MAPPING QTL CONFERRING RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE SPRING WHEAT CULTIVAR 'UI STONE'

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Abstract

Fusarium head blight (FHB) is one of the most destructive diseases of wheat causing significant yield losses and quality reduction in the humid areas of the world. It is also an emerging wheat disease in Southeastern Idaho of the United States. Resistance to FHB is known to be a quantitatively inherited trait and highly influenced by confounding environmental factors. The objectives of this study were to map and characterize QTL associated with FHB resistance in 151 ($F_{4:6}$) recombinant inbred lines (RILs) derived from the cross between a resistant cultivar 'UI Stone' and a moderately susceptible cultivar 'Alturas'. The population was evaluated for type II FHB resistance by measuring disease severity expressed as a percentage of infected spikelets (PIS) in four greenhouse experiments over three years. Two major QTL for type II FHB resistance, *QFhbuis.ab-2B* and *QFhbuis.ab-3B*, were identified by both single marker and composite interval mapping (CIM) methods and together these QTL explained 23.6 to 24.8% of phenotypic variation. Four additional QTL, *QFhbuis.ab-1D*, *QFhbuis.ab-2D.1, QFhbuis.ab-2D.2,* and *QFhbalt.ab-4A*, were identified by either the single marker or CIM methods and were associated with disease severity in specific experiments. The two major QTL identified in this study have potential application in marker-assisted breeding of FHB resistance. This study also identified 4 lines with better FHB resistance and higher grain yield than UI Stone and these lines could be used as germplasm and/or released as new resistant cultivars after further testing.

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Chapter 1

Literature Review

1. Wheat: Evolution and Importance

Wheat (*Triticum* spp.) is believed to have originated around ten thousand years ago in the Fertile Crescent of the Tigris and Euphrates rivers, in present day Iraq, Israel, Jordan, Syria and Turkey and is one of the first domesticated crops (Lev-Yadun et al., 2000; Gustafson et al., 2009). The most widely cultivated wheat species is bread wheat (*Triticum aestivum* L.). Bread wheat is a hexaploid (2n=6x=42) which is known to have three subgenomes (A, B and D) that are organized in seven homoelogous groups. It is believed that hybridization followed by chromosome doubling between two grass species *T. urartu* (the A genome donor) and a close relative of *Aegilops speltoides* (the B genome donor) produced the tetraploid species *T. turgidum*. A second hybridization followed by chromosome doubling between this tetraploid species and *A. tauschii* (diploid species, the D genome donor) resulted in hexaploid bread wheat (Petersen et al., 2006).

Wheat is a widely cultivated crop across the world and is one of the most important staple food crops, occupying 17% of crop acreage worldwide, and feeding about 40% of the world's population (Gupta et al., 2008). The United States (US) is the third largest producer of wheat after China and India, but it occupies first place among wheat exporting countries. In 2013, the total US wheat production was 2,114.08 million bushels representing 8.12% of the world's total wheat production (26,047.28 million bushels) and the US exported nearly 52% of its total production (http://www.ers.usda.gov). Idaho produced 101.8 million bushels in 2013 which is 4.8% of total US wheat production.

Idaho is consistently one of the US's top wheat growing states and exceptional in per acre yield (82.1 bushels per acre) when compared to the national average yield (47.1 bushels per acre) (NASS 2013). Typically two main types of wheat are planted in the US: winter and spring wheat. Winter wheats are planted in the fall, undergo vernalization in the winter, flower in the following spring, and are harvested in early summer. In contrast, spring wheats are planted in spring, flower in early summer, and are harvested in late summer. Wheat can be further classified based on grain color (red or white) and grain texture (soft or hard). There are six market classes of wheat: Hard Red Spring, Soft White, Hard Red Winter, Soft Red Winter, Durum and Hard White (http://www.idahowheat.org). Soft White wheat is used in making flat breads, cakes, biscuits, pastries and crackers; Hard Red Winter is used in pan breads, hard rolls and general purpose flour; Hard Red Spring is used in rolls, croissants, bagels and pizza crust; Durum is used in pasta and Mediterranean breads; Soft Red Winter is used in pastries, cookies and pretzels; and Hard White is used in Asian noodles, whole wheat and high extraction flour applications (http://www.idahowheat.org). Overall, wheat is a major source of nutrition in the human diet: 100 grams (g) of wheat provides about 340 kcal of energy, 13.2 g proteins, 2.5 g lipids, 72 g carbohydrates, 10.7 g dietary fiber, 34 microgram (mg) calcium, 3.6 mg iron and vitamins such as niacin, riboflavin, folate, thiamin and alpha-tocopherol (http://ndb.nal.usda.gov).

2. Fusarium Head Blight

A significant increase in total wheat production has been achieved in the last few decades; however, production stability remains fragile because of challenges such as

susceptibility to diseases, pest damages and changes in climate (Gupta et al., 2008; McMullen et al., 1997). Among several diseases, Fusarium Head Blight (FHB), also known as 'Scab', is one of the most devastating fungal diseases of wheat and barley worldwide (McMullen et al., 1997; Bai and Shaner, 2004). FHB leads to severe losses not only to grain yield but also in quality, since contamination with mycotoxin renders harvested grain harmful for humans and animals upon consumption. In recent years, FHB has received much attention because of its ability to completely destroy a wheat crop within a few weeks of harvest (McMullen et al., 1997).

FHB was first recorded in England by Smith in 1884 (Smith, 1884; Parry et al., 1995). Chester (1890) and Arthur (1891) independently reported scab for the first time in the US and emphasized its importance. Since then reports from China, England, Australia, Brazil, India, Canada and other major wheat growing countries have identified damage up to 70% due to FHB (Parry et al., 1995). From 1990 to 2002 US wheat and barley farmers lost over $3 billion due to FHB epidemics (Schmale III and Bergstrom, 2003). The FHB epidemic struck the tri-state area of Minnesota, North Dakota and South Dakota in 1993, causing an estimated $1 billion loss, one of the biggest losses due to any plant disease in a single year in the US (McMullen et al., 1997). Historically, FHB is not a common problem in Idaho because the climate is not favorable for establishment of this disease. However, occasionally local or regional epidemics of FHB have been recorded since the disease was first reported in Idaho in 1980 (Mihuta-Grimm and Forster, 1989). The United States Department of Agriculture (USDA) has ranked FHB as the worst plant disease to hit the US since the rust epidemics in the 1950s (Schmale III and Bergstrom, 2003), suggesting the need for further research in FHB. In the US, the threat from FHB has led to the

formation of the US Wheat and Barley Scab Initiative (USWBSI), which is a collaborative research initiative with the goal of developing management and control strategies to reduce the devastating impact of this disease (http://www.scabusa.org).

At least 17 *Fusarium* species are known to infect wheat or barley spikes with various level of virulence, but in most areas of the world *Fusarium graminearum* (teleomorph *Gibberella zeae*), *Fusarium culmorum* and *Fusarium avenaceum* (teleomorph *Gibberella avenaceae*) appear to predominate depending on climatic conditions (Parry et al., 1995). The genus *Fusarium* belongs to the Ascomycete branch of the fungal kingdom, as these fungi produce ascospores in sexual reproduction. However most *Fusarium* species lack a sexual stage and produce either micro-and/or macroconidia as a means of asexual reproduction. Different species can be morphologically recognized on the basis of colony color, size, shape and septation of macroconidia; presence or absence of microconidia, chlamydospores and perithecia as well as species' specific primers used in the polymerase chain reaction (PCR) assay (Summerell et al., 2003). There are 5-6 septae in macroconidia of *F. graminearum* and 3-4 septae in *F. culmorum*. The shape of macroconidia in *F. graminearum* is long and narrow compared to macroconidia in *F. culmorum* which is thick and bluntly pointed at the apex (Leslie and Summerell, 2006). Identification of *Fusarium* species on the basis of morphological characteristics has been used in various studies (Leslie and Summerell, 2006), however, PCR based identification is preferred depending upon the objective of research (Summerell et al., 2003).

Ascospores and macroconidia both can be used as the principal source of inoculum and may be disseminated by aerial dispersal (Bai and Shaner, 1994). Infection is initiated when airborne ascospores or macroconidia are deposited on wheat spikelets, and

subsequently germinate and infect. The fungus may infect the glume, palea or rachila by direct penetration. Soon after infection, visible symptoms develop with tan or brown discoloration at the base of the spikelets. A few days later, this bleached symptom will spread to the entire infected spikelet if in susceptible hosts. For resistant cultivars, symptoms could be limited to the inoculated spikelet without spreading to adjacent uninoculated spikelets. However, for susceptible plants, the fungus invades the rachis and spreads up and down the entire spike if the weather is favorable for disease development. Infected spikelets on the spike can be infertile, or kernels can become shriveled, bleached and chalky, also known as "tombstone" kernels (Bai and Shaner, 1994). Therefore, FHB can cause high yield reductions.

Environmental factors play a pivotal role in FHB initiation, development and severity. Infection can occur at any point after anthesis up to the soft dough stage. However, flowering is the most susceptible stage for wheat as anthers function as initial infection points for the fungus to enter spike tissues and anthers contain high levels of compounds such as choline and betaine that can facilitate the growth of *Fusarium* species (Bai and Shaner, 1994). In general, warm and humid weather is required for fungal infection. The infection is usually favored by moderate temperature and high humidity coinciding with wheat flowering. The optimum infection temperature and relative humidity by *F. graminearum* are reported to be 25°C and 100% respectively for 24 hours (Parry et al., 1995). Temperatures below 16°C or above 36°C are reported to inhibit macroconidia production (Tschanz et al., 1976). Parry et al. (1995) suggested some key factors that may generate an FHB epidemic: (i) warm dry soil conditions during the early part of the growing season, (ii) intense rainfall during the period of anthesis and (iii) prolonged

periods of warm humid conditions. Agricultural practices such as crop rotation and crop management also have effects on FHB. Growing susceptible cultivars can increase initial inoculum and therefore FHB incidence (Dill-Macky and Jones, 2000). Crop rotations with non-hosts may reduce the head blight incidence (Champeil et al., 2004). Reduced soil tillage increases initial inoculum survival rate and raises the FHB incidence, whereas ploughing reduces inoculum to some extent and modifies microclimate of the soil, and therefore reduces the development of FHB. Irrigation may also encourage development of pathogen and, hence, influence FHB frequency and severity (Champeil et al., 2004). Additionally, sowing date, wind speed**,** weeds, and canopy density can all affect FHB pathogen establishment and hence the disease (Champeil et al., 2004).

3. FHB Management

FHB occurs as a result of the combined effects of several factors such as weather conditions, plant growth stage and agronomic practices. Weather cannot be controlled but there are several other factors which can be manipulated to prevent disease establishment (McMullen et al., 1997). Management of losses caused by FHB requires an integrated approach. A single control strategy is often not sufficient at obtaining adequate control. FHB control strategies consist of agronomic and cultural practices, biological control, fungicide applications and most importantly host resistance.

3.1. Agronomic and Cultural Practices

Various agronomic and cultural practices including crop rotation and soil tillage, plays important roles in development of FHB (Champeil et al., 2004). Intensive cultivation of

cereal crops such as maize, wheat and barley increases the abundance of *F. graminearum* inoculum (Shaner, 2003). Dill-Macky and Jones (2000) reported that FHB contamination is more severe when maize is the preceding crop. Therefore, FHB in wheat can be significantly reduced by alternating planting of cereal crops following non-cereal crops. Pereyra et al. (2004) described that decomposition of crop residues reduces the survival and recovery of fungi. Conventional tillage compared to no-till or minimum tillage buries crop residues and enhances the decomposition process (Pereyra et al., 2004). FHB severity and deoxynevanol (DON) contamination can be significantly reduced by deep-ploughing (Blandino et al., 2010).

3.2. Biological Control

Several studies have been done to investigate the possibility of using biological agents to control FHB. Bleakley et al. (2012) reported *Bacillus* strains as having potential for biological control of FHB. Chen et al. (2012) showed that the fungus *Clonostachys rosea* can be used as a bio-fungicide in combination with chemical fungicides. Gilbert and Fernando (2004) recognized *Lysobacter* spp. as a promising biocontrol agent for having ability to induce resistance in the host. Antagonistic action of *Pseudomonas* spp. against *F. graminearum* has been recognized *in vitro* (Gilbert et al., 2004). Similarly, Perez et al. (2008) reported that incorporation of green manure enhances *Streptomyces* spp. population in the soil including other microorganisms which is also antagonistic against *F. graminearum*. Therefore, *Pseudomonas* and *Streptomyces* both could be utilized as possible biocontrol agent. However, due to several constraints, biological methods are not currently practical for FHB control in the field.

