A NOVEL QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT CHROMOSOME 7A

by

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Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum,* is an important cereal disease in humid and semi-humid wheat growing regions. In recent FHB epidemics in the USA, FHB dramatically reduced wheat yields and grain quality due to mycotoxin contamination. Five types of FHB resistance have been reported, but resistance to disease spread within a spike (Type II) and low deoxynivalenol (DON) accumulation in infected kernels (Type III) have drawn the most attention. A Chinese Spring-Sumai3 chromosome 7A substitution line (CS-SM3-7ADSL) was reported to have a high level of Type II resistance, but quantitative trait locus (QTL) on chromosome 7A has never been mapped. To characterize QTL on chromosome 7A, we developed 191 Chinese Spring-Sumai3-7A chromosome recombinant inbred lines (CRIL) from a cross between Chinese Spring and CS-SM3-7ADSL and evaluated the CRIL in a greenhouse for both types of resistance in three experiments. Two major QTL with Sumai 3 (SM3) origin, conditioning Type II and Type III resistance were mapped in chromosomes 3BS and 7AC. QTL on chromosome 3BS corresponds to *Fhb1*, previously reported from SM3, whereas 7AC QTL, designated as *Fhb5,* is a novel QTL identified from SM3 in this study. *Fhb5* explains 22% phenotypic variation for Type II resistance and 24% for Type III resistance. Marker *Xwmc17* is the closest marker to *Fhb5* for both types of resistance. *Fhb1* and *Fhb5* were additive and together explained 56% variation for Type II and 41% for Type III resistance and resulted in 66% reduction in FHB severity and 84% in DON content. Both QTL showed significant pleiotropy effects on Type II and Type III resistance, suggesting both types of resistance may be controlled by the same gene(s). Haplotype analysis of SM3's parents revealed that *Fhb5* originated from Funo, an Italian cultivar. A survey of worldwide germplasm collection of 400 accessions showed that *Fhb5* is present mainly in Chinese cultivars, especially in Funo-related accessions. Further, *Fhb5* is the second major QTL from SM3 and have potential to be used in improving wheat cultivars for both types of resistance.

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CHAPTER 1 - Literature review

Fusarium head blight

Fusarium Head Blight (FHB), commonly attributed as "scab", has been an important disease in tropical and subtropical regions of the world for a century (Bai and Shaner 1994; Goswami and Kistler 2004). FHB has a devastative impact not only on wheat (*Triticum aestivum* L.) production, but also on other economically important cereal crops such as durum (*T. turgidum* L. var. *durum*) and barley (*Hordeum vulgare* L.*)*, as it causes both yield and quality losses (Leslie and Summerell 2006). FHB epidemics have been reported in many countries including USA, Canada, Europe and China (Bai and Shaner 1994; Goswami and Kistler 2004; Parry et al. 1995; Sutton 1982; Tuite et al. 1990). Although FHB epidemics are sporadic, they were responsible for over 40 – 70% yield losses in the past (Parry et al. 1995). A positive correlation has been observed between FHB severity and yield loss (Mesterházy et al. 1999). Apart from the heavy losses in grain yields, FHB infection causes a significant impact on the quality of the cereals due to mycotoxin contamination, that lead to downgrading of wheat grains (Parry et al. 1995; Sutton 1982). Consumption of such toxin contaminated products is hazardous for humans and animals (Canady et al. 2001). Regulations on acceptable toxin levels in food products are imposed in many countries (Anonymous 2006). Furthermore FHB infected florets often fail to produce grains, or grains are poorly filled if produced (Bai and Shaner 1994). FHB infected seeds tend to have a low germination rate and poor seedling vigor if they are used as planting material (McMullen et al. 1997; Tuite et al. 1990). Since FHB causes many difficulties during production, processing, marketing and exporting of cereal grains, it has received more and more attention in recent years (Goswami and Kistler 2004).

Causal organism, inoculum sources and dispersion

FHB is caused by members of the genus *Fusarium*, a filamentous ascomycetes fungus (Goswami and Kistler 2004). *F. graminearum, F. culmorum* and *F. avenaceum* are major species that have been reported to cause FHB (Nyvall 1999; Sutton 1982). However *F. graminearum* Schwabe (telomorph *Gibberella zeae* (Schw.) Petch) is the primary pathogen responsible for FHB epidemics in most regions of the world (Leslie and Summerell 2006; Nyvall 1999; Parry et al. 1995; Sutton 1982; Xu and Nicholson 2009). In nature ascospores, macroconidia,

chlamydospores and mycelium can all serve as inoculum for epidemics (Sutton 1982). The mycelium can survive saprophytically and chlamydospores can stay viable as over wintery structures due to thick walls (Xu and Nicholson 2009). However, ascospores of *F. graminearum* released from a specialized structure known as perithecia produced by the fungus have been considered as the major initial inoculum in the field (Leslie and Summerell 2006). Stubble and debris from previous crop seasons are the main carriers of the initial inoculums. But chaff and soil are also important sources of inoculum (McMullen et al. 1997; Sutton 1982). Wind and water splash is considered to be the main mode of disease spread (McMullen et al. 1997; Xu and Nicholson 2009). The ascospores are forcibly discharged in to the air increasing their disperse range (Xu and Nicholson 2009). Upon reaching the host, ascospores starts a new disease cycle in flowered spikes and mycelium grows within a spike to spread FHB to other spikelets and eventually take over the entire plant (Xu and Nicholson 2009).

Symptoms and disease development

FHB symptoms observed among the main causal *Fusarium* spacies or strains are similar (Bai and Shaner 1996; Parry et al. 1995). Initial symptoms of FHB infection includes a water soaked appearance on the glume and a tan or brown color discoloration appearing at the base of the infected floret (Bai and Shaner 1996; Nyvall 1999; Parry et al. 1995). Later on due to the presence of conidia, a characteristic orange color can be observed in infected florets (Ribichich et al. 2000). With time the infection would spread within a spike and infected spike will show a bleached appearance, or blight (Bai and Shaner 1996; Parry et al. 1995). Infected florets could become sterile, or produce shriveled kernels or chalky appearance known as “tombstone grains” (Bai and Shaner 1994; McMullen et al. 1997). Further in to disease development, brown discolorations can be observed in rachis and culm. As the pathogen continue to colonize the head, entire spike could die off giving it a straw color appearance (Bai and Shaner 1996; Bai and Shaner 1994).

For initial infection, conidia will start to germinate within a 6 - 12 hour period upon contact and then hyphae will arise from the germ tube (Xu and Nicholson 2009). Usually anthers are the first floral component to get infected, but other parts the of floret could also be the target sites for initial infection (Pritsch et al. 2000). During disease development horizontal disease progression occurs from anthers to glumes and vertical disease progression occur from anthers to

rachis (Ribichich et al. 2000). During a period of 24 -36 hours, mycelium will densely colonize the glume, lemma, palea and ovary, but is rarely observed on the surface (Xu and Nicholson 2009). Histological evidence shows that with disease progression, pollen grains, lemma, glume, rachilla, xylem and phloem get infected. Chlorenchyma tissues are said to be the most damaged tissues and this lead to chlorosis and necrosis of the affected areas (Ribichich et al. 2000). In caryopsis, fungus invades inner layers of pericarp, testa, aleurone and endosperm (Jansen et al. 2005). Fungus reaches neighboring spikelets by different means, such as germinated conidia, hyphae advancing through rachilla and hyphae colonizing the vascular bundle. Fungal hyphae can also grow through natural openings or by direct penetration. Thereby spikelets both in distal or basal proximity to the initially infected spikelet could get infected (Ribichich et al. 2000). Upon sufficient colonization, the fungus engages in mycotoxin production. These mycotoxins are translocated through phloem and xylem tissues in to other parts of the plant. Thus, mycotoxin contamination of uninfected tissues are inevitable (Xu and Nicholson 2009).

Under a high disease pressure, the infection could spread to neighboring heads. But rate of spread and severity depends on other factors such as, cultivar and environment. In susceptible cultivars entire spike can get bleached. Often tan or brown discolorations are observed on infected rachis and culms (Bai and Shaner 1996; Bai and Shaner 1994). In resistant cultivars, symptoms are seen only on the inoculated spikelet or in few others. Sudden desiccation of the terminal spikelets are not observed in highly resistant cultivars. Disease symptoms of resistant cultivars remain confined to the inoculated spikelet. But in highly susceptible cultivars and in moderately susceptible cultivars the infection could spread to non-inoculated spikelets through rachis. Even though it's not common in resistant varieties, spreading to non-inoculated spikelets can occur at a much slower rate towards later stages. This marks a major difference between a highly resistant cultivar and a moderately resistant cultivar (Bai et al. 1999). Overall resistant cultivars show no spread of FHB to uninoculated spikelets and therefore have a low level of disease severity (Ribichich et al. 2000). Disease development is boosted by high precipitation or humidity that coincide with flowering or early kernel development stages (Bai and Shaner 1994; McMullen et al. 1997). Anthesis is the most vulnerable stage for FHB infection. But infection can occur even as late as soft dough stage (Bai and Shaner 1996; McMullen et al. 1997). However disease susceptibility declines towards later stages of caryposis development (Bai and Shaner 1994).

Factors affecting FHB disease development

FHB disease incidence, development and final severity are heavily influenced by environment (Bai and Shaner 1994; Fernandez et al. 2005; Verges et al. 2006). Temperature and moisture are critical for growth and conidiation of the pathogen (Xu and Nicholson 2009). *F. graminearum* can grow in a temperature range of 10 - 25˚C, but tend to perform poorly above 25˚C. The optimal growth temperature is considered to be 25˚C (Brennan et al. 2005). This coupled with high humidity would create a perfect condition for disease development. Thereby provision of ideal environmental conditions is crucial in FHB studies (Bai and Shaner 1994). Other than environmental factors, plant/cultivar characteristics have an effect on FHB incidence and severity. Flower morphology is thought to have an impact on FHB incidence. Florets that open wider are more susceptible to FHB compared to florets with narrow openings. Florets that open wider, remain opened for longer duration and thereby allow more time and more space for the inoculum to enter the floret (Gilsinger et al. 2005). Awned cultivars with short peduncles and compact spikes create a humid environment around the spikelets fueling disease incidence. Taller plants with rapid grain filling ability are less prone to FHB (Rudd et al. 2001; Somers et al. 2003). These cultivar characteristics act as the first line of defense to minimize the potential disease incidences (Kolb et al. 2001).

Agronomic practices such as tillage and crop rotation often have resulted an upsurge of FHB incidences. No tillage or minimum tillage leaves more crop residue in fields. This increases the potential for a FHB epidemic (Dill-Macky and Jones 2000). Crop succession history of a field has a major impact on FHB incidence (Bai and Shaner 2004; Dill-Macky and Jones 2000). Frequent use of susceptible crops in crop rotation increases disease occurrence (Dill-Macky and Jones 2000). Irrigation may create a microenvironment suitable for pathogen colonization and cause the disease to progress regardless of the persisting climatic conditions. Sowing date can have an effect on disease development, as sowing date along with the cultivar and environment decides the flowering days. If flowering days coincide with an ideal environment it would promote disease epidemics in the field. Weeds in the crop field serve as alternate hosts and thus lead to higher disease incidence. Factors that affect the canopy density such as, row spacing, sowing density and nitrogen fertilizer application may influence the occurrence of FHB (Champeil et al. 2004).

Mycotoxins

Fusarium species produces different types of mycotoxins. A primary role of these mycotoxins is to compete against other pathogens (Xu and Nicholson 2009). Deoxynivalenol (DON), Zearalenone and Nivalenol are some of the mycotoxins reported to be associated with FHB (Anonymous 2006; Xu and Nicholson 2009). Vomitoxin, deoxynivalenol is the most important mycotoxin produced by *F. graminearum* (Mesterházy et al. 1999). Deoxynivalenol is a type B trichothecene, which is less toxic to humans and animals compared to type A trichothecenes (Xu and Nicholson 2009). DON does not accumulate in body tissues and thereby are not found in animal based food as residues (Pestka and Smolinski 2004). Most important health implication of deoxynivalenol in humans is its potential to induce acute gastroenteritis with vomiting (Pestka and Smolinski 2004). Nausea, reduction in food intake, dizziness and headache have also been reported as symptoms of DON toxicity (Canady et al. 2001). Based on animal studies, it is predicted that in a chronic exposure, effects on growth, immunity and reproduction could be added to the list of possibilities (Pestka and Smolinski 2004). Feed contaminated with *Fusarium* mycotoxins lead to feed refusal induced nutritional deficiencies, growth retardation, adversities in metabolic functions and poor immunity in livestock (Korosteleva et al. 2007; Smith et al. 1997; Swamy et al. 2003). Therefore proper regulation of *Fusarium* mycotoxins in food and feed is essential. European Union has defined maximum limits for *Fusarium* toxins for cereals and cereal based products by Commission Regulation (EC) No 1881/2006. According to the regulations, tolerable daily human consumption is set at 1µg/kg body weight per day for DON, 0.2µg/kg for zearalenone and 0.7µg/kg for nivalenol (Anonymous 2006).

In wheat plants, DON is proven to be important for disease spread, but not for initial infection (Bai et al. 2002; Lemmens et al. 2005). Thereby DON is not a requirement for infection initiation, but is a virulence factor of *F. graminearum* (Jansen et al. 2005; Proctor et al. 1995). However in DON producing pathogen isolates, the amount of toxin produced is allied to its aggressiveness (Tóth et al. 2008). DON accumulation depends on number of factors such as cultivar, pathogen strain, and existing environmental conditions (Mesterházy et al. 1999). The general understanding is that moderately susceptible and susceptible cultivars would have a higher DON concentration than resistant cultivars (Bai et al. 2001). Favorable environments result early accumulation of DON (Zhou et al. 2002a). The relationship of FHB severity and

DON accumulation is still unclear. Some studies report high correlations between FHB symptom spread and DON accumulation (Bai et al. 2001; Lemmens et al. 2005; Yu et al. 2008b), but others fail to find a strong relationship between the two traits (Ma et al. 2006c; Mesterházy et al. 1999). Prediction of DON contamination through FHB severity or kernel infection data may not always be accurate. There are reports of lower DON concentrations in some susceptible genotypes associated with higher kernel infection. Lower DON content can be speculated as a result of mechanisms that prevent synthesis, degrade DON and /or prevent its accumulation (Mesterházy et al. 1999; Miller et al. 1985). Furthermore it is also possible to have DON contaminated seeds without showing any visible symptoms (Lacey et al. 1999). Therefore to evaluate resistance to DON accumulation it is important to accurately quantify the DON concentration. This can be done by gas chromatography - mass spectrometry (GC-MS) method (Mirocha et al. 1998), thin layer chromatographic (TLC) method (Trucksess et al. 1984), high pressure liquid chromatography (HPLC) method (Chang et al. 1984) or by enzyme linked immunosorbent assay (ELISA) method (Hart et al. 1998). However measurements of DON concentration is expensive and thereby it is not a feasible tool to use in everyday breeding practice (Bai et al. 2001).

FHB resistance mechanisms

Resistance for FHB is considered to be horizontal and it is observed to be non-race and non-species specific. So far no race differentiation has been reported for *F. graminearum* or for *F. culmorum* (Eeuwijk et al. 1995; Tóth et al. 2008), suggesting that wheat has a common resistance mechanism (Tóth et al. 2008). Resistance to FHB could be morphological or physiological. Morphological characters to avoid initial infection such as plant height, awnedness, peduncle length, flower opening duration and level of opening, compactness of the spike remain less important compared to physiological resistance (Rudd et al. 2001). Physiological resistance to FHB has been characterized in to several categories. Commonly accepted types of resistance against FHB are: resistance to initial penetration of the pathogen (Type I), resistance to disease spread (Type II, (Schroeder and Christensen 1963) and low DON accumulation (Miller et al. 1985). Mesterházy et al., (1999) proposed five resistance types by changing type III to resistance to kernel infection, type IV to tolerance FHB infection and type V to resistance to accumulation of DON (Mesterházy et al. 1999). To date type I resistance

is not well characterized due to lack of a reliable evaluation method (Yu et al. 2008b). Type II resistance is extensively studied and thought to be the most stable type of resistance against FHB (Bai and Shaner 2004; Kolb et al. 2001). Type II resistance may be affected by the rate of symptom spread, spread frequency and time taken for the symptom to appear in non-inoculated spikes. These parameters would be useful in categorizing the germplasm based on the resistance level (Bai et al. 1999). Furthermore, single genotype may not contain complete type I or type II resistance, instead would contain a combination of both (Miedaner et al. 2003).

Resistance against FHB can be scrutinized at different levels. According to Ribichich et al. (2000) thickening of cell wall and deposition of amorphous material at inter or intra cellular spaces create physical barriers delaying the disease progression. But eventually the fungus trespass these physical barriers. A study done with *F. graminearum* expressing a green florescent pigment (GFP) showed that, when fungal hyphae penetrates the host cell, the cell undergoes plasmolysis that leads to cell disintegration and cell death (Jansen et al. 2005). There is speculation suggesting an existence of a substance that is capable of suppressing the mycelium growth within the spike (Bai and Shaner 1996). It is evident that less virulent strains of *F. graminearum* that do not produce DON can cause initial infection, but cannot cause disease spread beyond the infected spikelet (Bai et al. 2002). Thereby DON could have a role to play in suppressing disease development. Jansen et al. (2005) observed an enhanced defense system in wheat against *F. graminearum* strains that do not produce DON. It was seen that in strains that do not produce DON, host retains the fungus at rachis nodes by inducing cell wall thickening. But in the presence of trichothecenes, the fungal hyphae overtake the defense system and enter the vascular bundle easily. Thus in wheat, trichothecenes are important for the movement of *F. graminearum* beyond the rachis node (Jansen et al. 2005). According to Lemmens et al. (2005), DON gets detoxified to a chemical compound called DON - 3- O- glucoside in highly resistant plant lines. This detoxification process could be an important link in the resistance mechanism against DON accumulation. Furthermore, they state that the quantitative trait loci (QTL) *Fhb1*, contains a gene region that encode for glucosyl transferase enzyme or regulates its expression. According to Lemmens et al. (2005), DON is an important compound in FHB resistance complex, but existence of DON resistance in plants and the role of DON in overall FHB resistance is not perfectly clear.

Biochemical composition of host plants may influence resistance / susceptibility of a cultivar against FHB (Brown and Brindle 2007). Brown and Brindle (2007) reported, that metabolic profiles show a significant correlation with latent period (delay in sporulation of the fungus in host tissue), an important factor influencing resistance. It was evident that choline was the single most prominent metabolite among the shorter latent period cultivars. Betaine, glutamine, glutamate and alanine and sucrose were also higher in susceptible cultivars. Findings of Brown and Brindle (2007) suggest an involvement of these metabolites, especially choline to disease susceptibility. But contradictory evidence was given by Engle et al. (2004), where no significant correlation of fungal hyphae growth or spore germination was associated with the levels of choline or betaine (Engle et al. 2004). These findings underscore the fact that molecular and biochemical mechanisms underlying FHB resistance are still not completely understood. According to Li and Yen (2008), jasmonate (a volatile fatty acid) mediated defense responses and ethylene mediated defense responses are important for FHB resistance in Sumai 3 (SM3). When the plant is under a pathogen attack, jasmonate activates proteinase inhibitor synthesis. Another defense mechanism is activated through ethylene mediated reactions where it induces senescence and ultimately results in necrosis (Li and Yen 2008). Therefore, an up regulation in jasmonate and ethylene biosynthesis was evident in resistant cultivar SM3. Furthermore application of jasmonate or ethylene on to the susceptible cultivar prior to inoculation made the cultivar resistant to FHB, suggesting a potential involvement in resistance development (Li and Yen 2008). General defense response against pathogen invasion is obtained through systemic acquired resistance (SAR) mediated by salicylic acid. SAR pathway includes different pathogenesis related proteins (PR). Defense responses of PR proteins includes secretion of protease enzymes to defuse the activity of pathogen secreted proteolytic enzymes, secreting enzymes to degrade the microbial cell wall and trigger hypersensitive responses in the host (Li and Yen 2008). PR proteins such as thaumatin like proteins (TLP) (Chen et al. 1999), chitinase and β-1,3-glucanase (Li et al. 2001) involved in SAR were reported to be associated with FHB resistance. Expression of PR transcripts of peroxidase, PR-1, PR-2 (β -1,3-glucanase), PR-3 (chitinase), PR-4, and PR 5 (TLP) in both resistant and susceptible cultivars were reported in a previous study and found temporal differences in their expression levels (Pritsch et al. 2000). Li and Yen (2008) reported expression of PR proteins in resistant and susceptible cultivars triggering general defense responses. But the associations of FHB resistance to PR proteins were

reported as insignificant, suggesting SAR may not be involved in FHB resistance. It's possible that these PR genes may be responsible for general host responses against an infection but they may not necessarily be the key component in FHB resistance (Bai and Shaner 2004). However modern molecular and biochemical analysis methods and technologies may pave paths for a better understanding of the mechanisms behind FHB resistance in time to come (Bai and Shaner 2004).

