

Implications of genome-wide studies for resistance to Fusarium head blight and foliar viruses in
US wheat breeding programs

by

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B.S., University of Ghana, 2016
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ABSTRACT

Fusarium head blight (FHB) and foliar viruses such as barley yellow dwarf virus (BYDV), and soil-borne wheat mosaic virus (SBWMV) are among the significant plant pathogenic diseases, causing billions of dollars in annual losses in wheat and small grain cereal production. FHB is particularly concerning due to its characteristic production of mycotoxins, which have substantial quality and health implications for both humans and animals. Genetic resistance remains the primary strategy for managing these complex plant pathogenic diseases. Resistance to FHB, BYD, and SBWMV in host wheat plants is a multifaceted trait influenced by numerous genes with small effect sizes. Over several decades, resistance to these diseases has been identified in studies in various wheat germplasms, including wild relatives. The application of genomic tools such as practical haplotype graphs (PHGs), genome-wide association studies (GWAS), and genomic selection (GS) has enabled the mapping and prediction of stable genomic regions associated with FHB, BYDV, and SBWMV resistance traits. High-resolution GWAS has been employed to identify loci associated with resistance to FHB, BYDV, and SBWMV in wheat. The development of shallow exome capture and imputation tools has facilitated the use of GS, which leverages genome-wide markers to predict FHB resistance traits and enhance genetic gain.

This study utilized genomic data to construct a customized wheat practical haplotype graph database, evaluate disease severity traits, map genomic regions conferring resistance to FHB, BYDV, and SBWMV, and predict FHB resistance using GS models in a diverse panel of hard winter wheat (HWW) and soft winter wheat (SWW) cultivars representing major United States public wheat breeding programs.

The results indicated significant correlations among FHB disease traits ($r = 0.32\text{--}0.70$, $P < 0.001$) across years and moderate broad-sense heritability (H^2) estimates ($0.65\text{--}0.74$). Twenty-six significant loci were identified in multiple GWAS models, many of which affected multiple traits

and represent breeding targets for prioritizing FHB resistance. Most associated loci are located within or near previously identified native quantitative trait loci (QTL). Notably, a 1BL haplotype from the soft winter wheat cultivar ‘Jamestown’ reduced the cumulative disease, the area under the disease progress curve (AUPDC) and Fusarium-damaged kernels (FDK) by 25% and, mycotoxin deoxynivalenol (DON) levels by 18%.

In the analysis of foliar viruses, 10 and 7 significant genomic regions associated with resistance to BYDV and SBWMV, respectively, were mapped. These regions included the *Bdv2* and *Sbwm1* loci that conferred resistance to BYDV and SBWMV. The effect sizes ranging 5% to 22% were reported for these novel rare associations. Disease surveys in the nursery confirmed the presence of both BYDV and SBWMV and revealed annual variation in virus populations. Co-infections with multiple viruses were common, underscoring the necessity for breeding lines with resistance to multiple viruses.

The application of genomic selection (GS) and forward genomic prediction in this study highlights the potential of a wheat diversity panel and uniform regional FHB nurseries as valuable resources for breeding programs targeting FHB resistance in the United States. Cross-validation prediction accuracies of the GS models in the diversity panel ranged from 0.51 to 0.68 across FHB traits and single nucleotide polymorphism (SNP) densities. Forward genomic prediction accuracy for FHB severity traits in the independent United States Wheat and Barley Scab Initiative (USWBSI) population, external to the training set, was consistent with cross-validation accuracy from the diversity panel in both 2023 and 2024, with accuracies of 0.52 and 0.55, respectively.

The integration of genomic selection with the deployment of novel genetic resistance regions, alongside existing resistance loci, is expected to enhance resistance, promote more sustainable disease control, and reduce the risk of wheat yield loss due to FHB, BYDV, and SBWMV.

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CHAPTER ONE

1.1 INTRODUCTION

Bread and durum wheat (*Triticum aestivum* and *Triticum turgidum*) are among the oldest and most important staple food crops (Erenstein et al., 2022). Global per capita wheat consumption averages 63.9 kg annually (Statista, 2025), and wheat-based products provide approximately 20% of the daily energy intake for the world's population (Weiser et al., 2020). In the 2024/2025 cropping season, global wheat production reached 800.86 million metric tons, with the United States ranking fifth with 53.65 million metric tons representing 7% of global wheat production (USDA, 2025). Demand for wheat is expected to continue increasing through 2034 (OECD/FAO, 2025), driven by population growth projected to reach 9.7 billion by 2050 (Wowrzeczka, 2021). However, meeting this demand is increasingly challenged by both abiotic and biotic stresses.

Among these constraints, wheat diseases in major production regions substantially reduce yield potential and vary by cultivar susceptibility, environmental conditions, and production practices (Friskop et al., 2023). In 2022 alone, diseases reduced US wheat yield by 3.6%, spanning root, stem, foliar, and head diseases (Crop Protection Network, 2025; Friskop et al., 2023). Globally, wheat losses due to diseases were estimated at \$2.9 billion over a four-year period (2018, 2019, 2020, 2021), with Fusarium head blight (FHB) being the most damaging disease (Friskop et al., 2025).

FHB is primarily caused by *Fusarium graminearum* as reported in many studies, although other *Fusarium* species have been associated with FHB and mycotoxin contamination (Mesterhazy, 1981; Arruda et al., 2016; Bai and Shaner, 2004; Karlsson et al., 2021). Common management strategies include the use of fungicides, agronomic practices, and host resistance (Moonjely et al., 2023). By far the most effective management strategy for FHB is host resistance

(Bai and Shaner, 2004; Bai et al., 2018; McMullen et al., 2012). Host resistance to FHB in wheat is a complex trait influenced by many genes (Boyles et al, 2023). There have been efforts to improve adapted cultivars with FHB resistance, including growing lines with native FHB resistance and introgressed resistance from wild relatives of wheat (Boyles et al, 2023).

Wheat is also affected by viral pathogens including *barley yellow dwarf virus* (BYDV) and *soil-borne wheat mosaic virus* (SBWMV) which for substantial yield losses in wheat production, with an estimated 1.8 million bushels lost due to BYDV and 274,000 bushels lost due to SBMV between 2019 and 2023 (Friskop *et al.*, 2022a, 2022b, 2022c, 2024).

BYDV is one of the most widespread viral diseases of cereal crops worldwide and belongs to the genus *Luteovirus*. Members of this group are transmitted by aphid vectors, with transmission efficiency influenced by gut morphology and cellular anatomy, particularly in species such as *Myzus persicae* (Ponsen, 1972). BYDV comprises several isolates, namely BYD-MAV, BYD-PAS, and BYD-PAV (Lapierre and Hariri, 2008). Among these, BYD-PAV is the most prevalent, although its distribution varies across environments. BYDV symptom expression varies in the field among wheat cultivars and environments. Common symptoms include purple or reddish flag leaves, chlorosis, and stunting of plants under high disease pressure (Choudhury *et al.*, 2019).

SBWMV, a member of genus *Furovirus*, is transmitted by *Polymyxa graminis*, a soilborne plasmodiophorid (Lapierre and Hariri, 2008). The virus persists within the vector and is transmitted to plants during root infection. SBWMV is one of the most important wheat diseases under favorable environmental conditions, particularly in the central and eastern United States (Schlegel et al., 2021). Infection of SBWMV is favored by prolonged wet conditions in fall followed by an extended and cool period in spring (15–20 °C) promoting disease development (Zhang et al., 2011; Hao et al., 2012). The symptoms of SBWMV in susceptible cultivars include

yellow mosaic patterns, leaf mottling, streaking, and plant stunting (Zhang et al., 2011). As temperatures increase later in the spring, foliar symptoms may fade, and plants can appear to recover but still have significant yield reduction. The most practical management for SBWMV is the use of resistant wheat lines.

Advances in genomic tools and resources over the past few decades have created new opportunities to identify robust, stable genomic regions associated with key traits, including disease resistance diagnostic markers, available to wheat breeders (Minter and Saunders, 2023). Genome-wide association studies (GWAS) are widely used to map genes of interest and identify genetic markers for marker-assisted selection (MAS) in both plant and animal breeding (Zuo et al., 2025). By leveraging recombination events within diverse, unrelated populations, GWAS can achieve high mapping resolution.

Genomic selection (GS) is increasingly vital tool for advancing materials in breeding programs. It uses statistical models trained on genomic and phenotypic data to predict performance and guide and selection decisions within the breeding pipeline. The emergence of low-cost next-generation DNA-sequencing, marker-based genotyping, and genome-wide single-nucleotide polymorphism (SNP) data made GS more accessible and affordable (Alemu et al., 2024). GS reduces breeding cycle length and improves the accuracy of genomic estimated breeding values (GEBV), even for lines with only genotypic data (Crossa et al., 2017). This approach is particularly valuable for complex traits such as FHB where resistance is quantitatively inherited and controlled by many small-effect loci, resulting in slow genetic gain through conventional methods (Moreno-Amores et al., 2020). Unlike MAS, which relies on a limited set of markers that may not be consistent across genetic backgrounds, GS uses genome-wide markers to predict GEBVs. GS has shown

strong potential in FHB resistance breeding, with studies reporting higher prediction accuracy and faster genetic gains over time (Moreno-Amores et al., 2020; Rutkoski et al., 2012).

Low-coverage sequencing data are increasingly valuable when coupled with imputation methods that generate more genomic variants at relatively low cost. Imputation involves inferring missing genomic variants that could not be sequenced on a low-coverage sequencing platform such as skim-sequencing. These approaches rely on statistical frameworks such as the hidden Markov Model (HMM), which enable phasing and imputation (Li and Stephens, 2003; Berdnikova et al., 2024). Imputation is critical in genome-wide association studies (GWAS) by increasing the number of variants (markers) available (Berdnikova et al., 2024) and enhances genomic selection/prediction models.

The practical haplotype graph (PHG) is a recently developed novel genomics tool used to improve the efficiency of low-coverage sequencing (Jordan et al., 2022; Jensen et al., 2020). Compared to other imputation tools like Beagle, the PHG offers high accuracy even with as low as 0.01x coverage of sequence data (Jordan et al., 2022).

The aims of this study were to use genomic data to construct a customized wheat practical haplotype graph (PHG) database, evaluate FHB, BYDV, and SBWMV resistance, and map resistance genomic regions using a diverse panel of HWW and SWW cultivars representing major US public wheat breeding programs.

Objectives:

1. Build a customized wheat practical haplotype graph (PHG) database for a diverse panel of modern wheat cultivars and breeding lines used in public U.S. breeding programs.

2. Evaluate hard and soft winter wheat cultivars for FHB response and phenotypic plasticity under inoculated nursery conditions.
3. Assess resistance to barley yellow dwarf virus (BYDV) and soil-borne wheat mosaic virus (SBWMV) under natural field infection to identify novel resistance regions.
4. Identify and prioritize critical targets for increasing FHB, BYDV, and SBWMV resistance within US public breeding programs.
5. Develop and validate genomic selection (GS) model and predict the genomic breeding estimated values (GBEV) for cross-validation, and predictability of the GS model,
6. Leverage U.S. Wheat and Barley Scab Initiative (USWBSI) regional nurseries as a resource for genomic prediction and genetic gain.

1.2 LITERATURE REVIEW

1.2.1 Wheat production, consumption and economic importance

Wheat is one of the most important crops globally, underpinning food security, economic stability, and both human and animal nutrition (Shewry and Hey, 2015). It occupies more land area than any other crop and ranks third in total production by harvested metric tons globally (Holman et al., 2024). Wheat grown for consumption is primarily represented by two species, namely *Triticum aestivum* (bread wheat) and *Triticum turgidum* var. *durum* (durum wheat). These are further classified based on end use, including bread wheat classes including hard and soft red winter, hard and soft white, and hard red spring and durum wheat, which is used for products such as pasta and couscous (Erenstein et al., 2022). Bread wheat accounts for approximately 95% of global production, while durum wheat comprises the remaining 5% (Wieser et al., 2020). Wheat and its products have been staple foods since their domestication 10,000 years ago and serve as an important source of 15-20% nutrient and energy for the world's population (Balfourier et al., 2019; Wieser et al., 2020). Worldwide, the cultivated area of harvested wheat stood at ~220 million hectares and was estimated at 798.9 million metric tons (FAO, 2025). Notably, from 1994 to 2023, global wheat production increased by approximately 257 million metric tons with relatively little expansion in harvested area, reflecting substantial gains in yield efficiency (Figure 1. 1) (FAOSTAT). Wheat consumption has also risen steadily, increasing by about 8% since 2018/2019, with Asia (particularly China and India) and the European Union representing the largest consumer regions (USDA and Statista, 2025). These trends underscore the growing need for continued genetic improvement, particularly for resistance to plant diseases and pests, which remain major constraints to sustainable production.

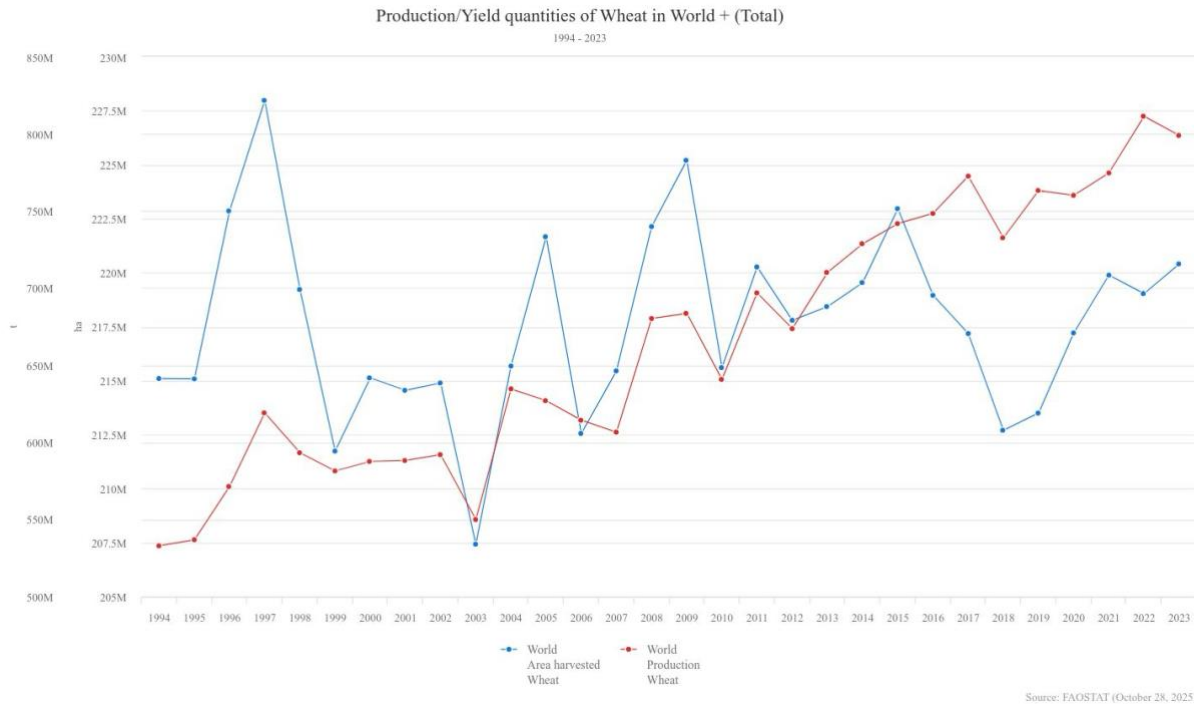


Figure 1. 1. Production/Yield quantities of the Wheat in World from 1994 to 2023. (Sourced from FAOSTAT).

The global wheat market is projected to reach \$270.88 billion by 2030, with 4.23% growth (Wheat Market Size, Share and Growth Analysis Report, 2030 (n.d.)). Global wheat production stood at 768 million metric tons in 2018, with 190 million metric tons (~25%) traded internationally, making it the most traded cereal, surpassing corn (Erenstein et al., 2022). Despite substantial gains in yield and production, both biotic and abiotic constraints continue to limit progress in global wheat systems. Among these, biotic stresses caused by pathogens, including viruses and fungi, pose major economic challenges to wheat production worldwide (Shiferaw et al., 2013).

Barley yellow dwarf virus (BYDV), soil-borne wheat mosaic virus (SBWMV), and Fusarium head blight (FHB) are among the most significant diseases affecting wheat production. FHB is particularly important due to its dual impact on yield and grain safety. The disease is associated with the accumulation of the mycotoxin deoxynivalenol (DON), or vomitoxin, which leads to substantial price penalties and poses serious concerns for food safety and international trade

(Shiferaw et al., 2013). These challenges highlight the critical need for continued genetic improvement and effective disease management strategies in wheat breeding programs.

1.2.2 The occurrence and importance of *Fusarium* head blight (FHB)

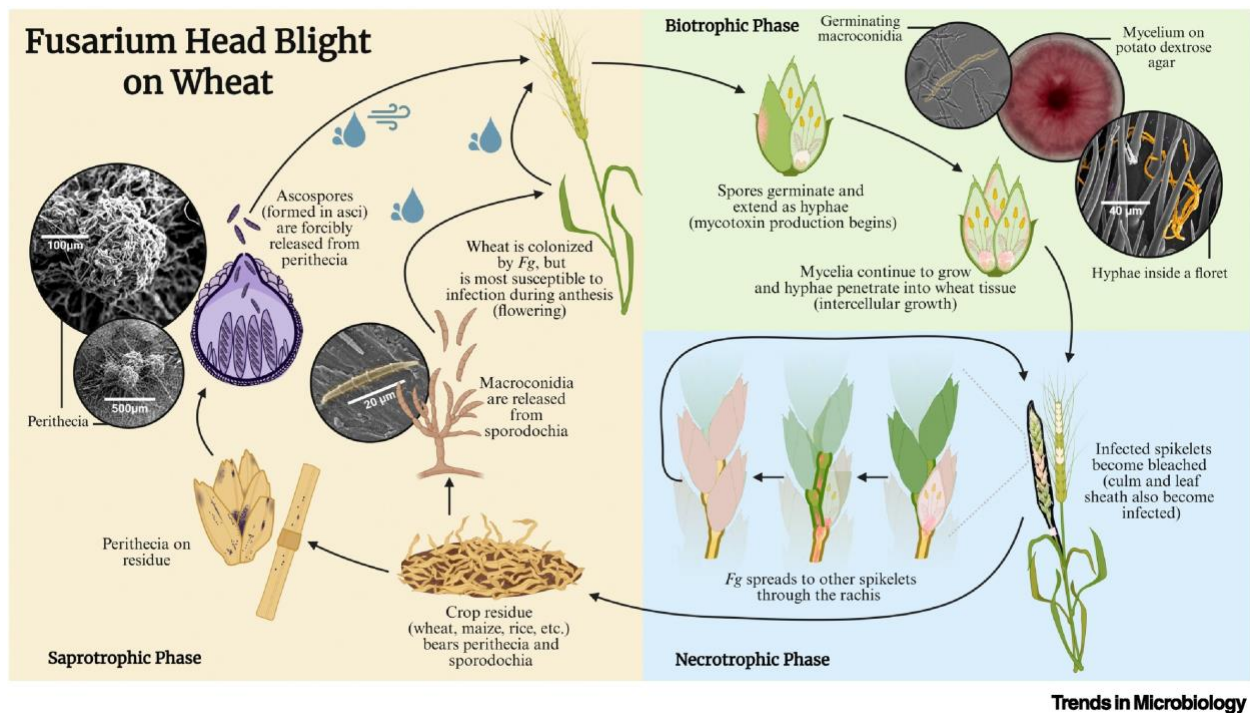
FHB, also known as scab, is a serious disease of small grain cereals, including wheat, barley, and maize caused by a complex of *Fusarium* species (Dweba et al., 2017). Globally, *Fusarium graminearum* is known to be the predominant causal agent of FHB, although several different *Fusarium* species are associated (Karlsson et al., 2021). A major concern associated with FHB is the contamination of grain with trichothecene mycotoxins, including DON and its acetylated derivatives, which are highly toxic to humans and animals and can cause diarrhea, nausea, vomiting, and, in some cases, feed refusal (Del Ponte et al., 2022).

W.G Smith first described FHB in England in 1884, and FHB was reported in Ohio, Indiana, and Delaware in 1890 and 1892 (Arthur, 1891; McInnes and Fogelman, 1923). Historical accounts suggest that FHB on wheat was already widespread in the U.S. wheat growing regions by the late 19th century. By 1917, FHB had been documented across 31 U.S. states, as well as Europe, Japan, and Australia (Stack, 2003). The economical and agricultural importance of FHB has grown substantially with increased awareness of mycotoxin contamination and its implication for food safety and trade. Severe epidemics in the Midwest and eastern U.S., as well as parts of Canada have caused substantial yield and quality losses, prompting coordinated research and management efforts such as the establishment of the US Wheat and Barley Scab Initiative (Stack, 2000).

1.2.3 Biology, symptoms, and signs of *Fusarium* head blight (FHB) in wheat

Fusarium head blight in wheat is primarily caused by *Fusarium graminearum*, a filamentous fungus belonging to the kingdom Fungi, phylum Ascomycota, class Sordariomycetes, order Hypocreales, and family Nectriaceae (Selvarajan et al., 2026). This species is part of the *Fusarium*

sambucinum species complex. Members of the *Fusarium* genus exhibit considerable biological diversity, with differences in life cycle, ecology and disease pathogenicity (Karlsson *et al.*, 2021). The fungus has asexual and homothallic sexual stages, namely *F. graminearum* and *Gibberella zeae*, respectively (Kant *et al.*, 2017). *Fusarium graminearum* isolates are mostly homothallic and can reproduce sexually without a partner, although a few isolates are heterothallic and require a compatible partner (Schmale and Bergstrom, 2003). The pathogen produces asexual spores called macroconidia, which are hyaline, canoe-shaped, and multicellular, formed on specialized structures called phialides (Kant *et al.*, 2017). Figure 1.2 demonstrates the typical life cycle of FHB disease, with the sexual spores produced in the perithecia referred to as the ascospores (contained in asci), which are typically forcefully ejected from the perithecia and dispersed by the turbulent air (Selvarajan *et al.*, 2026). *F. graminearum* is also characterized by the production of chlamydospores that can survive in the soil for subsequent infection of the host (Karlsson *et al.*, 2021; Leslie and Summerell, 2006).



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Figure 1. 2. Life cycle of *Fusarium graminearum* demonstrated in wheat FHB (From Selvarajan et al., 2026).

F. graminearum is the primary causal agent of FHB in small grain cereals and is also responsible for *Gibberella* ear rot and stalk rot in cultivated maize in North America and many other countries. Disease development is favored by warm, humid environmental conditions (Kant et al., 2017). The pathogen has diverse ecological and metabolic capabilities across variable lifestyles, including as saprotrophs on crop residues and as endophytes in some wild grasses (Selvarajan et al., 2026). Infection typically occurs through natural openings of host plants, after which the fungus penetrates and spreads systematically, progressing from the biotrophic to the necrotrophic phase associated with toxin synthesis (Kant et al., 2017). In wheat, FHB symptoms are characterized by premature bleaching of spikelets that can spread both upward and downward from the infection point. Diagnostic signs include pink to orange sporulation at the base of infected spikelets, discolored or necrotic tissues, and, under severe infection, kernel abortion. At maturity, infected grain often contains shriveled, lightweight, chalky kernels, referred to as Fusarium-damaged

kernels (FDK), or “tombstones.”. Symptom severity and expression vary depending on the resistance level of the wheat cultivar. FHB disease is characterized by premature clear bleaching on the infected spikelets both upward and downward from the point of infection, presence of pink or orange spore masses at the base of infected heads/spikelets, pink or white discoloration, lesions, kernel abortion, and dark fruiting structures on the infected areas (Crop Protection Network; Alisaac et al., 2023).

F. graminearum and related species are endophytic colonizers of the host (i.e., cereals and grasses) and can be found in tissues from the roots to seeds (Viviani et al., 2024). Overwintered crop residues are the primary source of *F. graminearum* inoculum for epidemics. Common crop rotations involving wheat, corn, or soybeans can therefore serve as important reservoirs of inoculum that can contribute to infection (Keller et al., 2013). The amount of inoculum influences disease severity at optimum environmental conditions, namely moisture (humidity) and temperature (Keller et al., 2013).

1.2.4 Mycotoxins

F. graminearum produces a diverse suite of specialized metabolites, including mycotoxins and hormone-like compounds, that exhibit strong biological activity in host tissues. Many of these metabolites function as virulence factors by facilitating host colonization and suppressing plant defenses, most notably through inhibition of eukaryotic protein synthesis (Selvarajan et al., 2026). Among these, trichothecenes represent the most economically and biologically important class of mycotoxins associated with Fusarium head blight (FHB). Trichothecenes are broadly categorized into type A (e.g., T-2 and HT-2 toxins) and type B (e.g., deoxynivalenol [DON] and nivalenol [NIV]), with type B trichothecenes predominating in *F. graminearum* populations (McCormick et al., 2013; Viviani et al., 2024). Deoxynivalenol (DON) is the principal mycotoxin associated with

FHB in wheat and plays a central role in pathogen virulence by promoting fungal spread within the spike, particularly during the transition from initial infection to systemic colonization (Dweba et al., 2017).

The ecological and evolutionary dynamics of the *F. graminearum* species complex are highly variable, with documented shifts in species composition and trichothecene chemotypes across regions and over time. For example, changes from DON to NIV production, as well as shifts between 15-acetyl-DON (15-ADON) and 3-acetyl-DON (3-ADON) chemotypes, have been observed in North America and elsewhere, reflecting ongoing adaptation to environmental conditions and host selection pressures (Kant et al., 2017). Despite this variability, 15-ADON remains the predominant chemotype globally (Dweba et al., 2017).

The rapid evolutionary capacity of fungal pathogens, including shifts in toxin producing profiles, can outpace the deployment of host resistance, complicating long-term disease management strategies. From a food and feed safety perspective, DON contamination is of particular concern due to its stability during processing and its adverse effects on human and animal health. Consequently, regulatory agencies have established maximum allowable DON concentrations, and grain exceeding these thresholds may be subject to price discounts or outright rejection at elevators and during processing (Selvarajan et al., 2026). Regulatory limits for deoxynivalenol (DON) vary by end use. In the United States, the U.S. Food and Drug Administration has established advisory levels of 1 ppm (1 mg kg⁻¹) for finished wheat products intended for human consumption, 10 ppm for grains and grain byproducts destined for ruminating beef and feedlot cattle older than four months and for poultry (with additional restrictions on diet inclusion rates), and 5 ppm for swine and other sensitive livestock, above which grain may be discounted or rejected in commercial channels (FDA, 2010).

1.2.5 Sources of FHB Resistance in Wheat

Resistance to FHB in wheat is complex and multifaceted and is commonly classified into five types (Type I-V). Type I refers to host resistance of initial infection, type II resistance limits the spread of the pathogen within the spike, type III confers resistance to kernel damage, type IV is the tolerance to infection with minimized yield penalty, and type V involves reduced accumulation of mycotoxins (Dweba et al., 2017; Kant et al., 2017; Mesterhazy, 2020). Among these, type II resistance has been the most extensively studied and utilized in breeding programs due to its relative stability and ease of phenotyping (Bai and Shaner, 2004; Bai et al, 2018).

Globally, major sources of FHB resistance have been identified in diverse germplasm pools. In the United States, key resistance sources have historically been derived from spring wheat lines originating in Asia and South America, as well as winter wheat from Europe (Kant et al., 2017). The Chinese Spring wheat cultivar ‘Sumai 3’ is one of the most widely used and well-characterized sources of resistance, particularly for type II resistance, and has been extensively incorporated into breeding programs worldwide. Additional resistance sources include Asian-derived lines such as ‘Ning 7840’ and ‘CM-82036’ from China and ‘Chokwang’ from Korea (Ghimire et al., 2020). ‘Frontana’ from South America offered type-I stable resistance on chromosome 3AL and 5A (Buerstmayr et al., 2009; Ghimire et al., 2020).

There are currently nine (9) major FHB resistance loci in the wheat gene pool, of which six are from adapted wheat accessions and three from the wild relatives of wheat (Viviani et al., 2024; Venske et al., 2019). These major loci for FHB resistance include *Fhb1* on chromosome 3BS, *Fhb2* on 6BS, *Fhb3* on 4BL derived from wild relative *Leymus racemosus*, *Fhb4* on 7AL with type-I resistance from ‘Wangshuibai’, *Fhb5* on 5A from ‘Sumai3’ and ‘Wangshuibai’, *Fhb6* on 1A from wild relative *Elymus tsukushiensis* with type-II resistance,

Fhb7 on chromosomes 7AL, 7BL, and 7DL from wheat wild relatives via chromosomal translocations, *Fhb8* on 7D from Wangshuibai and *Fhb9* on chromosome 2DL from bread wheat ‘Ji5265’. The *Fhb1* gene is by far the most studied FHB resistance locus. *Fhb1* from bread wheat cv. Sumai3 is widely used in most breeding programs and confers strong type II resistance, with a phenotypic effect size on disease severity of 24.8%-41.6% in a wheat population (Anderson et al., 2001; Viviani et al., 2024). Studies show that *Fhb1*-mediated resistance is encoded by putative localized-nuclear histidine-rich calcium-binding proteins (Su et al., 2019). The wild-type allele and the mutant allele for *Fhb1* resistance differs in the deletion of a 752-bp region (Su et al., 2019; Viviani et al., 2024), where the deletion confers resistance to FHB. *Fhb7* on chromosomes 7AL, 7BL, and 7DL derived from *Thinopyrum ponticum* or *Thinopyrum elongatum*, offers up to 90% phenotypic reduction in FHB severity and this major resistance locus is currently a point of focus for further studies with its promising resistance and stronger effect size than *Fhb1* (Kuzmanovic et al., 2019; Viviani et al., 2024). The latest known major locus is *Fhb9* on 2DL from bread wheat ‘Ji5265’, with strong type-II resistance reported by Zhang et al. (2024), and accounts for 26-30% of phenotypic variation. FHB resistance in other alien species, including *Ae. ventricosa*, *Ae. speltoides*, *Elymus tsukushiensis*, wild emmer (*T. dicoccoides*), etc., have been reported (Bai et al., 2018).

Native sources have demonstrated useful levels of partial resistance that have been utilized in regional breeding programs (Buerstmayr et al., 2009; McKendry et al., 1995). Native FHB resistance present in U.S.-adapted germplasm is particularly valuable because it is already incorporated within agronomically competitive backgrounds, minimizing issues such as linkage drag while maintaining high adaptability, good quality, and phenotypic traits (Ghimire et al., 2020). Although this resistance is generally moderate and quantitatively inherited, it has played an

important role in long-term genetic improvement and complements major resistance loci from exotic sources.

Numerous U.S. cultivars have been documented to carry native sources of FHB resistance distributed across multiple genomic regions. These include ‘Massey’ (3BL) (1981), ‘COKER 9474’ (3BS), ‘Ernie’ (1A/2B/3BL/4A/4BL/5A/6A) (1994), ‘Patton’ (*Fhb2*/3B/6B), ‘Roane’, ‘Hondo’, ‘Heyne’ (3AS/4DL/4AL) (1998), ‘Goldfield’ (2B/7B), ‘Wesley’ (*Fhb1*) (1999), ‘McCormick’ (3BS/2D/ 5A), ‘Tribute’ (2002), ‘NC-Neuse’ (1A/4A/6A), ‘Truman’ (2ASc/2DS/ 3DS), ‘INW0304’ (2B), ‘IL94-1653’ (2B/4B) (2003), ‘Cecil’, ‘INW0411’ (*Fhb1*/1B/3A) (2004), ‘Bess’ (1A/2B/3B), ‘NY88046-8138’, ‘COKER 9511’, ‘WestBred X00-1079’ (2005), ‘USG3555’ (2007) (Ghimire et al., 2020) and ‘Everest’ (Clinesmith et al., 2019). The soft red winter wheat (SRWW) cultivar ‘Jamestown’ is currently considered a strong donor of native FHB resistance on chromosome 1BL (Ghimire et al., 2020; Gaire et al., 2021; Wright, 2014) and is used as a donor parent in breeding programs.

1.2.6 Genome-wide association studies (GWAS)

Genome-wide association studies (GWAS) use thousands to millions of genetic variants, known as single-nucleotide polymorphisms (SNPs), across the entire genome of related species to identify associations between genotypes and phenotypes (Tam et al., 2019). These SNPs are units of genetic variation in the genome of related individuals and are by far the most abundant form of variation (Bush and Moore, 2012). There are two allelic forms of an SNP within a population, which are the common base-pair possibilities at a SNP site (Bush and Moore, 2012). The ultimate goal of GWAS is to identify associations between alleles or genotype frequencies and the trait status of the individuals being studied (Tam et al., 2019). GWAS was involved in identifying the trait of interest (e.g., *Fusarium* head blight in wheat), selecting a diverse panel of wheat lines, and including checks

for disease susceptibility and resistance to the trait. Genotyping with advanced genotyping platforms for the individuals in the study is done to generate the genetic units, or SNPs, for the association studies using statistical models. The correlation of a SNP with a trait, such as disease resistance, in a study suggests the presence of causal variants in the vicinity of the SNP (Sul et al., 2018). GWAS is noted to be a reliable and enhanced approach for mapping at higher resolution, estimating allele frequencies, achieving comprehensive genome coverage, and detecting numerous recombination events than QTL mapping (Sahito et al., 2024). Standard GWAS models require correcting for factors that increase the rates of false positives in associations. The power of GWAS is linked to its marker resolution with a higher resolution boosting the accuracy of identifying casual genes associated with traits. Other factors that significantly impact GWAS accuracy includes phenotypic variation of the panel, panel size, population structure, and linkage disequilibrium (LD) (Sahito et al., 2024; Young, 2024; Sul et al., 2018; Bush and Moore, 2012).

1.2.7 Genomic Selection (GS)

Genomic selection (GS) applications in practical breeding have become a powerful approach for advancing breeding materials using readily available SNPs (Alemu et al., 2024). GS integrates dense genotypic marker information across the whole-genome level to predict trait performance with sufficient accuracy to support selection decisions for advancement in breeding pipelines (Lorenz et al., 2011). Unlike marker-assisted selection (MAS), which assumes that the effect of known alleles on the phenotype is known, which relies on a limited number of loci with known effects and is most effective for major-gene traits, GS captures the combined effects of many loci, including those with small individual contributions (Bernardo and Charcosset, 2006; Lorenz et al., 2011). GS or GP model building relies on high-throughput phenotypic data or reliable data. GS uses statistical models under various scenarios to select breeding population material for

advancement based on estimated genetic breeding values (Alemu et al., 2024). The genetic breeding values estimated from phenotypic data in GS represent the combined effects of all loci (Escamilla et al., 2025). GS selection entails four main steps: training population selection, GS model building, genomic prediction of independent lines, and selection of material to advance based on GBEV, as depicted below. The effectiveness of GS relies on prediction accuracy, commonly measured as the Pearson correlations between GEBVs and observed phenotypic values.

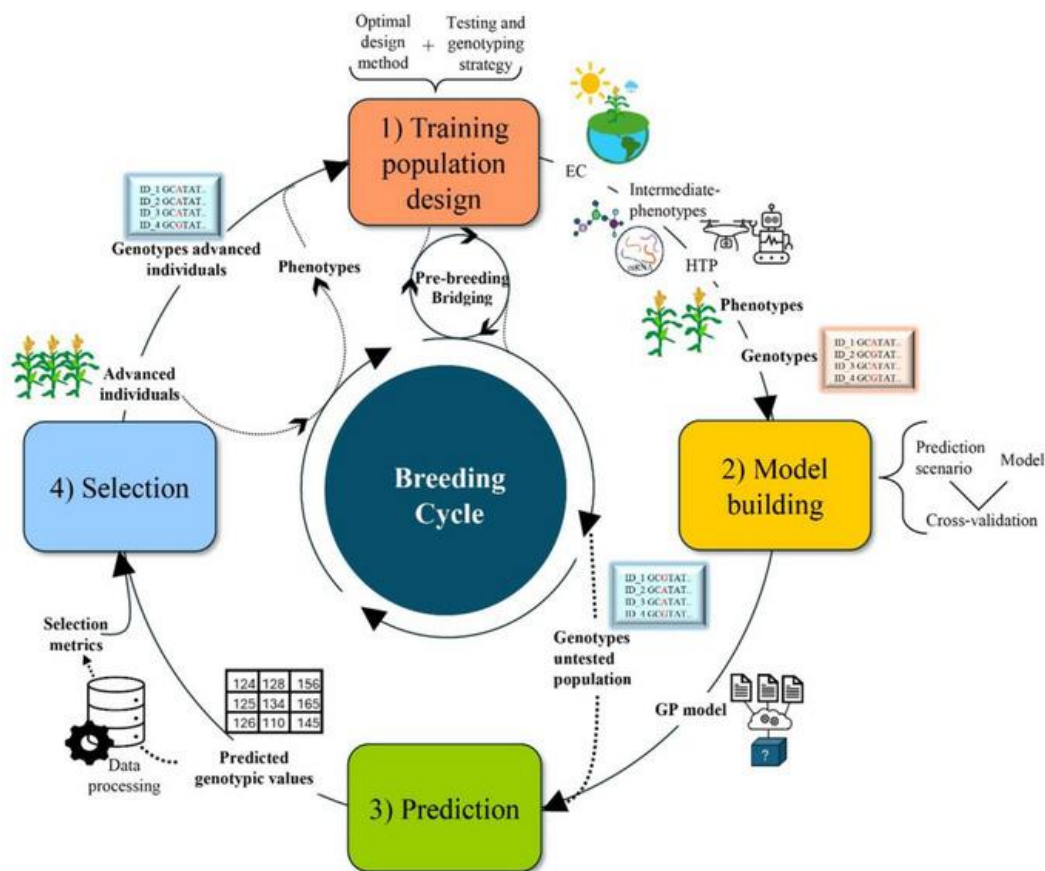


Figure 1. 3. Schematic representation of genomic selection (GS) steps in plant breeding (From Escamilla et al., 2025).

1.2.8 Agronomic traits and FHB Resistance in wheat

1.2.8.1 Plant Height

The resistance in wheat for FHB is a complex quantitative trait controlled by multiple genes, influenced by agronomic traits, and genotype-by-environment interactions (Malihipour *et al.*, 2016). Numerous FHB resistance loci (QTL) are associated with morphological traits, such as plant height, and this trait is thought to contribute to FHB resistance through disease escape, supported by the negative association between plant height and FHB incidence (Zhang *et al.*, 2022; Buerstmayr and Buerstmayr, 2016, Akohoue *et al.*, 2022). The negative association between FHB incidence and plant height is often attributed to increased distance between taller plants and the inoculum source (Schroeder and Christensen, 1963; Yan *et al.*, 2011; Bai *et al.*, 2018). However, controlled greenhouse studies using artificial point inoculations have confirmed this relationship independent of distance effects (Hilton *et al.*, 199). Plant height morphological traits have been shown to passively influence breeding efforts in FHB resistance (Akohoue *et al.*, 2022; Xu *et al.*, 2020).

Plant height in adapted wheat is controlled by at least 150 QTLs, which are scattered across the complex wheat genome (Mao *et al.*, 2010; Akohoue *et al.*, 2022). Two major semi-dwarf (*Rht*) genes, *Rht-B1* (*Rht1*) and *Rht-D1* (*Rht2*) on chromosomes 4B and 4D, respectively (Akohoue *et al.*, 2022; Zhao *et al.*, 2022; Wurschum *et al.*, 2017), have been associated with increased FHB susceptibility (Hales *et al.*, 2020). Several semi-dwarf minor QTLs on chromosomes 2A, 2B, 4A, 5A, 6A, 6B, and 7B were also associated with FHB severity variation (Akohoue *et al.*, 2022). *Rht24*, also located on chromosome 6A is a vital height-reducing gene (Tian *et al.*, 2018; Wurschum *et al.*, 2013) and is known to influence plant height and is not associated with FHB resistance in segregating lines from biparental crosses (Tian *et al.*, 2022; Herter *et al.*, 2018). *Rht24* has been shown in European wheat germplasm not to influence FHB resistance, and researchers

suggest that, in breeding efforts for FHB resistance, the choice of *Rht* gene in the selection stage is very important (Miedaner et al., 2022), notwithstanding the complexity of interactions among various traits in wheat. There is a moderate negative correlation between plant height at both the phenotypic and genotypic levels, as indicated by data analyses (Buerstmayr, 2015; Akohoue et al., 2022; Zhang et al., 2022). It is also noted by Akohoue et al. (2022) who reported that genotypes with *Rht24* and No *Rht* (not carrying any known *Rht* loci) had statistically lower FHB severity compared to *Rht2* and *Rht2* + *Rht24* genetic backgrounds, and identified a total of eight multi-trait genome-wide association studies (MT-GWAS) for plant height, which explained nearly 70% of the genetic variation for plant height. Their work identified six pleiotropic loci that affect height and FHB severity located on chromosomes 2A, 4D, 5A, 6B, and 7B (Akohoue et al., 2022). Wild-type alleles of *Rht-B1a* and *Rht-Da1* (i.e., *Rht1* and *Rht2*) contribute to FHB resistance, with *Rht1* directly controlling the influence of plant height through pleiotropy and/or through tight linkage (Buerstmayr and Buerstmayr, 2016; Zhang et al., 2022). Major QTL of plant height on chromosome 4BS coincided with the *Rht-B1*(*Rht1*) locus and accounted for 40% phenotypic variation as noted by Lu et al. 2013. A major FHB resistance QTL on 4B co-localizes with the *Rht-B1* locus (Prat et al., 2016). There are several small loci of *Rht-D1* (*Rht2*) that control FHB severity, identified in wheat genomic regions on chromosomes 2A, 2B, 4A, 5A, 6A, 6B, and 7B, respectively (Akohoue et al., 2022).

1.2.8.2 Vernalization in Winter Wheat

The major genetic factors controlling plant responses to phenological characteristics, such as low temperature and photoperiod, in wheat are the vernalization response genes (*VRN*), which respond to the cold period to activate the reproductive phase (Dubcovsky et al., 1998). There are three identified major vernalization genes, namely *VRN-1*, *VRN-2*, and *VRN-3*, respectively, that control vernalization requirements in wheat (Whittall et al., 2018). *VRN-1* and *VRN-3* are involved in the

transitioning of the apex from vegetative to reproductive phase, while VRN-2 mutants accelerate flowering (Yan et al., 2003; Whittall et al., 2018). The *VRN-1* gene comprises three homologous loci, namely *VRN-A1*, *VRN-B1*, and *VRN-D1*, located on the long arms of chromosomes 5A, 5B, and 5D, respectively (Guedira et al., 2016; Law et al., 1978; Yan et al., 2003). Allele combinations of *VRN-1* and the photoperiod gene (*PPD-1*) result in variations in agronomic traits and physiological development, such as flowering time (Cane et al., 2013), tillering, plant height, and spikelet number (Dyck et al., 2004). The difference in genotypic groups at the *VRN-A1* locus accounts for significant differences in height and time to maturity (Whittall et al., 2013). The vernalization gene *VRN-B1*, located on 5BL, is also a QTL region that influences plant height and days to anthesis and confers FHB resistance, with a larger effect on incidence and FHB severity (Zhang et al., 2020). *VRN-A1* is located at 584.7 Mb using the Chinese Spring reference genome version 2.1 (Zhu et al., 2021), and the first of two major FHB resistance loci is mapped on chromosome 5AL (Prat et al., 2016), and the second is located around 658.1 Mb (Zhang et al., 2020).

1.2.8.3 Photoperiod

Reproductive phase of winter wheat is switched on after the vernalization requirements as the spring temperature increases (Guedira et al., 2016). The response of wheat to photoperiod is controlled by the PHOTPERIOD (*Ppd1*) gene loci on chromosomes 2AS, 2BS and 2DS (Guedira et al., 2016). *Ppd2* is an additional major photoperiod gene in wheat on chromosome 2BS (Scarath and Law, 1983). Two photoperiod genes, designated *Ppd1* and *Ppd2*, respectively, are noted to influence wheat sensitivity to day length (Pugsley, 1966; Keim et al., 1973). FHB resistance QTL for syntenic regions on chromosomes 2AS and 2BS were identified to colocalize with the photoperiod/flower control genes *Ppd-A1* and *Ppd-B1* (Zhang et al., 2020). There are separate designations for photosensitive (long-day) and insensitive (day-neutral) phenotypes, with *Ppd-*

A1a and *Ppd-A1b*; *Ppd-B1a* and *Ppd-B1b*; and *Ppd-D1a* and *Ppd-AD1b* designating insensitive and sensitive phenotypes, respectively (Whittall et al., 2018). The photoperiod-insensitive phenotype is mainly controlled by the dominant *Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a* alleles (Kiss et al., 2014). Allelic variation in *PPD-I* loci shows significant differences in plant height (Whittall et al., 2018), and this could influence passive FHB resistance in wheat. The *Ppd-D1*, *Rht-B1*, and *Rht-D1* genes have pleiotropic effects on height and FHB traits, and alleles *Ppd-D1b*, *Rht-B1a*, and *Rht-D1a* reduce FHB and increase height (Liu et al., 2013). The alleles for reduced plant height (*Rht-B1b* and *Rht-D1b*) and photoperiod insensitivity (*Ppd-D1a*) had pleiotropic effects, reducing height and increasing FHB susceptibility (Liu et al., 2013).

1.2.9 Soilborne Wheat Mosaic Virus (SBWMV), Transmission and Symptoms Biology

Changes in traditional weather patterns, with increased precipitation and prolonged cold temperatures in temperate wheat-growing regions, increases the transmission of soilborne viruses, such as SBWMV, in Canada, the United States, Asia, and Europe (Nopsa et al., 2014). SBWMV, a member of the *Furoviruses*, is transmitted by the ubiquitous *Polymyxa graminis* zoospores or germinating zoospores released from the cytosori that can survive harsh conditions in the soil (Niehl and Koenig, 2008). SBWMV in the United States was reported in Illinois and Indiana in 1919 (Clover et al., 2001) and described by McKinney et al. in 1923, as noted by Barbosa et al. (2001). The viruliferous fungus *Polymyxa graminis*, which vectors the virus, colonizes and infects the root hairs and epidermal cells of the host (wheat) under high soil moisture and favorable conditions (Myers et al., 1993; Barbosa et al., 2001). During systemic infection and movement, SBWMV uses xylem vessels to move from the roots to the leaves. Chlorotic leaf streaking, stunting, and yield losses in winter wheat are associated with SBWMV. The host range includes wheat, barley, rye, and triticale. Studying and phenotyping SBWMV in the control environment is

difficult. Mechanical transmission of SBWMV is successful in *Chenopodium* and *Nicotiana* genus but unsuccessful in winter wheat (Niehl and Koenig, 2008).

1.2.10 Soilborne Wheat Mosaic Virus (SBWMV) Resistance

SBWMV can cause severe yield losses in with reductions of up to 80% reported under favorable conditions (Myers et al., 1993). Because the virus is transmitted by the soilborne vector *Polymyxa graminis* and can persist in soils for extended periods, chemical or cultural control options are limited. Consequently, the use of resistant cultivars remains the most practical and effective management strategy. Earlier approaches such as formaldehyde soil treatments and soil heating have been explored but are largely impractical for modern production systems (Hao et al., 2012). Field resistance to SBWMV is thought to arise from multiple mechanisms, including reduced susceptibility to the fungal vector, hypersensitive responses that limit infection, or restricted systemic virus movement in resistant cultivars (Myers et al., 1993). The major QTL for SBWMV resistance in hard winter wheat was reported in several wheat cultivars, including ‘Karl 92’ (PI 564245), ‘Pioneer 26R61’, ‘AGS 2020’, and Heyne (Liu et al., 2020; Hao et al., 2012). Other winter wheat cultivars, including ‘Shawnee’, ‘Centurk’, and ‘KS73256’, were reported to have dominant resistance genes to SBWMV (Hall et al., 2009).

1.2.11 Barley yellow dwarf virus (BYDV), Transmission and Symptoms Biology

Barley yellow dwarf (BYD) is a viral disease of small grain cereals that is transmitted by several aphid species and caused by a two closely related viruses, namely *Barley yellow dwarf virus* (genus *Luteovirus*) and *Cereal yellow dwarf virus* (genus *Poleovirus*) (Zhang et al., 2009). BYD infects wheat, oat, barley, and many wild relatives of wheat. This is an important, commonly occurring wheat disease in the Southeastern and Central regions of Kansas, with sporadic occurrences in other areas of the state (Erick DeWolf, 2018). Crop losses to BYD are higher in

common wheat (*Triticum aestivum*), with up to 46% losses compared to barley (*Hordeum vulgare*) and oats (*Avena sativa*) with up to 25% and 15% losses, respectively (Zhang et al., 2009).

1.2.12 Barley yellow dwarf virus Resistance Sources in Wheat

In domesticated wheat, four major BYDV resistance QTLs (genes) have been identified, including *Bdv1*, *Bdv2*, *Bdv3*, and *Bdv4*, which are mainly derived from wild wheat relatives (Bian et al., 2024). The *Bdv1* locus on chromosome 7DS shares a location in close proximity to the pleiotropic adult plant resistance complex the *Lr34/Yr18/Sr57*, which confers partial resistance to multiple rust diseases (Bian et al., 2024). ‘Frontana,’ a spring wheat variety from Brazil, is a key source of resistance, carrying *Bdv1* and this rust resistance complex (Singh et al., 1993). Beyond major loci, numerous minor-effect QTLs contributing to BYDV tolerance have been identified. Twenty-two BYDV resistance QTLs have been identified in a biparental cross between ‘Opata’ x ‘Synthetic’ (Ayala et al., 2002). Frontana x ‘INIA66’ biparental cross mapped seven QTLs (Ayala et al., 2002). Additionally, a recombinant inbred line (RIL) population generated from a cross between ‘Jagger’ (P1 593688) and a Jagger mutant (JagMut1095) mapped several QTLs for yield-related traits and BYDV resistance QTL *QByd.hwwg-2DL* on chromosome 2DL with up to 44.5% phenotypic variation (Bian et al., 2024). Highly effective resistance to BYDV is rare, with the dominant gene *Bdv1* conferring only tolerance to the virus based on field observations across environments (Zhang et al., 2009). Wild relatives of wheat, including *Thinopyrum*, *Agropyron*, *Elymus*, *Leymus*, *Roegneria*, and *Psathyrostachys*, have been reported to have resistance to BYDV (Zhang et al., 2009). The *Bdv2* resistance gene derived from *Thinopyrum intermedium* was introgressed into common wheat backgrounds using the *ph1b* mutant, which allows homoeologous chromosome pairing (Zhang et al., 2009). *Bdv2* gene is known to confer high level of BYD resistance suppressing virus replication and movement (Aradottir and Crespo-Herrera et al., 2021).

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2.0 CHAPTER TWO

A winter wheat genome-wide association studies (GWAS) identifies large effect Fusarium head-blight (FHB) resistance haplotypes using diverse lines from U.S. breeding programs

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2.1 ABSTRACT

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* in North America, is a devastating disease in bread wheat (*Triticum aestivum* L.) and other cereal crops. The characteristic mycotoxin production and reduced grain fill cause several million metric tons of annual crop and economic losses. To identify single nucleotide polymorphisms (SNPs) associated with FHB resistance, a genome-wide association study (GWAS) was conducted using exome capture-generated SNPs from a diverse panel of 269 winter wheat breeding lines and cultivars representing most U.S. wheat-growing regions. The panel was evaluated for FHB disease symptoms in the Rocky Ford FHB nursery (Manhattan, KS) across three field seasons (2022, 2023, and 2024). Significant variation was observed among wheat lines for FHB disease metrics, including FHB severity (%), area under the disease progress curve (AUDPC), *Fusarium*-damaged kernel (FDK, %), and deoxynivalenol (DON, ppm). Data analysis revealed significant FHB disease trait correlations ($r = 0.32\text{--}0.70$, $P < 0.001$) across years and moderate broad-sense heritability (H^2) estimates (0.65–0.74). We identified 26 significant loci in multiple models and/or affected multiple traits representing breeding targets to prioritize combating FHB damage. Most of the associated loci reside in or are within close proximity to previously identified native quantitative trait loci (QTL). One region identified was a 1BL haplotype from the soft winter wheat cultivar ‘Jamestown’ reducing AUDPC and FDK by 25% and DON by 18%, demonstrating FHB resistance in an environment different from its native environment. Our findings highlight FHB resistance breeding progress in U.S. programs as demonstrated by the increased frequency of many resistant alleles in adapted wheat varieties and breeding lines and emphasizes the significance of pyramiding native FHB resistance regions as the next step to prevent FHB losses.

2.2 INTRODUCTION

Fusarium head blight (FHB), also known as scab, is a seriously damaging disease to bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* ssp. *durum*), and barley (*Hordeum vulgare* L.). In North America, it is primarily caused by the fungus *Fusarium graminearum* Schwabe (Arruda et al., 2016; Bai et al., 2018; Zhang et al., 2020). Other *Fusarium* species also have been reported to cause FHB to a lesser extent (Buerstmayr et al., 2009). The characteristic production of mycotoxins, grain damage, and significant yield reductions result in high economic losses (Janaviciene et al., 2023). For example, from 2020 to 2023, FHB caused an accumulated \$317 million loss, representing 1.38 million metric tons (51 million bushels of yield) in the United States (Crop Protection Network website). These losses are mainly due to a reduction in grain fill, quality, and grain contamination by major type B trichothecenes, such as deoxynivalenol (DON). Deoxynivalenol in infected wheat grain is harmful to both humans and monogastric animals and is considered a significant food and feed safety concern worldwide (Arruda et al., 2016, Gab-Allah et al., 2022).