3.3. Chemical Control

Several fungicides are reported to be effective against FHB but with variable results. The composition of fungicide, application timing and resistance level of cultivars are related to the variability of fungicide efficacy (Mesterhazy et al., 2003; Mesterházy et al., 2011). Some fungicides are effective in reducing the disease but may have an antagonistic effect on mycotoxin accumulation. Therefore, considerations should be made to suppress both disease severity and mycotoxin level with the application of fungicide (Mesterhazy et al., 2003). In general, triazole fungicides are considered as the most effective fungicide for controlling both disease severity and minimizing DON concentration (Bradley, 2011). However, none of the triazole fungicides offer complete control of the disease.

3.4. Host Plant Resistance

Host plant resistance is the most effective method to control FHB (McMullen et al., 1997; Sneller et al., 2010). However the greatest challenge in breeding for FHB resistance is to release adapted FHB resistant cultivars that combine competitive yield and acceptable end-use quality (Bai and Shaner, 2004; Buerstmayr et al., 2009). No wheat cultivars have been identified that have complete immunity to FHB, however, a few cultivars with moderate to high level tolerance to FHB have been identified and are used as parents in breeding programs. Cultivars with moderate resistance may improve fungicidal efficacy and provide better protection against FHB (Mesterhazy et al., 2003).

To date, conventional breeding methods are mainly used to develop resistant cultivars but are time consuming and expensive (Buerstmayr et al., 2002, 2009). However, it has been found that resistance to FHB is governed by major and minor quantitative trait loci

(QTL). Identification of major QTL and markers linked to the QTL may open the door for accelerating breeding programs through marker assisted selection (MAS) (Buerstmayr et al., 2002).

4. Mechanisms and Type of FHB Resistance

Mechanisms of resistance can be classified as morphological (passive) and physiological (active) (Mesterhazy, 1995; Gilsinger et al., 2005). Morphological mechanisms refer to those crop traits which lead to unfavorable conditions for FHB to initiate infection, such as plant height, presence of awns, and degree to which the flower opens during anthesis. Plants with wide open flowers are more susceptible to FHB. Physiological mechanisms involve biochemical pathways that produce compounds to inhibit the pathogen growth after initial infection (Gilsinger et al., 2005).

Resistance to FHB is considered to be race non-specific, since resistant wheat genotypes show similar reactions against different isolates of *F. graminearum* (Mesterházy et al., 2005; Tóth et al., 2008). To date five types of resistance to FHB have been described and are summarized in Table 1.1. Among them, type I, II and III are commonly accepted. Type I resistance is a major type of resistance in barley (Steffenson, 2003), type II is more stable resistance in wheat (Bai and Shaner, 2004), while type III is found in both barley and wheat. Type I resistance is usually evaluated as FHB incidence after spray-inoculation generally conducted in field experiments and occasionally in greenhouse studies. Type II resistance is generally evaluated by point inoculation to a single spikelet and rating of symptom spread within a spike. Type III resistance is usually assessed as DON content of grains from naturally infected field trials or single-floret and

spray-inoculated experiments conducted in both the greenhouse and field. Among all the types of resistance, type II resistance is considered the major type of resistance and has been studied most extensively.

Table 1.1. Type of FHB Resistance

Type of Resistance	Description	Reference
I	Resistance to invasion	Shroeder and Chriestensen, 1963
II	Resistance to spreading	Shroeder and Chriestensen, 1963
III	Resistance to toxin accumulation	Miller et al., 1985
IV	Resistance to kernel infection	Mesterhazy, 1995; Mesterhazy et al., 1999
V	Tolerance to yield loss	Mesterhazy, 1995; Mesterhazy et al., 1999

5. FHB Resistance Sources

Use of FHB resistant cultivars is the most effective and eco-friendly strategy in FHB management (Bai and Shaner, 2004). Arthur (1891) was the first to denote differences in susceptibility to FHB among wheat cultivars. Since then considerable attention has been devoted to finding sources of resistance that can be used in breeding programs. Although FHB-immune cultivars have not been reported (Fang et al., 1997), cultivars with various levels of resistance have been identified worldwide (Bai and Shaner, 2004).

Identified resistance sources in common wheat can be divided into four groups (Chen, 2005). Group I consists of highly resistant sources, which mainly include spring wheat genotypes, such as 'Sumai3', 'Ning7840', 'Wangshuibai' and 'W14' from China, and 'Nobeokabouzu-komugi' and 'NyuBai' from Japan. Sumai3 and its derivatives are the most widely used FHB resistance sources worldwide. These sources have been characterized as having type II resistance. Group II resistance sources include the Brazilian cultivar 'Frontana', which has both type I resistance and type III resistance. Group III resistance sources include adapted winter wheat cultivars or lines of diverse origin that are also referred to as native resistance sources. North American genotypes such as 'Ernie', 'Freedom' and 'Roane', and European genotypes such as 'Arina' and 'Renan' have expressed high levels of resistance with good agronomic traits. Group IV resistance sources include wild relatives of wheat, such as *Triticum tauschii* (Coss.) Schmal, *Roegneria kamoji* C. Koch, and *Thinopyrum elongongatum* (Host) D.R. Dewey. These sources can play an important role in enriching the gene pool and providing novel and complementary sources of FHB resistance.

FHB resistance is race non-specific, at least for the most prevalent species like *F. culmorum* and *F. graminearum* (Mesterhazy et al., 1999). Therefore, Sumai3 and its derivatives are still the major sources of resistance to FHB in wheat breeding programs (Bai and Shaner, 2004). Although the resistance genes in Sumai3 and other sources of resistance currently used in breeding programs are not expected to be overcome by new isolates of the pathogen in the near future, the utilization of at least a few different resistance genes in a wheat breeding program would be a wise approach (Buerstmayr et al., 2009).

6. Inheritance of FHB and the Relationship of FHB Resistance with Agronomic Traits

Previous FHB studies have concluded that resistance is quantitative, however, there is no consensus concerning its genetic control. Resistance to FHB has been reported to be oligogenic and polygenic with high heritability (Bai et al., 2000). Different numbers of genes have been proposed in the same resistant cultivar in different studies. Kolb et al. (2001) pointed out several possible reasons for these inconsistent results including polygenic control of FHB resistance in wheat, effects of different genetic backgrounds, different types of resistance evaluated, genotype and environment interactions, heterogeneous sources of a resistant parent, or the use of different inoculation techniques used in various studies. Inheritance of type II resistance in wheat has been extensively studied (Buerstmayr et al., 1999; Bai et al., 2000). Additive gene effects play a major role in inheritance of type II resistance, but non-additive gene effects may also be important in some cases (Snijders, 1990; Bai et al., 2000). Dominance appears to be the most important component of non-additive gene effects (Bai et al., 2000). Using a set of diallel crosses among different genotypes including Sumai3, Yibo et al. (1992) indicated that inheritance of resistance to a strain of *F. graminearum* is governed by the additive-dominance model with additive gene action being the most important factor. Two to four genes were estimated to contribute to resistance in this population. Singh et al. (1995) reported additive interaction of three minor genes in a population derived from Frontana and, in their study, transgressive segregation was observed indicating that a susceptible parent can also carry one (or two) minor genes. The combination of these genes with the genes in Frontana generated progenies with better FHB resistance than that of Frontana.

FHB resistance genes have been assigned to several chromosomes using cytogenetic analysis. Since only a few cultivars have a high degree of resistance, and these materials have many other undesired traits, the use of resistance genes from moderately resistant or moderately susceptible cultivars in a breeding program may permit combining different resistance genes in a genetic background that results in desired agronomic traits (Bai et al., 2000). It is also possible to select FHB resistant lines from among transgressive segregants that would be superior to the resistant parent (Bai et al., 2000). Development of adapted cultivars combining good agronomic performance, acceptable end use quality, resistance to FHB and other diseases is a great challenge for plant breeders (Bai and Shaner, 2004; Buerstmayr et al., 2009).

The quantitative nature of inheritance of FHB resistance, its frequent association with undesirable agronomic traits and the large effect of the environment make breeding for this trait very difficult (Bai and Shaner, 2004). Previous research showed that there is some relationship between plant height and resistance to FHB in wheat (Mesterhazy, 1995). A negative correlation between plant height and FHB symptoms was reported by Buerstmayr et al. (2000) in two different populations of wheat. Somers et al. (2003) showed that taller plants had less FHB infection under field conditions. Mesterhazy (1995) stated that the presence of awns in wheat enhances the development of FHB. It is also evident that wheat plants with a narrow flower opening or a short duration of flower opening will have a lower incidence of FHB by reducing the area or time in which *Fusarium* spores can enter the spikelet and initiate infection (Gilsinger et al., 2005). *Fhb*1 is a major QTL in Sumai3 and its descendants and has been utilized in many mapping studies. Salameh et al. (2011) tested the possible side effects (linkage drag) of

introgression of *Fhb*1 and *Qfhs.ifa*-5A (a QTL on chromosome 5A) into European wheat lines and found that there is no systematic negative effect on grain yield, thousand grain weight, hectoliter weight and protein content. In contrast, McMullen et al. (2012) pointed out that incorporation of *Fhb*1 has been very limited because it is associated with many undesirable traits responsible for low yield and increased susceptibility to other diseases. Because of such inconsistent result, the use of resistance sources from Asian cultivars should be minimized and increase the use of native resistance sources for breeding FHB resistance (Gilbert and Haber, 2013).

7. QTL, Molecular Markers and Marker Assisted Selection (MAS) for FHB Resistance

The quantitative nature of inheritance, the large genotype by environment interaction, arduous phenotyping procedures, and the poor agronomic qualities often associated with resistance sources create challenges for breeders developing FHB resistant germplasm especially when relying on conventional phenotypic selection. Employing marker assisted selection (MAS) could be an alternative. Waldron et al. (1999) and Bai et al. (1999) were the first to undertake QTL mapping of FHB resistance. The basic concept of QTL mapping is to test the association of genomic regions with the quantitative traits of interest (Mohan et al., 1997). If a marker is tightly linked to a QTL, the QTL will co-segregate with the marker. If a recombinant inbred population is separated into two groups based on two alleles of the marker, a significant difference in the trait values between the two groups indicates that the DNA marker is linked to the QTL (Collard et al., 2005). The major types of DNA markers which have been used for QTL mapping of FHB resistance

include RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), microsatellite or simple-sequence repeats (SSR) and SNP (single-nucleotide polymorphism). RFLPs and AFLPs were the first molecular markers used for construction of genetic linkage map; however SSR markers have been the most frequently used because of their simplicity, reproducibility and co-dominant nature. SNP markers which can detect individual nucleotide variation and are suitable for high-throughput detection, are considered the future markers of choice for genetics research and breeding (Buerstmayr et al., 2009).

More than 100 QTL for FHB resistance have been reported in wheat (Buerstmayr et al., 2009). However, only a few of these QTL provide stable resistance across environments. Previously identified QTL and markers associated with those QTL are summarized in Table 1.2 at the end of this section. Some QTL have been detected in at least two studies, validating the probability that the QTL is a real effect. Those QTL are found on chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4B, 4D, 5A, 5B, 6A, 6B, 7A and 7B. A few of these have been formally named including *Fhb*1 on chromosome 3BS (Cuthbert et al., 2006), *Fhb*2 on chromosome 6B (Cuthbert et al., 2007), *Fhb*3 on Chromosome 7AS from a Wheat-Leymus introgression line (Qi et al., 2008), *Fhb*4 on Chromosome 4B (Xue et al., 2010), and *Fhb*5 on Chromosome 5A (Xue et al., 2011). However, only the Sumai3-derived *Fhb*1 is now extensively used in breeding programs due to its stable effect on type II resistance across different genetic backgrounds (Anderson et al., 2001; Shen et al., 2003a). Another QTL on chromosome 5A (*Qfhs.ifa-5A*) has also been known as a consistent QTL associated primarily with reduced FHB

incidence (type I resistance) (Buerstmayr et al., 2009) and have been successfully used for MAS (Salameh et al., 2011).