Genetics of FHB resistance

Polygenic inheritance and quantitative variation of FHB is reported even within an individual resistance type (Buerstmayr et al. 2000; Grausgruber et al. 1999; Miedaner et al. 2003). FHB resistance is mainly due to additive effects (Bai et al. 2000; Buerstmayr et al. 2000), but epistatic interactions between QTL have also been reported to have a significant enhancing effect on the overall FHB resistance in some crosses. These epistatic QTL would be more sensitive to the environmental variation (Bai et al. 2000; Ma et al. 2006b). In some crosses, dominance could also contribute to the phenotypic variation (Bai et al. 2000). FHB resistance is thought to be controlled by a complicated gene network (Ma et al. 2006b) involving two to six QTL (Table A.1) in most resistant cultivars. According to Kolb et al. (2001), the number of genes detected in a study can vary due to several reasons: (i) FHB resistance is controlled by many genes that segregate differently among different crosses, (ii) segregation could depend on the genetic background of the parents in the cross and their disease resistance levels, (iii) if the source of resistance is heterogeneous, the resistance genes carried by successive lines could differ from each other, so that the same set of genes may not be detected at all times, (iv) use of different *Fusarium* species for disease induction could lead to differences in the final gene count, (v) genes controlling other resistance types can make an impact on the assessment, (vi) different techniques used in phenotypic evaluations could cause a difference and (vii) different environmental conditions where the experiment was conducted can render the number of detected genes due to gene by environment interaction (Kolb et al. 2001).

Control strategies

Traditionally farmers adopted different cultural practices to minimize the damage caused by FHB infection. Tillage is an important agronomic practice to manage FHB in the field.

Tillage incorporates crop residue into the soil. Crop residue is a major source of initial inoculum for epidemics. Clearing them from fields decrease inoculum levels and thereby lowers the potential of an epidemic outbreak (Bai and Shaner 2004; Dill-Macky 2008; Dill-Macky and Jones 2000). Crop rotation is a cultural practice that helps control FHB. Crop rotation with less susceptible crops reduces FHB incidence (Dill-Macky and Jones 2000). Deciding a proper sowing date is critical to reduce disease occurrence. Sowing date needs to be decided in such a way to ensure that less favorable field conditions coincides with the flowering stage (Champeil et al. 2004). Suspending irrigation during the time of flowering, until after anthesis and removal of infected seeds from seed lots are few other traditionally adopted cultural practices (Bai and Shaner 2004). Use of certified seeds conditioned with fungicides and application of fungicides to fields are other types of control strategies (Bai and Shaner 2004). Application of a suitable fungicide will reduce FHB severity and DON accumulation (Bai and Shaner 2004; Miriam et al. 2005). Fungicide application, with tebuconazole and/or prochloraz as active ingredient, at the beginning of the flowering season or later is found to be effective. This reduces FHB severity and causes a significant reduction in DON accumulation (Homdork et al. 2000; Miriam et al. 2005). But high cost associated with fungicide treatments, difficulty in determining the optimal time of application, and lack of highly effective fungicides for FHB are some of the major draw backs (Bai and Shaner 2004). Attempts to find biological control agents against *F. graminearum* have been reported in several cases (Khan and Doohan 2009; Khan et al. 2001; Schisler et al. 2002). Biological control agents are prospective candidates to be used in an integrated FHB management program. But further research is needed to effectively use these biological control agents successfully in the field. So far use of cultivars with FHB resistance and low DON accumulation is the most economical and effective way to address the problem. But development of a highly resistant cultivar for complete control of FHB has not been possible even with the enormous efforts put in to it by breeders for decades (Bai and Shaner 2004). Therefore the best available approach so far is to go for an integrative approach of cultural practices, chemical control and use of resistant cultivars.

Resistance sources

FHB resistance sources have been reported from different geographical regions such as, Asia, Europe, North and South America (Bai and Shaner 2004; McCartney et al. 2004). Asian

resistant sources are mostly land races from China and Japan (Yang et al. 2005a). Chinese cultivar SM3 is considered to be the most used FHB resistance source in breeding programs worldwide (Bai and Shaner 1996; Kolb et al. 2001; Rudd et al. 2001). Wide use of SM3 is credited to its high resistance to disease spread and low DON accumulation (Bai et al. 2001). Thereby resistance QTL in SM3 are commonly seen in its successors around the world (McCartney et al. 2004). Breeders have found SM3 to be a highly heritable, stable and consistent resistance source. However, even though it's a valuable source of FHB resistance, SM3 drags along few undesirable traits such as susceptibility to other disease and shattering (Rudd et al. 2001) that makes it difficult to be directly used as resistant parent. Some of its derivatives such as Ning7840, Sumai49 and Fu5114 have some improved traits than SM3 and are better parents for crosses (Bai and Shaner 1996). In addition, some other sources unrelated to SM3 have been identified from China such as Wangshuibai (Jia et al. 2005a; Lin et al. 2006; Yu et al. 2008c; Zhou et al. 2004), CJ W14 and CJ 9306 (Jiang et al. 2006), Chokwang from Korea (Yang et al. 2005a) with a high level of resistance and improved agronomic traits.

Resistant cultivars were widely used as resistance sources to pyramid resistance genes into cultivars (Bai et al. 1999). But resistant cultivars are not the only ones with breeding importance. Even moderately resistant cultivars can have a better breeding potential. According to Waldrone et al. (1999) moderately resistant parents may contain resistance genes that are often not found in resistant parents. When these resistant alleles combine, a progeny with higher level of resistance can be expected. The best example would be SM3. The cultivar was developed by a cross between moderately resistant parents Taiwan wheat and Funo (Bai et al. 2000). This combination significantly improved the resistance level against FHB and also broadened the diversity resulting a better adaptation (Bai et al. 2003). Thereby resistance genes in moderately resistant cultivars can be efficiently utilized for the development of new cultivars (Ma et al. 2006b). Some of the known moderately resistant cultivars such as Ning 8306, Ning 8331 (Bai and Shaner 1996), Stoa (Waldron et al. 1999), Frontana (Mardi et al. 2006), Chinese Spring (Grausgruber et al. 1999) and Ernie (Liu et al. 2007) were used in breeding programs as sources of resistance. Some moderately susceptible cultivars like Alondra (Shen et al. 2003) and Maringa (Somers et al. 2003) have been reported to have resistance QTL in them. In addition to the conservative breeding strategies, alien chromosomes carrying resistance to FHB has successfully been used as a novel source of FHB resistance (Oliver et al. 2005). Methods such as

homoeologous chromosome pairing, induced chromosome pairing or translocation through chromosome manipulation is used to transfer resistance from alien sources to wheat (Cai et al. 2005). Development of transgenic wheat is another novel strategy that can be used to introduce FHB resistance to breeding lines. Transgenic wheat expressing barley class II chitinase gene (Shin et al. 2008), or over expressing defense related genes such as α-1-purothionin, thaumatin-like protein 1 (tlp-1), β-1,3-glucanase (Mackintosh et al. 2007), and NPR 1 gene (Makandar et al. 2006) has been reported to elevate FHB resistance. However, transgenic plants with better resistance than SM3 have not been found to date.

Level of resistance of individual resistance components varies with cultivar. A cultivar with high Type II FHB resistance may or may not have high Type I resistance. Therefore detailed studies on individual resistance components needs to be carried out for each resistance source prior to their use in breeding programs (Yu et al. 2008b). To date, resistance from SM3 remains stable across different regions of the world. Reason behind this could be the non-species and non-race specific nature of FHB resistance in wheat. Therefore even the most aggressive race has limitations when infecting wheat (Tóth et al. 2008). This enables the use of resistance source across breeding programs worldwide. However one important consideration when using a resistance source from another region would be the adaptability of the cultivar to the existing climatic conditions.

Molecular markers and genetic maps

Molecular marker technology is an indispensible tool in modern plant breeding. Selections in earlier breeding programs were based on morphological markers. However with the recent developments in molecular marker technology more emphasis has been given to adjoin a molecular based selection method to the existing breeding program. Some of the added advantages of molecular markers over morphological markers are (i) molecular marker analysis can be done at any growth stage where as morphological markers often are distinguishable at the adult stages, (ii) molecular markers can be used to detect polymorphism among alleles in most regions of the genome, but such polymorphism occurring at alleles that are morphologically distinguishable are rare and are often accompanied with deleterious effects. Therefore compared to morphological markers, molecular markers allow monitoring many segregating markers simultaneously (Tanksley 1983) and can accelerate selection for both simple and quantitative

traits in breeding programs. Molecular markers have been used in different applications such as, marker assisted selection (MAS), phylogenetic and evolutionary studies, disease diagnostics, varietal identification and cultivar characterization (Gupta et al. 1999). Molecular markers can be protein or DNA markers. In plant breeding the most commonly used protein markers were isozymes (Tanksley 1983). Use of enzyme markers at that time had several advantage such as, co-dominant nature of protein markers that allowed proper identification of the genotype and lack of epistasis that allowed classification of several markers that segregate simultaneously (Tanksley et al. 1982). The main drawback of protein markers was the limited availability (Tanksley 1983), therefore they was quickly replaced by DNA markers, that are practically unlimited in plants.

Molecular markers can be broadly categorized into three groups as hybridization based, PCR based and sequencing based. Restricted Fragment Length Polymorphism (RFLP) and oligonucleotide fingerprinting are hybridization based molecular markers. In recent years PCR based molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) have become more popular. The newest addition would be the sequencing based markers such as Single Nucleotide Polymorphism (SNP), Sequence Tagged Sites (STS) and Expressed Sequence Tags (EST) (Gupta et al. 1999). Throughout the years many different types of DNA markers, such as RAPD (Bai 1995; Ban 2000), RFLP (Anderson et al. 2001; Waldron et al. 1999), AFLP (Bai et al. 2003; Bai et al. 1999; Zhang et al. 2004), SSR (Kumar et al. 2007; Ma et al. 2006b), STS (Cuthbert et al. 2006; Liu and Anderson 2003a) , have been used in QTL mapping of FHB. Some of the newer additions would be the use of Single nucleotide polymorphism (SNP) and Single-strand conformational polymorphism (SSCP) (Yu et al. 2008a). The earliest type of molecular marker RFLP, was successfully used in detecting QTL for FHB resistance (Anderson et al. 2001; Waldron et al. 1999). It is a co-dominant marker. The use of radioactive labeling in RFLP which often seems like a disadvantage can be avoided if non radioactive labeling methods are available. PCR-based markers have made molecular work more time, effort and cost effective (Gupta et al. 1999). RAPD has been used as a molecular marker in QTL mapping of FHB resistance (Bai 1995; Ban 2000). However low polymorphism, lack of reproducibility and complications arising due to complex genomic structure are some of the disadvantages of RAPD markers (Gupta et al. 1999). AFLP has been successfully used in QTL mapping studies (Bai et al. 2003; Bai et al.

1999; Zhang et al. 2004). A high level of polymorphism and reproducibility has made AFLP an attractive marker system (Gupta et al. 1999). However, it is not a breeder-friendly marker for MAS due to extra step of digestion and pre-amplification. Therefore, conversion of AFLP into STS markers is essential to make the markers useful in MAS (Bai et al. 1999; Guo et al. 2003). In wheat, microsatellites are fairly abundant, highly polymorphic, evenly distributed in chromosomes (except in centromeric regions) and many are locus specific (Gupta et al. 1999; Roder et al. 1998a). Analysis using microsatellite requires small amounts of genomic DNA and can be easily integrated into a high throughput analysis system. It can be efficiently used to study quantitative traits in segregating breeding populations. Thus, microsatellites are highly suitable molecular markers for mapping studies in wheat and have been used as major markers to map many important traits (Roder et al. 1998b). Microsatellite markers can be successfully used to track down previously reported FHB resistance QTL and to quarry for novel QTL (McCartney et al. 2004). These markers are highly polymorphic between FHB resistant Chinese land races and are an efficient tool to study their genetic diversity and conduct QTL mapping (Wei et al. 2005). Another type of a maker that can be used in a mapping study is STS. STS markers are short unique sequence with a specific location on the chromosome. These are developed by sequencing polymorphic RFLP or AFLP markers (Gupta et al. 1999).

For mapping studies a good consensus map is a necessity (Somers et al. 2004). One of the earliest consensus genetic maps for bread wheat (based on microsatellite markers) was developed by Roder et al. (1998). Later a more comprehensive map covering 1,235 SSR markers were developed by Somers et al. (2004). A high density marker map is beneficial for mapping studies, as it provides an adequate marker coverage to detect polymorphic markers in a given region of interest (Somers et al. 2004). A frequent revision of the available consensus map is necessary to implement successful MAS in a breeding program. This is especially crucial when dealing with a complex traits (Banks et al. 2009). At present SSR markers are still the most used marker for mapping studies in wheat, but in the near future its place may be taken over by SNPs (Anderson et al. 2007). In recent years several single feature polymorphism (SFP) based maps have been created for wheat, and would be very useful for the detection of SNPs and QTL mapping of wheat (Banks et al. 2009; Bernardo et al. 2009).

QTL Mapping of FHB resistance

A quantitative trait shows a continuous variation in the population (Kearsey 1998). QTL mapping is a highly effective way to map genes responsible for quantitative traits (Young 1996). QTL mapping has become a very important tool in modern genetics to understand gene network underlying quantitative traits. These mapping studies identify loci associated with the trait and explain the variation to which it is held responsible (Kearsey 1998). The basic concept behind QTL mapping is looking for associations of segregating molecular markers with the quantitative trait of interest (Liu 1997). When a marker is closely linked to a QTL, the marker and the QTL will not undergo independent segregation. Thereby a marker polymorphism could be associated with a phenotypic difference (Kearsey 1998). QTL mapping has lead to a better understanding of disease resistance in complex traits and provides the framework for MAS and positional cloning (Young 1996). However there are few problems related to QTL mapping such as, (i) when there are many minor QTL it's often hard to detect them and could end up detecting only the major QTL. This gives the idea that the trait is controlled by few QTL with major effects, (ii) it is often difficult to narrow down the QTL region to a map distance of less than 10cM unless the QTL has a large effect and environmental effects are greatly reduced. This makes it difficult to do positional cloning, but often this level of precision is sufficient enough for MAS and (iii) separating two QTL that are closely located would be tricky. If those QTL are interacting with each other that could lead to detection of false QTL (Kearsey 1998).

Statistical methods need to be used to predict QTL and to calculate their effects reliably (Kearsey 1998). There are many different QTL mapping methods available. In very earlier mapping studies the QTL were identified by comparing the means between homozygous and heterozygous groups within the population. If the two means are significantly different it was declared that a QTL is associated with the marker (Tanksley et al. 1982). Single locus association or single marker regression tries to find associations between the phenotypic trait data and the genotypic data at an individual marker locus. If the association is significant it was assumed that a QTL is present (Darvasi et al. 1993). However, single marker analysis cannot determine the location of a QTL, but interval mapping has the function to do so. Interval mapping allows efficient detection of strong QTL and minimizes the detection of false positives. Furthermore it accurately estimates the contribution of the QTL to the phenotypic variation and gives the location of the QTL on the chromosome (Lander and Botstein 1989). The power of

detecting QTL in interval mapping is greater than single marker analysis (Darvasi et al. 1993). But one of the errors associated with early versions of interval mapping is that two linked QTL could often be reported as one. This could include QTL that lay in the same marker interval as well as in different marker intervals, but very close to each other (Manly and Olson 1999). At present, several different interval mapping methods are used. Simple interval mapping (SIM) is based on figuring out an association between the trait and a hypothetical QTL. This is done by looking for the likelihood of having such association between any adjacent markers at multiple points and coming up with a LOD score for each point. If higher LOD scores were seen, the location is considered to house a putative QTL (Darvasi et al. 1993; Manly and Olson 1999). However SIM had some problems associated with it such as, (i) the test statistic can be affected by QTL located at other loci and (ii) it's considered less efficient because at a given time, only the information of the two markers flanking the interval are utilized and other marker information is excluded from analysis (Zeng 1993). Composite interval mapping (CIM) is an extension of simple interval mapping. CIM uses multiple regression analysis. CIM gives a more refined mapping result as the model is capable of minimizing the impact of neighboring QTL manifesting effects on the QTL of interest. The precision of the test is comparatively higher. The method also utilizes the information of few selected markers as cofactors. Thereby it is more efficient in utilizing the available marker information to avoid detection of false QTL (Zeng 1994). Multiple interval mapping (MIM) is another type of interval mapping that uses multiple marker intervals simultaneously in analysis. MIM has a high precision and a power to detect putative QTL. Despite the QTL mapping methods used, the reliability of an identified QTL need to be validated in multiple genetic backgrounds (Kao et al. 1999).

In QTL mapping the term logarithm of the odds (LOD score) is often used to report the QTL effect at a chromosome position. LOD score is the ratio between the base 10 logarithm of the likelihood of having a QTL, to the base 10 logarithm of not having a QTL at a particular point. Position with the highest LOD score is taken as a possible QTL position (Manly and Olson 1999). Often in QTL mapping a permutation is done to set a significant threshold value for likelihood ratio statistics (LRS). This allows the distinguishing of strong QTL from others. Such a random permutation results in a distortion of the relationships between trait data and genotypic data. QTL parameters and likelihood ratio statistics are generated for each permutated data set at regular intervals. Procedure is repeated 300 to 1000 times giving rise to an LRS distribution,

given the condition that no QTL are associated with the markers. This is used to determine the permutation threshold at a given confidence level (Manly and Olson 1999). Permutation results in an empirical threshold that is statistically powerful and robust enough for the given data set (Churchill and Doerge 1994). In QTL mapping it is very important to have higher marker density in the region of interest to ensure getting a marker that is tightly associated with the trait (Banks et al. 2009). Further accurate and reproducible phenotypic data has a grave importance in mapping studies (Cuthbert et al. 2006; Kolb et al. 2001). Researchers often face difficulties in getting reproducible and reliable data in FHB evaluations. Use of large population size, mixture of pathogen isolates, single spikelet inoculation, phenotyping in multiple locations and years, replication and conducting evaluations in a controlled environment can be done to minimize this variability (Cuthbert et al. 2007; Cuthbert et al. 2006; Verges et al. 2006). As mentioned above the error associated with FHB phenotypic evaluation can be greatly reduced by the use of replicates and by conducting multi location experiments (Campbell and Lipps 1998; Fuentes et al. 2005). Since repeating experiments in multiple locations is expensive, the most cost effective way to improve the accuracy is to go for more replicates (Campbell and Lipps 1998).