Increased acreage of maize provides abundant crop residue for *Fusarium* inoculum accumulation for the primary infection, leading to frequent FHB epidemics in major wheat production areas globally (Boyles et al., 2024; Bai et al., 2018; McMullen et al., 2012; Shude et al., 2020). Overall, *Fusarium* Head Blight (FHB) was more prevalent in 2024 than in recent years, especially in the northern and eastern United States (FHB Disease Impact update |USWBSI, 2024).

Advancing genetic resistance is the most sustainable way to manage FHB in adapted wheat. Over 500 FHB resistance quantitative trait loci (QTLs) across 21 wheat chromosomes have been identified through numerous genetic studies (Venske et al., 2019; Ghimire et al., 2022; Buerstmayr et al., 2019, Buerstmayr et al., 2009). These QTLs offer various types of resistance including *type*

I (resistance to initial infection), *type II* (resistance to disease spread within a spike), *type III* (low mycotoxin accumulation in kernel), and *type IV* (resistance to Fusarium-damaged kernel, FDK) (Bai et al., 2018; Mesterházy, 1995; Mesterházy et al., 1999; Ghimire et al., 2022). There are nine formally named FHB resistance QTLs (Wu et al., 2024) and many are derived from Chinese landraces. Both *Fhb1* on chromosome 3BS (Cuthbert et al., 2006), which was characterized as a loss of function of a susceptibility locus (Su et al., 2019) and *Fhb2* on 6BS (Cuthbert et al., 2007; Steiner et al., 2019), were derived from ‘Sumai3’. *Fhb4* on 4BL was detected in ‘Wangshuibai’, while *Fhb5* on 5AS (Xue et al., 2010, 2011) and *Fhb8* on 7DL were identified in both Sumai3 and Wangshuibai (Wang et al., 2023; Wu et al., 2024). *Fhb9* on 2DL was identified from ‘Ji5265’ (Zhang et al., 2024). Wheat wild relatives contribute other sources of major FHB resistance genes including *Fhb3* on 7AS derived from *Leymus racemosus* (Qi et al., 2008), *Fhb6* on 1AS from *Elymus tsukushiensis* (Cainong et al., 2015), and *Fhb7* from *Thinopyrum ponticum* (Wang et al., 2020). *Fhb7* encodes a *glutathione S-transferase* (GST) which detoxifies trichothecene toxins including DON.

Incorporating landrace and alien FHB resistance QTLs into adapted wheat varieties with limited linkage drag on desired agronomic traits remains difficult. However, a number of FHB-resistant soft and hard winter wheat cultivars without *Fhb1* have been reported, including ‘Truman’, ‘Massey’, ‘Ernie’, ‘Roane’, ‘Bess’, ‘Jamestown’, ‘NC-Neuse’, ‘Freedom’, ‘Everest’, ‘Overland’, ‘Lyman’, ‘Heyne’, and ‘Hondo’ (Rudd et al., 2001; Bai et al., 2018; Gaire et al., 2021), suggesting the existence of native resistance in adapted wheat. The reported resistance loci include regions on chromosomes 3BL in Massey (Liu et al., 2013), 3B and 5A in Ernie (Liu et al., 2007), 2B and 3B in Bess (Peterson et al., 2017), 1A, 4A, and 6A in NC-Neuse (Peterson et al., 2016), 1B in Jamestown (Wright, 2024), and 2D in Everest (Clinesmith et al., 2019).

Wheat FHB resistance is a complex quantitative trait controlled by many genes, influenced by agronomic traits, and genotype-by-environment interactions (Malihipour et al., 2016). Numerous FHB resistance loci (QTL) are associated with morphological traits such as plant height, where taller plants are generally believed to have increased resistance through disease escape (Zhang et al., 2022; Buerstmayr and Buerstmayr, 2016). Two major semi-dwarf (*Rht*) genes, *Rht-B1* (*Rht1*) and *Rht-D1* (*Rht2*) on chromosomes 4B and 4D, respectively (Akohoue et al., 2022), have been associated with increased FHB susceptibility (Hales et al., 2020). Several semi-dwarf minor QTLs on chromosomes 2A, 2B, 4A, 5A, 6A, 6B, and 7B were also associated with FHB severity variation (Akohoue et al., 2022). Flowering date, head compactness, and anther extrusion are correlated with passive FHB resistance (Malihipour et al., 2016; Mesterházy, 1995). The inconsistency of replicated effects of wheat FHB resistance QTLs across environments and studies demonstrates the complexity of the inheritance of this trait (Rudd et al., 2001).

Genomic and bioinformatic tools have reduced the challenges of sequence-based analyses of wheat diversity panels despite the wheat genome's size (15Gb) and complexity (Jordan et al., 2022). The increase in FHB epidemics and economic losses call for alternative and advanced approaches to identifying resistance and susceptibility genes in breeding programs (Boyles et al., 2024; Wang et al., 2020). The goals for this project were to: a) build a diverse population of hard and soft winter wheat breeding lines and cultivars that are enriched for lines carrying native FHB resistance loci, b) evaluate moderately resistant SWW cultivars in the hard winter wheat region to analyze phenotypic plasticity of resistance, and c) identify and prioritize critical targets increasing FHB resistance within U.S. public breeding programs. The panel was phenotyped for three years (2022, 2023, 2024) at the Manhattan, Kansas FHB nursery. Each line was assessed for FHB severity through the grain-filling period, *Fusarium*-damaged kernels (FDK), and DON concentration. We

performed a genome-wide association study (GWAS) combining phenotyping data with ~15x exome capture-based genotypic data to identify genomic regions associated with resistance to FHB.

2.3 MATERIALS AND METHODS

2.3.1 Wheat diversity panel

A diversity panel of 269 wheat breeding lines and cultivars, including soft and hard winter wheat was evaluated for FHB disease resistance (Supplemental Table 2. 1). This germplasm was sourced from public breeders across the Great Plains, Midwest, Northeast, and Southern U.S. wheat-growing regions. The panel captures a broad range of both genetic and phenotypic diversity for both disease resistance and agronomic traits.

2.3.2 Field nursery

The field experiment followed a randomized complete block design (RCBD) utilizing two replicates over three crop seasons: 2022, 2023, and 2024. Five check varieties, ‘Everest’ (PI 659807), ‘Karl 92’ (PI 564245), ‘Overley’ (PI 634934), ‘Emerson’ (PI 676108), and ‘Flourish’ (PI 673352), with known FHB resistance levels were included. Plots measured 2.3 m in length and 0.51 m in width. Seeds were planted at a seeding rate approximately 67 kg/ha on October 7, 2021 (2022 season), October 17, 2022 (2023 season), and October 12, 2023 (2024 season) and plants were harvested on June 27, 2022, July 11, 2023, and June 25, 2024, respectively.

2.3.3 Inoculum production

The aggressive Kansas native *Fusarium graminearum* isolate GZ3639 (Bowden and Leslie, 1992; Desjardins et al., 1996) was grown on homemade potato dextrose agar (hmPDA) that supports rapid growth and produces the greatest quantity of mycelium used in mung bean broth inoculation.

Mung bean broth was prepared with 84 g of mung bean per 2.1 L of distilled water and used for conidia production. The mung-bean broth containing the conidia and mycelium plugs was used to inoculate corn kernels for corn spawn as described by Rudd et al. (2001) as a source of primary inoculum to mimic natural conditions.

2.3.4 Field inoculum application

Three field applications of the inoculum (corn spawn) were completed at a rate of 53 g/m² at approximately 35, 20, and 5 days before the estimated mean heading date (50% heading), which generally occurred between Feekes 6 to Feekes 10.1 wheat growth stages. The three inoculum applications ensured a constant flow of fungal spores for the diverse wheat panel's heading dates. An additional inoculum (fourth) application was spread within late heading plots as needed. To ensure damp heads and spikelets for pathogen establishment, mist sprinklers were placed across the nursery and night irrigated for 15 minutes at 4-hour intervals starting at 10:00 pm and ending at 6:00 am. The early moist conditions also ensure perithecia formation on the corn spawn to produce sufficient spores for infection.

2.3.5 Phenotyping

Heading date was recorded as the day when 50% of wheat heads fully emerged within each plot. Once FHB symptoms were present in the field, disease severity (estimated as percentage of infected wheat spikes within the plot) was visually scored at two- or three-day intervals until spike maturity. To correct for heading date differences among lines, severity scores were normalized using each line's own severity scores between 20/21 days post-heading until the onset of senescence. The initial 20/21-day post-heading severity phenotype is henceforth referred as SEV. Cumulative disease severity, or area under the disease progress curve (AUDPC), was calculated using normalized severity scores across a 4 time-point window beginning at 20/21 days post-

heading and incorporated the next three consecutive severity observations per line. Wheat accessions also were evaluated for FDK and DON concentration from harvested seeds. DON concentration was measured using gas chromatography-mass spectroscopy (GC-MS) at the U.S. Wheat and Barley Scab Initiative DON testing lab (North Dakota State University, Fargo, ND) and reported as ppm within the quantification limits of 0.2 - 40 ppm.

2.3.6 Kompetitive allele-specific polymorphism (KASP) genotyping

About 50 mg of leaf tissue was collected for each of 269 lines, and DNA was prepared and genotyped using exome capture libraries (Wang *et al.* 2023) as previously described (Tidakbi *et al.* 2025). The panel was KASP genotyped for the presence of *Rht1* (*Rht-B1b*) and *Rht2* (*Rht-D1b*) dwarfing alleles to evaluate confounding resistance effects due to plant height in the GWAS model. KASP assays followed the protocol described in Tidakbi *et al.* (2025), and primers and conditions are listed in Supplemental Table 2. 2. Following the GWAS analysis, the panel also was genotyped for *Fhb1*, *Fhb-2DL* (*Fhb9*), *Fhb7*, and *Ppd-A1* assays to confirm if these loci were significantly associated with the detected SNPs.

2.3.7 Statistical analysis and GWAS

Raw field phenotypic observations were analyzed separately by year as the mean of the two replications each year. Data relationships were compared using Pearson correlations between years. To analyze data across years, we calculated best linear unbiased estimates (BLUEs) for the overall performance of each disease severity metric per line using the lme4 R package (Bates *et al.*, 2015) using the model:

$$Y_{ijkl} = \mu + G_i + HD_j + y_k + (G*y)_{ik} + r_l(y_k) + \varepsilon_{ijkl},$$

where Y represents the FHB-related trait (AUDPC, SEV, FDK, and DON), μ is the overall mean, G_i is the fixed effect of the i^{th} genotype, HD_j is the fixed effect of the j^{th} heading date, y_k is random effect of k^{th} year, $G*y$ is the random interaction of i^{th} genotype and k^{th} year, r is the random effect of l^{th} replication nested within year, and ε is the residual error. Variance components were generated using the same model except genotype (G_i) was treated as a random variable. The broad-sense heritability (H^2) for each FHB-related trait was calculated as:

$$H^2 = \frac{\sigma^2_G}{(\sigma^2_G + (\sigma^2_{G*Y}/y) + (\sigma^2_r/(n*y)))}$$

where σ^2_G is the variance attributed to genotype, σ^2_{G*Y} is the variance attributed to the genotype by year interaction divided by the number of years (y), and σ^2_r is the residual variance divided by the number of observations per genotype (replications by years, $n*y$). GWAS were conducted using trait BLUEs with the GAPIT3 R package (Wang and Zhang, 2021) and were run using mixed linear model (MLM), multi-locus mixed model (MLMM), fixed and random model circulating probability unification (FarmCPU), and Bayesian information and linkage-disequilibrium iteratively nested keyway (BLINK) models. The population structure was determined using principal component analysis, calculated within GAPIT3, (Supplementary Table 1).

Each model accounted for population structure (Q), kinship (K), and *Rht1* status (0/1) as a covariate. To account for multiple testing, formal statistically significant SNP-trait associations are reported as Benjamini-Hochberg $FDR \leq 0.05$. As a less conservative approach, we also retained notable SNP associations if a SNP achieved $P < 1.0 \times 10^{-5}$ in more than one GWAS model, or with more than one FHB-related trait, or is in proximity to or overlaps known FHB resistance genes (QTLs) and/or agronomic trait genes. GWAS models without using the *Rht1* status as a covariate

also were run, but data is not reported, as the results were similar with *Rht1* status included due to its 4.3% effect on phenotypic variation of AUDPC (see Results, Figure 2a).

2.4 RESULTS

2.4.1 Phenotyping

FHB-related traits including SEV (%), AUDPC, FDK (%), and DON, (ppm), were analyzed over three growing seasons (2022, 2023, and 2024) at the Rocky Ford field research station (Manhattan, KS). The mean heading dates for the entire panel were May 10, May 12, and April 27 in 2022, 2023, and 2024, respectively, reflecting annual environmental variation. FHB infection was visibly detectable in each variety around 20/21 days post-heading each year. Significant FHB-related trait variation (Supplementary Table 2. 3) was detected by ANOVA. A few accessions were present with a more resistant phenotype than the moderately resistant check (Everest). Genotype trait values showed moderate correlations across years, with Pearson correlation coefficients (r) ranging from 0.38–0.69 for SEV, 0.32–0.70 for AUDPC, 0.36–0.61 for FDK, and 0.40–0.53 for DON. All correlations were statistically significant ($P \leq 2.67 \times 10^{-7}$, Supplementary Table 2. 4). Generally, trait correlations were moderate between 2022 and 2023-2024, while higher correlations were observed between 2023 and 2024. These correlations support the moderate to high broad-sense heritability (H^2) estimates (Table 2.1) for the three-year dataset: 0.68 for SEV, 0.74 for AUDPC, 0.72 for FDK, and 0.65 for DON.

Table 2. 1. Variance Components and Heritability Estimates.

Variance Component	SEV	AUDPC	FDK	DON
Genotype	91.46	13647.10	241.43	15.13
Year	245.60	24252.71	6.30	5.58
Genotype x Year	78.90	9610.74	154.15	12.31
Rep (Year)	4.50	766.61	2.24	1.13
Residual	101.00	9339.59	263.91	24.19
Heritability (H^2)	0.68	0.74	0.72	0.65

SEV: FHB severity, **AUDPC**: area under the disease progress curve, **FDK**: Fusarium-damaged kernel, **DON**: deoxynivalenol.

Best linear unbiased estimates (BLUEs) for each FHB-related trait were obtained and analyzed across years (Supplementary Table 2. 1). ANOVA revealed significant levels of phenotypic variance (Figure 2. 1, Supplementary Table 2. 3) for each trait. The Pearson correlations of the trait BLUEs were moderate to high, ranging from 0.61 to 0.94 (Supplementary Table 2. 5, Supplementary Figure 2. 1). AUDPC is most highly correlated with SEV ($r = 0.93$), while post-harvest seed metrics of FDK and DON were moderately correlated with each other ($r = 0.67$), suggesting different genetic loci could be responsible for variation in these traits.

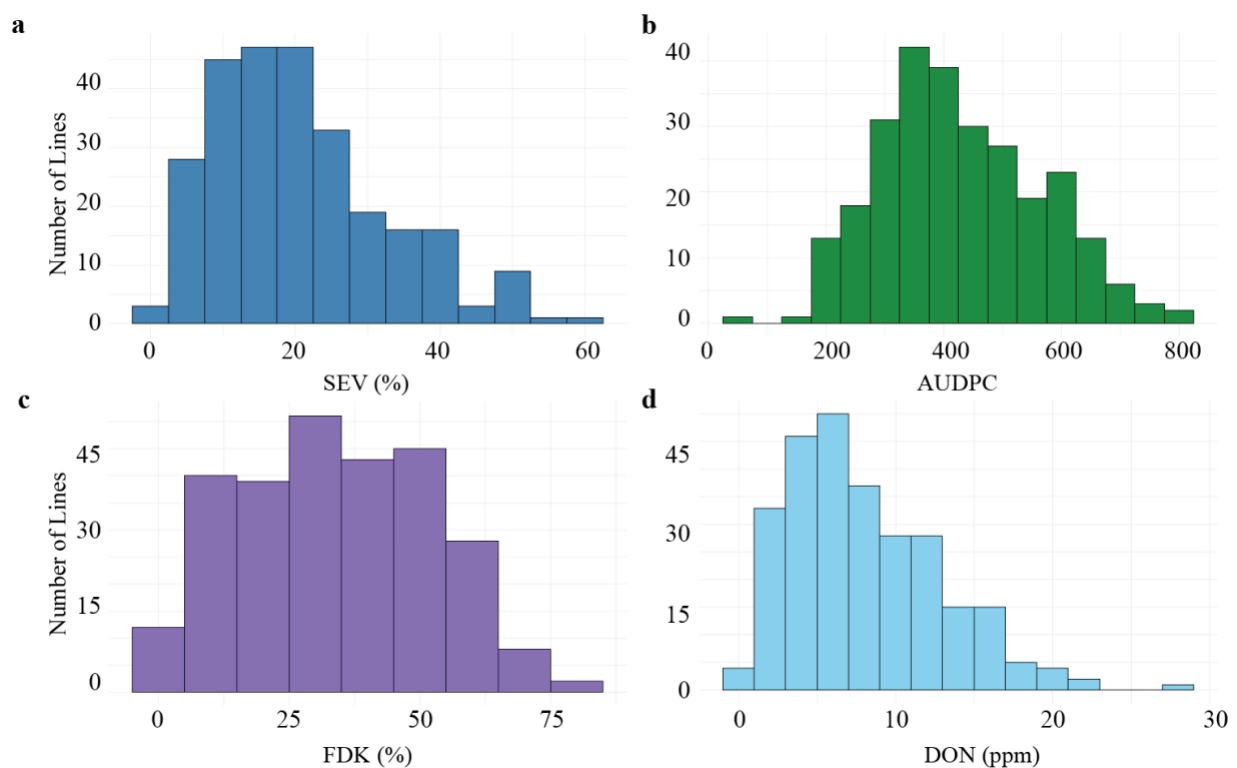


Figure 2. 1. Distribution of best linear unbiased estimates for (a) SEV, (b) AUDPC, (c) FDK, and (d) DON in the panel of 269 wheat accessions. **BLUE**, Best Linear Unbiased Estimates; **SEV**, FHB severity; **AUDPC**, Area Under the Disease Progress Curve; **FDK**, Fusarium-Damaged Kernels; **DON**, Deoxynivalenol.

2.4.2 Effect of major reduced-height genes on FHB disease progression

Due to the widely reported correlation of FHB severity with reduced plant height, we genotyped the lines for the presence of the major reduced-height alleles *RhtB1b* (*Rht1*) and *RhtD1b* (*Rht2*) (Supplementary Table 2. 1). The panel carries both semi-dwarfing alleles. The *RhtB1b* dwarf allele was present in 67% of lines, followed by the *RhtD1b* dwarf allele in 24%, and wildtype (WT) alleles at both loci (*Rht-B1a* and *Rht-D1a*) in approximately 10% of the lines. As expected, we observed a significant plant height difference (*RhtB1b* vs WT, P -value = 0.028; *RhtD1b* vs WT, P -value = 6.11e-05; Mann Whitney) between the wildtype and either of the semi-dwarf alleles, with the *RhtD1b* locus displaying a larger reduced height effect than *RhtB1b* (Figure 2. 2a). For FHB disease progression, the *Rht1* dwarfing allele (*Rht-B1b*) accounted for a 4.29% increase in AUDPC, while the *RhtD1b* allele (*Rht-D1b*) accounted for 0.77% (Figure 2. 2b). A similar trend was observed for SEV, where *RhtB1b* increased SEV by ~3%, while *RhtD1b* had no effect (Supplementary Figure 2. 2). Though not formally significant (P -value = 0.3, Mann Whitney), the non-zero *Rht1* effect size in the population on both severity metrics suggests a possibility of a biological difference between the two groups, therefore we included the *Rht1* allele status as a covariate in our GWAS model, but not *Rht2*. Our results revealed a low correlation between the plant height (HT) and the FHB disease severity traits (Supplementary Figure 2. 1; 0.29 for AUDPC and 0.38 for SEV; P -values < 0.01).

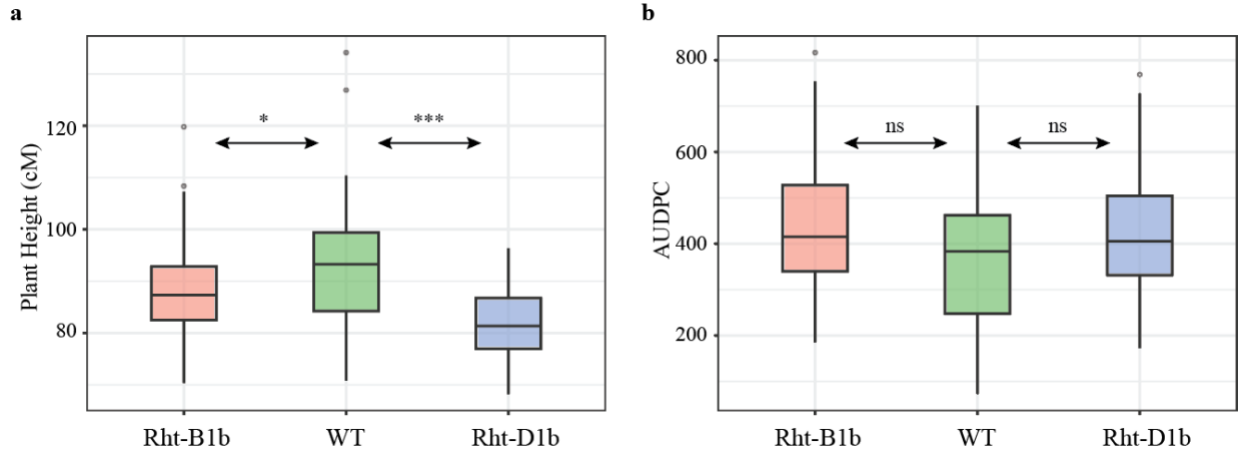


Figure 2. 2. Effect of dwarfing alleles on (a) height and (b) AUDPC. Mann Whitney test for each Rht dwarf allele versus wildtype (WT) allele depicted by arrow between groups, *: P-value < 0.05; *** P-value < 0.0001; ns: P-value > 0.05. Rht-B1b: n= 174, WT: n = 24, Rht-D1b: n=61.

2.4.3 Genome-wide association analysis

We performed GWAS using multiple statistical models in GAPIT3 (Wang and Zhang, 2021) on the FHB-related trait BLUEs with 620,704 high-quality exome-capture generated SNPs previously described in this population (Tidakbi *et al.*, 2025; Supplementary Figure 2. 3) and identified 44 SNP-trait associations at FDR < 0.05 (Figure 2. 3, Table 2. 2, Supplementary Table 2. 6). Most (36%) of these associations were for DON content, suggesting important targets for mycotoxin control were identified. We included 35 additional SNP associations based on possible biological relevance as they were identified in multiple scans or traits with $P < 1.0 \times 10^{-5}$ and fall within previously identified FHB QTL (Supplementary Table 2. 6). Of the 44 significant and 35 putative loci, we prioritized for further analysis those associations detected in multiple models, in multiple traits, or those highly significant ($P < 1 \times 10^{-10}$). Among them, twenty-three were notable in at least two models and thirteen were detected for multiple traits, suggesting the possibility of pleiotropic effects (Supplementary Table 2. 7). Nine associations were highly significant ($P < 1 \times 10^{-10}$) in at least one trait and model (Supplementary Table 2. 6). The prioritized SNPs resulted in 26 unique

genomic loci that impact FHB resistance (Table 2. 3). Average effect sizes for the significant associations ranged between 6.1% for FDK and 8.1% for AUDPC (Table 2. 2). The most significant SNPs were mapped on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 5D, 6A and 7A, with the most significant association detected on 1B at 569.16 Mb with an effect size of 14.6% on DON content (Table 2. 3, Supplementary Table 2. 6).

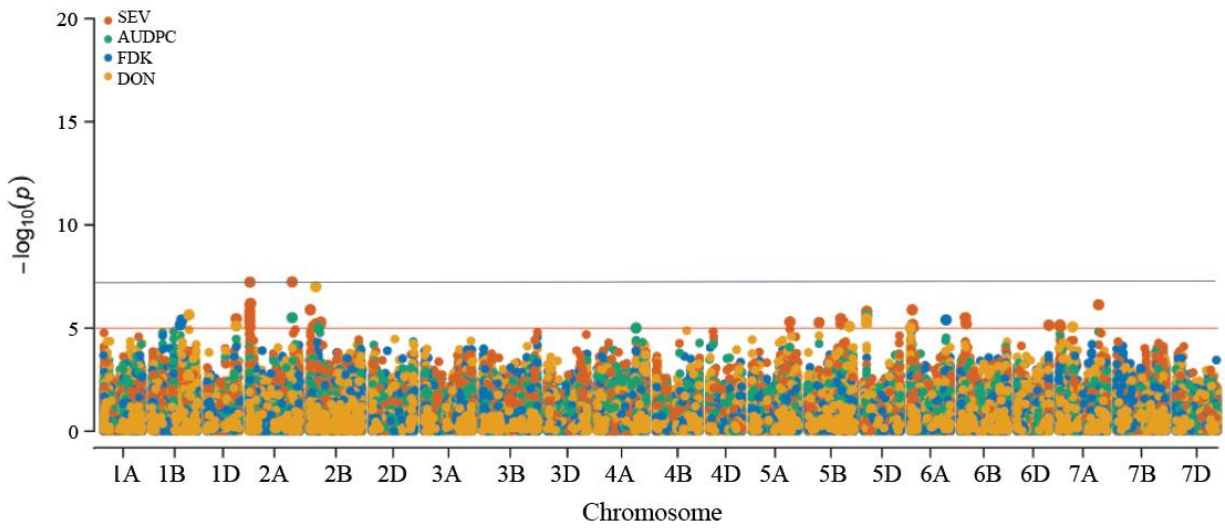
Table 2. 2. Summary of significant (FDR < 0.05) GWAS results across traits and models

Trait	Number of SNPs	Average effect size (%) [#]	Maximum effect size (%) [#]	Minimum effect size (%) [#]
SEV	14	7.67	16.88	3.39
AUDPC	9	8.08	11.64	3.84
FDK	5	6.08	9.24	4.49
DON	16	6.49	14.64	2.21

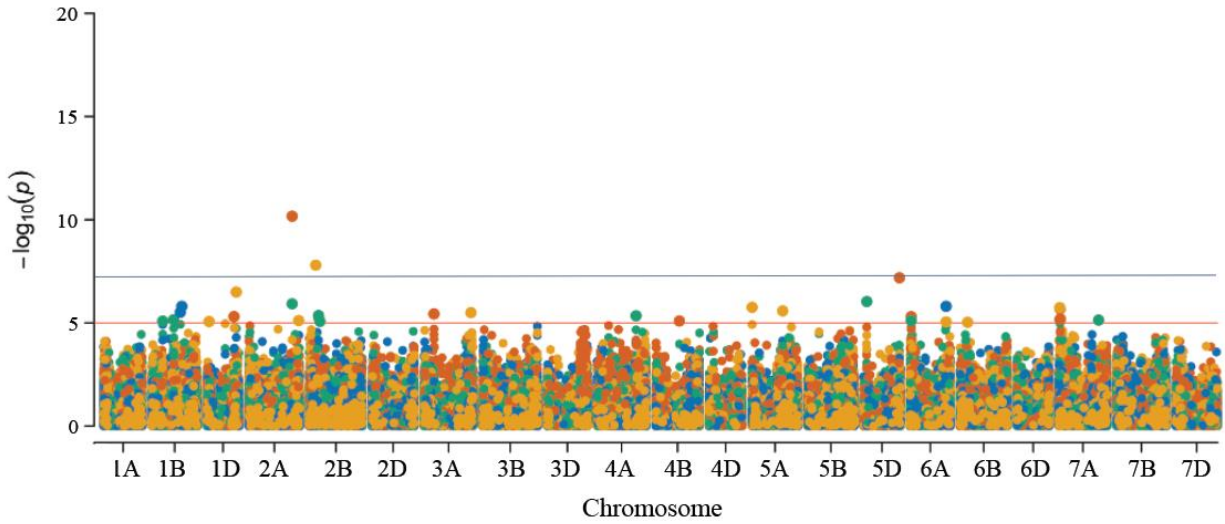
[#] Average effect size (%) determined as the percentage of the allele effect size divided by the trait phenotypic range, which is determined by highest BLUE minus lowest BLUE for each trait.

Of the thirteen putatively pleiotropic loci (Supplementary Table 2. 7), two loci each are on chromosomes 1B, 2B, 5B, 5D, and 7A. The most common combination (9 of 13) of multiple traits was AUDPC and SEV, which reflects the high trait correlation. Effect sizes for some of these putatively pleiotropic SNPs represented more than 10% of the trait variation range, including the association on chromosome 1D at 450.7 Mb, which was associated with both SEV and DON accumulation, accounting for 17.6% and 15.7% of the phenotypic variation, respectively.

a



b



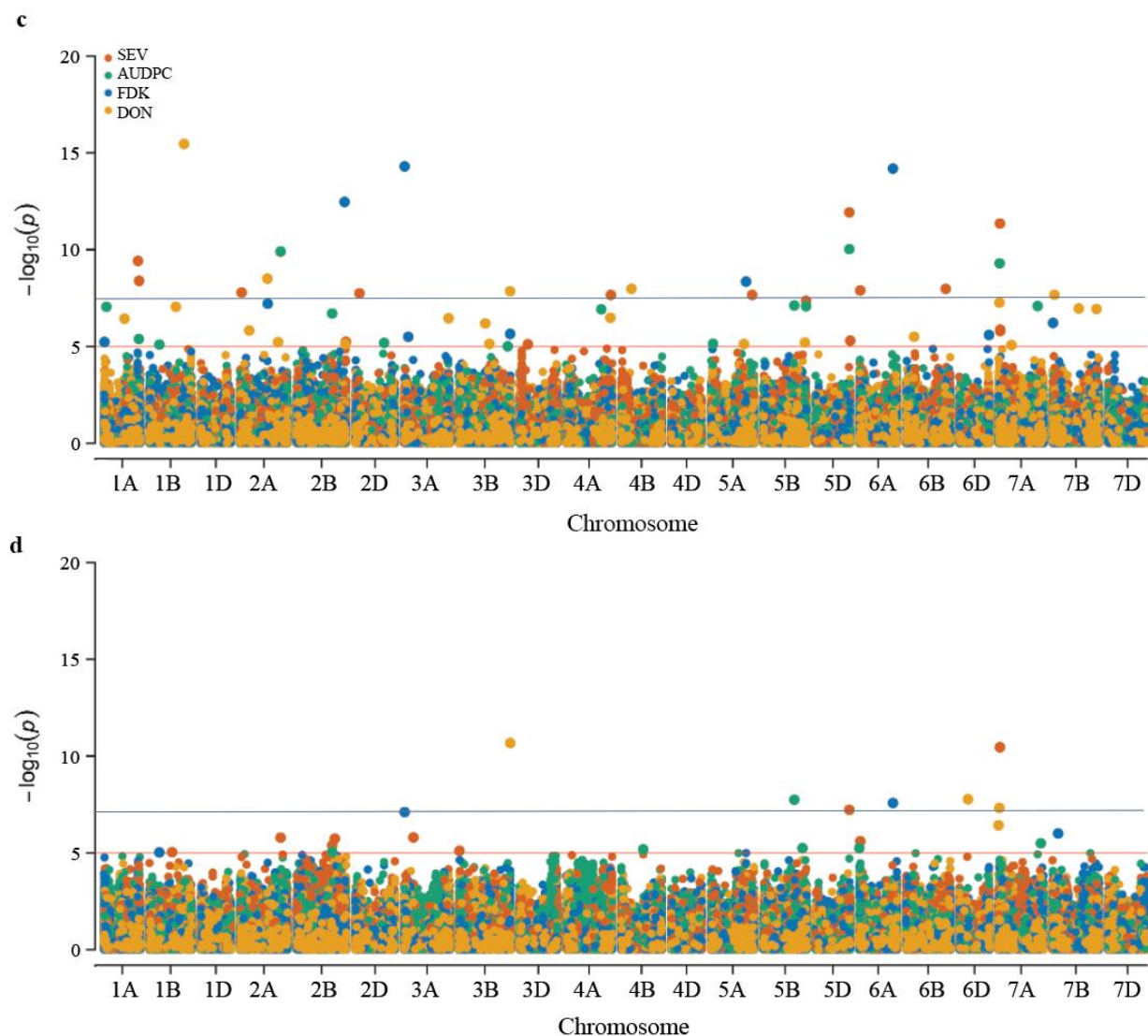


Figure 2. 3. Multi-trait Manhattan plots using BLUEs for FHB-related traits for (a) the MLM model, (b) the MLMM model, (c) FarmCPU model, and (d) the BLINK model. The red line represents $P = 1.0 \times 10^{-5}$, the blue line represents $FDR < 0.05$, and colored circles above the lines denote significant markers by traits detected using the specified significance threshold and model. **BLUE**, best linear unbiased estimates; **MLM**, mixed linear model; **MLMM**, multi-locus mixed linear model; **FarmCPU**, Fixed and Random Model Circulating Probability Unification; **BLINK**, Bayesian-information and linkage-disequilibrium iteratively nested keyway; **AUDPC**, area under the disease progress curve; **SEV**, FHB disease severity; **FDK**, Fusarium-damaged kernel; **DON**, deoxynivalenol; **SNP**, single nucleotide polymorphism.

Table 2. 3. Prioritized SNP associations based on GWAS in multiple models and multiple traits or highly significant.

FHB Trait	Chromosome	Position (bp)	MAF	Model*
AUDPC	1B	166444551 [#]	0.142	2, 3
FDK	1B	166444551 [#]	0.142	4
AUDPC	1B	332262821	0.123	1, 2
DON	1B	435466844 ^{#*}	0.168	3
FDK	1B	435466844 [#]	0.168	1, 2
FDK	1B	458665282 [#]	0.138	1,2
DON	1B	569163320 ^{#*}	0.03 [^]	1, 3*
SEV	1D	450758304	0.019 [^]	1
DON	1D	450758304	0.019 [^]	1, 2
SEV	2A	28823494 ^{#*}	0.045	1, 3
SEV	2A	665979623 ^{#*}	0.052 [^]	1, 2*, 3, 4
AUDPC	2A	665979623 ^{#*}	0.052 [^]	1, 2, 3
DON	2B	98006278*	0.037	1, 2
AUDPC	2B	140904969 [#]	0.063	1, 2
SEV	2B	162824813 [#]	0.037 [^]	1
AUDPC	2B	162824813 [#]	0.037 [^]	2
SEV	2B	580030865	0.071	4
AUDPC	2B	580030865*	0.071	3, 4
FDK	2B	782993929*	0.119	3*
FDK	3A	11532385*	0.31	3*, 4
DON	3B	843038669*	0.358	4*
SEV	5B	509272985 [#]	0.082 [^]	1
AUDPC	5B	509272985 ^{#*}	0.082 [^]	3, 4
SEV	5B	701047429 ^{#*}	0.03	3
AUDPC	5B	701047429 ^{#*}	0.03	3
SEV	5D	50810559 [#]	0.041 [^]	1
AUDPC	5D	50810559 [#]	0.041 [^]	1, 2
SEV	5D	550632004*	0.366	2, 3*, 4
AUDPC	5D	550632004*	0.366	3*
SEV	6A	18452189 ^{#*}	0.134	2, 3, 4
DON	6A	553038439 [#]	0.414	2
FDK	6A	553038439 ^{#*}	0.414	1, 2, 3*, 4
DON	7A	18049084*	0.146	2, 3, 4
SEV	7A	21559496 ^{#*}	0.097	1, 2
AUDPC	7A	21559496 ^{#*}	0.097	3
SEV	7A	26275779*	0.119	1, 2, 3*, 4*

DON	7A	215238121	0.052	1, 3
SEV	7A	612449697 [#]	0.049 [^]	1
AUDPC	7A	612449697 [#]	0.049 [^]	2

*GWAS models loci were found significant at P -value 1×10^{-5} : **1. MLM**: Mixed linear model, **2. MLM**: Multi-locus mixed model, **3. FarmCPU**: Fixed and random model circulating probability unification, **4. BLINK**: Bayesian information and linkage-disequilibrium iteratively nested keyway. * SNPs are formally significant for Benjamini-Hochberg correction $FDR \leq 5\%$. [#] represents repeatedly significant SNP associations in more than one year. **MAF**: Minor allele frequency, **bp**: base pair; [^] cases where the rare allele increases susceptibility over the common allele when $MAF < 0.095$.

2.4.4 FHB resistance regions of interest detected by GWAS

Fhb1 is the most widely reported FHB resistance QTL on 3BS. *Fhb1* (*TaHRC*)-KASP marker genotyping detected positive *Fhb1* locus in 27 wheat accessions (Supplementary Table 2. 1). Mann Whitney tests comparing lines carrying the *Fhb1* locus showed a significantly lower AUDPC than those without (P -value = 0.0085, Figure 2. 4a), with an effect size of 10%. Interestingly, the GWAS scan identified an association ($P = 7.8 \times 10^{-6}$) on chromosome 3B at 11.3 Mb for SEV, but not for AUDPC. However, among the five lines carrying the resistant SNP allele from the GWAS, only one carries the *Fhb1* KASP marker allele, suggesting that the *Fhb1* locus is likely not the source of this association. Similar results were found for *Fhb9* at 553 Mb on chromosome 2DL and *Fhb7* on the distal end of homoeologous chromosome group 7. KASP data identified only three *Fhb9* positive lines (Supplementary Table 2. 1) and none for *Fhb7*. Given the minor allele frequency cutoff for SNP inclusion in the models was ≥ 5 lines, *Fhb9* (three lines) was not evaluated.

Of the SNPs identified in multiple models and multiple traits (Table 2. 3), one of the most detected regions was on chromosome 1B (166.44Mb, 332.36 Mb, and 435.47Mb) affecting AUDPC, DON, and FDK. A previous soft winter wheat FHB study (Carpenter *et al.*, 2020) identified a native QTL in the SWW cultivar Jamestown affecting SEV, FDK, and DON using 90K SNP arrays. The 90K

SNPs identified from this study (IWA6259, IWA7594, IWB43992) map to chromosome 1B at 195Mb, 336Mb, and 477Mb based on the Chinese Spring 2.1 reference map (Zhu *et al.*, 2021). We traced the favorable alleles at the three loci identified in this study, and found Jamestown carries each FHB decreasing allele at all three sites. In addition to Jamestown, twenty-one other lines carry the Jamestown haplotype (Supplementary Table 2. 1) exhibiting large effects, including nearly a 25% decrease on AUDPC and FDK (Figure 2. 4b, c), and an 18% reduction in DON concentration (Figure 2. 4d; all $P \leq 1.9\text{e-}07$, Mann Whitney test).

Similarly, the 2B 162.82 Mb association lies adjacent to a previously reported SWW cultivar Bess QTL between 173-218 Mb (Peterson *et al.*, 2017). Bess possesses the common allele at this locus, and the rare allele in the GWAS increases AUDPC by 27% (Supplementary Figure 2. 4a), suggesting the Bess allele could be an important source of FHB resistance. A region on 6A at 553.04 Mb was identified for both FDK and DON and falls within a previously identified QTL from NC-Neuse (Peterson *et al.*, 2016). The NC-Neuse allele, which is more common in the panel, decreased both DON and FDK compared to the non-NC Neuse allele by 9% and 14% (Supplementary Figure 2. 4b, c), respectively. These results suggest that the previously identified regions from SWW on 2B from Bess and 6A from NC-Neuse could be the underlying QTLs detected in this study.

Despite normalizing the phenotyping window around heading date to retain uniform disease pressure in the panel, twelve SNPs were identified affecting SEV ($P < 1.0 \times 10^{-5}$) on chromosome 2A between 17-29 Mb (Supplementary Table 2. 6). We hypothesized it could be linked to *Ppd-A1* due to the high correlations between the FHB traits and the heading dates (Supplementary Figure 2. 1), and its proximity to the region. The panel was genotyped using two diagnostic KASP markers *Ppd-A1-GS100* and *Ppd-A1-GS105* (Royo, *et al.*, 2016) and the results of this genotyping revealed

more lines carrying the photoperiod insensitivity alleles ($Ppd-A1-GS100 = 35/269$; $Ppd-A1-GS105 = 29/269$) than lines responsible for the association where $MAF \leq 0.045$ (Figure 2. 5a, b). Analyzing the lines that carry the rare 2A haplotype, all are photoperiod sensitive at *Ppd-A1* (Supplementary Table 2.1). Interestingly, of these 2A rare lines responsible for the association, most are adapted to Kansas or more northern climates. They have later heading dates (Figure 2. 5c) than the rest of the panel, but all have higher SEV (Figure 2. 5d). These results indicate that the *Ppd-A1* locus is not responsible for the associations, but there could be an environmental clinal effect where the lines adapted for longer day length are correlated with FHB sensitivity.

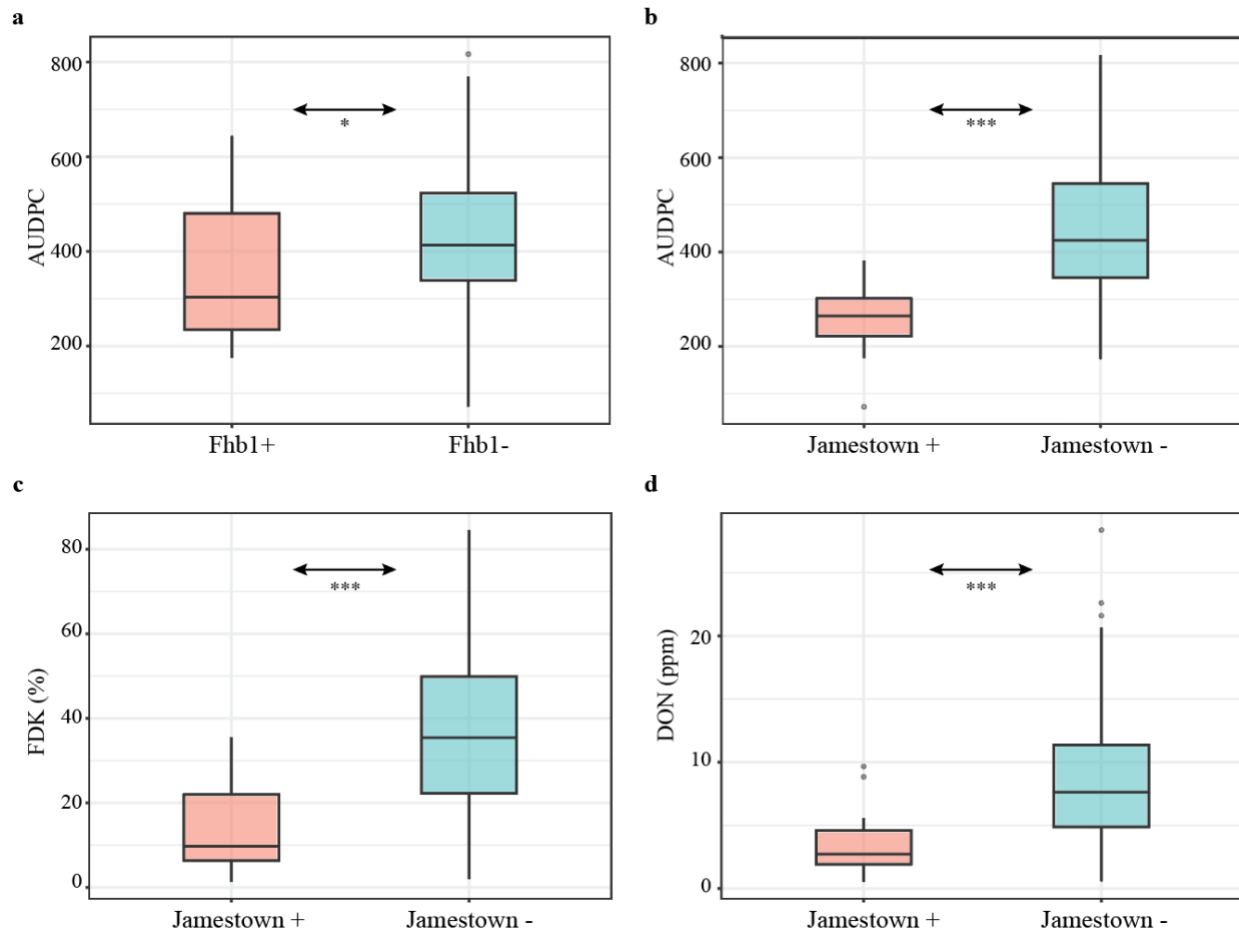


Figure 2. 4. Distribution of FHB significant phenotypes at (a) *Fhb1* locus or (b-d) carrying the Jamestown haplotype. Mann Whitney significance levels for each comparison depicted by arrow between groups, *: $P < 0.01$; *** $P < 1 \times 10^{-6}$; Fhb1+: $n = 27$, Fhb1-: $n = 232$, Jamestown +: $n = 22$, Jamestown -: $n = 247$.

Historically, the repeatability of QTL and associations for FHB resistance has been inconsistent across breeding backgrounds and environments, likely due to the trait's complex genetic control. In addition to the associations already discussed from Jamestown, Bess, and NC-Neuse, one of the highly significant SNPs on 2B associated with FDK was ~5 Mb from a region reported by Gaire *et al.* (2021) at 787 Mb affecting FHB index. Here, there are 33 wheat lines (Supplementary Table 2. 1) which carry the favorable FDK-reducing allele at 782.99 Mb, and all FHB-related traits

evaluated in this study were reduced by ~20% (Supplementary Figure 2. 5), suggesting a high-priority locus to incorporate into breeding programs.

Allele frequency for the prioritized regions ranges from very common $MAF = 0.41$ (553 Mb on 6A) to quite rare $MAF = 0.02$ (450 Mb on 1D, Table 2. 3). Thirteen of the twenty-six regions (50%) have a $MAF < 0.095$. Eight of those rare loci are putatively pleiotropic, identified in more than one trait. Coupled with the observation common alleles increased FHB resistance at previously identified NC-Neuse and Bess QTL regions, and the rare alleles on 2A increased susceptibility, we evaluated the effects of rare associations on FHB resistance ($MAF < 0.095$). In six of the eight (75%) rare pleiotropic loci, the rare allele significantly increased FHB susceptibility compared to the common allele (Supplementary Figure 2. 6), suggesting breeding programs are generally retaining neutral or beneficial alleles within their programs.

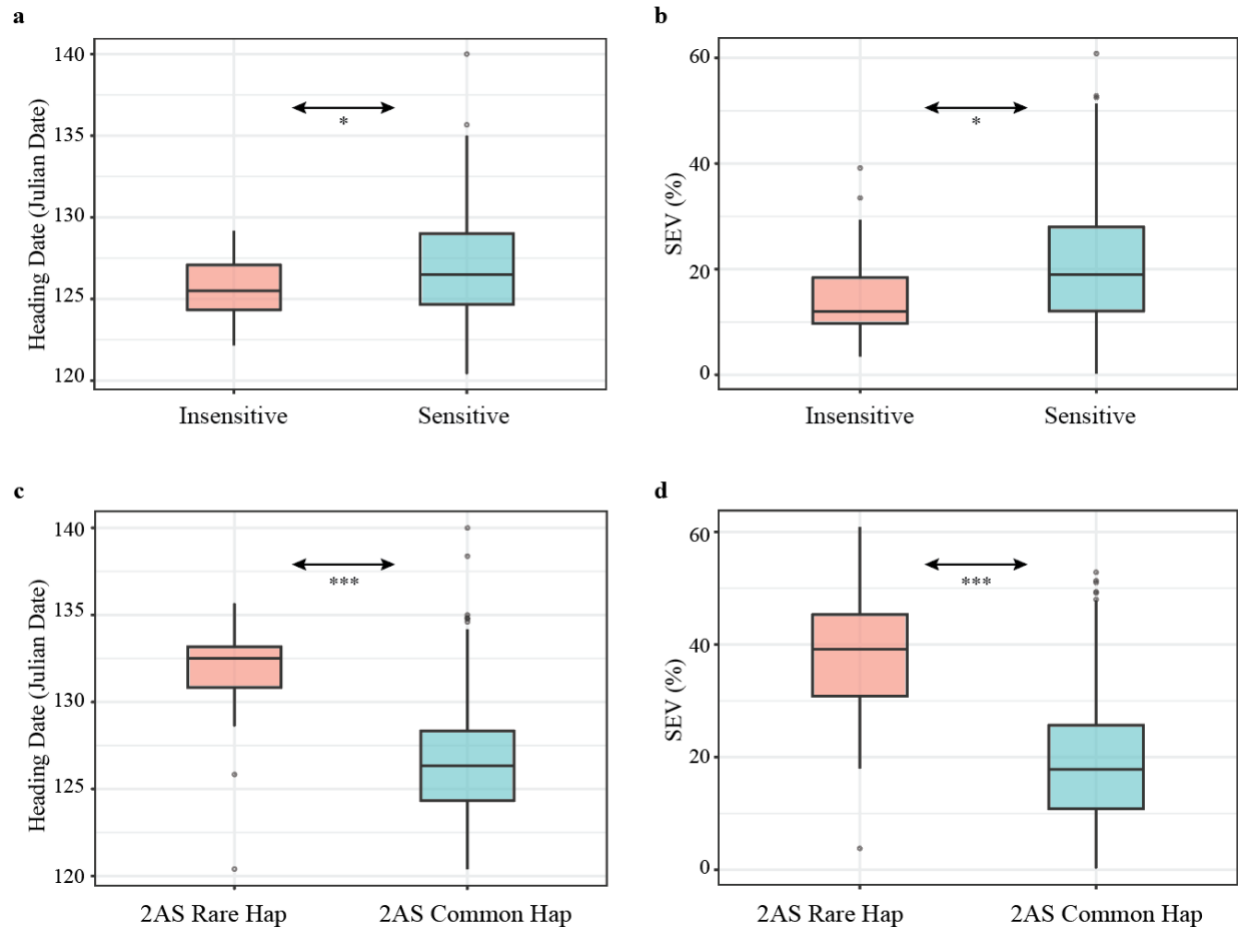


Figure 2. 5. Distribution of (a) heading dates and (b) SEV for lines carrying sensitive and insensitive Ppd-A1 GS100 KASP marker alleles, and (c) heading date and (d) SEV for lines carrying rare significant 2AS haplotype associated with SEV from 17-28 Mb. Mann Whitney significance test for each comparison depicted by arrow between groups, *: $P < 0.05$; **: $P < 0.01$; ***: $P < 1 \times 10^{-5}$. Insensitive GS100 allele, $n = 35$; sensitive GS100 allele, $n = 228$; 2AS rare haplotype $n = 12$; 2AS common haplotype, $n = 257$.

2.5 DISCUSSION

This study combined both hard and soft winter wheat lines from major U.S. wheat breeding programs aiming to produce well adapted cultivars capable of producing high-yielding and high-quality cultivars with FHB resistance. This diverse panel of accessions exhibited significant phenotypic variation in FHB-related traits measured over three years in the field. The heritability

estimates were moderate to high (0.65-0.74), reflecting the genetic diversity and the extent to which genetic factors affect winter wheat disease resistance traits. Using this data, we successfully identified 44 SNP associations in the panel, and twenty-six prioritized FHB resistance regions.

The most common timepoint reported in the literature for field phenotyping FHB severity is 21 days post-heading (Sutton, 1982; Dill-Macky, 2003; Liu et al., 2019; McCallum et al., 2024). In this study, we detected 14 associations for SEV, and two fall on 2AS in proximity to the *Ppd-A1* locus. However, our results revealed the breeding lines responsible for this rare association (mainly the northern programs in this study) are all photoperiod sensitive with late heading dates and have higher disease severity (Figure 2. 5). This finding is also supported by a high positive correlation between the heading dates (HD) and SEV (0.87), which is somewhat at odds with the belief that late heading lines usually “escape” FHB pressure. It is possible that by combining well-adapted, geographically distinct lines from Louisiana and Texas to Montana, despite normalizing for heading date, the later heading northern lines were subjected to higher disease pressure in our inoculated field than the earlier heading southern varieties. Perhaps by using longer day length adapted cultivars from different environments, coupled with optimal conditions for fungal development, more infection was present in these later heading lines than usually experienced in their native environments. This highlights the intricacies of combining diverse lines in a single population where the testing environment is different than adapted environment, resulting in genotype by environment interactions.

Shorter plant height has been correlated with increased FHB susceptibility (Akohoue et al., 2022; Hales et al., 2020). Semi-dwarfing alleles *RhtB1b* and *RhtD1b* reduce plant height but differ in their FHB susceptibility impact on wheat. Previous studies suggest *RhtD1b* had a greater effect on susceptibility than *RhtB1b* (Buerstmayr and Buerstmayr, 2016). Here, *RhtD1b* had a greater effect

on height than *RhtB1b* (Figure 2. 2) but had a negligible effect on AUDPC and SEV. Significant plant height differences and the lack of significant variation in disease severity traits between semi-dwarf and tall genotypes suggest other genetic (possibly minor *Rht* genes) and environment factors beyond *Rht1* and *Rht2* influence FHB susceptibility. Contrary to a recent HWW GWAS (Zhang et al., 2022) sharing some cultivars with this study, we did not detect an *Rht1* locus association on 4B from 35-40 Mb, further supporting height is not an FHB resistance contributing factor in this study. To check if this was an artifact of using *Rht1* status as covariate, we reran the analysis without the *Rht1* status and did not identify an association in this area. To confirm signal was detected, a GWAS using height phenotype detected six SNPs from 37.4 - 40.04 Mb (data not shown). These results taken together suggest height was not a contributing FHB resistance/sensitivity factor in this study.

