University of Kentucky
UKnowledge

Theses and Dissertations--Plant and Soil Sciences

Plant and Soil Sciences

2014

FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT POPULATIONS

Daniela Sarti Dvorjak
University of Kentucky, danisarti@gmail.com

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Dvorjak, Daniela Sarti, "FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT POPULATIONS" (2014). *Theses and Dissertations--Plant and Soil Sciences*. 38.
https://uknowledge.uky.edu/pss_etds/38

This Doctoral Dissertation is brought to you for free and open access by the Plant and Soil Sciences at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Plant and Soil Sciences by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Daniela Sarti Dvorjak, Student

Dr. David A. Van Sanford, Major Professor

Dr. Mark Coyne, Director of Graduate Studies

FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT POPULATIONS

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture, Food and Environment at the University of Kentucky

By

Daniela Sarti Dvorjak

Lexington, Kentucky

Director: Dr. David A. Van Sanford, Professor of Agronomy

Lexington, Kentucky

2014

Copyright © Daniela Sarti Dvorjak 2014

ABSTRACT OF DISSERTATION

FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT POPULATIONS

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schwein. (Petch)], is recognized as one of the most destructive diseases of wheat (*Triticum aestivum* L. and *T. durum* L.) and barley (*Hordeum vulgare* L.) worldwide. Breeding for FHB resistance must be accompanied by selection for desirable agronomic traits. Donor parents with two FHB resistance quantitative trait loci (QTL) *Fhb1* (chromosome 3BS) and *QFhs.nau-2DL* (chromosome 2DL) were crossed to four adapted SRW wheat lines to generate backcross and forward cross progeny. F_2 individuals were genotyped and assigned to 4 different groups according to presence/ absence of one or both QTL. The effectiveness of these QTL in reducing FHB in F_2 derived lines was assessed in a misted, inoculated scab nursery.

Resistance alleles and the interaction among FHB resistance QTL have distinct behavior in different genetic backgrounds in wheat. *Fhb1* showed an average disease reduction of 12%, however it did not result in significant improvement of FHB resistance in all populations. In general, for the four backgrounds studied, the *QFhs.nau-2DL* QTL was more effective reducing FHB (19% average reduction). The combination of *Fhb1* and *QFhs.nau-2DL* is not necessary, but recommended and it improved resistance in all populations.

Backcross derived (BC) progeny from diverse backgrounds were planted in replicated plots (2011 and 2012) and in the scab nursery in 2012. Population 2 had its progeny characterized by 961 DArT markers distributed throughout the genome. Several high-quality polymorphic markers were identified and listed as good predictors of phenotypic traits like disease resistance, and improved agronomic and quality characteristics. Backcross and forward cross derived progenies were tested for FHB resistance, agronomic and baking quality performance for 4 different populations sharing the same donor parent for resistance QTL.

The results confirmed that F_2 populations were effective indicators of expression levels of QTL prior to extensive backcrossing. QTL *Fhb1* and *QFhs.nau-2DL* increased

FHB resistance without detriments on agronomic and quality traits on wheat populations investigated. BC populations were assessed as breeding populations and established as being rewarding tools for derivation of inbred lines in a breeding program, being BC_2 the most recommended from our results.

KEYWORDS: *Triticum aestivum*, Wheat breeding, Fusarium head blight, Deoxynivalenol, Backcross.

Daniela Sarti Dvorjak

January, 31st 2014

FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT POPULATIONS

By

Daniela Sarti Dvorjak

Dr. David A. Van Sanford

Director of Dissertation

Dr. Mark Coyne

Director of Graduate Studies

January, 31st 2014

ACKNOWLEDGEMENTS

The following dissertation, while an individual work, benefited from the insights and direction of several people. First, my Dissertation Chair and Adviser, Dr. David Van Sanford, exemplifies the high quality scholarship to which I aspire. He has been extraordinarily supportive and his selfless time and care were an enormous help to me. Next, I wish to thank the complete Dissertation Committee: each individual provided insights that guided and challenged my thinking, substantially improving the finished product. Dr. Todd Pfeiffer provided timely and instructive comments, and contributed to my formation as a Plant Breeder. Dr. Timothy Phillips, for his constructive explanations, warm encouragement, and extraordinary knowledge of plant sciences and breeding. Dr. Hongyan Zhu, for helpful observations and suggestions. Dr. John Lhotka, for the great feedback and generous support. In addition to the technical and instrumental assistance above, I received equally important assistance from family and friends. My husband, Daniel Dvorjak, whose faith and patience provided on-going support throughout the dissertation process. My parents, Luiz Carlos and Tania Mara Sarti, instilled in me, from an early age, the desire and skills to pursue higher education. I am also deeply grateful to my brother Bruno Sarti, for constant encouragement and for always finding the positive side of things and situations. Special thanks to my officemate Kenneth Cropper, for his friendship and support during Graduate School. Finally, I wish to thank the Wheat Breeding Group at University of Kentucky: Dr. David Van Sanford, Dr. Anthony Clark, John Connelley, Sandy Swanson, Richard Green and M.Sc. Ana Balut. Their insights and help created a positive work environment and the constructive team work was an essential motivation on the development of this interesting project.

Table of Contents

List of Tables

List of Figures

Chapter 1.

Introduction

Wheat is a widely cultivated crop with 2010 world production greater than 641 million tons according to USDA (2010). Fusarium head blight, caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schwein.(Petch)], is recognized as one of the most-destructive diseases of wheat. The cumulative direct economic losses from FHB in hard red spring (HRS) wheat, soft red winter (SRW) wheat, durum wheat, and barley is estimated at $870 million from 1998 through 2000. The combined direct and secondary economic losses for all the crops were estimated at $2.7 billion (Nganje et al., 2002).

Genetic resistance to *Fusarium* head blight (FHB) is considered the best strategy to reduce the wheat grain yield and quality losses caused by this disease (McMullen et al., 1997). Therefore, the availability of genetically diverse germplasm with broad resistance to FHB is important to the success of wheat improvement programs.

This study evaluated lines derived from different breeding methods, with a defined source of resistance to FHB, aiming to investigate the effect of resistance alleles and the interaction among FHB resistance QTL, in different genetic backgrounds in wheat. The objectives of the study were to: 1) validate two FHB resistance Quantitative Trait Loci (QTL) in diverse backgrounds; 2) compare performance of breeding lines

derived from forward and backcrossing and 3) explore the utility of Diversity Arrays Technology (DArT) markers as a tool to accelerate breeding projects.

Copyright © Daniela Sarti Dvorjak 2014

Chapter 2.

Literature Review

2.1. Wheat Crop

Wheat (*Triticum aestivum* L.) is grown worldwide with an annual average production of 23.7 billion bushels in 2010 (USDA, 2011). In United States (U.S.), wheat is grown commercially in nearly every State, with the majority of the 2.0 billion bushels (USDA, 2013) being produced in the Great Plains (from Texas to Montana), with Kansas as the leader.

Wheat grown in the United States is either “winter wheat” or “spring wheat” depending on the season in which it is planted. Winter wheat varieties are sown in the fall and make some preliminary growth before cold weather arrives. The plants lie dormant through the winter. In the spring, they resume growth and grow rapidly until summer harvest. Winter wheat usually accounts for about two thirds of U.S. production. Spring wheat varieties are planted in the spring, when the ground is tillable, and grow continuously until harvest in July – August (Ali et al., 2000).

Wheat in the U.S. can be grouped into six market classes. Each class of wheat has its own characteristic and can be recognized by the time of the year it is planted, by the hardness, color and kernel shape, and by milling and baking quality traits. Where each class of wheat is grown depends largely upon rainfall, temperature, soil conditions and tradition. The market classes are Hard Red Winter (HRW), Soft Red Winter (SRW), Hard

Red Spring (HRS), Hard White (HW), Soft White (SW), and Durum wheat (Ali et al., 2000).

Hard Red Winter is the dominant class in U.S. exports and the largest class produced each year. This class presents a wide range of protein content in seeds, good milling and baking characteristics, and is mainly produced in the Great Plains states, a large interior area extending from the Mississippi River west to the Rocky Mountains and from Canada to Mexico. Hard Red Spring wheat contains the highest percentage of protein, making excellent bread wheat with superior milling and baking characteristics. The majority of this class is grown in Montana, North Dakota, South Dakota and Minnesota. Soft Red Winter is grown primarily east of the Mississippi River, including Kentucky. It is high yielding, but relatively low in protein content with versatile but weak gluten. Hard White wheat is the newest class of wheat to be grown in the United States and is closely related to red wheat, with a milder sweet flavor, equal fiber and similar milling and baking properties. Soft White wheat is low moisture with high extraction rates, it has low protein, but it is a high yielding wheat used in much the same way as SRW for baking products other than bread. SW wheat is grown mainly in the Pacific Northwest and to a lesser extent in California, Michigan, Wisconsin and New York. Durum is the hardest of all U.S. wheat and consistently the class with the lowest export volume, accounting for less than 5% of all U.S. wheat exports. It has a rich amber color and high gluten content. Durum wheat is grown in the same northern states as Hard Red Spring, with 70 to 80 percent of the production coming from North Dakota (http://www.smallgrains.org/WHFACTS.HTM).

In 2012/2013, U.S farmers planted wheat on 55.7 million acres and produced 2,266 million bushels, down 11.8 percent from 2011/2012. The average yield per harvest acre was 46.3 bushels and the exports reached 1.0 million bushels (USDA, 2013).

Wheat is especially adaptable to extreme weather conditions and grows best on well-drained soils, but yields generally improve if irrigation is used. The quantity of nitrogen applied varies among regions, ranging from 50 pounds per acre in the Northern Plains to 85 pounds per acre in the North Central Region. Fertilizers are generally applied at higher rates in the eastern regions because of double-cropping and the large amount of wheat acreage harvested for straw (Ali et al., 2000).

The fact that wheat is the principal cereal grain crop used for food consumption in the United States and most of the world; thus additional knowledge on wheat production and its major variables such as agricultural management and disease control is always desirable.

2.2 *Fusarium* Head Blight in Wheat

Fusarium head blight (FHB), generically known as scab, is a significant disease of small grain cereals and has been reported throughout the world, severely limiting crop productivity, particularly in wheat (*Triticum aestivum* L. and *T. durum* L.) and barley (*Hordeum vulgare* L.).

The first description of FHB was in 1884 in England and it was considered to be a major hazard to the production of small grains during the early years of last century (Stack, 2003). In 1890, an outbreak associated with important yield loss in wheat was reported in Indiana (Bai & Shaner, 1994). Severe FHB epidemics have been reported throughout United States, Canada, South America, Europe and Asia during the twentieth century (McMullen et al., 1997).

According to McKay (1957), a severe head blight occurrence in Ireland in 1942, decreased wheat yield by between 21 - 55%. During the 1990's devastating outbreaks of FHB occurred in United States, causing significant economical and sociological harm to the affected areas. Losses due to FHB in the small grain producing areas of Minnesota, North Dakota, South Dakota and Manitoba in 1993 were estimated in the range of $1 billion (McMullen et al., 1997). According to Sayler (1998), in nine US states between 1991 and 1996, wheat producers lost 501 million bushels of grain, equivalent to $2.6 billion.

The causal agent is an ascomycete fungus (in the U.S., primarily *Fusarium graminearum* Schwabe) that infects developing wheat spikes and results in kernels with varying degrees of infection (Bechtel et al., 1985). Initial symptoms of FHB appear as slightly brown water-soaked spots present on the glumes. The lesions increase in size until the whole spikelet is covered and, depending on weather conditions, spread to the neighboring spikelets. Eventually, the fungi-infected spikelets become necrotic and bleached (Pirgozliev et al., 2003). Also, under humid conditions, pink mold can be seen on the surface of the glumes. Later in the season, black raised spots formed by perithecia

may appear (Bai & Shaner, 1994). Grain harvested from FHB-affected heads is often shriveled and may have a red discoloration due to the presence of fungal growth (Pirgozliev et al., 2003).

The presence of *Fusarium* spp. in wheat can cause deleterious effects on grain processing qualities. The fungus can destroy starch granules, storage proteins and cell walls during invasion of wheat grains (Bechtel et al., 1985). Dexter et al. (1997) evidenced in Canada that hard red spring wheat grain samples that contained *Fusarium* damaged kernels (FDK) exhibited weak dough properties and unsatisfactory baking quality. Studies of the effects of fungal proteases on wheat storage proteins suggest that *F. graminearum* and *F. avenaceum* produce proteolytic enzymes. These enzymes hydrolyze endosperm proteins during dough mixing and fermentation and result in weaker dough and decreased loaf volume (Nightingale et al., 1999). In barley, infection of grains with *Fusarium* spp. reduces malt yield and quality, as well as causing reduced gas stability and uncontrolled foaming of beer (gushing) during the malting process (Bechtel et al., 1985); Schwarz et al., 2002). In addition to quantitative losses, *F. graminearum* also causes a reduction in grain quality due to the production of trichothecene mycotoxins.

2.3 Deoxynivalenol Production and Impact

Apart from the effects on seed and grain processing qualities, *Fusarium* species produce a range of toxic metabolites. These include a number of mycotoxins belonging to

the trichothecene group. The mycotoxin deoxynivalenol (DON) is a vomitoxin and poses a serious hazard to human and animal health because it is potent inhibitor of eukaryotic protein biosynthesis (Bai & Shaner, 2004).

If grain contaminated with *Fusarium* is used as feed for animal or human consumption, numerous adverse toxicoses as well as anorexia, emesis and other health disorders are observed. In ruminant animals, the effect of DON-contaminated feed grain is vomiting and serious feeding problems (McMullen et al., 1997). Among farm animals, pigs show greatest sensitivity to DON, causing a series of reproductive disorders in young pigs, increase in stillborn and small litters (Miller et al., 1973).

The level of DON concentration in grain is extremely variable and difficult to predict, depending on the wheat variety, the fungal genotype and the environmental conditions (Mesterhazy et al., 1999). As result, several countries have adopted advisory limits to ensure minimum levels of DON in finished products intended for human consumption and for animal feeds (Van Egmond, 1989). The Food and Drug Administration (FDA) in the United States recommends that DON levels should not exceed 1 ppm in finished wheat products for human consumption and should not exceed 5 ppm for grain and grain byproducts destined for swine and other animal species (except cattle and chickens) and should not exceed 10 ppm for feed intended for chicken and cattle, respectively (http://www.gipsa.usda.gov/GIPSA/documents/GIPSA_Documents/b-vomitox.pdf; verified 10 October, 2010). The Chinese advisory limit of DON in grains is 1 ppm. The proposed advisory limits for trichothecene mycotoxins to be adopted within

the European Union are 0.5 ppm for retail product such as breakfast cereals, bread and pasta and 0.75 ppm for flour and grain (Pirgozliev et al., 2003; Pricket et al., 2000).

2.4. Breeding for FHB Resistance

FHB may cause serious losses, both in terms of lower grain yields and significantly reduced test weights. The majority of the yield losses sustained is the result of loss of kernel development, reduced grain set and lightweight kernels that are shriveled and discolored, and easily blown out the back of the combine during harvest operations (Hershman, 1997). The greatest concern with FHB- infected wheat is that fungal toxins (mycotoxins) may be produced in the infected grain (McMulen et al., 1997; Agostinelli et al., 2011). The mycotoxin, deoxynivalenol (DON) causes feed refusal or poor weight gain in animals and may cause immunological and teratogenic problems in humans (Desjardins, 2006).

Since the 1990s, devastating outbreaks of FHB have occurred in United States with important economic losses. In 2003, a significant epidemic occurred on soft red winter wheat grown in Kentucky, Maryland, North Carolina, Pennsylvania, southern Illinois, Indiana and Ohio; west Tennessee; and Virginia, with estimated loss of $13.6 million (Cowger & Sutton, 2005). The authors suggested that several million additional dollars were lost by millers in the region due to increased shipping costs, DON testing, and additional handling expenses (related to grain cleaning and blending).

The development of resistant varieties is considered to be the single best strategy to control the disease (McMullen et al., 1997; Rudd et al., 2001). Breeding programs have identified new resistance sources and also searched for a better ways to incorporate the existing kinds of resistance into breeding lines. Resistance to FHB is highly complex due to its quantitative inheritance and the high genotype by environment interactions, and additionally is sometimes associated with undesirable agronomic characteristics (Snijders, 1990; Bai & Shaner, 2004).

Backcross and doubled haploid approaches, as well as traditional breeding methods have been used in conjunction with phenotypic and marker assisted selection (MAS). Backcross breeding is the method of choice for gene introduction when a cultivar possesses many desirable properties, but lacks a specific trait that is known to reside in a crossable relative, or donor parent (Young & Tanksley, 1989).

Quantitative trait loci (QTL) have been detected in several mapping populations of both spring and winter wheat. FHB resistance QTL have been reported on all wheat chromosomes, with the exception of 7D (Buerstmayr et al., 2009). *Fhb1* is a major QTL identified on chromosome 3BS (Qfhs.ndsu-3BS) and has been incorporated and pyramided with FHB resistance QTL located on wheat chromosomes 1B, 2B, 2D, 3A, 3BSc, 4B, 5A, and 6B as well as from wheat relatives including *Qfhs.ndsu-3AS* from *T. dicoccoides* and *Qfhs.pur-7EL* from tall wheatgrass (Garvin et al., 2009; McCartney et al., 2007).

While accurate phenotyping remains essential, wheat breeding programs are adding scab resistance alleles to breeding material, and marker assisted selection (MAS)

is progressively being used to select for FHB resistance. Although challenges remain and progress has been steady, we can expect substantive genetic progress for the mid- and long-term (Bai & Shaner, 2004).

2.5. Soft Winter Wheat Flour Milling and Baking Quality

Wheat is the primary cereal consumed by humans around the world. Several products are derived from soft red winter (SRW) wheat including cakes, cookies, crackers, donuts, flat breads and breakfast cereals. These products require specific flour composition and rheological functionality that are collectively referred as quality. Milling quality is governed by flour yield and flour particle size. Baking quality is mainly function of gluten strength and water absorption (Smith et al., 2011). Selection for milling and baking quality in wheat head-rows could increase the efficiency of most breeding programs (Knott et al., 2009).

Understanding the different components allow breeders to identify SRW wheat lines with desirable quality. In North America, desired milling and baking targets include greater milling yield, reduced flour particle size, reduced flour water absorption, and a range of gluten strengths to facilitate manufacture of a diverse variety of products (Souza et al., 2012). Required flour functionality varies depending on the final end product, for example, flour for bread is very different from that from cookies or crackers or cakes. Flour for cookies generally requires low water absorption, minimal gluten strength, and low damaged starch and arabinoxylans. The biscuit industry generally prefers soft wheat

flours with high gluten strength but low water-holding capacity (WHC), especially for use in commercial cracker production (Kweon et al., 2011). Flour water holding capacity is increased by damaged starch (generated during flour milling) and arabinoxylans (originated from the aleurone bran layers of the wheat kernel) which are undesirable for good quality cookies and cracker flour, since they increase baking time and temperature, resulting in increased energy costs (Slade & Levine, 1994).

Gluten strength is determined primarily by flour protein concentration and the ability of the proteins to form viscoelastic networks, which is related to elasticity and the strength of the dough (Smith et al., 2011; Souza et al., 2012). The oxidative linking of proteins into large networks significantly reduces the length of time a cookie or other baked products spreads during baking, reducing the diameter of sugar-snap cookies (Pareyt et al., 2010).

Flour water absorption is affected by the amount of damaged starch in flour, the concentration and structure of non-starch polysaccharides, and the water binding properties of flour proteins. During the milling process, starch granules can be sheared and broken. The amount of damaged starch produced during milling is related to the hardness of the wheat kernel (Hogg et al., 2004). Greater concentration of water soluble arabinoxylans results in smaller (less desirable) cookie diameters (Guttieri et al., 2001).

Many assays have been developed to classify wheat lines. The most common flour-based assays include flour yield, softness equivalent, flour protein, solvent retention capacity, and sugar-snap cookies diameter. Flour yield and flour protein measure the percent of flour produced from a grain sample and protein content. Softness equivalent

measures flour particle size (associated with break flour yields) and is correlated to damaged starch. The sugar-snap cookie integrates the functionality of flour as a global indicator of milling quality, grain hardness, starch damaged by milling, flour protein, gluten strength, and water soluble non-starch polysaccharides (Souza et al., 2012).

Solvent retention capacity (SRC) tests predict commercial baking performance of soft wheat by measuring the weight of different solvents retained by the flour. The SRC test is a solvation assay for flours that is based on the enhanced swelling behavior of individual polymer materials in selected single diagnostic solvents – water, 5% w/w lactic acid (LA) in water (for glutenin), 5% w/w sodium carbonate (Na_2CO_3) in water (for damage starch), and 50% w/w sucrose in water (for pentosans) – which are measured by changes in weight, to predict the functional contribution of each individual flour component (Guttieri et al., 2001; Kweon et al., 2011). The water SRC is a test of global water absorption, sodium carbonate SRC tests damaged starch levels, flour sucrose SRC is a test for arabinoxylan (related to water absorption and cookie quality) and partially hydrated gliadin content. Sucrose SRC is considered the best predictor for cookie quality (Souza et al., 2012). The flour lactic acid SRC predicts gluten strength and it is correlated with the Sodium Dodecyl Sulfate (SDS) sedimentation test and flour protein concentration (Souza et al., 2011).

Guttieri et al. (2004) evaluated early generation soft wheat experimental lines, investigating two quality assays: whole grain-wheat meal SDS sedimentation volume (WM-SDS) and the whole grain-wheat meal sodium carbonate solvent retention capacity (WM-SRC). The WM-SDS test predicts gluten strength and flour lactic acid SRC. The

WM-SRC test measures the amount of damaged starch, predicts flour sodium carbonate and sucrose SRCs, flour yield, and sugar-snap cookie diameter, in irrigated soft white spring wheat (Guttieri et al., 2004). Whole grain-wheat meal quality assays require small amounts of grain and the sample mills that grind the whole grain are inexpensive, making WM assays appropriate and accessible for most soft wheat breeding programs (Knott et al., 2009).

Among soft wheat lines, milling characteristics such as break flour and flour yields are highly heritable and map to the same quantitative trait locus (QTL) within the wheat genome depending on the genetic background (Guttieri & Souza, 2003; Smith et al., 2011). McCartney et al. (2006) found a QTL related to flour protein, mixograph results and dietary fiber on chromosome 2B and a QTL associated with water absorption and sucrose on chromosome 3B. Smith et al. (2011) detected several QTL for quality traits, mostly also on wheat genome B, all heritable and transgressive segregants were noted, which suggests that marker-assisted selection for quality traits could have a significant impact on soft wheat quality breeding programs.

Copyright © Daniela Sarti Dvorjak 2014

Chapter 3.

Quantitative Trait Loci Analysis of Resistance to Fusarium Head Blight in Wheat Populations

3.1. Introduction

Fusarium head blight (FHB), caused by several *Fusarium* species, is a destructive disease of wheat (*Triticum aestivum* L. and *T. durum* L.) and barley (*Hordeum vulgare* L.) worldwide (Bai & Shaner, 1994; Mesterhazy, 1995). In North America, *Fusarium graminearum* is the causal agent primarily responsible for recent scab epidemics, generating losses over $13.6 million in Maryland, Virginia and North Carolina (Cowger & Sutton, 2005). Nganje et al. (2004) determined that the cumulative direct economic loss attributable to FHB for the period 1993 to 2001 for nine states in the Northern Great Plains and Central United States was $2.5 billion, with an even larger number for secondary (indirect) losses.

Many wheat breeding programs focus on, along with high yield, the development of FHB resistance in commercial cultivars. The incorporation of genetic resistance reduces the need for fungicide applications and, consequently, reduces production costs and environmental pollution while increasing food safety.

A major concern associated with FHB in wheat and barley is the production of mycotoxins, especially deoxynivalenol (DON) and its derivatives. High levels of DON in grains have negative effects on animal production, causing vomiting in ruminants animals (also known as vomitoxin) leading to serious feeding problems and economic losses

(McMullen et al., 1997). Recent literature supports the premise of a close linear relationship between FHB resistance and DON concentration in the infected grain. Regulation of DON accumulation is challenging and depends on the host and fungal genotypes as well as environmental conditions (Mesterhazy et al., 1999).

Resistance to FHB is complex and significantly affected by the environment (Bai & Shaner, 2004) and diverse wheat genotypes can present differential responses to FHB (Arthur, 1891). Most of the studies on the genetics of FHB resistance report it as being under oligogenic or polygenic control with additive gene effects (Snijders, 1990). Previous research indicates that FHB resistance is quantitatively inherited and while genes with major effects have been identified, none confer complete resistance. The level of FHB resistance conferred by single genes is not sufficient to satisfactorily reduce losses in grain yield and quality. QTL limitations involve genotype by environment (G * E) interactions and the effects of different genetic backgrounds (Van Sanford et at., 2001).

As the majority of common wheat cultivars are susceptible to FHB (Mesterhazy, 1995), the few available resistance sources are poorly adapted. Sources originating from China (e.g. Sumai-3 and Wangshuibai), South America (e.g. Frontana and Encruzilhada) and Europe (e.g. Arina and Praag-8) have been used in different studies (Ruckenbauer et al., 2001) and as sources of resistance in different breeding programs.

The Chinese cultivar Sumai-3, derived from a cross between Funo and Taiwan Xiaomai, and its derivatives such as Ning7840, confer a high level of Type II resistance to FHB, by restricting spread of the disease within a spike (Rudd et al., 2001; McCartney

et al., 2004). This cultivar incorporates several resistance QTL including *Fhb1* (Liu et al., 2006), which is located on chromosome 3BS and explains 20 to 60% of the phenotypic variation in FHB, depending on genetic background (Anderson et al., 2001; Buerstmayr et al., 2002; 2003; Zhou et al., 2002; Agostinelli et al., 2012; Balut et al., 2013).

The development of DNA-based markers provides a powerful method for the dissection of complex traits, including FHB resistance in wheat (Gupta et al., 1999). Diagnostic markers for *Fhb1* exist, and therefore, this quantitative trait locus (QTL) has been used worldwide, linked to restriction fragment length polymorphisms (RFLP), simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLP), single nucleotide polymorphisms (SNP) and sequence tagged site (STS) markers, for mapping and marker assisted selection (MAS) (Bernardo et al., 2009).

A previous study from Somers et al. (2003) with SSR markers identified five QTL on chromosomes 2DL, 3BS (2 QTL), 4B, and 5AS, respectively, in a double haploid (DH) population derived from Wuhan-1 (resistant) x Maringa. Lines with resistance alleles on 2DL and 3BS reduced fungal spread by 32% after single-floret inoculation. The presence of 3BS and 4B QTL reduced the disease by 27% in the field, and QTL on 3BS and 5A reduced DON accumulation by 17%.

The 2DL allele is likely derived from the Chinese landrace Wangshuibai and it can explain up to 11% of the phenotypic variation in scab resistance (Mardi et al., 2005). This same investigation identified two QTL on chromosomes 3BS and 2DL in Wangshuibai with AFLP and SSR markers. Compared to Sumai-3 and its relatives, Wangshuibai most likely carries a common QTL for FHB resistance on chromosome 3BS

(*Qfhs.ndsu-3BS*) and a different QTL on 2DL (Mardi et al., 2005). The *QFhs.nau-2DL* differs from the one present in the same chromosome in Wuhan 1, known as *QFhs.crc-2D* (Somers et al., 2003; Jiang et al., 2007a). Jiang et al. (2007a; 2007b) reported that *QFhs.nau-2DL*, derived from CJ 9306, explained on average 20% of the variation in DON and 15.5 % of the variation in Type II resistance. In a validation study for *Fhb1* and *QFhs.nau-2DL* in diverse genetic backgrounds, Balut et al. (2013) encountered reductions of 32% on Fusarium Damaged Kernels (FDK) and 20% for DON concentrations when the QTL *Fhb1* was present for all populations studied. The QTL *QFhs.nau-2DL* reduced FDK and DON by 29% and 24%, however, it was only significant for some populations and the effectiveness was changed on different backgrounds. In other study, the QTL *QFhs.nau-2DL* showed more pronounced effect, reducing 40% on FDK and 55% on DON levels, in comparison to *Fhb1* reductions of 32% and 25% of FDK and DON respectively (Agostinelli et al., 2012)

Kang et al. (2011) evaluated the effects of exotic FHB resistance QTL in soft red winter wheat, singly and in combination, introgressing 3 FHB resistance genes (3BS, 2DL and 5A) in backcross lines derived from crossing Ning7840 (resistant/donor parent) and McCormick (moderately resistant/ recurrent parent) crosses. The 3BS+2DL NIL showed higher resistance and lower deoxynivalenol (DON) content than other NILs in most of the greenhouse and field studies. The 3BS and 2DL NIL had the lowest FDK, reducing visually infected seeds even more than the 3 QTL altogether. In this case, 5A had little effect and possibly even a reverse effect on FDK (Kang et al., 2011).

In a similar study, Agostinelli (2009) investigated genotypic selection for QTL on the 3BS and the 2DL chromosomes and concluded that 3BS conferred a moderate but stable FHB resistance while the 2DL QTL conferred high levels of resistance but with significant QTL by environment interaction. Although *QFhs.nau-2DL* showed a significant QTL by environment interaction, consistent increases on resistance conferred by this QTL made it a promising non-Sumai-3 source of resistance, with reductions of 40% on FDK and 55% on DON levels. Furthermore, given the fact that the Sumai-3 2D chromosome appears to have negative effect on FHB resistance, it is possible that *QFhs.nau-2DL* would complement Sumai-3 derived resistance Zhou et al. (2002).