Environment plays a phenomenal role in the initiation and development of FHB (Bai and Shaner 1994). Since the phenotypic data is sensitive to environment, the QTL analysis will also be greatly affected by it. Under these circumstances, the same locus may express different resistance levels in different environments. QTL detected under greenhouse conditions may not be significant under field conditions due to environmental influence (Yu et al. 2008c). Therefore, even though some QTL are not showing highly significant associations in a given environment, they could play an important role in enhancing the overall performance of the cultivar in the field (Ma et al. 2006b). Therefore, for an environment sensitive trait like FHB, it is important to repeatedly evaluate the mapping population, to accurately detect QTL and to quantify the phenotypic variation explained by them (Kolb et al. 2001). Thus carrying out experiments in an appropriate environment is very important to get better phenotypic data for mapping studies (Ma et al. 2006b). Furthermore it is essential to validate the position and effect of putative QTL prior to recommending them for further use. To do so additional mapping or validation populations need to be assessed. QTL can also be validated by creating near isogenic lines (NIL) by backcrossing (Anderson et al. 2007; Pumphrey et al. 2007). A more reliable method for

validation is through QTL - NILs, where the NILs are created using lines that segregate for the QTL of interest in the current population (Pumphrey et al. 2007).

Important QTL for resistance to FHB

QTL for FHB resistance have been found in almost all wheat chromosomes (Table A.1). For Type II resistance, QTL on chromosome 3BS, 6BS and 5AS has been the most consistent (Buerstmayr et al. 2009). According to Cuthbert et al. (2006), *Fhb 1* on chromosome 3BS is thought to be the most important QTL for FHB resistance. Waldrone et al. (1999) mapped this major QTL with SM3 origin for Type II FHB resistance using a recombinant inbred population derived from a cross between SM3 and Stoa. This major QTL was validated later by several different studies (Anderson et al. 2001; Chen et al. 2006) and was successfully fine mapped to the same location (Cuthbert et al. 2006). This major resistance QTL *Fhb 1* is donated by Taiwan wheat parent to SM3 (Bai et al. 2003; Shen et al. 2003). A *Fhb 1* QTL was reported in other resistant cultivars such as in Wangshuibai (Mardi et al. 2005; Yu et al. 2008c), but this may not be the same *Fhb 1* QTL (Bai et al. 2003). Commonly used markers to track *Fhb 1* are *Xgwm 533*, *Xbarc 133*, *Xgwm493* (Cuthbert et al. 2006) and *Xumn 10* (Liu et al. 2008). Out of these marker *Xumn 10* is the best marker for MAS (Liu et al. 2008). Markers associated with *Fhb1* has explained 15% (Waldron et al. 1999), 25% and 42% (Anderson et al. 2001), 60% (Buerstmayr et al. 2002) of the phenotypic variation for disease spread. To date this major QTL in chromosome 3BS is the single most important and consistent QTL affecting FHB Type II resistance (Anderson et al. 2007). Other than *Fhb 1,* chromosome 6B QTL (*Fhb 2*) (Anderson et al. 2001; Yang et al. 2003) is also noteworthy. *Fhb 2* was mapped closer to *Xgwm644* and it was successfully fine mapped to the same location published earlier (Cuthbert et al. 2007). According to Yang et al. (2003), the QTL explained 21% of the phenotypic variation. The QTL on chromosome 5A explained 4% (Yu et al. 2008c), 11% (Buerstmayr et al. 2002), 27% (Lin et al. 2006) and 16% (Chen et al. 2006) of the phenotypic variation of disease spread. Another major QTL for FHB was also mapped on chromosome 4B (Lin et al. 2006), which was later fine mapped as *Fhb4* (Xue et al. 2010b). *Fhb3* is another important FHB resistance gene donated by alien species *Leymus racemosus* to a wheat-*Leymus* integration line (Qi et al. 2008). Low accumulation of DON was also reported in several chromosomes (Table A.1). Most frequently reported QTL were on chromosome 2D (Semagn et al. 2007; Somers et al. 2003), 3B (Chen et al.

2006; Lemmens et al. 2005; Somers et al. 2003; Yu et al. 2008c) and 5A (Chen et al. 2006; Somers et al. 2003; Yu et al. 2008c). The QTL on 3BS is reported to have a very close association with the *Fhb 1* QTL for symptom spread (Lemmens et al. 2005). *Fhb 1* QTL has explained 92% (Lemmens et al. 2005), 11% (Somers et al. 2003), 9 – 30% (Yu et al. 2008c) of the phenotypic variation in different studies.

Mapping populations

When selecting parents to create a mapping population it is important to select parents with adequate level of diversity at both genotypic and phenotypic levels (Liu 1997). Different types of mapping populations have been used in QTL mapping experiments. Most commonly used mapping populations include, F_2, Backcross populations (Buerstmayr et al. 1999), recombinant inbred lines (RIL) (Cuthbert et al. 2006; Waldron et al. 1999; Yu et al. 2008c), double haploid lines (DH) (Chen et al. 2006; Jia et al. 2005b; Yang et al. 2005b) and near isogenic lines (Pumphrey et al. 2007). In recent years many experiments have used chromosome recombinant inbred lines (CRIL) as the mapping population (Garvin et al. 2009; Grausgruber et al. 1999; Kumar et al. 2007; Ma et al. 2006b). Chromosome Recombinant Inbred lines (CRIL) are developed by substituting a chromosome of one cultivar with a corresponding chromosome from another cultivar. Substituting a chromosome from a susceptible cultivar with its analogous from a resistant cultivar has become an important tool to study individual chromosomes in isolation (Kumar et al. 2007; Ma et al. 2006b). These inter-varietal chromosome substitution lines can efficiently be used to identify QTL segregating in a particular chromosome of interest and explain much of the variation (Kumar et al. 2007). Recombinant inbred lines have been successfully used to map QTL for Type II resistance against FHB in previous studies targeting chromosome 2A (Garvin et al. 2009) and 7A in common wheat (Ma et al. 2006b) and also chromosome 7A in durum wheat (Kumar et al. 2007).

Disease inoculation and phenotypic evaluation

Under natural conditions the occurrence of FHB is unpredictable. Thereby in a mapping study, it is essential to artificially inoculate the plants for a more reliable disease evaluation (Buerstmayr et al. 2002). Disease inoculation can be done by spray inoculation or by point inoculation. Point inoculation targets FHB resistance against disease spread (Miedaner et al.

2003). Point inoculation is done by injecting the inoculum in to the glume of the center floret. This ensures a uniform inoculation among the plants. Such uniformity is essential for clear differentiation of more resistant genotypes from others for disease spread. Single spikelet inoculation reduces variation among test lines, but most importantly it targets Type II FHB resistance and simplifies the evaluation of a complex trait (Bai et al. 1999). Point inoculation also results in a high correlation between scab severity data among different generations and between different assessment methods (Bai et al. 1999). The early anthesis stage is considered to be the ideal time for scab inoculation. But since each tiller comes into anthesis on their own terms the general thumb rule is to delay the inoculation until the main culm attains anthesis (Bai and Shaner 1994). Spray inoculation is done by spraying a spore suspension on to wheat head and re-sprayed again to infect the plants that were not in anthesis at the time of first spraying. Thus it is a less laborious task than performing point inoculation. Spray Inoculation enables the detection of both resistance against FHB Type I and Type II, however the contribution of each type cannot be distinguished (Miedaner et al. 2003; Rudd et al. 2001). Grain spawn inoculation is widely used for inoculation in the field, where a large number of plants are subjected to evaluation (Rudd et al. 2001). In this method the inoculum is introduced via already colonized grains (Verges et al. 2006). These artificially inoculated wheat or corn grains are allowed to colonize prior to their distribution in the fields. In some cases FHB infected kernels itself have been used. Grain spawn is done around the boot stage and reintroduced few times in desired time intervals. In time perithecia are formed and ascospores are released around anthesis. Grain spawn inoculation method is the closest to natural situation but with an enriched inoculum level (Rudd et al. 2001).

Type I FHB resistance can be quantified by spray inoculation followed by taking counts of the infected spikelets 7 – 21 days after inoculation. Type II FHB resistance is measured in a similar way but the inoculation method used is point inoculation. Kernel damage, test weight and kernel number reduction are few parameters that can be used to measure the resistance to kernel infection. Tolerance can be quantified by comparing grain yield of infected plots to uninfected plots. DON accumulation is measured by determining the DON concentration at a given level of FHB infection (Rudd et al. 2001). Selection of the inoculation technique would depend upon several factors such as expected level of precision, population size and resource availability. Then again for routine screening of large populations a much faster, cheaper and reliable

inoculation method needs to be selected. Spray inoculation have added advantages over point inoculation as it is a fast and cheaper method with much similarity to natural inoculation in the field (Rudd et al. 2001). The disease levels observed in spray and spawn inoculation is often sufficient to distinguish between the genotypes (Fuentes et al. 2005). But when evaluating for Type II and /or Type III FHB resistance, point inoculation gives more reliable data than spray inoculation (Bai and Shaner 1996; Miedaner et al. 2003; Rudd et al. 2001). For inoculation, either a single aggressive strain or a mixture of available strains can be used. A mixture of pathogen strains are preferred because the virulence of the pathogen seems to be affected by the persisting environmental conditions (Rudd et al. 2001). FHB phenotyping is often difficult because it behaves as a quantitative trait, heavily influenced by the environment and based on several different resistance mechanisms (Bai and Shaner 1996). Controlled greenhouse conditions can be used to provide the best suited environmental conditions. Doing so enables a better separation of genotypes with different resistance levels and permits a more precise phenotypic evaluation (Bai et al. 2000).

Marker assisted selection

To develop a cultivar with resistance against FHB, the breeder need to stay in line with the objective of minimizing yield loss as well as quality deterioration (Zhou et al. 2002a). According to Mesterházy et al. (1999), most resistant genotypes correlate well with visual symptoms of FHB and furthermore breeding for high resistance against pathogen invasion will ultimately result in lower DON accumulation. The aim of a plant breeder should be to achieve a high FHB resistance level which guarantees a lower disease incidence, symptom spread and low DON accumulation (Bai et al. 2001). But breeders are often challenged when trying to develop FHB resistant cultivars. With FHB it is difficult to perform early generation selection in field, as it is unpredictable and needs to be replicated several times in order to get more accurate phenotypic data. MAS provide a way to identify the plants with desired genotypes in early generations and discard the unwanted. This enables breeders to come up with cultivars with FHB resistance in a shorter time than the conventional methods (Waldron et al. 1999). For the selection of some traits that are difficult to reliably phenotype such as FHB, MAS is a promising approach (Gupta et al. 1999). With the aid of MAS, selections can be performed at an early generation and can pyramid QTL associated with desired traits in to breeding lines (Mohan et al.

1997). Therefore a MAS scheme can be efficiently used to accelerate cultivar release (Ma et al. 2006c). It is important to note that a MAS program can complimentary an existing breeding program that is based on phenotypic evaluation, but in no way can replace it (Anderson et al. 2007). However it has enabled the breeders to target the genes of interest with higher precision (Mohan et al. 1997).

For a successful MAS program it is necessary to have markers tightly linked to the trait of interest. Further it also requires an efficient, reproducible and economical method to screen large populations (Gupta et al. 1999). For MAS to work it is a necessity to have prior knowledge about DNA markers that are closely linked to the preferred QTL (Waldron et al. 1999). Thus a proper QTL mapping programs needs to be conducted concurrently, to identify QTL and to fine map them to find markers that are closely linked to the genes. Thereby the first step would be to identify major QTL associated with FHB resistance and map them. Then the QTL position needs to be validated and the magnitude of its effect needs to be assessed. Fine mapping needs to be done in order to get a higher resolution map in the QTL region, so that closely linked markers can be identified. Such closely linked markers come very handy in MAS, as these can be used to select against any progeny that underwent recombination between the marker and the QTL (Kolb et al. 2001). They would also increase the precision of MAS (McCartney et al. 2004). But if the marker is not close enough to the gene of interest, use of MAS could trigger false positive selections (Mohan et al. 1997). However the use of flanking markers as oppose to a single marker in MAS could increase the selection precision (Ma et al. 2006c). Complex inheritance of FHB has often made it difficult to perform selection in breeding programs based on phenotype. This has prioritize the development of appropriate molecular markers to be used in MAS for FHB (Anderson et al. 2007). MAS facilitate large scale evaluation of breeding lines. However, just the assurance that a marker is tightly linked to the QTL is not enough to qualify a marker be included in a program. In order for a marker to be most effective in MAS it needs to be easily used in the selection process. This requirement makes markers like SSR, STS and SNPs fit in to the picture better. Breeders would prefer to use the same set of markers across different populations. Therefore it is a necessity to make sure that the recommended marker is polymorphic not only in the evaluated population, but also in other populations (Kolb et al. 2001). It is equally important to select markers that are linked to QTL that gives significant effects in multiple environments. The inconsistence of QTL effects across population has

become the number one concern in a MAS program. Therefore it is important to look for QTL that remain consistent across populations (Anderson et al. 2007). At all times the breeders need to avoid targeting QTL that are linked to genes that would confound phenotypic selection and also QTL that are associated with undesirable traits needs to be excluded (Kolb et al. 2001); (Lander and Botstein 1989). Anderson et al. (2004), reports a successful implementation of MAS using the *Fhb1* QTL. MAS has been successfully used to transfer QTL at *Qfh.nau-2B, Qfhs.nau-3B, Qfhi.nau-4B* and *Qfhi.nau-5A* on to susceptible cultivar Mianyang 99-323 (Xue et al. 2010a). Development of more efficient high throughput DNA extraction methods and marker platforms will enable effective use of MAS to track down these QTL in future breeding programs (Anderson et al. 2007).

Breeding strategies to develop FHB resistant cultivars

For FHB resistance, it is the additive effect that accounts for most of the variation, but even epistasis and dominance effects can have an significant effect when it comes to enhancing the resistance as a whole in some crosses (Bai et al. 2000). When resistance is mainly due to additive effects, the breeding strategy should be to pyramid resistance genes from diverse sources and remove susceptibility genes to enhance the resistance level of the cultivar (Ma et al. 2006b; Rudd et al. 2001). For the cause, it is important to target major QTL than minor QTL, as unlike major QTL, minor QTL may not be consistent due to environmental influence on them (Ma et al. 2006b). Thus it is important to select markers associated with the major QTL as they will be closely linked to a gene(s) with large effects for gene pyramiding (Kolb et al. 2001). Pyramiding different QTL in to a cultivar does not always result in the desired increase in resistance level, as epistatic interactions among the different QTL could act negatively (Jia et al. 2005a). But epistatic interactions always remain subordinate to QTL effects (Anderson et al. 2001). If a QTL with significant epistatic effect is used, the QTL could behave differently than expected in another genetic background (Anderson et al. 2007). A combination of two to three major QTL representing different resistance mechanisms can make all the difference in withstanding an FHB outbreak (Grausgruber et al. 1999). It is better to have a combination of QTL coming from different origins as it will increases the genetic diversity, while restoring resistance (Bai et al. 2003). Cultivar WSY developed by pyramiding QTL from a three way cross between SM3, Wangshuibai, and Nobeokabouzu parents is a good example for a pyramided

FHB resistance line (Shi et al. 2008). Continuous selection for desired FHB resistant alleles will favor the development of more resistant breeding populations in time (Yang et al. 2003). As for the doubts whether the selection of resistant phenotype progeny based on the genotypic data is effective or not, the study of Yang et al. (2003) provided evidence in favor of it. Transgressive segregation was reported in several FHB studies (Buerstmayr et al. 2000; Waldron et al. 1999). This transgressive segregation can be used to develop resistant cultivars, as it inherits a combination of resistance genes from the parent sources. The best examples known for such an effort is development of SM3 (Bai et al. 2000). FHB resistance QTL in SM3 has been widely used in breeding programs worldwide (McCartney et al. 2004). The diversity of USA cultivars with FHB resistance is low, thereby in order to have a better diversity it's important to use cultivars from other regions (Bai et al. 2003). Final goal of a breeding program is to acquire the highest possible resistance level against FHB (Bai and Shaner 1996). For that finding novel genes for FHB resistance is crucial (Liu and Anderson 2003a).

CHAPTER 2 - Characterization of a novel quantitative trait loci for Fusarium head blight resistance in wheat chromosome 7A

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum,* is an important wheat disease in humid and semi-humid wheat growing regions of the world. In a severe epidemic, FHB can drastically reduce grain yield and quality (Bai and Shaner 1994). During the 1990s, US wheat industry suffered a cumulative loss of $1.3 billion to FHB epidemics (Johnson et al. 1998). Such major outbreaks have been reported in several other countries including China, Canada and Europe, making FHB a global issue affecting wheat production worldwide (Parry et al. 1995). Apart from the yield losses, grain quality degradation due to mycotoxin contamination is another key concern. Mycotoxins such as deoxynivalenol (DON) produced by *Fusarium* spp. makes the grains unsuitable for human and animal consumption (Canady et al. 2001; Korosteleva et al. 2007). Given the fact that wheat is one of the most important cereal crops in the world, severe global outbreaks of FHB can fuel the food crisis worldwide.

High humidity coupled with high temperatures creates an ideal environment for severe FHB epidemics (McMullen et al. 1997). An integrative approach of cultural practices, chemical application and use of resistant cultivars is the best measure to prevent such an outbreak (Bai and Shaner 2004). However use of FHB resistant cultivars with low toxin accumulation is the most efficient and economical strategy for FHB control. Therefore over the last decade, one of the major objectives of a breeding program, has been to improve wheat cultivars with high FHB resistance (Bai and Shaner 2004). Although a wheat germplasm with complete immunity to FHB has not been identified, three types of FHB resistance have been proposed: resistance to initial penetration by the pathogen (Type I), resistance to symptom spread within a spike (Type II), (Schroeder and Christensen 1963) and low DON accumulation (Type III) in infected seeds (Miller et al. 1985). Among these, Type II and III resistance have been considered as more stable measurements of FHB resistance and used as major targets for cultivar improvement.

All three types of resistance to FHB in wheat are quantitative traits (Grausgruber et al. 1999). Over the past few decades, many major and minor quantitative trait loci (QTL) associated with FHB resistance have been identified in almost all wheat chromosomes through QTL mapping (Anderson et al. 2001; Buerstmayr et al. 2009; Lin et al. 2006; Liu and Anderson

2003a; Ma et al. 2006c; Waldron et al. 1999). *Fhb1* mapped on chromosome 3BS is a QTL with a stable major effect on FHB Type II (Anderson et al. 2007) and Type III resistance (Lemmens et al. 2005) across different genetic backgrounds. Thus it has been used in breeding programs worldwide for genetic improvement of wheat resistance to FHB. However, *Fhb1* alone is not sufficient to protect yield losses in severe epidemics. Other resistance QTL reported in wheat includes a QTL on chromosome 5A (Chen et al. 2006; Lin et al. 2006), *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al. 2007) and *Fhb4* on chromosome 4B (Lin et al. 2006; Xue et al. 2010b). Further an *Fhb3* gene was also reported in wheat- *Leymus* integration lines, donated by alien species *Leymus racemosus* (Qi et al. 2008). In addition, QTL on chromosomes 2D (Somers et al. 2003) and 5A (Chen et al. 2006) have been reported to have major contributions towards Type III resistance. However to date, only *Fhb1* showed a stable major effect on Type II or III resistance, all other QTL have either only minor effects or unstable effects in different genetic backgrounds. Thus, to significantly enhance the levels of FHB resistance in a breeding line, several such QTL need to be pyramided with *Fhb1*, which is a challenge even with marker-assisted selection (MAS). Thus additional QTL with a major effect on FHB resistance are urgently needed to improve resistance levels of breeding materials.

FHB resistant germplasm have been reported from different geographical regions such as, Asia, Europe, Africa, North and South America. Among the Asian germplasm, Chinese landraces are important source materials for mining FHB resistance QTL (Bai and Shaner 2004). Chinese cultivar Sumai 3 (SM3) is one such highly resistant germplasm that is often used in many breeding programs as a donor parent (Bai and Shaner 1996). Several QTL were identified from SM3, including *Fhb1* (Anderson et al. 2001; Waldron et al. 1999), *Fhb2* (Anderson et al. 2001; Cuthbert et al. 2007) and QTL on chromosome 5A (Buerstmayr et al. 2002). These QTL were further confirmed to be derived from one of SM3's parent Taiwan wheat (Yu et al. 2006). Transgressive segregation has been reported in the segregating population of Taiwan wheat/Funo, the cross from which SM3 was selected (Liu and Wang 1990). But a FHB resistance QTL from Funo has never been detected.