The most reported FHB resistance locus is *Fhb1*, however it may have undesired linkage drag (Viviani et al., 2025; Anderson et al., 2001), leading to it not being widely established in most U.S. wheat breeding programs (Zhang et al., 2022). Twenty-seven lines carry the *Fhb1*⁺ resistance allele in this panel, resulting in a 10% reduction in AUDPC compared to *Fhb1*⁻ lines. We reported an *Fhb1* region for SEV association at 11.3 Mb on 3B. However, only one of the five lines carrying the rare allele has *Fhb1*. Studies have shown that FHB resistance QTLs are not always reproducible. For example, *Fhb1*, originally identified in Sumai3, accounts for up to 60% phenotypic FHB disease severity variation but can be ineffective in some breeding backgrounds (Zhang et al., 2020; Miedaner and Korzun, 2012). Newer FHB resistance loci *Fhb7* and *Fhb9* are currently being pyramided into breeding lines for better resistance, however these pyramids were not available when this panel was composed. Testing these pyramided lines may bring more stable sources of FHB resistance in the future.

Cultivars in this panel have been previously reported carrying native FHB resistance QTL. Despite not identifying *Fhb1* associations, we identified regions previously associated with resistance QTL with larger effects than *Fhb1*. The best example is the 1BL region that harbors the three associations ($P < 1.0 \times 10^{-5}$; 166.4 Mb, 332.3 Mb, 435.5 Mb) which comprise the Jamestown haplotype affecting multiple traits. Jamestown has been used as source of moderate FHB resistance (Boyles et al., 2023) in soft winter wheat. Here, in a climate that favors hard winter wheat, the Jamestown haplotype significantly increased FHB resistance accounting for 25% reduction in cumulative FHB severity (AUDPC) and FDK, and 18% reduction in DON, with effect sizes larger than what was observed for *Fhb1*. The 18% DON reduction translates to a ~2.50 ppm reduction, which has important implications in the food industry and thus on the marketability of harvested grain. A threshold of 1 ppm DON is set by the Food and Drug Administration (FDA) for finished wheat products intended for human consumption, while a limit of 5 ppm is established for grain and grain products for animal feed. Grain elevators reject or penalize farmers that produce grain with DON content above 5 ppm, which results in economic losses. These findings lead us to conclude the Jamestown 1BL region is an important FHB resistance region affecting DON accumulation and should be incorporated and evaluated in U.S. breeding pipelines. Perhaps pyramiding the 1B haplotype of Jamestown in the presence of *Fhb1* could provide improved DON mycotoxin resistance. It is noteworthy that using the stricter Benjamin-Hochberg FDR value < 0.05 would have missed two of the SNPs comprising the beneficial haplotype, suggesting biological relevance can be context dependent without formal significance. Future experiments should be conducted to check for replication of these effects.

For a trait like FHB resistance, widely studied with more than 500 QTL reported, novel loci are rare. In this work, 26 genomic loci were detected in multiple models, associated with multiple

traits, or have large effects, and are worthy candidates to introduce and propagate into breeding pipelines. A previous GWAS study by Ghimire et al, 2020, identified an association at 333 Mb on 1B affecting multiple traits including severity, incidence, and FDK and falls within the previously discussed Jamestown 1B region (Wright, 2014; Peterson et al., 2016). There are five loci on 2B, including two (140.9-162.8 Mb) that are ~27 Mb from an association for FHB incidence (189 Mb) reported by Larkin et al. (2020) in soft red winter wheat, and includes the common Bess 2B QTL (Peterson *et al.*, 2017, Fig S4). The remaining two 2B associations (580 Mb and 783 Mb) fall within 35 and 15 Mb to associations reported by Gaire et al. (2021) that affect incidence, severity, and disease index. The association on 2BL revealed a large effect lowering all FHB-related traits between 18-25% in this study. Other chromosomes that have multiple prioritized regions include 6A and 7A. An association on 6A at 18.4 Mb, which is in proximity to associations reported by Gaire et al. (2021) and Larkin et al. (2020) for incidence and disease index. In addition, an association on 6AL at 553 Mb that affects FDK and DON is likely the reported NC-Neuse QTL (Peterson et al 2016, Supplementary Table 2. 7). Three loci on 7AS between 18 - 26 Mb affecting DON and AUDPC, fall near associations reported by Gaire et al. (2021), Larkin et al. (2020), and Zhang et al. (2010) and the QTL interval of *QFdk.nc-7A* associated with FDK (Winn et al., 2025). On chromosome 5D, a SNP at 550.6 Mb was associated with SEV and AUDPC. These results are consistent with findings by Mandal et al. (2025), who reported an association for FHB resistance at 561 Mb.

Ranking the lowest 10% of lines for AUDPC, the moderately resistant HWW cultivar Everest ranked seventh, and Jamestown ranked 24th (Supplemental Table 2. 1). Both HWW and SWW cultivars comprise the top twenty-five resistant lines. Five carry *Fhb1*, seven carry the 1B Jamestown haplotype, and twenty share the more common NC-Neuse 6A haplotype. Nine of the

twenty-five lines with the lowest AUPDC scores carry at least two of the *Fhb1*, Jamestown, or NC Neuse haplotypes, suggesting there is benefit in pyramiding these regions of resistance to increase overall resistance.

This study highlighted existing FHB resistant haplotypes in adapted wheat and some potential additional FHB resistance allele resources using a diversity panel sourced from various U.S. public wheat breeding programs. It combined both hard and soft winter wheat in a single population, finding SWW lines that perform well in the southeastern US had similar resistance in the Central Great Plains, demonstrating degrees of phenotypic plasticity. We identified twenty-six breeding targets where thirteen are putatively pleiotropic and should be given priority for improved FHB resistance. In general, common alleles decreased susceptibility or increased resistance with effect sizes greater to or similar to *Fhb1* in this study, suggesting U.S. breeding efforts are utilizing these regions in breeding pipelines. More work is needed to fine-map and identify which loci are ultimately responsible for these effects and to understand the resistance mechanisms but, for breeding purposes, pyramiding these regions into newly developed varieties coupled with fungal management practices is an effective approach to reducing the economic losses attributable to this disease.

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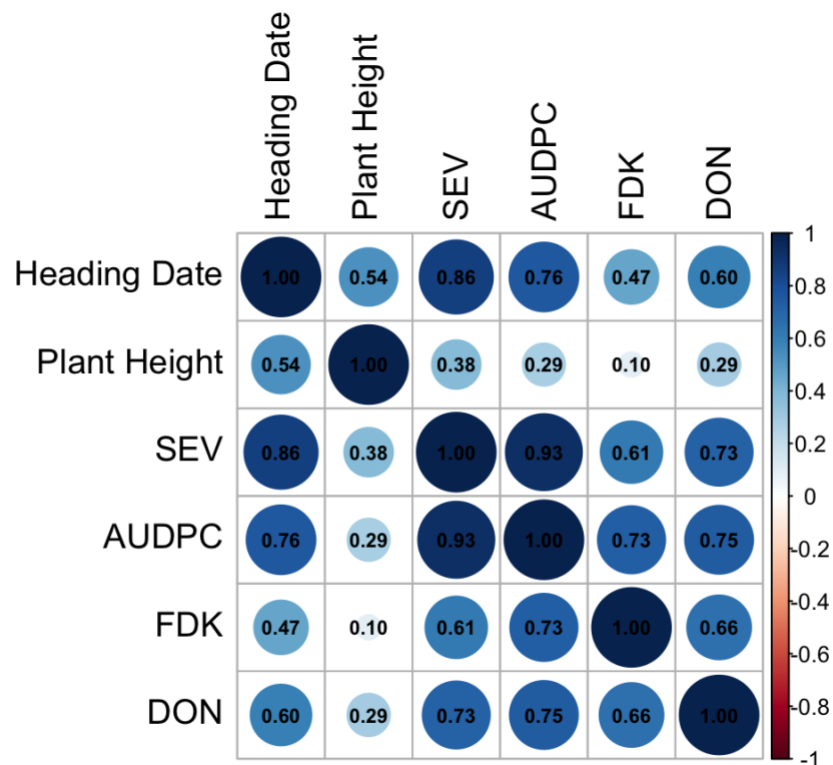
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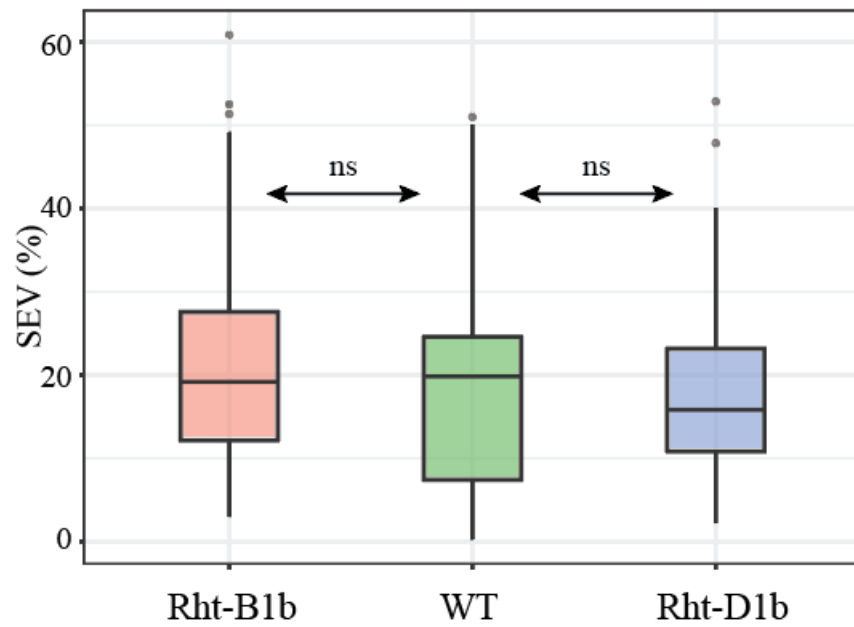
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2.7 SUPPLEMENTARY FIGURES



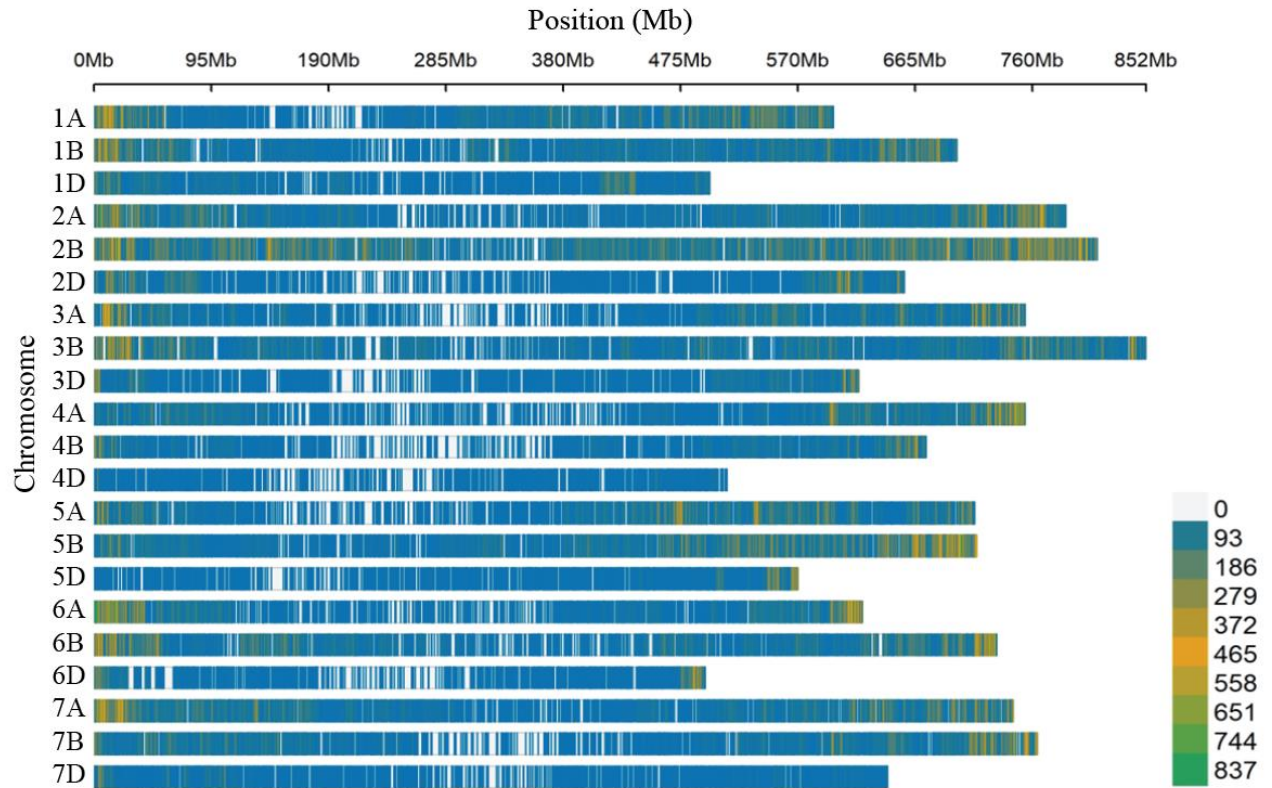
Supplementary Figure 2. 1. Trait pairwise correlations using best linear unbiased estimates.

Supplementary Figure 2.1. Trait pairwise correlations using best linear unbiased estimates.

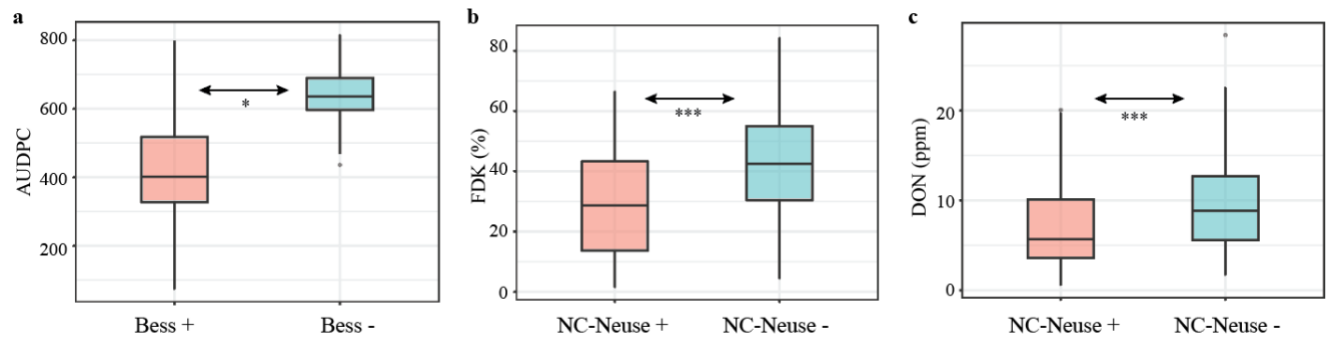


Supplementary Figure 2. 2. Effect of dwarfing alleles on FHB severity (SEV %) best linear unbiased estimates.

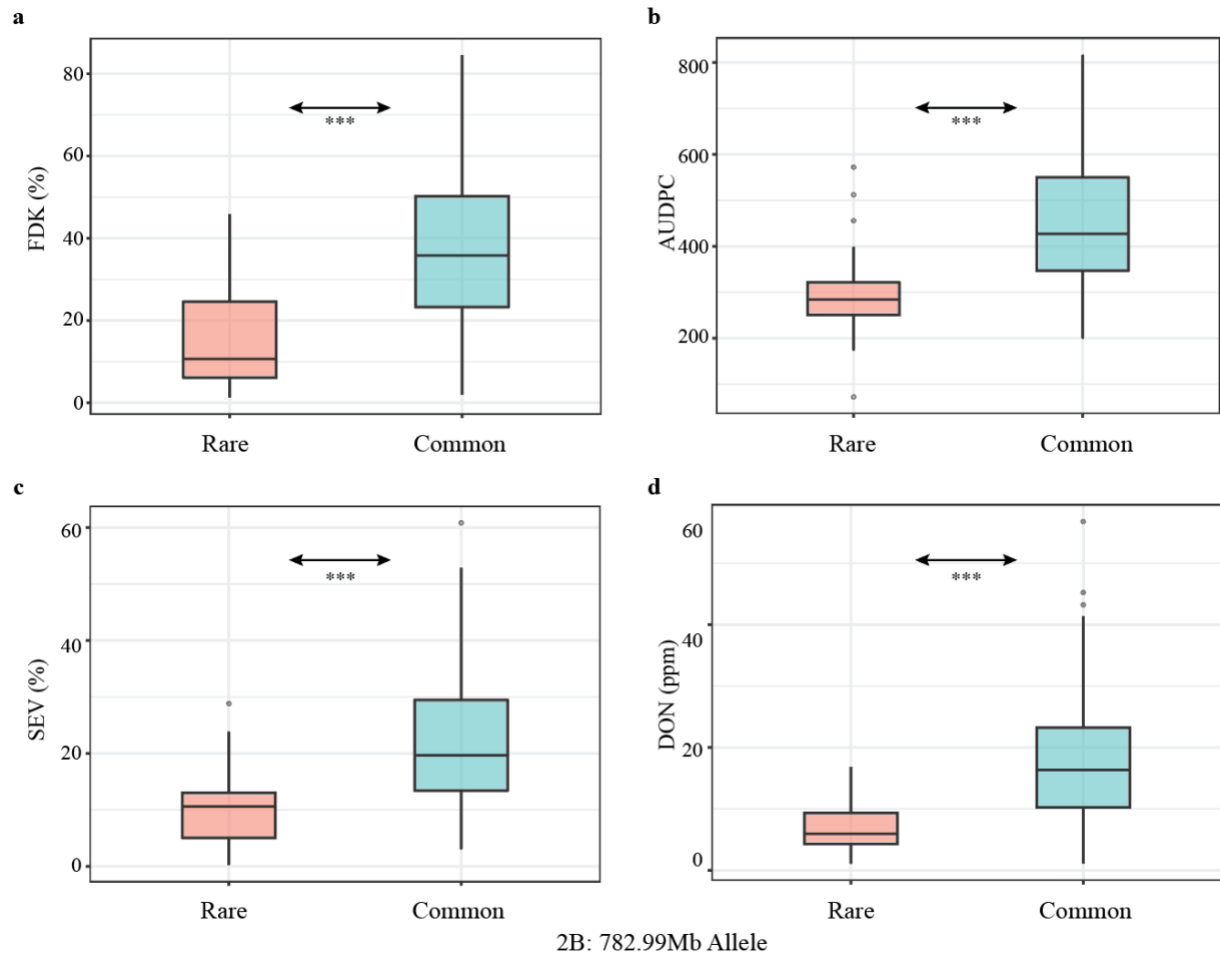
Mann Whitney test for each Rht dwarf allele versus wildtype (WT) allele depicted by arrow between groups, ns: P-value > 0.05. *Rht-B1b*: n= 174, WT: n = 24, *Rht-D1b*: n=61.



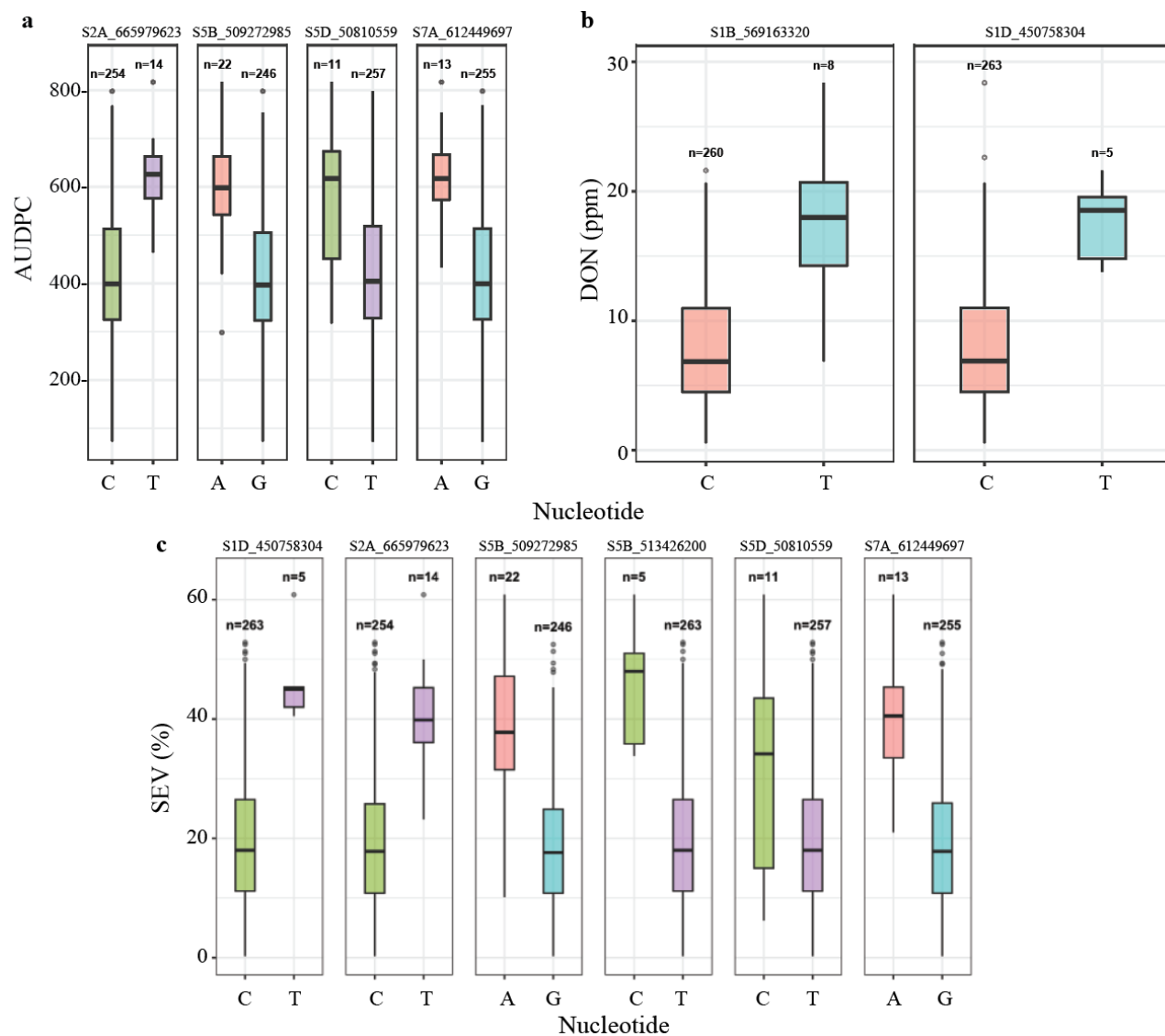
Supplementary Figure 2. 3. Distribution of the exome-generated SNPs on 21 wheat chromosomes based on Chinese Spring v2.1 reference genome within 1 Mb windows. The heatmap (bottom right) shows the number of SNPs within each window.



Supplementary Figure 2. 4. Distribution of FHB significant phenotypes carrying (a) Bess 2B allele or (b, c) carrying NC-Neuse 6A allele. Mann Whitney significance test for each comparison depicted by arrow between groups, *: $P < 0.05$; *** $P < 1 \times 10^{-4}$. Bess 2B + : $n = 259$, Bess 2B - : $n = 10$; NC Neuse 6A + : $n = 158$; NC Neuse 6A - : $n = 111$.



Supplementary Figure 2. 5. Distribution of FHB significant phenotypes at 2B: 782.99Mb (a) FDK (b) AUDPC, (c) SEV, (d) DON. Mann Whitney significance test for each comparison depicted by arrow between groups, *** $P < 1 \times 10^{-5}$. Rare allele: n =33, Common allele: n = 236.



Supplementary Figure 2. 6. Boxplots showing the effect of two alleles of the rare prioritized SNPs in the panel. (a) AUDPC, (b) DON, and (c) SEV.

2.8 SUPPLEMENTARY TABLES

Supplementary Table 2. 1. Panel information (Excel sheet, refer to

<https://access.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fesc2.70016&file=csc270016-sup-0002-TableS1.xlsx>)

Supplementary Table 2. 2. Primer sequences and KASP PCR profiles used in this study

Fhb1	Fhb1 GAAGGTCGGAGTCAACGGATTGCTCAGTCGTGCAAATGGT CTTCCAGTTTCTGCTGCCAT	KASP60XXR 1. 94°C, 15 min 2. 94°C, 20 sec 3. 60°C 1 min 4. Go to 2, 49 times 5. 59°C, 30 sec -1°C/cycle 6. Go to 5, 34 times 7. 10°C, 5 min	Theor Appl Genet (2018) 131: 2371. https://doi.org/10.1007/s00122-018-3159-6
Rht1-B1	GAAGGTGACCAAGTTCATGCTCCCATGGCCATCTCSAGCTG GAAGGTCGGAGTCAACGGATTCCCATGGCCATCTCSAGCTA TCGGGTACAAGGTGCGGGCG	KASP60XXR	Ellis et al. 2002. Theor Appl Gen 105:1038-1042.. CIMMYT-SDreisigacker
Rht2-D1	GAAGGTGACCAAGTTCATGCTCATGGCCATCTCGAGCTRCTC GAAGGTCGGAGTCAACGGATTATGGCCATCTCGAGCTRCTA CGGGTACAAGGTGCGCGCC	HK60T3XR 1. 94°C, 15 min 2. 97°C, 20 sec 3. 68°C, 1 min -0.8°C/cycle 4. Go to 2, 9 times 5. 97°C, 20 sec 6. 60°C, 1 min 7. Go to 5, 49 times 8. 59°C, 30 sec -1°C/cycle 9. Go to 8, 33 times 10. 10°C, 5 min	Ellis et al. 2002. Theor Appl Gen 105:1038-1042.. USDA- Brown-Guedira
Fhb7	GAAGGTGACCAAGTTCATGCTAGCGCATCATGCAGCTGG GAAGGTCGGAGTCAACGGATTAGCGCATCATGCAGCTGC GTGAGTGAGTGGCAGGAG	HK55T3XR 1. 94°C, 15 min 2. 97°C, 20 sec 3. 65°C 1 min, -0.8°C/cycle 4. Go to 2, 12 times 5. 97°C, 20 sec 6. 55°C, 1 min 7. Go to 5, 49 times 8. 54°C, 30 sec, -1°C/cycle 9. Go to 8, 28 times 10. 10°C, 5 min	Lanfei Zhao, pers. com.
Fhb-2DL (Fhb9)	GAAGGTGACCAAGTTCATGCTTCGCTGCAGCTTAACATATGT GAAGGTCGGAGTCAACGGATTTCGCTGCAGCTTAACATATGC TGGAAGGCAGATCTGCCACG	HK60T3XR 1. 94°C, 15 min 2. 97°C, 20 sec 3. 68°C 1 min, -0.8°C/cycle 4. Go to 2, 9 times 5. 97°C, 20 sec 6. 60°C, 1 min 7. Go to 5, 49 times 8. 59°C, 30 sec, -1°C/cycle 9. Go to 8, 33 times 10. 10°C, 5 min	Su: Theoretical and Applied Genetics volume 135, 1867–1877 (2022) doi.org/10.1007/s00122-022-04080-5
Ppd-A1 (GS100)	GAAGGTGACCAAGTTCATGCTCCAGTATCTTTAGATGCACCATGC GAAGGTCGGAGTCAACGGATTGCCGGCGGTAAAGG CTATACAATGCTAAAGTCGCACAT	HK60T3XR 1. 94°C, 15 min 2. 97°C, 20 sec 3. 68°C 1 min, -0.8°C/cycle 4. Go to 2, 9 times 5. 97°C, 20 sec 6. 60°C, 1 min 7. Go to 5, 29 times 8. 59°C, 30 sec, -1°C/cycle 9. Go to 8, 33 times	JIC-DL, TAG 2007, 115:721–733 , TAG 2016, Vol 129, Issue 10, pp 1843–1860. 10.1007/s00122-016-2743-x

		10. 10°C, 5 min	
Ppd-A1 (GS105)	GAAGGTGACCAAGTTCATGCTGGGGACCAAATACCGCTCG GAAGGTCGGAGTCAACGGATTCTGTTGGTGGGACGGG GAAACAGAGGGGTGTTTGAAT	KASPXXR 1. 94°C, 15 min 2. 94°C, 20 sec 3. 65°C 1 min, - 0.8°C/cycle 4. Go to 2, 9 times 5. 94°C, 20 sec 6. 57°C, 1 min 7. Go to 5, 49 times 8. 56°C, 30 sec, - 1°C/cycle 9. Go to 8, 31 times 10. 10°C, 5 min	JIC-DL, TAG 2007, 115:721–733 , TAG 2016, Vol 129, Issue 10, pp 1843–1860. 10.1007/s00122-016- 2743-x

Supplementary Table 2. 3. Population statistics for measured trait BLUEs across three field seasons.

Population Range				
Trait	Min	Max	Overall Mean	ANOVA (<i>P</i> -value)
SEV (%)	0.25	60.83	20.56	< 0.001
AUDPC	72.25	816.75	428.27	< 0.001
FDK (%)	1.33	84.50	34.25	< 0.001
DON (ppm)	0.54	28.38	8.08	< 0.001

ANOVA: Analysis of Variance, BLUEs: Best linear unbiased estimates. ANOVA model = μ + Genotype + Heading Date + Year + Genotype x Year + Rep(Year); ANOVA (*P*-value) represents the *P*-value for the fixed effect of genotype in the model. SEV: FHB disease severity, AUDPC: Area Under Disease Progress Curve, FDK: Fusarium Damaged Kernel, DON: Deoxynivalenol

Supplementary Table 2. 4. Pearson Correlations of Phenotypic FHB Disease Severity Trait Metrics by Year

Trait Year	SEV 2023	SEV 2024
SEV 2022	$r = 0.38$ $P = 3.31 \times 10^{-10}$	$r = 0.47$ $P = 1.78 \times 10^{-15}$
SEV 2023		$r = 0.69$ $P = 0$
	AUDPC 2023	AUDPC 2024
AUDPC 2022	$r = 0.32$ $P = 2.66 \times 10^{-07}$	$r = 0.40$ $P = 2.87 \times 10^{-11}$
AUDPC 2023		$r = 0.70$ $P = 0$
	FDK 2023	FDK 2024
FDK 2022	$r = 0.39$ $P = 1.43 \times 10^{-10}$	$r = 0.36$ $P = 5.52 \times 10^{-9}$
FDK 2023		$r = 0.61$ $P = 0$
	DON 2023	DON 2024
DON 2022	$r = 0.40$ $P = 3.93 \times 10^{-11}$	$r = 0.33$ $P = 9.86 \times 10^{-08}$
DON 2023		$r = 0.53$ $P = 0$

SEV: FHB disease severity, AUDPC: Area Under Disease Progress Curve, FDK: Fusarium Damaged Kernel, DON: Deoxynivalenol.

Supplementary Table 2. 5. Traits correlations using best linear unbiased estimates (BLUES) calculated across the three years of the study.

Trait	Correlation coefficient (<i>r</i>)			
	SEV	AUDPC	FDK	DON
SEV	1.00			
AUDPC	0.93	1.00		
FDK	0.61	0.73	1.00	
DON	0.74	0.77	0.67	1.00

SEV: FHB disease severity, AUDPC: Area Under Disease Progress Curve, FDK: Fusarium Damaged Kernel, DON: Deoxynivalenol. All correlation coefficients are significant at $P < 1 \times 10^{-12}$.

Supplementary Table 2. 6. GWAS significant associations from all models.

Trait	Chr	Position (bp)	MAF	B H P-value	P-value (MLM)	Effect (MLM)	Effect Size (%) MLM	P-value (MLMM)	Effect (MLM)	Effect Size (%) MLMM	P-value (Farm CPU)	Effect (FarmC PU)	Effect Size (%) FarmC PU	P-value (BLINK)	Effect (BLINK)	Effect Size (%) BLINK
AUDPC	1A	40633813	0.205	0.008*							8.97E-08	28.59	3.84			
DON	1A	336583306	0.366	0.019*							3.71E-07	-0.61	2.21			
SEV	1A	557635036	0.444	5.92E-05*							3.82E-10	-2.2	3.63			
SEV	1A	575776487	0.101	5.09E-04*							4.10E-09	-2.97	4.9			
AUDPC	1B	166444551 _s	0.142	0.382				8.22E-06	61.02	8.2	8.01E-06	36.12	4.85			
FDK	1B	166444551 _s	0.142	0.583										9.51E-06	6.24	7.5
AUDPC	1B	332262821 _s	0.123	0.583	9.45E-06	58.65	7.88	7.08E-06	58.65	7.88						
DON	1B	435466844	0.168	0.008*							9.00E-08	0.84	3.01			
FDK	1B	435466844 _{ss}	0.168	0.633	7.06E-06	7.68	9.23	3.06E-06	7.68	9.23						
FDK	1B	458665282 _#	0.138	0.491	4.02E-06	8.6	10.34	1.58E-06	8.6	10.34						
DON	1B	569163320	0.03	2.12E-10*	2.28E-06	-4.06	14.58				3.44E-16	-4.08	14.64			
SEV	1D	450758304 _#	0.019	0.154	3.53E-06	-10.66	17.59									
DON	1D	450758304 _#	0.019	0.099	7.94E-06	-4.38	15.72	3.20E-07	-4.52	16.25						
SEV	2A	17404901 ^s	0.045	0.154	4.16E-06	-7.47	12.33									
SEV	2A	17404903 ^s	0.045	0.154	4.16E-06	-7.47	12.33									
SEV	2A	17404919 ^s	0.045	0.154	2.42E-06	-7.68	12.67									
SEV	2A	19021407 ^s	0.041	0.095	7.62E-07	-8.06	13.31									
SEV	2A	19022080 ^s	0.019	0.154	4.81E-06	-10.1	16.67									
SEV	2A	19022081 ^s	0.019	0.154	4.81E-06	-10.1	16.67									
SEV	2A	19022087 ^s	0.026	0.161	8.68E-06	-8.58	14.16									
SEV	2A	19582312 ^s	0.045	0.113	1.64E-06	-7.59	12.53									

SEV	2A	20111591	0.026	0.018*	5.96E-08	-10.22	16.88									
SEV	2A	21120246 ⁵	0.041	0.161	8.81E-06	-6.97	11.51									
SEV	2A	21120251 ⁵	0.041	0.161	8.81E-06	-6.97	11.51									
SEV	2A	28823494	0.045	0.001*	6.39E-07	-7.95	13.12				1.63E-08	-4.52	7.46			
DON	2A	153856694 _s	0.078	0.066							1.50E-06	-1.13	4.06			
DON	2A	450072576	0.168	9.75E-04*							3.14E-09	-0.94	3.37			
FDK	2A	455783638	0.179	0.007*							6.18E-08	3.74	4.49			
SEV	2A	665979623	0.052	2.63E-05*	5.78E-08	-8.49	14.01	6.78E-11	-9.23	15.24	1.27E-10	-5.37	8.86	1.64E-06	-4.58	7.56
AUDPC	2A	665979623	0.052	3.79E-05*	3.16E-06	-86.7	11.64	1.19E-06	-86.7	11.64	1.22E-10	-70.57	9.48			
DON	2B	98006278	0.037	0.010*	9.94E-08	-3.76	13.49	1.60E-08	-3.76	13.52						
AUDPC	2B	140904969 _#	0.063	0.583	9.66E-06	-71.76	9.64	4.41E-06	-71.76	9.64						
SEV	2B	162824813 _{es}	0.037	0.161	7.66E-06	-8.16	13.47									
AUDPC	2B	162824813 _{es}	0.037	0.583				8.45E-06	-93.86	12.61						
SEV	2B	580030865 _#	0.071	0.366										4.14E-06	-5.89	9.72
AUDPC	2B	580030865	0.071	0.014*							1.98E-07	-63.77	8.57	8.83E-06	-84.54	11.36
FDK	2B	782993929	0.119	7.10E-08*							3.43E-13	7.68	9.24			
SEV	2D	71944329	0.101	0.001*							1.80E-08	-2.86	4.72			
FDK	3A	11532385	0.31	2.01E-09*							4.99E-15	5.37	6.45	7.82E-08	5	6.01
DON	3A	730558518	0.093	0.019*							3.51E-07	-1.31	4.69			
SEV	3B	11377446 ⁵	0.019	0.58										7.82E-06	-7	11.56
DON	3B	434048674	0.045	0.031*							6.44E-07	1.32	4.73			
DON	3B	839879787	0.239	0.002*							1.40E-08	0.79	2.85			
DON	3B	843038669	0.358	1.31E-05*										2.10E-11	-1.17	4.21
AUDPC	4A	575489362	0.026	0.009*							1.21E-07	68.15	9.15			
AUDPC	4A	597403139 _#	0.071	0.582	9.93E-06	57.28	7.69	4.55E-06	57.28	7.69						
DON	4A	724908154	0.474	0.019*							3.32E-07	-0.6	2.16			
SEV	4A	731537370	0.03	0.001*							2.19E-08	-4.78	7.9			
DON	4B	171563644	0.041	0.002*							1.05E-08	-1.8	6.46			
FDK	5A	575025589	0.295	6.96E-04*							4.49E-09	3.74	4.49			
SEV	5A	675298722	0.328	0.001*							2.19E-08	-2.05	3.39			
SEV	5B	509272985 _#	0.082	0.154	3.48E-06	-5.99	9.89									
AUDPC	5B	509272985	0.082	0.008*							7.66E-08	-47.14	6.33	1.79E-08	-59.5	7.99
SEV	5B	701047429	0.03	0.002*							4.43E-08	-4.87	8.05			
AUDPC	5B	701047429	0.03	0.007*							8.37E-08	-66.85	8.98			
SEV	5D	50810559 [#]	0.041	0.113	1.55E-06	-7.59	12.52									
AUDPC	5D	50810559 [#]	0.041	0.37	2.55E-06	-88.92	11.94	9.25E-07	-88.92	11.94						
SEV	5D	550632004	0.366	7.34E-07*				6.51E-08	-3.62	5.97	1.18E-12	-2.59	4.28	5.95E-08	-2.45	4.05
AUDPC	5D	550632004	0.366	3.79E-05*							9.43E-11	-32.3	4.34			
SEV	5D	550632004	0.366	7.34E-07*				6.51E-08	-3.62	5.97	1.87E-10	-62.01	4.4	5.95E-08	-2.45	4.05
SEV	6A	18452189	0.134	0.001*				5.02E-06	-3.84	6.34	1.28E-08	-2.73	4.51	2.50E-06	-2.81	4.63

DON	6A	553038439 _{#S}	0.414	0.487				9.00E-06	-1.16	4.16						
FDK	6A	553038439	0.414	2.01E-09*	4.02E-06	-5.2	6.25	1.58E-06	-5.2	6.25	6.46E-15	-4.98	5.99	2.65E-08	-4.67	5.61
SEV	6B	651169565	0.019	0.001*							1.06E-08	-5.95	9.82			
DON	6D	142180862	0.104	0.005*										1.69E-08	-1.61	5.78
DON	7A	4026398	0.127	0.058										3.79E-07	-1.35	4.85
DON	7A	18049084	0.146	0.005*				1.83E-06	-1.97	7.07	5.41E-08	-1.03	3.69	4.79E-08	-1.44	5.16
SEV	7A	21559496 [#]	0.097	0.161	7.09E-06	-5.41	8.92	2.11E-06	-4.92	8.12						
AUDPC	7A	21559496	0.097	1.05E-04*							5.09E-10	-47.89	6.43			
SEV	7A	26275779	0.119	1.38E-06*	8.66E-06	-5.24	8.66	6.49E-06	-4.58	7.56	4.46E-12	-3.65	6.02	3.50E-11	-4.55	7.51
SEV	7A	30794288	0.131	0.07							1.59E-06	-2.28	3.76			
SEV	7A	30794298	0.127	0.065							1.37E-06	-2.32	3.82			
DON	7A	215238121 _#	0.052	0.251	8.74E-06	-2.92	10.5				8.51E-06	-1.22	4.4			
SEV	7A	612449697	0.049	0.095	7.43E-07	-7.87	12.99									
AUDPC	7A	612449697	0.049	0.583				7.34E-06	-81.99	11.01						
AUDPC	7A	643528910	0.104	0.008*							8.22E-08	39.4	5.29			
FDK	7B	8040403	0.194	0.063							6.11E-07	3.22	3.87			
DON	7B	30792302	0.037	0.003*							2.17E-08	-1.64	5.88			
DON	7B	429350245	0.019	0.008*							1.10E-07	-2.17	7.78			
DON	7B	718765746	0.056	0.008*							1.16E-07	-1.44	5.17			

SEV: FHB disease severity, **AUDPC:** area under the disease progress curve, **FDK:** Fusarium-damaged kernel, **DON:** deoxynivalenol. **MLM:** mixed linear model, **MLMM:** multi-locus mixed model, **FarmCPU:** Fixed and random model circulating probability unification, **BLINK:** bayesian information and linkage-disequilibrium iteratively nested keyway. *SNPs are significant for Benjamini-Hochberg correction P -value to control the false discovery rate (5%) and are highlighted in light gray. SNPs highlighted in dark gray are found for multiple traits at least P -value $< 1.0 \times 10^{-5}$. #Additional SNPs were retained if they had a P -value $< 1.0 \times 10^{-5}$, and co-located with SNP with FDR < 0.5 , were identified in 2 scans, or ^sfall within a region containing previous FHB-related QTL or agronomic traits.

Supplementary Table 2. 7. List of SNPs associated with two FHB disease severity metrics at $P < 1.0 \times 10^{-5}$

Multi-Traits SNPs	Chromosome	Traits	Min. Effect Size (%)	Max. Effect Size (%)	Candidate Loci
S1B_166444551	1B	AUDPC, FDK	7.5	8.2	1B Jamestown (Wright, 2014)
S1B_435466844	1B	FDK, DON*	3.01	9.23	1B Jamestown (Wright, 2014)
S1D_450758304	1D	SEV, DON [#]	15.72	17.59	
S2A_665979623	2A	SEV*, AUDPC*	9.48	15.24	DIS, 2A 722 Mb (Zhang <i>et al.</i> , 2022)
S2B_162824813	2B	SEV, AUDPC	12.61	13.47	2B Bess (Peterson <i>et al.</i> , 2017)
S2B_580030865	2B	SEV, AUDPC*	8.57	11.36	INC, FHBdx 555Mb (Gaire <i>et al.</i> , 2021); SEV, 2B 653 Mb (Larkin <i>et al.</i> , 2020)
S5B_509272985	5B	SEV, AUDPC*	6.33	9.89	
S5B_701047429	5B	SEV*, AUDPC*	8.05	8.98	

S5D_50810559	5D	SEV, AUDPC	11.94	12.52	
S5D_550632004	5D	SEV*, AUDPC*	4.05	5.97	
S6A_553038439	6A	FDK*, DON	4.16	6.25	6A NC-Neuse (Peterson <i>et al.</i> , 2016)
S7A_21559496	7A	SEV, AUDPC*	6.43	8.92	INC, SEV, 7A 42 Mb (Ghimire <i>et al.</i> , 2022); DIS, 7A 48 Mb (Zhang <i>et al.</i> , 2022); QFdk.nc-7A (Winn <i>et al.</i> , 2025)
S7A_612449697	7A	SEV [#] , AUDPC	11.01	12.99	IND, SEV, 7A 545 Mb (Ghimire <i>et al.</i> , 2022)

SEV: FHB disease severity, **AUDPC**: area under the disease progress curve, **FDK**: Fusarium-damaged

kernel, **DON**: deoxynivalenol. Min and max effect size (%) determined as the percentage of the allele effect size

divided by the trait phenotypic range, which is determined by highest BLUE minus lowest BLUE for each trait. *

FDR < 0.05; [#] FDR < 0.1.

3.0 CHAPTER THREE

Genome-Wide Association Study Identifies Novel Associations with Barley Yellow Dwarf

Virus and Soil-borne Wheat Mosaic Virus Resistance in Winter Wheat Association

Mapping Panel

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3.1 ABSTRACT

Viral pathogens adversely affect wheat (*Triticum aestivum* L.) development and are responsible for significant wheat yield losses. *Barley yellow dwarf virus* (BYDV) is one of the most serious worldwide virus threats to cereal crops. *Soil-borne wheat mosaic virus* (SBWMV) has been present in the Great Plains and responsible for wheat damage for over a century. Identification of additional sources of genetic resistance is paramount to combat the potential damage from these viruses. We constructed a panel of 269 winter wheat cultivars and breeding lines to assess the resistance to naturally occurring BYDV and SBWMV in a Kansas nursery. These lines were sequenced using exome and promoter capture identifying over 640,000 variants for association analysis with visual disease severity ratings. We found 10 and 7 significant regions affecting resistance to BYDV and SBWMV respectively. These regions include the *Bdv2* and *Sbwm1* loci, as well as novel loci affecting virus resistance. Most of the novel associations are rare, with effect sizes ranging from 5-22%. We performed a survey of the viral population present in the disease nursery, which confirmed the presence of both BYDV and SBWMV and revealed differences in virus population from year to year. Additionally, it suggested that co-infections of multiple viruses are common, demonstrating the need for breeding lines harboring resistance to multiple viruses. Deployment of these novel genetic resistance regions in combination with existing resistance loci should allow for increased resistance and potentially more sustainable viral control and reduce the risks associated with wheat yield loss due to these viruses.

3.2 INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple food crop accounting for 20% of the human energy intake worldwide (Aradottir and Crespo-Herrera, 2021). The United States (U.S.) annually produces more than 49.4 million metric tons (1.8 billion bushels) of wheat (Friskop, *et. al.*, 2022, 2023, 2024). Kansas is one of the leading U.S. wheat producers and has produced nearly 7.9 M metric tons (290 million bushels), which generated nearly \$1.75 billion in annual revenue over the past 5 years (2019-2023; Crop Protection Network, 2024). Both biotic vectors and viral pathogens cause stress and disease, resulting in significant yield and economic losses. Two common viral wheat pathogens causing serious foliar diseases are *barley yellow dwarf virus* (BYDV) and *soil-borne wheat mosaic virus* (SBWMV). Annual U.S. yield loss estimates account for ~49,000 metric tons (1.8 million bushels) due to BYDV and ~7,500 metric tons (274 thousand bushels) due to SBWMV in the past 5 years (2019-2023), although the estimates vary with environmental and temperature conditions (Friskop, *et. al.*, 2022, 2023, 2024).

Barley yellow dwarf disease is a common cereal crop disease in the U.S. Great Plains and Midwestern regions affecting wheat, oat (*Avena sativa* L.), and barley (*Hordeum vulgare* L.). BYDV is caused by a group of *Luteoviridae* family viruses and is becoming one of the most severe global viral cereal diseases (Walls *et. al.*, 2019). In Kansas alone, BYDV has accounted for nearly 20.4 K metric tons (750,000 bushels) in yield losses costing producers over \$5.5 million annually (2021-2023; Crop Protection Network, 2024). BYDV is transmitted by aphids, and the most common vectors are the bird cherry-oat aphid (*Rhopalosiphum padi*, Aradottir and Crespo-Herrera, 2021) and the English grain aphid (*Sitobion avenae*, Parry *et. al.*, 2012). There are many BYDV isolates including BYDV-PAV, BYDV-MAV, and BYDV-PAS capable of infecting grass species, where BYDV-PAV is the prevalent isolate in wheat and barley (Chrpová *et. al.*, 2020).

The BYDV disease symptoms vary among wheat cultivars and include yellowing, reddening discoloration of leaf blades and tips, stunting of plants, and purple flag leaves (Choudhury *et al.*, 2018). Interestingly, the evaluation of wheat resistance to BYDV based on visual symptoms may not necessarily represent actual viral titer. The idea of tolerance to BYDV was presented in 1993 (Singh *et. al.* 1993) where similar virus titers between susceptible and tolerant varieties were reported. In that study the tolerant varieties represented less visually symptomatic wheat plants than the susceptible varieties, yet viral titer was unable to distinguish the classes from each other. Alternative methods such as RT-qPCR (Chrpová *et. al.*, 2020), ELISA, and sequencing methods could be used to confirm the presence of BYDV for the clarification of BYDV resistance versus tolerance. BYDV can be managed by delaying the time of planting to avoid aphid vectors and/or using pesticides but using genetic resistance and tolerance to BYDV infection remains the most effective and environmentally safe form of control.

To date, only a few genes for BYDV resistance have been identified. Among them, *Bdv1* originated from a common Brazilian spring bread wheat (*Triticum aestivum*) cultivar “Frontana” (PI 500147) and provides moderate resistance to BYDV (Jarošová *et. al.*, 2016, Aradottir and Crespo-Herrera, 2021). *Bdv1* was reported to be effectively tolerant to BYDV-MAV infection and is closely linked to the *Lr34/Yr18* complex on the short arm of chromosome 7D that confers resistance to leaf and stripe rusts (Singh *et. al.*, 1993, Jarošová *et. al.*, 2016). *Bdv2*, *Bdv3*, and *Bdv4* originated from the wheat wild relative, intermediate wheatgrass (*Thinopyrum intermedium* (Host.) Barkworth & D.R. Dewey) and have been introgressed into common wheat cultivars through chromosomal translocations. These translocations include 7D-7Ai#1 (*Bdv2*), 7B-7Ai#1, (*Bdv3*), and 2D-2Ai-2 (*Bdv4*) (Zhang *et. al.*, 2009). These genes confer moderate resistance to different BYDV strains

under different environments (Choudhury *et al.*, 2019, Aradottir and Crespo-Herrera, 2021, Silva *et. al.*, 2022, Brettle *et. al.*, 1988, Larkin *et. al.*, 1995).

Soil-borne wheat mosaic virus belongs to the family *Virgaviridae* and the genus *Furovirus* (Niehl and Koenig, 2021), and has been observed in the Great Plains since 1919 (McKinney, 1923). SBWMV is a rod-shaped virus transmitted via the zoospores of *Polymyxa graminis*, an endoparasitic slime mold, that infects wheat roots under moist soil conditions (Jones *et al.*, 2024, Jianpiag, 2005, Zhang *et al.*, 2014). Virus infection occurs during the early root development stages just after seed germination, and its spread is affected by the soil conditions, particularly free water in soils. The free-soil water is required for the movement of the fungus that carries the virus through the soil to the roots (Liu *et. al.*, 2020). Temperature can also affect virus movement through plant tissues as movement generally ceases when temperatures reach 20° C (Shirako *et al.*, 2000). Visible SBWMV symptoms, such as irregular chlorotic or mosaic patches usually occur from wheat seedlings to early tillering stages (Feekes 2 - 5 stages) and disappear as the temperature increases later in the spring in winter wheat (Liu *et. al.*, 2020). Although SBWMV is not consistently detected in Kansas due to the requirement of moisture and temperatures for the fungus infections and the virus movement in plants, it was present in 2019, 2021 and 2023 in Kansas and caused over \$1.6 million in economic losses in 2019 (Crop Protection Network, 2024).

Polymyxa graminis is a soil-borne fungus and its resting spores can remain dormant and invasive for more than 20 years and become active when conditions are favorable (Liu *et. al.*, 2020), suggesting that once the soil is infected it is nearly impossible to be removed. Therefore, the best strategy for SBWMV management is growing resistant cultivars. To date, only *Sbwm1* has been identified with a major effect on resistance to SBWMV in bread wheat (Merkle and Smith 1983, Liu *et. al.*, 2020). *Sbwm1* is located on chromosome 5DL and has been widely deployed in many

U.S. winter wheat cultivars (Hao *et. al.* 2012, Narasimhamoorthy *et. al.* 2006, Liu *et. al.* 2014, Liu *et. al.* 2020). A gene of similar effect has also been detected in *Aegilops tauschii* derived wheat materials (Bass *et. al.*, 2007, Hall *et. al.*, 2009), which might be either the same gene or a tightly linked gene to *Sbwm1* residing on 5DL.

The differences in BYDV resistance gene specificity and the quantitative nature of its resistance have made repeatability difficult across BYDV studies (Choudhury *et. al.*, 2019, Silva *et. al.*, 2022). The variability in environmental conditions makes it difficult to consistently detect SBWMV's presence and identify additional regions of interest in SBWMV resistance. These factors have resulted in only a few resistance genes available for deployment in wheat to combat these diseases, which constrains gene pyramiding schemes to build durable resistance, which is important for sustained resistance. In this study, we evaluated a panel of winter accessions from the major U.S. winter wheat breeding programs relying on natural field infections of both BYDV and SBWMV in the field disease nursery in Kansas. The panel was genotyped using exome and promoter capture to identify SNPs for association testing. In addition, we employed Oxford Nanopore sequencing to identify the viruses present and the predominant strains to better understand the nature of resistance existing in the field nursery. We expected to detect the known sources of resistance previously identified for both these viruses, with the hope of identifying novel sources of resistance. This information will be useful to the breeding programs to incorporate these novel sources of resistance for deployment, as well as to identify the population of viruses that exist in the field to ensure the materials breeders are growing carry sufficient genetic resistance components to combat these two naturally vectored viruses.

3.3 MATERIALS AND METHODS

3.3.1 Wheat lines

A panel of 269 winter wheat lines was obtained from the Central Plains, Midwest, Northeast, and Southern U.S. wheat growing regions for evaluation of disease resistance (Supplementary Table 3. 1, Supplementary Figure 3.1). These lines were selected to represent the genetic diversity in disease resistance within their breeding programs.