Table 1.2. Summary of mapping studies for FHB resistance including the type of FHB resistance, chromosome (Chr) location, marker used in study, significant markers identified, study population, study population type and literature reference

Type of FHB Resistance	Chr	Marker used in Study	Significant Markers	Study Population	Population Type	References
Type II	2AL, 3BS	RFLP	Xbcd907, XksuH16	Sumai3 (R)/Stoa (MS)		Waldron et al., 1999
Type II	7BL	AFLP	ACT/TGC7	Ning7840(R)/Clark (S)		Bai et al., 1999
Type II	3AL, 6AS, 3BS	RFLP, AFLP, SSR	Xgwm493, Xgwm533, Xbcd941	ND2603(R)/Butte86(MS)	139 RIL	Anderson et al., 2001
Type II	2AL, 3BS, 4BS, 6BS	RFLP, AFLP, SSR	Xgwm493, Xgwm533, XksuH4	Sumai3(R)/Stoa(MS)	112 RIL	Anderson et al., 2001
Type II	2AS, 2BL, 3BS	AFLP, SSR	Xgwm533,Xbarc147, Xgwm120, Xgwm614	Ning7840(R)/Clark(S)	133 RIL	Zhou et al., 2002
Type II	5A, 1B, 3BS	AFLP, SSR	Xgwm493, Xgwm533, Xgwm293,Xgwm304, XgluB1	CM-82036(R)/Remus(S)	239 DH	Buerstmayr et al., 2002
Type II	3BS	SSR	Xgwm533,Xgwm274	Sumai3 derived HRSW lines	36 NIL	Blanco et al., 2003
Type II	3BS	SSR	Xgwm389,Xgwm533, Xgwm493	Ning7840(R)/Wheaton(S)	185 $F_{2:3}$	Zhou et al., 2003

Type II	3BS	SSR	Xgwm389, Xbarc147	Ning7840(R)/IL89-7978(S)	63 $F_{3:4}$	Zhou et al., 2003
Type II	3BS	SSR	Xgwm493,Xgwm533	CM-82036(R)/Remus(S)	DH	Buerstmayr et al., 2003
Type I	5A	SSR	Xgwm293,Xgwm156	CM-82036(R)/Remus(S)	DH	Buerstmayr et al., 2003
Type II	3BS, 6BS, 2DS	SSR	Xbarc133, Xgwm261, Xgwm644	Ning894037(R)/Alondra(MS)	218 RIL	Shen et al., 2003b
Type II	3AS, 3BS, 3BL, 5BL	SSR	Xbarc133,Xgwm247, Xgwm5, Xbarc59	Huapei57-2(R)/Patterson (MS)	163 RIL	Bourdoncle and Ohm, 2003
Type I,II and III	5AS, 3BS, 4B, 2D	SSR	Xgwm96, Xgwm533, Xwmc238, Xgwm539	Wuhan-1(R)/Maringa (MS)	91 DH	Somers et al., 2003
Type II	1B, 3BS	AFLP, SSR	Xgwm161, Xgwm285, XEtcgMctc11, XEtcg.Magc-7	Wangshuibai(R)/Alondra(S)	104 RIL	Zhang et al., 2004
Type II	7AL, 3BS, 1BL, 3BSc	AFLP, SSR	Xbarc344, Xwms1083, Xwms759, pAG/mCTGA149, pAGG/mCAA316, pCGA/mTGCG23	Wangshuibai(R)/Wheaton(S)	139 RIL	Zhou et al., 2004
Type I and II	3A, 5A	SSR	Xgwm720, Xdupw227, Xgwm129, Xbarc197	Frontana(MR) and Remus(S)	210 DH	Steiner et al., 2004

Type II	2B, 3B, 4B, 5A	AFLP, SSR	Xgwm271, Xgwm319, Xgwm77,Xe8m1_1, Xbarc495, Xgwm149, Xbarc56, Xbarc165	Ernie / MO 94-317	243 RILs	McKendry et al., 2004
Type II	3BS, 4BL, 5DL	SSR, TRAP	Xbarc 239, Xbarc 1096, Xgwm 533	Chokwang(R)/Clark(S)	79 RIL	Yang et al., 2005a
Type I	3AS, 5AS, 3BS, 3BSc, 6BS, 2DS, 4DL	SSR	Xwm539, Xwmc16, Xwmc533, Xwmc52, Xwmc612, Xwmc331, Xwm293, Xwmc397, Xgwm644	DH181(R)/AC Foremost(S)	174 DH	Yang et al., 2005b
Type II	3BS, 6BS, 2DS, 7BL	SSR	Xwmc144, Xwmc533, Xwmc397, Xwmc526	DH181(R)/AC Foremost(S)	174 DH	Yang et al., 2005b
Type IV	1DL, 2DS, 3BS, 3BSc, 6BS, 4DL	SSR	Xgdm126 , Xwmc144, Xgwm533,Xwmc527, Xwmc397,Xwmc331	DH181(R)/AC Foremost(S)	174 DH	Yang et al., 2005b
Type I and II	6AL, 1B, 2BL, 7BS	AFLP, SSR	XP66M55_242, XS25M12_206, XS26M23_365, XS23M21_497	Dream(R)/Lynx(S)	145 RIL	Schmolke et al., 2005
Type II	3B, 5B	SSR	Xgwm533, Xgwm335	Wangshuibai(R)/Alondra‘s’(S)	134 DH	Jia et al., 2005a

Type III	3BS	SSR	Xbarc147	CM-82036(R)/and Remus	96 DH	Lemmens et al., 2005
Type I, II and III	5AS, 3BS	SSR	Xbarc 133, Xgwm 493, Xbarc 117, Xbarc 56	W14(R)/Pion2684(S)	96 DH	Chen et al., 2006
Type II	6A, 3B, 2D, 4D	AFLP, SSR	XmCTG.pACT132, Xgwm533,Xgwm493, XmACAG.pACT134, Xcfd84-X, Xwmc331	Chinese spring Sumai3 disomic substitution line(R)/Annong 8455(S)	92 RIL	Ma et al., 2006b
Type II	3BS	SSR, STS	STS3B-80, STS3B-142, STS3B-66	Sumai3*5(R)/Thatcher(S) and HC374(R)/3*98B69-L47(S)	RIL	Cuthbert et al., 2006
Type II	3AL, 7AS, 1BL	AFLP, SSR	Xe32m65_10, Xgwm720, Xgwm1121, Xgwm233	Frontana (MR)/Seri82(S)	171 $F_{3:5}$	Mardi et al., 2006
Type I	5A, 4B, 5B	SSR	Xwmc96, Xgwm513, Xgwm149	Wangshuibai (R)/Nanda2419	RIL	Lin et al., 2006
Type II	1AS, 3BS, 7BS, 2DL	SSR	Xwmc24, Xbarc148, Xgwm533, Xgwm493, Xgwm400, Xgwm573, Xgwm157, Xwmc41	CJ9306(R)/Veery(S)	152 RIL	Jiang et al., 2007a; b
Type III	1AS, 5AS, 3BS, 2DL,	SSR	Xgwm533, Xgwm539, Xbarc148, Xgwm425	CJ9306(R)/Veery(S)	152 RIL	Jiang et al., 2007a; b
Type II	6B	SSR	Xgwm133, Xgwm644	BW278(R)/AC Foremost(S)	89 RIL	Cuthbert et al., 2007

Type II and III	1AL, 7AL 1BL, 2AS, 6BS	AFLP, DArT SSR	Xbarc213, XDuPw2, wPt-3475, P45/M60-265, barc124	Arina (MR)/NK93604(MR)	93 DH	Semagn et al., 2007
Type II	5A, 2B, 3B, 4BL	AFLP, SSR	Xbarc165, Xgwm276, Xgwm285, Xgwm495	Ernie(MR)/MO 94-317(S)	233 RIL	Liu et al., 2007
Type II	3AS, 5AS, 3BS, 4B, 5DL	AFLP, SSR	Xbarc 147, Xwmc 47, XpCGA-mGTG352, Xgwm 292, Xbarc 180	Wangshuibai(R)/Wheaton(S)	139 RIL	Yu et al., 2008
Type II	1A, 5AS, 7AL, 3BS, 3DL, 5DL	AFLP, SSR	Xbarc 147, Xgwm 376, Xwms 1083, XpCAT-mTGCG188, Xgwm97, Xbarc 180, XpAG-mTCGA338	Wangshuibai(R)/Wheaton(S)	139 RIL	Yu et al., 2008
Type III	1A, 5AS, 7AL, 1BL, 3BS, 5DL	AFLP, SSR	Xbarc 147, Xbarc 376, XpACTG-mTGC521, Xbarc 180, Xgwm 212, Xwms 759, Xwms 1083	Wangshuibai(R)/Wheaton(S)	139 RIL	Yu et al., 2008
Type II	1A, 2BL	AFLP, SSR	XS26M13_329, XS17M16_115	G16-92(R)/Hussar(S)	136 RIL	Schmolke et al., 2008

Type II	2DS		Xgwm261, Xgwm296	Sumai3(R)/Gamenya(S)	118 DH	Handa et al., 2008
Type I, II and IV	2B, 3B, 4B, 6B	SSR	Xbarc55,Xbarc139, Xgwm495, Xwmc47, Xgwm513,Xwmc494	IL94-1653/Patton	269 RIL	Bonin and Kolb, 2009
Type II	5BL, 6BS, 7BS	AFLP, SSR	Barc72, Xgwm335, Xp75M60-563, Xgwm644	Pelikan(S)/G93010(R)	122 $F_{6:8}$	Haberle et al., 2009
Type II	7A, 1B, 3B, 6B, 2D	AFLP, SSR	Xwmc479, Xwmc737, Xwmc231, Xwmc503, Xwmc134	Wangshuibai(R)/Sy95-7(S)	194 $F_{2:3}$	Zhang et al., 2010
Type II	2A, 5A, 2B, 5B	AFLP, SSR	Xs11m24_10, Xs20m13_4, Xs24m19_6, Xgwm497	*T. macha*(R)/Furore(S)	321 RIL	Buerstmayr et al., 2011
Type II and type III	7AC, 3BS	SSR, STS	Xumn10, Xwmc17	CS-Sumai3-7ADSL	191 CRIL	Jayatilake et al., 2011
Type II	7DL	SSR	Xwmc121	Haiyanzhong (R)/Wheaton	136 RIL	Li et al., 2011
Type II	7AL, 3BS	SSR	Xbarc147, Xgwm276, Xbarc121	Huangfangzhu(R)/Wheaton	106 RIL	Li et al., 2012
Type II	3B, 3A, 5A	SSR	Xgwm533, Xgwm493 Xgwwm566, Xwmc307, Xbarc141, Xwmc651	Baishanyuehuang(R)/Jagger(S)	188 RIL	Zhang et al., 2012

Type II	3B, 4B, 6B, 7B	SSR, AFLP	Rht-B1, Xgwm356, Xgwm816	*T. dicoccum*-161/ DS-131621	117 Backcross	Buerstmayr et al., 2012
Type II	4B, 6B, 7B	SSR, AFLP	Xbarc133, Rht-B1, Xs24m25_f4, Xs24m12_f6h5	*T. dicoccum*-161/ Floradur	120 Backcross	Buerstmayr et al., 2012
Type II	4B, 7B	SSR, AFLP	Rht-B1, Xs24m12_f6h5	*T. dicoccum*-161/ Helidur	120 Backcross	Buerstmayr et al., 2012
Type II	3B	SNP	Xsnp3BS-11, Xsnp3BS-8	Ning 7840/Clark	71 RILs	Bernardo et al., 2012

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Chapter 2

Mapping QTL Conferring Resistance to Fusarium Head Blight in the Spring Wheat Cultivar 'UI Stone'

1. Introduction

Fusarium head blight (FHB) is one of the most destructive diseases of wheat causing significant yield losses and quality reduction in the humid areas of the world (Bai and Shaner, 2004; Ma et al., 2006a). FHB is also an emerging wheat disease in Southeastern Idaho of the US (Windes, 2007; Chen et al., 2013). Breeding of wheat cultivars resistant to FHB is one of the best strategies to minimize crop and grain quality losses due to this disease (Buerstmayr et al., 2009). Resistance to FHB is a quantitative trait and strongly influenced by environments (Bai and Shaner, 2004) which is a great challenge for breeders if relying only on phenotypic selection. Identification of QTL, tagging QTL with suitable molecular markers and adopting MAS is an alternative approach which can be implemented to enhance the phenotypic selection in numerous quantitative traits including FHB (Buerstmayr et al., 2009). QTL mapping studies have been conducted using known resistance sources Sumai3 and its derivatives, in which two major QTL on 3BS and 5AS were identified and highly recommended for use in MAS (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2009; Salameh et al., 2011). The soft white spring wheat cultivar 'UI Stone' (PI660550) has good resistance to FHB, high yield potential, excellent end-use quality, and tolerance to cereal cyst nematodes (Chen et al., 2013). UI Stone was crossed with another high yielding cultivar 'Alturas' (PI 620631, Souza et al., 2004), which is less resistant to FHB than UI Stone (Chen et al., 2013). The

objectives of the present study were to map and characterize QTL associated with FHB resistance in RILs derived from the cross between UI Stone and Alturas.