To achieve a high level of FHB resistance in a wheat genotype, the combination of several resistance genes is recommended. Improvement for FHB resistance should be made by combining resistance genes from different sources and simultaneous selection for resistance and desirable agronomic traits. However, transfer of non-adapted resistance QTL into cultivated wheat generally involves unpredictable effects on agronomic and quality performance. The introgression of target QTL is often found to be associated with linkage drag from the donor, which is the genetic linkage of the gene of interest to genes that can have a negative impact (Jacobsen & Schouten, 2007). It is known that expression levels of FHB resistance genes like *Fhb1* can vary tremendously according to genetic background (Pumphrey et al., 2007; Balut et al., 2013). Before undertaking a lengthy backcrossing program, it would be useful to know if the background of the recurrent parent was amenable to sufficient expression of the resistance QTL.

This study had as main objectives: i) investigation of the relative interaction between FHB resistance QTL and the improvement of disease resistance components, when present alone or combined in the plant; ii) evaluation of the disease resistance outcomes from *Fhb1* and *QFhs.nau-2DL* QTL combinations in diverse genetic background; iii) exploration of the utility of F_2 populations as indicators of expression levels of QTL prior to extensive backcrossing.

3.2. Materials and Methods

3.2.1. Population Development

F_2 populations were initially developed as part of a backcrossing project, in which the objective was the introgression of favorable resistance alleles into existing breeding lines. The intent was to use F_2 derived lines for genotyping and selection, ensuring QTL for FHB resistance were effective before continuing time-consuming and laborious backcrossing.

High yielding, FHB-susceptible wheat lines were crossed to the FHB-resistant line VA01W-476, a double haploid line derived from ‘Roane’ and ‘W14’ (Perugini, 2007; Agostinelli et al., 2011). The resistant line’s parents provide different sources of resistance to FHB. ‘W14’, had many different FHB resistant parents in its pedigree and most likely many different FHB resistance alleles (Jiang et al., 2006). ‘Roane’ is also known to have some level of native resistance (Griffey et al., 2001).

A total of 7 populations were created (Table 3.1) derived from these single crosses which were advanced to the F_2 generation in a greenhouse at Spindletop Research Farm, near Lexington, KY, 2008. In spring 2009, F_2 seeds were planted in nursery flats and leaf samples were collected for DNA isolation and genotyping.

Hanson's (1997) simulation study suggests that the number of segregating progeny required to establishing the genotype of an individual when two or more genotypes are possible is 16 to 17 individuals per progeny row. There were four homozygous possibilities, in seven populations, with four potential QTL combinations. If 17 lines per QTL combination were desired, it would require about 1,632 plants according to Hanson's family size planning. After considering germination and growth difficulties that can occur and prevent seeds from developing, I decided to aim for 30 individual plants per QTL combination, and a total of 3,612 F_2 wheat seeds were planted. Seedlings were germinated in nursery flats at the laboratory and samples were collected when seedlings reached one to two weeks old, at the two leaf stage. Approximately 25 mg of tissue per sample was collected and placed into 1.1 ml 8-strip tubes in racked boxes (ISC BioExpress P-8705-2 [racked tubes]) preloaded with silica gel for drying tissue.

3.2.2. Genotyping

The DNA was isolated according to Pallota et al. (2003). Simple Sequence Repeats (SSR) used were *UMN10* (Liu et al., 2008) and *Xgwm533Pd* (Röder et al., 1998)

for *Fhb1*; and *Xcfd233* (Grain genes 2.0 at http://wheat.pw.usda.gov/GG2/index.shtml, verified May 2010) and *Xgwm539* (Röder et al., 1998) for *2DL*. These markers have been shown to be useful for selecting *Fhb1* and *QFhs.nau-2DL* (Anderson et al., 2001, Agostinelli et al., 2011). The genotyping process for the 3,538 F_2 genotypes was divided between two laboratories: 1,120 leaf samples were processed in the University of Kentucky Wheat Breeding Laboratory and 2,418 samples were submitted to the USDA/ARS Regional Small Grains Genotyping Lab (RSGGL) (http://www.ars.usda.gov/Main/docs.htm?docid=19522) at Raleigh, NC, in 2009 for DNA extraction and marker amplification.

All PCR products were submitted to capillary electrophoresis fragment analysis using *ABI 3730 DNA Analyzer* by *Applied Biosystems* and the results investigated using the software *GeneMapper v4.0*, in which the peaks were identified and the fragments were sized. When the fragment peaks were difficult to distinguish, the software *PeakScanner v1.0* was used and the DNA peaks were identified individually.

After fragment analysis, I separated the genotypes into four groups, according to the resistance alleles of each QTL (Table 3.2). All heterozygous plants were not used for further analysis, since the effect of genes in the heterozygous state is of minor importance for breeding purposes in autogamous species like wheat. Selected homozygous seedlings for four combinations were transplanted to the greenhouse. From all individuals transplanted, some seedlings had no success in producing heads or viable seeds and a final 810 heads were harvested.

3.2.3. Scab Nursery

For the 2011 season, 469 heads from Populations 2, 3, 4, 6 and respective parents were planted in replicated $F_{2:3}$ head-rows (930 head-rows total) in a misted, inoculated scab nursery at the UK Spindletop Research Farm (38°7’37.81’’N, 84°29’44.85’’W; Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs]) near Lexington, KY on 11 October, 2010. Each row from all four populations, plus respective parents, was evaluated for FHB traits, harvested by row, and screened for grain disease levels. In the following year, all 930 $F_{2:4}$ head-rows were planted again in the 2012 scab nursery on 17 October, 2011. Each population was planted in a randomized complete block design (RCBD) with 2 replications, in which head-rows were the replicated experimental unit. Lines were planted in rows 1m long, spaced 30 cm apart. Rows were misted with an overhead mist irrigation system on an automatic timer, from May to June, for periods of 5 minutes, every quarter hour from 8:00 to 8:45 pm, 11:00 pm to 11:45 pm, 2:00 am to 2:45 am, 5:00 am to 5:30 am and 8:30 am (e.g. the equipment operated from 8:00 pm to 8:05 pm the first time and the last time in the misting cycle was from 8:30 am to 8:35 am).

The scab nursery was inoculated with *Fusarium graminearum* - infected corn (*Zea mays* L.) (Verges et al., 2006). Inoculum consisted of twenty-seven isolates taken from scabby wheat seed collected from 2007 to 2010 in multiple locations across Kentucky. For the inoculum preparation, dry corn was imbibed in water for 16 hours and placed in an autoclave for sterilization. After autoclaving, the corn was inoculated with PDA plugs of *Fusarium graminearum* mixed with 0.2 g streptomycin in 50 ml sterile

water, covered and incubated at room temperature for 3 weeks until it was fully colonized by the fungus. At this point, the corn was manually spread on a sterilized plastic sheet until dry, put in mesh bags and stored in the freezer until used. The corn was spread between rows at a rate of 11.86 g/m^{-2}, approximately 3 weeks prior to heading, on 14th April and 31st March of 2011 and 2012, respectively. Liquid nitrogen fertilizer (28% UAN) was applied in the spring at a rate of 105 kg N/ha in split applications. Harmony Extra® herbicide was applied on 20th April, 2011 and 20th March for the 2012 season.

3.2.4. Phenotyping

Weather conditions information was observed and extracted from the University of Kentucky Ag Weather Center (http://www.agwx.ca.uky.edu/). Heading dates were recorded for each head-row in the scab nursery, when 50% of the spikes in the row had emerged. Plant height was measured at the soft dough stage. Effectiveness of QTL in reducing FHB in $F_{2:3}$ and $F_{2:4}$ lines was assessed through several resistance traits. These traits were measured approximately 24 days after heading date and consisted of: Rating, Severity, Incidence, and FHB index. Ratings were visually scored as 1-9 scale, where 1 < 10% and 9 > 90% of diseased plants. Incidence was measured from the number of diseased spikes among the 20 randomly selected spikes per head-row. Severity consisted of the proportion of visually infected spikelets per total spikelets per spike, in 10 randomly selected heads per row. Disease Incidence and Severity were assessed approximately 21 to 24 days after anthesis. FHB index is the product of Severity * Incidence.

Each head-row was hand harvested with a sickle and threshed in a small thresher with low air flow to avoid loss of tombstones (infected kernels, blighted and lighter than healthy grains). Fusarium damaged kernels (FDK) percentages were estimated from carefully cleaned samples that were run through an air separation machine specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from healthy ones (Agostinelli et al., 2008, 2012). FDK was expressed as the weight of scabby kernels divided by total weight.

FDK and DON were predicted using the Near-Infrared Reflectance spectroscopy (NIR - Delwiche & Hareland, 2004) from Perten Instruments, DA7200. FDKNIR and DONNIR measurements from 15 to 20 g samples were compared with actual values. The purpose of this comparison is to estimate the correlation between scab damage and DON levels with NIR predictions, through the idea that NIR might eventually replace expensive, time-consuming techniques like DON gas chromatography analysis. NIR calibrations have been assembled and updated every year since 2007 by the University of Kentucky Wheat Breeding Program and the manufacturers, and have shown strong positive correlations between FDK and DON values measured with traditional methods and NIR estimations. Balut et al. (2013) had FDK - FDKNIR correlations of 0.70 and 0.73 and DON – DONNIR of 0.56 and 0.63 in 2010 and 2011, respectively. NIR was more effective in predicting DON than FDK methods for several populations (R^2 = 0.24 - 0.62) and the correlations between NIR predictions and actual chromatography data for DON ranged from r = 0.56 to 0.63 (Balut et al., 2013). Tibola et al. (2010) tested the ability of NIR to predict DON levels in both 125 grams whole grain and milled samples in southern Brazil and reported 0.89 and 0.91 coefficient of determination (R^2).

DON concentrations on samples were analyzed at University of Minnesota DON Testing Laboratory using gas chromatography with mass spectrometry (GC-MS) (Mirocha et al., 1998).

3.2.5. Data Analysis

Analysis of variance (ANOVA) was performed by population using the General Linear Model procedure (PROC GLM; SAS 9.3). The model used was:

$$Y_{ij} = \mu + ENV_i + R\,(ENV)_{ij} + QTL + G_k\,(QTL) + ENV_i * QTL + E_{ij}$$

Where:

- Y_{ij} = observation in the k^{th} genotype in the j^{th} rep in the i^{th} environment,
- μ = overall mean,
- G_k (QTL) = effect of the k^{th} genotype within QTL,
- QTL= effect of the QTL,
- ENV_i= effect of the i^{th} environment (year),
- $R(ENV)_{ij}$ = effect of j^{th} rep within i^{th} environment,
- ENV_i * QTL = effect of the interaction of the i^{th} environment with the QTL,
- E_{ij} = residual error.

Fisher's Least Significant Difference (LSD) was used to indicate significant differences among QTL combination classes.

Because 2011 and 2012 were very distinct years in regards to weather and wheat development, we considered the QTL by environment interaction from the previous model and also calculated genotype x environment interaction using the model below. Broad sense heritability of FHB and agronomic traits estimations were based on entry means using the following model:

$$Y_{ij} = \mu + ENV_i + R(ENV)_{ij} + G_k + G_k * ENV_i + E_{ij}$$

Where:

- Y_{ij} = the observation in the k^{th} genotype in the j^{th} rep in the i^{th} environment,
- μ = the overall mean,
- ENV_i= effect of the i^{th} environment,
- G_k = the effect of the k^{th} genotype,
- $R(ENV)_{ij}$ = the effect of j^{th} rep within i^{th} environment,
- $G_k * ENV_i$ = the effect of the interaction of the k^{th} genotype with the i^{th} environment,
- E_{ij} = the residual error.

Data were analyzed using the General Linear Models procedure (PROC GLM; SAS 9.3). Genotypic and phenotypic variances were estimated from the expected mean squares (EMS) and heritability estimates were computed as:

$$h^2 = V_g / V_p$$

Where:

- h^2 = broad sense heritability,
- V_g = genotypic variance,
- V_p = phenotypic variance.

Confidence intervals (90 %) were calculated after Knapp et al. (1985) as:

$$UL= 1- [MS3/MS2 * F_{UL\ (0.10,\ v1\ and\ v2\ df)}]^{-1}$$

$$LL= 1- [\ MS3/MS2 * F_{LL\ (0.90,\ v1\ and\ v2\ df)}]^{-1}$$

Where:

- UL = upper limit of the confidence interval,
- MS3 = entry mean square,
- MS2 = residual mean square,
- LL = lower limit of the confidence interval,
- F_{UL} and F_{LL} = F value for the upper and lower limits calculated using the FINV function of Microsoft Excel (2010).

PROC CORR (SAS 9.3) was used to analyze the relationship among traits on an entry mean basis. Entry means were plotted using Microsoft Excel (2010) to study the relationship among traits and calculate the coefficient of determination R^2. Degrees of freedom ranged from 79 to 136, depending on population.

3.3. Results and Discussion

3.3.1. Genotyping and Fragment Analysis

The fragment screening was done for a total of 3,538 F_2 genotypes with the two major QTL for FHB (3BS and 2DL). Of the four markers, *UMN10* and *Xcfd233* worked more effectively in separating resistant and susceptible individuals. For the *UMN10* marker (dinucleotide) the fragment size for susceptible alleles was 228 or 236 bp and resistant plants showed 238 bp fragment size. The marker *Xcfd233* (dinucleotide) had fragment sizes of 271 bp and 276 bp for susceptible and resistant alleles, respectively.

Both *GeneMapper* and *Peak Scanner* successfully analyzed microsatellite fragments data. From all the wheat genotypes evaluated, we selected 806 homozygous genotypes:

- 205 double recessive homozygotes for both markers *UMN10* and *Xcfd233* [SS or (--;--)] or (228/236bp and 271 bp),
- 271 homozygous dominant for *UMN10* and homozygous recessive for *Xcfd233* and [RS or (++; --)] or (238bp and 271 bp),
- 144 homozygous recessive for *UMN10* and homozygous dominant for *Xcfd233* [SR or (--; ++)] or (228/236 bp and 276 bp),
- 186 homozygous dominant for both *UMN10* and *Xcfd233* markers [RR or (++;++)] or (238 bp and 276 bp).

The heterozygous plants were discarded and the homozygotes were separated into four groups according to the QTL combination (Table 3.3) and transplanted to the

greenhouse. From these seven populations, we selected Populations 2, 3, 4 and 6 as the most promising populations to be planted in the scab nursery. Population 5 was not considered due to marker distribution issues.

3.3.2. Weather Conditions and Disease Levels

Weather conditions are a key element in determining FHB disease levels. The year 2011 experienced record warm and dry conditions, which stimulated early plant growth, in March. This same year presented frequent rainfall in April and May, which favored disease pressure throughout the state of Kentucky. In 2012, there were unusually warm temperatures for March through May, which accelerated growth and reproductive development in the wheat crop. The 2012 wheat crop in Kentucky headed about three to four weeks earlier than normal and was harvested approximately three weeks earlier than normal. Low temperatures in April caused freeze damage throughout the state of Kentucky in 2012. In both years, rainfall fell considerably below the 30-year normal of 116.6 cm. The drought-like conditions during April and May 2012 minimized disease pressure and scab levels were much lower than in 2011 (Bruenning et al., 2012).

Low to moderate levels of leaf blotch (*Septoria tritici* and *Phaeosphaeria nodorum* -*Stagonospora nodorum* synonym, formerly *Septoria nodorum*) and low levels of leaf rust (*Puccinia triticina*) were observed in yield plots in Lexington 2011. In 2012, moderate levels of leaf and glume blotch (*Phaeosphaeria nodorum*) but moderate to high levels of leaf rust and barley yellow dwarf virus (BYDV) were detected, resulting in

reduced ability of photosynthesis in several plants. The very mild winter and early spring must have allowed aphids to remain active in the field and transmit BYDV causing leaf yellowing and/or purpling and stunted plants.

As noted, disease levels in 2011 were higher than in 2012 and, for both years, susceptible parents showed higher disease levels than the resistant parent, in all populations. Transgressive segregates with DON levels lower than the resistant parent were observed in three populations in 2011 and four populations in 2012 (Table 3.4).

3.3.3. QTL effects on Fusarium Head Blight traits

Disease related traits (Rating, Incidence, Severity, FHB index, FDK and DON) were scored and results suggest that the QTL *Fhb1* and *QFhs.nau-2DL* significantly reduced disease in all 4 populations tested (Table 3.5). *QFhs.nau-2DL* and *Fhb1* complemented one another in reducing high DON and FDK. Reductions ranged from 12 to 34% in FHB Rating, 16 to 34% in FDK and 23 to 36% in DON levels. The combination of *Fhb1* and *2DL* in a same genotype reduced significantly FHB levels in all populations. *QFhs.nau-2DL* alone was significant for all populations, but for Population 4 in 2011. The magnitude of resistance was increased when combined with *Fhb1* (Table 3.6). In 2012, when FHB infections were clearly less pronounced, *QFhs.nau-2DL* effectively showed highly significant ($P < 0.01$) reductions in FDK and DON for Populations 3, 4 and 6 (Table 3.7).

The resistance conferred by *Fhb1* has been suggested not sufficient under heavy epidemics (Verges et al., 2006). The year 2011 was a highly scabby and some reduction in FHB can be noticed, but overall the addition of QTL was not enough to achieve complete resistance. In 2012, neither *Fhb1* nor *QFhs.nau-2DL* showed significant differences between resistant and susceptible lines in Population 2. In all other populations, the presence of one or both QTL was associated with significant reductions in disease, especially DON concentrations (Table 3.7).

In general, DON levels were not significantly diminished in Population 2, which had the most promising high yielding parent (KY97C-0321-05-2), but no apparent background resistance. Previous studies have discussed the question of whether exotic QTL will provide sufficient resistance to progeny in the absence of native resistance (Balut et al., 2013). For Population 2, the presence of exotic QTL did not always lead to adequate levels of FHB resistance. This fact helps to validate the importance of the presence of at least some native resistance level in the population background. A foundation of native resistance should lead to the acceleration of the release of resistant cultivars therefore lessening the economic losses caused by FHB. Preserving a base of native FHB resistance can provide a solid foundation upon which to pyramid resistance genes from more exotic sources (McKendry, 2008).

Additive effects play a major part in genetic effects of FHB resistance (especially type II resistance), thus pyramiding of different genes in a wheat cultivar can increase FHB resistance in wheat (Bai & Shaner, 2000). Studies suggest that the best way to get highly resistant parents is to cross moderately resistant to moderately susceptible parents

that might contain native resistant genes from locally adapted parents. They may either contain a few major QTL or some minor QTL for FHB resistance. Some of them are native resistance genes that may be different from the Asian resistance sources. This would allow a combination of genetically diverse resistance genes in more adapted genetic backgrounds (Cai, 2012). Native alleles for resistance to FHB that apparently differ from those in the more widely used exotic sources of resistance including Sumai-3 and its derivatives have been identified in Missouri winter wheat germplasm. Out of 250 advanced Missouri lines carrying only native resistance, 45% had FHB index that were equal to or better than Truman (<10%). Of 1700 preliminary lines with complex pedigrees involving both native and exotic FHB sources of resistance, 31.5% also had resistance levels similar to or better than Truman (McKendry, 2008).

Although Sumai-3 is the major source of FHB resistance in wheat breeding programs worldwide, being found in 60% of the pedigrees of entries in the Uniform Regional Spring Wheat Scab Nursery (Garvin & Anderson, 2002), some other resistance should also be explored. In addition to incorporating the QTL *Fhb1*, breeders should also use native resistance in breeding, such as Heyne, Ernie and Freedom (Cai, 2012). These winter cultivars show good FHB resistance, but do not have *Fhb1* (Bai & Shaner, 2004). Truman family haplotypes are suggested to be different from either Ernie or Sumai-3 and several are yet unstudied (McKendry, 2008).

Wheat breeding programs often aim on breeding for native FHB resistance. Selection based on marker based prediction models could lead to greater genetic gain per cycle and greater genetic gain per unit time. In this study, Population 2 had an

outstanding agronomic line as high yielding parent, but clearly the QTL selected *Fhb1* and *QFhs.nau-2DL* are not enough to introduce sufficient FHB resistance in the population. In 2011, FDK was reduced by 17.3% under the effect of *QFhs.nau-2DL* in Population 2. No significant resistance was added in 2012 for the same population. When 2011 and 2012 results were averaged, FDK percentages were reduced by 13.5% by *QFhs.nau-2DL* and 15.7% by the addition of *Fhb1* and *QFhs.nau-2DL*. DON levels had no significant changes with or without the presence of one or both QTL in 2011 and 2012 for Population 2.

QTL by environment interaction occurs when the QTL are more effective in some environments than in others. QTL by environment interaction has been revealed by inconsistent detection and variable effects of QTL across environments in maize (Austin & Lee, 1998; Crossa et al., 1999), barley (Hayes et al., 1993), and rice (Zhuang et al., 1997). In soybean, QTL were inconsistent across environments for plant height and lodging resistance, but consistent for maturity, indicating that QTL by environment interaction is trait dependent (Lee et al., 1996). Wheat studies revealed significant QTL by year interaction in several populations for *Fhb1* and *QFhs.nau-2DL* (Agostinelli et al., 2012; Balut et al., 2013). The nature of such interaction is important. Changes in rank (crossover) across environments could have significant impacts on marker assisted selection. Changes in the magnitude of QTL effects should be of less consequence (Hayes et al., 1993).

Significant QTL by year interaction in FDK was found in Population 3 for *QFhs.nau-2DL* and in Population 6 for *Fhb1* and both QTL with respect to DON

concentration (Table 3.8). However, when all genotypes were compared for each individual year's results, there were no rank changes and the resistant lines (with QTL present) tended to cluster within the group with low FDK and DON values, suggesting that *Fhb1* and *QFhs.nau-2DL* QTL were consistent across environments. Overall, our results are in accordance with Agostinelli et al. (2012) where *QFhs.nau-2DL* more often presented QTL by environment interaction, however, the high level of resistance conferred by this QTL offers promising results on lessening FHB for all populations studied.

Transgressive segregates were observed for FDK and DON in all populations, but were much more frequent in Populations 3 and 4, where several lines were observed with FDK values and DON levels lower than the resistant parent VA01W-476, especially in 2012, when the scab infection was less pronounced.

Overall, *QFhs.nau-2DL* was more effective at reducing disease in all populations, in both years. Agostinelli et al. (2012) also found for their soft winter wheat populations that the QTL *QFhs.nau-2DL* conferred more pronounced disease resistance than *Fhb1*. The R^2 of the model indicates that a high percentage of the variation in FHB resistance can be explained by the QTL. For FHB Rating, the R^2 ranged from 48 to 70%. R^2 for Severity ranged from 74 to 88% and from 46 to 61% for Incidence. R^2 for FHB index was between 63 and 77%. FDK and DON also presented high R^2, from 58 to 72% and 64 to 83%, respectively (Table 3.4).

FHB index was reduced 17.4% by the presence of *Fhb1* + *QFhs.nau-2DL* in population 3. FDK was significantly reduced by *Fhb1* + *QFhs.nau-2DL* by 16, 22, 32 and

34 % in Populations 2, 3, 4 and 6. When combined, *Fhb1* + *QFhs.nau-2DL* reduced DON by 23, 32 and 36% in Populations 3, 4 and 6, respectively (Table 3.5).

Significant QTL effects on FDK were also detected using NIR. Correlations between FDKNIR and actual FDK were 0.48 for all populations combined. Correlations between DONNIR and FDK were 0.53 (Table 3.10). Correlations between DONNIR and DON ranged from 0.55 to 0.82, with a value of 0.63 among all the population combined (Table 3.9 and 3.10). Visual ratings also proved to be predictive, with correlations of 0.65, 0.69, 0.71, 0.43 and 0.67 for Incidence, Severity, FHB index, FDK and DON. Interestingly, FDKNIR was better correlated to DON (0.63) than FDK – DON (0.47) (Table 3.9).

In these 4 backgrounds, *QFhs.nau-2DL* was more effective than *Fhb1* in reducing FDK, in contrast to Balut et al. (2013), where *Fhb1* was more effective over 5 populations when susceptible and resistant plants were compared. Averaged over years and populations, *Fhb1* reduced FDK 15% while *QFhs.nau-2DL* brought about a 22% reduction in FDK. *QFhs.nau-2DL* was associated with a more generalized response in FDK reduction (all populations) than *Fhb1* (2 of 4 populations). *Fhb1* alone reduced DON an average of 15% and *2DL* was responsible for 19% reduction in DON levels. When combined both QTL reduced FDK by 26% and DON by 30%. The highest reduction on FDK was in 2011 for Population 6, with a FDK reduction of 36% when both QTL were present. DON was diminished by the presence of *Fhb1* + *QFhs.nau-2DL* by 45% in Populations 4 and 6 in 2012. The results agree with Agostinelli et al. (2012) and

the effect of *QFhs.nau-2DL* was more pronounced than that of *Fhb1*, 55% and 25% on DON reductions and 40% vs. 32% FDK reduction.

3.3.4. Fhb1 effects on Fusarium Head Blight traits

The QTL *Fhb1* individually reduced disease when comparing susceptible and resistant lines, with significant effects in 3 of 4 populations, the exception being Population 2. The reduction in FHB depended on trait and on population. FHB Ratings presented reductions ranging from 9 to 17% in Populations 4 and 6. Incidence and Severity was reduced only in Population 4, with about 7% less FHB Incidence and Severity in resistant lines (Table 3.5).

The presence of *Fhb1* resistance alleles significantly reduced FDK in Population 4 by 15%, which is inferior to the 31, 32 and 32% reported respectively by Cardwell (2011), Agostinelli et al. (2012) and Balut et al. (2013). However, in 2011 alone, the reduction was 27% in Population 4 which showed the largest effect of *Fhb1* on FDK values (Table 3.6). This same QTL reduced DON levels by an average of 15% in Populations 3, 4 and 6. These results are still lower when compared to the 20% reduction in DON observed by Balut et al. (2013), 25% reported by Agostinelli et al. (2012) and 40% according to Cardwell (2011).

NIR estimates of scab traits were well correlated with the actual values. The correlation of FDKNIR and FDK was 0.48 over all populations, while the r^2 of DONNIR and DON was 0.63 (Table 3.9). The presence of *Fhb1* significantly reduced FDKNIR and

DONNIR by 5 and 6% in Population 3 (Table 3.5), comparing to non-significant reductions on FDK, but 11.5% reductions in DON for the same population.

These disease reductions indicate that *Fhb1* QTL is effective in lowering FHB impact in diverse genetic backgrounds, being more successful in some populations than others. When looking more closely at each year, *Fhb1* had affected more disease traits in 2011(Rating, Severity, FDKNIR, DONNIR and DON) but the magnitudes of the disease reductions were larger in 2012, when disease pressure was not so pronounced (Table 3.7). This fact might suggest that the QTL is reasonably effective under low disease pressure but when the disease pressure is high, there is a need for extra background resistance or native resistance. Natural field infections typically result in lower disease pressure than the inoculated scab nursery; therefore the results suggests that one can expect *Fhb1* to be effective in reducing typical FHB levels in farmer's fields.

3.3.5. QFhs.nau-2DL effects on Fusarium Head Blight traits

The presence of *QFhs.nau-2DL* significantly reduced FHB traits in all populations. Rating was decreased at about 13% on average, in Populations 3, 4 and 6 (Table 3.5). For Population 3, *QFhs.nau-2DL* alone was responsible for a reduction of 28% on Ratings in 2012 (Table 3.7). In 2011 + 2012, Severity was significantly reduced in Populations 2, 4 and 6 by 7, 14 and 13% respectively. Incidence was only significantly reduced in Population 3 (15%). In the case of FHB Incidence, it was not expected that the presence of either *Fhb1* or *QFhs.nau-2DL* alleles would reduce Incidence levels, since

their impact is on Type II resistance (spread of the disease throughout a spike). FHB Index was significantly reduced by the presence of *QFhs.nau-2DL* in all populations but Population 2, with an average decrease of 16% for that disease trait. FDKNIR was reduced by 12, 15 and 14% in Populations 3, 4 and 6. DONNIR was reduced in all populations, by an average of 12%. The actual FDK and DON reductions were 14, 24, 26 and 24% for FDK in Populations 2 to 6, and 13, 23 and 20% for DON in Populations 3, 4 and 6 (Table 3.5).

QFhs.nau-2DL effects were observed in all populations for FDK, reducing 14, 24, 26 and 24% in Populations 2, 3, 4 and 6, respectively for 2011 and 2012 averaged. DON levels were significantly reduced in Populations 3 (16%), 4 (23%) and 6 (20%). The largest DON reduction was observed in Population 3 in 2012, where DON levels were 33% lower in lines with the *QFhs.nau-2DL* resistance allele (Table 3.7). These DON reductions are in agreement with Balut et al. (2013) who obtained 24% reductions in several populations. Agostinelli et al. (2012), under different levels of FHB pressure (2007 and 2008) showed 50% less DON by *QFhs.nau-2DL* presence. FDK results are also in agreement with other studies which detected 29% and 25% FDK decreases by the presence of *QFhs.nau-2DL* QTL (Balut et al., 2013; Agostinelli et al., 2012).

3.3.6. NIR Predictions

Near infrared reflectance (NIR) can be successfully employed to nondestructively sort kernels with FHB damage and to estimate DON levels of those kernels. The NIR

technology will detect the absorbance of reflected energy when a kernel is illuminated with visible-NIR light. Wheat spectra (950 – 1640 nm) are examined to automatically identify sound kernels and Fusarium damaged kernels (FDK), and predict deoxynivalenol (DON) concentrations. The difference in absorption may arise due to differences in chemical (changes in carbohydrate, lipid, protein and DON levels) and physical properties of the kernels (Peiris et al., 2010).