3.3.2 Disease phenotyping

The panel, including 173 hard and 96 soft winter wheat accessions was planted at the Rocky Ford Experiment Station disease nursery in Manhattan, Kansas utilizing a randomized complete block design of two replicates per accession, and six replicates of three check varieties, “Everest” (PI 659807), “Karl92” (PI 564245), and “Overley” (PI 634934). Each accession was planted as a 1.5 meter single-row plot using ~3 g of seed, with ~0.3 m spacing between rows. The panel was planted on October 8, 2021, October 14, 2022, and October 10, 2023, and evaluated during the spring of 2022, 2023, and 2024.

Lines were visually evaluated for SBWMV severity at the tillering stage (Feekes 2-4) in the early spring of 2023 and 2024, and BYDV severity was evaluated at the post flowering growth stage (Feekes 10.5-11) all three seasons. Due to differences in weather patterns, including air and soil temperatures and precipitation (Figure S2), phenotyping times varied from year to year. SBWMV was scored April 5, 2023 (Julian date 95), and March 13, 2024 (Julian date 73), while BYDV was evaluated May 31, 2022 (Julian date 151), June 2, 2023 (Julian date 153), and May 10, 2024 (Julian date 132). Heading dates, defined as when 50% of the heads within each plot emerged, were recorded for all lines allowing for comparison between years and germplasm. Both diseases were rated on a scale from 0-5 (Choudhury *et. al.*, 2019), where a rating of 0 represents no infection, and a rating of 5 represents a severe infection. Figures 1a and 1b illustrate the natural field infection

of BYDV and SBWMV, respectively. Data were collected from all 269 accessions except for 2022, where data were collected only from 255 lines due to poor seed germination and mortality.

3.3.3 Genomic DNA extraction and normalization

Genomic DNA extraction for the 269 lines was carried out using the sbeadex maxi plant kit following the manufacturer's instructions (LGC Biosearch Technologies, Herts, UK) using ~50 mg of leaf tissue from a single wheat plant at the two-leaf stage grown under greenhouse conditions. The collected tissues were dehydrated using a FreeZone Freeze Dry Systems (Labconco) lipholizer for 48 hours. Dry leaf tissues were combined with 2-3 2.0 mm diameter zirconia beads (BioSpec Products) and ground into a fine powder using a 2010 Geno/Grinder (Spex SamplePrep). Genomic DNA was extracted with sbeadex reagents on an oKtopure automated DNA extraction platform (LGC Biosearch Technologies). Genomic stock DNA for all samples was quantified using Quant-iT PicoGreen dsDNA Assay Reagent (Thermo Fisher Scientific) and was diluted accordingly for genotyping assay inputs (KASP and captures).

3.3.4 Kompetitive allele-specific polymorphism (KASP) genotyping

For KASP assays, 3 μ L (ranging ~ 6 - 30 ng) of each genomic DNA sample was loaded into a 384-well PCR plate (Thermo Scientific) and dehydrated at 65° C for an hour. A MANTIS® liquid handler loaded 4 μ L of reaction mixture containing 1.7 μ M of each allele-specific forward primer, 4.2 μ M common reverse primer, and 1x PACE™ Genotyping Master (3CR Bioscience, Essex, UK). PCR was carried out in a C1000 Touch™ thermal cycler (Bio-Rad, Hercules, CA) using primer sequences and PCR profiles in Supplementary Table 3. 2. Fluorescence was read using FLUOstar Omega Reader (BMG LABTECH, Cary, NC), and the results were analyzed using KlusterCaller Version 3.4.1.39 (LGC Biosearch Technologies, Herts, UK).

3.3.5 Exome and promoter capture assays

Exome and promoter capture libraries were generated using genomic DNA diluted to 1 ng/μl following the previously reported protocol (Wang *et al.*, 2023). Multiplexed library DNA of 48 samples was purified using QIAquick PCR spin columns (Qiagen, Germantown, MD). Library DNA (8 μg) was concentrated to 7 μL with vacuum centrifugation. Hybridizations for exome and promoter captures were carried out following the manufacturer's instructions using the Wheat Exome v1 Kit (Cat# 309108.V5, Arbor Biosciences, Ann Arbor, MI) and Wheat Promoter Kit, (Cat# 309308.V5, Arbor Biosciences) respectively. Post-capture amplification previously described in Wang *et al.* (2023), was run for 10 cycles. The amplified DNA library was purified using QIAquick PCR spin columns prior to quantification using Qubit dsDNA HS Assay (Thermo Fisher Scientific). Size distributions of library DNA were visualized using an Agilent 2100 Bioanalyzer with a high sensitivity DNA kit (Agilent, Santa Clara, CA).

3.3.6 Illumina sequencing protocols

High-quality post-capture libraries were pooled in equimolar amounts based on the Qubit and Bioanalyzer data, diluted to 550 pM and pair-end sequenced on a NextSeq 2000 sequencer using P2 and P3 300-cycle standard reagent kits (Illumina, San Diego, CA, USA). One P2 and four P3 sequencing kits were used to sequence 6 exome capture libraries and one kit of each was used to sequence the 6 promoter capture libraries.

3.3.7 Sequencing analysis

Illumina paired-end raw fastq files were filtered for average read quality ≥ 20 using bbdduk tools v39.01 (Bushnell, 2014). Reads passing the quality filter were aligned to the IWGSC Chinese Spring reference genome RefSeq v2.1 (Zhu *et al.*, 2021) using the HiSat2 aligner v2.2.1 (Kim, Langmead, Salzberg, 2015), which produced a .sam file for each accession. A Perl script was used to filter the alignments to retain only uniquely mapped reads. The resulting unique alignments

(.sam file) were processed into a .bam file for each accession using Samtools v1.17 (Li, *et. al.* 2009). The resulting unique alignments (.bam file) were analyzed using the best practices workflow in GATK v4.4.0 (McKenna *et. al.* 2010), which produced a g.vcf file for each sample using GATK's Haplotype Caller algorithm.

For the exome capture data, each accession's g.vcf file was loaded into a practical haplotype graph (PHG) (Jensen *et. al.*, 2020, Valdez-Franco *et. al.*, 2020, Jordan *et. al.*, 2022) to build a PHG database (v0.0.31) specific for this panel. Reference ranges were constructed using ± 2 kb up- and down-stream of CSv2.1 high confidence annotated gene coordinates (IWGSC, 2018). All variants identified within the PHG database were exported to a vcf file, and subsequently filtered for minor allele frequency (MAF) ≥ 0.0185 , representing five wheat accessions carrying the alternative allele in the population. This criterion was set to retain alleles that might be specific to a single program for analyses as breeding programs sent 10-15 wheat lines each. This resulted in 620,704 variants for genome-wide association analysis (GWAS) using the exome capture data.

For the promoter capture data analysis, the g.vcf files were combined into a single .vcf file using CombineGVCFs and GenotypeGVCFs tools within GATK v4.4.0. The resulting .vcf file was filtered to retain bi-allelic sites present in $\geq 75\%$ of the accessions (202 accessions) with a Perl script. The resulting filtered sites were imputed using Beagle v 5.4 (Browning *et. al.*, 2018) with default parameters to impute missing data points. Variant sites were further filtered for a MAF ≥ 0.0185 , as was done for the exome capture data. This resulted in a total of 44,563 high-quality (HQ) SNPs used for GWAS in the promoter capture.

Coverage for each method by sample was calculated using bedtools coverage v2.30.0 (Quinlan & Hall 2010) that compared a .bed file of bait locations in the IWGSC RefSeq v2.1 reference genome to the .bam alignment file for each sample. To calculate the depth of target coverage, the total

number of reads that aligned to bait regions was multiplied by read length (151bp) and divided by the target size from the IWGSC RefSeq v2.1 reference genome. The exome capture target size was 226.3 Mb and the promoter capture target size was 163.9 Mb for hexaploid bread wheat. Depth of coverage for each sample is listed in Supplementary Table 4. 3 (exome) and Supplementary Table 4. 4 (promoter data).

3.3.8 Statistical analysis and GWAS

Raw field phenotypic observations were analyzed separately by year and then as an aggregate to obtain the best linear unbiased estimates (BLUEs) for the overall performance to each virus across the 2- or 3-year span (Supplementary Table 3. 1). Correlations and means between years were computed using base R (R Core Team, 2021). BLUEs were computed using the polyqtl R package (Bourke, 2022). Variance components for heritabilities were computed using the lme4 package in R using the following equation:

$$H^2 = \frac{\sigma^2_G}{(\sigma^2_G + (\sigma^2_{G*Y}/y) + (\sigma^2_r/n)}$$

where σ^2_G is the variance attributed to genotype, σ^2_{G*Y} is the variance attributed to genotype by year interaction divided by the number of years (y), and σ^2_r is the variance attributed to residuals divided by the number of replications (n). Principal components and kinship matrices were calculated using TASSEL v5.2.93 (Bradbury *et. al.*, 2007). GWAS was conducted using BLUE values in the GAPIT3 R package in R (Wang and Zhang, 2021) and run using mixed linear model (MLM) and multiple locus mixed model (MLMM) for both viruses and PCA components 1-3, which contributed to 9.7% of the variation. Associations were considered significant if $p \leq 5.0 \times 10^{-5}$. The most significant SNPs per region that were found to be significant for both models (MLM and MLMM) are reported in Table 1.

3.3.9 Virus tissue sampling and RNA extraction

Leaf tissue samples were randomly collected in 2023 and 2024 from 24 row samples including both asymptomatic and symptomatic coloration or mosaic patterns. The leaf tissue was sampled within one week of BYDV evaluation (Feekes 10.5-11). The tissues were collected in tubes containing RNAlater™ (Thermo Fisher) and stored at -80 °C. Total RNA was extracted using the mirVana miRNA Isolation Kit (cat# AM1560; Ambion Life Technologies California USA) from 70 – 85 mg of tissue following manufacturer's instructions. Two volumes of RNA binding buffer were added to each sample and equal volume of ethanol (95-100%) and mixed. The reactions were purified using a Zymo-Spin IICR Column (cat# R1018, Zymo Research, USA). The concentrations of total RNAs were estimated with a NanoDrop Spectrophotometer and stored at -20°C. Samples that met the concentration ≥ 100 ng/μL and 260/280 ratio (1.8-2.0) were used for cDNA library preparation.

3.3.10 Nanopore sequencing

The RNA samples were prepared for Oxford nanopore sequencing (Nanopore, Oxford UK) using the cDNA kit (cat# SQK-PCB109, Oxford Nanopore), following the manufacturer's instructions. Samples were barcoded by adding 5 μL reverse-transcribed cDNA, 1.5 μL barcode primers (cat# BP01-BP12, Oxford Nanopore), 18.5 μL nuclease-free water, 25 μL of 2x Long Amp *Taq* Master Mix. The reaction was amplified at 95°C for 30 sec for initial denaturation, followed by 15 cycles of annealing for 15 sec at 62°C and extension at 62°C for 50 secs per kb (15 cycles) with a final extension step of 65°C for 6 min. One microliter of exonuclease was directly added to the PCR reaction in each tube and the reaction mix was incubated at 37 °C for 15 min, followed by 80°C for 15 min in a thermal cycle. After incubation, 40 μL (0.8X) of resuspended AMPure XP beads was added to each of the reaction tubes and the tubes were incubated for 5 min at room temperature (~20 °C) on a rotation mixer. The beads were washed with freshly prepared 70% ethanol (Oxford

Nanopore). Residual ethanol was pipetted off, and the pellets were resuspended in 12 μ L of Elution Buffer (EB) and incubated for 10 min at a room temperature in a rotation mixer. The 12 μ L of the eluate which contained the cDNA library was transferred into a clear 1.5 mL Eppendorf tube and 1 μ L of the amplified cDNA was taken from each tube to determine the concentration and quality using a NanoDrop Spectrophotometer. The remaining 11 μ L high-quality cDNA library samples were added with 1 μ L of Rapid Adaptor (RAP), and then incubated for 5 minutes before they were loaded on the flow cells (Oxford Nanopore, FLO-MIN114) following the priming and loading instructions. Only 21 selected samples from 2023 season passed quality and concentration requirements, and a total of three flow cells (FLO-MIN114) were run for those samples.

The 24 RNA samples from 2024 were prepared for sequencing using a new Oxford Nanopore cDNA kit (cat# SQK-PCB111.24, Oxford Nanopore). Compared to the previously used kit, the new kit multiplexed 24 samples in a single run using barcodes and used RNAXP beads for size selection. The addition of RNAXP beads allowed for more RNA molecules to be retained for sequencing compared to the previous year's kit, which contained both RNA and DNA, thereby diluting the informative RNA reads. Three flow cells (FLO-MIN114) were run on the 2024 samples.

3.3.11 Analysis of Nanopore sequencing data

MinKNOW 24.02.8, Bream 7.9.8, Configuration 5.9.18, Dorado 7.3.11 and MinKNOW Core 5.9.12 data processing software were used to generate the raw fast5 files and perform base-calling. The option of high-accuracy modified base detection for 5mC in CG contexts of the base-caller (Dorado 7.3.11) was used to create the fastq files for each library. The adapter sequence was removed using Porechop v0.24 (Wick, 2018). The reads were filtered for ≥ 75 bp and ≤ 30 kb using Nanofilt 2.8.0 (De Coster, 2018) and mapped to a cereal virus reference genome (Ranabhat

et al., 2023) using CLC genomic Workbench® software v24.0.1 (QIAGEN, USA). Single reads were mapped with resequencing analyses toolbox of CLC genomic workbench using the following parameters: masking mode = no masking, match score = 1, mismatch cost = 2, cost of insertions and deletions = Linear gap cost, insertion cost = 3, deletion cost = 3, length fraction = 0.5, similarity fraction = 0.8, global alignment = no, non-specific match handling = map randomly, output mode = create stand-alone read mappings, create report = yes, and collect unmapped reads = no. Samples containing ≥ 2 reads that mapped to ≥ 750 bp of a virus reference sequence were considered present for that virus in the sample.

3.4 RESULTS

3.4.1 Phenotyping

Infection severity was evaluated for BYDV and SBWMV for 3 and 2 years respectively for 269 winter wheat accessions (Figure 3. 1a, and 1b, Supplementary Table 3. 1). The variation in weather conditions affected plant development (Supplementary Figure 3. 2) and created differences in phenotyping dates. In 2022, the average heading date for the panel was May 10 (Julian day 130), in 2023 was May 12 (Julian date 132) and in 2024 was April 27 (Julian date 118). The largest difference in heading dates (21 days between Julian dates 125-146) was observed in 2023 among all lines, while the smallest variation (15 days between Julian dates 122 and 137) was observed in 2022. 2024 had a similar range of heading dates (20 days between Julian dates 113 and 133) as in 2023. Both diseases were scored when the susceptible plants showed maximum disease symptoms in the field, with SBWMV symptoms scored ~5-6 weeks prior to flowering (Feekes stage 2-4) and BYDV scored ~1-2 weeks post-heading (Feekes stages 10.5-11).

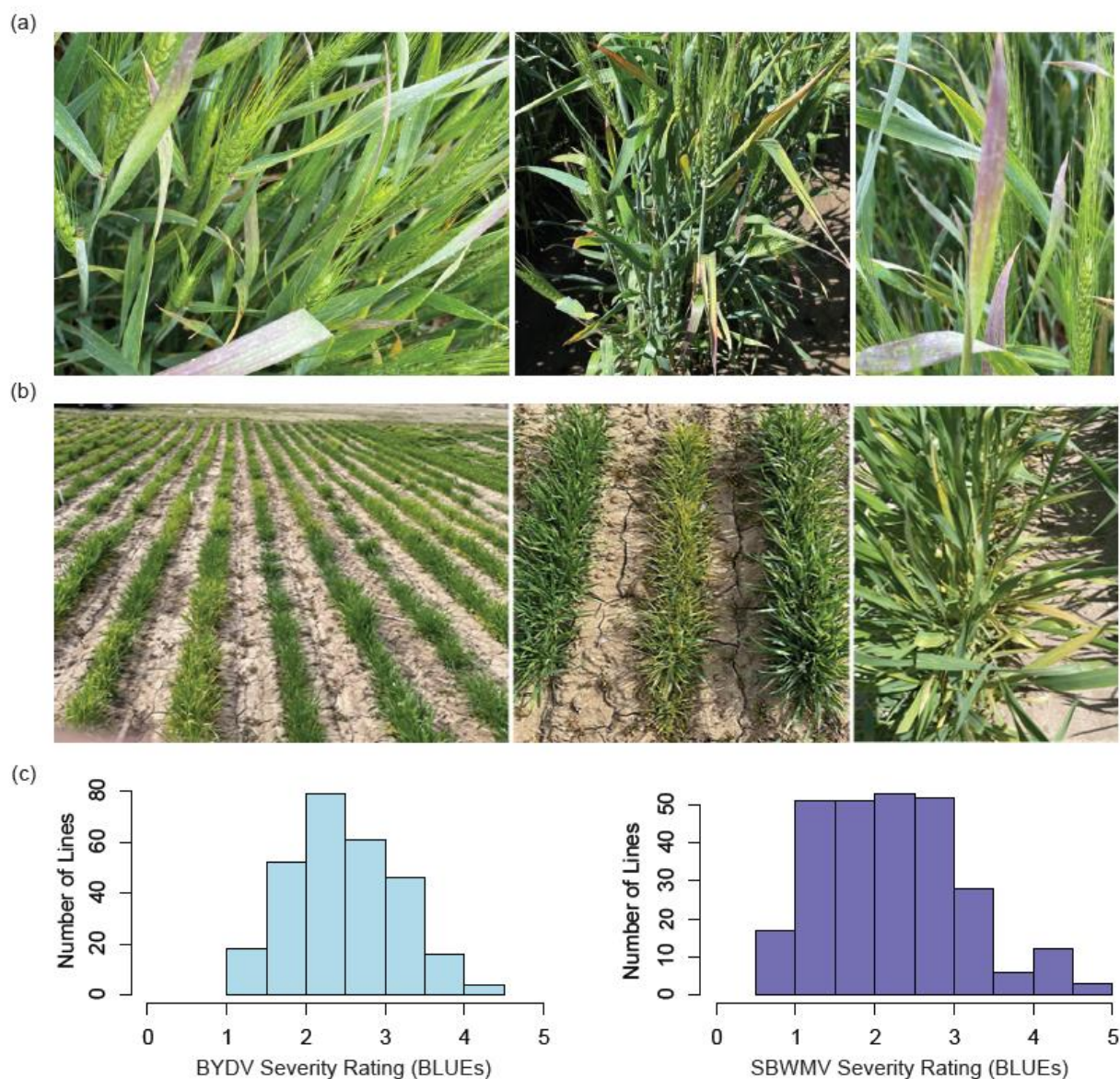


Figure 3. 1. (a) BYDV and (b) SBWMV infected winter wheat plants in the Rocky Ford Experiment Station disease nursery. (c) Histograms of BLUEs for BYDV (left) and SBWMV (right) in the panel of 269 winter wheat lines. BYDV: barley yellow dwarf virus, SBWMV: soil-borne wheat *mosaic* virus, BLUEs: Best Linear Unbiased Estimates.

Correlations among the years for visual disease ratings were modest, but significant for BYDV (ranging $r = 0.18$ - 0.30 , Supplementary Table 3. 5), and for SBWMV ($r = 0.23$, $p = 1.1 \times 10^{-4}$), resulting in broad-sense heritability estimates of 0.47 and 0.37 respectively (Supplementary Table 3. 6). We calculated the best linear unbiased estimates (BLUEs) of disease severity ratings for each

virus across years. BYDV severity ratings followed a normal distribution in the population (Figure 3. 1c), with 66 moderately resistant lines (severity rating ≤ 2) and 80 susceptible lines (severity rating ≥ 3) across the years, and an overall panel mean of 2.5. SBWMV displayed a slightly skewed distribution towards more resistance with 115 moderately resistant lines (severity rating ≤ 2) and only 61 susceptible lines (severity rating ≥ 3) (Figure 3. 1c). This skewness toward resistance was also reflected by the panel mean for SBWMV severity of 2.3.

3.4.2 Genotyping results

KASP assays of the known genes conferring resistance to BYDV and SBWMV (Supplementary Table 3. 2) detected seven positive lines for *Bdv2* and 181 positive lines for *Sbwm1* (Supplementary Table 3. 1). Screening with two KASP markers (Supplementary Table 3. 2) differentiated the functional presence of *Lr34*; where wildtype (*Lr34+*) is represented by *Lr34 exon11+/Lr34 exon22-* KASP notation, heterozygous and functional *Lr34* (*hetLr34+*) represented by *hetLr34 exon11+/Lr34 exon22-*, mutated non-functional *Lr34* protein (*mutLr34*) is represented by *Lr34 exon11+/Lr34 exon22+*, and the absence of *Lr34* (*Lr34-*) is represented by *Lr34 exon11-* (Supplementary Table 3. 1). Comparing BYDV ratings for these groups gives mean BYDV severity ratings of 2.51, 2.28, 2.75, and 2.55 respectively (Figure 3. 2a, $p = 0.35$, *Kruskal-Wallis*). Excluding the non-functional *Lr34* (*mutLr34*) allele from comparison does not reveal a difference between the wildtype *Lr34* and the absence of *Lr34* either ($p = 0.34$, *Wilcoxon test*). These results suggest there is not a significant association of BYDV rating and *Lr34* presence in this mapping panel.

Analysis of both exome and promoter capture sequences produced more than 12 billion paired-end reads. On average, 41.4 million paired-end reads were generated for each sample and 88% of the reads passed quality filtering for exome capture. The alignment of high-quality reads to the

Chinese Spring RefSeq v2.1 (Zhu *et.al.*, 2021) aligned nearly 70% of the reads to a single location on the reference genome. On-target read coverage was estimated to be ~14x of the exome targets (Supplementary Table 3. 3). SNPs called by the practical haplotype graph tool (PHG) generated 620,704 variants with a $MAF \geq 0.0185$. PCA component analysis using a subset of the exome capture SNPs (Supplementary Table 3. 1) found that the first three PCA components contributed 9.7% of the total genetic variation, with components 1 and 2 generally separating hard winter wheat from soft winter wheat and PC2 and PC3 separating soft winter wheat germplasm into two pools (Figure 3. 2b, c).

The 620,704 exome SNPs were mapped across all wheat chromosomes, with the most SNPs identified on chromosome 2B and the least SNPs on chromosome 4D (Figure 3. 2d). To expand the number of genomic variants outside the exome regions, promoter capture libraries were also used to identify SNPs. The promoter capture generated an average of 6.4 million paired-end reads per sample with 85% of those that passed quality filtering (Supplementary Table 3. 4). Comparing the alignments to the design space showed only ~0.75x target coverage per sample, which was lower than expected. This discrepancy could be due to lower quality of overall reads from the higher G/C content outside the genic regions, the inability of an alignment in the more repetitive upstream regions of genes, or the unique mapping filtering criteria allowing a read pair to only map once to the reference. These promoter captures generated 44,563 high-quality SNPs (Supplementary Figure 3. 3), which were called using GATK and imputed using Beagle. Interestingly, a substantial number of high-quality SNPs between promoter and exome SNPs overlapped, with only 24,510 (55%) SNPs unique to the promoter capture (Figure 3. 2e). The MAF for the unique promoter filtered SNPs was slightly higher ($MAF=0.11$) than what was found in the exome capture SNPs ($MAF=0.09$, Figure 3. 2f), however, the promoter MAF distribution was

bimodal and its first peak also at $MAF = 0.09$. A comparison of genotypic datasets between exome and promoter capture and between algorithms (PHG and GATK) utilizing the shared SNPs revealed a high genotype concordance of 98% (Supplementary Table 3. 7).

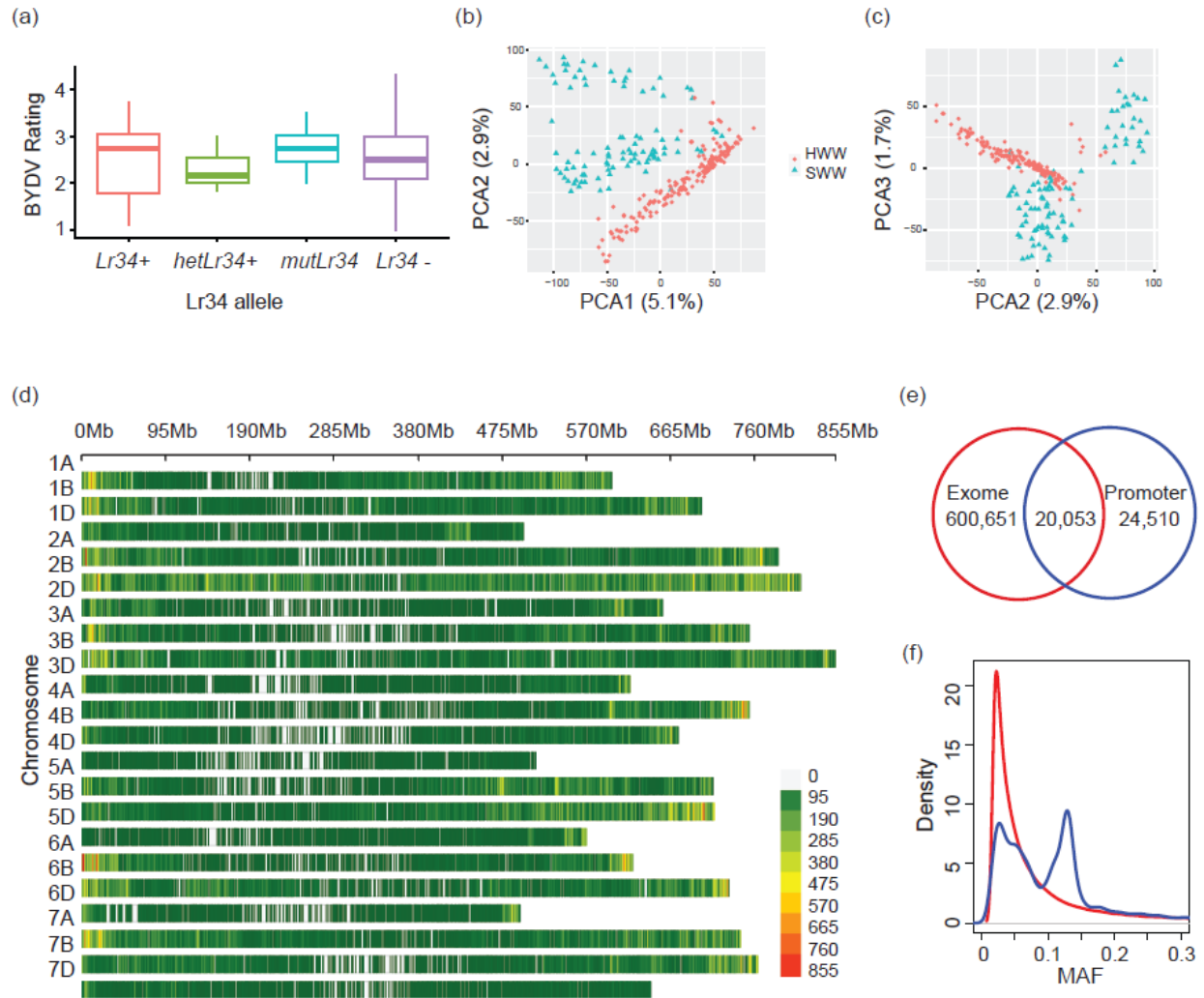


Figure 3. 2. Genotyping results of the 269 U.S. winter wheat accessions. (a) Boxplot of BYDV ratings by Lr34 KASP allele: wildtype (Lr34 +), heterozygous (hetLr34+), non functional mutation of Lr34 (mutLr34), and absence of Lr34 (Lr34-), ($p = 0.35$, Kruskal Wallis). (b) PCA1 vs PCA2 shows separation of HWW (orange circles) lines from SWW lines (blue triangles). Proportion of variance attributal to each principal component is listed on axes. (c) PCA2 vs PCA3 shows separation of SWW into two subgroups. (d) Distribution of the exome-generated SNPs on 21 wheat chromosomes based on Chinese Spring reference genome within 1Mb windows. The heatmap (bottom right)

shows the number of SNPs within each window. (e) Comparison of shared and unique HQ SNPs between the two capture methods. (f) The distribution of MAF of SNPs generated from the exome (red) and promoter (blue) capture. PCA: Principal Component Analysis, HWW: hard winter wheat, SWW: soft winter wheat, SNP: Single Nucleotide Polymorphism, Mb: million basepairs, HQ: high-quality, MAF: minor allele frequency.

3.4.3 Genome wide association analysis

Genotypic data were combined with the BYDV and SBWMV BLUEs in GAPIT3 to generate associations using both mixed linear model (MLM) and multiple loci mixed model (MLMM), which incorporated both population structure (Q) and kinship (K) into the models. The MLMM model adds an additional cofactor of associated SNPs (S) to the model with Q and K (Segura *et al.*, 2012). Association analysis on BYDV BLUEs using $p \leq 5.0 \times 10^{-5}$ based on the knowledge of the presence of KASP marker for *Bdv2* detected 49 trait associated SNPs using MLM (Figure 3. 3a) and 54 SNPs using MLMM. Among these significant SNPs, 46 were common between the two models (Supplementary Table 3. 8). The significant SNPs were found on chromosomes 2A, 2D, 3B, 4B, 5A, 5B, 6A, 7B, and 7D (Figure 3. 3a, Table 3. 1). The regions on chromosomes 2DL (588-592Mb), 3BL (621-631Mb), and 7DL (575-621Mb), were detected by multiple significant SNPs indicating strong LD and signals in these regions in the population. At least two SNPs each identified significant marker-trait associations for BYDV resistance were detected on chromosomes 4B (92.5Mb), 5B (558 Mb) and 6A (504-582Mb) (Supplementary Table 3. 8) using the exome-derived SNPs.

Seven significant marker-trait associations for BYDV resistance were detected on chromosomes 3B, 4D, 5A, and 7D by promoter-derived SNPs using both models (Figure 3.3b, Supplementary Table 3. 8). Of these associations, only the 4D region was exclusively identified by the promoter-derived SNPs (Table 3. 1). The significant region on 3B with 4 SNPs flanks the region detected by the exome-derived SNPs. The significant SNP on chromosome 5A was a common SNP between

exome and promoter datasets and was significant in both. The SNP on 7DL falls within the *Bdv2* region. Overall, these results suggest these approaches are somewhat complementary, however the power to detect associations seems more limited with promoter data.

Table 3. 1. Significant SNPs associated with wheat resistance to BYDV and SBWMV identified in both models (MLM and MLMM).

Virus	SNP ID †	Chr	Pos (bp)	MAF	<i>P</i> value (MLM)	<i>P</i> value (MLMM)	Allele Effect (MLM)	Effect size* (%) MLM	Allele Effect (MLMM)	Effect size* (%) MLMM
BYDV	S2A_649582924	2A	649,582,924	0.030	2.83E-05	1.11E-05	-0.520	10.4%	-0.529	10.6%
	S2D_592479264*	2D	592,479,264	0.067	1.15E-06	8.37E-07	0.431	8.6%	0.415	8.3%
	S3B_621676340	3B	621,676,340	0.026	5.04E-06	2.06E-06	-0.586	11.7%	-0.586	11.7%
	S3B_622432768*	3B	622,432,768	0.026	8.23E-06	7.00E-06	0.577	11.5%	0.560	11.2%
	S4B_92522697*	4B	92,522,697	0.063	6.31E-06	1.13E-06	0.402	8.0%	0.418	8.4%
	S4D_410210136	4D	410,210,136	0.022	6.99E-05	4.23E-05	-0.524	10.5%	-0.524	10.5%
	S5A_488449438**	5A	488,449,438	0.052	1.84E-05	9.31E-06	-0.423	8.5%	-0.423	8.5%
	S5B_558442645	5B	558,442,645	0.049	1.76E-05	5.02E-06	-0.376	7.5%	-0.390	7.8%
	S6A_582762857*	6A	582,762,857	0.045	2.08E-05	1.90E-05	-0.413	8.3%	-0.403	8.1%
	S7B_755919036	7B	755,919,036	0.019	4.89E-05	1.87E-05	-0.593	11.9%	-0.611	12.2%
	S7D_614846889*	7D	614,846,889	0.019	4.78E-05	2.97E-05	-0.610	12.2%	-0.617	12.3%
SBWMV	S2A_63168303	2A	63,168,303	0.492	7.59E-06	5.45E-06	-0.238	4.8%	-0.197	3.9%
	S3B_652661487	3B	652,661,487	0.218	4.29E-06	1.17E-06	-0.297	5.9%	-0.262	5.2%
	S4B_46040839*	4B	46,040,839	0.023	5.16E-06	8.62E-06	-0.831	16.6%	-0.647	12.9%
	S4D_438048125*	4D	438,048,125	0.038	2.18E-07	7.25E-06	0.839	16.8%	0.560	11.2%
	S5D_550632004	5D	550,632,004	0.361	3.56E-16	2.32E-26	0.545	10.9%	0.597	11.9%
	S5D_551468827	5D	551,468,827	0.320	3.56E-22	2.17E-36	-0.683	13.7%	-0.690	13.8%
	S6B_77897254	6B	77,897,254	0.019	8.79E-06	9.93E-06	0.857	17.1%	0.691	13.8%
	S7B_179144386	7B	179,144,386	0.019	2.85E-08	5.61E-07	-1.117	22.3%	-0.801	16.0%

† Exome SNPs, *promoter SNPs*

* Most significant SNPs identified in the significant chromosome regions.

** SNP detected by both exome and promoter captures.

& Allelic effect (%) with respect to total scale = (allele effect / 5 units)

The effect sizes for the detected marker-trait associations for BYDV resistance ranged from 0.38 (7.5%) on chromosome 5B to 0.61 (12.2%) on chromosome 7D, with the largest effects observed in the *Bdv2* region (Table1). Most of the marker-trait associations affecting BYDV resistance are rare in the population with MAF ranging from 0.0187 to 0.067 (5 - 18 accessions), consistent with

previous GWAS studies on BYD resistance in winter wheat (Choudhury *et. al.* 2019 and Silva *et. al.* 2020).

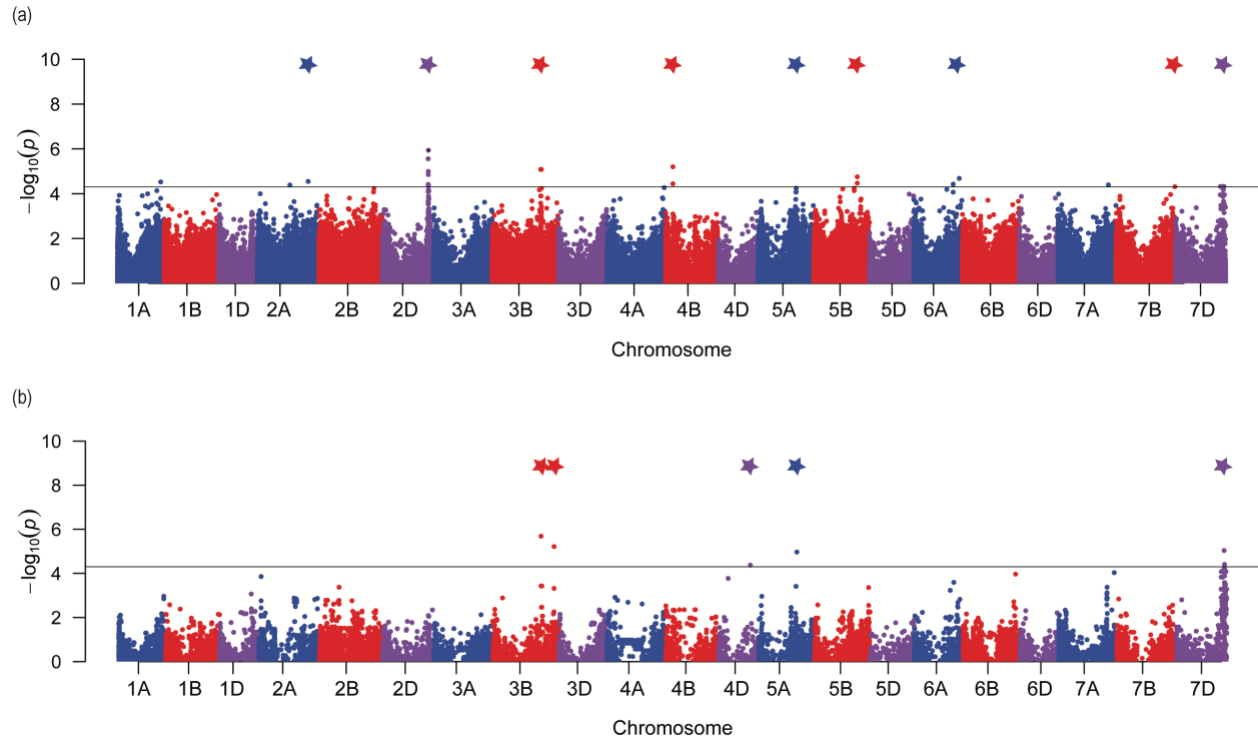


Figure 3. 3. Manhattan plots using BYDV BLUE values for, (a) exome-derived SNPs using MLM model (b) and promoter-derived SNPs using MLMM model. The black line represents $p = 5.0 \times 10^{-5}$, and colored stars above the SNPs denote significant regions detected by both MLM and MLMM models. BYDV: *barley yellow dwarf virus*, BLUE: Best Linear Unbiased Estimates, SNP: Single Nucleotide Polymorphism, MLM: mixed linear model, MLMM: multiple loci mixed model.

Similar GWAS analyses were conducted on SBWMV using BLUEs. MLM identified 72 SNPs with $p \leq 1.0 \times 10^{-5}$, and MLMM detected 23 significant marker-trait associated SNPs (Figure 3. 4a). There were 17 common associations on chromosomes 2A, 3B, 4B, 4D, 5D, 6B, and 7B between the two models (Supplementary Table 3. 9). The SNP region on 5DL, which overlaps *Sbwm1* was highly significant in both models. Multiple SNPs were detected in the 50kb region on 4B (45.9 – 46.0 Mb) and the 3.5 Mb region 4D (435.0 - 438.5Mb) (Supplementary Table 3. 9).

Using promoter-derived SNPs, we detected 5 significant associations on chromosomes 4D and 5D using MLM and 2 significant associations on 5D and 6D using MLMM (Figure 3. 4b). The *Sbwm1* region on 5D is the only region in common from both exome and promoter captures, where the promoter-derived associated SNP is about 837 kb downstream from the exome-derived SNP.

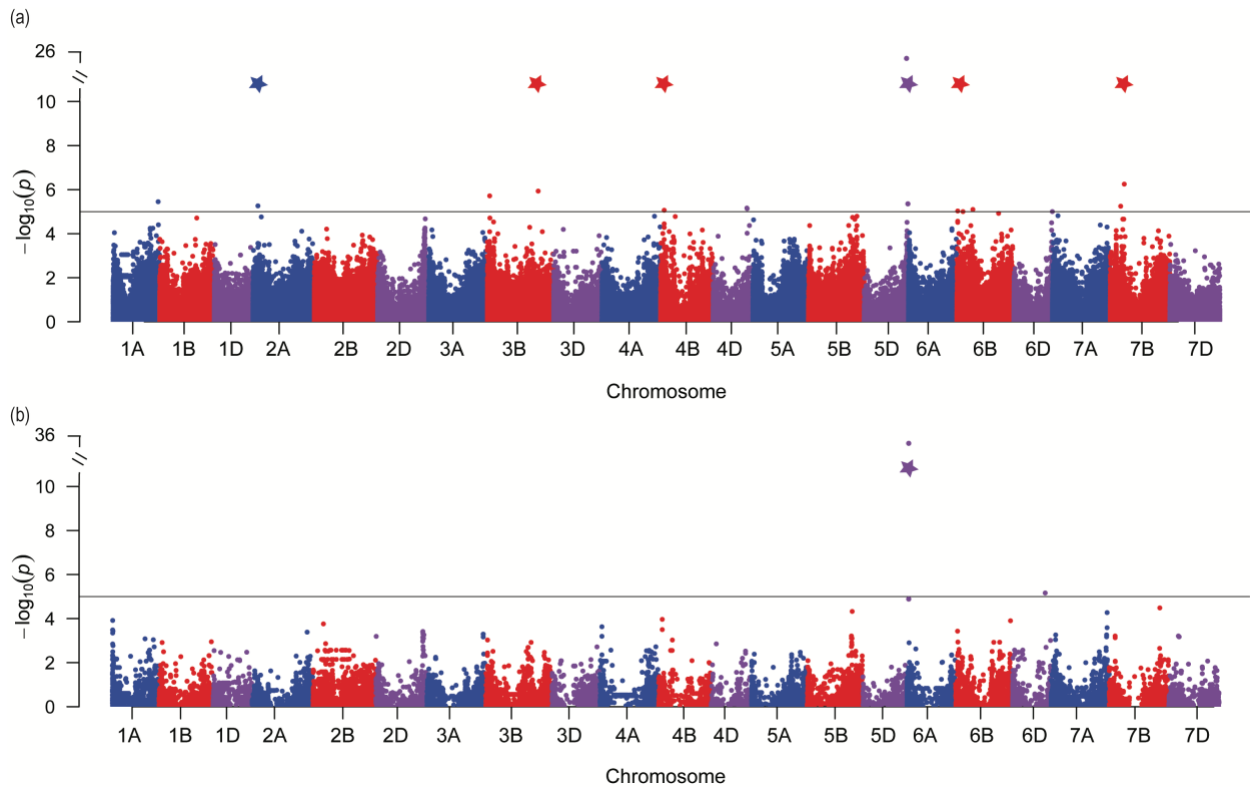


Figure 3. 4. Manhattan plots using of SBWMV BLUE values for (a) exome-derived SNPs using MLMM model, (b) promoter-derived SNPs using MLMM model. Black line represents $p = 1.0 \times 10^{-5}$, and colored stars above the SNPs denote significant regions detected by both MLM and MLMM models. SBWMV: soil-borne wheat mosaic virus, BLUEs: Best Linear Unbiased Estimates, SNP: Single Nucleotide Polymorphism, MLM: mixed linear model, MLMM: multiple loci mixed model.

The effect sizes of SNPs for SBWMV resistance ranged from 0.24 (4.8%) on chromosome 2A to 1.12 (22.3%) on chromosome 7B (Table 1). MAF at the significant SNPs varied greatly, the SNP on 7B with the largest effect showed the lowest MAF in the population (MAF= 0.0188, 5

accessions) whereas the SNP on 2A showed the highest MAF of 0.49. These results suggest that the SNP on 7B might associate with a resistance gene of major effect that has not been widely deployed in US breeding programs.

3.4.4 Detection of viruses in infected plants using Nanopore sequencing

Given the presence of BYDV and SBWMV symptoms we visibly observed, we selected samples across the field to evaluate the virus composition present in our field trials. The selected plant samples included both moderately resistant and susceptible lines from both the BYDV and SBWMV visual screening (Table 2). OXN sequencing generated over 6.6 million barcoded quality reads for the samples from 2023 and 2024 with average reads per sample of 268,466 for 2023 and 42,015 for 2024. The OXN reads were mapped to a cereal disease reference file of common cereal diseases downloaded from NCBI (Supplementary Table 3. 10), which identified sequences of multiple viruses including BYDV, SBWMV, *wheat streak mosaic virus* (WSMV), *wheat spindle streak mosaic virus* (WSSMV), and *brome mosaic virus* (BMV) (Tables 2, S11).

Table 3. 2. Viruses detected in the field by Oxford Nanopore Sequencing.

Entry Name	Year	BYDV Rating (0-5) †	SBWMV Rating (0-5) †	Cereal Virus(es) Mapped
KS Hatchett	2023	2	1	BYDV_PAV
T153*	2023	5	1	BYDV_PAV
Fultz	2023	3	2	BYDV_PAV
NC07-24-337*	2023	5	3	BYDV_PAV+BYDV_MAV
Roane	2023	1	1	BYDV_PAV+BYDV_MAV
KS16FHB0211-201-8	2023	5	2	BYDV_PAV+BYDV_MAV
Emerson	2023	4	5	BYDV_PAV+SBWMV
Flourish*	2023	2	5	BYDV_PAV+SBWMV
Chokwang*	2023	5	4.5	SBWMV
FL302	2023	1	5	SBWMV
KS19U16MD4132R2.10	2023	3	5	SBWMV
KS20UMD4144R4	2023	5	5	SBWMV+BMV
KS16DH0010-17*	2023	1	1	None detected
KS19H84	2023	1	4.5	None detected
Overley*	2023	4	1	None detected
KS18HW106-4*	2023	4	4.5	None detected

NE18573*	2023	0	1	None detected
KS Venada	2023	1	1	None detected
Ernie	2023	1	5	None detected
MT19175	2023	5	1.5	None detected
TAM 205	2023	4	1	None detected
Loma	2024	5	3	BYDV_PAS
Flourish*	2024	3	1	BYDV_PAV
MTFH21169	2024	5	1	BYDV_PAV
21NORD-155	2024	5	2	BYDV_PAV
TAM 204	2024	4	1	BYDV_PAV
MTF20189	2024	4	2	BYDV_PAV+BYDV_PAS
MTFH19132	2024	5	1	BYDV_PAV+BYDV_PAS+WSMV
21NORD-164	2024	2	1	BYDV_PAV+ WSMV
Overley*	2024	2	1	BYDV_PAV+WSMV
Atlass66	2024	3	4	BYDV_PAV+SBWMV+WSMV
Rampart	2024	5	5	CYD
MTS1903	2024	3	2	CYD
KS16DH0010-17*	2024	1	1	None detected
Chokwang*	2024	2	1	None detected
T153*	2024	1	4	None detected
NE18573*	2024	1	1	None detected
KS18HW106-4*	2024	1	2	None detected
MT19175*	2024	1	1	None detected
Pioneer-26R61	2024	2	1	None detected
NC07-24-337*	2024	1	4	WSMV
NC13-20076	2024	1	4	WSMV
SD Andes	2024	5	1	WSMV
Everest	2024	1	2	WSMV
Northern	2024	1	1	WSSMV

† Visual Severity Rating (0-1: resistant, 1-2: moderately resistant, 3-5: susceptible)

* Sampled both years (2023 and 2024)

BYDV: *Barley Yellow Dwarf Virus*.

SBWMV: *Wheat Soil-borne Mosaic Virus*.

WSMV: *Wheat Streak Mosaic virus*

WSSMV: *Wheat Spindle Streak Mosaic Virus*

CYD: *Cereal Yellow Dwarf*

BMV: *Brome Mosaic Virus*

Among the 21 samples from 2023, BYDV, SBWMV, and BMV sequences were detected (Table 2). Eight samples (38%) were infected with BYDV and six samples (29%) were infected with SBWMV. A co-infection of both BYDV and SBWMV was identified in two samples and a co-infection of both SBWMV and BMV in one sample. Virus sequences were not detected in nine (43%) accessions. For BYDV, both BYDV-PAV and BYDV-MAV strains were detected in the samples, with BYDV-PAV being the predominant strain (Table2).

In the 2024 samples, 10 lines (42%) showed a BYDV infection and only 1 line detected a SBWMV infection although five samples showed susceptible symptoms in the field (Table 2). Virus sequence was not detected in seven lines (29%). Several other viruses were detected in the 2024 samples including WSMV in eight (33%) samples, *cereal yellow dwarf virus* (CYDV) in two samples, and WSSMV in one sample (Supplementary Table 3. 11). In addition, co-infection of multiple viruses was observed in four samples, where three samples were co-infected with BYDV and WSMV, and one sample was co-infected with three viruses including BYDV, SBWMV, and WSMV. For BYDV strains, BYDV-PAV was the most detected strain, but three samples showed an infection of BYDV-PAS, and BYDV-MAV was not detected in any samples.

Taken together, we observed variation in viral composition across the years (Figure 3. 5). Viruses were detected in 64% of the samples selected. Eighteen samples (40%) had detectable BYDV and seven (16%) of the samples identified SBWMV. Some plants with no visible disease symptoms were positive for viruses and some plants with viral disease symptoms did not detect any viral presence. A total of seven samples (16%; Supplementary Table 3. 11) revealed a co-infection with two or more viruses with one having three viruses, suggesting co-infection is fairly common. For the virus confirmation survey, we only sampled a small portion of the population and did not collect all the same genotypes between years, which could contribute to a sampling bias. However,

under the natural infection conditions, besides BYDV and SBWMV, WSMV, WSSMV, CYD and BMV were also detected, which could complicate visual disease severity scoring for BYDV and SBWMV.

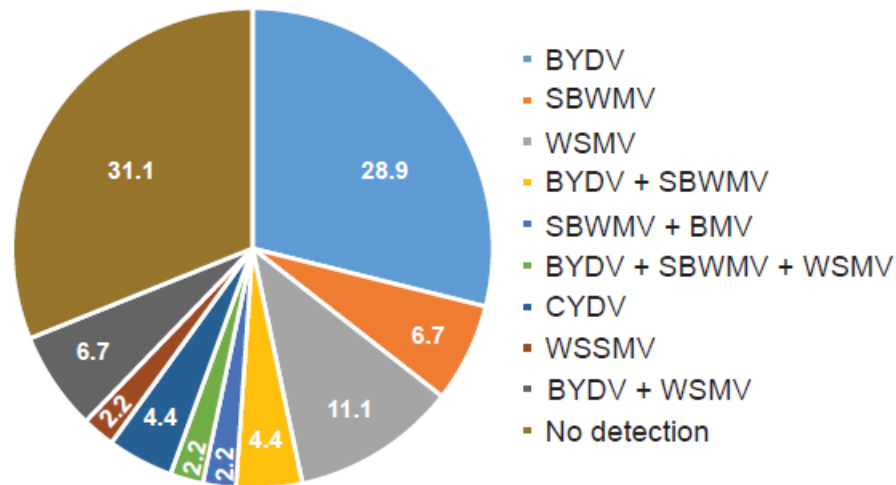


Figure 3. 5. Viruses present (%) in samples (n=45) using Nanopore sequencing and sequence mapping to cereal viruses' reference genomes. BYDV: barley yellow dwarf virus, SBWMV: soil-borne wheat mosaic virus, WSMV: wheat streak mosaic virus, WSSMV, wheat spindle streak mosaic virus, BMV: brome mosaic virus, and CYDV: cereal yellow dwarf virus.

3.5 DISCUSSION

In this study, we evaluated a panel of adapted wheat cultivars and advanced breeding lines from the U.S. winter wheat breeding programs for BYDV and SBWMV resistance in Manhattan, Kansas. Due to the nature of field experiments and natural infection, correlations between years were modest compared with controlled greenhouse experiments or inoculated studies. However, the variability and moderate heritability we observed for BYDV is consistent with previously reported studies conducted under natural infection conditions across multiple years and fields (Choudhury *et. al.* 2019, Silva *et. al.* 2022). A result of the naturally vectored BYDV infection is the infection of the host plant relies on aphids for field transmission. Differences in aphid spread

and dispersion can create an uneven disease pressure across the field within and between years. Another random factor affecting natural infection is which virus isolate is present from year to year, which was highlighted in our nanopore sequencing results for BYDV infection. We observed different BYDV isolates present between years, with BYDV-MAV in 2023 and BYDV-PAS in 2024, and BYDV-PAV in both years. This could be due to a sampling bias as the entire panel was not sequenced to verify all virus presence, however, differences in BYDV isolates were observed which could lead to variability over the years and is dependent on the aphid population present in the field. A third factor affecting variability is the use of a single human evaluation at one timepoint in a season. In a previous study, Silva *et. al.* (2022) observed better repeatability estimates for BYDV severity, with high-throughput phenotyping technology of normalized difference vegetative index (NDVI) to obtain estimates of the seasonal progression of the disease for multiple rounds of phenotyping per season. Both repeated measurements and instrumentation to estimate vegetative index would decrease variance in BYDV ratings.

Another explanation of the variation across the field seasons is the weather conditions (Supplementary Figure 3. 2) and patterns that dictate the timing of wheat development. The observed heading dates were similar between 2022 and 2023 seasons, but in 2024 plants headed two weeks earlier. This was likely attributed to a warmer February in 2024 that allowed for plant development to begin earlier than the previous two years (Supplementary Figure 3. 2), which can disrupt the timing of natural infection under favorable environmental conditions. Soil precipitation and temperature affect SBWMV infection in wheat. On average, late fall and winter 2021 was much drier than in 2022 and 2023, which is consistent with less SBWMV detected in our study in 2022 than in 2023 and 2024 field seasons. Lack of sufficient soil water limits the movement of

SBWMV vector spores to the wheat root tissues, which could partially explain the variability in disease scores over the years.

Overall, we observed more SBWMV resistant accessions than BYDV resistant accessions based on their BLUEs, which is likely due to the major effect of the *Sbwm1* gene on SBWMV resistance and its wide deployment in the US winter wheat (Hao *et. al.* 2012, Narasimhamoorthy *et. al.* 2006, Liu *et. al.* 2014, Liu *et. al.* 2020). This was supported by the KASP marker data for *Sbwm1*, visual disease ratings, and the high MAF for the 5D marker-trait associations identified for SBWMV resistance. The major effect gene on 5DL has been associated with SBWMV resistance in other studies (Liu *et. al.*, 2020). Highly significant SNPs ($p < 10^{-25}$) on 5D for SBWMV resistance were identified from both promoter and exome capture sequences, emphasizing the major effect of this locus. This significant region is ~25 kb away from one of the flanking markers, BS00073116_51, for *Sbwm1* and about 140 kb away from the candidate gene *TraesCS5D01G531200* (*TraesCS5D03G1159900* in CS RefSeq v2.1) proposed in the previous study (Liu *et. al.*, 2020). These results suggest the 5D region represents *Sbwm1*. Further fine mapping in the region will facilitate final determination of the causal gene for *Sbwm1*.

