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Plant Microbial Interactions in Wheat: Fusarium Head Blight and Arbuscular Mycorrhizal Fungi

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PLANT MICROBIAL INTERACTIONS IN WHEAT: FUSARIUM HEAD BLIGHT AND ARBUSCULAR MYCORRIHZAL FUNGI

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DISSERTATION ACCEPTANCE PAGE

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

PLANT MICROBIAL INTERACTIONS IN WHEAT: FUSARIUM HEAD BLIGHT AND ARBUSCULAR MYCORRIHZAL FUNGI

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Plant microbial interactions consist of the many relationships between plants and microbes which involve studies that observe the biology and molecular genetics of pathological, symbiotic, and associative interactions. Worldwide studies involving these interactions are scarcely available in wheat (*Triticum aestivum* L.). In South Dakota (SD), wheat research is a major platform used to understand the nature and consequences of these interactions. Specifically, our research efforts here at South Dakota State University (SDSU) concentrate on two different, but valuable, interactions in wheat: the fungal pathogen that causes fusarium head blight (FHB) and the symbiotic interaction of arbuscular mycorrhizae fungi (AMF) with wheat. These interactions were selected to help provide a better understanding of plant microbial interactions in wheat.

In our first project, we studied FHB, which is one of the most devastating plant diseases in the world. It is responsible for significant economic loss due to lower crop yield and quality, as well as human health concern due to mycotoxin accumulation in infected grains. To date, no sources of resistance conferring complete resistance to FHB have been identified in wheat. Using double haploid (DH) populations derived from

selected four-way crosses combining several sources of resistance, we developed wheat lines that display resistance to FHB. Screening evaluations followed by selections were conducted using both DH spring and winter wheat populations to further evaluate the potential usage of this material to enhance adapted wheat germplasms.

Selection for resistance quantitative trait loci (QTL's) and the use of fungicide (Prosaro) are two different approaches, which when combined, may present a better way of minimizing disease damage. We conducted a field experiment to evaluate the effect of combining resistance QTL's and fungicide application on FHB severity.

In our second project we studied AMF, which forms a mutualistic symbiotic interaction with the majority of land plants. Like many plant microbial interactions, not much information is available on AMF and wheat. Consequently, we conducted a field study to examine the contribution of AMF to nutrient uptake and biomass yields of spring wheat genotypes. Our results demonstrate that there are differences in mycorrhizal responsiveness and nutrient efficiency with the presence of AMF on wheat. This could suggest that there is a genetic control of these genotypic differences.

Overall, our findings assist ongoing efforts aimed to describe the causes and benefits of these plant microbial interactions. Our studies are potential baselines that can assist both development and production of wheat and other major crops.

Chapter 1

INTRODUCTION AND BACKGROUND

1. WHEAT

The development of humanity, as we know it, is centered around the origin of farming and the evolution of food crops. Domestication of many crops occurred around approximately 10,500 years ago (Balter, M. 2007). Crops like wheat, maize and rice are major food supplies for the world, providing 44% of the total edible dry matter and 40% of the food consumed in developing countries. Wheat is grown in more than 70 countries on five continents and is the most widely grown crop in the world (Baenziger et al., 2009). It is second (only to rice) among the world's most important food crops in many aspects including nutritive profile, market value, trade, and ease of harvest. It is inexpensive to store, transport and process.

Given the essential role that wheat plays in human nutrition, it is suggested that wheat may be monumental with assisting the vast challenges associated with food security and quality. Assuming the global population will increase by 2050, the wheat community's continued aims are to improve and develop wheat resources and varieties in hopes of assisting world hunger and sustainable wheat production (Hubert et al., 2010).

Wheat is a cereal grass of the genus *Triticum* with about 10,000 species that represents one of the largest families of flowering plants (Sakuma et at., 2011). Wheat was domesticated about 10,000 years ago and was initially cultivated in the regions of the Fertile Crescent of the Near East, which encompasses the eastern Mediterranean, southeastern Turkey, northern Iraq and western Iran, and its neighboring regions of the Transcaucasus, and northern Iran (Faris, 2013).

Wheat species can be diploid, with two sets of chromosomes, but several are polyploid, with four (tetraploid) or six (hexaploid) sets of chromosomes (Debes, 2014). Wheat is known for being an excellent versatile crop with two growing season types: winter and spring wheat. Winter wheat is planted in the fall and matures in the summer. Spring wheat is planted after the danger of frost is over and matures in the summer. In the United States (US), winter wheat accounts for 70-80 % of total wheat production. Spring wheat on the other hand is the most produced and exported in the world, which includes over 70 countries (Bond, 2017).

Beyond the growing season, there are six different wheat classes grown in the U.S., namely, Hard Red Winter (HRW), Hard Red Spring (HRS), Hard White (HW), Durum, Soft White (SW), and Soft Red Winter (SRW) (Debes, 2014). Of the thousands of varieties known, hexaploid species (*Triticum aestivum*) (Bread wheat or Common wheat) (AABBDD) (6x) is the most prominent type and widely cultivated in the world. Tetraploid species (*Triticum durum*) (Pasta wheat) (Durum) (AABB) (4x) is the only tetraploid species used as of today, and diploid species (*Triticum monococcum*) (AA) (2x) is the least commonly used but was domesticated at the same time as Emmer and

durum wheat (Reynolds et al., 2001). Additionally, club wheat (*Triticum compactum)* is a softer type, used for cake, crackers, cookies, pastries, and flours (Reynolds et al., 2001).

Wheat belongs to the family Poacea (also called Gramineae family or true grasses) and is a monocotyledon indicating that the seed has one embryonic leaf that initially grows out of the seed coat beneath the soil, has pollen with a single furrow or pore, flower parts in multiples of three, major leaf veins are parallel, roots are adventitious and secondary growth is absent.

Briefly, wheat plants are herbaceous annual plants that have two parts (petiole and limbo) and grow to be two to four feet tall. Wheat plants have long slender leaves that are surrounded by a lean stalk. Spikes (or ears) can be found at the top ends of stalks. Each spikelet is made up of many spikelet's that are distributed laterally. Wheat flowers (grain) are gathered in spikelets between the lemma and the palea. Wheat requires adequate sun and sufficient moisture during the growing season for ideal yields. Although wheat is grown in many different climates, optimum growing temperature ranges from 20 °C - 25°C, with minimum temperatures of 3- 4 °C (Briggle, 1980).

Over the years, wheat has been monumental given its relatively easy harvesting, storing, transportation and processing, as compared to other grains. Wheat is grown on 25% of the global agricultural land for its grain's properties (mostly protein, iron and manganese) making it the largest food crop regarding growing area (Panguluri et al., 2013). Wheat is an economically important staple food for 40% of the world's populations (Panguluri et al., 2013).

In 2016, wheat production exceeded annual amounts (roughly 735 million tons) and is predicted to rise (to 752 million tons) in 2017 (Bond, 2017). The US is ranked first in wheat exports globally, fifth among the top wheat producing countries, and is second only to rice in regard to overall production worldwide (Bond, 2017). To date, 50% of the total US wheat production is exported with a gross value of about nine billion dollars. Of all the wheat grown worldwide, 95% is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum wheat (Shewry, 2009). South Dakota is the sixth largest producer of wheat in the US.

In 2016, South Dakota accounted for 5.0 % of the total US production of wheat (103,406,000 bushels). By type, South Dakota spring accounted for 60,480,000 bushels (10 % of the total US production of wheat) and winter accounted for 42,680,000 bushels (3.1% of the total US production of wheat).

2. FUSARIUM HEAD BLIGHT OF WHEAT

Wheat in the Great Plains region faces many unfavorable conditions because of abiotic and biotic stresses like salinity, drought, pathogens and insects. These conditions affect wheat growth and limit agronomical yields. Fusarium head blight (FHB) (or scab) is one of the major conditions (biotic stresses) limiting wheat production.

Fusarium head blight is primarily caused by the fungal species *Fusarium graminearum* and is one of the most devastating plant diseases in the world due to the large reductions in grain yield and baking quality which poses a risk to human and animal health. Fusarium head blight was first recognized as a fungal disease in North America about 120 years ago. *Fusarium acuminatum* and *F. reticulatum* are other important

Fusarium species that have also been identified in FHB infected wheat (Nielsen, 2014). However, FHB can be associated with at least seventeen *Fusarium* species.

Fusarium head blight occurs almost every year but is generally limited to relatively few wheat and barley crops. Thus, FHB is not recorded as a widespread disease (Parry et al., 1995). In recent years, however, FHB has re-emerged worldwide as a disease of economic importance (Windels, 2000) with enormous economic impact because of its multifaceted effects on crops (Atanasoff, 1920). The impact of FHB starts right after germination as Fusarium infection of seed can result in reduced germination and post-emergence seedling blight (Bechtel et al., 1985; Jones, 1999). FHB, however, cannot be transmitted through Fusarium-infected seeds (Jones, 1999). In addition to yield losses caused by FHB, the presence of mycotoxins in infected grain further exacerbates losses from disease (McMullen et al, 1997).

In the 1917 disease outbreak, wheat yield loss was 288.8 megagram (Mg, one million grams) and was attributed to several species of Fusarium (Atanasoff, 1920). In the 1928 epidemic, there were yield losses of 20% and 15% in barley and wheat, respectively (Stack, 2003). Yield losses in wheat due to the FHB epidemics during the 1990's in the US was over 18.4 Mg valued at ca. $2.5 billion. Similarly, barley producers lost $400 million at the same time (Windels, 2000). The epidemics of the 1990's in the tri-state area were so serious that there was a net loss in revenue per harvested acre of wheat in the Red River Valley area of North Dakota and Minnesota every year from 1993 to 1998, with the exception of Minnesota in 1996 (Windels, 2000). Estimated direct and secondary economic losses by FHB in wheat and barley in the northern Great Plains and central US was valued at $2,679 million from 1998 to 2000 (Nganje et al., 2002). As a

consequence of these losses to FHB, land planted to barley from 1991 to 1999, decreased by 77%, 53% and 84% in Minnesota, North Dakota and South Dakota, respectively. Similarly, the area planted to wheat decreased by 6%, 5% and 7% in Minnesota, North Dakota and South Dakota, respectively (NASS, 2009).

Many farmers abandoned farming as an occupation and wheat crops became rotational crops and the barley crop was almost wiped out of Minnesota entirely (McMullen, 2003). The decrease in wheat and barley planting from 1991 to 1999 can be attributed primarily due to yield losses caused by FHB and associated quality losses due to mycotoxin accumulation in the infected grain (Windels, 2000).

3. EPIDEMICS OF FUSARIUM HEAD BLIGHT

FHB has increased its importance as a wheat disease given the many epidemics in the U.S and especially in the central Great Plains dating back to the mid 1990's (Baenziger et al., 2009). Several of these major epidemics occurred and caused hundreds of thousands to millions of metric tons (MT) of grain loss. In fact, records show that epidemics in the US took place in the years of 1917, 1919, 1928, 1937, and 1982, but 1993 was the first major epidemic that affected North Dakota, South Dakota, Minnesota and Manitoba (Qu, 2008). In the late 1990's, epidemics were estimated to cause losses around 1.3 MT. (McMullen et al., 1997) (Stack, R. W. 2003). Epidemics have become more frequent in the Great Plain regions and other wheat growing areas in the United States, causing significant economic losses in Kansas, Nebraska and South Dakota since

the early 1990s (Dill-Macky, R. 2010). FHB has most recently occurred yearly to varying levels of severity since 2007.

The increase of FHB epidemics is thought to be associated with many different factors; more frequent precipitation during spring and summer, the use of cultivars with high susceptibility to FHB, and an increase in area under corn cultivation which, together with reduced or no-tillage practices, has favored development of epidemics.

4. **CAUSAL AGENT AND LIFE CYCLE OF DISEASE**

Fusarium head blight, like most diseases, requires a host, a pathogen, and favorable conditions for the disease to develop. Fusarium head blight has a wide host range that includes all small grain crops, corn, and many wild and domesticated grass species. Specifically, host plants such as wheat (straw) and corn (stalks) are amongst the most common plant parts that allow the pathogen to overwinter (Bai, 2004). There are two main ways in which the spread of fungi happens; short distance spread occurs via the dispersal of fungal spores that are blown by the wind from one cereal field to the next, while long distance spread occurs through the transportation of infected crop residue or seed (Rieux, 2014). After either procedure of spreading, the fungus then produces perithecia on colonized residues resulting in the perithecia releasing ascospores into the air which infect the wheat or barley plant (Rieux, 2014).

Fusarium head blight outbreak typically occurs when there are optimal conditions such as high humidity, rainfall at or near heading, and warm temperatures. As this process transpires in wheat, kernels are infected in head and grain with a noticeable bleaching color (then turning pink or orange) and can include tan or brown lesions on

some or all of the spikes. Seed from infected spikelets may be small, shriveled, and white or chalky in appearance. Fusarium head blight infected seeds are commonly referred to as tombstones. Fusarium head blight progresses in many different forms, but true infection of FHB is at the time of flowering or anthesis.

More specifically, *Fusarium graminearum* overwinters saprophytically as perithecia on decaying crop residues, particularly long-lasting corn residues, and residing on the soil surface. The adoption of no-till agricultural practices for the prevention of soil erosion and increasing corn acreages have therefore substantially increased the source of inoculum and prevalence of FHB in the US (Markell, 2003). The primary source of inoculum are airborne ascospores, but infection can also be initiated from macroconidia spores and hyphal fragments that are splash dispersed from the soil surface by rainfall.

5. DISEASE ASSESSMENT

The assessment of FHB is commonly carried out using four measurements to quantify disease (Paul et al., 2005a), (Paul et al., 2005b), (Paul et al., 2006): incidence - defined as the proportion of disease spikes in a sample; severity - defined as the proportion of diseased spikelets per spike; index - defined as the product of incidence and severity; and fusarium damaged kernels (FDK), defined as the proportion of visually scabby kernels in a sample of harvested grain.

6. FHB MANAGEMENT

FHB occurs as a result of the combined effects of several factors such as weather conditions, plant growth stage, and agronomic practices. Weather cannot be controlled, but there are several other factors which can be manipulated to prevent disease

establishment (McMullen et al., 1997). Management of losses caused by FHB requires an integrated approach. A single control strategy is often not sufficient at obtaining adequate control. FHB control strategies consist of agronomic and cultural practices, biological control, fungicide applications and most importantly host resistance.

6.1. Agronomic and Cultural Practices

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

6.2. Biological Control

Several studies have been carried out to investigate the possibility of using biological agents to control FHB. Bleakley et al. (2012) reported Bacillus strains as having potential for biological control of FHB. Chen et al. (2012) showed that the fungus *Clonostachys rosea* can be used as a bio-fungicide in combination with chemical fungicides. Gilbert and Fernando (2004) recognized *Lysobacter spp*. as a promising

biocontrol agent for having ability to induce resistance in the host. Antagonistic action of Pseudomonas spp. against *F. graminearum* has been recognized in vitro (Gilbert et al., 2004). Similarly, green manure enhances *Streptomyces spp.* population in the soil, including other microorganisms which are also antagonistic against *F. graminearum*. Therefore, Pseudomonas and Streptomyces both could be utilized as possible biocontrol agents. However, due to several constraints, biological methods are not currently practical for FHB control in the field.

6.3. Chemical Control

Several fungicides are reported to be effective against FHB but with variable results. The composition of fungicide, application timing, and resistance level of cultivars are related to the variability of fungicide efficacy (Mesterhazy et al., 2002). Some fungicides are effective in reducing the disease but may also have an antagonistic effect on mycotoxin accumulation. Therefore, considerations should be made to suppress both disease severity and mycotoxin level with the application of fungicide (Mesterhazy et al., 2003). In general, triazole fungicides are considered as the most effective fungicide for controlling both disease severity and minimizing deoxynivalenol (DON) concentration. However, none of the triazole fungicides offer complete control of the disease.

6.4. Host Plant Resistance

Host plant resistance is the most effective method to control FHB (McMullen et al., 1997; Sneller et al., 2010). However, the greatest challenge in breeding for FHB resistance is to release adapted FHB resistant cultivars that combine competitive yield and acceptable end-use quality (Bai and Shaner, 2004; Buerstmayr et al., 2009). No

wheat cultivars have been identified that have complete immunity to FHB, however, a few cultivars with moderate to high level tolerance to FHB have been identified and are used as parents in breeding programs. Cultivars with moderate resistance may improve fungicidal efficacy and provide better protection against FHB (Mesterhazy et al., 2003). To date, conventional breeding methods are mainly used to develop resistant cultivars but they are time consuming and expensive (Buerstmayr et al., 2002, 2009). However, it has been found that resistance to FHB is governed by nine major and minor quantitative trait loci (QTL). Identification of major QTLs and markers linked to the QTLs may open the door for accelerating breeding programs through marker assisted selection (MAS) (Buerstmayr et al., 2002).

7. RESISTNACE TO FHB

Given the impacts of FHB epidemics, worldwide efforts have been conducted to develop resistant varieties in the past three decades. To date, not much has changed regarding FHB. We still are unable to control favorable weather conditions and provide areas where tillage trends are predicted to better rotate wheat and corn, which remain two of the most grown crops in the US. Diversity in crop management practices are only partly effective in controlling the disease, and therefore, the development of FHB resistant varieties is important for disease control and the prevention of mycotoxin contamination. Resistant varieties play an important role in controlling FHB. It is important to note however that there have been many breakthroughs in the wheat community that have occurred due to the many epidemics to help manage FHB. Chinese

wheat cultivar Sumai 3 and its derivatives represent the greatest degree of resistance to FHB (Bai, 2004). A major quantitative trait locus (QTL) on chromosome 3BS and other minor QTLs for FHB resistance have been identified in these cultivars and used in wheat-breeding programs worldwide (Bai, 2004). A combination of breeding lines with the 3BS resistance QTL and improved agronomic traits and marker-assisted selection for FHB resistance has been incorporated in many breeding programs in the northern plains (Randhawa, 2013). There are also many wheat accessions that have been produced and reported in places like Japan and US for resistance to FHB in which are used as parental line in breeding programs worldwide.

Fusarium head blight resistance is a quantitative trait controlled by major and minor genes and located on all wheat chromosomes, except 7D (Buerstmayr, 2009). Several components exist for FHB but we generally classified resistance into two types: Type 1 (resistance to initial infection after spray inoculation) and Type 2 (resistance to spread after point inoculation of a single floret on the wheat head) (Schroeder and Christensen 1963). Through research, quantitative trait loci (QTL) for resistance have been identified mostly conferring Type 2 resistance (Buerstmayr, 2009). There have been QTLs that confer Type 1 resistance as well, but identification and selection is difficult (Mesterházy, 2008). Although we have seen many leaps in research in the past three decades to provide a high level of resistance to FHB in wheat, marker assisted selection (MAS) of these QTL should be used to pyramid these resistances into an agronomically desirable background. Currently, pyramiding multiple resistance resources combined with double haploids, is an example of wheat research that can help develop resistant varieties for lowering the impact of FHB, increasing the efficiency of selection in plant

breeding programs, faster route to homozygosity, screening for resistance, and increase cultivar production by three to five years (Eckard, 2015).

8. MYCOTOXIS AND F. graminearum INFECTION

While FHB has caused many problems (yield loss, low-test weights, and low seed germination), animal and human consumption are still the major concern in regard to the contamination of the grain by mycotoxins (Miller, 2002). The fungus produces a mycotoxin known as deoxynivalenol (DON) that poses a significant threat to the health of domestic animals and humans. The U.S. Food and Drug Administration (FDA) has set maximum levels of DON which is associated with FHB. DON levels exceeding these standards have been known to reduce body weight and have reproductive and immunotoxin effects. DON can also affect baking quality and flavors in foods.

