cause FHB (Liddell, 2003). Among major causal species, including *F. culmorum, F. graminearum, Microdochium nivale, M. majus, F. avenaceum,* and *F. poae* as (Xu and Nicholson, 2009), *F. graminearum* is the predominant FHB causal species in most areas of the world. Within *F. graminearum,* isolates may differ in virulence. For example, Chinese isolates may be more virulent than the isolates from U.S.A. (Bai et al., 2001; Lu et al., 2001). However, consistent specificity of cultivar resistance and pathogen virulence was not observed and proof evidence for race differentiation has not been found (Lu et al., 2001; Bai et al., 1996). Hence use of a mixture of different *F. graminearum* isolates as inoculums to screen FHB resistance is a common practice for inoculation (Bai et al., 1996, Zhou et al., 2002b).

Fusarium can survive in crop residues between host crop cycles. Ascospores, macroconidia, chlamydospores, and hyphal fragments can be all used as initial inoculums for infection (Bai and Shaner 2004, Dill-Macky 2003) with ascospores as the primary inocula during natural infection. However, *F. graminearum* conidia are often used as inoculums for experimental inoculation due to its easiness for production (Dill-Macky, 2003). In nature, *F. graminearum* forms perithecia to produce ascospores (*Gibberrella zeae* (Schw.) Petch). Very thick wall of perithecia can keep the fungus viable throughout the winter, which provides the pathogen a potential epidemiological advantage to overwinter (Xu and Nicholson, 2009). In late spring, matured perithecia forcibly discharge their ascospores into air when high moisture is available to initiate initial infection in wheat during wheat flowering (Webster and Weber, 2007). Thus, crop residuals from previous crop seasons are major sources of inoculum, and increased tillage may lower residue retention and the amount of overwintering inocula.

Wind blowing and rain splash are considered to be common mode of disease spread although birds and insects can also be the vectors of inoculum dispersion. Wind blows spores for long distance and rain splash can transfers them from crop debris on ground level to wheat heads (Frances et al., 2009). Upon reaching wheat head, ascospores will germinate and colonize in the wheat tissues of spikes to start infection.

FHB symptoms and infection pathway

Visible FHB infection symptom starts with tan or brown discoloration at the base of an inoculated floret (Wolf et al., 2003). A few days later, this light tan or bleached symptom will spread to entire inoculated spikelets. For resistant cultivars, the symptom could be limited to the inoculated spikelet without spread to adjacent uninoculated spikelets. However, for susceptible plants, the fungus invades rachis and spreads up and down to the entire spike if the weather is favorable for disease development. Infected florets on the spike can be infertile, or kernels become shriveled, bleached and chalky, also known as "tombstone", if they are produced (Bai and Shaner, 1994).

During initial infection, conidia begin to germinate 6-12 h after the initial contact, and then germ tubes give rise to hyphae that will grow and extend on the interior surface to form dense mycelium networks (Xu and Nicholson, 2009). Hyphae grows through the interior surfaces of lemma, glume, and palea. After 24 to 36 h, hyphae may reach ovary. This infection process throughout floral parts is nonselective (Argyris et al., 2005). The fungus may enter the host tissue through stomata. Upon pathogen penetrating rachilla and rachis, disease will spread upward and downward on heads through vascular bundles and cortical parenchyma tissue (Goswami et al., 2004; Bushnell et al., 2003). Mycelium would clog the vascular tissue in the rachis that can cause head to premature and grains to be shriveled due to lacking of supply of water and nutrition (Xu and Nicholson, 2009). Other than that, stomata on glumes can be another entry point (Pritsch et al., 1999). Although anther can be the first part to be infected during FHB development. Then the disease spread horizontally from anthers to glumes, and vertically from anthers to rachis (Rinichich et al., 2000). However, the infection process normally occurred on the inner surfaces of lemma, glume, palea and rachis, not necessary through anthers (Xu and Nicholson, 2009). During colonization of wheat heads, the pathogen may secrete cell wall degradation enzymes that can decompose the host cells including cell wall, cytoplasm and cell organelles (Xu and Nicholson, 2009).

The pathogen hyphae may reach adjacent spikelets from initial infection site in two ways: through vascular bundles or stomata. When the weather is favorable, the fungal hyphae may penetrate rachilla and rachis, spread inter- or intra-cellularlly upward and downward on heads through vascular bundles and cortical parenchyma tissue to infect other neighboring spikelets (Goswami et al., 2004). Mycelium may also spread through outside glum from initially diseased

spikelets to those uninfected spikelets (Ribichich et al., 2000). The hyphae can produce mycotoxins in 36 h after inoculation, which could be transferred upward and downward to the neighboring uninfected spikelets through xylem vessels and phloem sieve tubes (Kang and Buchenauer, 1999). Thus, given favorable environments and adequate time, toxins contamination between spikelets is unavoidable (Xu and Nicholson, 2009). Infection spread from spikes to spikes (secondary infection) is rare if any.

Disease symptoms are different between resistant and susceptible cultivars. In resistant plants, a dark-brown discoloration appears on an inoculated spikelet. In some cases, only a small dark brown spot could be observed on the lemma (Bai and Shanner, 1994). In FHB favorable conditions, the symptoms may spread to neighboring spikelets through vascular bundle, but it occurs very late, at least two weeks, and most spikelets in the spikes remains uninfected and still set normal seeds. However, in susceptible plants, entire inoculated spikes can be blighted with bleach discolorations on spikelets and dark brown rachis and culm. Infection spreads quickly to uninoculated spikes, usually in a week after inoculation and whole spike can be blighted in 7-10 days after inoculation. Thus resistant cultivars show much lower final disease severity than susceptible cultivars (Ribichich et al., 2000).

Factors affecting FHB infection and development

Environmental factors have a significant impact on expression of FHB resistance as reflected by FHB incidence and severity (Parry et al. 1995, Bai and Shanner, 1994). Warm temperature and high humidity coinciding with wheat anthesis favor FHB development. For greenhouse experiments, 20- 25 ^{o}C has been considered as a favorable temperature (Bai and Shaner, 1994, Brennan et al., 2005). Wet period during anthesis is also necessary for initial infection. The wet period that required for a high infection rate may vary with temperatures. It may take longer time for symptom development under low temperature (16 ^{o}C); and incubation period can be decreased with the increase of temperature (Rossi et al., 2001).

Flower stage is the most susceptible stage for wheat to get infection by FHB pathogen although some cultivars may be susceptible at the beginning of caryopsis development (Bai and Shaner, 2004; Lu et al., 2001). Anthers contain a high level of chemicals such as choline and betaine that can facilitate the growth of *Fusarium* and function as initial infection points for fungus to enter spike tissues (Bai and Shanner, 1994). Given abundance of primary inoculums

and optimum weather condition during anthesis stage, *F. graminearum* can cause severe FHB epidemics (Bai and Shaner, 1994). A positive relationship between width and duration of flower opening and incidence of FHB in wheat has been reported, because wider flower opening allows a larger area for *Fusarium* spores to enter a floret to initiate infection (Gilsinger et al., 2005).

Agriculture practices such as crop rotation and crop management also have effects on FHB. Continuing to grow susceptible cultivars can increase initial inocula thus the FHB incidence (Dill-Macky and Jones, 2000). Wheat and non-host crop rotations may reduce the head blight incidence (Champeil et al., 2004). Limited soil tillage increases initial inoculum survival rate, and raises the FHB incidence, while ploughing (deep tillage) reduce inocula (McMullen et al. 1997, Dill-Macky et al. 2000, Teich et al. 1989, Krebs et al. 2000), to some extent, also modifies microclimate of the soil, and therefore reduces the development of *Fusarium.* Irrigation may also influence soil structure, and increases FHB frequency and severity. In addition to sowing date, wind speed, weeds, canopy (crop residue) density (Dill-Macky et al. 2000) can all affect FHB pathogen development.

FHB resistance mechanism

Mechanisms of resistance to *Fusarium* in wheat are classified as morphological or physiological (Gilsinger et al., 2005). Morphological mechanisms refer to these crop traits that lead to unfavorable conditions for FHB to initiate infection, such as plant height, awnness, and degree of flower opening during flowering (Gilsinger et al., 2005). Plants with wide opening florets are more susceptible to FHB. Physiological mechanism involves biochemical pathways that produce chemicals to prohibit pathogens growth after initial infections. Resistance to FHB is considered to be non-race specific. Resistant wheat genotypes show similar reactions against different isolates of *F. graminearum* (Tóth et al., 2008).

FHB resistance can be phenotypically classified into five categories: type I, resistance to initial penetration of the pathogen; type II: resistance to disease spread within a spike; type III: resistance to kernel infection; type IV: tolerance and type V: resistance to accumulation of DON (Mesterhazy et al., 1999). Among them, type I, II and V are commonly accepted (Schroeder and Christensen, 1963, Miller, et al., 1985), but type V is usually referred as type III. Type I resistance is a major type of resistance in barley (Steffenson et al., 2003), type II is more stable resistance in wheat (Bai and Shaner, 2004; Kolb, 2001), while type III is evaluated for both

barley and wheat. Type II resistance can be evaluated by point inoculation to a single floret of a spike and rating of symptom spread, within a spike. Percentage of symptom spread (PSS) within a spike usually used as the measurement for the level of type II resistance. Using this measurement, highly resistant cultivars may have as low as 5% PSS, while highly susceptible cultivars could reach 100% PSS. Moderate resistant and susceptible cultivars are in between (Bai et al., 1999). Significant correlations between the PSS and DON content were observed in single-point inoculation experiments (Bai et al., 2001; Yu et al., 2008b). All infected grains contain DON, even in a resistant cultivar. Contradicted results have been reported in different studies (Ma et al., 2006c; Mesterhazy et al., 1999). However, DON measurement is complicated, and inoculation time, harvesting and DON testing methods may significantly alter DON measurement. Early inoculation produces small highly-infected kernels and combine threshing may not be able to keep most of infected DON containing kernels for DON measurement, which all lead to underestimation of DON in susceptible cultivars (Ma et al., 2006c; Mesterhazy et al., 1999; Bai and Shaner, 2004).

Morphological variation is more likely to be associated with primary difference in initial infection, generally referred as Type I resistance, between cultivars, while difference in biochemical pathways is associated with symptom spread variation within a spike (Type II resistance). Although the processes of infection through anthers, floral bracts, rachilla and rachis are non-selective between resistant and susceptible wheat cultivars, biochemical responses to the infection are different between resistant and susceptible cultivars. Resistant wheat cultivars may produce substances that inhibit rapid growth of mycilium within a spike to prevent sudden desiccation on the spikelets above initial infected spikelets of a spike. Resistant wheat plants may form a physical barrier or accumulate chemical compounds such as phenols and triticens that are toxic to *F. graminearum* (Ribichich et al., 2000). These physical barriers include thickened cell wall and deposition of amorphous materials that can delay the disease progression. Trichothecenes may not be virulence factors for FHB initial infection of wheat floret (Bai et al., 2001; Jansen et al., 2005). When green fluorescence protein (GFP) labeled wild type and trichothecene knock-out mutant of *F. graminearum* strains were used to inoculate wheat, hyphae enters the cytosol of the epicarp cells in wheat, and leads to a cell death no matter inoculated with wild or mutant type of *F. graminearum* (Jansen et al., 2005). However, the action of DON may promote the spreading of *F.graminearum* in wheat from on infected spikelet to other ones

by hyphae growth through rachis nodes (Bai et al., 2001; Jansen et al., 2005). However, DON may be detected in the infected kernels no matter the level of FHB resistance. Low DON content in resistant cultivars may be due to small amount of DON produced by the *F. graminearum,* DON degradation enzymes produced by wheat, and accumulation in spike tissues rather than kernels (Bai and Shaner, 2004). Some mapping studies detected that major resistance QTL for low FHB severity was associated to low DON content in wheat. (Jayatilake et al., 2011; Bai et al., 2000; Pena et al., 1999).

FHB pathogen development induces defense response genes during early infection of wheat spikes. The induced genes include pathogen resistant proteins PR-1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4, and PR-5 (thaumatin-like protein) (Pritsch et al., 1999). Transcripts for the five defense-related genes are detected 6 to 12 h after inoculation, and peaked at 36 to 48 h after inoculation. More and earlier accumulation of PR-4 and PR-5 transcripts was observed in resistant cultivars than susceptible cultivars (Bai and Shaner, 2004). A recent study reveals that a plant cytochrome P450 gene, CYP709CI, was associated with resistance in both spikes and seedlings (Li et al., 2010). Li and Yen (2008) indicated PR proteins might have nothing to do with FHB resistance, instead, Jasmonate (JA) and Ethylene (ET) mediated defense responses are important in wheat resistance to FHB based on observation that resistant wheat plants can have elevated JA or ET biosynthesis after inoculation (Li and Yen, 2008, Ding et al. 2011). JA and ET are proposed to be important signaling pathways in plant defense response to FHB pathogen infection (Ding et al. 2011). In JA pathway, lipoxygenase (LOX2) and chalcone synthase are up-regulated in resistant wheat plants but not in susceptible ones. ET can stimulate plant organs to senescence, which leads to cell wall dissolving and cell death (Li and Yen, 2008).

Biochemical composition in wheat may affect the resistance level of a cultivar (Brown and Brindle, 2007). Choline was considered to be the most influential metabolite. Other than that, betaine, the amino acids glutamine, glutamate and alanine, trans-aconitate and sucrose are correlated with FHB fungus (Brown and Brindle, 2007). Brown and Brindle (2007) reported a significant correlation between metabolic profiles and fungal hyphae growth. However, Engle et al. (2004) did not find any significant correlation of fungal hyphae or spore growth associated with the metabolic levels. Thus, the biochemical mechanisms of FHB resistance are still debatable.

FHB resistant sources

Growing FHB resistant wheat cultivars is the most effective and economic method in FHB management (Bai and Shaner, 2004). Differences between cultivars in susceptibility to FHB were firstly reported in 19th century in US (Arthur et al., 1891). Since then, many breeders have attempted to find resistant sources. Although completely FHB immune cultivars have not been found (Fang et al., 1997), cultivars with various levels of resistance have been reported worldwide (Bai and Shaner, 2004). Most of highly resistant sources are from China and Japan (Bai et al., 2003b, Lu et al., 2001). In China, more than 30,000 *Triticum* accessions have been screened since 1980s (Fang et al., 1997), but only a small portion of them have good resistance, including Sumai3 and Wangshuibai (Bai and Shaner, 2004, Fang et al., 1997, Rudd et al. 2001). Sumai3, as well as its derivatives such as 'Ning7840', are the mostly used FHB resistance source in breeding programs (Bai and Shaner, 1996, Kolb et al., 2001, Rudd et al., 2001), because of its high heritability, stable resistance across environments (Rudd et al., 2001). Chinese landrace Wangshuibai is another highly FHB resistant source that is unrelated to Sumai3 (Jia et al., 2005b; Lin et al., 2006; Yu et al., 2008c; Zhou et al., 2004). However, attempt to use this source as resistant parent in breeding was not successful due to its many undesired agronomic traits (Bai and Shanner, 2004). Some Japanese cultivars such as Shinchunaga, Nobeokabouzu and Nyu Bai also showed a high level of FHB resistance (Bai and Shanner, 2004; Ban, 2000), but they all have poor agronomic traits which are difficult to be separated from resistance using conventional breeding. Besides resistance from Asia, many germplasm from American also show a good level of resistance, e.g, Frontana and Encruzilhada from Brazil (Ban, 2001; Mesterhazy, 1995; Singh et al. 1997), Ernie and Freedom from the U.S.A. (Rudd et al., 2001) were all reported to have a reasonable level of FHB resistance.