In addition to the *Sbwm1* region on 5D, we detected four other significant associations for SBWMV resistance with effects sizes $> 10\%$ (Supplementary Table 3. 12). The largest effect of these comes from 7BS but is very rare in the population. Genes or QTL have not been reported on 7BS previously; therefore this region likely carries a new major gene for SBWMV resistance. Further mapping of the gene in biparental populations will confirm the QTL and its effect on SBWMV resistance. Another significant association with a large effect was found on chromosome 4D. A QTL on the same chromosome was previously reported by Zhang *et. al.* (2011). Only two of the associated markers from that study (Xwmc89, Xwmc48) were successfully blasted to CS

RefSeq v2.1 at the position 67.6 Mb, which is 370 Mb away from our peak, therefore this marker-trait association likely represents a different resistance gene of major effect. In addition to 7B and 4D, significant SNPs on chromosomes 4B and 6B explained 16-17% of the phenotypic variation for SBWMV resistance and appear to be novel QTL that warrant further evaluation. Currently, only *Sbwm1* has been used in the U.S. winter wheat breeding programs. To diversify sources of SBWMV resistance genes, the four putative novel genes identified on 4B, 4D, 6B, and 7B need to be confirmed and each are important resistance genes for improving the level of durability of wheat resistance to SBWMV.

Unlike the results for SBWMV, significant SNPs showed smaller effects for BYDV resistance, and those SNPs had a low MAF in the population, consistent with previous studies (Choudhury *et al.* 2019 and Silva *et al.* 2022). The *Bdv2* locus region (575.6 – 621.8 Mb) on 7DL, which possesses the largest effect identified in GWAS, was present in only five lines. The *Bdv2*-gene specific KASP marker assays confirmed the *Bdv2* presence in all five of these lines. KASP analysis also identified a breeding line from USDA HWWGRU breeding program carrying the *Bdv2* translocated into chromosome 7A (T7AS.7AL-7Ae#1L-7Ai#1L), which is described as *Lr19-Bdv2*, no yellow phenotype (Adam Lukaszewski, Dalhoe Koo, personal communication). Each of these *Bdv2* positive lines identified by the GWAS or KASP assays exhibited consistent moderate BYDV resistance across years with visual disease severity ratings ranging from 1.25 - 2.25. The observation that only a few accessions in the panel carry the *Bdv2* genes might be due to linkage drag of large alien translocations that are unable to recombine thus hampering their deployment in modern cultivars. Further cytogenetic work to reduce the size of the alien segment may facilitate broader deployment of these genes. Identification of native BYDV resistance genes from elite

breeding lines or cultivars is an alternative use of alien-derived genes that may speed deployment of resistance genes in modern cultivars.

There is some evidence that the *Lr34/Yr18* locus could be pleiotropic for *Bdv1* in the literature (Singh 1993). Singh (1993) found a weak significant statistical correlation ($0.01 < p < 0.05$) of BYDV tolerance co-segregating with *Lr34/Yr18* resistance and concluded it was either due to a possible pleiotropic effect or due to tightly linked genes on chromosome 7D. We tested this hypothesis in our panel using *Lr34* KASP genotyping and BYDV visual severity ratings and were unable to detect a significant difference between groups ($p = 0.35$, Kruskal-Wallis), leading us to suggest that *Lr34* locus does not confer resistance to BYDV. However, it is important to highlight the previous observation was from an inoculated experiment with the BYDV-MAV serotype, where this study is naturally infected, and the predominant strain in the field was BYDV-PAV. Further evaluation with more equal sample sizes of alleles, and/or recombinants and fine-mapping testing should be conducted to confirm this observation.

In this study, we identified several QTL for BYD resistance from modern wheat cultivars and breeding lines. One QTL region on chromosome 6A at 582 Mb is located 15Mb away from the significant association of the 90K marker IWB69770 reported by Choudhury *et. al.* (2019). Given this significant replicated signal on chromosome 6AL was identified by independent markers in separate populations, we suggest this locus is a suitable target for further investigation into BYDV resistance. Two marker-trait associations were identified at 622Mb on 3BL and 756 Mb on 7BL in this study and are similar to regions presented in Silva *et. al.* (2022). However, that study only focused on the associations at *Bdv2* locus on 7DL, and two minor regions on 5AS and 7AL. The current study did not detect the significant regions on 5AS and 7AL, which could be due to a difference in allele frequencies between the two sets of materials. The Silva *et. al.* (2022) panel

exclusively consisted of breeding materials from Kansas State University, and while this panel included some breeding lines from Kansas State University, they only represented a small portion in the current study and originated from different cohorts within the KSU program.

In addition to the common significant associations for BYD resistance between this and previous studies, we detected some novel regions on chromosomes 2AL, 2DL, 4BS, 5AL, and 5BL (Supplementary Table 3. 12). The presence of the largest peak detected by multiple SNPs on chromosome 2DL where *Bdv4* was located, suggests this could be a *Bdv4* translocation or a novel QTL from winter wheat. The Choudhury *et. al.* (2019) study reported a significant marker-trait association of IWA3520 on chromosome 2A, but it maps to the 2AS arm in the Chinese Spring RefSeq v2.1 reference genome. Except for the possibility of the 2DL region that could be *Bdv4*, where more analyses are needed to confirm the presence or absence of a translocation, most of these novel regions originate from winter wheat, and not from a wheat wild relative. These newly identified regions associated with BYDV resistance warrant further evaluation to confirm resistance and trace the source.

This study combined wheat SNPs from both exome capture and promoter capture, but the exome capture data identified more significant marker-trait associations in the panel. Perhaps this is an artifact of the larger number of SNPs and higher coverage to confidently call SNPs in the exome dataset, or perhaps this is a biological signal where the source of the resistance is found in exomes/genic regions. These answers will come from further investigation into identifying the causal genes for resistance. Another difference between these two sets of data is the promoter data variants were called using GATK and imputed with Beagle, while the exome variants were derived from the PHG. We detected 45% of the variants from the promoter capture in the exome dataset, suggesting substantial off-target reads in the promoter dataset. We observed 98% genotyping

concordance between the overlapped SNPs using PHG vs GATK/Beagle, similar to the difference previously found comparing PHG and Beagle (Jordan *et. al.*, 2021). These observations suggest that unless a specific regulatory variant is the target, there is greater confidence and utility to working with exome capture data.

We employed Oxford Nanopore sequencing to generate a survey of viruses present in the field where visual disease severity ratings of BYDV and SBWMV were evaluated. Traditional immunological methods can be effective and sensitive to symptomatic tissues with high viral titers, but also can be ineffective if specific antibodies are required or a very high titer is needed for a positive test (Fellers *et. al.*, 2019). This nanopore survey is a method to detect virus presence in the field without bias of predetermining which viruses are tested and choosing specific targets needed for RT-PCR or ELISA methods. Building on this, the sequencing results identified other wheat viruses present in our field and suggest 13% of the samples were coinfecting. Co-infection of viruses has previously been reported in literature notably BMV, BYDV, WSMV, SBWMV, TriMV, and WSSMV with two or more of these viruses co-infecting wheat samples from several counties in Kansas (Ranabhat *et. al.*, 2023). Our results revealed some cases where viral infection was found in asymptomatic plants, suggesting BYDV viral tolerance might be present in our population, supporting previous studies by CIMMYT (Singh *et. al.* 1993). Further studies investigating the nature of resistance/tolerance in this panel should be conducted using the more traditional quantitative assays, such as RT-PCR to confirm. The nanopore analysis also found some symptomatic plants with no virus detected, which could be due to the timing of leaf sampling. The optimal time to sample would be when virus symptoms are highest, which would be early spring for SBWMV, and around heading for BYDV, but could vary for other viruses present in the field. An additional value to using a sequencing platform like OXN is to help determine which strain of

viruses are present unlike the immunological method which only detects the viruses present. Our data revealed that the BYDV-PAV strain commonly infecting wheat and oats (Chrpová *et. al.*, 2020) is the prevalent isolate in the Rocky Ford Experiment Station disease nursery, but other BYDV strains were present. This sequencing survey represents a method to inform breeders of the resistance/tolerance background of lines in the context of the population of viruses present so that better selection decisions can be made within breeding programs to combat viruses that negatively affect development and have the potential to cause yield loss.

3.6 CONCLUSION

Viral pathogens negatively affect wheat yield by stunting plant growth, reducing leaf photosynthesis capacity, reducing the number of tillers, thereby reducing the grain number and weight produced per acre. These yield reductions adversely affect the economic revenue for wheat producers and the food supply chain for consumers. Understanding genetic resistance to these viruses will inform breeding selection and ultimately reduce yield loss and improve land use efficiency. This study evaluated naturally occurring BYDV and SBWMV disease resistance in adapted wheat cultivars and elite breeding lines in a Kansas nursery over multiple years. We detected associations with known sources of resistance for both BYDV and SBWMV that coincide with *Bdv2* and *Sbwm1* resistance genes currently utilized to fight viral infection. In addition, we identified novel regions representing new potential breeding targets for improved resistance through gene pyramiding for both viruses. These novel regions generally are rare in this panel and have a diverse range of effects on reducing visual symptomology. Associated effect sizes for SBWMV were larger than for BYDV, but the average effect for BYDV resistance was nearly 10%. More research needs to be conducted to evaluate if this reduction in visual symptoms comes with an increase in yield. We also performed a survey of the viral pathogens from the field samples and

found co-infections of multiple viruses. Ultimately the knowledge of which viruses are present coupled with genetic resistance to those viruses, will help reduce the risks associated with grain yield loss due to these viruses.

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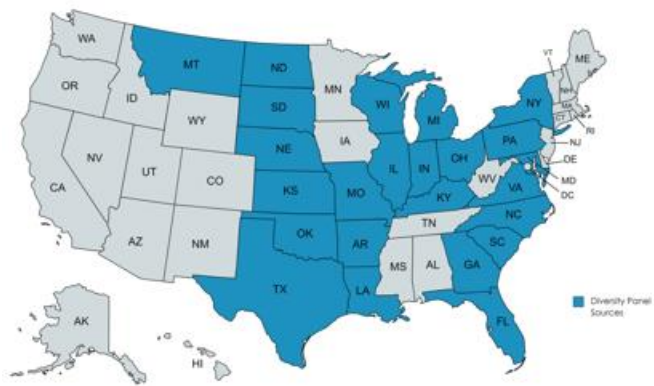
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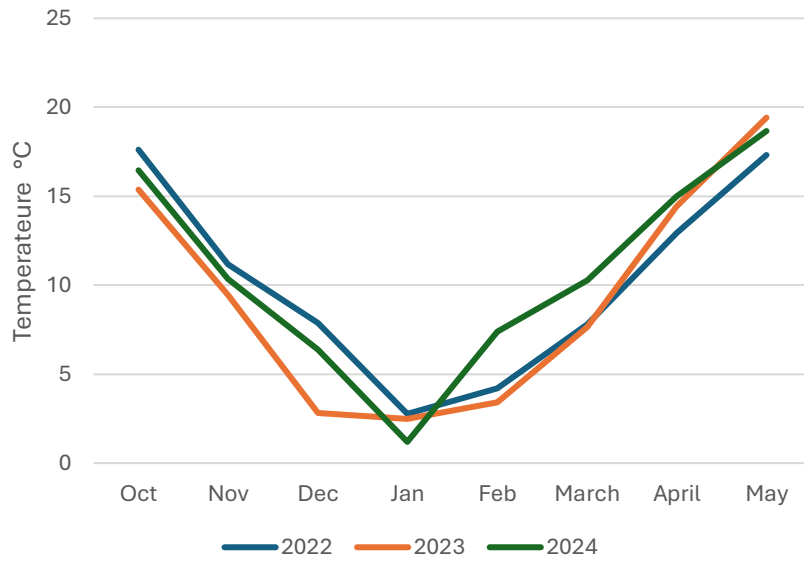
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3. 8 SUPPLEMENTAL FIGURES

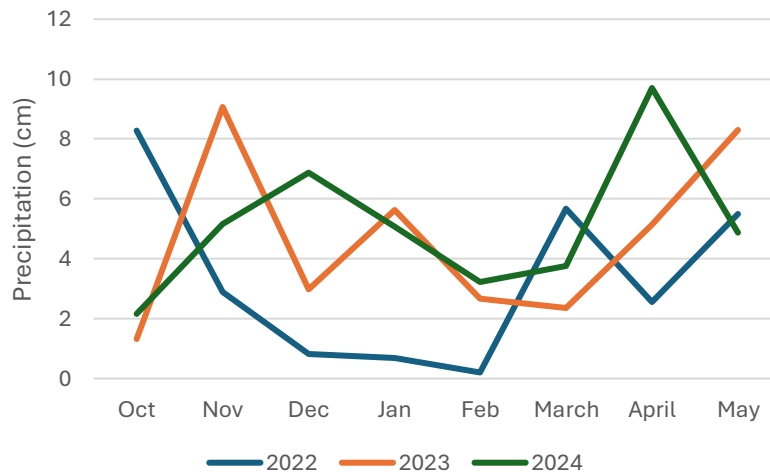


Supplementary Figure 3. 1. Geographic distribution of winter wheat lines evaluated in this study.

(a)



(b)



Supplementary Figure 3. 2. Weather Patterns per Season. (a) Soil Temperature (°C) at 5cm depth, (b) Precipitation (cm)



Supplementary Figure 3. 3. Distribution of unique SNPs from promoter capture SNPs for GWAS by chromosome.

SNP; Single nucleotide polymorphism, GWAS: genome-wide association study.

3.9 SUPPLEMENTARY TABLES

Supplementary Table 3. 1. List of lines, States, Types, BLUEs, PCA, KASP. (Separate excel file,

<https://access.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fesc2.70016&file=csc270016-sup-0002-TableS1.xlsx>)

Supplementary Table 3. 2. KASP primer sequences and PCR profiles.

Gene	Primer (5' → 3')	PCR Profile
Bdv2 [#]	GAAGGTGACCAAGTTCATGCTGATGCACACCCGGACGA (Susceptible) GAAGGTCGGAGTCAACGGATTAGGATACACATCCAGACGGTTA (Resistant) TGAAGGAAGATGATMTTGTGGTCA	KASP60XXXR 8. 94°C, 15 min 9. 94°C, 20 sec 10. 60°C 1 min 11. Go to 2, 49 times 12. 59°C, 30 sec -1°C/cycle 13. Go to 5, 34 times 14. 10°C, 5 min
Lr34 (exon 11) [§]	GAAGGTGACCAAGTTCATGCTCTGGTATGCCATTTAACATAATCATGAA (Susceptible) GAAGGTCGGAGTCAACGGATTCTGGTATGCCATTTAACATAATCATGAT (Resistant) CGCATGACAATAAGTTTCACTCATGCAAA	KASPXC 1. 94°C, 15 min 2. 94°C, 20 sec 3. 65°C, 1 min, - 0.8°C/cycle 4. Go to 2, 9 times 5. 94°C, 20 sec 6. 57°C, 1 min 7. Go to 5, 39 times
Lr34 (exon 22) [§]	GAAGGTGACCAAGTTCATGCTTGTAATGTATCGTGAGAGATTTCGAG (Susceptible/Resistant) GAAGGTCGGAGTCAACGGATTATTGTAATGTATCGTGAGAGATTTCGAT (Susceptible) GATCATTATCTGACCTGTGCGAATGAATA	KASP60XXXR
Sbwm1 [*]	GAAGGTGACCAAGTTCATGCTCACGCCATTAGCAGACGTACGTA (resistant) GAAGGTCGGAGTCAACGGATTACGCCATTAGCAGACGTACGTG (susceptible) GGGGAGTTCCCGTGTATATGTAAATAAAT	KASP60XXXR

[#] Bernardo and St. Amand, personal communication.

[§] Yan, Liuling; liuling.yan@okstate.edu, personal communication.

^{*} SNP198467, DOI 10.1007/s00122-014-2277-z

Supplementary Table 3. 3. Coverage and reads stats for Exome Capture data (separate excel file,

<https://access.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fesc2.70016&file=csc270016-sup-0003-TableS3.xlsx>)

Supplementary Table 3. 4. Coverage and reads stats for Promoter Capture data (separate excel file,

<https://access.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fesc2.70016&file=csc270016-sup-0004-TableS4.xlsx>)

Supplementary Table 3. 5. BYDV Correlations of Visual Severity Phenotypes by Year.

Year/Virus	2022 BYDV BLUEs	2023 BYDV BLUEs
2023	$r = 0.300$ $p = 1.11 \times 10^{-06}$	-
2024	$r = 0.299$ $p = 1.33 \times 10^{-06}$	$r = 0.179$ $p = 0.004$

BYDV: *barley yellow dwarf virus*, BLUEs: Best Linear Unbiased Estimates

Supplementary Table 3. 6. Variance Components and Heritability Estimates.

Variance Component	BYDV	SBWMV
Genotype	0.210	0.393
Year	0.636	0.439
Genotype x Year	0.298	0.975
Rep (Year)	0.003	0.001
Residual	0.829	0.776
Heritability (H^2)	0.469	0.365

BYDV: *barley yellow dwarf virus*, SBWMV: *soil-borne wheat mosaic virus*. Variance components were

derived using the model: $y = \mu + G + Y + G*Y + R(Y) + \varepsilon$; where μ is the overall mean, G is the random effect of genotype, Y is the random effect of year, G*Y is the random interaction effect of genotype by year, R is the random effect of replication nested within year, and ε is the residual.

Supplementary Table 3. 7. Concordance of Exome and Promoter SNP Genotypes. (Separate excel file)

Supplementary Table 3. 8. GWAS Significant Associations for BYDV Identified by MLM and MLMM

SNP ID #	Chr	Pos (bp)	MAF	P value (MLM)	P value (MLMM)	Allele Effect (MLM)	Allele Effect (MLMM)
S2A_649582924	2A	649,582,924	0.030	2.83E-05	1.11E-05	-0.520	-0.529
S2D_588686943	2D	588,686,943	0.056	2.76E-06	1.41E-06	-0.424	-0.419
S2D_588893182	2D	588,893,182	0.086	3.85E-05	3.72E-05	-0.321	-0.313
S2D_590268046	2D	590,268,046	0.078	1.01E-05	8.80E-06	-0.353	-0.341
S2D_590268073	2D	590,268,073	0.097	1.40E-05	7.14E-06	-0.324	-0.323
S2D_590268084	2D	590,268,084	0.097	1.40E-05	7.14E-06	0.324	0.323
S2D_592479264	2D	592,479,264	0.067	1.15E-06	8.37E-07	0.431	0.415
S2D_592479274	2D	592,479,274	0.067	1.15E-06	8.37E-07	0.431	0.415
S2D_592479290	2D	592,479,290	0.067	1.15E-06	8.37E-07	0.431	0.415
S2D_592479323	2D	592,479,323	0.075	4.32E-05	3.64E-05	0.346	0.338
S3B_621676340	3B	621,676,340	0.026	5.04E-06	2.06E-06	-0.586	-0.586
S3B_621676424	3B	621,676,424	0.026	8.23E-06	7.00E-06	-0.577	-0.560
S3B_621677874	3B	621,677,874	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_621677875	3B	621,677,875	0.026	8.23E-06	7.00E-06	-0.577	-0.560
S3B_621677897	3B	621,677,897	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_622432768	3B	622,432,768	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_626171268	3B	626,171,268	0.026	8.23E-06	7.00E-06	-0.577	-0.560
S3B_626681666	3B	626,681,666	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_626717029	3B	626,717,029	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_631228756	3B	631,228,756	0.026	8.23E-06	7.00E-06	0.577	0.560
S3B_788320512	3B	788,320,512	0.026	1.27E-05	6.08E-06	-0.549	-0.549
S3B_788325246	3B	788,325,246	0.026	1.27E-05	6.08E-06	0.549	0.549
S3B_788325273	3B	788,325,273	0.026	1.27E-05	6.08E-06	0.549	0.549
S4B_92522679	4B	92,522,679	0.060	3.60E-05	8.16E-06	-0.376	-0.394
S4B_92522697	4B	92,522,697	0.063	6.31E-06	1.13E-06	0.402	0.418
S4D_410210136	4D	410,210,136	0.022	6.99E-05	4.23E-05	-0.524	-0.524
S5A_488449438	5A	488,449,438	0.052	1.84E-05	9.31E-06	-0.423	-0.423
S5A_488449438	5A	488,449,438	0.075	2.09E-05	1.08E-05	-0.368	-0.368
S5B_558442645	5B	558,442,645	0.049	1.76E-05	5.02E-06	-0.376	-0.390
S5B_558442651	5B	558,442,651	0.052	3.38E-05	9.24E-06	0.350	0.367
S6A_504968418	6A	504,968,418	0.078	3.76E-05	3.31E-05	-0.336	-0.341
S6A_582762857	6A	582,762,857	0.045	2.08E-05	1.90E-05	-0.413	-0.403
S7B_755919036	7B	755,919,036	0.019	4.89E-05	1.87E-05	-0.593	-0.611
S7D_575624025	7D	575,624,025	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_575624026	7D	575,624,026	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_575624082	7D	575,624,082	0.019	4.78E-05	2.97E-05	0.610	0.617

S7D_598409224	7D	598,409,224	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_609554784	7D	609,554,784	0.030	9.19E-06	1.83E-05	0.572	0.572
S7D_614157843	7D	614,157,843	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_614262307	7D	614,262,307	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_614262308	7D	614,262,308	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_614846821	7D	614,846,821	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_614846889	7D	614,846,889	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_615852629	7D	615,852,629	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_615852631	7D	615,852,631	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_617947271	7D	617,947,271	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_617947329	7D	617,947,329	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_619694417	7D	619,694,417	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_620204985	7D	620,204,985	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_620243629	7D	620,243,629	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_621752828	7D	621,752,828	0.019	4.78E-05	2.97E-05	-0.610	-0.617
S7D_621752873	7D	621,752,873	0.019	4.78E-05	2.97E-05	0.610	0.617
S7D_621800109	7D	621,800,109	0.019	4.78E-05	2.97E-05	0.610	0.617

Exome SNPs, *promoter SNPs*

MLM- mixed linear model

MLMM- multiple locus linear model

Supplementary Table 3. 9. GWAS Significant Associations for SBWMV Identified by MLM and MLMM

SNP ID #	Chr	Pos (bp)	MAF	p value (MLM)	p value (MLMM)	Allele Effect (MLM)	Allele Effect (MLMM)
S2A_63168303	2A	63,168,303	0.492	7.59E-06	5.45E-06	-0.238	-0.197
S3B_652661487	3B	652,661,487	0.218	4.29E-06	1.17E-06	-0.297	-0.262
S4B_45998427	4B	45,998,427	0.023	5.16E-06	8.62E-06	0.831	0.647
S4B_46037543	4B	46,037,543	0.023	5.16E-06	8.62E-06	-0.831	-0.647
S4B_46038565	4B	46,038,565	0.023	5.16E-06	8.62E-06	-0.831	-0.647
S4B_46038928	4B	46,038,928	0.023	5.16E-06	8.62E-06	0.831	0.647
S4B_46040839	4B	46,040,839	0.023	5.16E-06	8.62E-06	-0.831	-0.647
S4B_46043983	4B	46,043,983	0.023	5.16E-06	8.62E-06	0.831	0.647
S4B_46044109	4B	46,044,109	0.023	5.16E-06	8.62E-06	-0.831	-0.647
S4B_46044335	4B	46,044,335	0.023	5.16E-06	8.62E-06	0.831	0.647
S4B_46046172	4B	46,046,172	0.023	5.16E-06	8.62E-06	0.831	0.647
S4D_435013291	4D	435,013,291	0.049	7.44E-07	6.86E-06	0.747	0.523
S4D_438048125	4D	438,048,125	0.038	2.18E-07	7.25E-06	0.839	0.560
S4D_438468280	4D	438,468,280	0.056	1.33E-06	7.25E-06	0.609	0.442
S5D_550632004	5D	550,632,004	0.361	3.56E-16	2.32E-26	0.545	0.597
S5D_551468827*	5D	551,468,827	0.320	3.56E-22	2.17E-36	-0.683	-0.690
S6B_77897254	6B	77,897,254	0.019	8.79E-06	9.93E-06	0.857	0.691
S7B_179144386	7B	179,144,386	0.019	2.85E-08	5.61E-07	-1.117	-0.801

Exome SNPs, *promoter SNPs*

MLM- mixed linear model

MLMM- multiple locus linear model

Supplementary Table 3. 10. Cereal Virus References for Oxford Nanopore (OXN) mapping.

Name of virus	Accession number
Ageratum yellow leaf curl betasatellite	KC305091.1
Agropyron mosaic virus	NC_005903.1
Barley mild mosaic virus	AJ544268.1
Barley stripe mosaic virus RNA1	NC_003469.1
Barley stripe mosaic virus RNA2	NC_003481.1
Barley stripe mosaic virus RNA3	NC_003478.1
Barley yellow dwarf virus-PAS	NC_002160.2
Barley yellow dwarf virus-MAV	NC_003680.1
Barley yellow dwarf virus-GAV	KF523382.1
Barley yellow dwarf virus-PAV	EF043235.1
Barley yellow striate mosaic virus, polymerase (L) gene	FJ665628
Barley yellow striate mosaic virus, glycoprotein (G) gene	KP163565.1
Cereal yellow dwarf virus	EF521830.1
Hordeum mosaic virus	NC_005904.1
Maize streak virus	AF239960.1
Oat golden stripe virus RNA1	NC_002358.1
Oat golden stripe virus RNA2	NC_002357.1
Oat necrotic mottle virus	NC_005136.1
Tobacco mosaic virus Queensland	AF332868
Rice black streaked dwarf virus (S1)	KC134289.1
Rice black streaked dwarf virus (S2)	KC134290.1
Rice black streaked dwarf virus (S3)	KC134291.1
Rice black streaked dwarf virus (S4)	KC134292.1
Rice black streaked dwarf virus (S5)	KC134293.1
Rice black streaked dwarf virus (S6)	KC134294.1
Rice black streaked dwarf virus (S7)	KC134295.1
Rice black streaked dwarf virus (S8)	KC134296.1
Rice black streaked dwarf virus (S9) cds_AFX68415.1_1	KC134297.1
Rice black streaked dwarf virus (S9) cds_AFX68415.1_2	KC134297.1
Rice black streaked dwarf virus (S10)	KC134298.1
Soil borne wheat mosaic virus RNA1	KT736088.1
Soil borne wheat mosaic virus RNA2	KT736089.1
Wheat streak mosaic virus type strain	AF285169
Wheat streak mosaic virus Hoym	HG810954.1
Wheat mosaic virus KS7 RNA1	KT988860.1
Wheat mosaic virus KS7 RNA2	KT988861.1
Wheat mosaic virus KS7 RNA3A	KT988862.1
Wheat mosaic virus KS7 RNA3B	KT988863.1
Wheat mosaic virus KS7 RNA4	KT988864.1
Wheat mosaic virus KS7 RNA5	KT988865.1
Wheat mosaic virus KS7 RNA6	KT988866.1
Wheat mosaic virus KS7 RNA7	KT988867.1
Wheat mosaic virus KS7 RNA8	KT988868.1
Wheat dwarf virus	KJ473705.1
Wheat eqolid mosaic virus	NC_009805.1
Wheat rosette stunt virus	AF059602.1

Name of virus	Accession number
Wheat spindle streak mosaic virus RNA1	NC_040508.1
Wheat spindle streak mosaic virus RNA2	NC_040507.1
Wheat stripe virus RNA2	AY312434.1
Wheat stripe virus RNA3	AY312435.1
Wheat stripe virus RNA4	AY312436.1
Wheat yellow mosaic virus RNA1	AB910332.1
Wheat yellow mosaic virus RNA2	AB910336.1
Foxtail mosaic virus	EF630359.1
Triticum mosaic virus KS	FJ263671.1
Barley virus G	KT962089.1
Barley yellow mosaic virus RNA1	AJ132268.1
Barley yellow mosaic virus RNA2	AJ132269.1
Brome mosaic virus RNA1	NC_002026.1
Brome mosaic virus RNA2	NC_002027.1
Brome mosaic virus RNA3	NC_002028.2
European wheat striate mosaic virus RNA1	MN044342.1
European wheat striate mosaic virus RNA2	MN044343.1
European wheat striate mosaic virus RNA3	MN044344.1
European wheat striate mosaic virus RNA4	MN044345.1
Chinese wheat mosaic virus RNA1	NC_002359.1
Chinese wheat mosaic virus RNA2	NC_002356.1
Johnsongrass mosaic virus	KX897165.1
Maize chlorotic mottle virus	X14736.2
Maize yellow dwarf virus-RMV	KC921392.1
Maize yellow mosaic virus-Morogoro	MW036244.1
Maize yellow striate virus	KY884303.1
Oat dwarf virus	KX533459.1
Panicum mosaic virus	MH885652.1
Ryegrass mosaic virus	MT005828.1
Sitobion miscanthi flavi-like virus	MH778148.1
Soil borne cereal mosaic virus RNA1	NC_002042.1
Soil borne cereal mosaic virus RNA2	NC_002041.1
Sugarcane mosaic virus	AJ297628.1
Wheat leaf yellowing-associated virus	KY605226.1
Wheat yellow dwarf virus-GPV	NC_012931.1
Wheat yellow striate virus	MG604920.1
Brome streak mosaic rymovirus	Z48506.1

Supplementary Table 3. 11. Oxford Nanopore (OXN) Read Mapping data to Cereal Virus Reference.

Entry Name	Year	Total Reads ^a	Mapped Reads ^b	Cereal Virus(es) Mapped	Reads Mapped to Virus ^c	Mapped Consensus Length ^d	Virus Reference Length ^e
KS Hatchett	2023	121295	40	BYDV_PAV	6	1.4 kb	5.6 kb
T153*	2023	368607	339	BYDV_PAV	173	5.4 kb	5.6 kb
Fultz	2023	203819	48	BYDV_PAV	24	3.8 kb	5.4 kb
NC07-24-337*	2023	324901	306	BYDV_PAV + BYDV_MAV	182	5.6 kb	5.6 kb
Roane	2023	276951	235	BYDV_PAV + BYDV_MAV	126	5.3 kb	5.6 kb
KS16FHB0211-201-8	2023	241403	371	BYDV_PAV + BYDV_MAV	322, 7	5.6 kb, 1 kb	5.6 kb
Flourish*	2023	141087	859	BYDV_PAV + SBWMV	182, 235	5.6 kb, 5.4 kb	5.6 kb, 5.4 kb
Emerson	2023	197930	8241	BYDV_PAV + SBWMV	129, 3523	5.6 kb, 5.4 kb	5.6 kb, 5.4 kb
Chokwang*	2023	277604	139	SBWMV	5	723 bp	5.4 kb
FL302	2023	165830	1978	SBWMV	850	5.4 kb	5.4 kb
KS19U16MD4132R2.10	2023	222187	45	SBWMV	3	2.18 kb	3.9 kb
KS20UMD4144R4	2023	175999	5462	SBWMV + BMV	2384, 1	5.4 kb, 1.2kb	5.4 kb, 2.1 kb
KS16DH0010-17*	2023	298627	120	None detected			
KS19H84	2023	313877	134	None detected			
Overley*	2023	278154	150	None detected			
KS18HW106-4*	2023	460859	284	None detected			
NE18573*	2023	329187	130	None detected			
KS Venada	2023	421970	164	None detected			
Ernie	2023	233235	107	None detected			
MT19175	2023	323599	41	None detected			
TAM 205	2023	260662	31	None detected			
Loma	2024	30244	64	BYDV_PAS	5	1.3 kb	5.6 kb
Flourish*	2024	61982	366	BYDV_PAV	17	3.2 kb	5.6 kb
MTFH21169	2024	44941	129	BYDV_PAV	12	2.8 kb	5.6 kb
21NORD-155	2024	30255	186	BYDV_PAV	12	2.6 kb	5.6 kb
TAM 204	2024	55679	300	BYDV_PAV	10	1.8 kb	5.6 kb
MTF20189	2024	43181	143	BYDV_PAV + BYDV_PAS	5, 4	2 kb, 1.8 kb	5.6 kb, 5.6 kb
MTFH19132	2024	34154	893	BYDV_PAS + BYDV_PAV + WSMV	11, 3, 21	2.5 kb, 1.8 kb, 4.2 kb	5.6 kb, 5.6 kb, 9.3 kb
21NORD-164	2024	38176	901	BYDV_PAV + WSMV	4, 204	2.3 kb, 7.8 kb	5.6 kb, 9.3 kb
Overley*	2024	30200	260	BYDV_PAV + WSMV	7, 57	2.8 kb, 3.2 kb	5.6 kb, 9.3 kb
Atlass66	2024	76367	1851	BYDV_PAV + SBWMV + WSMV	42, 8, 328	3.1 kb, 2.5kb, 7 kb	5.6 kb, 3.9 kb, 9.3 kb
Rampart	2024	58445	267	CYD	3	1.2 kb	5.7 kb
MTS1903	2024	44352	159	CYD	4	1.5 kb	5.7 kb
KS16DH0010-17*	2024	38612	67	None detected			
Chokwang*	2024	56310	173	None detected			
T153*	2024	43057	231	None detected			
NE18573*	2024	53128	129	None detected			
KS18HW106-4*	2024	55893	245	None detected			

MT19175*	2024	34396	118	None detected			
Pioneer-26R61	2024	29846	145	None detected			
NC07-24-337*	2024	28195	81	WSMV	2	1.3 kb	9.3 kb
NC13-20076	2024	40057	1361	WSMV	56	6.9 kb	9.3 kb
SD Andes	2024	16380	300	WSMV	147	2.3 kb	9.3 kb
Everest	2024	16220	26	WSMV	6	1 kb	10 kb
Northern	2024	48289	253	WSSMV	35	757 bp	7.5 kb

BYDV: *Barley Yellow Dwarf Virus*.

SBWMV: *Wheat Soil-borne Mosaic Virus*.

WSMV: *Wheat Streak Mosaic virus*

CYD: *Cereal Yellow Dwarf*

WSSMV: *Wheat Spindle Streak Mosaic Virus*

BMV: *Brome Mosaic Virus*

^a Total reads generated by OXN per sample

^b Total mapped reads to the cereal virus reference file (Supplementary Table 3. 10)

^c Read mapped to specific virus listed. Commas separate reads mapped to different viruses with co-infection.

^d Mapped consensus length to specific virus listed. Commas separate reads mapped to different viruses with co-infection

^e Reference length (kb) from NCBI accessions used to construct the cereal virus reference

*Repeated sampling in 2023 and 2024

Supplementary Table 3. 12. Accessions Carrying Rare Associations at Novel Loci Continued

Virus	SBWMV	SBWMV	SBWMV	SBWMV
Chromosome	4B	4D	6B	7B
Position	46040839	438048125	77897254	179144386
MAF	0.0226	0.0376	0.0188	0.0188
Alleles	C/A	G/T	A/G	C/A
Rare Allele	A	T	G	A
	Frontana	AGS2000	21NORD-153	Atlass66
	Hope	Coker9663	D6234	Frontana
	NE17441	FL302	Frontana	Hope
	NI17410	Frontana	MTFH2289	Xiaoyan22
	Rampart	LA09264C-P2	Rampart	Zhengmai9023
Accessions	Zhengmai9023	LA841		
		LA95135		
		NC09BGTS16		
		Oakes		
		Royal		
		U7487-614		

4. 0 CHAPTER FOUR

**Genomic selection for Fusarium head-blight (FHB) resistance in a diversity panel of wheat
cultivars in US wheat breeding programs**

4.1 ABSTRACT

Genomic selection is increasingly vital and adopted in wheat breeding programs for complex quantitative traits. This study evaluated the potential of genomic selection and forward genomic prediction to help predict and/or increase genetic gains for increased FHB resistance in winter wheat for public breeding programs. We further explored the potential of forward genomic prediction using the diversity panel (DP) as a training population to predict the genetic estimated breeding values of independent populations from the United States Wheat and Barley Scab Initiative uniform regional nurseries' lines (USWBSI) and the Great Plains Lines from the Kansas State University wheat breeding program (GPL). Phenotypic FHB traits used in the model building and cross-validation (CV) prediction accuracies (PA) were evaluated in three crop growing seasons (2023, 2024, and 2025) at the Rocky Ford Manhattan, KS research station under inoculated mist-irrigated FHB nurseries. The significant correlations among FHB traits ranged from 0.59 to 0.93 (P -value < 0.05). The CV prediction accuracies of the GS models in the DP ranged from 0.51 to 0.68 across the FHB traits, depending on SNP density. RR-BLUP and GBLUP GS models had the highest prediction accuracies for traits across the different SNP densities. The forward genomic prediction accuracy of FHB SEV in the USWBSI was consistent within the CV PA from the DP population in both 2023 and 2024, with prediction accuracies of 0.52 and 0.55, respectively. The PA for FHB SEV in the GPL population was inconsistent with the DP prediction accuracies. The PA in the forward prediction of DON for the USWBSI was inconsistent between 2023 and 2024, with PA values of -0.04 and 0.42 respectively, although the PA was not significant in 2023. The applications of GS and forward genomic prediction in this study show the potential of a wheat diversity panel and the uniform regional FHB nurseries as a resource for genomic selection in FHB resistance in breeding programs in the United States.

4. 2 INTRODUCTION

Global usage of wheat is expected to increase by 57 million metric tons, and production is expected to go up by 74 million metric tons to 874 million metric tons by 2034, relying on improved seed varieties (OECD/FAO, 2025), demanding better resistance to plant diseases and pests, which are a critical risk. Wheat production globally has increased by ~257 million metric tons, with nearly no significant change in total production area (220 million hectares) (FAOSTAT), which can be attributed to improved technologies, including low-cost genotyping and genomic selection (GS). GS is increasingly vital for selecting materials to advance breeding programs. Breeding efforts are enhanced by the practical applications of genomic tools. Genomic selection (GS) applications in practical breeding have been a powerful tool for advancing breeding materials using readily available single-nucleotide polymorphisms (SNPs) (Alemu et al., 2024). GS involves methods that combine genotypic data (marker information) at a whole-genome level to predict traits with sufficient accuracy to enable selection for advancement in a breeding pipeline (Lorenz et al., 2011). Selection can't rely entirely on methods like marker-assisted selection (MAS), which assume that the effect of known alleles on the phenotype is known and viable for major-gene traits (Lorenz et al., 2011; Bernardo and Charcosset, 2006). GS model building relies on high-throughput phenotypic data or reliable genotypic data and uses statistical models under various scenarios to select breeding population material for advancement based on estimated genetic breeding values (Alemu et al., 2024). In GS, the assumptions are that QTLs underlying a complex trait like FHB are in linkage disequilibrium with the casual variant/gene and can be explained by the markers used in GS with the marker effects estimated from a training population with available combined phenotype and genotype and subsequent prediction of phenotypes for a population with only genotype data using the marker effects from the training population (Arruda et al., 2015; Goddard and Hayes, 2007). The genetic breeding values estimated from phenotypic data in GS represent

the combined effects of all SNP/marker loci (Escamilla et al., 2025). GS selection comprises four main steps: (1) training population selection, (2) GS model building, (3) genomic prediction of testing population of lines, and (4) selection of material to advance based on GBEV. GBEV for traits associated with FHB disease resistance can be used to select and advance wheat lines with higher resistance or remove ones that are highly susceptible (Arruda et al., 2015).

High-quality, dense genotyping data that captures a large proportion of genetic variation (SNPs) for complex traits has improved genomic selection accuracy (Howard and Likpa, 2025). Targeted sequencing of markers, using technologies such as AgriSeq platforms, can further increase prediction accuracy in genomic selection. However, several factors limit genomic selection and cause inconsistent repeatability of predictions across models. SNP array platforms, genotyping protocols, data processing pipelines, and standardization methods affect the accuracy and reproducibility of genomic selection (Howard and Likpa, 2025).

GS is particularly promising for complex traits like Fusarium head blight (FHB), which is controlled by minor-effect alleles that contribute to resistance (Arruda et al., 2015). FHB is one of the single most important diseases in wheat breeding programs in the Midwestern United States. The use of GS leverages linkage disequilibrium between markers and quantitative trait loci (QTL) linked to a causal gene controlling the trait (Arruda et al., 2015; Goddard and Hayes, 2007), such as FHB resistance in wheat. The use of large marker data (SNPs) together with population phenotypic data in GS results in small p and large n , leading to limited degrees of freedom and low statistical power (Merrick and Carter, 2021).

The parametric models, namely ridge regression best linear unbiased prediction (RR-BLUP) and Bayesian models, are the most widely used methods for GS (Merrick and Carter, 2021). The parametric approach assumes a linear relationship between the phenotype and genotype,

accounting only for additive marker effects (Pérez-Rodríguez et al., 2012; Merrick and Carter, 2021). Various statistical and machine learning models are also used for genomic selection, including Genomic best linear unbiased prediction (G-BLUP), Bayesian models (Bayesian ridge regression (BRR), Bayesian Lasso, BayesA, BayesB, BayesC, RHKS (reproducing kernel Hilbert spaces)). All the models operate under certain assumptions. The RR-BLUP GS model assumes random effects for markers and sets a common variance based on an infinitesimal model, which shrinks marker effects to zero (Meuwissen et al., 2001). The G-BLUP method in genomic selection uses genomic relationships to estimate the genomic estimate values and merit of an individual for selection based on the genotype (Clark and van der Werf, 2013). The G-BLUP model assumes a single variance across all SNP loci, unlike Bayesian models, which allow variance differences between SNP loci (Clark and van der Werf, 2013). BRR uses priors for dense marker effects centered at zero, with common variance across all markers, and assumes marker effects are independent and identically distributed *a priori* (Pérez-Rodríguez et al., 2012). There is a common prior variance for marker effects in Bayesian LASSO, Bayes A, and Bayes B, which relaxes the assumption of a common prior variance (Pérez-Rodríguez et al., 2012). Bayesian LASSO with large p and small n selects at most n variables and sets the effects of the remaining markers to zero (Arruda et al., 2015).

This study aimed to evaluate the potential of genomic selection and forward genomic prediction to help yield genetic gains for FHB resistance in winter wheat, using prior conventional phenotyping of a diversity panel of winter wheat cultivars for model building and testing. The objectives of this study were to (1) build a genomic selection (GS) model using a diversity panel of wheat lines, (2) predict the genomic breeding estimated values (GBEV) for cross-validation and predictability of the GS model, (3) to utilize United States Wheat and Barley Scab Initiative

(USWBSI) uniform regional FHB nurseries as a resource for forward genomic prediction and genetic gain.

4.3 MATERIALS AND METHODS

4.3.1 Plant Materials

The dataset used in this study consisted of three populations making up the datasets: a diversity panel of 253 lines (Supplementary Table 4. 1) used in genomic selection model building, model training, and cross-validation (CV); an independent 416 Great Plains lines (GPL) from the KSU wheat breeding program (Supplementary Table 4. 2); and 283 lines (combination of 2023 and 2024 lines) (Supplementary Table 4. 3) from the USWBSI uniform regional nursery (USWBSI) for forward genomic prediction to cross-validate the GS model built with the diversity panel. These wheat lines in the diversity panel were sourced from public breeders across the Central Plains, Midwest, Northeast, and Southern United States wheat-growing regions. Most of the lines in the diversity panel were used in building a customized wheat practical haplotype graph (PHG) for *Fusarium* head blight resistance prediction and genome-wide association studies of barley yellow dwarf and soil-borne wheat mosaic virus resistance in foliar viruses (Tidakbi et al., 2025).

4.3.2 Marker Data

The 253 DP wheat population were genotyped with exome capture libraries at ~15x coverage, the 416 GPL population were genotyped using skim exome capture protocol (Wang et al., 2023), and the subset of 283 USWBSI uniform regional nursery population were genotyped based on the target sequencing of markers using the AgriSeq platform. Approximately 50 mg of leaf tissue was collected from each line, and DNA was prepared as previously described (Tidakbi *et al.* 2025). The DNA of USWBSI populations was extracted using the CTAB protocol and quantified using Picogreen DNA (ThermoFisher). For each sample, 32 μ L (11 ng/ μ L) was sent to North American Genomics for AgriSeq target sequencing. The marker data for the diversity panel was generated

from genome analysis toolkit (GATK) variant calling. The GATK variant calling was done using raw, unmapped exome capture sequencing reads. The analysis pipeline followed the Broad Institute's GATK Best Practices workflow, resulting in the generation of high-quality Variant Call Format (VCF) files for downstream analyses. The low-pass exome capture data was imputed using the customized wheat practical haplotype graph (PHG) database as described by Jordan et al. (2022). AgriSeq used the "USDA-Wheat-AgriSeqV2" panel, with 4K markers. AgriSeq was received in VCF files format, one per sample. These VCF files were merged using bcftools version 1.22 to generate single VCF files for filtering. For the USWBSI uniform regional lines, on which the GS model was also tested by predicting and comparing phenotypic scores and GEBV, the genotype data generated from AgriSeq, comprising 3,002 SNPs. The VCF file was later imputed in Tassel using the mean score. Of the 3,002 SNPs, 776 SNPs were commonly shared between the USWBSI and the DP populations. The 776 SNPs in this population were used to predict GEBV for the USWBSI lines. The Pearson correlation test was used to assess the prediction accuracies within the DP population and the forward genomic prediction of the USWBSI population.

4.3.3 Inoculum Preparation

Corn spawn inoculum was prepared in the lab using isolate GZ3639, the native and aggressive isolate of *Fusarium graminearum* in Kansas. *Fusarium graminearum* isolate GZ3639 (Bowden and Leslie, 1992; Desjardins et al., 1996) was grown on a homemade potato dextrose agar (hmPDA). hmPDA supports rapid growth, producing the greatest amount of fungal mycelium within ~4 days. Plugs of hmPDA with fungal mycelium growing on it were inoculated into mung bean broth (prepared with 84 g of mung bean per 2.1 boiling distilled water to cool and sieved with cheesecloth). The plugs of mycelium in the mung bean broth were allowed to sporulate and producing conidia after ~7 days, it was used to inoculate autoclaved corn kernels to produce the

corn spawn. The corn spawn inoculum mimics the field debris and natural inoculum on the field as described by Rudd *et al.* (2001) as a source of primary inoculum under natural conditions. Colonized corn spawn was then spread in the field at a rate of 53 g/m² starting 5 weeks before anthesis and applied 3 times (at 2 weeks intervals). The FHB disease nursery was mist irrigated 15 minutes twice per night starting from the first spread of the corn spawn on the field FHB nursery.

4.3.4 Field Nursery Phenotyping of Populations

FHB disease severity of the diversity panel of wheat lines was evaluated in field trials conducted at KSU Manhattan Rocky Ford Station in three years (2022, 2023, and 2024). Each line was replicated twice each year. Plots measured 2.3 meters in length and 0.51 meters. Heading dates, FHB disease severity 21 days post-heading date (SEV), cumulative disease severity area under disease progress curve (AUDPC), Fusarium-damage kernels (FDK), and deoxynivalenol (DON). Heading dates for each line were recorded as the day when 50% of tillers within each plot head emerged. Disease severity (estimated as the percentage of wheat heads/spikelets infected) was visually scored every other day until wheat heads reached ripening/maturity. The severity at 21 days post-heading (SEV) and the cumulative disease severity (AUDPC) were determined and used in the downstream analysis. Fusarium-damaged kernels (FDK) and deoxynivalenol (DON) concentrations in the harvested seeds were visually estimated and measured by in the lab in North Dakota State University using gas chromatography/mass spectrometry (GC/MS), respectively. For forward genomic prediction accuracy of the GPL and USWBSI populations as prediction sets, the actual phenotypic FHB disease severity trait data were scored to compare with the predicted GBEV generated using the DP trained GS models. The GPL data was unbalanced, meaning that most lines were not replicated across the years (2021, 2022, 2023) of the field evaluation for FHB. For the

USWBSI uniform regional nursery lines, we used severity scores from two years, namely the 2023 and 2024 cropping seasons, for the forward genomic prediction validation.

4.3.5 Best Linear Unbiased Estimates (BLUE) Model

BLUE values for the training and testing sets were calculated using the lme4 package (Bates et al., 2015) in R for each genotype, accounting for variation due to years, replications, and heading dates. Genotype-by-environment (year) interaction (G×E) was included in the BLUE model. For the diversity panel used in building the GS model, the years of the trials were considered as an environment. The equation of the BLUE model is denoted by,

$$Y_{ijkl} = \mu + g_i + HD_j + y_k + (G \times y)_{ik} + r_l(y_k) + \varepsilon_{ijkl} \dots \dots \dots \text{equation (1)},$$

where Y represents the FHB traits AUDPC, SEV, FDK, and DON, μ is the overall mean, g_i is the fixed effect of the i th genotype, HD_j is the fixed effect of the j th heading date, y_k is random effect of k th year, $G \times y$ is the random interaction of i th genotype and k th year (G×E), r is the random effect of l th replication nested within year, and ε is the residual error.

4.3.6 Genomic Selection Models

The additive relationship and the genomic relationship matrix (GRM) were estimated using the A.mat function in RR-BLUP. Parametric and nonparametric models were used in the genomic prediction model, including ridge regression best linear unbiased prediction (RR-BLUP), Bayesian ridge regression (BRR), Bayes A, Bayes B, Bayes C, Bayesian Lasso, and RHKS. The genomic prediction models were evaluated for four FHB disease severity metrics: SEV, AUDPC, FDK, and DON levels. The RR-BLUP GS selection was based on mixed.solve using RR-BLUP package in R software (R version R 4.5.1). The equation is denoted by,

$$y = X\beta + Zu + \varepsilon \dots \dots \dots \text{equation (2)},$$

where β is a vector of fixed effects (mean FHB trait) and u is a vector of random effects (marker effects). The Bayesian models were run using BGLR package in R software (R version R 4.5.1). The breeding values were determined from genomic estimated breeding values (GEBVs) calculated from marker effects in the models using the FHB trait BLUES. GEBV is denoted;

$$g(x_i) = \sum_{k=1}^p x_{ik} \beta_k \dots \dots \dots \text{equation (3)},$$

Where $g(x_i)$ is the genomic estimated breeding values, p is the total number of markers, x_{ik} is the genotype of individual i at a marker k , and β_k is the effect of marker k . The GEBV was in simple terms extracted from the GS model with model\$g, where \$g is the best linear unbiased predictor. (BLUP) solution for the genetic values.

4.3.7 Heritability

Heritability was estimated for each trait using the RR-BLUP/G-BLUP package (Endelman, 2011), G-BLUP (Clark and Van der Werf, 2012), and Bayesian models (Bayesian ridge regression, Bayesian in R). The heritability of the traits was derived from the GS models using the package functions. The heritability and genetic variance or error variance was estimated from the GS model from,

$$\text{Heritability} = \frac{\text{model\$Vu}}{\text{model\$Vu} + \text{model\$Ve}} \dots \dots \dots \text{equation (4)}$$

Where \$Vu is the estimate of the genetic variance, and \$Ve is the estimate of the error variance.

4.3.8 Training, Testing and Prediction Size

The diversity panel comprising of 253 lines was used as the training and testing set in a five-fold scheme (i.e. 80% training set and 20% testing set). The DP was then used as a trained model for forward genomic prediction of GEBV of GPL and the USWBSI population. In the forward

genomic prediction, DP was used as the training set to predict GBEVs for the two independent populations.

4.3.9 Genomic Selection Cross-Validation Scheme

In the GS model-building using the diversity panel, the data set was randomly partitioned into five sets: four for training set (80%) and one for testing set (20%). GBEV was calculated for the testing set population (TP) for cross-validation. The cross-validation (CV) prediction accuracies (PA) of each model within the diversity panel were calculated as the Pearson correlation between observed phenotypic values (BLUEs) and predicted GEBVs, as denoted in equation (3).

4.3.10 Forward Prediction of Independent Set of Lines

Forward genomic predictions were cross-validated on lines outside the diversity panel, referred to as the predicting independent populations (GPL and USWBSI). Forward genomic prediction refers to predicting the breeding values of the unphenotyped independent lines outside the diversity panel (training population) using SNP marker data. The entire diversity panel (n = 253) was used as the training population. Marker effects were estimated using only the shared common SNP markers between the training and forward prediction populations (GPL and USWBSI) to ensure compatibility across genotyping platforms. These estimated marker effects were then used to compute Genomic Estimated Breeding Values (GEBVs) for the GPL and USWBSI lines. The prediction accuracies were determined using Pearson's correlation of the GBEVs and the real field phenotypic scores of FHB disease severity trait divided by the square root of the heritability of each trait, as described by (Aruda et al., 2015). This is denoted by the equation,

$$\frac{r(\text{GBEV:PV})}{\sqrt{h^2}} \dots\dots\dots \text{equation (5)}$$

where PV is the phenotypic values of scored FHB traits (i.e. AUDPC, SEV, FDK, DON) and h^2 is the heritability. We report the comparison mainly between the GBEV and PV of the FHB SEV and DON.