Different Fusarium species have been described as producers of toxic secondary metabolites that affect human and animal health (Bennett, 2003). However, trichothecenes have been identified as one of the most important types (Schollenberger et al., 2007). Trichothecenes are extremely potent inhibitors of eukaryotic proteins synthesis; They are toxic to both animals and plants (McCormick, 2003). *F. graminearum* produces several mycotoxins, including nivalenol, DON and its derivatives, zearalenone, fusarin C, and aurofusarin (McCormick, 2003), (Bennett, 2003) (Trail, 2009).

The primary economic and health consequence of FHB is due to DON contamination even with its relatively low acute toxicity (Paul et al., 2005b). DON is a potent protein biosynthesis inhibitor affecting the digestive system and major organ

function in humans and animals. When ingested in sufficient doses, it causes nausea, vomiting, and diarrhea. Farm animals fed with contaminated grain have weight loss and food refusal (Bennett, 2003) and for this reason DON is also called vomitoxin or food refusal factor. It is a virulence factor in wheat, causing tissue necrosis (Desjardins et al., 1996) (Proctor et al., 1995). DON is the only mycotoxin shown to be a virulence factor (McCormick, 2003) (Trail, 2009). Tolerance limits of DON in the U.S. are 1, 10, 5, and 5 ppm, respectively, in finished wheat products, grain and grain byproducts destined for ruminating beef and feedlot cattle older than 4 months and chickens (not exceeding 50 % of the total diet), grain and grain byproducts destined for swine (not exceeding 20 % of their diet), and grain and grain byproducts for other animals (not exceeding 40 % of their diet) (Dexter et al., 2003).

In the upper Midwestern region of the United States, DON levels frequently exceed this limit (Trail, 2009). In addition to the health consequences, wheat grain with DON concentrations exceeding the minimum limits allowed may be rejected or devalued at grain intake points (Cowger et al., 2009).

9. RESARCH OBJECTIVES

The overall objective of this research was to investigate the interaction between the fungal pathogen (*F.graminearum*) that causes FHB with wheat to better understand host and pathogen interactions. Our work provided us opportunities to observe plant host and pathogen interactions under varied environments and conditions. From my studies, we were optimistic that our findings would allow us to improve upon current technologies, practices, and methodologies to assist in crop improvement and development. Ultimately, we hope through our efforts, resources (including baseline information) are developed to

help crops with their ability to uptake nutrients in both promising and undesirable conditions and withstand abiotic and biotic stresses. The following chapters describe the causes and benefits of the interaction of *Fusarium graminearum* and wheat.

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Chapter 2

USING DOUBLED HAPLOID WHEAT (*TRITICUM AESTIVUM* L.) LINES DERIVED FROM F1 PLANTS WITH MULTIPLE SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT FOR SCREENING AND GERMPLASM SELECTION

1. Abstract

The double haploid (DH) system is a key technology that is currently being used to speed up the breeding process. Unlike, the traditional practices of variety development, double haploids are a practical approach of creating new varieties due to the ability to obtain homozygous plants in just one generation. The development of double haploid wheat lines may be monumental in assisting wheat demands considering the predicted increase in the world's populations and the need for food. The goal of this study was to screen previously developed double haploid lines derived using multiple sources of resistance to Fusarium head blight in hopes of selection germplasm.

In the first part of this study, we screened 225 double haploid spring wheat lines in South and North Dakota from 2014-2016. Using field nurseries, we were able to assess this DH population for FHB severity. After multiple years (assessing FHB severity) followed by selections based on FHB resistance performance, we conducted a one-year

study in 2015 to assess the combined ability of these DH lines and the use of a fungicide (Prosaro). In 2016, with a smaller population, we assessed both FHB severity and DON content. A portion of the DH lines each year had severity ratings that were as good or better than many of the commercial used resistant line in the screening areas.

In the second part of this study, we screened roughly 200 double haploid winter wheat lines at similar locations as the spring wheat DH population (South and North Dakota) from 2015-2016. Using both greenhouse and fields nurseries, we were able to assess this DH population for FHB severity. Like the spring wheat material, the DH winter wheat lines were assessed and had severity ratings similar to or better than, commercially used resistant lines in screening areas.

Results from both studies have allowed many of the DH lines to be used throughout the region as parental lines and some will be submitted as germplasm. This study attempted to develop and validate wheat lines that should display resistant characteristics to FHB given the materials genetic background.

2. Introduction

Common or bread wheat (*Triticum aestivum L.,* AABBDD, 2n = 42) is an important crop worldwide having originated from hybridization of tetraploid wheat (*Triticum turgidum L.,* AABB, 2n = 28) and diploid *Aegilops tauschii Coss*. (DD, 2n = 14) (Chantret et al. 2005). The United States is a major wheat producing country, and one of the world's leading wheat exporter (https://www.statista.com/topics/1668/wheat/). With wheats' global production at 743.2 million tons (2016-2107) and the US production

at roughly 9.1 billion over 15 years, wheat continues to thrive as a worldwide staple crop (https://www.statista.com/statistics/190358/total-us-wheat-production-from-2000/).

Producing wheat with desirable traits such as disease and pest resistance is of great importance. One of the major limiting factors in wheat production is fusarium head blight (FHB), which is one of the most important diseases due to its ability to decrease yields (losses that include reduced kernel number, kernel weight and grain quality may cause losses of up to 100%), contaminates grain with mycotoxins Deoxynivalenol (DON) and reduces the profitability for producers (Stein et al., 2009).

FHB, also known as ear blight, scab, white heads and pink mold, is mainly caused by Fusarium graminearum. Since the disease was initially recorded on wheat, barley and other small grains, 17 different Fusarium species have been associated with the disease (Schmale III et al., 2003). Over the years, many FHB outbreaks have caused major damages to wheat production across Europe, America and Asia during the 20th and 21st centuries (Elias et al. 2005; Oliver et al. 2007; McMullen et al. 2012; Girouxetal et al. 2016). FHB is difficult to control, so it is imperative to prevent the disease from becoming established in a field.

Minimizing FHB can only be achieved through an integrated approach including cultivation practices, fungicide application, and the use of resistant cultivars. Since limited varieties and resistant resources are available in the wheat community to control FHB, it is crucial to continue building upon already resistant sources to develop new varieties and the usage of integrated management strategies. To date, the best-known cultivar that has resistance to FHB is Sumai 3 (Chinese origin), an improved cultivar with good combining abilities for yield and FHB resistance and used frequently in breeding

programs worldwide (McKendry, 2000; Bai & Shaner, 2003; Mesterházy, 2003). Other cultivars, including the Brazilian cultivars Frontana and Encruzilhada have also been reported to possess FHB resistance and are therefore utilized in breeding programs (Bai & Shaner, 2004).

In South Dakota, FHB has occurred yearly at varying levels of severity and prevalence since the early 90's, with the worst epidemics in over 20 years occurring in 1993 and 1997. *F. graminearum,* and several other species of Fusarium, are causal agents of FHB in the U.S. (specifically South Dakota). Knowledge of the major species of Fusarium causing FHB in South Dakota will be useful to researchers in devising management strategies for the disease.

Developing resistant cultivars with conventional breeding has become outdated. In the past, it took up to 15 years for a resistant cultivar to be developed with all the desirable agronomic and quality traits. With the complex task of accelerating breeding efforts to create resistant varieties, double haploids technology dramatically increases complete homozysity of wheat lines in a single year, making the selection process efficient in plant breeding (Rudd et al., 2001). DH technology reduces several time-intensive generations of inbreeding and phenotyping and genotyping more predictive. Therefore, the release time of a variety is reduced to half, or less than half, and desirable characteristics of diverse wheat genotypes can be combined as compared to conventional breeding methods like back cross, pedigree or bulk method.

To date, no sources conferring complete resistance to FHB have been identified in wheat. We are using double haploid (DH) wheat lines derived from selected four-way

crosses combining several sources of resistance to validate putative QTLs (Xmc758. Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB for the producers, processors, and consumers of wheat. These studies were conducted to achieve the following objectives:

1. To screen both spring and winter wheat DH populations in multiple locations for FHB severity and selection of best lines for advancement as FHB germplasm lines
2. To assess if DH lines combined with fungicide could reduce FHB severity
3. To validate putative QTLs (Xmc758. Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296

3. Materials and Methods

3.1 Plant material

Two hundred and twenty-five spring wheat DH lines were developed using F2 seeds derived from 28 four-way crosses (from 565 four-way F1 plants, with an average of 20 four-way F1 plants per cross). Each four-way F1 plants were selfed to derive F2 seed. Crosses were made using 10 winter wheat parental lines to develop 28 segregating four-way F1 populations. Parental lines included two backcross-derived lines carrying Fhb1 resistance allele in a ‘Wesley’ background (Wesley-Fhb1-BC06 and Wesley-Fhb1-BC56) and an experimental line AL-107-6106 (Alsen / NE00403 // NE02583-107) were used as donors of Fhb1 in each cross. Founders providing native sources of resistance were the HWW varieties ‘Lyman’ (KS93U134/Arapahoe) and ‘Overland’ (Millennium 133 sib // Seward / Archer) and the SWW varieties ‘Ernie’ (Pike / MO9965) and ‘Freedom’ (GR876 / OH217). The remaining founders, which provided desirable agronomic traits

were NE06545 (KS92-946-B-I5-1 / Alliance), NI08708 (CO980829 / Wesley), and ‘McGill’ (NE92458 / Ike) (Eckard et al., 2015).

FHB severity evaluations were conducted in greenhouse trials (Brookings, SD) and field trials (South and North Dakota) from 2014-2016 respectively. Two SD resistant checks (Advance and Brick resistant), one ND resistant check (2710), and two susceptible cultivars (Forefront SD) (2398 ND) were used in the experiments as well. These nurseries have been used annually by respective breeding programs in each location.

200 winter wheat DH lines were developed using F2 seeds derived from 28 four-way crosses from 565 four-way F1 plants, with an average of 20 four-way F1 plants per cross. Each four-way F1 plant were selfed to derive F2 seed. Crosses were made using 10 winter wheat parental lines to develop 28 segregating four-way F1 populations. Parental lines included two backcross-derived lines carrying Fhb1 resistance allele in a ‘Wesley’ background (Wesley-Fhb1-BC06 and Wesley-Fhb1-BC56) and an experimental line AL-107-6106 (Alsen / NE00403 // NE02583-107) was used as donors of Fhb1 in each cross. Founders providing native sources of resistance were the HWW varieties ‘Lyman’ (KS93U134/Arapahoe) and ‘Overland’ (Millennium 133 sib // Seward / Archer) and the SWW varieties ‘Ernie’ (Pike / MO9965) and ‘Freedom’ (GR876 / OH217). The remaining founders, which provided desirable agronomic traits, were NE06545 (KS92-946-B-I5-1 / Alliance), NI08708 (CO980829 / Wesley), and ‘McGill’ (NE92458 / Ike) (Eckard et al., 2015). Cultivars ‘Lyman’, BC06 and ‘Overland’ which have been shown

to moderate levels of FHB resistance, were used in all experiments as a measurement of resistance. Cultivar Wesley was used in the same manner but for susceptibility.

3.2 Double haploid development

Double haploids were created at the Heartland Plant Innovations wheat breeding laboratory (Manhattan, KS) using the protocol below (Barkley and Chumley 2011). F2 seeds were grown to the stage where they are developing pollen within the male part of the plant, which is easily seen as the anthers that protrude from the head at flowering time. The plants are emasculated, with the glumes clipped and the tiny anthers removed with tweezers. Roughly two days later the wheat ovaries were pollinated with fresh corn pollen, which were grown in an adjoining greenhouse at Heartland Plant Innovations. At this point, the corn pollen stimulates wheat embryo development, but because the wheat and corn are so different, no corn genetic material remains in the wheat embryo after the first few cell divisions. The developing embryo now is given 2, 4-D and a key plant hormone called gibberellic acid. These act as growth stimulants to encourage the immature embryo to continue to develop (Barkley and Chumley 2011). The embryo is not viable since it only has the single copy of chromosomes from the wheat plant. This embryo would not develop into a seed if allowed to continue development on its own. So, the embryos are removed from the plants using tweezers, a microscope, and are cultured in a growth medium. This process is known as embryo rescue (Barkley and Chumley 2011). After the embryos grow in this medium, they may regenerate into whole plants that are haploids, meaning they only contain one copy of the chromosomes of a normal wheat plant (Barkley and Chumley 2011). Following early seedling development, the

haploid plants are treated with colchicine, which serves to duplicate the chromosomes in the cells. The plants were then raised in a greenhouse, and the seeds harvested and returned to SDSU (Barkley and Chumley 2011).

3.3 Phenotyping (FHB screening nursery design) spring wheat

DH lines were planted in mid-April as randomized replicated row environments, where each environment was established with a minimum of 20 seeds in South and North Dakota. Inoculum was cultured on ½ PDA using a mixture of 9 *Fusarium graminearum* isolates (Fg1, Fg4, Fg5, Fg6, Fg30, Fg41, Fg56, Fg62, Fg63, Fg64 and Fg70). DH lines were spray inoculated with infested corn kernels spread on the soil surface about 1 month prior to heading, and heads were mist irrigated beginning at heading at all sites to provide constant disease pressure (Paillard et al., 2004).

At 50% anthesis, DH were sprayed and inoculated directly using a conidial spore suspension containing 100,000 spores/mL. DH lines were assessed for FHB infection 21 days after flowering. To determine FHB severity, infections were scored on 20 heads per environment using a 10-point visual scale described by where each score corresponds to a percent of the spike infected (Eckard et al., 2015). FHB severity was measured as a binomial trait by counting the number of spikelets with disease symptoms out of the total number of spikelets on each inoculated spike (Stack et al., 1998).

3.4 Phenotyping (FHB screening nursery design) winter wheat

Double haploids lines were vernalized for roughly 8 weeks and then transplanted as individual plants in 8x8 pots. Each line was replicated three times and organized in a complete randomized design. Five seeds per line were planted and grown in the

greenhouse as per standard conditions for seed increase, and leaves were collected for isolating DNA and screening of FHB. As the plants approached anthesis, an aggressive Fusarium graminearum isolate Fg4, was prepared for FHB inoculations in the greenhouse. The fungus was cultured on ½ 146 strength PDA (12 g potato dextrose, 15 g agar, 1 L dH20) with 0.2% lactic acid. Conidial pore suspensions containing 50,000 spores/mL were prepared and stored at -20 °C. Individual spikes were spray inoculated using a 0.5liter sprayer when approximately 50% spikelet's had extruded anthers (50% anthesis). Approximately 1 mL of the spore suspension was sprayed from top to bottom on two opposite sides. Polyethylene (small zip-lock bags) bags were placed over the spikes for 48 hours after inoculation to provide adequate humidity for infection. The temperature in the greenhouse was maintained between 21 and 26 °C.

To further assess the DH population, phenotypic evaluations were carried out in field nurseries in South and North Dakota breeding program. DH lines were planted in mid-September as randomized replicated row environments, where each environment was established with a minimum of 20 seed. Inoculum was cultured on ½ PDA using a mixture of 9 *F. graminearum* isolates (Fg1, Fg4, Fg5, Fg6, Fg30, Fg41, Fg56, Fg62, Fg63, Fg64 and Fg70). DH lines were spawn inoculated with infested corn kernels spread on the soil surface about 1 month prior to heading and heads were mist irrigated beginning at heading at all sites to provide constant disease pressure (Eckard et al., 2015). At 50% anthesis, DH were sprayed and inoculated directly using a conidial spore suspension containing 100,000 spores/mL. DH lines were assessed for FHB infection 21 days after flowering. To determine FHB severity, infections was scored on 20 heads per

environment using a 10-point visual scale described by, where each score corresponds to a percent of the spike infected (Eckard et al., 2015).

3.5 Genotyping

DH plants for both the spring and winter wheat population were grown in greenhouse long enough to collect at least ten totally leaves. After collection, plants were maintained for seed growth, screening and propagation. DNA was isolated from the leaf tissue using a midiprep phenol: chloroform extraction protocol adapted from (Eckard et al 2015). Plant tissue from multiple plants were pooled and collected by line and flash frozen in liquid nitrogen and stored at -80˚C until use. Occasionally, leaves were processed immediately after collection, being first flash frozen in liquid nitrogen.

Approximately two grams of each leaf tissue were ground to a fine powder using a mortar and pestle and added into a 50 milliliters (ml) screw cap tube with 5 ml of preheated 60˚C extraction buffer 1 % n-lauroylsarcosine, 100 Mm Tris-base, 100 mm NaCl, 10 mm EDTA, 2% polyvinyl-polyprrolidone, (pH 8.5). The mixture tissue and buffer in tubes was incubated at 60˚C for 30 minutes in a hot water bath. A mixture of phenol: chloroform: isoamyl alcohol 25:24:1 saturated with 10 mm Tris (pH 8.0) for nuclei acid separation was added to tube and shaken vigorously. Pressure was released by uncapping the tubes periodically after shaking. The tubes were centrifuged at 3500 reps per minute (rpm) for 15 minutes using a refrigerated ultracentrifuge. The supernatant was removed from each of the tubes and placed in clean 50 ml centrifuge tubes. Cheese cloth was used to avoid getting large pieces of leaf tissue in the clean supernatant. Next, 5 ml of isopropyl alcohol was added to precipitate the DNA strands. Here, the samples are usually stored in the -20°C freezer for a half hour to overnight to precipitate the DNA as

much as possible. Next, the samples were centrifuged at 3,000 rpm for 15 minutes. The supernatant was discarded and the pellet was washed with 10 ml of DNA wash solution (7.5 M Ammonium Acetate (pH 7.7), 95% Ethanol, brought up to one liter in distilled water) and kept in the cooler for at least 20 minutes. This step removes traces of chloroform. A second wash step was done. The supernatant was removed from each sample and the pellet DNA was placed upside down and allowed to dry overnight. The pellets were then re-suspended in 200 microliters (µl) of TE buffer with RNase A (10 µl of 10 mg/ml RNase A per ml of TE) and incubated for an hour to activate RNase and dissolve DNA in liquid.

3.6 DNA Clean Up

All samples were then cleaned up using a 50:50 combination of phenol: chloroform (Sambrook and Russell 2001). An equal amount of combination was added to each of the tubes and shaken until the solutions were one. Samples were then centrifuged for one minute to separate the layer containing the DNA from the other. The top layer of liquid containing the DNA was then transferred to a clean micro centrifuge tube and kept for further clean-up. An equal amount of chloroform was added to the sample and shaken until an emulsion forms. The tubes were centrifuged for one minute at 3,000 rpm. The top layer was then transferred into a fresh micro centrifuge tube. Ethanol precipitation was then performed on the samples. Two times (2x) the amount of ice-cold ethanol was added to each tube and mixed well. The samples were precipitated for at least half an hour on ice. DNA recovery occurred by centrifugation at 0˚C for one minute at 3,000 rpm. The supernatant was then discarded. Seventy percent ethanol was added to each 1.5ml tube hallway and centrifuged at 4˚C at top speed for 2 minutes. This step was repeated a

second time and supernatant was removed. The liquid was then allowed to evaporate from the pelleted DNA in an open area. After the liquid evaporated, TE buffer was added to each tube. The amount of TE buffer added was depended upon the size of the DNA pellet at the end of the isolation process. The amount usually ranged from 200-500 µl.

3.7 DNA Quantification

After extraction, all DNAs were quantified by NanoDrop 2000 spectrophotometer (ThermoScientific, US), and diluted to a standard concentration of 50 ng/µl. Simultaneously, the DNA was quantified by gel electrophoresis to ensure the quantity and quality of each genotype can be visualized in agarose.