For FHB type II resistance in most of sources, additive effects play a major part in genetic effects, thus pyramiding of different genes in a wheat cultivar can upsurge FHB resistance in wheat (Bai and Shaner, 2000). In addition to highly resistant cultivars, moderately resistant cultivars are also good sources of breeding parents (Waldron et al., 1999). Moderate resistance may be easier to be achieved and can be easily to combine with desired agronomic traits. 'Alsen' was the first moderately resistant spring wheat cultivar released by NDSU (Mergoum et al., 2007). Alsen contains the *Fhb1* QTL for FHB resistance from Sumai3 with other QTL from native backgrounds as well as great agronomic performance, such as yield

potential and end use quality (Mergoum et al., 2007). In US hard spring wheat region, 54% of acreage was grown moderately resistant cultivars in 2011 (Connie, 2012). Some of the moderate resistant cultivars contain QTL from known FHB resistant parents, such as *Fhb1* from Sumai 3, while others may contain native resistant genes from local adapted parents. A cross between moderately resistant or moderately susceptible parents may generate highly resistant progenies. A good example is the well-known highly resistant cultivar Sumai3. It was developed by crossing two moderately susceptible parents, Funo and Taiwanmai (Bai et al., 2000). Thus, the resistance was derived by selection from transgressive segregation of resistance. One of advantages to use moderately resistant or moderately susceptible cultivars as a source of resistance is to allow a quick combination of genetically diverse resistant genes in more adapted genetic backgrounds. Some of the moderate resistant cultivars were mapped in breeding programs, such as Chokwang (Yang et al., 2005b), Frontana (Mardi et al., 2006) and Chinese spring (Grausgruber et al., 1999).

In addition, alien chromosome introgressions were used as an effective way to breed resistant cultivars. The same as homologous chromosome pairing during meiosis stage, the alien chromatin from monosomic alien addition line will recombine or translocate with the homologous to transfer resistant genes from alien sources to adapted common wheat (Cai et al., 2008). Such as *Fhb3* on chromosome 7A translocated from an alien species *leymus racemosus* (Qi et al., 2008). However, the biggest issues of this method are linkage drag and epistatic effects (Cai et al., 2005). Besides, genetic engineering provides novel approaches to develop transgenic wheat to enhance FHB resistance. Some examples are transgenic wheat expressing a barley class II chitinase (Shin et al., 2008), over-expressing the defense response genes α-1-purothionin, thaumatin-like protein1 and β-1.3-glucanase (Caroline et al., 2007), expression of an antibody fusion protein comprising a *Fusarium* –specific recombinant antibody derived from chicken and an antifungal peptide from *Aspergillus giganteus*, expression of Arabidopsis *NPR1* gene (Makandar et al., 2006) etc.

Other FHB control measures

Besides cultivar resistance, weather is one of the key factors that determine epidemics of FHB. Wet and warm conditions facilitate all stages of development of the fungus (Champeil et al., 2004). Cultural practices that minimize initial inoculum can reduce the FHB epidemics. Crop

rotation (Dill-Macky and Jones, 2000) and growing less susceptible crops (Champeil et al., 2004, McMullen et al., 1997, Dill-Macky et al., 2000) can be effective practices. Crop residues left on the soil surface are the major reservoir of inoculums (Bai and Shaner, 2004, Shaner, 2002). Traditional tillage practices left crop residues at the surface of soil after harvesting, which brings a high potential of FHB outbreak (Dill-Macky, 2008). Deep tillage can decrease the frequency of FHB outbreak (McMullen et al., 1997, Dill-Macky et al., 2000, Krebs et al., 2000) because ploughing buries inocula. Irrigation of wheat field may increase FHB infection, especially during anthesis, by providing moisture for spores development and release as well as establishing initial infection in wheat florets. Besides, early sowing or growing early maturity cultivars may escape heavy FHB infection in some locations (Champeil et al., 2004). Prior inoculation using other weak pathogen isolates (*F. culmorum, F. avenaceum, F. poae* etc.) has also been proposed to induce resistance by activating the host's defense response to provide cross protection to host plants (Diamond et al., 2003). Application of fungicides is still a major method for FHB control in commercial production. The effectiveness of fungicide application varies by active ingredient, method and date of application (Homdork et al., 2000). Using fungicides with specific active ingredients (triazoles containing tebuconazole) at a couple of days before a flowering season can be effective. However, difficulties in determination of an optimal time for fungicide application, high cost and lacking of fungicides with specific active ingredient are all problems involved with fungicide application (Bai and Shanner, 2004, Homdork et al., 2000). Suitable fungicide will reduce FHB severity and DON accumulation, especially in moderately resistant cultivars (Wegulo et al., 2011).

Inhibition of FHB through biological control agents is more environmental friendly compared with chemical control. Attempts to use biological control agents against FHB have been reported from several studies (Henkes et al., 2011, Petti et al., 2010, Khan and Doohan, 2009). But it is only a prosperous addition to current FHB manage-programs, and further studies are needed for a large-scale application in field. Therefore, no single solution is available for FHB control. A combination of cultural practices, chemical treatments, and use of resistant cultivars should be the best solution for FHB control.

Genetics of FHB resistance and heritability

FHB resistance is a quantitative trait that is controlled by multiple QTL or genes, and the expression of the resistance is greatly affected by environments (Jia et al., 2005b; Bai et al., 2000; Parry et al., 1995). Some reports showed that many minor genes controlled the resistance (Chen et al., 1983; Liao and Yu, 1985), while others concluded that a few major genes plus some minor genes might control the resistance (Bai et al., 1990; Yao et al., 1997). The minimum number of genes for FHB resistance was estimated to be one to three (Bai et al., 2000). Additive effect is a major component of genetic variation for FHB resistance (Bai et al. 2000) although epistasis might also play important role in some populations. Thus it is possible to pyramid several genes from different resistant sources to enhance wheat FHB resistance. It is also possible to select FHB resistant lines from transgressive segregations that would be superior to the lines that they derived from (Yang et al., 2005a; Bai et al., 2000).

Molecular markers and genetic maps

Molecular marker is becoming the most popular tool in modern plant breeding. Conventional crop breeding mainly relies on direct selection of morphological variation in breeding populations. Earliest marker used in breeding was morphological markers (Stadler et al., 1929). The phenotypes associated with phenotypic variation such as pigment differences, vernalization habit and plant height etc. were used as indirect selection criteria. However, morphological markers were not extensively used due to its limitation on number of available markers (Worland et al. 1987). Protein isozymes replaced morphological markers in 1970s (Market and Moller, 1959), but it has not been widely used in breeding (Tanksley, 1983). DNA markers are abundant, easy to be assayed and have become popular since 1980s.

Molecular markers can be classified into three categories: hybridization based, PCR-based and sequencing based. Hybridization- based markers include restriction fragment length polymorphism (RFLP; Botstein et al., 1980), fluorescent in situ hybridization (FISH), and microarray for marker detection. In 1990's, PCR-based markers are invented, which includes random amplified polymorphic DNA (RAPD; Williams et al., 1990), amplified fragment length polymorphism (AFLP; Vos et al., 1995), and simple sequence repeats (SSRs; Akkaya et al., 1992) etc. PCR-based markers quickly became popular because it needs small amount of DNA, without needing of radioisotopes, ability to amplify DNA from preserved tissues; high level of

polymorphism that enables to generate molecular markers very fast; and ability to screen many genes simultaneously. The sequence-based markers include single nucleotide polymorphism (SNPs; Jordan and Humphries 1994), sequence tag sites (STSs) and expressed sequence tags (ESTs; Gupta et al., 1999). Microsatellite markers (SSRs) are 1-6bp tandem repeats, highly abundant, high polymorphic, and widely distributed throughout genomes. Many SSRs are locus specific and can be used as framework for linkage mapping (Gupta et al., 1999). SSR analysis requires small amount of DNA, fits high throughput analysis and has high reproducibility. Thus SSR is suitable for QTL mapping and validation (McCartney et al., 2004). Another type of DNA marker is STS (Olsen et al., 1989), a unique DNA fragment derived from known sequences (Farooq et al., 2002; Gupta et al., 1999). More recently, the newest type marker is SNP (Jordan and Humphries 1994), which can detect individual nucleotide variation, and is suitable for high-throughput marker detection, thus it should be unlimited and is the future of markers for genetics research and breeding.

All types of molecular markers discussed above have been used for QTL mapping of FHB resistance (Anderson et al., 2001; Buerstmayr et al., 2002; Waldron et al., 1999; Mardi et al., 2005; Bai et al., 2003a; Bai et al., 1999; Zhang et al., 2004; Sun et al., 2003; Ban et al., 2000; Buerstmayr et al., 2011; Somers et al., 2003; Chen et al. 2007; Liu et al., 2007; Steiner et al., 2004; Ma et al., 2006c; Cuthbert et al., 2006; Liu and Anderson, 2003; Bernardo et al., 2012; Yu et al., 2008a). These markers have been used to construct genetic linkage maps to locate QTL positions for FHB resistance. Relative positions of genetic markers are arranged in linkage maps according to recombination frequency (RF) among markers. According to marker trait relationship in the map, QTL are located to certain map locations.

Because each map is developed using different populations, marker positions in different maps from different populations may be different. A map combining all map information from several different populations (a consensus map) may be more useful reference for determining consensus chromosome locations of markers and QTL. In wheat, the first genetic linkage map with 279 SSR markers were constructed in 1990s' (Roder et al. 1998). In 2004, Somers used 4 populations and developed a wheat consensus map with 1,235 SSR markers (Somers et al. 2004). This consensus map provides framework for mapping QTL for traits of interest in different mapping populations and for map based cloning of different genes/QTL (Somers et al. 2004).

QTL for FHB resistance

Quantitative trait locus (QTL) mapping using DNA marker is a highly effective approach for studying quantitative traits (Young, 1996; Tanksley, 1993). QTL mapping is used to dissect complicated traits, locate QTL underlining these traits in a genetic map, and determine their effects and interactions between QTL (Kearsey, 1998). Quantitative traits may be conditioned by several individual QTL that each may segregate in a Mendelian manner and affected by environments. By fitting phenotypic data with predicted genetic models, it is possible to estimate gene number, genotype by environment effect and heritability.

QTL mapping was firstly proposed by Sax in 1923 (Sax, 1923), and elaborated by Thoday later (Thoday, 1961). The basic concept of QTL mapping is to test the association of genomic region with the quantitative traits of interest (Mohan et al., 1997; Young, 1996). If a marker tightly linked to a QTL, the QTL will co-segregate with the marker. If a recombinant inbred population is separated into two groups based on two alleles of the marker, significant difference ($P<0.05$) in the trait values between groups indicates that the DNA marker is more likely linked to the QTL (Collard et al., 2005; Young, 1996). QTL mapping has been widely used to develop markers for marker-assisted selection (MAS) and map-base cloning (Buerstmayr et al., 2009; Mohan et al., 1997; Collard et al., 2005). Several factors may affect accuracy of QTL mapping. First, high-density map may provide more power for QTL detection; second, minor QTL may not be detectable especially when heritability is low; third, low heritability also results in a large confidence interval of QTL (Hyne et al., 1995); fourth, it is difficult to discriminate two QTL that are not far apart on the same chromosome (Kearsey, 1998; Young, 1996).

QTL mapping starts with mapping population. For mapping FHB resistance QTL, the parents for mapping population should show significant contrast in FHB resistance (Liu, 1998; Collard et al., 2005). Population sizes used for preliminary genetic mapping construction have been reported from 70 to 250 lines (Mohan et al., 1997), however, larger populations are required for high-resolution QTL mapping (Collard et al., 2005). Several types of populations have been utilized in QTL mapping experiments. F_2, backcross (BC) (Buerstmayr et al., 1999), and recombinant inbred lines (RILs) (Waldron et al., 1999; Yu et al., 2008c), double haploid (DH) (Chen et al., 2006; Jia et al., 2005b; Yang et al., 2005b) and chromosome recombinant inbred lines (CRILs; Garvin et al., 2009; Ma et al., 2006a) can all be used for QTL mapping. RIL

has been more commonly used population type for FHB mapping because the same genotypes can be repeatedly evaluated for FHB in different years or locations (Collard et al., 2005), although its construction takes several years.

Several methods have been used in routine QTL mapping; single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), and multiple interval mapping (MIM) (Tanksley, 1993). SMA (single locus regression) calculates phenotypic difference between two allelic groups of each marker. A t-test at each marker can be used to identify significant trait difference between two allelic groups. If the difference is significant, the marker is assumed to link to the QTL. At the same time, genome wide type I error has to be taken into account (Lander and Botstein 1989). However, SMA cannot determine QTL locations in a map. SIM, firstly described by Lander and Botstein (1989) can determine map location (or marker interval) of a QTL. SIM, the earlier version of interval mapping, evaluates the association between the phenotypic values and a target QTL between multiple pairs of adjacent markers. The QTL genotype is estimated by flanking marker genotypes as well as marker-QTL distance (Manly and Olson, 1999). Compared with SMA, SIM improves the power of detecting QTL to some extent; and QTL locations can be better resolved in SIM. However, the disadvantages of SIM are that it cannot detect a QTL outside of the defined interval , it can not distinguish two linked QTL if they located in the same or close marker intervals (Manly and Olsen, 1999), and QTL and background effects, and only two markers are tested at each time (Zeng et al, 1993; 1994). Composite interval mapping (CIM) can solve some of the problems SIM has. It can detect hypothetic QTL by setting a certain number of markers as window size, while utilizing the background markers as cofactors to control background noises (Manly and Olsen, 1999; Jansen, 1993; Zeng et al., 1993, 1994). This refined mapping model enable us to distinguish one target QTL from the ones in adjacent intervals and thus is more efficient and precise. MIM is another method that utilizes multiple marker intervals simultaneously. It can also be used to estimate epistasis between QTL, genotypic values of individuals, and heritability of quantitative traits (Chen et al., 1999). However, the identified QTL still need to be further validated.

To detect significant QTL, a t-statistic (Zeng et al., 1994), the logarithmic of odds (LOD , Lander and Botstein, 1989) and the likelihood ratio statistics (LRS, Haley and Knott 1992) are commonly used. LOD score is a ratio between the 10-base-logarithm of likelihood of having linked QTL to the 10-base-logarithm of not having the linked QTL. LOD scores and LRS are

convertible to each other with LRS=4.6×LOD (Liu, 1998) and both are commonly used in interval mapping to identify the most likely position of a QTL in a linkage map. If the peak or the highest point exceeds a LOD/LRS threshold the QTL is claimed to be a significant. Significant threshold of LOD or LRS is usually determined by 1000 random permutations (Churchill and Doerge, 1994). The permutation test breaks all the marker-trait associations, and shuffles all the phenotypic data while marker data remain constant. Permutation is performed to calculate the level of false positive QTL. Parameters such as LOD or LRS generated from the permutation on the random data form a distribution of LRS or LOD with H。= no QTL associated with the markers. This process is then repeated one thousand times to determine significant threshold at a given confidence level, usually 95% (Manly and Olsen, 1999). A conservative threshold at LOD of 3.0 is also used for claiming significant QTL (Collard et al., 2005). A QTL can be located in an interval between two markers called 'flanking' markers. This QTL can be 'major' or 'minor' QTL depends on the proportion of the phenotypic variation explained by the QTL (R^2). A major QTL usually explains a large portion of phenotypic variation (>10%) while a minor QTL accounts for a relatively small portion of phenotypic variation (<10%). Empirically, major QTL are more stable across environments and locations, especially for disease resistance QTL (Li et al., 2001; Collard et al., 2005).

