# IDENTIFICATION AND DEPLOYMENT OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN U.S. HARD WINTER WHEAT

by

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# Abstract

Fusarium head blight (FHB) is one of the most damaging diseases in wheat, which impacts both grain yield and quality drastically. Recently, the disease has become more prevalent in the hard winter wheat (HWW) grown areas of the United States including Oklahoma where FHB has not been reported before. Growing resistant cultivars is the most economical and effective strategy for disease management. To dissect quantitative trait loci (QTL) for FHB resistance in a moderately resistant hard winter wheat (HWW) cultivar, Overland, a population of 186 recombinant inbred lines (RILs) was developed from the cross between Overland and Overley, a susceptible HWW cultivar from Kansas. The RILs were evaluated for FHB type II resistance in one field and three greenhouse experiments and genotyped using genotyping-by-sequencing (GBS) markers. Three FHB resistance QTLs were mapped on Chromosomes 4DL, 4AL, and 5BL. The QTL on 4DL was the most consistent one and explained up to 13% of the phenotypic variation for type II resistance and 14 % for low Fusarium damaged kernels (FDK). Two GBS markers closely linked to the 4DL QTL were successfully converted to Kbioscience competitive allelic specific PCR (KASP) assays and can be used in marker-assisted breeding.

In breeding, a single QTL may provide only partial resistance and pyramiding of several resistance QTLs in a cultivar can provide more protection in FHB epidemics. *Fhb1* is a major QTL for FHB resistance from a Chinese source and *Fhb3* is an alien gene from wild rye grass (*Leymus racemosus).* To study the effects of these QTLs individually and cumulatively in hard winter wheat backgrounds, they were transferred into two HWW cultivars Overland and Jagger. The results show that *Fhb1* significantly increased FHB resistance, but *Fhb3* did not. Thus, *Fhb3* is not an effective gene for improvement of FHB resistance in HWW.

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# Dedication

This work is dedicated to every single farmer in the field for their hard work, spirit, and enthusiasm. To every researcher in agriculture no matter where they belong, it's their tiresome work that keeps the economic machinery running despite all odds. All the teachers devoted to equip the young researchers with knowledge and skill, to those young minds who keep inspiring through their efforts. In short to an honorable team united under one word "AGRICULTURE".

# Chapter 1 - Literature Review

## Wheat Production in the United States

Wheat (*Triticum aestivum* L.) is one of the oldest and important cereal crops grown worldwide. With a yield of 660 million tons in 2012 (FAO statistics 2013), it is third to maize and rice in global cereal production. Wheat in the U.S.A serves as a source of domestic food and feed, its production usually ranges from 55 to 60 m tons and U.S. is a major exporter of wheat and almost half of wheat produced is used for export (MacFall and Fowler 2009). To meet the increasing wheat demand by growing population, it is important not only to increase the wheat yields but also to sustain the current productivity by minimizing the losses due to biotic and abiotic stresses. Generally wheat grown in the U.S.A. can be classified as "winter wheat" and "spring wheat" on the basis of their growth habit and planting season. Winter wheat is usually sown in fall, and establishes itself before it becomes dormant in severe cold weather. In spring, it resumes its growth and is ready to harvest in the summer. One of the major differences between winter and spring wheat is that the former one requires a vernalization period in order to produce the seeds while later one doesn't require vernalization (Chouard 1960; Amasino 2004). Vernalization is a process to expose plant seedlings to a certain period of low temperature, which is required by some plant species including winter wheat to enter in the reproductive stage subsequently for grain production (Streck et al. 2003). Wheat can further be classified into five classes in the U.S.A. This classification is based on several factors, including planting time, growth habit, grain color, and grain hardiness. Each class has its own unique characteristics and differs from the others in the end-use quality.

Winter wheat contributes 70 - 80% of the total wheat production in the U.S.A. Hard red winter wheat (HRWW) is primarily grown in the states of Great Plains including Texas, Nebraska, Oklahoma, Colorado, Kansas, North and South Dakota and some in neighboring states such as

Montana, New Mexico and Wyoming (Carver et al. 2001). It is mainly used for bread-making and accounts for 40 % of total wheat production in the U.S.A.

Hard white winter wheat (HWWW) is relatively new in U.S.A., it is similar to hard red winter wheat but has a white outer covering which is supposed to improve the color in certain products. This wheat is majorly produced in the states of California, Colorado, Idaho, Kansas, and Montana. Durum wheat is the only tetraploid wheat grown in the northern areas just like hard red spring wheat comprising 3-5% of total production. It provides semolina for spaghetti and other pasta products (National Association of Wheat Growers, ND).

Soft red winter wheat (SRWW) is produced in the eastern third of the U.S., i.e. North and South Carolina, Louisiana, Georgia, Virginia, Maryland, Pennsylvania, Arkansas, Missouri, Illinois, Indiana, Tennessee, Kentucky, Ohio, and Michigan (Bacon, 2001).This type of wheat is low in protein contents and is majorly used in baking cakes, pastries and snacks. Breeding efforts in this region are focused on grain yield, winter hardiness and resistance to diseases.

Hard red spring wheat (HRSW) is important bread wheat containing 13 - 14% protein, and has almost 20% share in wheat exports. It has excellent milling and baking quality. HRS wheat is predominantly grown in north central states i.e. Dakotas, Minnesota and Montana (Busch and Thomas 2001). Soft white wheat is primarily produced in the Pacific Northwest states of Washington, Oregon, Idaho, and parts of Northern California, and the Northern states of Michigan and New York. This has a white outer layer with a soft endosperm and has low protein contents. Soft white wheat has both winter and spring types; its end-use is mainly for the baking purposes. It is exported to Asia and Middle East (National Association of Wheat Growers, ND).

Improving wheat yield has always been a prime objective in order to meet the needs of ever growing population. Like other crops, wheat productivity has also been constantly hampered by

different biotic (i.e. pathogens, insects and weeds) and abiotic (i.e. waterlogging damage, heat, mineral deficiency, high salinity, drought etc.) stresses. The biotic factors can cause around 28 % of yield losses while the abiotic stress accounts for 82% yield losses in wheat (Oerke E.C. 2006).

## Impact of Fusarium head blight on wheat production

Fusarium head blight (FHB), also referred to as scab, is one of the most damaging diseases in wheat. It directly impacts grain quality and quantity and has become a serious concern for the cereal growers in humid and semi-humid areas of the world (Bai and Shaner 2004). Warm weather coupled with high humidity at wheat anthesis stage in the presence of sufficient natural inocula can result in severe epidemics resulting in dramatic reduction of wheat yield and quality (Bai and Shaner 1994). Florets that get infected by *Fusarium* either produce tombstone, or don't produce any collectable grain at all. Such tombstone kernels are so light in weight that they are more likely to blown away during the threshing process, thus the yield from the infected wheat is significantly reduced. FHB infection also affects the grain quality by mycotoxins produced by the pathogens. The toxins are harmful when contaminated grains are consumed by the humans and animals. Infected FHB kernels in wheat mostly contain the tricothecence, deoxynivalenol (DON) and its derivatives (De Wolf 2003). Different species of Fusarium produce different types of trichothecences. Amongst those, *F. graminearum* generally produces Type B trichothecences (Placinta et al. 1999). With more frequent and intense occurrences of FHB epidemics in many parts of the world, DON contamination in wheat is a seriously growing concern for the animal production and human health. In case of human consumption, DON ranging from 0.5 to 2 mg/kg is considered to be maximum acceptable levels in wheat grains (Yu et al. 2008). According to a review of FHB occurrences in U.S. relatively low but varying levels of FHB were recorded in many states in 2010, the mean FHB incidence was about 25% according to the survey conducted at 145 fields in 32 Ohio counties (Lilleboe D. 2011) while in

some fields incidences up to 60% and DON content up to 18mg/kg (ppm) were recorded. In the Great Plains, FHB mainly occurred in Nebraska, South and North Dakota and Minnesota (Lilleboe D.2011). In Kansas FHB epidemics occurred for four consecutive years (2007 - 2010), with the FHB index [(incidence × head severity)/100] ranging from 2 to 10% in 2010, around 3.3 million bushels of the infected grains in Kansas was estimated to value at $13 million. In 2010 FHB was also reported in Oklahoma, where FHB was not a wheat disease before (Lilleboe D. 2011). Therefore, FHB in Great Plains is becoming more frequent and severe and expanding to Southern Great Plains.

## Causal organism, symptoms and infection pathway

Several species in the genus *Fusarium* can cause Fusarium head blight in cereal crops. Some of the important ones possessing the ability to produce different types of mycotoxins in the genus *Fusarium* are *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* (Parry et al. 1995). Among 17 different species of *Fusarium* associated with FHB in small grain cereals, *Fusarium graminearum* and *F. culmorum* are the most common pathogenic ones. *F. culmorum* is prevalent in cooler wheat growing regions of the world like U.K., Northern Europe and Canada (Desjardin 2006) while *F. graminearum* is the most predominant one in North America, China and many other warm humid and semi-humid areas of the world (Osborne and Stein 2007). Their prevalence in a certain environment is highly dependent on the temperature and moisture. *F. graminearum* is known to be complex specie with multiple lineages (O Donnell et al. 2004). Also the Isolates within *F.graminearum* differ in pathogenicity, there is so much cultural variation found. However specific interaction between the wheat cultivars and pathogen isolates in different geographic regions was not found and resistance against FHB in wheat is considered race nonspecific (Van Eeuwijk et al. 1995). Therefore it became a common practice in FHB screening to use a mixture *F. graminearum* isolates as inocula (Zhou et al. 2002).

*Fusarium* has the potential to survive in crop residues in multiple modes such as mycelium, ascospores, macroconidia, chlamydospores and perithicia. The pathogen modes and host ranges dictates the potential feature of pathogen survival, proliferation and dispersal. Crop residues of wheat, corn and rice can serve as the potential reservoirs of the fungus. Two types of *F. graminearum* spores serve as inoculum: macroconidia (asexual stage) and ascospores (sexual stage) (Wagacha and Muthomi 2007). The life cycle is comprised of two major phases, saprophytic and pathogenic. In the saprophytic phase the fungus depends entirely on the crop debris for nutrients while in the pathogenic phase it depends on a living host such as wheat for nutrition. Naturally *F. graminearum* produces ascospores in perithecia, the thick walls of perithecia helps the fungus to survive in the winter (Xu and Nicholson 2009). During the late spring, matured perithecia burst discharging the ascospores in air under high moisture conditions (Webster and Weber 2007). In wheat fields, infection is initiated by these ascospores driven by the air, landing on the flowering spikelets. The spores enter through the natural openings like stomata or anthers during anthesis. Among various other factors fungal adhesion is one of the important factor to maintain the physical contact with the host surface in order to get entry and penetration in the host tissues (Bushnell et al. 2003). While speaking of wheat, anthers may also generally provide an easy route of entry as invading the inner surface of spikelet palea, lemma and glumes is much more effective than invading the external surface of host (Kang and Buchenauer 2000). This process is further facilitated by the secretion of hydrolyzing enzymes like cutinasese and lipases by the pathogen (Walter et al. 2010).

After the pathogen penetrates rachilla and rachis disease will start spreading upward and downward of the spike through vascular bundles and cortical parenchyma tissues (Goswami et al. 2004; Bushnell et al. 2003). Kernel development gets affected as the mycelium clogs the vascular tissues to deprive the developing kernel of nutrients and water. Thus the infection spreads from

anthers to glumes and then rachis (Xu and Nicholson, 2009). One of the most characteristic symptoms of early FHB infection is the presence of small water-soaked areas near the bottom of the glume and colonized tissue then becomes bleached or discolored. Bleached heads in a field is a sign of FHB infection. However symptoms vary in resistant and susceptible cultivars , unlike susceptible plants which show the symptoms as described above resistant plants can be differentiated by a dark brown discoloration on the infected spikelet or in some cases there is a small dark brown spot on the lemma only (Bai and Shanner 1994).

## Environmental factors affecting FHB infection

The infection process and germination of Fusarium conidia is highly influenced by and often dependent on several environmental factors like moisture and temperature (Colhoun et al. 1968). Even different stages in a disease cycle are greatly influenced by the particular conditions of weather. For example high relative humidity and warm temperatures favor the formation of spores and maturation of perithecia in infested maize and wheat residues (Gilbert et al. 2008). Moreover production of ascospores also depend on the soil moisture, usually if the soil moisture is < 30 % ascospores cannot be produced while the production is maximum if soil moisture is > 80 %, the optimum temp range is around 15-20˚C. The inoculum potential can be greatly reduced by burning the crop residues where spores overwinters (Dill-Macky and Salas 2001). It was found that the infection of wheat spikes was maximum at 25˚C in moist conditions and symptoms start to develop from 36 -72 h (Bai and Shaner 1994 and Brennan et al. 2005).

Wheat anthesis is the most prone stage to the FHB infection. However, initial caryopsis development is also suspected to be a critical stage in some cultivars (Bai and Shaner 2004; Lu et al. 2001). Given optimum weather conditions and abundant inocula at these stages, *F. graminearum* can cause severe epidemics (Bai and Shaner 1994). Certain agronomic cultural practices are also

associated with FHB incidence. As mentioned above burning of crop residues coupled with deep tillage not only reduces the FHB incidence but also improves the soil texture. Crop rotations especially with crops that are non-host of the Fusarium help to minimize the development of the *Fusarium* spores. Similarly irrigation may affect the soil moisture which in turn facilitates the spore production (Champeil et al. 2004).

## Resistance mechanisms against FHB

Resistance mechanisms in wheat have not been well understood at molecular and biochemical levels. However, several studies were done to understand the differences in induced expression of chemical compounds between resistant and susceptible cultivars. It was first reported by Chen et al. (1999) that FHB resistance in transgenic wheat was associated with the expression of a pathogenesis related (PR) protein which resembled a protein found in rice i.e. thaumatin. These PR proteins were expressed in both resistant and susceptible varieties after being infected with FHB, including PR-1, PR-2 (β-1,3-glucanases), PR-3 (chitinase), PR-4, and PR-5 (thaumatin-like protein) (Pritsch et al. 2001). A cDNA microarrays study that was conducted on Sumai-3 and two near isogenic lines by Golkari et al. (2009) revealed 25 differentially expressed genes. Genes that encode for PR-2, PR-4 and PR-5 showed up regulation in the cultivars containing *Fhb1* from Sumai-3. Some enzymes were also found to be important in imparting FHB resistance in wheat such as ascorbic acid oxidase, ascorbic acid peroxidase, catalase, phenylalanine ammonia-lyase, and superoxide dismutase (Chen et al. 2000).

DON produced during the Fusarium infection is considered to be a virulence factor. *TRI5* gene encoding trichodiene triggers the catalysis of trichothecene biosynthesis (Hohn and Beremand, 1989). Desjardins et al. (1996) conducted a study using *TRI5* gene mutants of *F. graminearum* strain and observed less severity of infection in mutants. However, contradictory results have been

reported in several studies stating that DON is not a necessary component to initialize the infection but plays a role in spreading of disease within the spike (Bai et al. 2002; Jansen et al. 2005). Jasmonate (JA) and ethylene mediated defense pathways are also important in resistance to FHB, as elevated levels of Jasmonate and ethylene were found in the plants after inoculation (Li and Yen, 2008). Two enzymes lipoxygenase and chalcone synthase were up regulated in the resistant plants, these enzymes represent JA defense pathway while elevated levels of ethylene are responsible for senescence, degradation of cell wall and ultimately cell death (Li and Yen, 2008). In short resistance mechanism against FHB is a complex signaling interplay between the pathogen and host biochemical features, much work is needed to be done in this area.

## Control Measures

### Host resistance

The resistance mechanism shown by the host plant can be broadly classified into two major categories morphological and physiological. The former category of resistance refers to the morphological characteristics of plant which imparts either susceptibility or resistance against FHB. Plant height, flowering and grain filling time have been found to be associated with the FHB resistance (Gervais et al. 2002; Gosman et al. 2009). Usually wheat varieties which flower early and take more time in grain filling are susceptible unlike varieties that rapidly fill grains and show late flowering (Mesterhazy 1995; Rudd et al. 2001). Plant height is negatively correlated with FHB resistance with shorter plants affected more than taller plants (Mesterhazy 1995). Some studies show that dwarfing genes *Rht-B1b (Rht1) and Rht-D1b (Rht2)* enhance FHB symptoms (Hilton et al. 1999). However, there are also reports of some resistance loci associated with *Rht-B1b* (Srinivassachary et al. 2009; Miedaner and Voss 2008). These morphological types come under the passive resistance as plant tries to escape the infection but it can cofound the results if varieties are

evaluated on this basis, as passive resistance is not consistent across the years and environments (Kolb et al. 2001; Parry et al. 1995). The second category refers to the physiological mechanisms involving biochemical pathways. It includes responses of the plant to inhibit the fungal growth after the initial infections through the production of chemicals, also mentioned as active resistance. This is considered to be an important indicator of resistance. This ability of plant to prevent infection on the basis of morphological and physiological features is divided into five types: Type I - resistance to pathogen penetration and initial infection (Schroeder and Christensen 1963), Type II – to the spread of infection within the spike (Schroeder and Christensen 1963), Type III – to mycotoxin accumulation in infected grains (Miller et al. 1985), Type IV –to the Fusarium damage kernels (Mesterhazy 1995), Type V – tolerance of the plant (Mesterhazy 1995). Presence of multiple types of resistance in the host plant including type I, type II, and type III helps in improving the overall plant's defense (Yu et al. 2008). Different cultivars exhibit varying levels of resistance and sometimes disease evaluations are also affected by the environmental factors especially in case of Type I resistance. Usually this type of resistance is not considered to be a reliable estimate for FHB evaluation as it's very hard to determine the initial infections in the fields (Yu et al. 2008). Therefore Type II resistance has been used worldwide by the breeders to evaluate disease severity because it is relatively easier to evaluate and more stable across different environments than the first type. To measure type II resistance it is recommended to use a mixture of fresh aggressive isolates while preparing inoculum (Dill-Macky 2003). Disease evaluation is usually done 18-21 days post inoculation but can be varied depending on the population being studied (Bai and Shaner, 1996); evaluation is done by scoring severity which is the percentage of scabby spikelets. By the use of grain spawn method, incidence which is the percentage of diseased heads and severity ratings are

used for measuring type I and type II FHB resistance. In addition of these two parameters FHB index is also calculated ((incidence × severity)/100), to aid the breeder to select for the resistant cultivars.

