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Studies on Genetic Characterization of Agronomic Traits and Disease Resistance in Bread Wheat

Jyotirmoy Halder
South Dakota State University, jyotirmoy.halder@sdstate.edu

STUDIES ON GENETIC CHARACTERIZATION OF AGRONOMIC TRAITS AND DISEASE RESISTANCE IN BREAD WHEAT

BY

JYOTIRMOY HALDER

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DISSERTATION ACCEPTANCE PAGE

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

Sunish Sehgal
Advisor Date

David Wright
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

To my mother and father for their never-ending love, support, and sacrifices!

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ABSTRACT

STUDIES ON GENETIC CHARACTERIZATION OF AGRONOMIC TRAITS AND DISEASE RESISTANCE IN BREAD WHEAT

JYOTIRMOY HALDER

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A steady increase in wheat yield is vital to feed the continuously rising world population. Systematic exploitation of wheat germplasm and a better understanding of the underlying genetic control could be pivotal in accelerating the genetic gain for yield and disease management. Various modern techniques such as genome-wide association study (GWAS), genomic selection (GS), fine mapping, and cloning can expedite wheat improvement and broaden our understanding of the complex wheat genome. In the first objective of this study, we evaluated the Watkins core set of 121 landrace cultivars (LCs) to identify novel sources of resistance against the tan spot, Stagonospora nodorum blotch (SNB), and Fusarium Head Blight (FHB). The phenotypic evaluation identified 13 LCs with multiple resistance to tan spot and SNB, while five other LCs were found to be a potential source for FHB resistance. A total of 30 significant marker-trait associations (MTAs) were identified in a GWAS for response to tan spot and SNB. In the second objective, we performed GWAS in a panel of 297 hard red winter wheat lines from the US Great Plains region to identify QTLs for various spike and kernel-related traits and evaluated the prediction accuracy (PA) of GS models for these traits. Most of the MTAs (47) were identified for spike-related traits, where 16, 15, 11, and 5 MTAs were

identified for spike length, spikelet per spike, spike density, and kernel per spike, respectively, while only 6 MTAs were identified for three kernel-related traits (kernel weight, kernel area, and thousand kernel weight). Fourteen MTAs were identified at two or more individual environments were considered stable QTLs. Univariate genomic selection (GS) models like genomic best linear unbiased prediction (GBLUP) were compared with multivariate models like Bayesian multi-trait multi-environment (BMTME) and we found that the multi-trait model (BMTME) outperformed the single-trait model (GBLUP) in terms of PA. In the last objective, we developed a fine map of a grain yield QTL on chromosome 7DS introgressed into bread wheat from *Aegilops tauschii* (D-Genome donor of wheat). Heterogeneous inbred families (HIFs) were developed. Eleven high-quality SNP markers were developed and mapped to the target region (3-17 Mb) on chromosome 7DS using recombination breakpoints (recombinants). A total of 29 homozygous recombinants (7 haplotype groups) were identified and evaluated in the greenhouse and field. KASP markers spanning to the QTL region can be used for marker-assisted selection of 7DS yield QTL. Overall, the finding of this study can be used for genetic improvement of wheat and accelerate the genetic gain.

Chapter 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops, playing a crucial role in feeding 35% of the world's population by providing 19% of calories and 21% of proteins (Tadesse et al., 2019). The yearly increase of 0.9% in wheat yield is the lowest among the four major food crops (maize, rice, wheat, and soybean), to double the global production for feeding an estimated world population of 9 billion by 2050 (Ray et al., 2013). However, wheat production facing increasing challenges from various factors including climate change, scarcity of natural resources like land and water, increasing biotic and abiotic stresses, etc. Therefore, to feed the growing world population with a gradual decrease in farmland, wheat breeding must focus on increasing yield through genetic improvement of wheat and minimizing yield losses due to biotic and abiotic stresses.

Throughout the world, biotic stress such as many fungal diseases of wheat like rusts, tan spot, Stagonospora nodorum blotch (SNB), powdery mildew, and Fusarium head blight (FHB) remains a constant threat for wheat production. These diseases can cause up to 50% of yield losses along with a significant reduction in end-use quality and mycotoxin contamination (Bai and Shaner, 2004; Gurung et al., 2009). Fungicides are considered an effective control measure to some extent, but their application adds additional production cost and may not inadequate control in diseases like FHB and may not be environment friendly (McMULLEN et al., 2012).

