GENETIC DISSECTION AND IMPROVEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Fusarium Head Blight (FHB) is a destructive and complex fungal disease in wheat. Durum wheat, an economically important crop for pasta production, is under the severe impact of FHB. While numerous favorable QTL/genes have been identified in hexaploid wheat, there are few resistance resources found in durum wheat. An interspecific cross between FHB resistant hard red spring wheat (HRSW) and durum wheat cultivar 'Riveland' has been conducted for the introgression of resistance resources into durum wheat. Given the complex quantitative trait of FHB resistance in wheat, a recurrent selection population was constructed by crossing durum wheat cultivars and durum lines deviated from resistant tetraploid wheat and common wheat. Several FHB resistant breeding lines with lower FHB severity, lower plant height, and shorter flowering date than 'Riveland' were obtained from interspecies crosses and recurrent selection populations. These breeding lines can be used for the development of new durum wheat cultivars with high resistance to FHB. To explore the implementation of genome-wide markers to screen FHB resistance in the durum wheat breeding program, a genomic prediction model was built using breeding lines from 2012-2018 advanced yield trials (AYT) evaluated in multiple environments of scab nurseries. The genomic prediction accuracies were 0.53 and 0.47, respectively, based on ten-fold cross-validation and forward prediction to untested breeding lines. The results indicated that genomic selection could enhance FHB resistance improvement in the durum wheat breeding program.

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DEDICATION

To my grandfather. Peace to his soul.

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CHAPTER I. LITERATURE REVIEW

Origin, domestication, and distribution of durum wheat

Durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn., 2*n* = 4*x* = 28, AABB] is an important food crop and is mainly used for pasta production. Durum wheat is resulted from two steps of domestication. First, domestication of wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. & Graebner) Thell.] in the Fertile Crescent about 10,000 years ago gave rise to cultivated emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell.] (Dubcovsky and Dvorak, 2007), where non-shattering trait determined by the *Br* (*brittle rachis*) loci on chromosomes 3A and 3B was selected (Nalam et al., 2006). Durum wheat was further domesticated from cultivated emmer wheat (Nesbitt and Samuel, 1996), where the loss of tough glumes converted wheat from hulled into free-threshing habit. The free-threshing was reported to be associated with recessive mutations at the *Tg* (*tenacious glume*) loci and a dominant mutation at the *Q* locus (Jantasuriyarat et al., 2004). From the Fertile Crescent, durum wheat was introduced to Asia, Europe, and Africa along with the expansion of agriculture.

Durum has been grown on approximately 13 million hectares globally (Kadkol and Sissons, 2016). According to the International Grains Council (IGC) in Grain Market Review, the world durum wheat production in 2017-2018 was about 38 million tons annually. The main durum-growing regions include Mediterranean basin countries and the northern Great Plains of the United States and Canada. Durum wheat was grown on 0.77 million hectares with an annual production of 1.7 million tons in the USA from 2017 to 2019 (NASS-USDA). In the USA, about 53% of durum wheat is grown in North Dakota with an annual average durum wheat production of 0.9 million tons (https://usda.library.cornell.edu/concern/publications/tm70mv177).

Fusarium head blight and its impacts on grain yield and quality

Fusarium head blight (FHB) is a major fungal disease that threatens wheat grain yield and quality globally. In the USA, FHB was first reported in the 1890s (Arthur, 1891; Chester, 1890; and Detmers, 1892). Since the 1990s, FHB epidemic has frequently occurred and caused severe loss of wheat worldwide including in the northern Great Plains, reviewed by McMullen et al. (1997, 2012). In 2019, spring wheat grown in southwestern and southeastern North Dakota had a reported yield loss of up to 50% due to FHB (Lilleboe, 2019). Additionally, mycotoxin deoxynivalenol (DON) produced by the FHB pathogens can disable the natural plant defense system and pose adverse effects on the health of humans and livestock. The U.S. Food and Drug Administration (FDA, 2010) has provided guidance on advisory levels for finished wheat products. Durum wheat was traditionally planted in eastern North Dakota in the USA. Due to the lack of FHB resistant cultivars, most of durum wheat is now planted in western North Dakota, where low rainfall reduces incidence of FHB.

FHB is caused by several *Fusarium* species including *Fusarium graminearum*, *F. culmorum* and *F. avenaceum* (Covarelli et al., 2015). In North America, *F. graminearum* is the dominant fungal species causing FHB (Gale, 2003; Shaner, 2003). The fungal pathogen stays in crop residues such as corn stalks, wheat straw, and other host plants to live through the winter. The asexual spore macroconidia produced from the infested residues spread around by wind or rain-splash. When conditions are warm, the sexual stage will develop and form bluish-black perithecia to produce sexual spore ascospores. Both macroconidia and ascospores can infect the wheat head. Moderately warm temperatures (15 - 30 °C) and prolonged humidity (> 90%) are favorable for the infection process. The infected spikelet is partially or fully bleached and continuously spreads to other spikelets. The stem becomes brown or has purple discoloration and

the color change may extend to the entire stem. Mature seeds infected by the pathogen shrink and wrinkle and seed colors range from pink, soft-gray, to light-brown (Gilbert and Fernando, 2004). Several mycotoxins including DON and derivatives (15-ADON, 3-ADON) are produced immediately after infection.

Controls of FHB disease in wheat

Cultivational practices, fungicide spray, biological control, and resistance cultivars can be used to control FHB disease in wheat. Cultivational practices are aimed at reducing pathogen inoculum (on infested residue) in wheat. Tillage and rotation with non-gramineaceous crops, like soybean, burial or burning of corn residue are very effective ways to remove inoculum (Yuen and Schoneweis, 2007). Fungicides have proved to be an efficient way to control FHB disease, but it is neither cost-effective nor environmentally friendly. Some biological control strategies including fungal, bacterial and yeast species in several studies are suggested to have the potential to decrease the FHB impact (Yuen and Schoneweis, 2007; Gilbert and Fernando, 2004; Jochum et al., 2006). However, the commercialization for biological control has not been successful. Compared to other approaches, growing FHB resistant cultivars is environmentally friendly and the most efficient approach in mitigating the effect of the disease.

Morphological and physiological traits related to FHB resistance

Many morphological and physiological traits in wheat were reported to have association with FHB resistance, such as anther retention, plant height, flower opening, heading and flowering date, spike compactness, presence/absence of awns, cell wall traits, canopy, and ear traits (Mesterházy, 1995; Ando et al., 2007; Lionetti et al., 2015; Lahlali et al., 2016; Jones et al., 2018). The retained anthers provide a significant advantage for the onset of fungus spores and facilitate the development of pathogen growth (Dickson et al., 1921). Microscopic analysis

indicated that more hyphal growth was observed on tissues such as the retained anther, pollen, and stigma, while infection progressed more slowly on harder tissues such as lemma and palea (Kang and Buchenauer, 2000). Anther extrusion or removal after pollination prior to spray inoculation can significantly reduce the initial infection and early disease development, but did not protect the plant from fungal spreading within the spike (Buerstmayr et al., 2020). In the field conditions, plant height relates closely with many key factors of FHB infection to wheat. The heads are generally infected by rain-splash-dispersed conidia or ejected ascospores which reside in crop debris on the soil surface, consequently, the taller the plant is, the more likely it can escape from infection (Jenkinson and Parry, 1994). Plant height affects disease severity by influencing microclimate around heads. The shorter plants have denser canopy structure that lowers the air circulation, resulting in relative high humidity and temperature and causes an increased disease pressure to promote infection and disease development (Buerstmayr & Buerstmayr, 2016; Hilton, Jenkinson, Hollins, & Parry, 1999; Jones, et al., 2018). It was demonstrated that tall plants have better type I resistance (resistance to initial infection) than short ones when assessed at natural conditions. (Yan et al., 2011). A narrow flower opening reduces the chance for pathogen invasion (Gilsinger et al., 2005). Both positive and negative correlations between FHB resistance and heading/flowering date were reported (Gervais et al., 2003; Somers et al., 2003; Steiner et al., 2004; Klahr et al., 2007; Buerstmayr et al., 2008), in which environmental factors, especially temperature and humidity, played a major role on the development of FHB (Buerstmayr et al., 2020). A significantly positive correlation exists between spike compactness and pathogen spreading on head (Giancaspro et al., 2016; Jantasuriyarat et al., 2004). The presence of awns catching the spore in the air increases the initial infection under natural epidemic conditions while having no influence on disease severity

during artificial inoculation (Mesterházy, 1995). Jones et al. (2018) demonstrated that lower tiller numbers, shorter flag leaves and less dense heads are positively associated with avoidance of FHB disease while alteration of these traits influence the potential trade-off with biomass and grain production.

The plant cell wall plays a critical role in FHB resistance of wheat. The plant cell wall has three layers. The primary cell wall is composed of polysaccharides including cellulose, hemicellulose and pectin; the secondary cell wall consists of cellulose, xylan and lignin; and the middle lamella is rich in pectins forming the interface between adjacent plant cells (Buchanan et. al., 2015). Three main cell wall degrading enzymes (CWDE) produced by *Fusarium graminearum* are pectinases, xylanases, and cellulases that degrade pectin, arabinoxylan (AX) and cellulose fiber, respectively (Wanjiru et al., 2002; Yang et al., 2012). During the early stages of infection, pectinases are first produced by the pathogen followed by hemicellulases and cellulases (Phalip et al., 2009; Bellincampi et al., 2014).

Pectins are complex polymers with different structural domains. The methylesterification of galacturonosyl residues of pectin backbones decreases its digestibility by pectinases (Volpi et al., 2011; Lionetti et al., 2010; Bonnin et al., 2002) and reduces growth of fungal pathogens (Volpi et al., 2011). Pectin in *Fusarium* resistant spikes is less susceptible to polygalacturonases of *F. graminearum* secreted at early stage infection (Tomassini et al., 2009). Lignin contains three types of monomers, p-hydroxyphenyl (H), guaiacy (G), and syringy (S) phenylpropanoid monolignols. Lionetti et al. (2015) demonstrated that higher S lignin content was associated with FHB resistance and proposed that genes regulating S-type lignin accumulation might be involved in FHB resistance. A higher degree of xylan arabinosylation was observed in resistant wheat spikes compared to the susceptible strain. This may be attributed to the ferulic acid-mediated

crosslinks, formed by arabinose residues of xylan, between xylan chains and lignin components that limit the digestibility of cell walls from CWDE, such as xylanases (Bily et al., 2003; Santiago and Malvar, 2010; Ralph et al., 2004; Molinari et al., 2013).

Additionally, many structural proteins in cell wall are known to be involved in pathogen infection. For example, the cell wall-bound thionins (Pelegrine and Franco, 2005) and cell wall reinforced by cross-links and insolubilization of structural proteins, e.g. hydroxyproline-rich glycoproteins (HRGPs), inhibits the pathogen growth (Blümke et al., 2014; Deepak et al., 2010; Walter et al., 2010). Furthermore, cell wall degradability during infection process is also influenced by a number of CWDEs inhibitors such as polygalacturonase inhibiting proteins (PGIPs), pectin methylesterase inhibitors (PMEIs), *Triticum aestivum* xylanase inhibitors (TAXIs) and xylanase inhibitor proteins (XIPs) (Moscetti et al., 2013; Bellincampi et al., 2014).

FHB resistance types

Several types of FHB resistance have been defined in wheat. Resistance to initial infection (Type I resistance) and resistance to pathogen spread within infected spikes (Type II resistance) defined by Schroeder and Christense (1963) are widely used in germplasm screening and genetic studies (Buerstmayr et al., 2020). Type III is resistance to mycotoxin accumulation; Type IV is resistance to kernel infection that is reflected by proportion of shriveled and misshaped grains; Type IV resistance can be measured either by grains that have been damaged by infection, termed as *Fusarium* damaged kernel (FDK), or by grains that are poorly filled, e.g. thousand kernel weight (TKW); Type V is tolerance to grain yield loss (Miller et al., 1985; Wang and Miller, 1988; Mesterházy, 1995).

FHB resistant germplasm in durum wheat and its relatives

Thousands of durum wheat accessions have been screened but no lines with high FHB resistance were found (Elias et al., 2005). Wild tetraploid relatives of durum wheat including wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*), and Persian wheat (*T. turgidum* ssp. *carthlicum*) are potential resources of FHB resistance because of their wide genetic adaptation to biotic stresses. A panel of 290 accessions of wild emmer wheat were evaluated and several of them with high type II resistance were identified (Miller et al., 1998). Another panel of 151 Israel originated wild emmer wheat lines were screened and eight of them were found with moderate resistance (Buerstmayr et al., 2003). Oliver et al. (2008) identified 16 Persian wheat and four cultivated emmer wheat lines with moderate to high level of resistance from 376 tetraploid wheat accessions.

Various hexaploid bread wheat accessions with high level of FHB resistance were discovered. Most validated FHB resistant bread wheat lines such as ‘Sumai 3’ and ‘Ning 7840’, landraces ‘Wangshuibai’ and 'Nobeokabouzu', as well as Brazilian cultivar ‘Frontana’ have been used in bread wheat breeding programs worldwide (Kubo and Kawada, 2009; Kubo et al., 2013; Kubo et al., 2014; Lamb et al., 2009; Li et al., 2016; Niwa et al., 2014; Zhang et al., 2010).

FHB resistance resources from tertiary gene pool have also been explored. Species that were used for alien introgressions include *Thinopyrum elongatum* (syn. *Lophopyrum elongatum*) (Ceoloni et al., 2017; Dai et al., 2017; Fu et al., 2012; Gou et al., 2015; Jauhar and Peterson, 2011, 2013; Jauhar et al., 2009; Miller et al., 2011; Turner et al, 2013; Wang et al., 2010), *Th. intermedium* (Bajgain et al., 2019; Zeng et al., 2013), *Th. bessarabicum* (Jauhar and Peterson, 2013), *Th. junceum* (McArthur et al., 2012; Turner et al., 2013), *Th. ponticum* (Forte et al., 2014; Guo et al., 2015; Turner et al., 2013), *Elymus repens* (Fedak et al., 2017), *E. rectisetus*

(McArthur et al., 2012), *E. tsukushiensis* (Cainong et al., 2015) and *Leymus racemosus* (Wang et al., 2009). Brisco et al. (2017) evaluated 109 accessions of *Aegilops tauschii* Cosson by single-floret inoculation and found that the resistance lines were generally originated from areas receiving high levels of rainfall, such as Caspian Sea. Despite the broad resistance resources identified in wheat wild relatives, breeders are cautious when utilizing exotic resistance donors considering the trade-off between resistance improvement and linkage drag (Brar et al., 2019).

Genetic basis of FHB resistance in wheat

To explore the genetic basis of FHB resistance in wheat, different measurements to certain FHB resistance types have been implemented in designed populations for resistance loci mapping and evaluation. The measurements of FHB resistance in wheat include FHB spreading (a measurement of type II resistance) using single-floret inoculation (SFI), FHB incidence (percentage of infected heads per plot, a measurement of type I resistance) using spray or gain spawn (SPI), FHB severity (percentage of spikelets that were necrotic in infected heads), area under the disease progress curve (AUDPC), FHB index (Incidence × Severity/100), *Fusarium* damaged kernels (FDK), and DON content (Buerstmayr et al., 2020).

Buerstmayr et al. (2020) summarized about 500 quantitative trait loci (QTL) related to FHB resistance identified from 159 QTL mapping studies of hexaploid wheat and tetraploid wheat. Type I and II resistances were analyzed in most studies. QTL detected using SPI (84 QTL) were about as twice number of QTL as detected by SFI (40 QTL) inoculation in 13 studies conducting both SFI and SPI (Buerstmayr et al., 2020). Only 18 QTL were identified in both SFI and SPI. This provided evidence that type I and type II resistance are under different genetic control (Steiner et al., 2004). To find more effective markers and candidate genes in these QTL regions, 65 meta-QTL were generated based on 556 QTL found in previous studies (Venske et

al., 2019). The most refined meta-QTL1/chr. 3B validated 10 genes responsive to FHB (Venske et al., 2019).

Only a limited number of moderate-effect QTL have been identified in tetraploid wheats, while numerous QTL for FHB resistance were reported for bread wheat (Buerstmayr et al., 2014, 2020; Prat et al., 2014). Thirteen QTL for FHB resistance with small to moderate effects were repeatedly detected on 11 chromosomes of tetraploid wheat (Buerstmayr et al., 2012, 2013; Chen et al. 2007; Ghavami et al., 2011; Gladysz et al., 2007; Kumar et al., 2007; Otto et al., 2002; Ruan et al., 2012). Several QTL with minor effects on chromosomes 1A, 2A, 2B, 3BL, 5A, and 5BL have been identified in a susceptible durum cultivar (Zhang et al., 2014, 2017; Sari et al., 2018) and Tunisian durum lines (Ghavami et al., 2011; Fakhfakh et al., 2011; Huhn et al., 2012; Zhang et al., 2014; Pirseyedi et al., 2019). These studies suggest that durum wheat does carry a certain level of FHB resistance.

Several QTL were commonly found in hexaploid wheat mapping studies with large genetic effects, such as *Fhb1, Fhb2, Qfhs.ifa-5A, Fhb4, Fhb5, and Fhb7* (Waldron, 1999; Bai et al., 1999; Buerstmayr et al., 2003; Xue et al., 2010, 2011). It was reported that *Fhb1* derived from 'Sumai 3' mainly contributes to type II resistance (Buerstmayr et at., 2020), while a few studies also found type I resistance to FHB in wheat (Basnet et al., 2012; Yang et al., 2005). *Fhb1* performed as a major QTL for type II resistance in greenhouse experiments, while it had minor effect on field test (Buerstmayr et al., 2020). Additionally, *Fhb1* is also related with FDK and kernel infection (Basnet et al., 2012). Several studies reported cloning of *Fhb1* gene but they disagreed on the causative gene. Rawat et al. (2016) identified a pore-forming toxin-like (PFT) gene as the causal gene behind *Fhb1*. However, Su et al. (2019) and Li et al. (2019) indicated that the histidine-rich calcium-binding-protein gene, which shares a common position with *Fhb1*

on chromosome 3BS, results in FHB resistance of *Fhb1*, but these two studies disagreed on the mode of gene action. Su et al. (2019) stated that the wild type allele caused susceptibility to FHB, and a rare deletion within this gene's 3′ exon resulted in the resistance of 'Sumai 3'; while Li et al. (2019) stated that the Sumai-3/Wangshuibai haplotype of the histidine-rich calcium-binding protein gene is unique, and therefore the Wangshuibai allele confers resistance in an active manner. Further studies need to clarify the causative gene of *Fhb1* and its mode of action.

Fhb7 is a stable QTL on FHB resistance introduced from *Thinopyrum elongatum* to Triticeae tribe wheat and shows a similar effect as *Fhb1* (Guo et al., 2015). Using assembled Triticeae E reference genome, *Fhb7* was genetically mapped and located to a 245-kb genomic region, and a gene encoding a glutathione S-transferase (GST) was determined as *Fhb7* using virus-induced gene silencing and evaluated mutants and transgenic plants (Wang et al., 2020). It was found that *Fhb7* detoxifies pathogen-produced trichothecene toxins by conjugating a glutathione (GSH) unit onto the epoxide moieties of type A and B trichothecenes (Wang et al., 2020). Interestingly, *Fhb7* was demonstrated to be horizontally transferred from *Epichloë* to *Th. elongatum*, because *Fhb7* GST homologs are absent in the plant kingdom but approximately 97% identical to a sequence from endophytic fungi of an *Epichloë* species that have symbiosis with temperate grasses (Wang et al., 2020). Furthermore, *Fhb7* was suggested to provide resistance to FHB without penalizing wheat yield (Wang et al., 2020).

Germplasm improvement and breeding of FHB resistance in durum wheat

Due to lack of resistant resources, most efforts of improving FHB resistance in durum wheat placed the focus on introgression of resistance QTL/genes from bread wheat or other tetraploid wheat relatives. *Fhb1* had been successfully introduced into three durum wheat cultivars (Prat et al., 2017). A major QTL *Qfhb.ndwp-5A* derived from hexaploid wheat resistant line PI277012

was successfully introgressed into durum wheat (Zhao et al., 2018). The efficient introgression of these major QTL contributes to a significant step forward for enhancing FHB resistance in durum wheat.

FHB resistance is a complex trait controlled by many genes in wheat. In addition to introgression of major QTL, integrating more favorable alleles at multiple loci with moderate/minor effects will provide high and stable resistance. Phenotypic selection is an effective way to pyramid the favorable alleles and is commonly used in wheat breeding programs for improving FHB resistance. Generally, phenotypic selection for FHB resistance is conducted on F_4 or later generations, where a number of lines were evaluated in field nursery with artificial inoculation at multiple locations. However, it is costly and time consuming. Genomic selection (GS) is a marker-aided selection method suitable for complex traits. By estimating genome wide marker effects in a training population to predict genomic estimated breeding values (GEBVs) of non-phenotyped individuals in a selection population, GS can increase selection intensity and accuracy for a complex quantitative trait like FHB resistance. A number of studies have explored GS for FHB resistance in wheat (Arruda et al., 2016; Hoffstetter et al., 2016; Rutkoski et al., 2012; Jiang et al., 2015; Miedaner et al., 2017; Steiner et al., 2019). Arruda et al. (2016) compared GS and MAS on the ability to predict six traits associated with FHB resistance in wheat and found that GS models greatly outperformed MAS models in both prediction accuracy and selection differential for parameters associated with FHB resistance. Steiner et al. (2019) reported greater prediction ability for FHB severity by phenotypic over genomic based selection. However, simulation tests showed higher selection responses of genomic over phenotypic selection when using genomic breeding values for early generation selection (Steiner et al., 2019). A strong association was observed between plant height and FHB resistance traits

(Miedaner et al., 2017; Steiner et al., 2019) which leads the preference of taller plants selection when aiming to increase FHB resistance. Consequently, employing a multi-trait genomic prediction models and including a GS index to account for plant height were recommended for FHB resistance improvement while keeping plant height constant (Steiner et al., 2019). With the availability of suitable prediction models and reliable phenotypic data, GS can make an earlier generation identification of the most promising lines and crossing parents. This noticeable advantage makes GS as a largely positive strategy for faster population improvement.

