GENETIC MAPPING OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT CULTIVARS ART AND EVEREST

by

MARSHALL CLINESMITH

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Major Professor
Allan Fritz

Abstract

Fusarium head blight (FHB) is a fungal disease, mostly commonly associated with *F. graminearum,* which affects cereal crops such as wheat resulting in substantial yield losses and reductions in grain quality. The onset of the disease can occur rapidly when warm, wet or humid weather coincides with flowering in the spring. The pathogen also produces mycotoxins such as deoxynivalenol (DON) that accumulate in the grain and can be toxic to humans and animals. This results in additional economic losses as contaminated grain must be discarded or blended to reduce the amount of toxin in order to meet federal regulatory limits. Development and deployment of resistant cultivars has proved to be an effective method to combat the disease, and many resistant sources have been reported in the literature with the majority of major resistance coming from Chinese landraces. Transferring resistance from these sources into cultivars adapted to the U.S. has been a slow process due to linkage of FHB resistance genes with poor agronomic traits. Therefore, it is important for breeders to search for sources of resistance in native material adapted to their local conditions. In this study, we aimed to identify quantitative trait loci (QTL) for resistance to spread of FHB within the head (Type II resistance), accumulation of DON toxin in grain (Type III resistance), and resistance to kernel infection (Type IV resistance). Plant material consisted of 148 doubled haploid (DH) lines from a cross between the two moderately resistant hard red winter wheat (HRWW) cultivars Art and Everest. The study was conducted for two years using a point inoculation technique in a greenhouse in Manhattan, KS. Three QTL conferring resistance to FHB traits were detected on chromosomes 2D, 4B, and 4D. The QTL on chromosomes 4B and 4D overlapped with the major height genes *Rht1* and *Rht2*, respectively. Plant height has shown previous associations with FHB, though the underlying cause of these associations is not well understood. The majority of

results have reported increased susceptibility associated with shorter plant types; however, in this study, the haplotype analysis for the *Rht-B1* and *Rht-D1* loci showed an association between the dwarfing alleles and increased resistance to FHB. This suggests either pleiotropic effects of these loci or perhaps linkage with nearby genes for FHB resistance. Markers close to the peaks of the FHB resistance QTL have the potential for Kompetitive Allele Specific PCR (KASP) marker development and subsequent use in marker assisted selection (MAS) to help improve overall FHB resistance within breeding programs.

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Chapter 1 - Literature Review

Wheat

History and Domestication

The practice of what we consider modern day agriculture began about 12,000 years ago when humans shifted from a nomadic, hunter-gatherer lifestyle and began raising plants and animals as a source of sustainable food. This event is known as the Neolithic revolution that led to an increase in food security and allowed humans to build permanent settlements. The earliest evidence of crop cultivation is found in Southwest Asia in an area known as the Fertile Crescent located in the Tigris and Euphrates River Basin (Salamini et al., 2002). This area is the center of origin for some important modern crops such as wheat and barley and is also the main source of diverse germplasm that is contained in wild relatives of these species. The cultivation of wheat and barley helped start the expanse of agriculture from the Fertile Crescent throughout Asia, Europe, and Africa. Wheat became an important crop during the Neolithic period due to its nutritive value with high amounts of starch and proteins such as gluten (Zohary et al., 2012). The domestication of wild species led to the selection of beneficial traits found in modern species such as free-threshing seeds and synchronous germination. These same characteristics have allowed wheat to maintain its position as one of the most important staple crops for developing countries (Ammerman & Cavalli-Sforza, 1984; Zohary et al., 2012).

The genus *Triticum* can be subdivided into three main groups based on ploidy level: diploid Einkorn wheats, tetraploid Emmer wheats, and hexaploid bread wheats. Modern bread wheat (*Triticum aestivum*) is an allohexaploid (6n = 42) composed of three homeologous diploid genomes (AABBDD). Each genome is comprised of seven chromosome pairs that result in 42 total chromosomes. The A genome originated from the diploid species *Triticum urartu* (2n = 14;

AA), the D genome from *Aegilops tauschii (*2n = 14; DD), and the B genome is thought to have originated from *Aegilops speltoides* (2n = 14; SS) (Kilian et al., 2009). The evolution of the hexaploid species *T. aestivum* was the result of two separate hybridization events. Formation of the wild tetraploid species *T. turgidum* spp. *dicoccoides* (4n = 28; AABB) was the result of an allopolyploidization event between *T. urartu* and *Ae. speltoides* followed by a doubling of the chromosomes (S. Huang et al., 2002; Kilian et al., 2007). Domestication of *T. dicoccoides* and its subsequent spread across Europe and Asia allowed for a second hybridization event with *A. tauschii* and the formation of the hexaploid wheat *T. aestivum* (Kilian et al., 2009; Luo et al., 2007).

Production

World

Wheat is one of the main staple crops that, along with rice and maize, feed around 4 billion people worldwide, or roughly sixty percent of the world's population (www.fao.org). In 2013 wheat ranked fifth in global production with 715,909,258 tonnes produced. The top three global producers of wheat are China (122 million tonnes), India (93.5 million tonnes), and the United States (58 million tonnes). The average worldwide yield in 2013 was 3.6 Hg/Ha which is nearly triple the yield achieved fifty years earlier in 1963 (Food and Agriculture Organization of the United Nations Statistics Division [FAOSTAT], 2013). This dramatic increase in yields is the result of a number of factors including better management practices, improved varieties, and addition of fertilizers and other chemicals such as pesticides and herbicides. The introduction of many of these factors began in the 1960's during a period known as the Green Revolution. During the twenty-five year period from 1963 to 1987, global wheat yields increased at an average of 2.74 percent annually. This increase helped prevent widespread famines that were

predicted to affect many developing countries and brought food security to millions of people around the globe. Over the next twenty-five year period, from 1988 to 2013, annual yield only increased at an average of 1.17 percent (Food and Agriculture Organization of the United Nations Statistics Division [FAOSTAT], 2013).

United States

Wheat is an important crop in the U.S. and is mostly grown in Midwestern and Pacific Northwestern states. Data from the USDA Economic Research Service ranks wheat as the third largest field crop in the U.S., behind corn and soybean, in both acreage planted and gross farm receipts. In the 2014-2015 growing season, U.S. wheat farmers planted 56.8 million acres, harvested 46.4 million acres, and had an average yield of 43.7 bushels per acre (United States Department of Agriculture Economic Research Service [USDA ERS], 2016). According to the USDA National Agricultural Statistics Service, in 2015, the largest wheat producing states by volume ranked as follows: North Dakota (370.02 million bushels), Kansas (321.90 million bushels), Montana (185.42 million bushels), Washington (111.54 million bushels) and Texas (106.50 million bushels) (United States Department of Agriculture National Agriculture Statistics Service [USDA/NASS], 2016a).

Fusarium Head Blight

Economic Impacts

Fusarium head blight (FHB), commonly referred to as “scab”, is a fungal disease of small grain crops, such as wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.), that can have severe impacts on yield and grain quality (Dill-Macky, 2008; Fernando et al., 1997; Osborne & Stein, 2007). This disease occurs in many of the major wheat growing areas around the world including Asia, Canada, Europe, and North and South America; and severe epidemics

have resulted in substantial economic losses (Stack, 1999). Severe FHB outbreaks have resulted in yield losses of more than 1 million metric tons in China with the disease affecting over 7 million hectares of wheat (Bai & Shaner, 2004). In 1919, the U.S. estimated a combined spring and winter wheat loss of 2.18 million metric tons. Significant yield losses were also reported during the period from 1928 to 1937 (Dickson, 1942). Optimal weather conditions in the 1980's led to severe outbreaks of FHB in the U.S. In 1982, an estimated 2.72 million metric tons of wheat was lost due to the disease (Boosalis et al., 1983). Several studies have been conducted in attempts to quantify economic losses resulting from FHB epidemics during the 1990's. Johnson *et al.* (2003) estimated direct losses of over $1.3 billion and total economic losses of $4.8 billion in the U.S. from 1991 to 1997, with the most severe losses occurring in North Dakota and Minnesota spring wheats. Nganje *et al.* (2004) conducted a similar study focusing on the impacts of FHB from 1993 to 2001. They reported cumulative direct economic losses of $2.492 billion for hard red spring wheat, soft red winter wheat, durum wheat, and barley. When combined with secondary losses, the total economic impact was estimated to be around $7.7 billion. More recent outbreaks occurred from 2007 to 2010 effecting specific U.S. wheat growing regions. Several states were affected during this time with Kansas seeing significant impacts all four years. In 2008 the total statewide losses for Kansas were estimated to be around $57 million (McMullen *et al.*, 2012). Significant regional outbreaks still continue to occur; however, the release of moderately resistant cultivars and adoption of better management practices has helped in reducing the impacts of the disease.

Causal Agents

The causal agents behind FHB are species of the genera *Fusarium* and *Microdochium* which can vary in prevalence depending on climate and agronomic practices. *F. graminearum*

Schwabe, *F. culmorum* (W.G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc., *F. sporotrichioides* Sherb., *F. poae* (Peck) Wollenw., *Microdochium majus* (Wollenw.), and *M. nivale* (Fr.) Samuels & I.C. Hallett (Aoki *et al.*, 2014; Glynn *et al.*, 2005) are among the most common species reported (Bai & Shaner, 2004; Dill-Macky & Jones, 2000; Osborne & Stein, 2007; Xu & Nicholson, 2009); however, up to 19 species have been associated with the disease (Liddell, 2003). FHB is found worldwide and can exist in a range of different climates with the presence of moisture or humidity being necessary for most species as it helps promote fungal growth. *F. graminearum* is the species most commonly associated with FHB. The appearance of this species in the majority of infected areas is likely due to its ability to reproduce over a wider array of temperatures and moisture conditions relative to other species (Osborne & Stein, 2007; Xu et al., 2008).

Disease Development

There are several plant hosts capable of harboring *Fusarium spp.* including wheat, barley, rye, oats, triticale, corn, sorghum, rice, and soybeans (Bai & Shaner, 2004; Pereyra & Dill-Macky, 2008; Wegulo et al., 2015). Crop residue, such as leftover kernels and chaff, can be used as a secondary survival or overwintering site for the pathogen and is therefore a main source of inoculum for the following crop season. *Fusarium* is able to survive saprophytically in the residue as mycelium, perithecium initials, and chlamydospores (Bai & Shaner, 2004; Pereyra & Dill-Macky, 2008). Main sources of inocula include asexual conidia that are produced in sporodochia and sexual ascospores which develop within perithecia. Fungal spores are initially produced in late spring or early summer. These spores are mainly dispersed by wind and water splash but certain fungal species have shown to be transported by insect and animal vectors as well (Paulitz, 1996; Pereyra *et al.*, 2004; Wegulo *et al.*, 2015). Ascospores are forcibly ejected

from perithecia at maturity which tends to occur at temperatures between 13 - 22°C and a relative humidity of 95 – 100% (Champeil *et al.*, 2004; Sutton, 1982).

Infection Pathway

F. graminearum is a hemibiotrophic pathogen that shows biotrophic traits during the early stages of infection (48 – 72 hours after infection (HAI)) before entering a necrotrophic phase at approximately 72 hai (Bushnell *et al.*, 2003). Infection of wheat heads can occur from head emergence until maturity; however, anthesis tends to be the most susceptible period. (Bai & Shaner, 2004; Wegulo et al., 2015). The short window of main exposure to infection during anthesis results in the pathogen typically being observed as monocyclic, with only one infection cycle per season. (Bai & Shaner, 1994). Germination of conidia can occur 6 – 12 hours after initial contact with the host surface. Fungal penetration initiates in anthers, glumes, palea, and rachilla. Penetration of the host tissue does not occur immediately; instead, the germ tubes produce hyphae that spread across the surfaces of the plant forming dense mycelial networks within the spikelet cavities along the ovary, lemma, glume, and palea (Xu & Nicholson, 2009). This lateral growth along the surface of the plant tissue appears to occur in order to allow the fungus to reach susceptible areas of the tissue such as stomata or other areas where the cell walls of the plant are thinner. The fungus can also spread from infected plant tissue into the developing kernels. Kernel susceptibility is highest when infection occurs early, during anthesis and kernel development, as the fungus can easily penetrate the pericarp of the developing kernels as well as spread inter- and intracellularly throughout the entire kernel (Pugh *et al.*, 1933).

At 24 – 48 hours after infection, the pathogen usually enters the host through direct penetration of the host tissue or via stomatal openings (Champeil et al., 2004; Goswami & Kistler, 2004). Infiltration of the host tissue is aided by the production of hydrolyzing enzymes

that can help degrade host cell walls (Ding *et al.*, 2011). Evidence of subcuticular hyphal growth after initial tissue penetration has been shown to occur. Flat hyphal branches have been observed spreading between the cuticle and epidermal cell walls of wheat glumes (Pritsch *et al.*, 2000). The pathogen can spread inter- and intracellularly in a vertical fashion through vascular bundles and cortical parenchyma tissue in the rachilla and rachis infecting adjacent florets and eventually neighboring spikelets (Ribichich *et al.*, 2000). Premature death of spikelets could be related to changes by the fungus to the vascular bundles of infected rachis causing xylem and phloem tissue to malfunction reducing the supply of water and nutrients to plant cells (Goswami & Kistler, 2004). The spread of the pathogen along the wheat head can also occur via the emergence of conidiogenic cells from the stomata allowing conidia and hyphae to reach the glumes of neighboring florets and spikelets (Ribichich *et al.*, 2000).

Symptoms

Shortly after infection, FHB symptoms appear as brown, water-soaked spots on glumes of infected florets. With heavy infestations of the pathogen, or when susceptible cultivars are planted, the symptoms will spread from initially infected spikelets towards the base and tip of the spike. The infected rachis will turn brownish in color and the entire spike can become blighted appearing whitish or bleached in color relative to green, uninfected spikes (Bai & Shaner, 1994). The symptoms of disease are lessened when resistant cultivars are planted. A decrease in the number of initially infected spikelets is seen as well as reductions in the spread of the fungus from spikelet to spikelet. In certain cases involving highly resistant lines the symptoms can be contained to the initially infected glume or lemma (Ribichich *et al.*, 2000). As the fungus develops, distinct orange/pink colored spores can appear on the glumes of infected spikelets. Early infections can prevent the formation of kernels in affected florets. Kernels that do develop

are typically shriveled and discolored appearing grey/brown or white/pink, referred to as 'tombstone' kernels, and have lower test weights than healthy kernels (Bai & Shaner, 1994; Parry et al., 1995). Kernels that become infected late in development can appear healthy and unaffected; however, these kernels could still contain harmful levels of mycotoxins produced by the fungus (Moretti *et al.*, 2014).

Mycotoxins are secondary metabolites produced by certain fungal species that can inhibit protein synthesis in addition to causing chlorosis and necrosis of plant tissue. *Fusarium* spp. can produce several different types of fungal metabolites that show varying levels of toxicity (Desjardins & Proctor, 2007). The mycotoxins mainly focused on are trichothecenes, zearalenone, and fumonisin B1 (Antonissen *et al.*, 2014). Studies looking at the relationship between trichothecene production and fungal pathogenicity have concluded that trichothecenes are a virulence factor for the pathogen and that their presence or absence can determine the aggressiveness of fungal infection and spread of symptoms along the wheat head (Bai *et al.*, 2002; Lemmens *et al.*, 2005; McCormick, 2003). Jansen *et al.* (2005) reported the spread of mutant trichothecene deficient strains of *F. graminearum*, into the rachis of wheat, was inhibited by the growth of dense cell wall tissue. They concluded that this plant defense response is inhibited by the mycotoxin when infected with wild-type strains of the fungus. The trichothecenes group is made up of over 200 toxins that can be divided into four groups: Types A, B, C, and D (McCormick *et al.*, 2011). There are several FHB causing fungal species that produce trichothecene toxins. Type B trichothecenes are produced by *F. culmorum, F. poae, and F. graminearum* and include deoxynivalenol (DON), nivalenol (NIV), 15-ADON, 3-acetyl-DON(3-ADON), and fusarenon-X (Edwards *et al.*, 2001; Placinta *et al.*, 1999). *F. graminearum* strains have been classified based on the chemotype of the mycotoxins they produce.

Chemotypes IA (deoxynivalenol, 3-acetyl) and 1B (deoxynivalenol, 15-acetyl) are associated with Chinese and North American strains, respectively. Chemotypes II (nivalenol) and IIA (nivalenol, 4-acetyl) have also been reported in strains of *F. graminearum*; however, the appearance of these chemotypes in this species is rare (Miller *et al.*, 1991; Paul *et al.*, 2005).

The accumulation of DON and NIV in FHB infected grain is a serious problem in major wheat growing areas of the world. These toxins are harmful to humans and animals which can make contaminated grain unfit for food or feed. In North America and Europe, DON is the most common trichothecene found to contaminate wheat grains (Placinta *et al.*, 1999). DON has a comparatively low acute toxicity compared to other trichothecene mycotoxins; however, it can accumulate to hazardous levels in grain samples and withstand high temperatures allowing it to remain above acceptable levels in unprocessed grain, as well as finished flour and malting products (Hughes *et al.*, 1999; Rotter *et al.*, 1996). In wheat and barley samples, DON concentrations can reach levels of up to 30 ppm (mg/kg) (Alberta Agriculture and Forestry, 2003). When mycotoxin levels accumulate to certain concentrations, ingestion of grain or grain products can result in mycotoxicosis (McCormick, 2003; McCormick *et al.*, 2011). The effects of ingestion can include both acute and chronic toxic effects and can vary based on several factors including the type of mycotoxin present (Antonissen *et al.*, 2014). Several symptoms have been associated with mycotoxicoses in humans and animals. Human ingestion of food products with high DON levels can result in nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever (Sobrova *et al.*, 2010). Symptoms reported in animals include feed refusal, emesis and anorexia; oral and gastro-intestinal lesions; ill-thrift; reproductive dysfunction; equine leukoencephalomalacia; and porcine pulmonary edema (D'Mello et al., 1999).

The potential health effects of DON ingestion on humans and animals has resulted in many countries establishing maximum allowable levels of the toxin in food or feed products. China limits the concentration of DON in grain and grain products (corn, corn flour, barley, wheat, wheat flour and cereal) to 1 ppm (United States Department of Agriculture Foreign Agricultural Service [USDA FAS], 2014). The European Union set the limit for cereals intended for direct human consumption at 0.75 ppm, bread and breakfast cereals at 0.5 ppm, and processed cereal-based foods and baby foods at 0.2 ppm (European Union [EU], 2006). In the U.S. the FDA has set a limit of 1 ppm for all finished wheat products intended for human consumption. The FDA also has limits for grain and grain by-products intended for use as animal feed: for swine, the limit is 5 ppm and this can only account for 20 percent or less of the total diet. Chickens are limited to 10 ppm and is not to exceed 50% or more of the total diet. Ruminating beef and feedlot cattle are restricted to 10 ppm (Federal Department of Agriculture [FDA], 2011).

Factors Effecting FHB Management

Plant Growth Stage

Research on optimal infection times have shown that the majority of infection tends to occur during or shortly after anthesis. (Pugh *et al.*, 1933). Several studies have been conducted to investigate the correlation between anthesis and susceptibility to FHB. Strange and Smith (1978) reported increased infection due to exposure of the anthers which they reported contained certain nutrients and the compounds choline chloride and betaine hydrochloride that help stimulate fungal growth by supporting the extension of hyphae. These compounds appear to be at higher concentrations in the anthers than in other parts of the plants making this the optimal site of initial infection (Champeil et al., 2004; Strange & Smith, 1971; Strange & Smith, 1978). Engel *et*

al. (2004) conducted a similar study and concluded that these compounds had no significant effect on FHB susceptibility. Skinnes *et al.* (2010) suggested that instead of these chemical compounds influencing fungal growth, the dead and degenerating tissue of the anthers could be the reason it is initially targeted as it allows for easier infection by the fungus. The degree of flower-openness during anthesis has also been reported to have an effect on the level of FHB infection. Gilsinger *et al.* (2005) described a significant relationship where plants with wider flower openings tended to have higher levels of FHB incidence while those with narrower flower openings experienced less infection. Other studies show that while flower opening is correlated with FHB infection, the extrusion of the anthers could also have a significant impact on changes in infection levels. In these studies, plants with anthers that were partially extruded tended to have higher levels of infection relative to plants with either no extrusion (closed-flowering) or full extrusion (open-flowering) (Kubo *et al.*, 2013; Lu *et al.*, 2013; Skinnes *et al.*, 2010)

Environment

Environmental factors can have a profound impact on fungal development and disease severity and can vary depending on the species present. *F. poae* tends to be favored in warmer and drier climates relative to other species. Cooler climates with more moisture and humidity tend to be dominated by *F. avenaceum and F. culmorum*. Species of the genera *Microdochium* appear more prevalent in environments with cool/moderate temperatures and short, frequent precipitation events. *F. graminearum* tends to be most productive in warmer climates with higher levels of humidity (Xu *et al.*, 2008). The timing of precipitation events can also affect disease potential. Previous studies have shown that rainfall is more important for initial infection than colonization. Prolonged wet periods that occur around anthesis can quickly move fungal spores from the soil and crop residue to developing wheat heads via water splash (Xu, 2003; Xu *et al.*,

2008). Significant infections typically occur when warm, wet weather coincides with anthesis (Bai & Shaner, 1994); however, the optimum temperature and moisture level can be dependent on the fungal species present (Osborne & Stein, 2007). *F. graminearum* tends to have higher levels of infection when the temperature is between 20 – 30°C with the optimal temperature being approximately 25°C. Periods of moisture ranging from 36 to 72 hours are typically needed for significant infection to occur although shorter periods can be sufficient if they occur at the optimal temperature and plant growth stage (Andersen, 1948).