In QTL mapping, several factors may affect the power of QTL detection. A high-density map is preferred, especially in the QTL region. A marker space less than 10 cM may have little effect on mapping result, however, marker space more than 20 cM may reduce the power of QTL detection to some extent (Collard et al., 2005). Accuracy and reproducibility of phenotypic data are important in mapping studies (Cuthbert et al., 2006; Kolb et al., 2001). For FHB resistance, environmental effect may have a huge influence on the trait scoring. Thus it is very difficult to get reproducible FHB data over different experiments, especially type I resistance because the variation can be mainly accounted by environments (Bai and Shanner, 1994). The same QTL may express various levels of resistance under different environments, and minor QTL is more sensitive to environments than a major QTL. Thus, it is necessary that QTL experiments should be done with replications across multiple locations and/or over times (George et al., 2003; Kolb et al., 2001, Collard et al., 2005; Haley and Anderson, 1997). Population size is another important factor influencing the power of detection. Larger population size can increase power in QTL detection (Darvasi et al., 1993). Quality of genotypic data may also important. Too many

missing marker scores may alter the marker orders and distances in a linkage map (Hackett, 2003). QTL can be validated using different mapping populations (Lander and Kruglyak, 1995) and near isogenic lines (NIL) that have uniform genetic background but contrasting in the QTL of interest (Pumphrey et al., 2007).

QTL for FHB resistance have been mapped on about 50 wheat cultivars covering all 21 wheat chromosomes (Table 1.1; Liu et al., 2009). Among them *Fhb1* on 3BS shows the largest effect on type II and type III resistance (Bai and Shaner, 2004). This major QTL was validated later by other studies (Anderson et al., 2001; Chen et al., 2006). The QTL on chromosome 3A, 5AS, 7A, 1B, 3BS, 4B,5B, 6BS and 2D have been mapped in more than two populations in previous studies (Liu et al., 2009). Five of them were formally named as *Fhb1* on chromosome 3BS, *Fhb2* on chromosome 6B (Anderson et al., 2001), *Fhb3* on Chromosome 7AS from a Wheat-Leymus introgression line (Qi et al., 2008), *Fhb4* on Chromosome 4B (Xue et al., 2010), and *Fhb5* on Chromosome 5A (Xue et al., 2011). However, only the Sumai3-derived *Fhb1* is now extensively used in breeding programs due to its stable effect on type II and type III resistance across different genetic backgrounds (Bai et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2003; Shen et al., 2003; Bourdoncle and Ohm, 2003; Yang et al., 2005; Chen et al., 2006; Jiang et al., 2007ab). The QTL on 3BS has been reported in more than 30 studies in which Sumai 3 was the major source of resistance. It was also reported in cultivars that are not related to Sumai 3 such as Chinese landrace Wangshuibai (Lin et al. 2004; Zhou et al. 2004) and Japanese landrace Nyu Bai (Somers et al. 2003; Cuthbert et al. 2006) etc. A wide range of R^2 – values has even been reported for *Fhb1*, ranging from 6% to 60% for type II resistance (Bai, et al, 1999, Waldron et al., 1999, Anderson et al., 2001, Buerstmayr et al., 2002, Shen et al., 2003, Bourdoncle and Ohm 2003, Somers et al., 2003, Chen et al., 2006, Jiang et al., 2007ab, Lin et al. 2004, Zhang et al., 2004, Ma et al., 2006c, Yu et al., 2008b, Yang et al., 2005b). Besides, QTL on 6B (*Fhb2*; Anderson et al., 2001; Yang et al., 2003, Shen et al., 2003; Cuthbert et al., 2007) flanked by *Xgwm133* and *Xgwm644* was another major QTL that explained a wide range of phenotypic variation for disease spread from 4.4% (Shen et al., 2003) to 23% (Somers et al., 2006). The QTL on 7AS was mapped close to *Xgwm276*, explained 3% in Wangshuibai (Zhou et al., 2004)., while according to Recently, a novel QTL on 7A was mapped close to *Xwmc17*, explained 41% of FHB type II resistance (Jayatilake et al., 2011). The QTL on 5A explained 4% (Li et al., 2011), 7% (Li et al., 2012; Zhang et al., 2012) and 11% (Buerstmayr et al., 2002) of

phenotypic variation and was related to type I resistance. QTL for FHB resistance were mapped on almost all wheat chromosomes even on 7D (Li et al., 2011, Table 1.1) where QTL was not detected before (Buerstmayr et al., 2009). The QTL on 7D was peaked at *Xwmc121*, flanked by the SSR markers *Xcfd46* and *Xwmc702*, and explained up to 22.6% disease spread variations (Li et al., 2011).

The inconsistent numbers and locations of FHB resistant genes reported in different studies could be due to (Kolb et al, 2001): (I) some genes may segregate in some crosses but not the others; (II) different genetic backgrounds and parents used for population development; (III) some resistant alleles from susceptible parents; (IV) heterogeneous source of resistance; (V) different *Fusarium* species or different isolates of *F. graminearum* as inoculums; (VI) different types of resistance phenotyped at different environments. In addition, population size and degree of map saturation are also affect QTL detection.

Breeding strategies

To breed FHB resistant wheat, breeders desire to combine a high level of FHB resistance with favorable agronomic traits, by accumulating different genes to upsurge FHB resistance because additive effect is a major component of resistance (Bai and Shaner, 2000). Thus pyramiding FHB resistance genes from diverse gene pools and removal of susceptible genes is an effective method to upsurge the level of resistance (Ma et al., 2006b; Rudd et al., 2001). Since major QTL have a stable effect on type II resistance, transferring *Fhb1* into commercial susceptible or moderately susceptible cultivars may significantly improve the FHB resistance of cultivars in commercial production (Kolb et al., 2001). This can be achieved by backcrossing and marker-assisted selection. However many highly FHB resistant sources usually have many unadapted agronomic traits. These sources are mainly landrace such as ‘Wangshuibai’ or un-adapted breeding lines such as Ning 7840 (Bai and Shanner, 2000). Thus, using backcross to move resistance QTL into adapted backgrounds to create middle parents may be critical for successful use of the QTL from these sources.

Owing to extensive breeding effort to improve wheat FHB resistance, many elite breeding lines and cultivars have moderate resistance or moderate susceptibility. They may either contain a few major QTL or some minor QTL for FHB resistance. Some of them are native resistance genes that may be different from the Asian resistance sources. These cultivars

can be directly used in commercial production to reduce the losses caused by FHB epidemics. They also can serve breeding parents for transferring these major QTL from Asian sources for better resistance. For example, In the U.S. spring wheat cultivars with moderately resistant were grown in more than half of the total acreage in 2011 (Connie, 2012). Some of the cultivars contain QTL from Asian sources, such as *Fhb1*, others may contain native resistance QTL.

Although Sumai3 is the major source of FHB resistance in wheat breeding programs worldwide, some other resistance sources should also be explored. Combining QTL that originated from different geographical regions can broaden the genetic diversity (Bai et al., 2003). For example, the US winter wheat cultivars Heyne, Ernie and Freedom may have different QTL from Asian sources (Bai and Shanner, 2000). An example of pyramided FHB resistance line has been reported. In that case, WSY was developed by pyramiding QTL from a 'three way crosses' among Sumai3, Wangshuibai and Nobeokabouzu (Shi et al., 2008). In the U.S.A., Some commercial soft wheat cultivars harbor *FHB1* have been released, including Pioneer Brands 25R18, 25R42, and 25R51, most of them are developed by marker-assisted backcross (Brown-Guedira et al., 2008). In addition to incorporating *Fhb*1 as well as other minor QTL from Asia into new wheat cultivars, breeders in US also use native resistance in breeding, such as Heyne, Ernie and Freedom. These winter wheat cultivars show good FHB resistance, but do not have *Fhb1* (Bai and Shaner, 2004). Truman and Bess developed at the University of Missouri, have better FHB resistance and overall performance on yield, test weight under disease pressure (Mckendry, 2008). Besides, many US hard red spring wheat cultivars also have FHB resistance, such as Bacup developed in Minnesota, and Alsen, Steele ND2710 and Glenn developed by North Dakota State University (Mergoum et al., 2007). Among them, Alsen was derived from a three way cross from "ND674/ND2710/ND688", where ND2710 was from a cross involving Sumai 3 (Mergoum et al., 2007).

Transgressive segregation has been successfully used in creating resistant cultivars. Examples of this are some wheat cultivars from Southern Chinese: Sumai 3, Zhen 7495, Xiangmai 2, Jingzhou 1 and Jingzhou 47 (Bechtel et al., 1985; Bai et al., 2000). Thus, selecting elite resistant lines from transgressive segregation of resistance may be able to improve the level of FHB resistance. Besides, many other breeding strategies have been applied in wheat FHB resistance (Bai and Shanner, 2004; Lu et al., 2001), such as introduction of alien resistance genes or chromosomes to develop new synthetic hexaploid wheat cultivars (Gilchrist et al., 1997, Rudd

et al., 2001). For example, wheat addition lines with chromosomes from *Roegneria ciliaris*, *R. kamoji*, or *Elymus giganteus* showed FHB resistance as Sumai 3 in China (Chen et al., 1993).

Table 1.1 Review of FHB resistance QTL

Type of FHB resistance	Chromosome	Population name	Population type	References
Type II resistance	2AL, 3BS	Sumai3 (R)/Stoa (MS)	RIL	Waldron et al., 1999
Type II resistance	7B	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., 2002b
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	CM-82036(R)/Remus(S)	DH	Buerstmayr et al., 2003
Type I resistance	5A	CM-82036(R)/Remus(S)	DH	Buestmayr et al., 2003
Type II resistance	3BS, 6B and 2D	Ning894037(R)/Alondra(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 III resistance	5AS, 3BS and 2DS	Wuhan-1(R)/Maringa (MS)	DH	Somers et al., 2003

Type II resistance	3BS 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, 3BS, 1BL and 3BSc	Wangshuibai(R)/Wheaton(S)	RIL	Zhou et al., 2004
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 II resistance	3BS, 6BS, 2DS and 7BL	DH181(R)/AC Foremost(S)	DH	Yang et al., 2005b
Type II resistance	3BS, 4BL and 5DL	Chokwang(R)/Clark(S)	RIL	Yang et al., 2005a
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., 2005b
Type III resistance	3BS	CM-82036(R)/and Remus	DH	Lemmens et al., 2005
Type II and Type III 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	RIL	Ma et al., 2006a

		(R)/Annong 8455(S)		
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., 2006
Type I resistance	5A, 4B and 5B	Wangshuibai (R)/Nanda2419	RIL	Lin et al., 2006
Type II resistance	1AS, 3BS, 7BS, 2DL	CJ9306(R)/Veery(S)	RIL	Jiang et al., 2007ab
Type III resistance	1AS, 5AS, 3BS and 2DL,	CJ9306(R)/Veery(S)	RIL	Jiang et al., 2007a and 2007b
Type II resistance	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 III resistance	1AL and 2AS	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	3AS, 5AS, 3BS, 4B, and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	Yu et al., 2008c
Type II resistance	1A, 5AS, 7AL 3BS, 3DL and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	Yu et al., 2008c
Type III resistance	1A, 5AS, 7AL, 1BL, 3BS and 5DL	Wangshuibai(R)/Wheaton(S)	RIL	Yu et al., 2008c
Type II	1A and 2BL	G16-92(R)/Hussar(S)	RIL	Schmolke et al.,

resistance				2008
Type II resistance, plant height	2DS	Sumai3(R)/Gamenya(S)	DH	Handa et al., 2008
Type II resistance	4DS	Spark(R)/Rialto(S)	DH	Srinivasachary et al., 2008
Type II resistance	2B, 3B, 4B and 6B	IL94-1653/Patton	RIL	Carolyn et al., 2009
Type II resistance	5BL, 6BS and 7BS	Pelikan(S)/G93010(R)	$F_{6:8}$ RIL	Haberle et al., 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 and type III 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	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., 2012

Chapter 2 - Mapping QTL for *Fusarium* head blight resistance in Chinese wheat landrace Huangcandou (HCD)

Introduction

FHB resistance inherits as a quantitative trait, which is usually controlled by a few major genes and several other minor genes (Bai and Shaner, 1994; Buerstmayr et al. 1997, 1999). Mapping of quantitative trait loci (QTL) has been widely used to determine the effect of QTL underlining quantitative traits. To date, FHB associated QTL have been reported from about 50 wheat cultivars covering all 21 chromosomes (Liu et al. 2009). Among them QTL on 3BS, formally designated as *Fhb1*, shows the largest effect on type II and type III resistance. Sumai 3 and its derivatives such as 'Ning7840' carry *Fhb1* and are the most used source of FHB resistance in breeding programs worldwide (Bai and Shaner; 1996, Bai et al.1999; Somers et al., 2003; Cuthbert et al., 2006; Buerstmayr et al. 2009). The QTL on chromosome 5AS, 6BS, 3A, 4B, 2D, 1B, 7A, 5B also have been mapped in more than two populations in previous studies and considered as stable QTL (Liu et al. 2009). Four of these resistance QTL were also formally named as *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al., 2007), *Fhb3* on Chromosome 7AS from Wheat-Leymus introgression lines (Qi et al. 2008), *Fhb4* on Chromosome 4B (Lin et al., 2006; Xue et al. 2010) and *Fhb5* on Chromosome 5A (Xue et al., 2011). To date, only Sumai3-derived resistance QTL on 3BS (*Fhb1*) has been extensively used in breeding programs due to its stable major effect on type II and type III resistance across different genetic backgrounds (Bai et al. 1999; Anderson et al. 2001). However, single resistance QTL may not provide sufficient protection from severe epidemics, thus, exploring new source of resistance to facilitate pyramiding of the QTL is urgently needed to enhance the level of resistance and diversity of resistance sources.

Huangcandou (HCD) is a Chinese landrace and showed a high level of type II resistance to FHB (Yu et al. 2008a). But QTL underlining the resistance in HCD has not yet been characterized. In this study, a population of F_5-derived recombinant inbred line (RIL) was developed from the cross HCD ×Jagger and used to characterize QTL associated with FHB type II resistance and to identify markers that tightly linked to the QTL for marker-assisted selection (MAS).

Materials and Methods

Plant materials and FHB evaluation

A population of 190 recombinant inbred lines (RILs) was derived from the cross HCD and Jagger by single-seed descent. HCD is a resistant wheat landrace from China, and Jagger is a FHB susceptible US hard red winter wheat (HRWW) cultivar from Kansas. $F_{5:6}$ and $F_{5:7}$ RILs were evaluated for FHB resistance at Kansas State University, Manhattan, KS. The experiments were conducted in the greenhouses of spring and fall 2010, and spring 2011 with two replications in each experiment. Two parents were used as checks in each experiment. Seeds of RILs and parents were planted in Plug Flat Trays filled with Metro-mix 360® soil mix (Hummer International, Earth City, MO). After vernalization at 5^{o}C in a cold chamber for 6 weeks, 5 seedlings per line were transplanted into each 13-cm squared Dura pot containing Metro-mix 360® (Hummer International, Earth City, MO). The pots were arranged on greenhouse benches in a randomized complete block design (RCBD). The greenhouse was maintained at 15-20 ^{o}C with 12 hours supplemental daylight. Miracle-Gro® (Hummer International, Earth City, MO) was applied four times at one week interval after transplanting.

F. graminearum (KS strain GZ3639) was used as the source of inoculums and conidia were prepared following Bai et al. (1999). The concentration of conidia was calculated using a hemocytometer under a light microscope. The final concentration of inoculums was adjusted to 100,000 conidia per mL. At early anthesis, 10µl conidial suspension (~1000 conidia/spike) was injected into a central spikelet of a spike using a syringe (Hamilton, Reno, Nevada). Five spikes per pot were inoculated and enclosed in a moist chamber at 100% relative humidity and 20-25^{o}C to initiate fungal initial infection. After 48 hours of incubation, the plants were move to greenhouse benches at 20-25^{o}C with 12h supplemental daylight. Type II resistance (symptoms spread within a spike) was evaluated by counting infected spikelets and total spikelets in a spike on the 18th day after inoculation. Percentage of symptomatic spikelets (PSS) of an inoculated spike was calculated by counting the number of infected spikelets and total number of spikelets. Mean PSS of RILs for each experiment and across all three experiments were calculated for QTL analysis.