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CHAPTER II. DEVELOPING DURUM WHEAT FHB RESISTANT GERMPLASM USING INTERSPECIFIC CROSSES WITH HARD RED SPRING WHEAT

Abstract

Fusarium Head Blight (FHB) is a devastating disease for wheat production. Due to the lack of resistant resources in durum wheat, introgression of resistance genes from wheat relatives is the primary choice to improve FHB resistance in durum wheat. Numerous FHB resistance QTL have been identified from hexaploid bread wheat. In this study, we aimed to introduce resistant genes/QTL from Hard Red Spring wheat with high genetic resistance diverse into durum wheat. Six families with the significant lower FHB severity were obtained by interspecific crossing between FHB resistant Hard Red Spring wheat and durum wheat cultivar 'Riveland'. These resistant families can be used for the development of FHB resistant durum germplasm.

Introduction

Wheat is one of the most important food crops and provides about 20% of the calories consumed by humans (Braun et al., 2010). According to FAO (2020), wheat has been grown on about 220 million hectares worldwide with an annual production of over 760 million tons. Hexaploid common wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, AABBDD) accounts for 95% of the total wheat production and the other 5% is tetraploid durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn., 2*n* = 4*x* = 28, AABB]. Durum wheat was domesticated from wild emmer wheat [*T. turgidum* L. ssp. *dicoccoides*, 2*n* = 4*x* = 28, AABB] and cultivated emmer wheat [*T. turgidum* L. ssp. *dicoccum*, 2*n* = 4*x* = 28, AABB] in the Fertile Crescent about 10,000 years ago (Luo et al., 2007). Hybridization of cultivated emmer wheat with *Aegilops tauschii* Cosson (2*n* = 2*x* =14, DD) resulted in hexaploid common wheat (Dvorak et al., 1998). Many diseases have threatened wheat production. Fusarium head blight (FHB) or scab is one of most devastating

diseases, caused by several *Fusarium* fungal species. The pathogen infection spikes during flowering and causes shriveled kernels. Infected kernels contaminated with mycotoxins like deoxynivalenol (DON) produced by the pathogen have adverse effects on human and animal health. Since the 1980s, due to climate changes, deployment of wheat-maize rotation, and no-tillage that favor FHB development (West et al., 2012; Zhang et al., 2012), outbreaks of FHB has frequently occurred in major wheat growing regions of the world and caused severe loss of grain yield and decrease in quality (Aponyi et al., 1998; Bilska et al., 2018; Kohli and de Ackemann, 2013; Liu et al., 2016; Ma et al., 2020; McMullen et al., 2012; Nganje et al., 2004).

Growing resistant cultivars, combined with other agronomic management, plays a key role in alleviating loss caused by FHB. However, this is challenging to durum wheat due to the lack of resistant sources. Thousands of durum wheat breeding lines and worldwide landrace collections were screened, but none was found with high FHB resistance (Elias et al., 2005; Huhn et al., 2012). Screening of tetraploid wheat relatives including wild emmer wheat, cultivated emmer wheat and Persian wheat (*Triticum turgidum* L. ssp. *carthlicum*) discovered a few accessions with moderate resistance to FHB (Buerstmayr et al., 2003; Miller et al., 1998; Oliver et al., 2007, 2008). A number of durum wheat lines with improved FHB resistance were developed from the crosses between those resistant lines of tetraploid wheat relatives and elite durum wheat breeding lines, from which numerous QTL with moderate effects were identified (Buersmayr et al., 2012, 2013; Sari et al., 2018; Zhang et al., 2014).

Breeding of FHB resistance in hexaploid common wheat was successful, largely attributed by the high resistant lines like Sumai 3 (Ma et al., 2020; Zhu et al., 2019). A few major QTL including *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5* were consistently identified in genetic dissection studies with various common wheat mapping populations (Anderson et al., 2001; Bai et al.,

1999; Buerstmayr et al., 2009; He et al., 2014; Liu et al., 2019; Loffler et al., 2009; Venske et al., 2019; Waldron et al., 1999). Those resistant germplasm and major QTL have been widely utilized in common wheat breeding programs and resulted in numerous resistant cultivars released (Buerstmayr et al., 2020; Ma et al., 2020; Zhu et al., 2019). Transferring the common wheat major QTL into durum wheat have been attempted. Recently, the well-known common wheat major QTL *Fhb1* has been successfully introgressed into three durum wheat cultivars and explained 5%, 11%, and 14% of total variation of the population under study, respectively (Prat et al., 2017). Two major QTL from common wheat PI 277012 were introgressed into durum wheat cultivar "Joppa" (Zhao, et al., 2018). A gene related to cell well structure was introgressed into durum wheat and increased resistance to FHB (Giancaspro et al., 2016, 2018). Those developed germplasms have greatly broadened genetic diversity of the durum wheat breeding pool for FHB resistance improvement.

It was documented that FHB resistance in wheat is a quantitatively inherited trait controlled by many genes with varied mechanisms. Several types of FHB resistance have been described in wheat: type I is defined as resistance to initial infection; type II is resistance to spread within infected spike; type III is resistance to mycotoxin accumulation; type IV is resistance to kernel infection measured by the proportion of FHB damaged kernel; type V is tolerance to FHB measured by grain yield loss (Mesterházy et al., 1999; Miller et al., 1985; Schroeder and Christense, 1963). Multiple morphological and physiological traits including plant height, flag leaf length, spike density, flowering time, anther extrusion, cell well structure and component, etc. have been reported as related to FHB resistance (Mesterházy, 1995; Ando et al., 2007; Lionetti et al., 2015; Lahlali et al., 2016; Jones et al., 2018). Over 600 QTL for FHB resistance have been identified and mapped on every chromosome of wheat from previous

genetic mapping studies (Buerstmayr et al., 2009, 2020; Prat et al., 2014; Venske et al., 2019), where type I and type II resistance were commonly evaluated. It was reported that the well-known type II resistance QTL *Fhb1* could only explain a part of the total variation and shows large interaction with genetic backgrounds and environments in common wheat (Bokore et al., 2017; Liu et al., 2019). To breed for durum wheat FHB resistant cultivars, developing new germplasm is still crucial and a top priority task, including diverse resistance types and genes with varied resistant mechanisms as well as enriching genetic diversity of the current breeding pool.

In this project, we created interspecific populations by crossing hard red spring (HRS) wheat FHB resistant lines to durum wheat cultivar 'Riveland". The objective was to develop durum wheat lines with improved FHB resistance from the interspecific population.

Materials and Methods

Plant materials

Diverse resistant resources have been used for FHB resistance improvement in the North Dakota State University (NDSU) HRS wheat breeding program. A set of cultivars with good FHB resistance have been released. For example, Alsen (Frohberg et al., 2000) and Faller (Mergoum et al., 2008) were developed using Sumai 3 and its derivatives as resistant donors. Steele-ND is a cultivar with its FHB resistance from wild emmer wheat (Mergoum et al., 2005). Glenn is a cultivar (Mergoum et al., 2006) with its resistance from combination of Steele-ND and Alsen. In a previous study, we performed genome wide association analysis using 427 breeding lines from the NDSU HRS wheat breeding program (Liu et al., 2019), where the phenotypic data of FHB resistance was collected from the FHB field nurseries from 2012 to 2019. We found no QTL with large effect, and even the well-known QTL *Fhb1* only explained

3.1% of total phenotypic variation (Liu et al., 2019). We selected the top 10 resistant lines out of the 427 lines and created an HRS wheat recurrent selection population (HRS-FHB-RS) by crossing them to another eight HRS wheat cultivars (with advantages of high protein, grain yield and partial FHB resistance) followed by two generations of random mating (Table 2.1).

Table 2.1. Development of base population of Hard Red Spring wheat recurrent selection.

Generation	Female	Male
1	Ms2-Faller and Ms2-Glenn	*Top 10 FHB resistant lines from NDSU HRS wheat AYT trials (2012-2019)*: 15-13-1101, 15-13-1115, 14-13-1111, 15-13-1088, 11-13-1063, 15-13-1093, 11-13-1003, 16-13-1013, 16-13-1029, 16-13-1089 *HRS wheat cultivars*: Bolles, Linkert, Shelly, Ingmar, Valda, ND828, VitPro, and Glenn
	Male-sterile plants	Male-fertile plants
2	Ms2-Faller/Bolles, Ms2-Faller/Ingmar, Ms2-Faller/ND828, Ms2-Faller/Glenn	Ms2-Glenn/15-13-1115, Ms2-Glenn/14-13-1111, Ms2-Glenn/11-13-1063, Ms2-Glenn/15-13-1093, Ms2-Glenn/11-13-1003
	Ms2-Faller/15-13-1101, Ms2-Faller/15-13-1088, Ms2-Faller/16-13-1013, Ms2-Faller/16-13-1029, Ms2-Faller/16-13-1089	Ms2-Glenn/Linkert, Ms2-Glenn/Shelly, Ms2-Glenn/Valda, Ms2-Glenn/VitPro

Cross between HRS and durum wheat

Three cycles of phenotypic selection for FHB resistance were conducted from 2019 to 2021, one cycle per year. In each cycle, about 190 S_1 families were evaluated in field nurseries for FHB resistance in the summer and top 10% families were selected and recombined to obtain the subsequent cycle population in the greenhouse in winter. The best families selected from field evaluation were pollinated by the durum wheat cultivar ‘Riveland’. Riveland was a durum wheat cultivar released by the NDSU durum wheat breeding program with a moderate

susceptibility to FHB (Elias and Manthey, 2019). Because of the unhealthy status of pentaploid F_1 generations from an interspecific cross between HRS wheat (hexaploidy) and durum wheat (tetraploidy), the F_1 population was advanced backcrossed with Riveland by pollinating F_1 by Riveland.

In the Fall of 2020, the two best families 20S558 and 20S760 selected from the HRS-FHB-RS Cycle1 population were crossed to Riveland. A total of 200 BC_1F_1 progenies were planted in the greenhouse, 45 BC_1F_1 progenies with poor growth were discarded, and the remaining 155 BC_1F_1 plants were self-pollinated. A total of 1,925 BC_1F_2 progenies (15 plants per family for 75 BC_1F_1 families and 10 plants per family for the other 80 BC_1F_1 families) were planted and evaluated for FHB severity in the greenhouse in the Fall of 2021. In the greenhouse experiment, plants were grown in plastic pots (15 × 15 ×15 cm diameter) with one plant per pot. The temperature was maintained between 22°C and 25°C with 16 hours light and 8 hours dark. The inoculum was prepared as a spore suspension at a concentration of 50,000 spores ml^{-1} by mixing equal spore concentrations of four pathogenic *F. graminearum* strains (Fg8-13, Fb10-124-1, Fg13-79, and Fg10-135-5) collected from North Dakota (Puri and Zhong 2010). Inoculation was performed using a spray inoculation method, where 400 microliters of spore suspension were sprayed on a spike at anthesis onset using an airbrush with 20 psi. For each plant, roughly two to five spikes were inoculated. The inoculated plants were put in a misting chamber after inoculation. After 48 hours, the plants were moved back to the greenhouse. Disease severity was visually scored using a 0-9 scale at 21 days post-inoculation as described in Table 2.2. Mean FHB severity was calculated for each plant, reflecting a combination of type I and type II resistances. The five checks used were spring wheat breeding lines/cultivars Alsen, ND2710, Wheaton, and durum wheat cultivars Grano (Elias et al., 2021), Riveland (Elias and

Manthey, 2019). Each check was planted in five pots, 3 plants per pot. The top 34 BC_1F_2 plants were selected based on mean FHB severity score and self-pollinated. A total of 340 BC_1F_3 progenies (10 plants per selected BC_1F_2) were evaluated for FHB severity in the greenhouse with the same protocol as described above in Spring 2022, which failed, however, due to low disease pressure. As a retest, 190 BC_1F_3 were randomly selected and self-pollinated and their $BC_1F_{3:4}$ families were evaluated in the greenhouse with two replications (3 plants in one pot for each replication) using the same protocol as described above. The 190 $BC_1F_{3:4}$ families were also evaluated in the field nurseries with hill plots (15 seeds/hill) in Fargo and Prosper, ND in the Summer of 2022. The experimental design was a lattice design with two replications in each location. Inoculum was applied using a grain-spawn inoculation method in the field nurseries. The autoclaved corn seed infected with four *F. graminearum* strains were evenly spread on the soil surface about three weeks prior to heading. The nurseries were misted for 3-5 min in 15-min intervals for 12 h daily, until 7 days after anthesis of the latest genotypes. Roughly ten spikes at anthesis were marked from each hill plot. Disease severity was visually scored using a 0-9 scale at 21 days post-anthesis as described in Table 2.2. Five checks, Alsen, Grano, ND2710, Riveland, and Wheaton were used.

Table 2.2. FHB severity visual score scale.

Score	Number of infected spikelet (IS)
0	0
1	1
2	1< IS ≤1/7 N
3	1/7N<IS≤2/7N
4	2/7N<IS≤3/7N
5	3/7N<IS≤4/7N
6	4/7N<IS≤5/7N
7	5/7N<IS≤6/7N
8	6/7N<IS<N
9	N

N=Total number of spikelets in one head.

In the Summer of 2021, we made the second-time crosses using four families (20S628, 20S760, 20S793, and 20S795) selected from the HRS-FHB-RS Cycle1 population with Riveland. A total of 350 BC_1F_1 progenies were evaluated for FHB severity in the greenhouse in the Spring of 2022 and the top 14 BC_1F_1 plants were selected and self-pollinated. A total of 280 BC_1F_2 progenies (20 plants per BC_1F_1 family) were evaluated for FHB severity in the greenhouse in the Summer of 2022 and the top 34 BC_1F_2 plants were selected and self-pollinated. A total of 680 BC_1F_3 progenies (20 plants per selected BC_1F_2 family) are currently being evaluated for FHB severity in the greenhouse (2022 Fall).

In the Fall of 2021, the top three S_1 families (21S047, 21S727, and 21S839) selected from the HRS-FHB-RS Cycle2 population were crossed to Riveland (third-time crosses). A total of 360 BC_1F_1 progenies were evaluated for FHB severity in the greenhouse in the Summer of 2022 and the top 15 plants were selected and self-pollinated. A total of 325 BC_1F_2 progenies (25 plants per selected BC1F1) are currently being evaluated for FHB severity in the greenhouse (2022 Fall).

The number of lines from each generation evaluated in the greenhouse and in the field nurseries and information about year and season of evaluation were listed in Table 2.3.

Table 2.3. The interspecific crosses, number of lines generated, evaluated, and selected at each generation in this study.

Cross	Generation	No. planted	Trial	FHB severity evaluation	No. selected
1	BC_1F_1	200	2021-Spring	No evaluation	155
	BC_1F_2	1925	2021-Fall	Evaluated in GH	34
	BC_1F_3	340	2022-Spring	Evaluated in GH but failed	190
	$BC_1F_{3:4}$	190	2022-Summer	Evaluated in GH and field	
2	BC_1F_1	350	2022-Spring	Evaluated in GH	14
	BC_1F_2	280	2022-Summer	Evaluated in GH	34
	BC_1F_3	680	2022-Fall	Evaluated in GH	
3	BC_1F_1	360	2022-Summer	Evaluated in GH	15
	BC_1F_2	325	2022-Fall	Evaluated in GH	

Phenotypic data analysis

For the evaluation of FHB severity in the greenhouse, we consider a combination of year and season as one trial. A total of three trials including 2021-Fall, 2022-Spring, and 2022-Summer were conducted. The best linear unbiased estimators (BLUEs) for FHB severity were estimated using the PROC MIXED procedure in SAS v.9.3 (SAS Institute, 2011). The statistical model was

$$y_{ij} = \mu + g_i + t_j + \varepsilon_{ij} \quad [2.1]$$

where y_{ij} is the vector of unadjusted phenotypes, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and t_j is the random effect of the j_{th} trial, and ε_{ij} is the random effect of the i_{th} genotype in the j_{th} trial. The variance components of error (σ^2) and genotype (σ^2_g) were estimated by considering all factors as random. The broad-sense heritability (H^2) was estimated as $\sigma^2_g/(\sigma^2 + \sigma^2_g)$.

For the phenotypic data of FHB severity, plant height, and days to flowering collected from the field nurseries for the 190 $BC_1F_{3:4}$ families, we performed a two-stage analysis. A combination of year and location was considered as one trial. There were two trials, 2022-Fargo and 2022-Prosper. In the first stage, the BLUEs were estimated for the entries within each trial. The statistical model was

$$y_{ijk} = \mu + g_i + r_j + b_k(r_j) + \varepsilon_{ijk} \quad [2.2]$$

where y_{ijk} is the vector of unadjusted phenotypes, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and r_j is the random effect of the j_{th} replication, and $b_k(r_j)$ is the random effect of the k_{th} block nested in the j_{th} replication.

The estimated BLUEs from each individual trial were then used for the second stage analysis to estimate BLUEs for all $BC_1F_{3:4}$ families across all trials. The statistical model was

$$y* = \mu + g_i + t_j + \varepsilon_{ij} \quad [2.3]$$

where $y*$ represents the estimated BLUEs from the first stage analysis, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and t_j is the random effect of the j_{th} trial. The estimated BLUEs were further used in genomic prediction analysis. The variance components of error (σ^2) and genotype (σ^2_g) were estimated by considering all factors as random. The broad-sense heritability (H^2) was estimated as $\sigma^2_g/(\sigma^2 + \sigma^2_g)$ for FHB severity, plant height, and days to flowering, respectively.

Results

Interspecific crosses were made three times between the durum wheat cultivar Riveland and resistant families selected from hard red spring wheat population. Their BC_1F_1, BC_1F_2, BC_1F_3, and $BC_1F_{3:4}$ progenies were evaluated for FHB severity in the greenhouse using a spray inoculation method. Broad sense heritability was estimated as 0.61. A wide range of FHB severity was found in all generations from all three times of crosses (Table 2.4).

Table 2.4. Means and ranges of FHB for the BC_1F_1, BC_1F_2, BC_1F_3, and $BC_1F_{3:4}$ progenies from the three times of interspecific crosses evaluated in the greenhouse.

Cross	Generation	FHB severity (Score of 0-9)
1	BC_1F_2	5.73 (0.00~8.31)
	$BC_1F_{3:4}$	6.99 (1.69~8.33)
2	BC_1F_1	6.65 (0.45~9.00)
	BC_1F_2	6.57 (1.21~8.33)
3	BC_1F_1	6.90 (0.50~8.33)
Check	Alsen	3.06
	Grano	7.06
	ND2710	1.41
	Riveland	6.18
	Wheaton	8.77

A total of 190 $BC_1F_{3:4}$ families from the first-time crosses were evaluated in field nurseries in Fargo and Prosper, ND in 2022. The broad sense heritability was 0.23, 0.71, and 0.55 for FHB severity, plant height, and days-to-flowering, respectively. FHB severity had an average score of 5.33 with a range of 2.89 to 9.00; plant height ranged from 57.7 to 105.6 cm; days-to-flowering ranged from 53 to 63 days (Table 2.5). A significant negative correlation between FHB severity and days-to-flowering was observed at Fargo field nursery (Figure 2.1). A significant negative correlation between FHB severity and plant height along with a positive correlation between plant height and days-to-flowering were observed at Prosper field nursery (Figure 2.2). Six $BC_1F_{3:4}$ families showed significantly lower FHB severity than Riveland, with their plant heights significantly shorter than Riveland. Days-to-flowering was not significantly different from Riveland (Table 2.6).

Table 2.5. Means and ranges of FHB severity (FHBsev), plant height (PH), and days to flowering (DTF) for the 190 $BC_1F_{3:4}$ families evaluated in Fargo and Prosper, ND in 2022

Population	**Trial**	**FHBsev (Score of 0-9)**	**PH (cm)**	**DTF (Day)**
$BC_1F_{3:4}$	2022-Fargo	5.62 (1.10~9.00)	78.2 (57.9~106.9)	58 (54~64)
$BC_1F_{3:4}$	2022-Prosper	7.19 (3.60~9.00)	74.9 (51.2~104.0)	59 (54~65)
$BC_1F_{3:4}$	All	6.03 (2.89~9.00)	81.5 (57.7~105.6)	57 (53~63)
Alsen		5.39	71.8	51
Grano		7.51	84.7	59
ND2710		3.67	87.0	50
Riveland		6.43	96.2	60
Wheaton		7.74	63.2	52

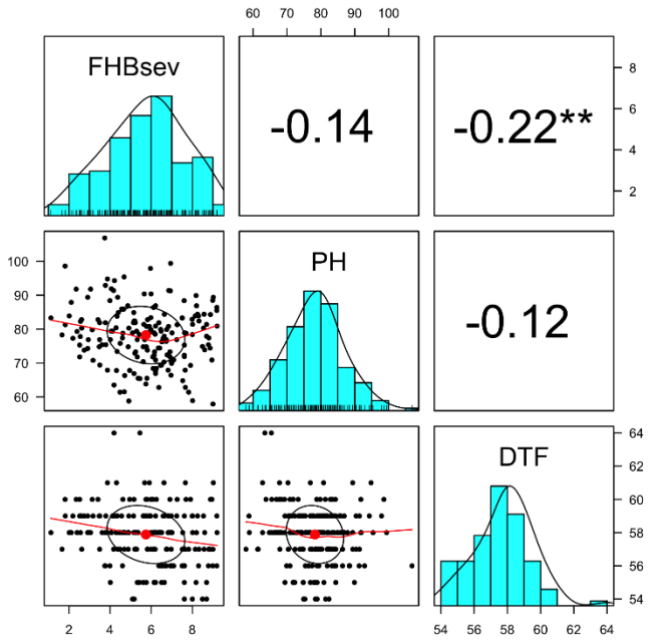

Figure 2.1. Distributions of the estimated BLUEs for FHB severity (FHBsev), plant height (PH), and days to flowering (DTF) in Fargo, ND in 2022 and Pearson correlations between the three traits.

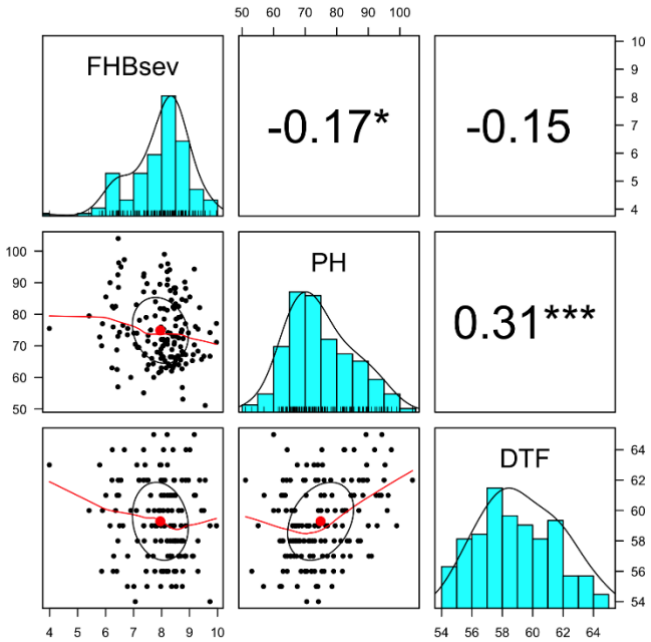

Figure 2.2. Distributions of the estimated BLUEs for FHB severity (FHBsev), plant height (PH), and days to flowering (DTF) in Prosper ND in 2022 and Pearson correlations between the three traits.