Breeders are specifically interested in the low DON content in infected grain and Fusarium damaged kernel (FDK) as they affect the quality and quantity of the yield. Generally there are two methods to measure FDK, comparing infected and reference samples visually (Jones and Mirocha 1999) and manually separating damaged and healthy kernels (Verges et al. 2006). Each method has its own shortcomings; visual comparison is quick but subjective to variations and personal error while manual separation is time consuming. Several other methods were devised and can be used now to measure FDK. Some of them include digital image analysis (Agostinelli et al. 2008), near infrared reflectance (Delwiche and Hareland 2004) and separation by air. Some immunochemical methods to measure DON in wheat include ELISAs (Maragos, S.P. McCormick 2000), LFDs, dipstick tests (Molinell et al. 2008), fluorescence polarization immunoassay (FPIA) (C.M. Maragos 2002), immunofiltration assays and biosensor assays (Tudos et al. 2003). Mass spectrometry-gas chromatography (Dill-Macky 2003) and Single Kernel Near Infrared (SKNIR) are also used to measure FDK and DON accurately (Jin, F et al. 2014).

## Sources of resistance

Breeders have always tried to look for the genotypes that show good resistance against FHB, it became an important breeding objective since 1970s (Bai and Shaner 1994). FHB resistant cultivars from different geographical regions have been identified so far, such as from Asia, Europe, South America and U.S. Among the Asian sources, some land races from China were identified as good sources of resistance i.e. Wangshuibai, Sumai-3 and Ning7840 are the notable ones. Amongst these landraces, Sumai-3 and Ning7840 are the most widely used resistance sources (Bai and Shaner 1996; Kolb et al. 2001). Sumai-3 resistance has been proved to be stable, heritable and consistent

across various environments but it also has some unfavorable characteristics (Rudd et al. 2001). Shinchunaga, Nobeokabouzu and Nyu Bai cultivars from Japan also showed high levels of FHB resistance but all of them have poor agronomic characteristics linked with their resistance and conventionally breeding has not been successful so far in parting them(Bai and Shanner 2004). Korean cultivar 'Chokwang' also carries type II resistance QTL, despite of its origin from the Asian germplasm, its pedigree and the resistant loci are different from Sumai-3 (Yang et al. 2005b).This cultivar because of its potential can be used in breeding programs complementing other resistance loci. Some winter wheat cultivars from Europe known for moderate FHB resistance include Arina, Renan, Dream, Cansas and Fundulea201R (Buerstmayr et al. 2009).Other Sources of resistance from South America that show good resistance include Brazilian lines Frontana and Encruzhilada (Ban 2001; Mesterhazy 1995; Singh et al. 1997) and U.S., cultivars Freedom, Truman, Bess and Ernie (Rudd et al. 2001). Tetraploid wheat Durum does not have many resistant cultivars. Buerstmayr et al. (2009) summarized some notable ones from durum and its relatives including *T. dicoccoides* accession FA-15-3, *T. dicoccoides* accession PI478742, *T. durum* cultivar Strongfield and *Triticum carthlicum* cultivar Blackbird. In addition to these, chromosome introgression from related species having FHB resistance has also been a strategy for breeders. For FHB such a translocation has been achieved from an alien species *Leymus racemosus. Fhb3* on chromosome 7A was translocated from this alien species (Qi et al. 2008), carrying some undesirable epistatic effects.

Presently different approaches of breeding for FHB resistance are in use including conventional selection procedures, Marker-assisted selection (MAS), and transformation of resistant genes. The last two approaches have long lasting effects but only if they can be refined and effectively utilized (Muehlbauer and Bushnell 2003).

## Other control measures

*Fusarium graminearum* has many host species which makes it ubiquitous; therefore it cannot be controlled by a single strategy (Bai and Shaner 1994). Multiple control strategies need to be implemented to control the fungus effectively. One of the control measures that farmers are applying traditionally is tillage. Tillage helps to bury the crop residues, as the fungus can overwinter in the crop residues and it is usually the initial source of inoculum therefore tillage is an important agronomic practice (Dill-Macky 2008). Although *Fusarium graminearum* has a wide host range yet growing non host crops can help in controlling the incidence of disease to some extent. It has been shown by studies that planting wheat after corn has higher chances for the incidences of diseases (Bai and Shaner, 1994). Adjusting the sowing date is also crucial as the right choice of sowing date can minimize the probability of favorable conditions coinciding with the antheis (Champeil et al. 2004). Application of fungicides and the use of fumigated certified seeds is also an important control measure. There is a wide range of fungicides available usually the ones that have tebuconazole and/or prochloraz as active ingredients are found to be effective (Homdork et al. 2000). Effectiveness of the fungicides is dependent on several factors such as the timing of application, resistance of cultivar itself and coverage of fungicides (Mesterhazy et al. 2003). Fungicide application at the beginning of anthesis usually yields good results. Hollingsworth et al (2008) found less severity and a significant reduction in DON accumulation after the use of fungicides. The use of fungicides is limited due to the cost of their use, determining right time of application and lack of availability of highly effective fungicides (Bai and Shaner 2004). Many studies were done to find the biological control of FHB (Khan and Doohan 2009). However, the use effective biological control in conjunction with integrated disease management is an area that still needs to be discovered. Currently, growing resistant cultivars is the best economical solution of the problem. Breeders have

been putting efforts for decades to develop fully resistant cultivars against FHB but are not successful yet. Therefore a good control strategy against FHB should be a combination of resistant cultivars, good agronomic practices, and chemical control.

## Genetic resistance and heritability

Studies have been done to dissect the genetic basis underlying FHB resistance and it was reported that resistance is polygenic with high heritability (Bai and Shaner 2004). Being a quantitative trait, it is highly influenced by environment (G X E interactions). Therefore breeders have been trying to find the genes for resistance and markers linked to these genes so that benefits from marker-assisted selection can be utilized to develop resistant cultivars efficiently. In Sumai-3 one dominant gene along with some modifiers for FHB resistance was proposed by Chen (1989), while two genes were proposed by Zhou et al (1987) and three genes were estimated by Bai et al. (1989). This disparity among the studies can be explained by quantitative nature of the disease, G x E interactions, inoculation techniques and types of resistance to be evaluated in different studies (Kolb et al. 2001). Bai (1995) also reported the presence of three genes with major effects on resistance in two Chinese varieties Sumai-3 and Ning7840. Singh et al. (1995) proposed three resistance genes in Frontana while Van Ginkel et al (1996) estimated two in Frontana as well as Ning 7840; all these four genes were different. Efforts first focused on finding FHB resistance genes, then assigning them to different chromosomes using monosomic analysis. Many resistance genes were found on chromosomes 4A, 5A, 7A, and 4D in Wangshuibai (Liao and Yu 1985). Generation mean analysis was used in multiple studies to analyze crosses between resistant and susceptible cultivars, and it was concluded that additive effects are important in FHB resistance while non-additive effects are also significant (Chen 1983; Snijders 1990). Dominance is the most important amongst the non-additive effects (Bai et al. 1990; Snijders 1990).

## Molecular markers

The development of molecular markers have revolutionized the breeding nowadays and is used as an indispensable tool in breeding programs. Earlier breeding programs relied only on morphological markers. With the advent of molecular age breeders have tried to integrate the molecular markers along with morphological markers in their breeding programs. Some of the advantages of using molecular markers are (1) screening can be done at any stage of development while morphological markers are usually assessed at an adult stage (2) molecular markers can detect polymorphisms occurring at alleles while the morphological markers are unable to detect these polymorphism unless these are lethal or deleterious or each allele has a phenotype associated. Hence molecular markers can be utilized to monitor segregating alleles in a population (Tanksley 1983). In general, molecular markers can be either proteins or DNA markers (Tanksley et al. 1982). Isozymes are the protein markers that were used in plant breeding earlier but due to their limited availability they were replaced by DNA markers (Tanksley 1983). DNA markers can be hybridization based, PCR based or sequence based. The most commonly used molecular markers are SSRs (Simple sequence repeats) which are PCR based markers. Relatively recently some new markers in sequence based category of markers were introduced and were proved to be greatly helpful. These include Single Nucleotide Polymorphism (SNP) and Sequence tagged sites (STS) markers such as Expressed Sequence Tags (EST) (Gupta et al. 1999).Expressed sequence tags are utilized in gene discovery, comparative mapping, and genome annotation (Rudd 2003). ESTs are generated from unedited single end reads from cDNA libraries, these cDNA libraries are highly informative as they trace back to the expressed genes. ESTs in many model plant species i.e. Arabidopsis and important crop plants like wheat, rice and soybean are generally available in public databases (Rudd 2003). The utility of ESTs is limited due to a number of factors; the representation of host genes is limited as not

all the genes are constitutively expressed, at the same time housekeeping genes are overexpressed in the library. To overcome these limitation mRNA libraries at different developmental stages from different cell types are made, this increases the ESTs range (Rudd 2003). Simple sequence repeats are widely used in plant breeding for marker-assisted selection, genotyping and for many other applications. Although these days SNPs are becoming the marker of choice yet SSRs are still important markers as they provide a direct link to existing linkage maps and other resources.

SSRs occur in different repeat motifs i.e. trinucleotides and dinucleotides etc. (Varshney et al. 2005). The most common dinucleotide repeats in cereal crops are (AC)n and (GA)n, which are shown to be distributed evenly in genome (Varshney et al. 2002). In case of wheat SSRs are highly polymorphic and evenly distributed, some are even locus specific (Gupta et al. 1999; Roder et al. 1998). These markers require only a small amount of genomic DNA, and are suitable for high throughput genotyping. Because SSR markers are highly polymorphic, easy to visualize, stable and co dominant (Song et al. 2005), they are widely used for FHB mapping (Wei et al. 2005). However, due to high cost associated with SSR discovery, the number of available SSR markers in many crops are limited (Kalia et al. 2011). There are two methods to develop SSRs, selection hybridization and primer extension enrichment (Kalia et al. 2011). Once they are developed their cost is highly reduced.

Since the discovery of next generation sequencing (NGS) technology like Roche 454 (Roche, Applied Science, Indianapolis, IN), HiSeq (Illumina, San Diego, CA), SOLid (Life Technologies, Carlsbad, CA) and Ion Torrent (Life Technologies, Carlsbad, CA) discovery of SNP even in complex genomes has become feasible. SNPs from exons, introns and promoter regions can be identified as markers (Khlestkina and Salina 2006). Theoretically four alleles can be present at a specific nucleotide locus but only two variants can occur in any organism (Brooks 1999) that's why

SNP markers are biallelic. Six mapping populations were used to develop a consensus map containing 7,504 polymorphic markers. Akhunov et al. (2013) developed 90,000 wheat SNP chip assay, this will increase the genotyping throughput dramatically. Sequencing of the large genomes like wheat is still a challenge therefore much efforts are done through the SNP discovery as this will help in generation of high density consensus map. Recently Genotyping-By-Sequencing (GBS) method is developed, which is a low cost but high throughput in SNPs detection method (Elshire et al. 2011). GBS first utilizes restriction digestion to reduce complexity of genomes, ligate digested fragment to adaptors with barcodes for multiplexing, and is followed by multiplexing PCR (Poland et al. 2012). It is a reduced representation of genome that targets a fraction of genome for sequencing (Altshuler et al. 2000) and a promising technology to develop SNPs for genotyping in all species.

## Genetic maps

Genetic or linkage maps are essential for identifying the locations of genes or quantitative trait loci (QTL) within the genome (Collard et al. 2005). Types and sizes of mapping populations are important in determining the quality of linkage map. For preliminary studies a mapping population of 100-200 individuals is generally used however it is recommended to use larger populations for high resolution genetic maps (Collard et al. 2005). Size of the population usually affects the marker density and coverage, and small populations result in undetected recombination events due to limited sampling (Liu 1997). Recombination fraction is used to calculate the order and distance between the markers, and the low recombination frequency between two markers indicates that the markers are in close proximity to each other (linked) while higher recombination frequency indicates greater distance between them (Collard et al. 2005). Usually 50 % or greater recombination frequency is the defined cut off for the unlinked markers. The marker distances are generally additives but mapping

functions have been developed to convert these recombination frequencies into distances. There are two types of mapping functions, Haldane function (Haldane 1919) and Kosambi function (Kosambi 1943). Each mapping function has its own characteristics, Haldane function assumes that crossover occur independently in the genome while Kosambi takes into the account interference effects i.e. one crossover inhibits or affects another crossover. These mapping functions should be considered while constructing the genetic maps as they directly affect the genetic distances. However studies have supported the interference effects and concluded that crossover do not occur randomly (Muller 1916). Different criteria such as choice of correct mapping function and grouping methods (Regression or Maximum likelihood ratio) exist for constructing a linkage map, one should be very careful while choosing them, as a wrong pattern and gene order can inflate the genetic map resulting in under or overestimation. Physical mapping is becoming more and more important as cost of genome sequencing is reduced; physical maps provide a link to join sequence data and markers along the chromosome (Meyers et al. 2004). Similarly cytogenetic mapping using deletion, aneuploid, and substitution lines is helpful in assigning the molecular markers to physical locations on chromosomes. However cytogenetic maps face some limitations of viable lines and true identification of deletions (Hass-Jacobus and Jackson 2005). Cytogenetic maps are still used for the validation of marker order and position on the chromosome (Qi et al. 2004). There is another method of generating physical maps utilizing radiations to fragment the chromosomes, which are than hybridized to the somatic cells of another species; this is called the Radiation hybridization method (Cox 1990). STS markers generated from either ESTs or any genomic sequences are used to analyze these hybridized fragments and consequently help in making physical maps. However radiation mapping not necessarily yields or represent the correct marker order on the chromosomes (Cox

1990). These genetic maps are further used in conjunction with the phenotyping data to locate the genomic regions associated with the trait under study.

## QTL Mapping

Quantitative traits exhibit a trend of continuous variation in the population. QTL mapping has become an important and instrumental technique to dissect the quantitative traits and uncover the genetics underlying the trait (Young 1996). QTL mapping based studies locate the loci in the genome associated with the trait and proportion of variation explained by them (Kearsey 1998). This technique's basic concept involves the co-segregation of molecular markers with the trait under study in a mapping population (Liu 1997) implying that the molecular marker and QTL are closely linked. It was first proposed by Sax (1923) and later on explained by Thoday (1961).

This marker trait associations help in understanding the gene network under the complex quantitative traits and directs the approaches for MAS and positional cloning (Young 1996). However there are some shortcomings related to this technique that affects its accuracy. This technique is very robust in detecting the QTLs with major effects but it is not efficient in detecting minor effect QTLs. It is hard to narrow down the QTL region into less than 10cM map distance unless QTL under study has major effects without much environmental affect, which in most cases is very unlikely. Lastly if two QTLs are present very close to each other and have some interaction as well it is very difficult to separate them (Kearsey 1998). Development and utilization of an appropriate mapping population is very essential in QTL mapping projects. Mapping populations can be categorized into two main groups (1)Populations developed for linkage based mapping i.e. inbred lines of self-pollinating crops and half sib and full sib families for cross pollinated crops (2)Natural populations used in linkage disequilibrium based mapping also known as association mapping population (Yu et al. 2006). Linkage based mapping populations include mostly

recombinant inbred lines (RILs), backcross lines, double haploids, $F_2$ derived families and near isogenic lines (NILs). These kinds of populations are artificially generated by a cross of two parents that exhibit contrasting phenotypes for the specific trait of interest. RILs, NILs and DH populations due to their homozygous and true breeding nature are considered as permanent populations and are mostly used in mapping studies. These can be reproduced and multiplied without any significant genetic changes (Young 1994; Paterson 1996 and He et al. 2001). Due to high cost associated with genotyping and phenotyping of the complex quantitative traits, size of the mapping populations is limited (Somers et al. 2003) and mapping populations of 100 - 200 progenies has been used in most of the mapping projects (Lynch and Walsh 1998).

QTL mapping relies on statistical methods for finding the associations between genotype and phenotype. These statistical methods are grouped in several categories based on the kind of mapping populations developed and whether a genetic map is required or not prior running QTL analysis (Manly and Olson 1999). The later one groups the methods in two categories (1) those methods that do require genetic map for the population prior QTL detection i.e. Simple interval mapping(SIM), composite interval mapping(CIM) and multiple interval mapping(MIM) (2) those that do not require genetic map (analysis of variance, linkage disequilibrium based mapping and partial least square regression mapping) (Semagn et al. 2006).For the first category scientists conduct linkage analysis of the genotypic data to construct genetic linkage maps.