2. Materials and Methods

2.1. Mapping Population

A population of recombinant inbred lines (RILs) was developed from the cross between UI Stone and Alturas using the modified single seed descent method (Figure 2.1). UI Stone and Alturas were both released by University of Idaho. UI stone has superior grain yield under non-irrigated and irrigated production conditions and better resistance to FHB than Alturas (Chen et al., 2013). Resistance to FHB in UI Stone was first documented among adapted cultivars in the Pacific Northwest based on data derived from multi-state screening of FHB resistance in Minnesota, Montana and Idaho (Chen et al., 2013). The original cross was made in the field in the summer of 2010 and UI Stone was used as the female parent. Six F_1 plants of the cross were planted in the greenhouse in the fall of 2010 to produce F_2 seeds. After harvest, seeds from the individual F_2 plants were planted in 4-row field plots in the spring of 2011. Fifty heads from the six F_2 plots were randomly harvested and threshed individually to acquire F_3 generations. One seed of each F_3 head was planted in 4" x 4" pots in the greenhouse in October, 2011 to obtain the F_4 generation, which comprised 300 plants. The F_4 to F_6 generations were advanced using the modified single seed descent and simultaneously screened for type II resistance to FHB in greenhouse in Aberdeen, ID (Figure 2.1).

2.2. Inoculum Preparation and FHB Evaluation

2.2.1. Preparation of Macroconidial Suspension

Macroconidial inoculum was used for FHB disease screening. FHB infected seeds were randomly collected from a research wheat field in Aberdeen, ID were used to develop cultures for the macroconidial suspension. Potato Dextrose Agar (PDA) media was used to grow the FHB fungus in the lab (Figure 2.2). FHB infected seeds were surface sterilized and then transferred to PDA media. Surface sterilization was done to kill any fungus present on the seed surface and to allow the mycelium of the pathogen to grow from the infected seed. The culture plates were kept in an incubator at 31±2°C to provide a favorable temperature for fungal growth. The plates were sub-cultured after 7 days of first culture to obtain multiple *Fusarium* cultures. Two sub-cultures were done before preparing the inoculum. After the second sub-culture, when the entire PDA plate was homogeneously covered by fungal mycelium, the plates were used to prepare a macroconidial suspension. A few drops of autoclaved double distilled water (dd H_20) was poured over mycelium growth and gently scrapped with the help of Cell Scrapper® to release macroconidia from hyphae. The concentration of the working solution was measured with a lumicyte® haemocytometer (Propper Manufacturing Co., Inc., Long Island City, New York), and the concentration was adjusted to 8-10 x 10^4 macroconidia per microliter.

2.2.2. Disease Screening in Greenhouse

A soil mixture was prepared by mixing vermiculite (Vermiculture, Therm-O-Rock, West, INC, Chandler, Arizona), peat moss (Sungro® Horticulture) and sand in a 1:1:1

ratio. Osmocote® Classic control release fertilizer (14-14-14) was added to provide optimum nutrition to plants.

A total of four disease screening experiments were conducted in greenhouse in the F_4, F_5, and F_6 generations in May 2012 (151 F_4 lines, FHB2012A), November 2012 (258 F_5, FHB2012B), March 2013 (258 F_5, FHB2013) and March 2014 (226 F_6, FHB2014), respectively (Figure 2.1). Both parents were used as checks and repeated five to seven times in individual experiment and randomly planted among the RILs within each experiment. All RILs and parental lines were planted with four replications. In this study 151 lines, that were common across the four experiments, were used in data analysis.

The inoculation was conducted during anthesis (Figure 2.3). Two to three spikes in each plant were inoculated in the central spikelet of a spike with approximately 5µl of macroconidial suspension (~250-500 conidia/spike) using a pipette dropper (Figure 2.4). The inoculated plants were put under automatic misting benches for 48 hours to provide favorable humidity for infection (Figure 2.5). After 48 hours of incubation, the plants were then moved to greenhouse benches at 65-70°F with 12 hour supplemental daylight. Type II resistance was evaluated as disease severity at 21 and 28 days post inoculation (DPI). Therefore, two data sets (21 and 28 DPI) were recorded in each greenhouse experiments.

The disease severity was calculated by the percentage of infected spikelets (PIS) of total spikelets in the spike (Figure 2.6). The mean disease severity of the two to three spikes was calculated for each line to be used in statistical analysis.

2.3. Phenotypic Data Analysis

The histograms of FHB severity in each data set were drawn to visualize the genetic variation among RILs (Figure 2.7). Spearman correlation coefficients for all data sets were estimated to verify similarity or difference among data sets to determine if a pooled data set could be derived.

Analyses of variance (ANOVA) were performed using JMP software (SAS Institute Inc.). Broad sense heritability (H^2) was calculated based on the ANOVA results using the formula $H^2=\sigma_g^2/\sigma_p^2$, where σ_g^2 is the genotypic variance and σ_p^2 is the phenotypic variance (Kobayashi and Koyama, 2002; Holland et al., 2003; Lu et al., 2013). These analyses were performed for each individual experiment as well as across all experiments. Phenotypic variance (σ_p^2) for individual experiments, and across all experiments, was calculated using formula $\sigma_p^2=\sigma_g^2+\sigma_e^2/r$ and $\sigma_p^2=\sigma_g^2+\sigma_{gy}^2/y+\sigma_e^2/ry$ respectively, where σ_e is error variance, σ_{gy} is genotype* experiment variance, r is number of replication and y is number of environments.

2.4. DNA Extraction and Genotyping

Five to six pieces of approximately 3 centimeter of leaf tissues were collected at the two leaf stage of plant for DNA extraction in 96 well plates according to protocol set by Western Regional Small Grain Genotyping Laboratory (WRSGGL), based in Pullman, Washington. Collected leaf tissues were lyophilized in a freeze dryer. The protocol for lyophilization was to set the freeze dryer to -50°C for 72 hours and then gradually increase the temperature to -20°C, -10°C, 0°C and 10°C for 5 hours, 2 hours, 1 hour and 1 hour respectively. Lyophilized tissues were stored at room temperature. DNA extraction

and genotyping of RIL were performed in WRSGGL. Lyophilized tissues were ground in a Mixer Mill and total DNA was isolated using a QIAGEN BioSprint 96 Robotic Workstation (Catalog no. 9000852; QIAGEN Inc., Valencia, CA) and the BioSprint 96 DNA Plant Kit (Catalog no. 941557; QIAGEN Inc., Valencia, CA). The DNA concentration was quantified with a spectrophotometer.

2.4.1. Simple Sequence Repeat (SSR)

SSR markers including BARC (Beltsville Agriculture Research Center; Song et al., 2005) , WMC (Wheat Microsatellite Consortium; Gupta et al., 2002; Somers et al., 2004), GWM (Gatersleben Wheat Microsatellites; Röder et al., 1995, 1998; Ganal and Röder, 2007), GDM (Gatersleben D-genome Microsatellites; Pestsova et al., 2000), CFA and CFD (INRA Clermont-Ferrand, France; Guyomarc'h et al., 2002; Sourdille et al., 2004) were used to screen the parents and polymorphic primer pairs were used to genotype 151 RILs. PCR amplification was done in a DYAD thermocycler (MJ Research, Waltham, MA). A 12 µl PCR mix contained 1.2 µl of 10X PCR buffer, 0.96 µl of 2.5 mM dNTP, 0.48 µl of 25 mM $MgCl_2$, 0.24 µl of 10 mM fluorescent dye (FAM, VIC, NED, PET), 0.3 µl of 10 mM reverse primer, 0.06 µl of 10 mM forward primer with M13-tail added to 5'-end (5'- ACGACGTTGTAAAACGAC), 0.2 µl of 5 u/µl of Taq polymerase, 5 µl of 25 ng genomic DNA and 3.56 µl of molecular grade water. An individual SSR marker was tagged with different fluorescent dye so that more than two PCR products could be pooled into a single plate for fragment analysis in an ABI (Applied Biosystems) genetic analyzer. The PCR were run in gene mate 384 well plate with parents being replicated four times per plate. The PCR program included initial denature at 94°C for 5 min; 41 cycles of 94°C

for 30 sec, 61°C annealing temperature (may vary for different primers as documented in GrainGenes 2.0) for 45 sec and 72°C for 1 min; a final extension 72°C for 10 min; and final incubation at 4°C for forever. Amplified PCR products from three separate PCR plates labeled with different florescent dyes (FAM, VIC and PET) were pooled using a Beckman Coulter Biomek® NX^P robotic platform and analyzed in an ABI 3730*xl* or 3130*xl* Genetic Analyzer. A robot program was set up so that each pool contained 3 µl of FAM, 3 µl of VIC, 6 µl of PET and 13 µl of water. Each well in ABI plate contained 1.5 µl of Cassul445 ladder, 8.5 µl of Formamide and 3 µl of pooled samples. The ABI plate was denatured at 95°C for 5 minutes before putting it into the Genetic Analyzer. Data scoring was done using GeneMarker 1.50 (SoftGenetics LLC. State College, PA, USA). Allele sizes (base pair) were converted to 'A' (UI Stone allele), 'B' (Alturas allele), 'H' (heterozygote), and '-' (missing data) for mapping purposes. All 'H' scores were converted to missing data points to avoid their treatment as another genomic class.

2.4.2. Single Nucleotide Polymorphism (SNP)

One hundred and fifty nine polymorphic SNP markers were selected based on Illumina Genome Studio data obtained from USDA, National Small Grains Research Facility located at Aberdeen, ID. However, only seventy seven polymorphic SNP markers were informative (polymorphic) using the Sequenom Platform and were used to genotype the RILs. All steps were performed using the standard protocol developed by WRSGGL.

2.5. Linkage Map Construction and QTL Analysis

MapMaker 3.0b (Lander et al., 1987) was used to construct linkage maps. Linkage groups were established using a two point 'group' command with a logarithm of odds (LOD) score threshold 3.0 and the recombination distance threshold 40.0. Kosambi mapping function (Kosambi, 1943) was employed to convert recombination distances between markers into centimorgan (cM) map units. For each linkage group, the marker order was determined in several steps. For linkage group 3-8 markers, the compare command was used for selecting the best order. The orders were then verified using the 'ripple' command. The 'try' command was used to place unassigned markers within a linkage group. The 'three point' command was used to examine the right order of markers within each linkage group.

The linkage map was drawn using the computer program Mapchart 2.2 (Voorrips, 2002). Linkage groups were assigned to chromosomes using GrainGenes 2.0 (http://wheat.pw.usda.gov) and other published consensus maps (Röder et al., 1998; Gupta et al., 2002; Somers et al., 2004; Song et al., 2005).

QTL analysis was performed using single marker analysis and composite interval mapping (CIM) methods. Single marker analysis is the simplest way to find marker association with putative QTL and could be performed without drawing a linkage map. Single marker analysis was done using one way ANOVA in JMP. CIM is considered to be more precise and effective at mapping QTL compared to single marker analysis because this method combines interval mapping with linear regression and includes additional markers in the statistical model in addition to an adjacent pair of linked markers (Collard et al., 2005). In this study, CIM was performed by using WinQTL Cartographer Ver. 2.5

(Wang et al., 2007). The CIM was run in a standard model with five markers as control in a forward regression method. The window size and walking speed were 10 cM and 1 cM, respectively. WinQTL Cartographer was also used to calculate coefficient of determination (R^2) values and additive effects of significant QTL.