In a previous study, a Chinese Spring-Sumai 3 – 7A disomic substitution line (CS-SM3-7ADSL) has been reported to show a very high level of Type II and Type III resistance (Ma et al. 2006a). However in a successive linkage mapping study using a population of Annong 8455/CS-SM3-7ADSL, a QTL was not detected on chromosome 7A (Ma et al. 2006b). In this study we

developed a Chinese Spring/CS-SM3-7ADSL mapping population, with the objectives to characterize QTL associated with Type II and Type III FHB resistance on chromosome 7A and to identify simple sequence repeat markers (SSR) associated with the QTL for MAS.

Materials and Methods

Planting materials

A population of 191 chromosome recombinant inbred lines (CRIL) were derived from a bi-parental cross between Chinese Spring (CS) and CS-SM3-7ADSL by single seed descent. Phenotypic data of $F_{2:5}$ and $F_{6:7}$ were used for QTL discovery and $F_{6:8}$ were used for QTL confirmation. CS parent is moderately susceptible to FHB and CS-SM3-7ADSL is highly resistant (Ma et al. 2006a; Ma et al. 2006b; Zhou et al. 2002a).

Planting and disease inoculation

F_5 and F_7 CRIL were evaluated for FHB resistance in a greenhouse at Kansas State University, Manhattan, Kansas, over spring 2009 (F_5) and fall 2009 (F_7) with three replications. Selected 89 F_8 CRILs representing four genotypes were tested for FHB using four replications in spring 2010. Two parents, CS and CS-SM3-7ADSL along with SM3 were included in the FHB test as checks. About 15 seeds from each CRIL and parent checks were planted in trays (Plug flat trays, Hummert International, St. Louis, MO) containing soil (Sungrow Metro-mix 360® growing medium, Hummert International, St. Louis, MO). In F_8 population, 20 seeds per line were planted. Trays were kept in a growth chamber at 4 °C and vernalized for three weeks. Seedlings were transplanted into three (F_5 and F_7) and four (F_8) Dura pots (Hummert International, St. Louis, MO) filled with soil mix with 5 plants per pot (replicate). The pots were placed on greenhouse benches in a randomized complete block design (RCBD). Greenhouse temperature was maintained at 20 °C. Plants were fertilized with Miracle-Gro® (The Scotts Miracle-Gro Company, Marysville, OH) four times at a 2 week interval and watered as necessary.

F. graminearum inoculum was prepared using a Kansas strain GZ3639. Inoculum was cultured in a mung been liquid medium described by Bai and Shaner (1996). The spore density was evaluated by counting the spores using a hemocytometer under a light microscope. The inoculum concentration was adjusted to 100,000 conidial spores/ ml by diluting with sterilized distilled water. At anthesis, a single spikelet residing in the center of the spike was inoculated by dispersing 10 µl inoculum into the spikelet using an inoculation syringe. In each pot 4 - 6 spikes at similar developmental stage was inoculated. Inoculated plants were placed in a humid

chamber and sealed by polythene sheets to facilitate disease development. After 48 hours of incubation, the plants were moved back to the greenhouse benches at 22±5 °C with 12 h supplemental daylight.

Evaluation of FHB symptoms spread and FHB infected kernels

The rate of FHB symptom spread within a spike was evaluated on the 18th day after inoculation by counting the number of infected spikelets and total number of spikelets per inoculated spike. Any spikelet with a dark brown water-soaked spot to a completely bleached spikelet was recorded as an infected spikelet (Figure 2.1). FHB data were collected from spring and fall 2009, and spring 2010 experiments.

Figure 2.1 Comparison of (a) susceptible with (b) resistant lines from the cross CS/CS-SM3-7ADSL, showing Fusarium head blight symptoms spread within a spikelet in a susceptible and resistant genotype

Percentage of symptomatic spikelets (PSS) in an inoculated spike was calculated to measure Type II resistance (Equation 2.1). PSS average for each CRIL and parent were calculated for each season. Combined average of spring 2009 and fall 2009 were calculated to be used in QTL mapping.

Equation 2.1 Percentage of symptomatic spikelets

$$Percentage\ of\ \ symptomatic\ spikelets\,(PSS) = \frac{Infected\ spikelets}{Total\ \ spikelets\ per\ head} \times 100$$

Percentage *Fusarium* damaged kernel (FDK) was calculated as an additional measurement to quantify Type II resistance in the two 2009 experiments. To calculate percentage of FDK, all inoculated spikes from each replicate were hand threshed, and the *Fusarium* damaged seeds were visually separated from healthy seeds and counted (Figure 2.2). Extra care was taken to prevent damaged kernels from getting blown away during threshing. Average percentage of FDK per head was calculated for each CRIL and parent for each season (Equation 2.2). Combined average from the two seasons data for each CRIL and parent were calculated for QTL analysis.

Figure 2.2 Comparison of Fusarium head blight (a) infected seeds and (b) normal seeds in an inoculated plant

Equation 2.2 Percentage of *Fusarium* damaged kernels

$$Percentage\ of\ \ Fusarium\ damaged\ kernels\ (FDK\%) = \frac{FHB\ infected\ kernels}{Total\ kernels\ per\ spike} \times 100$$

Evaluation of deoxynivalenol (DON)

Seeds from the inoculated spikes of each CRIL and parent from two 2009 experiments were individually weighed using an electric balance (SCIENTECH SP150, Scientech Inc., Boulder, CO). DON concentration in infected spikes was determined at University of Minnesota, St. Paul, MN using Gas chromatography – Mass spectrometry (GC-MS) as described by Mirocha et al. (1998). Line average was calculated for each CRIL and parent for each season and combined average of each CRIL from the two seasons were calculated for QTL analysis.

DNA extraction

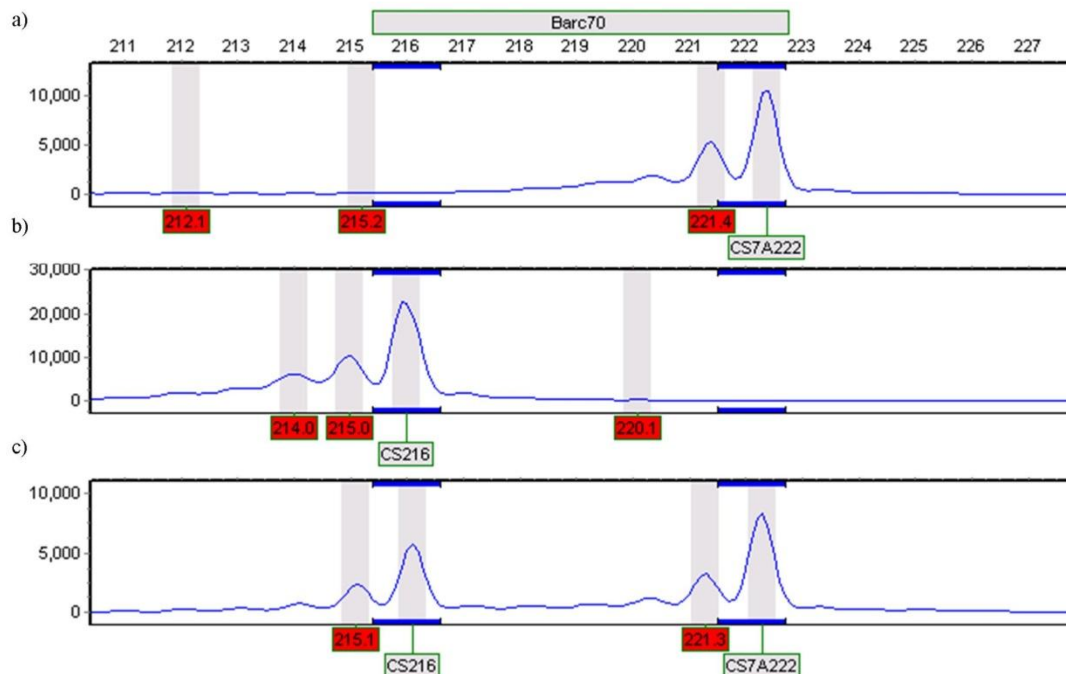
DNA was extracted from F_6 CRIL using a modified cetyltrimethylammonium bromide method (Saghai-Maroof et al. 1984). Two-weeks-old leaf tissue samples were collected in 1.1 ml strip tubes and dried in a freeze dryer (Thermo Fisher Scientific Inc., Waltham, MA) for three days at –50 °C and 150 mbar pressure. The dried tissue was ground in a Mixer Mill (Retsch Inc., Newtown, PA) to a fine powder by shaking the tubes at 1200 rpm for 6 minutes with a 3.2 mm stainless steel bead in each strip tube. DNA concentration was determined by randomly selecting samples in each DNA plate and running 5 µl of raw DNA in a 1.0% agarose gel. Electrophoresis was done at an 80 V constant voltage in a horizontal electrophoresis system (Bio-Rad Laboratories, Hercules, CA). Agarose gel was visualized under UV, using Gel Doc system (Bio-Rad Laboratories, Hercules, CA). DNA concentration was estimated by comparing the intensity of raw DNA with known concentration series of λ phage DNA and adjusted by diluting to 20 ng/µL with 5 mM Tris / Triton 100X (pH 8) solution.

Marker analysis

A genome-wide background screening was done using 84 locus-specific SSR markers representing 42 chromosome arms with at least two unlinked markers per arm, selected from GrainGenes 2.0 database (http://wheat.pw.usda.gov). The entire chromosome 7A and a small fragment of the chromosome 3BS were found to be of SM3 origin in CS-SM3-7ADSL. Therefore parents CS, CS-SM3-7ADSL and SM3 were screened with markers that were mapped on chromosome 7A and 3BS. A total of 75 SSR markers from chromosome 7A and 30 SSR and 28 sequence tagged sites (STS) from the chromosome 3BS were screened between the parents. The markers included 23 BARC, 21 GWM, 7 CFD, 6 CFA, 47 WMC (Somers et al. 2004; Song et al. 2005), and 29 STS markers (Liu and Anderson 2003b). Population screening was carried

out using 33 polymorphic SSR markers from chromosome 7A and 3 SSR and 4 STS markers from chromosome 3BS. All polymerase chain reactions (PCR) were carried out in 384-PCR plates. A PCR mix contained 14 µl of 10X ASB buffer, 2.5 mM of $MgCl_2$, 200 µM of dNTP, 100 nM of tailed forward primer, 200 nM of reverse primer, 100 nM of M13 fluorescent-dye labeled primer, 1 U of *Taq* DNA polymerase and 50 ng of template DNA. PCR amplification was done in Gene Amp® PCR System 9700 (Applied Biosystems Inc., Foster City, CA) using a touchdown program with an initial denaturing step at 95 °C for 5 min, 5 cycles of 96 °C for 1 min, 68 °C for 3 min with a reduction of 2 °C in each following cycle and 72 °C for 1 min, followed by 4 cycles of annealing temperature of 58 °C for 2 min with a reduction of 2 °C in each following cycle. The final step consisted of 40 cycles of 96 °C for 20 sec., 50 °C for 20 sec., 72 °C for 30 sec. and ended with a final extension step of 72 °C for 5 min. PCR products from 4 separated reactions labeled with different florescent dyes (FAM, VIC, NED and PET) were pooled together using 96-channels Biomek NXp liquid handling system (Beckman Coulter, Inc., Brea, CA) and analyzed using ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Data was analyzed by GeneMarker v1.75 (SoftGenetics LLC. State College, PA, USA) and CRILs were scored for the polymorphic alleles (Figure 2.3).

Figure 2.3 Output of GeneMarker v1.75 for alleles of marker *Xbarc70* from parents Chinese Spring-Sumai 3-7A substitution line (a), Chinese Spring (b), and a heterozygous progeny (c)



QTL mapping

Linkage maps were developed using JoinMap v3.0 (Van Ooijen and Voorrips 2001) using a LOD score of 3.00 and Kosambi mapping function (Kosambi 1944). QTL for PSS, FDK and DON concentration in infected spikes were analyzed by single trait multiple interval mapping feature of Qgene v4.3 (Joehanes and Nelson 2008) using data from two 2009 experiments and combined averages. An additional QTL confirmation was done using spring 2010 PSS data. A threshold value was set at $p < 0.05$ to claim significant QTL by performing 1000 permutation. Multiple-trait-multiple-interval mapping feature of Qgene was used to identify pleiotropy effects between the traits. PROC REG function of SAS v9.1 (SAS Institute Inc. Cary, NC) was used to calculate R^2 value for QTL effect and to determine the interactions between QTL.

Statistical analysis

The frequency distribution histograms of PSS, FDK and DON concentration in infected spikes were drawn using combined averages of each CRIL in the population. Since PSS was

normally distributed, it was directly used in QTL analysis. However, FDK and DON concentration in infected spikes were normalized to obtain a normal distribution by a common logarithmic transformation (base 10). PROC CORR function of SAS v9.1 (SAS Institute Inc. Cary, NC) was used to calculate the correlation among PSS, FDK and DON concentration in infected spikes. Using PSS, FDK and DON concentration in infected spikes from both 2009 experiments, an ANOVA was done with PROC GLM function of SAS v9.1 (SAS Institute Inc. Cary, NC) for an RCBD design to determine the significance of environmental effect, genotypic variation, genotype × environment interaction and variation between replicates. Because PSS data from spring 2010 were derived from a smaller population of selected genotypes, they were not used in this analysis. Broad sense heritability (H^2) was calculated for trait PSS based on the ANOVA results (Equation 2.3). where σ^2_G= genotypic variance, σ^2_ε = residual error variance, σ^2_{GE} = genotype x environment variance, r = number of replicates (pots) and e = number of experiments (seasons). Broad sense heritability was not calculated for FDK and DON concentration in infected spikes due to unbalanced replication.

Equation 2.3 Broad sense heritability (H^2)

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + \left(\frac{\sigma^2_{GE}}{e}\right) + \left(\frac{\sigma^2_\varepsilon}{re}\right)}$$ (Kumar et al. 2007)

Trait averages were calculated for genotypes AABB, AAbb, aaBB and aabb with AB alleles from SM3 and ab alleles from CS for 7AC and 3BS loci, respectively. The percentage disease/ toxin reduction due to substitution of CS alleles (a/b) by SM3 alleles (A/B) were calculated using equation 2.4.

Equation 2.4 Disease / toxin reduction estimate

$$Disease\ /\ toxin\ reduction\,\% = \frac{Average\ of\ \ desired\ genotype - Average\ of\ \ aabb}{Average\ of\ \ aabb} \times 100$$

Validation of QTL in a diverse germplasm collection

To validate the QTL identified in the mapping study and to identify polymorphic levels of markers linked to the new QTL from chromosome 7AC, a diverse collection of 400 wheat accessions representing USA, China, Japan, Korea, Austria, Argentina, Brazil, Italy, France, Ukraine, Chili and Russia (Table B.1) were genotyped using flanking markers *Xwmc17* and *Xwmc9* from chromosome 7AC QTL. The parents of SM3, Funo and Taiwan wheat were included in the study to trace the origin of 7AC QTL. Cultivar Annong 8455 was included to check for a possible cause that prevented the 7AC QTL from getting detected in the study of Ma et al. (2006b). Allele frequency of accessions with 7AC QTL was determined by equation 2.5:

Equation 2.5 Allele frequency of 7A quantitative locus

$$Allele\ frequency of\ 7A\ QTL = \frac{Accessions\ with\ 7A\ QTL}{Total\ accessions\ evaluated}$$

Among the 400 accessions, 339 were evaluated for PSS under greenhouse conditions. PSS data of the 339 accessions were used to calculate the average disease reduction contributed by the QTL at chromosomes 3BS, 7AC and their combination.

Results

FHB symptom spread and DON accumulation of CRIL population

The frequency distribution of PSS, FDK and DON concentration showed a continuous variation in the CRIL population (Figure 2.4). Mean for PSS, FDK and DON concentration in infected spikes of resistant parent CS-SM3-7ADSL were 13%, 5% and 1.3 ppm (Table 2.1) respectively, whereas CS parent had 60% of PSS, 32% of FDK and 11.1 ppm DON concentration in infected kernels. Therefore the three measurements of FHB resistance used in this study PSS, FDK and DON concentration in infected spikes were higher in CS-SM3-7ADSL parent than in CS parent.

The correlation for PSS of CRILs was significant between experiments (r = 0.62, $P < 0.01$). A significant ($P < 0.01$) correlation was observed between all three traits, where correlation of PSS with FDK and DON concentration in infected spikes was 0.84 and 0.83 respectively. FDK and DON concentration in infected spikes had a significant correlation ($P < 0.01$) of 0.91. The correlations between experiments were 0.61 for FDK and 0.63 for DON concentration in infected kernels. ANOVA indicated a significant ($P < 0.01$) genotypic effect, environment and genotype × environment effect for trait PSS (Table 2.2) from all 2009 experiments. The mean PSS in spring 2009 was significantly higher than that of fall 2009. Broad sense heritability for PSS was 0.71 across the seasons.

Genome-wide background check

The genome-wide background check using evenly distributed SSR markers across genomes confirmed that CS-SM3-7ADSL carried SM3 alleles in a small fragment (2 cM) in the short arm of chromosome 3B and in the entire chromosome 7A (Figure 2.5). All other chromosome regions were of CS origin. The linkage map of chromosome 7A spanned over a genetic distance of 181.7 cM and had a marker density of 5.5 cM per marker (Figure 2.5).

QTL mapping

Two major QTL were detected with significant effects on PSS ($P < 0.05$) in short arm of chromosome 3B (3BS) and in chromosome 7A (7AC), close proximity to centromere (Figure 2.6a). The QTL on 3BS was most likely the same QTL as previously reported *Fhb1* because the

closest marker for the QTL was *Xumn10*. The flanking markers for QTL 7AC were *Xwmc17* and *Xwmc9*. The QTL on 3BS and 7AC were consistently detected across three experiments. The variation explained by individual QTL (R^2) varied from 17% (QTL on 7AC) to 35% (QTL on 3BS). However both QTL in a combination explained up to 56% of the total phenotypic variation of Type II resistance (Table 2.3). In the confirmation study using selective genotyping method with a smaller population of 89 CRIL, the *Fhb1* was mapped at the same chromosomal position. However the 7AC QTL was mapped between *Xwmc596 – Xwmc65*, about 4 cM shift towards the long arm (Figure 2.7).

For FDK, *Fhb1* and 7AC QTL were both significant ($P < 0.05$) (Table 2.3). *Fhb1* was mapped to the same position as that of PSS. As for FDK, the position of 7AC QTL was slightly shifted (Figure 2.6b) in spring 2009 experiment, but the closest marker for FDK QTL remained to be *Xwmc17* across experiments. Each QTL explained 14% to 21% of the variation and together they accounted for 36% of the FDK variation (Table 2.3). The same set of QTL was significant ($P < 0.05$) for Type III resistance (Figure 2.6c) and explained 16% to 24% variation individually and 41% variation together (Table 2.3). A significant pleiotropy effect ($P < 0.05$) was detected between the three traits, PSS, FDK and DON concentration in infected spikes (Figure 2.8). The pleiotropy of *Fhb1* was mapped at *Xumn10* and the pleiotropy effect of the 7AC QTL was mapped between *Xwmc17* and *Xwmc9* (Table 2.3).