DNA extraction and genotyping

Leaf tissues were collected from $F_{5:6}$ RILs at 3-leaf stage in 96-deepwell plates. Harvested tissues were dried in a freeze dryer (Thermo Savant, Holbrook, NY) for 48 h and ground in a Mixer Mill (MM 400, Retsch, Germany). Genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). A core set of 384 pairs of SSR primers including BARC, WMC, GWM, STS, CFA and CFD (http://wheat.pw.usda.gov) were used to screen the parents. This primer set were originally selected from 2000 primer pairs according to previous studies conducted at USDA Central Small Grain Genotyping Lab, Manhattan, KS. These primers are evenly distributed on all 21 chromosomes (Sommer et al. 2004). Primer pairs that detected polymorphism between parents were used to genotype a randomly selected subset of 96 RILs. Coefficient of determination (R^2) was calculated for each of the markers to determine significant markers associated with FHB type II resistance. To increase the marker density in QTL regions, another 70 markers linked to known QTL that have been reported in previous studies were screened for polymorphism (Liu et al. 2009; Buerstmayr et al. 2009; http://wheat.pw.usda.gov) and polymorphic markers were added to the linkage map for further QTL analysis.

Polymorphic SSR markers between parents were used to screen RILs. PCR amplification was done in a DNA engine Tetrad Peltier thermal cycler (MJ Research, Waltham, MA). A 10 µl PCR master mix contained 1X ASB buffer, 2.5 mM of $MgCl_2$, 200 µM of dNTP, 100 nM each of a fluorescent-dye-labeled M13 primer (5'- ACGACGTTGTAAAACGAC) and a forward primer with M13-tail added to 5'-end, and 200 nM of a reverse primer, 0.6 U of Tag polymerase, and 40 ng template genomic DNA. PCR amplification was done using a touchdown program. Initially, PCR reaction mixture was incubated 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 cycles; 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 ended with a final extension at 72^{o}C for 5 min. Amplified PCR products from four PCR labeled with different florescent dyes (FAM, VIC, NED and PET) were pooled and analyzed in ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Data scoring were done using GeneMarker v1.75 (SoftGenetics LLC. State College, PA, USA)

Data analysis

Heritability (h^2) of PSS was calculated using $h^2=\sigma^2_g/(\sigma^2_e/re+\sigma^2_{ge}/e+\sigma^2_g)$, where σ^2_g is an estimate of genetic variance, σ^2_{ge} is an estimate of G by E variance, and σ^2_e is an estimate of environmental variance. The variances σ^2_g, σ^2_{ge}, and σ^2_e were estimated by analysis of variance (ANOVA) using PROC GLM function in SAS v 9.1.2 (SAS Institute Inc. Cary. NC).

Linkage maps were constructed by IciMapping v3.1 (Wang et al. 2011) using Kosambi mapping function (Kosambi 1944) and a logarithm of odds (LOD) threshold of 3.0. QTL for PSS were analyzed using composite interval mapping (CIM) module in WINQTL Cartographer Ver. 2.5 (Wang et al. 2006). Five SSR markers were used as controls with a window size of 10 cM and 2 cM walking speed. Permutation test was performed 1000 times to determine the LOD threshold for claiming the significance of QTL at P <0.05 (Doerge et al. 1996). Coefficients of determination (R^2) for each QTL were calculated through multiple linear regressions in QTL IciMapping v3.1 (Wang et al., 2011). The joint QTL effects for each individual experiment were calculated using stepwise regression in both additive and epistasis mapping (Wang et al., 2011).

To compare the effect of different QTL combinations on FHB Type II resistance, all RILs were separated into 2^n genotypic groups based on allele constitution of all QTL (n) identified in the population. Alleles for each QTL were represented by the alleles of the closest marker to the QTL. Multiple comparisons among the groups were conducted by Tukey-Kramer method (Miller, 1981).

Results

FHB in HCD × Jagger population

The resistant parent HCD showed moderate resistance with a mean PSS of 16.7%, ranging from 9.63% to 27.19% across the three experiments, while the susceptible parent (Jagger) had a mean PSS of 84.4%, ranging from 69.9% to 98.3% (Figure 2.1). The mean PSS of each of the three experiments ranged from 4.7% to 100% for RILs. Different patterns of PSS frequency distributions were observed among the three experiments. In general it showed a continuous variation with two peaks, but with a major peak skewed toward HCD in 2010 spring and fall experiments and toward Jagger in 2011 spring experiment (Figure. 2.1). Mean PSS over all RILs was 44.8%, ranged from 29.8% (2010 fall) to 54.99% (2011 spring). Thus disease pressure was the highest in 2011 spring and the lowest in 2010 fall. Significant transgressive segregation was observed in all the three experiments, suggested that the susceptible parent might contribute resistance QTL in the population. The positive correlations of PSS were highly significant among the three greenhouse experiments ranging from 0.48 to 0.69 (P <0.001). Analysis of variance (ANOVA) indicated significant variations for genotypes, environments and genotype by environment interactions (Table 2.1). Heritability for PSS across three greenhouse experiments was high (0.80).

Linkage maps and QTL for FHB resistance

After screening 454 selected SSR primers between parents, 261 primers were found to be polymorphic, indicating a high level of polymorphism (57.5%) for the set of primers. All polymorphic primers were used to screen a subset of 96 RILs. Among them, 242 markers were mapped to 43 linkage groups that covered 953.7 cM in genetic distance. Composite interval mapping (CIM) using the resulting map identified five chromosomes regions in 3A, 3B, 4B, 5A and 2D that were significantly associated with PSS. All polymorphic markers from the five chromosomes were used to screen the rest of 94 RILs in HCD/Jagger population. The final linkage map was constructed using 190 RILs and used for final QTL detection. However, QTL on 4B and 5A was not significant in the final map, thus not analyzed further.

Three significant QTL were detected in HCD/Jagger mapping population. One was mapped in the distal end of the short arm of chromosome 3B (3BS), one on the short arm of chromosome 3A (3AS) and the other on chromosome 2DS. The one on 3BS showed a significant

major effect on Type II resistance in all the three experiments (Figure 2.2), and coincided with previously reported *Fhb1* according to the haplotype of tightly linked markers *Xumn10* and *Xbarc133*. This QTL explained 10.8 to 36.9% of phenotypic variations across the three experiments (Table 2.2). The QTL on 3AS was flanked by *Xcfa2134* and *Xgwm2*, and was significant in two 2010 experiments and mean PSS over the three experiments. This QTL explained 7.3% to 14.5% of phenotypic variation (Table 2.2). The third QTL on 2D was tightly linked to the marker *Xgwm261* and significant only in spring 2011 experiment. This QTL explained 6.5% of phenotypic variations (Table 2.2). The QTL on 3BS (*Fhb1*) and 3AS were from HCD and QTL on 2D was from Jagger. The total effects of the QTL in the population were 26.5% in 2010 Spring, 25.1% in 2010 Fall, 38.3% in 2011 Spring and 37.3 in combined average.

Effects of QTL on FHB resistance

To demonstrate the effect of each QTL, RILs that carry different allele combinations at the three QTL were grouped and compared for their allele substitution effects. Three QTL contain eight genotype groups: group1 (AABBCC) carries resistance alleles in all the three QTL (3BS, 3AS and 2D), group2 (AABBcc) carries resistance alleles in 3BS and 3AS, group3 (AAbbCC) carries resistance alleles on 3BS and 2D; and group4 (AAbbcc) carries resistance allele only in *Fhb1*, group5 (aaBBCC) carries resistance alleles in 3AS and 2D, group6 (aaBBcc) carries resistance alleles on 3AS only, group7 (aabbCC) carries resistance allele on 2D only; group8 (aabbcc) does not carry any of these resistant alleles. Markers tightly linked to each QTL (*Xumn10* on 3BS, *Xgwm2* on 3AS and *Xgwm261* on 2D) were selected to represent these QTL. Two contrasting alleles at each of the three SSR loci exhibited a 1:1 segregation ratio. For 190 RILs, mean PSS for each groups of RILs ranged from 22.4% to 69.3%. The mean PSS for RILs carrying a marker allele linked to only one of the three resistant QTL were 44.3% for *Xumn10* (3BSd), 53.8% *Xgwm2* (3A) and 58.4% for *Xgwm261* (2D). In contrast, the averaged PSS of RILs carrying all three susceptible alleles was 69.3% (Null). The mean PSS with *Fhb1* or 3A allele alone was significantly lower than that of 'null' group. Meanwhile, the mean PSS of RILs with resistant alleles at *Fhb1* plus an additional QTL (3AS or 2D QTL) were consistently lower than those with two or three susceptible alleles. Thus the QTL *Fhb1* showed the largest effect on FHB resistance and substitution of a resistant allele at *Fhb1* by a susceptible allele increased PSS significantly. Also RILs carrying a resistance allele on 3A with or without additional QTL had

significantly lower PSS than that of the RILs without any resistance allele at all three QTL, suggested that substitution of resistance alleles on 3A QTL by susceptible allele can significantly increase FHB severity.

Discussion

Although many Chinese wheat cultivars or landraces have been reported to show a high level of FHB resistance (Yu et al. 2008a, Li et al. 2011, Li et al. 2012, Yang et al. 2005ab), only a few of them, mainly Sumai3 and its derivative Ning7840, have been well characterized to carry QTL for type II resistance (Bai and Shaner, 2004; Rudd et al. 2001; Yu et al. 2008b, Zhang et al. 2004, Somer et al. 2003). QTL in many other Chinese sources, especially landraces, have not been well characterized. Characterization and utilization of QTL in different sources of resistance will enhance genetic diversity of FHB resistance QTL. HCD is one of the resistant Chinese landraces. The frequency distributions of mean PSS from the three experiments showed two peaks of unequal sizes (Figure 2.1), suggesting at least two QTL segregating for FHB resistance in the population. Transgressive segregation observed in all the three experiments suggested that Jagger might also contribute a QTL for resistance. QTL mapping using HCD/Jagger RILs indicated that FHB resistance in HCD is mainly conditioned by a combination of two QTL for FHB resistance on 3BS and 3AS and Jagger also contributes a minor QTL for resistance. This result supports the prediction based on the PSS frequency distributions of the mapping population.

FHB resistance is a quantitative trait that is controlled by several genes/QTL, and its resistance expression is also severely influenced by environments where plants are evaluated (Jia et al. 2005a; Bai et al. 2000; Parry et al. 1995). Difference in FHB inoculation techniques and environmental conditions may contribute significantly to the differences in QTL detection. Thus, it is essential to evaluate FHB resistance repeatedly in different seasons and environments to improve the repeatability of QTL detection (Kolb et al. 2001). In this study, the RIL population was evaluated repeatedly in three-greenhouse experiments. Although variation in the patterns of frequency distributions was observed for different experiments (Figure 2.1), the correlation coefficients of PSS for RILs were high among experiments (r= 0.48 -0.69, $P<0.001$). A high heritability (0.80) was also observed. These results suggested that the phenotypic variation was mainly due to genotypic variation and the data were reliable for QTL analysis in this study (Bai

et al. 1999). The current study was conducted in greenhouses under controlled temperature and moisture environments and the population is relatively large with 190 RILs in comparison with some studies that used smaller size of populations (Lemmens et al., 2005; Ma et al., 2006b), thus detected QTL should be reliable.

The QTL on 3BS explained 10.8% to 36.9% of total phenotypic variation in the HCD/ Jagger population. This QTL was flanked by *Xgwm493* and *Xgwm533* with the peak at marker *Xumn10*, thus it coincides with the location of a previously mapped QTL *Fhb1* from Sumai3 (Cuthbert et al. 2006; Waldron et al. 1999). This QTL has been reported in more than 30 studies in which Sumai3 and its relatives were the major sources of the resistant parents. This QTL showed a stable major effect on FHB type II resistance across different genetic backgrounds (Buestmayr et al., 2009; Bai et al., 1999; Zhou et al., 2002b; Buerstmayr et al., 2003; Shen et al., 2003; Bourdoncle and Ohm, 2003; Yang et al., 2005; Chen et al., 2006; Jiang et al., 2007ab). It was also reported in cultivars that are not related to Sumai3 such as Wangshuibai (Lin et al., 2004; Zhou et al., 2004) and Nyu Bai (Somers et al., 2003; Cuthbert et al., 2006) etc. Thus, the QTL on 3BS of HCD is *Fhb1*.

Effect of *Fhb1* on type II resistance varied among studies, ranging from 6% in DH181 (Yang et al. 2005a) to 60% in Ning7840 (Bai et al. 1999). A wide range of R^2 –values has even been reported for the same source of resistance used in different studies (Waldron et al. 1999, Anderson et al., 2001, Zhou et al., 2002a, Buerstmayr et al., 2002, 2003, Shen et al., 2003, Bourdoncle and Ohm 2003, Somers et al., 2003, Chen et al., 2006a, Jiang et al., 2007ab, Lin et al., 2004, Zhang et al., 2004, Ma et al., 2006a, Yu et al., 2008b, Yang et al., 2005b). In the current study, effect of *Fhb1* on type II resistance in HCD was highly significant in all the three-greenhouse experiments with a R^2-value up to 0.283 for mean PSS across the three experiments. However, when individual experiments were examined, the phenotypic variation explained by *Fhb1* varied from 10.8% to 36.9%, indicating that QTL effect may vary significantly with environments even the same population is used. This may be due to difference in times of a year for inoculation in different experiments. Inoculation during winter season is usually under lower temperature conditions than that is done in early summer, which may significantly affect disease levels of inoculated plants, especially resistant and moderately resistant plants may have a lower level of disease. In field conditions, the situation even worse than in a greenhouse because inoculum amount available, flowering times, temperature and moisture conditions during

infection period can differ dramatically among locations and years, which will lead to significant variations in effect of QTL from different studies. Thus the discrepancy in effect of *Fhb1* reported from different studies could be due to difference in FHB evaluation environments, genetic backgrounds of different populations, population sizes and inoculation methods. It may also be possible that different sources of resistance may harbor different alleles of *Fhb1*.

The second QTL located on 3AS was significant in two greenhouse experiments. This QTL, closest to *Xgwm2* was flanked by *Xcfa2134* and *Xgwm5*, and explained 10.0% the phenotypic variation for the mean PSS over the three experiments. A QTL in similar location was firstly reported on 'Huapei 57-2' centered at *Xgwm5*, which explained 8.1% of variation for type II resistance (Bourdoncle and Ohm 2003). In F201R, this QTL was flanked by *Xbarc76* and *Xgwm674*, and explained 13.4% FHB spread variation (Shen et al., 2003). Meanwhile a QTL from *Triticum dicoccoides* was mapped close to *Xgwm2*, and explained 37% of phenotypic variation (Chen et al. 2007). Because *Xgwm2* and *Xgwm5* were 2.8cM apart (Somers et al. 2004), the QTL mapped in this study is most likely at the same position as these 3A QTL reported previously from different studies. In this study, the QTL on 3AS was not as stable as *Fhb1*, and significant in only two (2010 Spring and Fall) of the three experiments and explained a much smaller proportion of phenotypic variance than *Fhb1*. However, this QTL contributes a moderate effect on type II resistance relative to other QTL with a minor effect. In this study, this QTL alone was able to significantly reduce FHB PSS from 69.3% (Null) to 53.8% (Figure 2.3). Thus, this QTL can be a good candidate for pyramiding of different QTL with major stable effects to improve FHB type II resistance.

