Quantitative Trait Loci for Resistance to Fusarium Head Blight in the Hulless Winter Barley Cultivar Eve

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating fungal disease that affects barley production worldwide through damaged kernels, low yields, and production of mycotoxins. The most effective means of control is through the use of FHB resistant cultivars that are developed through gene pyramiding and incorporation of various sources of resistance. Resistance identified in winter barley cultivar Eve has been identified in Virginia Tech’s Small Grains Breeding Program. The objectives of this study are to validate resistance and identify additional resistance QTL, and identify tightly linked and diagnostic markers for use in MAS programs. A population of F$_{5:7}$ RILs derived from the cross Eve/ ‘Doyce’ were evaluated for FHB resistance. FHB incidence (Inc), FHB severity (Sev), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) were assessed over 2014-15 and 2015-16 at Blacksburg, VA, Mount Holly, VA, Kinston, NC, and Lexington, KY. A QTL on chromosome 6H associated with FHB Sev, FDK, and DON was identified. The QTL accounted for as high as 14.5% of the phenotypic variation for DON accumulation, 8.3% for FHB Sev, and 11.87% to 17.63% for FDK. The QTL marker region spans 56.5 to 66.6 cM with flanking markers SCRL_RS_147342 and Bmag0613. QTL for morphological traits, heading date and height, were found in the same region as the FHB resistance traits. Diagnostic SNP makers associated with the FHB resistance QTL identified can be used for FHB resistance identification in MAS breeding programs to incorporate and pyramid resistance QTL and genes into other plant material.
Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating fungal disease that affects barley production worldwide through damaged kernels, low yields, and production of mycotoxins. The most effective means of control is through the use of FHB resistant varieties that are developed through gene pyramiding and incorporation of various sources of resistance. Resistance identified in winter barley cultivar Eve has been identified in Virginia Tech’s Small Grains Breeding Program. The objectives of this study are to validate resistance and identify additional resistance gene regions, and identify tightly linked and diagnostic DNA markers for use in breeding programs. A population individuals derived from the cross Eve/ ‘Doyce’ were evaluated for FHB resistance. FHB incidence (Inc), FHB severity (Sev), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) were assessed over 2014-15 and 2015-16 at Blacksburg, VA, Mount Holly, VA, Kinston, NC, and Lexington, KY. A gene region on chromosome 6H associated with FHB Sev, FDK, and DON was identified. The region accounted for high variation in DON accumulation, FHB Sev, and FDK. The molecular marker region spans 56.5 to 66.6 cM with flanking markers SCRI_RS_147342 and Bmag0613. The physical traits, heading date and height, were found in the same gene region as the FHB resistance traits. Diagnostic DNA makers associated with the FHB resistance genes identified can be used for FHB resistance identification in breeding programs to incorporate and pyramid resistance genes into other plant material.
DEDICATION

This master’s thesis is dedicated to my parents, Brian and Carla Ullrich, as well to my younger sister, Mallory. Without their love and support over the years, I would not have been able pursue and achieve my goals. They have always been my biggest supporters in everything I strived for and I would not be the person I am today without them.
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ATTRIBUTIONS

Many collaborators and colleagues assisted in the collection and analysis of the data I am presenting in this thesis. A description of their contributions is described below.

**Chapter 2: Quantitative Trait Loci for Resistance to Fusarium Head Blight in the Hulless Winter Barley Cultivar Eve**

Carl Griffey, PhD is currently the W.G. Wysor of Agriculture at Virginia Tech. Dr. Griffey was one of the co-principal investigators for the grants that supported this research.

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Chapter 1

Literature Review
INTRODUCTION

Barley (*Hordeum vulgare*) is an extremely important cereal grain worldwide. It is ranked fifth for crops grown across the globe (Goyal and Ahmed, 2012). In the United States barley is ranked the fourth most important grain crop that is grown (Magness et al., 1971). Barley is a widely adapted grain that is cold, salt, and drought tolerant (Goyal and Ahmed, 2012). Barley is an annual cereal crop that favors cool, dry growing conditions (Clark, 2007). Some breeding programs have developed more adaptable cultivars that thrive in environmental conditions that may not be conducive to typical barley growth. In geographical regions, such as the mid-Atlantic region, where there is high humidity and variable rain fall, barley is a valuable crop to farmers due to its ability to grow in a variety of environmental conditions (Thomason et al., 2009). Two classes of barley are primarily grown, spring and winter types, depending on the geographical location. Winter barley has been traditionally grown in the mid-Atlantic region for livestock feed.

Barley production in the United States can generate about $3 billion annually (Goyal and Ahmed, 2012). It is a very important grain that is used for malt, animal feed, and human consumption (Baik and Ullrich, 2008). According to the U.S Grains Council, roughly 66% of the barley produced in the United States is used for human consumption, 22% is used for animal feed, and roughly 12% is exported (Cordero, 2013). Barley can be a source of vital nutrients as well as provide other health benefits when consumed by humans. Soluble fiber, which can be found in barley, is able to lower risk of cardiovascular disease, diabetes mellitus, reduce levels of plasma cholesterol, and provide many other health benefits (Hallfrisch and Behall, 2005). With the rise of craft brewing, the demand for malt barley has risen exponentially due to the increase in the amount of malt required to create craft beers. Three to seven times more malt is needed to
create craft beer versus non-craft beer (Sheet, 2015). Due to this dramatic increase in the brewing industry, maltsters and brewers are requesting local production of superior varieties that are able to meet their demands and requirements for malting and brewing. Barley exports have been predicted to rise through 2024 to developing countries such as China and India, for livestock feed as well as for using in brewing. Development of superior locally adapted cultivars is essential in order for this commodity to meet the rising economical demands for this important grain crop.

Aside from the two different growth habits of spring and winter barley, there are also other characteristics that are taken into account when deciding which genotypes to plant. These genotype characteristics include: hulled versus hulless barley and two versus six rowed barley. Hulled and hulless barley types vary significantly in kernel weight, starch, β-glucan, oil and ash content (Griffey et al., 2010). Hulless barley has lower ash and oil concentrations than hulled barley, but has higher starch concentrations (Griffey et al., 2010). Both hulled and hulless types can be used as livestock feed. Hulless barley tends to have higher digestible energy and nutritional value than hulled barley, while hulled barley tends to have a lower energy content due to its high fiber content (Bhatty, 1993, Wang et al., 1997). Hulled barley that is grown in the mid-Atlantic region is typically used as livestock feed (Brann et al., 2009).

Fusarium Head Blight (FHB), caused by *Fusarium graminearum* Schwabe, is one of the most detrimental diseases of the barley production industry in the United States, especially in regions that are prone to have long periods of wet weather during the flowering stage (Zhu et al., 1999). Economic losses caused by FHB have exceeded more than $3 billion in wheat and barley since 1996 in the U.S. (McMullen et al., 1997). FHB causes yield losses, low test weights, discolored shriveled kernels, low seed germination, reduced seed quality, and contamination of grain with mycotoxins (McMullen et al., 1997). Low test weights and yield are primarily due to
floret sterility and deformed seed that is produced by infected barley plants (Zhu et al., 1999). Deoxynivalenol (DON) or vomitoxin, is the main mycotoxin that is most problematic in barley and wheat production (McMullen et al., 1997). DON accumulation in kernels results in unacceptable grain quality due to its ability to inhibit protein synthesis (Berger et al., 2014, Pestka, 2007). Even at low levels, the mycotoxin renders the grain unacceptable for animal feed, human consumption, and malting (Zhu et al., 1999).

Barley breeding programs worldwide are working towards developing high yielding, disease resistant and economically viable cultivars. Multiple methods are used in breeding to select desired individuals such as marker assisted selection (MAS) and genomic selection. The process of MAS uses molecular markers to identify and select individual genotypes having genes and quantitative trait loci (QTL) associated with critical phenotypic traits. It is mainly used to identify traits that show simple inheritance or QTL that explain a large proportion of the phenotypic variation within a population (Massman et al., 2011). These traits are often complex, meaning that genotype x environment interactions as well as interactions with other genes influence trait expression. MAS can also improve the efficiency of transferring resistance by using gene specific or tightly linked markers to avoid transferring unwanted traits to the improved progeny (Kou and Wang, 2010). Using molecular breeding methods such as MAS is critical to incorporating and pyramiding genes for resistance to diseases in improved cultivars.

**Disease Cycle and Pathogen**

FHB is caused by the fungal pathogen *Fusarium graminearum* Schwabe and is the most common causal pathogen of this disease in North America as well as many other small grain growing regions in the world (Goswami and Kistler, 2004). It affects a variety of small grain
crops such as wheat (*Triticum aestivum* L.), barley, triticale (*X Triticosecale* Wittmack), rye (*Secale cereale*), and oats (*Avena sativa*) (Wegulo et al., 2008). The fungus may also cause ear rot and *Gibberella* stalk rot disease in maize (*Zea mays*) (Goswami and Kistler, 2004). FHB can cause seed to be shriveled and chalky with low test weight, and produce mycotoxins that affect seed quality. The two mycotoxins that are mainly produced are, a trichothece toxin known as deoxynivalenol (DON) or vomitoxin and the oestrogenic toxin known as zearalenone (Goswami and Kistler, 2004).

According to Bai et al. (2001), DON is essential to FHB virulence and disease spread in wheat, but not in barley. This mycotoxin affects an eukaryotic cell’s ability to synthesize protein by binding to ribosomes that are involved in essential cellular activities such as cell differentiation and death (Goswami and Kistler, 2004, Pestka, 2007, Pestka and Smolinski, 2005). According to Bennett et al. (2003), DON can cause detrimental effects in livestock such as feed refusal, diarrhea, hemorrhaging, among other acute symptoms. Poor weight gain is often a result due to the adverse effects of this mycotoxin which is detrimental to producers (Wise and Woloshuk, 2010). Not only does DON affect livestock, but can adversely affect humans as well. Symptoms such as nausea, vomiting, convulsions, and anorexia are usually associated with humans who have digested significant amounts of this mycotoxin (Goswami and Kistler, 2004). DON is usually concentrated in the shriveled, chalky kernels (Wise and Woloshuk, 2010). This mycotoxin is regulated by the U.S. Food and Drug Administration (FDA), who sets acceptable and safe concentration limits for human consumption, swine feed, cattle feed and feed destined to other animals. The FDA set a 1 part per million (ppm) limit in finished products but in malt barley there is zero tolerance for DON (Food and Administration, 2010). The maximum DON concentration is 5 ppm for feed to be used by swine, 10 ppm for feed to be used by cattle, and
5ppm for feed to be used by other non-ruminant animals (Food and Administration, 2010, Wise and Woloshuk, 2010). For brewers and maltsters, DON can be very problematic and can affect both the malting and brewing process. DON is can typically be washed out of infected grains during the steeping stage of malting, but Fusarium regrowth can occur during germination leading to unacceptable DON levels in both the finished malt and beer (Schwarz et al., 1995). The other mycotoxin, zearalenone, is no common in small grains showing symptoms of FHB.