Table 2.6. FHB severity (FHBsev), plant height (PH), and days-to-flowering (DTF) of the six $BC_1F_{3:4}$ families with significantly lower FHB severity than Riveland.

Population	Entry	FHBsev (Score of 0-9)	PH (cm)	DTF (Day)
BC_1F_3	22S2259	4.15**	80.6**	55**
BC_1F_3	22S2393	3.13**	81.3**	59
BC_1F_3	22S2407	4.11**	83.3**	55**
BC_1F_3	22S2408	3.41**	88.0	57
BC_1F_3	22S2474	3.95**	94.6	58
BC_1F_3	22S2520	3.98**	89.3	58
	Riveland	6.43	96.2	60

Note: * significant at $p<0.05$; ** significant at $p<0.01$

Discussion

FHB resistance in wheat is a multifaceted trait controlled by many genes. Even a few of major QTL have been introgressed into durum wheat (Prat et al., 2017, Zhao, et al., 2018; Giancaspro et al., 2016, 2018), the effect of introgressed QTL considerably depends on the genetic background of durum wheat. This was evidence that the resistance expression of single major QTL in the tetraploid background was limited, and even totally inhibited, but the situation was ameliorated when combined with the other QTL (Somer et al., 2006; Zhao et al., 2018). Therefore, pyramiding more favorable QTL is an efficient way to improve FHB resistance in durum wheat. In NDSU HRS wheat breeding population, a diverse FHB resistance resource was found in 427 AYT lines (Liu et al., 2019). To increase the frequency of favorable alleles, we created an HRS wheat recurrent phenotypic selection population by crossing the selected top 10 resistant lines out of the 427 lines with another eight HRS wheat cultivars. The best resistant lines from each cycle of recurrent selection were used to cross and backcross with durum wheat cultivar, 'Riveland'. Following the evaluation of early generations of progenies, a set of $BC_1F_{3:4}$ lines were selected and further evaluated in field nurseries. Six $BC_1F_{3:4}$ families derived from first-time crosses showed significantly lower FHB severity than Riveland. We expect to identify

more resistant lines from the second- and third-time crosses. We will genotype the selected BC_1F_3 lines with 40K SNP array and compare them to the durum wheat recurrent selection population (see Chapter III). The genetically distinct lines will be integrated into the durum wheat recurrent selection population.

Pentaploid hybridization is an efficient strategy to transfer favorable alleles between bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* spp. *durum* Desf.) from each other. However, it has low pollen compatibility, poor seed quality, failed seedling development, and frequent sterility in F_1 hybrids (Padmanaban et al., 2017). Additionally, the number of fertile F_1 progenies is extremely different depending on the cross direction (Hexaploid/Tetraploid or Tetraploid/Hexaploid). Kihara (1982) proposed that the maternal parent should be from the higher ploidy level species to attain the highest number of fertile F_1 progenies from an interspecific cross. It was demonstrated in our study that all the pentaploid F_1 progenies were from the cross of Hexaploid/Tetraploid, and no progeny was generated from Tetraploid/Hexaploid. In the backcross, the pentaploid F_1 progenies were used as maternal parents as well. The species cytoplasmic specific (*scs*) genes located on chromosomes 1DL of *T. aestivum* (*scsae*) and 1A of *T. turgidum spp. durum* (Simons et al., 2003) express in interspecific hybrid to retain the nuclear-cytoplasmic interaction (NCI) and provide appropriate vigor and viability to the hybrid lines (Maan, 1992). More studies need to be done on the NCI mechanism in pentaploid hybrid wheat.

Creating segregating populations between resistant sources and elite breeding lines followed by phenotypic screening is a common way to develop a locally adapted FHB resistant germplasm in wheat. A phenotypic evaluation method that can accurately assess FHB resistance for a large number of progenies is crucial. FHB disease development is highly dependent on high

temperature and humidity during flowering, so artificial inoculation is generally recommended. Testing DON accumulation (type III resistance), damaged kernel (type IV resistance), and grain yield loss (type V resistance) are costly, while visually scoring of FHB incidence (percentage of infected heads), FHB severity (averaged percentage of infected spikelets per plant or plot) is much easier. It was found that the visual score of FHB severity was significantly correlated with DON accumulation and grain yield loss (Mesterházy et al., 1998; Mesterházy 2003) and therefore has been widely adopted. The visual score following single-spikelet injection inoculation can accurately access the spread (type II resistance). However, single-spikelet injection is time/labor intensive and not practical for large scale of germplasm screening. To evaluate a large number of lines, spray or grain-spawn inoculation in the field nursery is commonly employed, where the visual score of FHB severity reflects both type I and type II resistances (Dill-Macky, 2003).

In this study, we used a spray inoculation protocol and evaluated early generations of heterozygous progenies from the interspecific crosses in the greenhouse. A high broad sense heritability for FHB severity was found with the spray inoculation protocol in this study. A total of 190 $BC_1F_{3:4}$ families were evaluated in both the greenhouse using the modified spray inoculation method and the field nurseries with classic grain-spawn inoculation method, between which a significant correlation for the FHB severity was observed (APPENDIX A). This body of evidence suggests that the spray inoculation is an effective approach for evaluating FHB severity in the greenhouse. Compared to single-spikelet injection, it is much easier to operate and enables us to evaluate more progenies at a time. Another advantage of this inoculation method is that it enables us to evaluate single plants, which is difficult to do with spray/grain-spawn inoculation in the field nursery.

Considered together, the results from this study suggest that interspecific cross with HRS wheat can effectively allow introgression of resistant resources and improve FHB resistance in durum wheat. Combined with spray inoculation of FHB screening method in the greenhouse, the selection efficiency can be increased than by single-spikelet injection.

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CHAPTER III. RECURRENT SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN A DURUM WHEAT POPULATION

Abstract

Fusarium Head Blight (FHB) is a devastating disease that can cause severe loss of grain yield and quality of durum wheat in the northern Great Plains of the U.S. FHB resistance in wheat is a complex trait controlled by many genes. Recurrent selection is an effective way to increase frequencies of favorable resistant alleles and to develop improved germplasm. In this study, four cycles of recurrent phenotypic selection were conducted for reducing FHB severity from 2019 to 2022 in a durum wheat population derived from intercrossing of 15 elite cultivars and breeding lines. The FHB severity was reduced 34.5% from Cycle 0 to Cycle 3 population. Significant negative correlations were found between FHB severity and both plant height and days to flowering in Cycle 0, Cycle 3 populations. Genomic selection can speed up selection and increase genetic gain in terms of time and cost. A total of 284 S0 parents from the Cycle 2 and Cycle 3 populations were genotyped using 90K SNP array and obtained 2,706 SNP markers. Using ridge regression best linear unbiased prediction (rrBLUP), the prediction accuracy for FHB severity was 0.53 with cross-validation. Our results indicate that recurrent phenotypic selection can improve FHB resistance in durum wheat. Implementing genomic-assisted selection in the recurrent selection is practical to accelerate genetic improvement.

Introduction

Durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn., $2n = 4x = 28$, AABB] is an important food crop and is mainly used for producing pasta. Durum wheat has been grown on approximately 13 million hectares globally with an annual production of 38 million tons (Kadkol and Sissons, 2016). Major durum wheat growing regions include Mediterranean basin countries

and the northern Great Plains of the United States and Canada. In the USA, durum wheat was grown on 0.77 million hectares with annual production of 1.7 million tons from 2017 to 2019 (USDA-NASS). Over 53% of which were grown in North Dakota with an annual production of 0.9 million tons (https://usda.library.cornell.edu/concern/publications/tm70mv177).

Since the early 1990s, epidemic of Fusarium head blight (FHB) has frequently occurred in the northern Great Plains and caused massive economic losses (Gilbert et al., 2000; McMullen, 2012). FHB is a fungal disease caused by *Fusarium* species. The pathogen spores infect spikes during flowering and spread within infected spikes, which can result in reduced grain yield, decreased thousand kernel weight, and lower malting and baking quality (Dubin et al., 1997; Kottapalli et al., 2005). In addition, mycotoxin deoxynivalenol (DON) produced by the pathogen has adverse effects on the health of human and livestock, causing feed refusal, vomiting, and suppressed immune functions (Rotter, 1996).

Cultivating resistant cultivars is a key strategy to alleviate the loss caused by FHB. However, the lack of FHB resistant resources has challenged breeding of resistant cultivars in durum wheat. In the past 20 years, FHB resistant germplasm improvement in durum wheat has been focused on utilizing of resistant resources found in hexaploid bread wheat (*T. aestivum* L.) and in tetraploid relatives such as wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn. ex Asch. & Graebner) Thell.], cultivated emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell.] and Persian wheat [*T. turgidum* ssp. *carthlicum* (Nevski) Á. Löve & D. Löve]). A number of durum wheat lines with improved FHB resistance have been developed from the crosses between the FHB resistant tetraploid wheat relatives and elite durum wheat lines, from which QTL with moderate effects were identified (Buerstmayr et al. 2012, 2013; Somer et al. 2006; Zhang et al., 2014). Different from tetraploid wheat, some high FHB resistant lines have

been identified in hexaploid bread wheat like Sumai 3 and several major QTL including *Fhb1* have been commonly identified in different studies and mapping populations (Anderson et al., 2001; Bai et al., 1999; Jia et al., 2018; Ma et al., 2020; Waldron et al., 1999). Those resistant germplasm and major QTL have been widely used in bread wheat breeding and resulted in numerous cultivars released (Zhu et al., 2019). Recently, the well-known hexaploid bread wheat major QTL *Fhb1* have been successfully introduced into three European durum wheat cultivars Karur, Durobonus, and SZD1029K (Prat et al., 2017). Zhao et al. (2018) reported that two QTL on chromosomes 5A and 7A were introgressed into durum wheat cultivar Joppa from bread wheat resistant line PI277012. A gene related to cell well structure was introgressed into durum wheat from a bread wheat resistant line and increased resistance to FHB (Giancaspro et al., 2016, 2018). Those durum wheat introgression lines derived from tetraploid wheat relatives and hexaploid bread wheat contribute a significant step forward to improve FHB resistance in durum wheat.

FHB resistance is a complex quantitative trait controlled by many genes and integration of many resistant QTL with major and medium even minor effects will provide high and durable resistance. Recurrent phenotypic selection is an integral tool to improve complex traits by increasing frequency of favorable alleles within a population. It has been demonstrated that recurrent selection can effectively improve FHB resistance in hexaploid bread wheat (Ittu et al., 1997; Milus, et al., 2001; Yang et al., 2000) and barley (Therrien, 2005).

Recurrent phenotypic selection for FHB resistance in wheat is laborious and time-consuming due to heavy inter-mating work, high cost of field nursery evaluation, and long growth period. In the northern Great Plains, it takes one year to conduct one cycle of selection, with evaluation in the field nursery in the summer and recombination in the greenhouse in the

winter. Implementing genomic selection (GS) in recurrent selection can enhance genetic improvement by shortening one year to four month per cycle and/or increasing selection intensity and accuracy. GS is to select the best lines based on the genomic estimated breeding values (GEBVs) calculated by a prediction model based on genome wide markers, which can capture most genes involved in a complex trait (Meuwissen et al., 2001). The genomic prediction model can be developed using phenotypic data and marker data collected from a training population. Implementing GS in recurrent selection has been evaluated for resistance to Fusarium ear rot in maize (Holland et al., 2020) and popping expansion/grain yield in popcorn (Schwantes et a., 2018) and is promising to accelerate genetic improvement.

We have developed a base population by using elite durum wheat breeding lines/cultivars and advanced durum wheat FHB resistant lines as founders. The objectives of this project are to (1) improve FHB resistance through recurrent phenotypic selection in this durum wheat population; (2) develop durum wheat FHB resistant germplasm adapted to the northern Great Plains; and (3) develop GS model and explore if implementing GS in recurrent selection can enhance genetic improvement of FHB resistance.

Materials and Methods

Development of a base population

A total of 10 durum wheat FHB resistant lines were chosen. The 10 resistant lines were selected from crosses between durum wheat cultivars and resistant accessions of tetraploid wheat relatives or hexaploid bread wheat (Dr. Steven Xu, unpublished data). The durum wheat cultivars used in the original crosses were Ben (Elias and Miller, 1998), Carpio (Elias et al., 2015), Divide (Elias and Manthey, 2007), Lebsock (Elias et al., 2001), and Maier (Elias and Miller, 2000). The

original tetraploid wheat FHB resistant accessions were PI41025, PI272527, PI61102, and PI94728. The common wheat FHB resistant accessions were Sumai 3 and PI277012 (Figure 3.1).

We created a base population by crossing the 10 durum wheat FHB resistant lines with five elite durum wheat breeding lines/cultivars followed by two generations of random mating. The five elite durum wheat breeding lines/cultivars were D12345, D13671, D13761, Riveland (Elias & Manthey, 2019), and Strongfield (Clarke et al., 2006). First, each of the five elite durum wheat breeding lines/cultivars was crossed to two FHB resistant lines, which resulted in 10 F_1s. Then, random mating was conducted using the 10 F_1s, where bulked pollens from the 10 F_1s were applied to emasculated spikes for each F_1, and three planting dates were used to minimize assortative mating due to flowering time variation. The second generation of random mating was conducted by using the seeds derived from the first random mating, where a bulk of 20 seeds harvested from each F_1 were planted and randomly intercrossed. After two generations of random mating, 190 S_0 seeds were planted and self-pollinated in the greenhouse, and the resulted 190 $S_{0:1}$ families served as the based population (C0) (Figure 3.1).

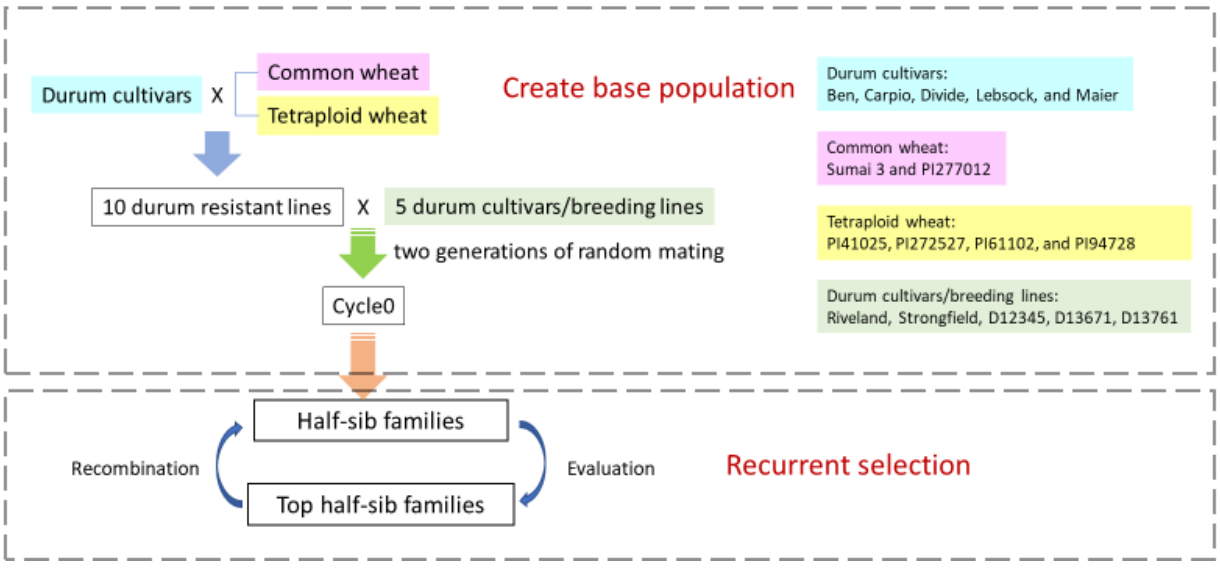

Figure 3.1. Scheme of development of base population and recurrent selection for FHB resistance improvement in durum wheat.

Evaluation, selection, and recombination within each cycle

In 2019, the 190 $S_{0:1}$ families of the C0 population were grown in hill plots (15 seeds/hill) in two locations, Fargo and Prosper, ND. The experimental design was a lattice design with two replications at each location. Hard red spring wheat breeding lines/cultivars ND2710 (Frohberg et al., 2004), Alsen (Frohberg et al., 2006), and Wheaton (Busch et al., 1984) and durum wheat cultivars Carpio, Grano (Elias et al., 2021), and Riveland were used as checks. Inoculum was applied using a grain-spawn inoculation method in the field nurseries. The autoclaved corn seed infected with four *F. graminearum* strains were evenly spread on the soil surface about three weeks prior to heading. The nurseries were misted for 3-5 min in 15-min intervals for 12 h daily, until 7 days after anthesis of the latest genotypes. About ten spikes at anthesis were marked from each hill plot. FHB severity was visually scored using a 0-9 scale at 21 days post-anthesis as described in Table 2.2. Plant height was measured in centimeters from the ground to the top of the spikes excluding awns for each hill plot. Days-to-flowering was measured in days from planting to 50% anthesis.

Best linear unbiased estimators (BLUEs) for FHB severity, plant height, and days-to-flowing were estimated for the 190 $S_{0:1}$ families at each field nursery (a combination of year and location) using the PROC MIXED procedure in SAS v.9.3 (SAS Institute, 2011). The statistic model was

$$y = \mu + g_i + r_j + b_k(r_i) + \varepsilon_{ijk} \quad [3.1]$$

where y is the vector of unadjusted phenotypes, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and r_j is the random effect of the j_{th} replication, and $b_k(r_i)$ is the random effect of the k_{th} block nested in the j_{th} replication. The variance components of error (σ^2) and genotype (σ^2_g)

were estimated by considering all factors as random. The broad-sense heritability (H^2) was estimated as $\sigma^2_g/(\sigma^2 + \sigma^2_g)$.

Top 19 resistant $S_{0:1}$ families were selected based on the mean estimated BLUEs from the two locations. Their remnant seeds were planted (10 plants per family) and randomly intercrossed in the greenhouse in 2019 winter. The seeds harvested from the 19 families were equivalently bulked, from which 240 S_0 seeds were planted and self-pollinated in the greenhouse in 2020 spring. The 240 $S_{0:1}$ families served as the C1 population and were evaluated in field nurseries in the summer of 2020. We conducted three cycles of phenotypic selection from 2019 to 2022 using the same procedure, one cycle selection per year. In each cycle of selection, the top ~10% of the $S_{0:1}$ families were selected and intercrossed to generate the subsequent cycle population.

Phenotypic data analysis across cycles and field nurseries

The estimated BLUEs for FHB severity, plant height, and days-to-flowering from each individual field nursery were used for the second stage analysis to estimate BLUEs for all four cycles of $S_{0:1}$ families across all environments. The field nurseries in Fargo in 2019 (2019-Fargo) and in Fargo in 2021 (2021-Fargo) with broad-sense heritability of less than 0.1 for FHB severity were removed from the second stage analysis.

The statistical model for the second stage analysis was

$$y* = \mu + g_i + t_j + \varepsilon_{ij} \quad [3.2]$$

where $y*$ represents the estimated BLUEs from the first stage analysis, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and t_j is the random effect of the j_{th} nursery. The estimated BLUEs were further used in genomic prediction analysis.

Genotyping

DNA of the S_0 parents from the C2 and C3 populations were isolated with the Wizard Genomic DNA Purification Kit (A1125; Promega) per the manufacturer's instructions and quantified with a Quant-iT PicoGreen dsDNA assay kit (P7589; Thermo Fisher Scientific). A total of 284 S_0 parents were genotyped using Illumina Infinium 90K SNP array (Wang et al., 2014). SNP genotype calling was performed using the software GenomeStudio (Romm et al., 2013). In total, 2,706 SNP markers were obtained with minor allele frequency greater than 1% and missing values less than 1%.

Using the marker data and estimated BLUEs for the 284 S_0 parents, additive variance component (*Va*) and error variance component (*Ve*) were calculated with the *mixed.solve* function in R package rrBLUP (Endelman, 2011). Genomic heritability (h^2) was calculated as *Va*/(*Va* + *Ve*).

Population structure and genome wide association mapping

Principal component (PC) analysis was conducted with the 2,706 SNPs using TASSEL v.5 (Bradbury et al., 2007). Based on the scree plots (APPENDIX B), the first three PCs were used for model-based cluster analysis with R package Mclust (Fraley et al., 2012).

Genome wide association analysis was performed using TASSEL v.5 (Bradbury et al., 2007). The first three PCs were chosen as covariates to capture population structure in the association analysis. A centered kinship (**K**) matrix was calculated based on the 2,706 SNPs using TASSEL (Bradbury et al., 2007). Four statistical models were tested: (i) simple association analysis using general linear model (naïve model); (ii) general linear model including the first three PCs as covariates (**P** model); (iii) linear mixed model including kinship matrix (**K** model); and (iv) linear mixed model including population structure and kinship matrix (**PK** model). The

mean of the squared difference (MSD) between observed and expected *p*-values of all SNP markers was estimated for each model. The best model for each trait was determined as the model returning the smallest MSD value. The false discovery rate (FDR) was calculated using the R function *p.adjust* (method = *fdr*; Benjamini and Hochberg, 1995). Significance of marker-trait association is defined by FDR as a *q*-value < 0.01.

Development and validation of GS model

Genomic prediction was evaluated with rrBLUP using R package rrBLUP (Endelman, 2011). Prediction accuracies were cross-validated, where 90% individuals were randomly selected as the training population and the remaining 10% individuals were used to validate the genomic prediction accuracy. Genomic prediction accuracy was estimated as the Pearson correlation (*r*) between GEBVs and BLUEs of phenotypic values. Random sampling of training and validation sets was repeated 100 times and the mean of *r* was defined as the genomic prediction accuracy.

Results

Phenotypic data

Means and ranges of FHB severity, plant height, and days-to-heading of the C0, C1, C2, C3 populations and check cultivars were listed in Table 3.1. A wide range of FHB severity was observed in all four cycles of populations. The mean of FHB severity was decreased from C0 (7.69) to C3 (5.04). The means of plant height and days-to-heading did not significantly change across the populations. C0 and C1 had no families with the significant lower FHB severity than the durum wheat check cultivar Riveland. One family from C2 and five families from C3 showed significantly lower FHB severity than Riveland at *p-value* < 0.05 (Table 3.2).