One possible solution to this problem is to grow resistant cultivars which is both an economically feasible and eco-friendly way to combat foliar and spike diseases in wheat. To develop high yielding resistant cultivar, wheat breeders can take advantage of wheat gene pools that contain a huge reservoir of diverse genes/alleles. Exploiting the genetic resources present in the primary, secondary, and tertiary gene pools of wheat could be a useful strategy to increase the genetic diversity of wheat (Hoisington et al., 1999). It was previously found that introgression of novel genes/alleles present in the diverse landraces can broaden the genetic base of bread wheat germplasm (Smale et al., 2002; Reif et al., 2005) and can enhance the level of disease resistance in modern wheat. Therefore, mining the underutilized and genetically diverse landrace collection for diseases resistance genes/alleles could be an effective strategy to improve wheat.

In addition to minimizing yield losses, enhancing wheat productivity is central to meeting the future wheat demand. Wheat yield is a complex polygenic trait influenced by various morphological, physiological, and environmental factors, making this trait challenging to manipulate and improve (Nadolska-Orczyk et al., 2017; Liu et al., 2018c). However, many individual traits such as spikelet number per spike (SNS), spike length (SL), spike number, kernels per spike (KPS), kernel size (KS), thousand kernel weights (TKW), etc., contribute to the yield and are less sensitive to the environment and have higher heritability than that of grain yield (Kato et al., 2000; Hai et al., 2008). Therefore, identification of important quantitative trait loci (QTLs) for yield contributing traits and further deployment is essential for the overall improvement of wheat. Further with the recent development of advanced techniques like genomic selection (GS), we can select

superior individuals based on genomic estimated breeding values and shorten the breeding cycle thus leading to an increase in the genetic gain per unit of time (Meuwissen et al., 2001).

Similar to disease resistance traits wild relatives of wheat can also be exploited for yield improvement (Yang et al., 2009). Introgression wild relatives can slow the progress due to undesirable linkage drag, however, such negative effects are limited when genes are transferred from wheat progenitor species *Aegilops tauschii*, D subgenome donor of wheat (Sehgal et al., 2011; Olson et al., 2013). *Aegilops tauschii* has far higher genetic diversity as compared to the wheat D genome and was found to be a useful source of genes for grain yield, end-use quality, and improved stress tolerance genes/alleles (Cox et al., 1995; Yang et al., 2009; Lopes and Reynolds, 2010; Jia et al., 2013).

Further, to enhance wheat productivity, it is very important to understand the regulatory mechanisms of genes controlling wheat yield and their interactions with the environment (Reynolds et al., 2012). Mapping the quantitative trait loci (QTL) and identification of linked molecular markers can facilitate rapid genes transfer and pyramiding of several genes/QTLs for various agronomic traits including yield and yield contributing traits. Genetic characterization through linkage mapping and GWAS and application of genome-wide selection could be useful in enhancing the rate of genetic gain. The objectives of the current studies are;

1. To evaluate the core set of Watkins landrace collection for resistance to tan spot (*P. tritici-repentis* race 1 and race 5), SNB, and FHB and explore the genetic basis of that resistance.
2. To understand the genetic control of various spike and kernel-related traits in hard red winter wheat using genome-wide association analysis (GWAS) and to evaluate the prediction accuracy of different GS models for predicting various kernel- and spike-related traits in hard winter wheat.
3. To characterize the grain yield QTL on the short arm of chromosome 7D from *Ae. tauschii* (ac. TA1615) transferred to hexaploid bread wheat line KS05HW14.

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Chapter 2. Literature review

2.1. General introduction of wheat

2.1.1. Wheat origin and domestication

Bread wheat which is also known as common wheat is a member of the tribe *Triticeae* in the family Poaceae. There are about 25 species (both wild and domesticated) in the genus *Triticum*, consisting of a series of diploid, tetraploid, and hexaploid forms such as diploid wild wheat, *Triticum urartu* (AA genome) and einkorn wheat, *Triticum monococcum* (AA genome), allotetraploid emmer wheat, *Triticum turgidum* var. durum (AABB genome), allohexaploid common wheat, *Triticum aestivum* L. (AABBDD genome) etc. (Kim et al., 2017).