NIR predictions were correlated with the actual values of FDK and DON. The correlation between FDK and FDKNIR was 0.48 over all populations, with highest values on Population 6 with r = 0.73. DON and DONNIR presented an overall correlation of 0.63, ranging from 0.55 (Population 2) to 0.82 (Population 6). FDKNIR predictions were also correlated with actual DON values (r = 0.54) and DONNIR was correlated with FDK percentages (r = 0.53) (Table 9 and 10). The results indicate NIR as a good predictor of both FDK and DON values of red winter wheat populations, indicating the possibility of substituting a fast and non-destructive method for the expensive and time consuming tests for FDK and DON that are conventionally used.

3.3.7. QTL by Environment interaction and Agronomic traits

Genotype by Environment (G * E) interaction occurs when some genotypes perform to a higher degree under certain environmental conditions while others perform poorly in that same environment, conversely the lower yielding lines may exceed the higher yielding genotypes when grown under different conditions, leading to genotype

rank changes. A significant interaction in an analysis of variance can be obtained even when there is no change of ranking of genotypes under study. If there are no rank changes, the interaction can usually be designated as a scalar effect. G * E can be examined by looking at the variance of the phenotypes over the range of environments and selecting for the lowest variance as being the most stable. 2011 and 2012 were two extremely different years and some G * E can be expected. When FDK and DON values for 2011 are regressed on 2012 values, resistant genotypes from all populations studied were consistent on the bottom portion, despite the fact averages for 2011 were higher for both FDK (Figure 3.1) and DON levels (Figure 3.2).

QTL by year (QTL * Y) interactions were observed for the wheat derived lines studied in this project, even though no significant patterns across populations and disease traits were found. 2011was wet, with high FHB infection while 2012 was extremely dry and disease levels were much lower than normal. Consequently, the evaluations reveal very different numbers between the two years for disease traits like Rating, Incidence, Severity, FHB index, FDK and DON. (Table 3.6 and Table 3.7). Mean values for FDK were 23.4% in 2011, while in 2012, the mean FDK was 8.5% among all populations. Average DON concentration was 13.7 ppm in 2011 and 2.3 ppm in 2012 for the same lines among all populations (Table 3.12).

QTL by year interaction interferes with the selection process and reduces the effectiveness of marker assisted selection. My evaluations do not reveal a crossover QTL * Y interaction, although the different genotypes changed places on the top-yielding list, they still had a good performance considering the surrounding environment each year.

The differences which were observed could be explained by the environmental variations observed in 2011 (very high FHB infection) and 2012 (early maturation, freeze damage, and low FHB levels in the field). When 2011 means were plotted against 2012 means, resistant genotypes tended to cluster within the lower FDK percentages and DON levels (Figure 3.1 and 3.2).

Considering both years together, yield averages for Populations 2, 3, 4 and 6 are around 63 bushels per acre. This value is lower, but not too far from the yield average for checks (65 bu/acre) and the susceptible high-yielding parents (78 bu/acre) (Table 3.15) Even though these lines are in an early generation, the cross with a low-yielding resistant parent did not severely compromise the yields of the progeny. Height was not drastically affected. When each row in the scab nursery was measured, height increased 3.6% in Population 3 with the addition of the two resistance QTL *Fhb1* and *QFhs.nau-2DL*. In Population 6, the addition of *Fhb1* into the genotypes caused a decrease of 2.4% in average height. When measured in yield plots (as opposed to head-rows in the scab nursery), Populations 2, 4, 6 and 7 revealed an increased height of 10, 9, 13 and 15% from the susceptible parent's average height, respectively (Table 3.15). Jiang et al. (2007a) also found significant QTL by year interaction, with *QFhs.nau-2DL* explaining 9.9 to 28.4% of phenotypic variation in several recombinant inbred lines and significant enhancing FHB resistance in selected environments.

In general, the addition of FHB resistance QTL into F_2 genotypes was regularly linked to lower levels of disease both in the inoculated scab nursery and in the yield plots. Some populations clearly had FHB resistance enhanced while other backgrounds, like

Population 2 did not perform equally in reducing disease levels. These favorable results are encouraging for the next step of this project: the creation of backcrossed lines (BC) from these initial crosses, using the high yielding Kentucky lines as recurrent parents.

3.3.8. Heritability Estimates and Response to Selection

Broad sense heritabilities and their corresponding 90% confidence intervals were estimated on an entry mean basis for each population separately and also for all populations combined on an overall heritability for each disease trait (Table 3.11). FDK h^2 ranged from 0.16 to 0.48, which were lower than the estimates reported by Agostinelli et al. (2012) and Balut et al. (2013), whose h^2 values were greater than 0.60. DON h^2 estimates were higher and less variable and ranged from 0.54 to 0.75, similar to results reached by Balut et al. (2013) but still lower than Agostinelli et al. (2012). FHB index h^2 estimates were moderate, with an average of 0.40. Heritability of FHB Rating varied among all populations, ranging from 0.19 (Population 2) to 0.65 (Population 6; Table 3.11).

To evaluate the effectiveness of selection for low disease traits in F_2 generations, the top 10% lines were selected within each population. Correlated responses of FHB index, FDK, DON, Rating and NIR were also evaluated. Direct phenotypic selection response for Rating, FHB index, FDK and DON were 23, 32, 25 and 48% reductions, respectively, over all four populations. Selection response for NIR predictions was 57% of the population's mean for FDKNIR and 74% for DONNIR (Table 3.12). When

selecting FDKNIR, the indirect progress in FDK was 0.5%. When selecting DONNIR, the indirect progress with selection was 1.4%. The indirect response when selecting FHB index was reduction of 0.5% in FDK and 1.6% in DON levels. Rating showed low FDK and DON reductions, 0.1 and 0.3, respectively, indicating that this trait is less effective for selecting for resistance to FHB kernel damage. Although less efficacious, visual Ratings and FHB index, are taken early before harvest and at a low cost. Finally, selecting for FDK percentages resulted in a 1.2% reduction in DON levels.

3.4. Conclusions

The presence of FHB resistance QTL often increases the resistance to *Fusarium* head blight and reduces FDK and DON, the two most direct measurements of FHB impact. The combination of *Fhb1* and *QFhs.nau-2DL* is not necessary, but recommended and it improved resistance in all populations.

Resistance alleles and the interaction among FHB resistance QTL have distinct behavior in different genetic backgrounds in wheat. The best validated gene for FHB resistance, *Fhb1* on chromosome 3BS, showed an average reduction of 12% in disease levels, however it did not result in significant improvement of FHB resistance in all populations. In general, for the four backgrounds studied, the *QFhs.nau-2DL* QTL was more effective reducing FHB (19% average reduction).

Although *Fhb1* and *QFhs.nau-2DL* have potential as tools to help differentiate resistant or moderately resistant germplasm, more diagnostic markers should be

developed, and possibly with closer linkage to resistance QTL. Marker assisted selection (MAS) is a helpful tool to assist plant breeders, and other methods should be explored, aiming to identifying resistance genes and interpreting the FHB resistance they confer.

Table 3.1. Wheat populations developed from single crosses among KY lines to an FHB resistant parent, with their respective pedigrees.

Population	Pedigree
1	KY 99C-1051-03-1 / VA01W-476
2	KY97C-0321-05-2 / VA01W-476
3	KY97C-0519-04-05 / VA01W-476
4	KY97C-0540-1-03 / VA01W-476
5	KY98C-1446-02-1 / VA01W-476
6	KY97C-0508-01-01A / VA01W-476
7	KY98C-1474-02 / VA01W-476

Table 3.2. Presence of resistance alleles in all possible combinations of the QTL *Fhb1* and *QFhs.nau-2DL* in the seven F_2 derived wheat populations genotyped.

Combinations	***Fhb1***	***2DL***	***Code***
Both QTL present	+ +	+ +	**RR = 1**
Fhb1 present and *2DL* absent	+ +	- -	**RS = 2**
Fhb1 absent and *2DL* present	- -	+ +	**SR = 3**
Both QTL absent	- -	- -	**SS = 4**

Table 3.3. Total number of plants obtained for each genotype group planted and number of plants according to QTL combinations for all seven F_2 derived wheat populations evaluated, where SS = no QTL present, SR = *QFhs.nau-2DL* present, RS = *Fhb1* present, RR = both QTL present (*Fhb1 + 2DL*).

Populations	QTL Combinations				Total
	SS (- -/- -)	SR (- -/++)	RS (++/ - -)	RR (++/ ++)	
1	27	26	26	15	94
2	36	23	40	30	129
3	28	18	25	21	92
4	48	25	48	28	149
5	7	6	83	49	145
6	24	25	32	18	99
7	35	21	17	25	98
Total	205	144	271	186	806

Table 3.4. Mean, Coefficients of Variance (CV), Coefficient of Determination (R^2) and Minimum (Min) and Maximum (Max) values of Fusarium Head Blight disease traits for four F_2 derived wheat populations and respective parents, according to presence of disease resistance alleles in Lexington, KY scab nursery in 2011 and 2012.

		POP2 (KY97C-0321-05-2/VA01W476)									
		HDate	Rating	Severity	Incidence	FHB index	Height	FDK	FDKNIR	DONNIR	DON
Fhb1	R^2 (%)	96.60	48.06	80.53	46.29	65.21	79.60	60.27	91.31	84.90	64.26
	CV	2.03	43.89	28.16	30.59	50.86	8.20	51.89	20.83	22.92	42.58
	Mean	120.78	3.89	35.50	53.97	20.25	29.68	17.02	17.70	19.63	10.83
2DL	R^2 (%)	96.56	48.89	80.54	45.63	64.77	79.60	61.04	91.44	84.94	64.50
	CV	2.05	43.54	28.14	30.78	51.17	8.20	51.38	20.68	22.89	42.44
	Mean	123.54	3.54	31.72	52.56	17.70	29.58	15.11	18.45	18.21	10.90
Fhb1 + 2DL	R^2 (%)	96.63	52.36	81.46	46.34	66.28	79.60	61.23	91.45	85.04	64.55
	CV	2.04	42.22	27.59	30.71	50.29	8.20	51.49	20.77	22.92	42.60
	Mean	123.39	3.64	33.83	54.27	19.88	29.11	14.20	17.71	17.73	10.57
VA01W-476		130.00	1.25	37.50	26.51	10.43	28.20	8.02	14.64	16.19	3.68
KY97C-0321-05-2		127.80	4.00	70.00	45.88	19.72	32.00	24.17	18.05	21.16	22.84
Population Mean		123.11	3.73	34.22	53.88	19.59	29.51	15.99	17.81	18.57	11.12
(Min - Max)		(107.0 - 148.0)	(0.0 - 9.0)	(5.0 - 100.0)	(7.0 - 83.5)	(0.7 - 75.1)	(20.0 - 38.0)	(2.8 - 75.0)	(0.7 - 49.6)	(0.2 - 42.1)	(0.8 - 41.6)

		POP3 (KY97C-0519-04-05/VA01W476)									
		HDate	Rating	Severity	Incidence	FHB index	Height	FDK	FDKNIR	DONNIR	DON
Fhb1	R^2 (%)	97.35	69.18	87.12	60.76	76.57	75.81	70.47	95.48	88.34	79.55
	CV	1.97	38.00	27.59	33.73	52.00	6.36	56.55	16.91	22.30	44.30
	Mean	122.14	3.49	48.02	52.50	22.80	29.93	19.62	16.84	19.16	8.04
2DL	R^2 (%)	97.36	69.11	87.19	61.10	76.82	75.81	71.37	95.41	88.38	79.63
	CV	1.96	38.05	27.51	33.59	51.72	6.36	55.69	17.03	22.27	44.21
	Mean	120.56	2.77	29.73	39.09	14.05	31.27	14.19	15.14	17.04	7.53
Fhb1 + 2DL	R^2 (%)	97.41	69.184	87.71	61.41	76.84	75.81	71.61	95.50	88.44	79.69
	CV	1.95	38.166	27.07	33.60	51.93	6.36	55.69	16.95	22.30	44.34
	Mean	122.55	3.01	29.57	44.63	16.19	30.43	13.22	15.36	17.07	7.37
VA01W-476		130.00	2.2	32.5	29.4	10.2	28.20	8.7	16.5	16.7	4.7
KY97C-0519-04-05		127.8	7.3	85.0	42.9	38.0	32.0	29.5	24.2	32.5	26.7
Population Mean		121.92	3.20	31.53	46.25	17.23	30.15	16.12	16.62	18.66	8.20
(Min - Max)		(106.0 - 147.0)	(0.0 - 8.0)	(5.0 - 100.0)	(2.3 - 78.8)	(0.3 - 70.9)	(21.0 - 36.0)	(1.9 - 100.0)	(0.5 - 40.0)	(2.7 - 55.6)	(0.5 - 47.6)

Table 3.4 (Continued). Mean, Coefficients of Variance (CV), Coefficient of Determination (R^2) and Minimum (Min) and Maximum (Max) values of Fusarium Head Blight disease traits for four F_2 derived populations and respective parents, divided by presence of disease resistance alleles in Lexington, KY scab nursery in 2011 and 2012.

		POP4 (KY97C-0540-01-03/VA01W476)									
		HDate	Rating	Severity	Incidence	FHB index	Height	FDK	FDKNIR	DONNIR	DON
Fhb1	R^2 (%)	97.99	61.23	85.86	56.81	73.05	63.39	57.90	88.10	78.21	78.14
	CV	1.79	45.37	30.15	36.69	58.86	10.84	77.91	28.69	29.15	46.62
	Mean	123.56	3.00	86.77	43.09	40.32	29.92	14.51	16.98	18.22	8.93
2DL	R^2 (%)	97.98	61.47	85.92	56.83	73.00	63.38	57.52	88.00	78.18	77.86
	CV	1.79	45.23	30.08	36.68	58.91	10.83	78.26	28.81	29.17	46.91
	Mean	118.50	2.99	25.78	43.77	13.62	30.25	12.34	13.48	15.08	8.12
Fhb1 + 2DL	R^2 (%)	98.02	61.80	86.13	56.97	73.56	63.39	58.12	88.16	78.30	78.14
	CV	1.78	45.16	29.93	36.72	58.45	10.84	77.92	28.70	29.17	46.75
	Mean	121.53	2.67	29.30	37.74	13.11	28.84	12.45	15.63	16.43	7.47
VA01W-476		130.00	1.5	55.0	12.4	7.8	28.20	5.5	6.3	10.5	1.8
KY97C-0540-1-03		117.5	7.0	65.0	29.5	19.5	29.0	13.2	11.2	15.9	8.6
Population Mean		121.54	3.04	29.41	42.35	14.57	29.67	15.09	16.30	17.46	9.17
(Min - Max)		(105.0 - 148.0)	(0.0 - 9.0)	(5.0 - 100.0)	(3.3 - 81.2)	(0.2 - 72.1)	(19.0 - 37.0)	(0.4 - 100.0)	(0.4 - 85.0)	(1.9 - 74.0)	(0.3 - 52.1)

		POP6 (KY97C-0508-01-01A/VA01476)									
		HDate	Rating	Severity	Incidence	FHB index	Height	FDK	FDKNIR	DONNIR	DON
Fhb1	R^2 (%)	98.50	69.79	74.19	49.87	62.69	66.88	63.70	88.57	76.53	82.50
	CV	1.51	43.28	33.97	38.59	63.57	8.80	50.57	22.74	23.81	33.55
	Mean	121.71	2.77	29.41	42.95	20.26	28.79	17.97	19.09	20.94	8.72
2DL	R^2 (%)	98.50	69.41	74.59	49.73	62.59	66.88	63.78	88.51	76.87	81.80
	CV	1.51	43.55	33.71	38.64	63.65	8.80	50.52	22.79	23.64	34.20
	Mean	117.19	2.95	23.78	37.33	10.05	29.52	15.25	16.48	18.99	8.53
Fhb1 + 2DL	R^2 (%)	98.51	69.98	74.96	49.91	62.95	66.88	64.25	88.61	77.17	82.56
	CV	1.51	43.3	33.59	38.72	63.59	8.80	50.38	22.79	23.58	33.62
	Mean	118.73	2.06	25.23	34.23	10.23	28.50	16.69	16.52	18.39	6.79
VA01W-476		130.00	1.75	32.50	0.24	9.19	28.20	10.09	15.94	16.88	4.41
KY97C-0508-01-01A		119.80	3.25	23.75	0.19	6.15	27.50	25.81	23.14	26.72	12.63
Population Mean		119.17	2.79	26.63	37.12	11.23	29.07	16.69	17.89	20.30	8.85
(Min - Max)		(103.0 - 146.0)	(0.0 - 8.0)	(5.0 - 100.0)	(5.0 - 65.0)	(0.2 - 60.5)	(20.0 - 37.0)	(2.1 - 75.0)	(1.0 - 52.6)	(6.8 - 52.0)	(0.6 - 28.0)

Table 3.5. Means for FHB traits evaluated according to the presence of resistance (R) or susceptible (S) alleles at two QTL (*Fhb1* and *QFhs.nau-2DL*), for four F_2 derived wheat populations, Lexington, KY, 2011 and 2012.

		N	Hdate (Julian days)	Rating (1-9)	Severity	Incidence	FHB index	Height	FDK	FDKNIR	DONNIR (ppm)	DON (ppm)
						(%)						
POP2 (KY97C-0321-05-2/VA01W476)												
Fhb1	S	154	123.8 **	3.7 NS	33.7 NS	53.6 NS	19.1 NS	29.6 NS	16.2 NS	17.9 NS	18.4 NS	11.5 NS
	R	164	122.5	3.8	34.7	54.1	20.1	29.4	15.8	17.7	18.7	10.7
2DL	S	175	122.8 *	3.8 NS	35.4 *	54.2 NS	20.2 NS	29.7 NS	17.0 *	17.6 NS	19.1 *	11.4 NS
	R	143	123.5	3.6	32.8	53.5	18.9	29.3	14.7	18.1	18.0	10.7
Fhb1 +2DL	S	87	124.1 **	3.8 NS	35.5 NS	54.5 NS	20.3 NS	29.7 NS	17.2 *	18.0 NS	18.9 *	12.3 NS
	R	76	123.4	3.6	34.1	54.5	20.1	29.1	14.5	18.1	18.0	10.7
POP3 (KY97C-0519-04-05/VA01W476)												
Fhb1	S	168	121.5 **	3.2 NS	31.8 NS	46.6 NS	17.3 NS	30.1 NS	15.9 NS	17.0 **	19.2 **	8.7 **
	R	162	122.3	3.2	31.2	45.9	17.1	30.2	16.4	16.2	18.1	7.7
2DL	S	182	122.1 NS	3.4 **	33.1 NS	49.6 **	18.9 **	29.7 **	18.1 **	17.6 **	20.0 **	8.8 **
	R	148	121.7	2.9	29.6	42.2	15.2	30.8	13.7	15.5	17.1	7.4
Fhb1 +2DL	S	102	122.2 NS	3.4 **	33.4 *	51.7 **	19.6 **	29.4 **	17.0 **	18.1 **	20.8 **	9.6 **
	R	82	122.4	3.0	29.3	44.7	16.2	30.5	13.2	15.2	16.9	7.4
POP4 (KY97C-0540-01-03/VA01W476)												
Fhb1	S	288	120.5 *	3.2 **	28.5 *	43.6 **	14.5 NS	29.8 NS	16.2 **	16.1 NS	17.4 NS	9.9 **
	R	249	122.7	2.9	30.5	40.9	14.7	29.5	13.8	16.6	17.5	8.3
2DL	S	327	122.6 **	3.2 **	31.1 **	43.3 NS	15.3 **	29.8 NS	16.8 *	17.3 **	18.6 **	10.1 **
	R	210	119.9	2.8	26.8	40.9	13.4	29.5	12.5	14.7	15.7	7.8
Fhb1 +2DL	S	177	122.0 NS	3.4 **	30.7 *	43.9 **	15.4 NS	29.6 NS	18.9 **	17.9 **	19.2 **	11.1 **
	R	100	121.5	2.7	28.0	37.7	13.1	28.8	12.8	15.8	16.5	7.5
POP6 (KY97C-0508-01-01A/VA01476)												
Fhb1	S	206	118.1 *	3.0 **	25.7 NS	37.5 NS	11.0 NS	29.4 *	17.2 NS	17.8 NS	20.6 NS	9.6 **
	R	170	120.5	2.5	27.0	36.7	11.5	28.7	15.9	18.0	19.9	7.9
2DL	S	198	120.4 *	2.9 **	28.0 **	38.0 NS	12.2 **	29.1 NS	18.8 **	19.1 *	21.7 **	9.7 **
	R	178	117.8	2.6	24.4	36.1	10.1	29.1	14.3	16.5	18.8	7.8
Fhb1 +2DL	S	98	119.3 NS	3.2 **	28.1 NS	38.0 NS	12.2 NS	29.4 NS	19.8 **	19.5 **	22.6 **	10.9 **
	R	70	119.1	2.1	25.6	34.5	10.4	28.5	13.1	17.0	18.7	7.0

NS : not significant; * : significant at 5% level ($P < 0.05$); ** : significant at 1% level ($P < 0.01$).

Table 3.6. Means for FHB traits evaluated according to the presence of resistance (R) or susceptible (S) alleles at two QTL (*Fhb1* and *QFhs.nau-2DL*), for four F_2 derived wheat population, Lexington, KY, 2011.

		N	HDATE (Julian days)	RATING (1-9)	SEVERITY (%)	INCIDENCE (%)	FHBINDEX (%)	FDK (%)	FDKNIR (%)	DONNIR (ppm)	DON (ppm)
POP2 (KY97C-0321-05-2/VA01W476)											
Fhb1	S	76	134.7 *	3.7 *	47.7 *	53.7 ^{NS}	26.5 ^{NS}	22.7 ^{NS}	28.0 ^{NS}	26.1 ^{NS}	14.6 ^{NS}
	R	82	133.7	4.2	51.2	56.8	29.9	22.1	27.6	26.7	13.4
2DL	S	85	133.9 ^{NS}	4.3 **	51.7 **	55.4 ^{NS}	29.6 ^{NS}	24.3 *	27.3 ^{NS}	27.3 *	14.5 ^{NS}
	R	70	134.6	3.6	46.8	55.2	26.6	20.1	28.3	25.5	13.4
Fhb1 +2DL	S	41	134.8 *	3.8 **	49.6 **	53.8 ^{NS}	27.5 ^{NS}	24.2 ^{NS}	27.5 ^{NS}	26.7 ^{NS}	15.4 ^{NS}
	R	37	134.5	3.5	48.1	57.6	28.2	19.4	28.3	25.4	13.0
POP3 (KY97C-0519-04-05/VA01W476)											
Fhb1	S	81	134.1 ^{NS}	4.3 ^{NS}	51.5 *	57.7 ^{NS}	30.2 ^{NS}	24.0 ^{NS}	28.0 **	28.6 **	14.1 **
	R	82	133.8	4.2	47.7	58.3	28.4	23.6	25.9	26.0	11.9
2DL	S	93	134.0 ^{NS}	4.4 ^{NS}	51.2 *	60.0 ^{NS}	31.3 *	26.7 **	27.8 **	28.8 **	13.5 ^{NS}
	R	70	133.8	4.1	47.5	55.5	26.6	20.1	25.9	25.4	12.4
Fhb1 +2DL	S	51	134.5 ^{NS}	4.3 ^{NS}	51.6 **	61.7 *	32.4 ^{NS}	25.0 **	28.9 **	30.2 **	14.7 **
	R	40	134.5	4.1	45.1	59.9	27.4	19.2	25.6	25.2	12.0
POP4 (KY97C-0540-01-03/VA01W476)											
Fhb1	S	127	134.3 ^{NS}	4.2 **	47.8 ^{NS}	52.3 ^{NS}	25.8 ^{NS}	27.3 **	27.9 **	25.7 *	16.2 **
	R	135	133.8	3.7	46.4	49.0	23.3	20.0	25.3	23.5	12.4
2DL	S	173	134.1 ^{NS}	4.0 ^{NS}	47.4 ^{NS}	50.2 ^{NS}	24.6 ^{NS}	24.8 ^{NS}	26.6 ^{NS}	24.9 ^{NS}	14.6 ^{NS}
	R	89	134.0	3.8	46.5	51.2	24.4	21.0	26.4	23.9	13.4
Fhb1 +2DL	S	87	134.3 ^{NS}	4.2 **	48.1 ^{NS}	50.3 *	25.2 ^{NS}	29.8 **	28.4 **	26.2 *	16.4 **
	R	49	133.5	3.5	45.7	46.5	21.7	20.3	26.0	23.6	11.9
POP6 (KY97C-0508-01-01A/VA01476)											
Fhb1	S	94	131.3 ^{NS}	4.4 **	37.8 ^{NS}	45.0 ^{NS}	17.6 ^{NS}	26.0 **	28.0 **	27.8 **	15.1 **
	R	92	131.7	3.4	35.7	42.3	15.9	21.7	25.9	25.0	11.5
2DL	S	105	131.7 ^{NS}	4.0 ^{NS}	38.5 **	44.0 ^{NS}	17.6 ^{NS}	26.0 **	27.5 ^{NS}	27.7 **	13.8 **
	R	80	131.2	3.7	34.5	43.3	15.6	21.0	26.2	24.6	12.6
Fhb1 +2DL	S	49	131.5 ^{NS}	4.4 **	40.7 **	44.5 ^{NS}	18.7 *	28.6 **	29.0 **	30.0 **	16.2 **
	R	36	131.4	2.9	34.0	40.2	14.5	18.2	25.3	23.6	10.6

^{NS} : not significant; * : significant at 5% level ($P < 0.05$); ** : significant at 1% level ($P < 0.01$).

Table 3.7. Means for FHB traits evaluated according to the presence of resistance (R) or susceptible (S) alleles at two QTL (*Fhb1* and *QFhs.nau-2DL*), for four F_2 derived wheat population, Lexington, KY, 2012.

		N	Hdate (Julian days)	Rating (1-9)	Severity (%)	Incidence (%)	FHB index (%)	FDK (%)	FDKNIR (%)	DONNIR (ppm)	DON (ppm)
POP2 (KY97C-0321-05-2/VA01W476)											
Fhb1	S	78	113.1 **	3.7 ^{NS}	20.1 ^{NS}	53.6 ^{NS}	11.9 ^{NS}	9.8 ^{NS}	8.6 ^{NS}	11.2 ^{NS}	8.7 ^{NS}
	R	82	110.3	3.4	18.7	51.5	10.5	9.6	8.44	11.2	8.2
2DL	S	88	111.0 ^{NS}	3.4 ^{NS}	19.4 ^{NS}	53.0 ^{NS}	11.0 ^{NS}	9.9 ^{NS}	8.7 ^{NS}	11.5 ^{NS}	8.6 ^{NS}
	R	72	112.5	3.6	19.3	51.8	11.4	9.5	8.3	10.9	8.2
Fhb1 +2DL	S	44	113.4 **	3.8 *	21.3 *	54.9 ^{NS}	12.9 *	10.2 ^{NS}	8.6 ^{NS}	11.3 ^{NS}	9.2 ^{NS}
	R	38	112.3	3.8	20.0	51.7	12.1	9.6	8.0	10.6	8.3
POP3 (KY97C-0519-04-05/VA01W476)											
Fhb1	S	87	109.8 ^{NS}	2.1 ^{NS}	13.5 ^{NS}	36.3 ^{NS}	5.4 ^{NS}	8.3 ^{NS}	6.7 *	10.6 ^{NS}	3.7 ^{NS}
	R	78	110.0	2.2	13.5	32.4	5.0	8.6	6.1	10.0	3.4
2DL	S	89	109.7 **	2.5 **	14.1 ^{NS}	38.7 **	5.8 *	9.2 **	7.3 **	11.3 **	4.2 **
	R	76	110.1	1.8	12.7	29.5	4.4	7.5	5.4	9.2	2.8
Fhb1 +2DL	S	51	109.8 **	2.6 **	15.3 *	41.7 **	6.8 **	9.0 **	7.3 **	11.4 **	4.4 **
	R	40	110.3	2.0	13.8	29.8	5.2	7.5	4.9	8.8	2.8
POP4 (KY97C-0540-01-03/VA01W476)											
Fhb1	S	160	109.5 *	2.3 **	13.2 ^{NS}	36.7 **	5.5 **	7.4 **	7.0 *	11.2 *	5.0 **
	R	112	109.2	1.9	12.2	31.1	4.2	6.3	6.3	10.6	3.6
2DL	S	152	109.3 ^{NS}	2.2 ^{NS}	12.4 ^{NS}	35.3 ^{NS}	4.8 ^{NS}	7.6 **	7.1 **	11.7 **	5.0 **
	R	120	109.4	2.1	13.2	33.2	5.1	6.1	6.2	9.9	3.7
Fhb1 +2DL	S	90	109.6 **	2.5 **	13.0 ^{NS}	37.1 **	5.4 **	8.2 **	7.3 **	12.0 **	5.8 **
	R	50	109.6	1.8	12.9	29.0	4.5	5.7	5.8	9.6	3.2
POP6 (KY97C-0508-01-01A/VA01476)											
Fhb1	S	112	107.0 ^{NS}	1.9 **	0.2 ^{NS}	31.1 ^{NS}	5.5 ^{NS}	10.2 *	9.5 ^{NS}	14.9 *	5.2 **
	R	78	107.3	1.4	0.2	30.1	6.3	9.1	8.8	13.9	3.8
2DL	S	92	107.4 **	1.8 ^{NS}	0.2 ^{NS}	31.1 ^{NS}	6.0 ^{NS}	10.6 **	9.6 ^{NS}	14.8 ^{NS}	5.1 **
	R	98	106.9	1.7	0.2	30.3	5.6	8.9	8.9	14.2	4.1
Fhb1 +2DL	S	50	107.1 **	1.92 *	0.2 ^{NS}	31.0 ^{NS}	5.7 ^{NS}	11.1 **	10.0 ^{NS}	15.4 *	5.8 **
	R	36	106.8	1.3	0.2	28.6	6.2	8.1	8.5	13.7	3.2

^{NS} : not significant; * : significant at 5% level ($P < 0.05$); ** : significant at 1% level ($P < 0.01$).