The alleles for flanking markers *Xbarc174*, *Xwmc17,* and *Xwmc9* amplified in CS-SM3-7ADSL parent were different from CS (Figure 2.9). But the amplified alleles of CS-SM3-7ADSL parent were similar to SM3 (data not shown). Two independent Chinese Spring –Sumai3 – 3B substitution lines (CS-SM3-3BDSL10 and CS-SM3-3BDSL31) with SM3 in chromosome 3B and with a CS background had the same haplotype as with CS parent for the three markers in chromosome 7A. This result confirms that 7AC QTL originated from SM3 and not from CS. In the CS-SM3-7ADSL parent the *Xumn10* marker for *Fhb1* carried alleles with SM3 origin (Table 2.4). CS-SM3-3BDSL10 and CS-SM3-3BDSL31 lines amplified alleles to similar SM3 at *Xumn10*. This is an additional evidence to conclude that CS-SM3-7ADSL parent has SM3 fragment at chromosome 3BS QTL region.

QTL effect on FHB / DON reduction

In the population, individuals with SM3 allele at the two QTL regions showed lower PSS, FDK and lower DON concentration in infected spikes compared to individuals with CS alleles (Table 2.5). Replacement of CS alleles by SM3 alleles led to a significant reduction in PSS, FDK and DON concentration in infected spikes (Table 2.5). Reduction in PSS and FDK was higher in the lines having only *Fhb1* than the lines with 7AC QTL alone. However for lower DON concentration in infected kernels, contribution of 7AC QTL alone was slightly higher than that of *Fhb1*. Chromosome 3BS and 7AC QTL together reduced PSS by 66%, FDK by 55% and DON concentration in infected spikes by 84% (Table 2.5).

Allele diversity of markers linked to7AC QTL

SM3 is a transgressive segregant with the best FHB resistance, selected from a cross of Taiwan wheat / Funo. Fingerprinting of both parents of SM3 with the markers that flanked 7AC QTL revealed the same haplotype between SM3 and Funo (Table 2.6), but a different haplotype between SM3 and Taiwan wheat (data not shown). The result suggested that the QTL on 7AC was derived from Funo, not from Taiwan wheat. To further survey the polymorphism of the 7AC markers in a diverse germplasm collection, 400 wheat accessions mainly collected from China, Japan and USA were evaluated with these markers (Table B.1). The result identified target alleles in 12% of total accessions and out of the accessions with 7AC QTL 76% were from China. Most of these Chinese accessions with the target alleles have Funo ancestry in its pedigree (Table 2.6) and had various levels of resistance to FHB (data not shown). This further confirmed that QTL 7AC was contributed by Funo and not by Taiwan wheat. Comparison of PSS between 339 accessions with FHB data revealed that genotypes with *Fhb1* or 7AC QTL showed an FHB symptom spread reduction of 41% and 20%, respectively. However, when both QTL were together, reduction in symptom spread could reach to 49% (Table 2.7).

Discussion

Evaluation of FHB resistance in the mapping populations

FHB is a complex disease and its occurrence and development is often heavily influenced by the environment (Jia et al. 2005b). Phenotypic data is a crucial factor that affect accurate determination of QTL effect and location (Kolb et al. 2001). Thus the quality of the phenotypic

data can be improved by evaluating the disease in a controlled greenhouse environment with multiple replications (Bai et al. 1999). Under field conditions an experiment can be done over multiple seasons/locations to minimize environmental variation. Further a large size population can be used to improve the precision of mapping work (Cuthbert et al. 2006; Kolb et al. 2001). In the present study, a population of 191 individuals was evaluated in three experiments over two years in a greenhouse at Kansas State University, Manhattan, Kansas. The mapping population size is relatively large in comparison to some other studies (Lemmens et al. 2005; Ma et al. 2006b). PSS and FDK were separately measured to reflect FHB symptom spread within a spikelet and DON concentration in infected spikes was quantified to reflect DON accumulation across experiments. In this study, a spikelet in the center of a spike was inoculated in each line. Point inoculation of this nature minimizes differences in disease incidence between lines and targets symptom spread within the spike. As this provides uniform inoculation among the plants, differentiation of genotypes based on the level of symptom spread within a spike is less complex (Bai et al. 1999). In addition, point inoculation in a greenhouse targets only Type II resistance and this avoids confounding effects generated due to difference in initial infection among genotypes as in field infection (Bai et al. 1999). Therefore FHB data obtained in this study can more precisely reproduce the actual Type II resistance of the evaluated genotypes. FHB disease scoring need to be precisely timed, so that plants show the highest level of phenotypic differences among resistant and susceptible genotypes at the time of scoring (Bai et al. 1999). In the current study scoring for symptomatic spikelets were done as early as on the 18th day after inoculation, when susceptible control reached 95% PSS. Some studies have reported such early scoring (Buerstmayr et al. 2002), whereas some have scored as late as 26 days after inoculation (Buerstmayr et al. 2003; Mardi et al. 2005).

FDK was quantified by categorizing the seeds into damaged and normal seed lots by visual evaluation of damage level. Similar quantification of FDK has been reported in other studies (Bai et al. 2001; Jones and Mirocha 1999; Verges et al. 2006). Bai et al. (2001) and Verges et al. (2006), reports quantification of FDK by visual inspection and selecting the discolored seeds out of 200 randomly selected seeds. In the current study to achieve a better estimate, all seeds from inoculated spikes were included in the sample as oppose to a random subset. Thus scoring all seeds avoided sampling error and improved data quality in this study. To quantify DON concentration in infected spikes GC-MS was used. GC-MS gives an accurate

measurement of DON concentration (Mirocha et al. 1998). Careful harvesting of inoculated spikes and manual threshing minimizes highly infected and shriveled seeds from getting blown away (Ma et al. 2006a; Yu et al. 2008b). Therefore in this study spikes were only harvested from inoculated spikes to prevent dilution of toxin levels and hand threshed to minimize the loss of infected seeds.

Parent CS-SM3-7ADSL was highly resistant and had a low PSS and DON concentration that was consistent with previous reports (Ma et al. 2006b; Zhou et al. 2002a). CS showed moderate resistance to moderate susceptibility, which is in accordance to previous reports (Ma et al. 2006a; Yu et al. 2006; Zhou et al. 2002a). Even though the PSS reported in this study is closer to the previous reports, DON concentration of infected kernels is lower than other reports. Zhou et al. (2002a) reported 17.6 ppm DON concentration in a greenhouse study using enzyme-linked immunosorbent assay (ELISA) for CS. However, Ma et al. (2006a) reported up to 35 ppm for CS cultivar with ELISA, in a greenhouse study. Unlike PSS which is reported as a percentage value, DON concentration tends to vary between experiments due to number of reasons including FHB evaluation conditions (greenhouse or field), disease pressure, sampling technique and methods for DON analysis. Therefore DON concentration between studies could change significantly even for the same cultivar among experiments.

The continuous distribution of all three traits, PSS, FDK and DON concentration, in the mapping population derived from CS/CS-SM3-7ADSL (Figure 2.4) supported previous reports that FHB resistance showed quantitative inheritance (Ma et al. 2006c; Yang et al. 2003). A higher broad sense heritability was reported for PSS in the current study ($H^2 = 0.71$). Such high broad sense heritability was also reported in several previous reports for PSS (Lin et al. 2006; Ma et al. 2006b; Ma et al. 2006c; Zhou et al. 2004). High heritability indicates consistency and repeatability of trait data (Bai et al. 1999). The result indicates that PSS is a highly inheritable trait, when evaluated using single point inoculation under greenhouse conditions. Therefore PSS can be effectively used for screening resistant lines in a breeding program.

DON concentration in infected kernels is an expensive trait to measure. The procedure is complicated and labor intensive as it involves hand threshing, weighing, grinding and detection by GC-MS. Further any losses of infected kernels during the procedure will significantly affect the DON concentration. Therefore, DON measurement is not feasible for routine breeding selection. Under greenhouse conditions, the visual FHB symptoms start spreading to

uninoculated spikelets in susceptible genotype in 4-7 days after inoculation and can be blighted within about 7-10 days after inoculation (Bai and Shaner 1996). Therefore by the time an inoculated spike gets bleached, it may still be in early stages of seed development. Such seeds may be too small to be collected and may get blown away easily during threshing due to light seed weight. Furthermore under such a condition it may be difficult to distinguish scabby seeds from uninfected shriveled seeds. These factors may significantly increase the variation of FDK across experiments. However given that the above constrains can be minimized, the higher correlations seen between FDK with PSS (r = 0.84, $P < 0.01$) and DON concentration (r = 0.91, $P < 0.01$) makes FDK a suitable and reliable alternative for quantifying FHB Type II resistance. Such high correlations have been observed by several previous studies (Bai et al. 2001; Verges et al. 2006). However the correlations reported in this study tend to be slightly higher than the previous reports. This could be a result of using the entire seed lot for FDK evaluation as oppose to the commonly practiced quantification based on 200 randomly selected seeds. Further the higher correlation of FDK and DON observed in this study agrees with the results of Verges et al. (2006) that indicate a greater correlation between FDK and DON (r=0.91, $P<0.01$) than DON with severity measurement PSS (r = 0.83, $P<0.01$). This could be due to the fact that FHB could cause some sterile and seedless spikelets, especially in terminal part of a spike. These spikelets were counted in PSS scoring, but not in FDK calculation (Zhou et al. 2002a). A higher correlation between the two traits FDK and DON might be due to fact that the same sets of seeds were used for both measurements. Thus FDK seems to be a better estimate of DON concentration than PSS.

Relationship between type II and type III FHB resistance

PSS is a reliable parameter to screen Type II FHB resistance in a large scale experiment (Bai and Shaner 1994). However DON concentration in infected spikes would be a critically important trait, as it impacts quality of wheat products (Verges et al. 2006), The association between PSS and DON concentration is still highly debatable. In this study a significantly high correlation (r = 0.83, $P < 0.01$) was observed between PSS and DON concentration in infected kernels, indicating that PSS is a reliable alternative measurement to predict DON concentration beforehand. Such high correlations were earlier observed in several studies (Bai et al. 2001; Lemmens et al. 2005; Yu et al. 2008b). However some other studies suggested a low correlation

between PSS and DON concentration in infected spikes (Ma et al. 2006c; Mesterházy et al. 1999). The discrepancy among reports can be caused by overestimation of PSS due to counting wilted terminal spikelets that suffered water and/or nutrient deficiency as infected spikelets (Argyris et al. 2005; Bai and Shaner 1996) or it could be due to underestimating DON content as a result of mishandling. These factors might directly or indirectly meddle with trait correlations. Thus accuracy in measuring DON accumulation in a cultivar can be affected by plant growth stage when infection occurs, means used for threshing and method used for DON measurement. However in the current study, PSS showed high correlation with DON content in RIL population and can be used as a reliable measurement to predict DON accumulation in kernels in breeding programs, which agrees with the previous study of Bai et al. (2001).

Novel quantitative trait loci in chromosome 7A

In this study two major FHB resistances QTL were mapped on chromosome 3BS and 7AC in CS/ CS-SM3-7ADSL derived population for Type II and Type III resistance (Figure 2.6). These QTL were consistent across all experiments. Zhou et al. (2002a) reported a very high level of Type II and Type III resistance in CS-SM3-7ADSL compared to original CS. This study confirmed the previous report and further identified that the high level of resistance in CS-SM3-7ADSL was due to CS-SM3-7ADSL carrying two major QTL from SM3 on chromosomes 7AC and chromosome 3BS. The QTL on chromosome 7AC was not mapped in the previous study with Annong 8455 / CS-SM3-7ADSL RILs (Ma et al., 2006b). This could be due to lack of marker polymorphism between Annong 8455 and CS-SM3-7ADSL, which was confirmed in this study through a haplotype comparison using the flanking markers of 7AC QTL (Table 2.6). The novel QTL near the centromere of chromosome 7A was mapped with a major effect on both Type II and Type III FHB resistance. So far four FHB resistance QTL have been designated including *Fhb1* on chromosome 3BS from SM3 (Cuthbert et al. 2006), *Fhb2* on chromosome 6BS from SM3 (Cuthbert et al. 2007), *Fhb3* on chromosome 7Lr1 from a wheat-*Leymus racemosus* translocation line (Qi et al. 2008) and *Fhb4* on chromosome 4B from Wangshuibai (Xue et al. 2010b). Here we designate the novel QTL on 7AC as *Fhb5*. *Fhb5* was flanked by *Xbarc174* and *Xwmc*9 and explained up to 22% phenotypic variation of PSS, 18% of FDK and 24% of DON accumulation in infected kernels. *Xwmc17* was the closest linked marker to *Fhb5*. In this study *Fhb5* QTL for both PSS and FDK were aligned to the same position. Thus both PSS

and FDK are good estimate of Type II resistance. The overlapped QTL detected for the two traits provided additional evidence for the authenticity of the *Fhb5* QTL.

Several QTL have been reported on chromosome 7A previously (Jia et al. 2005b; Mardi et al. 2006; Semagn et al. 2007; Yu et al. 2008c; Zhou et al. 2004). However, chromosome locations of these reported QTL were different from *Fhb5* (Table A.2). A QTL on short arm of chromosome 7A was mapped at marker interval *Xe77m47_22 – Xgwm233* from Frontana (Mardi et al. 2006). This 7AS QTL were positioned distal to *Fhb5* and thereby is different QTL. Semagn et al. (2007) mapped a FHB severity QTL from NK93604 (WW//M26/'Runar'/3/Runar//'Møystad'/'Els') at the marker interval *Xgwm276* and *XDuPw226.* A QTL for FHB severity was also mapped proximal to marker *Xgwm282* (Jia et al. 2005b) in Wangshuibai. Zhou et al. (2004) and Yu et al. (2008c) also identified a QTL proximal to centromere of chromosome 7A for FHB Type II resistance in Wangshuibai. This QTL was tightly linked to *Xwms1083*, which is closer to marker *Xgwm276* in our linkage map (Figure 2.5). This QTL might be the same as the one reported previously (Jia et al. 2005b, Semagn et al., 2007), but different from the one reported in this study because it is about 30 cM away from *Fhb*5. In addition, *Fhb*5 showed a major effect on Type II resistance and previously reported QTL had minor effects for Type II resistance. The alleles of flanking markers for *Fhb5* were different between Wangshuibai and SM3. Therefore we believe that *Fhb5* is a different locus from that of previously reported on chromosome 7A and has a larger contribution towards FHB resistance. The findings of this study further emphasis the importance of wheat chromosome 7A to FHB resistance.

Relationship between Fhb1 and Fhb5

Based on the closely linked marker *Xumn10,* QTL on chromosome 3BS is most likely the same QTL as *Fhb1* (Anderson et al. 2001; Cuthbert et al. 2006). *Fhb1* is the single most important QTL mapped so far for both Type II and Type III FHB resistance (Anderson et al. 2007; Lemmens et al. 2005). The contribution of *Fhb1* in this study for Type II FHB resistance is greater than that of *Fhb5*. Thereby *Fhb1* remains to be the highest contributor to FHB Type II resistance so far. In this study the variation explained by the *Fhb5* is greater than that of *Fhb1* for lower DON accumulation in infected spikes across the experiment (Table 2.3), which disagrees with Lemmens et al. (2005), where *Fhb1* explained almost all phenotypic variation for DON

accumulation. The differences in R^2 reported from different studies could be due to differences in populations, test conditions, phenotypic evaluation method and interaction of QTL (Yang et al. 2003). Even though marker *Xwmc17* remained to be the closest linked marker for *Fhb5* throughout the experiment (Figure 2.6), a 1 cM shift in the QTL position was observed from one season to another for FDK and DON concentration in infected spikes as compared to the PSS QTL. This minor shift could be due to sampling errors. A 4 cM shift in QTL position was observed in PSS QTL in the confirmation experiment of spring 2010, which could be due to the smaller population size used in the confirmation experiment (Figure 2.7).

Multiple regression analysis on PSS, FDK and DON concentration of infected kernels in CRILs did not detect any significant ($P< 0.01$) interaction between *Fhb1* and *Fhb5* in the mapping experiments, suggesting that the additive effect contributed mostly to FHB resistance. This result agrees with several previous reports that concluded additive effects to be the main effect for FHB resistance (Bai et al. 2000; Jia et al. 2005b). In this study *Fhb1* and *Fhb5* together explained 56% of PSS (data not shown), 36% of FDK and 41% of DON concentration in infected spikes (Table 2.3). The unexplained variation of phenotype could be due to environmental effects and QTL that have not been detected in this study. A QTL could go unnoticed if it has minor effects, poor marker density around it or lack of marker polymorphism between the two parents (Bai et al. 1999). Presence of *Fhb5* provided an additional 34% reduction in PSS besides the 41% reduction provided by *Fhb1* and together reduces PSS by 66% (Table 2.5). A similar trend was observed among the 339 accessions in the diversity study (Table 2.7). In terms of reducing DON concentration in infected spikes *Fhb5* alone contributed to 61% reduction, whereas *Fhb1* contributed to 54% reduction and together an 84% reduction in DON concentration in infected spikes (Table 2.5). *Fhb1* and *Fhb5* QTL show additive effects and can be effectively pyramided on to a cultivar to build up a high level of resistance to FHB (Table 2.5). In this study, the QTL for Type II and Type III resistance was mapped to the same locations in both chromosome 3BS and chromosome 7AC (Table 2.3). This agrees with previous studies that reported similar overlapping that predicted a possible tight linkage between the QTL or an existence of pleiotropy (Lemmens et al. 2005; Ma et al. 2006c; Semagn et al. 2007). Results of this study, agree with previous reports (Figure 2.8) and suggests a possibility that the traits are controlled by the same gene(s), which disagrees with Somers et al. (2003). Therefore tight linkage detected in this study for Type II and Type III resistance suggested that selecting both

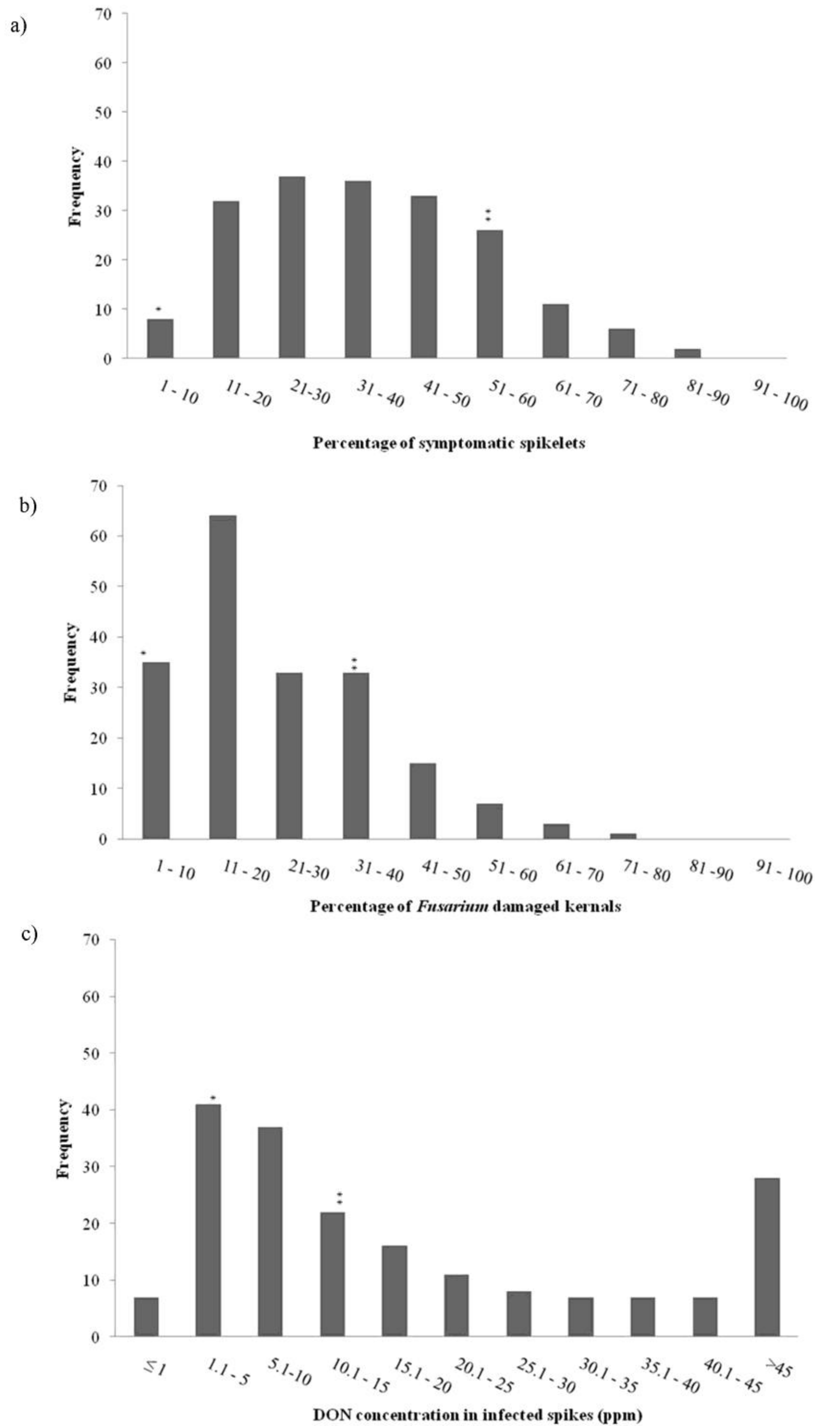
Fhb1 and *Fhb5* QTLs for Type II resistance can significantly enhance Type III resistance when SM3 is used as the FHB resistance source.