Modern-day wheat/bread wheat is an allohexaploid (6x) species with three sets of homeologous chromosomes designated as A, B, and D sub genomes, which made it the largest genome (~ 17 gigabases, Gb) among cereals (William et al., 2007). This huge genome of hexaploid bread wheat (AABBDD, 2n = 6x = 42) was the consequence of the hybridization between diploid genome of grass species *Aegilops tauschii* (DD) and the tetraploid genome of *T. turgidum* (AABB) (Dubcovsky and Dvorak, 2007). *T. urartu* is considered as the progenitor of A sub genome of bread wheat, while several S genome species in genus *Aegilops* sect. *sitopsis* were believed to be the contributor of B genome (Feldman and Levy, 2015). Around 9,000 years ago, a hybridization event between tetraploid (*T. turgidum* subsp. *dicoccon*) wheat and the D sub genome donor species- *Ae. tauschii* (2n=2x=14, DD) was spontaneously happened that gave rise to the modern bread

wheat, *T. aestivum* (2n=6x=42, AABBDD) (Shewry, 2009). Shifting from the tetraploid wheat to the modern hexaploid form through the addition of D sub genome brought enhanced geographic and environmental adaptability in bread wheat, as well as increased the grain yield and quality. The event of wheat domestication that occurred in the present-day Middle East, played a significant role in the development and evolution of human civilization, as it shifted human civilization to a more agrarian society from the hunter-gatherer and nomadic pastoral one (Eckardt, 2010).

2.1.2. Species of wheat

There are three major species of wheat namely bread, club, and durum wheat make up 90% of the wheat grown today (Englund, 2019). The most common species of wheat grown in the world are:

I. Common or Bread wheat (*T. aestivum*, subsp. *aestivum*): the most widely cultivated group in the world.
II. Club Wheat (*T. aestivum* subspecies *compactum*): It can be distinguished by its more compact ear due to shorter rachis segments.
III. Durum (*T. durum*): It is also called pasta wheat or macaroni wheat, a tetraploid form that is the second most widely cultivated wheat.
IV. Einkorn (*T. monococcum*): Einkorn wheat is a diploid species, can refer either to the wild species of wheat, *Triticum boeoticum*, or to the domesticated form, *Triticum monococcum*.
V. Emmer (*T. dicoccum*): Emmer wheat or hulled wheat is a tetraploid species, which has been cultivated since ancient times.

VI. Spelt (*T. spelta*): also known as dinkel wheat or hulled wheat, is a hexaploid species cultivated in limited quantities.

2.1.3. Wheat classes

Wheat varieties grown in the USA, are divided into six distinct classes according to their growth habits (winter or spring), kernel color (red or white), and texture of the ripened grain (hard or soft).

I. Hard Red Winter: It is a versatile class of wheat with excellent milling and baking characteristics and is mostly used for various bread, all-purpose flour, and even Asian style noodles. Winter wheat is planted in the fall and completes its life cycle in the spring. Hard Red Winter Wheat is grown in the Great Plains, Northern, and Pacific Northwest regions.

II. Hard Red Spring: This class contains the highest protein among the classes and is used for various specialty items like hearth breads, rolls, croissants, bagels, and pizza crust. This class of wheat is mostly grown in Montana, North and South Dakota, and Minnesota.

III. Soft Red Winter: This is a versatile weak-gluten wheat with excellent milling and baking characteristics. This type of wheat is generally high yielding and produces flour with relatively low protein content, which is suited for crackers, pastries, cookies, pretzels, and flat breads. Soft Red Winter Wheat is primarily grown east of the Mississippi River.

IV. Hard White: This is the newest class of wheat grown in many Great Plains states of the USA. This class is closely related to red wheat in its' milling and baking qualities but comparatively sweeter in flavor. It is used for hard rolls, noodles, yeast breads, and tortillas.

V. Soft White: It is primarily grown in the Eastern and Pacific Northwest regions. This class of wheat is ideal for Middle Eastern flat breads, crackers, pastries, cakes, and Asian-style noodles.

VI. Durum: This is the hardest of all classes and mainly used for making high quality pasta. Durum wheat is grown in the Northern and Pacific Northwest regions.

2.1.4. Climatic Requirement of wheat

Plant growth and development largely depend upon the surrounding climatic conditions, and each crop species has its optimum climatic requirement for better growth, development, and reproduction. Crop phenology genes are significantly associated with yield physiology as grain yield is strongly influenced by the timing of developmental stages in a specific environment (Slafer et al., 2009). Wheat varieties with a winter growth habit need to vernalize (exposures to low temperatures) for a certain period to accelerate flowering (Kippes et al., 2015) but spring wheat does not require this cold exposure. Based on the different durations of vernalization requirements and geographical locations, winter wheat cultivars are typically categorized into three types: weak winter type (need brief exposure to low temperature), semi-winter type (requires 2–4 weeks of cold exposure), and strong winter type (needs 4–6 weeks of cold exposure)