FHB is a monocyclic disease, indicating that after initial infection often via airborne ascospores, little to no secondary infection occurs from the conidia that form on the heads (Wegulo et al., 2008). Some of the secondary infection that can occur after initial infection is due to rain splash that can disperse ascospores or macroconidia throughout the plant canopy (Paul et al., 2004). The fungus overwinters in residues such as wheat, barley, maize, or in the soil (Nyvall, 1970, Reis, 1990, Shaner, 2003, Sutton, 1982, Wiese, 1987). Perithecia form on the overwintering material and produce conidia and sexual spores called ascospores, which are the primary inoculum in the spring and early summer (Schmale III et al., 2005, Wegulo et al., 2008). FHB infection begins with the introduction of ascospores into a field under favorable conditions (Markell and Francl, 2003). The ascospores are spread by wind, rain, or insects to susceptible plants within a field (Parry et al., 1995, Sutton, 1982, Wegulo et al., 2008). Ascospores transported long distances by atmospheric motions often have an essential role in initial disease development on susceptible hosts when optimal environmental conditions are present (Francl et al., 1999, Schmale III and Bergstrom, 2003, Shaner, 2003). Initiation of fungal growth begins when the ascospores land on or near spikelets on the host plant (Sutton, 1982). Host plants are most susceptible to Fusarium spore infection during anthesis, but infection can also occur during the soft dough stage of kernel development (Del Ponte et al., 2007, McMullen et al., 1997).
Wegulo et al., 2008). Host plants are most susceptible to infection during anthesis because the anthers and pollen serve as a source of nutrients and provide stimuliates to the developing fungal spores (Savi et al., 2015, Strange and Smith, 1971). The fungal hyphae develop on the spikelet where they grow towards stomata or other areas of weakness, such as exposed anthers, in order to enter inside the inflorescence (Bushnell et al., 2003). Not only does fungal growth occur from the outside to inside of inflorescences, mycelia growth can also form on glumes and spread to surrounding plant tissue (Bushnell et al., 2003). Later in the growing season during or after maturity, perithecia may form on seed heads (Wegulo et al., 2008).

Moist, warm temperatures are critical for fungal infection and growth (Wegulo et al., 2008). Temperatures from 77°F to 86°F are ideal for infection and symptom development (Wegulo et al., 2008). Along with warm temperatures, wet conditions are needed for spore germination, infection and production as well. Frequent wet conditions, especially during anthesis, combined with warm temperatures create ideal conditions for fungal infection and growth on susceptible plants (McMullen et al., 1997). Usually fields that are irrigated have more consistent and severe infection than rain fed fields due to the consistent supply of water (Wegulo et al., 2008).

FHB symptoms can start to appear two to four days after infection if optimal conditions for fungal growth are present (Wegulo, 2012). Initial infection can occur from anthesis to soft dough stages under conditions conducive to spore growth, although infection that occurs during anthesis is most damaging to the host plant (Wegulo, 2012). The inflorescences on an infected head will bleach and when severe infection occurs, premature bleaching or blight of the entire seed head occurs (Goswami and Kistler, 2004). Infection often occurs first in the middle of the head where flowering initiates (Wegulo et al., 2008). Browning or purpling of the peduncle
beneath an inflorescence is also an indication where infection has occurred (Goswami and Kistler, 2004, Wegulo et al., 2008). Under moist conditions, orange to salmon pink groups of spores will form at the base of the florets (Wegulo et al., 2008). Brown to tan discoloration or dark water soaking can also occur at the site of infection (Goswami and Kistler, 2004). Damaged kernels become “tombstone” like in appearance: white, chalky, shriveled seed that may also have pink discoloration depending on severity (McMullen et al., 1997).

The best management practices for control of FHB include an integration of good cultural techniques, fungicides, and resistant varieties (Mesfin et al., 2003). Fungicide applications can be very useful in controlling infections of FHB if they are applied at the appropriate time. Fungicides are generally applied during the critical time of infection, which is from flowering to soft dough stage depending on the level of risk for FHB infection. Although fungicides can be beneficial in control of FHB, studies have shown that fungicides usually only provide up to 50% protection against this fungal pathogen (Pirgozliev et al., 2003). Producers must take care to read fungicide labels to ensure proper dose and to ensure that the fungicides will not promote FHB or toxin development. Strobilurin fungicides, such as azoxystrobin, are not recommended since this class of fungicide has been reported to enhance FHB development and increase DON accumulation roughly by 41% in barley kernels (Pirgozliev et al., 2003).

The most economical method of FHB control is the use of resistant varieties (Mesfin et al., 2003). Completely resistant varieties are not available, and varieties vary from moderately resistant to susceptible. Producers are recommended to choose the most resistant variety available for their needs (Wise and Woloshuk, 2010). Another means to lower risks from FHB is use of a combination of varieties that have different flowering times. This staggers flowering dates of the crop and therefore, the times of peak infection to reduce the risk of total crop
infection. Other cultural control management options include reduction of inoculum through tillage or by non-host crop rotation (Kleczewski, 2014, Pirgozliev et al., 2003). Through tillage, farmers are able to bury and potentially reduce within-field inoculum sources as well as sources for long distance transport of fungal spores, in fields before planting their small grains crop. Reduction of inoculum within a field as well as inoculum that is transported long distances, through tillage is an impractical means of control since in recent years minimal to no-till methods have become more common in farming systems in an effort to reduce erosion and improve soil tilth. Non-host crop rotation is an effective means of control. By rotating non-host crops, such as soybeans or alfalfa, into crop rotation after host plants, farmers can reduce FHB incidence within a field by 15% (Pirgozliev et al., 2003). By using a combination of resistant varieties, crop rotation, and proper application of fungicides, the risk of FHB can be reduced.

**Host Resistance to Fusarium Head Blight**

Resistance to FHB has been difficult to breed for due to multiple factors including, limited information, sources and forms of genetic resistance, genotype x environment interactions, and the cost of phenotyping (Zhu et al., 1999). Resistance to FHB is race non-specific, which means that the resistance is quantitative or horizontal in nature (Zhu et al., 1999). FHB resistance is a complex trait that has relatively low heritability and is strongly influenced by environmental factors (Massman et al., 2011).

There are five types of FHB resistance in wheat and barley. Type I, type II, and type III resistance are generally considered to be the main resistance types. Type I resistance is the “restriction of initial pathogen infection”, which has been identified in both wheat and barley (Schroeder and Christensen, 1963). Type II resistance is the “restriction of spread of the
pathogen in the spike after initial infection” (Rudd et al., 2001). Type II resistance varies among wheat genotypes, while in barley it is innate and the fungus generally does not move up or down the spike after initial infection (Steffenson et al., 2003). Type III resistance is the ability to prevent DON synthesis and enhance its degradation (Zhu et al., 1999).

Quantitative trait loci (QTL) have been identified for both qualitative and quantitative disease resistance in both wheat and barley (Zhu et al., 1999). It is extremely important when introducing and pyramiding disease resistance genes to use molecular markers to select for and/or verify the presence of resistance QTL (De la Pena et al., 1999, Zhu et al., 1999). Several methods have been employed to identify QTL regions. Linkage maps saturated with restriction fragment length polymorphisms (RFLP) have been used to postulate the chromosome region where a given QTL most likely resides (De la Pena et al., 1999). Before implementing MAS into a breeding program, the effects and consistency of a QTL should be validated in multiple populations and environments (Mesfin et al., 2003). When identifying and selecting QTL of interest, certain criteria should be considered in the detection of other traits associated with the desired QTL, such as association of FHB resistance with heading date or plant height, in the targeted region of interest and to set thresholds for the detection of significant QTLs in repeated environments and populations (De la Pena et al., 1999). These criteria can aid in effectively identifying and validating desired QTL regions in the donor or resistant germplasm.

Resistance to FHB has been identified and mapped in spring barley. The challenge with most germplasm sources that provide resistance to FHB and DON are that they are either non-adapted to the area of interest and/or possess poor agronomic or quality traits. Such traits can include poor straw strength, late maturity, high protein, and susceptibility to other diseases. In the case of malting barley, FHB resistance has been found to be associated with an increase grain
protein and nitrogen content which can cause poor malting and brewing qualities (Sarlin et al., 2005). Incorporation of resistance from diverse sources into breeding programs is essential to enhance the level of FHB resistance. Some common sources of FHB resistance are ‘Chevron’ (CIho 1111), ‘Fredrickson’ (CIho 13647), ‘Zhedar 2’ (Prom et al., 1997), and ‘CIho 4196’.

Chevron is a non-malting, six row cultivar from Switzerland that has expressed resistance to FHB, kernel discoloration (KD), and DON in various field tests (De la Pena et al., 1999, Gebhardt et al., 1992, Rudd et al., 2001). Drawbacks from using Chevron as a source of FHB and DON resistance are its tall plant height, weak straw strength, late maturity, low yields, and small kernels (De la Pena et al., 1999). Resistance in Chevron has been reported to be inherited quantitatively (De la Pena et al., 1999). Fredrickson, a two row cultivar from Japan, has been moderately resistant to FHB in field tests (Mesfin et al., 2003). Zhedar 2, a two row variety that originated from China, has shown resistance to FHB severity and reduced DON production and accumulation (Dahleen et al., 2003, Prom et al., 1997) CIho 4196, a two row Chinese land race, also expresses resistance to FHB (Rudd et al., 2001). These are a few of the main sources of resistance that are being used in breeding programs.