Agronomic Practices

Tillage method, fertilizer applications, irrigation, and crop rotation can all have effects on species prevalence and a given field's inoculum potential. Water conservation efforts over the last 30 years have resulted in the adoption of no-till or reduced tillage practices by many producers (Dill-Macky, 2008). This has led to an increase in residue biomass on the soil surface which can create a more conducive environment for fungal pathogens to survive and reproduce. An increase in crop residue provides better protection from erosion for soil and soil organic matter, which can lead to higher levels of soil carbon and nitrogen. This can have positive effects on crop growth but can also lead to increased development of fungi and subsequent increases in the occurrence of residue-borne diseases (Champeil *et al.*, 2004; Dill-Macky, 2008). Dill-Mackey and Jones (2000) reported evidence of lower disease pressure in fields with conventional tillage (moldboard plow) compared to no-till. Moving the residue deeper into the soil profile can hinder certain stages of fungal development and reduce the amount of fungal spores that can be splashed onto host tissue.

The species of the current crop as well as the previous crop can play important roles in determining the amount of inoculum produced, the severity of the disease, and the amount of

mycotoxins that accumulate in the kernels. Noticeable decreases in severity and mycotoxin concentrations have been observed in wheat planted after soybeans compared to wheat planted after corn or wheat (Dill-Macky & Jones, 2000; Sutton, 1982). The effect the previous crop plays on disease level can be related to its ability to host certain fungal species, the amount of residue it produces, and possibly the amount of nitrogen in the residue. Corn residue has shown to be highly correlated with greater levels of FHB. The high nitrogen levels in corn residue could allow for extended periods of colonization by the fungus (Champeil *et al.*, 2004). Corn also produces more residue than certain other crops such as soybean which could lead to increased sources of inocula (Dill-Macky & Jones, 2000; Mesterházy et al., 1999).

Fungicide Applications

The use of fungicide applications to combat FHB and DON is still an important tool used by producers in commercial wheat production. Combining resistant cultivars with appropriate fungicide applications can help producers to minimize losses. Factors affecting the success of fungicide applications include fungicide efficacy, application timing, and coverage (Wegulo *et al.*, 2015). The efficacy of early developed fungicides was a major issue during the FHB epidemics in the 1990's. Few labeled fungicide products were available and their ability to limit FHB was reportedly low (McMullen *et al.*, 2012). Since then, several new fungicides have emerged showing promising levels of control. The most popular fungicides in use belong to the demethylation inhibitor (DMI) class which includes: propiconazole, tebuconazole, metconazole, prothioconazole, and prothioconazole + tebuconazole (McMullen *et al.*, 2012; Wegulo *et al.*, 2015), with the latter four being the most effective (Paul *et al.*, 2008; Paul *et al.*, 2010). Proper timing of fungicide applications is also crucial to ensure adequate control and can be a difficult decision for producers. The most effective application period for wheat is usually around

anthesis with several studies reporting adequate control immediately prior to or soon after flowering occurs (Bradley *et al.*, 2010; D'Angelo *et al.*, 2014). The occurrence of optimal conditions for infection, such as wet weather during anthesis, can prevent producers from applying fungicides at the appropriate time. While studies have shown that applications as late as 20 days following anthesis can reduce the accumulation of mycotoxins in the grain, it is reported to have no effect on other FHB symptoms (Yoshida *et al.*, 2012). Late applications can also push producers past the 30-day preharvest intervals that many fungicide labels require (McMullen *et al.*, 2012).

FHB Resistance

Host Specificity

Resistance of wheat genotypes to FHB causing pathogens has been observed to be non-species specific. This has been extensively tested for the species *F. graminearum* and *F. culmorum* (Mesterházy, 1987; Mesterházy, 1995; Mesterházy *et al.*, 1999). Mesterházy (1987) concluded that *F. graminearum* and *F. culmorum* appear to have similar genetic backgrounds. Different isolates of these two species have been observed in several studies which all reported similar cultivar responses regarding FHB infection. (Bai & Shaner, 1996; Eeuwijk et al., 1995; Mesterházy, 1987; Mesterházy et al., 1999). A more recent paper reports that the non-specificity of resistance encompasses disease severity, kernel infection, yield response, and DON accumulation (Mesterházy *et al.*, 1999). Scott and Benedikz (1986)meste also observed similar resistance rankings for eight winter wheat cultivars when inoculated wmestith *F. avenaceum, F. culmorum, F. graminearum, F. nivale,* and *F. poae*.

Genetics of FHB Resistance

Resistance to FHB is suggested to be complex and to be further confounded by the presence of a large environmental variance component (Rudd *et al.*, 2001). Many studies have reported the inheritance of FHB to be quantitative in nature, controlled by many minor genes (H. Buerstmayr *et al.*, 2009). Additive effects have been shown to account for the majority of the genetic effects; however, non-additive effects such as dominance and epistasis can also be significant (Bai *et al.*, 2000; Liu *et al.*, 2013; Snijders, 1990).

Mechanisms and Quantification of Resistance

Resistance mechanisms of FHB have been categorized into two general groups, morphological (passive) and physiological (active) (Gilsinger *et al.*, 2005; Mesterházy, 1995; Rudd *et al.*, 2001). Morphological mechanisms are those related to plant features such as plant height, awnedness, heading date, and anther extrusion. Studies have shown correlations between these traits and infection levels (Mesterházy, 1995; Miedaner, 1997). Mesterházy (1995) observed that short plants tend to have higher disease severity relative to taller genotypes likely due to shorter distance between the wheat heads and inoculum sources in the soil. Awned genotypes can also show higher levels of infection compared to awnless genotypes possibly caused by increased surface area resulting in greater accumulation of fungal spores (Mesterházy, 1995; Miedaner, 1997). Gilsinger *et al.* (2005) uses the term morphological "avoidance" instead of morphological resistance since these traits do not necessarily inhibit the pathogen after infection but rather help the plant evade initial infection. Physiological or active mechanisms of resistance are categorized as plant responses typically involving biochemical pathways that can produce fungal inhibiting compounds (Gilsinger *et al.*, 2005).

Components of resistance have been classified into five types based on the different phenotypes associated with the disease. Mesterházy (1999) selected the following designation which will be used in this paper: Type I – resistance to initial infection (incidence), Type II – resistance to disease spread (severity), Type III – resistance to mycotoxin accumulation, Type IV – resistance to kernel infection, Type V – tolerance (Mesterházy, 1989; Mesterházy, 1995; Mesterházy et al., 1999; Miller et al., 1985; Schroeder & Christensen, 1963). Types I and II were first proposed by Schroeder and Christensen (1963). Type I resistance is commonly measured as the percent of visually infected heads. Screening can be done in the field under natural or artificial infection or in the greenhouse by using a sprayable spore suspension. The phenotype associated with Type I resistance is generally associated with morphological mechanisms as variation in these plant features typically affects the number of infected heads. Type II resistance is evaluated as the percent of symptomatic spikelets within infected heads. Many of the studies for genetic resistance in wheat have focused on Type II resistance and it appears to be more stable than Type I resistance (Bai & Shaner, 2004). Type II resistance can be evaluated in the field by averaging the percent of infected spikelets for all the heads in a plot or row. It can also be done in a greenhouse by using a point inoculation technique where a central floret is selected and inoculated. Type III and IV resistances tend to be more difficult to accurately measure, especially in field settings where combine harvesting is used. Highly infected kernels tend to be smaller and lighter making it easier for them to be lost during mechanical threshing. The measurement for Type III resistance is usually expressed as mg/kg or ppm and is typically determined using either thin-layer chromatography, gas chromatography-mass spectrometry (GC-MS), or high pressure liquid chromatography (HPLC) (Dowell *et al.*, 1999; Peiris *et al.*, 2013). These methods are fairly expensive and typically require bulk samples and the destruction

of the kernels in order to estimate DON levels (Wegulo & Dowell, 2008). Type IV resistance to kernel infection is normally expressed as the percent of *Fusarium* damaged kernels (FDK) which is typically measured by experienced personnel via visual sorting of sound (non-infected) kernels and FDK. This method is time consuming and can result in biased results. Studies on the use of single kernel near infrared spectroscopy (SKNIR) for DON and FDK estimation have shown promising results when compared with traditional methods (Dowell et al., 2006; Peiris et al., 2010; Peiris et al., 2013; Wegulo & Dowell, 2008). Analyzing DON content in seed samples using SKNIR allows for a more in depth look at how DON is distributed among FDK and sound kernels (Peiris *et al.*, 2010; Peiris *et al.*, 2013). Furthermore, this technology has the advantage of being non-destructive to the seed and having a lower cost when compared to DON estimation using GC-MS (Dowell *et al.*, 2006). Sorting of infected kernels by SKNIR was also reported to have a wider range of FDK detection as well as showing higher levels of consistency (Wegulo & Dowell, 2008). Type V resistance (tolerance) is commonly defined as the ability for certain genotypes to yield higher than others when both have the same level of visual disease relative to uninoculated controls (Mesterházy *et al.*, 1999; Shaner, 2002). Mesterházy (1999) reported observing tolerance at different resistance levels and concluded that it is not a form of partial resistance and that is a separate mechanism of resistance. However, tolerance is a mechanism that is not widely accepted as most studies have not completely accounted for certain factors that could affect the results, such as timing of infection and the degree of kernel invasion (Shaner, 2002). Results obtained from screening for tolerance can also be inconsistent across years, possibly due to changes in environmental factors and isolate aggressiveness during the infection period (Mesterházy *et al.*, 1999).

Biochemical Responses

When an interaction with a pathogen occurs, plants can induce defense genes that provide a variety of biochemical responses. These reactions can help prevent initial infection as well as slow down the growth and spread of the pathogen (Bowles, 1990). Some studies have shown that certain defense genes encoding pathogenesis-related (PR) proteins are induced upon infection (Bowles, 1990; Pritsch *et al.*, 2000; Zhou *et al.*, 2005; Zhou *et al.*, 2006). These proteins have been observed to begin accumulating 6 to 12 hai (Pritsch *et al.*, 2000). Genes related to the production of PR proteins include: PR-1, PR-2 (b-1,3-glucanase), PR-3 (chitinase), PR-4, and PR-5 (thaumatin-like protein). Pritsch *et al.* (2000) observed that a resistant cultivar accumulated PR-4 and PR-5 transcripts earlier and in greater number than a susceptible cultivar. Li and Yen (2008) conducted a similar study and reported contrasting results where PR proteins showed similar expression levels in both resistant and susceptible cultivars. They suggested that PR proteins do not play a role in FHB resistance.

Through proteomic and transcriptomic analysis, 163 genes and 37 proteins have been observed to be induced upon infection with *F. graminearum* (Ding *et al.*, 2011). The authors proposed a resistance model composed of two phases. The early phase (within 6 hai) is associated with the biotrophic stage of the pathogen. During this phase, salicylic acid (SA) signaling, phosphatidic acid (PA) signaling, and reactive oxygen species (ROS) production and scavenging are thought to occur. These activities are associated with hyper-sensitive responses (HR) and plant cell death (PCD) which could help reduce the growth of the pathogen. The second phase is observed to occur between 6 and 24 hai and is likely associated with the necrotrophic stage of *F.* graminearum. Activation of the jasmonic acid (JA) and ethylene (ET) signaling pathways occur. It is thought that the ET pathways helps facilitate the transition from

SA to JA defense signaling (Ding *et al.*, 2011). Li and Yen (2008) observed up-regulation of lipoxygenase (LOX2) and chalcone synthase via the JA signaling pathway in resistant cultivars compared to susceptible ones. Further research will be needed to elucidate the exact roles of specific genes and proteins regarding FHB resistance.

Sources of Resistance

Planting resistant cultivars is regarded as the most economical means of controlling FHB. Reviews of over 50 QTL studies have found more than 250 QTL associated with FHB resistance types I-IV from over 40 sources on all 21 chromosomes of the wheat genome (H. Buerstmayr *et al.*, 2009; Liu *et al.*, 2009; Löffler *et al.*, 2009). The majority of the QTL reported were associated with Type II resistance. This could be due to the difficulty or cost of screening for other resistant types compared to Type II. A large portion of the QTL were also observed to be derived from Asian spring wheat sources which could be a result of fewer studies in other genetic pools (Liu *et al.*, 2009).

One of the most commonly used QTL is *Fhb1*, a major QTL on chromosome 3BS for Type II and Type III resistance derived from the Chinese spring wheat Sumai-3 (Anderson *et al.*, 2001; Liu *et al.*, 2006; Waldron *et al.*, 1999). *Fhb1* has been shown to explain up to 56% of the phenotypic variance for Type II resistance to disease spread (Bai *et al.*, 1999) and up to 93% of the phenotypic variation for the accumulation of DON (Lemmens *et al.*, 2005). Lemmens *et al.* (2005) observed that lines carrying *Fhb1* were able to convert DON into the less phytotoxic DON-3-O-glycoside. Sumai-3 is also the source for a major Type I resistance QTL (Qfhs.ifa-5A) on chromosome 5AS. Mesterházy *et al.* (2007) noted that most breeding efforts had focused on integrating *Fhb1* and other Type II resistance QTL into adapted lines. This seemed to result in slow progress at increasing resistance beyond the levels of donor parents. In their study, they

reported that the QTL on 5AS had a similar effect for Type I resistance as that of 3BS on Type II resistance. When each of these QTL are present alone only a moderate level of resistance is seen but when they are combined they result in higher levels of resistance. Compared to *Fhb1*, most QTL explain much lower levels of phenotypic variation with the majority of them explaining less than 20% (H. Buerstmayr *et al.*, 2009).

Besides *Fhb1* and Qfhs.ifa-5A, several other FHB QTL have been discovered in Asian wheat lines. A QTL for Type II resistance derived from Sumai-3 was found on chromosome 6BS (*Fhb2)* (Cuthbert *et al.*, 2007; Waldron *et al.*, 1999). Two QTL for Type II resistance were discovered on chromosomes 2DL and 4BS (*Fhb4*) originating from the cultivar Wuhan 1 (Somers *et al.*, 2003). QTL for multiple resistance types have also been found on chromosomes 2D, 3BS, 4B, 5A, 5B, and 7A in the cultivar Wangshuibai (Jia *et al.*, 2005).

Sources of resistance have also been discovered in germplasm from South America and Europe. The Brazilian spring wheat cultivar Frontana has been shown to have a major QTL for Type I resistance on chromosome 3A. Smaller effect QTL for Type I and Type II resistance have also been reported on chromosomes 5A, 2B, 6B (Steiner *et al.*, 2004), and 7AS (Mardi *et al.*, 2006). Resistance QTL in the Swiss winter wheat cultivar Arina have been studied in several different mapping populations. Paillard *et al.* (2004) crossed Arina with the susceptible cultivar Forno. They found two major QTL for Type II resistance on chromosomes 6D and 4A of Arina and one major QTL on chromosome 5B of Forno. Other minor QTL for Type II resistance were also observed on chromosomes 3B, 5A, and 2A of Arina, and on chromosomes 3A and 3D of Forno. Draeger *et al.* (2007) analyzed the mapping population ‘Arina x Riband’ and reported two major QTL for Type II resistance on chromosomes 4D and 6B of Arina.

Although the majority of early studies focused on exotic sources, FHB resistance has also been observed in both soft (SWW) and hard (HWW) winter wheat germplasm native to the U.S. The ability to find FHB resistance QTL in sources already adapted to winter wheat growing regions can improve the efficiency of breeding projects and shorten the time needed to incorporate this resistance into elite materials. Native lines and cultivars without *Fhb1* but reported to have some levels of resistance were tested for Type II resistance by Jin *et al.* (2013). Cultivars with higher levels of resistance include Freedom (SWW), Roane (SWW), T154 (HWW), Bess (SWW), Century (HWW), Heyne (HWW), Lyman (HWW), Everest (HWW), Harry (HWW), Atlas66 (SWW), and Husker (HWW). Cultivars reported to have moderate levels of resistance include T153 (HWW), Ernie (SWW), Aspen (HWW), Endurance (HWW), Winterhawk (HWW), Arapahoe (HWW), Overland (HWW), and Jerry (HWW) (Jin *et al.*, 2013). Mapping populations have been developed for several of these sources and resistance QTL have been reported on the following chromosomes: 2B, 3B, 4B, and 5A from Ernie (Liu *et al.*, 2007); 2A from Freedom (Gupta *et al.*, 2001); 1A, 1B, 3A, 4A, and 6A from Lyman; 1A, 1B, 2A, 5A, and 6A from Overland (Eckard *et al.*, 2015); 1A, 2B, 3B, 4D, and 5D from Bess (Petersen, 2015). The cultivar Truman (SWW), a full-sib of the cultivar Bess, has also shown high levels of resistance (McKendry *et al.*, 2005).

Current Strategies for Breeding FHB Resistant Cultivars

Breeding for increased FHB resistance has proven to be a successful method to reduce the risk of yield and quality loss in many countries around the world (Ruckenbauer *et al.*, 2001). The diverse genetic variation for FHB resistance in differing wheat gene pools allows breeders the opportunity to improve levels of resistance within their programs. Introduction of resistance QTL from these sources into germplasm native to the U.S. can help improve resistance but can

be costly and time consuming. What makes this job difficult is that a lot of the sources that show high levels of resistance are often unadapted and have poor agronomic traits. Attempts to incorporate this resistance into winter wheat has been especially tough due to lack of winter hardiness and adaption to winter wheat growing regions. Advances in molecular marker technology has helped breeders overcome some of these issues. Creating dense genetics maps with many markers and high levels of recombination can allow for 'fine-mapping' of QTL and reduce the size of the DNA segment needed for introgression. Multi-parent advanced generation inter-cross (MAGIC) populations is one method that can help reduce the size of these segments. The diverse parental base of these populations could in theory increase recombination levels by 25 and 75%, compared to bi-parental populations, for four-parent and eight-parent populations, respectively (B. E. Huang *et al.*, 2012). These 'fine-mapped' QTL can then be used with strategies such as backcrossing and marker-assisted selection (MAS) to transfer the gene of interest into elite materials. Recent work in these areas has aimed at backcrossing *Fhb1* into winter wheat lines and pyramiding it with native sources of resistance.

Eckard *et al.* (2015) mapped FHB QTL in early generation breeding populations using an identical-by-descent (IBD) based linkage mapping approach. They developed 28 four-way segregating F1 populations using ten winter wheat parental lines that contained varying sources of native resistance and agronomic traits; as well as, two backcross-derived lines that carrying the *Fhb1* resistance allele. They were able to map 15 total QTL on 12 chromosomes and the developed lines contained gene pyramids ranging from 0 to 14 resistance alleles. Comparing the FHB severity scores for these lines showed a cumulative additive effect of the QTL. These lines have the potential to be used as germplasm sources in breeding programs with MAS to improve resistance (Eckard *et al.*, 2015).

The discovery of large numbers of QTL affecting FHB resistance and the development of high quality linked markers can provide breeders with the opportunity to use tools such as genomic selection (GS) and cross prediction. Arruda *et al.* (2015) reported moderate to high cross-validated prediction accuracies for six FHB traits ranging from 0.35 to 0.82. Statistical modeling can be used to predict genetic estimated breeding values (GEBVs) for FHB traits. These values can then be used to help breeders select which lines should be most resistant in their populations and can extend to helping breeders choose which parental lines should initially be crossed to produce the best performing and most resistant lines.