Multiple linear regression analysis was performed in JMP to test the combined effect of major QTL identified in this study.

3. Results

3.1. FHB Severity in Parents and RILs

UI Stone consistently showed superior resistance to FHB compared to Alturas across all four greenhouse experiments. The two FHB severity readings (21 DPI and 28 DPI) were highly correlated, therefore, the mean FHB severity of the two readings was calculated which ranged from 7.3% to 24.6% for UI Stone and 15.0% to 67.9% for Alturas (Table 2.1) across all experiments. The mean FHB severity of UI stone was significantly different ($p<0.05$) from Alturas in the 2012 experiments, however, the difference was not statistically significant in the 2013 and 2014 experiments (Table 2.2).

3.2. Phenotype Data Analysis

Eight data sets were generated from four experiments. Each experiment contained two data sets from 21 days and 28 DPI. FHB severity at 21 and 28 DPI in each individual experiment was highly correlated ($p<0.0001$) (Table 2.4), while the FHB severity between the four experiments was not significant. Therefore, the mean of the two readings of all RILs in the four experiments was calculated and their frequency distribution histograms

were displayed in Figure 2.7. The mean disease severity of RILs ranged from 10.4% to 46.3%, while the histograms varied in different experiments, suggesting the confounding effect of environment. More variation of disease severity was observed in the summer 2012 experiment (FHB2012A) with 46.3% of the mean severity of all RILs (Table 2.3). Transgressive segregation was observed in all experiments.

Significant genotype effects were observed ($p<0.0001$) for FHB severity in each individual experiment as well as across all experiments (Table 2.5, 2.6). A significant environment and genotype by environment effect was also observed. However, the replication effect was not significant (Table 2.6). Broad sense heritability ranged from 0.49 to 0.71 in individual experiments with overall heritability of 0.33 across all experiments (Table 2.5, 2.6).

3.3. Linkage Map

Seventy Seven SSR markers, polymorphic between two parents UI Stone and Alturas, were utilized to genotype RILs population. Additionally, 159 polymorphic SNP markers selected based on Illumina Genome Studio data were also utilized to genotype the RILs population. Out of 159 polymorphic SNP markers originally selected only 77 were able to be genotyped using the Sequenom platform. Therefore, 154 markers were used in the linkage analysis. A total of 23 linkage groups were determined at LOD 3.0 consisting of 118 markers while 36 markers were unlinked. The 23 linkage groups covered a genetic distance of 2789.2 cM and comprised of 640.3 cM (23%), 1132.8 cM (41%) and 1016.1 cM (36%) for the A, B and D genome, respectively. The 23 linkage group ranged from 13.6 cM to 220.5 cM accommodating 2 to 10 markers (Figure 2.8).

3.4. QTL Analysis

With the limited markers mapped in this population it was necessary to conduct both single marker analysis and CIM to identify potential QTL and markers associated with type II FHB resistance.

Single marker analysis identified 17 significant ($p<0.01$) markers on 9 different chromosomes 1A, 1B, 1D, 2B, 2D, 3B, 3D, 6D and 7D associated with type II FHB resistance (Table 2.7). However, only two SSR markers (umn10 and barc133) were significant in all experiments at the significance level (p-value) 0.05.

CIM analysis identified 4 significant QTL (LOD> 2.5) on four different chromosomes, 1D, 2B, 2D, and 4A, when disease severity data sets from individual experiments were used (Table 2.8, Figure 2.9). The four QTLs accounted for 6.9 to 46.9% of phenotypic variation among the four experiments (Table 2.8). Two QTL (*QFhbuis.ab-2B* and *QFhbuis.ab-2D.1)* were detected from data set FHB2012A, one from each of the FHB2012B (*QFhbalt.ab-4A*) and FHB2014 (*QFhbuis.ab-1D*) data sets, and none from data set FHB2013 (Table 2.8). When the mean disease severity of the four experiments was used, three more significant QTL (*QFhbuis.ab-2B*, *QFhbuis.ab-2D.2, QFhbuis.ab-3B*) were identified, which explained 8.73 to 22.85% of phenotypic variation of the mean disease severity.

The resistant alleles at the five QTL, *QFhbuis.ab-1D*, *QFhbuis.ab-2B* and *QFhbuis.ab-2D.1, QFhbuis.ab-2D.2, QFhbuis.ab-3B* were contributed by UI Stone; whereas the resistant allele at QTL, *QFhbalt.ab-4A* was derived from Alturas (Table 2.8).

Two QTL *QFhbuis.ab-2B* and *QFhbuis.ab-3B* were identified in both single marker and CIM methods and together explained 23.6 to 24.8% (data not shown).

4. Discussion

FHB is a complex disease and is profoundly influenced by the environment (Parry et al., 1995; Jia et al., 2005b). The variation in heritability from a low to moderate value (0.49 to 0.71) suggests that environmental factors play an important role in FHB severity in addition to the genotypic effect. Additional factors such as population size, genetic marker data and error in phenotypic evaluation may influence the detection of QTL (Collard et al., 2005; Cuthbert et al., 2006). In this study phenotypic evaluation of 151 RILs were replicated and done at multiple times to examine the influence of environment. The population size used in this study was larger than in some studies (Anderson et al., 2001; Jia et al., 2005b; Lemmens et al., 2005; Semagn et al., 2007) but smaller than in others (McKendry et al., 2004; Liu et al., 2007; Bonin and Kolb, 2009; Buerstmayr et al., 2012). Error in phenotypic evaluation was minimized by performing experiments in a greenhouse with the point inoculation method (Bai et al., 1999) (Figure 2.4). The frequency distribution of disease severity in RILs was significantly different, indicating environment and genotype x environment effects (Figure 2.7).

Because the two parents are adapted cultivars, there was low polymorphism and therefore a limited number of markers were mapped in this population. The linkage maps constructed using 154 markers only cover a genetic distance of 2789.2 cM. However, the order of mapped markers are in agreement with previously published consensus maps (GrainGenes 2.0; Somers et al., 2004).

Using the unsaturated genetic maps, two QTL *QFhbuis.ab-2B* and *QFhbuis.ab-3B* were identified by both a single marker and CIM and significantly associated with the mean disease severity of the four experiments. *QFhbuis.ab-2B* was also associated with

disease severity in experiment FHB2012A by both a single marker and CIM. *QFhbuis.ab-2B* explained up to 32.4% of mean disease severity and was flanked by markers IWA4866 and barc18. QTL on chromosome 2B have been reported in several papers. Almost the entire length of chromosome 2B has been associated with FHB resistance (Buerstmayr et al., 2009), however, QTL found in this study is in the centromeric region of a chromosome as reported in studies of Gervais et al. (2003), Somers et al. (2006) and Liu et al. (2007). The resistant sources in all three of these studies (Gervais et al., 2003; Somers et al., 2006; Liu et al. 2007) were not related to Sumai3, in fact, they were an adapted winter wheat or tetraploid wheat cultivars. These results suggest that chromosome 2B has a major QTL contributing to FHB resistance from non-Sumai3 sources.

The *QFhbuis.ab-3B* explained up to 12% of mean disease severity and was flanked by markers umn10 and barc133 which could be similar to the *Fhb1* locus identified in Sumai3 and its derived sources. However, both UI Stone and Alturas lack genetic background from Sumai3. Therefore, the resistant allele of *QFhbuis.ab-3B* could be different from the one derived from Sumai3. So, it is necessary to conduct additional studies to uncover the actual allelic effect of the UI Stone 3B QTL on FHB resistance.

The QTL *QFhbuis.ab-1D*, *QFhbuis.ab-2D.1, QFhbuis.ab-2D.2,* and *QFhbalt.ab-4A* were identified by either single marker or CIM methods and associated with disease severity in a specific environment. To ascertain the real effect of these QTL it is necessary to saturate the genetic maps using additional markers and assess disease severity of RILs in additional experiments. The QTL on 1D explained 6.9% phenotypic variation in RIL for type II FHB resistance and was flanked by markers IWA7276 and cfd48 with the peak at 23.21cM and may be similar to the QTL as reported by Ittu et al. (2000) and Klahr et al.

(2007) in winter wheat cultivars. The QTL on 2D distal to the centromere accounted 28.2% of the phenotypic variation and was flanked by markers gwm539 and IWA7117, which is the same QTL as that published by Somers et al. (2003) who also reported gwm539 as a significant marker. The other QTL on 2D proximal to centromere explained 10.2% of phenotypic variation and was flanked by markers barc353 and IWA4865, and it could be the same QTL as reported by Handa et al. (2008). QTL on chromosome 4A proximal to the centromere was contributed by Alturas which explained up to 46.9% of the phenotypic variation and was flanked by markers IWA2723 and IWA5200. Paillard et al. (2004) reported a QTL on chromosome 4A in the Swiss winter wheat 'Arina', however they found it distal to the centromere. The QTL identified in this study is more in agreement with the region identified by Steed et al. (2005) derived from *Triticum macha*, but this QTL region was reported to contribute to type I resistance. This suggests that QTL on 4A of Alturas could be a valuable source of FHB resistance and needs further investigation.

This study also identified 4 lines that consistently showed better resistance to FHB than UI Stone across all experiments with very high yield based on one year replicated yield trial data (data not shown). Therefore, these RILs could be used as either germplasm or released as new FHB resistant cultivars after further evaluation.

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Figure 2.1. Schematic diagram of UI Stone/Alturas mapping population development and FHB evaluation

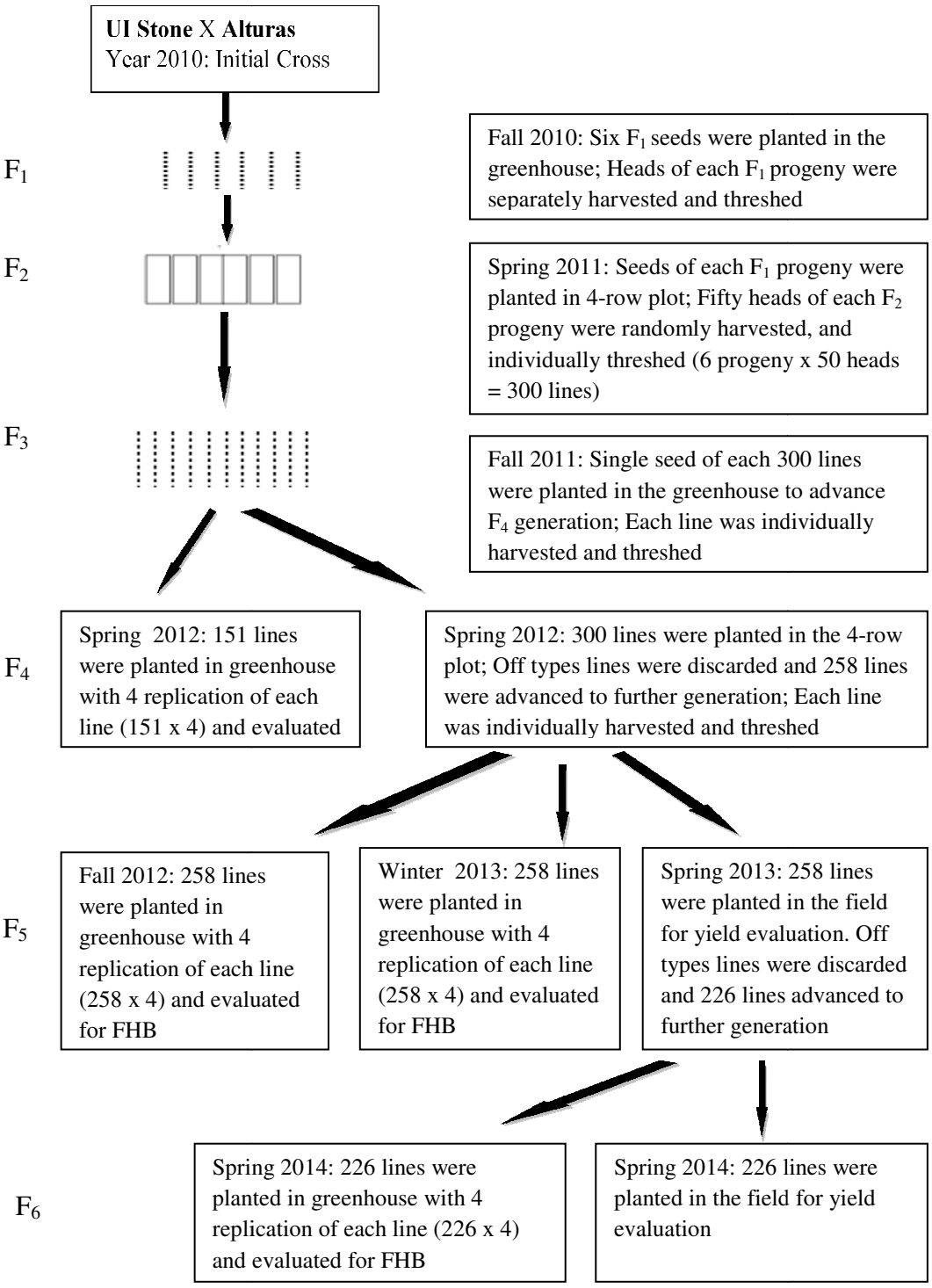


Figure 2.2. Growing FHB fungi as a source of inoculum in potato dextrose agar in the lab