4.4 RESULTS

The genotyping data used for the genomic selection and forward genomic predictions comprised three data sets, namely 613317 SNPs based on the whole DP population, 34K SNPs based on shared common markers shared between the DP and GPL populations from skim exome capture, and 776 SNPs based on shared common markers between the DP and USWBSI populations from exome capture, and AgriSeq. AgriSeq is targeted genotyping-by-sequencing (GBS) of markers. GATK variant calling analysis generated 613K SNPs for the diversity panel (DP) used in the GS model building, with training (80%) and testing (20%) sets comprising 202 and 51 lines, respectively. The genotypes of the GPL population were generated from skim exome capture and imputed from the wheat-customized practical haplotype graph (PHG) database as described by Jordan et al. (2022) and subsequently filtered for linkage disequilibrium (LD) of 0.2 and minor allele frequency (MAF) of 0.3 using PLINK, yielding 162,643 SNPs. Of the 162,643 SNPs, 34K SNPs were commonly shared with the DP genotyping marker data. GS/GP models were built, and predictions were done on the original DP 613K SNPs and the 34K SNPs shared with GPL.

4.4.1 Genetic structure of the diversity panel

There was a genetic structure in the diversity panel, as shown in the heatmap (Figure 4. 1). The genetic structure revealed two main populations in the diversity panel, which was expected given that the panel comprised HWW and SWW populations. The principal component analysis (PCA) plot of the similar population revealed two primary clusters, namely HWW and SWW, as reported by Tidakbi et al. (2025). There was noticeable overlap between the PCA clustering of the diversity

panel population and the USWBSI and GPL populations (Figure 4. 2, left & right panels). The first and second PCAs accounted for only 7.67% and 6.39% of the total genetic variation in the diversity panel and the USWBSI and GPL populations, respectively.

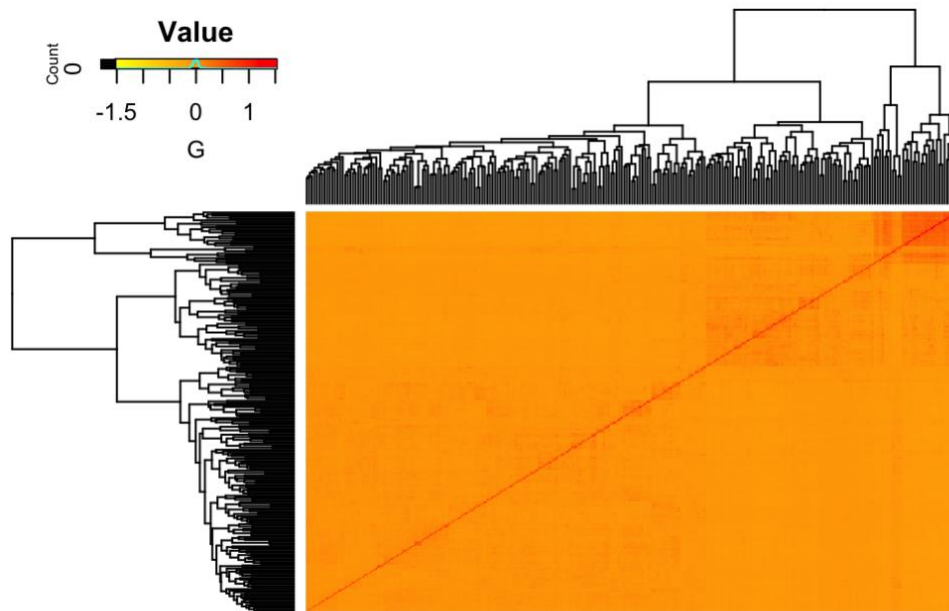


Figure 4. 1 Heat map of the marker-based additive relationship matrix for 253 wheat lines. Matrix obtained from 613k SNPs using A.mat function in RR-BLUP package in R.

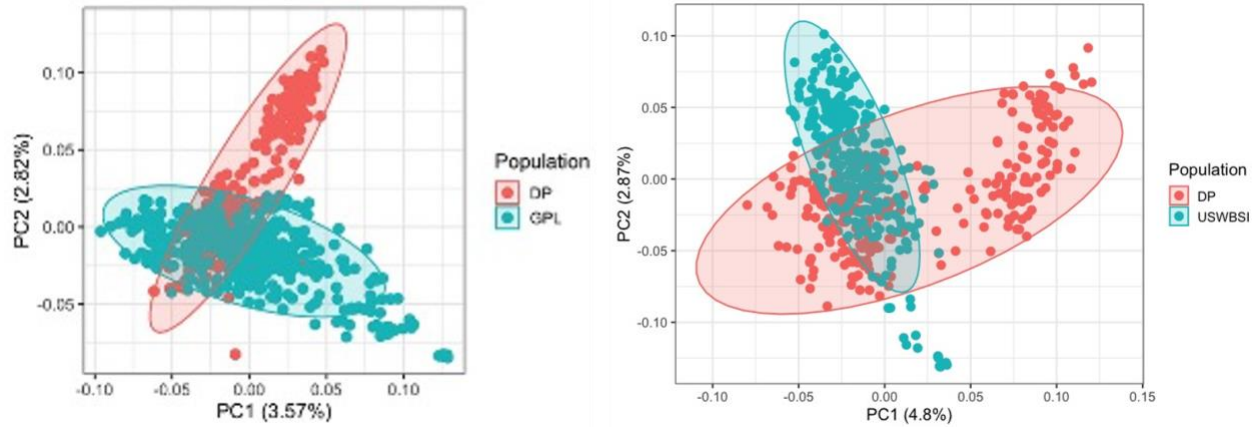


Figure 4. 2 Principal component analysis of the training population (DP) and the forward genomic predicted populations (GPL (left) USWBSI (right)). USWBSI: Population from the USWBSI uniform regional nursery; GPL: Great Plain lines. Left panel: DP vs GPL, Right panel: DP vs USWBSI

4.4.2 Phenotypes

The FHB traits, namely AUDPC, SEV, FDK, and DON, were evaluated from field FHB nursery trials in three crop growing seasons (2022, 2023, 2024) at the Rocky Ford Manhattan, KS, field research station. The field data indicated variations including selected accessions the moderately resistant check (Everest PI 659807), the susceptible check (Overley PI 634974), the moderately susceptible check (Karl92 PI 564245), and late heading (Emerson PI and Flourish PI) which showed varied disease severity across years, an indication of annual environmental variation and genotype-by-environment interaction (Supplementary Figure 4. 1). Significant ($p\text{-value} < 0.05$) Pearson correlations of the trait BLUEs were moderate to high, ranging between 0.59 and 0.93 (Figure 4. 3).

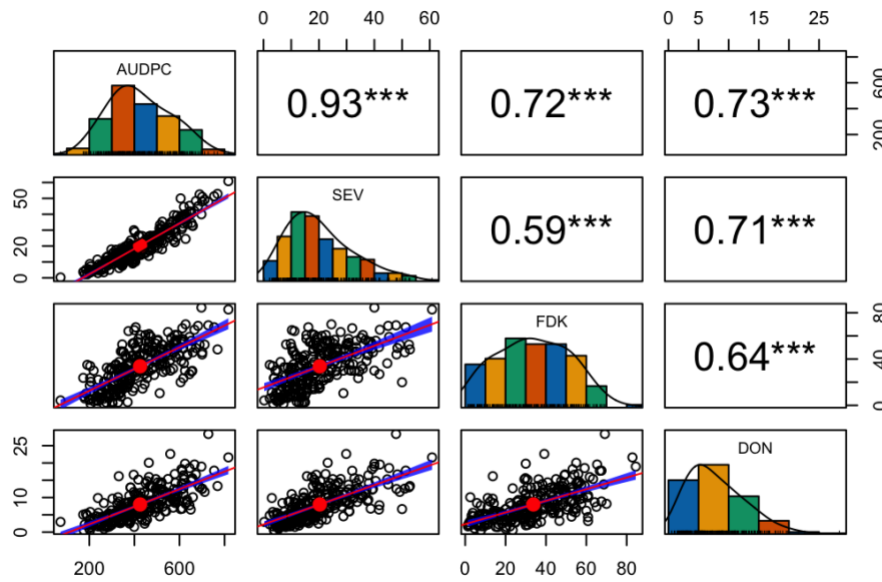


Figure 4. 3 FHB-related traits correlations. AUDPC: Area Under Disease Progress Curve, SEV: FHB disease severity 21 days post-heading date, FDK: Fusarium-damaged kernels, DON: Deoxynivalenol. ***p-value <0.05, n = 253.

4.4.3 Genomic Selection/Prediction Accuracy

We plotted the marker effects to demonstrate that FHB traits are largely influenced by small-effect loci with most loci effects centered around or close to zero, as shown in Figure 4. 4. The marker effects of the SNPs shared between the DP and the USWBSI AgriSeq data were larger for the FHB traits, as shown in Figure 4. 4 (left panel). The DON FHB-resistant traits from the marker effect plots indicate that most SNPs contribute small effects in both panels.

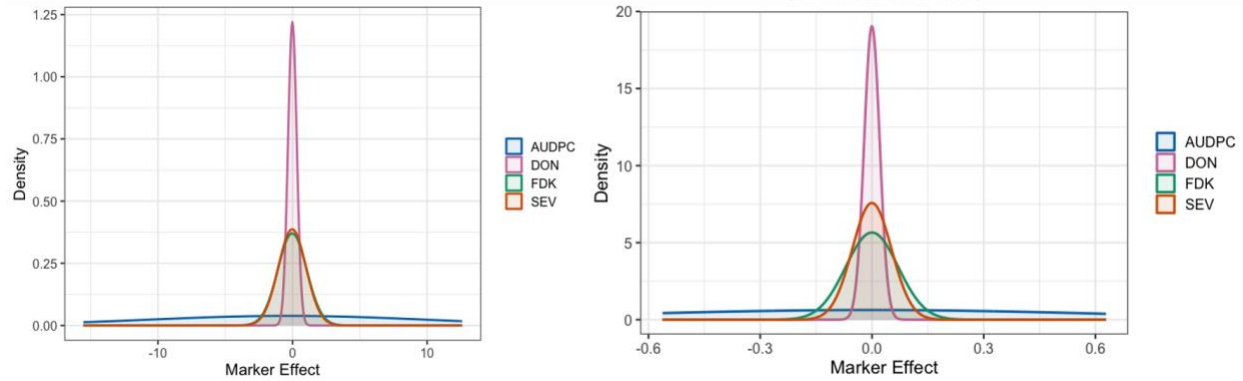


Figure 4. 4 Distribution of RR-BLUP marker effects for FHB traits estimated from two genotyping platforms using shared marker sets between the diversity panel (DP) and the independent Great Plains Lines (GPL) and USWBSI populations. Density plots of marker effects for AUDPC, DON, FDK, and SEV estimated using RR-BLUP. The left panel represents the AgriSeq targeted sequencing marker set, while the right panel represents the genome-wide SNP marker set. Both analyses were performed using markers shared between the DP and the independent populations (GPL and USWBSI) to ensure comparable genomic prediction.

The GS prediction accuracies for FHB traits (SEV, AUDPC, DON, FDK) were calculated as the Pearson correlation between observed phenotypic values (BLUEs) and predicted GBEVs (Figure 4. 5). In the GS model and cross-validation scheme, an 80% training set (202 wheat lines) and a 20 % testing set (51 wheat lines) were used. The prediction accuracies (PAs) shown in Table 1 varied across traits, marker densities, and GS models. The GS models and SNP densities varied across the FHB traits. Notably, the observed higher significance of PA at higher SNP density (p-value <0.05). Two traits, SEV and AUDPC, which were highly correlated (Figure 3), showed that higher SNP density consistently resulted in higher prediction accuracies, particularly for RR-BLUP and G-BLUP GS models (Table 1). Reductions in marker density to 34k and 776 markers led to a slight but varied significant reduction in the PAs among FHB traits and GS models.

Among the GS models, RR-BLUP and G-BLUP performed comparably, with no significant differences in PA across traits or models at the same marker density (Table 4. 1). The PA for FDK and DON was lowest across SNP densities and GS models, decreasing significantly with a reduction in marker density from 613k to 776 markers.

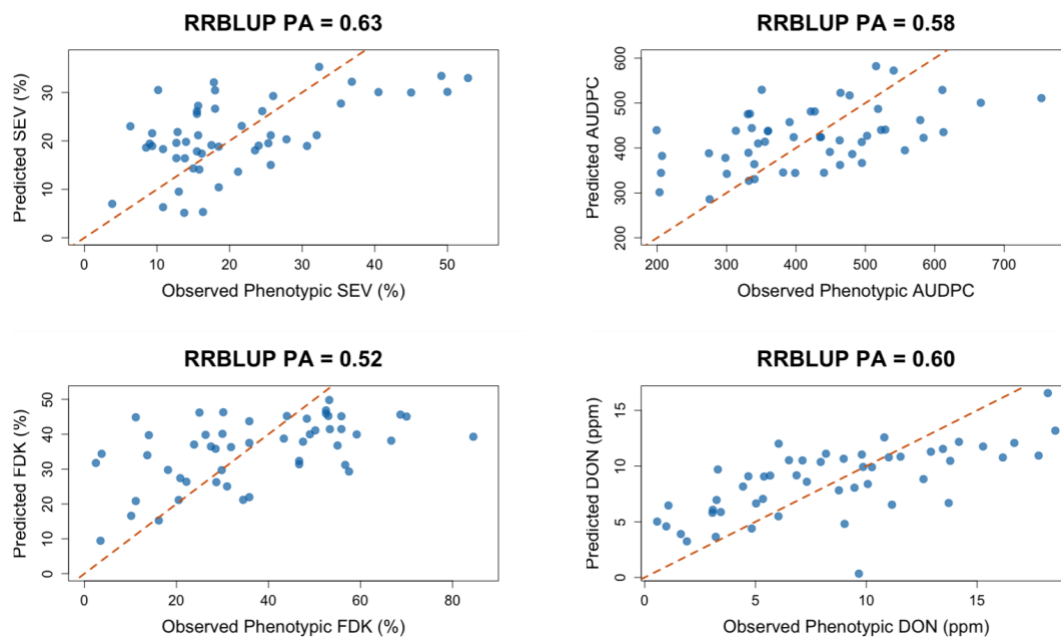


Figure 4. 5 Illustrated correlations between the predicted FHB traits and the observed the phenotypic scores. (PA is the correlation coefficient of the predicted trait vs the observed phenotypic score of the DP population). TP = 201; TS = 52. TP: training population, TS: Testing set.

Interestingly, RR-BLUP and G-BLUP models performed better with significantly higher PAs than the Bayesian and RHKS models across FHB traits at higher SNP density, but the significant differences diminished at lower marker densities, with results showing no significant differences in PA at lower marker densities at 34k and 776 marker densities, as shown in Table 4. 1.

Table 4. 1 Cross-validated prediction accuracies ($\pm$ standard deviation from the mean) for FHB-related traits according to GS models and SNPs for the diversity panel.

Trait	SNPs	RR-BLUP	G-BLUP	BRR	LASSO	BayesA	BayesB	BayesC	RKHS
SEV	613k	0.662 $\pm$ 0.073 ^{abAB*}	0.684 $\pm$ 0.015 ^{aA}	0.671 $\pm$ 0.072 ^{aAB}	0.639 $\pm$ 0.091 ^{aABC}	0.646 $\pm$ 0.089 ^{aABC}	0.650 $\pm$ 0.067 ^{abABC}	0.594 $\pm$ 0.089 ^{aC}	0.615 $\pm$ 0.105 ^{aBC}
	34k	0.658 $\pm$ 0.083 ^{aA}	0.653 $\pm$ 0.016 ^{bA}	0.654 $\pm$ 0.087 ^{aA}	0.658 $\pm$ 0.076 ^{aA}	0.679 $\pm$ 0.082 ^{aA}	0.678 $\pm$ 0.077 ^{aA}	0.663 $\pm$ 0.078 ^{bA}	0.643 $\pm$ 0.018 ^{aA}
	<1k	0.626 $\pm$ 0.088 ^{bAB}	0.631 $\pm$ 0.021 ^{cAB}	0.628 $\pm$ 0.073 ^{aAB}	0.605 $\pm$ 0.074 ^{aB}	0.637 $\pm$ 0.088 ^{aAB}	0.609 $\pm$ 0.074 ^{bB}	0.670 $\pm$ 0.060 ^{bA}	0.622 $\pm$ 0.082 ^{aAB}
AUDPC	613k	0.657 $\pm$ 0.068 ^{aA}	0.671 $\pm$ 0.015 ^{aA}	0.642 $\pm$ 0.081 ^{aAB}	0.613 $\pm$ 0.084 ^{aB}	0.638 $\pm$ 0.071 ^{aAB}	0.642 $\pm$ 0.078 ^{aAB}	0.591 $\pm$ 0.086 ^{aB}	0.649 $\pm$ 0.060 ^{aB}
	34k	0.624 $\pm$ 0.076 ^{bA}	0.629 $\pm$ 0.020 ^{bA}	0.625 $\pm$ 0.076 ^{aA}	0.609 $\pm$ 0.059 ^{aA}	0.638 $\pm$ 0.054 ^{aA}	0.646 $\pm$ 0.076 ^{aA}	0.638 $\pm$ 0.065 ^{aA}	0.611 $\pm$ 0.069 ^{aA}
	<1k	0.571 $\pm$ 0.079 ^{aA}	0.576 $\pm$ 0.021 ^{aA}	0.570 $\pm$ 0.067 ^{bA}	0.582 $\pm$ 0.071 ^{aA}	0.588 $\pm$ 0.089 ^{bA}	0.565 $\pm$ 0.070 ^{bA}	0.612 $\pm$ 0.082 ^{aA}	0.613 $\pm$ 0.088 ^{aA}
FDK	613k	0.612 $\pm$ 0.067 ^{aA}	0.606 $\pm$ 0.017 ^{aA}	0.551 $\pm$ 0.089 ^{aBC}	0.552 $\pm$ 0.103 ^{aBC}	0.576 $\pm$ 0.069 ^{aAB}	0.569 $\pm$ 0.09 ^{aABC}	0.510 $\pm$ 0.090 ^{aC}	0.531 $\pm$ 0.089 ^{aBC}
	34k	0.554 $\pm$ 0.081 ^{bA}	0.549 $\pm$ 0.022 ^{bA}	0.554 $\pm$ 0.076 ^{aA}	0.566 $\pm$ 0.070 ^{aA}	0.576 $\pm$ 0.076 ^{aA}	0.521 $\pm$ 0.077 ^{aA}	0.546 $\pm$ 0.080 ^{aA}	0.554 $\pm$ 0.600 ^{aA}
	<1k	0.515 $\pm$ 0.09 ^{aA}	0.514 $\pm$ 0.022 ^{aA}	0.519 $\pm$ 0.069 ^{aA}	0.531 $\pm$ 0.081 ^{aA}	0.534 $\pm$ 0.105 ^{aA}	0.527 $\pm$ 0.075 ^{aA}	0.533 $\pm$ 0.079 ^{aA}	0.559 $\pm$ 0.085 ^{aA}
DON	613k	0.642 $\pm$ 0.090 ^{aA}	0.631 $\pm$ 0.021 ^{aA}	0.574 $\pm$ 0.089 ^{aB}	0.579 $\pm$ 0.065 ^{aB}	0.596 $\pm$ 0.070 ^{aAB}	0.602 $\pm$ 0.058 ^{aAB}	0.578 $\pm$ 0.093 ^{aB}	0.596 $\pm$ 0.090 ^{aAB}
	34k	0.600 $\pm$ 0.081 ^{bA}	0.600 $\pm$ 0.021 ^{bA}	0.597 $\pm$ 0.078 ^{aA}	0.618 $\pm$ 0.066 ^{aA}	0.610 $\pm$ 0.085 ^{aA}	0.590 $\pm$ 0.055 ^{aA}	0.602 $\pm$ 0.095 ^{aA}	0.584 $\pm$ 0.013 ^{aA}
	<1k	0.597 $\pm$ 0.078 ^{bA}	0.591 $\pm$ 0.022 ^{aA}	0.575 $\pm$ 0.086 ^{aA}	0.601 $\pm$ 0.078 ^{aA}	0.592 $\pm$ 0.087 ^{aA}	0.576 $\pm$ 0.076 ^{aA}	0.593 $\pm$ 0.065 ^{aA}	0.604 $\pm$ 0.072 ^{aA}

SEV: FHB severity 21 post heading date; **AUDPC:** Area Under Disease Progress Curve; **FDK:** Fusarium Damage-
Kernel; **DON:** Deoxynivalenol. [#]Within rows, means followed by the same capital letter are not significantly different using the Tukey test to compare group means (p -value > 0.05). ^{*}Within columns, means followed by the same lowercase letter are not significantly different using the Tukey test to compare group means (p -value > 0.05).

4.4.4 Forward genomic prediction accuracy

Much of the genomic selection research has focused on cross-validation (CV) prediction accuracy in validating GS models. In this study, a step further was done to evaluate the trained GS model from the DP population using forward prediction. Forward genomic prediction of FHB severity (SEV) and DON accumulation of GPL and USWBSI was done using the diversity panel as the

training population. The predictions were estimated for the 2023 and 2024 USWBSI uniform regional nursery lines with FHB severity (SEV) and DON. For the GPL, only FHB SEV was used in the estimation of Pearson's correlation coefficients, as shown in Table 4. 2. The forward prediction accuracy was significant for SEV in 2023, SEV in 2024, and DON 2024 for breeding values estimated using both RRBLUP and GBLUP (Table 4. 2). PA for SEV was significant in both 2023 and 2024 using GBLUP and RRBLUP models. Significant PA was observed for DON in 2024, whereas DON prediction accuracy in 2023 was low and non-significant across the two GBEV estimation models.

Table 4. 2 Forward genomic prediction accuracies for two populations.

Population	Year	FHB Trait	Heritability	GS Model	PA
USWBSI	2023	SEV	0.82	GBLUP	0.52***
	2023	DON	0.76	GBLUP	-0.04
	2024	SEV	0.82	GBLUP	0.44***
	2024	DON	0.76	GBLUP	0.42***
	2023	SEV	0.82	RRBLUP	0.55***
	2023	DON	0.76	RRBLUP	-0.08
	2024	SEV	0.82	RRBLUP	0.42***
	2024	DON	0.76	RRBLUP	0.43***
GPL	2021	SEV	0.95	GBLUP	0.09
	2021	SEV	0.95	RRBLUP	0.07

USWBSI: USWBSI lines (2023 and 2024 lines); GPL: Great Plain lines; SEV: FHB severity 21 post heading date; DON: Deoxynivalenol; PA: Prediction Accuracy. *** significantly different Pearson's correlation coefficient (p -value < 0.05).

4.5 DISCUSSION

The ultimate goal of adapting GS/GP is to increase the prospects of genetic gain in breeding for complex traits through improved selection accuracy. GS, unlike QTL mapping or genome-wide association studies, does not map or identify marker loci that are statistically significant for traits. GS, by its approach, helps identify, within a pool of genetic material, the best candidates for

improved traits in breeding programs using dense genome-wide marker panel (Bernado, 2026). A step further in the study was done using forward genomic prediction to evaluate the GS model.

4.5.1 Prediction accuracy of the diversity panel

In this study, using a diversity panel comprising wheat lines from different breeding programs and geographical wheat growing regions in the US, we investigated the genomic selection and prediction capabilities of the diversity panel and used the USWBSI regional uniform nursery as a resource for cross-validating GS models for FHB-related traits using different SNP/marker densities and different genomic selection models. In the GS models and the cross-validation scheme, we noted that a marker density below 1k resulted in a consistent significant reduction in the PA across traits, and that GS models showed varied levels among the FHB traits with average PA of 0.5. In this study, a higher marker density was associated with increased prediction accuracy in the DP population. Analytical results from initial studies of GS in the early 2000s and beyond indicated higher prediction accuracies with increased marker density (Bernando, 2016). Our study reports PAs values ranging from ~0.5 to ~0.7 across different marker densities, using varied GS statistical models. These observations of moderate to high PA in the DP were similar to those reported in studies on GS and FHB resistance in the past (Aruda et al., 2015; Rutkoski et al., 2012). These results were consistent with findings from previous studies using diverse germplasm, including lines from 17 different wheat breeding programs in the Midwestern and Eastern United States, with cross-validation prediction accuracies ranging from 0.35 to 0.82 across different FHB traits and SNP densities and GS models (Aruda et al., 2015). In another study involving GS in FHB traits by Rutkoski et al. (2012), a set of 322 wheat lines from 15 public and three private breeding programs across the Eastern United States and Canada, using different SNP data generated from diverse genotyping platforms, observed mean low, moderate to high CV prediction

accuracies (between -0.12 to 0.64) using linear and nonlinear GS models. The higher prediction accuracies observed, even at marker densities below 1k, in this study could be attributed to the fact that this set of SNPs is the matching SNP set derived from target sequencing using FHB resistance QTLs. This outcome is supported by Rutkoski et al. (2012), noting that prediction accuracies based on targeted markers were higher.

4. 5.2 Forward prediction of the independent population

Overall, RRBLUP and GBLUP used in the forward GS produced comparable prediction accuracies across traits and years. However, forward prediction accuracy for the 2021 GPL was low and non-significant for both models despite the high trait heritability (Table 4. 2). Consistent with the cross-validation analysis of the DP, the correlation between the calculated GEBVs and phenotypic FHB SEV was moderate for 2023 and 2023 USWBSI populations. In contrast, prediction accuracies for DON in the USWBSI population and for FHB severity in the GPL population were inconsistent with the cross-validation accuracies obtained within the DP. The relatively stable and consistent correlation observed in the USWBSI population could be attributed to the fact that lines making up this populations are wheat lines that contributed to developing the diversity panel (DP). The low forward prediction accuracy for the GPL population could be attributed to the fact that the best-performing lines in the DP population for FHB resistance were mostly traditional SSW germplasm, and most of these lines also have spring wheat background from intermating a bunch of FHB-resistant sources, largely from China. Only a few HWW lines in the DP have the *Fhb1* allele and the Jamestown haplotype for FHB resistance. The GPL HWW population from the KSU wheat breeding program had no contributions from these germplasms, which explains the low PA for forward genomic prediction. These findings emphasize the importance of genetic relatedness between the training and validation populations for effective

genomic prediction. The forward genomic prediction accuracies for the SEV and DON traits ranged between -0.08 and 0.55. This range of forward prediction accuracies is within that reported in previous studies for FHB genomic prediction as a complex trait and for yield genomic selection in wheat (Larkin et al., 2021; Calvert et al., 2020)). Forward predictions of populations outside the training population (TP) have been challenging due to inconsistent PAs for complex traits like FHB resistance and yield. A reported study by the Kansas State University wheat breeding program, observed CV PA with the TP between 0.31 and 0.47, however in forward genomic prediction, the correlation (PA) between GEBV and the phenotypic yield component declined to -0.16 for different years as a prediction population (Calvert et al., 2020) and this is consistent with the insignificant low correlation we observed in the GPL population. In a separate study, Larkin et al. (2021) reported forward prediction correlations ranging from -0.01 to 0.83 between GEBVs and FHB-related traits in advanced and elite wheat breeding lines from the University of Arkansas breeding program. The low to moderate forward prediction accuracies observed in the present study are therefore consistent with previous reports for complex quantitative traits.

The cross-validation prediction accuracies (Pearson's correlation coefficient between the GBEV and the PBEV) for the GPL based on the FHB SEV were low, and this was most likely attributed to and reflect the pedigrees used (mostly HWW) of this population not having the major QTLs for FHB resistance as picked up in our GWAS study in the SWW lines using the same diversity panel, hereby reducing the effectiveness of forward genomic prediction across populations.

4. 6 CONCLUSION

The use of a diversity panel to build the GS model and of USWBSI uniform regional nurseries as a resource in breeding programs will be valuable tools for improving genetic gain for FHB resistance in wheat. In the context of natural changes in weather patterns or resource-limited

conditions, GS models are a practically valuable approach in breeding programs to identify the best candidates across multiple environments.

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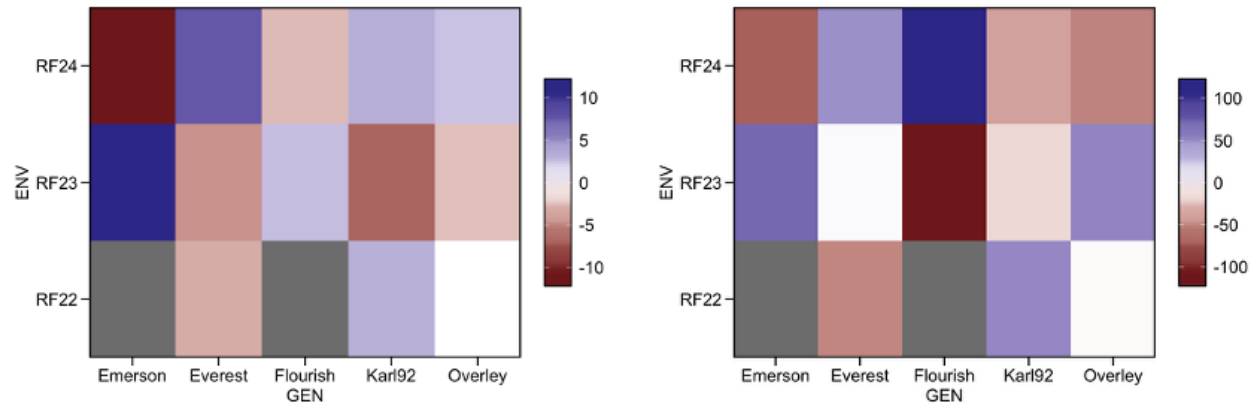
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4.8 SUPPLEMENTARY FIGURES



Supplementary Figure 4. 1 Genotype-by-environment interaction effects on the resistant/susceptible checks used in the 3 years trials of the diversity panel used in the GS model. ENV: environment (year); GEN: genotypes (entries/cultivars). The heatmap shows the effects of each environment on the genotypes.

4.9 SUPPLEMENTARY TABLES

Supplementary Table 4. 1 Type of wheat, state, PI numbers and FHB severity scores used in the GS model training

Taxa	Type	State/Source	PI Number	Fhb1	SEV	AUDPC	FDK	DON
0762A1-2-8	SWW	MI		Fhb1+	10.3333333	297	32.6666667	2.82
17Nord-96	HWW	ND		Fhb1-	44.6666667	619.583333	36.3333333	15.265
19Nord-124	HWW	ND		Fhb1-	18.5	390.833333	27.5	7.94166667
19Nord-127	HWW	ND		Fhb1-	20.5	383.333333	42.5	12.905
2019SEL-107	SWW	KY		Fhb1-	2.25	179.75	6	2.225
20NORD-138	HWW	ND		Fhb1-	30.8333333	540.75	30.5	6.045
20NORD-148	HWW	ND		Fhb1+	21	433.166667	29.75	3.54
21NORD-152	HWW	ND		Fhb1-	32.6666667	659.25	45.8333333	10.0983333
21NORD-153	HWW	ND		Fhb1-	28.8333333	401.916667	30	10.9883333
21NORD-154	HWW	ND		Fhb1+	35.3333333	524	34	10.9516667
21NORD-155	HWW	ND		Fhb1-	52.5	689.166667	52.5	12.955
21NORD-157	HWW	ND		Fhb1-	51.3333333	753.666667	67.5	17.7783333
21NORD-162	HWW	ND		Fhb1-	38.6666667	612.5	55	6.79166667
21NORD-163	HWW	ND		Fhb1-	33.5	581.916667	55.8333333	14.2416667
21NORD-164	HWW	ND		Fhb1+	26.8	515.4	49	14.168
21NORD-167	HWW	ND		Fhb1-	17.6666667	421.333333	30.8333333	8.98333333
AGS2000	SWW	GA	PI 612956	Fhb1-	29.8333333	559	39.1666667	22.6083333
AGS2020	SWW	GA	PI 658050	Fhb1-	9.5	331.5	25.5	8.84166667
AGS2033	SWW	GA		Fhb1-	11.8333333	392.833333	43.3333333	8.225
AMPSY003	HWW	TX		Fhb1-	14.5	427.083333	37.5	11.3816667

AMPSY077	HWW	TX		Fhb1-	11.3333333	359.833333	43.3333333	6.51
AMPSY515	HWW	TX		Fhb1-	23	502.416667	55.8333333	11.015
AMPSY588	HWW	TX		Fhb1-	13.1666667	401	40.5	8.15166667
AP05T2413	HWW	KS		Fhb1-	22.4	579.1	56	16.816
Arapahoe	HWW	NE	PI 518591	Fhb1-	18	411.9	49	10.708
ARGA051160-14LE31	SWW	NC		Fhb1-	22.5	579.083333	60.8333333	10.0616667
ARGA06473-9-4-4	SWW	NC		Fhb1-	23.8333333	572.083333	45.8333333	7.08333333
Art	HWW	KS	PI 648468	Fhb1-	10.8333333	274.583333	16.1666667	4.51166667
Atlas66	HWW	KS?		Fhb1-	41.6666667	649.666667	50.3333333	6.52666667
Bess	SWW	MO	PI 642794	Fhb1-	15	369.833333	2.83333333	3.49166667
Blueboy	SWW	NC	CItr 14031	Fhb1-	26.5	561.166667	50	11.13
Branson	SWW	OH	PI 639227	Fhb1-	4.66666667	251.5	11.1666667	2.16666667
Caldwell	SWW	IN	CItr 17897	Fhb1-	10.8333333	378.333333	35.5	2.48833333
Caledonia	SWW	NY	PI 610188	Fhb1-	34.5	653.083333	29.3333333	15.27
Camelot	HWW	NE	PI 653832	Fhb1-	19.5	443.916667	49.1666667	11.545
Catawba	SWW	NC		Fhb1-	4.33333333	219.083333	30	1.96666667
Century	HWW	OK	PI 502912	Fhb1-	25.8333333	517.416667	38.1666667	13.8816667
Chesapeake	SWW	MD	PI 643935	Fhb1-	15.5	448.833333	60.8333333	3.205
CI13227	HWW			Fhb1-	31	510.833333	35.1666667	8.025
Clark	SWW	IN	PI 512337	Fhb1-	11.3333333	398.833333	15.1666667	1.515
Coker68-15	SWW	SC		Fhb1-	17	467.583333	55	6.895
Coker9663	SWW	AR	PI 596345	Fhb1-	25.6666667	492.333333	38.3333333	6.85333333
D6234	SWW	MI	PI 633575	Fhb1-	29.3333333	583.833333	41.6666667	8.76333333
DG1800	SWW	SE		Fhb1-	0.25	72.25	4.25	2.95
Endurance	HWW	OK	PI 639233	Fhb1-	17.6666667	371.916667	34	6.035
Epoch	HWW		PI 699379	Fhb1-	26.3333333	434.583333	29.6666667	9.09166667
Ernie	SWW	MO	PI 584525	Fhb1-	3	238.083333	11.1666667	9.85666667
Everest	HWW	KS	PI 659807	Fhb1-	4.21052632	198.921053	5.92105263	2.43578947
FL302	SWW	FL	PI 601163	Fhb1-	47.8333333	727.833333	69.1666667	28.3833333
Flathead	HWW	MT	PI 693237	Fhb1-	26.6666667	419.083333	35.8333333	10.6233333
Foster	SWW	KY	PI 593689	Fhb1-	14	377.666667	19.3333333	3.13333333
Freedom	SWW	OH	PI 562382	Fhb1-	33.5	556.5	50.1666667	11.3266667
Frontana	HWW	Brazil	PI 500147	Fhb1-	21.1666667	440.333333	31.8636458	8.43333333
Fultz	SWW	PA	PI 5493	Fhb1-	20	436	14.5	5.37166667
G61505	HWW	KS		Fhb1-	18.5	340.083333	14.1666667	3.43166667
GA001138-8E36	SWW	GA		Fhb1-	26	423.416667	43.3333333	8.41666667
GA00190-7A14	SWW	GA		Fhb1-	17.5	464.333333	61.6666667	15.935
GA03185-12LE29	SWW	GA		Fhb1-	34.1666667	617.166667	23.5	12.6633333
GA05450-EL52	SWW	GA		Fhb1-	9.83333333	391.416667	53.3333333	6.045
GA06493-13LE6	SWW	GA		Fhb1-	40	697.083333	84.5	18.9566667
Gallagher	HWW	OK	PI 667569	Fhb1-	11.1666667	373.583333	64.1666667	8.62833333

Heyne	HWW	KS	PI 612577	Fhb1-	12.6666667	340.25	7.66666667	7.67166667
Hilliard	SWW	VA	PI 676271	Fhb1-	8.5	254.583333	6.91666667	2.45166667
Hitch	HWW	KS	PI 655954	Fhb1-	15.6666667	355.25	22	4.525
Holley	SWW	GA	CItr 14579	Fhb1-	17.6666667	318.083333	35.5	4.45166667
Hondo	HWW	KS	PI 603958	Fhb1-	18	372.5	18.6666667	4.90833333
Hope	HWW	SD	PI 192612	Fhb1-	30	397.5	53.3333333	11.6033333
Howell	SWW	IL	PI 552816	Fhb1-	10.6666667	352	46.1666667	5.385
Hunter	SWW	SC	PI 468977	Fhb1-	8.16666667	345.916667	52.6666667	4.47833333
Iba	HWW	OK	PI 667570	Fhb1-	19.6666667	464.166667	50	11.03
IL00-8061	SWW	IL		Fhb1-	9.66666667	289.833333	9.33333333	1.66
IL00-8530	SWW	IL		Fhb1-	6.83333333	264.333333	5.83333333	8.41166667
IL02-18228	SWW	IL		Fhb1-	3.5	263.833333	1.33333333	1.65666667
IL07-20728	SWW	IL		Fhb1-	12	300.333333	16.6666667	2.22666667
IL07-4415	SWW	IL		Fhb1+	5.16666667	185.333333	10.1666667	5.27166667
IL96-6472	SWW	IL		Fhb1-	5.16666667	219.416667	3.5	3.34166667
IL97-1828	SWW	IL		Fhb1-	12	302.25	3.16666667	4.755
INW0316	SWW	IN		Fhb1-	14.1666667	380.666667	21.3333333	4.65166667
INW0411	SWW	IN		Fhb1+	10.1666667	298.333333	31	2.07666667
Jamestown	SWW	VA	PI 653731	Fhb1-	7.33333333	248.916667	6.16666667	1.845
Jerry	HWW	SD	PI 632433	Fhb1-	37.8333333	617	49.1666667	16.4366667
Ji5625Fhb1ABCD3	HWW	KS		Fhb1+	6.16666667	229.833333	7.16666667	2.36
Ji5625Fhb1AD3	HWW	KS		Fhb1+	8.66666667	202	17.1666667	3.705
Joe	HWW	KS	PI 676270	Fhb1-	23.1666667	466.833333	35.8333333	13.3316667
Jupiter	SWW	MI	PI 664079	Fhb1-	32.8333333	656.75	29.8333333	15.6
Karl92	HWW	KS	PI 564245	Fhb1-	8.66666667	304	13.812125	5.02466667
KS Ahearn	HWW	KS	PI 701142	Unknown	21.8333333	491	44.1666667	5.71833333
KS08FHB-31	HWW	KS		Fhb1+	14.5	327.083333	2.5	10.0866667
KS120215K-6	HWW	KS		Fhb1-	23.3333333	522.666667	52.5	8.875
KS12FHB-A-1	HWW	KS		Fhb1+	10.8333333	284.75	7.5	2.77333333
KS130318K-3	HWW	KS		Fhb1-	6.33333333	257.666667	12.3333333	4.43666667
KS13DH0041-35	HWW	KS		Fhb1-	5	288.666667	30.5	5.02666667
KS140064M-1	HWW	KS		Fhb1-	16.3333333	355.333333	22.1666667	8.42166667
KS140732K-2	HWW	KS		Fhb1-	23.6666667	462.916667	29.1666667	8.98333333
KS140794K-8	HWW	KS		Fhb1-	23.1666667	505.5	44.3333333	7.12
KS15DH0055-11	HWW	KS		Fhb1-	20.5	456.166667	47.5	14.7416667
KS160476S-6	HWW	KS		Fhb1-	10.5	286.5	15.5	5.42833333
KS16DH0002-12	HWW	KS		Fhb1-	9	392.5	12.3333333	8.825
KS16DH0010-17	HWW	KS		Fhb1-	6.83333333	265.75	20.8333333	4.55666667
KS16FHB0211-201-8	HWW	KS		Fhb1+	4.8	207	20.4	1.51
KS16FHB0338-101-8	HWW	KS		Fhb1+	3.8	175.3	7.8	0.54
KS16FHB0478-101-1	HWW	KS		Fhb1+	7.2	205.5	1.4	2.11

KS18HW106-4	HWW	KS		Fhb1-	24.5	481.083333	32.6666667	12.915
KS19H10	HWW	KS		Fhb1-	25.6666667	586.75	56.6666667	9.46666667
KS19H84	HWW	KS		Fhb1-	15.6666667	330.583333	13.6666667	16.1633333
KS19U16MD4132R2-10	HWW	KS		Fhb1-	33.3333333	513.583333	46.6666667	12.51
KS20U15M0020R4	HWW	KS		Fhb1-	17.8333333	477.25	46.6666667	13.325
KS20U15M0042R2	HWW	KS		Fhb1-	18.1666667	377.166667	37.5	10.5116667
KS20UMD4144R4	HWW	KS		Fhb1-	28.1666667	525.333333	41.6666667	7.35166667
KS20UMD4272R5	HWW	KS		Fhb1-	40	624.416667	30	19.79
KS21U7266-E1-B2	HWW	KS		Fhb1-	38	610.916667	55	14.1816667
KS21U7445-H9-C3	HWW	KS		Fhb1-	3.33333333	203.916667	34	5.82666667
KS Dallas	HWW	KS	PI 693934	Fhb1-	32	612.5	66.6666667	17.745
KS Hamilton	HWW	KS	PI 699003	Fhb1-	21.6666667	376.166667	21.6666667	5.74166667
KS Hatchett	HWW	KS	PI 699114	Fhb1-	10.5	339.916667	46.6666667	5.55833333
KS Venada	HWW	KS	PI 690467	Fhb1-	20	382.333333	30.6666667	12.895
KS WesternStar	HWW	KS	PI 693935	Fhb1-	25.1666667	552.583333	43.3333333	10.255
KY020-3005-25	SWW	KY		Fhb1+	13.75	303.25	2	2.825
KY96C-0769-7-3	HWW	KY		Fhb1-	15.8333333	306.083333	18.1666667	5.25833333
LA09264C-P2	SWW	LA		Fhb1-	12.8333333	313.416667	24.5	4.67666667
LA16020LDH-22	SWW	LA		Fhb1-	3.75	173	23.75	5.04570625
LA841	SWW	LA		Fhb1-	37.5	598.75	43.6666667	9.00666667
LA95135	SWW	LA		Fhb1-	39.1666667	608.75	53.1969792	18.1938042
LANC19-12-5-48	SWW	NC		Fhb1-	2.25	191	35	4.825
KS18H111-3	HWW	KS		Fhb1-	19.6666667	357.833333	32.6666667	5.385
Loma	HWW	MT	PI 680576	Fhb1-	45	682	68.6666667	13.7883333
Lyman	HWW	SD	PI 658067	Fhb1-	17.8333333	297.75	25	6.29333333
M03-3616-c	HWW			Fhb1-	15.6666667	381.25	29.3333333	2.97166667
Massey	SWW	VA	PI 664481	Fhb1-	12.6666667	314.333333	34.5	5.55833333
McCormick	SWW	VA	PI 632691	Fhb1-	8	281.916667	16.1666667	1.465
MD01W233-06-1	SWW	MD		Fhb1-	8.66666667	271.666667	7.83333333	1.72166667
MD01W28-08-11	SWW	NC		Fhb1-	13.8333333	310.166667	9.33333333	4.87666667
MPV57	SWW	VA		Fhb1-	21	468.75	60	6.175
MT1745	HWW	MT		Fhb1-	60.8333333	816.75	82.8333333	21.6083333
MT19175	HWW	MT		Fhb1-	42	666.333333	65.3333333	19.5533333
MTF20189	HWW	MT		Fhb1-	45.3333333	644.333333	59.1666667	18.5316667
MTFH19132	HWW	MT		Fhb1-	30.1666667	572.916667	53	8.97833333
MTFH20164	HWW	MT		Fhb1+	18.8333333	390.166667	27.5	4.10833333
MTFH21169	HWW	MT		Fhb1+	35.8333333	591.083333	60.8333333	10.61
MTFH21178	HWW	MT		Fhb1+	35.8333333	571.666667	31.6666667	10.3333333
MTFH2289	HWW	MT		Fhb1+	35.1666667	563.666667	58.3333333	9.79833333
MTS1810	HWW	MT		Fhb1-	48	700.333333	44	12.48
MTS18149	HWW	MT		Fhb1-	39.1666667	589.333333	49.1666667	12.2566667

MTS1903	HWW	MT		Fhb1-	36.8333333	618.083333	56.6666667	11.0983333
N16MD9261-F02-3	HWW			Fhb1-	7.5	345.583333	33	5.91833333
NC-Neuse	SWW	NC		Fhb1-	18.5	443.666667	26.8333333	5.62
NC05-19896	SWW	NC		Fhb1-	16.3333333	337.666667	32.6666667	2.57666667
NC07-24-337	SWW	NC		Fhb1-	11.1666667	331.5	32	3.17
NC08-23383	SWW	NC		Fhb1-	15	316.75	8.5	5.13
NC09-20986	SWW	NC		Fhb1+	3.83333333	203.416667	23.5	0.56166667
NC09GBTUM15	SWW	NC		Fhb1-	13.75	463.5	35	5.875
NC11-22289	SWW	NC		Fhb1-	5.5	265.333333	20	0.97333333
NC11-331-6	SWW	NC		Fhb1+	6	239.5	22.1666667	1.63
NC12-22844	SWW	NC		Fhb1-	6.16666667	332.083333	37.5	2.98333333
NC13-20076	SWW	NC		Fhb1-	12.8333333	275.583333	2.41666667	1.90333333
NC21-13341	SWW	NC		Fhb1-	4.75	240	3.75	5.575
NC8248-1	SWW	NC		Fhb1-	7.83333333	283.333333	24.8333333	3.00166667
NC8248-20	SWW	NC		Fhb1-	6	230.333333	4.16666667	4.615
NC99GBTAG11	SWW	NC		Fhb1-	10.25	399.25	65	7.325
NE14494	HWW	NE		Fhb1-	26	463.666667	48.3333333	7.85333333
NE16424	HWW	NE		Fhb1-	10.6666667	345.416667	24.5	5.56833333
NE16562	HWW	NE		Fhb1-	9.33333333	353.166667	22.5	3.24333333
NE17441	HWW	NE		Fhb1-	8.83333333	288.833333	6.5	4.71
NE17443	HWW	NE		Fhb1-	18.3333333	417.333333	30.8333333	15.19
NE17544	HWW	NE		Fhb1-	39.5	575.333333	45.8333333	12.4716667
NE18455	HWW	NE		Fhb1-	14.3333333	427	39.1666667	9.69
NE18573	HWW	NE		Fhb1-	30	556.916667	52	8.85166667
NE19406	HWW	NE		Fhb1-	25.1666667	509.5	42.5	11.05
NE19454	HWW	NE		Fhb1-	20	364.333333	17.5	5.75
NE19590	HWW	NE		Fhb1-	21	413.333333	35.8333333	7.17166667
NI13706	HWW	NE		Fhb1-	15	359.416667	30.1666667	6.50666667
NI17410	HWW	NE		Fhb1-	13	333.666667	11.1666667	2.665
Northern	HWW	MT	PI 676026	Fhb1-	40.5	652.666667	55.8333333	14.8016667
Oakes	SWW	AR	PI 658040	Fhb1-	28.8333333	512.166667	10.5	4.565
OakleyCL	HWW	KS	PI 670170	Fhb1-	26.5	487.666667	26.3333333	9.29
Oasis	SWW	IN	CItr 15929	Fhb1-	22.6666667	455.833333	23.5	3.64666667
Oglethorpe	SWW	GA	PI 657986	Fhb1-	16.1666667	405.75	67.5	8.85166667
Overland	HWW	NE		Fhb1-	20.1666667	442.25	43.3333333	5.45166667
Overlay	HWW	KS	PI 634974	Fhb1-	10.1578947	404.315789	50.2631579	10.9873684
P02444A1-23-9	SWW			Fhb1+	8.5	242.916667	13.3333333	4.325
P03112A1-7-14	SWW			Fhb1+	13.5	281.833333	6.83333333	5.06333333
Pembroke	SWW	KY		Fhb1-	23.5	383.5	23.8333333	5.455
Pioneer-25R55	SWW	NC		Fhb1-	18.5	434.166667	30	7.58333333
Pioneer-25R80	SWW	NC		Fhb1-	15.8333333	405.333333	28.5	4.86833333

Pioneer-26R61	SWW	IN		Fhb1-	18.6666667	504.5	56.6666667	14.4116667
Rampart	HWW	MT	PI 593889	Fhb1-	51	700.833333	45.8333333	12.5816667
Roane	SWW	VA	PI 612958	Fhb1-	11.6666667	289.583333	10.8333333	4.50666667
Saluda	SWW	VA	PI 480474	Fhb1-	20.1666667	496.916667	39.1666667	6.99
SCTX98-27C1	SWW	SC		Fhb1-	21.3333333	461.416667	50.8333333	7.31666667
SD05085-1	HWW	SD		Fhb1-	11.5	235.666667	7.58333333	3.74333333
SD05210	HWW	SD		Unknown	19	396.666667	54.0303125	16.5916667
SD15007-5	HWW	SD		Fhb1-	48.3333333	672.75	49.5	16.6816667
SD15035-2	HWW	SD		Fhb1-	49.1666667	711.416667	52.5	11.6383333
SD17B032-1	HWW	SD		Fhb1-	30.8333333	518.25	48.3333333	12.4266667
SD17B210-2	HWW	SD		Fhb1-	37.8333333	633.75	43.3333333	9.35833333
SD18B025-8	HWW	SD		Fhb1-	23.1666667	463.833333	55	20.055
SD18B072-2	HWW	SD		Fhb1-	15.5	333.666667	30	5.08666667
SD18B083-8	HWW	SD		Fhb1-	11.1666667	302.583333	13.5833333	9.665
SD19B019-2	HWW	SD		Fhb1-	20.3333333	369.666667	15.5	6.02833333
SD19B033-2	HWW	SD		Fhb1-	31.6666667	559.333333	30	11.1583333
SD Andes	HWW	SD	PI 699244	Fhb1-	52.8333333	768.583333	48.3333333	13.3216667
SDMidland	HWW	SD	PI 700962	Fhb1-	29.3333333	546.416667	49.1666667	10.8133333
Shirley	SWW	VA	PI 656753	Fhb1-	18.3333333	426.333333	60.8333333	4.35333333
Siege	HWW		PI 693222	Fhb1-	17.5	349.166667	14	6.15333333
SS8412	SWW	VA		Fhb1-	15	438.583333	43.3333333	8.61666667
SS8641	SWW	GA		Fhb1-	14.3333333	421.75	38.3333333	5.20833333
T153	HWW	KS		Fhb1-	5.4	199.2	4.6	1.07
T154	HWW	KS		Fhb1-	7.5	247.916667	9.66666667	2.24
TAM113	HWW	TX	PI 666125	Fhb1-	37.1666667	607.666667	38.3333333	12.255
TAM114	HWW	TX	PI 686860	Fhb1-	23.5	513	40	10.0683333
TAM115	HWW	TX	PI 695149	Fhb1-	33.8333333	635.416667	44.1666667	8.70333333
TAM204	HWW	TX	PI 686859	Fhb1-	15.1666667	376.333333	28.6666667	5.68333333
TAM205	HWW	TX	PI 695150	Fhb1-	13.6666667	324.166667	14.1666667	8.32833333
Tatanka	HWW	KS	PI 681595	Fhb1-	13	350.666667	25.3333333	7.265
Tribute	SWW	VA	PI 632689	Fhb1-	13.6666667	340.333333	24.8333333	2.72833333
TurkeyRed	HWW	KS		Fhb1-	50	605	20	6.66
TX1112-108	HWW	TX		Fhb1-	14.1666667	365	42.1666667	7.37
TX1204896	SWW	TX		Fhb1-	27	521	40.8333333	14.3816667
TX14A001112-18AZ1	HWW	TX		Fhb1-	24.5	627	70	8.18
TX14A001215-18AZ295	HWW	TX		Fhb1-	31	535.25	35.8333333	5.97166667
TX14V70214	HWW	TX		Fhb1-	9.33333333	347.25	28.5	3.79
TX16M9155	HWW	TX		Fhb1-	20.8333333	410.333333	42.5	6.41166667
TX17A001295	HWW	TX		Fhb1-	18	522.583333	52.5	9.12
TX17M1572	HWW	TX		Fhb1-	13.5	366.333333	28.6666667	5.295
TX18A001129	HWW	TX		Fhb1-	9.33333333	323	14	3.94

TX18A001397	HWW	TX		Fhb1-	19	423.333333	30	5.69166667
TX18A001399	HWW	TX		Fhb1-	24.5	477.666667	31.1666667	9.02
TX18M2080	HWW	TX		Fhb1-	15.5	418.166667	57.5	8.19666667
TX18M2137	HWW	TX		Fhb1-	30.8333333	580.083333	54.1666667	8.435
TX18M2168	HWW	TX		Fhb1-	8.83333333	348.916667	32.8333333	4.41
TX18M2205	HWW	TX		Fhb1-	19.3333333	473.75	59.1666667	13.4583333
TX18M2209	HWW	TX		Fhb1-	27.8333333	549	63.3333333	15.3616667
TX18M2219	HWW	TX		Fhb1-	24	449.5	55.8333333	14.2116667
TXAMPSY473-18AZ816	HWW	TX		Fhb1-	25.3333333	494.75	53.3333333	12.5883333
U07-698-9	HWW	KS		Fhb1-	13.6666667	336.333333	11.1666667	7.315
U7494-E11	HWW	KS		Fhb1-	21.8333333	528.666667	28.8333333	12.0683333
U7710-E-2-2	HWW	KS		Fhb1-	32.3333333	523.166667	35.6666667	9.64
U8086-13-14	HWW	KS		Fhb1+	41.8333333	644	27	7.77880417
U8584-C-A3	HWW	KS		Fhb1-	17.8333333	346.166667	16.1666667	5.33333333
U9001-G-H05	HWW	KS		Fhb1-	15.5	305.416667	11	3.3
USG-3555	SWW	SE		Fhb1-	16.3333333	405.416667	25.5	6.37166667
USG3555	SWW	VA		Fhb1-	9.83333333	277.5	32.5	3.075
VA05W-151	SWW	VA		Fhb1-	10.8333333	345.333333	46.6666667	3.25666667
VA09W-52	SWW	VA		Fhb1-	15.3333333	345.916667	20.3333333	4.63833333
VA10W-21	SWW	VA		Fhb1-	16	381.5	22.6666667	3.05666667
VA12W-241	SWW	VA		Fhb1-	10.6666667	328	20.8333333	3.025
Wesley	HWW	NE	PI 605742	Fhb1-	30.6666667	610.333333	57.5	13.7183333
WesleyFhb1	HWW	NE		Fhb1+	19.8333333	445.416667	18.1666667	2.45
Winner	HWW	SD	PI 695101	Fhb1-	26.5	470.5	45.8333333	9.11166667
Winterhawk	HWW	KS	PI 652927	Fhb1-	14	335.416667	10	3.60166667
Xiaoyan22	HWW			Fhb1-	16.6	417	54.67275	5.66113
Yorktown	SWW	VA	PI 667643	Fhb1-	11.5	348.75	20.5	4.82833333
Zhengmai9023	HWW			Fhb1-	25	495	45	15.32

HWW: hard winter wheat; SWW: soft winter wheat; PI: production introduction

Supplementary Table 4. 2 Line pedigree, total sequence reads, QC, and coverage information of the Great Plain lines (GPL) from the KSU wheat breeding program.