Table 3.1. Means and ranges of the estimated BLUEs for FHB severity (FHBsev), plant height (PH), days-to-flowering (DTF) in the C0, C1, C2, and C3 populations.

Population (No. families)	Trial (Planting date)	FHBsev (Score of 0-9)	PH (cm)	DTF (Day)
C0 (188)	2019-Fargo (May 30, 2019)	7.69 (4.82~9.00)	89.6 (60.7~118.2)	56 (52~62)
C1 (236)	2020-Fargo (May 12, 2020) 2020-Prosper (May 22, 2020)	6.58 (3.65~7.50)	84.1 (54.1~106.4)	57 (51~63)
C2 (190)	2021-Prosper (May 19, 2021)	5.76 (3.58~7.45)	87.9 (44.5~108.0)	57 (50~64)
C3 (123)	2022-Prosper (May 26, 2022)	5.04 (2.54~6.99)	88.3 (62.8~116.6)	57 (52~63)
Alsen		5.39	71.8	51
Carpio		8.89	88.6	58
Grano		7.51	84.7	59
ND2710		3.67	87.0	50
Riveland		6.43	96.2	60
Wheaton		7.74	63.2	52

Table 3.2. FHB severity (FHBsev), plant height (PH), and days-to-flowering (DTF) of the six $S_{0:1}$ families with significantly lower FHB severity than Riveland.

Population	Family	FHBsev (Scores of 0-9)	PH (cm)	DTF (Days)
C2	21S1521	4.38*	92.3	58
C3	22S1664	3.82*	88.6	61
C3	22S1573	3.65*	96.6	59
C3	22S1550	3.32**	101.6	60
C3	22S1548	4.16*	85.6	60
C3	22S1581	3.12**	94.6	58
Check	Riveland	6.43	96.2	60

Note: * significant at $p<0.05$; ** significant at $p<0.01$

Pearson correlation coefficients among the three traits in C0, C1, C2, and C3 were exhibited in APPENDIX C, D, E, F, respectively. Significant negative correlations between FHB severity and plant height were observed in C0, C2, and C3, but not in C1. Significant negative correlations between FHB severity and days-to-heading were observed in C0 and C3, but not in C1 or C2. Plant height was positively correlated with days-to-heading in all populations.

Population structure

A total of 284 S_0 parents from the C2 and C3 populations were genotyped using 90K SNP array. PC analysis was performed using 2,706 SNP markers. The first three PCs explained 19.4%, 14.0%, and 7.0% of the total variation, respectively. Model-based cluster analysis with the first three PCs suggested that there were nine subgroups (APPENDIX G). The parents from C2 and C3 were intermixed among the subgroups (APPENDIX G), suggesting selection for the FHB severity did not lead toward any specific subgroups.

Genome wide association mapping

Genome wide association mapping was conducted for FHB severity with four statistical models: naïve, P, K, and PK models. Based on MSD values, the PK model was the best. The significant marker-trait association was determined by FDR as a *q*-value smaller than 0.01. No SNP was identified for FHB severity.

Genomic prediction

The genomic heritability was estimated as 0.74, 0.88, and 0.79 for FHB severity, plant height, and days to flowering, respectively. The prediction accuracies with cross-validation were 0.53, 0.67, and 0.57 for FHB severity, plant height, and days to flowering, respectively.

Discussion

Improvement of FHB resistance through recurrent phenotypic selection

We have developed a durum wheat population using diverse resistant resources and conducted three cycles of phenotypic selection for FHB severity from 2019 to 2022. The mean of FHB severity decreased about 34% from C0 to C3. Consistent with previous studies in hexaploid bread wheat (Jiang et al., 1994; Yang et al., 2000), the result suggested that recurrent phenotypic selection could also effectively improve FHB resistance in durum wheat. A total of six families

from C2 and C3 showed significantly lower FHB severity than the moderate susceptible durum wheat cultivar, Riveland. The six families were not significantly taller than Riveland and were not significantly flowering later than Riveland either. The six $S_{0:1}$ families are promising germplasm to conduct further selection in their progenies and to be integrated into the NDSU durum wheat breeding population.

We observed significant correlations between FHB severity and plant height in all four cycles of populations except for C1 evaluated in 2020. FHB resistance was observed frequently associated with plant height, especially under field evaluation using grain-spawn inoculation (Buerstmayr et al., 2020), where fungal spores residing on soil surface and spikes of shorter plants have higher chance to be reached and infected (Jenkinson and Parry, 1994). Besides, relatively higher humidity and temperate could promote disease development for shorter plants (Buerstmayr and Buerstmayr, 2016; Hilton et al., 1999; Jones et al., 2018).

Some studies reported that days-to-flowering was associated with FHB severity (Buerstmayr et al., 2012; Liu et al., 2013; Peterson et al., 2016), while others found no correlation or inconsistent correlations across environments/populations between the two traits (Buerstmayr and Buerstmayr, 2015; Chu et al., 2011; Prat et al., 2017). Fungal spores infect spikes during flowering and spread within the infected spike, so the temperature and humidity during/after flowering can affect infection and disease development. In this study, significant negative correlations between days-to-flowering and FHB severity were observed in C0 evaluated in 2019 and C3 in 2022, but not found in C1 evaluated in 2020 nor C2 in 2021. North Dakota has a short growing season, where the temperature generally decreases after late July. Delayed planting could cause flowering time of late matured families postponed to late July and exposed to relative lower disease pressure. Therefore, we believed that late planting dates in

2019 and 2022 (Table 3.1) might have caused uneven disease pressure for the early-flowered and late-flowered families and explained the observed correlations between FHB severity and days-to-flowering.

Increase in plant height can cause lodging while increase in days-to-flowering may result in immature seeds harvested in the northern Great Plains. To avoid selection towards increased plant height and days-to-flowering, any of the selected top 10% families within a cycle that were taller than Riveland or flowered later than Riveland were excluded for recombination and replaced by the next available families in line. This could partially explain why there was a significant change in plant height and days-to-flowering from C0 to C3.

Genome wide association mapping and genomic prediction

While the genomic heritability was estimated as 0.74 for FHB severity, no major QTL was identified. This indicated that many genes with moderate or minor effects were involved in FHB severity in this durum wheat recurrent selection population. Consistent with previous GS studies in wheat (Arruda et al., 2015, 2016; Dong et al., 2018; Miedaner et al., 2017; Mirdita et al., 2015; Rutkoski et al., 2012; Schulthess et al., 2018; Steiner et al., 2019), we found a moderate prediction accuracy of 0.53 for FHB severity in this durum wheat population.

There are multiple ways to implement GS in the recurrent selection. One is genomics-assisted recurrent selection to increase selection intensity. For example, a large number of S_0 plants (e.g., 1000 plants) from the C4 population are genotyped and 100 of them are phenotyped using their $S_{0:1}$ families in field nurseries; then using the updated prediction model by adding the 100 $S_{0:1}$ families in the current training population (C2 and C3) to predict GEBVs of all 1000 S_0 plants and make selection accordingly (Figure 3.1). Only one cycle of genomics-assisted

selection can be conducted in a year but can increase selection intensity (about 5-fold if genotyping 1,000 S_0 plants) compared to the traditional phenotypic selection.

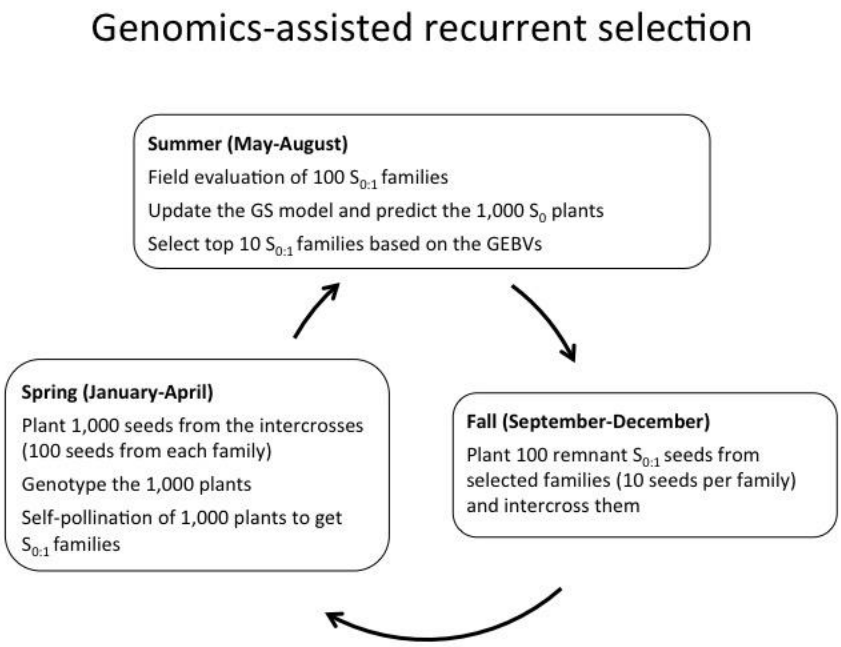

Figure 3.2. Genomics-assisted recurrent selection for FHB severity.

Another way to implement GS is genomic recurrent selection that can shorten selection cycle. For example, a number of S_0 plants (e.g., 200 S_0 plants) from C4 are genotyped and their GEBVs are predicted using the current GS model; top 10% S_0 plants are selected at seedling stage based on the GEBVs and recombined to generate the next cycle population; three cycles of selection can be done within one year. At the same time, a number of $S_{0:1}$ families are evaluated for FHB severity in field nurseries in the summer for updating the GS model each year (Figure 3.2). Genomic recurrent selection can potentially accelerate genetic improvement more effectively than genomics-assisted recurrent selection, but requires a GS model with high and stable prediction accuracy. Given the moderate prediction accuracy from the current genomic prediction model, genomics-assisted recurrent selection may be more practical. As more families

are evaluated at more environments and added to the training population, prediction accuracy and stability will keep increasing to make genomic recurrent selection practical in the future for FHB resistance improvement in the durum wheat population.

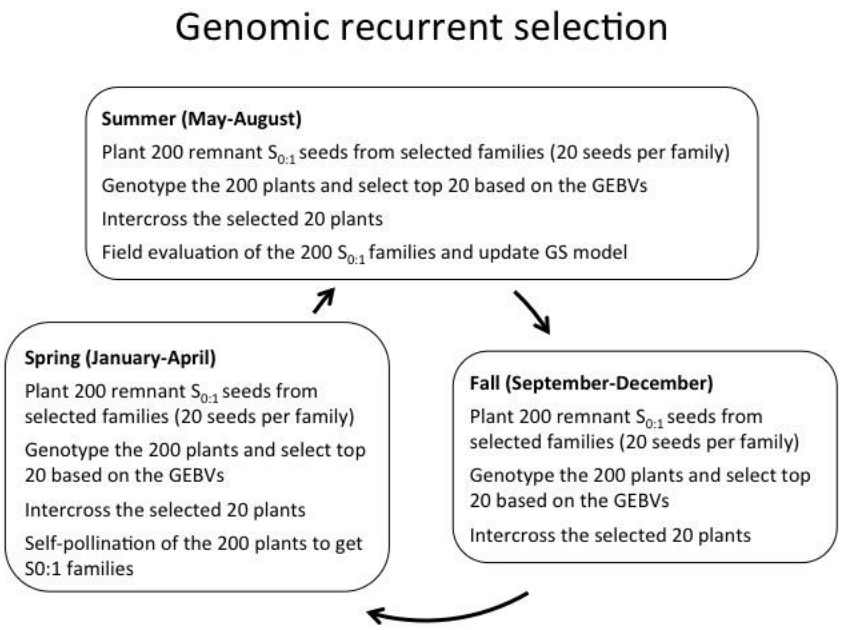

Figure 3.3. Genomic recurrent selection for FHB severity.

We observed that FHB severity was correlated with plant height and days-to-flowering in this durum wheat population. Selection based on FHB severity only may lead to increases in plant height and days-to-flowering, which may further cause lodging and yield loss. Multiple-traits GS selection should be considered (independent culling selection or index selection) when implementing GS in recurrent selection.

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CHAPTER IV. GENOMIC PREDICTION OF FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT BREEDING POPULATIONS

Abstract

Fusarium Head Blight (FHB) is a devastating fungal disease of wheat worldwide. It is documented that FHB resistance is a complex trait controlled by many genes and shows significant interactions with environments. Phenotypic selection on late generations of breeding lines under replicated field trials is commonly used to improve FHB resistance in wheat breeding programs; however, it is costly and time-consuming. Genomic selection (GS) can enhance genetic improvement of complex traits by shortening breeding cycles as well as increasing selection intensity and accuracy and has been widely adopted in animal and plant breeding. In this study, we developed a genomic prediction model for FHB severity using 588 breeding lines from the North Dakota State University (NDSU) durum wheat breeding program. The phenotypic data of FHB severity was collected from 62 unbalanced field trials at three locations from 2012 to 2019. The 588 breeding lines were genotyped using genotyping-by-sequencing (GBS), where a total of 37,752 SNP markers were obtained. Genomic heritability of FHB severity was estimated at 0.72. The genomic prediction accuracy was 0.53 based on a ten-fold cross-validation. Using previous breeding lines as the training population, the forward prediction accuracy was 0.47 for newly developed and untested breeding lines. The results suggested that GS on early generations of breeding lines could enhance FHB resistance improvement in durum wheat breeding program.

Introduction

Fusarium head blight (FHB) is a devastating fungal disease of wheat worldwide. FHB is caused by multiple fungal species, including *Fusarium graminearum*, *F. culmorum* and *F.*

avenaceum (Parry et al., 1995; Xu et al., 2005). Among them, *F. graminearum* is the major species that affects wheat production in North America (Gale, 2003; Shaner, 2003). In the last two decades, FHB epidemics have frequently occurred in the northern Great Plains of the United States (McMullen et al., 1997, 2012; McMullen, 2003; Nganje et al., 2004) and Canada (Gilbert et al., 1994) and caused massive economic losses. Furthermore, the mycotoxin deoxynivalenol (DON) produced by the pathogen can cause symptoms such as diarrhea, nausea, vomiting, and weight loss in humans and livestock (FDA 2010; Sobrova et al., 2010) .

Developing and growing resistant cultivars is the most effective and environmentally friendly approach to mitigate the effects of FHB. In durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn., $2n = 4x = 28$, AABB], however, this is challenging due to the lack of resistant resources. Thousands of durum wheat breeding lines and landrace accessions have been evaluated for FHB resistance; a few accessions with moderate resistance were reported (Ban et al., 2005; Elias et al., 2005; Huhn et al., 2012; Talas et al., 2011). The scarcity of FHB resistance sources in durum wheat could be due to most of them being derived from the warm and summer-dry Mediterranean basin (Ban et al., 2005), where there was little FHB pressure. Screening for resistance sources in tetraploid wheat relatives including cultivated emmer (*T. turgidum* subsp. *dicoccum*), wild emmer wheat (*T. turgidum* subsp. *dicoccoides*), and Persian wheat (*T. turgidum* subsp. *carthlicum*) discovered more moderate resistance lines like PI41025, PI 272527, Blackbird, etc. (Buerstmayr et al., 2003; Miller et al., 1998; Oliver et al., 2007, 2008). In order to introgress resistant alleles from exotic lines into elite breeding lines, once major QTL are identified, marker-assisted selection (MAS) is an effective and cost-efficient approach while minimizing linkage drags for other important traits. Numerous FHB resistance QTL have been identified in the tetraploid wheat accessions via QTL mapping (Buerstmayr et al., 2012, 2013;

Ghavami et al., 2011; Ruan et al., 2012; Somers et al., 2006; Zhang et al., 2014). Unfortunately, most of the QTL showed minor to moderate effects and few of them have been used for MAS (Prat et al., 2014).

Compared to durum wheat, breeding for FHB resistance in hexaploid bread wheat (*Triticum aestivum* L.; 2*n* = 6*x* = 42, AABBDD) is much more successful, where numerous FHB resistant cultivars have been developed and released. This is mainly attributable to the accessions identified with high FHB resistance, like Sumai 3 and Wangsuibai (Ma et al., 2020; Prat et al., 2014), from which a few major QTL with large effect were identified and widely used in bread wheat breeding populations. A recent study reported that the bread wheat major QTL, *Fhb1* was successfully introgressed into durum wheat and explained 5%, 11%, and 14% of total phenotypic variance in three mapping populations derived from *Fhb1* introgressed lines crossed with three durum wheat cultivars, respectively (Prat et al., 2017). Another major QTL *Qfhb.ndwp-5A* was introgressed into durum wheat from bread wheat resistant line PI277012, which explained 10-19% of FHB severity variation and 7% of DON content variation (Chu et al., 2011; Zhao et al., 2018). Those newly developed germplasms have broadened genetic diversity of FHB resistance in durum wheat breeding pool and promise to contribute to the development of resistant cultivars.

Previous genetic mapping studies documented that FHB resistance in wheat is a complex trait controlled by many genes (Buerstmayr et al., 2020; Haile et al., 2019; Ma et al., 2020). In China, some FHB resistant bread wheat cultivars were developed using parents with neither high levels of resistance nor presence of major QTL (Liu et al., 1992). In the USA, soft winter wheat cultivars with good FHB resistance, such as Ernie (McKendry et al., 1995) and Bess (McKendray et al., 2007), were developed by accumulating native resistance genes present in

local breeding lines through phenotypic selection (McKendry, 2008). A complementary approach to develop FHB resistant cultivars is to integrate more unknown resistance genes with moderate and even minor effects. Phenotypic selection is an effective way to improve complex trait and is commonly used for FHB resistance improvement in wheat breeding programs. However, due to the interaction of FHB resistance with environments, phenotypic evaluation is generally conducted on late generation breeding lines under replicated field trials with artificial inoculation across multiple years and at multiple locations, which makes the process costly and time-consuming.

Genomic selection (GS) is an approach that relies on genomic estimated breeding values (GEBVs) predicted from genome wide markers, which can capture most genes involved in a complex trait (Meuwissen et al., 2001). GS can enhance genetic improvement for complex traits by shortening breeding cycles as well as increasing selection intensity and accuracy compared to phenotypic selection (Heffner et al., 2010). In GS, a prediction model is developed using phenotypic data and marker data collected from a training population; then the resulted model, fed with marker data from the population under selection, is used to predict GEBVs. As genotyping technologies advance, GS has been increasingly adopted in animal and plant breeding (Gaffney et al., 2015; Garcia-Ruiz et al., 2016; Guzman et al., 2016; Meuwissen et al., 2016). Genomic prediction for FHB resistance has been widely investigated in bread wheat (Arruda et al., 2015, 2016; Dong et al., 2018; Liu et al., 2019; Mirdita et al., 2015; Rutkoski et al., 2012; Schulthess et al., 2018), where prediction accuracies of 0.22 to 0.60 were reported for FHB severity. A few studies have also attempted genomic prediction of FHB resistance in durum wheat. Miedaner et al. (2017) reported a genomic prediction accuracy of 0.70 for FHB severity using a durum wheat diversity panel comprised of 170 winter and 14 spring types. Steiner et al.

(2019) reported a prediction accuracy of 0.39 for FHB severity using a collection of 288 international elite cultivars. Previous empirical studies indicated that a close genetic relatedness between training population and selection population is key to achieve high genomic prediction accuracy (Brandariz and Bernardo, 2019; Crossa et al., 2014; Lorenz and Smith, 2015). Therefore, using breeding populations from an active breeding program for genomic prediction would be ideal. The objectives of this study were to (1) investigate genetic variation and genetic structure of FHB resistance in the NDSU durum wheat breeding population; and (2) to develop a genomic prediction model for FHB resistance and assess its prediction accuracy.

Materials and Methods

Plant materials and genotyping

In a previous study, a total of 1,184 $F_{4:7}$ breeding lines from the 2012-2016 advanced yield trials (AYTs) of the NDSU durum wheat breeding program were used for evaluating genomic prediction of grain/semolina quality related traits (Fiedler et al., 2017). The 1,184 breeding lines had been genotyped using genotyping-by-sequencing (GBS). Using the same protocol, we later genotyped 469 $F_{4:7}$ breeding lines from 2017 and 2018 AYTs. Using the raw sequence data from all 1,653 AYT lines (2012-2018 AYTs), we performed SNP discovery and genotype calling with the same method as described in Fiedler et al. (2017). SNP markers were filtered with an individual read depth > 2, minor allele frequency (MAF) > 0.05, and missing data < 50%. After filtering, a total of 37,752 SNP markers were obtained.

Field experimental design and phenotyping of FHB severity

In the NDSU durum wheat breeding program, a subset of breeding lines from preliminary yield trial (PYT), AYT, elite durum advanced trial (EDA), and uniform regional durum nursery (URDN) were also evaluated for FHB severity each year. A total of 2,570 breeding lines plus 14

check cultivars were evaluated for FHB severity in the FHB field nurseries from 2012 to 2019, in three locations, Carrington, Langdon, and Prosper, ND (APPENDIX H). Within each FHB field nursery (a combination of year and location), the breeding lines from different yield trials of PYT, AYT, EDA, and URDN were planted into separate FHB trials, which resulted in 62 FHB trials (a combination of year, location, and original yield trial) within 17 FHB field nurseries (APPENDIX H).

Out of the 2,570 breeding lines, 588 were from the 1,653 2012-2018 AYT lines that were genotyped with GBS as mentioned above and used in this study for investigating genomic prediction accuracy of FHB severity. The numbers of breeding lines with GBS marker data evaluated at each individual FHB trial were listed in APPENDIX I. Some breeding lines were evaluated for FHB severity at multiple years and locations. For example, there were 119 breeding lines genotyped from the 2013 AYT, of which 89 were evaluated in two FHB trials in 2012, 2012-Langdon-fhbPYT and 2012-Prosper-fhbPYT; 89 were evaluated in three FHB trials in 2013, 2013-Carrington-fhbAYT, 2013-Langdon-fhbAYT, and 2013-Prosper-fhbAYT; 53 were evaluated in three FHB trials in 2014, 2014-Carrington-fhbEDA, 2014-Langdon-fhbEDA, and 2014-Prosper-fhbEDA; 14 were evaluated in two FHB trials in 2015, 2015-Langdon-fhbURDN and 2015-Prosper-fhbURDN; five were evaluated in one FHB trial in 2016, 2016-Prosper-fhbURDN; two were evaluated in two FHB trials in 2017, 2017-Langdon-fhbURDN and 2017-Prosper-fhbURDN (Table 4.1; APPENDIX I).