Table 3.8. QTL by Year interaction measured on F_2 derived wheat populations 2, 3, 4 and 6, evaluated in the scab nursery 2011 and 2012. Lexington, KY. The measurements were taken for all three different situations: only QTL *Fhb1* was present (*Fhb1* * year), only QTL *QFhs.nau-2DL* was present (*2DL* * year), and when both *Fhb1* and *QFhs.nau-2DL* were present (both QTL * year) in each line studied for the FHB traits: Rating, HDate, Incidence, Severity, FHB index, FDK, FDKNIR, DONNIR, DON.

	Pr > F											
	Fhb1 * year				*2DL* * year				both QTL * year			
	POP2	POP3	POP4	POP6	POP2	POP3	POP4	POP6	POP2	POP3	POP4	POP6
Rating	0.02	0.46	0.27	0.03	0.00 **	0.88	0.06	0.27	0.00 **	0.91	0.08	0.10
HDate	0.08	0.49	0.29	0.89	0.33	0.31	0.42	0.60	0.09	0.13	0.09	0.59
Incidence	0.10	0.21	0.40	0.36	0.91	0.06	0.35	0.76	0.39	0.14	0.56	0.02
Severity	0.01	0.17	0.61	0.13	0.01 **	0.07	0.40	0.01	0.00 **	0.01 **	0.43	0.79
FHB index	0.01	0.57	0.35	0.12	0.07	0.09	0.66	0.19	0.00 **	0.40	0.05	0.24
FDK	0.94	0.99	0.01	0.07	0.03	0.01 **	0.09	0.05	0.14	0.03	0.04	0.06
FDKNIR	0.92	0.07	0.08	0.17	0.07	0.82	0.87	0.41	0.34	0.23	0.19	0.44
DONNIR	0.49	0.15	0.50	0.10	0.28	0.09	0.86	0.01	0.46	0.25	0.59	0.02
DON	0.88	0.50	0.02	0.00 **	0.22	0.24	0.41	0.45	0.62	0.58	0.16	0.01 **

^{NS} : not significant; * : significant at 5% level ($P < 0.05$); ** : significant at 1% level ($P < 0.01$).

Table 3.9. Pearson correlation coefficients of four F_2 derived wheat populations combined, for 2 years entry means of Height, heading date (HDate) and disease traits: Rating (Rat), Incidence (Inc), Severity (Sev), FHB index, Fusarium damaged kernels (FDK) and deoxynivalenol level (DON), with statistical significances. Lexington, KY 2011 and 2012.

	HDate	Rat	Inc	Sev	FHB index	FDK	DON	FDKNIR	DONNIR
Height	-0.07 ^{NS}	-0.12 *	-0.10 *	-0.06 ^{NS}	-0.09 ^{NS}	-0.40 **	-0.05 ^{NS}	-0.24 **	-0.22 **
Hdate	1	0.31 **	0.48 **	0.48 **	0.53 **	0.22 **	0.38 **	0.32 **	0.12 *
Rat		1	0.65 **	0.69 **	0.71 **	0.43 **	0.67 **	0.50 **	0.52 **
Inc			1	0.66 **	0.88 **	0.25 **	0.45 **	0.32 **	0.29 **
Sev				1	0.87 **	0.33 **	0.54 **	0.45 **	0.44 **
FHB index					1	0.31 **	0.51 **	0.38 **	0.37 **
FDK						1	0.47 **	0.48 **	0.53 **
DON							1	0.63 **	0.63 **
FDKNIR								1	0.84 **
DONNIR									1

^{NS} : not significant; * : significant at 5% level ($P < 0.05$); ** : significant at 1% level ($P < 0.01$)

Table 3.10. Pearson correlation coefficients of F_2 derived wheat populations 2, 3, 4 and 6, for 2 years entry means of Fusarium damaged kernels (FDK) and deoxynivalenol level (DON) and their predictions by Near Infrared Reflectance (FDKNIR and DONNIR), with statistical significances. Lexington, KY 2011 and 2012.

	FDK	FDKNIR	DONNIR	DON	Population
FDK		0.38 **	0.50 **	0.54 **	2
		0.35 **	0.51 **	0.42 **	3
		0.44 **	0.46 **	0.46 **	4
		0.73 **	0.69 **	0.61 **	6
FDKNIR			0.78 **	0.54 **	2
			0.90 **	0.70 **	3
			0.81 **	0.57 **	4
			0.87 **	0.77 **	6
DONNIR				0.55 **	2
				0.77 **	3
				0.64 **	4
				0.82 **	6

** : significant at 1% level ($P < 0.01$).

Table 3.11. Heritabilities and their 90% confidence interval (in parentheses) of four F_2 derived wheat populations based on 2 years entry means. Disease traits evaluated were: Rating, Incidence, Severity, FHB index, Fusarium damaged kernels (FDK) and deoxynivalenol level (DON), Lexington, KY 2011 and 2012.

	Rating	Incidence	Severity	FHB index	FDK	DON
Overall	0.37 (0.28 - 0.44)	0.51 (0.44 - 0.57)	0.24 (0.14 - 0.34)	0.41 (0.33 - 0.48)	0.32 (0.23 - 0.41)	0.65 (0.60 - 0.69)
POP2	0.19 (-0.08 - 0.39)	0.49 (0.32 - 0.62)	0.14 (-0.14 - 0.36)	0.42 (0.23 - 0.57)	0.41 (0.21 - 0.56)	0.66 (0.54 - 0.74)
POP3	0.48 (0.31 - 0.61)	0.42 (0.22 - 0.56)	0.30 (0.07 - 0.47)	0.35 (0.14 - 0.51)	0.48 (0.31 - 0.61)	0.54 (0.38 - 0.65)
POP4	0.36 (0.20 - 0.48)	0.57 (0.47 - 0.66)	0.26 (0.07 - 0.41)	0.43 (0.29 - 0.54)	0.16 (-0.05 - 0.33)	0.75 (0.69 - 0.80)
POP6	0.65 (0.54 - 0.73)	0.34 (0.14 - 0.66)	0.26 (0.41 - 0.66)	0.43 (0.25 - 0.56)	0.38 (0.19 - 0.53)	0.61 (0.49 - 0.70)

Table 3.12. Means of FDK, DON, Rating, Severity, Incidence, FHB index, FDKNIR and DONNIR from 2011 and 2012 head-rows of F_2 derived wheat populations 2, 3, 4 and 6 averaged. Heritability for each disease trait and Direct Response to Selection percentages, with 10 and 20% intensity selection (p = 0.1 and 0.2, respectively) of population mean.

Disease Traits		Mean		Heritability	Predicted Direct Response to selection (%) (p = 0.1)	Predicted Direct Response to selection (%) (p = 0.2)
		2011	2012			
FDK	(%)	23.43	8.50	0.32	24.74	19.68
DON	(ppm)	13.68	2.33	0.65	47.45	37.79
Rating	(1 - 9)	4.00	3.72	0.36	22.55	17.97
Severity	(%)	45.61	15.05	0.24	9.89	7.86
Incidence	(%)	51.45	6.47	0.51	25.24	20.11
FHBindex	(%)	24.35	7.55	0.41	31.97	25.44
FDKNIR	(%)	26.98	11.71	0.51	56.52	13.79
DONNIR	(ppm)	25.97	5.10	0.48	73.50	14.51

Table 3.13. Means, Heritabilities and Direct Response to Selection percentages of population mean, measured from F_2 derived wheat populations 2, 3, 4 and 6 averaged, in Lexington, KY 2011.

Disease Traits		Mean	Heritability	Predicted Direct Response to selection (%) (p = 0.1)
FDK	(%)	23.43	0.84	97.77
DON	(ppm)	13.68	0.91	97.77
Rating	(1 - 9)	4.00	0.76	42.54
Severity	(%)	45.61	0.07	3.87
Incidence	(%)	51.45	-	-
FHBindex	(%)	24.35	0.21	9.14
FDKNIR	(%)	26.98	0.81	160.66
DONNIR	(ppm)	25.97	0.83	308.46

Table 3.14. Means, Heritabilities and Direct Response to Selection percentages of population mean, measured from F_2 derived wheat populations 2, 3, 4 and 6 averaged, in Lexington, KY 2012.

Disease Traits		Mean	Heritability	Predicted Direct Response to selection (%) (p = 0.1)
FDK	(%)	8.50	0.84	68.61
DON	(ppm)	2.33	0.77	101.93
Rating	(1 - 9)	3.72	0.42	33.32
Severity	(%)	15.05	0.36	30.36
Incidence	(%)	6.47	0.57	32.73
FHBindex	(%)	7.55	0.07	5.80
FDKNIR	(%)	11.71	0.78	51.97
DONNIR	(ppm)	5.10	0.82	48.03

Table 3.15. Yield, test weight and height averages for F_2 derived wheat populations 2, 3, 4 and 6 planted in yield plots in Lexington, KY, 2012. Mean of FHB resistant parents (VA01W-476); FHB susceptible high-yielding parents (KY lines) and checks (Truman and Pembroke).

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	HEIGHT (in)
2	KY97C-0321-05-2 / VA01W476	R^2 (%)	70.9	81.8	83.2
		CV	11.1	1.4	5.8
		Mean	63.8	59.8	33.7
		Range	52.8 - 73.6	58.4 - 70.0	30.5 - 36.3
3	KY97C-0519-04-05 / VA01W476	R^2 (%)	86.6	98.6	89.7
		CV	11.9	0.5	4.6
		Mean	48.4	59.3	33.1
		Range	33.6 - 72.1	57.3 - 61.6	28.0 - 39.0
4	KY97C0540-0103 / VA01W476	R^2 (%)	75.5	86.8	85.7
		CV	10.6	1.3	5.0
		Mean	63.4	60.0	33.4
		Range	53.0 - 77.5	58.0 - 61.3	27.8 - 37.0
6	KY97C-0508-01-01A / VA01W476	R^2 (%)	90.1	89.5	90.6
		CV	8.0	1.2	4.7
		Mean	63.0	59.8	34.9
		Range	49.3 - 73.2	58.3 - 60.9	30.3 - 39.3
FHB Resistant Parents		Mean	46.2	60.4	31.0
FHB Susceptible Parents			78.1	56.2	30.3
Checks			66.5	57.7	33.3

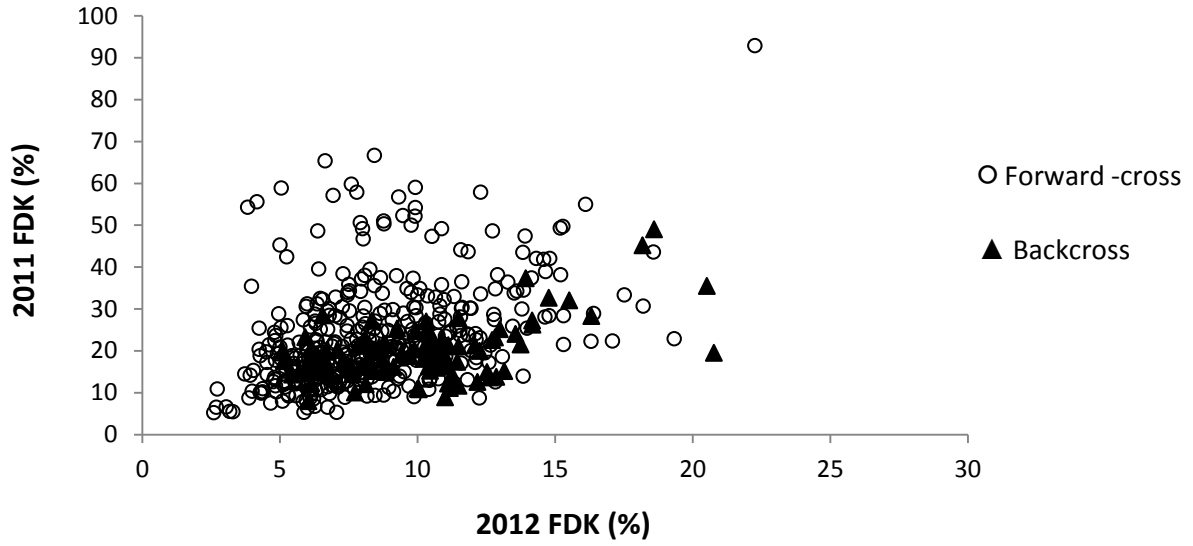

Figure 3.1: Relationship Between 2011 and 2012 measurements of FDK percentages, by Forward-cross and Backcross derived wheat populations.

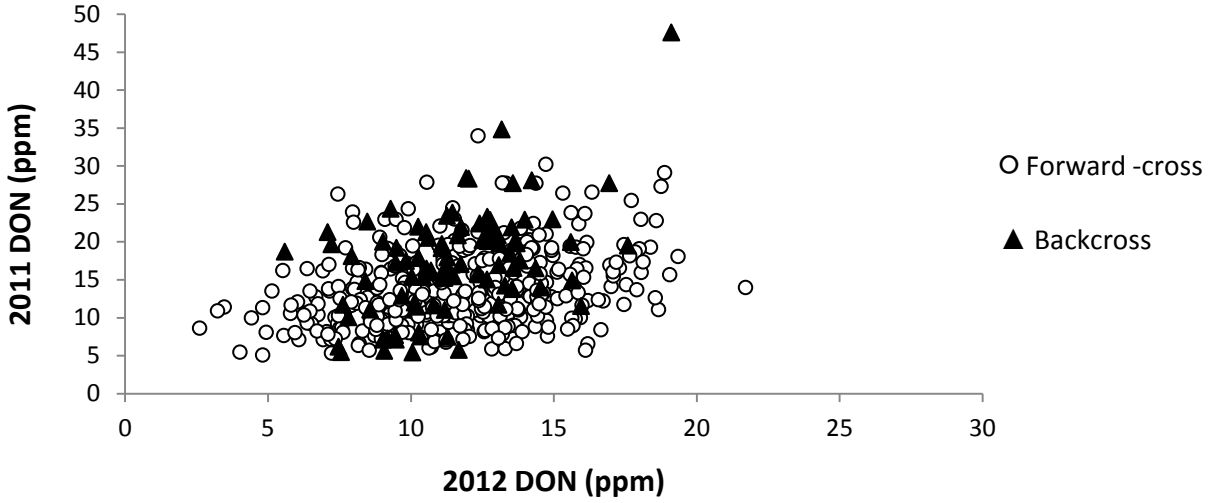

Figure 3.2: Relationship between 2011 and 2012 measurements of DON levels in ppm, by Forward-cross and Backcross derived wheat populations.

Copyright © Daniela Sarti Dvorjak 2014

Chapter 4

Diversity Arrays Technology (DArT) for genetic diversity and DNA polymorphism analysis in a wheat backcross population

4.1. Introduction

Molecular markers have contributed greatly to our understanding of the genetic basis of economically important crops. Numerous DNA-based genetic marker analysis methods have been developed over the last three decades, including restriction fragment length polymorphism (RFLP) (Heun et al., 1997), simple-sequence repeats (SSR) (Zohary & Hopf, 1993), random amplified polymorphic DNA (RAPD) (Kilian et al., 2007), amplified fragment length polymorphism (AFLP) (Wicker et al., 2003), nucleic acid indexing (Huang et al., 2002; Dvorak et al., 2004), restriction enzyme amplification display system (READS) (James et al., 2006), and single nucleotide polymorphisms (SNPs) (Jing et al., 2007). Although these genotyping methods have been an important tool in genome analysis and plant breeding, they are constrained by their dependence on gel electrophoresis or by the fact that some of these methods require pre-identification of a polymorphism, or even because a number of them are too expensive and laborious (Jaccoud et al., 2001).

Diversity Arrays Technology (DArT) is a powerful method developed as a sequence-independent and micro-array hybridization- based marker system, offering a low cost and high-throughput, robust method (Jaccoud et al., 2001). DArT generates medium density genome scans by scoring the presence versus absence of DNA fragments in representations of genomic DNA samples. It simultaneously determines hundreds to

thousands of polymorphic loci in a single assay, being able to detect single base pair changes and to provide comprehensive genome coverage even in organisms without any DNA sequence information (Jaccoud et al., 2001; Wenzl et al., 2004). Since its initial development in rice, DArT has been employed in genetic mapping, genotyping and diversity assessment in several crops such as barley (Wenzl et al., 2004; Hearnden et al., 2007; Wenzl et al., 2006; Wenzl et al., 2007), arabidopsis (Wittenberg et al., 2005), cassava (Xia et al., 2005), sorghum (Mace et al., 2008), rice (Reinke & Kilian, 2006) and hexaploid and durum wheat (Akbari et al., 2006; Peleg et al., 2008; Semagn et al., 2006; White et al., 2008).

DArT markers can also be used in breeding programs to compare DNA of different lines, measuring genetic diversity among breeding lines and cultivars, or parent lines and their progeny, at the molecular level. DArT markers are able to distinguish the genetic differences amongst genomes and facilitate the localization and map-based cloning of genes of interest (Reinke & Kilian, 2006; Jing et al., 2009). The technique has also been shown to be reproducible and cost effective (Gupta et al., 2008).

Akbari et al. (2006) showed in their studies that DArT can be successfully applied to the large hexaploid genome of bread wheat and could be used for genetic mapping, with the possibility of demonstrating unique segregation patterns between different wheat cultivars. There was still a concern that polymorphism frequency would be low and that polyploidy would affect the precision of DArT markers, however, no significant research linking DArT markers to phenotypic traits in wheat had been done at that point.

It was thought that DArT markers might be used in this study for identifying QTL associated with native (non-exotic) FHB resistance as well as QTL affecting agronomic and milling and baking quality traits. Fusarium head blight (FHB) is one of the most-destructive diseases of wheat worldwide and high yielding cultivars with complete resistance have yet to be developed in North America. Most soft wheat breeding programs make selections based upon agronomic characteristics such as yield, test weight, height, heading date, and disease resistance. It is only after identifying suitable lines in the field that the grains are tested and selected for quality characteristics. Usually, analysis for flour quality happens in later generations because traditional flour-based quality tests require large grain samples, expensive equipment and labor intensive activities.

Gutierri et al. (2004) investigated new methods for evaluating wheat quality using wheat meal (WM) instead of wheat flour. WM-based assays are highly efficient in predicting straight-grade flour tests and require small samples (1 gram of wheat meal) and common laboratory equipment. This way, evaluations can be done in early generations and include several experimental lines. Basic traits evaluated for baking quality include flour yield, flour protein, softness equivalent, water, sucrose and lactic acid SRC, and cookie diameter. Flours with high water retention produces a less tender product and require increased baking times during cookie and cracker manufacturing, which increases energy costs (Gutierri et al., 2004). A set of assays called solvent retention capacity (SRC) tests are used to predict the baking performance of soft wheat as they require little grain and less labor and expenses than traditional baking test (Smith et al., 2011). Balut et al. (2013) applied SRC tests to several populations of F_6 derived lines

of wheat and successfully correlated the results with chemically analyzed values and FHB resistance alleles. They were able to show FHB disease levels reduction with negligible impact on agronomic and quality traits.

Marker assisted selection could be an important tool to facilitate the selection of resistant cultivars and to enhance breeding efficiency. Several maps have been reported and DArT markers have demonstrated the potential for increasing marker density within a short time and at low cost (Jaccoud et al., 2001; Wenzl et al., 2004). The objective of this study was i) to evaluate the effectiveness of DArT markers in polymorphism detection in the wheat genome, ii) to apply DArT markers to backcross derived lines and their parents, computing the percentage of recurrent parent in each round of backcross performed, iii) to compare yield x recurrent parent percentage and evaluate this correlation and the effects on the genotypes, iv) to investigate possible linkage between markers and agronomic, disease resistance, milling and quality traits of economic importance in wheat.

4.2. Material and Methods

The population selected for DArT analysis was the backcross Population 2 (DN2) from the cross KY97C-0321-05-2/ VA01W-476// KY97C-0321-05-2. This population was selected because of the superior agronomic characteristics of the recurrent parent. BC_1F_2 seeds from Population 2 lines were planted in greenhouse trays and the leaves collected for DNA extraction. Genomic material was isolated according to “Plant DNA Extraction Protocol for DArT” (www.triticarte.com.au/pdf/DArT_DNA_isolation.pdf)

and a total of 94 genotypes were submitted to Diversity Arrays Technology P/L - Triticarte P/L Australia (www.triticarte.com.au). 961 DArT markers were applied, covering all the chromosomes on the wheat genome. DArT technology reduces the complexity of a DNA sample by digestion with a combination of restriction enzymes to obtain a representation of that sample. Digestion is followed by adapter ligation and amplification. The resulting representations of our lines were precipitated, denatured and labeled according to Wenzl et al. (2004). Libraries of genomic representations were prepared essentially as by Jaccoud et al. (2001).

Representations of each line to be genotyped were labeled and hybridized to the array. The polymorphisms scored the presence versus absence of hybridization to individual array elements. They reflect DNA sequence variation that determines which genomic sequences are present in the genomic representations of each genotype. The microarray platform makes the discovery process very efficient because all markers on a particular DArT array are scored simultaneously. A binary matrix was generated in which "1" indicates presence and "0" indicates absence of the allele. 50 samples were analyzed in duplicate and the discordance in 50 pairs of scores was reported. When a sample was assayed in duplicate, the consensus of scores was used. Informative markers that show polymorphic fragments were selected and the differences between means were compared for statistical significance through PROC ANOVA variance analysis. Markers with significant values for chi-square ($P<0.05$) were identified and from those, the mean values and coefficient of determination (R^2) were considered. Each DArT marker was evaluated with respect to individual phenotypic traits. The proportion of observed phenotypic variance explained by each marker was estimated as the coefficient of

determination (R^2). Markers with a coefficient of determination greater than or equal to 0.25 ($R^2 \geq 0.25$) were selected as the most promising markers for that specific characteristic.

Individual maps were constructed using EasyMap, a program developed at Diversity Arrays P/L for high-throughput mapping of populations. EasyMap distributes markers into linkage groups based on the record algorithm, the detection of potential genotyping errors, the re-optimization of marker orders after replacing potential errors, and the estimation of map distance. The Synthetic/Opata map was conducted with JoinMap 4.0 (Van Ooijen & Voorrips, 2006) using the "maximum likelihood" algorithm. Linkage groups were assigned to chromosomes based on comparison across populations and the existing chromosome assignments available on Synthetic / Opata and Cranbrook / Halberd maps, available online at GrainGenes map data report (GrainGenes: A Database for Triticeae and Avena, available at <http://wheat.pw.usda.gov/ggpages/map_summary.html>)

From the DArT markers results, the percentage of recurrent parent of each BC_1 line was estimated. Subsequently, the yield x recurrent parent percentage and FHB resistance x recurrent parent percentage was compared and the correlation between them was evaluated.

After the DArT screening, 86 lines were selected and planted in $BC_1F_{2:3}$ 1.2 m long head-rows, spaced 30 cm apart in the misted inoculated scab nursery in Lexington, KY, 10 October, 2010 and also planted in plots at Lexington, KY in a 2 rep RCB. The randomized complete blocks in the field plots were 6 rows wide; 3 m long each row,

planted on 25 October, 2010. Phenotypic disease notes were taken in both replications of all the lines planted in the scab nursery. The traits evaluated in 2011 were: Rating, Incidence, Severity, FHB index (Incidence * Severity), FDK, DON, FDK and DON predictions using Near Infrared Reflectance Spectroscopy (FDKNIR and DONNIR), plant height, yield and test weight (TWT).

The DArT lines were planted in the 2012 scab nursery again (22 October, 2011) and also in yield plots (5 November, 2011), in a randomized complete block design, with 2 replications, in two locations (Lexington and Princeton, KY). From 2012 plots, yield, test weight and plant height were one more time evaluated, as well as NIR measurements. Grain samples were collected from each line within Population 2 and baking quality tests for all genotypes were done at the USDA Soft Wheat Quality Lab in Wooster Ohio. Wheat meal (WM) assays with sodium carbonate (SRC) and sodium dodecyl sulphate sedimentation (SDS) tests were evaluated at the Wheat Breeding Molecular Marker Laboratory at University of Kentucky. Traits measured in 2012 included:

- Rating / Field Scab Rating: Fusarium Head Blight was visually scored as 1-9 scale, where 1 is <10% and 9 is > 90% of diseased plants.
- FHB incidence: At 21 to 24 days after anthesis, 20 random spikes were selected per head-row in the scab nursery and the number of diseased spikes were recorded and expressed as a percentage.
- FHB severity: Severity consisted of the proportion of visually infected spikelets per total number of spikelets per spike, in 10 randomly selected

spikes per row. FHB severity was also measured approximately 21 to 24 days after anthesis.

- FHB index: FHB index is the product of Severity * Incidence.
- FDK: Fusarium Damaged Kernels, expressed as the percentage weight of scabby kernels divided by total weight of kernels in a sample.
- DON: Deoxynivalenol mycotoxins levels were measured from a 20 g sample tested in laboratory using gas chromatography with mass spectrometry (GC-MS), at University of Minnesota.
- Yield: measured in bushels per acre.
- Test Weight: adjusted for moisture content and measured in pounds per bushel.
- Flour Yield: calculated as the bran weight subtracted from the grain weight, divided by the grain weight times 100 as described in Souza et al. (2008).
- Flour Protein: measures protein content at 14% humidity.
- Softness Equivalent: calculated from the fraction of mill product that is in the mids that is subtracted from the adjusted flour yield.
- Lactic Acid SRC, Sucrose SRC, Water SRC, and Sodium Carbonate SRC were estimated using approved AACC Method 56-11.02 (AACC, 2010) and were used to calculate GPI as described by Kweon et al. (2011).
- Flour sucrose SRC: the best predictor of cookie quality. It is a measure of arabinoxylans content that affect water absorption in baked products.

- Flour water SRC: measures global water affinity of starch, arabinoxylans, gluten, and gliadins.
- Sodium Carbonate SRC: measures starch damage in each sample.
- Wheat Meal SDS: this test is used to predict flour lactic acid SRC.
- Wheat Meal SRC: this flour solvent retention capacity (SRC) tests predict baking performance by measuring the weight of solvent retained as a percentage of the flour weight (Smith et al., 2011).
- Cookie Diameter: depending on the gluten strength, the cookie will be more spread or more compact, affecting its diameter.

Wheat Meal SDS sedimentation volume was measured as described in Knott et al. (2009) at the Wheat Breeding Laboratory at University of Kentucky, Lexington, KY. Duplicate evaluations were conducted for each sample. 25 g of BC_1F_2 derived seed were milled with a Cyclone sample mill (UDY, Fort Collins, CO, 80524) using a 1 mm sieve. Ten milliliters of deionized water were dispensed with a bottle-top dispenser into 25 mL glass graduated cylinders, with ground glass stoppers caps. 1 g of wheat meal was added to each graduated cylinder, shaken vigorously for approximately 15 s, and placed onto a test tube rocker to rest for 2 min. After the rest period, the cylinders were inverted four times, allowed to rest for 2 min, and inverted four times again. Sodium lauryl sulfate (10 mL, 2.5% w/v) was added to each cylinder with a bottle-top dispenser. The cylinders were inverted four times and allowed to rest 2 min. The procedure was repeated three times for a total of four cycles. Lactic acid (5 mL of 1.1% w/v) was added using a bottle-top dispenser. Four cycles of inverting the cylinders four times followed by a 2 min rest were completed. After the final inversion, the cylinders were removed from the rocker

and allowed to settle for 20 min before sedimentation volume was measured. Entry means values were calculated and the results compared.

Wheat meal sodium carbonate SRC was done for the same BC_1F_2 lines in Population 2 at the Wheat Breeding Laboratory at University of Kentucky, Lexington, KY. As described in Knott et al. (2009), 5 g wheat meal sample wheat meal were placed into disposable 50 mL centrifuge tubes and 25 mL of 5% (w/w) sodium carbonate was added using a bottle-top dispenser. Tubes were shaken horizontally 40 times to suspend the wheat meal into the sodium carbonate. Tubes were placed horizontally onto an orbital shaker and agitated for 20 min at 100 rpm. The tubes were centrifuged at 1000 x *g* for 15 min. The supernatant was decanted and the tubes were allowed to drain on absorbent towels for 10 min. The tubes were weighed and solvent retention capacity was calculated with the following equation:

SRC = 100 × {(Pellet weight/Flour weight) ×

[86/(100 − Wheat Meal Moisture)] − 1}

PROC CORR (SAS, 2013) was used to analyze the entry means relationship between the traits evaluated.

4.3. Results and Discussion

DArT markers are biallelic dominant markers. Each marker is scored for each sample: 0, 1 or "–" (missing data): the marker could not be reliably scored for that

sample. The marker quality (Q) was evaluated for all 961 markers through ANOVA and the values obtained were above 80, suggesting a good and reliable marker quality. Because Q is based on ANOVA and it is not an appropriate quality measure for markers with a very asymmetric distribution of 0 and 1 scores (low PIC), some markers with lower Q but high call rate and low PIC were also reported for the first inquiry.