**QTL for FHB Resistance**

Traditionally, barley breeding programs have experienced difficulty incorporating FHB resistance into their programs since resistance is complex and often involves a combination of many QTL as well as being greatly influenced by genotype x environment interactions and physiological traits such as heading date and height (Mesfin et al., 2003). Identification and validation of QTL is important since individual QTL may only have small effects on the overall level of resistance exhibited by an individual (Mesfin et al., 2003). Resistance QTL are also often
linked to agronomic traits, such as heading date or height, that confound FHB resistance and its assessment (Mesfin et al., 2003). Field studies conducted by Ma et al. (2000), de la Pena et al. (1999), Dahleen et al. (2003), and Zhu et al. (1999) indicate that FHB resistance is linked to multiple loci and certain morphological or physiological traits.

Resistance to FHB and DON accumulation has been mapped to all seven barley chromosomes, with resistance QTL on chromosomes 2H and 6H being the most common (Table 1.1 and 1.2) (Berger et al., 2014, Mesfin et al., 2003). De la Pena et al. (1999) identified FHB resistance QTL on multiple barley chromosomes: 1(7H), 2(2H), 3(3H), 4(4H), 5(1H), and 7(5H) in a Chevron/M69 mapping population (Mesfin et al., 2003). According to Mesfin et al. (2003) a major region on chromosome 2H is associated with FHB resistance, DON accumulation, and kernel discoloration and accounts for “13.5% of the phenotypic variation for FHB resistance” (De la Pena et al., 1999). Ma et al. (2000) identified nine QTL for FHB resistance in a Chevron/‘Stander’ (PI 564743) mapping population. Resistant QTL were found on chromosomes 1(7H), 2(2H), 3(3H), 6(6H), and 7(5H) (Ma et al., 2000). The QTL found on chromosome 2(2H) accounts for 11.8% to 20.8% of phenotypic variation in FHB resistance across the five environments that the Chevron/Stander population was tested (Mesfin et al., 2003). Zhu et al. (1999) identified FHB resistance on all barley chromosomes except 7(5H) in a ‘Gobernadora’ (OC640/Mari//Pioneer/3/Maris Canon) / CMB643 (Shyri/Gloria//Copal///Shyri/Grit) two-rowed barley mapping population (Meszaros et al., 2007). The largest FHB resistance QTL was found on chromosome 2(2H) and accounted for 33% of the phenotypic variation (Mesfin et al., 2003, Zhu et al., 1999). The resistance QTL found on chromosome 4(4H) accounted for 4% to 12% of the FHB phenotypic variation and was associated with “plant height, seeds per inflorescences, inflorescence density, and lateral floret size” (Mesfin et al., 2003). Dahleen et al. (2003)
identified nine resistance QTL for FHB severity and five resistance QTL for reduced DON accumulation on all chromosomes except 4(4H) and 7(1H), in a Zhedar 2/ND9712//Foster double haploid population. Among the QTL identified for FHB severity, only two significant QTL identified on 6H were unique to the resistant parent Zhedar 2 (Dahleen et al., 2003). Three resistance QTL associated with low FHB severity were identified in a study of ‘Harbin’ (accession No. C649) / Turkey 6 (accession No. T602) recombinant inbred mapping population (Hori et al., 2006). Resistant QTL were found on chromosomes 2H, 4H, and 6H across multiple years and locations with the QTL located on chromosome 6H contributing the highest amount of phenotypic variance (18.6%) (Hori et al., 2006).

Resistance to DON development and accumulation has been found on various chromosomes across all the studies mentioned above and is frequently associated with FHB resistance QTL. De la Pena (1999) identified four QTL regions in a Chevron / M69 population that were associated with DON accumulation resistance on chromosomes 1(7H), 2(2H), and 7(1H). The QTL found on chromosome 2(2H) that is related to DON accumulation is linked to FHB resistance as well (De la Pena et al., 1999). Dahleen et al. (2003) identified one unique QTL on chromosome 6 that is associated with low DON accumulation, as well as reduced FHB severity, in the Zhedar 2/ND9712//Foster population. Ma et al. (2000) identified nine QTL for lower FHB severity and DON accumulation in a Chevron / Stander population. Of the nine QTL identified, eight were shared between the two traits, which further supports that FHB resistance and DON accumulation QTL are often linked together or the same.
Morphological and Physiological Traits Associated with FHB

As discussed in the previous section, very often major morphological or physiological traits are linked with FHB resistance. Spike type, flowering type, and heading date are important traits that are often found to be associated with FHB severity (Mesfin et al., 2003). The Vrs1, main influential loci located on chromosome 2H, and Int-c loci control whether barley heads will be two or six rowed (Mesfin et al., 2003). Two rowed spikes are controlled by Vrs1; int-c/int-c (Lundqvist and Franckowiack, 1997), while six rowed spikes are controlled by vrs1/vrs1; Int-c. Several studies have been conducted and identified a link between the Vrs1 locus and resistance to FHB and DON accumulation. Mesfin et al. (2003) discovered that the Vrs1 locus was associated to FHB resistance in a Fredrickson/Stander (PI 564743) population as well as a Fredrickson/Stander//M81 population that was used to validate the QTL. Although they discovered that the Vrs1 locus is associated with FHB resistance, they were unable to determine if the regions were tightly linked or if it was due to a pleiotropic effect of the Vrs1 locus (Mesfin et al., 2003). FHB resistance in barley has more frequently been discovered in two row barley cultivars versus six row barley cultivars (Steffenson et al., 1996). Chevron is one of the few six row barley cultivars that exhibits resistance, while CIho 4196, Zhedar 1, Zhedar 2, Harbin, Fredrickson, ‘Harrington’ from Canada, ‘Kitchin’ from the U.S. and ‘Shyri’ from Mexico, are some of the two row barley varieties that exhibit moderate to high resistance to FHB and DON accumulation (Rudd et al., 2001). A study performed with five RIL mapping populations composed of Harbin by susceptible crosses identified 13 QTL regions across all seven barley chromosomes (Sato et al., 2008). All of the QTL found in the study were found to be associated with flowering type on chromosome 2H, but none near the vrs1 locus (Sato et al., 2008). Since
none of the resistance was found near the \textit{vrs1} locus, this suggests that the resistance seen in two row by six row may be due to a pleiotropic effect (Sato et al., 2008).

Flowering type, whether the flower is open or closed, is controlled by the \textit{cly1/Cly2} gene that is located on the long arm of chromosome 2H (Turuspekov et al., 2004). Previous studies performed by Steffenson (2003) and Yoshida et al. (2005) identified a link between flowering type and FHB resistance. Yoshida et al. (2005) observed that the difference in FHB resistance between cleistogamous and chasmogamous pairs was greater and more stable in the NIL populations, suggesting that flowering type does affect the amount of FHB resistance exhibited. Two studies performed by Hori et al. (2005, 2006), using Harbin derived populations, identified FHB resistance QTL on chromosome 2H that were near the same region as \textit{cly1/Cly2} locus. It is still to be determined if there is a linkage effect between FHB resistance and the \textit{cly1/Cly2} locus.

The \textit{Vrs1} locus often has been found to be associated with other agronomic traits such as heading date and spike morphology (Massman et al., 2011). Heading date has been associated with FHB resistance and DON accumulation in multiple studies. Plant height and heading date are crucial components to FHB development (Ma et al., 2000). Late heading date may be associated with FHB resistance since the heads experience less exposure time to the fungal spores (Mesfin et al., 2003). Ma et al. (2000) reported a negative correlation between late heading date and FHB severity and DON accumulation in a Chevron / Stander double haploid population. The resistance QTL identified by Dahleen et al. (2003) in the Zhedar 2 population were also found to be linked to later heading. The association between heading date and FHB resistance may be due to linkage between the genes for heading date and FHB resistance or due to pleiotropic effects (Ma et al., 2000). In a similar study performed by de la Pena et al. (1999), in a Chevron / M69 population, they also identified an association between late heading and tall
plants with lower FHB severity and DON concentrations. The linkage between heading date and FHB resistance has caused a barrier for plant breeders since they often do not select later heading individuals and therefore may be eliminating potential resistance genes. A study was conducted using near isogenic lines (NILs) derived from a Chevron / M69 mapping population to assess the Qrgz-2H-8 region on chromosome 2(2H) that is associated with heading date and FHB resistance (Nduulu et al., 2007). They discovered one recombinant that did showed uncoupling between late heading and FHB resistance which proves that the linkage between heading date and FHB resistance can be broken (Nduulu et al., 2007). Further research and fine mapping of the QTL regions in question are needed to confirm the association between FHB resistance and morphological traits as well as if the linkage can be unbroken in other mapping populations.

Within the Virginia Tech barley breeding program, sources of FHB resistance were identified in winter barley cultivars ‘Eve’ (PI659067) and ‘Nomini’ (PI566929). Eve is a six rowed hulless winter barley that express moderate resistant to FHB and has reduced accumulation of DON toxins (Brooks et al., 2013). Eve likely received its resistance from the parent VA94-42-13, which has Chevron in its background. Pedigree information for Chevron is available at The Triticeae Toolbox database (http://triticeaetoolbox.org/ [accessed 02 Dec. 2016]. Nomini is a six rowed hulled barley cultivar that has exhibited FHB resistance in field trials (Berger et al., 2014). The FHB resistance expressed by Nomini is likely derived from the parent ‘Henry’ (CI15690), which has Chevron in its background as well. However, some of the QTL flanking markers in Eve and Nomini do not match those of Chevron or Fredrickson, which indicates that their resistance may be unique. Further analysis and QTL mapping of elite lines such as these is critical in identifying and validating QTL for resistance to FHB and DON accumulation.
Proposed Research

Experimental

A hulless winter-barley mapping population derived from a resistant by susceptible cross will be evaluated for FHB resistance, FDK, and DON accumulation. The mapping population includes: Eve / ‘Doyce’ (PI634932). Recombinant inbred lines (RILs) will be planted in randomized complete blocks within inoculated and mist irrigated nurseries at Kinston, North Carolina, Lake Wheeler, North Carolina, Lexington, Kentucky, Blacksburg, Virginia, and Mt. Holly, Virginia during the 2014-2015 growing season. In 2015-2016, the populations will be planted in randomized complete blocks within inoculated and mist irrigated nurseries at Kinston, North Carolina, Lexington, Kentucky, Blacksburg, VA, and Mt. Holly, VA.