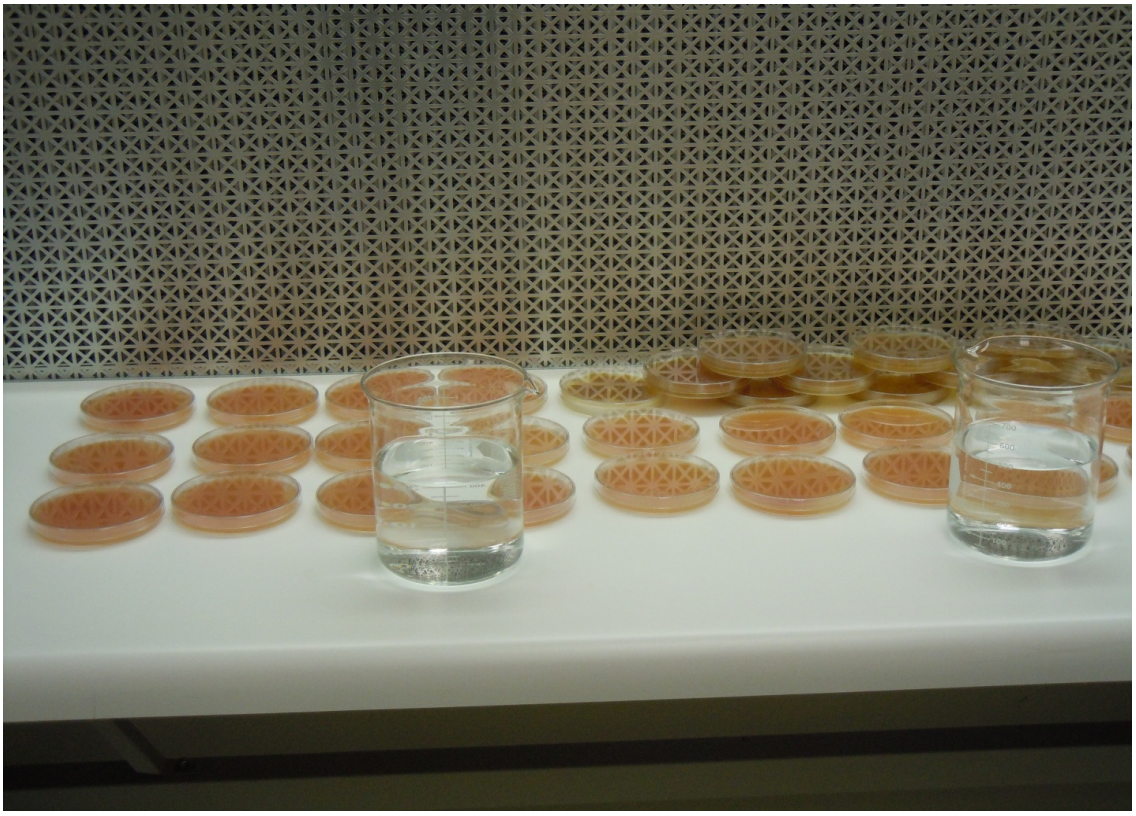


Figure 2.3. FHB disease screening in greenhouse

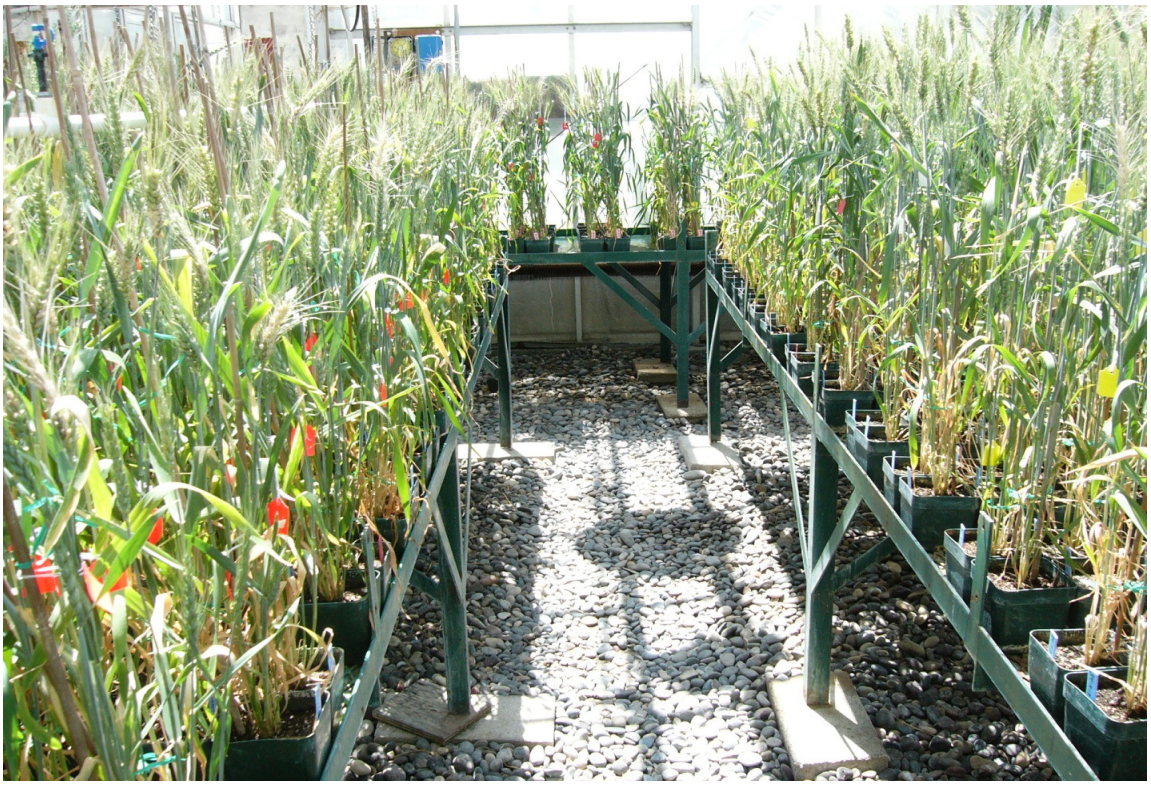


Figure 2.4. Point inoculation of FHB macroconidial suspension into a central spikelet

Figure 2.5. FHB inoculated plants under automatic misting system to provide favorable humidity for disease development