Genetics and Mapping

Molecular Markers

The advent of molecular marker technology has greatly improved the precision and efficiency of plant and animal breeding. Molecular markers are certain pieces of DNA that are associated with specific places in the genome. These markers often differ slightly between individuals (polymorphic markers) and can be used as tools to better visualize inter and intra-species evolution. Molecular markers are divided into three main groups: hybridization-based markers, PCR-based markers, and sequence-based markers (Caixeta *et al.*, 2014).

Hybridization-based markers were first popularized in the 1970's. The most well know of these markers was the restriction fragment length polymorphism (RFLP), which was first used in 1975 (Caixeta *et al.*, 2014). Analysis with RFLP markers uses restriction enzymes to digest genomic DNA at specific sequences (restriction sites) and subsequent DNA separation using electrophoresis. Differences between genotypes in DNA sequences at the restriction sites results in variations in the length of the DNA fragments produced. Probes can then be added to detect the fragments that contain certain sequences allowing researchers to detect polymorphisms

between individuals (Henry, 2012). RFLP markers were highly adopted in the 1980's but their high cost, need for large amounts of DNA, and time consuming process made them inefficient and they were eventually replaced by PCR-based markers in the 1990's (Caixeta *et al.*, 2014; Henry, 2012).

PCR-based markers use a molecular technique known as polymerase chain reaction (PCR). This technique uses replication enzymes, DNA primers, and nucleotide bases to amplify target DNA sequences. One of the first PCR-based marker technologies developed was random amplified DNA polymorphism (RAPD). This marker assay amplifies random DNA segments with small, single primers composed of random sequence which allows polymorphisms to be detected based on presence or absence of DNA amplification (Williams *et al.*, 1990). Amplified fragment length polymorphism (AFLP) markers, as described by Vos *et al.* (1995), allow for simultaneous analysis of randomly distributed DNA regions using selective amplification. Restriction enzymes are used to digest DNA and sequence adapters are ligated to the resulting fragments. PCR is then used to amplify selected fragments and the selected fragments are analyzed following gel electrophoresis. This technique proved to be much more reproducible compared to RAPD markers and also allowed for analysis of a large number of fragments in a single assay. However, this technique still requires several time-consuming steps and is unable to identify specific allelic variants to determine heterozygous genotypes (Vos *et al.*, 1995). DArT markers are another PCR based marker technology that are also able to genotype large numbers of loci in a single assay. What separates them from AFLP markers is that they are developed using a DNA array called a "diversity panel" which is composed of DNA from different individuals that represent the species of interest. Restriction enzymes are selected based on the species of interest in order to determine the most efficient way to reduce the genome complexity.

PCR is performed using selective primers, and the DNA fragments are cloned and arrayed. The DNA library that is created is made up of common fragments of DNA that are present in all individuals as well as polymorphic fragments that are only present in certain genotypes (Caixeta *et al.*, 2014). Microsatellite markers, also known as simple sequence repeats (SSR), are sequences varying from 1 to 6 base pairs (bp) that are repeated adjacently. These repeats are fairly evenly distributed and can be highly polymorphic in many populations. Conservation of the sequences flanking these repeats among species allows for the development of specific primers and ability to amplify the DNA using PCR (Henry, 2012). The high level of variation and ease of PCR make these markers popular for QTL studies; however, they lack straightforward multi-plexing capabilities and can be cost prohibitive for large sample sizes (Henry, 2008).

With the advent of DNA sequencing, new marker technologies have emerged allowing for higher throughput and greater genome coverage. The automated Sanger sequencing method is one of the most well-known technologies on this front. Developed by Frederick Sanger in 1977 (Sanger *et al.*, 1977), this method became widely adopted for sequencing studies (Metzker, 2009) eventually leading to the completion of the first human genome sequence (International Human Genome, 2004). More recently, next-generation sequencing tools have allowed for the development of an unprecedented amount of sequencing data. Substantial interest has been placed in markers based on variations in a single DNA nucleotide base (A, G, C, or T) at a specific location in the genome, known as single nucleotide polymorphisms (SNP). These are the most commonly occurring variant in the DNA sequence between individuals of the same species (Brookes, 1999). SNPs are created via mutations in single organisms that can then be inherited by their offspring. Since mutations give rise to SNPs, the number of SNPs present in a

population can vary widely both within and between species (Henry, 2008). SNPs can occur within genes (coding regions) but the majority are found in non-coding regions of the genome. When SNPs occur in coding regions, the base alteration can result in a change in the amino acid produced (non-synonymous mutation) or the amino acid can remain unchanged (synonymous mutation) (Caixeta *et al.*, 2014). Non-synonymous SNPs tend to be most useful for plant breeders and geneticists as the amino acid change can result in a change in protein function and ultimately lead to a difference in phenotype (Henry, 2008).

Genetic Mapping and QTL Analysis

Genetic based linkage maps are maps generated for specific populations that show the relative position and order of markers, genes, and QTL based on the rate of recombination (recombination frequency) between them. The recombination frequency is the frequency in which a single crossover of the chromosome occurs between two markers, genes, or QTL. Genetic positions are given in centimorgans (cM) where 1 cM is equal to a recombination frequency of 1%. The positions for specific markers, genes, and QTL can vary when maps are developed for different populations since the recombination frequency can differ. Linkage maps have been constructed using all of the markers discussed in the previous section and are usually developed for bi-parental populations such as backcrosses, doubled haploids (DH), recombinant inbred lines (RIL) (H. Buerstmayr *et al.*, 2009), and F2 populations. The development of a large number of maps for a certain species can allow researchers to build a consensus map by combining all of the information from the individual maps which can lead to a more accurate reference.

QTL mapping (Sax, 1923; Thoday, 1961) is a tool based on DNA markers that can be very useful for studying complex and polygenic traits. DH and RIL populations are commonly

used for QTL mapping studies since these populations are genetically fixed and can be phenotyped over multiple years and environments (H. Buerstmayr *et al.*, 2009). QTL mapping makes use of genetic marker data for the population of interest and phenotypic data for the trait of interest to scan the genome for marker-trait associations. Individuals in the population are grouped based on their relative genotype at each marker locus. If the groups differ for alleles at a certain locus and the mean value for the trait of interest is statistically significantly different between the alleles then there is a high probability for a QTL in this region (Young, 1996). Usually the parents of the population are chosen to have contrasting phenotypes for the trait of interest. For example, when studying disease traits it is common to select a resistant parent and a susceptible parent (Kolb *et al.*, 2001). This helps prevent issues such as identity-by-descent where both parents contain the same QTL; therefore, making it difficult to detect.

A number of factors can influence the power to detect QTL. Population sizes of less than 500 individuals can make it difficult to detect small effect QTL and two QTL that are less than 20 cM apart, making them appear as one single QTL. Proximity of the markers to the QTL is important. The closer a QTL is to a marker, the easier it is to detect, even if it has a small effect (Tanksley, 1993). As a result, using maps that contain greater numbers of markers that are more evenly distributed across the genome should increase the power to detect regions affecting the trait. The quality of these markers should be of consideration however. A genotypic dataset that has a greater number of markers but contains a lot of missing data could affect the accuracy of the linkage map compared to one with fewer, higher quality markers (Hackett & Luo, 2003). QTL with small effect may be too weak to detect and can fall below the significance threshold; especially when the population size or number of markers is low. Traits with low heritability values are more affected by environmental factors and the QTL governing these traits will

therefore be harder to detect. (Tanksley, 1993). The collection of accurate phenotypic data that can be reproduced across years is also important (Cuthbert *et al.*, 2006) and errors can result in spurious QTL; for that reason, it is common for researchers to repeat the experiment across space or time to validate QTL found in each experiment (Kolb *et al.*, 2001).

Mapping of QTL can be conducted using a range of different statistical techniques for analysis such as mean and variance analysis, regression and correlation analysis, moments and maximum likelihood analysis, and use of varying marker intervals including single and multiple interval analysis. The two most commonly used approaches to QTL mapping are based on classical statistics or Bayesian statistics that commonly relies on a Markov chain Monte Carlo (MCMC) approach. There are a couple main differences between these two approaches. First, the classical approach maximizes over QTL positions and effects; whereas, the Bayesian approach averages over the positions and effects since these parameters are unknown. Second, a maximum likelihood is considered for the model in the classical approach, while the Bayesian approach specifies a preceding distribution for QTL models before considering the subsequent distribution obtained from the data (Broman, 2009).

Several methods have been developed for use in QTL mapping studies including methods based on the classical approach: single marker analysis (SMA) (Tanksley et al., 1982), standard interval mapping (SIM) (Lander & Botstein, 1989), and composite interval mapping (CIM) (R. C. Jansen, 1993; R. C. Jansen, 1994; Zeng, 1993; Zeng, 1994); as well as, multiple imputation based on Bayesian statistics (Sen & Churchill, 2001). Many of the methods used in these analyses have also been extended for use in multiple QTL mapping (MQM) strategies (Broman, 2009). SMA looks at the difference between phenotypic means for two alleles at each locus using an additive-dominant model and statistical techniques including t-tests, ANOVA,

regression, maximum likelihood estimations, and log likelihood ratios. If a significant difference is detected then a QTL can be assumed to be present at that locus. One downfall of SMA is that genotyping data must be complete in order for accurate analysis. Tian (2015) also listed the following disadvantages associated with using SMA: it is unable to distinguish a single QTL from multiple linked QTL; the position of the QTL on a genetic map cannot be estimated; the effects of the QTL can be appear smaller than they truly are due to recombination between the marker and the QTL; false positives (Type I errors) can be an issue; a large population is required; and its efficiency at QTL detection is low. SIM overcomes many of the weakness associated with SMA and has been one of the most widely used methods to perform QTL analysis. This method is based on the maximum likelihood approach proposed by Lander and Botstein (1989) and improves the power to detect QTL compared to SMA by testing the intervals between markers. The genotypes in these intervals are still unknown; however, the probability of a certain genotype occurring can be estimated using linkage map distances and the genotypes at the closest flanking markers. This is most effective when there is a relatively even distribution of markers throughout the genome (Tanksley, 1993). Also, if the genotyping is incomplete at certain flanking markers then it moves down the line to the nearest genotyped marker (Broman, 2001). SIM is an improvement over SMA; however, it is still limited to looking at a single QTL per chromosome. Use of SIM also increases the computation time required compared to SMA. Haley and Knott (1992) developed a method for approximating interval mapping that greatly reduced the computation time; however, it is less reliable with low genotype information (Broman, 2001; Broman, 2009). Sen and Churchill (2001) proposed another method for QTL mapping using Bayesian QTL analysis employed through a simple Monte Carlo algorithm. This algorithm overcomes the issue with missing genotypic information by combining the results of

multiple imputations to fill in the missing values. In order to improve upon the weaknesses of SIM, Zeng (1994) proposed the CIM method that combines interval mapping with multiple regression. The general basis of CIM involves interval testing where the test statistic for a given marker interval remains unaffected by other QTL outside of a defined interval. To do this, certain markers in the model are used as covariates during the interval mapping analysis. There are several advantages reported to be associated with CIM. (1) The search for multiple QTL is reduced from a multiple dimensional search to a one dimensional search since only one interval is tested at a time. (2) The sensitivity of the test statistic to the position of individual QTL and precision of mapping is increased due to conditioning of linked markers. (3) The use of selected markers as covariates improves the efficiency of detecting QTL of lesser effect (Zeng, 1994). One major issue with this method is determining the proper markers to use as covariates in the analysis. The most appropriate markers to choose would be those closest to the actual QTL; however, errors in this choice can lead to spurious results (Broman, 2001). This method is also limited in that it cannot deal with certain genetic factors such as epistasis and QTL by environment interactions (Tian, 2015). Multiple QTL mapping (MQM) involves a model selection approach to QTL mapping. Methods used in single QTL analysis can be extended for use in a multiple QTL model (Broman, 2009). The MIM strategy (Kao *et al.*, 1999; Zeng *et al.*, 1999) extends the power of interval mapping to analyze multiple QTL at once through the use of QTL modeling. The location of QTL can be extrapolated to positions between markers and interactions between QTL can be included. This method also accurately accounts for missing genotypic data. Haley-Knott regression has also been extended for use in MQM. In this approach, indicators for QTL genotypes are replaced with their expected values using the available marker data and a linear regression is performed on the multiple QTL genotype

probabilities. This method is advantageous in terms of the computational time required; however, it still falls short with regard to the use of genotypic data and is less suited to data sets with high numbers of missing genotypes. Multiple imputation can also be used in MQM analysis without any modification. (Broman, 2009).

Chapter 2 - Genetic Mapping of QTL for Fusarium Head Blight Resistance in Winter Wheat Cultivars Art and Everest

Introduction

Fusarium head blight (FHB), commonly referred to as head scab, is a devastating fungal disease infecting wheat. In North America this disease is mainly caused by the pathogen *Fusarium graminearum* Schwabe (Osborne & Stein, 2007). Fusarium head blight can result in significant reductions in yield and grain quality. In 2008, FHB infection in Kansas resulted in estimated yield reductions between 8.75 and 17.6% for the eastern third of the state. Total statewide losses were valued at $57 million (McMullen *et al.*, 2012). The effect of infection can be further exacerbated due to the production of mycotoxins, such as deoxynivalenol (DON), which accumulate in the grain and are harmful to humans and animals when ingested (Desjardins & Proctor, 2007).

One of the best strategies for managing FHB is through the development and deployment of genetically resistant cultivars (Bai & Shaner, 2004). This can be a challenging task as FHB resistance has been shown to be quantitative in nature, being governed by a complex genetic system involving many genes of varying effect. Five different types of resistance are commonly accepted: Type I – resistance to initial infection (incidence), Type II – resistance to disease spread within the head (severity), Type III – resistance to mycotoxin accumulation in the grain,

Type IV – resistance to kernel infection, Type V – tolerance (Mesterházy *et al.*, 1999). Resistant sources native to the U.S. have been discovered; however, the most well-known genes of major effect have commonly been present in exotic germplasm that is typically unadapted and associated with poor agronomic traits. Several studies have aimed at transferring these major genes into native winter wheat material adapted to the Great Plains; however, this can be a time consuming process with no guarantee that the gene will have the same effect in the new genetic background. Therefore, discovering resistance genes in sources native to these regions and combining them to produce highly resistant cultivars has become a major priority for many breeders.

Mapping populations are commonly developed from parents that are genetically divergent to reduce the chance that the parents will show identity-by-descent at the genes affecting the trait of interest. The problem that arises is that the genetic relationship between the parents is usually unknown. Therefore, cultivars that show significant phenotypic differences are often selected. The cultivars used as parents in this study have similar phenotypes for FHB resistance; however, their pedigrees appeared to be genetically unrelated. Therefore, we expect the genes conveying resistance to be different between them. The objectives of this study were: (1) map QTL associated with FHB resistance types II, III, and IV in a population derived from the cultivars Art and Everest; (2) identify markers closely linked to these QTL that can be used in future marker assisted selection (MAS) efforts; and (3) identify individuals in the population with high levels of FHB resistance that accumulated resistance QTL from both parents.

Materials and Methods

Plant Materials

A doubled haploid (DH) population consisting of 148 individuals was derived from the cross between the hard red winter wheat (HRWW) cultivars Art and Everest. Everest is a hard red winter wheat (HRWW) variety developed by Kansas State University and released in 2009 through the Kansas Wheat Alliance. Everest was developed from a cross between the hard white winter wheat cultivar Betty and Pioneer experimental lines. It has shown an intermediate level of resistance to FHB with a designation of 4 on a 1-to-9 scale in KSU Extension publications where 1 is highly resistant and 9 is highly susceptible (De Wolf *et al.*, 2015). Everest accounted for the highest percentage of planted wheat acres in Kansas in 2013, 2014, 2015, and 2016 (United States Department of Agriculture National Agriculture Statistics Service [USDA/NASS], 2016b). The widespread adoption of Everest by Kansas wheat growers has had a significant impact on lowering the statewide vulnerability to FHB (Bockus et al., 2012). Art is another HRWW variety that was developed through the Agripro division of Syngenta cereals (Syngenta Cereals, Berthoud, CO) and was released in 2007. Art was developed from a cross between the hard red winter wheat cultivar Jagger and Agripro experimental lines. It has also shown intermediate levels of FHB resistance but is more susceptible than Everest with a rating of 6 in KSU Extension publications (De Wolf *et al.*, 2015).

Experimental Design

The DH population and parental lines Art and Everest were evaluated for FHB response in Fall 2013 and Fall 2014. Seeds were planted into germination trays containing Sunshine® professional peat lite growing mix and resulting seedlings placed in a vernalization chamber for a period of six weeks at 4°C. Seedlings for DH lines and parents were then removed from

vernalization and transplanted into 6 ¼" by 6 7/8" one gallon pots (National Polymers Inc.). The experiment was arranged in a completely randomized design across four greenhouse benches with four replications (pots) per line and four plants per pot. Marathon® 1% granular insecticide was applied ten days after transplanting to control insect pests. The greenhouse was set at a 14 hour photoperiod with a daytime temperature of 24°C and a nighttime temperature of 19°C. At anthesis the daytime temperature was increased to 30°C to provide an optimal temperature for fungal growth. Wheat spikes were inoculated at anthesis via a point inoculation technique.

Phenotypic Evaluations

Type II Resistance

Inocula of *F. graminearum* consisted of a conidial suspension using field isolate GZ 3639 native to Kansas (Desjardins *et al.*, 1996). The conidial suspension was developed from cubes of potato dextrose agar in a solution of mung bean broth (Jin *et al.*, 2013). The conidial suspension was adjusted to 100 spores μL^{-1} for inoculations. Wheat spikes were inoculated at anthesis by selecting 2 to 3 spikes per plant with a goal of 10 spikes per pot and 40 total spikes per DH line. Spikes were injected with 10 µL of conidial suspension, via a micropipette; into a single floret on a central spikelet (approximately the tenth fully developed spikelet from the base of the head). Inoculated spikes were immediately misted with water and a 7.62 cm by 12.7 cm clear, two mil, re-sealable bag (Uline co.) was placed over the spike to provide a humid environment for fungal development. The bags were removed after 48 hours and spikes were rated for percent symptomatic spikelets (PSS) after 14 days. Previous studies have reported infection of the wheat rachis can result in death of spikelets above the inoculation point, without the spikelets actually becoming infected. This can lead to over-estimation of disease spread. To account for this,

ratings were calculated by counting the number of infected, fully developed spikelets from the inoculated spikelet downward to the base of the spike and multiplying this number by ten. Each infected spikelet represented ten percent infection.

Type III and Type IV Resistances

Inoculated wheat heads from each pot were harvested at maturity. One head was randomly selected from each plant within each pot. Heads were threshed and the seed from each spikelet was bulked according to Peiris *et al.* (2011). Kernels in each spikelet were manually removed starting with the first fully developed spikelet from the base and progressing up to the tip of the wheat head. The kernels from each spikelet were placed in separate bins and each spikelet was given a number designation. The inoculated spikelet was designated as spikelet 0 with spikelets below the inoculated spikelet being designated by sequential negative numbers (-1 to -10) and spikelets above being designated by sequential positive numbers (+1 to +10).

Estimation of DON and FDK values was performed according to Peiris *et al.* (2010) using a single kernel near-infrared spectroscopy (SKNIR) machine developed by the USDA, ARS, CGAHR, Engineering and Wind Erosion Research Unit, Manhattan, KS, and commercialized by Perten Instruments (Stockholm, Sweden). Single kernels are automatically fed into a spectrometer viewing area via vacuum suction. A fiber optic bundle then exposes the kernel to visible-NIR light and the reflected energy is collected and transmitted to a spectrometer with an indium-gallium-arsenide detector that is able to measure light absorbance between 950 and 1650 nm. Calibration models were developed to allow estimation of different FHB factors. Spectra values can be collected once for each kernel and then different calibration models can be used to predict the traits of interest. The DON calibration model is used to estimate the concentration of DON in parts per million (ppm) for each kernel. The FHB calibration model is

used to sort kernels as either FDK or sound. Kernels with predicted values between -1.00 and 1.50 are classified as sound kernels while kernels with predicted values of 1.51 and above are classified as FDK.