(Crofts, 1989). Phenology plays a significant role in crop adaptation to a particular environment (Fatima et al., 2020). The diversity of phenology genes present in the wheat genome helps it grow in a vast range of environmental conditions (Nazim Ud Dowla et al., 2018). Better understanding of the genetic control of phenological traits can help breeders to develop crops varieties with improved adaptive abilities and increase wheat productivity. Wheat adaptation and synchrony of flowering to different climatic conditions are largely controlled by vernalization (*VRN1, VRN2,* and *VRN3*) (Yan et al., 2003, 2004, 2006), photoperiod sensitivity (*Ppd1, Ppd2,* and *Ppd3*) (Beales et al., 2007; Nishida et al., 2013), and autonomous earliness per se (*Eps*) genes (Nazim Ud Dowla et al., 2018). In general, wheat is a long day plant with a requirement of more than 14 h of light for flowering, whereas photoperiod-insensitive varieties can flower in short days (10 h or less light) (Beales et al., 2007; Kumar et al., 2012). Photoperiod-sensitive wheat cannot be grown as an overwinter crop in tropical regions because of the short-day length (Worland and Snape, 2001), whereas photoperiod-insensitive wheat flowers independently of day length and can grow in long- or short-day environments.

2.2. Wheat genetic resources

It is very important to understand the complex nature of the bread wheat (*T. aestivum* L.) genome and its wild relatives for future improvement of bread wheat particularly in the light of climate change. The genetic diversity in the modern wheat varieties is relatively low that has been recognized as a significant drawback for future wheat yield improvement. Wheat's close or distant relative species present in the secondary and tertiary gene pools harbor a higher level of genetic diversity and can be a valuable source

of genes/alleles to broaden the genetic base of modern wheat (Winfield et al., 2016). Gene pool can be referred to the complete set of genes or genetic information found in a particular species of population, also includes its wild relatives as genetic information can be shared between them. The traditional classification of plants according to the gene pool concept was based on the relatedness and easiness of sharing genetic information between cultivated species with their wild relatives (Harlan and de Wet, 1971). The primary gene pool usually consists of closely related species including landrace cultivar, wild and weedy forms, breeding lines, etc. Crossing and useful gene/allele transfer between the species in the primary gene pool is considered easy and F1 hybrid is generally fertile with effective chromosome pairing. While the secondary gene pool includes fewer close relatives, hybridization with the primary gene pool is difficult but F1 hybrid still may have some fertile progenies. The tertiary gene pool is composed of distant relatives or species with genomes non-homoeologous to bread wheat. Gene transfer with natural crossing is not possible, however, special methods such as the use of bridging materials or ionizing radiation treatments can be used to create hybrid (Hysing, 2007). The gene pools of bread wheat are shown in (Figure 2.1).

2.2.1. Wheat landraces; a valuable genetic resource

More than 25,000 types of bread wheat have been developed for a wide range of environments throughout the globe (Shewry, 2009). However, the genetic diversity of modern wheat is narrow in general because of the repeated selection and intercrosses of existing elite wheat germplasm in each breeding cycle, resulting in the depletion of alleles from a diverse gene pool (Cox, 1997). Such a narrow genetic base of modern

wheat will be a challenge for sustainable wheat production and may create vulnerability to the ever-changing world climatic conditions and various other biotic and abiotic stresses (Bhatta et al., 2018).

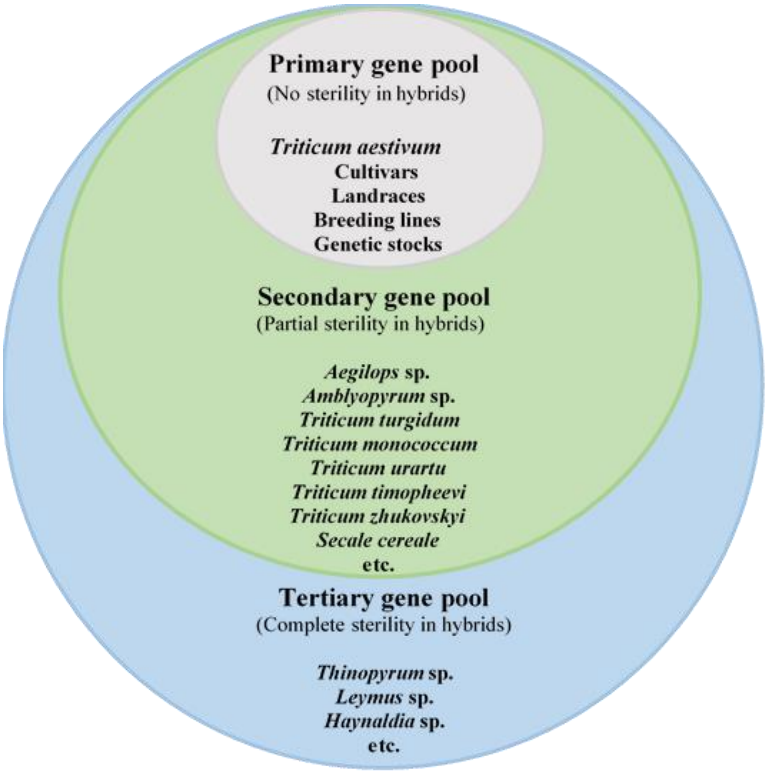

Figure 2.1. The gene pools of bread wheat (*Triticum aestivum*) according to the ease of gene transfer adopted from (Hysing, 2007).