Experimental units in this will study consist of one or two adjacent 1.2m head rows, which constitutes a plot, with 0.3m spacing between each head row. Each experimental unit will be used in the evaluation of phenotypic responses to FHB and to produce enough grain for subsequent analyses of fusarium damaged kernels (FDK) and DON concentration. Corn kernels inoculated with *Fusarium graminearum* will be applied to head rows at the early boot stage to enhance the development of disease at all locations (Khatibi et al., 2011). A combination of six *Fusarium graminearum* isolates will be used in the preparation of inoculum to incite infection of barley genotypes in the Blacksburg and Mt. Holly, VA scab nurseries. *Fusarium* isolates, that are native to this region, will be obtained from Dr. D. Schmale, department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. At 50% flowering, plots at Blacksburg will be spray inoculated using 50,000 spores/ml of macroconidia (Berger et al., 2014). Plots within nurseries will be mist irrigated for one hour each day between the times of 3:00pm and 4:00pm, except when natural precipitation events occur, for three weeks after the first inoculation. Plots
will be harvested at maturity (<16% moisture content) using Wintersteiger plot combines (Wintersteiger, Salt Lake City, UT) with low fan speeds to minimize loss of Fusarium damaged kernels during harvest.

*FHB INC, SEV, and Quantification of FDK and DON in Grain Samples*

FHB incidence (INC) and severity (SEV) will be rated in the field nurseries to assess genotypes for resistance. FHB incidence, the percentage of FHB infected heads, will be determined by arbitrarily choosing and evaluating 20 FHB infected heads per experimental unit. FHB severity, the number of FHB infected spikelets divided by the total number of spikelets, is determined by selecting and evaluating 20 FHB infected heads per experimental unit. Both incidence and severity will be rated at each of the locations roughly three weeks after flowering, to ensure that ratings occur at the appropriate time for early and late flowering RILs in within the population.

After harvest is completed a subsample of grain from each genotype will be taken from each rep. These subsamples will be cleaned using a screen cleaner without air, to limit the removal of infected kernels, to remove debris. A smaller subsample of 100 grams will be taken for quantification of DON concentration and about five grams to assess FDK (Berger et al., 2014). As described by Khatibi et al. (2012), DON concentration will be determined by grinding 100 grams of kernels into whole grain flour subsamples and then using gas chromatography and mass spectrometry to analyze the ground samples. FDK is calculated as a percentage of infected kernels per subsample of 100 kernels. Through these methods, further analysis of resistance to FHB within the RIL populations is conducted.
Molecular Marker Genotyping

Previous work conducted by Greg Berger et al. (2014) on FHB resistance of Virginia barley postulated that the winter barley cultivars Eve and Nomini may possess unique QTL for resistance on chromosomes 2H and 6H. The fragment sizes of multiple SSR and/or SNP markers associated with QTL for FHB resistance found in the resistant sources Nomini, Eve, and VA06H-48 were not similar to those identified in Fredrickson and Chevron. This indicates that these cultivars may contain unique FHB resistance QTLs. Genotyping will be conducted at Virginia Tech as well as at the USDA lab in Fargo, North Dakota. The genotypes will be screened with markers for resistance QTL across the whole genome, with focus on chromosomes 2H and 6H, using single nucleotide polymorphisms (SNP) and single sequence repeats (SSR). SNP screening will be conducted by both Virginia Tech and the USDA lab in Fargo, ND. An Illumina 9K iSelect SNP Chip (Illumina, San Diego, CA) will be performed in the USDA lab in Fargo, ND. Tissue grinding and DNA extraction will be performed at the USDA lab in Raleigh, NC prior to the 9K iSelect analysis. The SNP makers that will be ran at Virginia Tech, will be designed and ordered based on the results of the 9K iSelect. Direct label SSR screening will be conducted within the Virginia Tech lab via an Applied Biosystem™ (ABI) (ThermoFisher Scientific, Waltham, MA) and GeneMarker® Software (SoftGenetics, State College, PA). Along with validation of resistance QTL on chromosomes 2H and 6H in Eve, search for other FHB resistance QTL across the entire genome will be conducted in an effort to map FHB resistance in Eve.
LITERATURE CITED


Nduulu, L., A. Mesfin, G. Muehlbauer and K. Smith. 2007. Analysis of the chromosome 2 (2H) region of barley associated with the correlated traits Fusarium head blight resistance and heading date. Theoretical and applied genetics 115: 561-570.


1996. Severity of Fusarium head blight and concentrations of deoxynivalenol in near-isogenic lines of barley differing for several agronomic characters. Proc. 7th International Barley Genetics Symposium, University Extension Press, Saskatoon, Saskatchewan.


Table 1.1 Chromosome, QTL Marker Interval/Loci, and Resistant Cultivar for FHB Severity

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Table 1.2 Chromosome, QTL Marker Interval/Loci, and Resistant Cultivar for DON Accumulation

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Chapter 2
Quantitative Trait Loci for Resistance to Fusarium Head Blight in the Hulless Winter Barley Cultivar Eve
ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating fungal disease that affects barley (*Hordeum vulgare*) production worldwide. This disease results in reduced yields, damaged kernels, and accumulation of mycotoxins in the grain. The most effective means of FHB control can be achieved by deployment of FHB resistant cultivars developed via incorporation and pyramiding of resistance genes from diverse sources. Resistance to FHB in the winter barley cultivar Eve has been validated in Virginia Tech’s Small Grains Breeding Program. The objectives of this study were to map and identify the quantitative trait loci (QTL) for FHB resistance in Eve, and to identify tightly linked, diagnostic, and high-throughput markers for use in marker assisted selection (MAS) in breeding programs. A population of 180 recombinant inbred lines (RILs) derived from the cross Eve/'Doyce’ was evaluated for FHB incidence (Inc), FHB severity (Sev), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) over two growing seasons (2014-15 and 2015-16) at Blacksburg, VA, Mount Holly, VA, Kinston, NC, and Lexington, KY. A QTL was identified on chromosome 6H associated with FHB SEV, FDK, and DON. The QTL accounted for as high as 14.5% of the phenotypic variation for DON accumulation, 8.3% for FHB SEV, and 11.9% to 17.6% for FDK. The QTL marker region spans 56.5 to 66.6 cM with flanking markers SCRI_RS_147342 and Bmag0613 that are 10.1 cM apart. Other QTL for morphological traits, heading date and height, were also found in the same region as the FHB resistance traits. Phenotypic variation explained by the QTL was 15.5% for heading date and 6.6% for plant height. Diagnostic single nucleotide polymorphism (SNP) markers associated with the FHB resistance QTL in Eve can be used to genotype and select barley parental lines and in MAS breeding to incorporate and pyramid diverse FHB resistance QTL and genes into adapted cultivars.
INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating fungal disease that affects barley production worldwide (Zhu et al., 1999). FHB can cause reductions in grain yields, test weights, seed germination, kernel quality, and contamination by mycotoxins (McMullen et al., 1997). The most effective way to manage FHB is through the use of resistant barley cultivars. Although this is the most effective way to manage FHB, completely resistant cultivars are not currently available to producers. Resistance to FHB is race non-specific, has low heritability, and is influenced by environmental factors, which makes breeding for resistance complex (Massman et al., 2011, Zhu et al., 1999). Breeding for FHB resistance requires gene pyramiding from various sources to achieve an adequate level of resistance. There are five types of resistance to FHB in wheat (*Triticum aestivum*) and barley. Types I, II, and III are considered the main types of resistance. Type I is resistance to initial infection (Schroeder and Christensen, 1963), type II is resistance to the spread of the pathogen throughout the spike (Rudd et al., 2001) and type III is resistance to the synthesis and accumulation of deoxynivalenol (DON) (Zhu et al., 1999). FHB resistance has been identified on all seven chromosomes in the barley genome, with the most common resistance found on chromosomes 2H and 6H (Berger et al., 2014, Mesfin et al., 2003). Resistance traits for FHB are often associated with each other, meaning that selection for resistance to severity may also indirectly select for reduced Fusarium damaged kernels (FDK) and DON accumulation as well. Resistance has been identified in spring barley varieties such as ‘Chevron’, ‘Fredrickson’, and CIho 4196. Although these varieties display resistance to FHB, they often have poor agronomic traits, such as poor yield, and therefore are undesirable for production. Research conducted by Berger et al. (2014), identified potential unique sources of FHB resistance in winter barley cultivars Eve and Nomini in Virginia Tech’s Small Grains Breeding Program. Based on the study performed by Berger et al. (2014), it was hypothesized that FHB resistance in the hulless winter barley cultivar Eve is different than in previously reported sources. The purpose of this research was to: 1) validate the potential uniqueness of QTL for resistance to FHB in Eve versus those previously mapped on chromosomes 2H and 6H; 2) search for additional resistance QTL within the Eve mapping population; and 3) identify tightly linked and diagnostic markers for use in MAS breeding programs.
MATERIALS AND METHODS

Plant Material

A recombinant inbred line (RIL) population, initially consisting of 459 individuals lines, was created to map QTL for FHB resistance in Eve (PI 659067), a Virginia Tech hulless winter barley cultivar (Brooks et al., 2013). Eve, which is moderately resistant to FHB, was crossed with the FHB susceptible barley cultivar Doyce (PI 634932) (Brooks et al., 2005). Eve was derived from the cross SC860974/VA94-42-13. Parentage of SC860974 is VA75-42-45 / SC793556 // CIho 2457. Ancestry of VA75-42-45 is ‘Jotun’ (PI 467357) / 4*’Rogers’ (PI 539127) / 3/ ’Cebada Capa’ (PI 539113) / ’Wong’ (PI 539117) // awnleted selection of ‘Hudson’ (CIho 8067). Line SC793556 is a reselection from ‘Redhill’ (CIho 15830) and ‘Lokian’ (CIho 2457) is a hulless winter-habit barley from China. VA94-42-13 was derived from a series of crosses comprised of (CIho's 9623, 9658, 9708, BYDV Resistant ‘Atlas’ / ’Hanover’ (CIho 13197) or ‘Rapidan’ (CIho 14006) /4/ 2*(‘Harrison’ (CIho 10667) /3/ Cebada Capa / Wong // awnleted selection of Hudson) /6/ (Harrison /3/ Cebada Capa / Wong // awnleted selection of Hudson)*4 /4/ ’La Mesita’ (CIho 7565) /5/ (CIho's 9623, 9658, 9708, BYDV Res Atlas / Hanover or Rapidan) /4/ (Harrison /3/ Cebada Capa / Wong // awnleted selection of Hudson). It is postulated that Eve likely inherited its FHB resistance from the parent VA94-42-13, since it parentage includes ‘Chevron’.