Origin of chromosome 7A QTL

Many Chinese cultivars are known to have a high level of Type II and Type III resistance and most of these cultivars inherit these two types of resistance from SM3 or its derivatives (Bai et al. 2001). *Fhb1* was identified from SM3 (Waldron et al. 1999) and was donated by Taiwan wheat (Bai et al. 2003; Yu et al. 2006). The result from the current study confirmed the finding. Furthermore it was found that *Fhb5* QTL also originated from SM3, but was contributed by Funo, the other parent of SM3. Funo is an old cultivar from Italy, and was widely used as a popular breeding parent in Chinese breeding programs in 1960s. To date the transgressive segregant contributed by Funo has been a mystery. This important finding solves the mystery stating that a resistance QTL from Funo contributed to transgressive segregation in Funo/Taiwan wheat population (Bai and Shaner, 2004). An extensive survey of Chinese and US germplasm using closely linked marker to *Fhb5* confirmed that *Fhb5* was present in most Chinese accessions with Funo in their lineage (Table 2.6). As expected, these alleles were rarely observed in US wheat germplasm. This high level of polymorphism between Funo alleles and US wheat lines for *Fhb5* provides a good opportunity to integrate *Fhb5* into US breeding lines using MAS. To date *Fhb1* is the only QTL with a major effect that has been successfully used in breeding programs with MAS (Somers et al. 2003). Given the additive effects between *Fhb1* and *Fhb5*, they can be pyramided to enhance the resistance levels of US wheat accessions.

In conclusion, the new QTL *Fhb5* on the chromosome 7AC is an important QTL with major effects on both Type II and III FHB resistance. By pyramiding the *Fhb5* and *Fhb1*, cultivars through MAS, a high level of Type II and Type III resistance can be achieved. However further studies are needed to validate the *Fhb5* QTL in different genetic backgrounds and fine map the *Fhb5* QTL to understand the gene network underlying FHB resistance.

Figure 2.4 Frequency distribution of recombinant inbred lines of a CS/CS-SM3-7ADSL mapping population for (a) combined average of percentage of symptomatic spikelet, (b) combined average of percentage of *Fusarium* infected kernel and (c) combined average of deoxynivalenol (DON) concentration in ppm



* * Chinese Spring parent and * Chinese Spring-Sumai 3 -7A disomic substitution line

Figure 2.5 Linkage map of (a) short arm of chromosome 3B and (b) chromosome 7A

Relative marker position in centimorgan (cM) distance is shown to the right and the maker name shown to the left in each linkage map

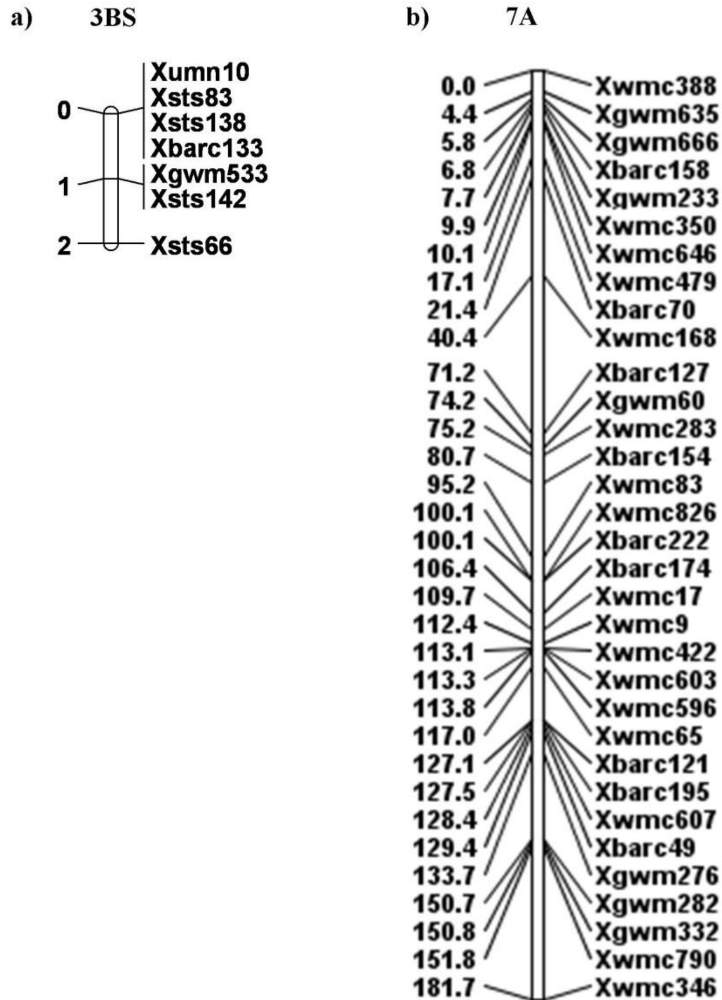


Figure 2.6 Single trait multiple interval mapping (SMIM) of quantitative trait loci associated with resistance to Fusarium head blight symptom spread and lower DON accumulation using (a) percentage of symptomatic spikelets (PSS %), (b) percentage of *Fusarium* damaged kernels (FDK %) and (c) deoxynivalenol (DON) concentration in ppm in CS/CS-SM3-7ADSL mapping population on chromosome 7A

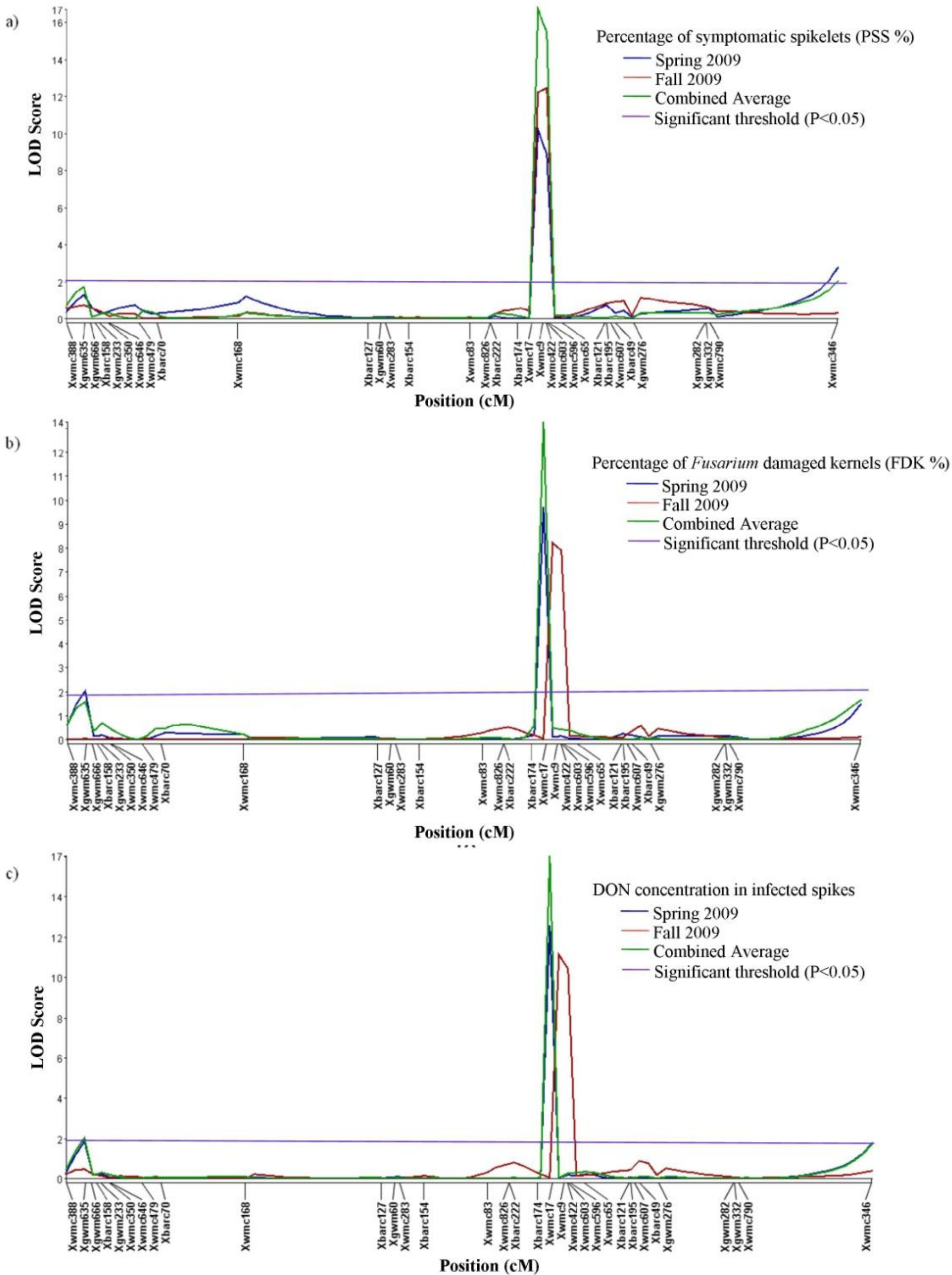


Figure 2.7 Single trait multiple interval mapping (SMIM) of 7AC quantitative trait loci for percentage of symptomatic spikelet in CS/CS-SM3-7ADSL confirmation population

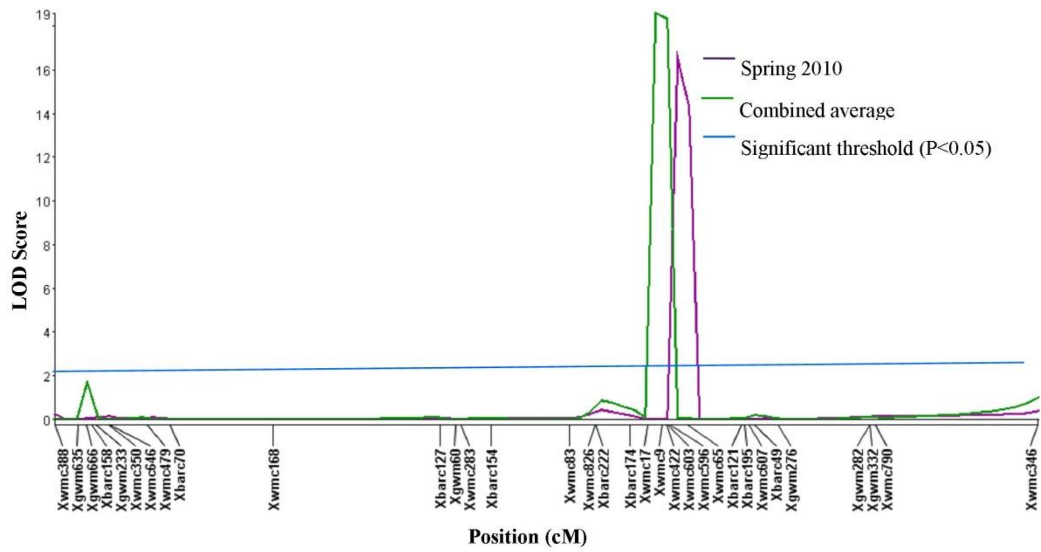


Figure 2.8 Pleiotropy effects between percentage of symptomatic spikelets, percentage of *Fusarium* damaged kernels and deoxynivalenol concentration in ppm in CS/CS-SM3-7ADSL mapping population using multiple trait multiple interval mapping (MMIM) on chromosome 7A

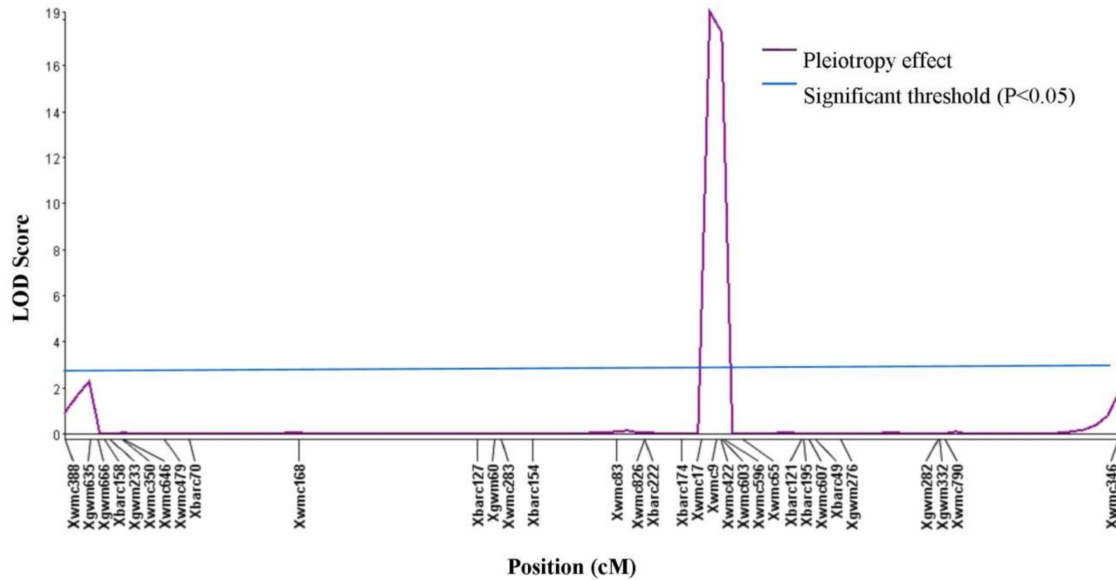


Figure 2.9 Output of GeneMarker v1.75 for alleles of marker (a) *Xbarc174* , (b) *Xwmc17* and (c) *Xwmc9* for parents Chinese Spring-Sumai 3-7A substitution line (CS7A) and Chinese Spring (CS)

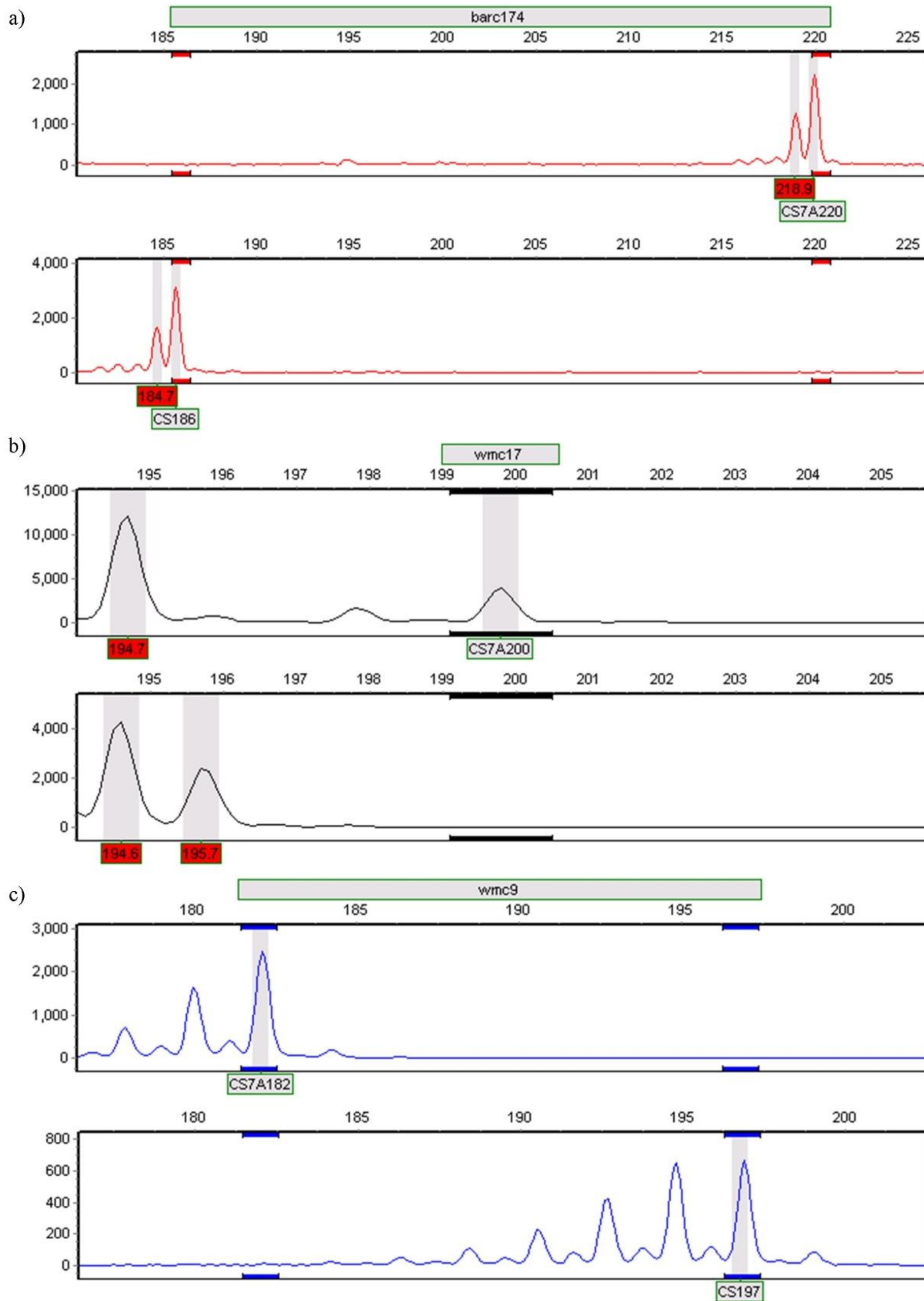


Table 2.1 Mean, range and broad sense heritability (H^2) of recombinant inbred lines (RIL) and parents based on combined line averages of percentage of symptomatic spikelets (PSS), percentage of *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) concentration in ppm

Trait	Parents			RIL			
	CS-SM3-7ADSL[a]	CS[b]	SM3[c]	Minimum	Maximum	Mean	H^2
PSS	13	60	7	5	84	36.5	0.71
FDK	5	32	1	1	73	23.9	-
DON	1.3	11.1	0.4	0.17	191.8	22.3	-

[a] Chinese Spring-Sumai 3-7A disomic substitution line; [b] Chinese Spring; [c] Sumai 3

Table 2.2 ANOVA table for CS/CS-SM3-7ADSL mapping population in spring 2009 and fall 2009 for percentage of symptomatic spikelets

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Environment	1	19689.91457	19689.91457	147.77	0.0067
Replication	2	712.33035	356.16518	2.67	0.2723
Environment × Replication	2	266.4876	133.2438		
Genotype	190	353583.3838	1860.9652	5.15	<.0001
Genotype × Environment	190	101776.1104	535.6637	1.48	0.0002
Error	749	270677.8477	361.3856		
Corrected Total	1134	743259.3833			