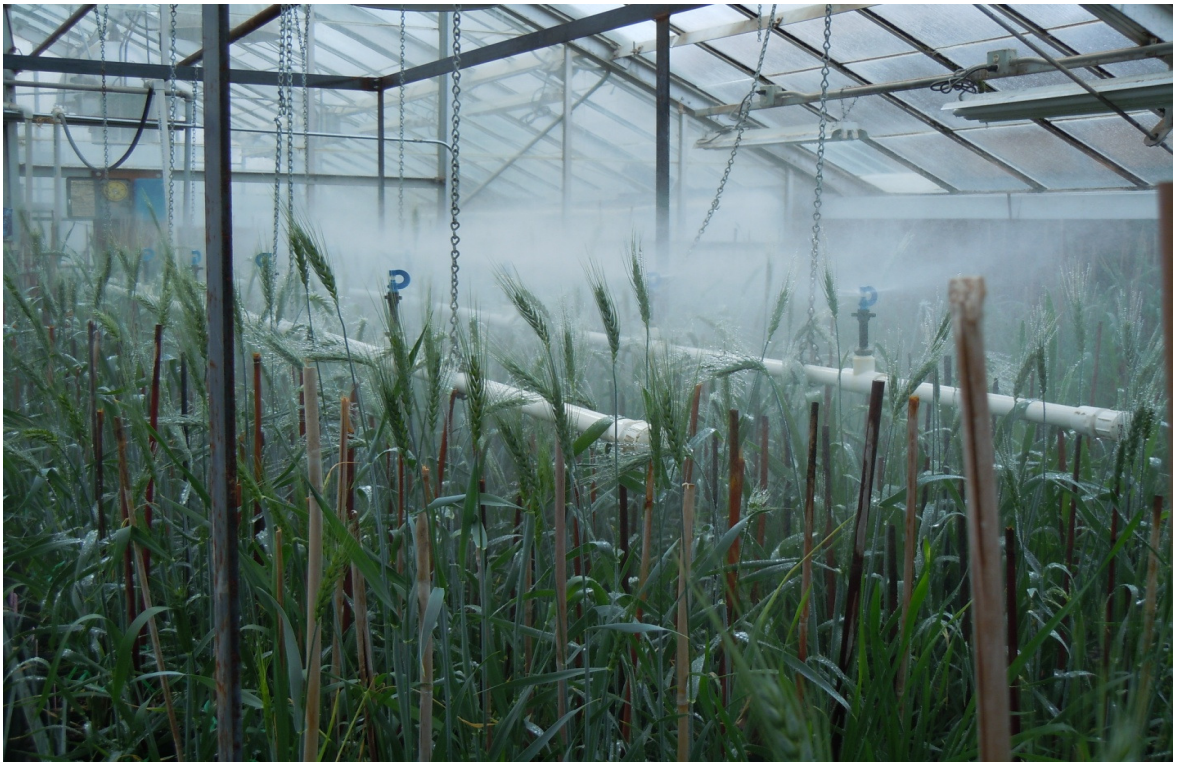


Figure 2.6. Variations in disease severity

Figure 2.7. Frequency distribution of 151 RILs for FHB severity

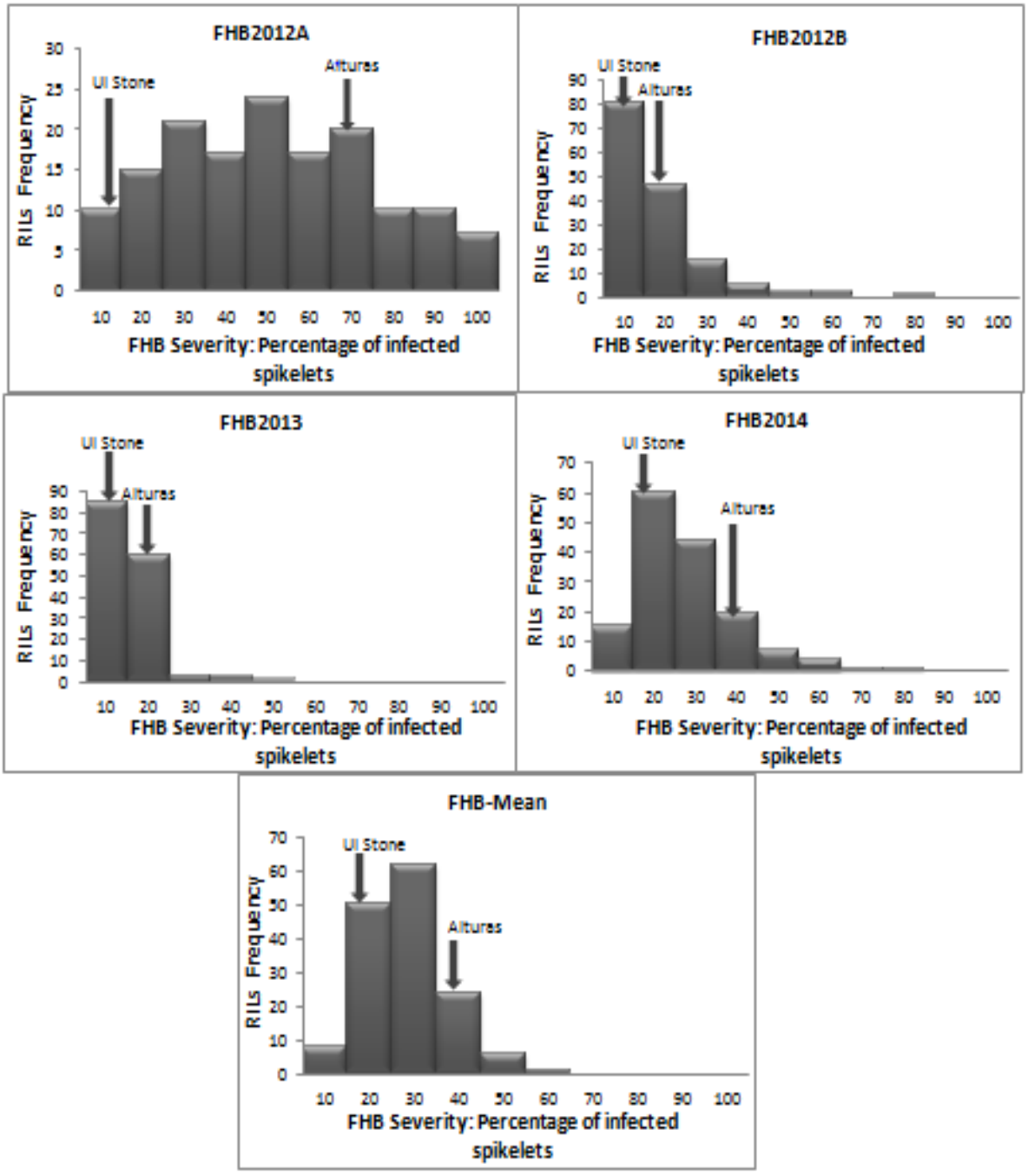


Figure 2.8. Linkage groups comprised of 118 SNP and SSR markers and their relative position in the genome

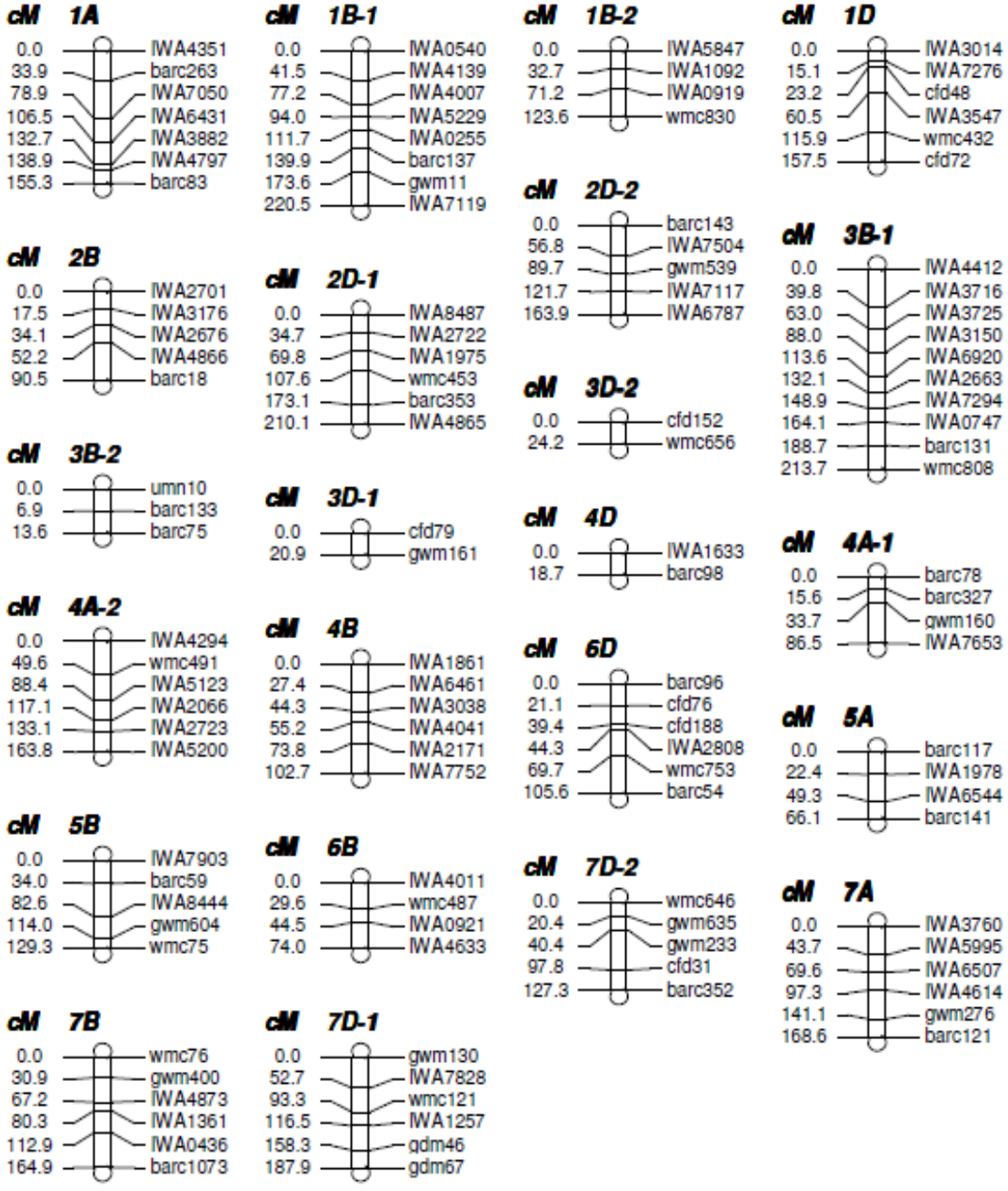
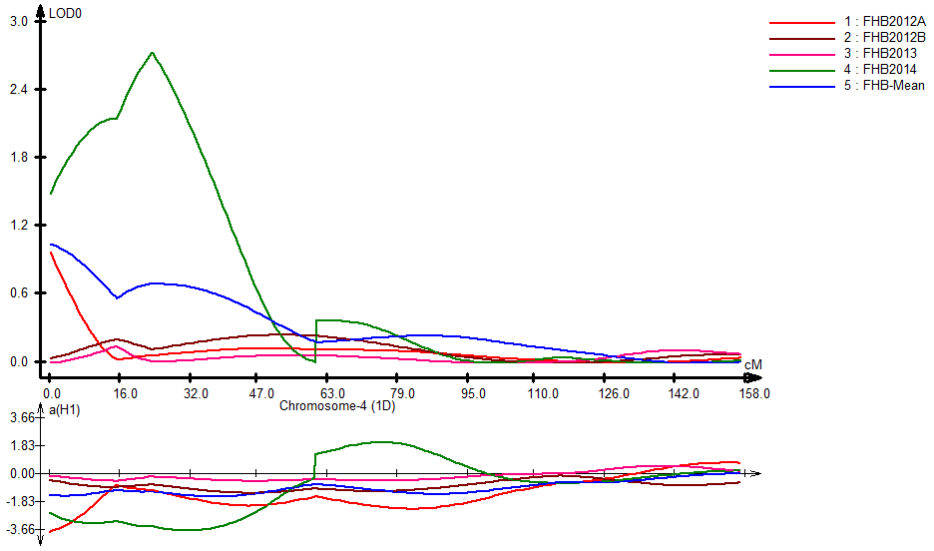
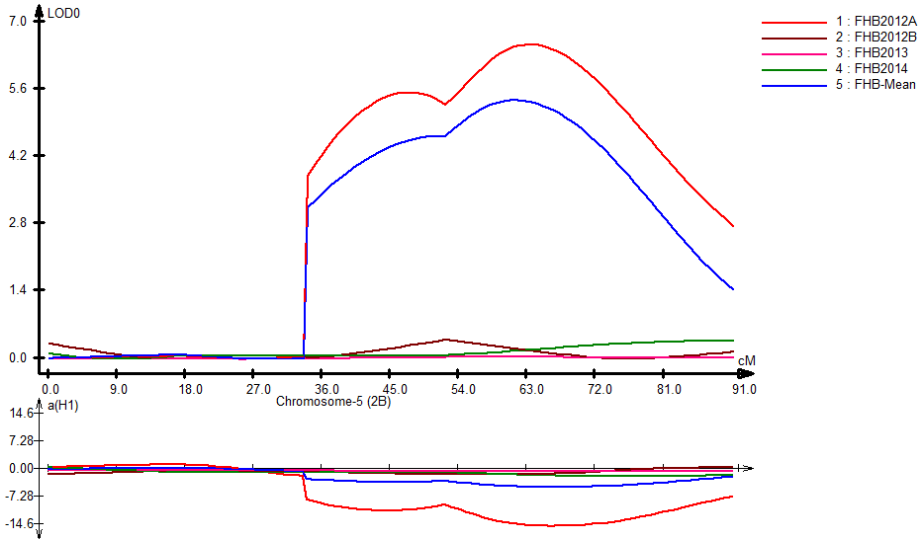


Figure 2.9. Composite interval mapping (CIM) of QTL associated with type II FHB resistance in RIL population derived from UI Stone/Alturas based on four greenhouse experiments over three years (a) chromosome 1D (b) chromosome 2B (c) chromosome 2D-1 (d) chromosome 2D-2 (e) chromosome 3B (f) chromosome 4A

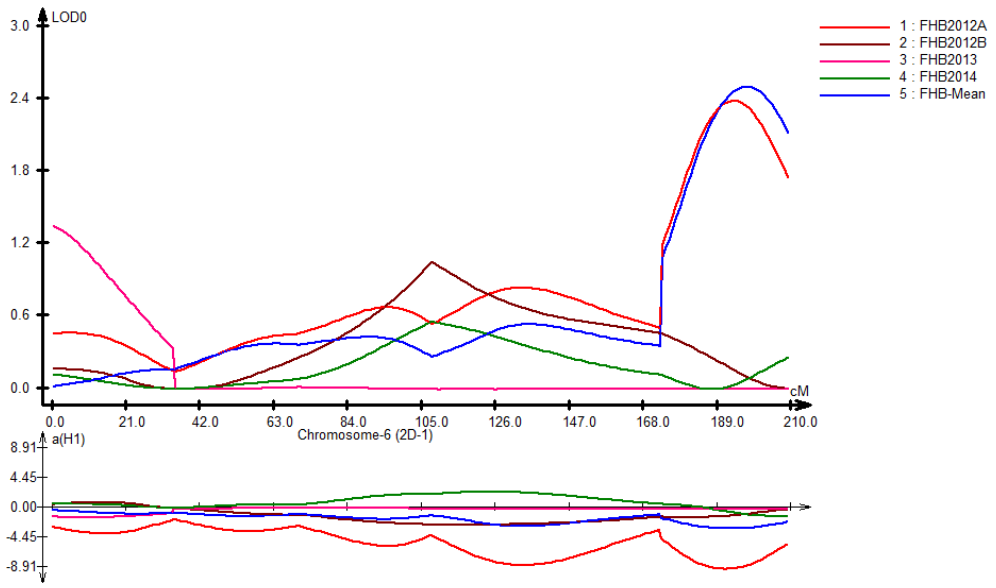
a)



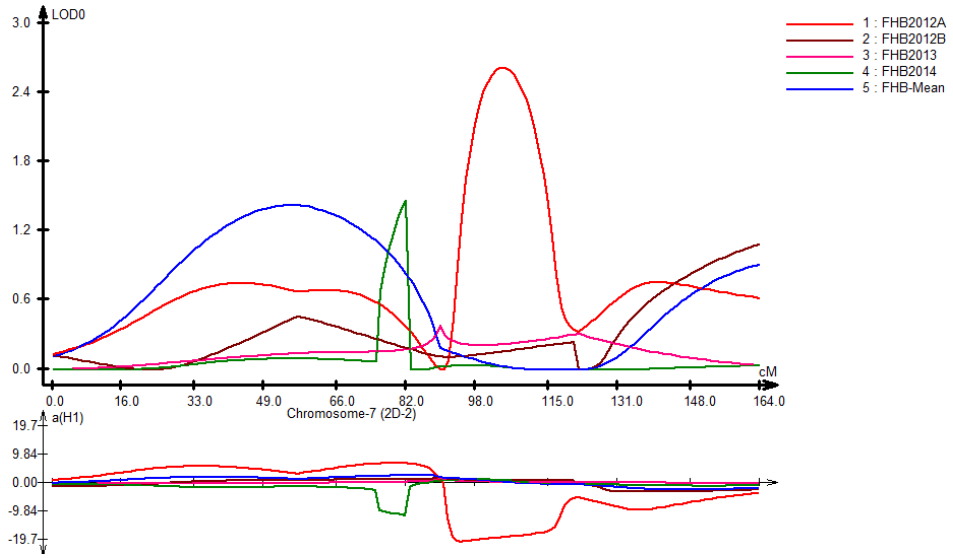
b)