From the initial population, a set of 284 F_5:6_ RILs (Virginia and Lake Wheeler, NC tests) and 212 RILs included in the former set (other tests in KY and NC) were randomly selected from the Eve/Doyce mapping population and evaluated for FHB in 2014-15 experiment. From the RILs evaluated during 2014-15, 180 F_6:7_ RILs were selected, based on FHB resistance and susceptibility, from the set of 284 RILs and evaluated for FHB during 2015-16.

Seed from each of the Eve/Doyce RILs included in the 2014-15 experiments was derived from individually harvested F_4:5_ head rows grown at the USDA-ARS research station in Aberdeen, Idaho (latitude 42° 57’ 25” N and longitude 112° 49’ 43’ W). Seed from the RILs used in the 2015-16 experiments was derived from four F_5:6_ head rows that were harvested in bulk using a Wintersteiger plot combine (Wintersteiger, Salt Lake City, UT) at Warsaw, VA. Seed of the RILs used in each of the experiments was treated with Raxil-MD® (Tebuconazole, Metalaxyl and Imazalil, Bayer Crop Science) and Gaucho® (Imidacloprid, Bayer Crop Science)
prior to planting each year. Treatment of the seed with Raxil-MD® and Gaucho® was used to
limit infection of plants with glume blotch (*Stagonospora nodorum* formerly *Septoria nodorum*)
and barley yellow dwarf (pathogen *Barley Yellow Dwarf Virus*). Subsamples of treated seed of
each RIL were used to plant tests in Virginia as well as at other testing sites by cooperators in
both 2014-15 and 2015-16 growing seasons.

**Inoculum**

The Fusarium inoculum used in this experiment was composed of various isolates from
the respective state or region in which each test was conducted. The Fusarium isolates used to
produce inoculum for field tests in Virginia were obtained from the lab of Dr. David Schmale, III
in the department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. The
native isolates used in this study are: GPS13#14, GPS8#12, GPS12#8, KBTappN5, KBTappN10,
and KBTappN27. Full strength potato dextrose agar (PDA) media (39g potato dextrose agar in
1L of deionized water; Becton, Dickinson, and Company, Sparks, MD) was used to culture
*Fusarium graminearum* for 14 days. Corn kernels (*Zea mays* L.) were soaked in water over night
and placed in autoclave bags (Fisher Scientific catalog # 018267) for sterilization. The corn
kernels were steam sterilized for 24 hours and allowed to cool to room temperature (21°C)
before being inoculated with fungal mycelium. Fungal mycelium was cut into 1cm² pieces,
placed into the autoclave bags with the sterilized corn kernels, and was allowed to incubate for
21 days at room temperature. After incubation, the mycelium colonized corn kernels were
allowed to dry on trays from four to seven days in the greenhouse. One quarter strength PDA (6g
potato dextrose broth and 15g granulated agar in 1L of deionized water; Becton, Dickinson and
Company, Sparks, MD) was used to culture macroconidia that were used to create inoculum
solutions sprayed onto barley spikes in field tests. Macroconidia were allowed to develop for 10
days, then harvested and adjusted to a concentration of 50,000 spores per mL⁻¹ using distilled
water. Spore suspensions were prepared the day of field inoculation.

**Field Tests**

During the 2014-15 growing season, 284 RILs (F₅₋₆), resistant and susceptible checks,
and parental lines were evaluated for FHB traits at three locations; Blacksburg, VA (BVA,
latitude 37° 11’ 59” N and longitude 80° 35’ 52” W; two replications, two head rows per entry)
Mount Holly, VA (MHV, latitude 38° 5’ 50” N and longitude 76° 43’ 30” W; two replications, two head rows per entry), and Lake Wheeler, NC (LWN, 35° 43’ 39” N and 78° 40’ 47” W; one replication, one head row per entry). A set of 212 RILs (F5:6), resistant and susceptible checks, and parental lines were evaluated for FHB traits during the 2014-15 growing season at two locations; Kinston, NC (KNC, latitude 35° 18’ 04” N and 77° 33’ 54” W longitude; two replications, one head row per entry) and Lexington, KY (LKY, latitude 38° 7’ 31” N and longitude 84° 29’ 44” W; two replications, one head row per entry). During the 2015-16 growing season 180 RILs, with resistant and susceptible checks, were evaluated, using the same design as in the previous experiments, for FHB traits at four locations: BVA and MHV, KNC, and LKY. Resistant checks included winter barley cultivars Dan (PI 659066) and Nomini (PI 566929). Susceptible checks included winter barley varieties ‘Atlantic’ (PI 665041), ‘Thoroughbred’ (PI 6349333), and VA07H-35WS (Thoroughbred/SC872143) (Berger et al., 2014). Heading date varied among the resistant and susceptible check varieties to monitor and assess disease incidence and severity among early and later heading lines at each test site.

The 2014-15 and 2015-16 scab nursery tests at all locations were composed of two replications in a randomized complete block design (RCBD) that included Eve/Doyce RILs, resistant and susceptible check cultivars, and parental lines dispersed throughout each replication. During both growing seasons at BVA and MHV, each experimental unit consisted of two adjacent head rows that were 1.2m in length and had 0.3m spacing in between each row. Test plots at KNC during both growing seasons consisted of two adjacent head rows that were 1.2 m in length and had 0.15 m spacing in between each row.

For both growing seasons at BVA, MHV, and KNC, scab nursery management practices included: a standard recommended application of fall pre-plant fertilizer, Harmony Extra SG® (Thifensulfuron methyl) herbicide, and at GS30 (Zadoks et al., 1974) a top dress application of nitrogen as UAN (30% Urea ammonium nitrate solution) (Table 2.1 and 2.2). During 2014-15 and 2015-16 in the BVA scab nursery, management practices also included application of Baythroid XL® (β-cyfluthrin) insecticide at a rate of 1.10 L ha⁻¹ and 1.46 L ha⁻¹ respectively; and an application of Tilt® fungicide at a rate of 1.46 L ha⁻¹. In the 2014-15 and 2015-16 scab nursery at MHV management practices included application of Starane® Ultra (fluoroxygur 1-methylhyepytal ester) herbicide applied at a rate of 9.866 L ha⁻¹ with Weedar® (2,4-D Amine)
herbicide at a rate of 11.692 L ha\(^{-1}\); Palisade\(^{\circledR}\) (Trinexapac-ethyl) growth regulator at 7.308 L ha\(^{-1}\); and Tombstone\(^{\circledR}\) insecticide (Cyfluthrin) at 1.462 L ha\(^{-1}\). For both growing seasons at KNC, the scab nursery management practices also included applications of potassium (K) and phosphorus (K), based on soil tests performed pre-planting and application of Osprey\(^{\circledR}\) herbicide (Mesosulfuron-Methyl) to control ryegrass (Lolium perenne) populations.

Fusarium colonized corn kernels were applied to experimental units at all locations at the early boot stage for each growing season (Tables 2.1 and 2.2). During both growing seasons at BVA, each plot was spray inoculated when 50% of the plants were at anthesis using a CO\(_2\)-pressurized sprayer (R and D Sprayers, Opelousas, LA) with 50mL of macroconidia spore suspension (concentration of 50,000 spores mL\(^{-1}\)). Fusarium isolates used for inoculation at KNC were randomly collected each year (Paul Murphy, personal communication, 2014).

The nurseries at all locations were mist irrigated, unless natural precipitation occurred, for three weeks after initial inoculation for both growing seasons. Both Virginia locations were then mist irrigated for one hour each day between the hours of 3:00pm to 4:00pm. At KNC the scab nursery was mist irrigated twice daily between the hours of 8:00am to 11:00am and 4:00pm and 7:00pm, for two minutes on, 18 minutes off intervals. At LKY the nursery was mist irrigated overnight, between the hours of 8:00pm and 7:00am, at 20 minute intervals.

During both test years and at all locations, 20 randomly chosen barley spikes were selected for FHB assessment 21 days after inoculation in each RIL, resistant and susceptible check, and parental experimental units. The phenotypic traits evaluated in each plot during both growing seasons included heading date (Julian), plant height (cm), FHB incidence (Inc), and FHB severity (Sev). Height was not recorded at MHV and KNC. FHB Inc is the percentage of FHB infected heads out of 20 arbitrarily selected heads per plot. FHB Sev is the number of FHB infected spikelets divided by the total number of spikelets per barley spike, for the 20 arbitrarily chosen barley heads per plot.

In 2015, both replications were harvested at MHV, KNC, and LKY, but only the first replication of RILs was harvested at BVA because of poor plant stands due to flooding that occurred throughout the second replication (Table 2.1). In 2016 both replications at BVA, MHV, and LKY were harvested (Table 2.2). In 2015-16, RILs at KNC were not harvested due to frost damage that occurred after heading making it impossible to accurately distinguish the difference
between damage caused by frost versus FHB. Harvest occurred when plots reached maturity (moisture <16%) using Wintersteiger plot combines set at low fan speeds to minimize loss of Fusarium damaged kernels during harvest. Samples from KNC and LKY were transported back to BVA where they were threshed using a Wintersteiger plot combine set at a low fan speeds. Upon completion of harvest, a subsample of all genotypes was taken from each experimental unit and cleaned using a screen cleaner without air, to limit the loss of Fusarium infected kernels, to remove any excess debris or chaff. A 5g subsample from each genotype was taken and used for FDK and a 100g was taken for DON analysis (Berger et al., 2014).

**FDK and Deoxynivalenol Evaluation**

FDK was calculated as the percentage of tombstone or scabby kernels out of 100 kernels. The 100g subsample taken for DON analysis was ground down into whole barley flour and a 1g subsample was sent to Dr. Schmale’s lab at Virginia Tech for analysis using a Shimadzu QP2010 GC/MS (gas chromatography/ mass spectrometry; Shimadzu Corporation, Kyoto, Japan; (Tacke and Casper, 1995) according to protocol describe by Mirocha et al. (1998).