Detection of polymorphisms of SSR markers: Polymorphisms of SSR markers, Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296 spring wheat parents and DH derived population from the wheat breeding programme at South Dakota State University were analyzed. These SSR markers were reported to be linked to the major scab resistance QTLs on multiple chromosomes including 3BS in mapping populations of wheat (Eckard et at., 2015).

4. Results

4.1 Field phenotypic analysis 2014 (spring wheat)

Overall, 225 DH lines were assessed for FHB in 2014. FHB severity was moderate to high in South and North Dakota 2014 with severity ratings ranging from 0-60 % of the total percentage of disease spikelet's. In both locations, over 70% of the DH populations had severity from 0-20% of the total percentage of disease spikelet's. In South Dakota (2014), 158 of the DH lines had severity ratings (0-20%) similar or better

that resistant check Forefront and Advance (fig 1). 116 of the DH lines had severity ranging from 0-10% the total percentage of disease spikelet's (fig 1).

In North Dakota, 158 of the DH lines had severity ratings from 0-20% p the total percentage of disease spikelet's (fig 2). 102 of the DH lines had severity ratings that were similar of better than resistant check 2710 ranging from 0-10% percentage of disease spikelet's (fig 2). A large portion of the DH lines had similar of equal rating for severity in both location. DH lines were screened in Minnesota in 2014 but due to germination (50% of the population destroyed results were not included in this study.

4.2 Field phenotypic analysis 2015 (spring wheat)

In 181 DH lines were assessed for FHB severity two locations in 2015. Severity rating in South and North Dakota in 2015 were similar to 2014 results. The overall severity ratings in both locations ranged from 0-60% the total percentage of disease spikelets. In South Dakota (2015), 135 of the DH lines had severity ratings (0-10%) similar to resistant check Forefront and Advance (fig 3). 18 of the DH lines had severity that were better than both resistant (fig 3). In North Dakota, 78 of the DH lines had severity ratings from 0-10% percentage of disease spikelet's (fig 4). All 78 of the DH lines had severity ratings that were similar or better than resistant check 2710.

In a one-year trials, 30 DH lines were selected from the entire DH population. We assessed effect of combining resistance QTL’s (DH lines) and fungicide application on FHB severity. Fungicide application reduce FHB severity (fig 5 &6). However, 50% of the selected DH lines showed reduction to FHB without fungicide when compared to resistant checks.

4.3 Field phenotypic analysis 2016 (spring wheat)

40 of the DH lines were selected for the entire spring wheat population based on previous years FHB severity. Severity ratings in South Dakota ranged from 0-30% percentage of disease spikelet's (fig 8). 15 of the DH lines had severity ratings (0-10% percentage of disease spikelets) that were better than resistant checks Forefront and Advance. 27 of the DH lines had severity ratings (10-20% the total percentage of disease spikelets) that were similar or equal to the both resistant checks.

4.4 Field phenotypic analysis 2015 (winter wheat)

Screening of DH population was severely affected by mid winters in both South and North Dakota. Due to this, roughly 50% of the population was destroyed in South Dakota and the entire population was destroyed in North Dakota. Overall, 112 DH lines were assessed for FHB severity in 2015. Severity was high in the field, rating ranged from 0- 80% the total percentage of disease spikelet's (fig 9). Fifty-one of the DH lines had severity ratings that ranged from 0-20% the total percentage of disease spikelet's. Only six of the DH lines had severity ratings that were similar or equal to resistant checks Lyman, Wesley/BC06 and overland (fig 9).

4.5 Greenhouse phenotypic analysis trial one

Screening in the greenhouse was our first attempt at screening the entire DH winter wheat population.

Greenhouse severity ranged from 0-60% percentage of disease spikelets. 153 of the DH lines had severity ratings ranging from 0-20% percentage of disease spikelets. 87 of the

DH line had severity rating less than 10% percentage of disease spikelets which was similar or better than resistant checks Lyman, Wesley/BC06 and overland (fig 10).

4.6 Greenhouse phenotypic analysis trial two (winter wheat)

Greenhouse trial two severity ranged from 0-60% percentage of disease spikelets. 133 of the DH lines had severity ratings ranging from 0-20% percentage of disease spikelets. 106 of the DH lines had severity ratings less than 10% percentage of disease spikelets which was similar or better than resistant checks Lyman, Wesley/BC06 and overland (fig 10).

4.7 Greenhouse phenotypic analysis trial three (winter wheat)

Greenhouse severity ranged from 0-80% percentage of disease spikelets. 135 of the DH lines had severity ratings ranging from 0-20% percentage of disease spikelets. 63 of the DH lines had severity ratings less than 10% percentage of disease spikelets which was similar or better than resistant checks Lyman, Wesley/BC06 and overland (fig11).

4.8 Field phenotypic analysis 2016 (winter wheat)

` Overall, severity was high in the field in 2016. Severity ratings ranged from 0-70% percentage of disease spikelet's (fig 10). 54 of the DH lines had severity ratings that ranged from 0-20% percentage of disease spikelet's and were similar or equal to resistant checks Lyman, Wesley/BC06 and overland (fig 12). 62 of the DH lines had severity ratings that were similar or better than resistant checks Flourish & Overly (fig 12).

5 Discussion

Developing FHB resistant wheat varieties serves to be the most practical approach for minimizing the overall impact of this disease. However, development of this type of material is difficult given the quantitative nature of the inheritance of FHB resistance (Miedaner 1997). Restraints associated with this process are mostly due to FHB being quantitatively inherited and is considerably affected by environment and pathogen populations (Miedaner 1997). In recent years, to solve many of the complications in development of resistance varieties, breeders have combined methods for quantifying the disease organism. Many of these methods revolve around, DNA-based markers, marker-assisted recurrent selection and genomic selection, coupled with high density and high throughput marker platforms (Landjeva et al., 2007). Detention of QTLs (identification) and validation studies are methods used to develop resistant varieties FHB and have been the most successful tool for breeders to use in conjunction with conventional techniques. As a result of marker technology, genes or QTL underlying control for FHB resistance have aided the rapid integration into elite material (Morgante et al., 2003) (Vaughan et al., 2007). Via Buerstmayr et al. (2009), 52 FHB QTL studies have been summarized. From this summary, QTLs for FHB resistance have been mapped on every chromosome except 7D. Provided this information, a study conducted by Eckard et at. (2015) summarized the findings/potential of pyramiding genes associated with FHB resistance. The Eckard et al. (2015) study provides information explaining how stacking or pyramiding multiple genes/QTL gives breeders the opportunity to select and combine multiple sources of resistance (Eckard et al, 2015). Therefore, gene pyramiding

is an enhanced method that can be used to assemble desirable alleles of multiple genes for one or more traits into a single genotype.

The phenotypic characterization of the DH lines suggested that the genetic background of this material was retained in these DH lines. By screening the DH population in multiple years, multiple locations with multiple replications of the DH populations (spring and winter) proves that this attempt of pyramiding multiple sources of resistance reduces FHB severity given we observed considerable infection. Because we used a multi-environmental trials approach, FHB severity was an appropriate assessment to screen DH population for FHB. In all studies, less than 10% of the population had severity over 35% of disease spikelets meaning that the combination of resistant donors was successful. In all studies, approximately 65% of the DH population had severity rating that concurred with commercial used resistant checks in respective locations (regions of study).

Fungicides are a commonly used approached used to assist with the impact of FHB. Given the good efficiency fungicide application are the only in-season option for control of FHB. However, application requires optimal timing and appropriate position but if applied correctly, studies have shown that roughly 70-75% reduction in FHB and mycotoxin accumulation can be obtained when using a combination of a moderately resistant wheat and fungicide at Feekes 10.5.1 (https://www.extension.purdue.edu/extmedia/id/id-422.pdf). In our one-year trials, 40 lines (including two resistant and two susceptible checks) made up the sample population (18 of the best and worst lines) used to assess the effect of combining resistance QTL's (DH lines) and fungicide application on FHB severity. Selections were based on prior

years FHB impact of lines with best and worst severity rating. Reduction occurred with and without the presents on the fungicide. Given that this was only a one-year trial, experiments set up in a similar manner should be conducted to further assess the benefits of having fungicides with this DH material.

In 2016, 40 DH spring wheat lines and 50 DH winter lines were analyzed for DON content. DON content was low in both populations with majority (95%) of the lines having DON content lower than 2.9ppm. In both populations, DON content of 75% met consumption guidelines for both humans and animals.

The spring and winter wheat population material have been submitted for SSR and SNP analysis respectively. SSR results are being finalized and we are currently waiting for the SNP results.

6. Conclusion

Wheat research proves that it takes several years, innovated approaches, financial stability, and a well put together research team to generation a variety with superior agronomical and defense traits for commercial use. However, DH derived lines are a feasible alternative providing the link between conventional breeding and genomics. DH lines, although considered labor and resource intensive, have key features in genomic programs for integrating genetic and physical maps. DH are most commonly used for their widespread applications in quantitative genetics and SNP discovery and in establishing chromosome maps, resulting in reliable information on the location of major genes and QTLs for important traits.

This study demonstrates a successful example of the ability to combine multiple sources of FHB resistance. A screening analysis established an overall severity index

similar to (Eckard et al., 2014) that allowed the selection of several DH lines for germplasm release and usage as parental lines. This valuable strategy can aid breeders in developing resistant materials, while reducing the amount of time to create new varieties. The incorporation of this methodology, combined with integrated pest management practices, present the most economical and efficient approaches to further build upon evidence to impact and reduce FHB worldwide. Therefore, DH lines selected from this study should be incorporated (crossed with other resistant material and used as parental lines) and screened for FHB and other desirable agronomical traits, and then further validated for other QTL's, fused into breeding programs across the country to evaluate the impact of different environmental conditions. There should also be further experiments conducted to evaluate the impact of combining different management practices.

7. Acknowledgements

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9. Figures and Tables

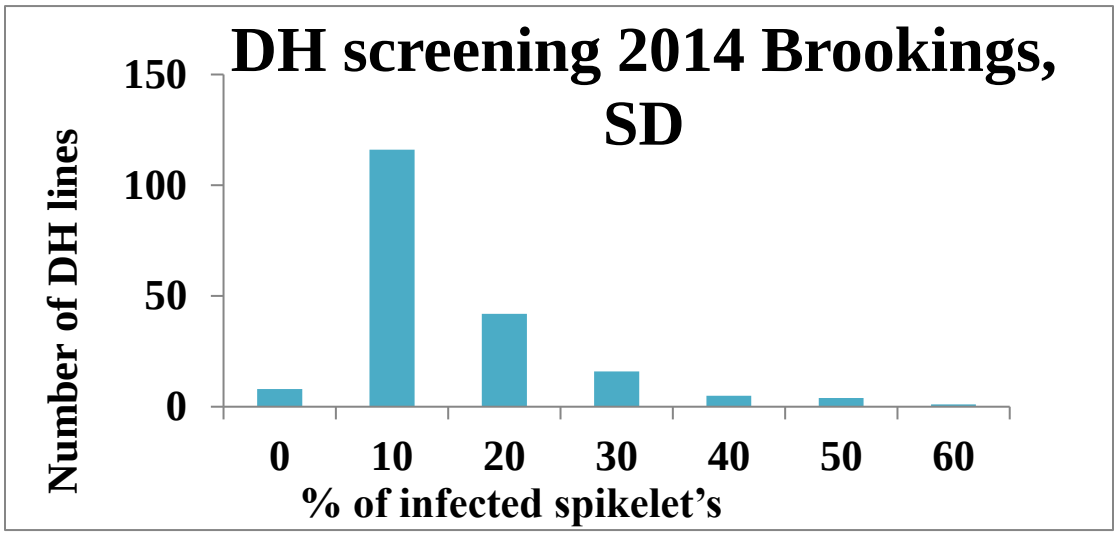

Figure 1: Percentage of disease spikelet's from DH lines with controls (Forefront & Advance resistant and Briggs Susceptible,)

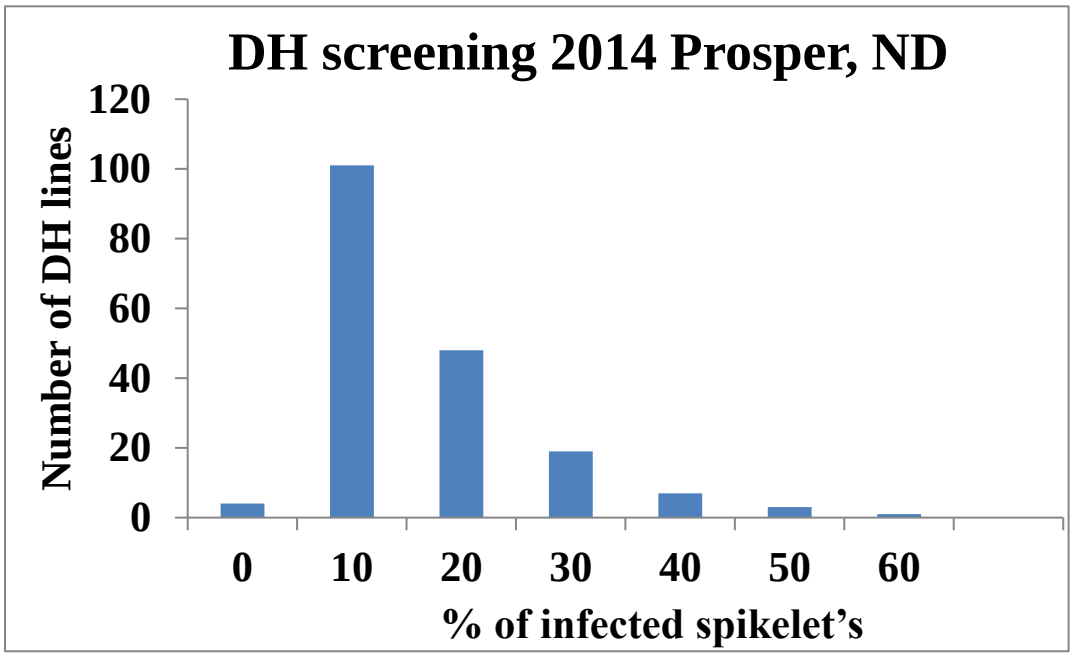

Figure 2: Percentage of disease spikelet's from DH lines with controls (2710 resistant and 2398 Susceptible,)

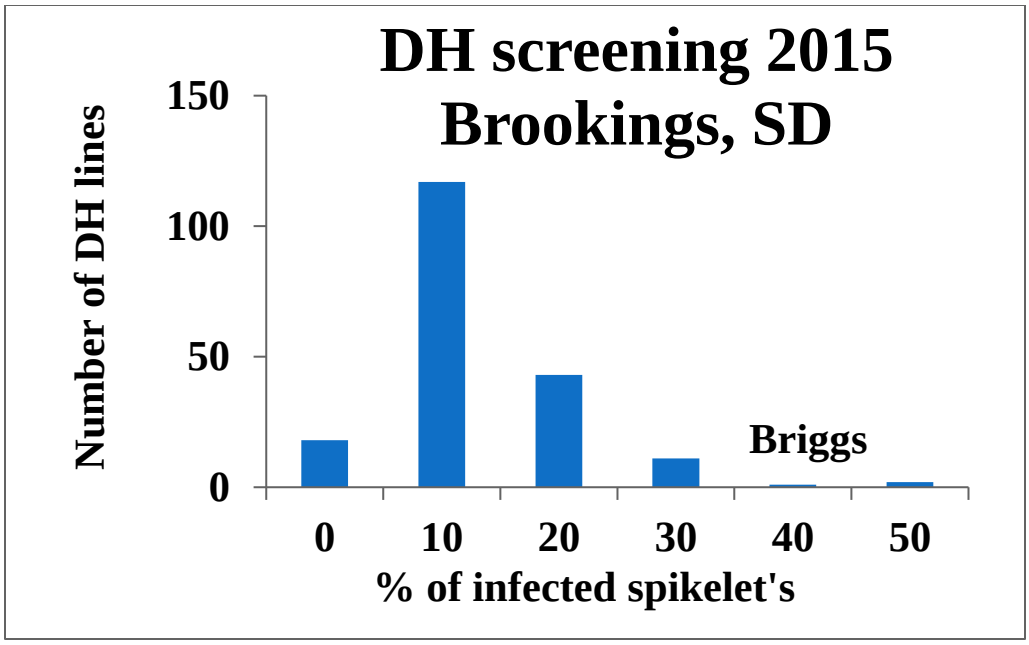

Figure 3: Percentage of disease spikelet's from DH lines with controls (Forefront & Advance resistant and Briggs Susceptible,)

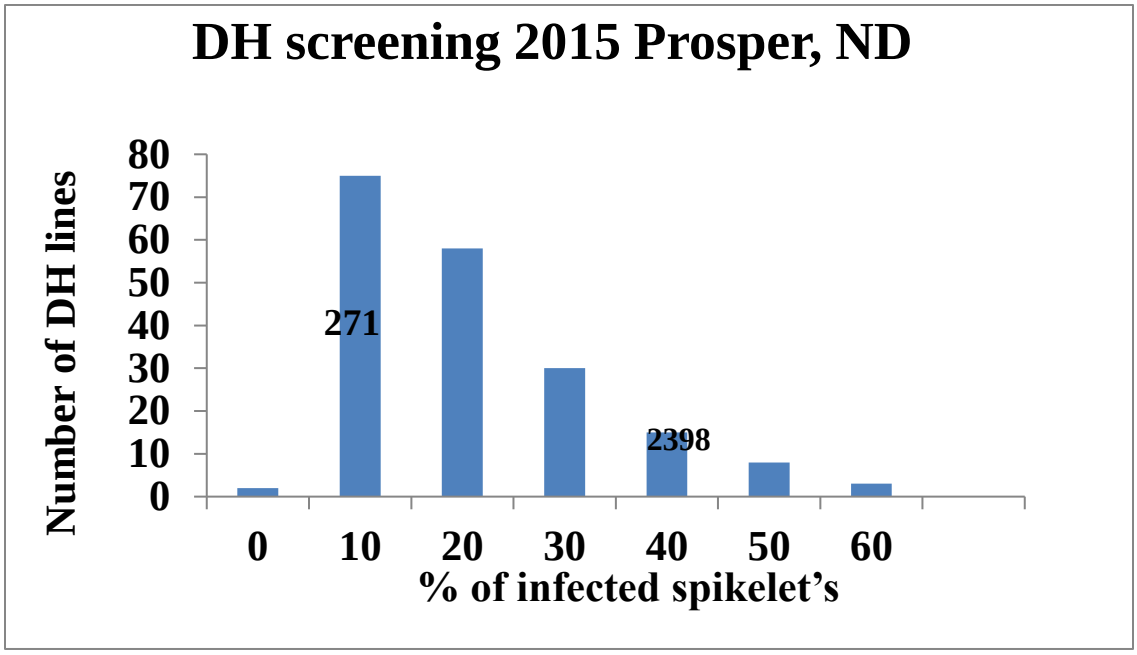

Figure 4: Percentage of disease spikelet's from DH lines with controls

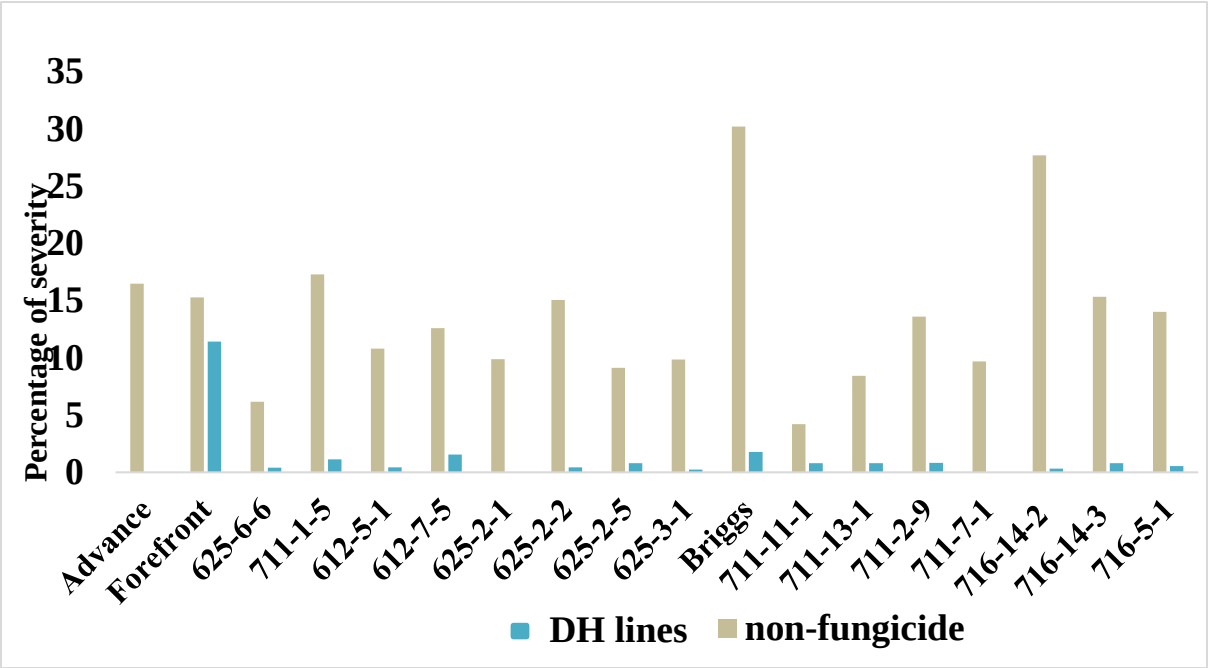

Figure 5: Observation of best 15 lines in respect to FHB reduction from previous experiments with both resistant (Advance and Forefront) and susceptible (Briggs) checks