Heading Date and Plant Height

Heading date was recorded separately for each inoculated wheat head during each individual experiment. Plant height was recorded in the 2014 experiment only, by measuring the height of the primary tiller from each plant in each pot.

DNA Collection and Marker Genotyping

Leaf tissue was collected from each DH line by harvesting tissue at the two leaf stage. Tissue was freeze dried at a temperature of -51°C for 48 hours using a Labconco Freezone 6 (Labconco, Kansas City, MO). Dried tissue was ground in a Retsch mm400 tissue grinder set at 25 cycles/sec (Restch, Haan, Germany). DNA purification was performed using the "BS96 DNA Plant" protocol for the Biosprint 96 workstation and the Biosprint 96 DNA plant kit (Qiagen, Hilden, Germany).

Genotyping by sequencing (GBS) (Elshire *et al.*, 2011) was performed according to the protocol of Poland *et al.* (2012). This protocol makes use of targeted complexity reduction via a two restriction enzyme system that involves one "rare-cutter" (*PstI)* and one "common-cutter" (*MspI).* Processing of raw GBS sequence data into SNP genotypes was performed using the TASSEL-GBS bioinformatics pipeline (Glaubitz *et al.*, 2014). SNPs with less than 80% missing data were retained. These markers were then loaded into Rstudio v. 0.98.1027 (RStudio, Boston, MA) and filtered for polymorphism between the parents using R code provided by Dr. Robert Gaynor. The polymorphic markers were then subjected to further filtering in Rstudio using code from the R/qtl package (Broman *et al.*, 2003) in Rstudio. Markers showing >95% similarity to

other markers, as well as markers showing significant segregation distortion ($P<0.001$) were removed.

The DH population was also genotyped for several markers linked to previously reported genes. The microsatellite marker Xgwm149 was previously reported to be linked to *Fhb4*, a major gene for type II resistance to FHB on chromosome 4BL (Xue *et al.*, 2010). The sequence for this SSR primer was obtained from GrainGenes2.0 (www.wheat.pw.usda.gov). Three Kompetitive Allele Specific PCR (KASP) markers were also used: GBSSNP1487, RhtB1_cim-KASP, and RhtD1-KASP. GBSSNP1487 has been reported to be linked to a QTL for type II resistance to FHB on chromosome 2DS (Cai, 2016). RhtB1_cim-KASP and RhtD1-KASP are linked to the major height genes Rht-B1 and Rht-D1 on chromosomes 4B and 4D, respectively (Ellis *et al.*, 2002). DNA amplification was performed for both SSR and KASP markers using a touchdown program on the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The PCR mixture for SSR markers was initially incubated at 95°C for 5 minutes, followed by five cycles at 96°C for 1 minute each. Each of the five cycles included an annealing step at 68°C for 3 minutes with a decrease of 2°C in each subsequent cycle and an extension step at 72°C for 1 minute. Another five cycles were performed with the annealing temperature starting at 58°C with a decrease of 2°C in each subsequent cycle. The PCR mixture then went through an additional forty cycles at 96°C for 20 seconds, 50°C for 20 seconds, and 72°C for 20 seconds, before ending with a final extension at 72°C for 5 minutes and cool down at 12°C for 5 minutes. For KASP markers, the PCR mixture was initially incubated at 94°C for 15 minutes followed by ten cycles with an initial step at 94°C for 20 seconds and an annealing step at 65°C for 1 minute with a decrease of 0.8°C in each subsequent cycle. The PCR mixture then went through fifty cycles with an initial step at 94°C for 20 seconds and annealing step at 57°C for 1 minute.

Amplified PCR products were analyzed for SSR markers using an ABI 3730 DNA Analyzer and for KASP markers using a 7900HT Fast Realtime PCR machine. KASP assays were scored using SAS 2.4 software (Applied Biosystems, Foster City, CA). Allele scoring of SSR markers were scored using GeneMarker 2.4.0 (SoftGenetics LLC. State College, PA, USA). The sequence for the photoperiod gene *Ppd-D1* was retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and added to the list of GBS snps. These sequences were then aligned with the recently published draft sequence of the wheat genome (International Wheat Genome, 2014) using POPSEQ (Chapman *et al.*, 2015).

Data Analysis

Phenotypic data were analyzed using SAS statistical software version 9.4. The SAS procedure PROC UNIVARIATE was used to evaluate datasets for normality. Distributions for PSS and DON were skewed to the right (toward susceptibility) within each individual experiment and between experiments. The distribution of the 2014 FDK dataset was also skewed to the right; therefore, a square root transformation was applied to PSS datasets and the 2014 FDK dataset, and a log transformation was applied to DON datasets for further analysis. The remaining datasets were not transformed. SAS procedure PROC GLIMMIX was used for statistical analysis. An analysis of variance was conducted to obtain variance components and calculate best linear unbiased estimates (BLUEs) for PSS, FDK, and DON in the individual experiments using the model $Y_{ij} = u + g_i + r(g)_{ij} + e_{ij}$ where Y_{ij} defines the trait, g_i defines the fixed effect of the i^{th} genotype, $r(g)_{ij}$ defines the random effect of j^{th} replicate nested within the i^{th} genotype, and e_{ij} defines the random error term. The model used to obtain variance components and calculate BLUEs across experiments was $Y_{ijk} = u + g_i + t_j + gt_{ij} + r(g)_{ik} + e_{ijk}$ where Y_{ijk} defines the trait, g_i defines the fixed effect of the i^{th} genotype, t_j defines

the random effect of the j^{th} experiment, gt_{ij} defines the random interaction of the i^{th} genotype with the j^{th} experiment, $r(g)_{ik}$ defines the random effect of the k^{th} replicate nested within the i^{th} genotype, and e_{ijk} defines the random error term. A broad range of heading dates were observed for DH lines, and lines with later heading dates were therefore inoculated later in the year where outdoor temperatures are typically lower. This experiment was conducted in a greenhouse with temperature control; however, period of extremely cold weather can still cause decreases in the relative humidity and temperature within the greenhouse. In order to account for the effects of these changes, heading date was included as a covariate in the analysis by adding HD into the model as a fixed effect factor. Analysis of variance was conducted treating genotype as a fixed effect with all other effects treated as random. For variance component estimation all effects were treated as random. Broad-sense heritability was calculated for each disease trait from variance components using $H^2 = \sigma^2_{\mathrm{G}}/(\sigma^2_{\mathrm{G}} + \sigma^2_{\mathrm{GxE}}/e + \sigma^2_{\mathrm{E}}/er)$ where σ^2_G = genotypic variance, σ^2_{GxE} = genotype-by-experiment interaction variance, σ^2_E = error variance, e = number of experiments and r = number of replications (M. Buerstmayr et al., 2012; Nyquist & Baker, 1991). Comparisons of adjusted means between parental lines and DH lines was performed using Dunnett's test (Dunnett, 1955). Pairwise comparisons between all DH lines were calculated using the Tukey-Kramer test (Tukey, 1949). Pearson correlation coefficients for each trait were calculated using the rcorr package in RStudio v. 0.98.1027 (RStudio, Boston, MA).

Genetic Map Construction

Genetic map construction was performed using MSTMap online software (Wu *et al.*, 2007; Wu *et al.*, 2008). A logarithm of odds (LOD) grouping criteria value of 7.0 was used to place markers into linkage groups, and the Kosambi mapping function (Kosambi, 1943) was used to estimate map distances from recombination values. A no mapping distance threshold of

15 cM, and a no mapping size threshold of 3.0 were used to detect and separate isolated markers. The genetic map was anchored to the physical wheat chromosomes using a Basic Local Alignment Search Tool (BLAST) (http://wheat-urgi.versailles.inra.fr/) (Deng *et al.*, 2007). To assign SNPs to wheat chromosomes, the DNA sequence for each GBS marker was blasted against the wheat sequence surveys for all 21 wheat chromosomes based on the draft sequence of the wheat genome published in 2014 (International Wheat Genome, 2014). BLAST results were filtered based on a similarity percentage of 98% or higher and a BLAST score of 102 or higher to eliminate partial matches.

QTL Mapping

QTL mapping for all traits was conducted using the R/qtl package (Broman *et al.*, 2003) in RStudio v. 0.98.1027 (RStudio, Boston, MA). All analyses were performed using Haley-Knott regression. The genome-wide LOD thresholds for each trait were estimated using a permutation test with 1,000 permutations to determine the significance of QTL at $P<0.05$ and $P<0.10$. The initial search for QTL was conducted using simple interval mapping (SIM). The results from SIM were used to obtain markers closely positioned to significant QTL. These markers were then used as initial covariates to search for additional QTL. CIM was implemented using forward selection of marker covariates and an infinite window size. The forward selection process identifies markers that best predict the phenotype by giving the greatest reduction in the residual sum of squares. The use of an infinite window size omits any marker covariates located on the same chromosome as the position currently being tested. Linkage of multiple QTL and epistatic interactions between QTL were investigated using the scantwo function in R/qtl. The results from these analyses were used in multiple QTL mapping (MQM) to fit a multiple QTL model to

the data. MQM was used to further test the significance of each QTL, refine the position of the QTL, and estimate the percent phenotypic variance explained by the QTL.

Haplotype Analysis

To test the effect of allele combinations for FHB resistance QTL, genotypes were placed into haplotype groups. The allelic state of the peak marker for each QTL was selected to represent the allelic state of each DH line in the analysis. Lines containing missing data at the peak marker were not included in the analysis. The SAS procedure PROC GLIMMIX was used to calculate BLUEs for each haplotype group using the model $Y_{ij} = u + h_i + g(h)_{ij} + e_{ij}$ where Y_{ij} defines the trait, h_i defines the fixed effect of the i^{th} haplotype, $g(h)_{ij}$ defines the random effect of j^{th} genotype nested within the i^{th} haplotype, and e_{ij} defines the random error term. DH lines were also placed into haplotype groups based on their allelic state at the *Rht-B1* and *Rht-D1* loci. BLUEs were calculated for the traits PSS, FDK, DON, and PH for individual experiments and across experiments. Pairwise comparisons between haplotype groups were conducted using the Tukey-Kramer method (Tukey, 1949).

Results

Phenotypic Data

The DH population showed normal distributions for adjusted means of the three disease traits; PSS, FDK, and DON, in each individual experiment and across experiments after transformation of non-normal datasets. Analysis of variance (ANOVA) indicated significant genotypic effects and genotype by experiment effects for all three traits in each individual experiment and across experiments (Table 2.1). Everest showed a significantly lower adjusted mean value for PSS compared to Art during the fall of 2013; however, no significant differences

were observed during the fall of 2014 or during the across-experiment analysis (Table 2.2). FDK and DON were not significantly different between the two parents. Datasets for PSS and DON for each individual experiment and across experiments; as well as, FDK during the 2014 experiment were skewed to the right. FDK data during the 2013 experiment appeared normally distributed. Different phenotypic frequency distributions for DH means were observed within each trait between the two experiments (Figure 2.1). In general, a lower range of phenotypic values were observed for all three traits during the 2014 experiment compared to the 2013 experiment indicating that disease pressure was higher in the first experiment. (Table 2.2). No transgressive segregation was observed in the DH lines for the three disease traits. While some lines had lower adjusted mean values compared to the parents, no significant differences occurred. The distribution of plant height was not significantly different between Everest and Art. The two parents carry different alleles at the *Rht-B1* and *Rht-D1* loci; therefore, double dwarf, semi-dwarf, and standard height plants were all present in the population. Significant differences were observed between the two parents for heading date during the 2014 experiment but not during the 2013 experiment. Pairwise Pearson correlations were significantly positive within each year between the three disease traits (r = 0.27 to 0.82, $P<0.001$). Significant negative correlations were observed between the three disease traits and HD during the 2013 experiment (r = -0.45 to -0.52, $P<0.001$). In the 2014 experiment, HD showed significant negative correlations with PSS and DON, and a significantly positive correlation with FDK. The overall trend appears to show higher disease scores associated with earlier heading dates. PH and PSS also showed a significant positive correlation in the 2014 experiment (r=0.28, $P<0.001$) suggesting that taller plants are associated with higher levels of disease. (Table 2.3). The broad-

sense heritability estimate for PSS was moderately high (0.58), while FDK and DON values were lower at 0.32 and 0.45, respectively.

Genetic Map and QTL Analysis

A total of 8,318 GBS SNPs were successfully called using the TASSEL-GBS pipeline. About 80% of these markers were found to be polymorphic between the parents. Filtering for duplicate markers and segregation distortion resulted in 4,231 GBS SNPs. Results of the SSR and KASP analyses showed that *Xgwm149, GBSsnp1487-KASP, Rht-B1,* and *Rht-D1* were all polymorphic between the parents. These markers were therefore included with the SNP markers in building the genetic map. A total of 2,268 markers were placed into 30 linkage groups representing all 21 wheat chromosomes and covering a total genetic distance of 3,683 cM. The distribution of markers on the wheat chromosomes was comparable to other studies with the majority of markers (~88%) located on the A and B genomes (Figure A.1). Marker *Xgwm149* was positioned at 82.13 cM on the long arm of chromosome 4B. Marker *GBSsnp1487-KASP* was positioned at 13.76 cM on the short arm of chromosome 2D. Markers *Rht-B1* and *Rht-D1* were positioned at 58.66 cM on the short arm of chromosome 4B and 35.55 cM on the short arm of chromosome 4D, respectively. The marker positions obtained from aligning *Ppd-D1* and the GBS snps with the wheat genome draft sequence using POPSEQ placed the *Ppd-D1* locus on the short arm of chromosome 2D in the interval between snp7155 and snp736.

Three significant FHB resistance QTL were discovered in the Art/Everest population. These QTL mapped to chromosomes 2D, 4B, and 4D (Figure 2.2). The QTL on 2D and 4B are considered to be stable, as they were detected in multiple experiments. In the 2014 experiment, the QTL on 4B fell slightly below the LOD threshold for significance ($P<0.05$); however, it was significant at ($P<0.1$) and was included in the results since it was significant in the other two

analyses (Figure A.2). The Art allele for the QTL *Qfhb.ksu-2D* decreased PSS in both individual experiments and the across-experiment analysis. The QTL *Qfhb.ksu-2D* also decreased FDK in the 2013 experiment and DON in the across-experiment analysis. This QTL explained 12.15% of the variation for PSS in the 2013 experiment, 12.30% of the variation in the 2014 experiment, and 13.63% in the across-experiment analysis. The QTL *Qfhb.ksu-2D* also explained 11.76% of the variation for FDK in 2013 and 11.24% of the variation for DON across-experiments. The position of this QTL appeared unstable between experiments and resulted in a wide 1.5 LOD support interval. In the 2013 experiment this QTL mapped to the long arm of chromosome 2D at 89 cM while in the 2014 experiment it mapped to the short arm of 2D at 28 cM. In the across-experiment analysis the peak position fell in between these two locations and was positioned on the long arm of 2D at 74 cM. The results from SIM in the 2013 experiment initially placed the location of the QTL at 35.9 cM; however, further analysis with CIM and MQM placed the QTL position at 89cM. No significant results indicated the presence of multiple QTL on this chromosome and no significant interaction was detected. These two peaks fall into fairly large intervals between markers; therefore, the inconsistency in the position could be a result of low marker density in certain regions of the 2D chromosome. In the 2014 experiment and across-experiment analysis the KASP marker *GBSsnp1487-KASP* was located within the 1.5 LOD support interval. The Art allele for the QTL *Qfhb.ksu-4B* decreased PSS in both individual experiments and the across-experiment analysis. This QTL explained 19.23% of the variation for PSS in the 2013 experiment, 8.31% of the variation in the 2014 experiment, and 17.80% of the variation in the across-experiment analysis. The position of this QTL was very stable with the peak of the QTL mapping to snp5422 on the long arm of chromosome 4B in all three analyses. The 1.5 LOD support interval was fairly large in the 2014 experiment compared to the other two

analyses. This could be a result of the reduced disease pressure in this experiment. The SSR marker *Xgwm149* was located within the support interval in all three analyses and was positioned ~10 cM from the peak marker. In the 2014 experiment, the KASP marker *Rht1-B1* was also located within the support interval and was positioned ~14 cM from the peak marker. The Everest allele for the QTL *Qfhb.ksu-4D* decreased PSS in the 2013 experiment and the across-experiment analysis explaining 10.20% and 9.74% of the phenotypic variation, respectively. In both analyses, the peak of the QTL was located in the interval between snp5131 and *Rht-D1*. Only seven markers mapped to this chromosome spanning a total distance of 36 cM. The low coverage and marker density of this chromosome is likely due to the limited amount of variability within the D genome of wheat.

Three significant QTL were detected for heading date on chromosomes 2B, 2D, and 4D. The QTL *Qhd.ksu-2B* mapped to the interval 102.34 – 125.27 on the short arm of chromosome 2B. The QTL *Qhd.ksu-2D* mapped to the interval 35.85 – 56.08 on chromosome 2D and overlapped with the resistance QTL *Qfhb.ksu-2D.* Comparison of the linkage map marker positions with the positions obtained from POPSEQ revealed that the *Ppd-D1* locus was positioned in the interval between the markers flanking the QTL *Qhd.ksu-2D.* The QTL *Qhd.ksu-4D* mapped to the interval 0.0 – 35.54 and overlapped with the resistance QTL *Qfhb.ksu-4D.* Two significant QTL associated with plant height were also detected. The QTL *Qph.ksu-4B* mapped to the interval 58.66 – 69.47 on chromosome 4B and overlapped with the *Rht-B1* locus and the resistance QTL *Qfhb.ksu-4B.* The QTL *Qph.ksu-4D* mapped to the interval 21.67 – 35.54 on chromosome 4D and overlapped with the *Rht-D1* locus and the resistance QTL *Qfhb.ksu-4D.*

Haplotype Effects

The haplotype analysis for the three FHB resistance QTL was conducted for each individual experiment and across experiments using the QTL detected in each respective analysis. The allelic state of each QTL is represented by the number and letter of the chromosome on which it was detected. Capitalized genome letters represent the resistance allele while lowercase letters represent the susceptible allele. These three QTL were represented by a total of eight haplotype groups in the 2013 experiment and in the across-experiment analysis (Table 2.5). In these two analyses the haplotype groups are designated as: haplotype 1 (4D 4B 2D), haplotype 2 (4D 4B 2d), haplotype 3 (4d 4B 2D), haplotype 4 (4D 4b 4D), haplotype 5 (4d 4b 2D), haplotype 6 (4d 4B 2d), haplotype 7 (4D 4b 2d), haplotype 8 (4d 4b 2d). Haplotype 1 containing all three resistance QTL showed significantly lower disease levels from haplotype 8, which included none of the resistance alleles, for all three disease traits in both analyses (Figure A.3 and Table 2.5). In the 2013 experiment the adjusted mean values for PSS, FDK, and DON ranged from 10.54 to 41.31, 21.21 to 49.03, and 6.68 to 26.74, respectively. In the across-experiment analysis the adjusted mean values for PSS, FDK and DON ranged from 10.87 to 29.10, 19.93 to 32.99, and 6.29 to 18.61, respectively. In the 2014 experiment, the two significant QTL on chromosomes 4B and 2D were represented by a total of four haplotype groups: haplotype 1 (4B 2D), haplotype 2 (4b 2D), haplotype 3 (4B 2d), and haplotype 4 (4b 2d) (Table 2.5). Haplotype 1 containing both resistance alleles showed significantly lower disease levels than haplotype four, containing neither resistance allele, for PSS; however, it did not show significantly lower disease levels for FDK or DON. In this experiment the adjusted mean values for PSS, FDK, and DON ranged from 11.09 to 18.71, 14.00 to 17.55, and 4.93 to 9.46, respectively.

DH lines were also placed into haplotype groups based on their allelic state at the *Rht-B1* and *Rht-D1* loci, and adjusted means were calculated for PSS, FDK, DON, and PH (Table 2.6). For PSS, *Rht-B1b/Rht-D1b* (double dwarf) lines showed significantly lower disease than semi-dwarf lines and standard height lines in 2013 and across experiments. In 2014, double dwarf lines were only significantly better than standard height lines (Figure A.4). For FDK, double dwarf lines showed significantly lower values than *Rht-b1a/Rht-D1b* (semi-dwarf) lines in the 2013 experiment. No other comparisons were significant for FDK. Double dwarf lines also showed significantly lower DON values than standard height lines in the 2013 experiment with no other significant comparisons. The dwarfing alleles of the *Rht-B1* and *Rht-D1* loci segregated with the peak markers for the FHB resistance QTL on their respective chromosomes at rates of 93.85% and 87.69%, respectively.