## Linkage based QTL mapping method

One of the simplest methods of QTL analysis is ANOVA (Soller et al. 1976); it tests the association of molecular markers and phenotypic data statistically. At each marker locus progenies are divided into two categories based on the genotype and then a comparison is made with phenotypic distributions. The marker locus under study is termed as target locus while additional

markers having known associations with the trait can also be added, these additional loci are called background markers. These background markers because of having known associations with the trait lie in proximity to QTL region. All the target loci are independently tested for associations in combination of these background markers by multiple regressions (Manly and Olson 1999). The strength of QTL at each target locus is assessed by the T statistic or F statistic and genetic map is not needed as it tests each marker locus separately. However, Lander and Botstein (1989), Manly and Olson, (1999) and Crosses in (2001) independently pointed out some limitations in this simple process. Some of them include the difficulty to conduct a separate analysis for QTL location and QTL effect, and removing lines with missing data or else using a different mixed model approach. Lander and Botstein (1989) came up with a technique known as Interval mapping or Simple Interval Mapping (SIM). It requires the genetic map and phenotypic data of the population, once these two are available SIM utilizes every marker interval in the map to detect the hypothetical QTL by testing the likelihood ratio on every possible position in a particular interval. Later Lander and Botstein (1989) suggested the method likelihood of odds (LOD score) as a rule to construct confidence interval for QTL. LOD score is the logarithmic ratio of two probabilities. The procedures used in SIM are regression or maximum likelihood and it increases the probability of single gene genetic model considering the mean of all the possible states at each probable QTL position (Haley and Knott 1992). SIM is more robust as it determines the map location of the gene and gene effect and requires less number of progeny lines as compared to ANOVA (Haley and Knott 1992) even though the method is robust it still have some issues. SIM only takes into the account one QTL at a time ignoring the effects of other QTLs that are yet mapped or not, thus it's a biased estimation of QTL effect and position in the presence of other QTLs in the same linkage group (Martinez and Curnow 1992; Zeng 1994). Presence of the QTLs beyond the interval of interest can influence the detection

of QTLs within the interval (Zeng 1993); therefore the analysis will lead to the false detection of QTL. Another approach for interval mapping using regression method was proposed by Harley and Knott (1992), the method produces the same results as maximum likelihood, but the residual variance and QTL detection power are affected (Xu 1995). Due to these limitations multiple QTL models were proposed due to their ability to estimate the detection and effects of linked QTLs (Schork 1993). The idea of Composite Interval mapping (CIM) which utilizes multiple regression analysis combined with SIM was presented independently by Jansen (1993), Zeng (1993) and Zeng (1994). CIM takes into account the effect of more background markers (sometimes referred as co factors) thus minimizing the effects of QTLs in the remainder genome while finding the QTL in a specific region. The background markers are helpful in the process depending on the probability of linkage between the background markers and interval under study. If the background markers and the interval are linked, background markers helps in separation of linked QTLs and if not linked, the detection process is made more sensitive by these background markers (Zeng 1994). CIM also have some limitations. It is affected by uneven marker distribution across the genome which means the test statistics calculated in marker deficient and marker rich regions cannot be compared, if multiple linked QTLs are present it is difficult to detect their joint contribution to the variance. The CIM analysis cannot be directly extended for the detection of epistasis (Zeng et al. 1999).

To tackle the problems of multiple QTLs the idea of multiple interval mapping (MIM) to map multiple QTLs at the same time was presented by Kao et al. (1999). The concept behind MIM is the integration of effects of multiple QTL and epistasis effects together in order to detect the position and interaction among multiple QTLs. MIM is more powerful and precise in the detection of QTLs in comparison to SIM and CIM. MIM utilizes Cockerham's model which is an interpretation of genetic parameters calculated by maximum likelihood (Kao et al. 1999). While Satagopan et al.

(1996) and Sillanpaa and Arjas (1998) to map multiple QTLs using Bayesian approach which relies on Markov chain Monto Carlo simulations.

## Challenges in QTL mapping

All these statistical methods mentioned above are based on an assumption, that the phenotype is normally distributed and has equal variance distribution in both the parents. These methods are generally considered to be robust against non-normality however robustness for normality is not well defined for QTL mapping methods. Sometimes the phenotype for the trait of interest is not normally distributed, in such cases the phenotypic data can be mathematically transformed i.e. log 10 or arcsine to follow the assumption of normality distribution (Wright 1968). As an alternative, nonparametric statistical methods can be used for QTL mapping (Kruglyak and Lander 1995). Determining an appropriate significance threshold is one of the critical points in QTL mapping. These thresholds are important to avoid type1 error or false positives (finding a QTL when there is not in reality) and type2 error (false negatives (there is not a QTL when in reality there is). It is problematic due to its sensitivity towards the variables involved in an experiment such as sample size, organism's genome size, linkage map density, proportion of missing values and total number of QTLs involved (Churchill and Doerge 1994). Many studies were designed and conducted to deal with the problem of identifying a statistical significance in QTL mapping and few solutions were proposed using various techniques which were (1) permutation test (Churchill and Doerge 1994), (2) bootstrap method of resampling (Efron 1979; Mammen 1993), (3) bootstrap procedure for model selection (Shao 1996) and (4) cross validation (Utz et al. 2000). Usually scientists use a minimum LOD value of 3 to declare a QTL (Collard et al. 2005). Permutation tests are done by shuffling the trait values along with the genotypic values in order to determine the threshold value for the test statistic. Statistical tests are performed on these permutated samples and maximum test statistics are

recorded. The permutation test is repeated many times usually 1000 times to determine the range of maximum test statistics and from this range LOD value consequently (Manly and Olsen 1999). Once the threshold is established original sample's peak value is checked if it's more than the threshold, QTL is declared. Whether the QTL is major or minor depends on the proportion of variation explained, major QTLs explains more variation usually >10 % while minor QTLs explains less variation <10 %. Moreover Major QTLs are usually stable across multiple environments and locations (Li et al. 2001; Collard et al. 2005).

## FHB resistance QTLs

To date, many FHB QTLs have been mapped on almost all chromosomes of wheat. Only one of them is a major effect QTL. Seven of them are formally named.

*Fhb1* (Qfhs.ndsu-3BS) is present on the distal end of chromosome 3BS and shows major effect towards FHB resistance (Cuthbert et al. 2006). This major QTL was mapped by Waldrone et al. (1999) for type II FHB resistance in a RIL population, develped from the cross of Stoa and Sumai-3. This QTL was donated by a Taiwan wheat parent to SM3 (Bai et al. 2003; Shen et al. 2003). Later on this QTL was mapped in different studies at the same location (Anderson et al. 2001; Chen et al. 2006). *Fhb1* QTL was also found in Chinese resistant cultivar Wangshuibai (Mardi et al. 2005; Yu et al. 2008c). Several markers can be used to detect *Fhb1* effectively, like *Xgwm533, Xbarc133, Xgwm493* (Cuthbert et al. 2006) but one of the important and widely used marker for *Fhb1* is *Xumn10* (Liu et al. 2008). To date, *Fhb1* is the most consistent and stable QTL for type II resistance (Anderson et al. 2007).

QTL on chromosome 6BS (*Fhb2*) was mapped between markers *Xgwm133* and *Xgwm644* and it explained 21 % of the phenotypic variation (Yang et al. 2003). It was also fine mapped successfully by Cuthbert et al (2007). *Fhb2* was detected in a RIL mapping population from the

cross of BW278 x AC Formost, and lines with *Fhb2* showed 56 % less severity in greenhouse studies.

*Fhb3* is another important QTL, and was derived from an alien species *Leymus racemosus.* This QTL was identified in wheat–Leymus introgression line (Qi et al. 2008). Translocation chromosome of wheat-Leymus T7AL·7Lr#1S was placed on the long arm of wheat chromosome 7A, by the help of cytogenetic studies (Qi et al. 2008). Studies done in the greenhouse suggested that the lines carrying *Fhb3* shows higher levels of Type II resistance but the effects are not significantly different from the resistant cultivar Sumai-3 .

*Fhb4* (Qfhi.nau-4B) conditioning for Type I resistance, is located on chromosome 4BL and it was fine mapped in a RIL population derived from the cross of Nanda2419 (susceptible parent) and Wangshuibai (resistant parent) (Xue et al. 2010). The *Fhb4* QTL region is marked by flanking markers, *Xhbg226 and Xgwm149* covering a distance of 1.7cM and resistant genotypes show 60% less infection.

*Fhb5* (Qfhi.nau-5A) is present on chromosome 5AS, flanked by *Xgwm30*4 and *Xgwm415* in a 0.3cM interval. It was mapped in two populations, one backcross and one RIL population. Both of them were derived from the cross of Nanda2419 and Wangshuibai. This QTL is for Type I resistance explaining 16.6 to 27% of phenotypic variation for incidence (Lin et al. 2006). *Fhb6* is located on Chromosome 1A was derived from *Elymus tukushiensis*, which is a wild relative of bread wheat (Cainong et al. 2015). And *Fhb7* was derived from *Thinopyrum ponticum* (Guo et al. 2015) it is located on Chromosome 7DS.

Amongst all these *Fhb1* on chromosome 3BS is the most studied major QTL explaining a wide range of variation from 6 % to 60 % for type II resistance (Anderson et al. 2001; Buerstmayr et al. 2002).

## Breeding for FHB resistance

Development and utilization of FHB resistant cultivars has become an important concern globally due to the economic losses and mycotoxin contamination associated with it. Moderate to high level of resistance can be achieved by transferring major resistance QTLs into susceptible / moderate susceptible elite or commercial lines and improving their resistance. Several breeding programs use backcrossing scheme to transfer the required genes in adapted background. Sumai-3 has been used as resistance donor for *Fhb1* in wheat breeding programs worldwide. Nearly 60 % of the advanced resistant lines tested in the U.S. Uniform Regional Scab Nursery from 1995 to 2002 had Sumai-3 in their pedigree (DF Garvin, JA Anderson 2002). Apart from Sumai-3, several other resistance sources in winter wheat like Ernie and Freedom have also been used in wheat breeding programs (Rudd et al. 2001). Owing to the additive gene action, pyramiding of several resistance genes in an elite cultivar has become another important breeding strategy. WSY is a pyramided FHB resistance line, developed by a three way cross among Sumai-3, Wangshuibai and Nobeokabouzu (Shi et al. 2008). Besides this, exploitation of native resistance is also important, breeders identified several native resistant sources in U.S. such as Heyne, Ernie and Freedom. These cultivars do not have *Fhb1* but show good level of resistance. Some resistant sources in U.S. hard red spring wheat include Steele, ND2710, Glenn and Bacup developed in North Dakota and Minnesota respectively (Mergoum et al. 2007). The most effective strategy against FHB would be to explore native resistance sources and followed by the introgressing of major FHB QTLs detected. Conventional breeding is difficult due to the significant amount of time required and repeated cycles of breeding to recover the desirable agronomic traits along with reasonable amount of FHB

resistance (Anderson 2007). Therefore Marker-assisted selection is used to make the breeding process more efficient.

## Marker-assisted Selection

The most effective strategy against FHB is to develop resistant cultivars. It is a challenging task for breeders as along with the resistance, yield and quality cannot be compromised. Marker-assisted selection (MAS) was predicted to be highly applicable in various crops including wheat due to certain advantages over conventional method of selection (Koebner and Summers 2003). Markers are highly helpful in selecting the traits that are difficult to phenotype, highly influenced by the environment and have low heritability. It helps in pyramiding of the resistance genes, which is a useful approach when resistance is mainly due to the additive effects as in the case of FHB. But before markers can be utilized in a breeding program for MAS, it is important to validate them first. Validation of markers is important to test their linkage or association with the QTL or desired gene and with the phenotype as well. A good marker should be able to select for the desired phenotype in different genetic backgrounds (Cakir et al. 2003). FHB resistance in wheat can be improved by MAS because of its quantitative nature that is highly influenced by the environment. According to Bai et al. (2001) breeders should aim for a cultivar with low incidence of infection and less symptoms spread, thus emphasizing breeding for high resistance. Selections for FHB is difficult to perform at early stages in field and a replicated test is required for the accurate results thus MAS can play a role in early generation selection discarding non desired genotypes. In this way, breeder proceeds only with the potential lines carrying resistance which saves resources (Waldron et al. 1999). Also screening results of FHB in greenhouse and field often are not correlated which leads to inconsistent measure of phenotype (Campbell and Lipps 1998). In such cases marker-assisted selection can enhance the genetic gain per unit time and cost (Bernardo 2009). It is important to mention here that

MAS only aids the breeding programs that heavily rely on phenotypic evaluations; it cannot replace phenotyping (Anderson et al. 2007). The success of MAS greatly depends on marker trait association therefore it is necessary to first identify major effect QTLs followed by fine mapping to identify closely linked markers (Kolb et al. 2001). Sometimes using more flanking markers increase the precision of selection (Ma et al. 2006c). Effective markers should be easily used, which makes SSRs, STS, and SNPs as ideal markers. Breeders often look for the markers that are polymorphic in various populations as they prefer to use the same set of markers over different populations (Kolb et al. 2001). Markers developed from the QTLs that show inconsistency in different environments can be problematic (Anderson et al. 2007). Therefore markers generated from the fine mapping of stable and major effect QTLs are ideal for MAS.

**Table 1-1 Summary of reported FHB QTLs from different studies.**

| Type of FHB resistance | Source | QTL Location | Population | References |
|---|---|---|---|---|
| **Type I resistance** | CM-82036 | 3B, 5A, 1B | CM-82036(R) x Remus(S) | (Buerstmayr et al. 2003) |
| | | | DH | |
| **Type I resistance** | Frontana | 3A, 5A | Frontana(R) and Remus(S) | (Steiner et al. 2004) |
| | | | DH | |
| **Type I resistance** | DH181 | 3AS, 5AS, 3BS, 3BSc, 6BS, 2DS and 4DL | DH181(R) x AC Foremost(S) | (Yang et al. 2005a) |
| | AC Foremost (3A) | | DH | |
| **Type I resistance** | Wangshuibai | 5A, 4B and 5B | Wangshuibai (R) x Nanda2419 | (Lin et al. 2006) |
| | | | RIL | |
| **Type I resistance** | Wangshuibai | 3AS, 5AS, 3BS, 4B, and 5DL | Wangshuibai(R) x Wheaton(S) | (Yu et al. 2008) |
| | | | RIL | |
| **Type I resistance** | RL4137 | 1B, 2B, 3A, 6A, 6B, 7A and 7D | RL4137 (R) x Timgalen(MR) | (Srinrvasachary et al. 2008) |
| | | | RIL | |
| **Type I resistance** | Sumai-3 | 3BS, 6BL, 2DS | Sumai-3 (R) x Y1193-6 | (Basnet et al. 2012) |
| | Y1193-6(2DS) | | RIL | |
| **Type I resistance** | Frontana | 3A, 6A, and 4D | Frontana (R) x Chris | (Yabwalo et al. 2011) |
| | | | Reciprocal backcross monosomic (RBCM) | |
| **Type I resistance** | T. dicoccum-161 | 4B, 6A, 6B | T. dicoccum-161 (R) x DS-131621 (durum wheat) | (Buerstmayr et al. 2012) |
| | | | BC1F4 | |
| **Type I resistance** | Floradur(3B) | 3B, 4B, 6B | T. dicoccum-161 (R) x Floradur (durum wheat) | (Buerstmayr et al. 2012) |
| | | | BC1F4 | |
| **Type I resistance** | T. dicoccum-161 | 4B, 7B | T. dicoccum-161 (R) x Helidur (durum wheat) | (Buerstmayr et al. 2012) |
| | | | BC1F4 | |
| **Type I resistance** | DT735 | 2A, 3B, 5B, 7A | BGRC3487 x 2*DT735 (MR) | (Ruan et al. 2012) |