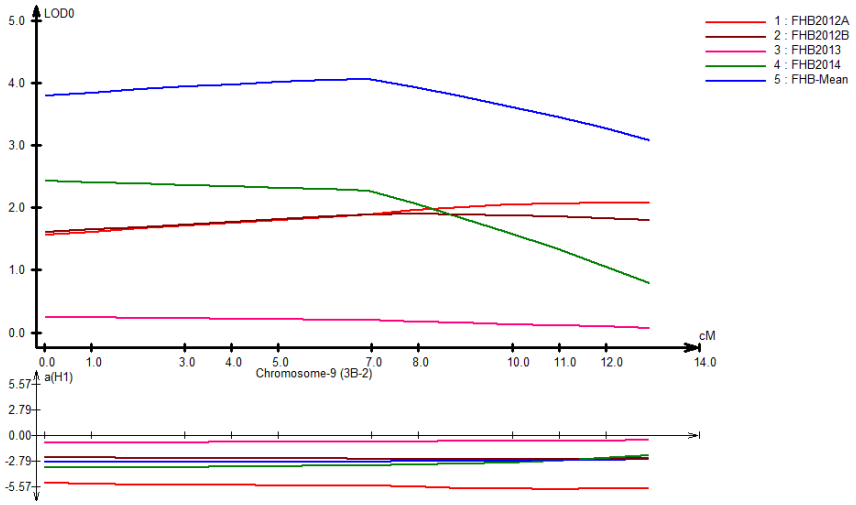
c)


LOD0
3.0
2.4
1.8
1.2
0.6
0.0
cM
0.0
21.0
42.0
63.0
84.0
105.0
126.0
147.0
168.0
189.0
210.0
Chromosome-6 (2D-1)
a(H1)
8.91
4.45
0.00
-4.45
-8.91
1 : FHB2012A
2 : FHB2012B
3 : FHB2013
4 : FHB2014
5 : FHB-Mean

d)


LOD0
3.0
2.4
1.8
1.2
0.6
0.0
cM
0.0
16.0
33.0
49.0
66.0
82.0
98.0
115.0
131.0
148.0
164.0
Chromosome-7 (2D-2)
a(H1)
19.7
9.84
0.00
-9.84
-19.7
1 : FHB2012A
2 : FHB2012B
3 : FHB2013
4 : FHB2014
5 : FHB-Mean

e)


LOD0
5.0
4.0
3.0
2.0
1.0
0.0
1 : FHB2012A
2 : FHB2012B
3 : FHB2013
4 : FHB2014
5 : FHB-Mean
cM
0.0 1.0 3.0 4.0 5.0 7.0 8.0 10.0 11.0 12.0 14.0
Chromosome-9 (3B-2)
a(H1)
5.57
2.79
0.00
-2.79
-5.57

f)

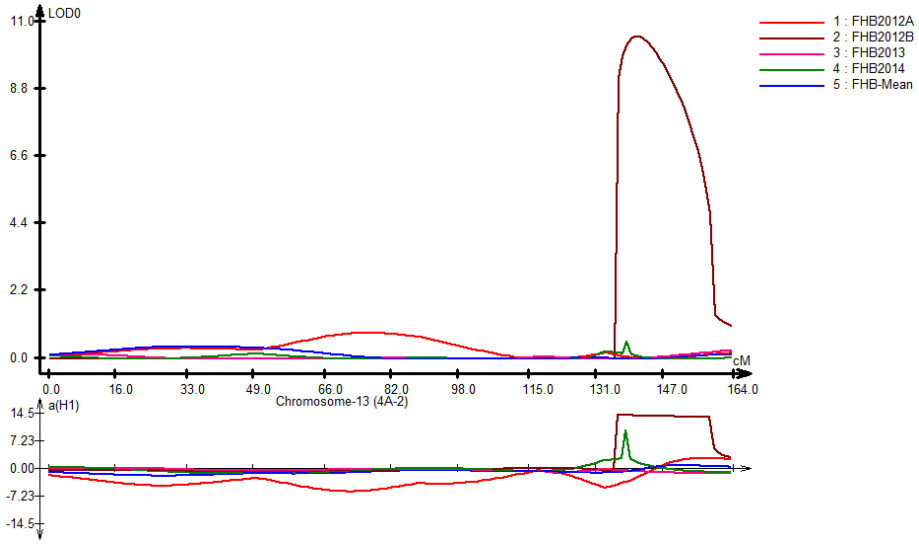

LOD0
11.0
8.8
6.6
4.4
2.2
0.0
1 : FHB2012A
2 : FHB2012B
3 : FHB2013
4 : FHB2014
5 : FHB-Mean
cM
0.0 16.0 33.0 49.0 66.0 82.0 98.0 115.0 131.0 147.0 164.0
Chromosome-13 (4A-2)
a(H1)
14.5
7.23
0.00
-7.23
-14.5

Table 2.1. Mean disease severity of the two parents in the four experiments in greenhouse over three years

Experiment	Mean Severity (%)	
	UI Stone	Alturas
FHB2012A	19.93	67.92
FHB2012B	7.33	15.01
FHB2013	8.59	17.29
FHB2014	24.63	40.50

Table 2.2. Analysis of Variance (ANOVA) for FHB severity (percentage of infected spikelets) between the two parents in each experiment over three year

Experiment	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
FHB2012A	Parent	1	8059.2487	8059.25	124.6871	<.0001***
	Error	12	775.6294	64.64		
	C. Total	13	8834.8781			
FHB2012B	Parent	1	190.4699	190.47	8.7966	0.0128*
	Error	11	238.1786	21.65		
	C. Total	12	428.6485			
FHB2013	Parent	1	265.2805	265.28	0.9534	0.3481 n.s.
	Error	12	3338.8686	278.24		
	C. Total	13	3604.1491			
FHB2014	Parent	1	629.7692	629.77	3.4002	0.1024 n.s.
	Error	8	1481.7245	185.22		
	C. Total	9	2111.4938			

*p=0.05, ***p=0.001, n.s.=nonsignificant

Table 2.3. Mean, standard deviation, maximum and minimum disease severity of recombinant inbred lines in the four greenhouse experiments over three years

Experiment	Mean	Std Dev	Maximum	Minimum
FHB2012A	46.30	25.01	100.00	5.74
FHB2012B	13.72	10.14	73.81	6.47
FHB2013	10.45	5.71	46.71	4.67
FHB2014	22.51	12.55	79.05	3.98

Table 2.4. Spearman correlation coefficients among each data set (percentage of infected spikelet of 151 RILs derived from UI Stone/Alturas) over three years

	FHB2012A[a]	FHB2012A[b]	FHB2012B[a]	FHB2012B[b]	FHB2013[a]	FHB2013[b]	FHB2014[a]	FHB2014[b]
FHB2012A[a]	1							
FHB2012A[b]	0.9140***	1						
FHB2012B[a]	0.3074***	0.2775***	1					
FHB2012B[b]	0.3250***	0.3009***	0.9106***	1				
FHB2013[a]	-0.0201	-0.0098	0.0830	0.0705	1			
FHB2013[b]	-0.0107	0.0107	0.0843	0.0653	0.8385***	1		
FHB2014[a]	0.1715*	0.2029*	0.1464	0.1705*	0.0977	0.0862	1	
FHB2014[b]	0.1102	0.1807*	0.1515	0.2144*	0.1311	0.1564	0.7145***	1

[a] 21 days post inoculation (DPI) data set, [b] 28 days post inoculation (DPI) data set
*p=0.05, ***p=0.001

Table 2.5. Analysis of variance (ANOVA) of disease severity (percentage of infected spikelets) evaluated for genotype effect and broad sense heritability (H^2) in each experiment over three year

Experiment	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	H^2
FHB2012A	Genotype	150	345951.50	2306.34	3.2374	<.0001	0.69
	Error	416	296359.40	712.40			
	C. Total	566	642310.90				
FHB2012B	Genotype	150	58474.90	389.83	3.5533	<.0001	0.71
	Error	418	45859.41	109.71			
	C. Total	568	104334.30				
FHB2013	Genotype	150	17990.12	119.93	1.9575	<.0001	0.49
	Error	430	26346.01	61.27			
	C. Total	580	44336.13				
FHB2014	Genotype	150	93945.13	626.3	2.3776	<.0001	0.57
	Error	449	118274.00	263.42			
	C. Total	599	212219.20				

Table 2.6. Analysis of variance (ANOVA) of FHB severity percentage of infected spikelets (PIS) data in 151 RIL, broad sense heritability (H^2) and coefficient of determination (R^2) across all experiment over three years

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	H^2	R^2
Genotype	150	172520.52	1150.14	4.041	<.0001	0.33	0.67
Replication	3	113.56	37.85	0.133	0.9404		
Experiment	3	432321.84	144107.28	506.29	<.0001		
Genotype*Experiment	450	347436.34	772.08	2.713	<.0001		
Error	1710	486725.1	284.63				
Corrected Total	2316	1461732.6					

Table 2.7. Coefficient of determination (R^2%) for markers significantly associated (*p=0.05, **p= 0.01, ***p=0.001) with FHB severity percentage of infected spikelets (PIS) using single marker analysis

Marker	Chromosome	FHB2012A	FHB2012B	FHB2013	FHB2014	FHB Mean
barc263	1A	-	-	4.98**	-	-
IWA0540	1B	6.21**	-	-	-	3.82*
IWA3014	1D	-	-	-	4.80**	5.01**
IWA7276	1D	-	-	-	5.60**	-
cfd48	1D	-	-	-	8.34***	4.21*
IWA2676	2B	10.88***	-	-	-	8.84***
IWA4866	2B	16.76***	-	-	-	12.37***
IWA2701	2B	6.96**	-	-	-	4.03*
IWA3176	2B	7.68**	-	-	-	4.71*
barc18	2B	8.64***	-	-	-	4.72**
barc353	2D	5.12**	-	-	-	2.96*
IWA4865	2D	4.63**	-	-	-	4.39*
umn10	3B	3.88*	4.31*	6.96**	8.97**	12.24***
barc133	3B	4.40*	5.09**	2.77*	6.98**	11.67***
wmc656	3D	3.58*	5.79**	-	3.65*	7.00**
barc96	6D	5.38**	-	-	-	3.61*
barc352	7D	6.67**	-	-	-	3.95*

Table 2.8. Quantitative trait loci (QTL) position, flanking markers, logarithm of the odds(LOD), additive effect(AE), and coefficient of determination (R^2) for QTL significantly associated with type II FHB resistance detected by composite interval mapping in UI Stone/Alturas RIL population

QTL	Chromosome	Data Set	Position	Flanking Marker	LOD	AE[a]	R^2 (%)
QFhbuis.ab-1D	1D	FHB2014	23.21	IWA7276-cfd48	2.73	-3.32	6.90
QFhbuis.ab-2B	2B	FHB2012A	63.21	IWA4866-barc18	6.54	-14.36	32.42
QFhbuis.ab-2B	2B	FHB-Mean	61.21	IWA4866-barc18	5.38	-4.19	22.85
QFhbuis.ab-2D.2	2D	FHB2012A	103.71	gwm539-IWA7117	2.62	-18.32	28.28
QFhbuis.ab-2D.1	2D	FHB-Mean	197.11	barc353-IWA4865	2.50	-2.84	10.23
QFhbuis.ab-3B	3B	FHB-Mean	6.91	umn10-barc133	4.08	-2.62	8.73
QFhbalt.ab-4A	4A	FHB2012B	140.11	IWA2723-IWA5200	10.52	14.19	46.93

[a]Negative additive effect values indicate that the resistance allele is derived from female parent 'UI Stone' and positive value indicates that resistance allele is donated by male parent 'Alturas' which contributes to reduce the spread of FHB infection within spike