Discussion

Three significant QTL associated with FHB resistance derived from the Art / Everest hard red winter wheat population were detected in this study on chromosomes 2D, 4B, and 4D using the phenotypic data for three disease traits collected in two replicated experiments in the greenhouse. Based on the chromosomal location and proximity to markers previously reported to be linked to FHB resistance, there are a few potential candidate QTL/genes for the QTL identified in this study.

The QTL *Qfhb.ksu-2D* on chromosome 2D, originating from Art, was significant in both experiments but mapped to different chromosome arms between the two experiments. This large discrepancy in position could be a result of low marker density in certain regions of the 2D chromosome. Another possibility is the presence of two separate QTL on this chromosome;

however, the results of the two-QTL scan did not show significant evidence for multiple QTL. The QTL peak present on the short arm mapped to position 28 cM. This QTL peak was significant in the 2014 experiment, and the SIM analysis during the 2013 experiment showed indication of a peak in this region. The 1.5 LOD support interval for this QTL included the KASP marker *GBSsnp1487-KASP* located on 2DS. This marker was previously reported to be linked to a resistance QTL in the hard red winter wheat cultivar Jagger (Cai & Bai, 2014; Cai, 2016). The results of the KASP assay revealed that Art inherited the Jagger allele at this marker. This evidence suggests that Jagger is a potential candidate as the source of the QTL found in this study; however, the unreliability of the QTL position makes this difficult to determine and further studies will be needed for confirmation. Several other studies have reported QTL on chromosome 2D. A resistance QTL explaining 12.1% of the phenotypic variance for disease spread was reported on 2DS in the CIMMYT wheat cultivar Alondra (Shen *et al.*, 2003). Jia *et al.* (2005) evaluated a doubled haploid wheat population developed from a cross between Alondra and the Chinese landrace Wangshuibai. They reported a QTL on chromosome 2DS explaining 8.0% to 13.0% of the phenotypic variation for PSS, with the source of resistance originating from Wangshuibai. This indicates the presence of another separate QTL for FHB resistance on 2DS. A QTL associated with FHB resistance has also been mapped to the Ppd-D1 locus in the soft winter wheat cultivars Ernie and Massey. The cultivar Ernie showed an association between the Ppd-D1b, photoperiod-sensitive allele, and increased resistance to SEV while in Massey this allele showed an increase in resistance to INC (Liu *et al.*, 2013). In another study, a QTL on 2DS, originating from the wheat cultivar Gamenya, was reported to explain 14% of the phenotypic variance for disease spread and 22% of the phenotypic variance for DON. Using a comparative genomic approach the authors identified a syntenous genomic region on

chromosome 4 of rice. Their results suggest that a gene coding for a multi-drug resistance-associated protein (MRP) could be the underlying mechanism resulting in increased FHB resistance in this genomic region (Handa *et al.*, 2008). Another well-known QTL for FHB resistance was reported on the long arm of chromosome 2D originating from the resistant spring wheat cultivar Wuhan-1 and explained 9% of the phenotypic variance for PSS. Petersen (2015) compared the phenotypic means for incidence (INC), severity (SEV), FDK, and DON using lines from the 2013 and 2014 Uniform Southern Winter Scab Nursery (USWWBN) which were grouped based on their allelic state at the Wuhan-1 2DL QTL. Lines carrying the resistance allele showed significantly lower disease scores for PSS, FDK, and DON, compared to lines carrying the susceptible allele.

The QTL *Qfhb.ksu-4B* on chromosome 4BS was significant in both the 2013 experiment and the across-experiment analysis, but fell just short of the significance threshold in the 2014 experiment. The LOD peak for this QTL mapped to the same position at 72 cM in all three analyses, with the peak marker mapping within the 30 cM interval between markers *Rht-B1* and *Xgwm149*. In the 2013 experiment and across-experiment analysis, the 1.5 LOD support interval overlapped with the *Xgwm149* locus, while in the 2014 experiment the support interval was much larger, and overlapped with both the *Xgwm149* and *Rht-B1* loci. A number of studies have reported QTL for FHB resistance on chromosome 4B of hexaploid wheat (Holzapfel *et al.*, 2008; Jia *et al.*, 2005; Li *et al.*, 2008; Lin *et al.*, 2006; Liu *et al.*, 2007; Somers *et al.*, 2003; Srinivasachary *et al.*, 2009; Xue *et al.*, 2010; Yu *et al.*, 2008). A QTL on this chromosome has also been detected in the tetraploid wheat *Triticum dicoccoides* (2n = 4x = 28) mapping at the position of the *Rht-B1* gene (M. Buerstmayr *et al.*, 2012). The marker *Xgwm149* was previously reported to be linked to the major FHB resistance gene, *Fhb4*, in the Chinese landrace

Wangshuibai. The FHB resistance QTL on chromosome 4B of Wangshuibai mapped to the interval *Xgwm368 – Xgwm149* and explained 9.9% to 10.5% of the phenotypic variation for PSS (Jia *et al.*, 2005). In the study by Xue *et al.* (2010), *Fhb4* was fine mapped to a 1.7 cM interval between *Xhbg226* and *Xgwm149*. Other studies focusing on FHB resistance in Wangshuibai associated this region on chromosome 4B with resistance to INC and FDK (Li *et al.*, 2008; Lin *et al.*, 2006); however, resistance to PSS on this chromosome was not detected (Lin *et al.*, 2004). The proximity of the QTL found in this study with *Xgwm149* suggests that *Fhb4* is a likely candidate for the gene underlying the resistance seen on chromosome 4B. The SSR assay showed a two base pair difference in peak size between Wangshuibai and Art for marker *Xgwm149* suggesting that the allele for this SSR marker is slightly different between the two cultivars. It is unlikely that these two cultivars share a common ancestor; therefore, resistance due to the same gene could be a result of identity-by-state.

The QTL *Qfhb.ksu-4D* on chromosome 4D was significant in both the 2013 experiment and across experiments. In both analyses, the closest marker to the QTL peak was *Rht-D1*. Chromosome 4D had very low marker coverage with only seven markers mapping a total distance of 35.54 cM. The 1.5 LOD support interval mapped from 10.93 cM to 35.54 cM in both analyses. QTL associated with FHB resistance on chromosome 4D have been reported in numerous studies (Draeger *et al.*, 2007; Holzapfel *et al.*, 2008; Ma *et al.*, 2006; Petersen, 2015; Srinivasachary *et al.*, 2009; Yang *et al.*, 2005). A number of European winter wheat varieties have shown FHB resistance co-localizing with the *Rht-D1* locus. The French cultivars Apache and Rubens; as well as the Dutch cultivar Romanus, were all reported to have QTL for FHB resistance mapping to *Rht-D1* (Holzapfel *et al.*, 2008). These QTL effectively reduced PSS by 16.3%, 29.2%, and 31.5%, respectively, relative to lines without the QTL. Kollers *et al.* (2013)

used a whole genome association mapping approach on a panel of European winter wheat varieties and reported 4 markers mapped to the region near *Rht-D1* that showed association with resistance to FHB score (INC x SEV). The Swiss cultivar Arina has also been reported to contain a QTL mapping to *Rht-D1* conferring FHB resistance for AUDPC (area under the disease progress curve) (Draeger *et al.*, 2007).

The QTL associated with heading date mapped to chromosomes 2B, 2D, and 4D. In winter wheat, heading date is associated with photoperiod sensitive (*Ppd*) genes and earliness per se (*Eps*) genes (Lukman, 2003). Two major genes associated with photoperiod sensitivity, *Ppd-B1* and *Ppd-D1*, have been reported on chromosomes 2B and 2D, respectively (Law *et al.*, 1978). The major heading date gene, *Ppd-B1*, was previously shown to be located on the short arm of chromosome 2B (Zhou *et al.*, 2016). The QTL *Qhd.ksu-2D* overlapped with the *Ppd-D1* locus on the short arm of chromosome 2D. These results suggests that *Ppd-B1* and *Ppd-D1* are the genes underlying the QTL on these chromosomes. Regions of chromosome 4D have also shown previous associations with heading date. Hoogendoorn (1985) located *Eps* genes associated with flowering time on several chromosomes, including 4D. The QTL on 2D and 4D co-localized with the FHB resistance QTL; however, the large support interval for *Qfhb.ksu-2D* makes it difficult to determine the true proximity of these QTL on chromosome 2D. Heading date mostly showed a significant negative correlation with the three disease traits; therefore, plants that headed at later dates generally showed less infection. It is possible that later inoculations had slower pathogen growth and subsequent spread of disease symptoms along the wheat spikes due to colder outside temperatures in the late fall which could have led to changes in the relative humidity and temperature within the greenhouse.

Associations between PH and FHB resistance have also been reported in several studies (Draeger et al., 2007; Gervais et al., 2003; Holzapfel et al., 2008; Klahr et al., 2007; Liu et al., 2013; Mesterházy, 1995; Miedaner, 1997; Srinivasachary et al., 2009; Steiner et al., 2004). Nearly all of these experiments have shown a correlation between the dwarfing allele and increased FHB susceptibility. In experiments involving field trials of FHB, it has been recognized that taller plants are commonly associated with lower levels of disease, possibly due to differences in micro-climates or a greater distance between the inoculum source in the soil residue and the wheat spikes of taller plants (Mesterházy, 1995; Miedaner, 1997; Srinivasachary *et al.*, 2009; Steiner *et al.*, 2004). A number of genetic studies have detected FHB QTL that co-localized with PH QTL (Draeger *et al.*, 2007; Gervais *et al.*, 2003; Holzapfel *et al.*, 2008; Klahr *et al.*, 2007; Liu *et al.*, 2013; Steiner *et al.*, 2004). These associations have been proposed to be due to disease escape; however, some QTL for PH do not co-localize with FHB QTL (Srinivasachary *et al.*, 2009) suggesting pleiotropic effects of height genes or linkage between separate genes for the two traits (Draeger *et al.*, 2007; Holzapfel *et al.*, 2008). The *Rht-D1b* dwarfing allele has been previously shown to have a negative effect on FHB resistance. Voss *et al.* (2008) observed a 22% to 53% increase in susceptibility to disease severity in European winter wheat lines carrying *Rht-D1b* compared to *Rht-D1a*. Other studies have reported slightly different results where the dwarfing allele negatively affected Type I resistance while having no effect on Type II resistance (Srinivasachary *et al.*, 2008). In a second study, the authors went on to show that lines carrying the *Rht-B1b* and *Rht-D1b* alleles significantly decreased Type I resistance; however, the *Rht-B1b* allele showed significant increases in Type II resistance while the *Rht-D1b* allele showed no association with Type II resistance. (Srinivasachary *et al.*, 2009). They postulated that these associations were possibly due to linkage of the PH loci with nearby

genes for FHB instead of pleiotropic effects, since the peak of the FHB resistance QTL consistently mapped a short distance from the *Rht-D1* locus. The study by Lui *et al.* (2013) reported that lines carrying the wild-type alleles showed increased resistance to FHB compared to lines carrying the double dwarf alleles. In the current study, the dwarfing alleles for both loci were associated with significant increases in resistance to PSS compared to the wild type alleles. Our results also appear to show support for the linkage hypothesis as the QTL peaks for FHB resistance mapped slightly away from both of the *Rht* loci, and a small number of lines appeared to show segregation of the alleles. This suggests that both cis and trans configurations between the *Rht* and FHB resistance loci on chromosomes 4B and 4D can occur.

The results of the haplotype analyses show significant reductions in disease level for lines carrying resistance alleles at the three FHB QTL, compared to lines carrying all susceptible alleles. While no transgressive segregation was reported in this study, eleven lines showed successful genetic pyramiding of FHB resistance QTL from the parents. Replicated field trials of selected lines and parental controls are currently planted for further screening under heavy disease pressure in the artificially inoculated scab nursery at the Rocky Ford Experiment Station in Manhattan, KS. Lines carrying dwarfing alleles at the *Rht-B1* and *Rht-D1* loci also showed significant reductions for PSS compared to lines carrying the wild-type alleles. This suggests that these markers could be suitable for use in marker assisted selection to help improve overall levels of resistance within breeding programs. The observed linkage between the dwarfing alleles at the *Rht-B1* and *Rht-D1* loci with increased FHB resistance indicates that the cultivars Art and Everest could be effective sources for incorporating these height genes into breeding programs without resulting in any negative effects on disease resistance. The small percentage of lines that appeared to segregate as semi-dwarf plants, while maintaining the resistance pyramid for all three FHB QTL, could also be

effective candidates for FHB resistant germplasm releases. *Rht-B1b/Rht-D1a* lines carrying all three FHB resistance QTL could be used in *Rht1* breeding programs and *Rht-B1a/Rht-D1b* lines carrying all three FHB resistance QTL could be used in *Rht2* breeding programs to incorporate these height alleles while simultaneously increasing scab resistance.

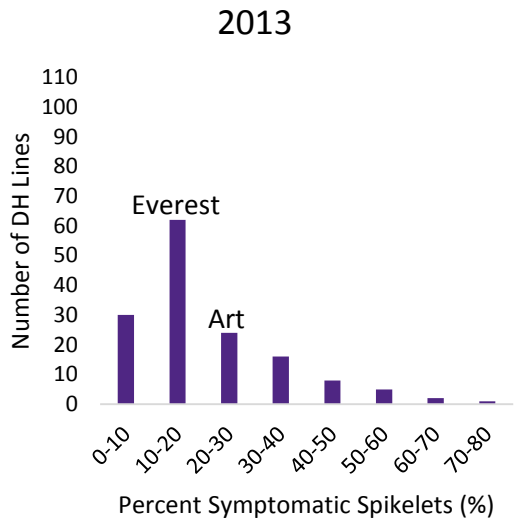
Table 2.1 Analysis of Variance (ANOVA) and broad-sense heritability for percentage of symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), and deoxynivalenol content (DON) for the Art/Everest doubled haploid (DH) population across two greenhouse experiments.

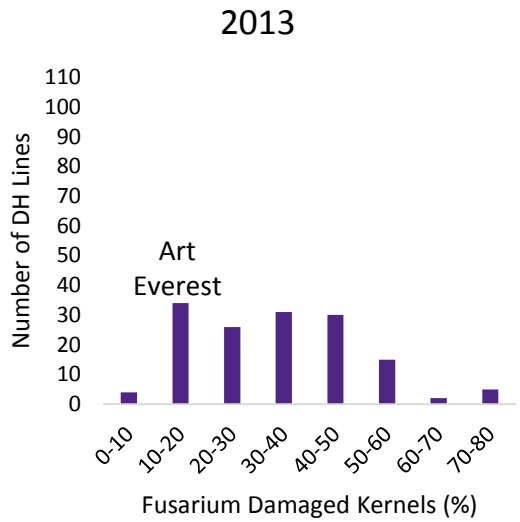
Source	PSS			FDK			DON		
	DF	Var (S.E.)	Pr>F	DF	Var (S.E.)	Pr>F	DF	Var (S.E.)	Pr>F
Experiment	1	0.0057 (0.0083)	NS	1	0.0341 (0.0487)	NS	1	0.0370 (.0550)	NS
Genotype	147	0.0046 (0.0010)	*P<0*.01	147	0.0029 (0.0013)	*P<0*.01	147	0.2292 (0.0700)	*P<0*.01
Replication(Genotype)	444	0.0001 (0.0002)	NS	444	0.0000 (-)	-	444	0.0000 (-)	-
Genotype*Experiment	146	0.0056 (0.0008)	*P<0*.01	146	0.0113 (0.0015)	*P<0*.01	146	0.3610 (0.0680)	*P<0*.01
Error	434	0.0042 (0.0003)	-	415	0.0038 (0.0002)	-	415	0.7743 (0.0377)	-
Corrected Total	1172	-	-	1153	-	-	1153	-	-
H^2	-	0.58	-	-	0.32	-	-	0.45	-

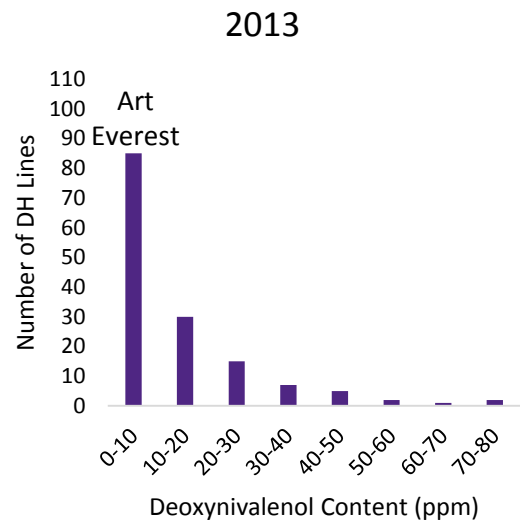
Table 2.2 Adjusted means of Art, Everest, and doubled haploid (DH) lines for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) content, heading date (HD), and plant height (PH). The traits PSS, FDK, DON, and HD were measured in both experiments. PH was measured in the 2014 experiment. The range of mean values for DH lines are listed for each trait.

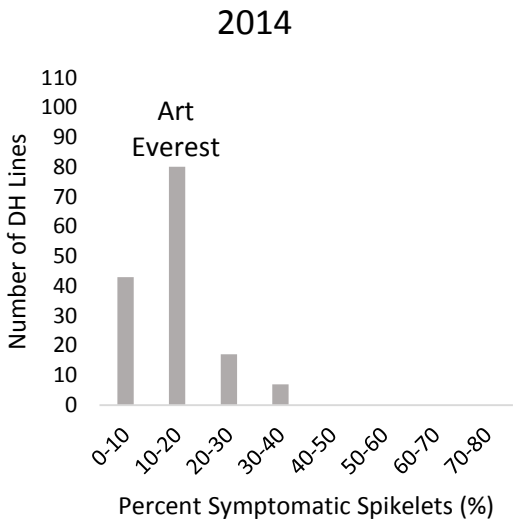
Trait	Art	Everest	DH Mean	DH Range
	2013			
PSS	20.69*†	11.35*	20.69	7.35 - 73.49
FDK	19.38	11.57	33.65	8.60 - 78.14
DON	5.59	2.73	13.81	0.99 - 79.48
HD	37.43	38.97	24.52	4.58 - 82.61
	2014			
PSS	14.46	12.84	14.59	6.57 - 39.89
FDK	19.00	10.86	14.74	1.48 - 46.34
DON	6.67	10.02	9.50	0.59 - 53.61
HD	24.10***	9.00***	17.35	5.84 - 40.36
PH	64.68	58.49	59.94	26.50 - 94.64
	Across Experiments			
PSS	18.19	13.01	17.40	7.99 – 44.95
FDK	20.10	10.31	25.39	7.35 – 51.02
DON	7.21	6.17	10.88	1.07 – 59.56
HD	30.04***	18.72***	19.97	6.09 - 47.97

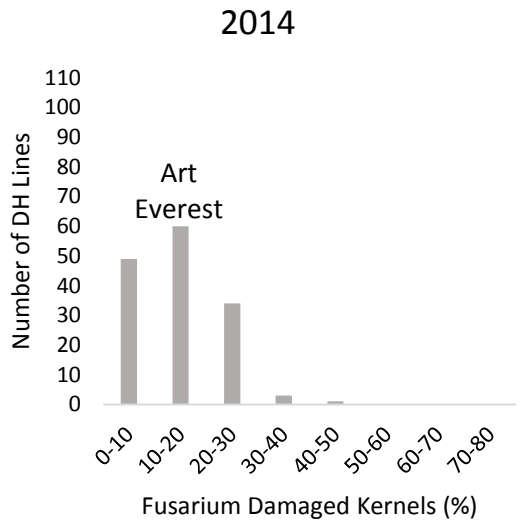
† Significant differences between parental means are designated as: * (*P<0*.05), ** (*P<0*.01), *** (*P<0*.001).