Taxa	Pedigree	Total reads	%QC	Coverage
21AYN1-KS20H105		2095482	88.26%	0.619754
21AYN1-KS20H106		1832294	87.25%	0.537169
21AYN1-KS20H120		2279076	87.40%	0.675985
21AYN1-KS20H122		2325276	87.69%	0.727647
21AYN1-KS20H124		1506968	86.23%	0.448201

21AYN1-KS20H126		2216294	88.77%	0.685139
21AYN1-KS20H21		2892474	87.85%	0.859955
21AYN1-KS20H3		2450778	89.16%	0.759373
21AYN1-KS20H61		2216018	86.71%	0.648648
21AYN1-KS20H62		1961602	85.99%	0.58426
21AYN1-KS20H89		2460300	86.92%	0.755438
21AYN1-KS20HD134		2588272	88.56%	0.793061
21AYN1-KS20HD135		2493970	87.32%	0.751246
21AYN1-KS20HD136		2968050	87.37%	0.907391
21AYN1-KS20HD140		2577090	87.57%	0.787814
21AYN1-KS20HD141		2178502	87.84%	0.658129
21AYN1-KS20HD143		2330686	85.52%	0.705411
21AYN1-KS20HD144		2457734	87.25%	0.749376
21AYN1-KS20HD146		2112624	86.09%	0.628604
21AYN1-KS20HD158		2558160	86.92%	0.789794
21AYN1-KS20HD168		1905478	86.23%	0.558966
21AYN1-KS20HD170		2496176	87.68%	0.716331
21AYN1-KS20HD175		2764168	88.01%	0.826238
21AYN1-KS20HD178		2469588	89.01%	0.740562
21AYN1-KS20HD179		2550294	88.36%	0.765977
21AYN1-KS20HD183		2891338	87.77%	0.876826
21AYN1-KS20HD184		2176106	89.08%	0.66582
21AYN1-KS20HDW127		2241028	87.86%	0.693895
21AYN1-KS20HM93		1992034	87.04%	0.607794
21AYN1-KS20HW103		2103406	89.68%	0.647748
21AYN1-KS20HW113		2392552	86.37%	0.704101
21AYN1-KS20HW14		2898384	88.50%	0.901408
21AYN1-KS20HW7		2452246	88.44%	0.726844
21AYN1-KS20HW98		2116200	86.63%	0.626656
21AYN1-SYMonument		1702780	87.39%	0.489213
21AYN1-SYWolverine		2227072	86.91%	0.681258
21AYN1-WB4699		2800422	88.49%	0.842974
21AYN1-Zenda	Zenda	2172896	87.48%	0.639918
21AYN2-KS130019C4-7	TX07A001505/Armour	1988194	86.79%	0.570161
21AYN2-KS130236C4-8	KS050525-K-4/KS061406-LN~39//WB-Grainfield	2010728	86.70%	0.564439
21AYN2-KS130249C4-3	KS030923K-5/TAM-111//KanMark	2458482	86.36%	0.741429
21AYN2-KS130291C4-1	KS060285-M-1/KS061406-LN~39//Joe	1863986	87.58%	0.562979
21AYN2-KS130291C4-6	KS060285-M-1/KS061406-LN~39//Joe	2570394	87.11%	0.747469
21AYN2-KS130293C4-3	Winterhawk/KanMark//Joe	2397192	86.80%	0.717571
21AYN2-KS130293C4-7	Winterhawk/KanMark//Joe	2259520	86.83%	0.641361
21AYN2-KS140009C4-4	Joe/Cedar	1684438	85.73%	0.496946

21AYN2-KS140138C4-4	KS061278M-4/Joe	1642018	83.59%	0.48952
21AYN2-KS140430C4-1	KS10DL0032-6/SY Wolf//KS12HW97-2	1231502	79.43%	0.348583
21AYN2-KS140443C4-5	BIO 10509/Antero//SY Wolf	1751642	80.76%	0.513326
21AYN2-KS140535C4-2	Clara CL/KanMark//Antero	2334774	86.75%	0.719016
21AYN2-KS140535C4-5	Clara CL/KanMark//Antero	2013872	89.12%	0.602444
21AYN2-KS140535C4-6	Clara CL/KanMark//Antero	2246540	86.06%	0.67282
21AYN2-KS140848C4-3	X070487-186-31/Cedar//Joe	1388320	86.59%	0.382464
21AYN2-KS140848C4-6	X070487-186-31/Cedar//Joe	1691264	86.77%	0.482703
21AYN2-KS140884C4-5	MERCATO//JNRB.5/PIFED/3/KS080394*C-32//Joe	2041918	88.55%	0.596021
21AYN2-KS140884C4-6	MERCATO//JNRB.5/PIFED/3/KS080394*C-32//Joe	2736572	87.32%	0.841061
21AYN2-KS140962C4-2	Byrd/KS061278K-1//KS061470M-4	1643266	87.36%	0.49251
21AYN2-KS140962C4-3	Byrd/KS061278K-1//KS061470M-4	2190888	87.99%	0.652274
21AYN2-KS140977C4-2	KS060167-M-8/OCW01S137T-1//Joe	2097896	86.95%	0.62459
21AYN2-KS150167S-10	Bob Dole/Joe	2330500	87.32%	0.700849
21AYN2-KS150365S-1	KS060808K-2/KS061705M-11//KS090529K-10	2473746	86.92%	0.760304
21AYN2-KS150405S-1	Pioneer 26R15/OCW03S426S-6//LCS Chrome	2432738	86.87%	0.706415
21AYN2-KS150575S-1	KS070093M-5/Joe//Joe	2431470	87.29%	0.737268
21AYN2-KS16GD0024-15	KS061278M-4/TA 2398//PI 478699	2369554	87.87%	0.725097
21AYN2-KS16GD0026-11	KS061278M-4/TA 2398//PI 538631	2523346	87.15%	0.769994
21AYN2-KS16GD0026-31	KS061278M-4/TA 2398//PI 538631	2048838	85.11%	0.608485
21AYN2-KS16GD0030-25	KS061278M-4/TA 10088//PI 471021	2596296	88.36%	0.814991
21AYN2-KS16GD0032-34	KS061278M-4/TA 10155//PI 471021	1877340	86.99%	0.525526
21AYN2-KS19RDM-110	KS19RDM-110	2288878	86.29%	0.670221
21AYN2-KS19RDM-88	KS19RDM-88	2913364	84.67%	0.848158
21AYN2-KSEvOv-1-13-1	Everest*2/Overley	2600578	87.44%	0.81512
21AYN2-KSEvOv-2-4-4	Everest*2/Overley	2508832	88.53%	0.767055
21KIN-KS120154K-5		2673284	87.40%	0.811624
21KIN-KS120215K-6		2541498	88.73%	0.79113
21KIN-KS120494M-1		2871848	87.23%	0.893441
21KIN-KS13DH0041-25		1928770	86.60%	0.582893
21KIN-KS14HD286		1538688	86.53%	0.458261
21KIN-KS15DH0055-11		2819866	87.63%	0.890688
21KIN-KS16DH0002-12		2857534	89.61%	0.907308
21KIN-KS16DH0004-30		2626116	86.37%	0.789731
21KIN-KS16DH0006-17		2795684	86.90%	0.836606
21KIN-KS16DH0008-32		2550248	88.55%	0.773941
21KIN-KS16DH0010-17	LCS Chrome/3/KS030810NT-9/90RN2491//3*KS020617~9	2214016	86.88%	0.658165
21KIN-KS16DH0011-12		2136792	88.99%	0.62971
21KIN-KS16DH0011-45		1450148	88.08%	0.423648
21KIN-KS18EVACK-327		2602670	87.93%	0.820707

21KIN-KS18EVACM-11		2613714	88.04%	0.802713
21KIN-KS18EVACM-34		2691632	89.69%	0.854738
21KIN-KS18H110-3		2724940	90.31%	0.839964
21KIN-KS18H111-3		2633634	87.68%	0.810746
21KIN-KS18H124-6		2706874	89.50%	0.821456
21KIN-KS18H19-1		1100476	86.47%	0.310941
21KIN-KS18H19-6		1685652	86.25%	0.516436
21KIN-KS19H10		2484312	87.10%	0.764094
21KIN-KS19H21		2398056	87.95%	0.736414
21KIN-KS19H68		1331096	79.12%	0.379809
21KIN-KS19H69		2321786	80.25%	0.679108
21KIN-KS19H74		2099022	87.46%	0.641812
21KIN-KS19H79		2486566	87.18%	0.76604
21KIN-KS19HW39		2284372	88.19%	0.691768
21KIN-KS19HW84		1776254	85.74%	0.534019
21KIN-NUSAKA15-3		2957386	88.59%	0.920636
21PYN2-KS130033M-3	KS060430-M-6/KS061406-LN~26	2253584	75.45%	0.638551
21PYN2-KS130036M-4	Iba/KS061406-LN~39	2803198	85.92%	0.830805
21PYN2-KS130036M-5	Iba/KS061406-LN~39	1599004	88.05%	0.484466
21PYN2-KS130039M-1	KS050525-K-4/KS061406-LN~39	2842902	88.64%	0.875124
21PYN2-KS130044M-2	KS060285-M-1/KS061406-LN~39	2348878	80.00%	0.678766
21PYN2-KS130044M-6	KS060285-M-1/KS061406-LN~39	1356868	87.42%	0.418912
21PYN2-KS130236K-2	KS050525-K-4/KS061406-LN~39//WB-Grainfield	2635302	75.48%	0.730906
21PYN2-KS130242M-1	KS060041-M-1/KS061406-LN~39//WB-Redhawk	2500214	80.94%	0.725714
21PYN2-KS130250K-5	KS050444-K-5/Overley//KS040640K-1	1620576	67.69%	0.424254
21PYN2-KS130258K-4	KS061406-LN~39/Clara CL//KS050278M-1	3010898	79.66%	0.883585
21PYN2-KS130291K-4	KS060285-M-1/KS061406-LN~39//Joe	2151590	79.89%	0.613087
21PYN2-KS130291M-6	KS060285-M-1/KS061406-LN~39//Joe	2202114	77.17%	0.626438
21PYN2-KS130291M-8	KS060285-M-1/KS061406-LN~39//Joe	2142818	72.83%	0.587194
21PYN2-KS130318K-4	Duster/KS060430-M-6//Bob Dole	2053892	78.07%	0.589718
21PYN2-KS130318M-2	Duster/KS060430-M-6//Bob Dole	2766238	85.61%	0.832564
21PYN2-KS130320M-3	KS050255K-6/KS061406-LN~39//Bob Dole	1649962	70.60%	0.443677
21PYN2-KS130322K-9	KS040810NT-8/KS061406-LN~39//Bob Dole	2824948	86.59%	0.852279
21PYN2-KS130324K-6	Everest/KanMark//Bob Dole	2186278	73.79%	0.610569
21PYN2-KS130324M-2	Everest/KanMark//Bob Dole	1862708	78.84%	0.531314
21PYN2-KS130326K-2	TAM-304/KanMark//Bob Dole	1505454	73.72%	0.410553
21PYN2-KS130326K-6	TAM-304/KanMark//Bob Dole	1307218	88.36%	0.387717
21PYN2-KS130336K-3	AP11T1115/KS061237K-1//Bob Dole	2216346	87.21%	0.645047
21PYN2-KS130339K-3	KS061406-LN~32/KS061237K-1//Bob Dole	2063144	73.98%	0.568497
21PYN2-KS130340K-4	KS060638-BE~C/KS061278M-4//Bob Dole	2576932	84.46%	0.790809
21PYN2-KS130346K-2	KS050278M-1/TAM-304//Bob Dole	1383350	69.97%	0.355763

21PYN2-KS130347K-6	KS050255K-7/Unknown//Bob Dole	2231134	89.49%	0.677963
21PYN2-KS130367S-1	OK12022003-138W/10BC107#29//Joe	3234350	88.00%	0.966221
21PYN2-KS130383M-1	KS061406-LN~26/Byrd//KS061860M-3	2365552	78.03%	0.688252
21PYN2-KS130403M-3	KS060606M-2/Antero//KS061860M-3	2556460	74.07%	0.706176
21PYN2-KS130403M-7	KS060606M-2/Antero//KS061860M-3	2216974	87.55%	0.659443
21PYN2-KS130410M-2	KS10HW78-1/KS061406-LN~32//KS061862M-17	2376794	77.68%	0.670881
21PYN2-KS130416K-1	Cedar/KS030923K-5//KS061862M-17	1487530	63.39%	0.370442
21PYN2-KS130416K-3	Cedar/KS030923K-5//KS061862M-17	2120210	79.67%	0.627945
21PYN2-KS130416K-4	Cedar/KS030923K-5//KS061862M-17	2715590	87.07%	0.837203
21PYN2-KS130416K-7	Cedar/KS030923K-5//KS061862M-17	1319394	73.69%	0.359827
21PYN2-KS130416K-8	Cedar/KS030923K-5//KS061862M-17	1322132	70.46%	0.355272
21PYN2-KS130424K-5	TAM-304/KS060808K-2//KS061862M-17	1339036	88.39%	0.400964
21PYN2-KS130460M-2	KS061237K-1/Jackpot//KS080448*-10	2709820	84.90%	0.815938
21PYN2-KS130467K-3	KS060430-M-1/KS061478M-10//KS080394*C-32	2253996	86.69%	0.678511
21PYN2-KS130500K-4	Aspen/KS061237K-1/3//KS060750~E//Garrison/Aspen	2242272	87.65%	0.683075
21PYN2-KS130509M-3	KS040640K-1/KS061406-LN~39//09BC450#128	3425854	87.07%	1.057267
21PYN2-KS130544M-2	KS050255K-6/KS061237K-1//10BC347#33	2310496	78.00%	0.663535
21PYN2-KS13FHB0320M-3	KS050255K-6/KS061406-LN~39//Bob Dole	2594580	88.68%	0.790584
21PYN2-KS13FHB0322M-1	KS040810NT-8/KS061406-LN~39//Bob Dole	1881818	87.33%	0.566205
21PYN2-KS140029M-4	Rustic/Duster	3251850	80.91%	0.945581
21PYN2-KS140060M-1	KanMark/WB-4458	3674772	87.59%	1.154474
21PYN2-KS140060M-4	KanMark/WB-4458	2299774	74.14%	0.612094
21PYN2-KS140064M-6	Basmati/WB-Redhawk	543680	66.07%	0.133013
21PYN2-KS140064M-7	Basmati/WB-Redhawk	1601844	88.72%	0.487911
21PYN2-KS140064M-8	Basmati/WB-Redhawk	2901504	88.12%	0.896132
21PYN2-KS140103M-3	KS060041-M-1/KS060808K-2	2440758	76.35%	0.687817
21PYN2-KS140104M-6	KS061456K-4/KS060808K-2	1447700	89.24%	0.436236
21PYN2-KS140141M-6	SY Wolf/KS050444-M-6	2714756	82.00%	0.79769
21PYN2-KS140148M-1	KS080040*C-13/Bob Dole	2826424	88.10%	0.877314
21PYN2-KS140148M-2	KS080040*C-13/Bob Dole	3093966	88.40%	0.928671
21PYN2-KS140148M-3	KS080040*C-13/Bob Dole	1640840	72.90%	0.453562
21PYN2-KS140153K-9	KWS Erasmus/KS061193K-3	1598444	87.32%	0.478952
21PYN2-KS140159M-5	KS060750-BE~B/KS061193K-1	1932276	87.29%	0.570663
21PYN2-KS140160M-5	KS061880M-3/KS061193K-1	2308040	74.06%	0.65341
21PYN2-KS140164K-6	KS050278M-1/KS061880M-3	1817492	68.50%	0.478911
21PYN2-KS140164K-7	KS050278M-1/KS061880M-3	2773234	76.90%	0.776406
21PYN2-KS140164M-1	KS050278M-1/KS061880M-3	1349508	87.04%	0.402547
21PYN2-KS140189K-4	KS061470K-2/KS061860M-3	1363118	87.18%	0.415678
21PYN2-KS140207K-9	KS060634M-5/KS061705M-11	2293870	82.35%	0.673196
21PYN2-KS140227M-2	SY Wolf/KS080448*-10	2213386	83.72%	0.658678
21PYN2-KS140272M-3	KS061582M-5/10BC347#33	1627090	69.92%	0.421778

21PYN2-KS140275M-5	BIO 10587/10BC347#33	1693838	89.64%	0.514107
21PYN2-KS140307M-11	TBIO Sinuelo/KS061837M-3	2085704	87.55%	0.630366
21PYN2-KS140323M-1	KS061162M-2/OCW01S137T-1	2342574	74.21%	0.653615
21PYN2-KS140382K-2	X070488-187B-52/KS061705M-11//KS080040*C-13	2251174	86.84%	0.682721
21PYN2-KS140394K-1	KS080394*C-32/KS061837M-2//Larry	2696082	84.17%	0.808664
21PYN2-KS140394K-3	KS080394*C-32/KS061837M-2//Larry	2878074	77.38%	0.806045
21PYN2-KS140394M-4	KS080394*C-32/KS061837M-2//Larry	1311166	69.88%	0.337839
21PYN2-KS140412M-3	KanMark/WB-4458//SY Wolf	1791456	83.07%	0.515109
21PYN2-KS140418M-3	BIO 10510/SY Wolf/KS061470M-4	2586220	90.31%	0.779085
21PYN2-KS140422M-7	X070487-186-27/Clara CL/KS061837M-2	2042226	73.58%	0.544234
21PYN2-KS140490M-3	BIO 10377/Overley//KS060476-M-6	1507870	70.39%	0.391011
21PYN2-KS140491K-4	BIO 10377/Overley//SY Wolf	2073982	88.15%	0.616761
21PYN2-KS140515K-1	KS060828M-10/Bob Dole//KanMark	1355582	87.06%	0.405189
21PYN2-KS140516K-3	KS060476-M-6/KS061837M-2//KS061470M-4	2705126	82.43%	0.808334
21PYN2-KS140535M-1	Clara CL/KanMark//Antero	2810726	81.48%	0.827711
21PYN2-KS140548K-2	KS050444-M-6/Bob Dole//KS061837M-2	1692740	84.93%	0.504146
21PYN2-KS140572M-1	KS060167-M-7/10BC347#33//KS080040*C-13	1766408	77.64%	0.489151
21PYN2-KS140572M-12	KS060167-M-7/10BC347#33//KS080040*C-13	2080162	79.23%	0.597495
21PYN2-KS140572M-3	KS060167-M-7/10BC347#33//KS080040*C-13	1467576	89.19%	0.432455
21PYN2-KS140572M-5	KS060167-M-7/10BC347#33//KS080040*C-13	812166	64.82%	0.2031
21PYN2-KS140575M-4	FFR-240/10BC347#33//Iba	2244328	88.92%	0.688789
21PYN2-KS140577K-2	TBIO Mestre/10BC347#33//KS060371-M-3	2839972	78.04%	0.829047
21PYN2-KS140577M-5	TBIO Mestre/10BC347#33//KS060371-M-3	1827444	87.66%	0.561304
21PYN2-KS140588M-6	BIO 10536/KS12FHB(A+)-44//Zenda	1420270	89.06%	0.437592
21PYN2-KS140613K-7	MERCATO//JNRB.5/PIFED/3/KanMark//Iba	1897152	81.29%	0.556694
21PYN2-KS140628M-2	KS061837M-3/KS040144M-3//KS060084-M-4	2140248	80.59%	0.635677
21PYN2-KS140663K-1	PFAU/SERI.1B//AMAD/3/WAXWING/4/ND643/2*WBLL1/5/KS061278K-2//WB-4458	3165536	81.68%	0.931286
21PYN2-KS140732K-2	KWS Erasmus/KS061193K-3//Bob Dole	1813544	87.55%	0.535205
21PYN2-KS140732M-5	KWS Erasmus/KS061193K-3//Bob Dole	2679288	87.62%	0.807876
21PYN2-KS140738M-6	WB-4458/KS061193K-1//KS060084-M-4	2529906	76.65%	0.702095
21PYN2-KS140740K-2	KS061161K-4/KS061193K-1//WB-Redhawk	2260564	78.53%	0.64359
21PYN2-KS140740M-1	KS061161K-4/KS061193K-1//WB-Redhawk	2590652	82.22%	0.770803
21PYN2-KS140758M-5	KS10DL0119-6/KS061880M-3//KS061470M-4	2449592	79.50%	0.70717
21PYN2-KS140758M-9	KS10DL0119-6/KS061880M-3//KS061470M-4	1115520	89.35%	0.331044
21PYN2-KS140759M-2	09BC450#128/KS061880M-3//KS061862M-17	2026708	80.28%	0.581452
21PYN2-KS140763K-3	BIO 10589/KS061880M-3//KS061705M-11	2617724	79.50%	0.772383
21PYN2-KS140792K-3	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/5/MUU/6/KS061456K-4//Cedar	1848914	88.52%	0.561999
21PYN2-KS140794K-8	KS050428-M-9/KS061860M-3//KS060476-M-6	2693548	89.43%	0.810131
21PYN2-KS140794M-1	KS050428-M-9/KS061860M-3//KS060476-M-6	2814270	79.82%	0.824118
21PYN2-KS140794M-2	KS050428-M-9/KS061860M-3//KS060476-M-6	1436724	89.10%	0.436341
21PYN2-KS140794M-8	KS050428-M-9/KS061860M-3//KS060476-M-6	2847226	77.03%	0.773975

21PYN2-KS140804K-5	KS060167-M-7/KS061862M-17//Joe	1882322	86.05%	0.566018
21PYN2-KS140809K-3	KS061131K-13/KS061862M-17//KS061393K-5	1646874	70.70%	0.432788
21PYN2-KS140817K-1	KS040144M-3/KS061705M-11//KS061837M-2	1982502	88.23%	0.608851
21PYN2-KS140817K-4	KS040144M-3/KS061705M-11//KS061837M-2	3052136	81.92%	0.901579
21PYN2-KS140823M-3	KS060634M-5/KS061705M-11//KS080002*-1	1465960	67.79%	0.382323
21PYN2-KS140848M-1	X070487-186-31/Cedar//Joe	2495584	79.45%	0.699804
21PYN2-KS140885K-5	OCW04S671T-3/KS080448*-10//KS080040*C-13	1386498	89.84%	0.415526
21PYN2-KS140891K-1	KS061789M-3/KS080448*-10//KS060084-M-4	2765216	88.33%	0.835039
21PYN2-KS140891K-3	KS061789M-3/KS080448*-10//KS060084-M-4	2725568	77.94%	0.753651
21PYN2-KS140907M-1	Skalmeje/WB-4458//Cedar	2439956	79.59%	0.700329
21PYN2-KS140919K-11	KS10DL0060-5/09BC450#128//KS061860M-3	1669242	89.15%	0.505194
21PYN2-KS140919K-6	KS10DL0060-5/09BC450#128//KS061860M-3	1802738	87.67%	0.525024
21PYN2-KS140921K-2	KS060167-M-7/10BC347#33//KS061393K-3	2018610	68.73%	0.530762
21PYN2-KS140924K-2	BIO 10192/10BC347#33//KS070004M-3	2697806	78.36%	0.770421
21PYN2-KS140933K-1	TBIO Sinuelo/KS061837M-3//Duster	2128994	88.37%	0.656496
21PYN2-KS140933M-2	TBIO Sinuelo/KS061837M-3//Duster	957946	88.78%	0.280705
21PYN2-KS140937M-3	TBIO Sintonia/KS061131K-13//Cedar	3038412	82.48%	0.857082
21PYN2-KS140953M-2	KS061162M-4/KS061278K-1//KS061862M-17	2306420	80.06%	0.662894
21PYN2-KS140966M-4	FFR-237/Nogal//KS061862M-17	2035654	89.62%	0.628995
21PYN2-KS140973M-1	KS060167-M-8/OCW01S137T-1//Cedar	2553186	78.96%	0.728241
21PYN2-KS140973M-3	KS060167-M-8/OCW01S137T-1//Cedar	2438580	87.43%	0.727919
21PYN2-KS140977K-4	KS060167-M-8/OCW01S137T-1//Joe	3002624	78.12%	0.865673
21PYN2-KS141049M-2	KS10DL0078-11/OCW04S386S-1//WB-Grainfield	1581860	87.30%	0.461979
21PYN2-KS141054K-4	KS080002*-14/OCW04S773T-3//KS080448*C-102	1383722	88.39%	0.404841
21PYN2-KS141057M-1	KS061658K-5/OCW04S773T-3//KS061837M-2	2086162	75.06%	0.58429
21PYN2-KS141074K-9	KS10DL0057-10/X070488-187B-52//WB-Redhawk	2524106	86.37%	0.739145
21PYN2-KS141074M-2	KS10DL0057-10/X070488-187B-52//WB-Redhawk	1836716	89.14%	0.554338
21PYN2-KS141076M-1	T158/X070488-187-75//KS080040*C-13	2781350	82.38%	0.809467
21PYN2-KS14FHB0004M-2	KWS Target/Everest	1088726	67.71%	0.276954
21PYN2-KS14FHB0005M-1	Nogal/Everest	2701028	81.30%	0.796305
21PYN2-KS14FHB0439K-1	KS10DL0057-11/Antero//Zenda	2299710	85.42%	0.669344
21PYN2-KS14FHB0565K-2	09BC450#128/Basmati//KS061470M-4	2686032	78.76%	0.768766
21PYN2-KS14FHB0565K-3	09BC450#128/Basmati//KS061470M-4	1535908	74.51%	0.425606
21PYN2-KS14FHB0599M-5	Basmati/WB-Redhawk//Duster	2011020	77.58%	0.570757
21PYN2-KS14FHB0732K-2-R001		3259984	78.69%	0.931548
21PYN2-KS14FHB0732K-2-R002		2966718	79.83%	0.866982
21PYN2-KS14FHB0732M-4	KWS Erasmus/KS061193K-3//Bob Dole	3008498	89.09%	0.931337
21PYN2-KS14FHB0785M-1	KWS Target/KS061456K-4//Zenda	1907238	89.20%	0.577726
21PYN2-KS150286S-3	KS061837M-2/Larry//Joe	3221726	79.35%	0.906372
21PYN2-KS16GD0030-25	KS061278M-4/TA 10088//PI 471021	2977412	87.66%	0.916112

21PYN3-KS130036M-6	Iba/KS061406-LN~39	2983896	86.70%	0.915354
21PYN3-KS130039M-2	KS050525-K-4/KS061406-LN~39	2557108	85.98%	0.780002
21PYN3-KS130039M-3	KS050525-K-4/KS061406-LN~39	3307448	88.29%	0.995799
21PYN3-KS130039M-5	KS050525-K-4/KS061406-LN~39	2550624	89.30%	0.780101
21PYN3-KS130059M-1	Byrd/KanMark	2662898	88.56%	0.809121
21PYN3-KS130059M-4	Byrd/KanMark	3287462	86.62%	0.99115
21PYN3-KS130069K-3	KS10HW78-1/KanMark	2955334	88.22%	0.900118
21PYN3-KS130069K-4	KS10HW78-1/KanMark	2643832	88.06%	0.802132
21PYN3-KS130092K-1	KS050444-K-5/KS040144M-3	1762378	87.07%	0.540313
21PYN3-KS130223K-2	Cedar/KanMark//Antero	2085754	88.66%	0.62112
21PYN3-KS130236M-1	KS050525-K-4/KS061406-LN~39//WB-Grainfield	3384544	89.06%	1.011181
21PYN3-KS130236M-2	KS050525-K-4/KS061406-LN~39//WB-Grainfield	2331848	86.48%	0.705745
21PYN3-KS130236M-7	KS050525-K-4/KS061406-LN~39//WB-Grainfield	3438280	86.89%	1.006236
21PYN3-KS130254K-2	AP11T2132/KS031009K-4//KS040640K-1	556484	89.48%	0.104164
21PYN3-KS130258K-2	KS061406-LN~39/Clara CL//KS050278M-1	2542338	88.26%	0.786744
21PYN3-KS130291M-7	KS060285-M-1/KS061406-LN~39//Joe	2220426	88.97%	0.688059
21PYN3-KS130292K-5	SY Wolf/KS061406-LN~39//Joe	2331078	89.50%	0.715985
21PYN3-KS130293M-2	Winterhawk/KanMark//Joe	2780898	87.91%	0.844462
21PYN3-KS130294K-1	AP11T1822/KanMark//Joe	2785638	87.90%	0.843422
21PYN3-KS130295K-1	TAM-111/KanMark//Joe	1912238	87.37%	0.541455
21PYN3-KS130306K-1	KS040810NT-8/KS061478M-10//Larry	2926108	89.80%	0.898818
21PYN3-KS130318K-3	Duster/KS060430-M-6//Bob Dole	2825534	88.41%	0.837608
21PYN3-KS130318M-3	Duster/KS060430-M-6//Bob Dole	2940406	88.22%	0.877692
21PYN3-KS130321M-1	KS040168M-2/KS061406-LN~39//Bob Dole	2277436	85.76%	0.66278
21PYN3-KS130326M-1	TAM-304/KanMark//Bob Dole	2738224	87.22%	0.840772
21PYN3-KS130346K-5	KS050278M-1/TAM-304//Bob Dole	2805464	89.11%	0.868805
21PYN3-KS130346K-6	KS050278M-1/TAM-304//Bob Dole	2930366	88.34%	0.884778
21PYN3-KS130385M-4	KS060041-M-1/KS060285-M-4//KS061860M-3	3544308	88.03%	1.075734
21PYN3-KS130410K-1	KS10HW78-1/KS061406-LN~32//KS061862M-17	2922290	87.58%	0.899647
21PYN3-KS130410K-3	KS10HW78-1/KS061406-LN~32//KS061862M-17	2781548	90.10%	0.86199
21PYN3-KS130439K-5	AP11T1115/KanMark//KS080448*-10	2991650	88.62%	0.925835
21PYN3-KS130446K-1	KS061837M-3/KS040144M-3//KS060084-M-4	2697534	88.28%	0.814006
21PYN3-KS130467K-4	KS060430-M-1/KS061478M-10//KS080394*C-32	2277822	87.88%	0.662358
21PYN3-KS130515M-5	Byrd/KanMark//09BC450#128	3157666	87.78%	0.958882
21PYN3-KS130528M-3	OK09634/KS061278M-4//09BC450#128	2484176	86.09%	0.73497
21PYN3-KS13FHB0321K-2	KS040168M-2/KS061406-LN~39//Bob Dole	2552532	88.68%	0.794686
21PYN3-KS13FHB0323K-3	Clara CL/KS061406-LN~39//Bob Dole	2201598	87.98%	0.627621
21PYN3-KS140004K-4	KWS Target/Everest	2434644	88.25%	0.740827
21PYN3-KS140010K-5	KS061131M-17/Cedar	2719776	88.36%	0.830416
21PYN3-KS140014M-4	Everest/Overley	3034816	87.42%	0.929691
21PYN3-KS140022K-2	Stadium/Overley	2698948	86.28%	0.822157

21PYN3-KS140036M-5	KS10DL0057-11/Antero	2475948	85.51%	0.727029
21PYN3-KS140052M-11	FFR-506/SY Wolf	3353692	88.40%	1.010031
21PYN3-KS140052M-3	FFR-506/SY Wolf	3030272	87.37%	0.922042
21PYN3-KS140060M-5	KanMark/WB-4458	2754874	86.42%	0.840441
21PYN3-KS140060M-6	KanMark/WB-4458	1312830	87.65%	0.378918
21PYN3-KS140064M-1	Basmati/WB-Redhawk	2491220	86.09%	0.756133
21PYN3-KS140064M-10	Basmati/WB-Redhawk	2229194	88.72%	0.685325
21PYN3-KS140092M-1	KS061837M-3/KS040144M-3	2767476	87.87%	0.848472
21PYN3-KS140138M-2	KS061278M-4/Joe	2623768	88.28%	0.797946
21PYN3-KS140148M-8	KS080040*C-13/Bob Dole	2569588	88.70%	0.759677
21PYN3-KS140153K-5	KWS Erasmus/KS061193K-3	2640104	89.19%	0.826773
21PYN3-KS140153K-6	KWS Erasmus/KS061193K-3	2563254	88.03%	0.773311
21PYN3-KS140159M-11	KS060750-BE~B/KS061193K-1	2558690	87.27%	0.77711
21PYN3-KS140159M-12	KS060750-BE~B/KS061193K-1	2365590	88.41%	0.727992
21PYN3-KS140159M-14	KS060750-BE~B/KS061193K-1	2366224	88.76%	0.730557
21PYN3-KS140159M-16	KS060750-BE~B/KS061193K-1	2458480	87.47%	0.73616
21PYN3-KS140159M-19	KS060750-BE~B/KS061193K-1	2635338	86.68%	0.77568
21PYN3-KS140159M-20	KS060750-BE~B/KS061193K-1	2090984	89.13%	0.663791
21PYN3-KS140159M-4	KS060750-BE~B/KS061193K-1	2175098	87.90%	0.64556
21PYN3-KS140160K-7	KS061880M-3/KS061193K-1	2800738	88.01%	0.834334
21PYN3-KS140164K-2	KS050278M-1/KS061880M-3	2588316	87.10%	0.761514
21PYN3-KS140178K-2	KS061860M-3/KS061456K-4	2804438	87.96%	0.804316
21PYN3-KS140189K-5	KS061470K-2/KS061860M-3	2532646	90.00%	0.769409
21PYN3-KS140206K-10	KS061237K-1/KS061705M-11	2087512	89.04%	0.649988
21PYN3-KS140206K-2	KS061237K-1/KS061705M-11	1608920	87.81%	0.474465
21PYN3-KS140206K-3	KS061237K-1/KS061705M-11	2845156	89.54%	0.868118
21PYN3-KS140207K-12	KS060634M-5/KS061705M-11	2332582	87.90%	0.695301
21PYN3-KS140234M-1	KS061162M-2/KS080448*-10	3428850	88.96%	1.061026
21PYN3-KS140273M-6	BIO 10192/10BC347#33	2864656	86.50%	0.851914
21PYN3-KS140274M-10	TBIO Mestre/10BC347#33	2277346	87.59%	0.677537
21PYN3-KS140307M-3	TBIO Sinuelo/KS061837M-3	3180886	89.73%	0.987969
21PYN3-KS140325M-1	Bob Dole/OCW04S773T-3	2152472	87.16%	0.614266
21PYN3-KS140394M-1	KS080394*C-32/KS061837M-2//Larry	2488646	87.78%	0.745887
21PYN3-KS140401K-5	SY Wolf/KS080448*-10//Iba	2529266	89.83%	0.765785
21PYN3-KS140401M-7	SY Wolf/KS080448*-10//Iba	2113184	85.00%	0.597487
21PYN3-KS140405K-1	FFR-237/KS050278M-1//Zenda	2937276	86.29%	0.882134
21PYN3-KS140469M-3	Stadium/Overley//KS061862M-17	2081260	88.11%	0.617422
21PYN3-KS140479M-2	KS040640K-1/Byrd//KS060084-M-4	3190248	88.18%	0.933988
21PYN3-KS140484K-4	FFR-230/Antero//KS061393K-3	3288464	87.93%	0.980388
21PYN3-KS140484M-2	FFR-230/Antero//KS061393K-3	3461996	89.28%	1.057952
21PYN3-KS140484M-5	FFR-230/Antero//KS061393K-3	2761266	88.67%	0.838333

21PYN3-KS140490K-10	BIO 10377/Overley//KS060476-M-6	2445560	88.41%	0.768574
21PYN3-KS140490K-3	BIO 10377/Overley//KS060476-M-6	2545012	87.38%	0.76746
21PYN3-KS140490K-4	BIO 10377/Overley//KS060476-M-6	2830768	90.48%	0.858815
21PYN3-KS140490K-9	BIO 10377/Overley//KS060476-M-6	2454944	87.27%	0.73433
21PYN3-KS140491K-13	BIO 10377/Overley//SY Wolf	2704090	88.55%	0.832169
21PYN3-KS140499K-1	KS060808K-2/Overley//KS061862M-17	2375974	88.37%	0.725728
21PYN3-KS140516K-2	KS060476-M-6/KS061837M-2//KS061470M-4	2063990	90.04%	0.6386
21PYN3-KS140572M-2	KS060167-M-7/10BC347#33//KS080040*C-13	2303666	87.93%	0.704475
21PYN3-KS140572M-6	KS060167-M-7/10BC347#33//KS080040*C-13	2601928	89.00%	0.773094
21PYN3-KS140572M-8	KS060167-M-7/10BC347#33//KS080040*C-13	2418792	90.19%	0.750011
21PYN3-KS140582K-4	BIO 10377/KS061837M-3//WB-Redhawk	2654254	87.17%	0.813255
21PYN3-KS140613K-4	MERCATO//JNRB.5/PIFED/3/KanMark//Iba	2429042	87.94%	0.733995
21PYN3-KS140624K-2	MERCATO//JNRB.5/PIFED/3/KanMark//KS061470M-4	3180762	89.43%	0.97097
21PYN3-KS140626K-9	OCW03S112T-6/KS040144M-3//SY Wolf	2121662	87.57%	0.623811
21PYN3-KS140628M-1	SY Wolf/KS061406-LN~39//Joe	2823254	88.58%	0.849644
21PYN3-KS140628M-4	KS061837M-3/KS040144M-3//KS060084-M-4	2582950	86.80%	0.757127
21PYN3-KS140725K-1	WB-Grainfield/KS061278M-4//KS061837M-2	2330726	87.34%	0.702452
21PYN3-KS140725K-3	WB-Grainfield/KS061278M-4//KS061837M-2	2436834	87.94%	0.744114
21PYN3-KS140732K-1	KWS Erasmus/KS061193K-3//Bob Dole	2262660	86.33%	0.67355
21PYN3-KS140732M-1	KWS Erasmus/KS061193K-3//Bob Dole	2770036	87.55%	0.808532
21PYN3-KS140732M-4	KWS Erasmus/KS061193K-3//Bob Dole	2659412	89.74%	0.798519
21PYN3-KS140763M-5	BIO 10589/KS061880M-3//KS061705M-11	2841066	89.68%	0.860134
21PYN3-KS140791K-10	MERCATO//JNRB.5/PIFED/3/KS061456K-4//KS071064K-1	1634240	90.14%	0.510233
21PYN3-KS140791K-11	MERCATO//JNRB.5/PIFED/3/KS061456K-4//KS071064K-1	2982224	88.39%	0.921401
21PYN3-KS140794K-4	KS050428-M-9/KS061860M-3//KS060476-M-6	2960642	88.16%	0.918575
21PYN3-KS140794K-6	KS050428-M-9/KS061860M-3//KS060476-M-6	1836832	86.26%	0.535993
21PYN3-KS140794K-9	KS050428-M-9/KS061860M-3//KS060476-M-6	3091848	86.23%	0.941398
21PYN3-KS140794M-3	KS050428-M-9/KS061860M-3//KS060476-M-6	2399834	86.89%	0.700155
21PYN3-KS140794M-6	KS050428-M-9/KS061860M-3//KS060476-M-6	1976648	87.85%	0.608156
21PYN3-KS140848K-3	X070487-186-31/Cedar//Joe	2455504	87.76%	0.732188
21PYN3-KS140873M-4	10BC347#33/KS080448*-10//Cedar	2694328	86.44%	0.791376
21PYN3-KS140883K-1	Larry/KS080394*C-32//KS070597M-4	2929868	85.32%	0.853638
21PYN3-KS140885K-3	OCW04S671T-3/KS080448*-10//KS080040*C-13	2345000	86.93%	0.717217
21PYN3-KS140885M-3	OCW04S671T-3/KS080448*-10//KS080040*C-13	1567460	87.66%	0.468095
21PYN3-KS140885M-5	OCW04S671T-3/KS080448*-10//KS080040*C-13	2945942	89.09%	0.895089
21PYN3-KS140890K-1	KS060634M-5/KS080394*C-32//KS061862M-17	3029250	89.13%	0.915519
21PYN3-KS140892M-5	KS061789M-3/KS080448*-10//KS061837M-2	2693052	88.11%	0.763388
21PYN3-KS140913K-2	KS10DL0080-3/09BC450#128//KS060371-M-3	2043114	85.03%	0.590197
21PYN3-KS140923K-1	KS061582M-5/10BC347#33//KanMark	2251150	87.86%	0.693855
21PYN3-KS140923K-4	KS061582M-5/10BC347#33//KanMark	2321706	87.83%	0.699353
21PYN3-KS140923M-3	KS061582M-5/10BC347#33//KanMark	2517002	87.38%	0.700199

21PYN3-KS140938M-2	BIO 10510/KS061837M-3//WB-Grainfield	2776106	88.00%	0.840712
21PYN3-KS140959K-5	FFR-237/Nogal//KS061470M-4	1773010	86.96%	0.485952
21PYN3-KS140973M-4	KS060167-M-8/OCW01S137T-1//Cedar	2886844	87.96%	0.896448
21PYN3-KS140992K-2	KS080448*-10/OCW01S137T-1//Larry	1890820	87.88%	0.552764
21PYN3-KS141009K-5	X070487-186-27/OCW04S658T-4//Joe	2982174	87.25%	0.872843
21PYN3-KS141009M-3	X070487-186-27/OCW04S658T-4//Joe	1686950	87.55%	0.488901
21PYN3-KS141032K-5	Basmati/OCW04S386S-1//KS061470M-4	1518280	90.71%	0.45264
21PYN3-KS141049M-3	KS10DL0078-11/OCW04S386S-1//WB-Grainfield	2288654	86.35%	0.702461
21PYN3-KS141050K-1	KS10DL0078-11/OCW04S386S-1//Clara CL	2393298	87.73%	0.693528
21PYN3-KS141050K-10	KS10DL0078-11/OCW04S386S-1//Clara CL	2644900	87.59%	0.832458
21PYN3-KS141050K-4	KS10DL0078-11/OCW04S386S-1//Clara CL	2948746	89.99%	0.887046
21PYN3-KS141057K-1	KS061658K-5/OCW04S773T-3//KS061837M-2	3185888	87.89%	0.949137
21PYN3-KS141057M-5	KS061658K-5/OCW04S773T-3//KS061837M-2	2469872	89.73%	0.762361
21PYN3-KS141068K-1	KS10DL0080-3/X070488-187B-52//KS061470M-4	3202328	89.07%	0.981128
21PYN3-KS14FHB0001K-1	KanMark/Everest	2159856	87.92%	0.645792
21PYN3-KS14FHB0064M-3	Basmati/WB-Redhawk	3252964	89.72%	0.984598
21PYN3-KS14FHB0565M-1	09BC450#128/Basmati//KS061470M-4	2801896	87.97%	0.831098
21PYN3-KS14FHB0565M-6	09BC450#128/Basmati//KS061470M-4	3028962	89.96%	0.908794
21PYN3-KS14FHB0588K-3	BIO 10536/KS12FHB(A+)-44//Zenda	3039334	89.39%	0.924715
21PYN3-KS14FHB0598K-11	Basmati/WB-Redhawk//KS061393K-3	2713064	88.21%	0.838425
21PYN3-KS14FHB0599M-2	Basmati/WB-Redhawk//Duster	2684640	88.23%	0.797271
21PYN3-KS14FHB0968M-5	09BC450#128/Basmati//KS070597M-4	2470258	89.53%	0.768439
22AYN3-KS130318K-3	Duster/KS060430-M-6//Bob Dole	1665356	85.20%	0.49224
22SCB-KS130318K-3	Duster/KS060430-M-6//Bob Dole	2351672	85.31%	0.676447
23AYN2-KS150286C-5	KS061837M-2/Larry//Joe	2236280	86.23%	0.683047
23AYN2-KS150286C-9	KS061837M-2/Larry//Joe	2802196	90.37%	0.829632
23AYN2-KS150721C-4	Fuller/Larry//Joe	2188034	87.59%	0.644887
23AYN2-KS150826C-2	WB-4458/KS040144M-3//Joe	1983244	85.33%	0.59894
23AYN3-KS150167-18	Bob Dole/Joe	2590728	84.72%	0.768814
23AYN3-KS150720-6	KS061278K-2/KS040144M-3//Joe	2063852	86.33%	0.637589
23AYN3-KS170604S-4	KS090387K-26/KS090391M-2//Bob Dole	1563564	86.35%	0.457561
23AYN3-KS170778S-6	KanMark/Sossego//KS120642M~8	1957438	87.36%	0.603394
23AYN4-KS150261-1	Pioneer 25R78/WB-4458	1874336	85.19%	0.538653
23AYN4-KS150417-3	OCW00S014S-1-1/Iba//Bob Dole	2338274	86.10%	0.721163
23AYN4-KS150417-5	OCW00S014S-1-1/Iba//Bob Dole	2095512	86.21%	0.624537
23AYN4-KS170778S-3	KanMark/Sossego//KS120642M~8	1661820	86.66%	0.490194
23AYN5-KS150167-17	Bob Dole/Joe	2557258	86.33%	0.74461
23AYN5-KS150167-19	Bob Dole/Joe	2437138	86.60%	0.736725
23AYN5-KS170453S-12	SY Flint/Bob Dole//KS12DH0230-47	2594158	87.82%	0.781462
23AYN5-KS170684S-9	Larry/Bob Dole//KS Hatchett	1709298	86.12%	0.483688

23KIN-KS160786S-6	SY Wolf/Joe//WB Grainfield	1785154	84.56%	0.516763
23KIN-KS170013D-19	Bob Dole/KS13H20-5	4416308	87.26%	1.35135
23KIN-KS170025D-11	KS061705M-11/KS Hatchett	2234960	88.78%	0.713541
KS140794M-4-S171		2645274	88.18%	0.774777

Supplementary Table 4. 3 Line pedigree information of the USWBSI population.