| | | | | |
|---|---|---|---|---|
| | BGRC3487(3B) | | BCRIL | |
| **Type I resistance** | Frontana | 3A, 4A, 6B, 2B, 4B, 5A, 7B | Frontana (R) x Remus | (Szabo-Hever et al. 2012) |
| | | | DH | |
| **Type I resistance** | Jamestown | 1A, 2B, 2D, 3B, 6A, 7A, and 7B | Jamestown x LA97113UC-124 | (Wright et al. 2012) |
| | | | RIL | |
| **Type I resistance** | Jamestown | 1B, 2B, 3A, and 6A | Pioneer25R47 x Jamestown | (Wright et al. 2012) |
| | | | RIL | |
| **Type I resistance** | Massey (4BS) | 2D and 4BS | Becker x Massey (MR) | (Liu et al. 2013) |
| | Becker (2D) | | RIL | |
| **Type I resistance** | Ernie | 4BS, 4DS, 5AL | Ernie (MR) x MO 94-317 | (Liu et al. 2013) |
| | MO 94-317(4BS) | | RIL | |
| **Type I resistance** | Frontana | 1A, 1B, 2D, 3B, 4A, 5A, 5B, 6A, 7B | GKMini Mano x Frontana | (Agnes et al. 2014) |
| | | | | |
| **Type I resistance** | NC-Neuse | 1A, 5B, 6A | NC-Neuse (Moderate R) x AGS | (Petersen et al. 2015) |
| | AGS(5B) | | RIL | |
| **Type II resistance** | Sumai-3 | 3BS, 6BS (Sumai-3) | Sumai-3 (R) x Stoa (MS) | (Waldron et al. 1999) |
| | Stoa(2AL, 4B) | | RIL | |
| **Type II resistance** | Ning7840 | 3BS | Ning7840(R) x Clark (S) | (Bai et al. 1999) |
| | | | RIL | |
| **Type II resistance** | ND2603 | 3AL,6AS, 3BS | ND2603(R) x Butte86(MS) | (Anderson et al. 2001) |
| | | | RIL | |
| **Type II resistance** | Sumai-3 | 2AL, 3BS, 4BS, 6BS | Sumai-3(R) x Stoa(MS) | (Anderson et al. 2001) |
| | Stoa(2AL, 4B) | | RIL | |
| **Type II resistance** | Ning7840 | 2AS, 2BL and 3BS | Ning7840(R) x Clark(S) | (Zhou et al. 2002) |
| | | | RIL | |
| **Type II resistance** | CM-82036 | 5A, 1B and 3BS | CM-82036(R) x Remus(S) | (Buerstmayr et al. 2002) |
| | | | DH | |
| **Type II resistance** | Ning7840 | 3BS | Ning7840(R) x Wheaton(S) | (Zhou et al. 2003) |

| | | | | |
|---|---|---|---|---|
| | | | F2:3 | |
| **Type II resistance** | Ning7840 | 3BS | Ning7840(R) x IL89-7978(S) | (Zhou et al. 2003) |
| | | | F3:4 | |
| **Type II resistance** | CM-82036 | 3BS, 5A | CM-82036(R) x Remus(S) | (Buerstmayr et al. 2003) |
| | | | DH | |
| **Type II resistance** | F201R | 1B, 3A, 3D, 5A | F201R(R) x cv. Patterson (MS) | (Shen et al. 2003) |
| | Patterson(3D) | | RIL | |
| **Type II resistance** | Huapei57-2 | 3A, 3BS, 3BL and 5B | Huapei57-2(R) x Patterson (MS) | (Bourdoncle and Ohm 2003) |
| | Patterson(5B) | | RIL | |
| **Type II resistance** | Wuhan-1(2DL, 4B) | 2DL, 3BSc and 4B | Wuhan-1(R) x Maringa(MS) | (Somers et al. 2003) |
| | Maringa(3BSc) | | DH | |
| **Type II resistance** | Wangshuibai | 1B and 3BS | Wangshuibai(R) x Alondra(S) | (Zhang et al. 2004) |
| | Alondra(1B) | | RIL | |
| **Type II resistance** | Wangshuibai | 7AL, 3BSd, 1BL and 3BSc | Wangshuibai(R) x Wheaton(S) | (Zhou et al. 2004) |
| | | | RIL | |
| **Type II resistance** | DH181 | 3BS, 6BS, 2DS and 7BL | DH181(R) x AC Foremost(S) | (Yang et al. 2005a) |
| | | | DH | |
| **Type II resistance** | Chokwang | 3BS, 4BL and 5DL | Chokwang(R) x Clark(S) | (Yang et al. 2005b) |
| | | | RIL | |
| **Type II resistance** | Dream | 6AL, 1B, 2BL and 7BS | Dream(R) x Lynx(S) | (Schmolke et al. 2005) |
| | Lynx(1B) | | RIL | |
| **Type II resistance** | Wangshuibai | 7A, 3B, 5B and 2D | Wangshuibai(R) x Alondra(S) | (Jia et al. 2006) |
| | | | DH | |
| **Type II resistance** | W14 | 5AS and 3BS | W14(R) x Poin2684(S) | (Chen et al. 2006) |
| | | | DH | |
| **Type II resistance** | CS-SM3-7ADS | 6A, 3B, 2D and 4D | CS-SM3-7ADS (R) x Annong 8455(S) | (Ma et al. 2006a) |
| | | | RIL | |

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| **Type II resistance** | Wangshuibai | 3B, 2A | Wangshuibai (R) x Annong 8455 | (Ma et al. 2006b) |
| | | | RIL | |
| **Type II resistance** | Sumai-3 | 3BS | Sumai-3*5(R) x Thatcher(S) and HC374(R) x 3*98B69-L47(S) | (Cuthbert et al. 2006) |
| | | | RIL | |
| **Type II resistance** | Frontana | 3AL, 7AS and 1BL | Frontana (MR) x Seri82(S) | (Mardi et al. 2006) |
| | Seri82(1BL) | | F3:5 | |
| **Type II resistance** | CJ9306 | 1AS, 3BS, 7BS, 2DL, 5AS | CJ9306(R) x Veery(S) | (Jiang et al. 2007) |
| | | | RIL | |
| **Type II resistance** | Sumai-3 | 3BSc, 5A, 6B | BW278(R) x AC Foremost(S) | (Cuthbert et al. 2007) |
| | | | RIL(from 1440) Sumai-3 | |
| **Type II resistance** | Arina | 1AL, 7AL, 1BL, and 6BS | Arina (MR) x NK93604(MR) | (Semagn et al. 2007) |
| | NK93604(1AL, 7AL) | | DH | |
| **Type II resistance** | Ernie | 5A, 2B, 3B, and 4BL | Ernie(MR) x MO 94-317(S) | (Liu et al. 2007) |
| | | | RIL | |
| **Type II resistance** | Wangshuibai | 5AS, 7AL, 3BS, 3DL and 5DL | Wangshuibai(R) x Wheaton(S) | (Yu et al. 2008) |
| | | | RIL | |
| **Type II resistance** | G16-92 | 1A and 2BL | G16-92(R) x Hussar(S) | (Schmolke et al. 2008) |
| | Hussar(1A) | | RIL | |
| **Type II resistance** | Gamenya | 2DS | Sumai-3(R) x Gamenya(S) | (Handa et al. 2008) |
| | | | DH | |
| **Type II resistance** | IL94-1653(2B, 4B) | 2B, 3B, 4B and 6B | IL94-1653 x Patton | (Bonin and Kolb 2009) |
| | Patton(3B, 6B) | | RIL | |
| **Type II resistance** | G93010 | 7BS /5BL, 6BS and | G93010 (R) x Pelikan | (Häberle et al. 2009) |
| | | | RIL | |
| **Type II resistance** | Wangshuibai | 7A, 1B, 3B, 6B and 2D | Wangshuibai(R) x Sy95-7(S) | (Zhang et al. 2010) |
| | | | F2:3 | |

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| **Type II resistance** | T. macha | 2A, 5A, 2B, 5B | T. macha(R) x Furore(S) | (Buerstmayr et al. 2011) |
| | | | RIL | |
| **Type II resistance** | Sumai-3 | 7AC and 3BS | CS-Sumai 3-7ADSLC | (Jayatilake et al. 2011) |
| | | | RIL | |
| **Type II resistance** | Haiyanzhong | 1AS, 5AS, 6BS(2) and 7DL | Haiyanzhong (R) x Wheaton | (Li et al. 2011) |
| | | | RIL | |
| **Type II resistance** | PI 277012 | 5AS, 5AL | PI 277012 (R) x Grandin | (Chu et al. 2011) |
| | | | DH | |
| **Type II resistance** | Frontana | 3A, 6A, and 4D | Frontana (R) x Chris | (Yabwalo et al. 2011) |
| | | | Reciprocal backcross monosomic (RBCM) | |
| **Type II resistance** | Huangfangzhu | 1AS, 5AS, 7AL, 1B and 3BS | Huangfangzhu(R) x Wheaton | (Li et al. 2012) |
| | | | RIL | |
| **Type II resistance** | Heyne | 3AS, 4AL and 4DL | Heyne(R) x Trego | (Zhang et al. 2012a) |
| | | | RIL | |
| **Type II** | Baishanyuehuang | 3BSd, 3BSc, 3A, 5A | Baishanyuehuang (R) x Jagger | (Zhang et al. 2012b) |
| | | | RIL | |
| **Type II resistance** | BGRC3487 | 3B, 5A, 5B, 7A, 7B | BGRC3487 x 2*DT735 (MR) | (Ruan et al. 2012) |
| | DT735(7A, 7B) | | BCRIL | |
| **Type II resistance** | RCATL33 | 3B, 5A, 3A | RCATL33(R) x RC Strategy | (Tamburic-Ilincic and Miedaner 2012) |
| | | | RIL | |
| **Type II resistance** | VA00W-38 | 1BL, 2A, 2DL, 5B, 6A, and 7A | VA00W-38 (MR) x 26R46 | (Liu et al. 2012) |
| | 26R46 (7A) | | RIL | |
| **Type II resistance** | Jamestown | 1A, 2B, 2D, 3B, 6A, 7A, and 7B | Jamestown x LA97113UC-124 | (Wright et al. 2012) |
| | | | RIL | |
| **Type II resistance** | Jamestown | 1B, 2B, 3A, and 6A | Pioneer25R47 x Jamestown | (Wright et al. 2012) |
| | | | RIL | |

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| **Type II resistance** | Mt. Gerizim #36 | 3A, 6B | Mt. Gerizim #36 (R) x Helidur BC | (Buerstmayr et al. 2013) |
| **Type II resistance** | Becker | 1DS, 3BL | Becker x Massey RIL | (Liu et al. 2013) |
| **Type II resistance** | Ernie | 2DS, 4BS, 4DS, 5AL, 3BL, 4BS | Ernie x MO 94-317 | (Liu et al. 2013) |
| | MO 94-317(4B) | | RIL | |
| **Type II resistance** | Catbird | 7DS, 3BS, 5DL | Catbird x Milan DH | (Cativelli et al. 2013) |
| **Type II resistance** | Huangcandou | 3BSc, 3BSd, 3AS, 2D, and 6D | Huangcandou(R) x Jagger | (Cai and Bai 2014) |
| | Jagger(2D, 6D) | | RIL | |
| **Type II resistance** | Ben (2A) | 2A, 3A, 5A | Ben(Durum) x PI41025 | (Zhang et al. 2014) |
| | PI41025(3A, 5A) | | RIL | |
| **Type II resistance** | NC-Neuse | 1A, 2A, 6A | NC-Neuse (Moderate R) x AGS RIL | (Petersen et al. 2015) |
| **Type III resistance** | Wuhan-1 (2D) | 5AS, 2D, 3BS | Wuhan-1(R) x Maringa (MS) | (Somers et al. 2003) |
| | Maringa (5AS, 3BS) | | DH | |
| **Type III resistance** | CM-82036 | 3BS | CM-82036(R) x and Remus DH | (Lemmens et al. 2005) |
| **Type III resistance** | W14 | 5AS and 3BS | W14(R) x Poin2684(S) DH | (Chen et al. 2006) |
| **Type III resistance** | CJ9306 | 2DL, 1AS, 3BS, 5AS | CJ9306(R) x Veery(S) RIL | (Jiang et al. 2007) |
| **Type III resistance** | NK93604 | 1AL and 2AS | Arina (MR) x NK93604(MR) DH | (Semagn et al. 2007) |
| **Type III resistance** | Wangshuibai | 1A, 5AS, 7AL, 1BL, 3BS and 5DL | Wangshuibai(R) x Wheaton(S) RIL | (Yu et al. 2008) |
| **type III resistance** | Sumai-3 | 7AC and 3BS | CS-Sumai-3 - 7ADSLC | (Jayatilake et al. 2011) |

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| | | | RIL | |
| **Type III resistance** | PI 277012 | 5AS, 5AL | PI 277012 (R) x Grandin | (Chu et al. 2011) |
| | | | DH | |
| **Type III resistance** | RCATL33 | 3B, 5A, 3A | RCATL33(R) x RC Strategy | (Tamburic-Ilincic and Miedaner 2012) |
| | | | RIL | |
| **Type III resistance** | VA00W-38 | 1BL, 2A, 2DL, 5B, 6A, and 7A | VA00W-38 (Moderate R) x 26R46 | (Liu et al. 2012) |
| | 26R46 (7A) | | RIL | |
| **Type III resistance** | Jamestown | 1A, 2B, 2D, 3B, 6A, 7A, and 7B | Jamestown x LA97113UC-124 | (Wright et al. 2012) |
| | | | RIL | |
| **Type III resistance** | Jamestown | 1B, 2B, 3A, and 6A | Pioneer25R47 x Jamestown | (Wright et al. 2012) |
| | | | RIL | |
| **Type III resistance** | Becker | 4DL | Becker x Massey | (Liu et al. 2013) |
| | | | RIL | |
| **Type III resistance** | Frontana | 1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D | GKMini Mano x Frontana | (Agnes et al. 2014) |
| | | | DH | |
| **Type III resistance** | NC-Neuse | 1A, 1B, 1D, 2A, 4A, 5B | NC-Neuse (MR) x AGS | (Petersen et al. 2015) |
| | AGS(1D, 5B) | | RIL | |
| **Type IV resistance** | W14 | 5AS and 3BS | W14(R) x Poin2684(S) | (Chen et al. 2006) |
| | | | DH | |
| **Type IV resistance** | IL94-1653 | 2B, 4B, and 6B | IL94-1653 x Patton | (Bonin and Kolb 2009) |
| | Patton(6B) | | RIL | |
| **Type IV resistance** | PI 277012 | 5AS, 5AL | PI 277012 (R) x Grandin | (Chu et al. 2011) |
| | | | DH | |
| **Type IV resistance** | Frontana | 3A, 6A, and 4D | Frontana (R) x Chris | (Yabwalo et al. 2011) |
| | | | Reciprocal backcross monosomic (RBCM) | |
| **Type IV resistance** | RCATL33 | 3B, 5A, 3A | RCATL33(R) x RC Strategy | (Tamburic-Ilincic and Miedaner 2012) |

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| | | | RIL | |
| **Type IV resistance** | VA00W-38 | 1BL, 2A, 2DL, 5B, 6A, and 7A | VA00W-38 (MR) x 26R46 | (Liu et al. 2012) |
| | 26R46 (7A) | | RIL | |
| **Type IV resistance** | Frontana | 2B, 4B, 5A, 7B | Frontana (R) x Remus | (Szabo-Hever et al. 2012) |
| | | | DH | |
| **Type IV resistance** | Massey | 4BS | Becker x Massey | (Liu et al. 2013) |
| | | | RIL | |
| **Type IV resistance** | Ernie | 4BS, 4DS, 3BL | Ernie x MO 94-317 | (Liu et al. 2013) |
| | MO 94-317(4B) | | RIL | |
| **Type IV resistance** | Frontana | 1B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D | GKMini Mano x Frontana | (Agnes et al. 2014) |
| | | | DH | |
| **Type IV resistance** | NC-Neuse | 1A, 1B, 1D, 4A, | NC-Neuse (Moderate R) x AGS | (Petersen et al. 2015) |
| | AGS(1D) | | RIL | |

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# Chapter 2 - Mapping Quantitative Trait Loci for Fusarium Head Blight Resistance in Overland using Genotyping-by-Sequencing Markers

## Introduction

Fusarium head blight (FHB), also known as scab, is one of the most destructive fungal diseases in wheat and other small grain cereals grown in humid and semi-humid areas of the world (Bai and Shaner, 1994). In the U.S.A., it is primarily caused by *Fusarium graminearum* Schwabe (telomorph, *Gibberella zeae* (Schw.) Petch). Infection usually occurs at wheat anthesis and results in bleaching of the infected spikes, and sometimes, premature plant death (Bai and Shaner, 1994). Infected spikes produce small shriveled kernels, also called tombstones, which reduce not only grain yield but also quality. Mycotoxins produced by the pathogen are also harmful to animal and human health (Bai and Shaner 2004). FHB was first reported in 1884 in England by Smith, and then in 1890 in Indiana, U.S.A., and both were associated with significant yield losses (Stack 2003; Bai and Shaner 1994).

Devastating outbreaks of FHB occurred in the U.S.A. during the 1990s, which caused severe yield losses and seriously impacted the U.S. economy. The United States Department of Agriculture (USDA) declared these epidemics to be the worst since the 1950s (Wood et al. 1999). Nganje et al. (2004) estimated economic losses due to the FHB epidemics to be $2.49 billion from 1993 to 2001. Although many approaches have been used to minimize the damage caused by the disease, including tillage practices, crop rotation, and fungicide application, yet the most effective and economical strategy is to use host plant resistance (McMullen et al. 1997). Since 1990s, improving FHB resistance has become one of the important breeding objectives in the breeding

programs of the major U.S. wheat producing states. FHB resistance in wheat is a complex trait and several types of resistance have been defined, including resistance to initial penetration and infection (type I), to infection spread within the spike (type II) (Schroeder and Christensen 1963) and to kernel damage, (type IV) (Mesterhazy 1995). In the 1980s after an extensive screening of Chinese germplasm for FHB resistance, a few landraces such as Sumai-3 and its derivatives such as Ning7840 were identified as the best available resistance sources for type II resistance (Bai and Shaner 1994, Yang 1994). Later, several other resistant germplasms from Europe and North America were reported (Klahr et al. 2007; Häberle et al. 2007; Paillard et al. 2004).