Table 2.3 Summery of multiple interval mapping (MIM) analysis of quantitative trait loci for Fusarium head blight resistance in a CS/CS-SM3-7ADSL mapping population using percentage of symptomatic spikelet (PSS), percentage of *Fusarium* infected kernel (FDK) and deoxynivalenol (DON) concentration of infected kernels in ppm

Trait	Chromosome	Marker interval	Spring 2009 (F_5)		Fall 2009 (F_7)		Combined average	
			LOD	R^2	LOD	R^2	LOD	R^2
PSS	3BS	*umn10*	18.0	0.28	17.5	0.28	24.9	0.35
	7AC	*wmc17 – wmc9*	0.2	0.17	12.2	0.18	16.7	0.22
	Combined		-	0.44	-	0.46	-	0.56
FDK	3BS	*umn10*	9.9	0.16	12.4	0.21	15.4	0.19
	7AC	*barc174 – wmc9*	9.7	0.17	8.2	0.14	13.9	0.18
	Combined		-	0.33	-	0.34	-	0.36
DON	3BS	*umn10*	10.0	0.16	8.6	0.16	12.3	0.18
	7AC	*barc174 - wmc9*	12.6	0.20	11.1	0.20	16.0	0.24
	Combined		-	0.35	-	0.35	-	0.41
Pleiotropy	3BS	*umn10*	18.8	-	18.6	-	26.6	-
	7AC	*wmc17 – wmc9*	11.6	-	7.1	-	18.3	-

Table 2.4 Validation of Sumai 3 (SM3) as the donor of 3BS and 7AC QTL in Chinese Spring-Sumai 3-7A disomic substitution line (CS-SM3-7ADSL) parent

Line	3B chromosome (*Xumn10* and *Xsts83*)		7A chromosome (*Xbarc174*, *Xwmc17* and *Xwmc9*)	
	Chinese Spring	SM3	Chinese Spring	SM3
CS-SM3-3BDSL31	-	X	X	-
CS-SM3-3BDSL10	-	X	X	-
CS-SM3-7ASDSL[a]	-	X	-	X

[a] CS-SM3-7ASDSL parent

Table 2.5 Average and disease /toxin reduction in percentage of symptomatic spikelets (PSS), percentage of *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) concentration with the replacement of Chinese Spring (CS) alleles by Sumai 3 (SM3) at 3BS and 7AC quantitative trait loci in CS/CS-SM3-7ADSL mapping population

Genotype[a]	Average PSS %	Average FDK %	Average DON concentration
aabb (No 3BS/7AC)	56	38	44.2
aaBB (3BS only)	33 (41) [b]	22 (42) [c]	19.6 (54%) [d]
AAbb (7A only)	37 (34) [b]	23 (39) [c]	17.5 (61%) [d]
AABB (3BS and 7A)	19 (66) [b]	17 (55) [c]	6.9 (84%) [d]

[a] a – CS allele at chromosome 7AC; A – SM3 allele at chromosome 7AC; b – CS allele at chromosome 3BS; B - SM3 allele at chromosome 3BS

In parenthesis: [b] PSS reduction %,[c] FDK reduction % and [d] % reduction of DON concentration in infected kernels

Table 2.6 Country of origin, wheat class, pedigree and haplotype for flanking markers of 7AC quantitative trait loci (QTL) in 49 wheat accessions with 7AC QTL

				Flanking markers[a]		
Accession	Origin	Class	Pedegree	*Xbarc174*	*Xwmc17*	*Xwmc9*
TX03A0148	USA	HRW	TX89A7137/TIPACNA	-	b	b
OK05903C	USA	HRW	TXGH12588-120*4/FS4//2174/3/Jagger F4:10 RC	b	b	b
OK05830	USA	HRW	OK93617/Jagger F6:12	a	b	b
KS010143K-11	USA	HRW	TAM-400/KS950301-DD-4	-	b	b
KS07HW81	USA	HWW	KS02HW25(TGO/JGR 8W)/KS00HW114-1-1(94HW117//JGR/94HW301)	-	b	b
U07-698-9	USA	HRW	Jagger*2/HD29	-	b	b
M04-4715	USA	SRW	Mason/Ernie	-	b	b
Zhongshan 11	China		Funo background	b	b	b
Sumai 1	China		Funo background	b	b	b
Sumai 2	China		Funo background	b	b	b
Wumai 1	China		Funo background	b	b	b
Yangmai 1	China		Funo background	b	b	b
Suyang 7-2	China		Funo background	b	b	b
Xuan 7	China		Funo background	-	b	b
Yangmai 2	China		Wumai/Yangmai1	b	b	b

Xiangmai 8	China	Funo background	b	b	b
Yixi 4	China	Funo background	b	b	b
Xiangmai 10	China	Funo background	b	b	b
Xiangmai 11	China	Funo background	b	b	b
Xiangmai 12	China	Funo background	-	b	b
Jingguangmai	China	Funo background	b	b	b
Xiannong 68	China	Funo background	b	b	b
Youyimai	China	Funo background	b	b	b
Yunmai 27	China	Funo background	b	b	b
Qianjiang 1	China	Funo background	b	b	b
Ai73	China	Funo background	b	b	b
Emai 6	China		b	b	b
Funo	Italy	Duecentodieci/Demiano	b	b	b
ND2419	China	Funo background	b	b	b
Ning 7840	China	Aurora/Anhui 11//Sumai 3	b	b	b
Caizihuang	China	Landrace form Jiangsu Province	a	b	b
Zhen 7495	China		b	b	b
Jingzhou 1	China		b	b	b
Sumai 3	China	Funo/Taiwan wheat	b	b	b
Wannian 2	China	Selection of Mentana	b	b	b
Fumai3	China	Orofen/Funo	b	b	b
Fu5114	China	LongXi 18/(Avrora/Anhui 11//Sumai 3)	b	b	b

Frontana	Brazil	Fronteira (=Polysu / Alfredo Chaves 6 - 21) / Mentana	b	b	b
Hua 512	China		b	b	b
Annong 8455	China	NPFP 73 × Annong 1	b	b	b
IL-89-7978	USA		b	b	b
IL9634-24851	USA	P76788G2-5-494/5/Caldwell/4/Coker 68 -15/3/IL69-1751/6/Caldwell/Tyler//Auburn/7/Ning 7840	h	b	b
Ning8026	China	Avrora/ Sumai 3// Yangmai 2	-	b	b
Ning8831	China	Yangmai 4/(Avrora/ Anhui 11//Sumai 3)	b	b	b
Xianmai1	China	Ardito/Trevere/Wannian 2	-	b	b
Dsumai3	China	Sumai 3/Tom Thumb// Tom Thumb	b	b	b
JG1	China	Mayo/ Armadillo// Yangmai 3/ Avrora/ Ningmai 3	-	b	b
Pc2	China	unknown	b	b	b
Poncheau	france	Selection from land race	-	b	b

HRW- Hard red winter wheat; SRW- Soft red winter wheat; a- Allele similar to Chinese Spring; b- Allele similar to Sumai 3

[a] Flanking markers of 7AC quantitative trait loci represent a 6 cM chromosome region closer to the centromere

Table 2.7 Reduction in percentage of symptomatic spikelets (PSS) in 339 wheat accessions with 3BS and/or 7AC quantitative trait loci

Accession genotype	PSS reduction %
aaBB (3BS only)	41
AAbb (7A only)	20
AABB (3BS and 7A)	49

a – CS allele at chromosome 7AC; A – SM3 allele at chromosome 7AC; b – CS allele at chromosome 3BS; B - SM3 allele at chromosome 3BS

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Appendix A - Published quantitative trait loci for Fusarium head blight resistance in wheat

Table A.1 List of published quantitative trait loci for Fusarium head blight (FHB) resistance in wheat for FHB severity, FHB disease incidence, FHB symptom spread and deoxynivalenol (DON) accumulation

FHB trait	Chromosome	Parents	Population	Reference
FHB symptom spread	3BS and 2AL	Sumai 3 (R)/Stoa (MS)	RIL	(Waldron et al. 1999)
FHB symptom spread	7B	Ning 7840 (R)/Clark (S)	RIL	(Bai et al. 1999)
FHB symptom spread	3AL	ND2603 (R)/Butte 86 (MS)	RIL	(Anderson et al. 2001)
FHB symptom spread	6AS, 3BS and 6BS	ND2603 (R)/Butte 86 (MS) and Sumai 3 (R)/Stoa (MS)	RIL	(Anderson et al. 2001)
FHB symptom spread	2AL and 4BS	Sumai 3 (R)/Stoa (MS)	RIL	(Anderson et al. 2001)
FHB symptom spread	3BS, 2BL and 2AS	Ning 7840 (R)/Clark (S)	RIL	(Zhou et al. 2002b)
FHB symptom spread	3B, 5A and 1B	CM-82036 (R)/Remus (S)	DH	(Buerstmayr et al. 2002)
FHB symptom spread	3BS	Ning 7840 (R)/Wheaton (S)	$F_{2:3}$	(Zhou et al. 2003)
FHB symptom spread	3BS	Ning 7840 (R)/IL89–7978 (S)	$F_{3:4}$	(Zhou et al. 2003)
FHB symptom spread	3B	CM-82036 (R)/Remus (S)	DH	(Buerstmayr et al. 2003)
FHB incidence	5A	CM-82036 (R)/Remus (S)	DH	(Buerstmayr et al. 2003)
FHB symptom spread	3BS, 2D and 6B	Ning 894037 (R) /Alondra (MS)	RIL	(Shen et al. 2003)
FHB symptom spread	3BS, 3BL, 3A and 5B	Huapei57-2 (R) /Patterson (MS)	RIL	(Bourdoncle and Ohm 2003)
DON accumulation	2DS, 3BS and 5AS	Wuhan-1 (R)/Maringa (MS)	DH	(Somers et al. 2003)

FHB symptom spread	3BS and 4B	Wuhan-1 (R)/Maringa (MS)	DH	(Somers et al. 2003)
FHB symptom spread	3BS and 1B	Wangshuibai (R)/Alondra (S).	RIL	(Zhang et al. 2004)
FHB symptom spread	3BS, 3BSC, 7AL and 1BL	Wangshuibai (R)/Wheaton (S)	RIL	(Zhou et al. 2004)
FHB incidence	3A and 5A	Frontana (MR)/Remus (S)	DH	(Steiner et al. 2004)
FHB incidence	2DS, 3AS, 3BS, 3BC, 4DL, 5AS, and 6BS	DH181 (R)/AC Foremost (S)	DH	(Yang et al. 2005b)
FHB symptom spread	2DS, 3BS, 6BS, and 7BL	DH181 (R)/AC Foremost (S)	DH	(Yang et al. 2005b)
FHB symptom spread	5DL, 4BL and 3BS	Chokwang (R)/Clark (S)	RIL	(Yang et al. 2005a)
FHB severity	3B, 5B, 2D, and 7A	Wangshuibai (R)/Alondra (S)	DH	(Jia et al. 2005b)
FHB severity	6AL,1B, 2BL and 7BS	Dream (R)/Lynx (S)	RIL	(Schmolke et al. 2005)
DON accumulation	3BS	CM-82036 (R)/Remus (S)	DH	(Lemmens et al. 2005)
FHB symptom spread and DON accumulation	3BS and 5AS	W14 (R)/Pion 2684 (S)	DH	(Chen et al. 2006)
FHB symptom spread	2D, 3B, 4D and 6A	Chinese Spring Sumai3 disomic substitution lines (R)/Annong 8455 (S)	RIL	(Ma et al. 2006b)
FHB symptom spread	3BS	Sumai3*5 (R)/Thatcher (S) and HC374 (R)/3*98B69-L47 (S)	RIL	(Cuthbert et al. 2006)

FHB severity	1BL, 3AL and 7AS	Frontana (MR)/Seri 82 (S)	$F_{3:5}$	(Mardi et al. 2006)
FHB incidence	4B, 5A and 5B	Wangshuibai (R)/Nanda 2419	RIL	(Lin et al. 2006)
FHB symptom spread	3BS, 2DL, 1AS, 7BS, 5AS, 2Bl and 1BC	CJ9306 (R)/Veery (S)	RIL	(Jiang et al. 2007)
FHB symptom spread	6B	BW278 (R)/AC Foremost (S)	RIL	(Cuthbert et al. 2007)
FHB severity	1AL, 1BL, 6BS and 7AL	Arina (MR)/NK93604(MR)	DH	(Semagn et al. 2007)
DON accumulation	1AL and 2AS	Arina (MR)/NK93604(MR)	DH	(Semagn et al. 2007)
FHB symptom spread	2B, 3B, 4BL, and 5A	Ernie (MR)/MO 94-317 (S)	RIL	(Liu et al. 2007)
FHB incidence	3AS, 3BS, 4B, 5AS and 5DL	Wangshuibai (R)/Wheaton (S)	RIL	(Yu et al. 2008c)
FHB symptom spread	1A, 3BS, 3DL, 5AS, 5DL, and 7AL	Wangshuibai (R)/Wheaton (S)	RIL	(Yu et al. 2008c)
DON accumulation	1A, 1BL, 3BS, 5AS, 5DL, and 7AL	Wangshuibai (R)/Wheaton (S)	RIL	(Yu et al. 2008c)

S – susceptible ; MS – moderately susceptible ; MR – moderately resistant; R – resistant; RIL – recombinant inbred line; DH – double haploid

Table A.2 List of quantitative trait loci for Fusarium head blight resistance (FHB) on chromosome 7A in wheat for FHB symptom spread, FHB severity and deoxynivalenol (DON) accumulation

Trait	Parents	Population	Flanking marker	R^2	LOD	Reference
FHB symptom spread	Wangshuibai / Wheaton	RIL	*wms1083*	9.8	-	(Zhou et al. 2004)
FHB severity	Wangshuibai/Alondra	DH	*gwm276-gwm282*	12.6	2.75	(Jia et al. 2005b)
FHB severity	Frontana / Seri 82	F_3	*e77m47_22 – gwm233*	7.6	2.7	(Mardi et al. 2006)
FHB severity	Arina/ NK93604	DH	*gwm276 – DuPw226*	14.8	4.9	(Semagn et al. 2007)
FHB symptom spread	Wangshuibai/Wheaton	RIL	*Xwms1083*	3.2	2.0	(Yu et al. 2008c)
DON accumulation	Wangshuibai/Wheaton	RIL	*Xwms1083*	7.1	3.3	(Yu et al. 2008c)

RIL – recombinant inbred line; DH – double haploid

Appendix B - Accessions evaluated in the diversity study

Table B.1 List of origin, class and pedigree of the 400 wheat accessions evaluated in the diversity study

Accession	Origin	Class	Pedigree
TX03A0148	USA	HRW	TX89A7137/TIPACNA
OK05903C	USA	HRW	TXGH12588-120*4/FS4//2174/3/Jagger F4:10 RC
OK05830	USA	HRW	OK93617/Jagger F6:12
KS010143K-11	USA	HRW	TAM-400/KS950301-DD-4
KS07HW81	USA	HWW	KS02HW25(TGO/JGR 8W)/KS00HW114-1-1(94HW117//JGR/94HW301)
U07-698-9	USA	HRW	Jagger*2/HD29
M04-4715	USA	SRW	Mason/Ernie
Zhongshan11	China		Funo background
Sumai1	China		Funo background
Sumai2	China		Funo background
Wumai1	China		Funo background
Yangmai1	China		Funo background
Suyang7-2	China		Funo background
Xuan7	China		Funo background
Yangmai2	China		Wumai/Yangmai1
Xiangmai8	China		Funo background
Yixi4	China		Funo background

Xiangmai10	China	Funo background
Xiangmai11	China	Funo background
Xiangmai12	China	Funo background
Jingguangmai	China	Funo background
Xiannong68	China	Funo background
Youyimai	China	Funo background
Yunmai27	China	Funo background
Qianjiang1	China	Funo background
Ai73	China	Funo background
Emai6	China	
Funo	Italy	Duecentodieci/Demiano
ND2419	China	Funo background
Ning7840	China	Aurora/Anhui 11//Sumai 3
Caizihuang	China	Landrace form Jiangsu Province
Zhen7495	China	
Jingzhou1	China	
Sumai3	China	Funo/Taiwan wheat
Wannian2	China	Selection of Mentana
Fumai3	China	Orofen/Funo
Fu5114	China	LongXi18/(Avrora/Anhui11//Sumai 3)
Frontana	Brazil	Fronteira (Polysu / Alfredo Chaves 6 - 21) / Mentana
Hua512	China	

Annong8433	China	NPFP73 × Annong 1
IL-89-7978	USA	
IL9634-24851	USA	P76788G2-5-494/5/Caldwell/4/Coker 68 -15/3/IL69-1751/6/Caldwell/Tyler//Auburn/7/Ning 7840
Ning8026	China	Avrora/ Sumai 3// Yangmai 2
Ning8831	China	Yangmai 4/(Avrora/ Anhui 11//Sumai 3)
Xianmai1	China	Ardito/Trevere/Wannian 2
Dsumai3	China	Sumai 3/Tom Thumb// Tom Thumb
JG1	China	Mayo/ Armadillo// Yangmai 3/ Avrora/ Ningmai 3
PC2	China	unknown
Poncheau	France	Selection from land race
PA8769-158	USA	
Kaskaskia	USA	
OH552	USA	
P93D1-10-2	USA	
MO94-312	USA	
OH569	USA	
Foster	USA	
IL95-1966	USA	
IL95-2066	USA	
IL95-2909	USA	
Pontiac	USA	

PB2555	USA
Cardinal	USA
IL94-6280	USA
IL93-2283	USA
IL94-1549	USA
IL94-1909	USA
IL94-2426	USA
38M.A.	Argentina
Bacup	USA
Wuhan3	China
MO-94-193	USA
Spartakus	Austria
Perlo	Austria
Expert	Austria
Karat	Austria
Coop-Cabildo	Argentina
Vilela-Sol	Argentina
111.92	Argentina
113.92	Argentina
117.92	Argentina
ShirasayaNo1	Japan
Wangshuibai	China

Sumai49	China		
F5125	China		
F60096	China		
FSW	China		
Jagger	USA		
Overland	USA		
Atlas66	USA	HRW	Frondoso // Redhart3 / Noll 28
OK04505	USA	HRW	OK91724/2*Jagger
KS05HW136-3	USA	HWW	KS98HW518(93HW91/93HW255)//KS98H245(IKE/TA2460//*3T200)/TREGO
T158	USA	HRW	KS93U206/ 2*T81
KS980554-12-~9	USA	HRW	2180*K/2163//?/3/W1062A*HVA114/W3416
KS980512-2-2	USA	HRW	T67/X84W063-9-45//K92/3/SNF/4/X86509-1-1/X84W063-9-39-2//K92
TX04M410211	USA	HRW	MASON/JAGGER//OGALLALA
N98L20040-44	USA	HRW	CS/PI467024//CS/3/SXLD/4/TAM202/5/SXLD
NI04420	USA	HRW	NE96644(=ODESSKAYA P./CODY)//PAVON/*3SCOUT66/3/WAHOO SIB
Duster	USA	HRW	W0405 / NE78488 // W7469C / TX81V6187
OK02522W	USA	HRW	OK02522W
Scout66	USA	HRW	Composite of 85 selections from Scout, Citr13546
AP04T8211	USA	HRW	W98-232/KS96WGRC38
HV9W96-1271R-1	USA	HRW	HV9W00-1551WP/KS94U326
NE04424	USA	HRW	KS92H363-2/COUGAR SIB(=NE85707/TBIRD)
CO02W237	USA	HWW	98HW519(93HW91/93HW255)/96HW94