The DArT methodology efficiently generated DArT fingerprints of wheat Population 2 and a large number of high-quality markers, with 95.5% genotype call rate. The genetic diversity was analyzed and the genotype x recurrent parent genetic similarity was estimated for each line. The correlation between percentage of recurrent parent and FDK was 30.5% and between percentage of recurrent parent (RP) and severity was 19.7%. Correlation between FHB index and percentage of recurrent parent was 24%. The line with lowest percentage of RP (30.6%) were not the lowest yielding line (yield = 57.3 bu/acre) however it was extremely susceptible to FHB (FDK = 26%, DON = 20 ppm). Line D216712 presented the highest recurrent parent percentage but it was not the highest yielding line of the population with scab profile towards the moderated / high infection with FDK averages of 19% and 15 ppm DON levels. This observation is important because scab infection will reduce yields even within lines with good high yielding backgrounds. Two lines with exactly same %RP (40.1) differed on yield and scab levels, being line D216517 showing low disease levels (FDK = 13%) and high yield (63.4 bu/acre), and line D200039 had yields reduced to 55 bu/acre and high scab infection, with FDK levels of 24% and average DON of 20 ppm. Several lines were identified as possible candidates for wheat breeding variety development, for example line D2166605

presented the lowest scab levels, with FDK of 8% and 9 ppm DON. Mean yields for this genotype was 63 bu/acre; DArT analysis indicated that it was 49% recurrent parent.

From the 2011 yield plot study, the correlation between BC_1F_2 derived line's yield and percentage of recurrent parent was 0.28. Yield and test weight measurements evidenced some promising lines, which were able to restore agronomic performance after one backcross (Figure 4.1). Disease traits such as Ratings, Incidence, Severity, FHB index, FDK and DON were also evaluated through the increase of recurrent parent proportions in each line, with no substantial association between FHB resistance and RP percentages (Figure A.4.1 to Figure A.4.6). There were 15 out of 26 lines (58%) the yield of which did not significantly differ ($P < 0.05$) from commercial checks' yields. For test weight, there were 16 out of 26 lines (61.5%) tested that were not significantly different from the checks ($P < 0.05$). These preliminary results indicated that BC_1 populations may be a useful source of breeding lines.

The DArT information can also be useful characterizing derivatives of these 86 lines to ascertain the importance of other individual markers. Several other traits related to disease resistance and baking quality were evaluated and the phenotypic data compared to genotypic polymorphisms from the DArT result panels, contributing to investigations of a possible linkage between phenotype and molecular marker. The possible linkage between phenotypic data and molecular markers were examined and some markers were good predictors of phenotypic traits (Tables 4.1 to 4.5).

After DArT screening, FHB ratings exhibited linkage with 4 potential markers, and two of them mapped to chromosome 2B. Incidence and FHB index each had their

variability attributed to one marker (wPt-5075 and wPT-733571, respectively), with good coefficient of determination (R^2= 0.8 and 0.6) but no known mapping location on the wheat genome. Severity was linked to 3 markers, one of them have been mapped to chromosome 7 on genome A. FDK and DON were significant for one marker each, with moderate R^2. DON levels are particularly important to the white wheat industry, for whole grain products and breakfast cereal production. DON is relatively stable throughout most processing and milling procedures, and it is highest in the bran and lowest in the flour. DON had a highly significant marker ($P < 0.001$) mapped to chromosome 6A. Field scab ratings on individual plots presented 11 significant markers located on chromosome 2B (Table 4.1).

From the scab related traits screened with DArT markers, marker wPt-5075 for Incidence ($R^2 = 0.8$) and marker wPt-733571 for FHB index ($R^2 = 0.6$) were two of the most informative for FHB resistance in Population 2. The next step would be to identify polymorphic lines and double check the reproducibility of these markers on traits based on type II FHB resistance, against the spreading of Fusarium within the wheat spike, like incidence. Because these markers show no known association with any specific region of the wheat genome, an alternative would be to determine the DArT marker segment sequence and locate within the genome.

Most traits important to agriculture, such as yield, are controlled by polygenes. Finding markers which positively influence agronomic traits could be a valuable tool to increase yield in several cultivars and breeding lines. We found 2 markers related to yield that had good R^2; however, they have not been mapped yet. For test weight, 38

significant markers were identified, mapped mainly to regions 4A, 5B and 7A (Table 4.2).

Quality traits were also screened with DArT markers and polymorphisms analyzed. Flour yield is essential for the milling industry, the higher the flour yield values, the higher its quality importance is. Softness equivalent is the measurement of flour particle size and it is negatively correlated with flour yield. As softness equivalent increases, the amount of break flour increase, which elevates energy costs. The 5 most significant markers for flour yield were not mapped, with exception of wPt-1554, mapped to chromosome 2D. The same marker was also found significant for softness equivalent and cookie diameter. Flour protein had 7 markers related to the trait, from 1D, 2A, 5A, 6D and 5B chromosomes. Softness equivalent presented several potential markers and we presented on the table the ones with coefficient of determination above 0.25, mapped mainly to chromosomes 2D and 5B (Table 4.3). Lactic Acid SRC predicts gluten strength, which is important to select bread quality in wheat. For lactic acid SRC and cookie diameter, 15 and 33 significant markers were pointed as the most substantial to predict the traits, respectively. Marker wPt-3647, on chromosome 2B, had a coefficient of determination equal to 0.97, being the most promising marker found for acid lactic SRC (Table 4.4).

Significant DArT markers for sucrose SRC were mapped on chromosomes 7A and 1A. Water and sodium carbonate SRC showed 6 and 4 significant markers each, at 1A, 1D, 2B, 2D and 6B for water SRC and 1B, 2B and 5B for sodium carbonate SRC. Wheat meal SDS and SRC were confirmed to be valuable tools to predict wheat quality

using fewer amounts of grains and less equipment. Wheat meal SDS had 7 significant markers, from chromosomes 1D, 2B, 6B, 7A and 7B. Wheat meal SRC had 2 significant predictive markers, mapped on 1A and 6A chromosomes (Table 4.5).

It has been shown that genes encoding D-genome of wheat are related to gliadins and glutenins. Gliadins are monomeric proteins that interact with gluten protein. Glutenins polymers are responsible for the elastic properties of gluten and dough. Dough strength is under genetic control (Lafiandra et al., 2007). Gluten proteins, encoded by a series of loci on the group 1 and 6 chromosomes are the major determinants of technological properties in bread and durum wheat (Shewry et al., 2003).

Liu et al. (1995) demonstrated large effects of chromosome 1D substitutions on glutenin amount, SDS sedimentation value, mixing time and peak resistance value. Most of the milling and quality traits studied in our experiment presented DArT markers linked to wheat D-genome, especially lactic acid SRC (related to glutenin characteristics), cookie diameter and softness equivalent (related to break flour and damaged starch).

Lafiandra et al. (2007) produced recombinant durum wheat lines with genes encoding D-genome related gliadins and glutenins. They introduced a segment on the short arm of chromosome 1A and 1D carrying genes for gliadins and alleles related to wheat dough strength. Sucrose SRC is a test associated to pentosans and gliadins proteins from wheat flour. We found 3 significant DArT markers mapped to chromosome 1A with R^2 ranging from 0.25 to 0.34.

4.4. Conclusions

Wheat is a globally important crop due to its adaptability to wide ranges of climate and improved grain quality for the production of baking goods. DArT markers are efficient tools to identify and locate polymorphisms in wheat genome. In this study, we were able to point out DNA regions possibly linked to important phenotypic economical and quality traits for wheat. The recommended next step for this investigation is to select the most promising markers for further mapping and progeny analysis.

Table 4.1. Significant DArT markers (P<0.05), coefficient of determination (R^2), marker name and mapped location in the wheat genome for Scab Rating, Incidence, Severity, FHB index, Fusarium Damaged Kernel (FDK), Deoxynivalenol (DON), and Field Scab, for BC_1 derived wheat Population 2.

Rating (mean= 3.71)				
Marker Number	Marker name	P (>F)	R^2	Map
54	wPt-730136	0.003	0.13	-
89	wPt-741571	0.001	0.15	-
216	wPt-7229	0.001	0.15	3B
220	wPt-9310	0.000	0.17	3B
Incidence (mean= 58.14)				
Marker Number	Marker name	P (>F)	R^2	Map
53	wPt-5075	0.030	0.80	-
Severity (mean= 35.53)				
Marker Number	Marker name	P (>F)	R^2	Map
47	tPt-513473	0.000	0.16	-
75	tPt-513279	0.004	0.12	-
319	wPt-6768	0.003	0.12	7A
FHBindex (mean= 21.54)				
Marker Number	Marker name	P (>F)	R^2	Map
40	wPt-733571	0.065	0.60	-
FDK (mean= 13.97)				
Marker Number	Marker name	P (>F)	R^2	Map
102	wPt-667472	0.004	0.12	-
DON (mean= 10.96)				
Marker Number	Marker name	P (>F)	R^2	Map
280	wPt-0698	0.000	0.18	6A
Field Scab Rating (mean= 1.38)				
Marker Number	Marker name	P (>F)	R^2	Map
51	wPt-741382	0.001	0.32	-
52	wPt-744808	0.001	0.32	-
151	wPt-2989	0.002	0.34	2B
157	wPt-6108	0.002	0.36	2B
159	wPt-0948	0.002	0.35	2B
160	wPt-375218	0.004	0.32	-
161	tPt-4248	0.004	0.32	2B
162	wPt-9736	0.001	0.31	2B
163	tPt-5438	0.001	0.32	2B
164	wPt-3647	0.001	0.31	2B
165	tPt-9486	0.004	0.32	2B

Table 4.2. Significant DArT markers (P<0.05), coefficient of determination (R^2), marker name and mapped location in the wheat genome for yield and test weight (TWT), for BC_1 derived wheat Population 2.

Yield (mean= 62.67)				
Marker Number	Marker name	P (>F)	R^2	Map
65	tPt-513575	0.035	0.26	-
66	tPt-514219	0.019	0.29	-
TWT (mean= 57.88)				
Marker Number	Marker name	P (>F)	R^2	Map
34	wPt-730660	0.014	0.26	-
41	wPt-740658	0.016	0.25	-
45	tPt-513004	0.006	0.30	-
53	wPt-5075	0.001	0.37	-
55	wPt-732603	0.006	0.30	-
57	wPt-7358	0.000	0.36	-
59	wPt-3754	0.014	0.26	2B
68	wPt-666939	0.008	0.29	-
75	tPt-513279	0.000	0.45	-
80	tPt-513409	0.004	0.32	-
100	tPt-513735	0.011	0.27	-
111	wPt-742051	0.002	0.36	-
128	wPt-4811	0.000	0.43	1A | 1B
205	wPt-2740	0.001	0.38	3A
208	wPt-1353	0.001	0.32	3A
227	wPt-3480	0.010	0.27	3D
238	wPt-3374	0.009	0.28	4A
241	wPt-7939	0.004	0.32	4A
242	wPt-6728	0.009	0.28	4A
243	wPt-0117	0.000	0.43	4A
244	wPt-375746	0.010	0.27	-
248	wPt-5857	0.004	0.32	4A
261	wPt-3334	0.000	0.42	5A | 6D
263	wPt-9814	0.005	0.30	5B
264	wPt-3457	0.005	0.30	5B
265	wPt-1250	0.003	0.33	5B
267	tPt-0228	0.017	0.25	5B
270	wPt-1951	0.004	0.25	5B
272	tPt-4875	0.004	0.25	5B
273	wPt-6135	0.004	0.31	5B
275	wPt-7114	0.013	0.26	5B
278	wPt-1853	0.001	0.37	5B | 7B
296	wPt-1547	0.001	0.39	6B
302	wPt-1089	0.016	0.25	6B
311	wPt-1601	0.007	0.29	7A
313	wPt-0687	0.016	0.25	7A
315	wPt-6495	0.016	0.25	7A
319	wPt-6768	0.009	0.28	7A

Table 4.3. Significant DArT markers (P<0.05), coefficient of determination (R^2), marker name and mapped location in the wheat genome for Flour Yield, Flour Protein, Softness Equivalent, for BC_1 derived wheat Population 2.

Flour Yield (mean= 67.86)				
Marker Number	Marker name	P(>F)	R^2	Map
39	wPt-733932	0.009	0.28	-
85	wPt-731617	0.010	0.27	-
101	wPt-667406	0.013	0.26	-
198	wPt-1554	0.008	0.28	2D
337	wPt-730934	0.011	0.27	-
Flour Protein (mean= 8.80)				
Marker Number	Marker name	P(>F)	R^2	Map
25	wPt-733674	0.008	0.29	-
56	wPt-733363	0.014	0.25	-
143	wPt-8866	0.014	0.26	1D
144	wPt-5915	0.012	0.26	1D
145	wPt-8490	0.046	0.13	2A
261	wPt-3334	0.012	0.26	5A|6D
263	wPt-9814	0.019	0.24	5B
Softness Equivalent (mean= 53.67)				
Marker Number	Marker name	P(>F)	R^2	Map
25	wPt-733674	0.001	0.37	-
34	wPt-730660	0.013	0.26	-
37	wPt-3258	0.001	0.31	-
39	wPt-733932	0.002	0.34	-
44	wPt-7465	0.001	0.31	-
55	wPt-732603	0.002	0.35	-
58	wPt-2224	0.010	0.27	-
61	wPt-730744	0.004	0.32	-
62	wPt-731406	0.001	0.31	-
63	wPt-731941	0.007	0.29	-
67	wPt-664805	0.001	0.31	-
69	wPt-671778	0.005	0.30	-
70	wPt-729860	0.004	0.32	-
75	tPt-513279	0.008	0.28	-
76	wPt-667476	0.001	0.31	-
77	wPt-731134	0.004	0.31	-
78	wPt-732270	0.004	0.32	-
84	wPt-671737	0.004	0.32	-
94	wPt-5954	0.001	0.32	-
100	tPt-513735	0.007	0.29	-
101	wPt-667406	0.002	0.34	-
191	wPt-8713	0.003	0.33	2B|2D
195	wPt-5586	0.010	0.27	2D
196	wPt-6704	0.001	0.31	2D
197	wPt-9950	0.001	0.31	2D
198	wPt-1554	0.002	0.34	2D
205	wPt-2740	0.008	0.28	3A
261	wPt-3334	0.004	0.32	5A|6D
263	wPt-9814	0.013	0.26	5B
264	wPt-3457	0.004	0.31	5B
267	tPt-0228	0.016	0.25	5B
276	wPt-3053	0.013	0.26	5B
278	wPt-1853	0.013	0.26	5B|7B
311	wPt-1601	0.009	0.28	7A
324	wPt-1080	0.001	0.32	7A|7D
325	wPt-7076	0.001	0.32	7A|7D

Table 4.4. Significant DArT markers (P<0.05), coefficient of determination (R^2), marker name and mapped location in the wheat genome for Lactic Acid SRC and Cookie Diameter, for BC_1 derived wheat Population 2.

Lactic Acid (mean= 91.28)				
Marker Number	Marker name	P(>F)	R^2	Map
15	tPt-514211	0.007	0.29	-
27	tPt-514011	0.001	0.41	-
41	wPt-740658	0.001	0.38	-
42	wPt-744595	0.013	0.26	-
46	tPt-513338	0.003	0.26	-
48	tPt-513652	0.003	0.26	-
49	wPt-732448	0.003	0.26	-
50	wPt-734310	0.003	0.26	-
56	wPt-733363	0.009	0.28	-
164	wPt-3647	0.083	0.97	2B
227	wPt-3480	0.001	0.40	3D
228	wPt-4991	0.008	0.28	3D
257	wPt-4280	0.003	0.26	4B
278	wPt-1853	0.012	0.26	5B|7B
321	wPt-8418	0.016	0.25	7A
Cookie Diameter (mean= 18.52)				
Marker Number	Marker name	P(>F)	R^2	Map
25	wPt-733674	0.003	0.33	-
34	wPt-730660	0.015	0.25	-
37	wPt-3258	0.002	0.28	-
39	wPt-733932	0.006	0.30	-
44	wPt-7465	0.002	0.28	-
55	wPt-732603	0.006	0.30	-
56	wPt-733363	0.006	0.30	-
58	wPt-2224	0.011	0.27	-
61	wPt-730744	0.007	0.29	-
62	wPt-731406	0.002	0.28	-
63	wPt-731941	0.013	0.26	-
67	wPt-664805	0.002	0.28	-
69	wPt-671778	0.010	0.27	-
70	wPt-729860	0.007	0.29	-
75	tPt-513279	0.007	0.29	-
76	wPt-667476	0.002	0.28	-
77	wPt-731134	0.007	0.29	-
78	wPt-732270	0.008	0.28	-
84	wPt-671737	0.008	0.28	-
94	wPt-5954	0.003	0.26	-
101	wPt-667406	0.008	0.29	-
191	wPt-8713	0.006	0.30	2B|2D
195	wPt-5586	0.010	0.27	2D
196	wPt-6704	0.002	0.28	2D
197	wPt-9950	0.002	0.28	2D
198	wPt-1554	0.006	0.30	2D
205	wPt-2740	0.009	0.28	3A
261	wPt-3334	0.006	0.30	5A|6D
263	wPt-9814	0.014	0.26	5B
264	wPt-3457	0.015	0.25	5B
276	wPt-3053	0.013	0.26	5B
324	wPt-1080	0.003	0.26	7A|7D
325	wPt-7076	0.003	0.26	7A|7D

Table 4.5. Significant DArT markers (P<0.05), coefficient of determination (R^2), marker name and mapped location in the wheat genome for Sucrose SRC, Water SRC, Wheat Meal SDS, Wheat Meal SRC, and Sodium Carbonate SRC, for BC_1 derived wheat Population 2.

Sucrose SRC (mean= 88.42)				
Marker Number	Marker name	P(>F)	R^2	Map
26	wPt-740561	0.003	0.26	-
45	tPt-513004	0.007	0.29	-
111	wPt-742051	0.010	0.27	-
308	wPt-8192	0.003	0.26	7A
309	wPt-2199	0.003	0.26	7A
310	wPt-3135	0.003	0.26	7A
316	wPt-1076	0.003	0.26	7A
317	wPt-6273	0.003	0.26	7A
318	wPt-7767	0.003	0.26	7A
321	wPt-5411	0.002	0.34	1A
322	wPt-5411	0.015	0.25	1A
323	wPt-5411	0.003	0.26	1A
Water SRC (mean= 57.69)				
Marker Number	Marker name	P(>F)	R^2	Map
121	wPt-5411	0.005	0.31	1A
143	wPt-8866	0.002	0.35	1D
161	tPt-4248	0.001	0.40	2B
296	wPt-6704	0.008	0.28	2D
302	wPt-1089	0.001	0.38	6B
307	wPt-7745	0.017	0.25	6B
Wheat Meal SDS (mean= 7.35)				
Marker Number	Marker name	P(>F)	R^2	Map
86	wPt-732704	0.034	0.26	-
143	wPt-8866	0.015	0.31	1D
161	tPt-4248	0.008	0.34	2B
300	wPt-6127	0.029	0.26	6B
302	wPt-1089	0.011	0.33	6B
312	wPt-7105	0.022	0.28	7A
335	wPt-5816	0.032	0.26	7B
Wheat Meal SRC (mean= 80.01)				
Marker Number	Marker name	P(>F)	R^2	Map
129	wPt-7541	0.016	0.30	1A|6A
337	wPt-730934	0.028	0.27	-
Sodium Carbonate SRC (mean= 68.15)				
Marker Number	Marker name	P(>F)	R^2	Map
135	wPt-2395	0.008	0.30	1B
157	wPt-6108	0.003	0.34	2B
267	tPt-0228	0.015	0.26	5B
276	wPt-3053	0.015	0.26	5B

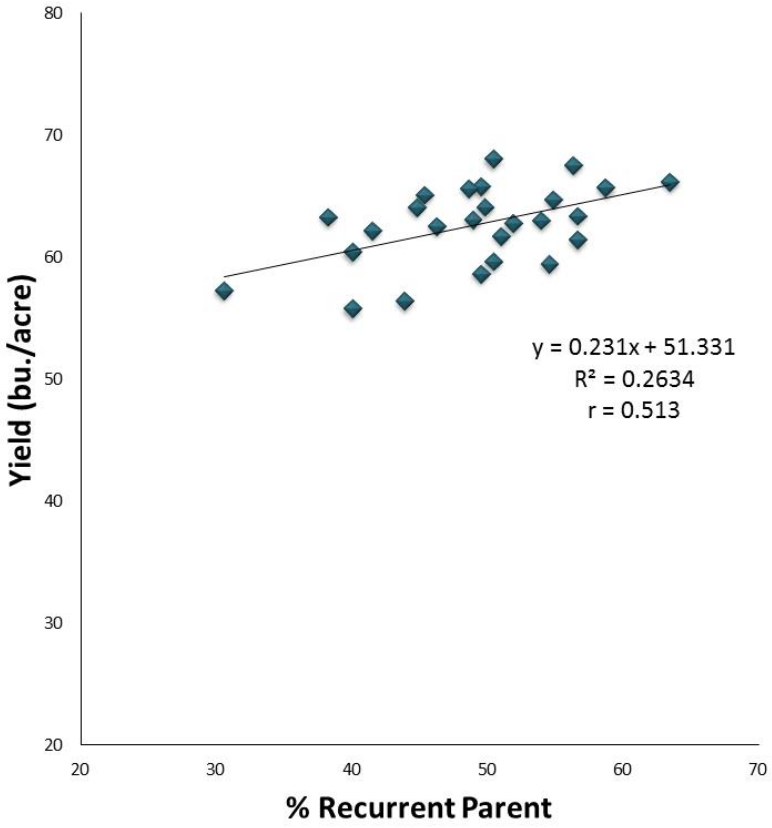

Figure 4.1: Relationship between yield averages and percentage of recurrent parent measured with DArT markers in Population 2, BC_1 derived wheat lines, over 3 environments (Lexington 2011; Lexington and Princeton 2012).

Copyright © Daniela Sarti Dvorjak 2014

Chapter 5

Evaluation of Fusarium Head Blight Resistance and Agronomic Performance in Backcross and Forward-Cross populations

5.1. Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph: *Giberella zeae* Schein. (Petch)], is recognized as one of the most destructive diseases of wheat (*Triticum aestivum L.* and *T. durum L.*) and barley (*Hordeum vulgare L.*) worldwide. Besides yield and quality losses, the most serious problem of FHB is the contamination of grains with mycotoxins including deoxynivalenol (DON).

The incorporation of genetic resistance to FHB reduces the need for fungicide applications, production costs and increases food safety, and is thus an important objective of most wheat breeding programs (Von der Ohe et al., 2010). Backcross (BC) breeding is used for gene transfer when an established elite line holds many desirable properties (recurrent parent), but lacks a specific trait that is known to exist in a non-adapted donor line (donor parent). Single gene introgressions are routinely performed by repeated backcrosses in an attempt to transfer the targeted gene into the recurrent parent genome. Transfer of these non-adapted genes generally involves the introgression of large chromosome segments carrying numerous non-adapted genes with unpredictable effects on agronomic and quality performance. This phenomenon is known as linkage drag and it is frequently thought to affect traits other than the one originally targeted (Young & Tanksley, 1989; Randhawa et al., 2009; Von der Ohe et al., 2010).

Backcross breeding is the method of choice for gene introduction. It has been used extensively for breeding several crops such as tomato (Young & Tanksley, 1989), soybean (Guzman et al., 2007), maize (Hoffbeck et al., 1995), cotton (Bayles et al., 2005), wheat (Von der Ohe et al., 2010; Sarti et al., 2011), for introgression of recessive and dominant target genes. When assembling genes onto the genome of a particular recipient parent, a certain number of backcrosses must be performed for the recovery of the recurrent parent genome and development of commercial varieties/ inbreds. Theoretically, it is possible to recover (on average) more than 93% of the genes of the recurrent parent after three backcrosses, over 96% after four, and over 98% after five (Fehr, 1987). In actual practice, the percentages obtained will always be lower, especially when transferring multiple traits. Empirical studies have shown that 5 to 10 cycles of selection are generally required for backcross breeding and still often results in yield drag (Briggs & Knowles, 1967; Hoffbeck et al., 1995).

Marker-assisted background selection (MABS) or marker assisted backcrossing (MAB) is a simple form of MAS, in which the goal is to incorporate a major gene from an agronomically inferior source (the donor parent) into an elite cultivar or breeding line (the recurrent parent). It is an important tool to facilitate the selection of resistant cultivars and to enhance breeding efficiency. The desired outcome is a line containing only the major gene from the donor parent, with the recurrent parent genotype present everywhere else in the genome.

MABS is commendable for selecting both the target gene as well as recurrent parent genotype for the rest of the genome. Von der Ohe et al. (2010) analyzed the effect

of two QTL (*Fhb1* and *5A*) in two $BC_3F_{2:5}$ German wheat populations and reported improved FHB resistance and similar high yield levels like the recurrent parent in at least one population. McCartney et al. (2007) investigated three Canadian spring wheat populations and results suggested that lines carrying *Fhb1* had increased test weight after two backcrosses.

Frisch & Melchinger (2001) previously studied the value of introgressing two genes simultaneously into new populations. Selecting both target genes and recurrent parent genome (RPG) proportions (background selection), they were able to reduce the number of backcross required for the introgression of one target gene from six to three generations of backcrossing.

Young & Tanksley (1989) focused on linkage drag, studying the size of chromosomal segment retained around a target locus of tomato after repeated backcrossing. They proposed that the use of molecular markers (RFLP) to monitor recombination around genes of interest can quickly and efficiently reduce the amount of linkage drag associated with introgression.

Besides the transfer of target genes, recovering the recurrent parent genome rapidly and as completely as possible is also a common goal of breeding programs. To get more information about the backcrossed lines background and to better estimate the recovery of the recurrent parent genome in this backcross study, I utilized the Diversity Arrays Technology. (DArT) method (http://www.diversityarrays.com/). DArT is a sequence-independent and micro-array hybridization- based marker system that generates medium density genome scans and whole genome fingerprints by scoring the presence

versus absence of DNA fragments in representations of genomic DNA samples. It simultaneously determines hundreds to thousands of polymorphic loci in a single assay, being able to detect single base pair changes and to provide comprehensive genome coverage even in organisms without any DNA sequence information (Jaccoud et al., 2001). This methodology provided good coverage of the population's genome, allowing for identifying parental alleles throughout the genome and comparing recurrent parent and breeding lines.

In this study, results on the efficiency of background selection for the introgression of target genes in early generation wheat populations were extended by considering alternative breeding plans, comparing the performance of forward cross and backcross derived populations on the basis of the presence of FHB resistant alleles and high yields.

The objectives of this study were i) to determine the possible effect of introgressed resistance alleles on agronomic and quality performance; ii) to investigate the number of backcrosses necessary to restore agronomic value to the populations tested; and iii) to evaluate the utility of backcross populations as breeding populations from which inbred lines can be derived.

5.2. Material and Methods

Plant material was developed in Lexington, KY in 2009, from single crosses and backcrosses between 7 FHB susceptible soft red winter wheat Kentucky lines and the

FHB resistant line VA01W-476, a double haploid line derived from ‘Roane’ and ‘W14’ (Perugini, 2007; Agostinelli et al., 2011). The resistant line’s parents provide different sources of resistance to FHB. W14 had many different FHB resistant parents in its pedigree and most likely many different FHB resistant alleles (Jiang et al., 2006). Roane is also known to have some level of native resistance (Griffey et al., 2001). Backcrosses were made in the greenhouse at Spindletop Research Farm (m (38°7’37.81’’ N, 84°29’44.85’’W) near Lexington, KY (LEX), and the populations named DN1, DN2, DN3, DN4, DN5, DN6, DN7, following the populations’ names from the forward crosses (Table 5.1).

5.2.1. Forward- Cross Population Development

Crosses were made in 2007, the F_1 generations were self-fertilized (2008) and the heads threshed in bulk and planted in F_2 plots in Lexington, KY, 2010. Each population was planted in a plot for seed increase and 60 to 100 heads were selected and planted the following year in 1.2 m long rows, spaced 30 cm apart, in 2011 in Princeton, KY (PRN) at the West Kentucky Research and Educational Center (37°6’7.37’’ N, 87°52’13.62’’ W; Crider silt loam [fine-silty, mixed, active, mesic Typic Paleudalfs]). In 2012, the $F_{2:3}$ head-rows were threshed independently and planted in a randomized complete block design with 2 replications in Princeton (29 October, 2011) and Lexington, KY (5 November, 2011). Plots were 6 rows wide and 3 m long. All experimental plots received 105 kg N/ha applied in the spring and recommended agricultural practices for wheat production in Kentucky were followed (Lee et al., 2009). Each population’s parents were

planted and also four commercial varieties were planted as checks. Notes on plant height, yield and test weight were taken from all plots and compared.

5.2.2. Backcross Population Development

After the single crosses between the high yielding Kentucky lines and the donor parent were made, the F derived plants were evaluated according to the presence of two FHB resistance QTL *Fhb1* and *QFhs.nau-2DL* using molecular markers assisted selection technique. Nuclear DNA was isolated from leaves according to Pallota et al. (2003). Simple Sequence Repeats (SSR) used were *UMN10* (Liu et al., 2008) and *Xgwm533Pd* (Röder et al., 1998) for *Fhb1*; and *Xcfd233* (Grain genes 2.0 at http://wheat.pw.usda.gov/GG2/index.shtml, verified May 2010) and *Xgwm539* (Röder et al., 1998) for *2DL*. These markers have been shown to be useful for selecting *Fhb1* and *2DL* loci (Anderson et al., 2001; Agostinelli et al., 2011). The genotyping process was done at the University of Kentucky Wheat Breeding Laboratory, in 2009. All PCR products were submitted to 1.5% agarose gel electrophoresis in order to determine the presence of resistance alleles segments.

Plants that were positive for the presence of both resistance alleles were selected and crossed with the high yielding recurrent parent. BC_1F_1 seeds were planted in the greenhouse and BC_1F_2 seeds were harvested and planted in head-rows in the field in 2009 at Spindletop Research Farm near Lexington, KY. Three head-rows were planted for each genotype, 1.2 m long, spaced 30 cm apart on the field near Lexington, KY. From the 3

head-rows, the best one was selected, in other words, the row with bigger stand and that visually offered greater seed number. Plants were bulk-harvested and the seeds cleaned carefully with minimal air in order not to remove tombstones.