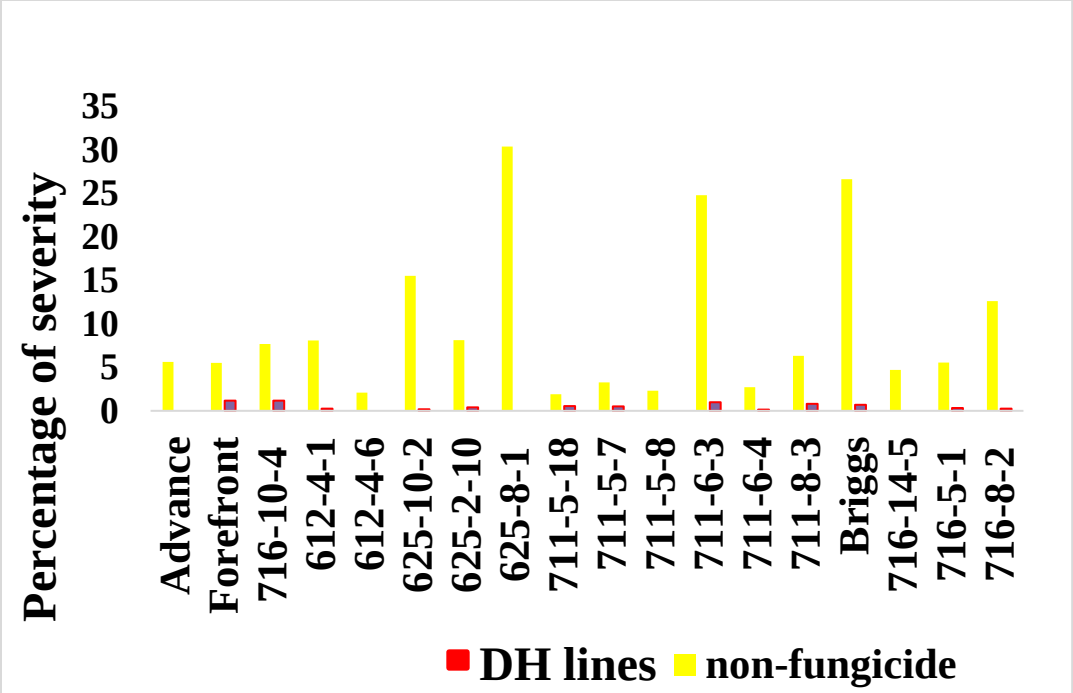

Figure 6: Observation of worst 15 lines in respect to FHB reduction from previous experiments with both resistant (Advance and Forefront) and susceptible (Briggs) checks

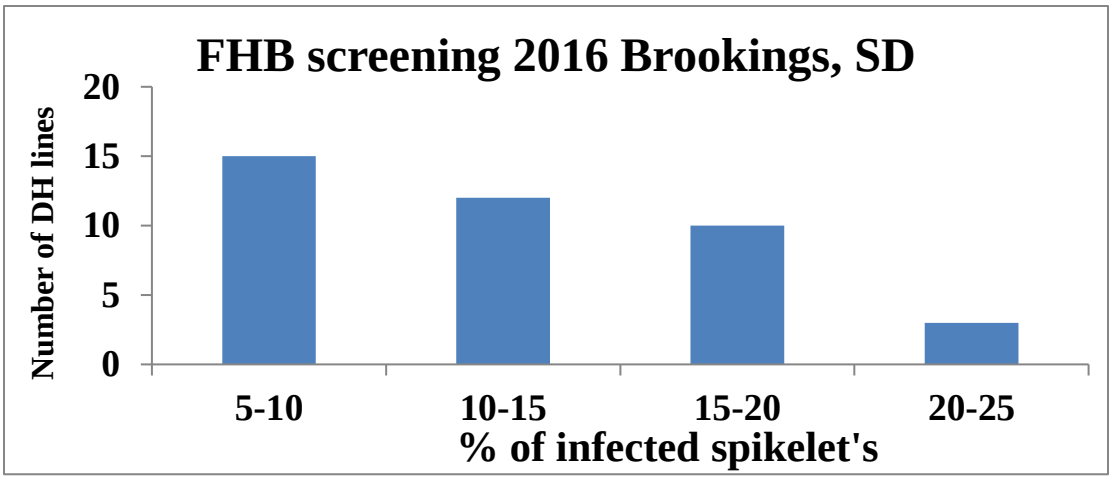

Figure 7: Percentage of disease spikelet's from best 40 DH lines selected for with controls (Forefront, Brick & Advance resistant)

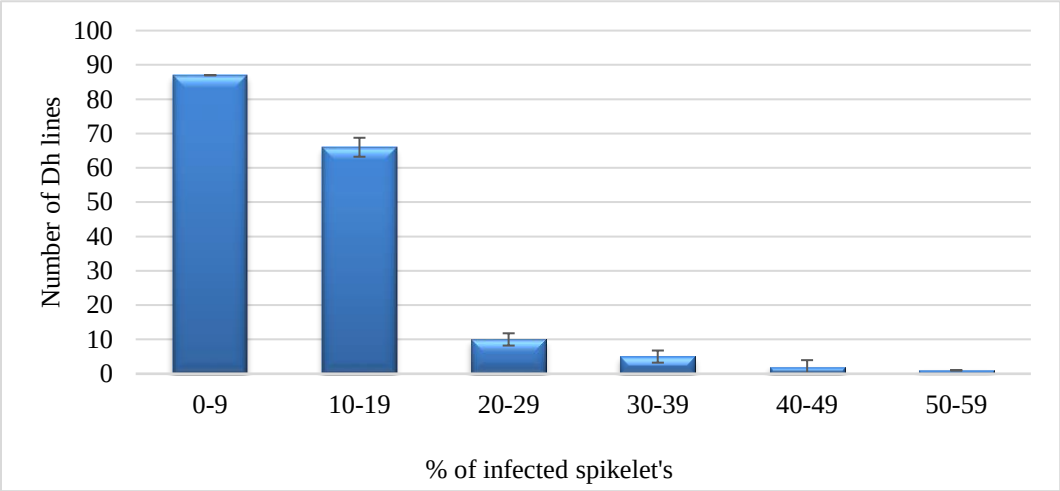

Fig 8: Field screening of DH lines in South Dakota (2015). Percentage of disease spikelet's from winter DH lines with resistant controls (Lyman, Wesley/BC06 & overland severity was 10%) (Susceptible control Wesley was 50%) in field

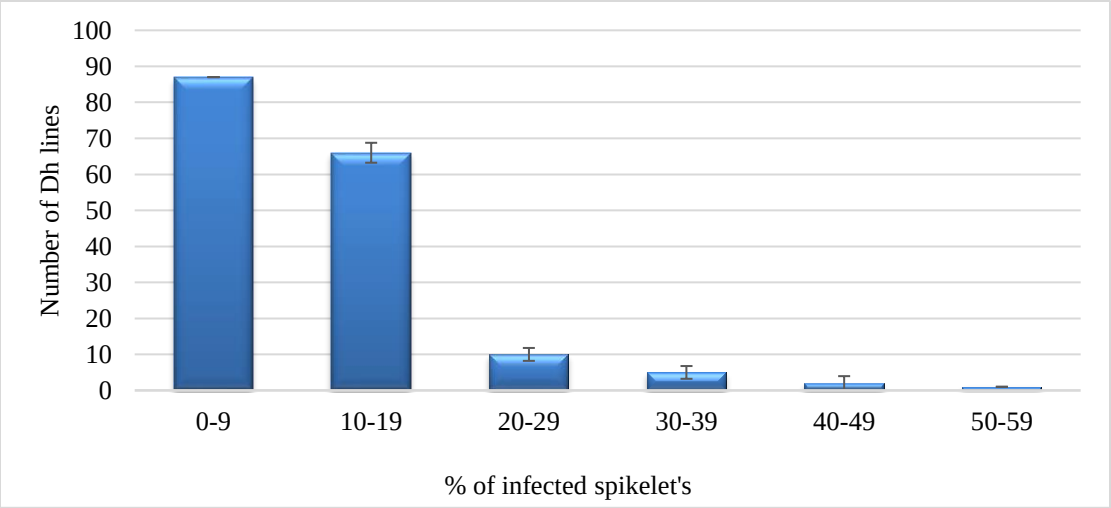

Figure 9: Greenhouse trial one screening of DH lines in South Dakota. Percentage of disease spikelet's from winter DH lines with resistant controls (Lyman, Wesley/BC06 & overland severity was roughly 10%) (Susceptible control Wesley was roughly 50%)

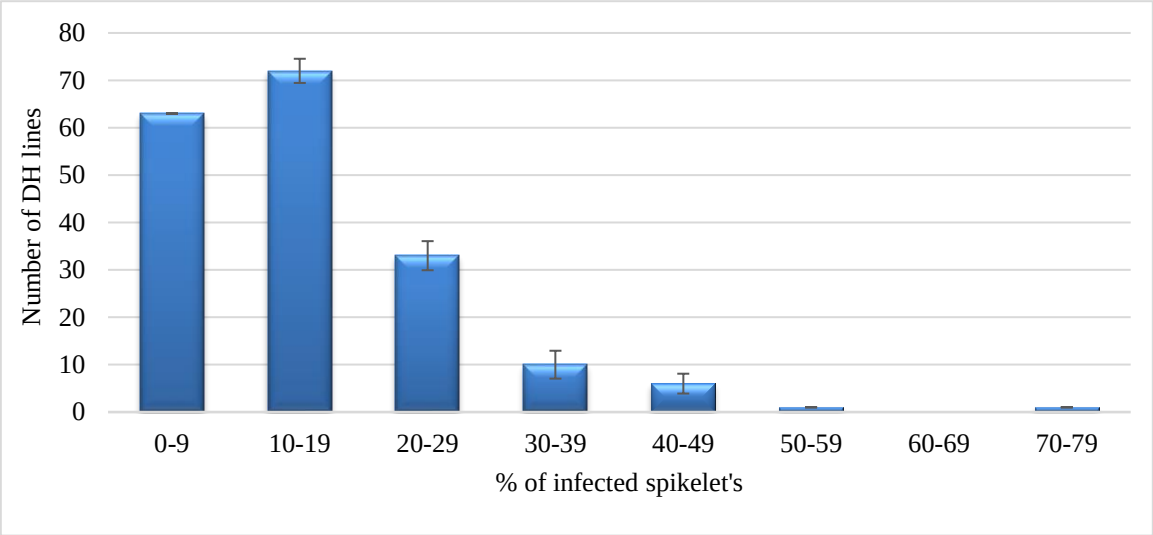

Figure 10: Greenhouse trial two screening of DH lines in South Dakota. Percentage of disease spikelet's from winter DH lines with resistant controls (Lyman, Wesley/BC06 & overland severity was roughly 10%)(Susceptible control Wesley was roughly 50%)

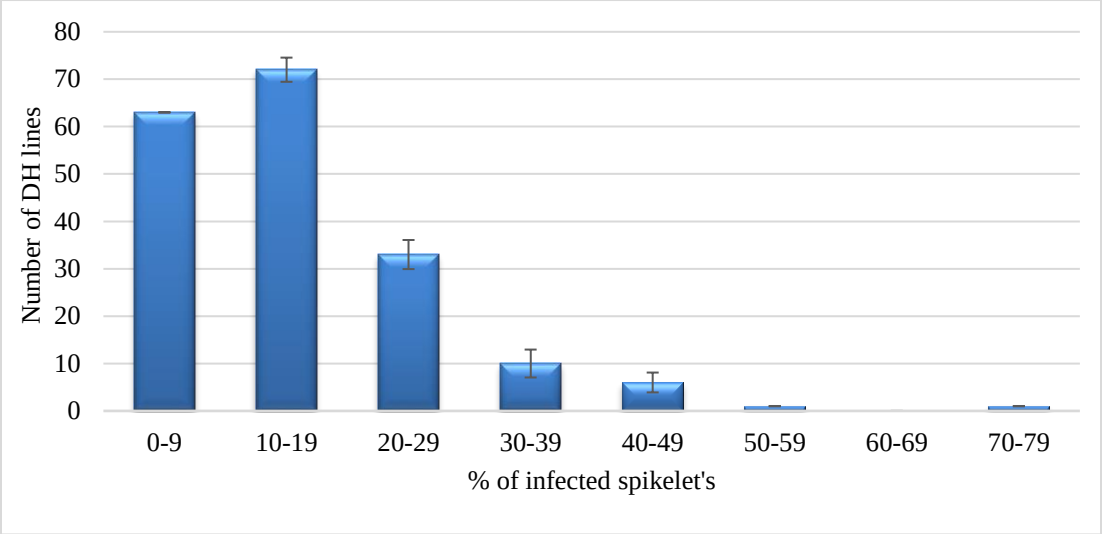

Figure 11: Greenhouse trial three screening of DH lines in South Dakota. Percentage of disease spikelet's from winter DH lines with resistant controls (Lyman, Wesley/BC06 & overland severity was roughly 10%) (Susceptible control Wesley was roughly 50%)

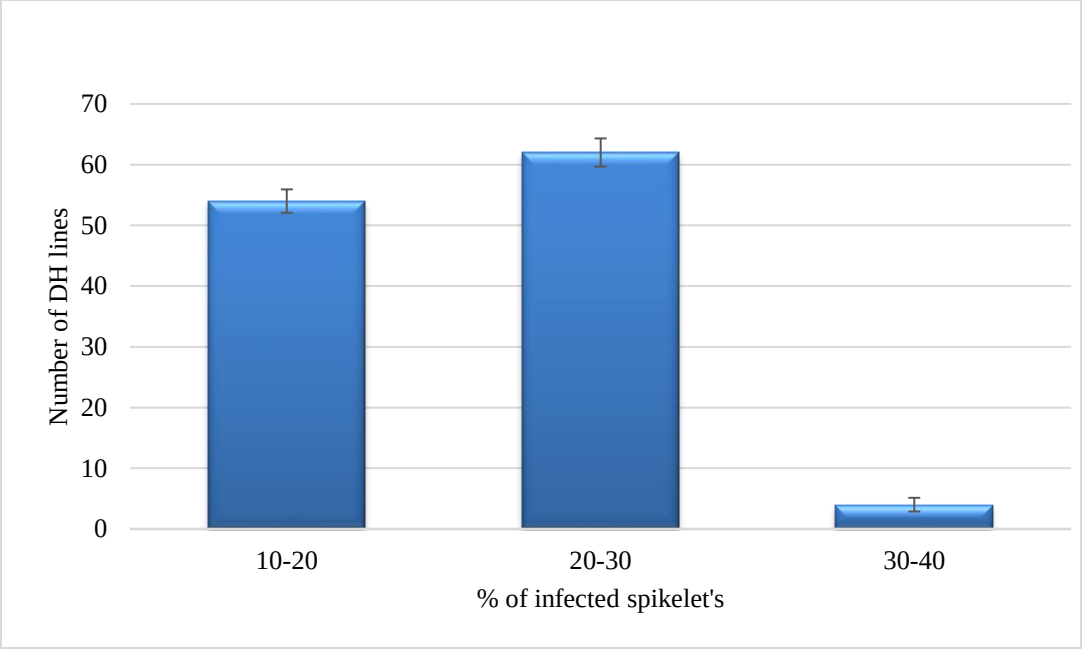

Figure 12: Field screening of DH lines in South Dakota (2016). Percentage of disease spikelet's from winter DH lines with resistant controls (Lyman, Overland& Emerson severity was roughly 10-20%)(Florish & Overly was roughly 20%)(Susceptible control Wesley was 40%) in field

Chapter 3

RELEASE OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED DOUBLE HAPLOID SPRING WHEAT BACKGROUND

1. Abstract

Breeding for resistance to FHB in wheat is important considering the scarce availability of varieties conveying adequate resistance to FHB. However, it has been demonstrated that pyramiding a variety of different resistance QTLs with Fhb1 provides enhanced resistance to FHB. A total of 225 spring wheat lines were initially screened in replicated field evaluation nurseries in 2014 and 2015 in two northern plains locations. Forty lines with low FHB severity were selected as putative resistant materials from the DH population based on FHB severity (evaluation for reaction to fusarium head blight). These lines had high levels of resistance to FHB, which were comparable to commercially used checks based on field nursery screening. The resistance of lines to FHB were further assessed in a screening to test the effectiveness of combining multiple sources of FHB resistance and fungicide.

The fungicide trial results demonstrate that lines were better than resistant check with or without the presence of a fungicide application. In 2016, these forty lines were rescreened for FHB, and of these seven Double Haploid (DH) spring wheat lines (s612-5-1, s625-3-1, s625-6-4, s625-7-2, s711-7-1, s716-11-11, and s716-12-4) were selected. All seven lines were developed for Fusarium Head Blight (FHB) resistance via 4-way crosses combining multiple sources of resistance at South Dakota State University. The characteristics displayed in these lines support recent discoveries of the value of

pyramiding different sources of FHB resistance with Fhb1. These serve as an opportunity to make valuable contributions towards breeding for enhanced FHB resistance, and to further enhanced FHB resistance of adapted wheat germplasm.