OK03825-5403-6	USA	HRW	(Custer*3/94M81)=STARS 0601W
TX04V075080	USA	HRW	JAGGER/TX93V5722//TX95D8905
SD06165	USA	HRW	Wesley/SD97049
NX03Y2489	USA	HWW	BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106
NI04427	USA	HRW	KS98HW22//W95-615W/N94L189
Endurance	USA	HRW	HBY756A/ Siouxland//2180
TAM-107	USA	HRW	
AP05T2413	USA	HRW	(KS95U522/TX95VA0011)F1/Jagger
HV9W03-539R	USA	HRW	KS94U275/1878//JAGGER
CO03064	USA	HRW	CO970547/Prowers 99
TX02A0252	USA	HRW	TX90V6313//TX94V3724(TAM-200 BC41254-1-8-1-1/TX86V1405
Kharkof	Ukraine	HRW	Landrace of Ukraine
SD06173	USA	HRW	BULK02R2B
NX04Y2107	USA	HWW	NW98S081/99Y1442
NE05548	USA	HRW	NE97426 (=BRIGANTINA.2*ARAPAHOE)/NE98574 (CO850267/RAWHIDE)
Deliver	USA	HRW	
Trego	USA	HWW	KS87H325/Rio Blanco
HV9W03-696R-1	USA	HRW	N94L027/TBOLT//KS89180B
NE05426	USA	HRW	W95-091 (=KS85-663-8-9//WI81-133/THUNDERBIRD)/AKRON
CO03W054	USA	HWW	KS96HW94//Trego/CO960293
Antelope	USA	HWW	

SD03164-1	USA	HRW	89118RC1-X-9-3-3/TX96D2845//Expedition
NW04Y2188	USA	HWW	MO8/REDLAND//KS91H184/3*RIO BLANCO
NE05549	USA	HRW	NI98414 (=NE90614/NE87612//NE87612)/WESLEY
OK Bullet	USA	HRW	KS96WGRC39/ Jagger
OK03716W	USA	HRW	Oro Blanco/OK92403 F4:11
OK00514-05806	USA	HRW	KS96WGRC39/Jagger
AP06T3832	USA	HRW	HBK0935-29-15/KS90W077-2-2/VBF0589-1
HV9W02-942R	USA	HRW	53/3/ABL/1113//K92/4/JAG/5/KS89180B
NE05430	USA	HRW	IN92823A1-1-4-5/NE92458
CO03W139	USA	HWW	CO980862/Lakin
TX03A0563	USA	HRW	X96V107/OGALLALA
Wesley	USA	HRW	Plainsman V / Odesskaya51 // Colt / Cody
NE02533	USA	HRW	NE94458 (=GK-SAGVARI/COLT//NE86582)/JAGGER
NE05569	USA	HRW	Wesley//Pronghorn/Arlin
Overley	USA	HRW	TAM-107 *3/ TA 2460/ Heyne 'S'// Jagger
Century	USA	HRW	
KS05HW15-2	USA	HWW	KS98HW452(KS91H153/KS93HW255)/CO960293//KS920709B-5-2(T67/X84W063-9-45//K92)
T151	USA	HRW	T81/ KS93U206
KS970093-8-9-#1	USA	HRW	HBK1064-3/KS84063-9-39-3-4W//X960103
CO03W239	USA	HWW	KS01-5539/CO99W165
TX04A001246	USA	HRW	TX95V4339/TX94VT938-6

Jerry	USA	HRW	
SD05118	USA	HRW	Wesley/NE93613
NE02558	USA	HRW	JAGGER/ALLIANCE
MT0495	USA	HRW	MT9640/NB1133
Fuller	USA	HRW	
OK03522	USA	HRW	N566/OK94P597
KS05HW121-2	USA	HWW	KS99-5-16(94HW98/91H153)//STANTON/KS98HW423(JAG/93HW242)
T153	USA	HRW	T136/ T151
KS970187-1-10	USA	HRW	TAM107*2/TA759//HBC197F-1/3/2145
CO03W043	USA	HWW	KS96HW94/CO980352
TX01V5134RC-3	USA	HRW	TAM-200/JAGGER
SD06W117	USA	HRW	Alice/SD00W024
SD05210	USA	HRW	SD98444/SD97060
NW03666	USA	HRW	N94S097KS/NE93459
MTS0531	USA	HRW	L'Govskaya167/Rampart//MT9409 (solid stem)
Centerfield	USA	HRW	TXGH12588-105*4 / FS4 // 2*2174
OK04525	USA	HRW	FFR525W/Hickok//Coronado F4:11
OK03305	USA	HRW	N40/OK94P455
MT0552	USA	HRW	N95L159/CDC Clair
T154	USA	HRW	T88/2180//T811
NE05496	USA	HRW	KS95HW62-6 (=KS87H325/RIO BLANCO)/HALLAM
TX04M410164	USA	HRW	MIT/TX93V5722//W95-301

SD06069	USA	HRW	Harry/Wesley//Jerry
SD05W030	USA	HWW	SD98W302/NW97S186
Chisholm	USA	HRW	Sturdy sib/Nicoma
Guymon	USA	HRW	
OK02405	USA	HRW	Tonkawa/GK50
KS010957K~4	USA	HRW	2145/Karl 92//KS940786-6-11
NE06619	USA	HRW	WESLEY/WAHOO
MTS04120	USA	HRW	L'Govskaya167/Rampart
TX06A001239	USA	HRW	OGALLALA/KS94U275
TXHT006F8-CS06/472-STA34	USA	HRW	Lockett/Halberd
MO011126	USA	SRW	MO94-103/Pio2552
OH02-7217	USA	SRW	92118B4-2/OH561
MD99W483-06-9	USA	SRW	VA97W358/Renwood3260
OK04507	USA	HRW	OK95593/Jagger //2174
KS020304K~3	USA	HRW	JAGGER/2137//KS940786-6-9
TX05A001334	USA	HRW	TX87V1233-3/U1254-4-6-6//K92/3/T200*2/TA2460*2//T202
TX06A001376	USA	HRW	NE94482/TX95A1161
VA03W-412	USA	SRW	Roane/Pio2643//SS520
OH03-41-45	USA	SRW	IL91-14167/OH599
OK05312	USA	HRW	TX93V5919/WGRC40//OK94P549/WGRC34
HV9W05-881R	USA	HRW	MASON/OGALLALA-vr/Betty

NE06436	USA	HRW	WESLEY/OK98699 (=TAM200/HBB313//2158)
NW05M6011-6-1	USA	HWW	Nuplains/Arrowsmith
TX06A001431	USA	HRW	T107//TX98V3620/Ctk78/3/TX87V1233/4/N87V106//TX86V1540/T200
TXHT023F7-CS06/607-STA07/40	USA	HRW	TX99U8544/Ogallala
AR97044-10-2	USA	SRW	Elkhart/AR494B-2-2
P02444A1-23-9	USA	SRW	981129/99793//INW0301/92145
VA05W-414	USA	SRW	Pio25W60//VA96W-606WS(FFR555W/Coker9803//Annette)/Pio2691
OK05511	USA	HRW	TAM 110/2174
SD07W041	USA	HWW	FALCON/SD99W042//TREGO
SD07204	USA	HRW	HARDING//SD98243/ALLIANCE
NW05M6015-25-4	USA	HWW	NW97S186/Rio Blanco
TXHT001F8-CS06/325-PRE07/75	USA	HRW	TX01M5009/Halberd
CO04W210	USA	HWW	NW97S343/Akron
KY96C-0769-7-3	USA	SRW	2552/Roane
P03207A1-7	USA	SRW	INW0304*2/RSI5//981281/3/INW0315/99794
LA01*425	USA	SRW	P2571/Y91-6B
KS07HW25	USA	HWW	KS025580(TREGO/CO960293)/KSO1HW152-6(TGO/BTY SIB)
SD07220	USA	HRW	TANDEM/Goodstreak
KS010379M-2	USA	HRW	KS920709-B-5-2-2/TAM-400
NE06472	USA	HRW	CO95043 (=HILL/PI294994//LAMAR)/KS89180B-2-1 (=KS8010-

			73/KS8010-1-4- 2//107349/KARL)//NE98574 (=CO850267/RAWHIDE)
Roane	USA	SRW	VA71-54-147(CI17449)/C68-15//IN65309C1-18-2-3-2 (formerly VA93-54-429)
OH02-12678	USA	SRW	Foster/Hopewell//OH581/OH569
LA02-923	USA	SRW	PS8424//XY90-1B/TX851212
SD05W148-1	USA	HWW	SD98153/SD98W117
KS010514-9TM-10	USA	HRW	CM98-42/3/HBF0290/X84W063-9-39-2//ARH/4/KS940786-6-4
N02Y5117	USA	HRW	YUMA//T-57/3/CO850034/4/4*YUMA/5/KS91H184/ARLIN S/KS91HW29//NE89526)
INW0411	USA	SRW	96204A1-12//Goldfield/92823A1-11 (formerly P97397E1-11-2-4-1-1)
MO040192	USA	SRW	IL85-2872/MO10501
NYCalR-L	USA	SRW	Reselection out of Caledonia
TX05V5614	USA	HRW	TX96V2427/TX98U8083
Branson	USA	SRW	Pio2737W/891-4584A (Pike/FL302) (formerly M00-3701)
IL00-8530	USA	SRW	IL89-1687//IL90-6364/IL93-2489
IL02-18228	USA	SRW	Pio25R26/IL9634-24437(IL90-4813/L85-3132/Ning7840)//IL95-4162
KS07HW117	USA	HWW	KS00HW151-4(94H871//VTA/94HW301)//KS98HW151-6/00HW114-1
NE06549	USA	HRW	HALLAM/WESLEY
TX06A001084	USA	HRW	KS90WGRC10//U1275-1-11-8/TA2455/3/KS93U69/4/Ogallala/TX89V4133
Bess	USA	SRW	MO11769/Madison (formerly MO981020)
IL02-19463	USA	SRW	Patton/Cardinal//IL96-2550

Mocha exp.	USA	SRW	OH489/OH490
Pioneer Brand 26R61	USA	SRW	Omega78/S76/4/Arthur71/3/Stadler//Redcoat/Wisc1/5/Coker747/6/2555sib (formerly XW663)
NC04-15533	USA	SRW	NC94-6275/P86958//VA96-54-234
M03-3616-C	USA	SRW	Hopewell/Patton
W98007V1	USA	SRW	F2IN82104B1-3-2(H14H15),W900003,Andy/Seneca/3/ Downy/F2IN82104B1-3-2(H14H15),Williams,IN86861-8(H18)/4/NC96BGTA6
Arena exp.	USA	SRW	NASW84-345/Coker9835//OH419/OH389
Coker 9553	USA	SRW	89M-4035A(IL77-2656/NK79W810/Pio2580 (formerly D00*6874-2)
VA05W-258	USA	SRW	VA98W-130(Savannah/VA87-54-558//VA88-54-328/Gore)//Coker9835/SS520
B030543	USA	SRW	VA93-54-429/LA85422
W98008J1	USA	SRW	IN82104B1-3-2(H14H15)/Williams,IN86861-8(H18)//NC96BGTA6
OK05122	USA	HRW	KS94U337/NE93427 F4:10
OK06210	USA	HRW	KS90175-1-2/CMSW89Y271//K92/3/ABI86*3414/X86035*-BB-34//HBC 302E RC F4:9 RC
India exp.	USA	SRW	KY85C-35-4/Karl/Madison
G69202	USA	SRW	VA91-54-219/OH413
USG 3555	USA	SRW	VA94-52-60/Pio2643//USG3209
LA01138D-52	USA	SRW	LA841/LA422//AGS2000
VA05W-78	USA	SRW	Tribute/AGS2000
OK05723W	USA	HRW	SWM866442/Betty F4:10 HW

OK06345	USA	HRW	FAWWON 06/2174//OK95548-26C F4:9
OK06319	USA	HRW	Enhancer/2174 F4:9
D04*5513	USA	SRW	DK1551W/D94-50228
M04-4566	USA	SRW	Bradley/Roane
NC03-6228	USA	SRW	A92-4452//NC96BGTD1sib/NC96BGTA6sib
AR96077-7-2	USA	SRW	Jackson/Pio2643
D04-5012	USA	SRW	NC96BGTD1/Mason
G59160	USA	SRW	T812/VA91-54-219
OK01420W	USA	HRW	KS93U206/Jagger RC
OK06528	USA	HRW	Vilma/Hickok//Heyne F4:9 A-
OK06518	USA	HRW	Palma/Hickok//2174 F4:9
KY97C-0321-02-01	USA	SRW	Kristy/VA94-52-25//2540
M04-4802	USA	SRW	FFR518//Elkhart/MV-18
AR97124-4-3	USA	SRW	P88288C1-6-1-2/Terra SR204
GA991336-6E9	USA	SRW	GA92432//AGS2000/Pio26R61
G61505	USA	SRW	ABI89-4584A/T814
OK05134	USA	HRW	OK97411/TX91D6825 F4:10
OK06313	USA	HRW	Emma/Karl 92//2174 F4:9
KY97C-0519-04-07	USA	SRW	SS555W/2540//2552
M04*5109	USA	SRW	VA94-54-479/Pio2628
VA04W-259	USA	SRW	VA97W-533 [FFR555W/Gore//Ck9803/VA87-54-636] /NC9511612(Stella/KS85WGRC01//C8433/3/C8629/FL7927)

MD01W233-06-1	USA	SRW	McCormick/Choptank
GA991209-6E33	USA	SRW	GA901146/GA96004//AGS2000
G41732	USA	SRW	T814/L900819
OK06848W	USA	HRW	OK94P461/Oro Blanco F6:11
W06-202B	USA	SRW	Ashland/Hopewell//OH546/L930605
TAM 110	USA	HRW	07Kochenower
LA99005UC-31-3-C	USA	SRW	Pio2548/Coker9835(LA90144B16-3-2)//AGS2000
P03112A1-7-14	USA	SRW	INW0411//INW0315/99794
TN801	USA	SRW	Cardinal/FL302//AR Exp 494B-2-2/3/Fillmore/Cardinal//Jackson
GA991371-6E13	USA	SRW	GA931521/2*AGS2000
OK05212	USA	HRW	OK95616-1/Hickok//Betty F4:10
OK06336	USA	HRW	Magvars/2174//Enhancer F4:9
MO040152	USA	SRW	MO 12278/Pio2571
AGS 2000	USA	SRW	Pio.2555/PF84301//FL 302 (formerly GA89482E7)
LA98214D-14-1-2-B	USA	SRW	Shelby/LA87167D8-10-2(FR81-19/FL302//Coker983)
P04287A1-10	USA	SRW	INW0315*2/4/INW0304//9346/CS 5Am/3/91202//INW0301/INW0315
GA991227-6A33	USA	SRW	VA97W-24/AGS2000
OK05128	USA	HRW	KS94U275/OK94P549 F4:10 RC
Jinagdu1	China		Funo background
Huai69-6	China		Funo background
Siyang117	China		Funo background
Fengmai2	China		Funo background

Qunzhong10	China		Funo background
Yangmai3	China		Wumai/Yangmai 1
Yangmai4	China		Nanda 2419/Triumph//Funo
Yangmai5	China		F4 (9-16)/St 1472/ 506
Yangmai158	China		Yangmai 4/St 1472/506
Linnong12	China		Funo background
Linnong14	China		Funo background
Zhongliang11	China		Funo background
Zhenmai17	China		Funo background
Beiquan565	China		Funo background
Wan7107	China		Funo background
Anxuan2	China		Funo background
Maoyingafu-2	China		Funo background
Huamai7	China		Funo background
Xiangnong3	China		Funo background
Wanya2	China		Funo background
Yunmai35	China		Funo background
Yunmai25	China		Funo background
Zhemai6	China		Funo background
Mengfeng8	China		Funo background
Taiwan wheat	China		Funo background
Clark	USA	SRW	Beau//65256A1-8-1/67137B5-16/Sullivan/Beau//5517B8-5-3-3/Logan

Sobakomugi 1B	Japan	Landrace	JGB99-61, accession no. 23662, unknown pedigree
Sobakomugi 1C	Japan	Landrace	JGB99-61, accession no. 23665, unknown pedigree
Aburakomugi	Japan		JGB99-12, accession no. 23516, unknown pedigree
Asozaira III	Japan		JGB99-16, accession no. 23524, unknown pedigree
AsoZairai(YuubouKappu)	Japan		JGB99-18, accession no. 23521, unknown pedigree
Chile	Chili		JGB99-20, accession no. 26869, unknown pedigree
Itoukomugi	Japan		JGB99-23, accession no. 23647, unknown pedigree
Kagoshima	Japan		JGB99-25, accession no. 23542, unknown pedigree
Kikuchi	Japan		JGB99-28, accession no. 23546, unknown pedigree
Nyuubai	Japan		JGB99-36, accession no. 22957, unknown pedigree
Qiaomaixiaomai	Japan		
Shironankin	Japan		JGB99-58, accession no. 23277, unknown pedigree
Shoukomugi II	Japan		JGB99-61, accession no. 23653, unknown pedigree
Sotome	Japan		JGB99-62, accession no. 23595, unknown pedigree
Asotomea	Japan	Landrace	
NobeokabouzuKomugi	Japan	Landrace	
Huoshaobairimai	China		
Hongjianzi	China		
Huangcandou	China		
Haiyanzhong	China		
Dafanliuzhu	China		
Huoshaomai	China		

Xueliqing	China		
Can Lao Mai	China		
Huang Fang Zhu	China		
Sanyuehuang	China		
Baisanyuehuang	China		
Dahongpao	China		
Heshangmai	China		
Fusuihuang	China		
Tawanhsiaomai	China		
PaiMaiTze	China		
TaFangShen	China		
SanChaHo	China		
MuTanChiang	China		
SapporoHaruKomugiJugo	Japan	Landrace	
Abura	Japan	Landrace	LV-Fukuoka
Minamikyushu69	Japan	Landrace	
NobeokaBozu	Japan	Landrace	
NyuBai	Japan	Landrace	
TokaI66	Japan	Landrace	
LiangGuangTou	China		
YouZiMai	China		
LingHaiMao Yang Mo	China		

ChanjiBaiDongMai	China		
JiangDongMen	China		
ShanhaiCaiZiHuang	China		
XingHuaBaiYuHua	China		
HuiShanYangMai	China		
ShuiLiZhan	China		
QiangShuiHuang	China		
YangLaZi	China		
FangTouBaiMang	China		
FangTouHongMang	China		
DaHuangPi	China		
HongHuaWu	China		
HongMongBai	China		
ChuShanBao	China		
HeiHangDongMai	China		
YouBaoMai	China		
MeiQianWu	China		
HongMangMai	China		
DaBaiPao	China		
Ernie	USA	SRW	Pike/3/Stoddard/Blueboy//Stoddard/D1707
Freedom	USA	SRW	GR876/OH217
Sanshukomugi	Japan		Land race from Mie

Shinchunaga	Japan		Land race from Mie
Shanasui	Japan		
WSB	China		Land race from Jiangsu Province
NTDHP	China	Land race	Land race from Jiangsu
Su49	China		N7922/(Aurora/Anhui 11//Sumai 3)
F60096	China		Jinzhou 1/Sumai 2
WZHHS	China		Land race from Zhejiang Province
Chinese Spring	China		Landrace
Chokwang	Korea		
Chukoku81	Korea	Indiana	
Jinmai33	China		
Wenmai6	China		
Luohan2	China		
Zhenghan1	China		
Suwon92	Korea		Purdue98-3450
Siyang936	China		
Y155	China		
Wheaton	USA	HRW	CRIM(CI-13465)/2*(CI-13986)ERA//BUITRE/GALLO
ND2928	USA	HSW	Ning 7840/ND706
NE04490	USA	HRW	NE95589/NE94632(=ABILENE/NORKAN//RAWHIDE)//NE95510 (=ABILENE/ARAPAHOE)
TXHT005F8-CS06/540-	USA	HRW	Halberd/Trego

STA07/14		
Huimaoafu	China	Funo background
Zairaiyuubou	Japan	JGB99-70, accession no. 22130, unknown pedigree
Aurora (Abpopa)	Russian	Lutescens314H147 / Bezostaja1