For each FHB trial, experimental design was randomized complete block design with three replications. Each breeding line was planted in a hill plot with 15 seeds. Grain-spawn inoculation method was used in the field nurseries. To prepare inoculum, autoclaved corn kernels were infected with a mixture of spores produced separately from 20 *F. graminearum* stains,

including ten 3ADON (3-acetyl deoxynivalenol) producers and ten 15ADON (15-acetyl deoxynivalenol) producers, collected from fields in North Dakota (Puri & Zhong, 2010), according to the procedure described by Zhang et al., (2008). At the boot stage of the earliest lines, inoculum was evenly sprinkled among plots at a rate of 0.2 kg/m^2, and repeated every two weeks until all wheat accessions completed anthesis. The nursery was misted for 5 min in 15-min intervals for 12 h daily (4:00 pm to 4:00 am), until 14 days after anthesis of the latest lines. FHB severity for each plot was scored at 21 days post anthesis using a visual scale 0-9 (0 stands for complete resistance and 9 for complete susceptibility).

Phenotypic data analysis

The number of breeding lines in each individual FHB trial ranged from 30 to 547 (APPENDIX H). Two-stage analysis was performed for the phenotypic data of FHB severity.

In the first stage, best linear unbiased estimators (BLUEs) were estimated for all breeding lines within each individual trial using R package lme4 (Bates et al., 2015). The model was

$$y = \mu + g_i + r_j + \varepsilon_{ij} \quad [4.1]$$

where y is the vector of unadjusted phenotypes, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and r_j is the random effect of the j_{th} replication.

The variance components of error (σ^2) and genotype (σ^2_g) were estimated by considering all factors as random. The broad-sense heritability (H^2) was estimated as $\sigma^2/(\sigma^2 + \sigma^2_g)$. The trials with broad-sense heritability of less than 0.1 were removed for further analyses. The remaining trials were used in the second stage analysis to estimate BLUEs for the breeding lines across all environments using R package lme4 (Bates et al., 2015). The model was

$$y* = \mu + g_i + t_j + \varepsilon_{ij} \quad [4.2]$$

where $y*$ represents the estimated BLUEs from the first stage analysis, μ is the overall mean, g_i is the fixed effect of the i_{th} genotype, and t_j is the random effect of the j_{th} trial. The estimated BLUEs were further used in genomic prediction analysis.

A relationship matrix was calculated using R package rrBLUP (Endelman, 2011) based on 37,752 SNP markers. Additive variance component (*Va*) and error variance component (*Ve*) were calculated with the *mixed.solve* function in the package rrBLUP (Endelman, 2011). Genomic heritability (h^2) was calculated as *Va*/(*Va* + *Ve*).

Population structure and genome wide association mapping

Principal component (PC) analysis was conducted with the 37,752 SNP markers using TASSEL v.5 (Bradbury et al., 2007). Based on the scree plots (APPENDIX J), the first seven PCs were used for model-based cluster analysis with R package Mclust (Fraley and Raftery, 2007).

Genome wide association analysis was performed using TASSEL v.5 (Bradbury et al., 2007). The first seven PCs were chosen as covariates to capture population structure in the association analysis. A centered kinship (**K**) matrix was calculated based on the 37,752 SNPs using TASSEL (Bradbury et al., 2007). Four statistical models were tested: (i) simple association analysis using general linear model (naïve model); (ii) general linear model including the first seven PCs as covariates (**P** model); (iii) linear mixed model including kinship matrix (**K** model); and (iv) linear mixed model including population structure and kinship matrix (**PK** model). The mean of the squared difference (MSD) between observed and expected *p*-values of all SNP markers was estimated for each model. The best model for each trait was determined as the model returning the smallest MSD value. The false discovery rate (FDR) was calculated using

the R function *p.adjust* (Benjamini and Hochberg, 1995). Significance of marker-trait association is defined by FDR as a *q*-value < 0.1.

Genomic prediction model development and validation

Genomic prediction was evaluated with rrBLUP using R package rrBLUP (Endelman, 2011). Prediction accuracies were validated using cross-validation, where 90% individuals were randomly selected as the training population and the remaining 10% individuals were used to validate the genomic prediction accuracy. Genomic prediction accuracy was estimated as the Pearson correlation (*r*) between genomic estimated breeding values (GEBVs) and BLUEs of phenotypic values. Random sampling of training and validation sets was repeated 100 times and the mean of *r* was defined as the genomic prediction accuracy.

Forward prediction validations were conducted using the breeding lines from 2018's AYT as test populations and the previous years' AYT lines as training population. There were no breeding lines overlapped between training population and test population. Euclidean genetic distances between AYT populations were calculated using the 37,752 SNP markers.

Results

Phenotyping data

Broad-sense heritability of FHB severity was estimated for each of the 62 FHB trials and ranged from 0 to 0.89 (APPENDIX H). Three FHB trials (2014-Carrington-fhbURDN, 2014-Langdon-fhbURDN, and 2015-Langdon-fhbAYT) with broad-sense heritability less than 0.1 were removed for further analysis. The total number of breeding lines did not change after removing the three FHB trials. BLUEs of FHB severity were estimated for the 2,570 breeding lines and 14 check cultivars using the phenotypic data collected from the remaining 59 FHB field

trials at three locations across eight years. The estimated BLUEs for the 2,570 breeding lines ranged from 0.73 to 8.42 with a mean of 4.44.

For the 588 breeding lines from 2012-2018 AYTs that were genotyped with GBS, the estimated BLUEs of FHB severity ranged from 1.79 to 7.15 with a mean of 4.79 (APPENDIX K); the estimated genomic heritability was 0.72. A wide range of FHB severity was found in all seven AYT populations (Table 4.1).

Table 4.1. Mean, minimum, and maximum of the estimated BLUEs for FHB severity within each of the 2012-2018 AYT populations.

Population	FHB severity (Score of 0-9)		
(No. Breeding lines)	Mean	Minimum	Maximum
2012 AYT (141)	4.81	1.83	7.08
2013 AYT (119)	4.85	3.26	5.63
2014 AYT (45)	5.34	2.76	7.08
2015 AYT (46)	4.31	3.38	5.41
2016 AYT (39)	4.66	3.33	5.78
2017 AYT (92)	5.04	3.66	7.15
2018 AYT (106)	4.20	1.79	6.41

Population structure and GWAS

The first two PCs explained 7.1% and 4.8% of the total variation, respectively (APPENDIX J). Model-based cluster analysis suggested that there were nine clusters (APPENDIX L). The breeding lines from different AYTs were intermixed within clusters (APPENDIX K).

GWAS was conducted for FHB severity using four statistical models: naïve, P, K, and PK models. The PK model exhibited the smallest MSD value. No SNPs significantly associated with FHB severity were identified at the threshold FDR of *q-value* < 0.1.

Genomic prediction

Genomic prediction model for FHB severity were developed using rrBLUP. Prediction accuracy was 0.53 based on a ten-fold cross-validation. We also evaluated the forward prediction accuracy for FHB severity by considering 2018 AYT breeding lines as the test population. There was no overlap between the 2018 AYT breeding lines and 2012-2017 AYT breeding lines. When using 351 breeding lines from the 2012-2015 AYTs, 390 breeding lines from the 2012-2016 AYTs, or 482 breeding lines from the 2012-2017 AYTs as the training population, the forward prediction accuracy was 0.17, 0.31 and 0.47, respectively (Table 4.2). The 2018 AYT breeding lines were evaluated for FHB severity in 2017-2019 FHB nurseries, where a subset of the 2012-2017 AYT breeding lines were also evaluated (APPENDIX I). This means that a subset of the test population was evaluated at the same environments with a subset of the training population. The training population size was increased when the 2016 AYT and 2017 AYT breeding lines were added. At the same time, the number of breeding lines in the training population evaluated at the same environments with the test population was also increased (APPENDIX I).

Table 4.2. Forward prediction accuracies for FHB severity with varied training populations when considering 2018 AYT breeding lines as testing population

Training population	Number of breeding line	Prediction accuracy
2012-2015 AYT	351	0.17
2012-2016 AYT	390	0.31
2012-2017 AYT	482	0.47
2012-2017 AYT	351 (randomly sampled)	0.44
2012-2017 AYT	390 (randomly sampled)	0.45

In order to test whether the increased forward prediction accuracy was due to increase in the training population size, we randomly sampled 351 and 390 out of the 482 breeding lines from 2012-2017 AYTs as the training population with 100 iterations, which resulted in the mean

forward prediction accuracy of 0.44 and 0.45, respectively (Table 4.2). In order to test whether the increased forward prediction accuracy was due to increase in the genetic relationship between training population and test population, Euclidean genetic distances between AYTs were estimated, which indicated no closer relationship between 2018 AYT and 2016-2017 AYTs than between 2018 AYT and 2012-2015 AYTs (Table 4.3). These results indicate that the increased forward prediction accuracy was primarily due to the training population containing more lines evaluated at the same environments with the test population, rather than increased training population size or increased genetic relationship.

Table 4.3. Euclidean genetic distances between different AYT populations

	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2012 AYT	18.1	26.2	41.4	28.7	29.1	22.3
2013 AYT		38.2	47.4	36.1	35.7	28.7
2014 AYT			33.0	23.7	26.3	20.2
2015 AYT				27.6	35.5	31.2
2016 AYT					26.4	18.9
2017 AYT						21.6

Discussion

Developing FHB resistant cultivars in durum wheat is lagging behind compared to bread wheat. One main cause is the lack of resistant sources in durum wheat. In the past 20 years, identifying resistant resources followed by introgression of resistant genes/QTL from exotic tetraploid relatives or bread wheat have resulted in some durum wheat advanced lines with improved FHB resistance (Prat et al., 2017; Somers et al., 2006; Zhao et al., 2018).

FHB resistance is a complex trait controlled by many genes and their interactions with genetic background and environments. Phenotypic selection is commonly used for FHB resistance improvement in wheat breeding programs. For example, in the NDSU durum wheat

breeding program, $F_{4:6}$ and later generation lines are evaluated in field nurseries with artificial inoculation at multiple locations and across multiple years. However, the number of breeding lines for screening are restricted by high-cost phenotyping and limited resources. Using MAS, breeders can integrate major resistance genes into breeding population, screen a large number of breeding lines at early generations and off-seasons, and select for promising lines more effectively, so that the genetic improvement of FHB resistance can be enhanced in terms of time and cost. In addition to the major QTL, integrating favorable alleles from resistance genes with moderate/minor effects promises to provide high levels of durable FHB resistance. Investigating the genetic basis and genomic prediction for FHB resistance in a breeding population can facilitate the implementation of MAS or GS directly in a breeding program. In this study, we first performed GWAS for FHB severity using historical phenotypic data and molecular marker data of 588 breeding lines from the NDSU durum wheat breeding program. The genomic heritability was estimated as 0.72; however, GWAS has found no significant QTL. This suggested that many genes with moderate or minor effects are involved in the plants' interaction with FHB in the NDSU durum wheat breeding population.

Furthermore, we developed a genomic prediction model for FHB severity and found that the cross-validation prediction accuracy was 0.53 and the forward prediction accuracy was up to 0.47. Given the moderate prediction accuracies, it will be challenging to select top resistant lines using markers only. It was reported that using GS to eliminate the most susceptible lines followed by phenotypic selection could enhance the improvement of FHB resistance, compared to phenotypic selection only (Steiner et al., 2019). Starting with GS to select against susceptible lines in newly developed populations may be practical in the NDSU durum wheat breeding program. Adeyemo et al. (2020) reported that 200 F_5 breeding lines selected with a stratified

sampling method as the training population could increase prediction accuracy for the other untested F_5 lines in a spring wheat breeding population, compared to the same number of lines randomly selected as the training population, presumably due to their closer genetic relationship with the test population. Including parents of the F_5 lines in the training population could further increase prediction accuracy (Adeyemo et al., 2020). An alternative way to implement GS in making selections in a newly developed population would be to phenotype the parents and a subset of test population and then update the current GS model for selection. For example, to implement GS in the NDSU durum wheat breeding program, where there are about 2,000 $F_{4:5}$ lines each year, it can be carried out sequentially in each cycle by genotyping all breeding lines, selecting 200 lines with stratified sampling, phenotyping the 200 lines, adding them in the current training population (2012-2018 AYTs) to update the GS model, then predicting all 2,000 lines with the updated model, and finally selecting top 200 lines for further field evaluation. The effectiveness and efficiency of different GS implementing strategies should be investigated.

In this study, we found that the forward prediction accuracy was increased when the training population contained more breeding lines evaluated with the test population at the same environments. The explanation to the increased forward prediction accuracy could be the genotype by environment interaction for FHB severity. Once GS is initiated in a breeding program, more breeding lines will be evaluated at various environments, adding which to update the genomic prediction model can increase not only prediction accuracy but also prediction stability. Phenotypic selection of FHB severity based on a single or a few environments (e.g., selection of early generation breeding lines in the first year FHB nursery) can be biased by the genotype by environment interactions. Increase in prediction stability will be even more beneficial. When the prediction accuracy is high enough, GS can be used to select top resistant

lines or even parents for a new crossing block, as evidenced in dairy cattle breeding (Garcia-Ruiz et al., 2016; Meuwissen et al., 2016), which will shorten breeding cycles and accelerate its genetic improvement. Furthermore, GS can be simultaneously used to target other traits like grain/semolina quality traits at early generations without additional genotyping cost (Fiedler et al., 2017).

Lowering the genotyping cost will make GS in wheat even more practical. One way this can be accomplished is to genotype the parents of the selection population with high density markers such as the TaBW280K high-throughput genotyping array (Rimbert al., 2018), wheat 90K SNP array (Wang et al., 2014), or 660K SNP array (Sun et al., 2020), and then genotype the selection population with a reduced number of markers. New low-cost genotyping platforms such as multi-species SNP array (Keeble-Gagnere et al., 2021) are continuously being improved and should be able to accelerate the implementation of GS in wheat and other crops.

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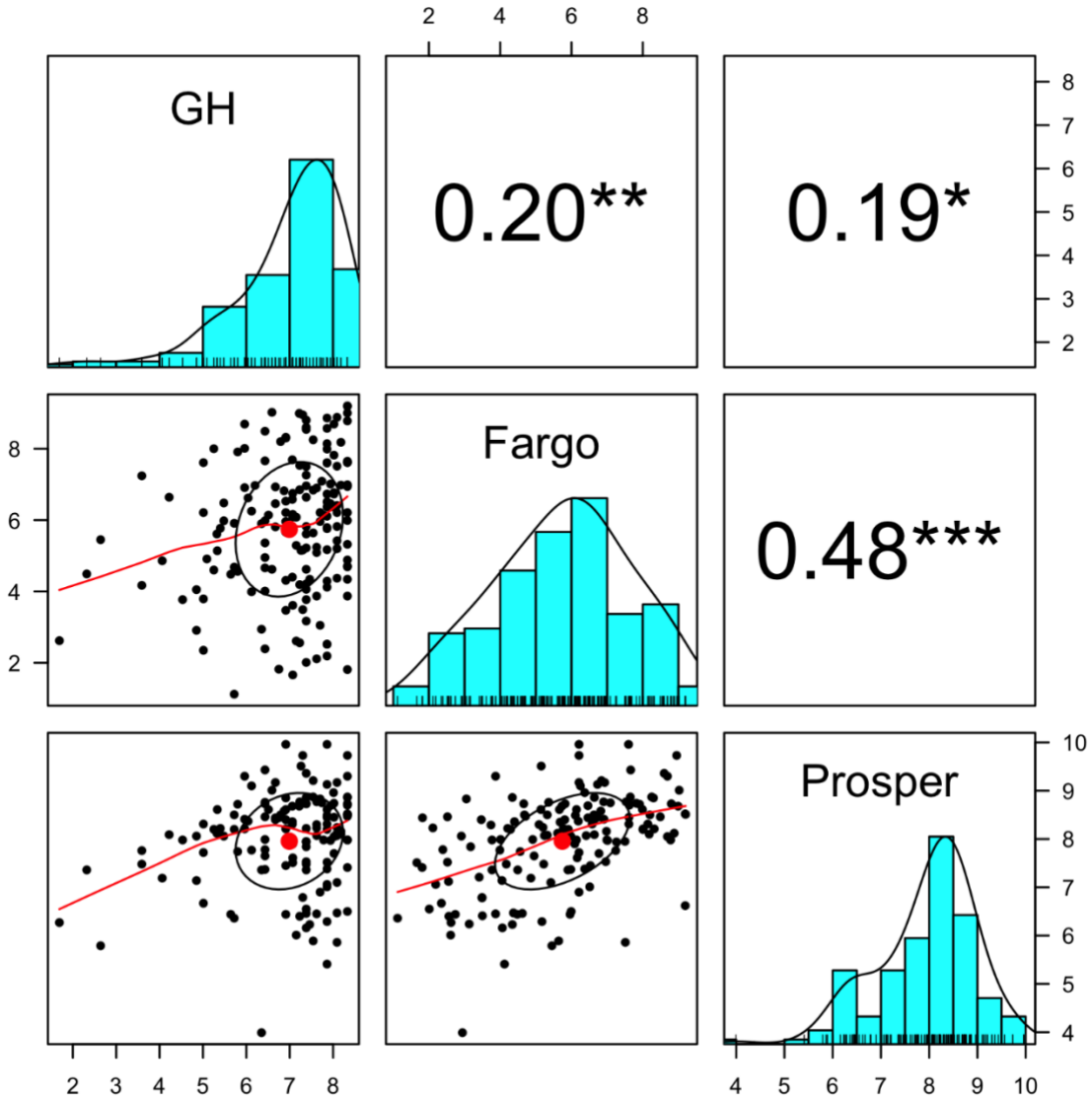
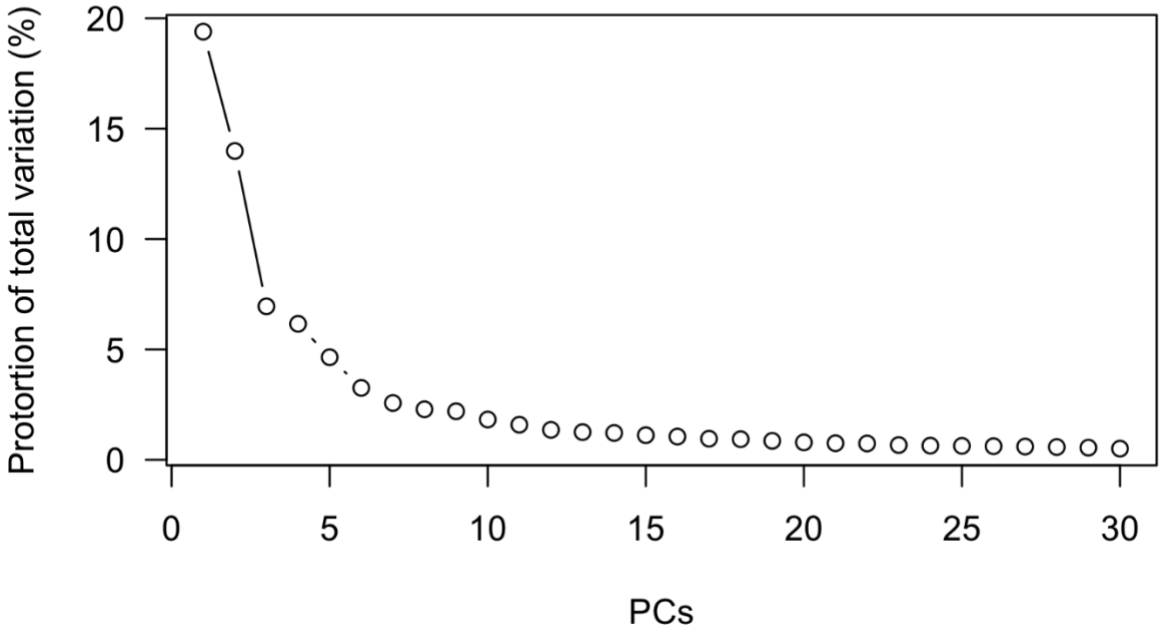
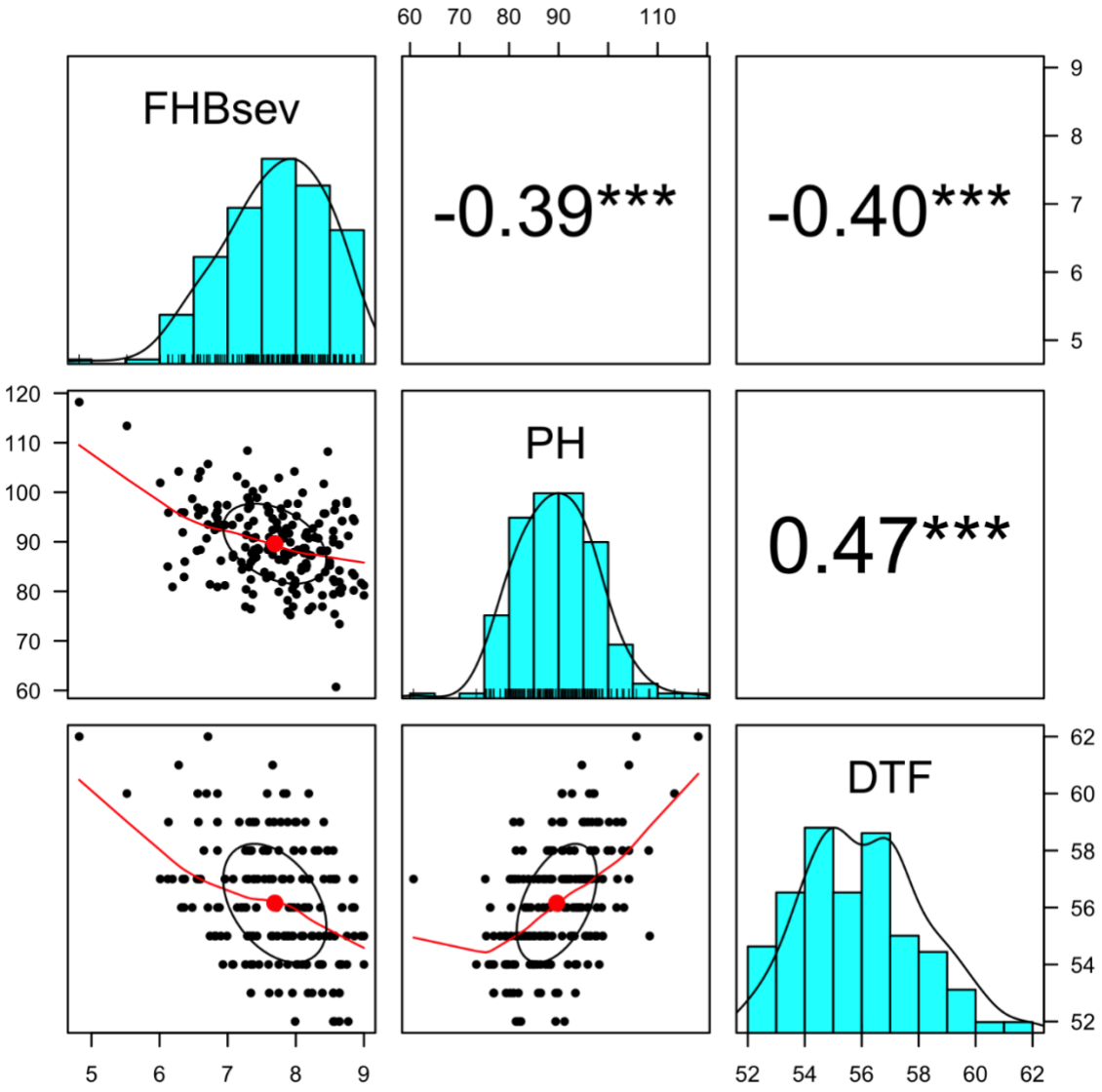
APPENDIX A. DISTRIBUTIONS OF THE ESTIMATED BLUES FOR FHB SEVERITY EVALUATED AT GREENHOUSE (GH), FARGO, AND PROSPER AND THEIR CORRELATIONS BETWEEN THE THREE ENVIRONMENTS.