2. Introduction

Fusarium head blight, (FHB or scab) which is caused by multiple *Fusarium species (*primarily by *Fusarium graminearum)* is a major concern for cereal production worldwide (Kazan et al., 2012) Areas worldwide where cereals are grown and produced have been mostly affected by epidemics of this disease. Geographical distribution and predominance of a Fusarium species is related to temperature requirements of the species (Okumu et al., 2016). The diversity in virulence *of F. graminearum* in many geographic regions has been reported (Okumu et al., 2016). The high genetic variability of the fungus is an important problem that challenges plant breeders to develop resistant varieties. Wheat is an agriculturally important crop that is recognized as the second most significant crop globally with production exceeding over 500 million annually (http://faostat.fao.org/) (http://www.igc.int/). To meet global food demand by 2020, wheat production should be increased by about 40% (Kolb et al., 2001).

Wheat suffers significant impacts due to FHB. This fungal disease has affected wheat causing significant yield losses due to floret sterility and reduced grain weight, as well as, quality reductions due to the production of mycotoxins. FHB has received much attention due to accumulation of trichothecene deoxynivalenol (DON or vomitoxin) which restricts wheat use for both human and animal consumption (McMullen et al., 1997). Recently, studies show that molecular mapping of FHB resistance, and combining Fhb1 with other resistant sources to developing populations with resistance, could benefit wheat disease

resistance. However, breeding for resistance can be difficult given that resistance to FHB is quantitatively inherited in common wheat (Zhang, 2016). In most cases, obtaining new breeding lines that are homozygous is a common problem due the inability to apply strong selection pressure on multiple genes at the same time, and because selection can be confounded by environmental factors and genetic backgrounds.

The objective of this study, therefore, was to screen for FHB severity using double haploid (DH) spring wheat lines derived from selected four-way crosses combining several sources of resistance to develop wheat germplasm that would have elite resistance.

3. Material and Methods

S612-5-1, s625-3-1, s625-6-4, s625-7-2, s711-7-1, s716-11-11, and s716-12-4 were selected from 225 DH lines screened in South and North Dakota from 2014 to 2016 respectively. These lines were also screened in 2015 for FHB while applying the fungicide Prosaro to test the combining ability of the DH lines and fungicide application. The DH material presented the best opportunity to minimize genetic variation while developing this new germplasm.

The DH lines were developed using F2 seeds derived from a total of 829 four-way F1 plants which were derived from the 43 four-way crosses, with an average of 20 four-way F1 plants per cross (Eckard et al., 2015). Four-way F1 plants were subsequently selfed, and F2 seed was collected in bulk for each plant for pyramiding loci for Fusarium head blight resistance (Eckard et al., 2015). Briefly, 15 experimental lines from the South Dakota State University wheat breeding program, were used for the elite *Fhb1* background in each cross. Founders providing novel sources of resistance were

experimental lines MN99112-10-2-4 (MN93377/MN94350) and MN99125-1-3-7-5 (MN94053/MN2514) from the spring wheat breeding program at University of Minnesota, two recombinant inbred lines RIL 35 and RIL 59 derived from the cross 'Wheaton' (PI 469271) × 'Sapporo Haru Komungi Jugo' (PI 81791), and the Peruvian line MULT 757 (PI 271127.

FHB severity evaluations were conducted in field trials (South and North Dakota) from 2014-2016 respectively. Briefly, evaluations were conducted of the entire DH population in 2014 in both locations. Both locations were used every year for FHB nursery screening. Several evaluations were carried out on the original DH population which allowed for the selection of these seven lines, and phenotypic evaluations were carried out in field nurseries in Minnesota, South and North Dakota breeding program. DH lines along with two SD resistant checks (Advance and Brick resistant), one ND resistant check (2710), and two susceptible cultivars (Forefront SD and 2398 ND), were planted in mid-April as randomized replicated row experiments, where each environment was established with a minimum of 20 seeds.

Inoculum was cultured on ½ PDA using a mixture of 9 *F. graminearum* isolates (Fg1, Fg4, Fg5, Fg6, Fg30, Fg41, Fg56, Fg62, Fg63, Fg64 and Fg70). DH lines were spawn inoculated with infested corn kernels spread on the soil surface about 1 month prior to heading, and heads were mist irrigated beginning at heading at all sites to provide constant disease pressure (Eckard et al., 2015). At 50% anthesis, DH were sprayed and inoculated directly using a conidial spore suspension containing 100,000 spores/mL. DH lines were assessed for FHB infection 21 days after flowering. To determine FHB severity, infections were scored on 20 heads per environment using a 10-point visual

scale where each score corresponds to a percent of the spike infected (Eckard et al., 2015). FHB severity was measured as a binomial trait by counting the number of spikelets with disease symptoms out of the total number of spikelet's on each inoculated spike (Stack et al., 1998).

The 30 lines (with respect to previous FHB screening results) from original DH were screened to test the combined ability of the elite DH lines with a fungicide application. This subset of the entire population was replicated three times and planted in four randomized blocks in the Brookings, SD spring wheat breeding program nursery. Prosaro fungicide was administered one week before plants were inoculated with Fusarium. Inoculum preparation and application and rating was the same as above methods.

A subset of the DH population was evaluated for DON concentration values. The subset included 39 lines that were replicated three times to establish a total of 119 samples. The concentration of DON in grain is measured in parts per millions. Ten-gram grain samples were ground to flour (South Dakota State University) and sent to the Virginia Polytechnic Institute and State University DON Testing Lab for DON analysis using gas chromatography with electron capture detection (GC/ECD).

4. Results and Discussions

The DH spring wheat population (225 lines) was planted so that it could be evaluated for FHB symptoms and severity following inoculations. Using diversity in growing areas, we assessed variation of FHB severity. This approach gave us the best

opportunity to observe the DH population. Overall, the DH population showed lower levels of FHB severity which concurs with the developmental process of the material carried out by Eckard et al. (2015). Our finding combined with Tyler's, support low to moderate FHB severity rating for this material. Disease severity was seen as a range of 0%-60% of spikelets infected 21 days after inoculation in the regular FHB nurseries in both South Dakota and North Dakota. In 2014, in both locations, a total of 86 lines including SD resistant checks, (Overland, Lyman, and BC06) and ND resistant checks (2710) scoring ratings of 0-19%. In the same experiments, 40 lines had lower FHB severity than the commercially resistant check used in this study. The DH population was also assessed in St. Paul MN in 2014. However, the results (scoring scale) were inconclusive. Therefore, that finding wasn't included in this study.

In 2015, the DH population (191 lines) were assessed at the South Dakota and North Dakota nurseries (experimental design was the same as 2014). The overall severity ranged from 0-60% of spikelet infected 21 dai. It was seen that in both locations, 50% of the population had a reduction in severity similar or better than known resistant cultivars 21 dai. As reported in 2014, the same 40 lines were observed as best lines with respect to FHB severity. Severity of the 40 lines ranged from 0-19% of spikelets infected 21 dai. However, severity in 2015 was somewhat higher than rating in 2014 in both locations.

To further assess the DH population, 15 of the best lines, 15 of the worst, and three commercially used checks with respect to FHB severity from previous screening were placed in a scab nursery and tested with a fungicide (Prosaro) application which was applied before inoculation of fusarium. Plants were replicated twice (72 total plants screened) in each testing block (4). The two blocks with the fungicide application had

very low symptoms of spikelets infected 21 dai. In these same blocks, all observed lines showed less than one percent of infection. FHB severity of the two blocks without fungicide was moderate to low. Severity ratings ranged from 0-30% of spikelet infected 21 dai. With regards to the blocks without fungicide, seven lines had FHB severity lower than commercially resistant checks used in this study (Table 2). However, FHB in the overall experiment was very low with or without the presence of the fungicide (Table 2).

Screening of the same seven lines conducted in a regular disease nursery resulted in severity ratings that were similar to findings in the fungicide studied (see fig or table).

In 2016, a subset (40 lines) of the entire DH population including three checks was selected and reevaluated for FHB severity in Brookings, SD. These 40 lines were planted and replicated (3x) in the South Dakota State University spring wheat breeding program nursery. FHB severity (moderate to low) ranged from 8-24% with 37 lines having ratings under 20%. 11 of the lines had FHB severity that was similar or lower than the commercially used check, Brick in this study. 19 lines were better than the resistant check Advance. Seven lines have been identified that can be used as germplasm sources given the result of all FHB screening trials. These seven lines have had some level of variation but remain the best performing lines in comparison to resistant checks (tables 1).

A total of 120 samples (40 lines, three replications) screened in 2016 for FHB were also evaluated for DON concentrations. The DON content ranged from >0.5 to 6 ppm for the entire population screened. 115 samples tested for DON concentration had ppm values that qualify for wheat usage (under 2ppm). Briefly, 48 of the total samples (16 lines) had DON values less than 0.5 ppm, 99 of the total samples (33 lines) had DON

values less than 1 ppm, 108 of the total samples (36lines) had DON values less than 2 ppm. There were nine samples (three lines) between 2-4 ppm and two samples (one line) above 5 ppm. DON analysis showed that 32 total lines were acceptable for the DON advisory level for human consumption of 1ppm (table 3). 7 lines selected had DON concentrations less than 1 ppm (table 3). When compared to the resistant check (Advance), all seven lines had DON concentrations were significantly better (Table 3). Four of the selected lines had DON concentrations that were better than the resistant line, Brick (table 3). Four of the selected lines had DON values less than or equivalent to the susceptible check, Forefront (table 3). All lines tested were contaminated with some level of DON. The seven lines selected had DON conc*e*ntrations that ranged from less than 0.5 to 1 (table2).

Phenotypic analysis revealed that these seven DH lines had severity similar or closely comparable to commercially used resistant cultivars observed in the study. These seven lines can now be investigated as parental sources and elite germplasm given that their severity rating was lower than resistant checks and DON concentrations.

5. Conclusions

Small seed quantities are available from the corresponding author for research purposes, including development and commercialization of new cultivars. It is requested that appropriate recognition be made if these lines contribute to the development of new germplasm, breeding lines, or cultivars.

6. Acknowledgements

The authors would like to thank Mike Trans and Rich Geppert for the field assistance with this project. We acknowledge funding from US Wheat & Barley SCAB

Initiative. We also acknowledge support provided by the South Dakota State University Agriculture Experiment Station.

7. References

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8. Tables and Figures

Table 1. Severity performance of best three DH lines in field trials

Lines	Brookings, SD 2014	Prosper, ND 2014	Brookings, SD 2015	Prosper, ND 2015	Brookings, SD 2016	DON content (ppm)
612-5-1	0.357	1.4	1.07	0.17	11.3	0.61
625-3-1	2.34	0	0	5.76	9.83	0.57
625-6-4	1.29	7.8	0.71	35.7	8.8	0.38 or >.5
ND Resistant check (2710)	n/a	6.15	n/a	16.7	n/a	n/a
ND Susceptible check (2398)	n/a	36.4	n/a	39	n/a	n/a
SD Resistant check (Advance)	0.7	n/a	4.6	n/a	10	1.28
SD Resistant check (Brick)	6.9	n/a	2.5	n/a	11.6	0.53
SD Susceptible check (Forefront)	5	n/a	45.6	n/a	10.22	0.48 or >.5

Table 2. Severity performance of selected (best seven) DH lines with fungicide treatment

Lines	Prosaro (Fungicide)	Non-Fungicide
612-5-1	0.44	10.8
625-3-1	0.24	9.8
625-6-4	n/a	n/a
625-7-2	n/a	n/a
711-7-1	0	9.69
716-11-11	0.8	4.2
716-12-4	n/a	n/a
ND Resistant check (2710)	n/a	n/a
ND Susceptible check (2398)	n/a	n/a
SD Resistant check (Advance)	0	4.6
SD Resistant check (Brick)	1.1	2.5
SD Susceptible check (Forefront)	0.4	45.6

Table 3. Severity and Agronomic performance of seven best DH lines in 2016 field trials

Lines	Severity	DON ppm
612-5-1	11.3	0.61
625-3-1	9.83	0.57
625-6-4	8.8	0.38 or >.5
625-7-2	8.67	0.32 or >.5
711-7-1	15.5	0.43 or >.5
716-11-11	17.3	0.48 or >.5
716-12-4	10.6	0.81
SD Resistant check (Advance)	10	1.28
SD Resistant check (Brick)	11.6	0.53
SD Susceptible check (Forefront)	10.33	0.48 or >.5

Chapter 4

RELEASE OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED DOUBLE HAPLOID WINTER WHEAT BACKGROUND

1. Abstract

Double Haploid winter wheat (Triticum aestivum L.) germplasm lines W651-2-1, W651-6-2, and W452-6-7 were developed at South Dakota State University, Brookings, SD using F2 seed derived from selected four-way crosses combining several sources of FHB resistance. All three lines were developed from parents (founder lines) with moderate to low levels of resistance to FHB. Evaluations were carried out over three years and in multiple environments.

The 3 lines that were selected demonstrated reduction in FHB severity and low DON concentrations, while maintaining a consistent number of heads which associated with good yield potential. The mean disease severity ratings for Fusarium in all evaluations were 0%- 80%, with 75% being less than all susceptible cultivars used in study. These three lines had severity and DON rating that were better or similar to resistant cultivars. We report that our lines have reduction to FHB which builds upon evidence accumulated from multiple studies in which pyramiding multiple sources and components of resistance with *Fhb1* serves to increase resistance to FHB.

2. Introduction

Fusarium head blight (FHB or scab) caused by Fusarium species is one of the most devastating diseases to effect wheat (Triticum aestivum L.) throughout many of the wheat producing regions of the United States, especially in South Dakota. Despite many of the negative impacts caused by FHB (reduced yields, discolored and shriveled kernels and overall seed quality), mycotoxin contamination in the infected grains is considered the most harmful and threatening to the crop and the health of human beings and livestock.

Breeding for resistance to FHB in wheat is considerably difficult, given that traditional breeding approaches require a considerable amount of time and resources to develop new varieties and the availability of varieties conveying adequate resistance to FHB. Therefore, the use of double haploid breeding populations as an alternative to traditional breeding represents a substantial pool (population) of genetic (complete homozygosity) information that can be used to develop new varieties in a shorter amount of time while still focusing on resistance characteristics (of a given cultivar) needed to assist with reducing FHB. Given the complexity of FHB resistance, a better understanding of the controlling components and mechanisms of host resistance remain the most economically and environmentally sound solutions to reduce FHB problems worldwide. Integrated pest management practices in collaboration with developing and growing resistant cultivars are alternative and practical approaches to control this disease as well (Yu et at., 2006).

Resistance to FHB in wheat is quantitatively inherited. The overall resistance of a given variety is the result of the combined effect of several resistance genes. Thus, there is a continuous variation in FHB resistance. Through extensive efforts, FHB resistance

has been identified in Asian and South America spring wheat, and European winter wheats (McCartney et at., 2007). Sumai 3 derived in China, is considered to have partial resistance but is the most widely used for resistance to FHB (Buerstmayr et at., 2003). However, no sources of resistance conferring complete resistance to FHB have yet to be identified in wheat. This is due to FHB resistance being influenced by multiple genes and environmental conditions (polygenic inheritance) (Eckard et at., 2015).

FHB resistance may also be driven by one gene that controls multiple genes (pleiotropy) (Eckard et at., 2015). Resistance to FHB has been shown to be correlated to the many discoveries with QTL mapping and identification. For instance, QTLs composed of one or more genes, such as *Fhb*1 derived from the Chinese wheat cultivar Sumai 3, have been identified in wheat (Van Ginkel et at., 1996). However, these genes confer only partial resistance to FHB, and many of the initial sources of resistance were not well adapted to most of the grain production regions of the United States (Van Ginkel et at., 1996). QTL mapping studies using populations derived from Sumai 3 have identified a number of FHB resistance QTLs, which have been mapped to almost all wheat chromosomes (mostly 3BS, 5AS, 6AS, 6BS, and 3BSc, a QTL region proximal to the centromere on 3BS) (Wilde et at., 2008)(Gervais et al., 2003)(Paillard et al., 2004)(Steiner et at., 2004)(Zhou et at., 2004) (Liu et al., 2009).

Although there have been many discoveries with QTL identification, FHB1 which describes up to 60% of the phenotypic variation in FHB resistance is still the most promising (Liu et al., 2006). Research using the fine-mapped QTL FHB1 has been used to discover alternative sources that could provide effective protection against FHB and

could complement Sumai 3-based resistance but comes at a cost given the gene that underlies *Fhb1* is still unknown (Cuthbert et al., 2007) (Ittu et al., 2000).

Breeding programs in the Midwest United States maintain many collective efforts to develop new wheat varieties and germplasm, improve upon selection methods and integrated pest practices, and identification of genes that can assist with FHB resistance. With no assessed collection of FHB resistant germplasm to date, breeders in our region remain focused on establishing resources that will allow for the establishment of a collection of FHB resistance germplasm. A combination of pyramiding quantitative trait loci (QTLs), and marker-assisted selection (MAS) could serve to be monumental in developing elite germplasms and breeding resistant cultivars (Eckard et al., 2015) (Jiang et at., 2007a). However, there are still many other difficulties given the complexity of genetic resistance and screening large nurseries (phenotyping). To overcome these issues, breeding programs should continue to identify and validate resistant QTLs using different populations. Combining several sources of resistance to FHB is a valid opportunity to develop strong FHB resistance. In South Dakota, moderate levels of FHB resistance in double haploid winter wheat lines derived using this type of approach have been reported.

Doubled haploids are genetically homozygous plants developed through a special cross-breeding and chemical process. This process speeds up traditional inbreeding and provides improved parents for higher performing hybrids. Specifically, the steps for double haploid production are as follows: conduct wheat by maize crosses and induce haploid induction by pollinating wheat with corn pollen, haploid embryo rescue and tissue culture, doubling with colchicine to produce fertile homozygous plantlets, and subsequent seed set. The doubled haploid technique saves at least three to four

generations of self-pollination for the fixation of homozygous pure lines cutting down the time from a range of 10 to 12 years, to 6-7 years to create a new variety (El-Hennawy et al., 2011).

Doubled haploids lead to the direct production of completely homozygous lines from heterozygous plants in a single generation (Hassawi et al., 1911). Double haploids (DH) have many applications in wheat research and breeding due to the production of homozygous wheat which has been shown to be time and labor intensive but are considerably vital. For instance, DH populations are being used to construct genetic maps, assist with new cultivars released worldwide, and to generate reference sequences of plant genomes (Devaux et al., 2016)].

With so many advantages DH is expected to play a significant role in efficient mapping QTL and genes controlling various traits of interest either through biparental populations, genome-wide association study (GWAS) and genomic selection (GS). In the present study, we combined multiple sources of resistance to develop FHB resistant germplasm with good agronomical traits. In addition, we also analyzed the DON levels after several screening trials. We hope that our study will provide potential solutions to improve the efficiency of pyramiding multiple resistance sources (genes), early generation mapping, and, screening and selection of elite FHB resistance wheat breeding

3. Materials and Methods

W651-2-1, W651-6-2, and W452-6-7 were selected after several years of FHB screening from a DH population (120 lines), which eliminates genetic variation that remains within a breeding line through conventional self-fertilization. DH lines were developed using F2 seeds derived from 28 four-way crosses from 565 four-way F1

plants, with an average of 20 four-way F1 plants per cross. Each four-way F1 plant was selfed to derive F2 seed. Crosses were made using 10 winter wheat parental lines to develop 28 segregating four-way F_1 populations. Parental lines included two backcross-derived lines carrying the Fhb1 resistance allele in a 'Wesley' background (Wesley-Fhb1-BC06 and Wesley-Fhb1-BC56) and an experimental line AL-107-6106 (Alsen / NE00403 // NE02583-107) which were used as donors of Fhb1 in each cross. Founders providing native sources of resistance were the HWW varieties 'Lyman' (KS93U134/Arapahoe) and 'Overland' (Millennium 133 sib // Seward / Archer) and the SWW varieties 'Ernie' (Pike / MO9965) and 'Freedom' (GR876 / OH217). The remaining founders, which provided desirable agronomic traits, were NE06545 (KS92-946-B-I5-1 / Alliance), NI08708 (CO980829 / Wesley), and 'McGill' (NE92458 / Ike) (Eckard et al., 2015).