BC_1 plants were submitted to additional rounds of backcrossing and crossed again with the recurrent parent for one or two generations, producing BC_2 and BC_3 plants. In 2011, BC_2 and BC_3 derived lines were planted in head-rows in a location where extreme weed pressure limited grain yields. Population 1 (DN1) had no BC_3 seedlings, however all BC_3 plants from populations 2, 3, 4, 5, 6 and 7 were submitted for genotyping at the USDA/ARS Regional Small Grains Genotyping Lab (RSGGL) (http://www.ars.usda.gov/Main/docs.htm?docid=19522) at Raleigh, NC, for DNA extraction and marker amplification. Simple Sequence Repeats (SSR) used were the same as previously used for FHB screenings: *UMN10* (Liu et al., 2008) and *Xgwm533Pd* (Röder et al., 1998) for *Fhb1*; and *Xcfd233* (Grain genes 2.0 at http://wheat.pw.usda.gov/GG2/index.shtml , verified May 2010) and *Xgwm539* (Röder et al., 1998) for *2DL*.

In 2010, BC_1F_3 plots were arranged in randomized complete block design, with two replications, located in Lexington, KY. Plots were 3 m long and constituted by 6 rows, subjected to phenotypic screening in 2011: field visual ratings, height, yield and test weight data were collected. In 2011, BC_1F_4 plots were planted again in a randomized complete block design, with two replications in two locations Lexington and Princeton, Kentucky. Other backcross populations BC_2F_4 and BC_3F_4, were also planted, in an Augmented Block Design (described below), with replicated commercial lines used as yield checks, in Lexington, Kentucky, 2011.

Representative BC_1F_3 and BC_1F_4 grain samples were taken after harvest for baking quality investigations. An 18 g sample from each plot was subjected to Near Infrared Reflectance Spectrometry (NIR) in order to obtain disease (FDK and DON) predictions and also milling and baking quality trait predictions.

All backcrossed lines were subjected to the same phenotypic and disease screening as the forward-cross populations, and the results compared, in order to determine if backcrossing would accomplish two goals: first, the use of QTL assisted backcross in an early generation (F_2 populations) as indicators of gene expression prior to extensive backcrossing, and second, the assessment of BC_1 populations as breeding populations (i.e. for derivation of inbred lines), removing donor parent segments linked to undesirable traits and restoring yields to an economically desirable level.

5.2.3 DArT markers analysis

The population selected for DArT analysis was the Backcross Population 2 (DN2) from the cross KY97C-0321-05-2/ VA01W-476// KY97C-0321-05-2. This population was selected because of the superior agronomic characteristics of the recurrent parent. BC_1F_2 seeds from population 2 lines were planted in greenhouse trays and the leaves collected for DNA extraction. Genomic material was isolated according to "Plant DNA Extraction Protocol for DArT" (www.triticarte.com.au/pdf/DArT_DNA_isolation.pdf) and a total of 94 genotypes were submitted to Diversity Arrays Technology P/L - Triticarte P/L Australia (www.triticarte.com.au). 961 DArT markers were applied,

covering all the chromosomes on the wheat genome. DArT technology reduces the complexity of a DNA sample by digestion with a combination of restriction enzymes to obtain a representation of that sample. Digestion is followed by adapter ligation and amplification. The resulting representations of our lines were precipitated, denatured and labeled according to Wenzl et al. (2004). Libraries of genomic representations were prepared essentially as by Jaccoud et al. (2001).

Representations of each line to be genotyped were labeled and hybridized to the array. The polymorphisms scored the presence versus absence of hybridization to individual array elements. They reflect DNA sequence variation that determines which genomic sequences are present in the genomic representations of each genotype. The microarray platform makes the discovery process very efficient because all markers on a particular DArT array are scored simultaneously. A binary matrix was generated in which "1" indicates presence and "0" indicates absence of the allele. 50 samples were analyzed in duplicate and the discordance in 50 pairs of scores was reported. When a sample was assayed in duplicate, the consensus of scores was used. Informative markers that show polymorphic fragments were selected and the differences between means were compared for statistical significance through analysis of variance. Markers with significant values for F-test ($P < 0.05$) were identified and from those, the mean values and coefficient of determination (R^2) were considered. Each DArT marker was evaluated with respect to individual phenotypic traits. The proportion of observed phenotypic variance explained by each marker was estimated as the coefficient of determination (R^2). Markers with a coefficient of determination greater than or equal to 0.25 ($R^2 \geq 0.25$) were selected as the most promising markers for that specific characteristic.

Individual maps were constructed using EasyMap, a program developed at Diversity Arrays P/L for high-throughput mapping of populations. EasyMap distributes markers into linkage groups based on the record algorithm, the detection of potential genotyping errors, the re-optimization of marker orders after replacing potential errors, and the estimation of map distance. The Synthetic/Opata map was conducted with JoinMap 4.0 (Van Ooijen & Voorrips, 2006) using the "maximum likelihood" algorithm. Linkage groups were assigned to chromosomes based on comparison across populations and the existing chromosome assignments available on Synthetic / Opata and Cranbrook / Halberd maps, available online at GrainGenes map data report (GrainGenes: A Database for Triticeae and Avena, available at <http://wheat.pw.usda.gov/ggpages/map_summary.html>)

From the DArT markers results, the percentage of recurrent parent of each BC_1 line was estimated. Subsequently, the yield * recurrent parent percentage and FHB resistance * recurrent parent percentage was compared and the correlation between them was evaluated.

After the DArT screening, 86 lines were selected and planted in $BC_1F_{2:3}$ head-rows, 1.2 m long, spaced 30 cm apart in the misted inoculated scab nursery in Lexington, KY, 10 October, 2010 and also planted in plots at Lexington, KY in a 2 rep RCB. The randomized complete blocks were 6 rows wide; 3 m long each row, planted on 25 October, 2010. Phenotypic disease notes were taken in both replications of all the lines planted in the scab nursery. The traits evaluated in 2011 were: Rating, Incidence, Severity, FHB index (Incidence*Severity), FDK, DON, FDK and DON predictions using

Near Infrared Reflectance Spectroscopy (FDKNIR and DONNIR), plant height, yield and test weight (TWT).

The 86 DArT lines were planted in the 2012 scab nursery again (22 October, 2011) and also in yield plots (5 November, 2011), in a randomized complete block design, with 2 replications, in two locations (Lexington and Princeton, KY). From 2012 plots, yield, test weight and plant height were one more time evaluated, as well as NIR measurements. Grain samples were collected from each line within Population 2 and milling and baking quality tests for all genotypes were done at the USDA Soft Wheat Quality Lab in Wooster Ohio. Wheat meal (WM) essays with sodium carbonate (SRC) and sodium dodecyl sulphate sedimentation (SDS) tests were evaluated at the Wheat Breeding Molecular Marker Laboratory at University of Kentucky. Traits measured in 2012 included: Rating, FHB incidence, FHB severity, FHB index, FDK: Fusarium Damaged Kernels, DON, Yield, Test Weight, Flour Yield, Four Protein, Softness Equivalent, Lactic Acid SRC, Sucrose SRC, Water SRC, Sodium Carbonate SRC, Wheat Meal SDS, Wheat Meal SRC, Cookie Diameter.

5.2.4 Milling and Baking Quality Traits

Wheat Meal SDS sedimentation volume was measured as described in Knott et al. (2009) at the Wheat Breeding Laboratory at University of Kentucky, Lexington, KY. Duplicate evaluations were conducted for each sample. 25 g of BC_1F_2 derived seed were milled with a Cyclone sample mill (UDY, Fort Collins, CO, 80524) using a 1 mm sieve.

Ten milliliters of deionized water were dispensed with a bottle-top dispenser into 25 mL glass graduated cylinders, with ground glass stoppers caps. 1 g of wheat meal was added to each graduated cylinder, shaken vigorously for approximately 15 s, and placed onto a test tube rocker to rest for 2 min. After the rest period, the cylinders were inverted four times, allowed to rest for 2 min, and inverted four times again. Sodium lauryl sulfate (10 mL, 2.5% w/v) was added to each cylinder with a bottle-top dispenser. The cylinders were inverted four times and allowed to rest 2 min. The procedure was repeated three times for a total of four cycles. Lactic acid (5 mL of 1.1% w/v) was added using a bottle-top dispenser. Four cycles of inverting the cylinders four times followed by a 2 min rest were completed. After the final inversion, the cylinders were removed from the rocker and allowed to settle for 20 min before sedimentation volume was measured. Entry means values were calculated and the results compared.

Wheat meal sodium carbonate SRC was done for the same BC_1F_2 lines in population 2 at the Wheat Breeding Laboratory at University of Kentucky, Lexington, KY. As described in Knott et al. (2009), 5 g wheat meal sample wheat meal were placed into disposable 50 mL centrifuge tubes and 25 mL of 5% (w/w) sodium carbonate was added using a bottle-top dispenser. Tubes were shaken horizontally 40 times to suspend the wheat meal into the sodium carbonate. Tubes were placed horizontally onto an orbital shaker and agitated for 20 min at 100 rpm. The tubes were centrifuged at 1000 x *g* for 15 min. The supernatant was decanted and the tubes were allowed to drain on absorbent towels for 10 min. The tubes were weighed and solvent retention capacity calculated.

5.2.5 Data Analysis

Analysis of variance (ANOVA) for BC_1 genotypes was performed using the General linear model procedure (PROC GLM; SAS 9.3). The model used was:

$$Y_{ij} = \mu + Block_j + Range\ (Block) + Pass\ (Block) + G_k + E_{ij}$$

Where:

- Y_{ij} = observation in the k^{th} genotype in the j^{th} rep in the i^{th} range in the l^{th} pass,
- μ = overall mean,
- G_k = effect of the k^{th} genotype,
- $Block_j$ = effect of j^{th} block,
- $Range\ (Block)_{ij}$ = effect of i^{th} range within the j^{th} block,
- $Pass\ (Block)_{lj}$ = effect of l^{th} pass within the j^{th} block,
- E_{ij} = residual error.

Fisher's Least Significant Difference (LSD) was used to corroborate significant differences among genotype classes.

For BC_2 and BC_3, because the seed number was limited, an augmented design was used, with incomplete blocks to remove some field variation from the plot residuals. In each incomplete block, a set of checks was included and every check occurred twice in each incomplete block, but the experimental lines were unreplicated and included in one

block. The analysis followed the general linear model procedure (PROC GLM; SAS 9.3). The model used was:

$$Y_{ij} = \mu + \beta_i + c_j + \tau_{k(i)} + E_{ij}$$

Where:

- Y_{ij} = observation in the k^{th} genotype with the j^{th} check in the i^{th} environment,
- μ = overall mean,
- β_i = effect of the i^{th} enviroment,
- c_j = effect of j^{th} check,
- $\tau_{k(i)}$ = effect of the k^{th} genotype within the i^{th} environment,
- E_{ij} = residual error.

The percentage of lines not significantly different from the yield checks were obtained from the calculated Least Significant Difference (LSD). Least significant difference was calculated according to:

$$LSD = t_{\upsilon,\alpha} * \sqrt{MS_{S(A)}\, 2/N}$$

Where:

- LSD = least significant difference
- $t_{\upsilon,\alpha} = t_{0.05/\upsilon}$ = t Table (where $\upsilon = N - A$ is the number of degrees of freedom of the error, this value can be obtained from a standard table).
- MS = mean square error

- N = number of observations on which a check mean is based

Wheat meal sodium carbonate solvent retention capacity was calculated with the following equation:

$$SRC = 100 \times \{(\text{Pellet weight}/\text{Flour weight}) \times [86/(100 - \text{Wheat Meal Moisture})] - 1\}$$

PROC CORR (SAS, 2013) was used to analyze the entry means relationship between the traits evaluated (Rating, Severity, Incidence, FHB index, FDK, DON, yield, test weight, height, whole grain protein, whole grain hardness, flour yield, softness equivalent, flour protein, lactic acid SRC, sucrose SRC, water SRC, sodium carbonate SRC, estimated cookie diameter).

5.3. Results and Discussion

In 2011 and 2012, BC_1F_3 and BC_1F_4 plots were harvested and the seeds were cleaned and weighed at the Foundation Seed Building at Spindletop Farm, close to Lexington, KY. Grain moisture and test weight were measured with a GAC grain analysis computer, and yield was estimated. The results evidence several promising lines, which were able to restore agronomic performance after one backcross (Table 5.2). For population 1, there were 6 out of 31 lines (19%) for which the yield average did not significantly differ ($P < 0.05$) from commercial check yields. Populations 2, 3, 4, 5, 6 and 7 had 23, 22, 13, 42, 13 and 58% of lines, respectively; whose yields were not

significantly different ($P < 0.05$) from commercial checks. These results indicated that BC_1 populations may be a useful source of breeding lines and these principles can be used for optimizing the breeding program and gene introgression strategies.

These results were specially promising due to the fact that I used adapted recurrent parents (KY lines) instead of using wild relatives or plant introgressions (PI). KY lines have known pedigrees and were bred to contain favorable genes for yield and test weight in Kentucky. They are relatively well-adapted lines that are important part of the breeding program, being used as parents for many of our crosses and backcrosses. According to Isleib (1999), when adapted recurrent parents and unadapted recurrent parents differences are big, the number of backcrosses required would likely to be greater. In this study, only one backcross was sufficient to restore high yields on FHB resistant populations. The adapted RP effectiveness is clear when we compare yields from BC_1 lines and commercial checks and observe several lines as high yielding as the yield checks. The attainment is confirmed with the presence of four significant transgressive segregants.

Bayles et al. (2005) estimated the measurement of degree and rate of recovery of RP traits through four backcross generations in upland cotton. After crossing one adapted recurrent parent and six unadapted recurrent parent cultivars, four backcrosses were not effective in the recovery of all recurrent parent traits. Depending on the trait, three, four, and often more backcrosses were required to recover desirable agronomical traits. The observed rate of recovery of RP traits was 4% less overall than the theoretical rate;

however, several instances of transgressive segregation were noted mainly in the earlier backcross generations.

Plots with BC_2F_3 and BC_3F_3 plants were also cultivated and yields were calculated. For BC_2 derived lines, 50% of the lines were not significantly different ($P > 0.05$) from the yield checks for Population 1 (Table 5.3). Populations 2, 3, 4, 5 and 6 had 97, 81, 50, 83 and 33% of lines as high yielding as the commercial varieties ($P > 0.05$). For population 7, all 12 BC_2 lines (100%) tested were not significantly different from the yield checks, with several lines exceeding the yield values for the checks Truman and Pembroke. In the BC_3 derived lines experiment, there were even higher percentages of lines with elevated yields and test weights (Table 5.4). Population 1 backcrosses produced no BC_3 plants; however, populations 2, 3, 4, 5, 6 and 7 revealed 88, 91, 72, 54, 33 and 100% of lines not significantly different from the check variety yields ($P > 0.05$) respectively.

Although generally more rounds of backcrossing led to a higher number of lines as high yielding as the commercial varieties checks, when mean yields are compared, the numbers are very similar among BC_1, BC_2 and BC_3. Yield averages for all populations are very similar among backcrossing generations and yield ranges are overlapping, suggesting that in this case, having more backcrosses does not assure that yields would increase every round of recurrent parent crossing. For populations 2, 3, 5 and 7, the higher yields are in BC_2. In populations 4 and 6, higher yields were observed in BC_1 lines. Overall, population 2 had the greatest number of promising high yielding lines and

population 7 had the second highest yielding lines and 100% of them being equivalent to the commercial varieties.

Molecular marker assays can be of advantage in backcross breeding for foreground selection and background selection (Hospital & Charcosset, 1997). Marker-assisted background selection was proposed by Tanksley et al. (1989) and has been established as a standard tool in plant breeding. The background selection accelerates recovery of the recurrent parent genome. Individuals are selected which are homozygous for the alleles of the recurrent parent at a large number of marker loci covering the entire genome. From Population 2, the highest performing population in our study, background investigations were made using DArT markers technique. The diversity array markers evidenced the percentage of markers each line shared with the donor parent and also with the recurrent parent. This provided a more accurate measurement of how much of the high yielding background was being inherited in Population 2 lines. The background percentages ranged from 65 to 89% of estimated percentage of recurrent parent in BC_1 lines. Average results from 2011 and 2012 reveal a coefficient of determination of R^2 = 0.26 and the correlation between yield and percentage of recurrent parent was r = 0.51, confirming that increases in high yielding background was positively associated with yield performance of lines, though the correlation was far from perfect (Figure 4.1, Chapter 4).

The procedure for backcrossing breeding is long and includes repeated backcrosses, needed to create a line that is similar to the recurrent parent with the trait of interest from the donor parent. The goal of backcrossing is to obtain a line as identical as

possible to the recurrent parent with the addition of the gene of interest or target gene. Not surprisingly, many examples of "linkage drag" are known in which undesirable traits that are closely linked to a target gene are carried along during the breeding program (Zeven et al., 1983).

It is well known that one of the disadvantages of this method is that many backcrosses are required to produce a new cultivar, which can take many years. The usual process includes 4^{th}, 5^{th} and 6^{th} backcrosses in succession, with an F_2 and F_3 being grown after the 6^{th} backcross with intense selection for both the desired trait and the recurrent parent plant phenotype. This entire process requires around eight generations and can take almost a decade to be completed.

Because of the long time taken by backcrossing breeding, most breeders do not make backcross populations for breeding purposes and generally the backcross is used for gene introgression or transfer target genes into elite lines. This experiment demonstrated that backcrossing is a valid breeding method and few backcrosses with the help of backcross assisted selection and background selections are enough to restore the high yielding potential in populations.

Analysis of DNA markers with a good coverage of the entire genome can help in identifying individuals with high proportion of the recurrent parent background and lead to a reduction on the number of backcrosses needed. The 961 DArT markers, made it possible to identify the percentages of recurrent parent in each line within Population 2 and assess its correlation with an increase on yield.

Not only for background marker selection, which has been proved to be highly effective for the recovery of recurrent parent genome, DArT markers could be applied for QTL mining, identifying potential genes or regions of the genome likely to be linked to other economically important traits. The discussion can be extended to detecting and assembling multiple genes using marker-based background selection, producing gene-pyramided lines and improving the selection efficacy on breeding programs.

Similar results were achieved based on computer simulations by Randhawa et al. (2009) when marker-assisted background selection was optimized in wheat for results of 97% or more of a recurrent parent genome recovering in just two backcross generations. Eller et al. (2010) applied backcross breeding using unadapted germplasm as a donor parent for improving quantitative disease resistance in maize, which are strongly influenced by the environment. After 4 backcrosses (BC_4) the results suggest that selection for reduced *Fusarium* ear rot improved resistance to that fungal disease without significantly lowering its yield potential, nevertheless with limited effectiveness.

When backcross populations were compared to forward cross populations derived from the same parents (donor line with 2 sources of resistance for FHB * high yielding Kentucky lines), the average yields were similar. For all seven populations, mean yields were 59 bu/acre for BC_1, 59 bu/acre for BC_2, 58 bu/acre for BC_3, 62 bu/acre for F_2 derived lines.

Considering each population, F_2 derived lines had a good performance in yield plots (Table 5.7) when compared to the commercial checks, however, backcross derived lines had higher population means for yield and test weight. Overall, BC_2 and BC_3

presented the highest population yield means and also percentages of lines not significant different from commercial checks.

F_2 derived Population 1 had 50% of lines not significantly different from the checks. Populations 2, 3, 4, 5, 6 and 7 had 57, 60, 41, 57, 72 and 87% of lines as high yielding as the commercial checks. Comparing to backcross derived lines, F_2 derived populations had smaller percentages, with the only exception being Population 6. Therefore, backcrossed lines offered consistently superior yields. Bayles et al. (2005), when backcrossing in cotton, evaluated lint yield from F_4, BC_1F_4, BC_2F_4, BC_3F_4 and BC_4F_4 populations. All F_4 populations were significantly lower yielding than the recurrent parent (yield check), as were three out of six BC_1F_4 and two out of six BC_2F_4 populations. For BC_3F_4, one population had lint yields 20% higher than the recurrent parent. It was only on BC_4F_4 that populations were not significantly different than the yield checks (high yielding recurrent parent), with no transgressive segregants recorded.

Backcross derived populations had high yields, with several promising lines for yields and test weights. BC_2 Population 2 presented 97% of lines as high yielding as the commercial checks. However, the same Population 2 demonstrated not to be very responsive over introduction of FHB resistance QTL *Fhb1* and *QFhs.nau-2DL* in that disease traits were not significantly reduced after 2011 and 2012 scab nursery inoculations. In the combined analysis (2011+2012), DON was not diminished but FDK was reduced by 16% when both QTL are present in each line. Milling and baking results were average and within the preferred values for Population 2. Population 3 presented 91% of lines with yields not significantly different from commercial checks by the third

round of backcrosses (BC_3). Addition of resistance QTL was effective and DON levels were 23% lower and FDK was reduced 22% in lines with *Fhb1* plus *QFhs.nau-2DL*. Flour yields were lower than desirable, and sucrose SRC results were higher than expected for good cookie quality in F_2 derived Population 3 lines.

DON and FDK were reduced by 32% with addition of *Fhb1* and *QFhs.nau-2DL* Q*TL* in Population 4. There were also high yielding lines for backcross and forward-cross derived lines, with 72% of BC_3 lines as high yielding as the checks. However, Population 4 did not have good milling and baking performance, with low flour yields and sucrose SRC results over than the desirable values of 87% or less. BC_1 derived lines presented high lactic acid SRC (103%), suggesting strong gluten content and F_2 derived lines showed low lactic acid SRC (87%) which means weak gluten strength. Sodium carbonate SRC percentages were higher than desirable, suggesting longer baking times and lower quality products.

Population 6 showed 33% of BC_1 derived lines and 72% of F_2 derived lines with yields as high as the commercial checks. The addition of FHB resistance QTL was very promising for this population background and the reductions for DON and FDK were 36 and 34% when both *Fhb1* and *QFhs.nau-2DL* Q*TL* were present combined in each line. Milling and baking results were good for Population 6, with good levels of flour yield and flour protein, softness equivalent on desirable ranges, yet sucrose SRC higher than preferable. For population 7, all 12 BC_2 lines (100%) tested were not significantly different from the yield checks, presenting the higher number of transgressive segregants and suggesting that it is a very promising population.

Marker assisted selection was effective in both backcross and forward cross derived populations. Disease was reduced in all populations and the average Rating, Severity, Incidence, FHB index, FDK and DON were lower than averages for the recurrent parents, in both years 2011 and 2012. F_2 derived lines were slightly more resistant than BC_1F_2 lines, for populations 2, 3, 4 and 6 (Table 5.8).

It is common for breeder's lines to have moderate resistance to FHB, good agronomic performance, but insufficiently high grain quality (Mergoum et al., 2007). Von der Ohe et al. (2010) tested $BC_3F_{2:5}$ winter wheat populations carrying two FHB resistance QTL (*Fhb1* and *Qfhs.ifa-5A*) for agronomic and quality performance. According to their results, variance within QTL classes allows selection for high quality performance in BC_3 derived populations. The introduction of FHB resistance QTL could affect quality traits but not necessarily in a negative direction. Balut et al. (2013) also studied the influence of exotic resistance QTL (*Fhb1* and *QFhs.nau-2DL*) on F_2 derived wheat breeding populations. Significant QTL effects on agronomic and quality traits were observed, although with small impact.

When milling and baking quality data were compared between BC_1F_2 and F_2 derived populations, the results were similar for the traits evaluated: whole grain hardness, flour yield, softness equivalent, flour protein, lactic acid SRC, sucrose SRC, water SRC, sodium carbonate SRC and estimated cookie diameter (Table 5.9). Flour yield values were around average (66%) with BC Populations 2 and 3 presenting values above 68%, more preferred than average. Forward cross derived populations had lower flour yield values, especially Population 4 (64% flour yield). Softness Equivalent was

within the acceptable 50 to 60% minimum range for this trait (Everts et al., 2001) for most of the populations, except for F_2 derived populations 4 and 6 that presented values below the minimum (48 and 49%). In this case, the donor parent had low SE (47%) in comparison to the 57% SE from averages from the recurrent parents. Sucrose SRC is related to pentosans, which are highly hydrophilic and affect cookie quality, consequently lower values of sucrose SRC are desirable. Average sucrose SRC was 89% and overall values were within the acceptable percentages, except for population 4, which had higher and less preferred results (above 91%). Water SRC is influenced by all constituents such as glutenin, pentosans, gliadins, and high numbers are known to be undesirable in baking quality assays. Results for the donor parent (59%) were considered high but the breeding populations presented acceptable water SRC percentages, being BC_1F_2 derived Population 3 showing most preferred values (55%). Sodium Carbonate SRC is correlated to damaged starch. Flours that possess high levels of damaged starch are undesirable because damaged starch increases water absorption and reduces sugar snap cookies quality, requiring longer baking times and producing less tender products. The average for sodium carbonate SRC was 68% and BC populations revealed some advantage with smaller values than F_2 derived populations (Table 5.9).

Knott et al. (2009) also found whole meal SRC tests efficient in predicting milling and baking quality and gluten strength in a wide array of soft winter populations. The $F_{4:7}$ experimental lines tested had similar flour yield (66.7%), softness equivalent (56%) and sucrose SRC (90.4%) values compared to our BC_1F_2 and F_2 derived lines. Results from water SRC were superior, with lower percentages (54%), however, their wheat meal

sodium carbonate results were less than preferred (70.5%) when compared to our results, 67.5% for backcross populations and 68.2% for forward cross populations.

Near Infrared Reflectance Spectrometry (NIR) was capable of predicting most quality traits effectively, except for lactic acid, sucrose and water SRC assays. Highly significant correlations were identified (r = 0.88 – 0.95) between whole grain protein and the NIR predictions for this trait and also whole grain hardness and whole grain hardness NIR (r = 0.46 – 0.80) (Table 5.10).

Disease evaluations and wheat flour and meal assays can be easily and economically incorporated to wheat breeding programs in backcross and forward cross early generations to increase frequency of experimental lines with not only good agronomic potential but carrying desirable disease resistance and acceptable milling and baking characteristics.

5.4. Conclusions

The investigation involved the comparison of all forward cross populations (F_2) with BC_1, BC_2 and BC_3 populations, aiming to evaluate their FHB resistance and agronomic performance. BC populations were assessed as breeding populations and were demonstrated to have utility for derivation of inbred lines in a breeding program. In the present study, the BC_2 generation was the most productive in that regard. F_2 populations could and should be used as indicators of gene expression prior to extensive backcrossing. Early generation selection for Fusarium head blight resistance and milling

and baking quality traits should increase the value of breeding lines and facilitate the selection for high yielding lines with desirable disease and quality characteristics.

Table 5.1. Wheat populations developed from Forward crosses and Backcrosses and respective pedigrees.

FORWARD CROSSES		BACKCROSSES	
Population	**Pedigree**	**Population**	**Pedigree**
POP 1	KY 99C-1051-03-1 / VA01W-476	DN1	KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1
POP 2	KY97C-0321-05-2 / VA01W-476	DN2	KY97C-0321-05-2 / VA01W-476 // KY97C-0321-05-2
POP 3	KY97C-0519-04-05 / VA01W-476	DN3	KY97C-0519-04-05 / VA01W-476 // KY97C-0519-04-05
POP 4	KY97C-0540-1-03 / VA01W-476	DN4	KY97C-0540-1-03 / VA01W-476 // KY97C-0540-1-03
POP 5	KY98C-1446-02-1 / VA01W-476	DN5	KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1
POP 6	KY97C-0508-01-01A / VA01W-476	DN6	KY97C-0508-01-01A / VA01W-476 // KY97C-0508-01-01A
POP 7	KY98C-1474-02 / VA01W-476	DN7	KY98C-1474-02 / VA01W-476 // KY98C-1474-02

Table 5.2. Yield and test weight of BC_1 derived wheat lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington, KY, 2011 and 2012 and Princeton, KY, 2012.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	Number of lines	% lines NS different yield from checks
1	(KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1)	Mean Range CV	61.8 53 - 78 10.6	57.9 33 - 62 1.3	31	18.8%
2	(KY97C-0321-05-2//KY97C-0321-05-2/VA01W476)	Mean Range CV	64.8 51 - 81 8.7	57.4 54 - 59 2.3	90	23.3%
3	(KY97C-0519-04-05//KY97C-0519-04-05/VA01W476)	Mean Range CV	51.6 43 - 77 15.1	54.5 55 - 59 19.5	32	21.9%
4	(KY97C-0540-01-03//KY97C0540-0103/VA01W476)	Mean Range CV	58.5 54 - 79 10.6	59.1 54 - 59 2.3	90	13.3%
5	(KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1)	Mean Range CV	56.0 54 - 77 13.0	55.7 55 - 60 2.6	34	41.7%
6	(KY97C-0508-01-01A//KY97C-0508-01-01A/VA01W476)	Mean Range CV	62.8 58 - 83 13.9	58.3 54 - 60 9.9	72	12.5%
7	(KY98C-1474-02 / VA01W-476 // KY98C-1474-02)	Mean Range CV	57.4 39 - 79 14.0	55.6 54 - 62 8.8	62	57.8%

Table 5.3. Yield and test weight of BC_2 derived wheat lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington, KY, 2012.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	Number of lines	% lines NS different yield from checks
1	(KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1)	Mean Range CV	51.5 48 - 55 6.6	58.1 57 - 59 1.9	2	50.0%
2	(KY97C-0321-05-2//KY97C-0321-05-2/VA01W476)	Mean Range CV	73.0 40 - 87 4.4	56.9 53 - 59 1.4	35	97.1%
3	(KY97C-0519-04-05//KY97C-0519-04-05/VA01W476)	Mean Range CV	61.2 41 - 83 8.0	57.2 55 - 59 2.4	16	81.3%
4	(KY97C-0540-01-03//KY97C0540-0103/VA01W476)	Mean Range CV	53.7 43 - 69 4.4	59.6 59 - 60 3.6	6	50.0%
5	(KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1)	Mean Range CV	61.4 52 - 68 4.1	57.1 56 - 58 3.7	6	83.3%
6	(KY97C-0508-01-01A//KY97C-0508-01-01A/VA01W476)	Mean Range CV	49.2 39 - 61 4.7	58.4 57 - 59 3.6	6	33.3%
7	(KY98C-1474-02 / VA01W-476 // KY98C-1474-02)	Mean Range CV	63.7 54 - 72 12.7	58.1 57 - 59 0.5	12	100.0%

Table 5.4. Yield and test weight of BC_3 derived wheat lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington, KY, 2012.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	Number of lines	% lines NS different yield from checks
1	(KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1)	Mean Range CV	†	†	0	0.0%
2	(KY97C-0321-05-2//KY97C-0321-05-2/VA01W476)	Mean Range CV	71.3 49 - 84 9.3	57.0 56 - 59 1.7	17	88.2%
3	(KY97C-0519-04-05//KY97C-0519-04-05/VA01W476)	Mean Range CV	59.4 48 - 66 9.6	54.0 51 - 57 2.7	11	90.9%
4	(KY97C-0540-01-03//KY97C0540-0103/VA01W476)	Mean Range CV	56.1 46 - 67 8.5	59.5 58 - 60 2.2	18	72.2%
5	(KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1)	Mean Range CV	52.6 42 - 69 9.2	56.7 55 - 58 2.4	13	53.9%
6	(KY97C-0508-01-01A//KY97C-0508-01-01A/VA01W476)	Mean Range CV	48.5 33 - 69 12.0	58.3 56 - 60 1.6	27	33.3%
7	(KY98C-1474-02 / VA01W-476 // KY98C-1474-02)	Mean Range CV	62.0 54 - 75 11.8	57.2 54 - 59 0.5	17	100.0%

†: no values obtained because there were no lines for this population.