The third QTL was identified on 2DS of Jagger. This QTL region was in the same position as the QTL previously reported to be responsible for FHB resistance and low DON accumulation in a Japanese cultivar (Handa et al., 2008). Comparative analysis with rice genome identified a candidate gene, multidrug resistance-associated protein (MPR), for FHB resistance located in rice chromosome 4 that is corresponding to the wheat genomic region on 2D (Handa et al., 2008). In current study, this QTL was close to *Xgwm261* in chromosome 2D, and flanked by *Xbarc95* and *Xgwm261*. it was especially significant in spring 2011 experiment, and explained 6.5% of phenotypic variation. Previous studies revealed that the SSR marker *Xgwm261* was linked to a reduced height locus *Rht8* (Korzum et al., 1998). Whether the resistance effect is due to plant height variation caused by *Rht8* is still unknown. The QTL was also reported in a double

haploid population from the cross Sumai3 × Gamenya and the 'susceptible parent' Gamenya contributed the resistance allele (Xu et al. 2001). The closest marker was *Xgwm261* and explained 14% to 25% phenotypic variations. Other studies also detected the QTL on 2DS of a moderate susceptible parent Alondra's that explained 12.1% phenotypic variation (Shen et al., 2003). Similar to previous report, the current study also identified the resistance locus on the same location of chromosome 2D from the susceptible parent, Jagger. This QTL in Jagger showed a minor effect on type II resistance but it is more likely a real QTL since it is consistent in the same location of different genetic backgrounds. This QTL often identified in susceptible parents suggested that some susceptible cultivars still carry resistance QTL. When transferring a major QTL to US wheat, use of these adapted susceptible parents with minor resistance alleles can improve the level of resistance of selected new cultivars. These cultivars without any minor resistance allele should be avoided as parents for breeding crosses. In addition, this QTL was also found in several other studies where resistant parents contributed the resistance allele (Yang et al. 2005a, Jia et al. 2005).

Figure 2.1 Frequency distribution of mean percentage of symptomatic spikelets (PSS) in a spike for RIL population derived from cross HCD × Jagger in three greenhouse experiments

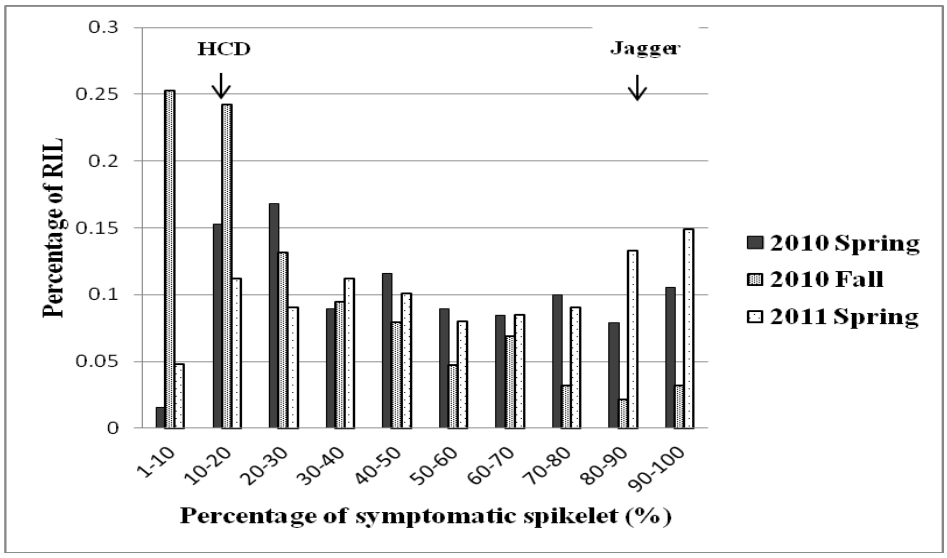
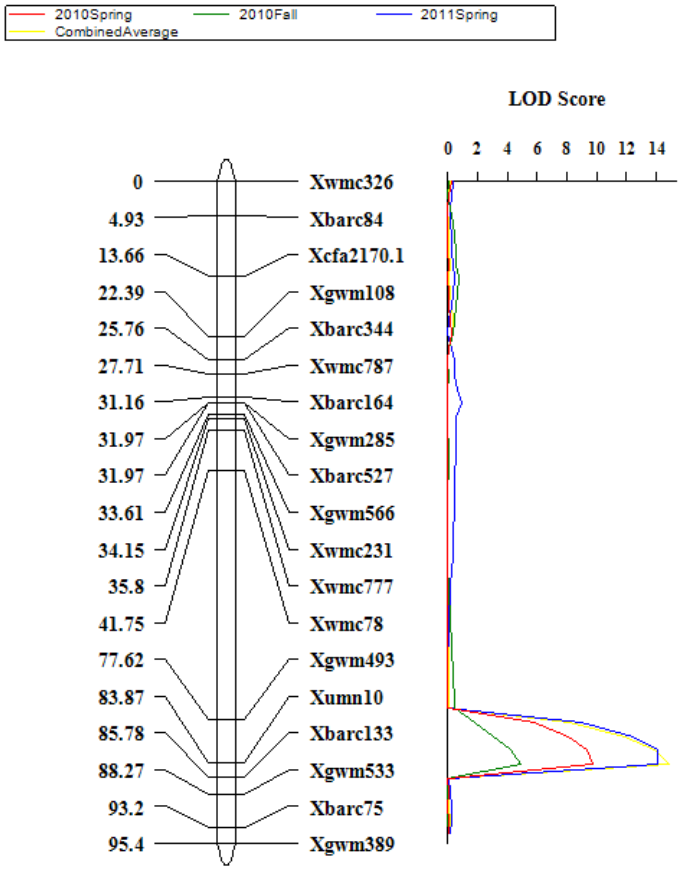
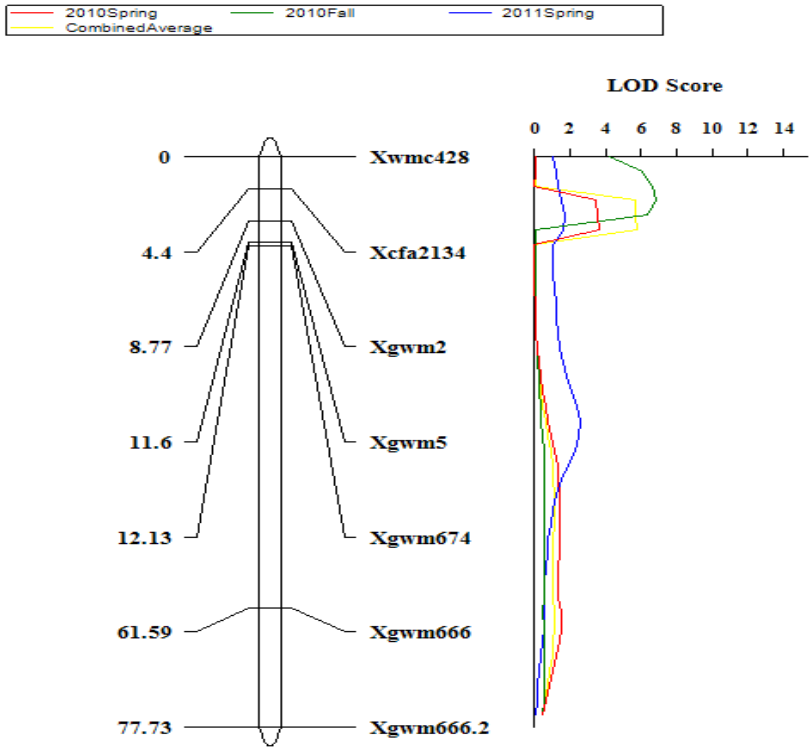


Figure 2.2 Composite interval maps of quantitative trait loci (QTL) for FHB type II resistance constructed from a recombinant inbred line (RIL) population derived from the cross HCD × Jagger based on three greenhouse experiments (2010 Spring, 2010 Fall, 2011 Spring) using percentage of symptomatic spikelets (PSS%) on (a) chromosome 3BS, (b) chromosome 3A and (c) chromosome 2D

a) 3BS



b) 3AS


2010Spring
2010Fall
2011Spring
CombinedAverage
LOD Score
0 2 4 6 8 10 12 14
0
4.4
8.77
11.6
12.13
61.59
77.73
Xwmc428
Xcfa2134
Xgwm2
Xgwm5
Xgwm674
Xgwm666
Xgwm666.2

c) Chromosome 2D

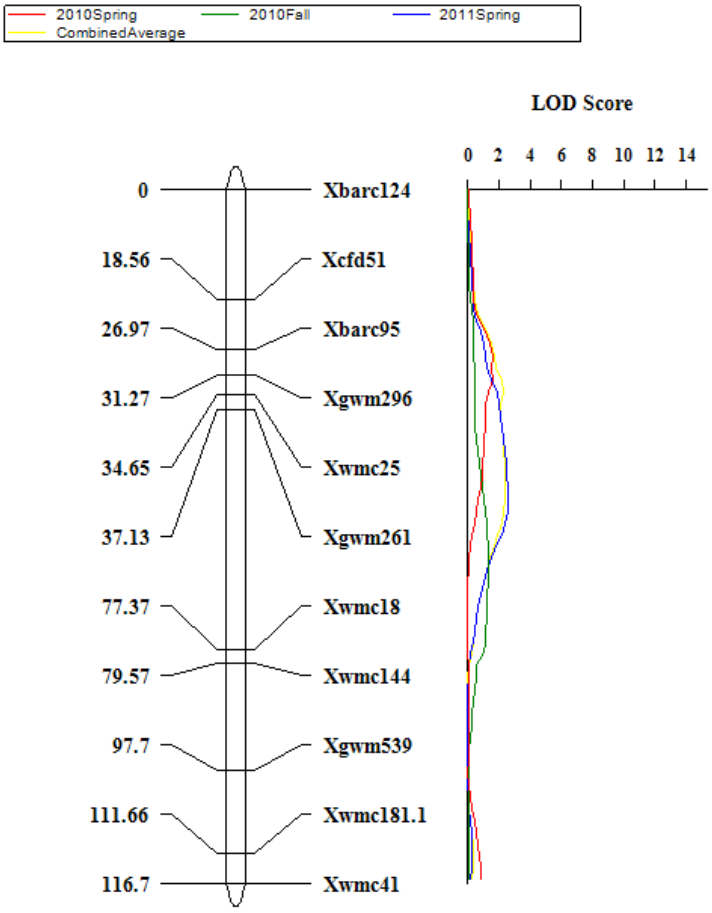


Figure 2.3 Effects of different combinations of QTL on percentage of symptomatic spikelets (PSS) of RIL derived from HCD × Jagger based on FHB resistance data collected from three greenhouse experiments. Group 1 resistance alleles from QTL on 3BS, 3AS and 2D; group 2 resistance alleles from QTL on 3BS and 3AS; group 3 resistance alleles from QTL on 3BS, and 2D; group 4 resistance allele from QTL on 3BS only; group 5 resistance alleles from QTL on 3AS+2D; group 6 resistance alleles from QTL on 3A only; group7 resistance alleles from QTL on 2D only; group 8 susceptible alleles from all three QTL. The hollow circles on the vertical lines are the mean PSS of each group, and lengths of the lines are 95% confidence intervals. If the confidence intervals have no overlap to each other, then the two groups are significantly different at LSD $_{0.05}$.

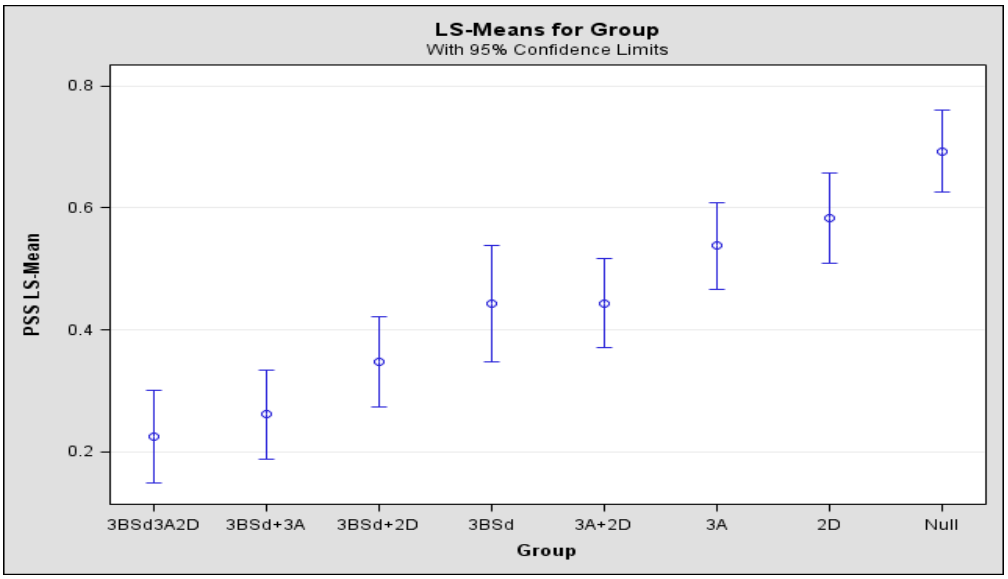


Table 2.1 Analysis of Variance (ANOVA) of percentage of symptomatic spikelets (PSS) data for the RIL based on three greenhouse experiments

Source	DF	Type III SS	Mean Square	F value	Pr>F
Experiment	2	127782.50	63891.25	218.94	<.0001
Replication (experiment)	3	1198.96	399.65	1.37	0.2512
Genotype	189	587702.09	3109.53	10.66	<.0001
Experiment*Genotype	376	230449.33	612.89	2.10	<.0001
Error	548	159920.26	291.82		
Corrected total	1118	1119363.67			

Table 2.2 Flanking markers, logarithm of the odds (LOD), coefficients of determination (R^2) of the significant QTL regions detected by composite interval mapping based on spring 2010, fall 2010 and spring 2011 greenhouse FHB data.

Locus	Flanking markers	2010 Spring		2010 Fall		2011 Spring		Combined mean	
		LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
Fhb1	Xgwm493~Xgwm533	9.75	20.4%	4.96	10.2%	14.73	32.4%	14.90	28.3%
3AS	Xcfa2134~Xgwm2	3.66	7.6%	6.71	13.5%	-	-	5.28	9.7%
2D	Xbarc95~Xwmc261	-	-	-	-	2.64	6.5%	-	-
Total		-	26.5%	-	25.1%	-	38.3%	-	37.3%

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

Introduction

In 1980s, 23434 Chinese landraces and breeding lines have been screened for FHB resistances, 1796 of them were reported to have high or at least moderately resistant to FHB, including Wangshuibai, Ning 7840, Huangfangzhu, Huangcandou and Baishanyuehuang (Huang et al., 1999; Yu et al., 2008b). However, only a few of them such as Sumai 3 are well characterized for FHB resistance QTL in multiple studies. QTL in many other Chinese landraces are poorly characterized and some were reported in only one population.

Meta-analysis, a statistic method to combine results across independent studies into one single result, has been widely applied in medical, social, and behavioral sciences (Veyrieras et al., 2007). Britten et al., (1996) successfully adapted the method in a genetics study on salmonid fishes. Meta-analysis has not been used extensively in FHB QTL mapping. To date, only two review articles reported such studies. Liu et al., (2009) conducted the meta-analysis study to cluster 249 FHB resistance QTL from 46 lines and confirmed 19 QTL on 8 chromosomes. Loffler et al. (2009) combined QTL from 30 mapping populations to conduct meta-QTL analysis, 9 meta-QTL were found on 12 chromosomes. Another review paper focused on updated knowledge on QTL mapping studies. Buerstmayr et al. (2009) reviewed 52 QTL mapping studies and concluded that FHB resistance QTL were found on all wheat chromosomes except 7D (Buerstmayr et al., 2009). In that review, some QTL detected in multiple studies were confirmed as stable QTL and proposed for use in breeding. However, all of the three review articles used the published partial maps from different studies without addition of new markers in the QTL regions, thus with no or poor marker coverage. Adding additional markers in the poor marker coverage regions with QTL in other populations can further validate whether the missing QTL is due to missing markers or QTL.