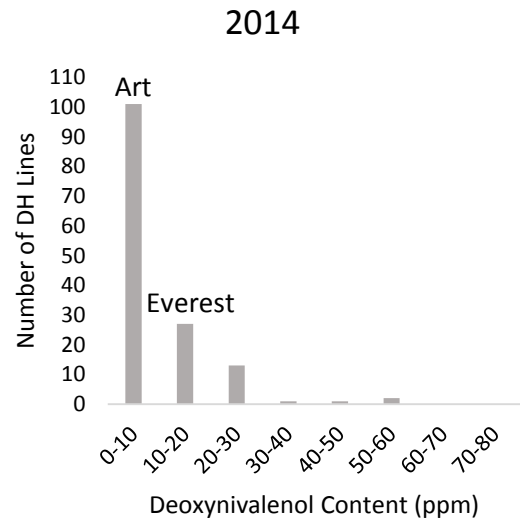

2013
Number of DH Lines
Everest
Art
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Percent Symptomatic Spikelets (%)


2013
Number of DH Lines
Art
Everest
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Fusarium Damaged Kernels (%)


2013
Number of DH Lines
Art
Everest
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Deoxynivalenol Content (ppm)


2014
Number of DH Lines
Art
Everest
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Percent Symptomatic Spikelets (%)


2014
Number of DH Lines
Art
Everest
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Fusarium Damaged Kernels (%)


2014
Number of DH Lines
Art
Everest
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
Deoxynivalenol Content (ppm)

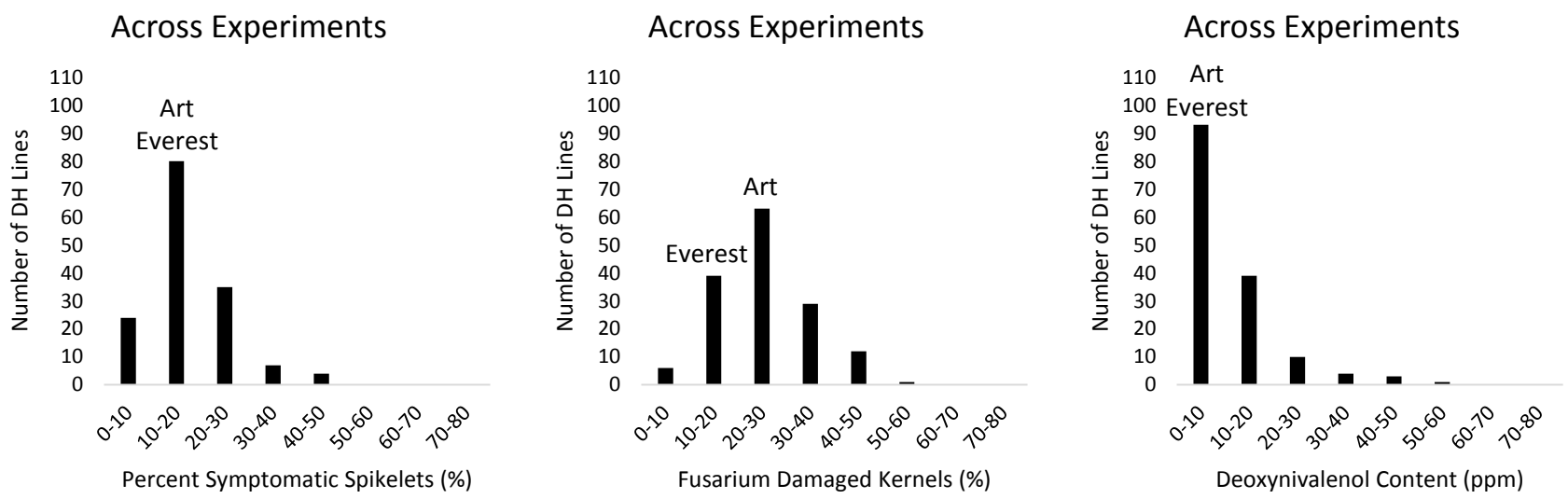


Figure 2.1 Histograms showing the frequency distributions of mean values for percent symptomatic spikelets, *Fusarium* damaged kernels, and deoxynivalenol content of each individual experiment and across experiments in the Art / Everest doubled haploid (DH) population. The names above bars represents the position of the parental means within the distribution.

Table 2.3 Pearson correlation coefficients between individual experiments adjusted means for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) content, heading date (HD), and plant height (PH).

Trait	PSS 2014	FDK 2013	FDK 2014	DON 2013	DON 2014	HD 2013	HD 2014	PH 2014
PSS 2013	0.51 ***†	0.74 ***	0.05 NS	0.82 ***	0.33 ***	-0.52 ***	-0.37 ***	0.39 ***
PSS 2014	-	0.43 ***	0.27 ***	0.47 ***	0.55 ***	-0.37 ***	-0.39 ***	0.28 ***
FDK 2013	-	-	0.19 *	0.74 ***	0.28 ***	-0.45 ***	-0.3 ***	0.16 NS
FDK 2014	-	-	-	0.02 NS	0.51 ***	0.09 NS	0.3 ***	-0.01 NS
DON 2013	-	-	-	-	0.34 ***	-0.45 ***	-0.36 ***	0.26 **
DON 2014	-	-	-	-	-	-0.17 *	-0.3 ***	0.04 NS
HD 2013	-	-	-	-	-	-	0.42 ***	-0.4 ***
HD 2014	-	-	-	-	-	-	-	-0.07 NS

† Significant differences between parental means are designated as: * (*P<0*.05), ** (*P<0*.01), *** (*P<0*.001).

Table 2.4 Quantitative trait loci (QTL) associated with percent symptomatic spikelets (PSS), *Fusarium* damages kernels (FDK), deoxynivalenol (DON) content, heading date (HD), and plant height (PH) in the Art / Everest doubled haploid (DH) population. QTL analysis was performed using adjusted means within individual experiments and across experiments.

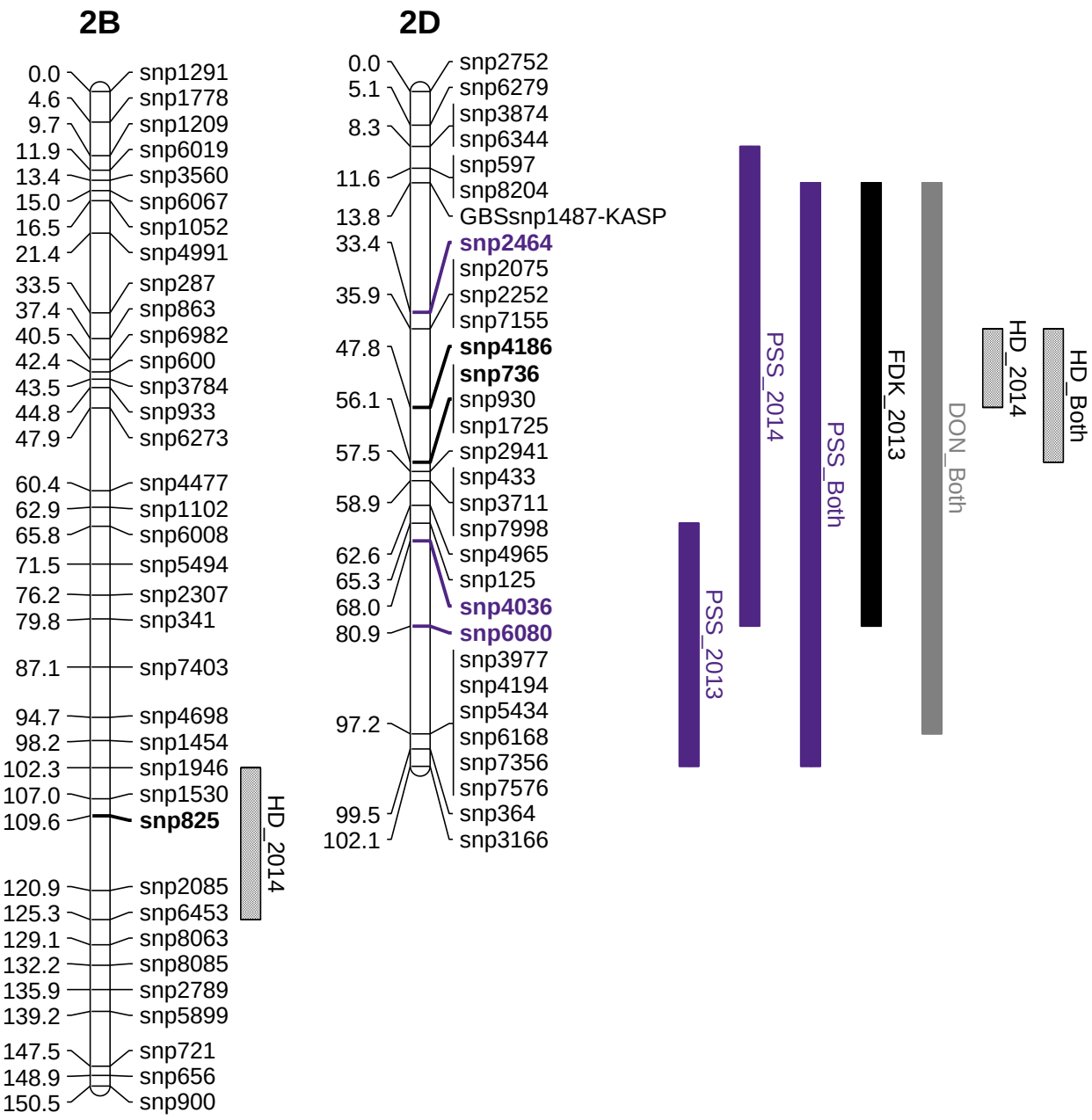
QTL	Overall Interval	Trait_Exp.	Position	1.5 LOD Position Interval	Peak Marker	LOD[†]	R^2	Additive Effect	Standard Error[#]	Source[‡]
Qfhb.ksu-2D	8.29 - 102.06 snp6344 - snp3166	PSS_2013	89.00	65.29 - 102.06	snp6080	5.30***	12.15	5.57	1.09	Art
		PSS_2014	28.00	8.29 - 80.9	snp2464	4.23**	12.30	2.70	0.61	Art
		PSS_Both	74.00	13.75 - 102.06	snp4036	5.99***	13.63	3.44	0.63	Art
		FDK_2013	54.00	13.75 - 80.9	snp736	3.61*	11.76	5.64	1.37	Art
		DON_Both	71.00	13.75 - 97.23	snp4036	3.47*	11.24	3.70	0.84	Art
Qfhb.ksu-4B	6.33 - 99.11 snp8012 - snp3285	PSS_2013	72.02	63.53 - 82.13	snp5422	7.99***	19.23	5.60	0.98	Art
		PSS_2014	72.02	6.33 - 99.11	snp5422	2.93	8.31	1.89	0.56	Art
		PSS_Both	72.02	63.53 - 82.13	snp5422	7.60***	17.80	3.33	0.59	Art
Qfhb.ksu-4D	10.93 - 35.54 snp7459 - Rht-D1	PSS_2013	28.00	10.93 - 35.54	snp5131	4.51**	10.20	-4.87	1.06	Everest
		PSS_Both	26.00	10.93 - 35.54	snp5131	4.40**	9.74	-2.89	0.64	Everest
Qhd.ksu-2D	35.85 - 56.08 snp7155 - snp736	HD_2014	42.00	35.85 - 47.82	snp4186	28.49***	56.15	-5.92	0.41	Everest
		HD_Both	44.00	35.85 - 56.08	snp4186	12.84***	31.87	-4.89	0.60	Everest

Qhd.ksu-4D	0 - 35.54 snp7128 - Rht-D1	HD_2013	32	0 - 35.54	Rht-D1	5.24***	16.47	6.10	1.19	Art
		HD_Both	34	21.67 - 35.54	Rht-D1	6.09***	13.37	3.20	0.59	Art
Qhd.ksu-2B	-	HD_2014	114	102.34 - 125.27	snp825	9.58***	13.14	2.83	0.40	Art
Rht-B1	-	PH_2014	64	58.66 - 69.47	snp4601	21.72***	28.55	7.87	0.68	Art
Rht-D1	-	PH_2014	35	21.67 - 35.54	Rht-D1	30.51***	47.77	-10.36	0.69	Everest

† Significance of QTL at the genome-wide LOD thresholds are designated as: * (*P<0*.05), ** (*P<0*.01), *** (*P<0*.001).

Positive values indicate the effect is from Art and negative values indicate the effect is from Everest

‡ The source of the QTL associated with FHB correspond with the resistance alleles, the source for HD QTL correspond with the allele for early HD, and the source for PH QTL correspond with the dwarfing alleles.


2B
0.0 snp1291
4.6 snp1778
9.7 snp1209
11.9 snp6019
13.4 snp3560
15.0 snp6067
16.5 snp1052
21.4 snp4991
33.5 snp287
37.4 snp863
40.5 snp6982
42.4 snp600
43.5 snp3784
44.8 snp933
47.9 snp6273
60.4 snp4477
62.9 snp1102
65.8 snp6008
71.5 snp5494
76.2 snp2307
79.8 snp341
87.1 snp7403
94.7 snp4698
98.2 snp1454
102.3 snp1946
107.0 snp1530
109.6 snp825
120.9 snp2085
125.3 snp6453
129.1 snp8063
132.2 snp8085
135.9 snp2789
139.2 snp5899
147.5 snp721
148.9 snp656
150.5 snp900
HD_2014
2D
0.0 snp2752
5.1 snp6279
8.3 snp3874
snp6344
11.6 snp597
snp8204
13.8 GBSsnp1487-KASP
33.4 snp2464
35.9 snp2075
snp2252
snp7155
47.8 snp4186
snp736
56.1 snp930
snp1725
57.5 snp2941
58.9 snp433
snp3711
snp7998
62.6 snp4965
65.3 snp125
68.0 snp4036
80.9 snp6080
97.2 snp3977
snp4194
snp5434
snp6168
snp7356
snp7576
99.5 snp364
102.1 snp3166
PSS_2013
PSS_2014
PSS_Both
FDK_2013
DON_Both
HD_2014
HD_Both

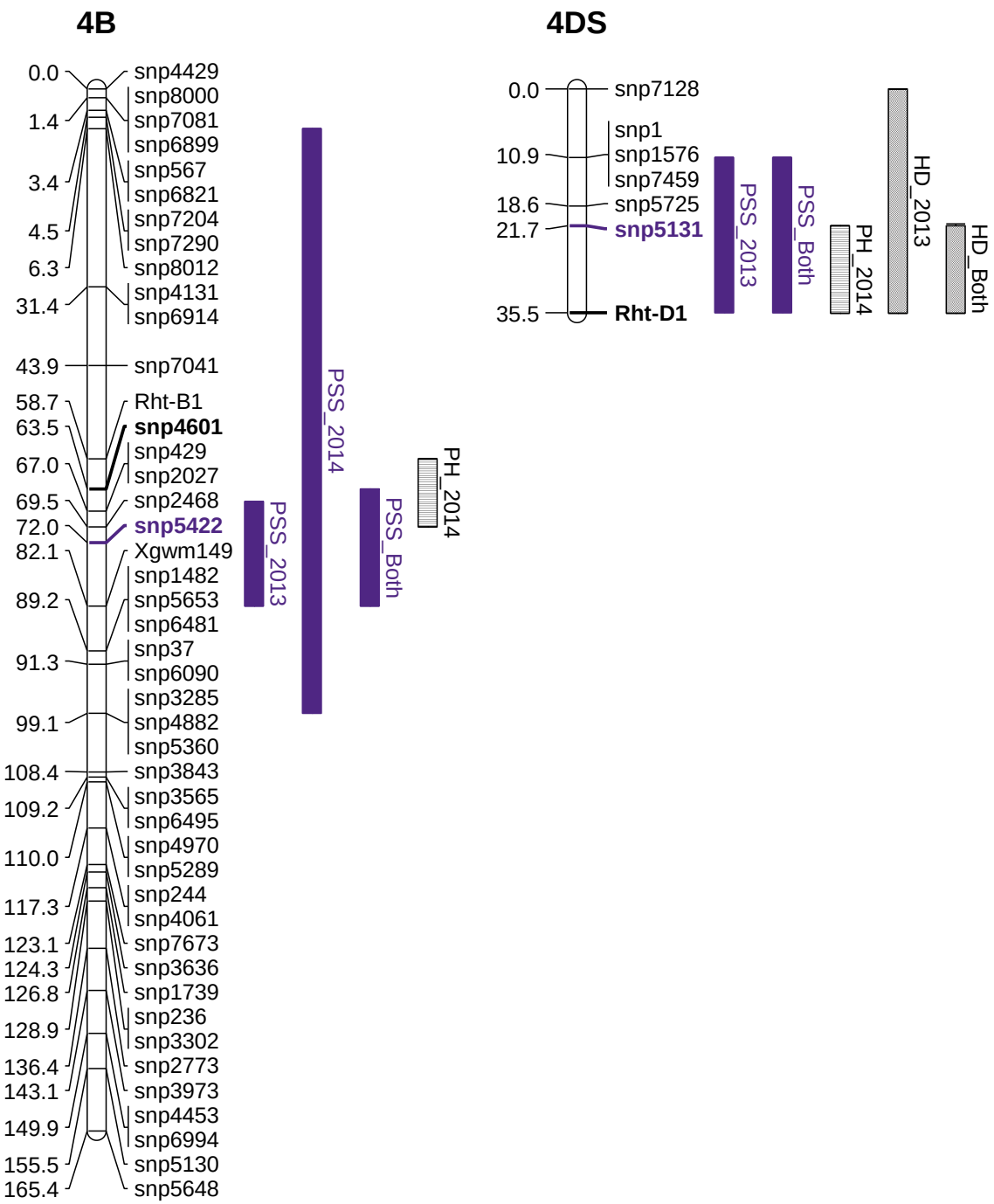


Figure 2.2 Chromosomal positions of quantitative trait loci (QTL) associated with percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) content, heading date (HD), and plant height (PH) for the Art/Everest doubled haploid (DH) population. QTL analysis was conducted within individual experiments and across experiments. Marker positions in centimorgans (cM) are positioned to the left of the chromosome and marker names are positioned to the right. Bars to the right of the chromosome represent the 1.5 LOD support interval for each QTL. Peak marker names are in bold and colored to match the bar with which they are associated.

Table 2.5 Adjusted means for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) content of doubled haploid (DH) haplotype groups containing different allelic combinations of QTL associated with Fusarium head blight found within each experiment and across experiments. The allelic state of each FHB QTL is represented by the number and letter of the chromosome on which it was detected. Capitalized genome letters represent the resistance allele while lowercase letters represent the susceptible allele. Within each individual year, or within the combined dataset, adjusted means of haplotypes in each column followed by different letters are significantly different at ($P<0.05$).

Haplotype	PSS	FDK	DON
		2013	
2D 4B 4D	10.54^{a}	21.21^{a}	6.68^{ab}
2d 4B 4D	10.79^{a}	22.86^{a}	3.79^{a}
2D 4B 4d	13.79^{ab}	24.85^{ab}	4.69^{ab}
2D 4b 4D	17.61^{ab}	26.84^{ab}	7.94^{abc}
2D 4b 4d	20.81^{ab}	32.06^{ab}	10.26^{abc}
2d 4B 4d	24.56^{bc}	33.71^{ab}	12.81^{bc}
2d 4b 4D	26.57^{bc}	42.19^{ab}	14.77^{bc}
2d 4b 4d	41.31^{c}	49.03^{b}	26.74^{c}
		2014	
2D 4B	11.09^{a}	14.00^{a}	4.93^{a}
2D 4b	13.86^{ab}	17.55^{a}	6.84^{a}
2d 4B	14.43^{ab}	12.50^{a}	7.41^{a}
2d 4b	18.71^{b}	12.64^{a}	9.46^{a}
		Across Experiments	
2D 4B 4D	10.87^{a}	19.93^{a}	6.29^{a}
2d 4B 4D	12.18^{ab}	24.28^{ab}	8.03^{ab}
2D 4B 4d	14.12^{abc}	22.05^{ab}	5.62^{a}
2D 4b 4D	16.47^{abc}	24.08^{ab}	7.24^{ab}
2D 4b 4d	17.26^{abc}	23.79^{ab}	8.41^{ab}
2d 4b 4D	21.13^{bcd}	30.59^{ab}	11.38^{ab}
2d 4B 4d	21.99^{cd}	27.66^{ab}	11.81^{ab}
2d 4b 4d	29.10^{d}	32.99^{b}	18.61^{b}

Table 2.6 Adjusted means for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) content, and plant height (PH) of doubled haploid (DH) haplotype groups containing different allelic combinations of QTL associated with the *Rht-B1* and *Rht-D1* loci both within each experiment and across experiments. *Rht-B1a* and *Rht-D1a* represent the wildtype alleles while *Rht-B1b* and *Rht-D1b* represent the dwarfing alleles. Adjusted means of haplotypes within each column followed by different letters are significantly different at ($P<0.05$).