Although more than 50 quantitative traits loci (QTL) for FHB resistance from different cultivars have been reported on almost all 21 chromosomes of wheat (Buerstmayr et al. 2009; Liu et al. 2009), yet the most stable QTL is *Fhb1* on chromosome arm 3BS derived from Sumai-3 (Bai and Shaner, 2004). Many cultivars in the U.S. have been found as potential native sources of resistance, i.e. soft winter wheat cultivars Roane, Ernie, and Freedom showed moderate FHB resistance in several studies (Rudd et al. 2001, Griffey et al. 2001). The resistance genes in these cultivars were found to be different from that in Chinese sources (Liu et al. 2005), with three QTLs on 3B, 4B and 5A identified in Ernie (Liu et al. 2007) and one on 2A from Freedom (Gupta et al in 2001). Screening for FHB resistance sources in hard winter wheat (HWW) germplasm was not done until recently. Several cultivars with moderate FHB resistance were identified, such as Heyne and Hondo (Bockus et al. 2009), in which the resistance genes were also characterized as different from Chinese sources (Zhang et al. 2012). Recent evaluations of HWW accessions for FHB resistance identified moderately to highly resistant accessions in both greenhouse and field experiments, including SD05210, Lyman, Overland, Everest, Harry and Hitch (Jin et al. 2013). Most of these accessions are adapted cultivars to the Great Plains. In Heyne, three major QTLs

were identified on chromosome arms 3AS, 4AL and 4DL (Zhang et al. 2012). Mapping studies in the other native resistance sources have not been reported yet (Jin et al. 2013).

Most of the mapping studies are based on low-density molecular maps. Recently genotyping-by-sequencing (GBS) has emerged as an efficient technique to develop SNP markers in diverse species (Poland et al. 2012). Abundance of SNP markers from GBS warrants construction of high-density genetic maps for QTL mapping. In the current study, we used GBS-SNP markers to map putative QTLs associated with FHB resistance in Overland, a moderately resistant hard red winter wheat cultivar from Nebraska. The objectives of the current study were to determine number of QTLs for FHB resistance and locate their chromosomal locations in Overland, to identify the SNPs closely linked to the QTLs, and convert the GBS-SNPs into Kbioscience competitive allele specific PCR (KASP) assays to be used in marker-assisted selection (MAS).

## Materials and methods

### Plant materials and FHB resistance evaluation

A mapping population of 186 $F_{5:6}$ recombinant inbred lines (RILs) was developed by single seed descent from the cross of Overland x Overley. Overland is a hard red winter wheat from Nebraska with moderate resistance to FHB, while Overley is a highly susceptible hard red winter wheat from Kansas. This RIL population was evaluated for type II FHB resistance in the greenhouse at Kansas State University, Manhattan KS. FHB evaluation was conducted in spring 2014, spring 2015 and fall 2015, with two replications in each experiment. Both RILs and the parents were planted in plastic trays filled with Metro-mix 360® soil mix (Hummert International, Topeka, KS). After vernalization in a cold room at 6$^{o}$C for 7 weeks, the seedlings were transplanted into a 4” x 4” Dura pot filled with Metro-mix 360® soil mixes. Five seedlings per RIL were planted in each pot. The pots were arranged on the greenhouse benches in a randomized complete block design (RCBD). To

control the powdery mildew infection, sulfur was burned for three hours each night in a closed greenhouse environment. This procedure has no impact on the development of FHB. The greenhouse temperature was maintained between 20-25$^{o}$C with 12 h of supplemental light.

A Kansas strain of *F. graminearum* (GZ3639) was used as the inoculum and a conidial suspension was prepared for inoculation following Bai et al. (1999). When the wheat spikes reached anthesis stage, five plants per pot were inoculated using the single floret inoculation method. A 10 µl conidial suspension containing ~1000 spores was injected into a floret in the central spikelet of a spike using a syringe (Hamilton, Reno, NV). The plants were kept in a moist chamber at 24$^{o}$C for 48 h with 100% humidity to facilitate disease initiation after inoculation, and then returned to the greenhouse benches for FHB development. FHB-symptoms were scored 14 d post inoculation when Overley reached about 95 % severity. FHB severity was calculated as

PSS= number of infected spikelets/ the total number of spikelets * 100

**FHB in field experiment**

FHB for RILs and the parents were evaluated at KSU Plant Pathology FHB Nursery, Rocky Ford, Manhattan, KS, in 2015 using the corn grain-spawn inoculation method (Tuite, 1969). The experiment was conducted in RCBD design with two replications per RIL, and a seeding rate of 1 g was planted in a 1.2 m single row plot. *F. graminearum* infected corn kernels were scattered in the field twice at booting stage and two weeks after. The field nursery was misted by sprinklers for 3 min per h between 21:00 h and 06:00 h daily from anthesis to early dough stage. FHB was visually scored 15 to 21 d post anthesis, varying with flowering times using a scale of 1% to 100% based on the overall performance in each row. Plant height and heading dates were also recorded for each entry. Fusarium damaged kernels (FDK) was visually scored by estimating the percentage of FDK

in total kernels harvested from each plot. Each row was harvested and manually threshed carefully for FDK evaluation.

## Statistical analysis

Analysis of variance (ANOVA) for greenhouse data and field data was performed separately using PROC GLM function in SAS v 9.1.2 (SAS Institute Inc. Cary. NC). The Pearson correlations were estimated in SAS by PROC CORR function. Genetic correlations were calculated with multivariate analysis using PROC MIXED in SAS as explained by Isik (2009). Heritability was estimated using the following formula;

$h^2 = \sigma^2_g / (\sigma^2_e / re + \sigma^2_{ge} / e + \sigma^2_{g)}$ where

$\sigma^2_g$ = Estimate of genetic variance

$\sigma^2_{ge}$ = Estimate of G x E variance

$\sigma^2_e$ = Estimate of environmental variance

## DNA extraction and genotyping

Leaf tissues were collected in 96-deepwell plates at three-leaf stage, dried in a freeze dryer (ThermoSavant, Holbrook, NY) for 72 h, and ground using Mixer Mill (MM 400, Retsch, Germany). DNA extraction was performed using modified cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). DNA quality was examined on 1% agarose gel using gel electrophoresis.

## GBS library construction

Genomic DNA from RILs and three replications of each parent were used to construct a library. The library was multiplexed at 192-plex using barcodes, following the protocol of Poland et al. (2012). DNA quantification was done using Quant-iT$^{TM}$ PicoGreen® dsDNA Assay (Life Technologies Inc., NY). Normalized DNA 20 ng/µl was digested using *HF-PstI* and *MspI* (New

England BioLabs Inc., Ipswich, MA 01938) at 37$^{o}$C for 2 h and then at 65$^{o}$C for 20 min to deactivate the enzymes.

The digested samples were then ligated to a set of 192 barcodes at 22$^{o}$C for 2 h followed by 65$^{o}$C for 20 min. The ligated samples were cleaned up with QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA,) before PCR. The PCR products were cleaned up again using the same Purification kit. The purified PCR products at the range of 250-300bp were size selected on an E-gel and the selected products were sequenced in an Ion Proton system (Life Technologies, Carlsbad, CA.).

## GBS data processing and SNP calling

The sequence reads from Ion Proton were filtered and trimmed to 64bp for SNP call. Only the reads with 80% of bases having a higher quality score of > 15 were retained. SNPs with < 20% missing data, > 5% minor allele frequency and < 10% heterozygotes were selected for further marker analysis. Sequences from both the parents (3 replicates) were aligned to identify SNPs and those with the least mismatches (≤ 3bp) were used as reference for the RILs. GBS data were analyzed using a reference independent UNEAK pipeline in TASSEL (Lu et al. 2013; Poland et al. 2012). SNPs tightly linked to QTLs were furthur validated using Kbioscience allele specific PCR (KASP) assays. These assays were designed using GBS-SNP sequences linked to the QTLs. The reaction master mix for each reaction included 3 µl of DNA, 2.9 µl of 2x KASP reaction mix and 0.08 µl of primer mix. PCR reactions were incubated at 94$^{o}$C for 15 min, 10 cycles of 94$^{o}$C for 20 sec, annealing at 65$^{o}$C for 60 sec with a decrease of 0.8 $^{o}$C every cycle followed by additional 40 cycles at 94 $^{o}$C for 20 sec and 57 $^{o}$C for 1 min. The plates were read using an Applied Biosystems 7900HT Fast Real-Time PCR system (Life Technologies Inc.,) after the PCR.

### Linkage map construction and detection of QTL

A genetic linkage map of GBS-SNPs was constructed using Kosambi mapping function (Kosambi 1994) and regression mapping algorithm in JoinMap v4.0 (Van Ooijen, 2006b). SNPs that were mapped at the same location were considered to be redundant. In such cases only the SNP with the lowest percentage of missing data was kept in the map. QTLs were mapped using Inclusive Interval Mapping in QTL ICiMapping software (Wang et al. 2011).

## Results

### FHB in RIL population

Frequency distribution of RILs was continuous across the three experiments (Fig 2-1). Mean PSS for the RILs over the three greenhouse experiments was 47.7%, ranging from 38.94% (fall 2015) to 62.18% (spring 2014). Overland consistently showed moderate FHB resistance with a mean PSS of 33.21% ranging from 20.99% to 45.50% in the three greenhouse experiments. Overley however had a mean PSS of 87.7% ranging from 81.33% to 93.14%. The positive correlations were significant *($P < 0.001$)* among the three experiments. Analysis of variance (ANOVA) showed significant effects of genotypes, experiments, and genotypes x experiments *($P < 0.0001$)* in all three greenhouse experiments (Table2-1) and a significant effect of genotypes in the field experiment (Table 2-2). Broad sense heritability for PSS was low (0.45).

In the 2015 field experiment, FHB scores showed continuous distribution (Fig 2-2). The mean PSS for RILs was 49.31%, ranging from 22% to 85%. Overland and Overley had a mean of 40% and 85%, respectively. The mean FDK was 49.31% for the RILs, 50% for Overland and 77.5 % for Overley.

Phenotypic correlations were calculated among plant height, heading date, PSS, FDK, and seed weight (SW) (Table 2-2). Field severity (FPSS) was significantly correlated with post-harvest

traits *(P < 0.001).* As expected, the seed weight (SW) was negatively correlated with the PSS. The correlations were not significant between PSS and heading date or plant height in either field or greenhouse experiments (Table 2-2). The genetic correlations overall were greater than phenotypic correlations, but the trend of correlation was similar between the traits. FPSS was significantly correlated with FDK *(r=0.87).* SW was negatively correlated with FPSS and FDK, while plant height was positively correlated to FDK (Table 2-3).

## Linkage Map

A total of 3079 markers, including 16 polymorphic SSRs and 3063 GBS SNP markers, were used for constructing the linkage map, and 2401 makers were mapped in 36 linkage groups covering 2267.36 cM of genetic distance. The average map density was 0.94 cM per marker. All the chromosomes were represented in this linkage map, with 45% markers mapped in the B genome, 38% in A genome and 17% in D genome. The marker number in each linkage group ranged from 4 to 226, spanning a distance from 8 cM to 146 cM.

## QTL analysis

Inclusive composite interval mapping detected three QTLs for PSS (type II resistance) on three different chromosomes 4A, 4D, and 5B when PSS data from the RIL population evaluated in three greenhouse experiments and one field experiment were analyzed (Table 2-4). The QTL for low PSS on 4DL was consistent in all three greenhouse experiments and the field experiment. It explained 7.66% - 9.73% of the phenotypic variation for low PSS and 14.39% of the phenotypic variation for low FDK. The QTLs on chromosome 4A, and 5B for low PSS were observed only in the field experiment, and explained 16.12%, and 5.43% of the phenotypic variation, respectively. Two QTLs for low FDK on chromosome 4D and 5B were also observed only in the field experiment, explaining 14.39% and 9.00 % of the phenotypic variation, respectively. The 4DL QTL

for low PSS and FDK overlapped each other at the same position, indicating the same QTL contributed to both types of resistance. However the QTL on 5B for low PSS and FDK were mapped in different locations and they were 64 cM from each other, therefore they are likely different QTLs. Overland contributed the low PSS or FDK alleles at all three QTLs.

Four SNPs were mapped in the 4DL QTL region, two SNPs, *XGbs1891* and *XGbs2702*, were consistently associated with low PSS in three greenhouse experiments. These two markers were successfully converted to KASP assays and remapped to the 4DL QTL region with identical SNP calls as the GBS SNPs in the RILs. The two KASP assays were validated using an association mapping population of 96 U.S. elite wheat lines and cultivars. Both KASP assays were well amplified in the association mapping population and separated the population in two unequal clusters, and showed that the allele associated with FHB resistance is present in most of the U.S. wheat accessions (Fig 2-2 – 2-3). These KASP assays (Table 2-5) can be very beneficial in marker-assisted selection, if the parents used in breeding programs are polymorphic at these SNP loci.

## Discussion

The quantitative nature of FHB resistance makes it a complex trait (Jia et al. 2005). The effect of these environmental factors can be minimized by increasing replications in each experiment, repeating the experiments for multiple times in different locations and using large size of mapping populations (Bai et al. 1999). In the current study, a mapping population of 186 RILs was used for QTL mapping, which is relatively larger as compared to some other studies (Anderson et al. 2001; Ma et al. 2006, Jia et al. 2005, Lemmens et al. 2005). FHB was evaluated in three greenhouse experiments with two replications in each experiment. Point inoculations in the greenhouse experiments allows measurement of FHB symptom spread from a single inoculation

site, to accurately estimate type II resistance (Bai et al. 1999). The positive correlations (0.45 - 0.52) among the greenhouse experiments were significant at $P < 0.001$.

In the field experiment, a strong positive correlation *(r = 0.71)* was observed between FPSS and FDK, and a negative correlation *(r = -0.6)* between field PSS and seed weight, which was consistent with several other studies (Lemmens et al. 2005, Somers et al. 2003, Bai et al. 2001,) indicating FHB infection significantly reduces seed weight. A weak correlation of PSS ($r = 0.12$) was observed between greenhouse and field experiments, which agrees with other studies ( Bai et al. 2001, Hall and Van Sanford 2003, Chen et al. 2006, Zwart et al. 2008). This can be due to different inoculation methods used between greenhouse and field experiments. In the greenhouse experiments, we used single point inoculation, whereas corn grain-spawn inoculation was used in the field experiments where multiple infection sites on the spikes are common. Similarly, in this study, correlations between greenhouse PSS and field FDK and seed weight were also weak as reported in other studies (Bai et al. 2001).

Three putative QTLs were identified in this study. The first QTL on 4DL was significant in all greenhouse and field experiments. The QTL from the field study data was mapped close to *Xwmc720*, while the ones from greenhouse experiments were mapped close to *XGbs2702*. The position variation can be due to the environmental effects of FHB evaluations in different experiments. This QTL explained 7.66% - 9.73% of the phenotypic variation for low PSS and 14.39% of the phenotypic variation for low FDK, thus the 4DL QTL is the most important QTL for FHB resistance in Overland. Two major QTLs have been reported on 4D in other studies; one on 4DS associated with plant height gene *Rht-D1 (Rht2)* in some European winter wheat including Arina (Draegar et al. 2007), Spark (Srinivasachary et al. 2008), Apache, History and Messay (Holzapfel et al. 2008). Another QTL was reported near the marker *Xwmc331* on 4DL that explain

10% - 13% of the phenotypic variation in DH181 (Yang et al. 2005), Arina (Draegar et al. 2007) and CS-SM3-7ADS (Ma et al. 2006). Yang et al (2005) reported a QTL on 4DL mapped in DH181 that was responsible for not only type II resistance but also low FDK. Recently Zhang et al (2012) also reported a QTL on 4DL associated with *Xwmc720* and *Xwmc331* in hard winter wheat Heyne that explained 13.8 % - 23.4 % of the phenotypic variation. The QTL mapped in this study is most likely the same QTL as mapped in Heyne, DH181 and Chinese Spring based on the common markers mapped in these studies.

The second QTL detected on 4AL was significant only in the field experiment. It explained 16.12% phenotypic variation for type I and type II FHB resistance. It was flanked by markers *XGbs1012* and *XGbs1117*. To date two QTLs on 4A have been reported, one for type I resistance on the distal end of 4AS that was flanked by *Xgwm165 –Xgwm601* in a wild wheat relative, *T. macha* (Steed et al. 2005); and another minor QTL on 4AL from Arina (Paillard et al. 2004) that was flanked by *Xcdo545 – Xgwm160,* in the distal end of 4AL. However, the relationship between the QTL identified in the current study and the QTL identified in Arina cannot be determined because common markers are not available between the QTLs.

The third QTL was a minor QTL detected on 5BL in the field experiment. This QTL explained 5.43% of the phenotypic variation for type I and type II FHB resistance. Five QTLs on 5B have been identified in previous studies and they were all detected in field experiments. Two QTLs on 5B were identified in Wangshuibai explaining 13.3% and 10.8% of the phenotypic variation (Jia et al. 2005). Paillard et al. (2004) reported a QTL on 5BL in Forno flanked by *Xgwm371 – Xgwm639* which explained 14.3% of the phenotypic variation for type II resistance. Klahr et al. 2007 reported a QTL on 5BL in Cansas, explaining 20% of the phenotypic variation for type II. Bourdoncle and Ohm (2003) also reported a minor QTL on 5BL associated with marker

*Xbarc59* in Patterson, explaining 7.1% for the phenotypic variation for type II resistance. It is not clear if the QTL on 5B in this study is similar to the ones reported previously, as there were no common markers available between the QTLs from these studies.