In this study, a set of five populations developed from different Chinese landraces was used for meta-analysis (Table 3.1) (Li et al., 2012, HYZ, Li et al., 2011, WSB, Yu et al., 2008c, BSYH, Zhang et al., 2012, and Chapter 2).We applied meta-analysis to estimate 95% CIs of FHB Type II resistance QTL according to the QTL locations and QTL effects from each study. This study focuses on five Chinese landraces as FHB resistance source to compare the QTL

across these Chinese landraces. Most QTL from previous studies were detected in only one or two of the five populations. These QTL were mainly located on chromosomes 3BS, 7D, 6BS, 5AS, 3A, 1AS and 7A in different populations (Yu et al., 2008c; Li et al., 2011; Li et al., 2012; Zhang et al., 2012; Chapter 2). In each population, 3 to 7 QTL were detected from a resistant parent. The objectives of this study are to I) identify rare unique and common QTL in Chinese landraces; II) conduct meta-analysis to pinpoint and narrow down the confidence intervals (CIs) of QTL; III) identify flanking markers for these QTL to facilitate marker-assisted selection (MAS).

Materials and Methods

Plant materials, FHB evaluation and marker analysis

Five mapping populations were developed by crossing five Chinese resistant landraces identified by Yu et al., (2008b) to two US susceptible cultivars Jagger and Wheaton (Table 3.1). These parents don't have any known pedigree relationship to Sumai 3, thus some may not carry the same FHB resistance QTL as these in Sumai 3. All the five populations are RILs (Table 3.2). FHB type II resistance (FHB spread) was evaluated in greenhouses at Kansas State University. Detailed methods for FHB (*F. graminarum*) inoculum preparation, single floret inoculation and FHB data collection were the same as used in Chapter 2.

Methods for DNA isolation and marker detection, and PCR conditions have been described in Chapter 2. To confirm that the absence of QTL in different populations are not due to poor marker coverage, markers from all the QTL regions in the chromosomes carrying QTL in at least one population were rescreened to construct a consensus map for further QTL analysis.

Map construction and meta-analysis of QTL from multiple populations

Newly generated marker data were merged with the original marker data file to construct linkage maps. Composite interval mapping (CIM) was performed using all the linkage maps developed from the five populations. Map construction and QTL analysis followed the same method described in Chapter 2.

To study the consistency of mapped QTL from different populations, five maps developed from the five populations were integrated into a consensus map for QTL analysis using Joinmap v 3.0 (Van Ooijen and voorrips 2001). The map calculations were based on mean recombination frequencies and combined LOD scores. Weighted least squares (WLS) were used to determine heterogeneity of recombination rates to optimize the consensus map results (Stam P. 1993). Consensus and linkage maps from individual studies with common markers were drawn using MapChart (Voorrips, 2002). The integrated maps with QTL were then projected onto the consensus map by referring the original QTL intervals and confidence intervals (CIs) on the consensus maps. A model choice criteria in the context of Gaussian mixture was selected to identify the most proper distribution of QTL location on consensus map (Veyrieras et al., 2007). Final consensus FHB-resistance QTL map with the 95% CIs was drawn using MQTLView command in MetaQTL (Veyrieras et al., 2005).

Results and Discussion

QTL for type II resistance and QTL Cluster

All SSR markers that were close to or at the previously reported QTL regions identified from different populations, but did not mapped previously, were rescreened between all parents for 5 populations. In the populations Wangshuibai (WSB)/Wheaton and Huangfangzhu (HFZ)/Wheaton, 21 additional SSR on 7D and 6B were screened between parents, respectively and 15 and 14 polymorphic primers were screened in two populations, respectively. In populations Baishanyuehuang (BSYH) and Huangcandou (HCD), 32 SSR markers from 7D, 6B, 1A and 7A were screened between parents, and 14 and 16 polymorphic primers were used to screen the populations, respectively. In Haiyanzhong (HYZ)/Wheaton population, 10 additional polymorphic markers on 3BS were analyzed. After screening those SSR markers on chromosomes 3BS, 7D, 6BS, 5AS, 3A, 1AS and 7A where QTL were detected in some studies in previous mapping studies, polymorphic markers were found in all these regions of 5 populations (Figure 3.1), suggesting that the new maps have reasonable marker coverage in all the QTL regions. However, additional QTL were not detected in any of these populations. The result indicates that absence of certain QTL in different populations was not due to missed markers in the regions, but to absence of the QTL in these regions.

Previous studies have mapped 24 QTL for FHB type II resistance on 11 chromosomes from the five Chinese landraces (Table 3.2). Among them, 20 QTL can be projected onto six consensus maps (1A, 3A, 5A, 7A, 3BS and 6B) using common markers and estimated meta-analysis QTL CIs (Figure 3.1). Based on the data from the five populations, chromosomes 1A, 3A, 5A, 7A, 1B, 2D, 3D, 5D and 7D have one QTL on each chromosome, while chromosomes 3BS and 6B have more than one QTL. The two QTL on 3BS were designated as separated QTL 3BSc and *Fhb1* because of the long genetic distance between them. The 19 QTL on 5 consensus linkage maps were classified into 10 clusters (Table 3.2; Figure 3.2). These clusters are more likely 'real' QTL that were detected in different mapping populations. QTL on each of the consensus linkage groups 1A, 3A, 3BS and 6B were classified into two clusters based on the Gaussian Mixture Model that gives how many QTL on the consensus maps can best define the QTL CIs detected from individual studies according to the sample sizes and R^2 of each QTL (Veyrieras et al., 2007). The QTL clusters were unevenly distributed among the three genomes,

with 6 on A, 4 on B and none on D genome (Table 3.2). Most of the detected QTL were derived from Chinese landraces, only one QTL each was detected on 1AS of susceptible parent Wheaton in the HFZ/Wheaton population and on 2DS of susceptible parent Jagger in HCD/Jagger population.

QTL with a Major Effect on FHB Resistance

In this study, Five QTL were confirmed on two loci of 3BS, 1A, 5A and 7A, which is consistent with previous studies (Yu et al., 2008c; Li et al., 2011,2012; Zhang et al., 2012; Chapter II). All of them were significant in at least two populations (Table 3.2). However, in all five populations, at least 3 QTL were identified and with one QTL showing a major effect. The QTL *Fhb1* on 3BS was identified in four of the five populations except in HYZ/Wheaton and located in the same cluster as the confirmed QTL in Sumai 3 (Liu et al., 2009). Through the current meta-analysis study, the CIs of *Fhb1* was narrowed down to 3.11cM, flanked by what markers *Xbarc147* and *Xbarc133*, centered by *Xumn10* (Table 3.3; Figure 3.2). These flanking markers should be useful markers for marker-assisted selection of *Fhb1*. *Fhb1* showed the largest effect on type II resistance in the four different populations. These Chinese landraces do not have known pedigree relationship with Sumai 3 (Yu et al., 2008b) or landrace Taiwan Wheat, the parent of Sumai 3. However, all of the Chinese landraces used in this study were collected from Jiangsu Province where Taiwan Wheat might originate from, thus they might be co-evolved in the same eco-environments. The *Fhb1* in different populations have huge difference in QTL effects as measured by R^2. The R^2 varied from 17% (Zhang et al., 2012) to 35.6% (Li et al., 2012) in the four populations that carry the QTL. These five populations only have two susceptible parents: Jagger and Wheaton. Thus, the differences in QTL effects may not be due to different genetic backgrounds. However, FHB type II resistance for HFZ, HYZ and WSB population were detected in both greenhouse and field, while the experiments on HCD and BSYH were conducted in greenhouses only. There might be larger environmental effects on type II resistance in the field than in the greenhouse (Yu et al., 2008b). Besides, the various R^2 may be due to genetic interactions between genes or QTL and the difference in alleles of the *Fhb1* in different parents because the haplotype of *Fhb1*-linked markers among these landraces are different (Jayatilake et al., 2011).

The *Fhb1* was not significant in HYZ/Wheaton population even after more markers were added in the region. However, a QTL on 7D showed a major effect on type II resistance in HYZ/Wheaton population, explained 20.4-22.6% of the phenotypic variation, and was stable across three greenhouse and one field experiments (Li et al., 2011). Other QTL in the population showed much smaller effects than the 7D QTL. The 7D QTL was not significant in other four populations even all the markers in the 7D QTL regions were polymorphic (Figure 3.1). Only one previous study reported a minor QTL on the same location of 7D in Arina/Riband population (Draeger et al., 2007). Thus, it is a unique major QTL in HYZ for improvement of level of FHB resistance by stacking with *Fhb1*.

QTL with a Minor, but stable Effect on FHB Resistance

Besides a major QTL in each population, 2 to 6 minor QTL with various effects were detected in each population. In addition to *Fhb1*, another QTL cluster was identified near the centromere of 3BS in two populations, designated as 3BSc. This QTL cluster was centered at *Xgwm566* and was flanked by *Xgwm285* and *Xwmc307* with a much smaller QTL CIs (3.47cM) than the original one (Table 3.3; Figure 3.2). This QTL showed much smaller effect than *Fhb1*, but it has been detected in several Chinese landraces or lines including 'Wuhan-1' (Somers et al., 2003) and U.S. winter wheat 'Ernie' (Liu et al., 2007). Chromosome 1A harbored two QTL clusters with one centered by *Xwmc120* in HFZ/Wheaton and HYZ/Wheaton populations and another flanked by SSR markers *Xcfd59* and *Xgwm611* in WSB (Yu et al., 2008c; Table 3.3). Two QTL in two clusters of 3A were flanked by *Xcfa2134* and *Xgwm2* in HCD/Jagger and another flanked by *Xgwm666* and *Xbarc356* in BSYH/Jagger (Table 3.3; Fugure 3.2). However, these two clusters are all close to SSR markers *Xgwm674* and *Xgwm5* , which was the same as previously reported in wheat 'Huapei 57-2', 'F201R' (Bourdoncle and Ohm, 2003; Shen et al., 2003) and durum wheat 'FA-15-3' (Chen et al., 2007). On 5A, a cluster of minor QTL was detected in four of the five populations. The cluster was flanked by *Xwmc705* and *Xgwm293*, centered by *Xbarc1* (Table 3.3; Figure 3.2), which coincides with the QTL reported in 'F201R' (Shen et al., 2003) and 'Wangshuibai' with FHB type II resistance from another study (Lin et al., 2006). The QTL confidence intervals ranged from 5.02-18.4 cM in each individual mapping studies, however, meta-analysis of QTL narrowed down the CIs and pinpointed the QTL to a 1.38 CM interval (Table 3.3; Figure 3.2). The narrower QTL CIs enable us to find markers that

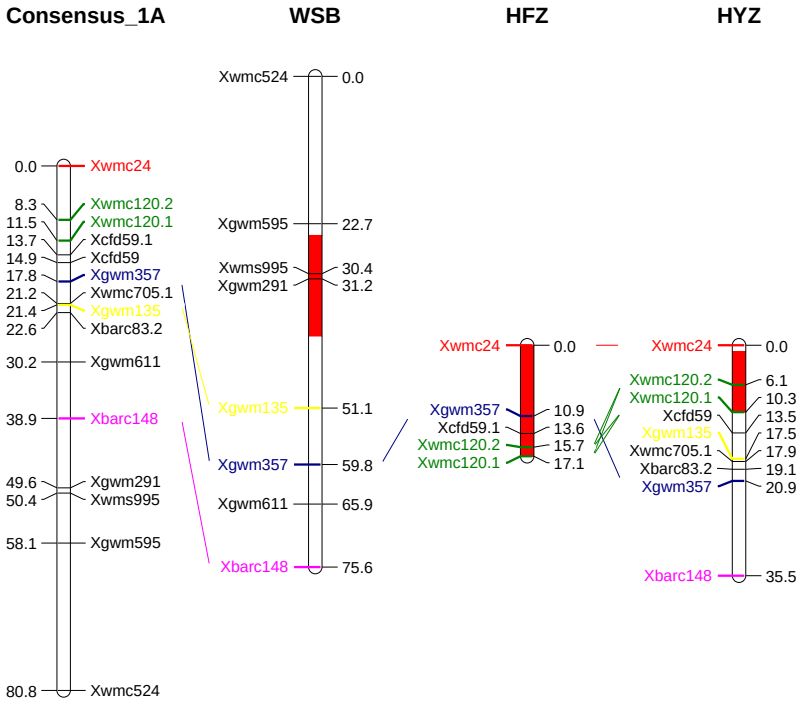
much closer to the resistant allele on 5A. The last QTL cluster on 7A was flanked by SSR markers *Xbarc165* and *Xwmc160*, centered by *Xbarc121* (Table 3.3). This QTL cluster location has been reported in different populations of Wangshuibai (Jia et al., 2005; Zhou et al., 2004), NK93604 (Semagn et al., 2007) and Ritmo (Klahr et al., 2007), as they shared the same markers *Xgwm276* and *Xbarc121*. These QTL clusters were detected in multiple populations, thus they are more likely stable QTL for type II resistance although they have minor effects. These QTL should also be important QTL to be used with major QTL in breeding programs for improvement of FHB resistance level of wheat cultivars.

Conclusions

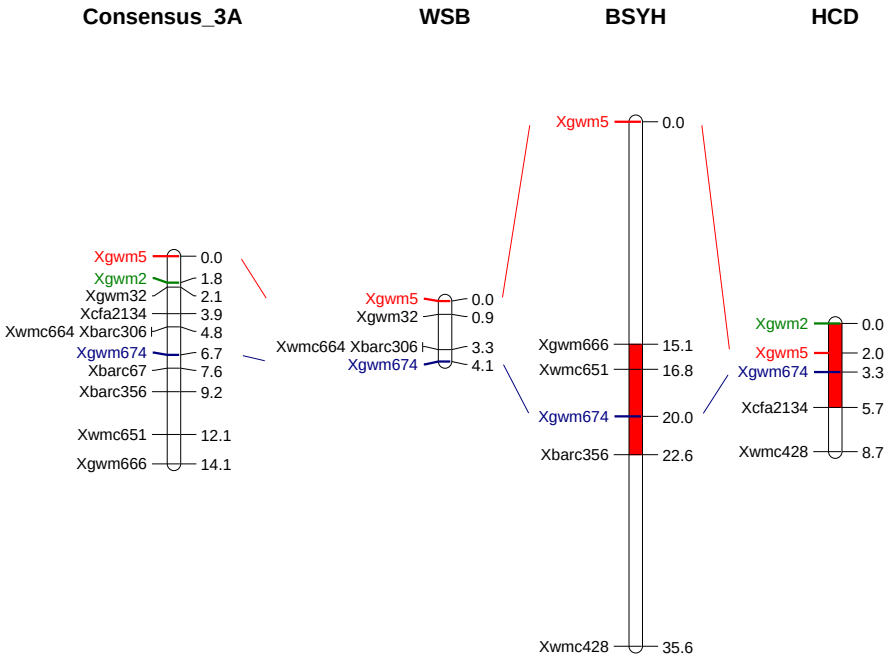
Meta-analyses were used to estimate 95% confidence intervals (CIs) of 24 mapped QTL in five populations derived from Chinese landraces. Nineteen QTL for FHB type II resistance were projected to 10 clusters. Five QTL (1A, 5A, 7A, 3BS (2)), especially, were identified as confirmed QTL to have stable and consistent effects on FHB resistance, should be good candidates for QTL pyramiding in breeding. The QTL on 7D is a unique QTL from HYZ, but it has a major effect on type II resistance, and should be used as another major source in breeding after further evaluation in different populations.