Haplotype	PSS 2013	PSS 2014	PSS Both	FDK 2013	FDK 2014	FDK Both	DON 2013	DON 2014	DON Both	PH
Rht-B1b/Rht-D1b	11.20^{a}	10.58^{a}	11.49^{a}	24.68^{a}	12.65^{a}	23.30^{a}	6.14^{a}	5.88^{a}	6.97^{a}	40.32^{a}
Rht-B1b/Rht-D1a	19.40^{b}	14.27^{a}	16.83^{b}	30.57^{ab}	13.08^{a}	24.16^{a}	8.90^{ab}	6.02^{a}	7.80^{a}	65.04^{bc}
Rht-B1a/Rht-D1b	21.36^{b}	13.86^{ab}	17.50^{b}	39.40^{b}	15.36^{a}	29.62^{a}	8.95^{ab}	6.21^{a}	7.98^{a}	60.04^{bc}
Rht-B1a/Rht-D1a	25.03^{b}	15.94^{b}	20.19^{b}	31.73^{ab}	11.50^{a}	23.49^{a}	12.62^{b}	8.10^{a}	10.15^{a}	77.34^{d}

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Appendix A - Supplementary Material for Chapter 2

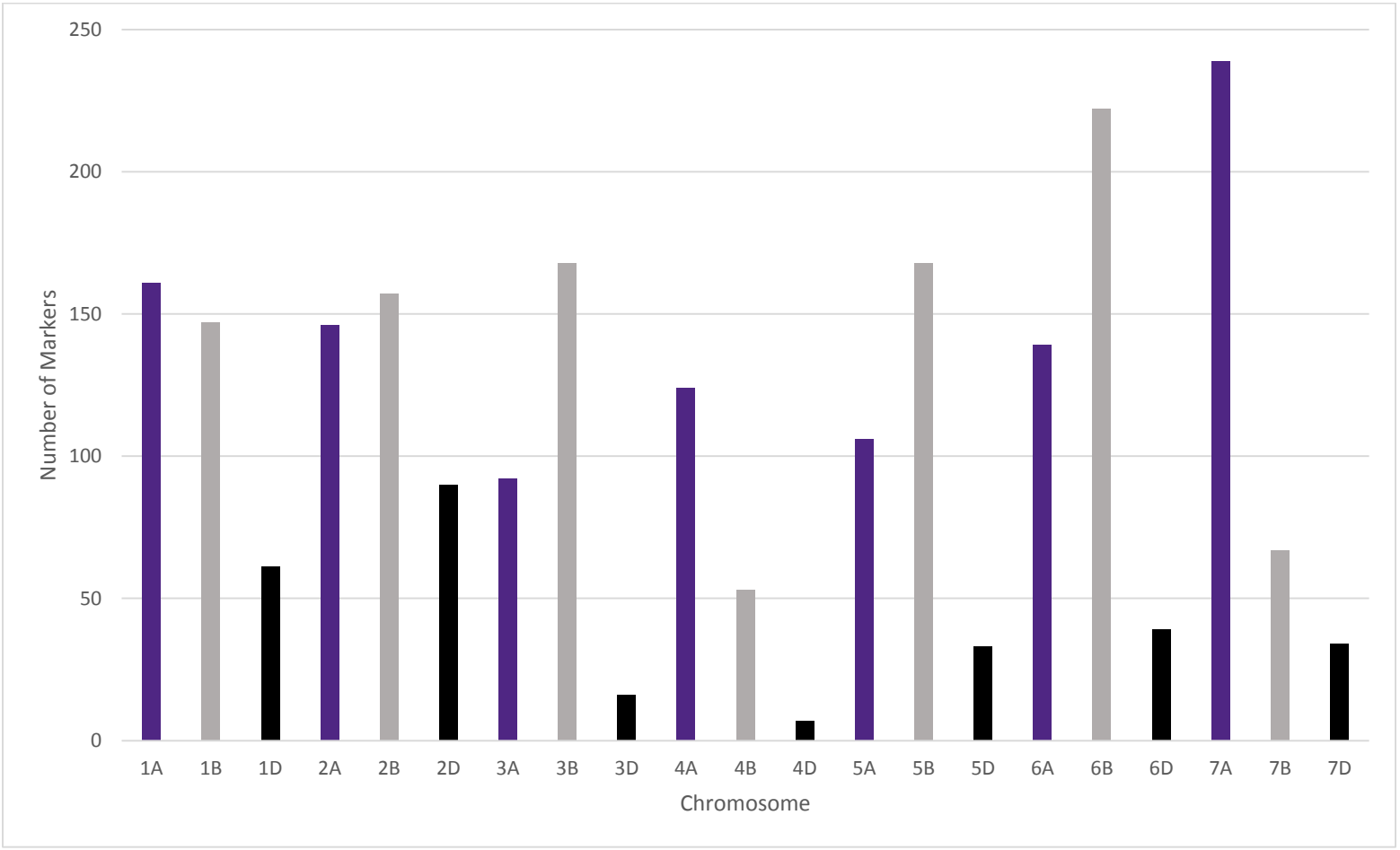
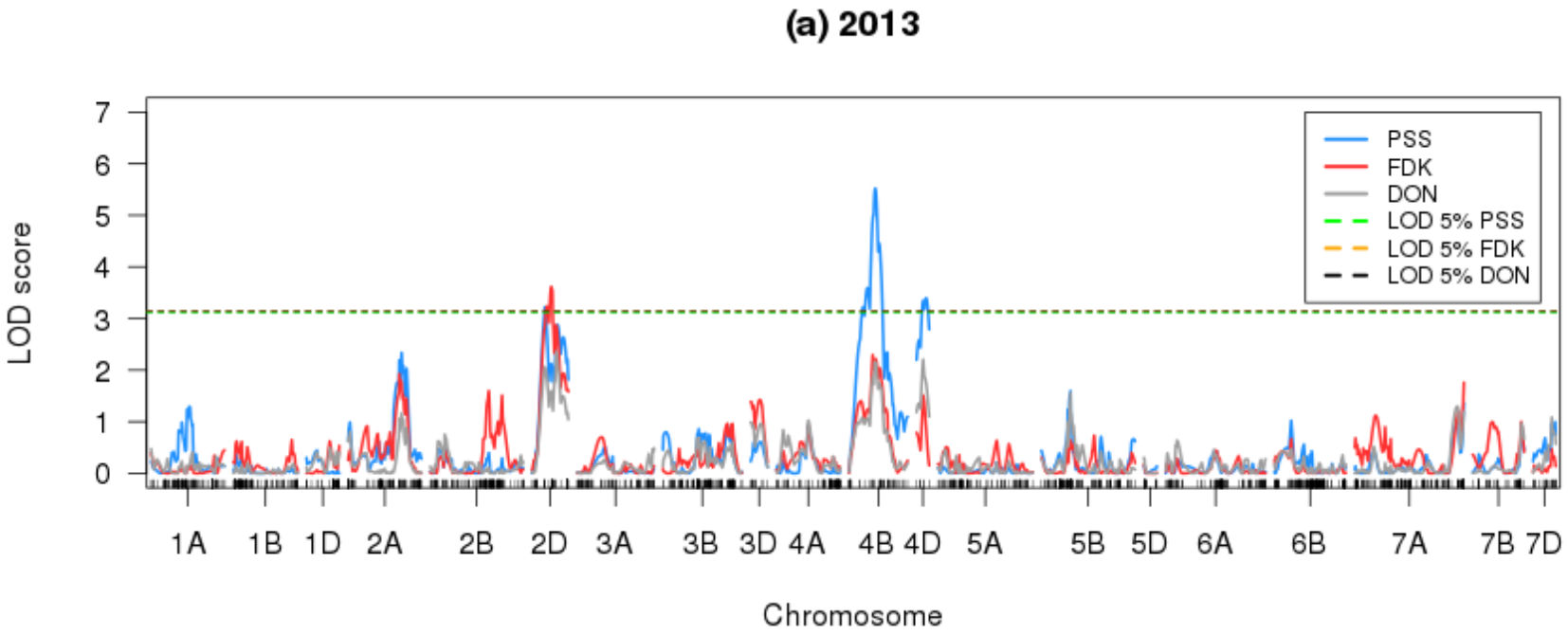
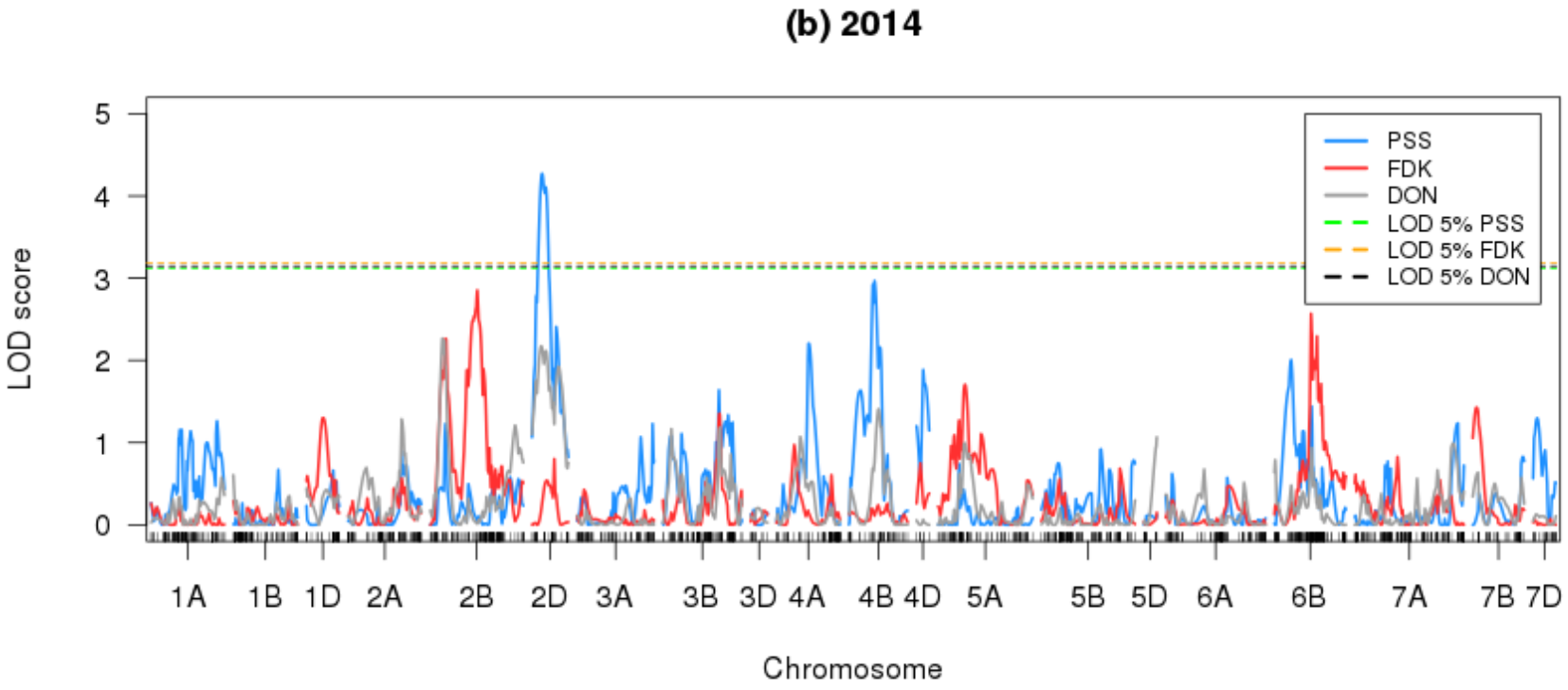


Figure A.1 Distribution of single nucleotide polymorphism (snp) markers across all twenty-one wheat chromosomes. The color of the bars represent the three genomes of hexaploid wheat: purple represents the A genome, gray represents the B genome, and black represents the D genome.


(a) 2013
LOD score
0
1
2
3
4
5
6
7
PSS
FDK
DON
LOD 5% PSS
LOD 5% FDK
LOD 5% DON
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
7A
7B
7D
Chromosome


(b) 2014
LOD score
0
1
2
3
4
5
PSS
FDK
DON
LOD 5% PSS
LOD 5% FDK
LOD 5% DON
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
7A
7B
7D
Chromosome

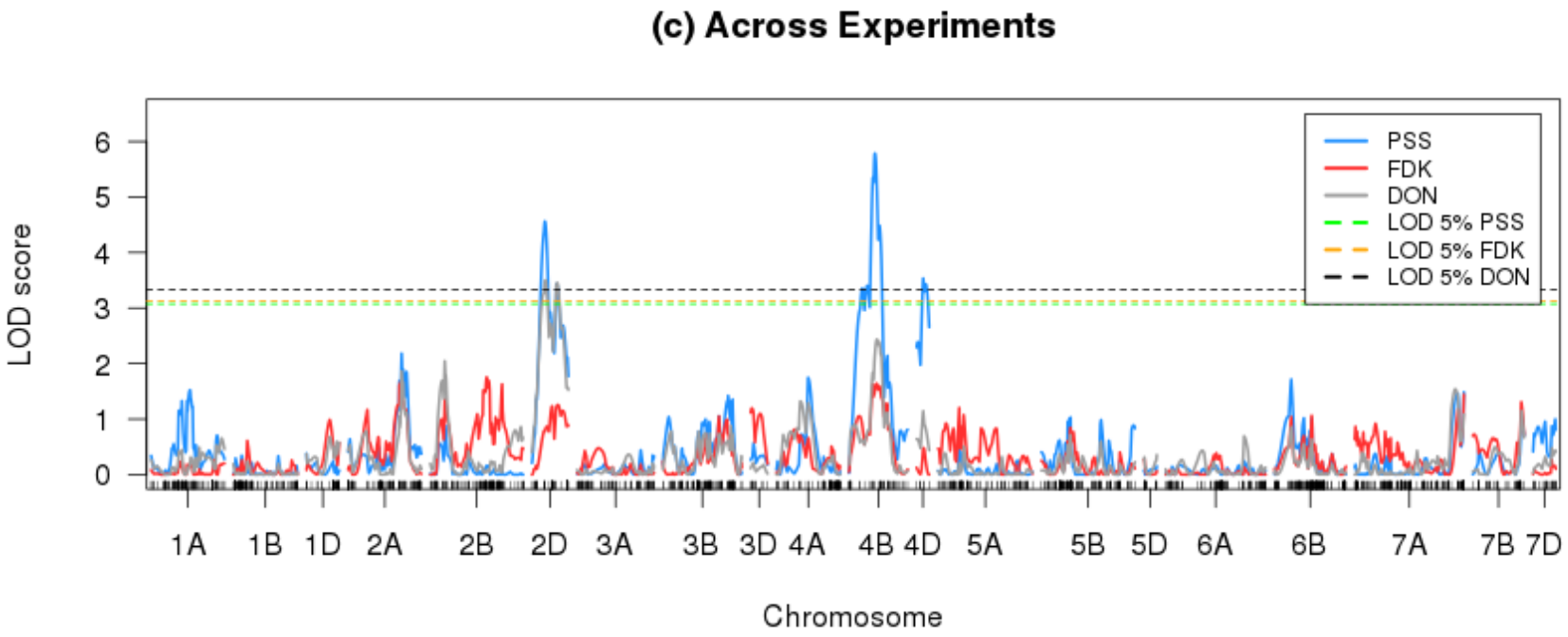
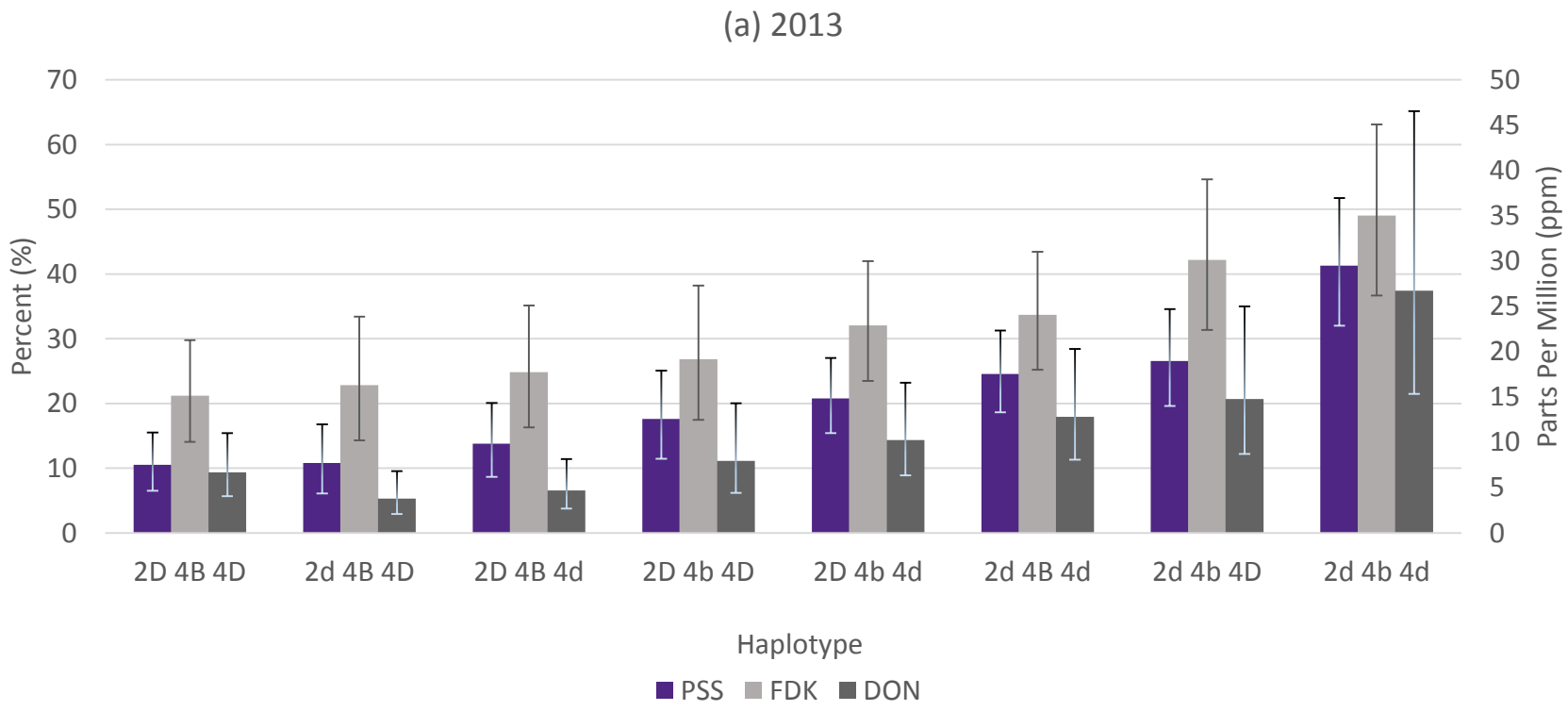
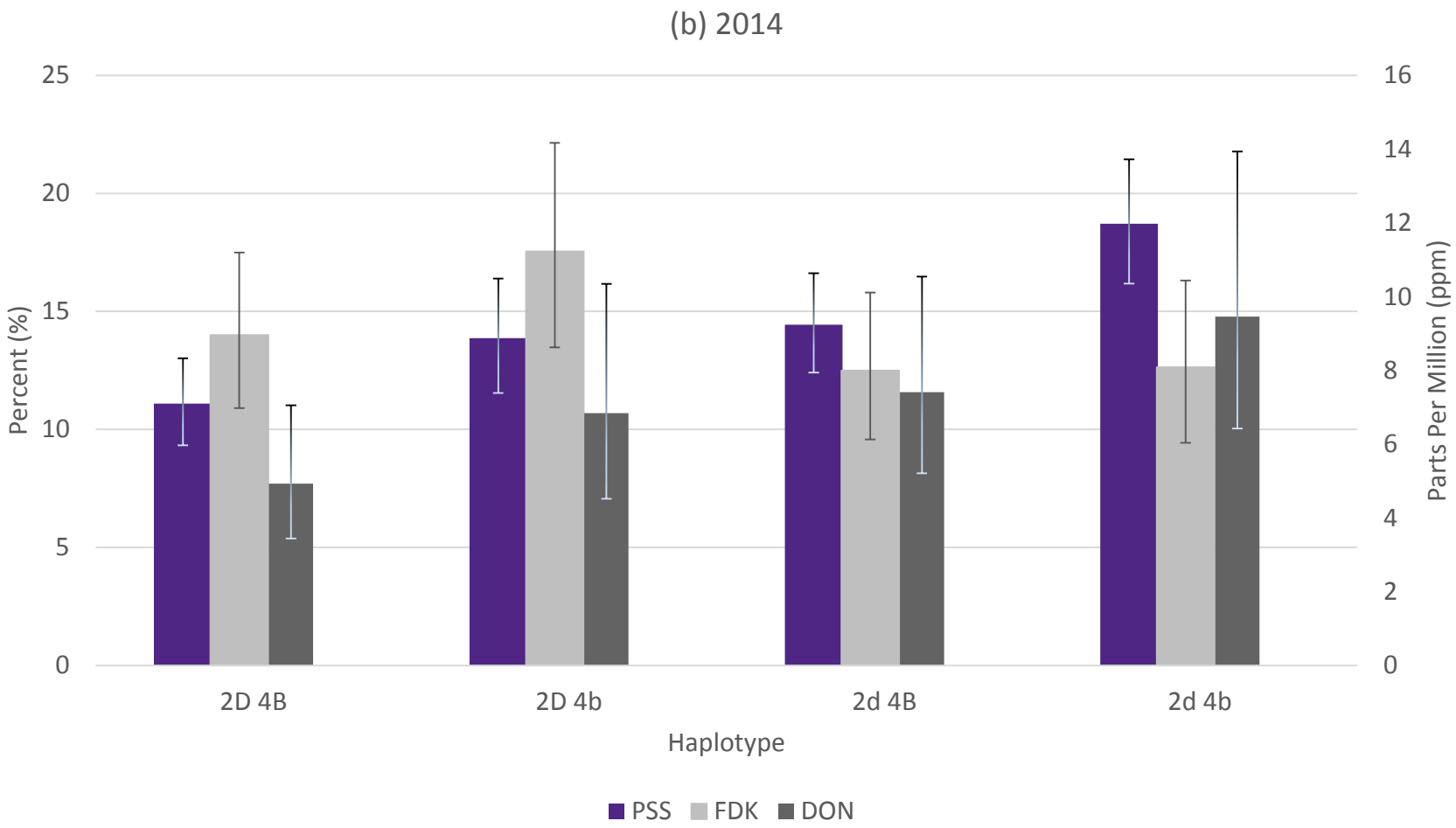


Figure A.2 Logarithm of odds (LOD) curves from composite interval mapping (CIM) for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels, and deoxynivalenol (DON) content for the Art / Everest doubled haploid (DH) population in (a) the 2013 experiment, (b) the 2014 experiment, and (c) across experiments. Solid lines represent the LOD curves for each trait and dashed lines represent the genome-wide LOD threshold for declaring significant QTL at ($P<0.05$) for each trait.