GBS technique on one hand is robust in identifying SNPs in diverse species but on the other hand GBS-SNPs often have a lot of missing data. The missing data can be reduced by increasing library runs, which also results in an increased cost per sample. Developing KASP assays for GBS SNPs that are closely linked to the QTL not only validates the SNP calls but also helps to eliminate the missing values to improve map quality. In this study, we developed two KASP assays for 4DL QTL that can be used by breeders to transfer the resistance alleles to new cultivars using marker-assisted selection. This study characterized the FHB resistance QTLs in HWW Overland. The dissection of the native resistance QTLs in HWW, and identifying the markers closely linked to these QTLs are important steps towards marker-assisted breeding. These native sources have potential to be used as parents in different breeding programs to pyramid minor resistance QTLs. These minor resistance alleles, together with *Fhb1*, can significantly improve FHB resistance in HWW germplasm.

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**Table 2-1 Aalysis of variance (ANOVA) of mean PSS data for RILs evaluated in three greenhouse experiments.**

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Experiments** | 2 | 119643.2537 | 59821.6268 | 128.44 | <.0001 |
| **Genotypes** | 185 | 227401.8478 | 1229.1992 | 2.64 | <.0001 |
| **Replicati**on **(Experiment)** | 3 | 3606.7970 | 1202.2657 | 2.58 | 0.0528 |
| **Experiment*Genotypes** | 357 | 241769.3255 | 677.2250 | 1.45 | <.0001 |
| **Error** | 506 | 235663.9791 | 465.7391 | | |
| **Corrected Total** | 1053 | 828716.2069 | | | |

**Table 2-2 Analysis of variance (ANOVA) of mean PSS for RILs in the field experiment conducted in spring 2015**

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Genotypes** | 185 | 64887.70161 | 350.74433 | 3.73 | <.0001 |
| **Replication** | 1 | 462.97043 | 462.97043 | 4.92 | 0.0277 |
| **Error** | 185 | 17399.52957 | 94.05151 | | |
| **Corrected Total** | 371 | 82750.20161 | | | |

**Table 2-3 Phenotypic correlations among seed weight (SW), fusarium damaged kernels (FDK), field severity (FPSS), greenhouse severity (GPSS), plant height (PH), and heading days (HD)**

| | FDK | FPSS | GPSS | PH | HD |
|---|---|---|---|---|---|
| **SW** | -0.573*** | -0.598*** | -0.014 | -0.016 | -0.420*** |
| **FDK** | | 0.706*** | 0.266** | 0.052 | 0.03 |
| **FPSS** | | | 0.125 | 0.01 | 0.047 |
| **GPSS** | | | | 0.093 | -0.049 |
| **PH** | | | | | 0.36*** |

FPSS = Percentage of infected spikelets evaluated in field experiment;
GPSS= Mean percentage of infected spikelets evaluated in greenhouse experiments;
SW = Seed weight; PH= plant height; HD = Heading date
FDK =Fusairum damaged Kernels
**P ≤ 0.05 **P ≤ 0.01 *** P ≤ 0.001*

**Table 2-4 Genetic correlations among the traits measured in the field experiment**

| | FPSS | SW | PH | HD |
|---|---|---|---|---|
| **FDK** | 0.875*** | -0.701*** | 0.12* | 0.046 |
| **FPSS** | | -0.826*** | 0.028 | 0.016 |
| **SW** | | | -0.121* | -0.524 |
| **PH** | | | | 0.367*** |

FPSS = Percentage of scabbed spikelets evaluated in field experiment; SW = Seed weight; FDK =Fusairum damaged Kernels;
PH = plant height; HD = heading date
**P ≤ 0.05 **P ≤ 0.01*** P ≤ 0.001*

**Table 2-5 Quantitative trait loci (QTLs) for Fusarium head blight resistance detected by composite interval mapping using mean PSS of RILs in three greenhouse and one field experiment.**

| Experiment | Locus | Flanking marker | LOD | $R^2$ % | Comments |
|---|---|---|---|---|---|
| **Greenhouse, Spring2014** | 4DL | Wmc720 – Gbs0725 | 3.36 | 8.74 | Type II |
| **Greenhouse, Fall2015** | 4DL | Gbs2702 - Gbs2460 | 2.84 | 7.92 | Type II |
| **Greenhouse, Spring2015** | 4DL | Wmc720 – Gbs0725 | 3.59 | 9.73 | Type II |
| **Mean PSS greenhouse** | 4DL | Gbs2702 – Gbs2460 | 5.54 | 13.85 | Type II |
| **2015 Field** | 4DL | Gbs1891 – Gbs2702 | 4.26 | 7.66 | Type II |
| | 4DL | Gbs3063 – Gbs1891 | 6.12 | 14.39 | Type IV |
| | 4AL | Gbs1012 - Gbs1117 | 8.35 | 16.12 | Type I and Type II |
| | 5BL | Gbs2344 - Gbs1715 | 2.96 | 5.43 | Type I and Type II |
| | 5BL | Gbs2885 - Gbs2446 | 3.94 | 9.00 | Type IV |

*Note*: PSS = Percentage of symptomatic spikelets; RILs = Recombinant inbred lines

**Table 2-6 List of KASP assays developed from GBS SNPSs**

| | | |
|---|---|---|
| **Gbs1891_Ovd** | 4DL | GAAGGTGACCAAGTTCATGCTCGCCTTCTTCACCTGGGAc |
| **Gbs1891_Ovy** | 4DL | GAAGGTCGGAGTCAACGGATTCGCCTTCTTCACCTGGGAt |
| **Gbs1891_R** | 4DL | ACCGCGCGACCCTGCT |
| **Gbs2702_Ovd** | 4DL | GAAGGTGACCAAGTTCATGCTTGCAGTCGTCCATCTTCg |
| **Gbs2702_Ovy** | 4DL | GAAGGTCGGAGTCAACGGATTTGCAGTCGTCCATCTTCa |
| **Gbs2702_R** | 4DL | GACTTCCAAACAATCAGACACGA |

*Note*: KASP = Kbioscience competitive allele specific PCR assay; Ovd forward primer with "Overland" allele; Ovy forward primer with "Overley" allele, R reverse primer

**Figure 2-1 Frequency distribution of mean percentage symptomatic spikelets (PSS) data of recombinent inbred lines derived from Overland x Overley evaluated in three greenhouse experiments.**

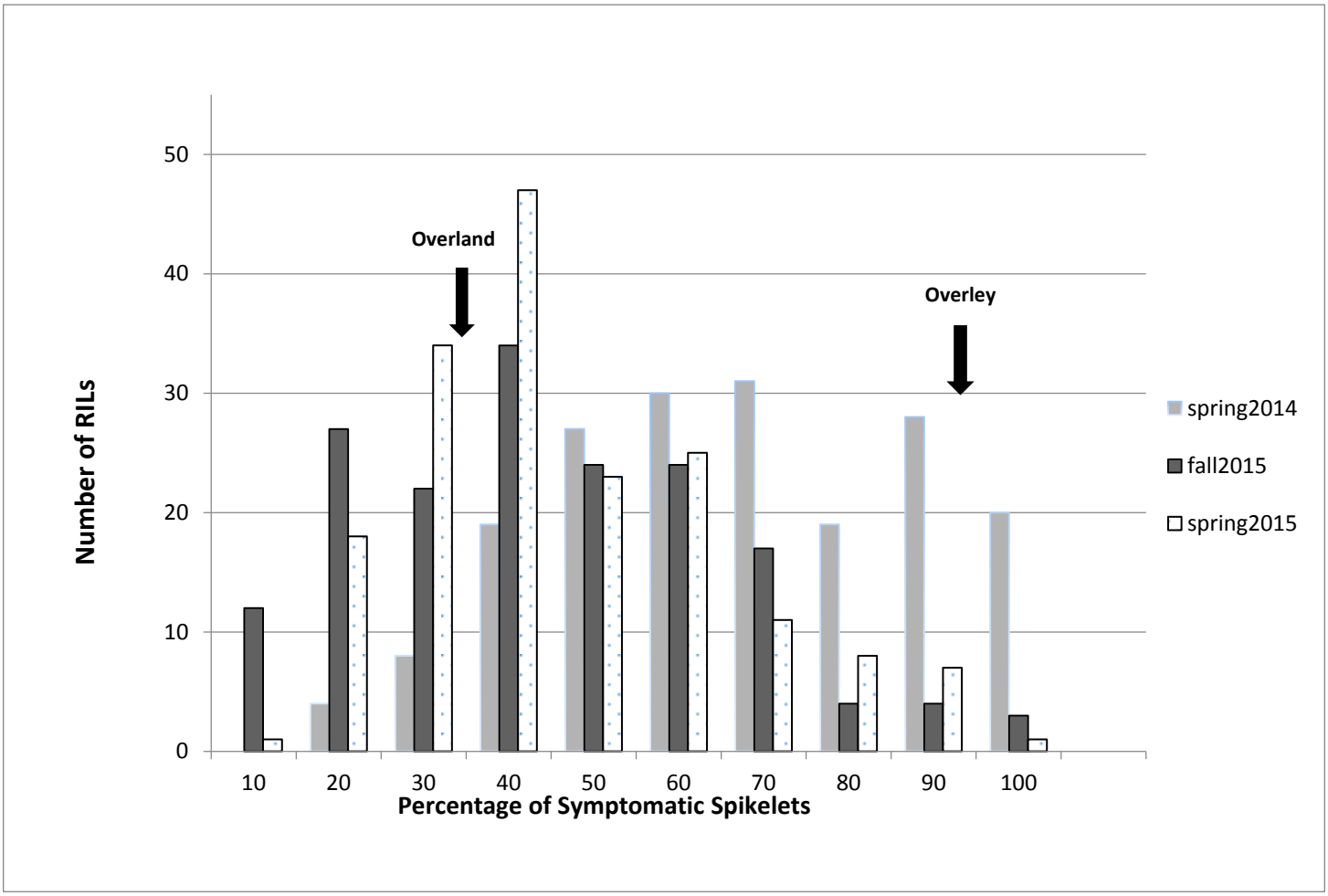


**Figure 2-2 Frequency distribution of mean percentage symptomatic spikelets for recombinent inbred lines in field experiment.**

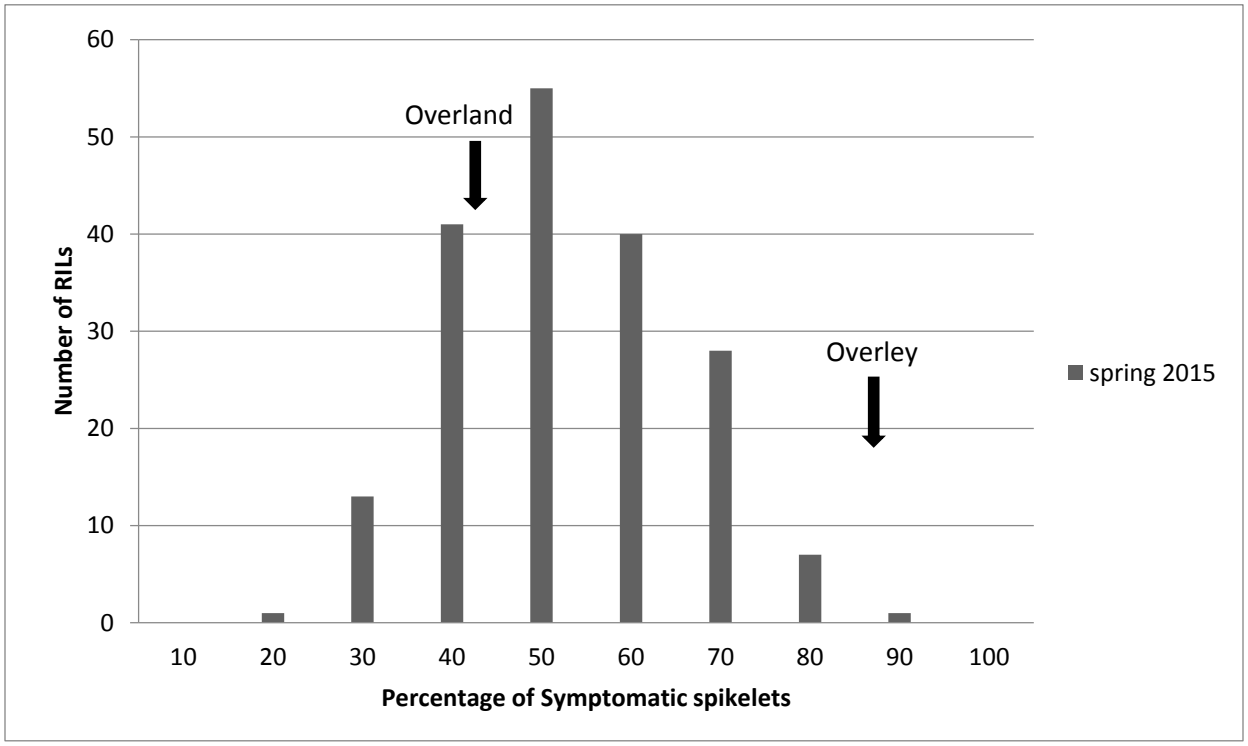
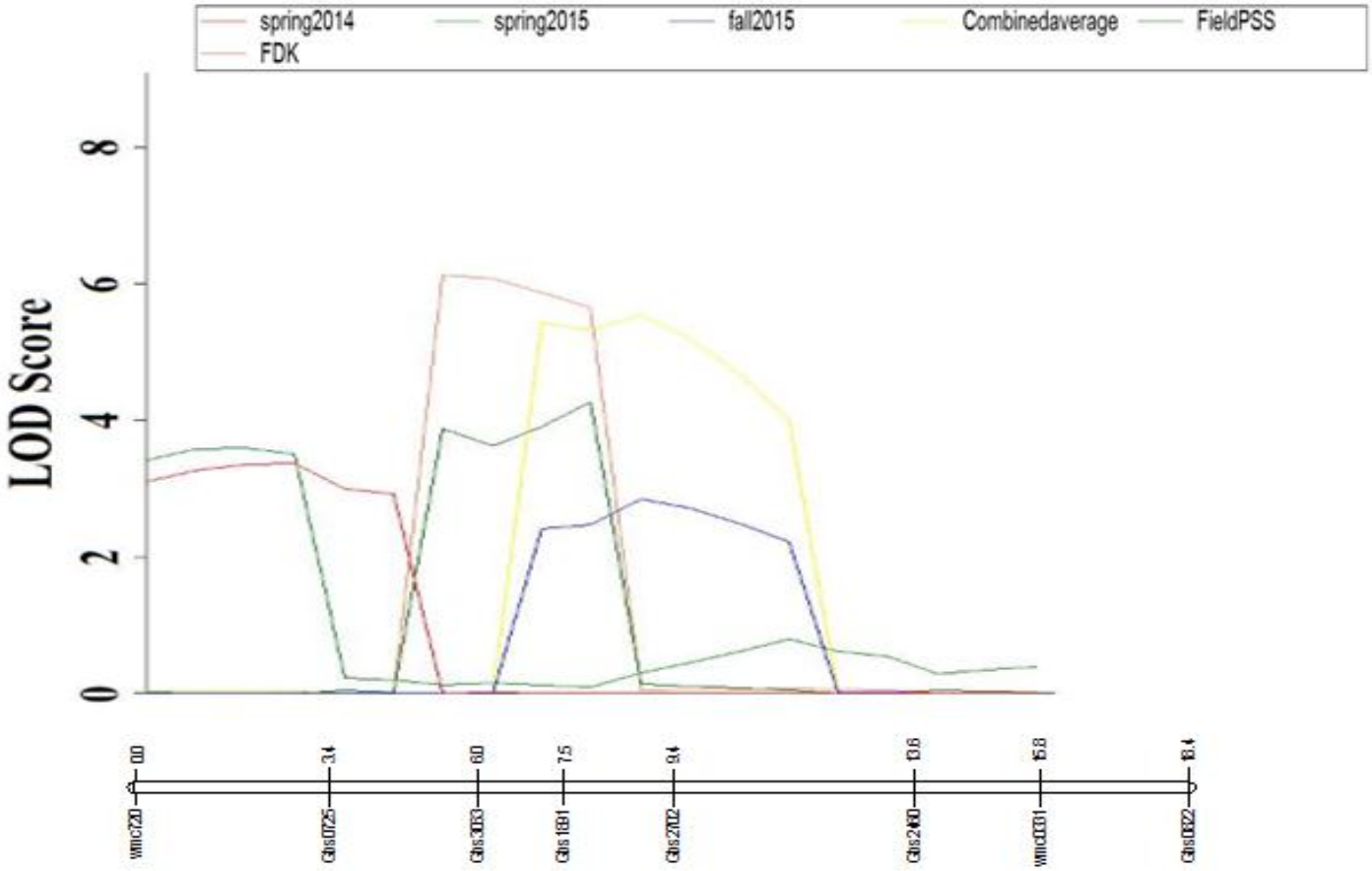
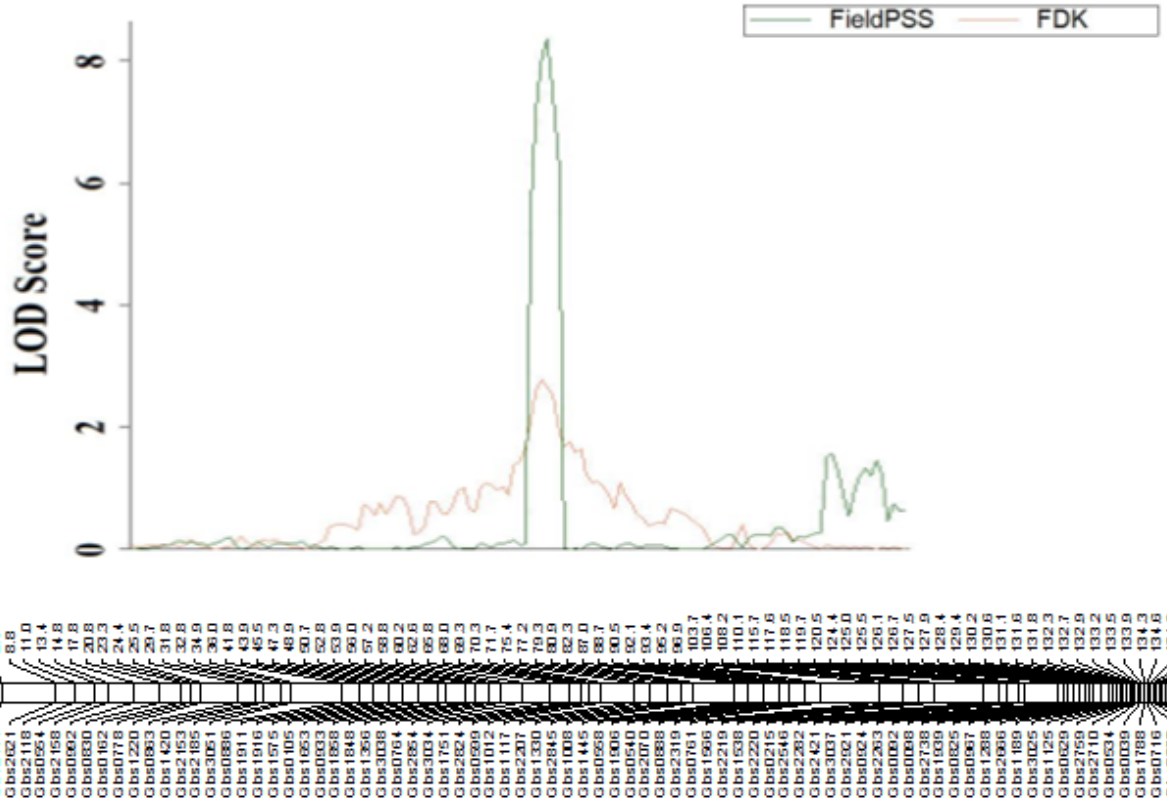