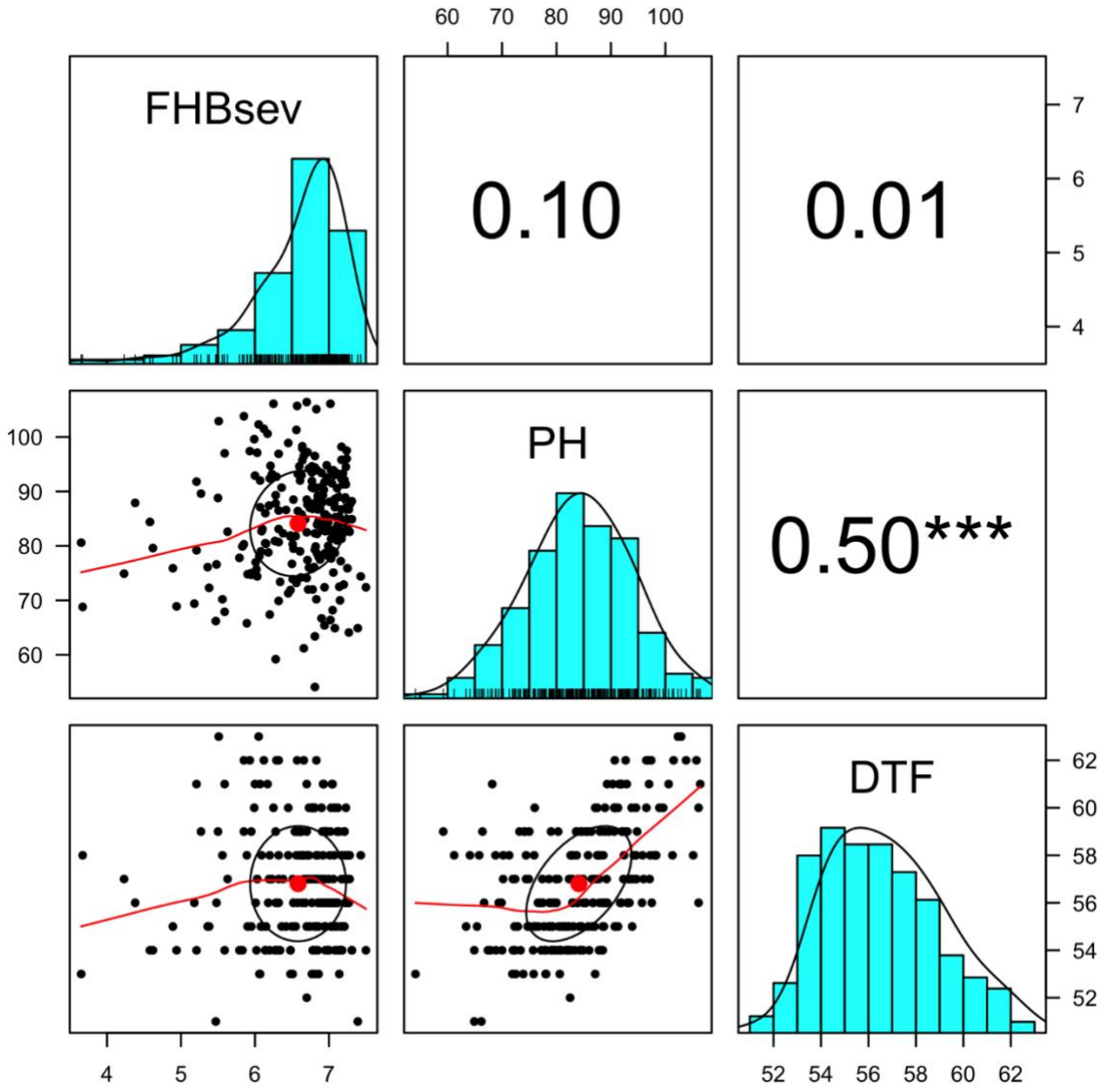
APPENDIX B. SCREE PLOTS OF THE FIRST 30 PCS DERIVED FROM A PRINCIPAL COMPONENT ANALYSIS.



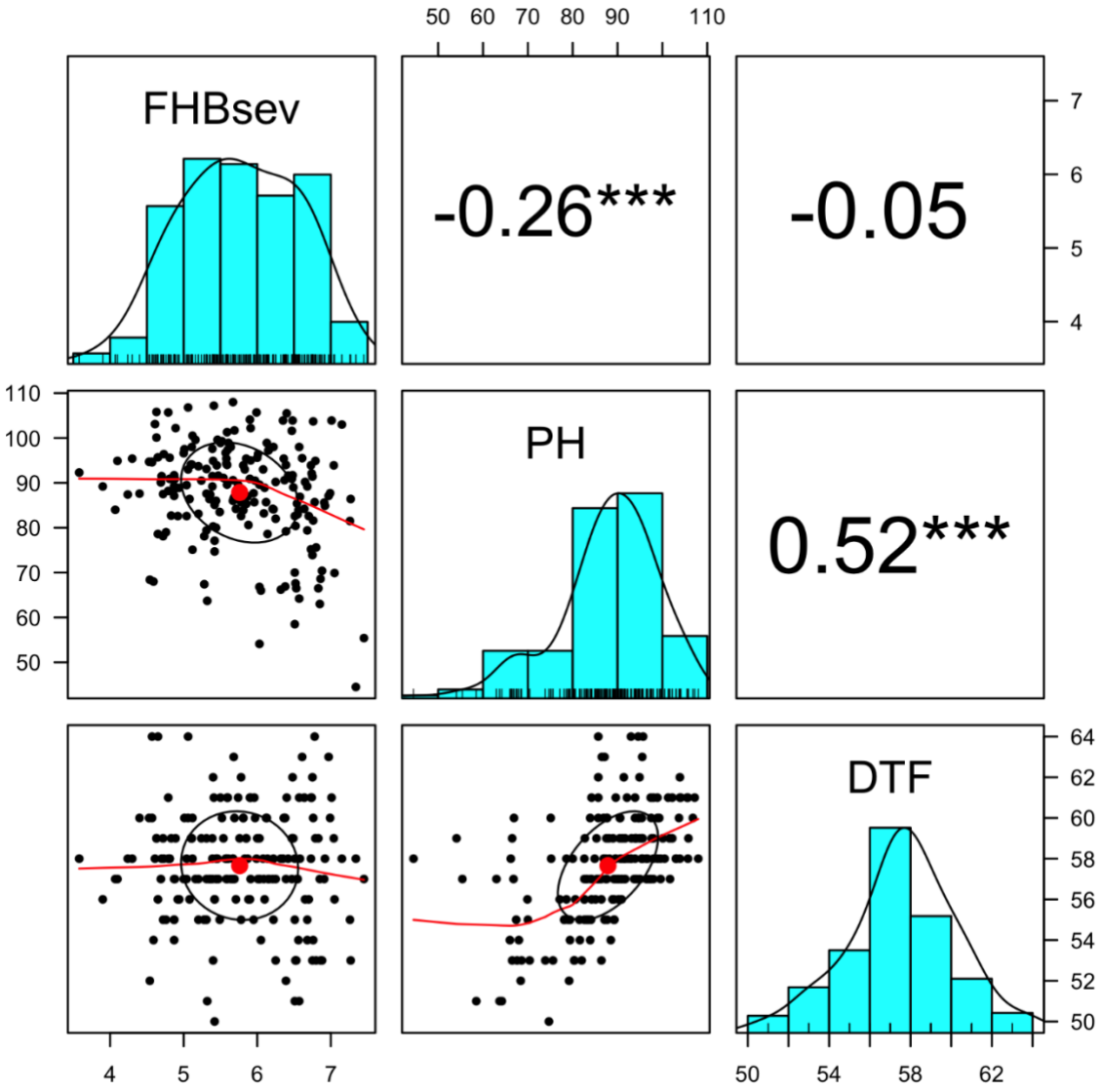
APPENDIX C. PHENOTYPIC CORRELATIONS BETWEEN THE THREE TRAITS IN THE C0 POPULATION.



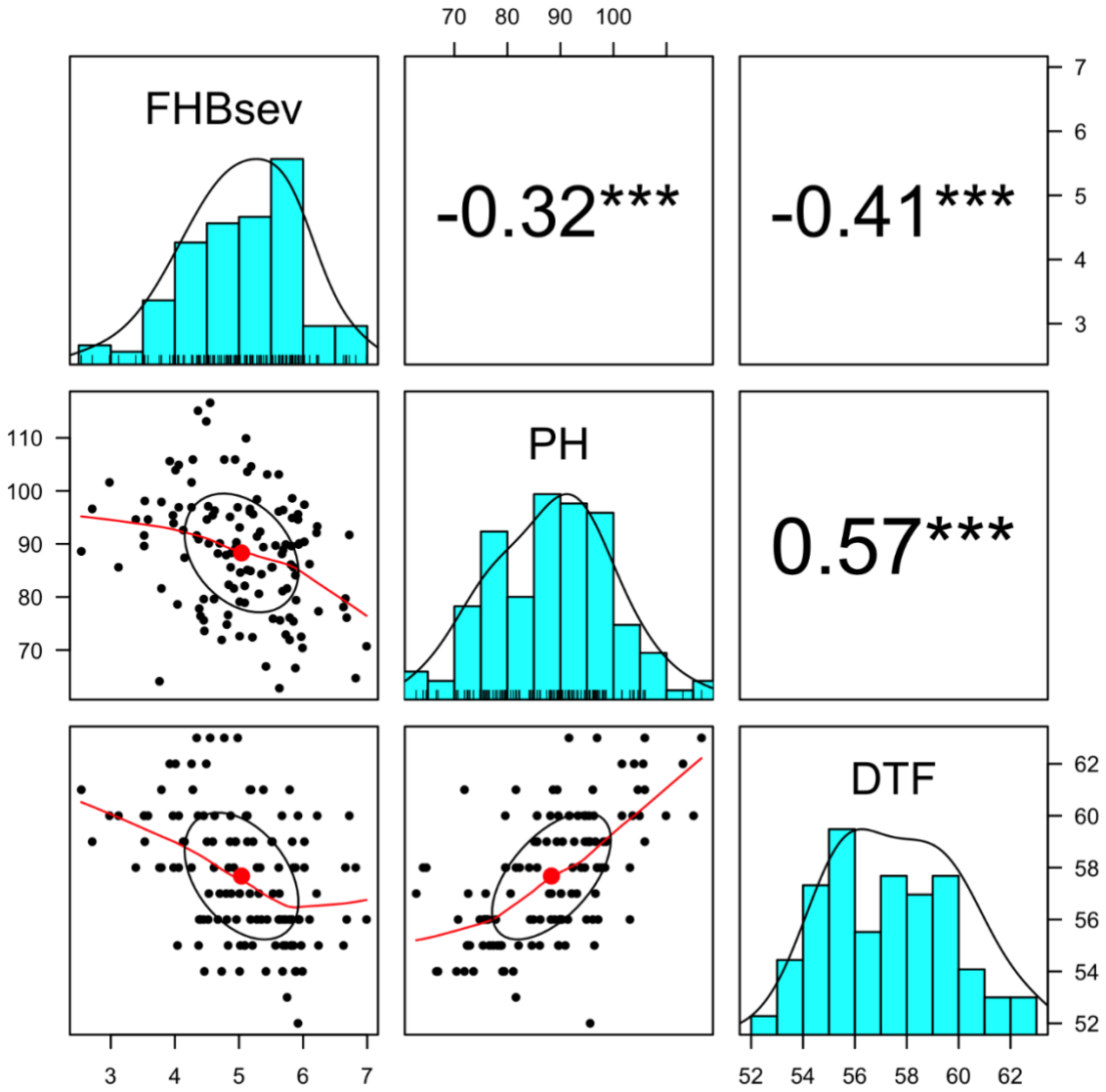
APPENDIX D. PHENOTYPIC CORRELATIONS BETWEEN THE THREE TRAITS IN THE C1 POPULATION.



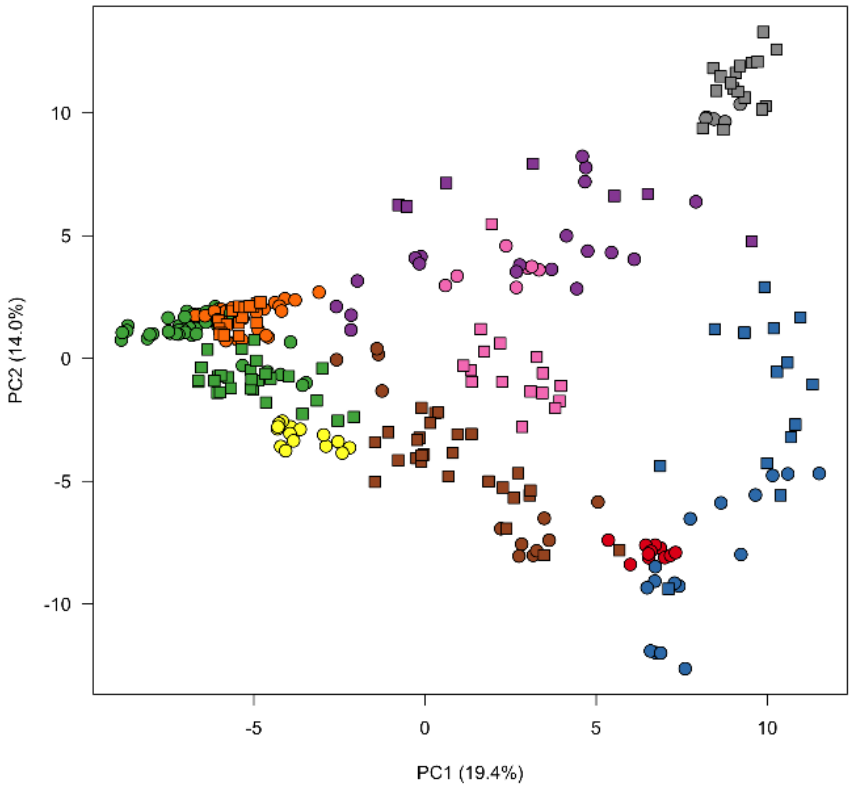
APPENDIX E. PHENOTYPIC CORRELATIONS BETWEEN THE THREE TRAITS IN THE C2 POPULATION.



APPENDIX F. PHENOTYPIC CORRELATIONS BETWEEN THE THREE TRAITS IN THE C3 POPULATION.



APPENDIX G. SCATTER PLOTS OF PC1 AND PC2 DERIVED FROM A PRINCIPAL COMPONENT ANALYSIS FOR THE 284 S0 PARENT LINES FROM THE C2 AND C3 POPULATIONS.



APPENDIX H. NUMBER OF BREEDING LINES, BROAD SENSE HERITABILITY, AND MEAN AND RANGE OF FHB SEVERITY AT EACH FHB FIELD TRIAL.

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2012-Langdon	2012-Langdon-fhbAYT	94	0.29	5.8	2.8	7.7
2012-Langdon	2012-Langdon-fhbEDA	94	0.26	4.9	3.3	7.3
2012-Langdon	2012-Langdon-fhbPYT	435	0.37	6.9	4.1	9.0
2012-Langdon	2012-Langdon-fhbURDN	32	0.21	5.2	2.9	6.3
2012-Prosper	2012-Prosper-fhbAYT	94	0.24	5.0	2.2	7.7
2012-Prosper	2012-Prosper-fhbEDA	94	0.32	3.5	2.1	6.0
2012-Prosper	2012-Prosper-fhbPYT	421	0.27	3.6	1.4	7.4
2012-Prosper	2012-Prosper-fhbURDN	32	0.29	4.7	2.6	6.8
2013-Carrington	2013-Carrington-fhbAYT	94	0.37	4.0	2.7	5.5
2013-Carrington	2013-Carrington-fhbEDA	64	0.57	4.2	2.0	5.8
2013-Carrington	2013-Carrington-fhbPYT	194	0.18	4.2	3.0	5.5

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2013-Carrington	2013-Carrington-fhbURDN	32	0.34	4.0	2.7	5.4
2013-Langdon	2013-Langdon-fhbAYT	94	0.71	7.0	4.6	9.0
2013-Langdon	2013-Langdon-fhbEDA	64	0.89	6.9	2.3	9.0
2013-Langdon	2013-Langdon-fhbPYT	191	0.55	7.3	4.5	9.0
2013-Langdon	2013-Langdon-fhbURDN	32	0.77	7.3	2.7	9.0
2013-Prosper	2013-Prosper-fhbAYT	94	0.29	3.5	2.3	5.7
2013-Prosper	2013-Prosper-fhbEDA	64	0.16	3.1	2.3	4.3
2013-Prosper	2013-Prosper-fhbPYT	194	0.20	4.2	1.9	6.6
2013-Prosper	2013-Prosper-fhbURDN	32	0.11	3.4	2.5	4.5
2014-Carrington	2014-Carrington-fhbAYT	49	0.38	3.7	2.0	6.5
2014-Carrington	2014-Carrington-fhbEDA	66	0.42	4.7	2.0	8.5

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2014-Carrington	2014-Carrington-fhbPYT	190	0.61	5.3	1.7	9.0
2014-Carrington	2014-Carrington-fhbURDN	32	0.06	5.8	4.5	7.0
2014-Langdon	2014-Langdon-fhbAYT	49	0.25	7.4	5.5	8.6
2014-Langdon	2014-Langdon-fhbEDA	66	0.43	6.9	4.7	9.0
2014-Langdon	2014-Langdon-fhbPYT	194	0.11	6.9	4.9	9.0
2014-Langdon	2014-Langdon-fhbURDN	32	0.06	7.2	5.7	8.5
2014-Prosper	2014-Prosper-fhbAYT	50	0.49	6.0	4.3	8.4
2014-Prosper	2014-Prosper-fhbEDA	66	0.30	7.0	5.5	8.7
2014-Prosper	2014-Prosper-fhbPYT	195	0.40	6.8	4.5	9.0
2014-Prosper	2014-Prosper-fhbURDN	32	0.45	6.8	4.5	8.8
2015-Langdon	2015-Langdon-fhbAYT	49	0.00	7.6	5.3	8.5

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2015-Langdon	2015-Langdon-fhbEDA	64	0.84	7.2	0.9	9.0
2015-Langdon	2015-Langdon-fhbURDN	32	0.56	6.5	4.1	9.0
2015-Prosper	2015-Prosper-fhbAYT	49	0.37	5.7	3.9	9.0
2015-Prosper	2015-Prosper-fhbEDA	64	0.49	6.4	1.2	8.9
2015-Prosper	2015-Prosper-fhbURDN	32	0.44	5.3	3.8	7.6
2016-Prosper	2016-Prosper-fhbEDA	64	0.17	6.3	4.3	8.2
2016-Prosper	2016-Prosper-fhbURDN	32	0.45	6.6	4.7	8.4
2017-Langdon	2017-Langdon-fhbAYT	49	0.45	3.2	1.3	6.6
2017-Langdon	2017-Langdon-fhbEDA	64	0.34	2.5	0.7	5.1
2017-Langdon	2017-Langdon-fhbPYT	343	0.34	3.3	1.1	6.3
2017-Langdon	2017-Langdon-fhbURDN	32	0.29	2.0	0.7	4.2
2017-Prosper	2017-Prosper-fhbAYT	49	0.27	6.4	4.5	9.0

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2017-Prosper	2017-Prosper-fhbEDA	64	0.43	6.3	4.3	7.7
2017-Prosper	2017-Prosper-fhbPYT	346	0.33	5.5	1.7	9.0
2017-Prosper	2017-Prosper-fhbURDN	32	0.63	5.7	3.9	8.0
2018-Langdon	2018-Langdon-fhbAYT	64	0.11	1.3	0.7	2.2
2018-Langdon	2018-Langdon-fhbEDA	64	0.51	1.0	0.4	4.2
2018-Langdon	2018-Langdon-fhbPYT	547	0.88	1.4	0.0	4.9
2018-Langdon	2018-Langdon-fhbURDN	32	0.30	0.8	0.2	1.9
2018-Prosper	2018-Prosper-fhbAYT	64	0.33	5.6	4.0	7.4
2018-Prosper	2018-Prosper-fhbEDA	63	0.45	5.7	3.1	7.5
2018-Prosper	2018-Prosper-fhbURDN	30	0.39	5.4	4.1	7.4
2019-Langdon	2019-Langdon-fhbAYT	113	0.35	2.3	0.5	4.3
2019-Langdon	2019-Langdon-fhbEDA	70	0.66	1.2	0.5	3.5
2019-Langdon	2019-Langdon-fhbPYT	255	0.36	3.1	0.6	5.8

FHB nursery	FHB trial	Number of lines evaluated	Broad heritability	FHB severity score		
				Mean	Minimum	Maximum
2019-Langdon	2019-Langdon-fhbURDN	37	0.72	1.0	0.3	2.8
2019-Prosper	2019-Prosper-fhbAYT	113	0.45	6.5	1.4	8.9
2019-Prosper	2019-Prosper-fhbEDA	70	0.63	6.2	1.5	8.4
2019-Prosper	2019-Prosper-fhbURDN	37	0.36	6.2	1.6	8.5

Table note: Broad heritability < 0.1 of FHB trails evaluated in the nurseries were marked as red font and removed for further analysis.