**DNA Collection**

The RILs, at the F5:6 generation, and parental lines were grown in a greenhouse and harvested at the two leaf stage. Leaf tissue was placed into 96 well plates, that were composed of twelve 1.2ml strip tubes (Corning catalog #4408; Corning, Corning, New York) containing a steel bead for grinding. A second steel bead was added to each tube after tissue was added to aid in the grinding process. The tissue was stored in a -80°C freezer until ready for extraction. To ensure that the tissue was completely frozen before grinding, the tissue was placed into liquid nitrogen for a minimum of five minutes. Once frozen, the tissue was ground down in a Spex® SamplePrep 2010 Geno/Grinder® (Metuchen, NJ) at 1,250 strokes for one minute or until finely ground. DNA isolation was performed using the CTAB extraction method, with minor modifications, as described by Saghai-Marroof et al. (1984, 1994).

**Genotyping**

For genotypic analysis, two types of molecular markers were used: single nucleotide polymorphisms (SNPs) and single sequence repeats (SSRs). Genotyping using SNP markers was conducted at both the USDA-ARS lab in Fargo, ND and at Virginia Tech, while SSR markers
were run and analyzed only at Virginia Tech. A set of 48 RILs were selected based on FHB assessments, from five locations between both growing seasons, 24 with high severity, FDK, and DON ratings and 24 with low severity, FDK, and DON ratings. These 48 RILs and parent DNA was sent to the USDA lab in Fargo, ND for SNP analysis on an Illumina® 9K iSelect SNP chip (Illumina, San Diego, CA). Data received from the 9K iSelect SNP chip was analyzed using GenomeStudio® software (Illumina, San Diego, CA). Based on the results from the 9K iSelect, eleven polymorphic SNP primers were designed by Dr. Luciana Rosso at Virginia Tech and produced by Integrated DNA Technologies (Integrated DNA Technologies, Coralville, IA). A bulk segregate analysis (Michelmore et al., 1991) was conducted on the full population, 180 RILs plus parent lines, at Virginia Tech using SNP and direct-labeled SSR molecular markers. Three out of nine SSR markers and the 11 SNP markers designed for this mapping population were polymorphic. Markers that were significant for FHB assessment traits were used to analyze all 180 RILs in the Eve/Doyce mapping population.

SNP polymerase chain reaction (PCR) was conducted at a volume of 8.11µL containing: 4 µL of 25ng (aqueous DNA), 4µL of KASP® master mix (LGC, Indianapolis, IN), and 0.11 µL of 72x assay mix (12 µL of 100 µM AL1 primer, 12 µL of 100 µM of AL2 primer, 30 µL of 100 µM C1 primer, and 46 µL of deionized water). PCR reactions were conducted in 0.2 mL semi-skirt PCR plates (USA Scientific, catalog # 1402-9790, Ocala, FL) using a Hydrocycler-4® (LGC, Indianapolis, IN). PCR reaction proceeded sequentially: 1) 94°C for 15 minutes, 1 cycle; 2) 94°C for 20 secs, 65°C for 1 min, 10 cycles with 0.8°C down at each cycle; and 3) 94°C for 20 secs, 57°C for 1 min, 35 cycles. PCR product was analyzed using FLUOstar Omega Microplate Reader® (BMG Labtech, Offenburg, Germany). Klustercaller™ (LGC, Indianapolis, IN) was used to call the alleles for each marker.

The PCR of direct labelled SSR markers was conducted, using a modified protocol described by Röder et al. (1998), at a volume of 12.5 µL containing: 2.5 µL of 25ng (aqueous DNA), 1.2 µL of 10 x standard reaction buffer with MgCl2, 0.3 µL of 10mM dNTP, 0.2 µL of forward primer, 0.2 µL of reverse primer, 7.42 µL of molecular grade H2O, and 0.18 µL of Taq polymerase (Apex, Genessee Scientific, San Diego, CA). The fluorescent dyes that were attached to the direct labeled primer are: FAM, VIC, NED, and PET (Applied Biosystems, Foster City, CA). PCR reactions were performed using GeneAmp® PCR System 9700 (Applied Biosystems,
Foster City, CA), iCycler Thermocycler™ (Bio-Rad, Hercules, CA), and C1000™ Thermocycler (Bio-Rad, Hercules, CA). PCR reaction proceeded sequentially: 1) 95°C for 5 min, 1 cycle; 2) 95°C for 45 sec, 60°C for 1 min, 72°C for 1.5 min, 34 cycles; and 3) a final extension at 72°C for 5 min, 1 cycle. The annealing temperatures optimized for each individual primer differed and were optimized for each primer pair.

SSR fragments were analyzed using a 3130lx Genetic Analyzer (Applied Biosystems, Foster City, CA). A 14 µL solution consisting of 9.75 µL of 95% formamide (Applied Biosystems, Foster City, CA), 0.25 µL of Gene Scan™ - 500 LIZ® size standard (Applied Biosystems, Foster City, CA) and 1 µL of FAM, VIC, NED, and PET PCR product. Alleles were called for each marker based on allele fragment size using Genemarker V1.6 software (SoftGenetics, State College, PA).

Statistical Analysis

The raw phenotypic data, that was averaged between repetitions at each location, was used for analysis. Data was not normalized since normalization could lead to the loss of transgressive segregates, which are individuals that exhibit extreme high or low FHB resistance within the mapping population (see Appendix Figure A1). A transgressive segregate is an individual that shows an extreme phenotype when compared to phenotype observed in the parent lines while an outlier is an observation that is found to abnormally distant from the sample population. When using a bi-parental mapping population, it is expected to observe transgressive segregates within the population. FHB assessments for each RIL were assessed for outliers through scatter plots developed in JMP Pro 13 (Cary, NC) (see Appendix Figure A2). Outliers were determined if there was abnormal variation between repetitions of the same RIL, i.e. if the RIL showed resistance in repetition one and susceptibility in repetition two. If a RIL was determine to be an outlier for the phenotypic data recorded in the field, that RIL was dropped from analysis for that specific environment. If the RIL was determined an outlier for any of the post-harvest disease assessments, the sample was re-sampled from the original source and retested for that specific disease assessment. If the result was the same as the original outlier result, the RIL was then removed from analysis for that given location. Outliers were removed from analysis to ensure an accurate depiction of FHB resistance was observed in each nursery at each location.
Phenotypic data was analyzed using R (version 3.3.3, Baltimore, MD). Pairwise correlations were estimated between heading date (HD), plant height (HT), FHB Inc, FHB Sev, FDK, and DON for the 48 individuals screened with the Illumina® 9K iSelect. The QTL analysis was performed using QTL IciMapping (ICIM, version 4.1.1) with Kosambi’s mapping function (Kosambi, 1943) and a LOD score of 3.0. A thousand permutations were conducted at a threshold p-value of 0.05 to determine LOD score. Consensus maps by Varshney et al. (2007) and Muñoz et al. (2011) were used to confirm marker order. Interval and composite interval mapping was performed to identify significant QTL regions. Construction of linkage maps was performed using ICIM and Mapchart 2.3 (Voorrips, 2002) was used to draw linkage maps.

RESULTS

Phenotypic Analysis

Distribution of RILs in the Eve/Doyce mapping population were constructed based on the phenotypic assessments of FHB Inc, FHB Sev, FDK and DON for each growing season (2014-15 and 2015-16) and each test location (BVA, LKY, KNC, and MHV). RILs typically displayed a normal distribution for each trait, with a few exceptions at some of the test locations. DON of RILs at KNC in 2014-15 was skewed towards lower concentrations (Figure 2.1). In the 2014-15 LKY and MHV experiments, incidence and FDK were skewed towards higher levels (Figures 2.2 and 2.3). In the 2015-16 BVA test, severity and DON were skewed towards lower levels (Figure 2.4). At LKY in 2015-16, incidence was skewed towards higher levels (Figure 2.5). Traits for which RIL distributions were skewed towards either higher (FDK and/or incidence) or lower (severity and/or DON) levels likely were due to environmental factors that influenced disease development and progression at the given test location. Parent lines displayed appropriate responses for each trait, i.e. Eve displayed a resistant response while Doyce displayed a susceptible response for all tests except for incidence at MHV in 2014-15 where both parents displayed the same response (Figure 2.3). High disease pressure at MHV may have caused parent lines to display the same response for incidence during 2014-15.

Data for FHB traits partitioned on the basis of heading date or height classes (high, medium and low values) was analyzed in an effort to determine possible interactions between these morphological characteristics and FHB traits (Figures 2.6 and 2.7). RILs were categorized
into classes (high, medium, or low mean values) based on either height or heading date. When grouped by height there was no statistical difference between the classes for heading date or incidence, but there were statistical differences between classes for severity, FDK and DON (Figure 2.6). When grouped by heading date there was no statistical difference between the classes for FDK, but there were statistical differences between classes for height, incidence, severity, and DON (Figure 2.7).

Significant positive correlations were found between incidence, severity and DON at BVA during 2015-16, MHV during 2014-15, and LKY in 2014-15 and 2015-16 (Figure 2.8). Morphological traits such as heading date and height were significantly correlated to each other as well as FHB traits across both growing seasons and locations except KNC (Figure 2.8). Generally, significant correlations were not observed between 2014-15 KNC and the other experimental sites. Stronger correlations were generally observed between FHB traits with heading date or height in the 2015-16 BVA and during both growing season at LKY (Figure 2.8).

**Genotypic Analysis**

From the Illumina® 9K iSelect SNP chip, 330 polymorphic markers were found between the parents, Eve and Doyce. Among those 330 polymorphic markers dispersed across all seven chromosomes, only 11 markers on chromosome 6H were significantly associated with FHB resistance. An additional nine SSR markers were analyzed to further facilitate construction of a linkage map, and three of them were polymorphic among the parents and RILs. All seven chromosomes were mapped, but the only significant linkage group was on chromosome 6H within the mapping population. Consensus maps constructed from Muñoz et al. (2011) and Varshney et al. (2007) were used to confirm the location of the identified QTL. The linkage map for chromosome 6H contains a resistance QTL associated with FHB Inc, FHB Sev, FDK, and DON as well as the morphological traits heading date and plant height (Figure 2.9).