(a) 2013
70
60
50
40
30
20
10
0
Percent (%)
50
45
40
35
30
25
20
15
10
5
0
Parts Per Million (ppm)
2D 4B 4D
2d 4B 4D
2D 4B 4d
2D 4b 4D
2D 4b 4d
2d 4B 4d
2d 4b 4D
2d 4b 4d
Haplotype
PSS
FDK
DON


(b) 2014
25
20
15
10
5
0
Percent (%)
16
14
12
10
8
6
4
2
0
Parts Per Million (ppm)
2D 4B
2D 4b
2d 4B
2d 4b
Haplotype
PSS
FDK
DON

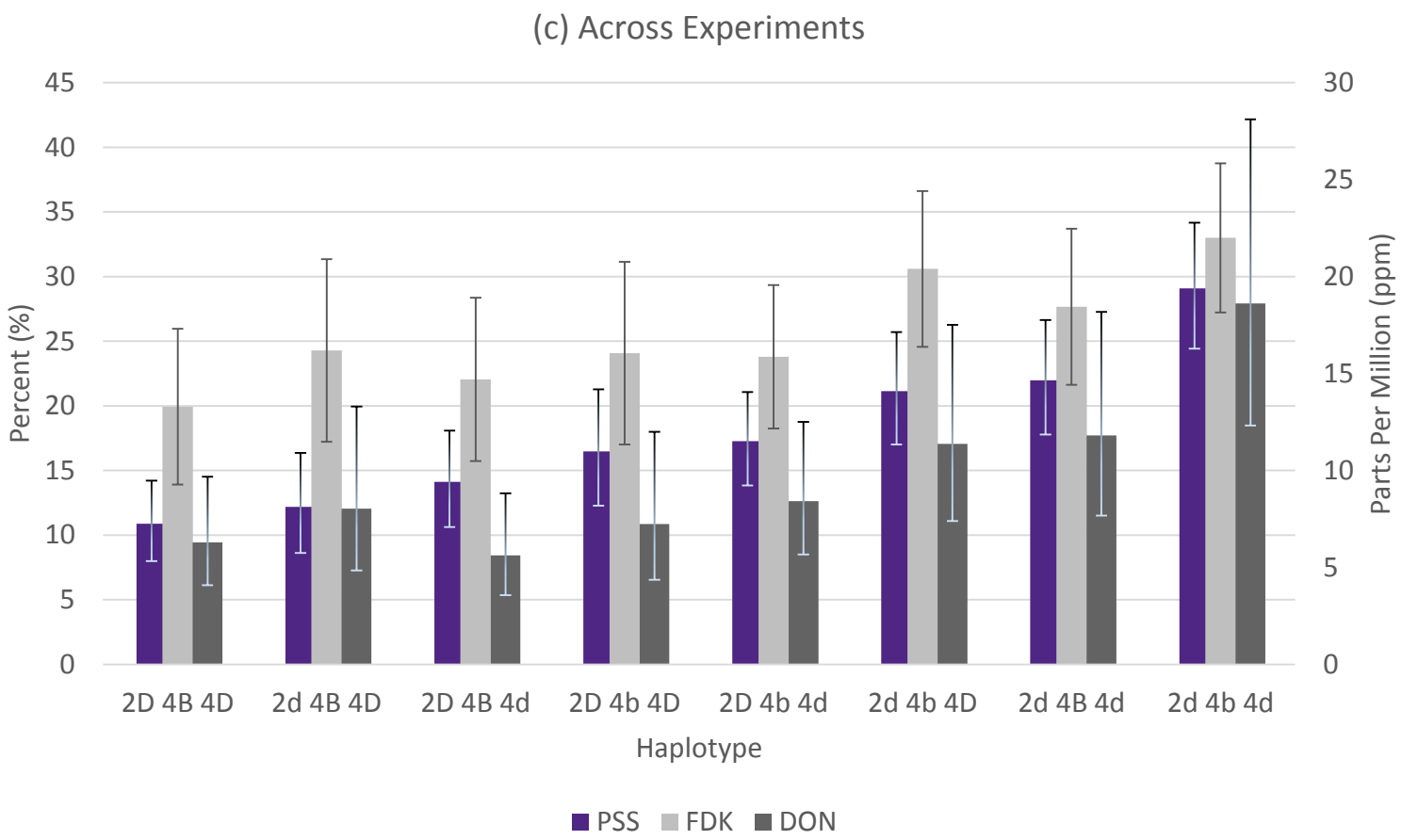
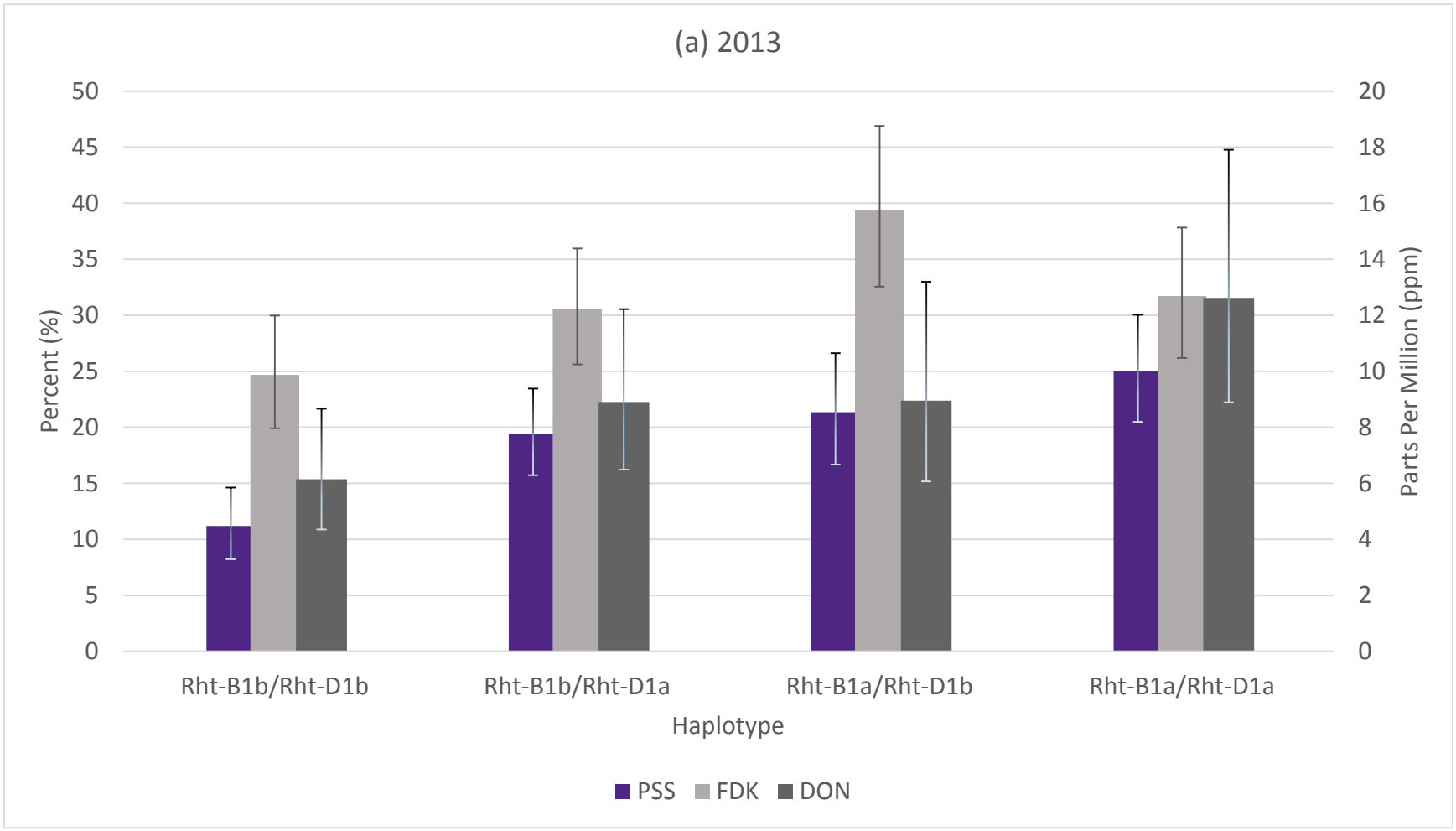
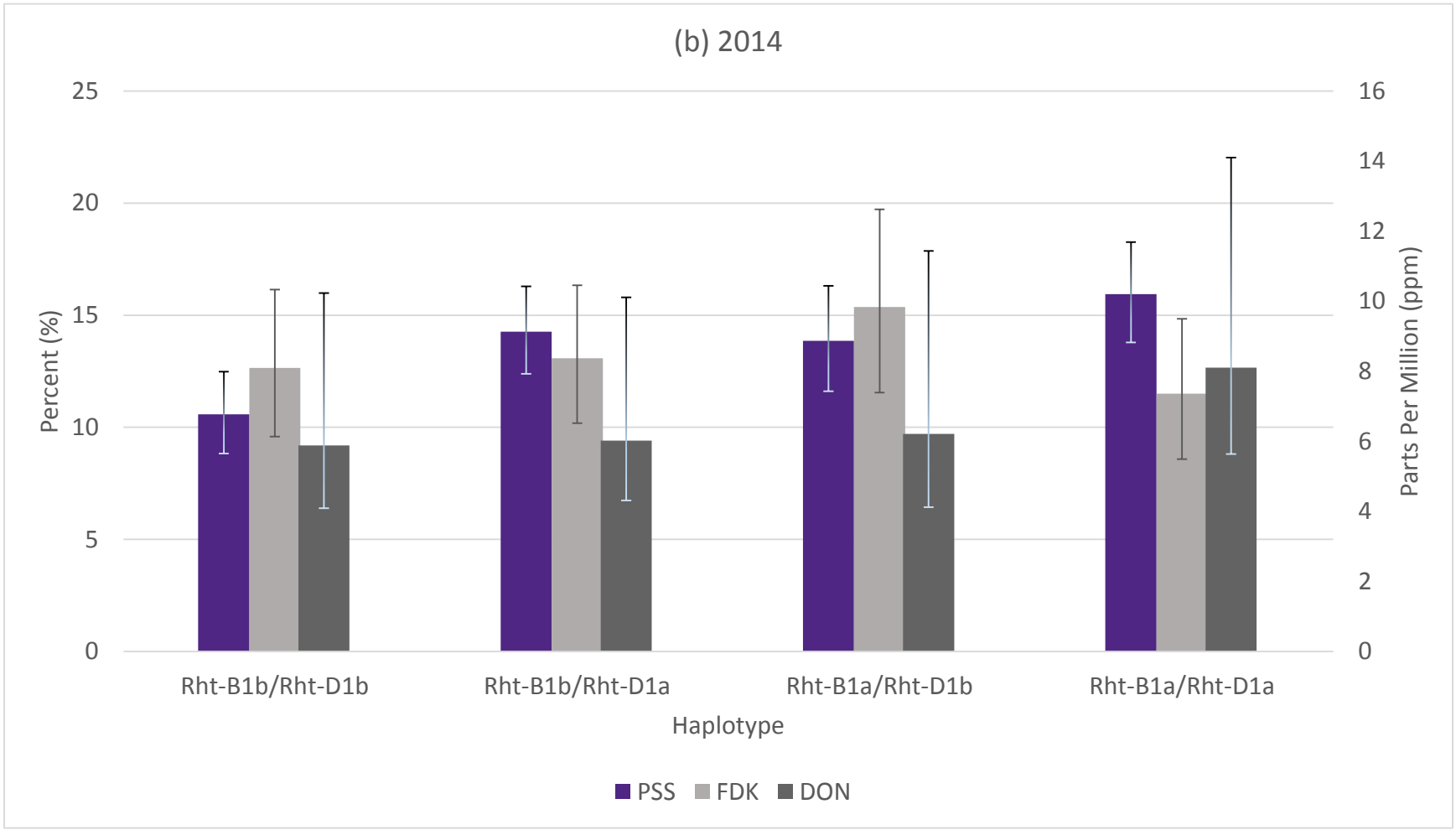


Figure A.3 Barplot representation of adjusted means for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) content of doubled haploid (DH) haplotype groups containing different allelic combinations of QTL associated with Fusarium head blight found in (a) the 2013 experiment, (b) the 2014 experiment, and (c) across experiments. The allelic state of each FHB QTL is represented by the number and letter of the chromosome on which it was detected. Capitalized genome letters represent the resistance allele while lowercase letters represent the susceptible allele. The horizontal axis to the left of the figure is on a percentage scale for PSS and FDK. The horizontal axis to the right of the figure is in parts per million (ppm) for DON. Error bars represent 95% confidence intervals.


(a) 2013
Percent (%)
Parts Per Million (ppm)
50
45
40
35
30
25
20
15
10
5
0
20
18
16
14
12
10
8
6
4
2
0
Rht-B1b/Rht-D1b
Rht-B1b/Rht-D1a
Rht-B1a/Rht-D1b
Rht-B1a/Rht-D1a
Haplotype
PSS
FDK
DON


(b) 2014
25
20
15
10
5
0
Percent (%)
16
14
12
10
8
6
4
2
0
Parts Per Million (ppm)
Rht-B1b/Rht-D1b
Rht-B1b/Rht-D1a
Rht-B1a/Rht-D1b
Rht-B1a/Rht-D1a
Haplotype
PSS
FDK
DON

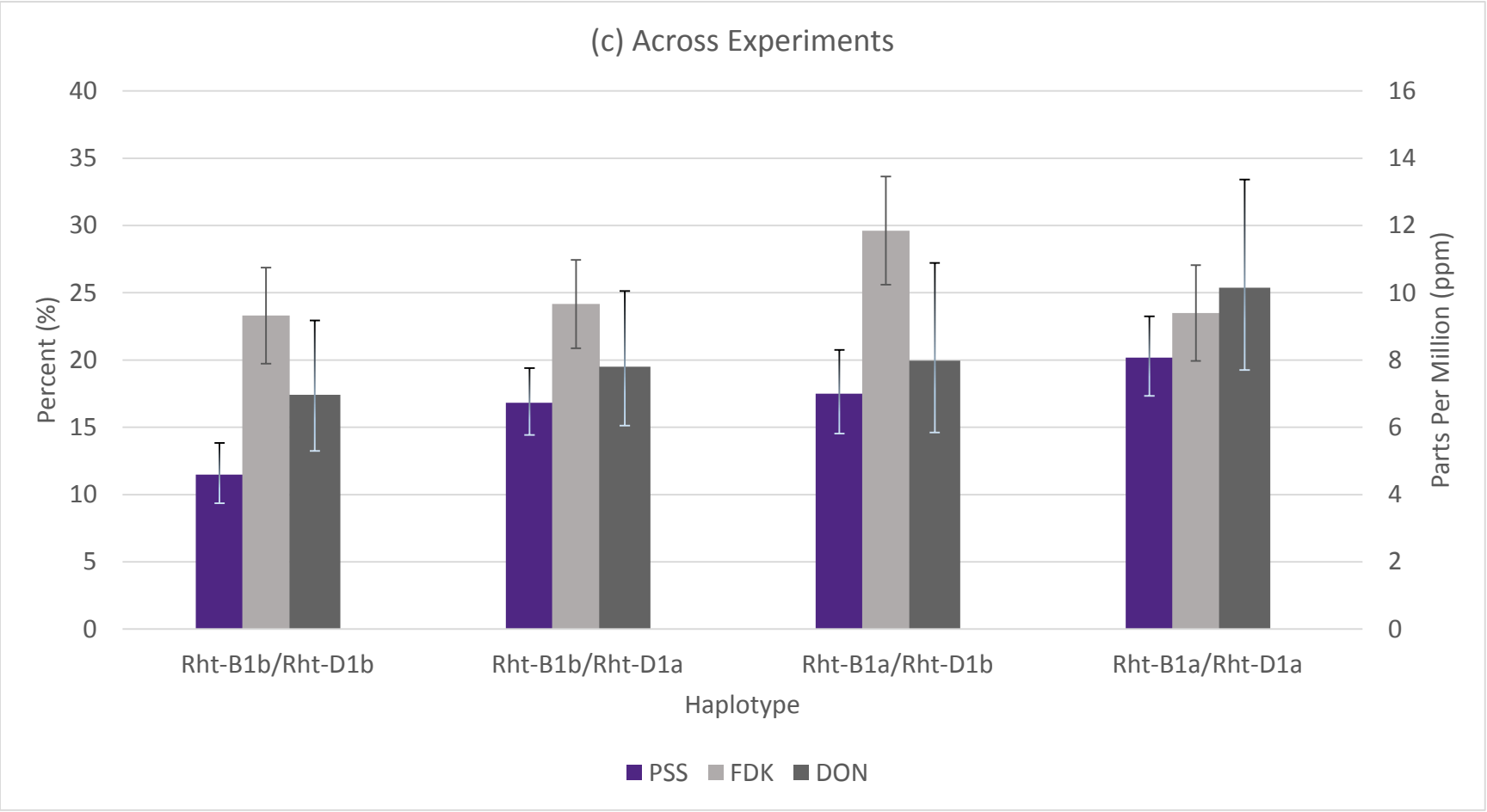


Figure A.4 Barplot representation of adjusted means for percent symptomatic spikelets (PSS), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) content of doubled haploid (DH) haplotype groups containing different allelic combinations of the *Rht-B1* and *Rht-D1* loci in (a) the 2013 experiment, (b) the 2014 experiment, and (c) across experiments. *Rht-B1a* and *Rht-D1a* represent the wildtype alleles while *Rht-B1b* and *Rht-D1b* represent the dwarfing alleles. The horizontal axis to the left of the figure is on a percentage scale for PSS and FDK. The horizontal axis to the right of the figure is in parts per million (ppm) for DON. Error bars represent 95% confidence intervals.