APPENDIX I. NUMBER OF BREEDING LINES WITH GBS MARKER DATA FROM EACH ADVANCED YIELD TRIAL (AYT) EVALUATED AT EACH FHB FIELD TRIAL FROM 2012 TO 2019.

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2012-Langdon	2012-Langdon-fhbPYT	0	89	0	0	0	0	0
2012-Langdon	2012-Langdon-fhbAYT	90	0	0	0	0	0	0
2012-Langdon	2012-Langdon-fhbEDA	0	0	0	0	0	0	0
2012-Langdon	2012-Langdon-fhbURDN	0	0	0	0	0	0	0
2012-Prosper	2012-Prosper-fhbPYT	0	89	0	0	0	0	0
2012-Prosper	2012-Prosper-fhbAYT	90	0	0	0	0	0	0
2012-Prosper	2012-Prosper-fhbEDA	0	0	0	0	0	0	0
2012-Prosper	2012-Prosper-fhbURDN	0	0	0	0	0	0	0
2013-Carrington	2013-Carrington-fhbPYT	0	0	0	0	0	0	0

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2013-Carrington	2013-Carrington-fhbAYT	0	89	0	0	0	0	0
2013-Carrington	2013-Carrington-fhbEDA	59	0	0	0	0	0	0
2013-Carrington	2013-Carrington-fhbURDN	1	0	0	0	0	0	0
2013-Langdon	2013-Langdon-fhbPYT	0	0	0	0	0	0	0
2013-Langdon	2013-Langdon-fhbAYT	0	89	0	0	0	0	0
2013-Langdon	2013-Langdon-fhbEDA	59	0	0	0	0	0	0
2013-Langdon	2013-Langdon-fhbURDN	0	0	0	0	0	0	0
2013-Prosper	2013-Prosper-fhbPYT	0	0	0	0	0	0	0
2013-Prosper	2013-Prosper-fhbAYT	0	89	0	0	0	0	0

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2013-Prosper	2013-Prosper-fhbEDA	59	0	0	0	0	0	0
2013-Prosper	2013-Prosper-fhbURDN	1	0	0	0	0	0	0
2014-Carrington	2014-Carrington-fhbPYT	0	0	0	0	0	0	0
2014-Carrington	2014-Carrington-fhbAYT	0	0	0	0	0	0	0
2014-Carrington	2014-Carrington-fhbEDA	0	53	0	0	0	0	0
2014-Carrington	2014-Carrington-fhbURDN	14	0	0	0	0	0	0
2014-Langdon	2014-Langdon-fhbPYT	0	0	0	0	0	0	0
2014-Langdon	2014-Langdon-fhbAYT	0	0	0	0	0	0	0
2014-Langdon	2014-Langdon-fhbEDA	0	53	0	0	0	0	0

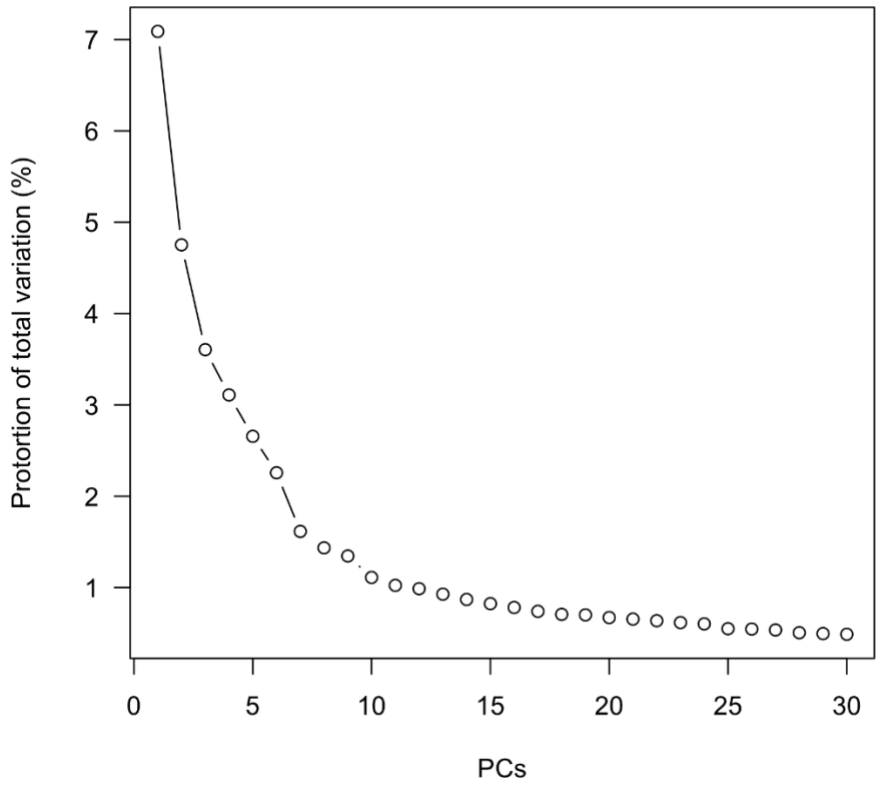
FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2014-Langdon	2014-Langdon-fhbURDN	12	0	0	0	0	0	0
2014-Prosper	2014-Prosper-fhbPYT	0	0	0	0	0	0	0
2014-Prosper	2014-Prosper-fhbAYT	0	0	0	0	0	0	0
2014-Prosper	2014-Prosper-fhbEDA	0	53	0	0	0	0	0
2014-Prosper	2014-Prosper-fhbURDN	14	0	0	0	0	0	0
2015-Langdon	2015-Langdon-fhbAYT	0	0	0	0	0	0	0
2015-Langdon	2015-Langdon-fhbEDA	0	0	45	0	0	0	0
2015-Langdon	2015-Langdon-fhbURDN	7	14	0	0	0	0	0
2015-Prosper	2015-Prosper-fhbAYT	0	0	0	0	0	0	0
2015-Prosper	2015-Prosper-fhbEDA	0	0	45	0	0	0	0

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2015-Prosper	2015-Prosper-fhbURDN	7	14	0	0	0	0	0
2016-Prosper	2016-Prosper-fhbEDA	0	0	0	46	0	0	0
2016-Prosper	2016-Prosper-fhbURDN	3	5	8	0	0	0	0
2017-Langdon	2017-Langdon-fhbPYT	0	0	0	0	0	0	60
2017-Langdon	2017-Langdon-fhbAYT	0	0	0	0	0	45	0
2017-Langdon	2017-Langdon-fhbEDA	0	0	0	0	39	3	0
2017-Langdon	2017-Langdon-fhbURDN	1	2	3	13	0	0	0
2017-Prosper	2017-Prosper-fhbPYT	0	0	0	0	0	0	60
2017-Prosper	2017-Prosper-fhbAYT	0	0	0	0	0	45	0

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2017-Prosper	2017-Prosper-fhbEDA	0	0	0	0	39	3	0
2017-Prosper	2017-Prosper-fhbURDN	1	2	3	13	0	0	0
2018-Langdon	2018-Langdon-fhbPYT	0	0	0	0	0	0	0
2018-Langdon	2018-Langdon-fhbAYT	0	0	0	0	0	0	60
2018-Langdon	2018-Langdon-fhbEDA	0	0	0	0	0	58	0
2018-Langdon	2018-Langdon-fhbURDN	0	2	2	8	12	0	0
2018-Prosper	2018-Prosper-fhbAYT	0	0	0	0	0	0	60
2018-Prosper	2018-Prosper-fhbEDA	0	0	0	0	0	58	0
2018-Prosper	2018-Prosper-fhbURDN	0	2	2	8	12	0	0
2019-Langdon	2019-Langdon-fhbPYT	0	0	0	0	0	0	0

FHB nursery	FHB trial	2012 AYT	2013 AYT	2014 AYT	2015 AYT	2016 AYT	2017 AYT	2018 AYT
2019-Langdon	2019-Langdon-fhbAYT	0	0	0	0	1	0	0
2019-Langdon	2019-Langdon-fhbEDA	0	0	0	0	0	3	56
2019-Langdon	2019-Langdon-fhbURDN	0	1	0	4	5	14	0
2019-Prosper	2019-Prosper-fhbAYT	0	0	0	0	0	0	0
2019-Prosper	2019-Prosper-fhbEDA	0	0	0	0	0	3	56
2019-Prosper	2019-Prosper-fhbURDN	0	1	0	4	5	14	0

APPENDIX J. SUPPLEMENTARY FIGURE S1. SCREE PLOTS OF PCS.



APPENDIX K. ESTIMATED BLUES OF FHB SEVERITY FOR THE 588 BREEDING LINES FROM THE 2012-2018 AYTS AND 14 CHECK CULTIVARS.

Entry	ID	FHB severity	Original AYT	Cluster
D101047	AYT12-124	4.87	2012 AYT	4
D101049	AYT12-126	4.17	2012 AYT	4
D101065	AYT12-128	5.37	2012 AYT	4
D101073	AYT12-130	4.92	2012 AYT	4
D101076	AYT12-131	5.95	2012 AYT	4
D101082	AYT12-132	4.80	2012 AYT	4
D101109	AYT12-137	5.32	2012 AYT	4
D101132	AYT12-139	5.15	2012 AYT	4
D101134	AYT12-140	5.29	2012 AYT	4
D101184	AYT12-150	5.37	2012 AYT	4
D101193	AYT12-152	5.46	2012 AYT	4
D101196	AYT12-153	4.62	2012 AYT	4
D101198	AYT12-155	5.87	2012 AYT	4
D101205	AYT12-159	4.58	2012 AYT	4
D101209	AYT12-160	5.62	2012 AYT	4
D101210	AYT12-161	5.54	2012 AYT	4
D101232	AYT12-164	4.58	2012 AYT	4
D101236	AYT12-166	5.04	2012 AYT	4
D101537	AYT12-216	4.25	2012 AYT	3
D101543	AYT12-218	3.83	2012 AYT	1
D101545	AYT12-219	3.03	2012 AYT	3
D101558	AYT12-220	4.06	2012 AYT	3
D101572	AYT12-221	3.87	2012 AYT	3
D101620	AYT12-229	3.07	2012 AYT	3
D101650	AYT12-235	4.82	2012 AYT	3
D101662	AYT12-238	2.51	2012 AYT	1
D101773	AYT12-257	1.83	2012 AYT	1
D101786	AYT12-262	4.48	2012 AYT	1
D101787	AYT12-263	3.29	2012 AYT	1
D101795	AYT12-265	3.76	2012 AYT	1
D101801	AYT12-269	4.74	2012 AYT	1
D101827	AYT12-271	2.29	2012 AYT	1

Entry	ID	FHB severity	Original AYT	Cluster
D101871	AYT12-284	3.79	2012 AYT	1
D101882	AYT12-289	2.96	2012 AYT	1
D101890	AYT12-295	4.61	2012 AYT	6
D101891	AYT12-296	4.76	2012 AYT	6
D101893	AYT12-297	2.77	2012 AYT	6
D101896	AYT12-298	4.76	2012 AYT	6
D101898	AYT12-299	3.76	2012 AYT	6
D101906	AYT12-300	4.01	2012 AYT	6
D101915	AYT12-301	4.13	2012 AYT	6
D101930	AYT12-302	5.01	2012 AYT	7
D101934	AYT12-303	5.13	2012 AYT	3
D101973	AYT12-304	4.26	2012 AYT	6
D101976	AYT12-305	6.01	2012 AYT	7
D101977	AYT12-306	5.01	2012 AYT	7
D101978	AYT12-307	4.26	2012 AYT	6
D101979	AYT12-308	6.01	2012 AYT	7
D101980	AYT12-309	5.76	2012 AYT	7
D101981	AYT12-310	5.01	2012 AYT	7
D101984	AYT12-311	4.76	2012 AYT	7
D101985	AYT12-312	5.51	2012 AYT	7
D101986	AYT12-313	5.63	2012 AYT	7
D102006	AYT12-314	4.88	2012 AYT	3
D102015	AYT12-315	5.26	2012 AYT	7
D102032	AYT12-316	2.71	2012 AYT	6
D102061	AYT12-317	3.95	2012 AYT	6
D102082	AYT12-318	4.88	2012 AYT	7
D102095	AYT12-319	4.26	2012 AYT	2
D102118	AYT12-320	5.37	2012 AYT	1
D102121	AYT12-321	6.51	2012 AYT	1
D102130	AYT12-322	4.13	2012 AYT	7
D102131	AYT12-323	4.63	2012 AYT	6
D102132	AYT12-324	5.03	2012 AYT	6
D102165	AYT12-325	5.26	2012 AYT	7
D102168	AYT12-326	3.51	2012 AYT	7
D102216	AYT12-327	5.88	2012 AYT	6
D102218	AYT12-328	5.13	2012 AYT	6

Entry	ID	FHB severity	Original AYT	Cluster
D102220	AYT12-329	5.38	2012 AYT	6
D102221	AYT12-330	6.63	2012 AYT	6
D102222	AYT12-331	5.76	2012 AYT	6
D102228	AYT12-332	5.26	2012 AYT	6
D102231	AYT12-333	5.13	2012 AYT	6
D102232	AYT12-334	5.13	2012 AYT	6
D102234	AYT12-335	6.26	2012 AYT	6
D102238	AYT12-336	5.63	2012 AYT	6
D102239	AYT12-337	4.83	2012 AYT	6
D102241	AYT12-338	4.46	2012 AYT	6
D102242	AYT12-339	4.76	2012 AYT	6
D102243	AYT12-344	5.58	2012 AYT	6
D102244	AYT12-345	4.58	2012 AYT	6
D102245	AYT12-346	5.92	2012 AYT	6
D102246	AYT12-347	5.16	2012 AYT	6
D102247	AYT12-348	4.08	2012 AYT	6
D102248	AYT12-349	4.83	2012 AYT	6
D102251	AYT12-350	5.67	2012 AYT	6
D102256	AYT12-351	5.02	2012 AYT	6
D102257	AYT12-352	4.83	2012 AYT	6
D102258	AYT12-353	4.96	2012 AYT	6
D102259	AYT12-354	4.83	2012 AYT	6
D102260	AYT12-355	4.14	2012 AYT	6
D102262	AYT12-356	4.33	2012 AYT	6
D102263	AYT12-357	3.83	2012 AYT	6
D102266	AYT12-358	4.43	2012 AYT	6
D102275	AYT12-359	4.58	2012 AYT	6
D102280	AYT12-360	5.83	2012 AYT	6
D102282	AYT12-361	5.83	2012 AYT	6
D102289	AYT12-362	5.46	2012 AYT	6
D102294	AYT12-363	6.33	2012 AYT	6
D102309	AYT12-364	5.33	2012 AYT	6
D102311	AYT12-365	6.50	2012 AYT	7
D102327	AYT12-366	5.21	2012 AYT	6
D102328	AYT12-367	5.33	2012 AYT	2
D102331	AYT12-368	6.58	2012 AYT	2

Entry	ID	FHB severity	Original AYT	Cluster
D102334	AYT12-369	6.46	2012 AYT	2
D102342	AYT12-370	7.08	2012 AYT	2
D102347	AYT12-371	4.96	2012 AYT	1
D102356	AYT12-372	3.41	2012 AYT	2
D102357	AYT12-373	4.96	2012 AYT	2
D102405	AYT12-374	5.96	2012 AYT	6
D102411	AYT12-375	4.66	2012 AYT	6
D102422	AYT12-376	5.96	2012 AYT	6
D102424	AYT12-377	5.33	2012 AYT	6
D102428	AYT12-378	4.33	2012 AYT	6
D102429	AYT12-379	5.41	2012 AYT	6
D102431	AYT12-380	4.83	2012 AYT	6
D102432	AYT12-381	4.96	2012 AYT	6
D102436	AYT12-382	5.46	2012 AYT	6
D102438	AYT12-383	5.96	2012 AYT	6
D102440	AYT12-384	3.83	2012 AYT	6
D102447	AYT12-385	5.76	2012 AYT	6
D102455	AYT12-386	4.60	2012 AYT	1
D102456	AYT12-387	5.83	2012 AYT	2
D102460	AYT12-388	4.21	2012 AYT	1
D10550	AYT12-013	3.45	2012 AYT	1
D10554	AYT12-015	4.79	2012 AYT	1
D10556	AYT12-017	4.96	2012 AYT	2
D10561	AYT12-019	5.12	2012 AYT	7
D10581	AYT12-023	4.06	2012 AYT	2
D10582	AYT12-024	4.06	2012 AYT	2
D10609	AYT12-031	5.54	2012 AYT	9
D10659	AYT12-039	5.12	2012 AYT	7
D10685	AYT12-044	4.51	2012 AYT	7
D10744	AYT12-056	4.79	2012 AYT	1
D10775	AYT12-066	4.99	2012 AYT	7
D10793	AYT12-067	4.71	2012 AYT	7
D10844	AYT12-072	3.99	2012 AYT	1
D10849	AYT12-073	3.87	2012 AYT	1
D10909	AYT12-081	4.20	2012 AYT	3
D10916	AYT12-083	3.12	2012 AYT	3

Entry	ID	FHB severity	Original AYT	Cluster
D10924	AYT12-085	3.48	2012 AYT	3
D111004	AYT13-538	4.72	2013 AYT	2
D111009	AYT13-539	5.63	2013 AYT	6
D111024	AYT13-540	5.33	2013 AYT	6
D111028	AYT13-541	4.78	2013 AYT	6
D111029	AYT13-542	4.99	2013 AYT	6
D111035	AYT13-543	5.18	2013 AYT	6
D111038	AYT13-544	4.98	2013 AYT	6
D111048	AYT13-545	5.06	2013 AYT	6
D111051	AYT13-546	3.91	2013 AYT	6
D111064	AYT13-547	5.25	2013 AYT	6
D111065	AYT13-548	4.63	2013 AYT	6
D111068	AYT13-549	4.69	2013 AYT	6
D111071	AYT13-550	5.02	2013 AYT	6
D111072	AYT13-551	4.73	2013 AYT	6
D111079	AYT13-552	5.17	2013 AYT	6
D111081	AYT13-553	4.77	2013 AYT	6
D111086	AYT13-554	5.03	2013 AYT	6
D111094	AYT13-555	5.23	2013 AYT	6
D111097	AYT13-556	4.67	2013 AYT	6
D111100	AYT13-557	5.22	2013 AYT	6
D111103	AYT13-563	6.16	2013 AYT	6
D111104	AYT13-564	4.61	2013 AYT	6
D111106	AYT13-565	5.15	2013 AYT	6
D111113	AYT13-566	4.94	2013 AYT	6
D111119	AYT13-567	5.41	2013 AYT	6
D111122	AYT13-568	4.53	2013 AYT	2
D111127	AYT13-569	4.56	2013 AYT	6
D111132	AYT13-570	5.36	2013 AYT	6
D111150	AYT13-571	5.06	2013 AYT	6
D111154	AYT13-572	5.51	2013 AYT	6
D111156	AYT13-573	4.60	2013 AYT	6
D111164	AYT13-574	5.69	2013 AYT	6
D111165	AYT13-575	5.72	2013 AYT	6
D111168	AYT13-576	4.36	2013 AYT	6
D111180	AYT13-577	5.59	2013 AYT	6

Entry	ID	FHB severity	Original AYT	Cluster
D111187	AYT13-578	4.91	2013 AYT	6
D111189	AYT13-579	3.79	2013 AYT	6
D111193	AYT13-580	4.67	2013 AYT	6
D111194	AYT13-581	4.81	2013 AYT	6
D111196	AYT13-582	4.01	2013 AYT	6
D111197	AYT13-583	4.39	2013 AYT	6
D111201	AYT13-584	4.52	2013 AYT	6
D111207	AYT13-585	4.67	2013 AYT	6
D111209	AYT13-586	5.11	2013 AYT	6
D111214	AYT13-587	5.41	2013 AYT	6
D111216	AYT13-588	3.94	2013 AYT	6
D111220	AYT13-589	4.91	2013 AYT	6
D111221	AYT13-590	5.27	2013 AYT	6
D111224	AYT13-591	5.16	2013 AYT	6
D111228	AYT13-592	4.39	2013 AYT	6
D111230	AYT13-593	5.31	2013 AYT	6
D111273	AYT13-594	4.70	2013 AYT	6
D111287	AYT13-595	3.86	2013 AYT	6
D111288	AYT13-596	4.22	2013 AYT	7
D111289	AYT13-597	4.44	2013 AYT	7
D111292	AYT13-598	4.45	2013 AYT	2
D111295	AYT13-599	4.19	2013 AYT	1
D111296	AYT13-600	3.69	2013 AYT	2
D111297	AYT13-601	3.74	2013 AYT	1
D111300	AYT13-602	4.55	2013 AYT	7
D111302	AYT13-603	4.13	2013 AYT	2
D111303	AYT13-604	4.45	2013 AYT	2
D111305	AYT13-605	4.53	2013 AYT	2
D111319	AYT13-606	5.20	2013 AYT	6
D111320	AYT13-607	5.16	2013 AYT	6
D111345	AYT13-631	5.62	2013 AYT	4
D111372	AYT13-636	5.06	2013 AYT	4
D111374	AYT13-637	5.39	2013 AYT	4
D111384	AYT13-639	5.34	2013 AYT	4
D111386	AYT13-640	5.23	2013 AYT	4
D111391	AYT13-641	4.58	2013 AYT	4

Entry	ID	FHB severity	Original AYT	Cluster
D111397	AYT13-642	4.88	2013 AYT	4
D111458	AYT13-654	4.19	2013 AYT	4
D11505	AYT13-429	5.22	2013 AYT	8
D11534	AYT13-437	5.32	2013 AYT	9
D11538	AYT13-439	4.81	2013 AYT	9
D11574	AYT13-445	5.03	2013 AYT	8
D11591	AYT13-448	4.82	2013 AYT	8
D11603	AYT13-450	4.44	2013 AYT	8
D11605	AYT13-451	5.26	2013 AYT	8
D11612	AYT13-453	4.43	2013 AYT	8
D11616	AYT13-455	5.06	2013 AYT	9
D11624	AYT13-456	5.12	2013 AYT	1
D11626	AYT13-457	5.28	2013 AYT	1
D11627	AYT13-458	5.46	2013 AYT	2
D11648	AYT13-467	3.26	2013 AYT	9
D11700	AYT13-472	3.50	2013 AYT	4
D11701	AYT13-473	3.79	2013 AYT	4
D11705	AYT13-476	4.49	2013 AYT	4
D11721	AYT13-479	5.20	2013 AYT	4
D11722	AYT13-480	5.47	2013 AYT	4
D11729	AYT13-482	4.25	2013 AYT	4
D11750	AYT13-484	4.54	2013 AYT	1
D11752	AYT13-485	4.55	2013 AYT	3
D11820	AYT13-502	7.12	2013 AYT	4
D11892	AYT13-514	5.33	2013 AYT	6
D11893	AYT13-515	4.94	2013 AYT	2
D11894	AYT13-516	5.19	2013 AYT	6
D11902	AYT13-517	4.91	2013 AYT	2
D11906	AYT13-518	5.04	2013 AYT	6
D11913	AYT13-519	3.84	2013 AYT	6
D11919	AYT13-520	3.59	2013 AYT	6
D11922	AYT13-521	5.04	2013 AYT	6
D11931	AYT13-522	4.62	2013 AYT	7
D11943	AYT13-523	3.97	2013 AYT	6
D11944	AYT13-524	6.01	2013 AYT	6
D11951	AYT13-525	5.60	2013 AYT	6