The QTL region identified on chromosome 6H is derived from the moderately resistant FHB parent Eve. The QTL marker region spans from SCRI_RS_147342 – Bmag0613 (56.5 – 66.6 cM), with significant LOD values ranging from 3.2 – 10.6 (Table 2.3). QTL associated with heading date and plant height at LKY during 2015-16 spans the marker interval SCRI_RS_147342 – BOPA1_4676-1-1-59 (56.5 – 60.0 cM). The QTL was significant with LODs of 10.6 and explained 25.2% of the phenotypic variation. In the same marker interval,
QTL associated with FHB Sev and FDK also were observed and explained 5.6% to 8.1% and 11.9% to 17.6% of the phenotypic variation, respectively. The QTL association with DON was consistent across multiple locations and years with the most significant locations being KNC (LOD 4.51) and MHV (LOD 3.59) during 2014-15. The marker interval associated with reduced DON concentration explained 8.95% and 14.5% of the phenotypic variation. In the same marker interval as DON, a QTL associated with height was also identified at LKY in 2014-15 and BVA in 2015-16 with LOD scores of 6.22 and 6.33, respectively.

**DISCUSSION**

FHB resistance has been previously identified on all seven chromosomes in the barley genome (Mesfin et al., 2003). Previous research has identified numerous FHB resistance QTL on chromosome 6H associated with lower FHB Sev and reduced DON accumulation in hulled, two row, spring barley cultivars such as Fredrickson (Canci et al., 2004, Dahleen et al., 2003, Ma et al., 2000, Mesfin et al., 2003). Research conducted by Berger et al. (2014), identified several potential sources of FHB resistance in the Virginia Tech Small Grains Breeding Program, including the hulless winter cultivar Eve. FHB resistance has been difficult to identify and incorporate into small grains breeding programs due to its quantitative nature, low heritability, association with morphological traits, and the strong influence by environmental factors (Massman et al., 2011, Zhu et al., 1999). In the current study, a FHB resistance QTL on chromosome 6H was identified in the hulless, winter, six row, cultivar Eve that is associated with reduced FHB Sev, DON accumulation, and FDK.

Previous mapping studies conducted on FHB resistance used SSR and restricted fragment length polymorphisms (RFLPs) to identify resistance QTL and construct linkage maps. Varshney et al. (2007) constructed a consensus map including identified FHB resistance QTL across the entire barley genome. Significant SSR markers, Bmag0613 and GBM1021, that are associated with FHB resistance, were identified and analyzed in the Eve/Doyce mapping population and reside in the same relative position on the constructed chromosome 6H consensus map. Recent studies have incorporated and identified SNP markers associated with FHB resistance due to their reduced cost and precision. Muñoz et al. (2011) developed an improved consensus map that contains 2,994 SNP loci that are mapped to 1,163 unique positions. Although this consensus map
contains numerous SNP markers, it does not primarily focus on SNP markers associated with FHB resistance. Diagnostic markers such as the 11 identified in the Eve/Doyce mapping population can be useful in incorporating and pyramiding QTL and/or genes from diverse sources of FHB resistance into adapted varieties.

The FHB resistance QTL identified in the Eve/Doyce mapping population was identified using SNP markers and covers the marker interval from SCRI_RS_147342 – BOPA1_8220-1223. This region spans approximately 3.5 cM (56.5 – 60.0 cM) with the SSR marker Bmag0613 as one of the flanking markers (66.6 cM). SSR marker positions for GBM1021 and Bmag0613 were compared to the consensus map created by Varshney et al. (2007) to confirm the identified FHB resistant QTL. The QTL was consistently associated with reduced DON accumulation, across multiple locations and years, and explained as much as 14.5% of the phenotypic variation. Reduced DON accumulation is a critical form of resistance due DON’s significant impact on barley producers. Research conducted by Canci et al. (2004), Dahleen et al. (2003), and Ma et al. (2000) reported resistance to DON accumulation on chromosome 6H. A QTL associated with lower FHB Sev was identified on 6H within the marker interval SCRI_RS_147342 – BOPA1_4676-1-1-59 across two locations and both growing seasons, and explained up to 8.1 % of the phenotypic variation. Canci et al. (2004), Dahleen et al. (2003), Ma et al. (2000), and Mesfin et al. (2003) reported resistance associated with FHB Sev on chromosome 6H. Canci et al. (2004) reported FHB resistance in a similar region on 6H, using a mapping population derived from Chevron that also was identified in the current study. Resistance to FDK was identified in the same marker interval as FHB Sev at two locations over both growing seasons and accounted for 11.9 % to 17.6% of the phenotypic variation. It is often common to see multiple FHB resistance traits identified in the same QTL region, which indicates that selection for resistance to FHB Sev or FDK may indirectly select for resistance to DON accumulation.

Although a significant QTL associated with FHB resistance was identified on chromosome 6H in this study, the morphological traits heading date and plant height were also associated with this region. A QTL associated with heading date and height was consistent between two locations over both growing seasons and mapped near the same interval region associated with reduced DON accumulation. Height was also identified in the same marker
region as FHB Sev and FDK. The question arises as to whether the QTL is conferring actual resistance or if heading date or height are indirectly influencing FHB resistance. Nduulu et al. (2007) performed a study to determine if the linkage between heading date and FHB resistance could be broken. Nduulu et al. (2007) identified only one recombinant line out 1,500 NILs screened in which the negative linkage between heading date and FHB resistance was broken. Although it was proven possible, breaking the linkage between morphological traits such as heading date and FHB resistance may be extremely difficult to achieve.

Through phenotypic and genotypic analysis performed in the current study, a QTL for FHB resistance on chromosome 6H in Eve was identified and confirmed to be similar to previously reported QTL on chromosome 6H. Based on these results, we reject the initial hypothesis proposed by Berger et al. (2014) and conclude that the FHB resistance identified in Eve is not uniquely different than previously identified FHB resistance. Previous studies have identified FHB resistance QTL near or in the same region on chromosome 6H (Canci et al., 2004, Dahleen et al., 2003, Ma et al., 2000, Mesfin et al., 2003). The QTL identified was found to be associated with lower FHB Sev, FDK, and DON accumulation as well as affecting heading date and height. Although the FHB resistant QTL identified is not unique, the diagnostic SNP makers identified in this study will facilitate rapid high-throughput genotyping and implementation of more efficient MAS breeding to incorporate and pyramid FHB resistance QTL and genes into adapted cultivars.
LITERATURE CITED


Nduulu, L., A. Mesfin, G. Muehlbauer and K. Smith. 2007. Analysis of the chromosome 2 (2H) region of barley associated with the correlated traits Fusarium head blight resistance and heading date. Theoretical and applied genetics 115: 561-570.


<table>
<thead>
<tr>
<th>Location</th>
<th>Planting Date</th>
<th>Harvest Date</th>
<th>Fusarium Inoculated Corn (g / plot)</th>
<th>Fusarium Corn Application</th>
<th>Pre-Plant Fertilizer (kg ha⁻¹)</th>
<th>Harmony Extra SG* (L ha⁻¹)</th>
<th>Fertilizer (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacksburg, VA</td>
<td>10/2/2014</td>
<td>6/14/15 - 6/15/15</td>
<td>25</td>
<td>4/22/15, 5/1/15</td>
<td>30-50-50†</td>
<td>0.73 (2)</td>
<td>56.0‡</td>
</tr>
<tr>
<td>Mount Holly, VA</td>
<td>10/16/2014</td>
<td>6/13/2015</td>
<td>25</td>
<td>4/7/15</td>
<td>35-75-110†</td>
<td>0.55</td>
<td>44.8†</td>
</tr>
<tr>
<td>Lexington, KY</td>
<td>10/23/2014</td>
<td>6/13/15 - 6/14/15</td>
<td>25</td>
<td>4/13/15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

†Fertilizer application of (N-P-K)
‡Fertilizer application of N
§Fertilizer application of UAN
¶Fertilizer application of N in (24-0-0-3) proportions
### Table 2.2 Management Practices for All Locations during 2015-16

<table>
<thead>
<tr>
<th>Location</th>
<th>Planting Date</th>
<th>Harvest Date</th>
<th>Fusarium Inoculated Corn (g / plot)</th>
<th>Fusarium Corn Application</th>
<th>Pre-Plant Fertilizer (kg ha⁻¹)</th>
<th>Harmony Extra SG (L ha⁻¹)</th>
<th>Fertilizer (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacksburg, VA</td>
<td>10/16/2015</td>
<td>6/11/16</td>
<td>25</td>
<td>4/12/16, 4/25/16</td>
<td>30-60-80⁺</td>
<td>0.44 / 0.55</td>
<td>50.4⁺</td>
</tr>
<tr>
<td>Mount Holly, VA</td>
<td>10/26/2015</td>
<td>6/13/16</td>
<td>25</td>
<td>4/11/16</td>
<td>30-60-60-5</td>
<td>0.55</td>
<td>28 / 67.3⁺</td>
</tr>
<tr>
<td>Kinston, NC</td>
<td>10/1/2015</td>
<td>-</td>
<td>4.13</td>
<td>3/14/16, 3/21/16, 3/28/16</td>
<td>30.6</td>
<td>applied w/ N</td>
<td>112.1⁺</td>
</tr>
<tr>
<td>Lexington, KY</td>
<td>10/19/2015</td>
<td>6/23/16 - 6/24/16</td>
<td>25</td>
<td>4/13/16</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Fertilizer application of (N-P-K)
²Fertilizer application of N
³Fertilizer application of UAN

---
Table 2.3 Resistance quantitative trait loci (QTL) on Chromosome 6H associated with Fusarium head blight (FHB) in the winter hulless barley mapping population Eve/Doyce