Table 5.5. Yield, test weight and height values of BC_1F_3 wheat lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington, KY, 2011.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	HEIGHT (in)	Number of lines	% lines NS different yield from check cultivars
1	(KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1)	R^2 (%)	99.9	99.9	99.9	15	86.7%
		CV	3.5	0.5	0.5		
		Mean	52.9	54.2	31.4		
		Range	35 - 77	49 - 59	23 - 38		
2	(KY97C-0321-05-2//KY97C-0321-05-2/VA01W476)	R^2 (%)	89.4	92.7	91.5	69	43.5%
		CV	11.4	2.5	5.0		
		Mean	51.6	53.4	32.9		
		Range	25 - 76	43 - 58	27 - 40		
3	(KY97C-0519-04-05//KY97C-0519-04-05/VA01W476)	R^2 (%)	98.2	97.2	92.3	27	29.6%
		CV	14.0	5.2	7.8		
		Mean	39.8	48.8	30.1		
		Range	14 - 85	43 - 57	25 - 32		
4	(KY97C-0540-01-03//KY97C0540-0103/VA01W476)	R^2 (%)	96.7	90.0	96.5	46	32.6%
		CV	7.9	2.9	3.5		
		Mean	53.8	55.5	33.4		
		Range	23 - 85	47 - 60	27 - 40		
5	(KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1)	R^2 (%)	98.8	97.3	96.2	26	19.2%
		CV	11.2	3.0	6.0		
		Mean	43.7	51.5	30.9		
		Range	13 - 80	42 - 58	23 - 37		
6	(KY97C-0508-01-01A//KY97C-0508-01-01A/VA01W476)	R^2 (%)	97.7	92.4	93.1	36	13.9%
		CV	6.5	3.0	5.9		
		Mean	56.5	54.6	31.6		
		Range	33 - 79	45 - 59	21 - 37		
7	(KY98C-1474-02 / VA01W-476 // KY98C-1474-02)	R^2 (%)	92.9	95.1	95.1	47	31.9%
		CV	13.3	2.7	4.6		
		Mean	46.6	52.5	32.0		
		Range	25 - 72	45 - 59	21 - 37		

Table 5.6. Yield, test weight and height values of BC_1F_4 lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington, KY, 2012.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	HEIGHT (in)	Number of lines	% lines NS different yield from checks
1	(KY99C-1051-03-1 / VA01W-476 // KY99C-1051-03-1)	R^2 (%)	95	64	96	16	51.6%
		CV	6.5	8.5	3.8		
		Mean	55.7	58.8	32.4		
		Range	36 - 80	55 - 64	25 - 40		
2	(KY97C-0321-05-2//KY97C-0321-05-2/VA01W476)	R^2 (%)	87	88	90	69	76.7%
		CV	6.6	1.6	5.2		
		Mean	68.2	59.0	33.7		
		Range	28 - 88	53 - 63	26 - 45		
3	(KY97C-0519-04-05//KY97C-0519-04-05/VA01W476)	R^2 (%)	95	95	96	20	62.5%
		CV	8.8	1.5	3.4		
		Mean	57.5	58.2	32.4		
		Range	34 - 88	51 - 62	28 - 40		
4	(KY97C-0540-01-03//KY97C0540-0103/VA01W476)	R^2 (%)	87	84	88	45	50.0%
		CV	8.0	1.3	5.0		
		Mean	59.7	60.0	33.3		
		Range	36 - 97	55 - 63	24 - 43		
5	(KY98C-1446-02-1 / VA01W-476 // KY98C-1446-02-1)	R^2 (%)	93	95	93	26	76.5%
		CV	9.8	2.2	3.9		
		Mean	60.7	57.3	34.5		
		Range	26 - 88	47 - 62	28 - 40		
6	(KY97C-0508-01-01A//KY97C-0508-01-01A/VA01W476)	R^2 (%)	90	89	91	53	73.6%
		CV	7.3	1.5	4.9		
		Mean	64.5	59.4	34.5		
		Range	28 - 89	49 - 63	25 - 43		
7	(KY98C-1474-02 / VA01W-476 // KY98C-1474-02)	R^2 (%)	71	91	93	38	61.3%
		CV	12.3	1.6	4.3		
		Mean	61.7	57.0	35.0		
		Range	23 - 88	52 - 61	27 - 44		

Table 5.7. Yield, test weight and height values of F_2 derived lines for the populations studied with total number of lines and percentage of lines with yields not significantly different from checks, Lexington and Princeton, KY, 2012.

Population	Pedigree	Statistics	YIELD (bu/acre)	TEST WEIGHT (lb/bu)	Number of lines	% lines NS different yield from checks
1	(KY99C-1051-03-1 / VA01W-476)	Mean Range CV	56.0 36 - 80 11.7	58.8 52 - 64 1.9	16	50.0%
2	(KY97C-0321-05-2/VA01W476)	Mean Range CV	63.8 28 - 82 11.1	59.8 55 - 63 1.5	21	57.1%
3	(KY97C-0519-04-05/VA01W476)	Mean Range CV	48.4 34 - 72 11.9	59.3 57 - 62 0.5	5	60.0%
4	(KY97C0540-0103/VA01W476)	Mean Range CV	59.7 41 - 97 10.6	60.0 55 - 63 1.3	44	40.9%
5	(KY98C-1446-02-1 / VA01W-476)	Mean Range CV	60.5 26 - 88 8.9	57.1 48 - 62 2.8	7	57.1%
6	(KY97C-0508-01-01A/VA01W476)	Mean Range CV	63.0 28 - 89 8.0	59.8 55 - 63 1.2	36	72.2%
7	(KY98C-1474-02 / VA01W-476)	Mean Range CV	63.6 42 - 78 6.7	57.8 53 - 61 1.3	15	86.7%

Table 5.8. Mean values by population entry means for disease related traits from backcross (BC_1) and forward cross (F_2) derived populations of wheat, and parents averages (donor and recurrent), planted in Lexington, KY, 2012.

Disease Traits		Population 2		Population 3		Population 4		Population 6		Parents	
		BC_1	F_2	BC_1	F_2	BC_1	F_2	BC_1	F_2	Donor	Recurrent
Rating	(1 - 9)	4.53	3.53	3.48	2.13	3.03	2.14	1.36	1.72	1.00	5.17
Severity	(%)	23.42	19.30	17.66	20.87	15.84	12.62	16.46	16.02	32.27	59.44
Incidence	(%)	62.74	52.63	54.71	34.26	41.65	34.01	31.36	30.97	15.84	23.21
FHB index	(%)	15.60	11.15	9.99	6.21	7.03	4.80	5.39	5.79	5.81	15.83
FDK	(%)	12.20	9.67	12.94	8.34	8.54	6.90	9.16	9.61	7.31	15.43
DON	(ppm)	9.93	8.26	7.20	3.49	6.00	4.39	3.19	4.57	1.62	11.78

Table 5.9. Mean values from backcross (BC_1) and forward cross (F_2) derived wheat populations, and parent averages (donor and recurrent), of wheat flour quality traits analyzed by chemistry tests and NIR predictions.

	Population 2		**Population 3**		**Population 4**		**Population 6**		**Parents**	
	BC_1	F_2	BC_1	F_2	BC_1	F_2	BC_1	F_2	**Donor**	**Recurrent**
Whole Grain Protein (at 12%)	10.4	11.0	10.9	12.1	11.8	12.2	11.5	12.0	13.2	10.2
Whole Grain Protein NIR	10.5	11.2	10.8	12.2	11.9	12.4	11.6	12.0		
Whole Grain Hardness (0-100)	33.9	32.7	26.5	33.0	35.3	33.4	32.4	32.7	32.0	28.1
Whole Grain Hardness NIR	29.4	28.7	27.4	35.4	37.0	36.4	34.4	35.8		
Flour Yield (%)	68.1	66.8	68.3	65.4	65.6	63.9	66.3	65.8	62.9	68.5
Flour Yield NIR	69.5	68.9	69.8	68.1	67.5	68.2	65.9	65.5		
Softness Equivalent (%)	54.0	51.8	51.9	50.3	51.8	47.6	51.7	49.3	47.0	57.0
Flour Softness Equivalent NIR	57.5	56.6	57.1	52.0	52.7	51.5	56.9	55.5		
Flour Protein (at 14%)	8.7	9.2	8.8	10.0	10.0	10.3	10.0	10.2	10.8	8.5
Flour Protein NIR	8.9	9.4	9.2	10.3	9.9	10.5	9.3	9.6		
As Is Lactic Acid SRC (%)	91.7	95.2	87.0	93.0	103.4	86.9	103.4	101.0	100.1	94.6
Lactic Acid SRC NIR	80.2	79.8	78.0	83.5	92.8	85.0	93.0	94.7		
Sucrose SRC (%)	88.4	89.1	86.1	90.0	91.3	91.0	90.2	89.3	88.8	89.5
Sucrose SRC NIR	87.2	89.1	85.7	98.8	88.2	100.9	83.7	84.4		
Water SRC (%)	57.7	58.2	55.1	57.2	58.0	58.3	56.9	57.5	59.4	56.3
Water SRC NIR	56.7	56.6	55.2	57.0	57.4	57.1	54.9	56.4		
Sodium Carbonate SRC (%)	68.1	68.5	66.5	68.7	68.9	69.2	66.3	66.5	68.9	66.7
Sodium Carbonate SRC NIR	67.5	66.9	65.4	67.7	68.4	67.6	67.0	68.6		
Estimated Cookie Diameter (cm)	18.6	18.3	18.5	18.0	18.0	17.8	18.0	17.9	17.7	18.7

Table 5.10. Correlations between wheat quality tests from backcross (BC_1) and forward cross (F_2) derived populations, obtained through chemistry analysis and predictions by NIR.

Correlations	Population 2		Population 3		Population 4		Population 6	
	BC_1	F_2	BC_1	F_2	BC_1	F_2	BC_1	F_2
Whole Grain Protein x Whole Grain Protein NIR	0.88 ***	0.93 ***	0.92 ***	0.94 ***	0.89 ***	0.93 ***	0.91 ***	0.95 ***
Whole Grain Hardness x Whole Grain Hardness NIR	0.57 ***	0.57 ***	0.68 ***	0.80 ***	0.46 **	0.56 ***	0.69 ***	0.71 ***
Flour Yield x Flour Yield NIR	0.54 ***	0.77 ***	0.29 ^{NS}	0.50 *	0.32 *	0.26 ^{NS}	0.56 **	0.72 ***
Flour Softness Equivalent x Softness Equivalent NIR	0.56 ***	0.37 **	0.76 ***	0.61 **	0.50 ***	0.06 ^{NS}	0.35 ^{NS}	0.37 *
Flour Protein x Flour Protein NIR	0.87 ***	0.91 ***	0.95 ***	0.93 ***	0.67 ***	0.90 ***	0.76 ***	0.74 ***
As Is Lactic Acid SRC x Lactic Acid SRC NIR	0.13 ^{NS}	0.13 ^{NS}	0.30 ^{NS}	0.13 ^{NS}	-0.11 ^{NS}	-0.16 ^{NS}	-0.04 ^{NS}	0.03 ^{NS}
Sucrose SRC x Sucrose SRC NIR	0.26 *	0.41 ***	0.14 ^{NS}	0.40 ^{NS}	-0.19 ^{NS}	0.28 ^{NS}	0.12 ^{NS}	0.42 *
Water SRC x Water SRC NIR	0.19 ^{NS}	0.20 ^{NS}	0.56 **	0.22 ^{NS}	0.04 ^{NS}	0.09 ^{NS}	0.18 ^{NS}	0.41 *
Sodium Carbonate SRC x Sodium Carbonate SRC NIR	0.16 ^{NS}	0.66 ***	0.49 *	0.09 ^{NS}	0.20 ^{NS}	0.08 ^{NS}	-0.01 ^{NS}	0.65 ***

^{NS}: not significant, *: significant at $P < 0.05$, **: significant at $P < 0.01$, ***: significant at $P < 0.001$.

Copyright © Daniela Sarti Dvorjak 2014

Appendix

List of Figures

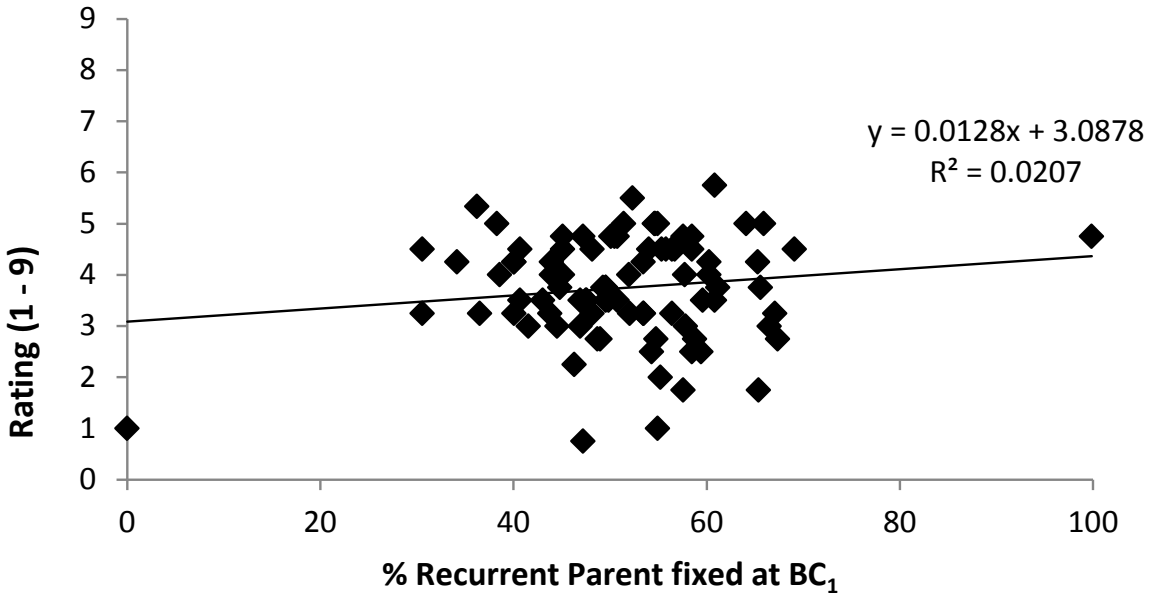

Figure A.4.1. Relationship between Rating averages and percentage of recurrent parent measured with DArT markers in Population 2, BC_1 derived wheat lines, in the scab nursery 2011 and 2012.

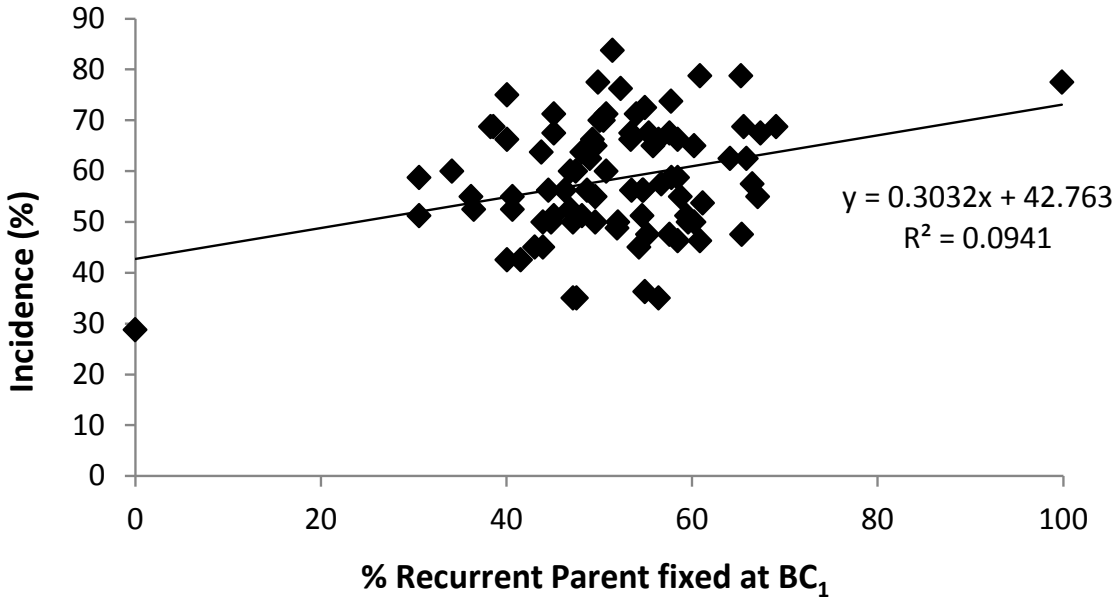

Figure A.4.2. Relationship between Incidence averages and percentage of recurrent parent measured with DArT markers in Population 2, BC_1 derived wheat lines, in the scab nursery 2011 and 2012.

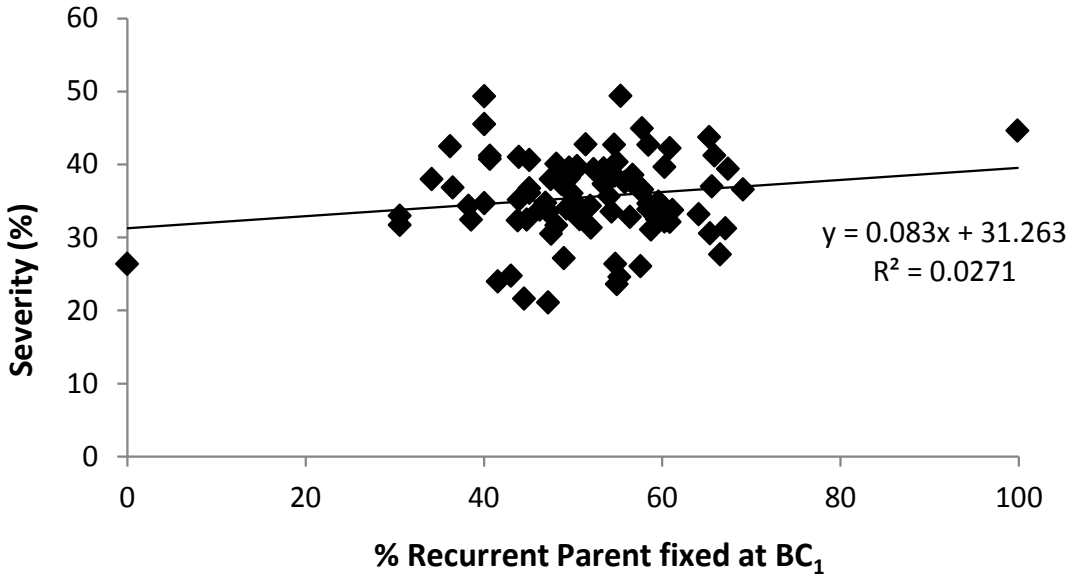

Figure A.4.3. Relationship between Severity averages and percentage of recurrent parent measured with DArT markers in Population 2, BC_1 derived wheat lines, in the scab nursery 2011 and 2012.

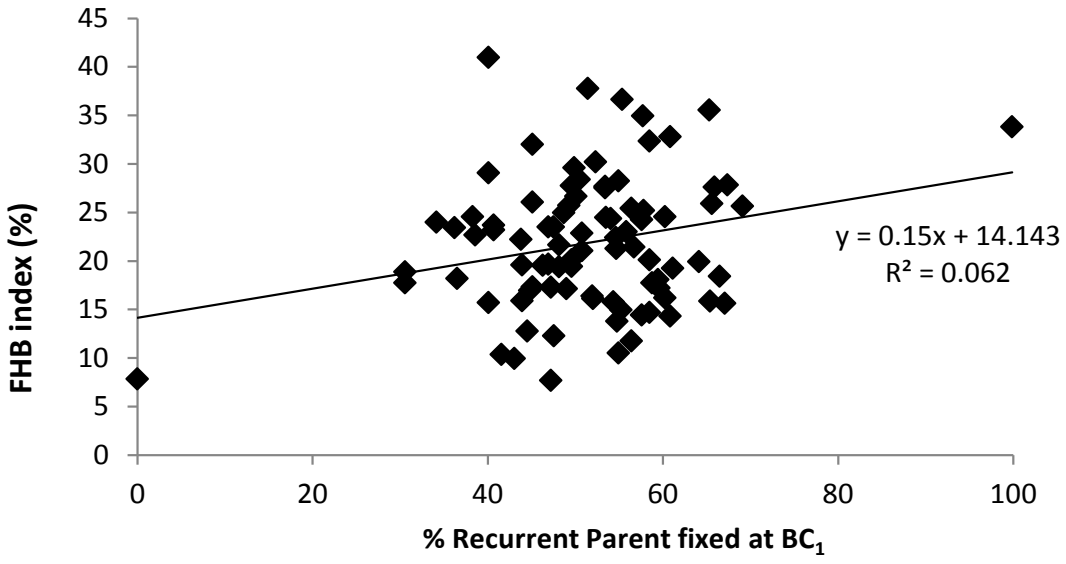

Figure A.4.4. Relationship between FHB index averages and percentage of recurrent parent measured with DArT markers in Population 2, BC1 derived wheat lines, in the scab nursery 2011 and 2012

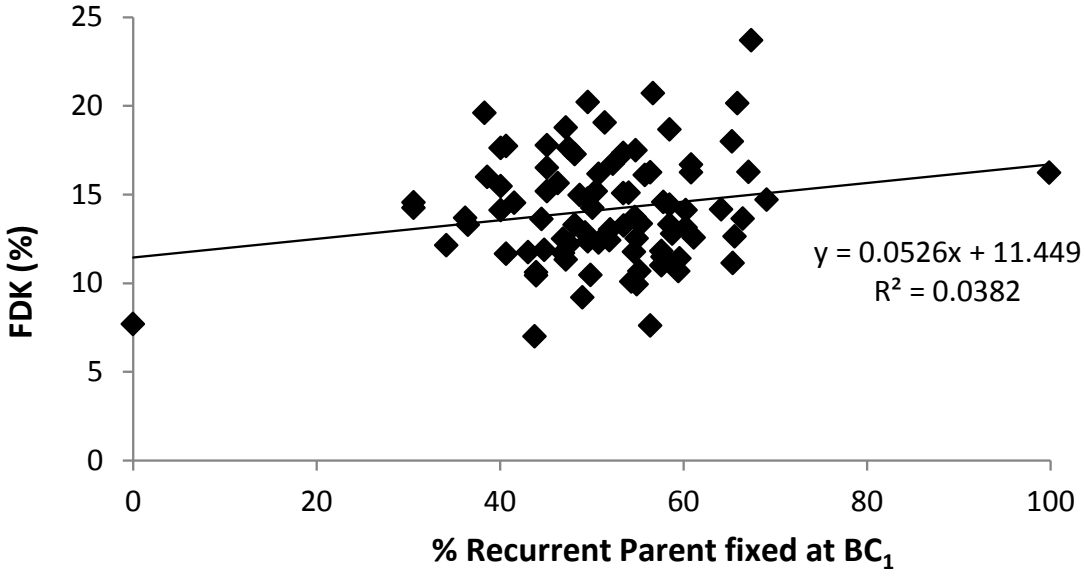

Figure A.4.5. Relationship between FDK averages and percentage of recurrent parent measured with DArT markers in Population 2, BC1 derived wheat lines, in the scab nursery 2011 and 2012

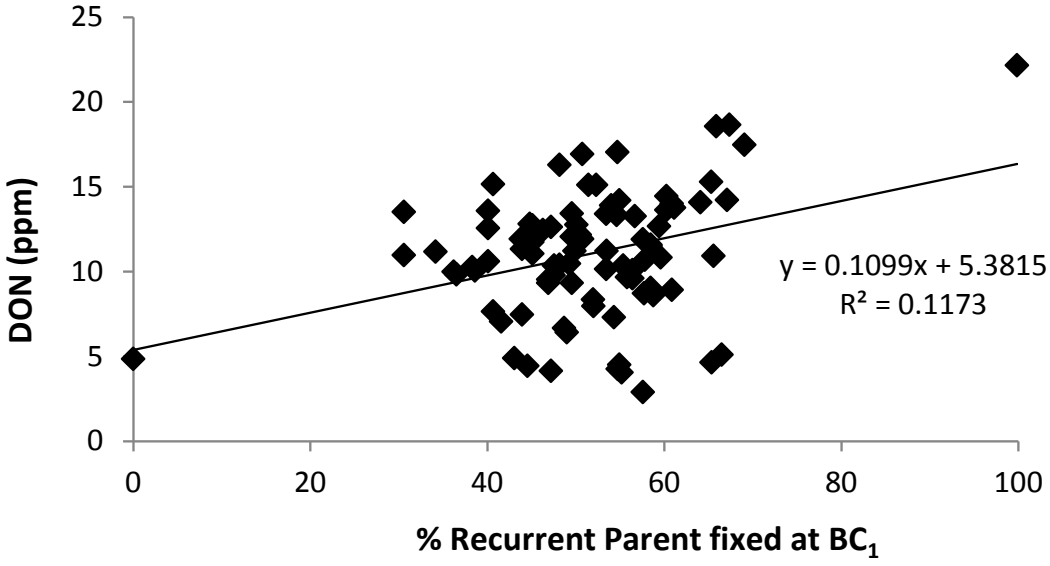

Figure A.4.6. Relationship between DON averages and percentage of recurrent parent measured with DArT markers in Population 2, BC1 derived wheat lines, in the scab nursery 2011 and 2012.

Copyright © Daniela Sarti Dvorjak 2014

Bibliography

Ackerman, M.D. 2006. Germplasm exchange in the southern cone of Latin America. In: Ban, T., Lewis, J.M., Phipps, E.E. (ed) The Global Fusarium Initiative for International Collaboration. Mexico, 2006 (pp 105-108).

Ali, M.B., Brooks, N.L., McElroy, R.G. 2000. Characteristics of U.S. Wheat Farming: A Snapshot. Resource Economics Division, Economic Research Service, U.S. Department of Agriculture. Statistical Bulletin No. 968, 68pp. June 2000.

Agostinelli, A.M., Clark, A.J., Brown-Guedira, G., Van Sanford, D.A. 2012. Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. Euphytica. 186: 115-126.

Agostinelli A., Clark A., Van Sanford, D. 2007. Air separation and digital photo analysis as novel methods to measure the percentage of Fusarium damaged kernels. Proceedings of the 2007 National Fusarium Head Blight Forum. Kansas City, Missouri.

Agostinelli A., Mundell N., Van Sanford, D. 2008. Percentage of Fusarium damaged kernels measured by air separation. Proceedings of the 2008 National Fusarium Head Blight Forum. Indianapolis, Indiana.

Agostinelli, A.M. 2009. Phenotypic and Genotypic Selection for Head Scab Resistance in Wheat. University of Kentucky. Thesis. 96 pp.

Akbari, M., Wenzl, P., Caig, V., Carling, J., Yang, S., Uszynski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M.J., Howes, N., Sharp, P., Vaughan, P., Rathmell, B.,·Huttner, E., Kilian, A. 2006. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet, 113(8):1409-1420.

Anderson, J.A., Stack, R.W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla,B., Fetch, J.M., Song,Q.J., Cregan, P.B., Frohberg, R.C. 2001. DNA

markers for Fusarium head blight resistance QTLs in two wheat populations. Theor. Appl. Genet. 102:1164-1168.

Arthur, J. C., 1891: Wheat scab. Indiana Agric. Exp. Stn. Bull. 36, 129-132.