Figure 3.1 Overview of the wheat chromosomes for the five mapping experiments involved in the meta-analysis of FHB Type II resistance. The filled marker intervals indicate that the standardized residual between the interval distance estimates of QTL on original chromosomes. (a. chromosome 1A; b. chromosome 3A; c. chromosome 3BC; d. chromosome 3BSd; e. chromosome 5A; f. chromosome 6B; g. chromosome 7A; h. chromosome 7D)

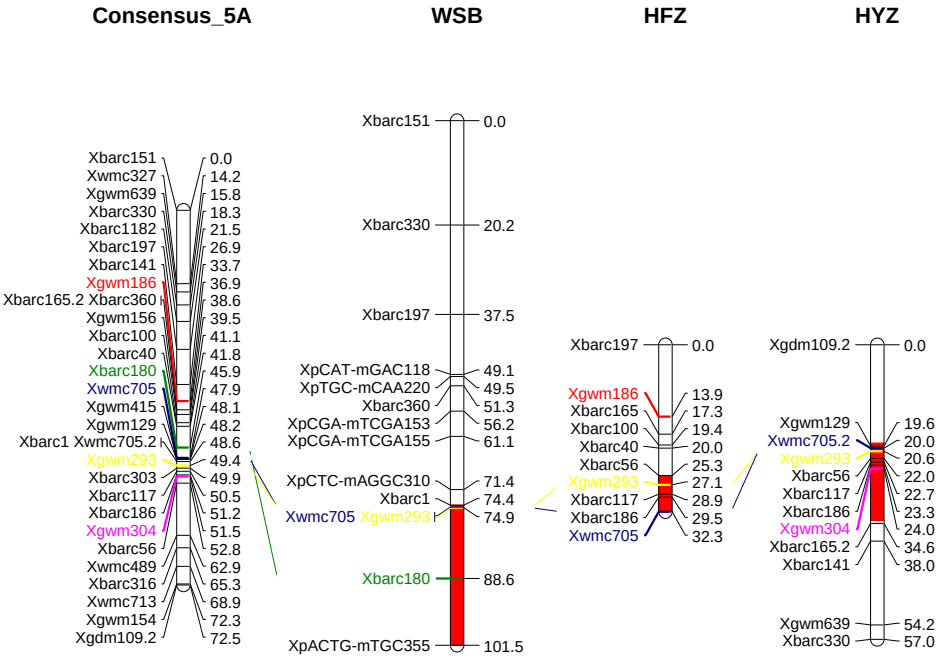
a) Chromosome 1A

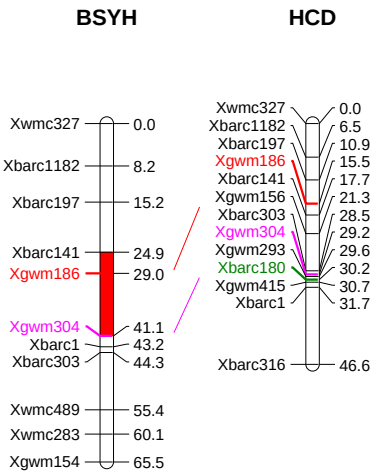


b) Chromosome 3A

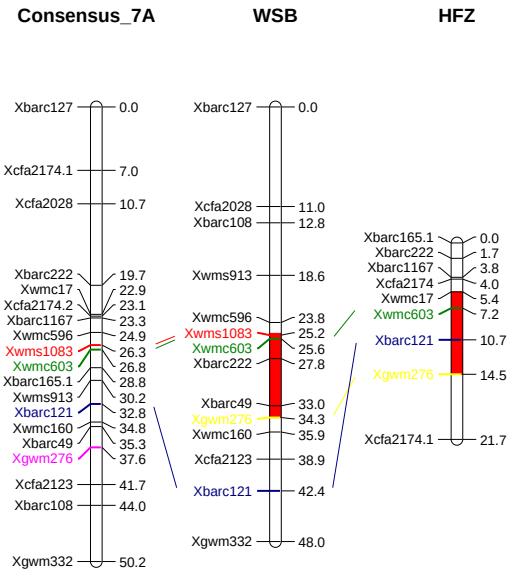


c) Chromosome 5A

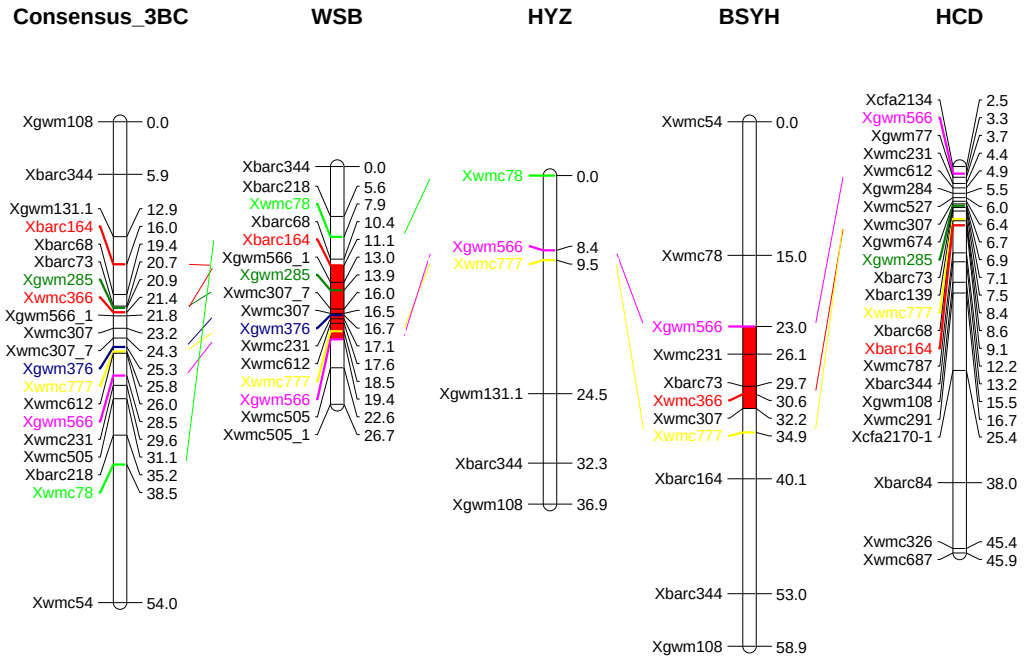


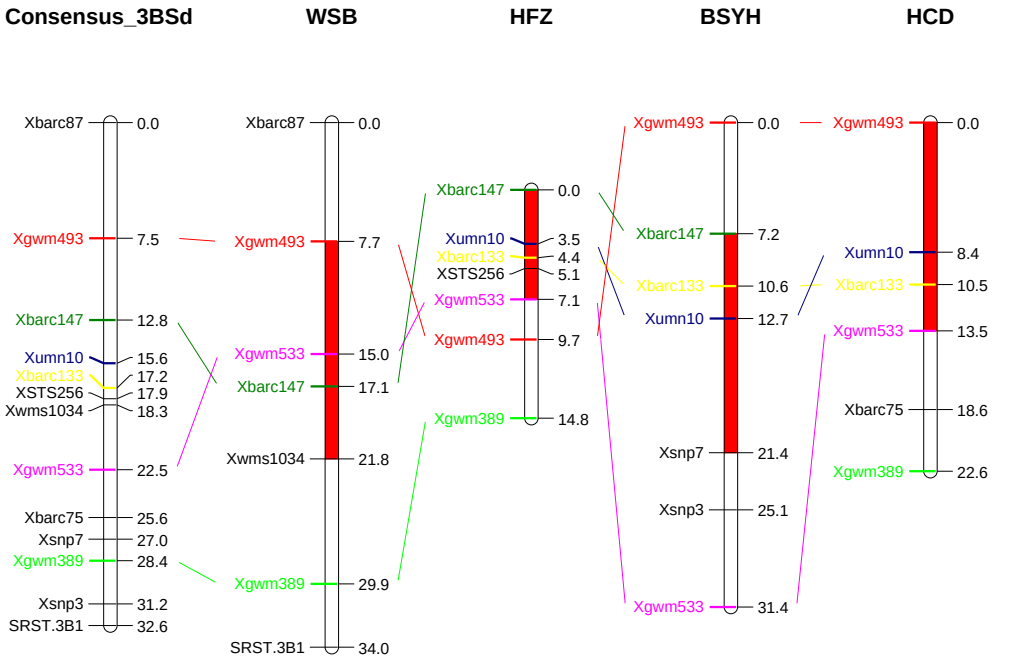


d) Chromosome 7A

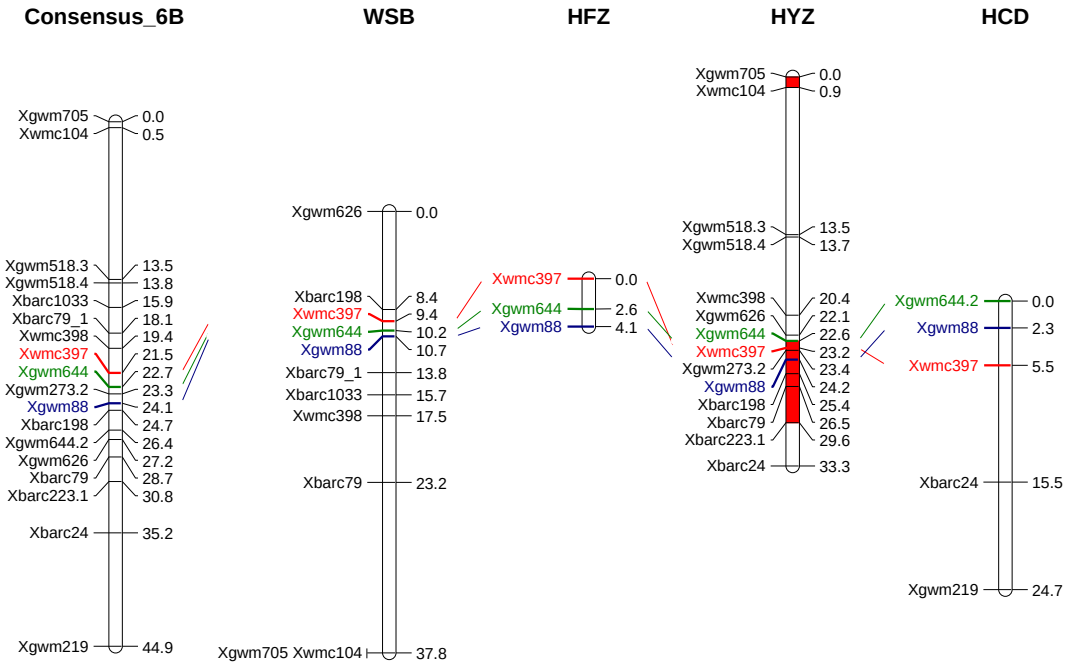


e) Chromosome 3BS





f) Chromosome 6B



g) Chromosome 7D

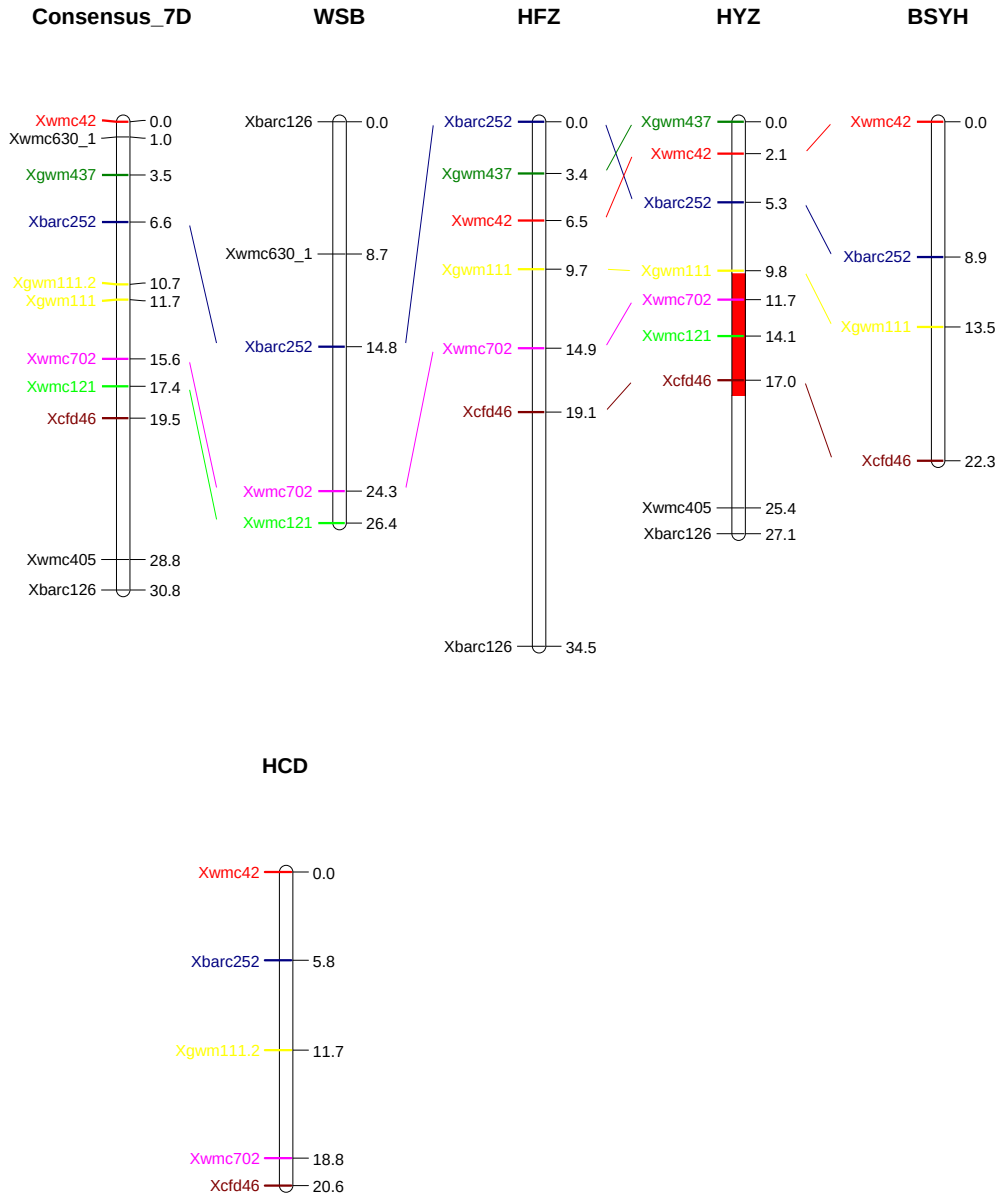
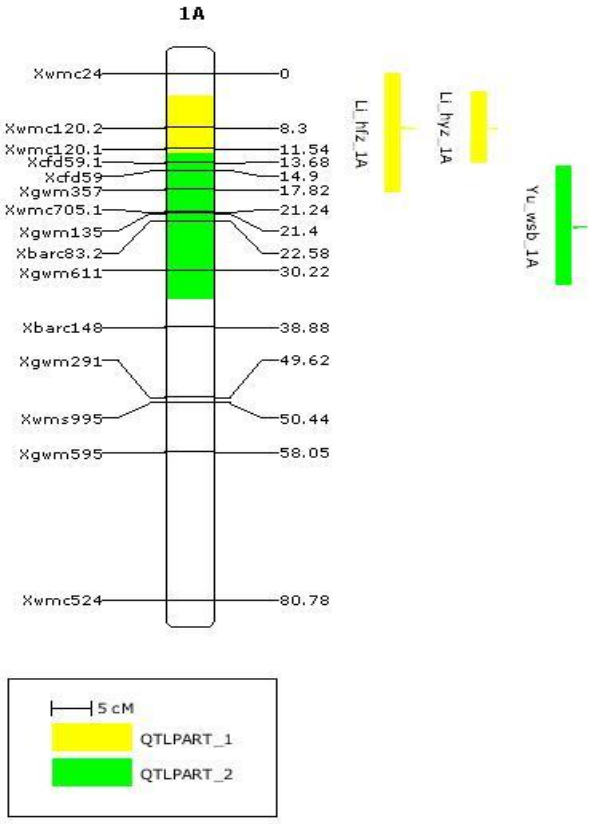
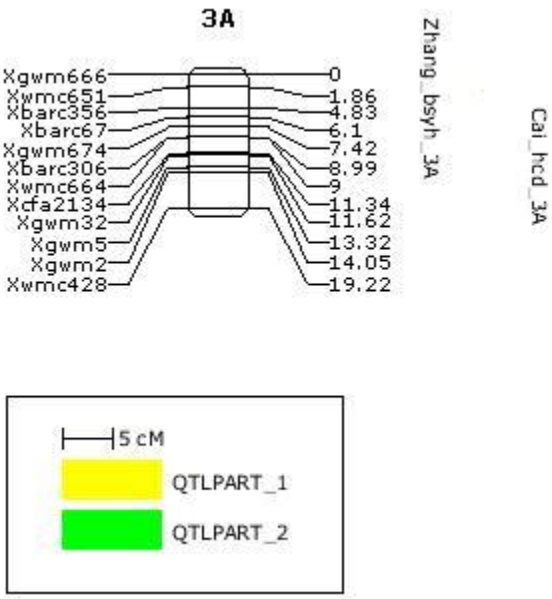


Figure 3.2 Chromosome locations of the 95% confidence interval of QTL associated with FHB Type II resistance in five Chinese wheat landraces on a. chromosome 1A; b. chromosome 3A; c. chromosome 5A; d. chromosome 7A; e. chromosome 3BS; f. chromosome 6B. Name of the markers on the right, the genetic distance in cM are drawn on the left.