FHB severity evaluations were conducted in greenhouse trials (Brookings, SD) and field trials (South and North Dakota) from 2014-2016 respectively. These nurseries have been used annually by respective breeding programs in each location. Multiple evaluations were completed on the original DH population (225 lines) to obtain these three lines.

Double haploid lines were vernalized for 6-8 weeks and then transplanted as individual plants in 8x8 pots. Each line was replicated three times and organized in a complete randomized design. Briefly, five seeds per line were planted and grown in the greenhouse as per standard conditions for seed increase, collections of leaves for isolating DNA and screening of FHB. As the plants approached anthesis an aggressive *F. graminearum* isolate Fg4, was prepared for FHB inoculations in the greenhouse. The

fungus was cultured on ½ 146 strength PDA (12 g potato dextrose, 15 g agar, 1 L dH20) with 0.2% lactic acid. Conidial pore suspensions containing 50,000 spores/mL were prepared and stored at -20 °C. As the plants approached anthesis, inoculum was prepared for FHB inoculations in the greenhouse. Individual spikes were spray inoculated using a 0.5 liter sprayer when approximately 50% spikelets had extruded anthers (50% anthesis). Approximately 1 mL of the spore suspension was sprayed from top to bottom on two opposite sides of the spikelet. Polyethylene (small zip-lock bags) bags were placed over the spikes for 48 hours after inoculation to provide adequate humidity for infection. The temperature in the greenhouse was maintained between 21-26 °C.

To further access DH population, phenotypic evaluations were carried out in field nurseries in both South and North Dakota breeding programs with assistance of collaborators. DH lines were planted in mid-September in randomized replicated row environments, where each environment was established with a minimum of 20 seeds. Inoculum was cultured on ½ PDA using a mixture of 9 *F. graminearum* isolates (Fg1, Fg4, Fg5, Fg6, Fg30, Fg41, Fg56, Fg62, Fg63, Fg64 and Fg70). DH lines were spawn inoculated with infested corn kernels spread on the soil surface about 1 month prior to heading and heads were mist irrigated beginning at heading at all sites to provide constant disease pressure. Additionally, at 50% anthesis, DH lines were sprayed and inoculated directly using a conidial spore suspension containing 100,000 spores/mL. DH lines were assessed for FHB infection 21 days after flowering. To determine FHB severity, infections were scored on 20 heads per environment using a 10-point visual scale described in Table 1 where each score corresponds to a percent of the spike infected [4].

A subset of the DH populations was also evaluated for DON concentrations values. The subset included 50 lines that were replicated twice to establish 100 samples total. The concentration of DON in grain is measured in parts per millions. Ten-gram grain samples were ground to flour (South Dakota State University) and sent to the North Dakota Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, ND for DON analysis using gas chromatography with electron capture detection (GC/ECD).

4. Results and Discussions

FHB disease severity was moderate to high in the field and greenhouse trials with 88% of spikelet's infected at 21 days after inoculation (dai). Each experiment presented variation with FHB severity ranging from 0% to 80% infected spikelets. FHB severity means ranged from 0%-80% among individual evaluation. Diversity in growing environments provided the best opportunity to assess the DH population.

FHB severity in the 2015 field trial overall ranged from 0-70% with 51 lines including resistant checks (Overland, Lyman, and BC06) scoring ratings of 0-19%. 5 of the DH lines had severity ratings better than the most resistant check Lyman, and 22 lines were better than the worst resistant check Overland. FHB severity in the greenhouse trial saw over 50% of the population having severity reductions similar to, or better than the known resistant cultivars 21 dai. Nearly 90% of the DH population had FHB severity under 20%. 87 of the DH lines had severity ratings under 5% which was better than the most resistant check Lyman and 66 lines were better than the worst resistant check Overland. FHB severity in greenhouse trial two ranged from 0 -60%.

In the greenhouse (GH) trial two, roughly 68% of the population had severity of 10% or less which was similar to the resistant checks. There was a difference of nearly 2% in severity between the best and worst resistant checks. Therefore, 101 lines had severity less than 5% which were better or equally comparable to the resistant checks. FHB severity in greenhouse trial three ranged from 0 -70%.

In greenhouse trial three, 63 lines of the population had severity of 10% or less which was similar to the resistant checks. From the above results, we identified 11 lines that were in the top 10 percentile in every evaluation. In 2016, we evaluated 120 of the best lines based on the severity ratings from our previous studies. Screening in the Brookings, SD field nursery (2016) severity was higher than previous observations but 45% of the lines had 10% severity. FHB severity ranged from 10 -40 % with 11 lines having lower severity rating than the best resistant check Emerson. 62 lines had lower severity rating with respect to worst resistant check Overly. Stripe rust evaluations of 120 lines in Brookings, SD in the 2015-2016 growing season were done using same 120 lines in South Dakota (2016). Stripe rust rating ranged from 0% to 90% infection. 11 lines showed MR to R responses to stripe rust.

FHB severity in the Prosper, ND (2016) field trial ranged from 8-65% with one line showing better resistance than the best resistant check. However, severity was higher in ND but 31 lines still had severity under 20% which was better than the worst resistant check BC06. Meanwhile, the susceptible check Wesley had a severity of ~22% which was lower than the resistant check BC6. Phenotypic greenhouse analysis shows that the DH lines had severity that was comparable to commercially used resistant cultivars observed in the study. Phenotypic analysis of severity also revealed that a large percent of

the DH population had undesirable severity ratings, leading us to conclude that these lines may have limited use in FHB resistant source development. The remaining lines have potential to be investigated as parental sources elite germplasm given that their severity rating was lower than the resistant checks. These lines with the lowest severity if further evaluated can be developed into potential new varieties. Three resistant DH lines were selected from both greenhouse and field trial evaluations of FHB. The severity ratings of all three lines were low, ranging from 0-25% in the greenhouse and 0-20% in the fields, whereas resistant checks had severity of 35% in greenhouse and 37% in the field. (Table 2 and 3). All 100 samples tested for DON concentration had ppm values that qualify for wheat usage. Briefly, a total of 62 samples (31 lines) had concentration under the acceptable DON advisory level for human consumption of 1ppm (Table 3). The remaining 19 samples (19 lines) had higher concentrations but none exceeded 3.5ppm. A portion of the samples (9 lines) had DON concentrations below 0.5 ppm. When compared to resistant checks (samples) Emerson (0.6 ppm) and Overland (0.85 ppm), a total of 34 samples (17 lines) had DON concentrations that were of equal or lower value. Only four samples (2 lines) were equivalent to or above the susceptible check Wesley (2.9 ppm).

5. Conclusions

This is the first release of our DH winter wheat lines. These lines are expected to be useful as parental lines to improve resistance to FHB and resources that serves as evidence of successfully pyramiding multiple sources of FHB resistance. Small seed quantities are available from the corresponding author for research purposes, including development and commercialization of new cultivars. It is requested that appropriate

recognition be made if these lines contribute to the development of new germplasm, breeding line or cultivar.

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8. Tables and Figures

Table 1: Summary of phenotypic evaluations setup of DH plants for FHB resistance

Evaluation	# of entries	Inoculation method	Biological replicated	Spikes evaluated per replicates
SD field 2015	84	Spawn/spray	1	20
GH trial 1	171	Spray	2	1-10
GH trial 2	156	Spray	2	1-10
GH trial 3	186	Spray	2	1-10
SD field 2016	120	Spawn/spray	3	20
ND field 2016	120	Spawn/spray	2	20

Table 2. Severity performance of best three DH lines in field and greenhouse trials

Lines	Volga, Field 2015	GH 1	GH 2	GH 3	Volga, SD Field 2016	Fargo, ND Field 2016
651-6-2	**3.2**	**4.95**	**0**	**8.5**	**24.4**	**12**
651-2-1	**0**	**0**	**0**	**8.9**	**14**	**34**
452-6-7	**0**	**0**	**0**	**6.3**	**19**	**14.4**
Resistant check (Overland)	**0**	**3.3**	**3.7**	**6.6**	**16.1**	**10.8**
Susceptible check (Wesley)	**28.7**	**36.1**	**50**	**55.5**	**29.8**	**22**

Table 3. Severity and Agronomic performance of three best DH lines in 2016 field trials

Lines	**Severity**	**Stripe rust**	**DON ppm**
651-6-2	**24.4**	**R**	**1.7**
651-2-1	**14**	**MR to R**	**>0.5**
452-6-7	**19**	**MR to R**	**>0.5**
Resistant check (Overland)	**10**	**N/A**	**.85**
Susceptible check (Wesley)	**22**	**N/A**	**2.9**

Table 4. Severity and Stripe Rust performance of DH lines overall evaluations

	Field 2015 severity	GH Trial 1 severity	GH Trial 2 severity	GH Trial 3 severity	Volga, SD 2016 severity	Prosper, ND 2016 severity	Stripe Rust in 2016
651-6-2	3.2	4.95	0	8.5	24.4	12	0 R
651-1-2	0	0	0	4.1	12.5	19.5	S
651-3-11	0	2.3	0	6.8	16.5	20.5	S
651-2-1	0	0	0	8.9	14	34	MR to R
452-1-7	4.1	0	10	11.3	14.2	11.3	S
452-4-9	3.5	n/a	9.5	18.1	17.3	10	S
452-1-10	0	n/a	0	12.6	15.5	29	S
452-4-10	0	n/a	n/a	0	14.2	24	S
651-3-1	10.5	2.5	2.0	4.4	16	23.5	S
452-6-7	0	0	0	6.3	19	14.4	MR TO R
Overland	5.3	3.3	0	4.15	16.1	10.8	30 S
Wesley	28.7	33.5	43.7	50.3	33.5	22	MS 40
BC06	2.5	4.1	12.5	8.3	n/a	33.7	n/a
Lyman	5.3	10.4	2.75	2.8	13.6	9.14	60 S
Freedom	n/a	n/a	n/a	n/a	n/a	31.9	n/a
Emerson	n/a	n/a	n/a	n/a	16.8	n/a	10 MR to MS
Flourish	n/a	n/a	n/a	n/a	23	n/a	n/a
Overley	n/a	n/a	n/a	n/a	27.5	n/a	n/a

Chapter 5

BENEFICIAL PLANT-MICROBE INTERACTIONS: RESPONSE OF WHEAT TO NATURAL COMMUNITIES OF ARBUSCULAR MYCORRHIZAL FUNGI

1 Abstract

Arbuscular mycorrhizal fungi form a mutualistic symbiosis with majority of land plants their role in improving plant growth is well-established. Arbuscular mycorrhizal fungi are ubiquitous in soils, allowing them to assist plants with the uptake of nutrients. Although the benefits from this interaction are enormous, the ecology of these fungi and usage as a potential alternative to fertilizer is not fully understood. To better understand this symbiosis, various agricultural and microbiology practices should be investigated qualitatively and quantitatively.

Previously conducted greenhouse experiments showed that mycorrhizal colonization among genotypes was varied. It has also been suggested that nutrient efficiency in wheat can be a direct response of AMF colonization. These differences in mycorrhizal responsiveness and nutrient efficiency could also suggest that there is genetic control of these genotypic differences. However, only a few studies have been conducted on arbuscular mycorrhizal communities in agricultural systems. Therefore, we conducted a field study to investigate the contribution of arbuscular mycorrhizal fungi

(AMF) to nutrient uptake and biomass yields of eight spring wheat (*Triticum aestivum* L.) genotypes. We used a split block design (with complete randomized environments) setup to develop three natural field growing environments. Each treatment environment represented a different observation to fully determine the contribution of arbuscular mycorrhizal fungi. The first treatment environment was treated with the fungicide Topsin M to suppress the colonization of the plants with naturally occurring arbuscular mycorrhizal communities; the second treatment environment was treated with commercially available inoculum containing arbuscular mycorrhizal fungi, and the third treatment environment was the natural community (control

Our results showed that all wheat genotypes that were tested had some level of mycorrhizal colonization. The inoculum treated environment and the natural environment became highly mycorrhizal with colonization rates that exceeded 60%. We observed that phosphate levels where highest with the presence of AMF and the fungicide application reduced arbuscular mycorrhizal fungi. Through many agronomical trait observations and statistical analysis, we can conclude that differences were seen amongst these genotypes with the presence and or absence of arbuscular mycorrhizal fungi. Our findings will assist ongoing efforts aimed to understand the potential of mycorrhizal fungi on growth, yield, and nutrient uptake in wheat.

2. Introduction

Despite progress in food production and security in the past century, it is estimated that in the coming years, food production will need to be increased over 50% while stabilizing yield irrespective of environmental constraints, with good quality crops

and a high nutritional value. A critical aim in resolving food production will be the development of crops that are resistant to abiotic and biotic stresses. There are many agriculturally important crops that may assist in solving some of the food requirements in the world. However, given its importance, wheat by far serves as the most meaningful, economically and practical food crop that could play a major role in food security.

In the US, wheat (*Triticum aestivum* L), ranks second among grains cultivated following corn, and third amongst U.S. field crops in both planted acreage and gross farm receipts (Shewry 2009*).* Wheat is cultivated for grain and contributes to the production of flour, pasta, breads, alcoholic beverages, beer, and industrial alcohol made into synthetic rubber and explosive.

The availability and uptake of nutrients is very important in crop production. Wheat depends on many essential nutrients to survive. Natural fertility of cropped agricultural soils is declining making it harder for crops to reach maximum yield potential. Ultimately, nutrients removed will need to be replaced at some point to sustain production. Nutrients like nitrogen and phosphorus are two major macronutrients that influence the overall development of wheat. In wheat, nitrogen is considered the most important given that it is the primary constituent of protein. An adequate soil nitrogen supply is essential for obtaining desirable yields and producing wheat with a high protein content (http://www1.agric.gov.ab.ca/%24department/deptdocs.nsf/all/crop1273). However, other nutrients like phosphorus play a vital role in wheat production as well.

Phosphorus is critical in the early development process of wheat. Early in the growing season, the wheat plant is dependent on uptake of sufficient phosphorus in order to establish tillers and ensure strong root growth. Phosphorus plays a critical in the

metabolism of plants, playing a role in cellular energy transfer, respiration, and photosynthesis (Glass et al., 1980). Nutrient deficiencies combined with adverse effects of many abiotic and biotic stresses are some of the factors limiting the development and production of wheat. As a result, a stagnation in wheat productivity has been seen due to these constant problems.

AMF are obligate symbionts, belonging to the phylum Glomeromycota, which are a key link between plants and soil minerals and nutrients (Fellbaum et al., 2014). AMF are primary biotic soil components that provide the host plant with minerals, nutrients and water, in exchange for photosynthetic products (Balestrini et al., 2015). Specifically, the mutualistic symbioses increase the plant uptake of P, N, S, K, Ca, Fe, Cu and Zn that are absorbed and translocated by the extraradical mycelial network spreading from colonized roots through the bulk soil (Kernaghan et al., 2017).

The most important benefits of this interaction are the host plants' increased resistance to biotic and abiotic stresses, and increased soil quality by enhancing soil aggregation and improving structure (Pellegrino et al., 2015). This process occurs by the growth of AMF mycelium (fungal hyphae) which are much thinner than roots, and therefore, are able to penetrate and acquire nutrients from soil inaccessible to roots (Finlay 2008). Furthermore, the functional site where nutrients are exchanged is in the root cortex where highly branched structures called arbuscules are formed (Balestrini et al., 2015).

Due to the beneficial plant-microbial relationship, use of AMF could be a natural and cost-efficient approach of enhancing the production of many agriculturally important crops like wheat. So far, AMF-wheat studies are scarce, given the high variability of

plant responses. However, application of AMF as a sustainable management approach is a promising direction to incorporate AMF considering the impact of the interaction. Therefore, in the present study we conducted a field trial aiming to understand the responsiveness of eight wheat genotypes (sample of genotypes) to different communities of AMF. We observed AMF colonization, plant height, shoot biomass, grain quantity and phosphate levels.

3. Materials and Methods

3.1 Plant materials

Eight South Dakota State University spring wheat genotypes (Advance, Brick, Briggs, Forefront, Granger, Grenora, Lebstock, and Oxen) were planted in Brookings, SD on 5/19/14 for the purpose of observing any genetic differences with respect to the interaction with AMF. These eight genotypes were selected randomly as a sample population to examine the responses of wheat to arbuscular mycorrhizal fungi.

3.2 Field setup

The field trial was setup as a split plot design with factorial arrangements with mycorrhizal treatment as the main plot and 8 wheat genotypes as subplots. The field design consisted of three treatments with 32 experimental units per treatment, which had specific application requirements. Briefly, we used the fungicide Topsin M application from Keystone Pest Solutions to suppress mycorrhizal colonization. (http://www.cdms.net/ldat/ld4EK000.pdf). We applied the first fungicide treatment on

6/10/14 and subsequently applied fungicide every two weeks, for a total of three applications. Fungicide was applied using a 5-gallon tank sprayer for 10 seconds equally over the entire row.

In our study, we added a mycorrhizal additive so that we had a clear knowledge of mycorrhizal species. The mycorrhizal additive was purchased from Millborn Seeds and is called MycoApply Ultrafine Endo which is a mycorrhizal inoculum powder consisting of four scientifically formulated species of endomycorrhizal fungi propagules (*Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, and *Glomus etunicatum*) (70 prop/g each). MycoApply is a wettable powder that has equal amounts of particle size < 300 microns, with each species accounting for 25% of the additive (http://www.millbornseeds.com/technical_specs/mycorrhizae/Endo.pdf). We applied the additive 6/18 /14 and another application roughly two weeks later. Using a 5-gallon tank sprayer we applied the mycorrhizal additive directly in the growing area (as close to the roots as possible) until the soil surrounding each row of plants was moist.

Given the consistent use of this field area, we assume that natural communities of mycorrhizal were present. Therefore, this area of the experiment was used as the natural (non-treated) control in the experiment.

3.3 Experimental Design and Statistical evaluations

The eight genotypes were grown in single rows in a factorial design, in a split plot arrangement with sampling. Factors included in the experiment were genotypes and treatments. Factor genotype was considered as random effects and treatments were fixed.

20 seeds per genotype were replicated four times and planted in each block. One week after the second application of the fungicide, and additive was applied, we randomly harvested three plants from each block to observe root colonization. Root colonization was studied by carefully rinsing roots with distilled water, cleared by 10% KOH, 30-45 min at 90°C and acidified in 1% HCl for 5-10 min. The roots were then stained using Trypan Blue (0.05% in lacto-glycerol) for 12 hours. Roots were again carefully cleaned and stored (Koskeet al., 1989). For quantification of AMF colonization, using a microscope under microscope (80×), we evaluated 50 (1cm) random selected sections to determine colonization percentage.

To determine the growth responses, we measured plant height, number of heads and shoot biomass. Plant heights were observed using a yard stick to measure an individual plant in the center of each row. Plant heights were taken two weeks prior to harvest. Each row was harvested individually in the first week of August 2015. Post-harvest, we weighed the total shoot biomass of each row with and without the heads. We then cut the head off and counted the total number of seeds.