Austin, D.F., Lee, M. 1998. Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and nonstress environments. Crop Sci 38:1296–1308

Bai, G.H., Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium Head Blight. Annual Review of Phytopathology, 42(1), 135-161.

Bai, G.H., Shaner, G., Ohm, H. 2000. Inheritance of resistance to Fusarium graminearum wheat. Theor. Appl. Genet. 100:1-8.

Bai, G. H., Shaner, G. 1994. Scab of Wheat - Prospects for Control. Plant Disease, 78(8), 760-766.

Balut, A.L., Clark, A.J., Brown-Guedira, G., Souza, E., Van Sanford, D.A. 2013. Validation of *Fhb1* and *QFhs.nau*-2DL in several soft red winter wheat populations. Crop Science, 53: 934-945.

Bayles, M.B., Verhalen, L.M., McCall, L.L., Johnson, W.M., Barnes, B.R. 2005. Recovery of recurrent parent traits when backcrossing in cotton. Crop Science 45:2087-2095.

Bechtel, D. B., Kaleikau, L. A., Gaines, R. L., Seitz, L. M. 1985. The Effects of Fusarium graminearum infection on wheat Kernels. Cereal Chemistry, 62(3), 7.

Bernardo, A.N., Zhang, D.D., Ma, H.X., Bai, G.H. 2009. Development, mapping and haplotype analysis of EST-based SNPs in the wheat Fhb1 region. In: Canty, S., Clark, A., Mundell, J., Walton, E., Ellis, D., VanSanford, D. (Eds.). Proceedings of the National Fusarium Head Blight Forum, 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp 111.

Briggs, F.N., Knowles, P.F. 1967. Introduction to Plant Breeding. Reinhold Publishing Corp. 1967. University of Winsconsin – Madison. 426 pp.

Bruening, B., Swanson, S., Connelley, J., Olson, G., Van Sanford, D. 2012. Kentucky Small Grain Variety Performance Test. Agricultural Experiment Station. University

of Kentucky, College of Agriculture, Lexington, KY, 40546. PR-640. Issued 7-2012. Available at http://www.uky.edu/Ag/wheatvarietytest/2012/pr640.pdf

Buerstmayr, H., Ban, T., Anderson, J.A. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat, a review. Plant Breed 128:1–26

Buerstmayr, H., Lemmens, M., Hartl, N., Doldi, L., Steiner, B., Stierschneider, M., Ruckenbauer, P. 2002. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor. Appl. Genet. 104:84-91.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B., Lemmens, M. 2003. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor. Appl. Genet. 107:503-508.

Cai, J. 2012. Mapping QTL for Fusarium Head Blight resistance in Chinese wheat landraces. Thesis. Master of Science. Kansas State University. Manhattan, Kansas.

Cardwell, L. A. 2011. Scab resistance QTLs are associated with quality and agronomic traits of soft red winter wheat. Dissertation, University of Maryland, College Park, MD. Available at http://hdl.handle.net/1903/11481.

Cowger, C., Sutton, A. L. 2005. The southeastern U.S. Fusarium head blight epidemic of 2003. Plant Health Progress. Published October 2005. doi:10.1094/PHP-2005-1026-01-RS Available at: http://www.plantmanagementnetwork.org/pub/php/research/2005/fhb/

Crossa, J., Vargas, M., Van Eeuwijk, F.A., Jiang, C., Edmeades, G.O., Hoisington, D. 1999. Interpreting genotype _ environment interaction in tropical maize using linked molecular markers and environmental covariables. Theor Appl Genet 99:611–625.

Delwiche, S.R., Hareland, G.A. 2004. Detection of scab-damaged hard red spring wheat kernels by near-infrared reflectance. Cereal Chem. 81(5): 643-649.

Desjardins, A.E. 2006. Selected mycotoxigenic Fuarium species. In, A. Desjardins. *Fusarium* Mycotoxins Chemistry, Genetics and Biology . The American Phytopath. Society. St. Paul, Minnesota. 259 pp.

Dexter, J.E., Marchylo, B.A., Clear, R.M., Clarke, J.M. 1997. Fusarium head blight: effect on semolina and pasta making quality of durum wheat. Cereal Chem. 74: 519-525.

Dvorak, J., Yang, Z.L., You, F.M., Luo, M.C. 2004. Deletion polymorphism in wheat chromosome regions with contrasting recombination rates. Genetics, 168(3):1665-1675.

Eller, M.S., Payne, G.A., Holland, J.B. 2010. Selection for Reduced Fusarium Ear Rot and Fumonisin Content in Advanced Backcross Maize Lines and Their Topcross Hybrids. Crop Science 50: 2249-2260.

Everts, K.L., Leath, S., Finney, P.L. 2001. Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. Plant Dis. 85:423-429. Doi:10.1094/PDIS.2001.85.4.423.

Fehr, W.R. 1987. Backcross method. P 360-376. In: Principles of cultivar development. Volume 1. Theory and technique. MacMillan, New York.

Frisch, M., Melchinger, A.E. 2001. Marker-assisted backcrossing for introgression of a recessive gene. Crop Sci. 41:1485-1494.

Garvin, D.F., Anderson, J.A. 2002. A historical analysis of the uniform regional scab nursery for spring wheat parents. In: S.M. Canty, J. Lewis, L. Siler, and R.W. Ward, editors, 2002 National Fusarium Head Blight Forum Proceedings, Erlanger, KY. 7–9 Dec. 2002. Michigan State Univ. Press, East Lansing, MI. p. 235–238.

Garvin, D. F., Stack, R. W., Hansen, J. M. 2009. Quantitative trait locus mapping of increased Fusarium head blight susceptibility associated with a wild emmer wheat chromosome. Phytopathology 99:447-452.

GeneMapper Software. Version 4.0. 2005. Microsatellite Analysis Getting Started Guide. Applied Biosystems. pp 72.

Griffey, C. A., Starlin, T. M., Price, A. M., Sisson, W. L., Das, M. K., Pridgen, T. H., Vaughn, M. E., Rohrer, W. L., Brann, D. E. 2001. Registration of ‘Roane’ wheat. Crop Sci. 41: 1359-1360.

Gupta, P.K.; Rustgi, S.; Mir, R.R. 2008. Array-based high-throughput DNA markers for crop improvement. Heredity 101, 5-18.

Gupta, P.K., Varshney, R.K., Sharma, P.C., Ramesh, B. 1999. Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390.

Guttieri, M.J., Becker, C., Souza, E. 2004. Application of wheat meal solvent retention capacity tests within soft wheat populations. Cereal Chem. 81:261‑266.

Guttieri, M. J., Bowen, D., Gannon, D., O’Brien, K., Souza, E. 2001. Solvent retention capacities of irrigated soft white spring wheat flours. Crop Sci. 41:1054-1061.

Guzman, P.S., Diers, B.W., Neece, D.J., St.Martin, S.K., LeRoy, A.R., Grau, C.R., Hughes, T.J., Nelson, R.L. 2007. QTL Associated with yield in three backcross-derived populations of soybeans. Crop Science 47: 111-122.

Hanson, W.D. 1959. Minimum Family Sizes for the Planning of Genetic Experiments**.** Agronomy Journal 51: 711-715.

Hayes, P.M., Liu, B.H., Knapp, S.J., Chen, F., Jones, B. 1993. Quantitative trait loci effects and environmental interaction in a sample of North American barley germplasm. Theor Appl Genet 87: 392– 401.

Hearnden, P.R., Eckermann, P.J., McMichael, G.L., Hayden, M.J., Eglinton, J.K., Chalmers, K.J. 2007. A genetics map of 1,000 SSR and DArT markers in a wide barley cross. Theor Appl Genet, 115(3):383-391.

Hershman, D.E. 1997. Head Scab of Small Grains in Kentucky. Cooperative Extension Service University of Kentucky PPA-38. Issued 4-92. 2pp.

Heun, M., Schafer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B., Salamini, F. 1997. Site of eikorn wheat domestication identified by DNA fingerprinting. Science, 278 (5341): 1313-1314.

Hoffbeck, M., Openshaw, S., Geadelmann, J., Peterson, R., Stuthman, D. 1995. Backcrossing and intermating in an exotic x adapted cross of maize. Crop Science. 35:1359-1364.

Hogg, A.C., Stripo, T., Beecher, B., Martin, J.M., Giroux, M.J. 2004. Wheat puroindolines interact to form friabilin and control wheat hardness. Theor. Appl. Genet, 108:1089-1097. Doi:10.1007/s00122-003-1518-3.

Hospital, F., Charcosset, A. 1997. Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485.

Huang, S., Sirikhachornkit, A., Su, X., Faris, J., Gill, B., Haselkorn, R., Gornicki, P. 2002. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. Proc Nat Acad Sci USA, 99(12):8133-8138.

Isleib, T.G. 1999. Recovery of superior homozygous progeny from biparental crosses and backcrosses. Crop Sci. 39:558–563.

Jaccoud, D., Peng, K., Feinstein, D., Kilian, A. 2001. Diversity arrays: a solid technology for sequence information independent genotyping. Nucleic Acids Research, 29(4):E25.

Jacobsen, E., Schouten, H.J. 2007. Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. Trends Biotechnology, 25:219-223.

James, R.A., Davenport, R.J., Munns, R. 2006. Physiological characterization of two genes for Na+ exclusion in durum wheat, Nax1 and Nax2. Plant Physiol, 142(4):1537-1547.

Jiang, G.L., Dong, Y., Shi, J., Ward, R. 2007b. QTL analysis of resistance to *Fusarium* head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxinivalenol accumulation and grain yield loss. Theor. Appl. Genet. 115:1043-1052.

Jiang, G.L., Shi, J., Ward, R. 2007a. QTL analysis of resistance to *Fusarium* head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. Theor. Appl. Genet. 116:3-13.

Jiang, G.L., Ward, R.W. 2006. Inheritance of resistance to Fusarium head blight in the wheat lines ‘CJ 9306’ and ‘CJ 9403’. Plant Breed 125:417–423.

Jing, H.C., Kornyukhin, D., Kanyuka, K., Orford, S., Zlatska, A., Mitrofanova, O.P., Koebner, R., Hammond-Kosack, K. 2007. Identification of variation in adaptively important traits and genome-wide analysis of trait-marker associations in Triticum monococcum. J Exp Bot, 58(13):3749-3764.

Jing, H.C., Bayon, C., Kanyuka, K., Berry, S., Wenzl, P., Huttner, E., Kilian, A., Hammond-Kosack, E. 2009. DArT markers: diversity analysis, genome comparison, mapping and integration with SSR markers in Triticum monococcum. BMC Genomics 10: 458. Available at http://www.biomedcentral.com/1471-2164/10/458

Kang, J., Clark, A., Van Sanford, D., Griffey, C., Brown-Guedira, G., Dong, Y., Costa, J. 2009. In: Canty, S., Clark, A., Mundell, J., Walton, E., Ellis, D., Van Sanford, D. (Eds.). Proceedings of the National Fusarium Head Blight Forum, 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp 128.

Kang, J., Clark, A., Van Sanford, D., Griffey, C., Brown-Guedira, G., Dong, Y., Murphy, P., Costa, J. 2011. Exotic scab resistance quantitative trait loci effects on soft red winter wheat. Crop Science, 51: 924-933.

Kilian, B., Ozkan, H.,Walter, A., Kohl, J., Salamini, F., Martin, W. 2007. Molecular diversity at 18 loci in 321 wild and 92 domesticated lines reveal no reduction of nucleotide diversity during *Triticum monococcum* (Einkorn) domestication: implications for the origin of agriculture. Mol Biol Evol.24 (12): 2657-2668.

Knapp, S. J., Stroup, W. W., Ross, W. M. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192‑195.

Knott, C. A., Van Sanford, D. A., Souza, E. J. 2009. Genetic variation and the effectiveness of early-generation selection for soft winter wheat quality and gluten strength. Crop Sci. 49:113-119.

Kweon, M., Slade, L., Levine, H. 2011. Solvent retention capacity (SRC) testing of wheat flour: principles and value in predicting flour functionality in different wheat based food processes in wheat breeding- A review. Cereal Chem. 88: 537-552.

Lafiandra, D., Ceoloni, C., Carozza, R., MArgiotta, B., Urbano, M., Colaprico, G., D'Egidio, M.G. 2007. Introduction of D-genome related gluten proteins into durum wheat. In: Buck, H.T., Nisi, J.E., Salomon, N. (eds.) Wheat Production in Stressed Environments, pp 449-454.

Lee, S.H., M.A. Bailey, M.A. Mian, E.R. Shipe, D.A. Ashley, W.A. Parrott, R.S. Hussey, and H.R. Boerma. 1996. Identification of quantitative trait loci for plant height, lodging, and maturity in a soybean population segregating for growth habit. Theor. Appl. Genet. 92:516–523. doi:10.1007/BF00224553.

Liu, C.I., Rathijen, A.J., Shepherd, K.W., Gras, P.W., Giles, L.C. 1995. Grain quality and yield characteristics of D-genome disomic substitution lines in Langdon (*Triticum turidum* var. *durum*). Plant Breeding 114:34-39.

Liu, S., Pumprey, M.O., Gill, B.S., Trick, H.N., Zhang, X., Dolezel, J., Chalhoub, B., Anderson, J.A. 2008. Toward positional cloning of FhB1, a major QTL for Fusarium head blight resistance in wheat. Cereal Res Commun 36: 195-201.

Liu, S., Zhang, X., Pumprey, M.O., Stack, R.W., Gill, B.S., Anderson, J.A. 2006. Complex microcolinearity among wheat, rice and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct. Integr. Genomics 6:83-89.

Mace, E.S., Xia, L., Jordan, D.R., Halloran, K., Parh, D.K., Huttner, E., Wenzl, P., Kilian, A. 2008. DArT markers: diversity analyses and mapping in *Sorghum bicolor*. BMC Genomics, 9:26.

Mardi, M., Buerstmayr, B., Ghareyazie, B., Lemmens, M., Mohemmadi, S.A., Nolz, R., Lemmens, M., Buerstmayr, H. 2005. QTL analysis of resistance to *Fusarium* head blight in wheat using a 'Wanshuibai'-derived population. Plant Breed. 124:329-333.

McCartney C.A., Somers, D.J., Fedak, G., Cao, W. 2004. Haplotype diversity at Fusarium head blight resistance QTLs in wheat. Theor. Appl. Genet. 109:261-271

McCartney, C.A., Somers, D.J., Fedak, G., DePauw, R.M., Thomas, J., Fox, S.L., Humphreys, D.G., Lukow, O., Savard, M.E., McCallum, B.D., Gilbert, J., Cao, W. 2007. The evaluation of FHB resistance QTL introgressed into elite Canadian spring wheat germplasm. Mol. Breed. 20:209-221.

McCartney, C.A., Somers, D.J., Lukow, O., Ames, N., Noll, J., Cloutier, S., Humphreys, D.G., McCallum, B.D. 2006. QTL analysis of quality traits in the spring wheat cross RL4452 x 'AC Domain'. Plant Breed. 125:565-575. Doi:10.1111/j.1439-0523.2006.01256.x

McKay, R. 1957. Ear blight, cereal scab, seedling blight of wheat and root rot oats. IN: McKay, R. (ed) Cereal disease in Ireland. Arthur Guinness, Dublin, pp 74-83.

McKendry, Anne. 2008. Native Resistance: An Essential Building Block for Accelerating the Development of Scab Resistant Soft Red Winter Wheat. In: Proceedings of the 3rd International Symposium on Fusarium Head Blight; 2008 Sept. 1-5; Szeged, Hungary, Cereal Research Communications (IF: 1.037).

McMullen, M., Jones, R., Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease, 81(12), 1340-1348.

Mergoum, M., Frohberg, R.C., Stack, R.W. 2007. Breeding hard red spring wheat for Fusarium head blight resistance, successes and challenges. p. 161‑167. *In* H.T. Buch (ed.) Wheat production in stressed environments. Springer Netherlands, the Netherlands.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114:377-386.

Mesterhazy, A., Bartok, T., Mirocha, C.G., Komoroczy, R. 1999. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding 118, 97-110.

Miller, J.K.; Hacking, A.; Harrison, J. and Gross, V.J. 1973. Stillbirths, neonatal mortality and small litters in pigs associated with the ingestion of Fusarium toxin by pregnant sows. Vet. Rec., 93: 555.

Mirocha, C.J., Kolaczkowski, E., Xie, W., Yu, H., Jelen, H. 1998. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. J. Agric. Food Chem. 46:1414‑1418.

Nganje, W.E., Bangsund, D.A., Leitritz, F.L., Wilson, W.W., Tiapo, N.M. 2002. Estimating the economic impacts a crop disease: the case of Fusarium head blight in U.S. wheat and barley. 2002 National Fusarium Head Blight Forum Proceedings. Other Reports. 245-281.

Nganje, W.E., Kaitibie, S., Wilson, W.W., Leistritz, F.L., Bangsund, D.A. 2004. Economic Impacts of Fusarium Head Blight in Wheat and Barley: 1993-2001. Agribusiness and Applied Economics Rep. No. 528. Online. U.S. Wheat and Barley Scab Initiative. Issued July 2004. Available at: http://ageconsearch.umn.edu/bitstream/23627/1/aer538.pdf

Nightingale, M. J., Marchylo, B. A., Clear, R. M., Dexter, J. E., and Preston, K. R. 1999. Fusarium head blight: effect of fungal proteases on wheat storage proteins. Cereal Chem. 76: 150-158.

Pallota, M.A., Warner, P., Fox, R.L., Kuchel, H., Jefferies, S.J., Langridge, P. 2003. Marker assisted wheat breeding in the southern region of Australia. Proceedings of the Tenth International Wheat Genetics Symposium (1-6 September, 2003, Paestum, Italy) p. 789-791.

Pareyt, B., Brunnel, C., Brus, K., Goesaert, H., Delcour, J.A. 2010. Flour sodium dodecyl (SDS) – extractable protein level as a cookie flour quality indicator. J. Agric. Food Chem. 58:353-360. Doi:10.1021/jf902879c.

PeakScanner Software. Version 1.0. 2006. Applied Biosystems.

Peiris, K. H. S., Pumphrey, M. O., Dong, Y., Maghirang, E. B., Berzonsky, W., Dowell, F. E. 2010. Near-infrared spectroscopy method for identification of Fusarium head blight damage and prediction of deoxynivalenol in single wheat kernels. Cereal Chem. 87: 511-517.

Peleg, Z., Saranga, Y., Suprunova, T., Ronin, Y., Roder, M.S., Kilian, A., Korol, A.B., Fahima, T. 2008. High-density genetic map of durum wheat x wild emmer wheat based SSr and DarT markers. Theor Appl Genet, 117(1):103-115.

Perugini, L.D. 2007. Genetic characterization of wheat germplasm with resistance to Fusarium head blight (FHB) and powdery mildew. Dissertation. North Carolina State University.

Pirgozliev, S. R., Edwards, S. G., Hare, M. C., Jenkinson, P. 2003. Strategies for the Control of Fusarium Head Blight in Cereals. European Journal of Plant Pathology, 109(7), 731-742.

Polak, J., Bartos, P. 2002. Natural sources of plant disease resistance and their importance in the breeding. Czech. J. Genet. Plant Breed. 38:146–149.

Pricket, A.J., MacDonald, S., Wiley, K.B. 2000 Survey of mycotoxins in stored grain from 1999 harvest in the UK. HGCA Project Report No. 230. Home-Grown Cereals Authority, London.

Pumphrey, M. O., Bernardo, R., Anderson, J. 2007. Validating the *Fhb1* QTL for Fusarium Head Blight Resistance in Near-Isogenic Wheat Lines Developed from Breeding Populations. Crop Sci. 47:200-206.

Randhawa,H.S., Mutti, J.S., Kidwell, K., Morris, C.F., Chen,X., Gill, K.S. 2009. Rapid and Targeted Introgression of Genes into Popular Wheat Cultivars Using Marker-Assisted Background Selection. PLoSONE. Vol4. Issue 6. e5752. 11pp.

Reinke, R., Kilian, A. 2006. Diversity array technology (DArT) for the rice breeding program. IREC Farmers' Newsletter, 171: 40-41.

Roder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M., Leroy, P., Ganal, M.W. 1998. A Microsatellite Map of Wheat. Genetics 149: 2007 – 2023.

Ruckenbauer, P., Buerstmayr, H., Lemmens, M. 2001. Present strategies in resistance breeding against scab (Fusarium spp.). Euphytica 119, 121-127.

Rudd, J.C., Horsley, R.D., McKendry, A.L. Elias, E.M. 2001. Host plant resistance genes for Fusarium head blight: sources, mechanisms and utility in conventional breeding. Crop Science 41:620-627.

Sarti, D., Clark, A., Brown-Guedira, G., Dong, Y., Van Sanford, D. 2011. Evaluation of FHB Resistance and Agronomic Performance in Backcross and Forward-Cross Wheat Populations. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (Eds.), Proceedings of the 2011 National Fusarium Head Blight Forum (pp. 48). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.

Sayler, T., 1998. Study: $ 2.6 billion, 501 million bushels lost to scab 1991–96. Prairie Grains 11: 12. Available at http://www.smallgrains.org/Springwh/jan98/scabloss.htm.

Schwarz, P.B., Jones, B.L., Steffenson, B.J. 2002. Enzymes associated with Fusarium infection of barley. J. Am. Soc. Brew. Chem. 60: 130-134.

Semagn, K., Bjørnstad, A., Skinnes, H., Marøy, A.G., Tarkegne, Y., William, M. 2006. Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome, 49(5):545-555.

Shewry, P.R., Halford, N.G., Lafiandra, D. 2003. The genetics of wheat gluten proteins. In: Hall JC, Dunlap JC, Friedman T, eds. Advances in genetics, Vol. 49. Academic Press, 111–184.

Slade, L. and Levine, H. 1994. Structure-function relationships of cookie and cracker ingredients. In: The Science of Cookie and Cracker Production, pp. 23–141. Faridi, H., Ed., Chapman & Hall, New York.

Smith, N., Guttieri, M., Souza, E., Shoots, J., Sorrells, M., Sneller, C. 2011. Identification and validation of QTL for grain quality traits in a cross of soft wheat cultivars Pioneer Brand 25R26 and Foster. Crop Sci. 51:1424-1436.

Snijders, C.H.A. 1990. The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:11-18.

Souza, E.J., Griffey, C., Kweon, M., Guttieri, M.J. 2008. Sources of variation for long-flow milling. Crop Sci. 48:1432‑1440.

Souza, E. J., Sneller, C., Gutierri, M. J., Sturbaum, A., Griffey, C., Sorrells, M., Ohm, H., Van Sanford, D. 2012. Basis for selecting soft wheat for end-use quality. Crop Sci. 52:21-31.

Souza, E. J., Gutierri, M. J., Sneller, C. 2011. Selecting Soft Wheat Genotypes for Whole Grain Cookies. Crop Science 51: 189-197.

Somers, D. J., Fedak, G., Savard, M. 2003. Molecular mapping of novel genes controlling *Fusarium* head blight resistance and deoxynivalenol accumulation in spring wheat . Genome 46:555-564.

Stack, R.W. 2003 History of Fusarium head blight with emphasis on North America. In Fusarium Head Blight of Wheat and Barley (Leonard, K.J. and Bushnell, W.R., eds). St. Paul, MN: APS Press, pp. 1–34.

Tanksley, S.D., Young, N.D., Patterson, A.H., Bonierbale, M.V. 1989. RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257–263.

Tibola, C. S., Fernandes, J. M. C., Delanora, R. 2010. Predicting wheat mycotoxin content using near-infrared reflectance spectroscopy. In: Canty, S., Clark, A., Anderson-Scully, A., Ellis, E., Van Sanford, D. (eds) Proceedings of the 2010 National Fusarium Head Blight Forum, December 7-9, Milwaukee, WI.

USDA, United States Department of Agriculture. 2011. Foreign Agricultural Service, Production, Supply, and Distribution Database and USDA, World Agricultural Outlook Board, World Agricultural Supply and Demand Estimates, 2011. http://www.ers.usda.gov/Data/Wheat/YBtable04.asp accessed by March 2011.

USDA, United States Department of Agriculture. 2013. Economic Research Service. Wheat Data Overview. Wheat year book. http://www.ers.usda.gov/data-products/wheat-data.aspx#.UtMS255dWSo accessed by Jan 2014.

Van Egmond, H. P. 1989. Aflatoxin M1: occurrence, toxicity, regulation. pp 11-55. In: Van Egmond, (Ed.) "Mycotoxins in Dairy Products". Elsevier Sci. Pub. Co., Ltd. New York.

Van Sanford, D., Anderson, J., Campbell, K., Costa, J., Cregan, P., Griffey, C., Hayes, P., Ward, R. 2001. Discovery and Deployment of Molecular Markers Linked to Fusarium Head Blight Resistance: An Integrated System for Wheat and Barley. Crop Science. 41: 638-644.

Van Ooijen, J.W., Voorrips. R.E. 2006. JoinMap 4.0. Software for the calculation of genetic linkage maps in experimental populations.

Verges, V.L., Van Sanford, D., Brown-Guedira, G. 2006. Heritability estimates and response to selection for Fusarium head blight resistance in soft red winter wheat. Crop Sci 46:1587–1594

Von Der Ohe, C., Ebmeyer, E., Korzun, V., Miedaner, T. 2010. Agronomic and quality performance of winter wheat backcross populations carrying non-adapted Fusarium head blight resistance QTL. Crop Science 50: 2283-2290.

Wenzl, P.; Caarling, J.; Kudrna, D.; Jaccoud, D.; Huttner, E.; Kleinhofs, A.; Kilian, A. 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA (PNAS), 101(26):9915-9920.

Wenzl, P.; Li, H.; Carling, J., Zhou, M.; Raman, H.; Paul, E.; Hearnden, P.; Maier, C.; Xia, L., Caig, V.; Ovesná, J.; Cakir, M.; Poulsen, D.; Wang, J.; Raman, R.; Smith, K.P.; Muehlbauer, G.J.; Chalmers, K.J.; Kleinhofs, A.; Huttner, E.; Kilian, A. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics, 7:206.

Wenzl, P., Raman, H., Wang, J., Zhou, M., Huttner, E., Kilian, A. 2007. A DArT platform for quantitative bulked segregant analysis. BMC Genomics, 8:196.

White, J., Law, J.R., MacKay, I., Chalmers, K.J., Smith, J.S., Kilian, A., Powell, W. 2008. The genetic diversity of UK, US and Australian cultivars of Triticum aestivum measured by DArT markers and considered by genome. Theor Appl Genet, 116(3):439-453.

Wicker, T., Yahiaoui, N., Guyot, R., Schlagenhauf, E., Liu Z-D., Dubcovsky, J., Keller, B. 2003. Rapid genome divergence at orthologous low molecular weight glutenin loci of the A and A^m genomes of wheat. Plant Cell. 15(5):1186-1197.

Wittenberg, A.H., Lee, T., Cayla, C., Kilian, A., Visser, R.G., Schouten, H.J. 2005. Validation of the high-throughput marker technology DArT using the model plant Arabidopsis thaliana. Mol Geneti Genomics, 274(1):30-39.

Xia, L., Peng, K., Yang, S., Wenzl, P., Vicente, M.C., Fregene, M., Kilian, A. 2005. DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. Theor Appl Genet, 110(6):1092-1098.

Young, N.D., Tanksley, S.D. 1989. RFLP analysis of the size of chromosomal segments retained around the TM-2 locus of tomato during backcross breeding. Theor Appl Genet 77: 353-359.

Zeven AC, Knott DR, Johnson R. 1983. Investigation of linkage drag in near isogenic lines of wheat by testing for seed- ling reaction to races of stem rust, leaf rust and yellow rust. Euphytica 32:319-327

Zhou, W.C., Kolb, F.L., Bai, G.H., Domier, L.L., Yao, J.B. 2002. Effect of individual Sumai-3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. Hereditas 137:81-89.

Zhuang, J.Y., Lin, H.X., Lu, J., Qian, H.R., Hittalmani, S., Huang, N., Zheng, K.L. 1997. Analysis of QTL * environment interaction for yield components and plant height in rice. 1997. Theor Appl Genet 95: 799– 808.

Zohary, D., Hopf, M. 1993. Domestication of plants in the Old World: The origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. 2nd edition, New York: Oxford University Press.

Copyright © Daniela Sarti Dvorjak 2014

VITA

Daniela Sarti Dvorjak was born in Orlandia, Sao Paulo, Brazil. She received her Biology Degree, double major in Education and Teaching License Degree from the State University of Sao Paulo, in June 2004. She got her Masters of Science in Agronomy/ Genetics and Plant Breeding from the same University in 2007, working with soybean breeding with assistantship from CAPES – Superior Level Improvement Personal Coordination, Brazil. In July of 2007, she moved to University of Kentucky as a student intern in the Plant and Soil Sciences Department, working with Soybean and Sweet Sorghum Breeding. She joined the Wheat Breeding Group in 2009, initiating her Doctoral Graduate Studies at University of Kentucky, working as a research assistant and also teaching assistant with the Plant and Soil Sciences Department, College of Agriculture, Food and Environment. During her studies as a PhD candidate, Daniela Sarti Dvorjak performed as Secretary of the University of Kentucky Graduate Students Association for two years; lead the Plant and Soil Sciences Journal Club for four years and the Agronomy Movie Club for two years; and published her research in major scientific meetings. Daniela Sarti Dvorjak was awarded with the "Delta Epsilon Iota Academic Honor Society's Recognition of Academic Excellence and Strong Leadership Qualities: Outstanding Graduate Student" in January 2013; and with the "Gamma Sigma Delta International Honor Society – The Honor Society of Agriculture's Recognition of Professional and Academic Accomplishments" with Lifetime Membership of the University of Kentucky Gamma Sigma Delta Chapter.