**Figure 2-3 Composite interval maps of quantitative trait loci (QTLs) for FHB type II and IV resistance constructed from recombinent inred lines derived from Overland x Overley based on three greenhouse and one field experiment on (a) chromosme 4DL (b) chromosome 4AL (c) chromosome 5BL**

(a) 4DL



(b) 4AL



(c) 5BL

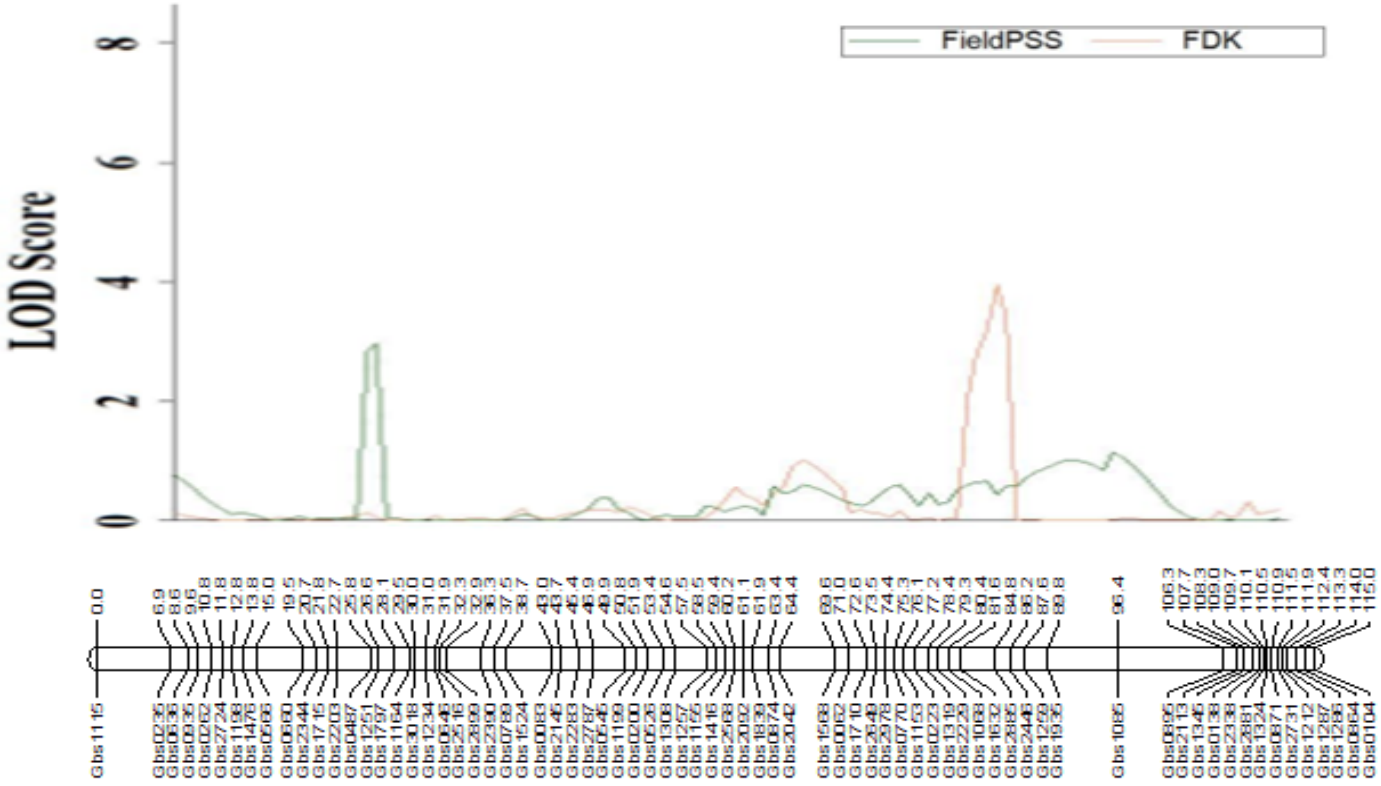


**Figure 2-4 KASP assay profile of GBS1891 on 4DL. Blue dots represesnt G allele, green dots represent A allele while red dots indicate heterozygotes.**
**Left panel ( SNP1891 in 186 RILs of Overland / Overley ) Right panel ( SNP 1891 in 96 U.S. wheat association mapping population**

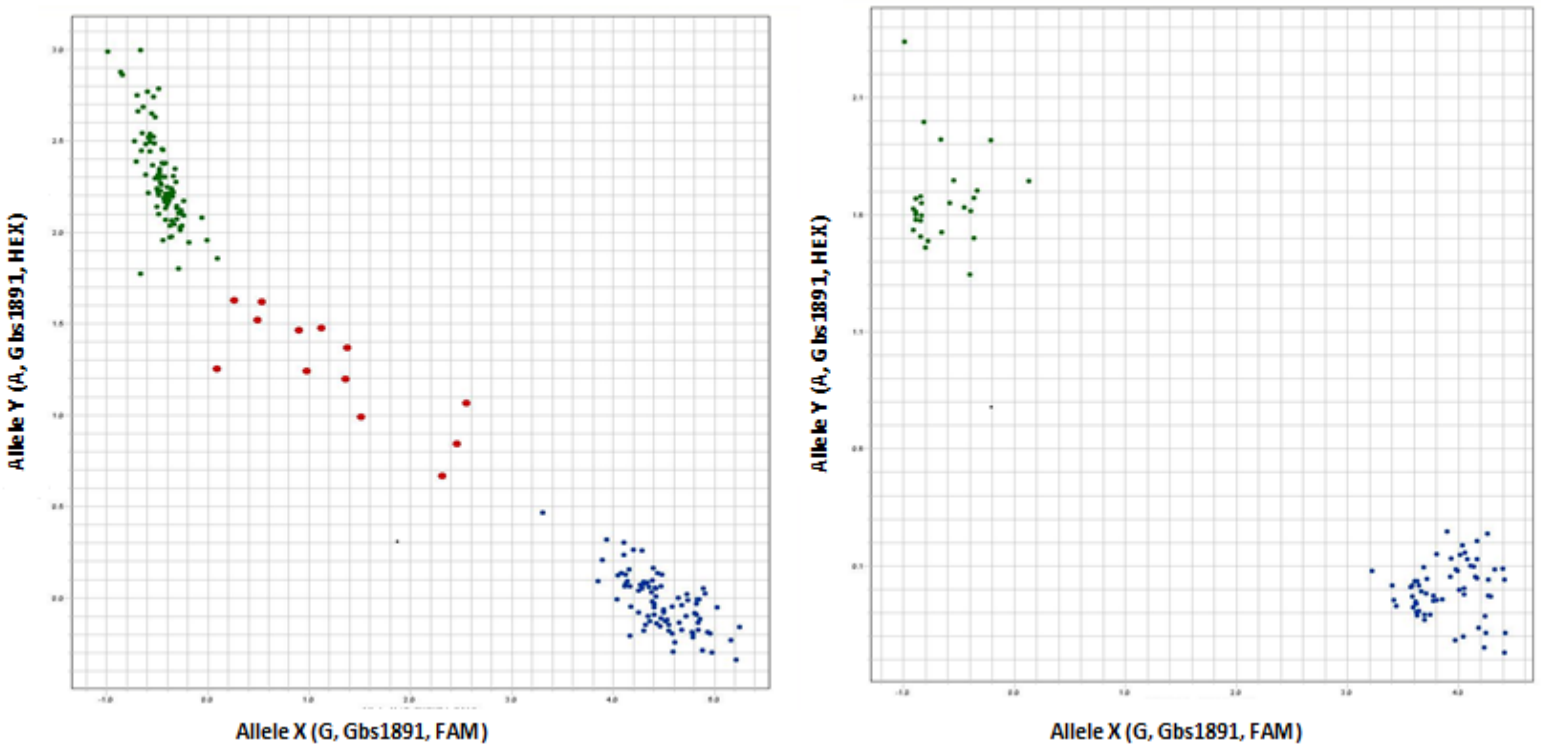
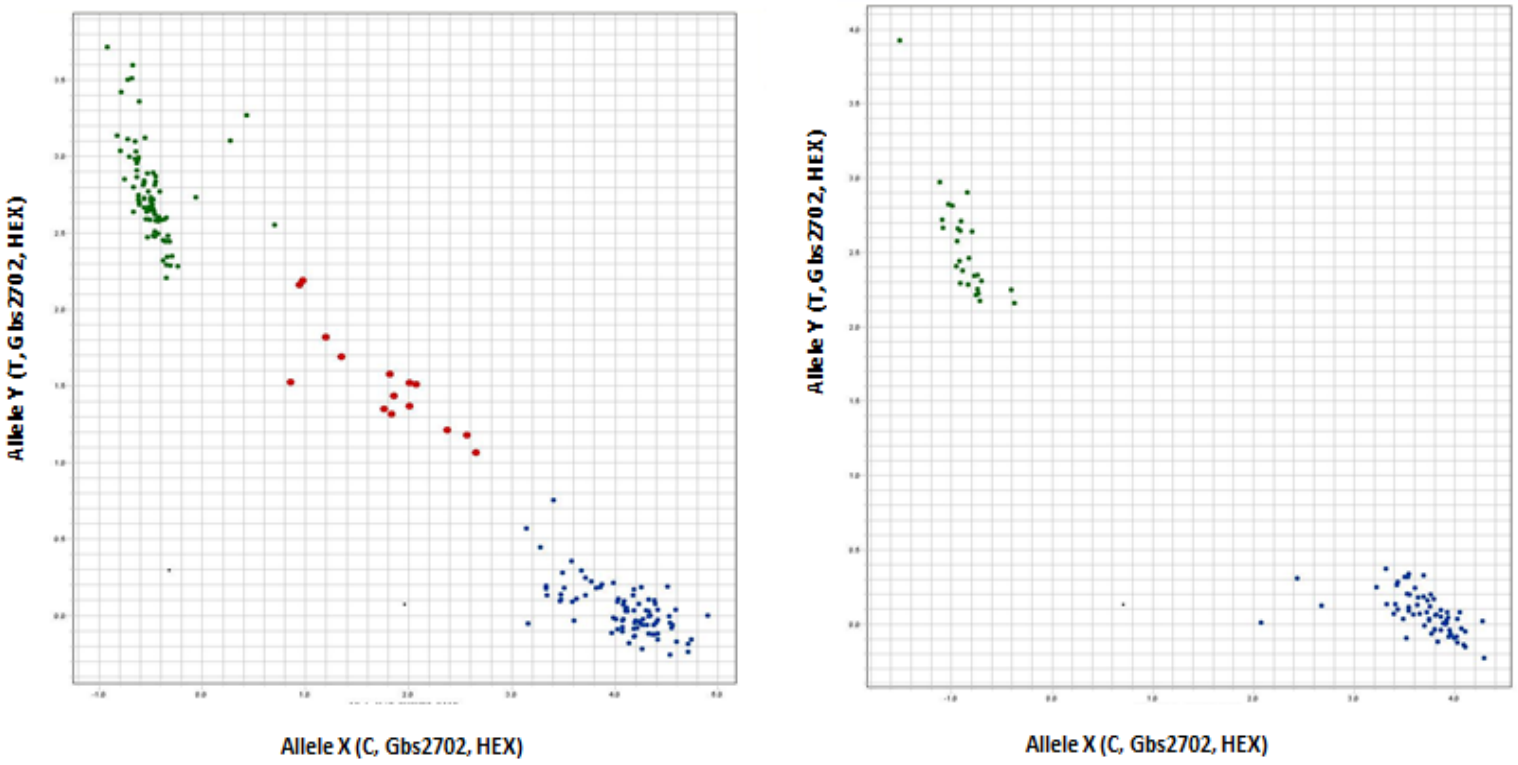


**Figure 2-5 KASP assay profile of GBS2702 on 4DL. Blue dots represesen t C allele, green dots represent T allele while red dots indicate heterozygotes.**
**Left panel ( SNP2702 in 186 RILs of Overland / Overley ) Right panel ( SNP2702 in 96 U.S. wheat association mapping population**



# Chapter 3 - Effects of *Fhb1* and *Fhb3* on FHB resistance in different HWW backgrounds

Fusarium head blight (FHB) in wheat is a growing concern for wheat breeders and producers all over the world. Identification of potential new sources of resistance is critical for success in breeding. Several sources with reasonable levels of FHB resistance have been identified around the world, including Sumai-3 and Wangshuibai from China (Fang et al. 1997; Rudd et al. 2001), Shinchunaga, Nobeokabouzu, and Nyu Bai from Japan (Bai and Shaner 2004), Frontana, and Encruzhilhada from Brazil (Ban, 2001; Singh et al. 1997). However, most of these sources are difficult to be used in breeding programs due to the undesirable traits associated with resistance genes (Bai and Shaner 2004). FHB resistance in wheat is a quantitative trait controlled by several QTLs with an additive effect (Bai et al. 2001). QTL mapping studies have revealed QTLs for FHB resistance on all 21 chromosomes of wheat however germplasm with immunity to FHB has not been found. Seven major QTLs from different sources have been formally named: *Fhb1* (Cuthbert et al. 2006), *Fhb2* (Cuthbert et al. 2007), *Fhb3* (Qi et al. 2008), *Fhb4* (Xue et al. 2010b) and *Fhb5* (Xue et al. 2011), *Fhb6* (Cainong et al.2015), and *Fhb7* (Guo et al. 2015).

*Fhb1* derived from Sumai-3 and its derivatives has been widely used in breeding due to its high heritability and stability across different environments and genetic backgrounds (Rudd et al. 2001). It is located on the distal end of chromosome 3BS and shows the largest effect on type II FHB resistance and low DON content among the QTLs identified so far (Bai et al. 1999; Zhou et al. 2002; Cuthbert et al. 2006). Several hard spring wheat cultivars with *Fhb1* have been released including Sabin and Alsen (Anderson et al. 2012), but *Fhb1* has not been deployed in hard winter wheat (HWW) cultivars.

Breeders also search exotic germplasm for new resistance sources. *Fhb3* derived from *Leymus racemosus* is such an example. *Leymus racemosus* is a wild tetraploid species showing FHB resistance (Mujeeb-Kazi et al. 1983; Wang et al. 1986). It has been transferred into wheat. Wheat-*Leymus* introgression lines (T01, T09 and T14) do not carry *Fhb1,* a major QTL from Sumai-3 ( Qi et al. 2008; Liu and Anderson 2003). Line T09 was identified to have a whole arm translocation T7AL.7Lr#1S and exhibited FHB resistance in greenhouse experiments. This gene was designated as *Fhb3* (Qi et al. 2008). Later this gene was transferred to two HWW cultivars, Jagger and Overley through marker-assisted backcrossing (Bockus et al. 2010).