<table>
<thead>
<tr>
<th>Trait†</th>
<th>Maker Interval‡</th>
<th>LOD</th>
<th>Variation (%)§</th>
<th>Additivity¶</th>
<th>Position Interval (cM)‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNC_HD_15</td>
<td>SCRI_RS_147666 - BOPA1_82220-1223</td>
<td>5.43</td>
<td>15.51</td>
<td>-0.97</td>
<td>57.5 - 60.5</td>
</tr>
<tr>
<td>LKY_HD_15</td>
<td>SCRI_RS_147666 - BOPA1_82220-1223</td>
<td>2.20</td>
<td>6.85</td>
<td>-0.40</td>
<td>56.5 - 59.5</td>
</tr>
<tr>
<td>BVA_HD_16</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>5.55</td>
<td>14.33</td>
<td>-0.48</td>
<td>56.5 - 59.5</td>
</tr>
<tr>
<td>LKY_HD_16</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>10.55</td>
<td>25.20</td>
<td>1.16</td>
<td>56.5 - 57.5</td>
</tr>
<tr>
<td>LKY_HT_15</td>
<td>BOPA1_82220 - 1223 - Bmag0613‡‡</td>
<td>6.22</td>
<td>17.05</td>
<td>3.24</td>
<td>58.5 - 64.5</td>
</tr>
<tr>
<td>BVA_HT_16</td>
<td>BOPA1_8220 - 1223 - Bmag0613‡‡</td>
<td>6.63</td>
<td>16.38</td>
<td>3.15</td>
<td>59.5 - 65.5</td>
</tr>
<tr>
<td>LKY_HT_16</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>10.55</td>
<td>25.20</td>
<td>2.93</td>
<td>56.5 - 57.5</td>
</tr>
<tr>
<td>LKY_Inc_16</td>
<td>SCRI_RS_114741 - SCRI_RS_147666</td>
<td>1.89</td>
<td>4.91</td>
<td>-1.08</td>
<td>51.5 - 59.5</td>
</tr>
<tr>
<td>LKY_Sev_15</td>
<td>BOPA1_82220 - 1223 - Bmag0613‡‡</td>
<td>2.76</td>
<td>11.49</td>
<td>-1.18</td>
<td>58.5 - 62.5</td>
</tr>
<tr>
<td>MHV_Sev_15</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>3.24</td>
<td>8.13</td>
<td>-1.56</td>
<td>56.5 - 57.5</td>
</tr>
<tr>
<td>LKY_Sev_16</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>1.76</td>
<td>5.56</td>
<td>-1.47</td>
<td>47.5 - 61.5</td>
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<tr>
<td>KNC_FDK_15</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>3.32</td>
<td>11.87</td>
<td>-4.68</td>
<td>56.5 - 57.5</td>
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<tr>
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<td>BOPA1_82220 - 1223 - Bmag0613‡‡</td>
<td>1.73</td>
<td>4.91</td>
<td>-1.28</td>
<td>47.5 - 67.5</td>
</tr>
<tr>
<td>LKY_FDK_16</td>
<td>SCRI_RS_147342 - BOPA1_4676-1-1-59</td>
<td>6.28</td>
<td>17.63</td>
<td>-3.36</td>
<td>56.5 - 57.5</td>
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<tr>
<td>KNC_DON_15</td>
<td>BOPA1_82220 - 1223 - Bmag0613‡‡</td>
<td>4.51</td>
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<td>-1.35</td>
<td>58.5 - 62.5</td>
</tr>
<tr>
<td>LKY_DON_15</td>
<td>BOPA1_8220 - 1223 - Bmag0613‡‡</td>
<td>2.57</td>
<td>7.76</td>
<td>-2.61</td>
<td>58.5 - 64.5</td>
</tr>
<tr>
<td>MHV_DON_15</td>
<td>BOPA1_8220 - 1223 - Bmag0613‡‡</td>
<td>3.59</td>
<td>8.95</td>
<td>-2.01</td>
<td>58.5 - 63.5</td>
</tr>
<tr>
<td>LKY_DON_16</td>
<td>BOPA1_8220 - 1223 - Bmag0613‡‡</td>
<td>1.94</td>
<td>5.32</td>
<td>-1.58</td>
<td>51.5 - 64.5</td>
</tr>
</tbody>
</table>

†Trait is formed at Location Trait Year where: HD = heading date, HT = height, Inc = incidence, Sev = severity, FDK = Fusarium damaged kernels, DON = deoxynivalenol; BVA = Blacksburg, VA, KNC = Kinston, NC, LKY = Lexington, KY, MHV = Mount Holly, VA; years are 15 = 2014-15, 16 = 2015-16.
‡Flanking markers for QTL.
§Percent variation explained by the individual QTL.
¶Additive effect (negative number = QTL infers resistance; positive number = QTL infers susceptibility)
‖Position of the highest LOD score in terms of genetic distance from the first maker of the interval.
‡‡Markers designated 'Bmag' are single sequence repeats (SSRs); all other markers are single nucleotide polymorphisms (SNPs)
Figure 2.1 Distribution of the RILs and parents derived from the Eve/Doyce Fusarium Head Blight (FHB) mapping population for assessment of FHB traits at Kinston, NC (KNC) during 2014-15. The data shown is the average value per FHB trait assessed at KNC. (a) FHB incidence (Inc; % of infected barley heads per plot). (b) FHB severity (Sev; % of infected spikelets). (c) The percentage of Fusarium damaged kernels from harvested samples (FDK). (d) Deoxynivalenol (DON) concentration in ppm from harvested samples. The arrows indicate the average value for parent lines, Eve (resistant) and Doyce (susceptible), for each of the FHB trait assessed.
Figure 2.2 Distribution of the RILs and parents derived from the Eve/Doyce Fusarium Head Blight (FHB) mapping population for assessment of FHB traits at Lexington, KY (LKY) during 2014-15. The data shown is the average value per FHB trait assessed at LKY. (a) FHB incidence (Inc; % of infected barley heads per plot). (b) FHB severity (Sev; % of infected spikelets). (c) The percentage of Fusarium damaged kernels from harvested samples (FDK). (d) Deoxynivalenol (DON) concentration in ppm from harvested samples. The arrows indicate the average value for parent lines, Eve (resistant) and Doyce (susceptible), for each of the FHB trait assessed.
Figure 2.3 Distribution of the RILs and parents derived from the Eve/Doyce Fusarium Head Blight (FHB) mapping population for assessment of FHB traits at Mount Holly, VA (MHV) during 2014-15. The data shown is the average value per FHB trait assessed at MHV. (a) FHB incidence (Inc; % of infected barley heads per plot). (b) FHB severity (Sev; % of infected spikelets). (c) The percentage of Fusarium damaged kernels from harvested samples (FDK). (d) Deoxynivalenol (DON) concentration in ppm from harvested samples. The arrows indicate the average value for parent lines, Eve (resistant) and Doyce (susceptible), for each of the FHB trait assessed.
Figure 2.4 Distribution of the RILs and parents derived from the Eve/Doyce Fusarium Head Blight (FHB) mapping population for assessment of FHB traits at Blacksburg, VA (BVA) during 2015-16. The data shown is the average value per FHB trait assessed at BVA. (a) FHB incidence (Inc; % of infected barley heads per plot). (b) FHB severity (Sev; % of infected spikelets). (c) The percentage of Fusarium damaged kernels from harvested samples (FDK). (d) Deoxynivalenol (DON) concentration in ppm from harvested samples. The arrows indicate the average value for parent lines, Eve (resistant) and Doyce (susceptible), for each of the FHB trait assessed.
Figure 2.5 Distribution of the RILs and parents derived from the Eve/Doyce Fusarium Head Blight (FHB) mapping population for assessment of FHB traits at Lexington, KY (LKY) during 2015-16. The data shown is the average value per FHB trait assessed at LKY. (a) FHB incidence (Inc; % of infected barley heads per plot). (b) FHB severity (Sev; % of infected spikelets). (c) The percentage of Fusarium damaged kernels from harvested samples (FDK). (d) Deoxynivalenol (DON) concentration in ppm from harvested samples. The arrows indicate the average value for parent lines, Eve (resistant) and Doyce (susceptible), for each of the FHB trait assessed.
Figure 2.6 Interaction between height (HT) in cm and the average of given Fusarium head blight (FHB) trait for each of the RILs in the Eve/Doyce mapping population across all locations (BVA, KNC, LKY, and MHV) and both growing seasons (2014-15 and 2015-16). Heading date (HD) is recorded in Julian. FHB incidence (Inc; % of infected barley heads per plot). FHB severity (Sev; % of infected spikelets). Fusarium damaged kernels (FDK) is the percentage of infected kernels per harvested sample. Deoxynivalenol (DON) content in ppm from harvested samples. Classes indicate the range in which each RIL’s height corresponded to: Low = 53.08 – 69.85 cm; Medium = 70.27 – 79.59 cm; and High = 80.01 – 88.90 cm. Letters designation above the bars indicate statistical differences between HT and each FHB trait, different letters indicate significant statistical differences.
Figure 2.7 Interaction between heading date (HD) in Julian and the average of given Fusarium head blight (FHB) trait for each of the RILs in the Eve/Doyce mapping population across all locations (BVA, KNC, LKY, and MHV) and both growing seasons (2014-15 and 2015-16). Height (HT) is record in cm. FHB incidence (Inc; % of infected barley heads per plot). FHB severity (Sev; % of infected spikelets). Fusarium damaged kernels (FDK) is the percentage of infected kernels per harvested sample. Deoxynivalenol (DON) content in ppm from harvested samples. Classes indicate the range in which each RIL’s HD corresponded to: Low = 114.0 – 117.90; Medium =118.0 – 119.90; and High = 120.0 – 121.50. Letters designation above the bars indicate statistical differences between HT and each FHB trait, different letters indicate significant statistical differences.
Figure 2.8 Correlation plot of Fusarium head blight (FHB) trait for each of the RILs in the Eve/Doyce mapping population across all locations (BVA, KNC, LKY, and MHV) and both growing seasons (2014-15 and 2015-16). Heading date (HD) was recorded in Julian. Height (HT) was recorded in cm. FHB incidence (Inc; % of infected barley heads per plot). FHB severity (Sev; % of infected spikelets). Fusarium damaged kernels (FDK) is the percentage of infected kernels per harvested sample. Deoxynivalenol (DON) content in ppm from harvested samples. Significant correlations are indicated by color: orange indicates positive correlation (value of 1.0) and purple indicates negative correlation (value of -1.0).
Figure A1. Comparison of Raw data and normalized data for the FHB Sev at Lexington, KY (LXY) during 2015-16. (a) Population distribution of the Recombinant Inbred Lines (RILs) constructed using the raw data for FHB severity (Sev; % of infected spikelets) at LXY. (b) Population distribution of the RILs constructed using normalized data for FHB Sev at LXY. Normalized data was transformed using log.
Figure A2. Comparison of Raw data with outliers and Raw data without outliers at FHB Sev at Lexington, KY (LXY) during 2015-16. (a) Recombinant Inbred Lines (RILs) plotted for each repetition at LXY for FHB severity with no outliers removed (Sev; % of infected spikelets). (b) RILs plotted for each repetition at LXY for FHB severity with outliers removed.