Taxa	Pedigree
KS Providence	
MTFH23309	Emerson/MT1469
MTFH23310	Emerson//MTS1577
KS170013D-19	
KS16DH0010-17	
MTFH2290	MTCL1502*3//MTCL1329/MN-11394-6
NE17441	Hitch/NE07409
NE18455	TX07A001505/NE06430
NE19619	TRCH/SRTU//KACHU/7/VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/163/8/NI08708
NE21448	NE09521/NE13483V//NE12561
NE21503	NE10589/KS11HW39-5
NE21524	NI08708/NE09517
NE21579	N11MD2172/NE06545 (FREEMAN)//NE09521
NE21646	11F-776/N11MD2172
NEB-145-12	LCH13NEDH-14-69/NE13483V
NEB-148-42	NW13493/LCH13NEDH-5-72
NEB-150-6	LCH13DH-20-87/NE14606
NEB-151-3	PSB13NEDH-14-83/LCH13DH-20-87
NHH19651	NI10703/NH09563//Brawl CL plus
NHH21415	OK1059060/NH11489//NHH144922-2
NW15443	OR 2060108/NW03681//NW03666
KS160855S-4	
KS140535C-7	
KS140924K-2	BIO 10192/10BC347#33//KS070004M-3
KS140064M-6	
KS140490K-10	BIO 10377/Overley//KS060476-M-6
KS160563S-3	
KS170025D-11	
KS160476S-9	
KS16FHB0211-101	Art/KS12FHB(A-)-1
CO20SF095R	CO15SFD030/Canvas

CO20SF227R	CO15SFD030/Canvas
CO20SF240R	CO15SFD030/CO12M112
CO20SF160R	CO15SFD030/Joe
CO20SF163R	CO15SFD030/Joe
CO20SF192R	CO15SFD030/LCS Chrome
CO20SF198R	CO15SFD030/LCS Chrome
CO20SFD033W	2333333333111111111111/
CO20SFD071R	CO16SF029/CO16SF078
CO20SFD011R	CO16SFD020/CO16SF029
CO20SFD013R	CO16SFD020/CO16SF029
CO20SFD019R	CO16SFD020/CO16SF029
CO20SF014W	Warhorse/Breck//CO12D1028
CO20SF141R	Warhorse/Breck//CO12D1028
MTFH2292	MTCL1502*3//MTCL1329/MN-11394-6
MT2019	MT10114/MT10128//MTW1251
KS160765S-6	
KS160786S-6	
KS14FHB0732M-4	
KS160786S-4	
KS19H10	KS11HW15-4-1/KS060476-M-6
KS20H89	TATANKA/14-50809(KS07HW52-5/09SGO2-14-6(KS03HW12-1/RonL SEL.))
KS20HW103	14-50809(KS07HW52-5/09SGO2-14-6(KS03HW12-1/RonL SEL.))/KS14HW25(TAM112/KS06HW46-3)
KS20H105	TX08A001249(TAM 112/TX98D1158 (TAM303 sib))/KS14H180(T151/KS08HW111-1)
KS20H106	TX08A001249(TAM 112/TX98D1158 (TAM303 sib))/KS14H180(T151/KS08HW111-1)
KS20H124	14-50809(KS07HW52-5(KS025580/KS02HW25)/09SGO2-14-6(KS03HW12-1/RonL SEL.))/Brawl CL Plus
KS20HD134	Joe/CO11D243(CO050173/Cowboy)//TATANKA/Bob Dole
KS20HDW185	KS11HW53/Snowmass
KS21H30	JOE/14-50809(KS07HW52-5/09SGO2-14-6(KS03HW12-1/RonL SEL.))//OK09125(OK95616-98-6756/Overlay)
KS21H31	KS12H18-3//KS14-50809/KS14HW25(TAM112/KS06HW46-3)
KS21H36	TATANKA/WP627(RonL/U5287-(4-5)-47-1-1//KS010209K-5)//KS13HW74(FRED-2-18-1/KS02HW35 TR)/Larry
KS21H38	KS13HW7(KS04HW101-3/KS04HW119-3)/Larry//KS13HW74(FRED-2-18-1/KS02HW35 TR)/CO11D1539(Byrd/Antero)
KS21HD147	TATANKA/CO11D1767(CO07MAS114/Cowboy)//KS14H205-6-6(TAM112/KS06HW46-3)
KS21HD158	TATANKA/OK12DP22002-042(Billings/OK08328)
KS21HD173	KS15H180-6(KS07HW52-5//BILL BROWN/KS08HW111-1)/17F1-7-19-5(CO11D1767/KS060476-M-6//KS17WGRC62)
D18B016-5	
D18B025-8	
D18B055-2	
D18B072-2	

D19B019-2	
D19B033-2	
D19B108-3	
D19B164-3	
D20B088-2	
D20D100-9	
D20C031-1	
D20D009-9	
D20D063-2W	
D20D064-3	
D20D108-6	
MTFH23301	MT1138//Colter/Emerson
MTFH23302	MT1138//Colter/Emerson
MTFH23303	MT1138//Colter/Emerson
MTFH23304	MT1265*2/Emerson
MTFH23305	MT1469//MT1360/Emerson
MTFH23306	MT1469//MT1360/Emerson
MTFH23307	MT1469//MT1360/Emerson
MTFH23308	Emerson//Northern/KS10HW78-1-1
BSYH- Baishanyuehuang	
BSYH- Baishanyuehuang_2	
CS	
CS_2	
FHB3-Positive	
FHB3-Positive_2	
MTS2068	(Judee sib, MTS0819)//08X350-A6/Warhorse
OK21534	
U7693-L-7	ZENDA//Zenda/KS09WGGRC51-J
U8345-B-4-1	KS0890099M-3//LCS_CHROME/Syn106
OK21120	
OK23414	
OK23416	
OK23422	
OK236B29	
OK236B30	
OK236B37	
OK236B40	
OK236B41	

OK22310	
OK16103083	Garrison*3/Wesley Fhb1-106
OK_Corral	
OK18217-19HR-4	OK07214/OK10338
OK20418-7C21	KS040640K-1/OK11D25056
OK16107133-19HR-3	OK12621/Doublestop CL+
OK16107133-19HR-4	OK12621/Doublestop CL+
OK20708	Miranda/OK11D25056
OK21538	OR2100037H/OK12620
OK198417C	OK10943C/OK09935C//OK09915C
OK20925WF	TX06A001186W/OK12716W
OK19515-20HR-2	BOW/FGK15//GEREK79(OCW00S014S)(OK08518)/3/GARZA/BOY//AE.SQ.(433)/5/GKARON/AGSECO7846//2180(OK99711)/4/SITE/MO/3/VORONA/BAU//BAU/5/CUTTER(OCW02S158T-5)
OK2189176WF	2*OK12621/WA8183(YR17)
OK2189178WF	2*OK12621/WA8183(YR17)
OK21227	MD01W233-06-1/OK07214-116190-24//OK13216
TX19A001030	TAM 113/CO07W245 (=KS01HW152-1/TAM 111)
TX15A001482-19AZ16	TX06A001431/TX06A001281
TX18DH129	Tatanka/TAM 114
TX18DH266	[(TX1112-108/TAM 113)/(TAM 1112-20/Gallagher)]/[(TX12A001044/TAM114)/(Joe/TX12A001078)]
TX18DH287	[(TX1112-108/TAM 113)/(TAM 1112-20/Gallagher)]/[(TX12A001044/TAM114)/(Joe/TX12A001078)]
TX18DH313	CO11D1767/TX12V7229
TX15M8456-19AZ435	X08A586S [=ME-1-1/TX04M410164]/TX01V5134RC-3
TX15M8456-19AZ504	X08A586S [=ME-1-1/TX04M410164]/TX01V5134RC-3
TX19M3183	TAM 304/TX10A001016 (=Doans/TX01M5009-28)
TX14A001035	OK03522/TX03A0563
TX14V70214	Art/TAM 401
TX15M8024	TAM 203/Duster
TX16M9216	X09A440S [=TX07A001482/TAM 401]/Duster
TX18A001119	TX08A001249 (=TAM 112/TX98D1158)/TX07A001418-YRR Resel#2
TX18A001132	TX09A001205 (=TAM 303/TX99U8618)/TAM 114
KS150826C-2	
KS150167-18	
KS150720-6	
KS170778S-6	
KS150167-19	
KS150286C-5	
KS16FHB0211-101-7	
KS16FHB0211-201-17	

KS16FHB0211-201-3	
KS16FHB0284-101-12	
KS16FHB0284-101-3	
KS16FHB0284-101-7	
KS15RLGDPOP30-1	
KS15RLGDPOP29-3	
KS15RLGDPOP61-2	
NE18435	TX07A001505/NE05496
NI17410	TX06A001281/NI04420
KS150286C-9	
KS150721C-4	
KS170604S-4	
NE21656	Denali/KS11HW39-6
KS150523-6	
KS150261-1	
NEB-147-53	OVERLAND-FHB10/LCH13-032
NE19406	OK07214/NE05496//WB-Grainfield
NHH21441	NHH144922-2/NHH144913-4
NE21489	MV36-13/NE09521//NE12571
NE21582	NE12443/NI04421 (Robidoux)
KS150380-1	
NE18445	NE09517/NE06430
CO19393R	24 Elite 13
CO19410R	24 Elite 14
CO1S129W	24 Elite 17
CO20D036R	24 Elite 8
CO20D068W	24 Elite 19
CO20D073W	24 Elite 20
CO20D079R	24 Elite 21
CO20D097R	24 Elite 22
CO20D108R	24 Elite 23
CO21D1411R	24 Elite 24
CO21D1790R	24 Elite 25
CO200247W	24 Elite 26
CO200215R	24 Elite 27
CO200168R	24 Elite 28
CO200037R	
HCD-Huangcandou	

HCD-Huangcandou 2	
HYZ-Haiyanzhong	
HYZ-Haiyanzhong_2	
JagFHB3-191	
JagFHB3-191_2	
23Nord-179	Flourish/Hawken//16DH1512
23Nord-180	Flourish/Hawken//16DH1632
23Nord-181	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-13
23Nord-182	F3:4 14K456-1-35F-9/15DH1287
23Nord-183	Novus 4/3/CM82036/Jerry//Jerry/3/Flourish/Hawken
23Nord-184	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-13
23Nord-185	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-14
23Nord-187	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-15
23Nord-189	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-16
23Nord-190	Norstar-Fhb1,Sr50/Monument/3/Norstar-Fhb1/Jerry//13RGON-17
23Nord-192	Crux/14Nord-01/5/CM82036/Jerry/4/Thatcher-Lr53/Jerry//Decade/3/Accipiter
23Nord-193	Crux/14Nord-01/5/CM82036/Jerry/4/Thatcher-Lr53/Jerry//Decade/3/Accipiter
23Nord-194	Crux/14Nord-01//Flourish/Hawken
24SeniorB-41	GP80/Novus-4//Monument/3/14Nord-01
23Nord-196	Flourish/Hawken//11M221A-56-3
KS150167-17	
KS150288-4	
Jagger-FHB3-2008-193	
Jagger-FHB3-2008-193_2	
D20B086-1	
D20B098-5	
D20B046-1	
D20B010-4	
D21D126-5	
D21D030-3	
D21C078-3	
D21B052-1	
D21C048-2	
D22B052-2	
D22C234-3	
MTV2164	MT1265*2/Joe
MTS22104	Bobcat/(CO15SFD092, Fortify SF sib)
MTS21103	Bobcat//LCS Jet/MTS1703

MTS2286	MT1090/WB-Quake//Spur
MT2270	MT1747*2/Flathead
MTS22100	Bobcat/(CO15SFD092, Fortify SF sib)
MT2219	MT1445/Spur
MT2230	MT1450/OK09528
MTS2298	Bobcat/(CO15SFD092, Fortify SF sib)
MTS22118	Bobcat*2/OR2120012R
MTS2272	MTS1305//MTS1303/04X494cE33-4
MT2262	MT1747*2/Flathead
MTFHS23315	MTS1591//Judee/Emerson
MTFHS23316	MTS1596//Judee/Emerson
MT2335	MV-Kolompos/MT1332
Ji5265	
OK21424F	25R56/OK12621
OK21428F	25R34/OK12621
OK21022F-22HR-3	25R34/OK12621
Ji5265_2	
Jimai22-Fhb7	
Jimai22-Fhb7_2	
Ning 7840	
OK20738	KS040477K-12/OK11D25056
OK21304	GT 7391-7 NT/OK13237//OK07214-116190-20
OK21PS821W_R	
OK19105134F	TCI012160/OK08413//OK10132
Ning 7840_2	
OK22123	OK14015/OK13411//OK118036
OK2234	
TX20A001189	TX08A001249 [=TAM 112/TX98D1158]/CO07W722-F5 [=KS02HW89-1/BC97ROM-41W]
TX20A001194W	TX08A001249 [=TAM 112/TX98D1158]/CO07W722-F5 [=KS02HW89-1/BC97ROM-41W]
TX18DH319	CO11D1767/TX12V7229
TX18DH132	Tatanka/TAM 114
TX18DH498	X16A406S {=TX04DH231-12/LCS Mint}/AMPSY473
TX21A001241	TX08A001249/TX12A001506
TX18DH858	TAM 204/TAM 114
TX20M4131	TX1112-74 (=TAM 112/TAM 111)/Gallagher (=OK07214=OK93P656-RMH3299/OK99711 F4:11)
TX20M4292	TX11D3043/TX12M4004
TX20M4294	TX11D3043/TX12M4004
TX20M4135	X12A671S [=AK11SPWCB-3087 (=BABAX/LR42//BABAX*2/3/BRAMBLING)/TAM 304 (=TX01D3232=TX92U3060/TX91D6564 (=X95U104-P66))]/TX08V7313 (=TX97V5300/TAM 111)
TX21M5145	TAM 114/Redhawk

TX21M5175	TAM 114/TX12M4063
TX21M5445	Redhawk/TAM 204 (=TX06V7266)//TX12M4065
TX21M5741	TAM 114 (=TX07A001505)/TX12M4065//TAM 305
U7321.B2_B.7	ZENDA/CO15D006R
U7710-E-2-2	NE10589//CULVER/KS09WGGRC51-C
Overland	
Overland_2	
OverlayFHB3-184	
KS22H88	TATANKA/CO12M112(Winterhawk/Danby//CO03W054)//KS15H137-2(KS08HW176-4//BILL BROWN/KS08HW61-2)
KS22H126	KS13H9-1-2/KS14HW111-6-1-2(KS05HW122-5-2//KS05HW15-2-2/KS02HW35 TR)
OverlayFHB3-184_2	
KS22H19	KS14H89-4(FRED-2-4-5/KS02HW35 TR//KS05HW122-5-2)/TAM114//KS15H137-2(KS08HW176-4//BILL BROWN/KS08HW61-2)
KS22H28	KS12HW104(KS05HW121/WILMA(LR))/TX09V7446(TAM 112/KS03HW156-3)//KS15H137-2(KS08HW176-4//BILL BROWN/KS08HW61-2)
KS22H40	KS12HW104(KS05HW121/WILMA(LR))/TAM114//KS Western Star
KS22H53	KS14H81-4(KS980554-12--9/DANBY TR//KS05HW122-5-2)/TATANKA//KS Western Star
KS22H84	JOE/TAM114//CO11D1767(CO07MAS114/Cowboy)/Larry
umai3	
umai3_2	
WGRC61	
WGRC61_2	
Wheaton	
Wheaton_2	

CHAPTER FIVE

5.0 CONCLUSION

This research identified existing haplotypes and rare genomic regions in domesticated wheat as additional sources of resistance to Fusarium head blight (FHB), *barley yellow dwarf virus* (BYDV), and *soil-borne wheat mosaic virus* (SBWMV) by analyzing a diverse panel of wheat cultivars from United States public breeding programs. The findings demonstrated the genetic complexity of plant disease resistance traits, which are influenced by multiple genes and agronomic factors such as plant height and heading date. The study revealed phenotypic plasticity within the diversity panel in response to FHB, BYDV, and SBWMV, and identified key targets for prioritizing increased resistance to these diseases in hard winter wheat (HWW) and soft winter wheat (SWW). These results contribute to a broader understanding of genetic resistance to FHB, BYDV, and SBWMV, thereby informing breeding selection strategies.

The potential of genomic selection and forward prediction models is high for increasing genetic gain in FHB resistance in wheat. The research illustrated the use of different GS models and SNP densities and their application for estimating breeding values across diverse wheat cultivars and germplasm backgrounds. The GS cross-validation and forward prediction accuracies demonstrated the use of diversity panel and uniform regional nursery lines as a resource for genomic selection coupled with a high-quality sequence data platform.

Whilst the identification of target regions to prioritize for improved FHB, BYDV, and SBWMV resistance, there is still further research to fine-map and identify the causal genes, and stack multiple resistance haplotypes for robust resistance. Genomic selection (GS) and forward genomic prediction represent promising approaches to support speed breeding initiatives aimed at enhancing Fusarium head blight (FHB) resistance. However, more extensive research is required

to ensure consistent prediction accuracies across complex FHB-related traits and multiple environments since genotype-by-environment interactions plays a critical role.

CHAPTER SIX

6.0 TECHNICAL REPORTS

Tidakbi, L., Davis, M., Bruce, M., Fritz, A., Zhang, G., and Rupp, J.L., (2023c). Reaction of Kansas Interstate Nursery winter wheat accessions to Fusarium head blight, 2022. Plant Disease Management Report (PDMR), 17:CF065,

<https://www.plantmanagementnetwork.org/pub/trial/PDMR/reports/2023/CF065.pdf>

Tidakbi, L., Davis, M.A., Bruce, M.A., Andersen Onofre, K., and Rupp, J.L., (2023b). Evaluation of foliar fungicides for the control of tan spot in winter wheat, 2022. Plant Disease Management Report (PDMR), 17:CF075

<https://www.plantmanagementnetwork.org/pub/trial/pdmr/reports/2023/CF075.pdf>

Tidakbi, L., Davis, M., Bruce, M., Fritz, A., Zhang, G., and Rupp, J.L., (2023a). Reaction of Kansas Interstate Nursery winter wheat accessions to Fusarium head blight, 2022. Plant Disease Management Report (PDMR), 17:CF080. <https://www-plantmanagementnetwork-org.er.lib.k-state.edu/pub/trial/PDMR/reports/2023/CF080.pdf>

Tidakbi, L., Bruce, M., Davis, M., Ibrahim, A., Rudd, J., Carver, B., and Rupp Noller, J. (2024) Disease Severity of Selected Southern Wheat Cultivars to Fusarium Head Blight (FHB), 2023.18:CF091, <https://www-plantmanagementnetwork-org.er.lib.k-state.edu/pub/trial/pdmr/volume18/abstracts/CF091.asp>

Tidakbi, L., Zhu, D., Bruce, M., Davis, M., Fritz, A., Zhang, G., and Rupp Noller, J. (2024) Reaction of Selected Winter Wheat Cultivars in Kansas to Fusarium Head Blight (FHB), 2023. Plant Disease Management Report, 18:CF099, <https://www-plantmanagementnetwork-org.er.lib.k-state.edu/pub/trial/pdmr/volume18/abstracts/CF099.asp>

Tidakbi, L., Bruce, M., Jordan, K., Davis, M., Davis, D., Marais, G., Seghal, S., Mondal, S. and Rupp Noller, J. (2024), Disease Severity of Selected Northern Wheat Cultivars to Fusarium Head Blight (FHB), 2023, Plant Disease Management Report,18:CF100, <https://www-plantmanagementnetwork-org.er.lib.k-state.edu/pub/trial/pdmr/volume18/abstracts/CF100.asp>

Tidakbi, L., Bruce, M., Jordan, K., Davis, M., Rupp Noller, J., (2024), Reaction of Selected Winter Wheat Cultivars to Fusarium Head Blight (FHB), 2023, Plant Disease Management Report, 18:CF101, <https://www-plantmanagementnetwork-org.er.lib.k-state.edu/pub/trial/PDMR/reports/2024/CF101.pdf>

Reaction of selected winter wheat cultivars to Fusarium head blight (FHB), 2022

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. The field soil type was Chase silty clay loam (pH = 6.5). A randomized complete block design was used with four replicates of 65 different wheat cultivars (entries) submitted for evaluation by private breeders and the checks namely ‘Everest’ (moderate resistance), ‘Overley’ (susceptible), and ‘Karl 92’ (moderately susceptible). Experimental plots were five rows 0.51 m wide and 0.1902 m long and were seeded on 7 Oct 21. Sterile corn kernels were used for inoculum production. Kernels were colonized with native, aggressive *Fusarium graminearum* isolate GZ-3639 and air-dried. Field application of the inoculum was done in early spring on 15 Apr, 1 May, and 15 May at a rate of 53 g/m². Moisture conditions on the nursery necessary for *Fusarium graminearum* perithecia formation, spore development, and infection were maintained with mist irrigation throughout the nursery for about 15 min at 4-hr intervals during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from natural infection of Fusarium head blight (FHB) was visually estimated for each plot during the flowering period. The FHB incidence (%) was rated every other

day namely 19 May, 23 May, 27 May, 3 Jun, 6 Jun, and 9 Jun by rating the percentage of infected spikelet with symptomatic head blight. The area under the disease progressive stairs (AUDPS) (quantitative intensity of FHB) was calculated for all entries. Plots for various entries were harvested on 14 Jul 22 and Fusarium damaged kernels (FDK) were estimated (in percentage) through visual inspection after cleaning. Kernels of all entries were evaluated for mycotoxin deoxynivalenol (DON) concentration using ultra-high-performance liquid chromatography. An analysis of variance (ANOVA) was performed to determine if AUDPS, FDK, and DON varied among cultivars (entries) and least significant differences (LSD) ($\alpha=0.05$) were determined using ‘Agricolae’ R package tool version 1.3-3 (R-Development Core Team). There was FHB infection across the nursery due to optimal conditions (temperature, humidity, and perithecia) necessary for colonization of the crop. However, there were significant differences between the entries and the checks. The early susceptible entry check ‘Overley’ had the second highest AUDPS of 853.75 after ‘PMWH2005800’ with an AUDPS of 864.75. The moderately resistant entry check ‘Everest’ had an AUDPS of 485, which was higher than the moderately susceptible line Karl92 with an AUDPS of 448.5. Entry 21BAY#1 had the lowest FHB severity with an AUDPS of 328.75. Thirty-two lines performed better than Everest with lesser FHB severity; however, Everest had the fourth lowest DON concentration among the cultivars evaluated (2.68 ppm). Only lines LCH16ACC424-33, 21BAY#4 and PMWH20512705 had lower DON concentrations compared to Everest. FDK ranged from 3% to 28.75% and was correlated with AUDPS and DON concentrations.

Entry	Heading	Average FHB (%) ^a	FDK ^x	DON ^w	AUDPS ^y
LCH16ACC403-1	125.75	20.96	9.50	5.25	382.25
LCH16ACC409-7	127.25	24.13	7.50	5.44	440.25
LCH16ACC421-64	126.50	26.67	11.25	8.75	486.25
LCH16ACC422-3	126.75	35.63	17.25	8.11	648.25
LCH16ACC424-33	126.50	23.04	3.00	2.30	419.75

LCH16ACC424-29	126.25	29.83	13.75	5.10	547.00
LCH16ACC425-19	127.50	18.67	4.25	4.56	341.75
LCH16ACC425-13	126.00	21.33	4.25	4.66	388.75
LCH16ACC444-12	125.75	29.75	7.75	5.32	545.00
LCH19DH-152-6	126.25	32.13	10.75	5.43	589.00
LCH19DH-152-25	126.50	30.75	17.50	6.52	563.50
LCH19DH-148-43	127.25	23.83	7.00	11.70	433.75
LCH19DH-149-13	127.25	32.21	10.00	4.94	589.00
LCH19DH-150-85	125.75	23.75	8.00	7.12	433.75
LCH19-1495	125.75	26.08	8.75	6.78	476.75
21BAY#1	127.00	18.00	12.50	4.20	328.75
21BAY#2	127.75	24.00	13.75	6.74	440.00
21BAY#3	126.25	29.92	10.75	2.82	548.50
21BAY#4	126.50	20.54	4.75	2.49	374.75
21BAY#5	126.50	38.29	13.00	7.39	702.25
21BAY#6	127.75	30.42	5.25	4.75	555.25
21BAY#7	125.75	25.33	11.25	5.71	461.00
21BAY#8	126.50	32.38	20.00	13.01	591.00
21BAY#9	127.00	29.04	13.00	8.56	529.50
21BAY#10	128.00	21.50	10.00	3.54	394.25
21BAY#11	126.25	25.75	6.50	2.75	472.75
21BAY#12	127.75	23.21	11.25	5.43	422.50
21BAY#13	127.75	29.33	7.50	9.11	534.00
21BAY#14	127.00	30.92	15.50	4.32	565.50
21BAY#15	131.50	29.79	13.25	4.33	547.50
SYN - Ext 85 - 1	126.25	20.00	7.50	6.24	366.25
SYN - Ext 85 - 2	127.25	26.67	13.25	5.55	492.00
SYN - Ext 85 - 3	125.75	41.13	13.75	7.15	759.75
SYN - Ext 85 - 4	127.75	36.21	17.50	7.32	657.75
SYN - Ext 85 - 5	126.25	30.71	5.75	4.31	560.25
SYN - Ext 85 - 6	126.50	41.46	28.75	8.69	775.75
SYN - Ext 85 - 7	125.75	33.67	15.00	7.32	618.75
SYN - Ext 85 - 8	127.00	29.38	20.00	7.31	540.00

SYN - Ext 85 - 9	129.00	22.63	18.75	6.82	413.00
SYN - Ext 85 - 10	126.25	36.33	13.75	6.35	668.50
SYN - Ext 85 - 11	126.25	33.17	13.75	7.96	605.00
SYN - Ext 85 - 12	126.25	29.08	20.00	7.30	536.25
SYN - Ext 85 - 13	126.75	30.92	18.75	6.39	565.50
SYN - Ext 85 - 14	125.25	32.13	20.25	6.15	585.50
SYN - Ext 85 - 15	125.50	30.33	10.75	5.16	553.75
PMWH89370073	126.50	22.63	6.50	4.02	413.00
PMWH89371594	126.75	21.42	6.50	4.37	390.75
PMWH78697383	126.50	28.67	5.75	6.36	523.75
PMWH20512705	126.25	26.38	4.00	2.68	479.75
PMWH89370883	128.50	22.38	8.25	6.61	412.50
PMWH89370053	127.00	22.38	8.75	3.81	411.75
PMWH89360597	126.75	21.42	3.50	2.96	391.50
PMWH131522266	127.5	23.00	8.75	4.19	418.75
PMWH89366917	127.00	25.00	11.25	4.74	457.00
PMWH89371574	128.25	18.67	9.00	3.69	341.00
PMWH20058001	125.75	45.79	23.75	9.86	864.75
PMWH78697085	126.25	23.46	5.75	5.49	428.00
PMWH131522269	127.25	25.58	12.00	4.50	467.25
PMWH89369463	126.25	21.33	3.50	5.25	388.75
PMWH78694915	127.00	21.92	9.50	8.04	399.00
Everest	125.75	26.42	4.00	2.68	485.00
Overley	125.25	44.83	18.75	10.64	853.75
Karl92	126.50	24.58	7.50	4.17	448.75
AP Roadrunner	127.75	24.50	18.75	5.78	446.75
LCS Atomic	125.75	37.88	9.50	4.37	701.75
Average	126.75	27.83	11.25	5.87	510.37
pval	-	-	< 0.001	< 0.001	< 0.001
LSD ($\alpha=0.05$) ^z	1.14	8.93	7.42	3.61	111.25

^zStatistical analysis was done using analysis of variance (ANOVA) to extract the p-value and least significant differences (LSD).

^yAverage percentage of wheat plants showing Fusarium head blight symptoms.

^xFusarium Damaged Kernel (FDK); is the damage to the wheat kernels.

^wDeoxynivalenol (DON); is the measure of mycotoxin levels in ppm.

^vArea Under Disease Progress Steps (AUDPS); the quantitative intensity of the Fusarium head blight

Reaction of Tan spot in winter wheat to fungicide treatments, 2022

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. Field soil type was Chase silty clay loam (pH = 6.5). A randomized complete block design was used with four replicates and fifteen treatments. Experimental plots were five rows 0.381 m long spaced 0.51 m apart. To encourage tan spot infection, plots were planted on 16 Sep, approximately two weeks earlier than the optimal planting date according to university recommendations. The fungicides, composed of fifteen treatments, were applied at 21 gallons per acre. The percentage of symptomatic plants from natural infection of tan spot was visually estimated on 19 May, 22 May, 25 May, 29 May, 31 May, 2 June, and 5 June. Incidence percentage was calculated by plot level percent infection and tan spot rating (1-9 scale). Tan spot severity ratings were used to calculate the Area Under Disease Progressive Steps (AUPDS) by using the “agricolae” package (version 1.3-3) in R (R-Development Core Team). AUPDS values were analyzed using the GLM procedure and (SAS Institute Inc.) and Fisher’s protected least significant difference (LSD; $\alpha = 0.05$) was used for mean comparisons. Correlation among heading date, yield, tan spot (TS) incidence and AUDPS were analyzed.

Mean AUDPS values varied with treatments ($P < 0.00122$). Treatment plots varied from moderately lower tan spot symptoms quantified by the high AUDPS compared to the untreated check with rather higher tan spot symptoms (higher severity). The fungicides’ treatments varied significantly for the average tan spot incidence and each incidence measurement contributed to the disease quantified intensity (AUDPS). There was a positive correlation ($n = 60$, $r = 0.32$ to 0.92 ,

$P < 0.001$) for each tan spot scoring date and tan spot incidence and a negative correlation ($r=-0.47$, $P < 0.001$) for yield measurements for the various treatments and the AUDPS

Tan Spot Severity (%) ^z										
Treatment	Yield/g m	19 May	22 May	25 May	29 May	31 May	2 June	5 June	Average TS Rating	AUDPS ^y
ALB4003B B 10	1932.66	2	9.75	15.75	16.25	23.75	40	45	21.79	433.25
ALB4003B B 7	1938.5	2.25	5	7.75	8.75	13.75	20.75	20.75	11.29	222.25
ALB4003B, ALB4003B A+B 10 10	2239.25	2.5	8.25	8.75	12.5	12.5	16.75	15	10.89	212.50
ALB4003B, ALB4003B A+B 7 7	2168.25	1.75	3.75	10.25	9.25	13	24.5	19.5	11.71	234.00
ALB4010B B 2.8	2011.75	3	7.75	14.25	15	21.25	29.5	43.75	19.21	381.50
ALB4010B B 4	2349	1.5	3.75	7.5	11.25	12.5	12.5	12	8.71	166.75
ALB4010B, ALB4003B A+B 2.8 7	2277.75	3	8.25	14.25	15.5	22.5	28.75	35	18.18	358.00
Cover XL B 10	2066.5	3	8.25	10.5	14.75	16.25	22.5	33.25	15.50	305.00
Cover XL B 7	1988.5	1.75	8.25	5	8	8.75	15.75	19.5	9.57	189.25
Cover XL, Cover XL A+B 10 10	2426	2.25	4	4.5	11.75	8.75	15.75	18.75	9.39	181.25
Cover XL, Cover XL A+B 7 7	2240.75	1.5	4.25	5.75	9.5	12.5	15.5	17.5	9.50	183.25
Propistar B 2.8	2119.5	1.25	2.5	4.75	6.25	12.5	14.5	16.25	8.29	160.00
Propistar B 4	2070.5	2.75	4.5	5.75	8.75	10	15	17.5	9.18	179.75
Propistar, Cover XL A+B 4 10	2506.25	1.75	3.75	4.5	7.25	11.25	12.5	13.75	7.82	150.25
Untreated Check	1853.75	4	10	17	24.5	33.75	55	60	29.18	571.50
Average	2145.93	2.28	6.13	9.08	11.95	15.53	22.62	25.83	13.35	261.90
P-value										<0.0012
LSD (p=0.05)	0.84	2.25	6.81	9.12	10.33	11.14	18.69	21.66	9.61	192.09

^z Percentage of plants showing leaf symptoms of Tan Spot

^y Area Under Disease Progress Steps

Reaction of Selected Winter Wheat Cultivars in Kansas to Fusarium Head Blight (FHB), 2022

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. Field soil type was Chase silty clay loam (pH = 6.5). A randomized complete

block design was used with four replicates of 35 wheat cultivars (entries) including the susceptible check Overley and moderately resistant check Everest. Plots were five rows, 0.51 m, wide and 0.1902 m long and were planted on October 7th. Corn kernel inoculum was prepared using a native *Fusarium graminearum* isolate, GZ-3639, and air-dried. Field application of the inoculum was done in early spring, 15 April, 1 May, and 15 May, at a rate of 53g/m². Environmental conditions necessary for *Fusarium graminearum* perithecia formation, spore development, and infection were maintained with mist irrigation throughout the nursery for about 15 minutes at 4-hour intervals during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from natural infection of Fusarium head blight (FHB) were visually estimated for each plot during the flowering period. The FHB severity (%) was determined by visual estimation of the percentage of infected spikelet with symptomatic head blight every other day, 19 May, 21 May, 23 May, 25 May, 27 May, 29 May, 31 May, 3 June, 6 June, 9 June, and 11 June. Area under the disease progressive stairs (AUDPS) was calculated for all entries and the least significant difference (LSD, $\alpha=0.05$) determined using the ‘Agricolae’ R package tool version 1.3-3 (R-Development Core Team). Plots for various entries were harvested on 12 July and Fusarium damaged kernels (FDK) were estimated (as a percentage) through visual inspection after cleaning. Kernels of all entries were evaluated for deoxynivalenol (DON) concentration (PPM) using ultra-high-performance liquid chromatography. The early susceptible check Overley had the highest disease severity with an AUDPS of 937.75. Entry AM505 had the lowest AUDPS (disease severity) of 254, which outperformed the moderately resistant check Everest (with AUDPS of 439.5). AM503 had the lowest DON concentration of 1 PPM and the moderately resistant line Everest placing 6th with a DON concentration of 2.75 PPM. Five lines, AM503, KS140924K-2, AM505, P25R76, and KS18HD447, compared with Everest have lower DON concentrations.

Average FDK estimations ranged between 6.25 % to 53.75 % and correlated (0.67 & 0.46 respectively) with evaluated AUDPS and DON concentrations.

Entry	Heading	Average FHB (%) ^z	FDK ^y	DON ^x	AUDPS ^w
AM505	129.25	10.39	10.00	2.00	254.00
KS140924K-2	132.00	10.50	15.00	1.75	257.75
KS19HW84	129.25	13.32	17.50	6.75	331.75
KS140064M-6	128.50	13.48	16.25	5.00	338.25
KS20HDW185	129.50	13.86	10.00	5.75	335.00
AM503	128.75	14.45	7.50	1.00	354.25
KS140064M-7	129.75	14.68	16.25	4.75	369.25
KS140953M-2	131.00	15.07	16.25	8.50	375.25
KS130039M-3	128.75	15.36	16.25	5.00	396.75
P25R76	127.25	15.55	11.25	2.25	389.50
KS18H111-3	128.25	15.66	16.25	5.00	390.75
KS13FHB0321	128.00	17.14	18.75	3.00	432.00
KS140913K-2	128.25	17.39	37.50	4.50	455.00
KS140732M-1	128.25	17.82	16.25	4.25	448.25
Everest	127.50	17.93	6.25	2.75	439.50
KS140732M-4	127.25	18.23	15.00	3.25	453.50
KS140794M-6	129.50	18.36	20.00	4.50	464.00
KS20HD178	128.67	18.45	20.00	6.00	476.00
KS130223K-2	126.50	18.70	16.25	3.50	471.50
KS20H124	127.75	19.05	16.25	7.25	487.75
KS130500K-4	127.25	19.98	28.75	2.75	502.00
KS19H10	128.25	21.50	13.75	4.75	569.75
KS130291K-4	127.25	23.80	31.25	3.75	621.00
KS130544M-2	129.00	24.70	26.25	4.50	642.25
KS20H89	128.25	25.05	18.75	10.50	658.50
KS20HD170	127.50	25.27	46.25	5.75	659.75
KS18HD499	130.75	25.80	17.50	4.50	706.25
KS19HD69	129.00	26.20	27.50	3.50	684.00
KS20HD134	129.50	26.41	53.75	7.25	708.50

KS19H21	128.75	26.52	28.75	4.25	686.00
KS130059M-4	128.50	26.68	51.25	4.75	682.00
KS18HD447	127.50	29.02	11.25	2.25	772.25
KS20HD144	127.75	29.18	50.00	8.75	787.00
KS20H106	127.75	31.02	32.50	5.00	832.50
Overley	126.75	34.61	45.00	11.00	937.75
Average	128.50	20.32	22.89	4.86	524.84
P-value	0.003	–	<0.001	<0.001	<0.001
LSD	1.293		52.61	42.66	23.14

^z Average percentage of wheat plants showing fusarium head blight symptoms

^y Fusarium Damaged Kernel (FDK)

^w Deoxynivalenol (DON)

^x Area Under Disease Progress Steps

Disease Severity of Selected Southern Wheat Cultivars to Fusarium Head Blight (FHB), 2023

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. The field soil type was Chase silty clay loam (pH = 6.5). A randomized complete block design was used with four replicates of 50 wheat cultivars (entries) including Emerson, Karl92, and Overly checks. Experimental plots were ten rows 0.51 m wide and 2.286 m long and were seeded on 7 October. Corn kernel inoculum was prepared using a native aggressive *Fusarium graminearum* isolate GZ-3639 and air-dried. Sterile corn kernels were used for inoculum production. Field application of the inoculum was done in early spring 15 April, 1 May, and 15 May at a rate of 53g/m². Moisture conditions on the nursery necessary for *Fusarium graminearum* perithecia, spore development, and infection were maintained with mist irrigation throughout the nursery for about 15 minutes at 4-hour intervals during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from natural infection of

Fusarium head blight (FHB) was visually estimated for each plot during the flowering period. Sterile corn kernels were used for inoculum production. The FHB incidence (%) was rated every other day namely 26 May, 28 May, 30 May, 1 June, 3 June, 5 June, 7 June, and 9 June by rating the percentage of infected spikelets with symptomatic head blight. The area under the disease progressive stairs (AUDPS) (quantitative intensity of FHB) was calculated for all entries and the least significant differences (LSD) ($\alpha = 0.05$) were determined using 'Agricolae' R package tool version 1.3-3 (R-Development Core Team). Plots for various entries were harvested on July 4th, 2023, and the Fusarium damaged kernel (FDK) was estimated (in percentage) through visual inspection after cleaning.

Pathogen infectivity across the nursery was due to optimal conditions necessary for pathogenicity. The early susceptible check Overly had the highest disease severity with an AUDPS (quantitative disease intensity/severity) of 587.5. Entry OK16103083 had the lowest AUDPS of 211, outperforming TX15M8456-19AZ504 (AUDPS 537) and Everest (AUDPS of 260) with the lowest concentrations of DON 5.60 PPM and 7.13 PPM respectively. TX15M8456-19AZ504 outperformed the moderately resistant entry Everest and other entries with lesser concentration mycotoxin DON levels but relatively higher AUDPS. The individual incidence at different dates contributed to the total area under the disease progression curve and Fusarium damaged kernel (FDK). Average FDK estimations range between 2.75 % (for entry Everest) to 58.75 % (for entry OK20708) and correlate with evaluated AUDPS and DON at 0.21 and 0.78 respectively.

Entry	Heading	Average FHB (%) w	FDK ^z	DON ^x	AUDPS ^y
OK16103083	131.50	13.19	17.00	19.57	211.00
OK Corral	131.25	31.59	31.25	17.23	505.50
OK18217-19HR-4	131.75	16.31	30.50	23.45	261.00
OK19225	131.67	20.54	32.33	32.57	328.67
OK20418-7C21	131.75	23.34	21.25	17.90	373.50
OK16107133-19HR-3	130.75	14.00	26.50	13.75	224.00

OK16107133-19HR-4	131.50	18.50	19.25	13.85	296.00
OK20708	129.00	27.25	58.75	27.38	436.00
OK21538	131.00	21.06	23.25	21.93	337.00
OK198417C	131.25	20.22	38.75	25.75	323.50
OK20925WF	129.75	15.97	2.75	11.00	255.50
OK19515-20HR-2	134.25	15.31	41.00	32.45	245.00
OK2189176WF	134.50	23.47	51.25	32.25	375.50
OK2189178WF	134.50	20.66	23.00	14.65	330.50
OK21227	130.50	35.72	27.00	10.85	571.50
TX19A001030	131.25	16.78	22.25	15.03	268.50
TX15A001482-19AZ16	134.00	24.06	38.75	28.85	385.00
TX18DH129	133.00	26.63	55.00	30.65	426.00
TX18DH266	130.75	25.16	26.50	17.33	402.50
TX18DH287	129.50	21.88	26.25	14.53	350.00
TX18DH313	131.50	21.06	20.33	12.93	337.00
TX15M8456-19AZ435	132.00	27.97	48.75	20.03	447.50
TX15M8456-19AZ504	126.50	33.56	26.25	5.60	537.00
TX19M3183	129.50	24.81	28.75	10.53	397.00
TX14A001035	130.00	21.97	18.75	14.20	351.50
TX14V70214	129.50	18.19	9.50	11.85	291.00
TX15M8024	130.50	28.09	35.75	19.75	449.50
TX16M9216	133.50	19.84	45.00	23.80	317.50
TX18A001119	131.00	20.06	14.25	13.83	321.00
TX18A001132	128.25	21.16	7.25	8.58	338.50
Everest	126.75	16.25	2.75	7.13	260.00
Karl92	127.50	13.47	6.25	11.68	215.50
Overly	126.50	36.72	10.00	12.30	587.50
AP Roadrunner	133.00	15.84	58.33	28.37	253.50
AP Bigfoot	128.75	22.75	22.75	13.50	364.00
Average	130.80	22.10	27.64	18.14	353.55
pval	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	1.14	22.58	54.50	33.81	22.58

w: Percentage of wheat plants showing Fusarium head blight symptoms

z: Fusarium damaged kernel (FDK)

x: Deoxynivalenol (DON)

y: Area Under Disease Progress Steps (AUDPS)

Reaction of selected winter wheat cultivars in Kansas to Fusarium head blight (FHB), 2023

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, KS. The field soil type was Chase silty clay loam (pH = 6.5). A randomized complete block design was used with four replicates of 35 wheat cultivars (entries) from Kansas State University wheat breeders, including Everest, Karl92, and Overley as checks. Experimental plots consisted of seven rows 0.5 m wide and 2.3 m long and were seeded on 17 Oct 2022. Corn kernel inoculum was prepared using native *Fusarium graminearum* isolate GZ-3639 and air-dried. Sterile corn kernels were used for inoculum production. Inoculum was spread in the field in early spring on 15 Apr, 1 May, and 15 May 2023 at a rate of 53 g/m². Moisture conditions in the nursery necessary for *F. graminearum* perithecia, spore development, and infection were maintained with mist irrigation throughout the nursery for approximately 15 min at 4-h intervals during the night during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from Fusarium head blight (FHB) was visually estimated multiple times for each plot during the flowering and grain fill period. The FHB incidence (%) was recorded on 28 May, 30 May, 3 Jun, 5 Jun, 7 Jun, and 9 Jun 2024 by visually estimating the percentage of infected spikelets presenting symptoms associated with head blight. The area under the disease progressive stairs (AUDPS) (quantitative intensity of FHB) was calculated for all entries and the least significant differences (LSD) ($\alpha = 0.05$) were determined using ‘Agricolae’ R package tool version 1.3-3 (R-Development Core Team). Plots for all entries were harvested on 11 Jul 2024, and the Fusarium damaged kernel (FDK) was estimated (in percentage) through visual inspection of 10g grain subsamples for all plots after cleaning. Subsamples used to estimate FDK for all harvested plots were ground to flour and evaluated for mycotoxin deoxynivalenol (DON)

concentration (ppm) by HPLC at the North Dakota State University Wheat Quality and Carbohydrate Research Laboratory.

Pathogen infectivity across the nursery was due to optimal conditions necessary for pathogenicity. The early susceptible check Overley had the greatest disease severity with an AUDPS of 697.30. Entry KS20HDW185 had the lowest AUDPS (disease severity) of 263.00, which outperformed the moderately resistant check Everest (with AUDPS of 379.10); however, KS20HDW185 had a greater DON concentration of 27.13 ppm compared to the lowest 5.50 ppm DON concentration of moderately susceptible check Karl92. The moderately resistant line Everest placed third with a DON concentration of 5.90 ppm. Karl92 and KS160786S-4 outperformed Everest with lower DON concentrations. Average FDK estimations range between 7 % to 85 %.

Entry	Heading ^z	Average FHB (%) ^y	FDK ^x	DON ^w	AUDPS ^v
KS130060C-2	132.50	40.54	41.25	23.78	538.20
KS130459C-4	128.75	36.13	35.00	11.93	476.40
KS140068C-6	132.25	30.83	20.00	17.88	402.15
KS140535C-7	136.50	23.13	21.25	25.50	300.80
KS140064M-6	131.00	27.92	40.00	18.43	360.35
KS160563S-3	132.25	42.00	38.75	11.55	554.90
KS170025D-11	132.50	36.79	85.00	39.30	485.15
KS170013D-19	130.50	30.21	23.75	15.68	405.15
KS170025D-1	132.50	35.79	61.25	38.45	444.47
KS160765S-6	130.50	29.88	23.75	9.80	394.05
KS160786S-6	130.50	28.54	37.50	19.33	378.30
KS170013D-16	133.00	28.83	31.25	16.08	382.35
KS160476S-9	130.75	28.04	22.50	22.58	367.25
KS14FHB0732M-4	131.00	20.46	41.25	20.25	265.60
KS160855S-4	129.00	31.08	32.50	14.50	411.65
KS160352S-1	129.50	36.63	37.00	17.68	481.25
KS160786S-4	126.75	34.63	8.75	5.78	460.60
KS19H10	134.00	41.92	65.00	30.35	563.55

KS20H89	134.00	33.58	76.25	93.23	434.60
KS20HW103	133.00	36.83	66.25	53.78	485.00
KS20H105	132.00	35.46	63.75	27.58	462.05
KS20H106	133.50	29.50	47.50	26.85	386.10
KS20H124	130.25	33.13	46.25	28.80	436.95
KS20HD134	131.50	35.35	37.50	21.78	449.27
KS20HDW185	135.25	20.38	35.00	27.13	263.00
KS21H30	131.50	33.71	53.75	25.55	448.60
KS21H31	131.75	29.17	41.25	63.98	381.25
KS21H36	131.25	38.79	51.25	24.60	516.55
KS21H38	130.00	30.83	28.75	16.35	409.90
KS21HD147	132.00	30.71	38.75	31.48	402.80
KS21HD158	137.00	31.96	58.75	55.95	419.00
KS21HD173	132.25	30.38	40.00	27.23	393.30
Everest	132.00	27.96	7.00	5.90	379.10
Karl92	130.00	26.00	13.75	5.50	346.70
Overlay	130.75	51.08	23.25	14.85	697.30
Average	131.76		39.85	25.98	428.10
<i>p</i> -value	-	-		<0.001	<0.001
LSD ($\alpha=0.05$)	1.82	18.66	49.38	47.81	18.78

^zAverage day per entry for 50% heading since 1 Jan

^yPercentage of wheat plants showing Fusarium head blight symptoms

^xFusarium Damaged Kernel (FDK)

^wDeoxynivalenol (DON)

^vArea Under the Disease Progress Stairs (AUDPS)

Disease severity of selected northern wheat cultivars to Fusarium head blight (FHB), 2023

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. The field soil type was Chase silty clay loam (pH = 6.5). A randomized

complete block design was used with four replicates of 50 wheat cultivars (entries) from public breeding programs at North Dakota State University, South Dakota State University, and Montana State University, including Emerson, Karl92, and Overlay checks. Experimental plots were ten rows 0.5 m wide and 2.3 m long and were seeded on 7 Oct 2022. Corn kernel inoculum was prepared using a native aggressive *Fusarium graminearum* isolate GZ-3639 and air-dried. Sterilized corn kernels were used for inoculum production. Field application of the inoculum was done in early spring 15 Apr, 1 May, and 15 May 2023 at a rate of 53 g/m². Moisture conditions on the nursery necessary for *F. graminearum* perithecia, spore development, and infection were maintained with mist irrigation throughout the nursery for approximately 15 min at 4-h intervals during the night during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from Fusarium head blight (FHB) was visually estimated for each plot during the flowering and grain-filling period. The FHB incidence (%) was recorded on 28 May, 30 May, 3 Jun, 5 Jun, 7 Jun, 9 Jun, and 12 Jun 2023 by rating the percentage of infected spikelets with FHB symptoms. The area under the disease progress stairs (AUDPS) (quantitative intensity of FHB) was calculated for all entries and the least significant differences (LSD) ($\alpha=0.05$) were determined using ‘Agricolae’ R package tool version 1.3-3 (R-Development Core Team). Plots for all entries were harvested on 11 Jul 2023, and the percent Fusarium damaged kernel (FDK) were estimated through visual inspection after cleaning 10g subsamples of grain. The same subsamples used to determine FDK percentage were ground to flour and used to determine deoxynivalenol (DON) concentration by HPLC at the North Dakota State University Wheat Quality and Carbohydrate Research Laboratory.

Pathogen infectivity across the nursery was ideal due to maintenance of optimal conditions necessary for pathogenicity. The early susceptible check Overlay had the highest disease severity

with an AUDPS of 1,100.56. Entry Emerson had the lowest AUDPS (quantitative disease intensity/severity) of 170.94, outperforming MTFH23308 (AUDPS of 267.44) with the lowest concentration of DON 8.68 ppm. MTFH23308 and 22Nord-171 outperformed the moderately resistant entry Emerson with lesser mycotoxin DON concentrations but relatively greater AUDPS. The individual incidence at different dates contributed to the total area under the disease progress stairs and FDK. Average FDK estimations range between 17.75% (for entry 22Nord-171) to 73.75% (for the check SD20D064-3).

Entry	Heading ^z	Average FHB (%) ^y	FDK ^x	DON ^w	AUDPS ^v
19Nord-124	140.75	25.04	37.50	13.20	439.88
20Nord-138	144.00	22.57	48.33	11.13	387.13
21Nord-155	143.00	14.64	27.50	11.80	253.63
21Nord-156	143.00	19.07	45.75	16.00	330.56
21Nord-161	137.00	31.11	56.25	41.15	531.38
22Nord-169	142.75	10.25	35.25	18.45	182.06
22Nord-170	144.00	15.00	37.50	27.53	272.94
22Nord-171	144.50	15.50	17.75	9.58	272.63
22Nord-172	144.75	13.43	31.50	18.23	238.44
22Nord-173	143.50	30.43	57.50	24.93	523.38
22Nord-174	129.67	45.86	23.33	15.10	795.67
22Nord-175	144.25	16.68	27.50	19.38	296.13
22Nord-176	142.75	21.00	42.50	23.58	366.31
22Nord-177	139.25	24.57	53.75	21.45	425.63
22Nord-178	141.25	13.86	18.75	14.80	248.69
SD18B016-5	138.25	24.57	55.00	32.65	424.13
SD18B025-8	140.00	26.00	40.00	28.68	449.44
SD18B055-2	130.75	47.89	56.25	25.58	832.81
SD18B072-2	134.00	41.50	51.25	18.38	718.25
SD19B019-2	136.00	38.32	53.75	16.33	648.50
SD19B033-2	137.75	32.39	38.75	21.70	556.50
SD19B108-3	139.75	33.82	65.00	28.00	579.75

SD19B164-3	137.00	26.75	52.50	28.20	469.25
SD20B088-2	137.75	33.93	38.75	18.23	572.75
SD20D100-9	129.75	46.64	32.75	19.28	806.19
SD20C031-1	141.50	50.75	52.50	27.18	867.44
SD20D009-9	145.00	22.29	51.67	19.67	383.56
SD20D063-2W	142.25	34.39	50.00	32.58	587.25
SD20D064-3	133.00	42.64	73.75	71.50	728.75
SD20D108-6	135.33	50.19	43.33	19.17	859.58
MTFH23301	145.25	16.25	59.00	15.30	286.88
MTFH23302	143.25	21.93	57.50	28.98	385.88
MTFH23303	143.75	22.21	20.00	15.60	383.63
MTFH23304	144.50	14.46	42.50	22.70	255.44
MTFH23305	145.75	16.71	57.50	14.20	297.38
MTFH23306	140.75	16.82	46.25	22.40	295.19
MTFH23307	143.25	19.89	70.00	15.95	347.81
MTFH23308	145.75	15.43	45.00	8.68	267.44
MTFH23309	111.50	21.82	50.00	16.13	376.06
MTFH23310	146.75	16.93	42.50	10.88	306.81
MTFH2290	142.50	35.89	52.50	25.40	610.00
MTFH2292	142.50	28.79	40.00	26.03	496.06
MTCL2010	144.00	16.07	31.25	14.10	277.56
MT2019	145.00	17.11	51.25	15.13	300.44
MTS2068	141.50	23.43	52.50	24.93	403.56
Emerson	145.25	9.57	32.50	9.75	170.94
Karl92	129.75	40.25	31.25	17.55	689.94
Overley	128.50	63.61	53.75	22.93	1,100.56
Flourish	142.75	28.21	51.25	34.68	488.13
LCS Steel AX	141.00	35.71	41.67	20.17	593.33
Average	139.92	27.04	44.88	21.50	467.63
p-value	<0.05	<0.001	<0.001	<0.001	<0.001
LSD	7.01	20.21	32.96	32.97	19.85

^zAverage day per entry for 50% heading since 1 Jan

^yPercentage of wheat plants showing Fusarium head blight symptoms

^xFusarium Damaged Kernel (FDK)

^wDeoxynivalenol (DON)

Reaction of selected winter wheat cultivars to Fusarium head blight (FHB), 2023

The experiment was conducted at Kansas State University Rocky Ford Research Station, Manhattan, Kansas. The field soil type was Chase silty clay loam (pH = 6.5). A randomized complete block design was used with four replicates of 50 wheat cultivars (entries) from private wheat breeders in the United States and included Everest (moderately resistant check), Karl92 (quality check), and Overley (susceptible check). Experimental plots were ten rows 0.5 m wide and 2.3 m long and were seeded on 7 Oct 2022. Corn kernel inoculum was prepared using a native *Fusarium graminearum* isolate GZ-3639 and air-dried. Sterile corn kernels were used for inoculum production. Field application of the inoculum was done in early spring 15 Apr, 1 May, and 15 May 2023 at a rate of 53 g/m². Moisture conditions in the nursery necessary for *F. graminearum* perithecia, spore development, and infection were maintained with mist irrigation throughout the nursery for 15 min at 4-h intervals during the night during flowering. Heading dates for entries were taken at 50% headed tillers. The incidence of symptomatic wheat plants from infection of Fusarium head blight (FHB) was visually estimated for each plot during the flowering and grain-filling period. The FHB incidence (%) was rated on 26 May, 28 May, 30 May, 3 Jun, 5 Jun, and 7 Jun 2023 by visually estimating the percentage of infected spikelets with head blight symptoms. The area under the disease progress stairs (AUDPS) (quantitative intensity of FHB) was calculated for all entries and the least significant differences (LSD) ($\alpha=0.05$) were determined using ‘Agricolae’ R package tool version 1.3-3 (R-Development Core Team). Plots for all entries were harvested on 11 Jul 2023, and the Fusarium damaged kernel (FDK) was estimated (in percentage) through visual inspection after cleaning 10g grain subsamples for each plot. The same subsamples

used to estimate FDK were ground to flour and assayed for deoxynivalenol (DON) at the North Dakota State University Cereal Quality and Carbohydrate Research Laboratory.

Pathogen infectivity across the nursery was consistent due to maintaining optimal conditions necessary for pathogenicity. The early susceptible check Overley had the highest disease severity with an AUDPS (quantitative disease intensity/severity) of 608.90. Entry PMWH186844383 had the lowest AUDPS of 178.35, outperforming seven other lines including the moderately resistant check Everest with AUDPS of 246.90. Karl92 and Everest had the lowest concentrations of DON of 8.55 ppm and 8.68 ppm, respectively. PMWH186844383, which outperformed all other entries in terms of severity (lower AUDPS) had a relatively greater concentration of the mycotoxin DON at 26.50 ppm. The individual incidence at different dates contributed to the total area under the disease progression curve and FDK. Average FDK estimations range between 9% (for entry 9 L01) to 62.50% (for entry 6 L01).

Entry	Heading ^z	Average FHB (%) ^y	FDK ^x	DON ^w	AUDPS ^v
LCH21-9398	130.50	21.83	60.50	23.23	301.85
LCH21-9436	130.00	25.25	44.75	28.90	353.45
LCH21-9485	131.25	16.79	52.00	26.00	229.00
LCH20-2165	128.25	29.46	20.25	14.60	414.90
LCH20-2026	128.00	21.00	19.75	15.63	293.85
LCH20-2031	130.75	22.33	60.00	24.65	306.75
LCH20-2264	128.75	21.33	32.50	23.85	298.15
LCH20-2054	130.75	21.88	27.00	24.35	311.10
LCH20-2091	130.50	27.29	50.50	28.90	385.10
PMWH186844385	130.75	13.21	32.00	24.40	187.35
PMWH186844383	131.25	12.79	35.50	26.50	178.35
PMWH186844376	130.75	19.67	46.50	30.48	274.35
PMWH186844380	130.25	21.17	19.00	22.50	293.90
PMWH186844381	130.00	21.75	34.75	22.63	301.10
PMWH186844379	130.50	20.58	24.00	21.98	287.80

PMWH186844377	130.25	24.79	35.50	27.25	345.65
PMWH186844378	131.25	22.88	58.25	29.90	315.40
PMWH186844382	130.25	22.71	24.50	21.20	314.15
PMWH186796700	129.75	17.46	31.50	20.25	242.40
1 L01	127.50	24.04	17.75	13.80	331.95
2 L01	131.25	17.38	10.50	16.10	236.60
3 L01	129.00	22.33	30.50	14.80	319.15
4 L01	130.00	25.67	48.00	21.25	362.05
5 L01	130.75	14.25	19.75	15.35	198.00
6 L01	130.50	23.00	62.50	44.88	313.80
7 L01	130.50	18.42	33.00	20.25	254.05
8 L01	129.75	32.50	48.25	21.35	454.40
9 L01	127.75	18.17	9.00	11.30	253.90
10 L01	131.00	18.00	11.25	20.80	249.05
Everest	128.50	17.79	9.75	8.68	246.90
Karl92	127.50	19.08	23.75	8.55	266.15
Overley	128.00	42.17	35.50	23.08	608.90
AP Prolific	130.25	21.63	25.50	14.70	303.75
LCS Runner	129.50	22.50	47.50	24.65	309.05
KS Territory	130.75	17.17	50.50	22.00	233.50
Average	129.89	21.66	34.05	21.68	302.17
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.90	23.54	46.89	28.82	24.04

^zAverage day per entry for 50% heading since 1 Jan

^yPercentage of wheat plants showing Fusarium head blight symptoms

^xFusarium Damaged Kernel (FDK)

^wDeoxynivalenol (DON)

^vArea Under the Disease Progress Stairs (AUDPS)