While harvesting, we collected and cleaned root samples from each row. Following the same protocol described above, roots were stained and colonization percentages were obtained. We also collected and stored (stored in -80○ using liquid nitrogen) a portion of each plant's shoot for phosphate analysis. Briefly, this portion of samples were dried in an oven for several hours, ground and then sample weights were taken. We then added 2N (25ul) HCI to each sample followed by incubation for two hours at 95 degrees (Olsen et al., 1954). After incubation, samples were placed on vortex to spin down samples. 25ul of sample solutions combined 450 ul of water was mixed

together. Finally, we add 500 ul of AMV solution to sample solution and measure the absorbance in the spectrophotometer. An analysis of variance (ANOVA) was calculated using RStudio to determine any significant difference among and between genotypes and treatments in respect to each of the observe phenotypic characteristics (http://www.rstudio.com/).

4. Results

4.1 Root colonization

Preliminary colonization of roots was assessed in all three observations (Fig. 1). AMF colonization was well established in additive and natural observation areas. In comparison to the above areas, the fungicide treated area which the application of Topsin M fungicide was applied had decreased colonization.

After harvest, root colonization occurred in the entire experiment (Fig. 2). Percentage of colonization ranged from 30 to 80% throughout the entire experiment. Results of root colonization concurred with preliminary assessment of root colonization to determine the presence of mycorrhizal. Results of root colonization provided evidence of the fungicide suppressing mycorrhizal colonization, additive presence and natural community's existence in field area.

The natural environment (environment three) had the highest colonization percentage (79.8 %), environment two followed with colonization percentage of (79.1 %), and environment one had worst root colonization percentage of (33.1%). In environment three, Grenora was the most colonized genotype and Advance was the least colonized (fig. 3). In environment two, Forefront was the most colonized genotype and

Briggs was the least colonized (fig. 3). In environment one, Advance was the most colonized genotype and Brick was the least colonized (fig. 3).

4.2 Plant Growth/ Agronomical traits evaluations

Plant height varied throughout the experiment (fig. 4). Overall, plant heights ranged from 27 to 50 inches (Table 1). The plants with highest plant heights occurred in environment three and the plants with lowest heights were in environment two. In environment one, genotype Briggs (37.5 in) had the highest plant height and genotype Oxen (29 in) lowest plant height (Fig. 4). In environment two, genotype Lebsock (36.5 in) had the highest plant height and genotype Oxen (29.75 in) lowest plant height (fig. 4). In environment three, genotype Lebsock (39.25 in) had the highest plant height and genotype Briggs (39 in) lowest plant height (fig. 4). Analysis of variance proved that there were no significant differences in respect to plant height (Table 1).

Shoot biomass overall ranged from 70 to 340 grams and was highest in fungicide treated environment and lowest in additive treated environment. However, the natural environment shoot biomasses were very similar to both the other environments. Genotype Advance had the highest shoot biomass in any of the environments (fig. 5) and genotype Brick had the lowest shoot biomasses (fig. 5). Analysis of variance proved that there were significant differences in respect to shoot biomass (table 2).

Grain yield (number of heads) on average ranged from 90 to 200 heads per row throughout the experiment (Fig 6). Specifically, fungicide treated environment had head count averages that ranged from 114-234 heads per row (per genotype). Additive-treated environment head count averages was 103-207 heads per row (per genotype) and the

natural environment head count averages was 117-272 heads per row (per genotype). Fungicide-treated environment had the highest head count in the experiment and additive treated environment had the lowest. Advance had the largest number of heads collected from either of the environments and Granger had the least number of heads collected (fig 6). Analysis of variance proved that there were significant differences in respect to head count (table 3).

4.3 Nutrient uptake analysis

Each genotype assessed in this experiment was observed to have some level of phosphate concentration and or content (fig 7 & 8). Overall, phosphate was highest in fungicide treated environment, with respect to both phosphorus concentration and phosphorus content and was lowest in the natural environment for both shoot concentration and shoot content (fig 7 & 8). Phosphate concentration ranged from 2.4 to 35 parts (p) per and shoot content 1500000.00 to 9600000.00 mg for the entire experiment. Briefly, shoot concentrations averages ranged from 3.5 to 10 parts (p) per in fungicide treatment, 4.1 to 11 additive treatment and 3.5 to 10 in the natural environment (table 4). Phosphate content averages ranged from 5700000.00 to 1800000.00 mg in fungicide treated environment, 490000.00 to 1300000.00 mg additive treated environment and 418000.00 to 1200000.00 mg in the natural environment (table 2).

4.4 Summary of results

Overall results support the assumption that there are genetic differences amongst these genotypes with respect to the interaction and presence of mycorrhizal. Results obtained showed that each environment in which these genotypes were assessed had

some level of different responsiveness to the presence of mycorrhizal (table 2). Common trend in this experiment was that the fungicide may play a role in responsiveness.

5. Discussion

5.1 The presence of Arbuscular mycorrhizal fungi

The preliminary assessment of root colonization was successfully carried out roughly when plants had four leaves (one week after the second application of the fungicide and additive was applied) to determine the presence of AMF, to observe the impact of the additive on colonization and to determine how much the fungicide suppressed AMF. By randomly selecting a few plants from each environment, we obtained unbiased observation of different genotypes, in different areas of each environment, which ultimately provides a strong prediction of the entire populations' root colonization.

Our selection method to assess preliminary root colonization was limited due to availability of space to plant more replicates (which would have allowed us to plant a replicate for the purpose of preliminary root colonization assessment) and the ability to sacrifice plants (which takes away from further assessments). Given more resources, a better design considering preliminary assessments would have allowed us to carry out root colonization assessment on a plant to plant bases.

The results as indicated in Figures 1 & 2 confirm that root colonization in this study was well established and achieved our goals of creating diverse environments to better understand the interaction of AMF and wheat. The same figures support the results showing suppression of root colonization by Topsin m (fungicide). However, the

mycorrhizal additive inoculation didn't increase the level of colonization in the roots. It was surprising, but not unexpected to observe that colonization was similar between environments two and three given the addition of the MYC additive in environment two. However, we did not have prior knowledge of presence of AMF in these environments. Therefore, the overall presence of mycorrhiza in these environments supports the idea that AMF forms a mutualistic symbiosis with 80% of all land plants (Behie et al., 2013).

Results from Figure 3 on the other hand provides a genotype by genotype breakdown of root colonization. These results suggest that each genotype interacted differently with AMF when colonizing root systems. However, given the complexity of the relationship, resources and information, such as generated in this research are needed to further understand what is truly happening as roots are colonized by AMF on a genotype by genotype interaction. In a previously conducted greenhouse study where these same genotypes were assessed for root colonization, we observed similar differences amongst these genotypes with respect to root colonization. Although, we observed well colonized roots, a greenhouse study is limited and does not fully contribute to understanding the interaction of AMF and wheat given that: there is no competition in the soil, many of the abiotic and biotic stresses are controlled, and we specifically control the availability of nutrients and water. Therefore, these results combined with our current findings from the field are useful resources that explain the genetic differences amongst these genotypes in regard to root colonization.

5.2 Plant Growth/ Agronomical traits evaluations

The agronomical traits data analysis was based on three field environments with various levels of AMF, and random selection and location of sampling.

Plant height is an intrinsic component use in breeding programs to develop new varieties of wheat. Considering the impact of dwarf wheat in the past century, plant height has played an important role in yield components given that plant architecture affects lodging stability, harvest index and yield (Curtis et al., 2014). In our experiment, plant height was calculated and analyzed to assess the impact of AMF having an influence on plant height. By measuring the middle row within the experimental rows, we were able to sample the best possible plants given that the plants located near the border/edge of each row had potential to be affected by surrounding projects, physical damage due to the frequency of contact with people, and chemical run off. Plants that neighboring other projects could also have to face more stress and competition in soil.

In a future experiment, it would be beneficial if all 20 plants for each row were assessed to get a range of plant height for each genotype. Nevertheless, our sampling from each row serves as a representation of each genotype given. We had four replicates in each environment in order to give us a thorough observation of each genotype throughout the entire experiment. Results from Figure 4 showed that each genotype responded differently in the diverse environments. However, our results suggested that mycorrhiza had no true effect on plant height but either the fungicide or the reduce mycorrhizal colonization allowed the plants to grow as good as or better than environments with best colonization. This research has produced a vast amount of resources that support the understanding of shoot characteristics such as plant height and

number of tillers that influence grain yield. However, the understanding of the relationship of shoot biomass and grain yield is scarce in wheat. Therefore, shoot biomass was the most important agronomical trait investigated in this studied given the potential shoots (tillers) have on increasing grain yield. Shoot biomass was greatest in fungicide treated environment in this experiment.

Several reasons could explain the phenomenon. First, environment one was treated with Topsin M fungicide to suppress mycorrhizal colonization. This is a well-known fungicide used in agricultural studies to assist against many biotic stresses (Wilson et at,. 2008). The presence of this fungicide may have allowed the plant to focus more resources on plant growth in environment one, while plants from other environments (2 & 3), were forced to defend and grow simultaneously limiting resources needed to produce greater shoot biomass. Secondly, the presence of mycorrhiza could have some effect on how the plant produces tillers given environment one had the least colonized roots but had the greatest shoot biomass. However, there is no evidence that clearly explains the role of mycorrhiza on tiller production in wheat. Therefore, continuous efforts need to be made to understand the interaction of mycorrhiza and wheat plant growth.

Finally, differences were found among the genotypes for shoot biomass in all observed environments. These findings can assist our efforts to produce resources that can potentially explain the genetic responsiveness of wheat to mycorrhiza. Like the above limitations, shoot biomass observations were constricted (by time and resources) and should be investigated on a more plant to plant basis under the experimental and

environmental conditions as this study. The availability of nutrients is also an important process that may have affect shoot biomass given that nitrogen and phosphorus when combined with other factors can create stresses that reduce the initiation of tillers.

Grain yield potential (number of heads) in this study gave us an opportunity to observe one of the factors that contribute to yield potential. Like many of our other results, each genotype had different numbers of total heads in respective environments (Fig 6). Many factors like variety, emergence time, tiller population density, fertility, foliar disease, insect infestations, weed control and moisture availability may have affected the total number of heads in this experiment. Physical and chemical damages are factors that also could have affected these results. Additionally, other agronomical traits such head weights and seed weight (1000 kernel weight) are important. These results weren't included in this study, because the experiment was designed to measure any differences amongst genotypes with respect to the presence of mycorrhizal, and not designed to obtain results that would predict yield potential. In the future, it would benefit to conduct a similar project to utilize all the observed agronomical traits for a better understanding of the influence mycorrhizal could have on yield potential.

5.3 Nutrient uptake analysis

Nutrient uptake is a very important process used to help farmers and producers determine optimal timing of fertilizer application. If we better understand the patterns of nutrient uptake, crops like wheat can properly develop and mature to ensure and assist with the growing food demands. In wheat studies, nutrients such as nitrogen, phosphorous, and sulfur macronutrients are the most commonly observed deficiencies.

Therefore, when considering uptake, nitrogen and phosphorus are considered the most important nutrients. Nitrogen studies have shown us that this nutrient is important in early seedling development, and nitrogen deficiency can cause delay in maturity as well (Splawinski 2001). Phosphorus, like nitrogen plays a crucial role in enhancing crop maturity and quality, and early developmental stages on plant growth. Phosphorus may only account for a portion of the process in which energy is stored but is critical in plant metabolism. Phosphorus is a key component for the processes of plant respiration and photosynthesis as well.

Many other nutrients are important in the overall plant growth process, but phosphorus is most needed to attain optimal yield potential (Ross 1998). In this study, we observed that genotypes with higher phosphorus content (mg) had the highest shoot biomass (fig 7 & 8). However, when considering each growing environment, fungicide treated environment had genotypes with highest phosphorus content which equaled genotypes with the highest shoot biomass, head number and plant height averages (table 4). And in respect to environment with the highest phosphate concentration, additive treated environment had genotypes with the greatest phosphorus concentrations which only equaled genotypes with the highest averages shoot biomass averages (table 2). In another observation, when phosphate levels were highest, root colonization was lowest (table 4); when root colonization was highest, phosphate levels lowest (table 4). Nutrient uptake analysis in this study was limited due to our ability to plant, harvest and analyze a large number of samples. With more resources and time, a study should be done to observe phosphate levels on a plant to plant basis and a thorough nitrogen analysis should be carried out as well.

6. Conclusions

Agricultural production in many areas have seen increasing threats due to soil degradation and nutrient depletion. Many of these ongoing issues revolve around crops having available nutrients especially phosphorous and water (Nagarathna et al., 2007). It is believed that incorporating AMF as an alternative means can assist crops withstand nutrient deficiency, toxicity, and abiotic and biotic stresses. Significant progress and discoveries in the understanding of the interaction of AMF to many different host (Nagarathna et al., 2007) have been made. In this study, we have summarized our current understanding of the interaction of AMF and wheat in respect to genetic differences in a field environment study. However, despite the findings presented in this study, many critical research questions in regard to the interaction of AMF with host plants (wheat) remain and should be addressed in future studies.

6.1 The effect of AMF on phenotypic characteristics (Plant height and biomass)

The incorporation of AMF into agricultural systems has affected plant responses and growth given the obligate symbiotic relationship (Habte 2000). Several factors remain unanswered about the relationship of AMF and wheat. However, the interaction mediates optimal opportunities for wheat to obtain nutrients such as N, P, K, Ca, S, Cu, Zn and other micro-elements from the soil which has some potential influence on phenotypic characteristics (Farahani et al., 2008). In this study, we observed that the availability of AMF showed differences amongst genotypes supporting that the presence of AMF have some effect on plant height and biomass. The genotype Advance is used most commonly in South Dakota due to its resistance to many wheat diseases. Advance,

used in this study serves as a good baseline genotype to further assess wheat's interaction with AMF and to study its performance in both greenhouse and field experiments. The findings of this experiment demonstrate that there are differences in mycorrhizal responsiveness and nutrient efficiency with the presence of AMF on wheat phenotypic characteristics which could suggest that there is a genetic control of these genotypic differences**.**

6.2 The effect of on root colonization and AMF nutrient uptake

The AMF symbiosis plays an important role in many environmental stresses. Root colonization is the first step of the interaction of mycorrhiza and wheat. In this project, root colonization was seen throughout the entire experiment, validating the presence of mycorrhiza. However, roots obtained from each environment supported various levels of root colonization which could potential be a result of genetic differences. In the future, an experiment with a control root system should be considered to measure the biomass of roots to observe whether root colonization is genetically or environmentally effected by mycorrhiza. On the other hand, we saw that nutrient uptake (phosphorous or phosphate) concentrations and content levels varied with respect to environment and or genotype. Our hope is that our finding will be used a potential resource for better understanding of plant microbial interactions (AMF symbiosis). Experiments like this one should be further conducted and evaluated to assess the contribution of AMF to nutrient uptake in wheat.

7. Acknowledgements

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9. Figures and Tables

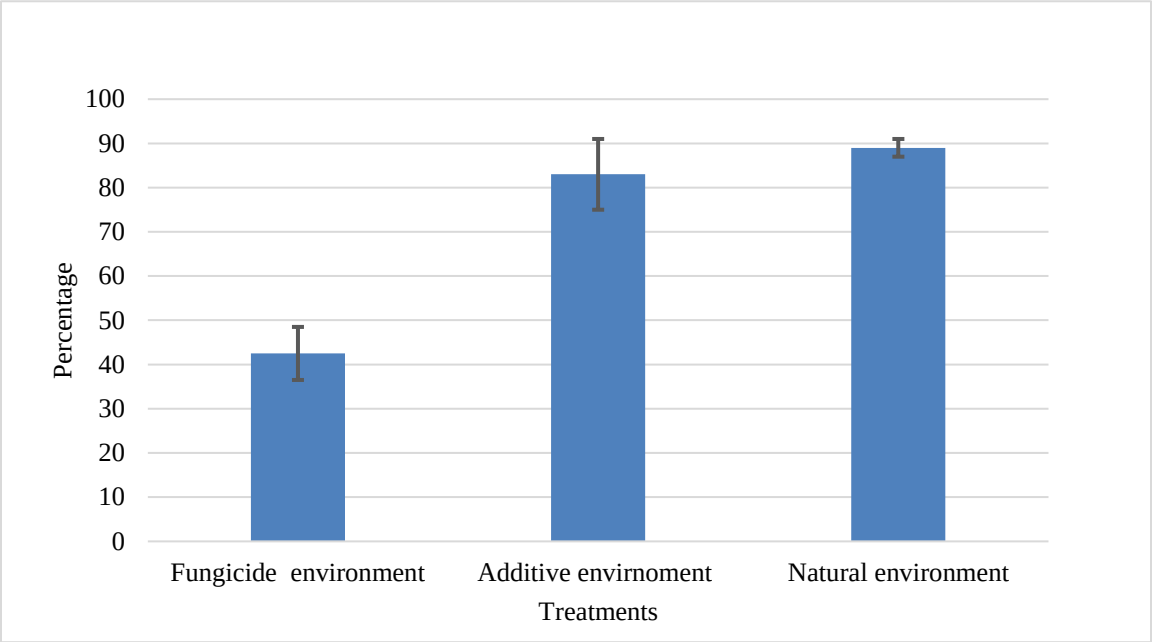

Figure 1: Assessment of root colonization (percentages) of field trial, after first application of fungicide and additive

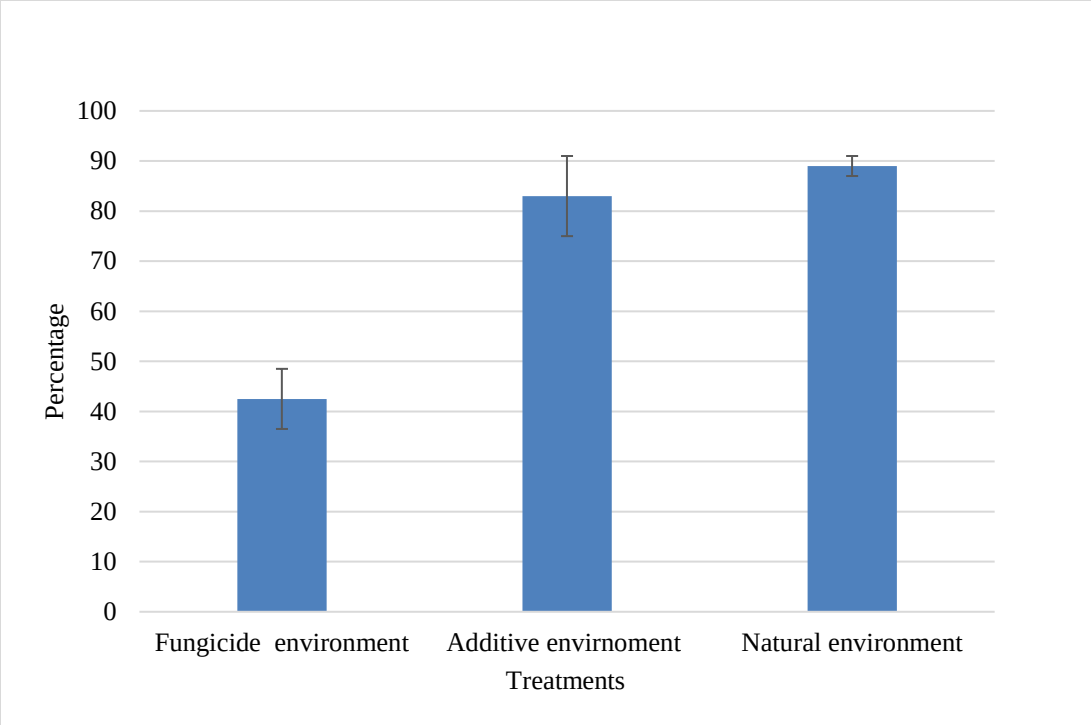

Figure 2: Assessment of root colonization (percentages) of field trial, after harvest