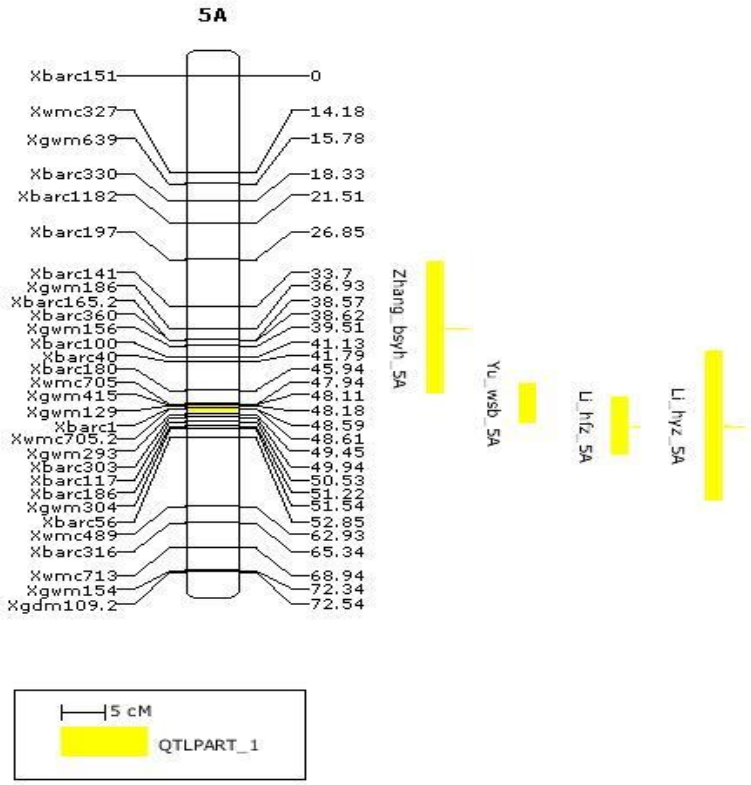
a)



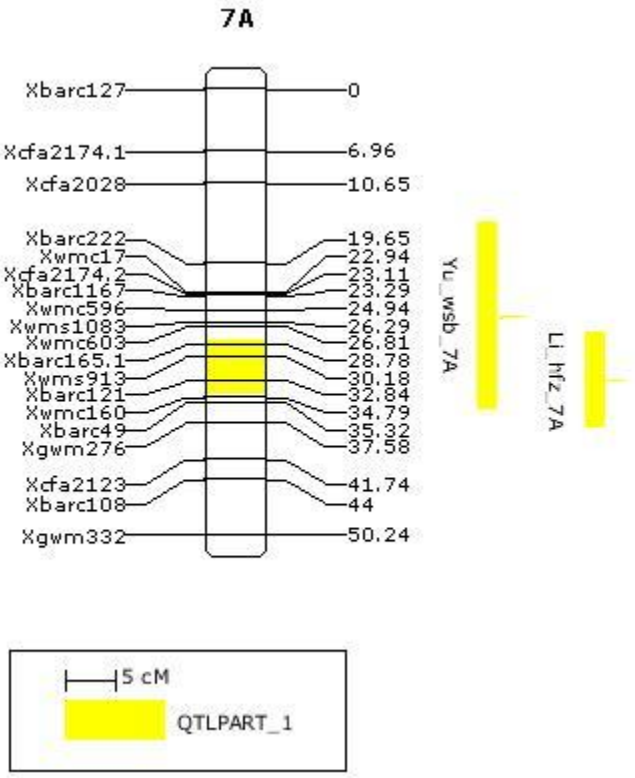
b)


3A
Xgwm666 0
Xwmc651 1.86
Xbarc356 4.83
Xbarc67 6.1
Xgwm674 7.42
Xbarc306 8.99
Xwmc664 9
Xcfa2134 11.34
Xgwm32 11.62
Xgwm5 13.32
Xgwm2 14.05
Xwmc428 19.22
Zhang_bsyh_3A
Cai_hcd_3A
5 cM
QTLPART_1
QTLPART_2

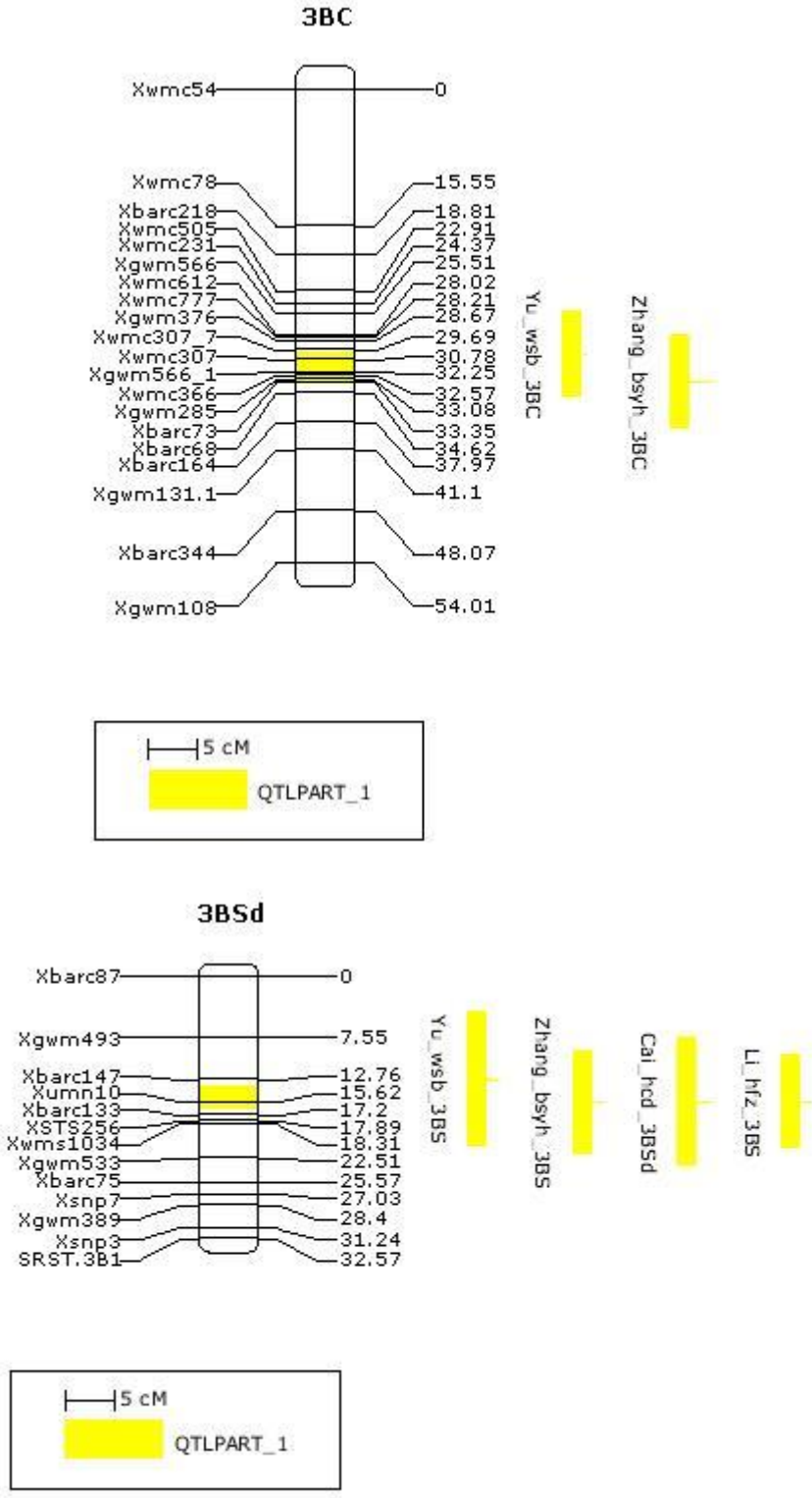
c)


5A
Xbarc151 0
Xwmc327 14.18
Xgwm639 15.78
Xbarc330 18.33
Xbarc1182 21.51
Xbarc197 26.85
Xbarc141 33.7
Xgwm186 36.93
Xbarc165.2 38.57
Xbarc360 38.62
Xgwm156 39.51
Xbarc100 41.13
Xbarc40 41.79
Xbarc180 45.94
Xwmc705 47.94
Xgwm415 48.11
Xgwm129 48.18
Xbarc1 48.59
Xwmc705.2 48.61
Xgwm293 49.45
Xbarc303 49.94
Xbarc117 50.53
Xbarc186 51.22
Xgwm304 51.54
Xbarc56 52.85
Xwmc489 62.93
Xbarc316 65.34
Xwmc713 68.94
Xgwm154 72.34
Xgdm109.2 72.54
Zhang_bsyh_5A
Yu_wsb_5A
Li_hfz_5A
Li_hyz_5A
5 cM
QTLPART_1

d)


7A
Xbarc127
0
Xcfa2174.1
6.96
Xcfa2028
10.65
Xbarc222
19.65
Xwmc17
22.94
Xcfa2174.2
23.11
Xbarc1167
23.29
Xwmc596
24.94
Xwms1083
26.29
Xwmc603
26.81
Xbarc165.1
28.78
Xwms913
30.18
Xbarc121
32.84
Xwmc160
34.79
Xbarc49
35.32
Xgwm276
37.58
Xcfa2123
41.74
Xbarc108
44
Xgwm332
50.24
Yu_wsb_7A
Li_hfz_7A
5 cM
QTLPART_1

e)


3BC
Xwmc54
0
Xwmc78
15.55
Xbarc218
18.81
Xwmc505
22.91
Xwmc231
24.37
Xgwm566
25.51
Xwmc612
28.02
Xwmc777
28.21
Xgwm376
28.67
Xwmc307_7
29.69
Xwmc307
30.78
Xgwm566_1
32.25
Xwmc366
32.57
Xgwm285
33.08
Xbarc73
33.35
Xbarc68
34.62
Xbarc164
37.97
Xgwm131.1
41.1
Xbarc344
48.07
Xgwm108
54.01
Yu_wsb_3BC
Zhang_bsyh_3BC
5 cM
QTLPART_1
3BSd
Xbarc87
0
Xgwm493
7.55
Xbarc147
12.76
Xumn10
15.62
Xbarc133
17.2
XSTS256
17.89
Xwms1034
18.31
Xgwm533
22.51
Xbarc75
25.57
Xsnp7
27.03
Xgwm389
28.4
Xsnp3
31.24
SRST.3B1
32.57
Yu_wsb_3BS
Zhang_bsyh_3BS
Cai_hcd_3BSd
Li_hfz_3BS
5 cM
QTLPART_1

f)

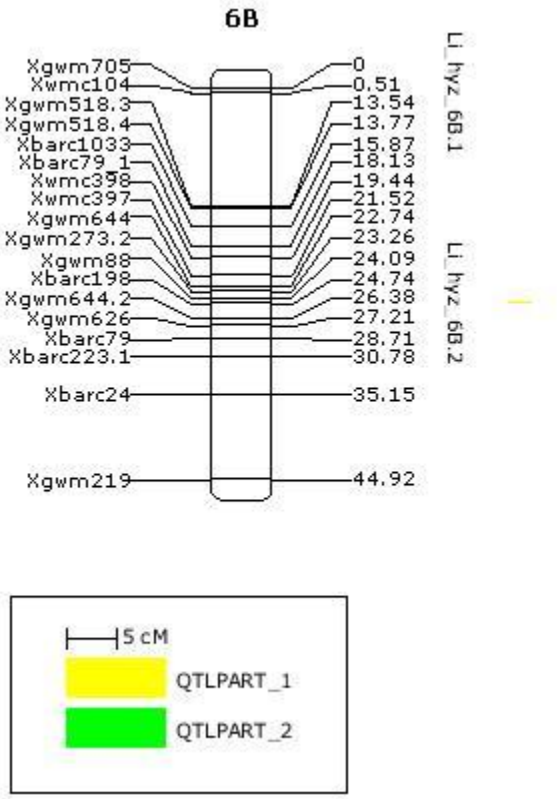

6B
Xgwm705
Xwmc104
Xgwm518.3
Xgwm518.4
Xbarc1033
Xbarc79_1
Xwmc398
Xwmc397
Xgwm644
Xgwm273.2
Xgwm88
Xbarc198
Xgwm644.2
Xgwm626
Xbarc79
Xbarc223.1
Xbarc24
Xgwm219
0
0.51
13.54
13.77
15.87
18.13
19.44
21.52
22.74
23.26
24.09
24.74
26.38
27.21
28.71
30.78
35.15
44.92
Li_hyz_6B.1
Li_hyz_6B.2
5 cM
QTLPART_1
QTLPART_2

Table 3.1 Previously published chromosome locations of QTL for Fusarium head blight (FHB) type II resistance in five Chinese landraces

Parents	Chromosome locations	No. of RIL	References
Wangshuibai /Wheaton	1AS, 5AS, 7AL, 3BS (2), 3DL, 5DL	124 F_6	(Yu et al., 2008c)
Huangfangzhu /Wheaton	1AS, 5AS, 7AL, 1BS, 3BS	102 F_8	(Li et al., 2011)
Haiyanzhong /Wheaton	1AS, 5AS, 6BS (2), 7DL	136 F_8	(Li et al., 2012)
Baishanyuehuang/Jagger	3A, 5AS, 3BS (2)	188 F_6	(Zhang et al., 2012)
Huangcandou /Jagger	3A, 3BS, 2D	190 F_6	(Chapter 2)

Table 3.2 Summary of Fusarium head blight (FHB) type II resistance Quantitative trai loci (QTL) in wheat and their chromosome location, number of QTL, and clusters identified in five Chinese wheat landraces

Chromosome locations	WSB/ Wheaton	HFZ/ Wheaton	HYZ/ Wheaton	BSYH/ Jagger	HCD/ Jagger	Total no. of clusters
1A	1	1	1	-	-	2
3A	-	-	-	1	1	2
5A	1	1	1	1	-	1
7A	1	1	-	-	-	1
1B	-	1	-	-	-	-
3B	2	1	-	2	1	2
6B	-	-	2	-	-	2
2D	-	-	-	-	1	-
3D	1	-	-	-	-	-
5D	1	-	-	-	-	-
7D	-	-	1	-	-	-
Total	7	5	5	4	3	10

Table 3.3 Summary of QTL locations and CIs from the ten QTL clusters

Chromosome locations	Cluster No.	QTL locations	QTLCIs (95%)	Original QTL CIs
1A	**C1**	8.35	9.83	9.33-17.1
1A	**C2**	23.42	22.34	15.5
3A	**C1**	2.23	13.14	7.49
3A	**C2**	6.79	5.18	5.73
5A	**C1**	48.46	1.38	8.9-18.4
7A	**C1**	31.35	6.09	9.1-17.9
3BS	**C1**	15.13	3.11	9.7-14.03
3BS	**C2**	31.66	3.47	8.2-9.1
6B	**C1**	0.27	8.29	6.9
6B	**C2**	24.53	0.05	0.7

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