Entry	ID	FHB severity	Original AYT	Cluster
D11952	AYT13-526	4.74	2013 AYT	6
D11954	AYT13-527	4.57	2013 AYT	6
D11955	AYT13-528	4.29	2013 AYT	6
D11958	AYT13-529	5.88	2013 AYT	6
D11959	AYT13-530	4.96	2013 AYT	6
D11960	AYT13-531	4.84	2013 AYT	6
D11962	AYT13-532	5.49	2013 AYT	6
D11974	AYT13-533	4.19	2013 AYT	6
D11975	AYT13-534	6.52	2013 AYT	6
D11990	AYT13-535	4.66	2013 AYT	2
D11994	AYT13-536	4.42	2013 AYT	2
D11995	AYT13-537	4.82	2013 AYT	2
D12001	AYT14-717	5.67	2014 AYT	4
D12055	AYT14-727	6.64	2014 AYT	4
D12066	AYT14-728	5.52	2014 AYT	1
D12076	AYT14-730	6.17	2014 AYT	4
D121068	AYT14-926	5.80	2014 AYT	7
D12108	AYT14-735	6.23	2014 AYT	4
D121089	AYT14-928	5.50	2014 AYT	8
D121091	AYT14-929	5.21	2014 AYT	8
D121112	AYT14-939	6.46	2014 AYT	7
D121118	AYT14-941	6.14	2014 AYT	7
D121120	AYT14-942	5.40	2014 AYT	7
D121121	AYT14-943	6.07	2014 AYT	7
D121140	AYT14-947	5.28	2014 AYT	7
D121152	AYT14-948	5.10	2014 AYT	7
D12130	AYT14-737	4.96	2014 AYT	4
D12134	AYT14-738	5.11	2014 AYT	4
D12185	AYT14-743	6.26	2014 AYT	4
D12288	AYT14-766	5.46	2014 AYT	5
D12373	AYT14-778	7.08	2014 AYT	7
D12433	AYT14-788	5.42	2014 AYT	9
D12445	AYT14-792	5.04	2014 AYT	9
D12484	AYT14-800	4.71	2014 AYT	8
D12507	AYT14-806	4.73	2014 AYT	8
D12559	AYT14-815	5.88	2014 AYT	8

Entry	ID	FHB severity	Original AYT	Cluster
D12629	AYT14-826	5.86	2014 AYT	7
D12645	AYT14-831	5.89	2014 AYT	7
D12678	AYT14-837	5.71	2014 AYT	8
D12679	AYT14-838	5.15	2014 AYT	8
D12704	AYT14-842	4.97	2014 AYT	8
D12727	AYT14-843	5.70	2014 AYT	7
D12733	AYT14-846	5.47	2014 AYT	8
D12773	AYT14-859	5.74	2014 AYT	8
D12816	AYT14-876	5.49	2014 AYT	8
D12842	AYT14-885	5.36	2014 AYT	5
D12846	AYT14-887	4.10	2014 AYT	1
D12863	AYT14-891	4.48	2014 AYT	1
D12875	AYT14-893	4.24	2014 AYT	5
D12886	AYT14-900	5.72	2014 AYT	5
D12894	AYT14-904	5.23	2014 AYT	5
D12897	AYT14-905	6.16	2014 AYT	5
D12929	AYT14-918	5.50	2014 AYT	7
D102621	AYT14-1038	3.28	2014 AYT	7
D102626	AYT14-1042	2.87	2014 AYT	7
D102629	AYT14-1045	4.85	2014 AYT	4
D102644	AYT14-1053	2.76	2014 AYT	7
D13084	AYT15-1099	4.51	2015 AYT	1
D13086	AYT15-1100	3.41	2015 AYT	1
D13089	AYT15-1102	4.61	2015 AYT	1
D13091	AYT15-1103	4.29	2015 AYT	1
D13102	AYT15-1104	3.94	2015 AYT	9
D13130	AYT15-1110	4.84	2015 AYT	7
D13132	AYT15-1111	4.19	2015 AYT	7
D13137	AYT15-1112	4.64	2015 AYT	7
D13204	AYT15-1132	3.51	2015 AYT	9
D13232	AYT15-1133	4.65	2015 AYT	7
D13334	AYT15-1124	5.41	2015 AYT	5
D13343	AYT15-1126	5.28	2015 AYT	3
D13344	AYT15-1127	4.44	2015 AYT	3
D13403	AYT15-1158	3.38	2015 AYT	1
D13489	AYT15-1167	3.45	2015 AYT	9

Entry	ID	FHB severity	Original AYT	Cluster
D13499	AYT15-1170	5.31	2015 AYT	9
D13500	AYT15-1171	4.00	2015 AYT	5
D13504	AYT15-1173	4.74	2015 AYT	9
D13507	AYT15-1174	4.74	2015 AYT	5
D13518	AYT15-1175	3.81	2015 AYT	9
D13521	AYT15-1176	4.36	2015 AYT	9
D13526	AYT15-1177	4.23	2015 AYT	9
D13541	AYT15-1179	4.10	2015 AYT	9
D13547	AYT15-1182	3.89	2015 AYT	9
D13561	AYT15-1184	4.81	2015 AYT	5
D13595	AYT15-1195	3.84	2015 AYT	8
D13615	AYT15-1200	4.41	2015 AYT	8
D13668	AYT15-1207	4.21	2015 AYT	5
D13673	AYT15-1210	4.56	2015 AYT	5
D13689	AYT15-1214	4.79	2015 AYT	5
D13720	AYT15-1220	4.06	2015 AYT	5
D13738	AYT15-1224	4.68	2015 AYT	5
D13750	AYT15-1226	4.09	2015 AYT	5
D13751	AYT15-1227	4.06	2015 AYT	5
D13761	AYT15-1230	3.48	2015 AYT	5
D13762	AYT15-1231	3.82	2015 AYT	5
D13771	AYT15-1239	4.79	2015 AYT	5
D13790	AYT15-1240	5.01	2015 AYT	5
D13823	AYT15-1246	4.46	2015 AYT	5
D13848	AYT15-1251	4.29	2015 AYT	5
D13896	AYT15-1262	4.68	2015 AYT	5
D13899	AYT15-1263	3.91	2015 AYT	5
D13900	AYT15-1264	4.28	2015 AYT	5
D13931	AYT15-1266	4.68	2015 AYT	5
D13943	AYT15-1269	3.71	2015 AYT	5
D13979	AYT15-1275	3.99	2015 AYT	9
D14053	AYT16-1345	4.14	2016 AYT	1
D14056	AYT16-1347	4.34	2016 AYT	1
D14065	AYT16-1350	4.84	2016 AYT	5
D141103	AYT16-1421	3.72	2016 AYT	8
D141104	AYT16-1422	4.65	2016 AYT	5

Entry	ID	FHB severity	Original AYT	Cluster
D141105	AYT16-1423	3.63	2016 AYT	8
D14115	AYT16-1357	4.36	2016 AYT	7
D141172	AYT16-1471	5.27	2016 AYT	3
D141264	AYT16-1518	5.04	2016 AYT	1
D141268	AYT16-1520	5.67	2016 AYT	1
D141279	AYT16-1523	5.35	2016 AYT	1
D14161	AYT16-1363	5.56	2016 AYT	7
D14171	AYT16-1364	3.44	2016 AYT	1
D14173	AYT16-1365	4.81	2016 AYT	1
D14246	AYT16-1376	4.50	2016 AYT	7
D14247	AYT16-1377	3.97	2016 AYT	7
D14251	AYT16-1379	4.64	2016 AYT	7
D14257	AYT16-1380	4.77	2016 AYT	7
D14258	AYT16-1381	4.74	2016 AYT	7
D14266	AYT16-1382	4.59	2016 AYT	7
D14324	AYT16-1387	3.33	2016 AYT	9
D14329	AYT16-1388	5.47	2016 AYT	9
D14354	AYT16-1393	5.11	2016 AYT	5
D14386	AYT16-1399	4.62	2016 AYT	5
D14401	AYT16-1400	5.22	2016 AYT	5
D14467	AYT16-1436	3.98	2016 AYT	8
D14511	AYT16-1440	4.99	2016 AYT	7
D14616	AYT16-1446	5.15	2016 AYT	3
D14649	AYT16-1449	5.78	2016 AYT	3
D14687	AYT16-1453	4.93	2016 AYT	3
D14703	AYT16-1458	4.47	2016 AYT	3
D14807	AYT16-1494	4.34	2016 AYT	3
D14812	AYT16-1462	4.72	2016 AYT	7
D14823	AYT16-1464	4.14	2016 AYT	7
D14852	AYT16-1497	3.90	2016 AYT	7
D14854	AYT16-1498	5.19	2016 AYT	7
D14881	AYT16-1504	5.41	2016 AYT	7
D14929	AYT16-1510	4.53	2016 AYT	5
D14935	AYT16-1514	4.51	2016 AYT	5
D15015	AYT17-1529	5.68	2017 AYT	8
D15051	AYT17-1534	4.36	2017 AYT	3

Entry	ID	FHB severity	Original AYT	Cluster
D15054	AYT17-1535	5.75	2017 AYT	8
D15078	AYT17-1542	4.81	2017 AYT	9
D151001	AYT17-1687	5.03	2017 AYT	2
D151012	AYT17-1690	4.39	2017 AYT	3
D15115	AYT17-1544	4.36	2017 AYT	7
D151165	AYT17-1708	5.18	2017 AYT	7
D151174	AYT17-1709	4.99	2017 AYT	8
D151181	AYT17-1710	4.51	2017 AYT	1
D151187	AYT17-1711	7.15	2017 AYT	1
D151190	AYT17-1712	4.47	2017 AYT	1
D151191	AYT17-1713	6.01	2017 AYT	1
D151193	AYT17-1714	4.93	2017 AYT	1
D151197	AYT17-1715	4.56	2017 AYT	1
D151202	AYT17-1716	5.39	2017 AYT	2
D151206	AYT17-1717	5.16	2017 AYT	7
D151212	AYT17-1718	5.61	2017 AYT	7
D151216	AYT17-1719	4.60	2017 AYT	7
D151220	AYT17-1720	5.62	2017 AYT	7
D151229	AYT17-1721	6.06	2017 AYT	1
D151231	AYT17-1722	5.26	2017 AYT	1
D151232	AYT17-1723	4.72	2017 AYT	1
D151234	AYT17-1724	4.40	2017 AYT	1
D151239	AYT17-1725	4.46	2017 AYT	1
D151243	AYT17-1726	4.34	2017 AYT	1
D151247	AYT17-1727	5.82	2017 AYT	1
D151248	AYT17-1728	4.42	2017 AYT	1
D151252	AYT17-1729	4.95	2017 AYT	1
D151255	AYT17-1730	5.60	2017 AYT	1
D151256	AYT17-1731	4.78	2017 AYT	1
D151258	AYT17-1732	6.05	2017 AYT	1
D151259	AYT17-1733	5.10	2017 AYT	1
D151260	AYT17-1734	5.51	2017 AYT	1
D151262	AYT17-1735	5.70	2017 AYT	1
D151264	AYT17-1736	6.07	2017 AYT	1
D151265	AYT17-1737	5.39	2017 AYT	1
D151266	AYT17-1738	5.47	2017 AYT	1

Entry	ID	FHB severity	Original AYT	Cluster
D151268	AYT17-1739	4.73	2017 AYT	1
D151276	AYT17-1740	4.12	2017 AYT	1
D151277	AYT17-1741	4.55	2017 AYT	1
D151278	AYT17-1742	6.02	2017 AYT	1
D151282	AYT17-1743	6.18	2017 AYT	1
D151283	AYT17-1744	6.43	2017 AYT	1
D151284	AYT17-1745	5.19	2017 AYT	1
D151285	AYT17-1746	5.50	2017 AYT	1
D151295	AYT17-1747	6.39	2017 AYT	3
D151299	AYT17-1748	5.05	2017 AYT	3
D151336	AYT17-1749	5.22	2017 AYT	9
D151343	AYT17-1750	3.97	2017 AYT	9
D151344	AYT17-1751	4.69	2017 AYT	9
D151345	AYT17-1752	4.07	2017 AYT	9
D15192	AYT17-1557	5.72	2017 AYT	7
D15262	AYT17-1563	3.66	2017 AYT	1
D15269	AYT17-1566	4.73	2017 AYT	1
D15279	AYT17-1569	6.28	2017 AYT	1
D15281	AYT17-1570	5.51	2017 AYT	1
D15282	AYT17-1571	5.65	2017 AYT	1
D15341	AYT17-1577	4.73	2017 AYT	5
D15354	AYT17-1580	5.34	2017 AYT	7
D15391	AYT17-1588	4.29	2017 AYT	5
D15428	AYT17-1593	4.15	2017 AYT	5
D15432	AYT17-1594	4.78	2017 AYT	5
D15433	AYT17-1595	5.34	2017 AYT	5
D15506	AYT17-1605	5.00	2017 AYT	5
D15508	AYT17-1606	5.07	2017 AYT	1
D15515	AYT17-1609	4.99	2017 AYT	5
D15568	AYT17-1615	4.96	2017 AYT	5
D15574	AYT17-1616	4.04	2017 AYT	4
D15588	AYT17-1619	5.65	2017 AYT	7
D15677	AYT17-1630	4.68	2017 AYT	7
D15722	AYT17-1637	4.74	2017 AYT	9
D15739	AYT17-1640	4.27	2017 AYT	9
D15752	AYT17-1642	4.95	2017 AYT	9

Entry	ID	FHB severity	Original AYT	Cluster
D15780	AYT17-1646	4.07	2017 AYT	4
D15787	AYT17-1650	4.79	2017 AYT	4
D15792	AYT17-1651	6.05	2017 AYT	4
D15836	AYT17-1654	5.32	2017 AYT	1
D15838	AYT17-1656	4.47	2017 AYT	1
D15841	AYT17-1657	4.40	2017 AYT	1
D15843	AYT17-1658	4.93	2017 AYT	1
D15851	AYT17-1661	4.48	2017 AYT	1
D15871	AYT17-1669	5.13	2017 AYT	1
D15872	AYT17-1670	5.37	2017 AYT	1
D15890	AYT17-1673	5.57	2017 AYT	1
D15898	AYT17-1674	4.88	2017 AYT	1
D15927	AYT17-1677	4.58	2017 AYT	4
D15933	AYT17-1678	4.27	2017 AYT	4
D15935	AYT17-1679	4.45	2017 AYT	4
D15937	AYT17-1680	4.72	2017 AYT	4
D15946	AYT17-1682	4.24	2017 AYT	9
D15963	AYT17-1685	4.94	2017 AYT	9
D16040	AYT18-1759	4.81	2018 AYT	7
D16058	AYT18-1762	4.39	2018 AYT	7
D16069	AYT18-1764	3.39	2018 AYT	3
D16093	AYT18-1771	3.79	2018 AYT	7
D161007	AYT18-1772	3.84	2018 AYT	8
D161012	AYT18-1773	4.10	2018 AYT	7
D161014	AYT18-1774	3.68	2018 AYT	7
D161018	AYT18-1775	4.84	2018 AYT	1
D161022	AYT18-1777	4.44	2018 AYT	1
D161025	AYT18-1778	5.08	2018 AYT	1
D161034	AYT18-1779	4.83	2018 AYT	3
D161043	AYT18-1781	5.18	2018 AYT	2
D161060	AYT18-1782	5.03	2018 AYT	7
D161062	AYT18-1783	6.41	2018 AYT	3
D161071	AYT18-1784	5.54	2018 AYT	3
D161072	AYT18-1785	5.32	2018 AYT	3
D161087	AYT18-1786	5.53	2018 AYT	1
D161095	AYT18-1787	4.47	2018 AYT	1

Entry	ID	FHB severity	Original AYT	Cluster
D161097	AYT18-1788	4.84	2018 AYT	1
D161099	AYT18-1789	5.70	2018 AYT	1
D161102	AYT18-1790	4.59	2018 AYT	2
D161104	AYT18-1791	5.42	2018 AYT	2
D161107	AYT18-1792	5.14	2018 AYT	3
D161115	AYT18-1793	4.86	2018 AYT	2
D161119	AYT18-1794	4.28	2018 AYT	2
D161128	AYT18-1795	4.78	2018 AYT	6
D161131	AYT18-1796	4.85	2018 AYT	6
D161132	AYT18-1797	4.67	2018 AYT	6
D161135	AYT18-1798	4.18	2018 AYT	2
D161143	AYT18-1799	4.62	2018 AYT	3
D161145	AYT18-1800	5.14	2018 AYT	1
D161147	AYT18-1801	4.62	2018 AYT	1
D161149	AYT18-1802	4.27	2018 AYT	1
D161151	AYT18-1803	4.59	2018 AYT	9
D161158	AYT18-1804	4.27	2018 AYT	4
D161172	AYT18-1805	5.00	2018 AYT	6
D161174	AYT18-1806	4.35	2018 AYT	1
D161175	AYT18-1807	4.51	2018 AYT	1
D161177	AYT18-1808	5.76	2018 AYT	2
D161181	AYT18-1809	4.80	2018 AYT	4
D161183	AYT18-1810	4.03	2018 AYT	4
D161184	AYT18-1811	5.00	2018 AYT	7
D161187	AYT18-1812	4.88	2018 AYT	7
D161190	AYT18-1813	4.54	2018 AYT	7
D161193	AYT18-1814	4.83	2018 AYT	8
D161195	AYT18-1815	4.15	2018 AYT	1
D161211	AYT18-1817	4.40	2018 AYT	1
D161213	AYT18-1818	5.46	2018 AYT	8
D161215	AYT18-1819	4.69	2018 AYT	7
D161221	AYT18-1820	4.95	2018 AYT	6
D161226	AYT18-1821	5.06	2018 AYT	4
D161227	AYT18-1822	4.34	2018 AYT	4
D16169	AYT18-1833	4.48	2018 AYT	9
D16177	AYT18-1836	3.62	2018 AYT	5

Entry	ID	FHB severity	Original AYT	Cluster
D16178	AYT18-1837	3.14	2018 AYT	5
D16185	AYT18-1839	2.50	2018 AYT	5
D16186	AYT18-1840	3.41	2018 AYT	5
D16195	AYT18-1844	3.60	2018 AYT	7
D16198	AYT18-1845	3.12	2018 AYT	7
D16205	AYT18-1846	2.34	2018 AYT	7
D16206	AYT18-1847	3.66	2018 AYT	6
D16218	AYT18-1849	3.93	2018 AYT	7
D16252	AYT18-1861	3.77	2018 AYT	5
D16259	AYT18-1864	1.79	2018 AYT	5
D16262	AYT18-1865	3.71	2018 AYT	5
D16292	AYT18-1866	2.80	2018 AYT	5
D16297	AYT18-1868	3.44	2018 AYT	5
D16305	AYT18-1870	3.97	2018 AYT	5
D16326	AYT18-1875	3.79	2018 AYT	5
D16328	AYT18-1876	3.36	2018 AYT	5
D16402	AYT18-1886	3.10	2018 AYT	5
D16409	AYT18-1888	3.12	2018 AYT	5
D16412	AYT18-1890	3.34	2018 AYT	5
D16425	AYT18-1892	2.36	2018 AYT	5
D16426	AYT18-1893	3.21	2018 AYT	5
D16443	AYT18-1895	3.14	2018 AYT	5
D16445	AYT18-1897	3.44	2018 AYT	5
D16448	AYT18-1898	3.09	2018 AYT	5
D16449	AYT18-1899	2.73	2018 AYT	5
D16454	AYT18-1901	2.11	2018 AYT	5
D16511	AYT18-1910	4.56	2018 AYT	7
D16540	AYT18-1916	4.22	2018 AYT	5
D16567	AYT18-1921	3.92	2018 AYT	1
D16618	AYT18-1930	4.27	2018 AYT	5
D16622	AYT18-1931	3.79	2018 AYT	5
D16650	AYT18-1943	3.34	2018 AYT	5
D16666	AYT18-1947	4.13	2018 AYT	9
D16716	AYT18-1956	3.69	2018 AYT	4
D16717	AYT18-1957	2.12	2018 AYT	4
D16730	AYT18-1960	4.98	2018 AYT	4

Entry	ID	FHB severity	Original AYT	Cluster
D16775	AYT18-1967	3.75	2018 AYT	9
D16781	AYT18-1969	3.43	2018 AYT	9
D16810	AYT18-1974	3.29	2018 AYT	9
D16882	AYT18-1981	4.12	2018 AYT	8
D16885	AYT18-1982	4.75	2018 AYT	7
D16894	AYT18-1983	4.37	2018 AYT	3
D16897	AYT18-1984	5.39	2018 AYT	3
D16900	AYT18-1985	4.60	2018 AYT	3
D16903	AYT18-1986	4.12	2018 AYT	1
D16905	AYT18-1987	5.60	2018 AYT	1
D16952	AYT18-1988	4.90	2018 AYT	3
D16967	AYT18-1989	4.13	2018 AYT	7
D16968	AYT18-1990	3.75	2018 AYT	7
D16971	AYT18-1991	4.03	2018 AYT	7
D16989	AYT18-1992	4.61	2018 AYT	8
D16999	AYT18-1993	4.85	2018 AYT	1
DG080348		3.01	Check	
DG081060		4.13	Check	
ALKABO		5.11	Check	
CARPIO		4.35	Check	
DIVIDE		4.55	Check	
GRENORA		5.33	Check	
JOPPA		4.84	Check	
LEBSOCK		3.17	Check	
MOUNTRAIL		5.46	Check	
ND2710		0.99	Check	
NDGRANO		4.39	Check	
NDRIVELAND		3.49	Check	
STRONGFIELD		5.75	Check	
TIOGA		4.66	Check	

APPENDIX L. SCATTER PLOTS OF PC1 AND PC2 DERIVED FROM A PRINCIPAL COMPONENT ANALYSIS.