Previously, FHB epidemics in the U.S. mainly occurred in the hard spring wheat, soft winter wheat regions and northern parts of HWW areas, but recently it has become more severe and frequent in southern HWW areas including Oklahoma where there was no FHB before. Thus increased frequency and severity of FHB epidemics makes breeding for FHB resistance as a major breeding objective in the US HWW breeding programs. Screening of cultivars grown in the HWW region has identified several native sources of resistance, including Hondo, Everest, and Heyne etc. However, most of these cultivars show moderate levels of resistance and cannot provide adequate protection. Pyramiding several FHB resistance QTLs in a cultivar that carry native resistance genes may provide a high level of protection. This study was designed to evaluate the individual and cumulative effects of two resistance QTLs, *Fhb1* and *Fhb3*, in HWW backgrounds, Jagger and Overland with some minor native QTLs for FHB resistance (Jin et al 2013).

## Materials and methods

### Plant materials

*Fhb1* from Ning7840 has been previously transferred to HWW cultivars, Jagger, and Overland through marker-assisted backcrossing in the USDA Central Small Grain Genotyping Lab. Overland is a moderately resistant cultivar from Nebraska, while Jagger is a moderately susceptible cultivar from Kansas. A Jagger*Fhb3* line with *Fhb3* was provided by Wheat Genetic Resource Center (WGRC). The Jagger*Fhb3* was crossed to Overland*Fhb1* and Jagger*Fhb1* and their progenies were advanced by selfing. Their $F_4$ progenies were screened for homozygous *Fhb1*, *Fhb3* and *Fhb1/Fhb3* and the selected lines were evaluated for FHB resistance in both greenhouse and field experiments. The homozygous genotypes of *Fhb1, Fhb3,* and *Fhb1/Fhb3* were selected using markers. A combination of *Xbarc127* and *Xgwm471* on the wheat 7AS and one marker BE585744 on 7Lr#1S (Qi et al. in 2008) was used to differentiate the absence and presence of *Fhb3* QTL, respectively. Homozygous genotype for *Fhb1* was selected using SSR marker *Xumn10.*

After selection, 25 lines that carry homozygous genotypes of *Fhb1*, *Fhb3*, and *Fhb1*/*Fhb*3 were identified in each cross. Also, 25 lines that do not carry any of the two QTLs in each cross were selected as controls. For marker screening, Jagger*Fhb3,* Jagger, and Overland were used as controls for *Fhb3,* while Ning7840 was used as the positive control for *Fhb1*.

### FHB in greenhouse and field experiments

FHB resistance was evaluated by point inoculations in the greenhouse experiments at Kansas State University, Manhattan KS in spring 2015 and fall 2015. Each line had two replications with five plants per replication. Detailed descriptions of inoculum preparation, inoculation procedure and FHB scoring were described in Chapter 2. The selected lines were also evaluated for FHB resistance in the field in the Plant Pathology FHB Nursery located at the Rocky Ford Research

Farm, Manhattan, KS in spring 2015 as described in Chapter 2. Plots of each line were manually harvested in the field and threshed using a plant thresher (Almaco, Nevada, IA). The threshed kernels were carefully hand-cleaned to retain maximum number of Fusarium damaged kernels (FDK). Harvested kernels from each plot were visually estimated for the percentage of FDK (1% - 100 %) over total harvested kernels. A random sample of 5 grams from each line was analyzed for DON concentrations in parts per million (ppm) using gas chromatography-mass spectrometry (GC-MS) (Mirocha et al. 1998) at the University of Minnesota, St. Paul, MN.

### Statistical analysis

Analysis of variance (ANOVA) was done using the general linear model in SAS v 9.1.2 (SAS Instit ute Inc. Cary. NC) followed by multiple pairwise comparisons of QTL means using Tukey's adjust ments. Boxplots of mean PSS of different QTL combinations were generated using R studio (RStud io Team 2015).

## Results

In the greenhouse experiments, the mean PSS of the lines carrying *Fhb1*was significantly lower than the lines carrying *Fhb3* from both the crosses. The mean PSS of Overland*Fhb1* lines was 12.57% with a range of 10% - 45%, while the mean PSS of Jagger*Fhb1* lines was 18.21% ranging from 18% to 55%. The mean PSS of *Fhb1* lines did not differ significantly between Overland and Jagger crosses. The mean PSS of lines having both *Fhb1*/*Fhb3* QTLs did not differ significantly from the lines carrying only *Fhb1* in both Overland Jagger crosses although Overland *Fhb1/Fhb3* lines had a slightly lower PSS of (14.78%) than Jagger *Fhb1/Fhb3* lines (17.7%). *Fhb3* lines from the Overland cross had a mean PSS of 38.30% and Jagger*Fhb3* lines had a mean PSS of 47.31%. The lines carrying *Fhb3* from Overland or Jagger crosses showed similar PSS to the lines without any of the *Fhb1* and *Fhb3* resistance alleles (46.23 %).

FDK and DON were only evaluated in the field experiment. *Fhb1* lines had significantly lower FDK and DON content than *Fhb3* lines. The mean FDK of *Fhb1* alone lines was 36.78%, which was similar to the mean FDK of lines with *Fhb1/Fhb3* (33.54%) from both the crosses. On the other hand, mean FDK of *Fhb3* lines was 53.90% which was close to the mean FDK of susceptible lines without any QTL from in crosses (55.78%). DON concentrations were lowest in *Fhb1* lines with the lowest DON concentration (13.3 ppm) in Jagger*Fhb1,* whereas DON concentration was high in Jagger*Fhb3* lines (27.85 ppm), which was similar to Jagger control (34 ppm). The correlation between mean PSS and mean FDK in our field experiment was high *(r = 0.61).* ANOVA results indicated significant effects of QTLs, while background effects were insignificant at *P = 0.0001* (Table 3-1). Multiple mean pairwise comparisons of different QTL combinations showed that overall mean PSS of the lines containing *Fhb1* was significantly lower than the lines containing *Fhb3,* while difference between the mean PSS of the lines with and without *Fhb3* was insignificant (Table 3-2). Parents including Jagger, Jagger*Fhb1*, Jagger*Fhb3,* Overland and Overland*Fhb1* were used in the field experiments as checks (Table 3-3). Jagger*Fhb1* parent had the lowest scores of FHB severity, FDK and DON concentration in the field experiment while Overland had highest FHB severity as compared to Jagger because of its late heading. The population was also analyzed for 4D QTL by KASP *XGbs2702* identified in the Chapter 2, but the marker showed no polymorphism between Overland*Fhb1* and Jagger*Fhb3* parents. Therefore further analysis was not done in the population for this QTL.

## Discussion

Most of the wheat traits that are of economic importance such as yield and resistance to biotic stress are quantitative in nature. Such traits are under the control of several genes or QTLs that contribute additive effects to the phenotype. Pyramiding of these genes into an elite cultivar can

improve the traits of interest by broadening the genetic base of the cultivar. Identification of such genes and transferring them to other cultivars using conventional methods of breeding is difficult and time consuming. Recent advancements in genomics and molecular markers have accelerated the process of genes identification from different sources and transfer them to elite breeding lines (Tanskley and McCouch 1997, Dubcovsky 2004). Molecular markers closely linked to the genes of interest help in marker-assisted breeding to improve the efficiency of transferring these genes to different genetic backgrounds. The process of pyramiding of genes involves recurrent marker-assisted backcrossing or marker-assisted foreground selection (Tanskley 1983) which is especially important for the traits that have difficulty to phenotype. An example of such trait is resistance to nematodes in soybean (Tanskley 1983) and in wheat (Eagles et al. 2001), in which the phenotyping is not only expensive but also unreliable. Gene pyramiding has been in practice since long time. Klopper and Pretorious (1997) studied the individual effects as well as the effects of different combinations of leaf rust resistance genes in several wheat lines and found that the combined effect of genes on the resistance was higher than the individual effects of these genes. Similarly Huang et al (1997) developed lines in rice containing several bacterial blight resistance genes. Some of the other recent examples include pyramiding of two cereal cyst nematode genes, *CreX* and *CreY*, in wheat (Barloy et al. 2007), two powdery mildew genes in wheat variety Yangmai158 (Liu et al. 2000), three soybean mosaic virus resistance genes, *Rsv1, Rsv3* and *Rsv4* in soybean (Shi et al. 2006), and three barley yellow mosaic virus resistance genes in barley (Werner et al. 2005). The success of gene or QTL-pyramiding depends on various factors including effects of individual QTLs, genetic distance between markers and target QTLs and stability of the QTLs in different genetic backgrounds. Fusarium head blight resistance in wheat is an ideal trait that can be improved by QTL-pyramiding. Bai et al (2007) conducted experiments involving the transfer of a major FHB

QTL *Fhb1* on chromosome 3BS derived from Sumai-3 in different genetic backgrounds of HWW. Miedaner et al. (2006) introgressed two FHB resistance QTLs, *Fhb1* and Qfhs.ifa-5A in European spring wheat lines with the help of molecular markers. They found the lines that carry both the resistance QTLs had lower PSS values and both QTLs contributed additively to FHB resistance. They reported reduction in FHB severity by 55% in lines carrying both the resistance QTLs in comparison of the lines lacking these QTLs. Marker-assisted selection is highly useful especially when markers closely linked to the QTLs are available, Xu et al. (2010a) successfully used molecular markers to develop and evaluate near isogenic lines for four FHB resistance QTLs in Chinese elite lines. These studies show that maker-assisted selection is a very useful approach for breeding FHB resistance in wheat.

To date *Fhb1* has been the most effective and stable QTL in FHB resistance and pyramiding *Fhb1* with other QTLs in different HWW varieties is the fast paced approach to combat FHB epidemics in Great Plains (McMullen et al. 2012). In this study we found that Jagger*Fhb1* had Overland*Fhb1* significant lower FHB than their susceptible parents which agrees with the previous findings (Bai et al. 2007). In the greenhouse experiment Overland*Fhb1* with a mean PSS of 12.71 % was better than Jagger*Fhb1* (PSS = 18.21%), while in the field experiment mean PSS of Jagger*Fhb1* (18.75 %) was lower than Overland*Fhb1* (35.62%). This discrepancy can be due to the difference in heading days in 2015, Overland flowered later than Jagger and was exposed to the higher disease pressure. Comparison of overall mean PSS among the four combinations of *Fhb1* and *Fhb3* lines showed that *Fhb1* depicted significantly lower PSS, while mean PSS of *Fhb3* containing lines was similar to the lines without *Fhb3*, thus *Fhb3* did not show any effect on FHB resistance in the crosses using Overland and Jagger as susceptible parents. These results were similar in greenhouse and field experiments (Fig 3-1 and Fig 3-2).

*Fhb3* is an alien gene for FHB resistance derived from *Leymus racemosus* (Qi et al. 2008) and has not been used in any breeding programs yet. In this study we find the use of *Fhb3* was ineffective for FHB resistance in Overland and Jagger. The *Fhb1* lines did not improve in FHB resistance after adding *Fhb3*. A similar result was reported for stacking of *Fhb1* with Qfhs.ifa-5A in European winter wheat lines (Salameh et al. 2010). Thus pyramiding of *Fhb3* with *Fhb1* in HWW is probably not a good practice to improve wheat FHB resistance. *Fhb3* was also found to be ineffective in some other backgrounds (Bockus et al. 2010). This may be due to low level of expression of the alien gene in wheat backgrounds. Other genes may need to be evaluated for gene pyramiding to improve wheat resistance to FHB.

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**Table 3-1 Analysis of variance (ANOVA) for QTL and background effects using PSS data of two greenhouse experiments conducted in spring 2015 and fall 2015**

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Experiment** | 1 | 12.45562 | 12.45562 | 0.05 | 0.8164 |
| **Background** | 1 | 500.96185 | 500.96185 | 2.17 | 0.1416 |
| **QTL** | 3 | 44231.07034 | 14743.69011 | 63.87 | <.0001 |
| **Background*QTL** | 3 | 5035.20062 | 1678.40021 | 7.27 | <.0001 |
| **Error** | 365 | 84253.8580 | 230.8325 | | |
| **Corrected Total** | 373 | 141906.9032 | | | |

**Table 3-2 Multiple pairwise comparions of QTL means at P < 0.0001 using mean PSS data of two greenhouse experiments**

| Tukey Grouping | Means | Trt | Range |
|---|---|---|---|
| **A** | 50.249 | Jagger*Fhb3* | 35 - 87 |
| **A** | 49.217 | JaggerNull | 31 - 78 |
| **B** | 39.877 | OverlandNull | 28 - 61 |
| **B** | 38.306 | Overland*Fhb3* | 25 - 75 |
| **C** | 18.209 | Jagger*Fhb1* | 18 - 55 |
| **C** | 17.702 | Jagger*Fhb1/3* | 15 - 40 |
| **C** | 14.786 | Overland*Fhb1/3* | 12 - 30 |
| **C** | 12.574 | Overland*Fhb1* | 10 - 45 |

*Note*: Means within the same grouping letter are not significantly different.

**Table 3-3 Comparison of FHB severity, FDK and DON in parents used as check in field experiment**

| Parents / Checks | FHB severity % | FDK % | DON (5g sample) ppm |
|---|---|---|---|
| **Jagger** | 45 | 65 | 34 |
| **Jagger*Fhb1*** | 25 | 25 | 13.3 |
| **Jagger*Fhb3*** | 42.5 | 60 | 27.9 |
| **Overland** | 52.5 | 50 | 28.8 |
| **Over1and*Fhb1*** | 35 | 30 | 18.5 |

**Figure 3-1 Effect of different QTL combinations on FHB severity using mean PSS data of two greenhouse experiments**

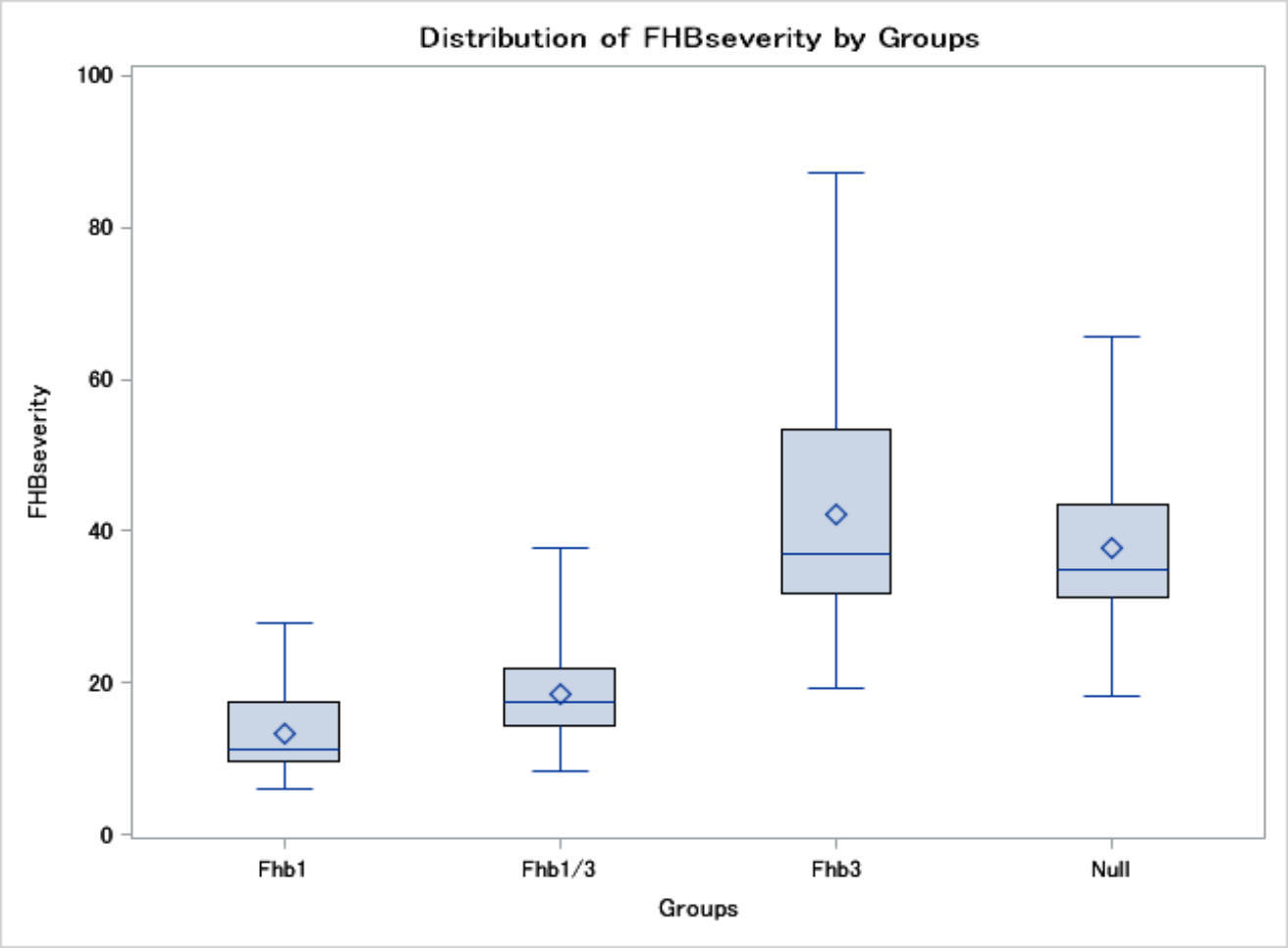

**Figure 3-2 Effect of different QTL combinations on FHB severity in the field experiment**