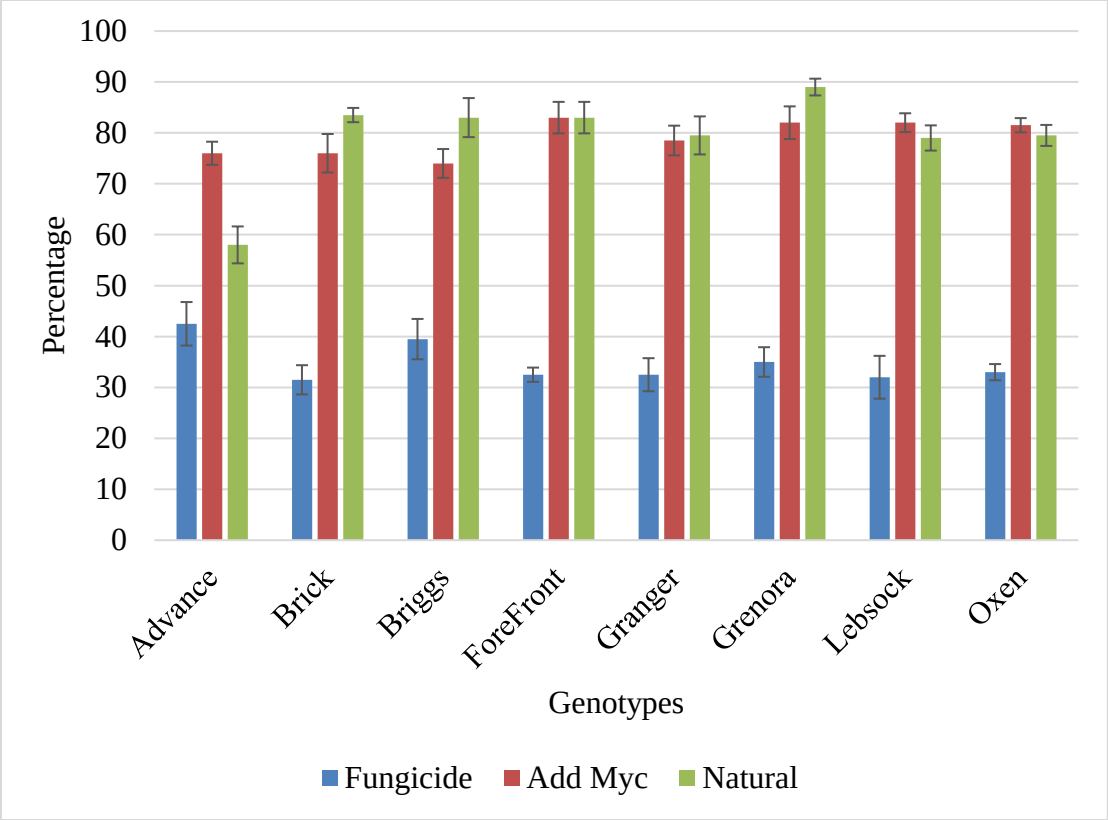

Figure 3: Total root colonization (percentage) of the eight genotypes observed in three different environments after harvest

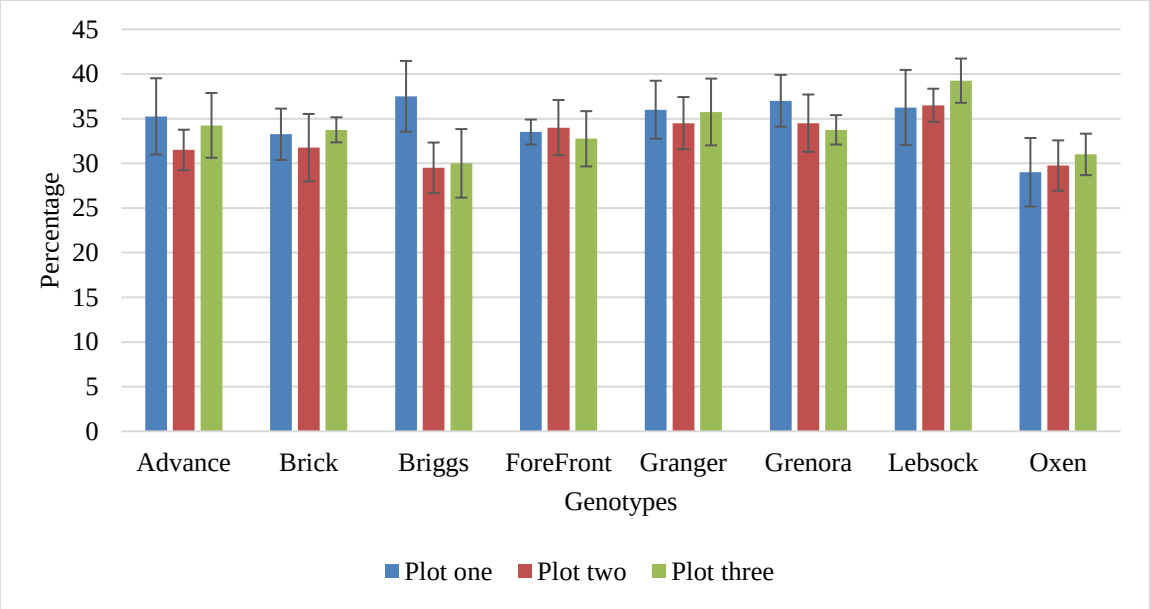

Figure 4: Plant height in each genotype in respective environments

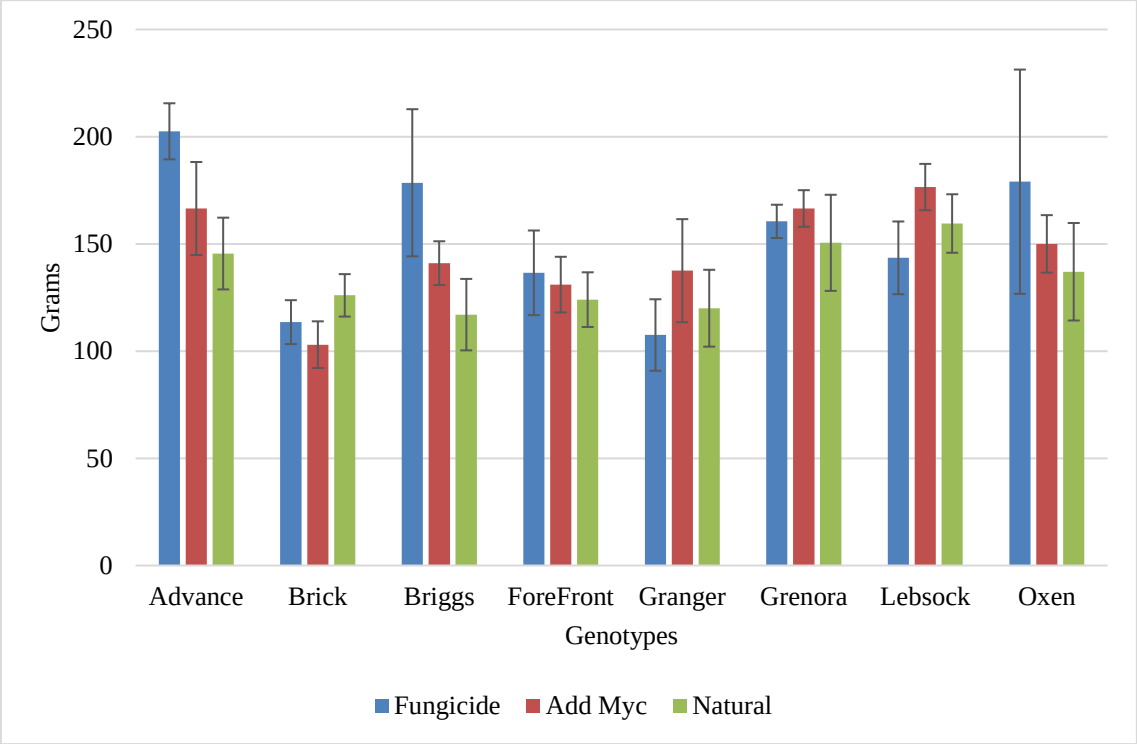

Figure 5: Shoot biomasses in grams of each genotype in respective environments

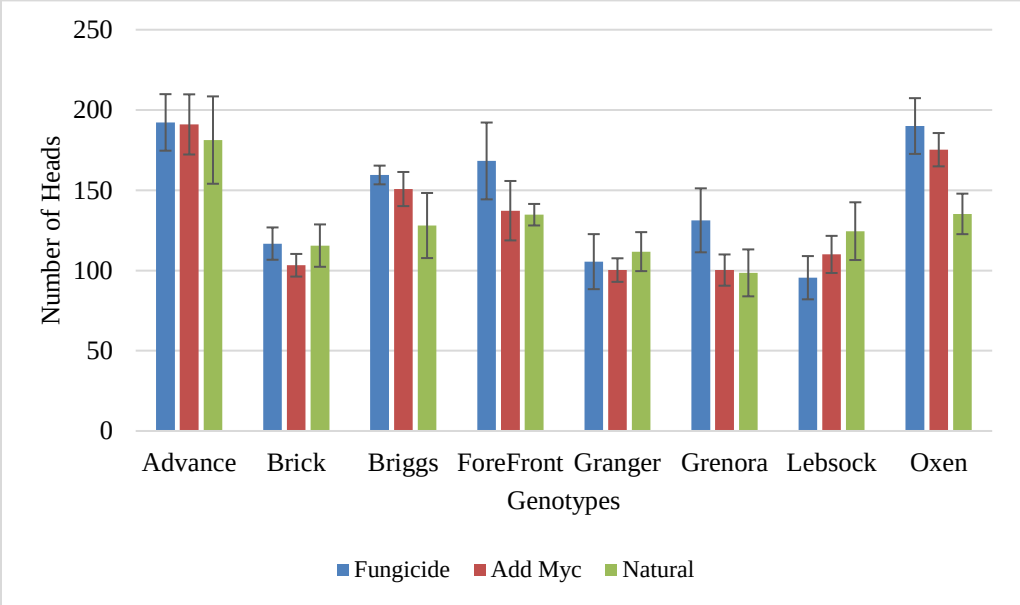

Figure 6: Average number of heads per genotype in respective environments

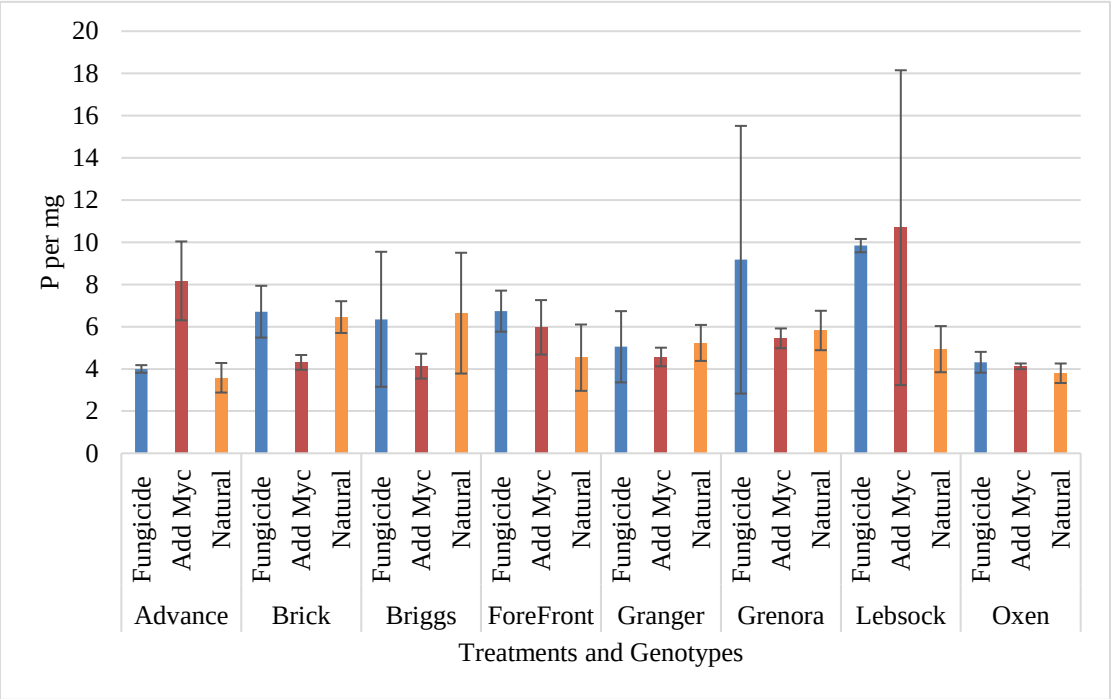

Figure 7: Shoot Concentration (P per mg) of phosphate of each genotypes in different environments

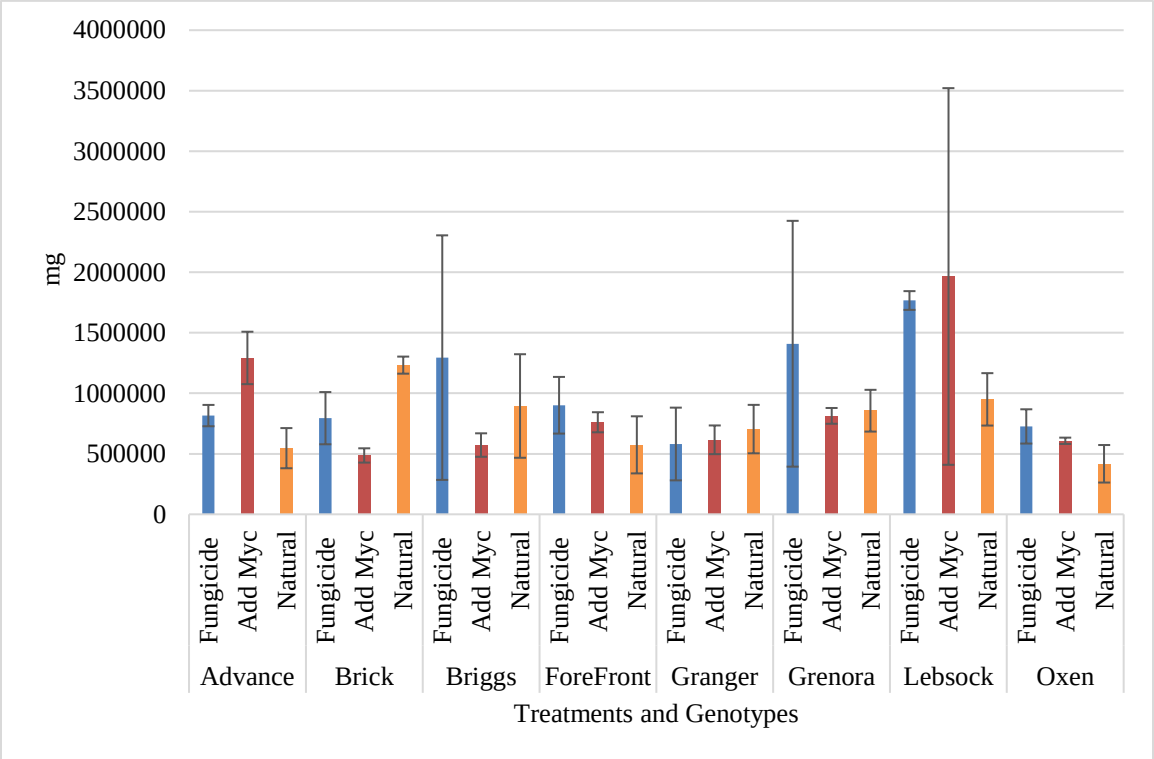

Figure 8: Shoot Content (Total P in Average Row) (mg)) phosphate of each genotype from respective environments

Table 1. ANOVA results for effects of arbuscular mycorrhizal on plant heights in Treatments and Genotypes

Analysis of variance	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	56.8	28.39	1.061	0.352
Mycorrhizal Treatment: Genotype	23	623.2	27.09	1.013	0.462
Error Residuals	70	1873.0	26.76		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 2: ANOVA results for effects of arbuscular mycorrhizal on shoot biomass in Treatments and Genotypes

Analysis of variance	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	5044	2522	2.038	0.138
Mycorrhizal Treatment : Genotype	23	49777	2164	1.749	0.039 *
Error Residuals	70	86631	1238		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 3: ANOVA results for effects of arbuscular mycorrhizal on head count in Treatments and Genotypes

Analysis of variance	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	4422	2211	2.091	0.131
Mycorrhizal Treatment : Genotype	23	94025	4088	3.867	6.54e-06 ***
Error Residuals	70	74009	1057		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4: Summary of averages (in range) phenotypic and agronomical trials (Fix)

	Number of Heads	Shoot biomass (grams)	1000 kernel weight (grams)	Phosphate content (mg)	Phosphate Concentration (p per)	Plant height (inches)	Root colonization (percentage of root)
Fungicide (Prosaro)	96-193	70-340	22.1-35	570000.00-1800000.00	3.5-10	27-54	33.1
MycoApply (additive)	100-191	74-244	24.4-35.5	490000.00 to 1300000.00	4.1-11	27-45	79/1
Natural	99-181	68-324	22.1-36	418000.00-1200000.00	3.5-10	27-50	79.8

Overall Conclusions

Plant host and pathogen interactions can be friendly or hostile. Rather the encounters, these interactions impact plants plant productivity, stress tolerance and disease resistance. Specifically, these interactions can impact the globally communities of both microbiology and plant breeding given that negative and positive effects. We have seen a broad range of scientific studies concerning how microbes interact with plants at the molecular biology and molecular genetics level. These studies have helped the understanding of the variables involved in determining the outcomes of plant host and pathogen interactions.

From this information, resources have been obtain allowing the creation of new plants or plant-microbe combinations that may serve as potential to overcome negative environmental factors and crop productivity limitations. This knowledge could also provide fundamental knowledge on plant-microbes interactions necessary for new innovations to increase farm productivity. Overall, the work conducted in this dissertation serves as a platform in unveiling many of the questions with plant host and pathogen interaction in wheat. Projects included in this dissertation were experimental designs that were project specific and were carried out during 2014 through 2016. The study sites were located in South Dakota, North Dakota and Minnesota. The resources develop in this dissertation may assist in plant development, plant defenses, soil properties, nutrient uptake, and essential breeding components.

The following conclusion were determined from the numerous experimental studies:

Study 2- Fusarium head blight for screening and germplasm selection

1). DH spring wheat lines had several lines with severity rating that were better that resistant cultivars used in study

2). DH winter wheat lines had several lines with severity rating that were better that resistant cultivars used in study

3). Combining DH material with fungicide reduce FHB severity

4.) DON content was low in both populations

Study 3 & 4 - Development of Fusarium head blight resistant germplasm wheat lines

1). Combining multiple sources of FHB resistance to develop double haploid was successful allowing us to establish screening populations (Eckard et al., 2015)

2). DH lines were assessed in multiple locations with significant decreases in FHB severity in comparison to specific commercially used resistant checks in experiment

3). The few DH lines selected as germplasm were assessed with severity rating better than commercially used resistant checks and now are being used a parent lines in multiple breeding programs throughout

Study 5 – Wheat responses to Arbuscular mycorrhizal fungi

1). Differences in mycorrhizal responsiveness and nutrient efficiency with the presence of AMF on wheat phenotypic characteristics suggest that there is a genetic control of these genotypic differences**.**

2). On the other hand, we saw that nutrient uptake (phosphorous or phosphate) concentrations and content levels varied with respect to environment and or genotype.