Researchers have been using the genetic resources from the primary gene pool to improve the elite wheat varieties or breeding lines. Alien or wild relative species from the secondary and tertiary gene pools have also been used for wheat improvement such as wide hybridization between wheat and non-Triticum species was successfully exhibited in many previous studies (Jiang et al., 1993; Mujeeb-Kazi and Rajaram, 2002; Gill et al.,

2011). However, the major drawbacks with the alien introgression are that the process is not easy to handle, alien chromosome segments often cannot compensate for the loss of wheat chromatin, or undesirable genes/alleles are often linked to the desirable genes. As a result, only very few successful hybridizations finally reached to the production of commercial level (Jiang et al., 1993; Friebe et al., 1996).

Wheat landraces are the locally adapted material that was not influenced by the modern breeding practices but had been gone through genetic improvement by traditional agricultural practice. Wheat landraces are very close to the elite breeding materials in terms of morphological similarities and genetic diversity that represent an important source of genetic variation in wheat (Harlan, 1975). Wheat landrace collections have much higher genetic diversity compared to the elite breeding populations and adaptability to diverse environmental conditions (Lopes et al., 2015). Wheat landraces not only have been traditionally cultivated for thousands of years throughout the globe under the most adverse environmental conditions but also in lower input farming systems (Lopes et al., 2015). In the early 20th century, wheat landraces were chosen as the starting materials for a systematic wheat breeding program, therefore the modern breeding population can be considered as the mosaics of landrace cultivars (Wingen et al., 2017).

Using wheat landraces for direct hybridization to transfer the useful genes/alleles to the advanced breeding materials could be an attractive breeding strategy when compared to the more complex genetic improvement strategies through the exploitation of genetic resources of wild species (Reynolds et al., 2007). Some of the successful examples of useful gene transfer from wheat landrace to modern breeding lines include reduced height

genes (*Rht*) in the wheat line ‘Norin 10’ which was integral to the ‘green revolution’ was inherited from Japanese landrace ‘Daruma’ that improved lodging and yield (Borlaug, 1988; Wilhelm et al., 2013), powdery mildew resistance gene *Pm24* from the Chinese landrace ‘Chiyacao’ (Huang et al., 1997), genes/QTLs for resistance to abiotic and biotic stresses (Hede et al., 1999; Skovmand et al., 2001; Halder et al., 2019).

2.2.2. *Aegilops tauschii;* D genome donor of wheat

Aegilops species which is from the tribe; Triticeae, have significant contributions in modern wheat origin and the current wheat breeding despite the handling difficulties that come along with the wild species (Kishii, 2019). This species mainly provided genetic resources conferring resistance to biotic stresses, but was also found as a useful source for other complex traits such as yield and abiotic stress tolerance (Rakszegi et al., 2020). Based on the *Aegilops* L. taxonomy, this genus consists of 23 species with C, D, M, N, S, and U genomes (Kishii, 2019). *Ae. tauschii* (2n = 2x = 14, genomes DD), the tausch's goatgrass is an annual grass species which is the donor of sub genome D to common wheat and an important genetic resource. *Ae. tauschii* is consists of two main lineage groups (subspecies), designated as lineage 1 (ssp. *tauschii*) and lineage 2 (ssp. *strangulata*) (Arora et al., 2017). However, while comparing the D sub genome of wheat and the known *Ae. tauschii* subspecies, it was found that lineage 2 contributed more than 99% of the genetic material to the wheat D genome (Wang et al., 2013). The genome of this diploid progenitor is found to be closely related to wheat sub genome D because of the recent origin of modern hexaploid wheat, that makes *Ae*. *tauschii* an important resource for wheat breeding (Luo et al., 2013) and has been most widely used in wheat

breeding (Rakszegi et al., 2020). *Ae. tauschii* tend to have much higher genetic diversity compared to the diversity present in bread wheat's D genome (Dvorak et al., 1998; Wang et al., 2013). The exploitation of the genetic variation of *Ae. tauschii* via introgression to the modern wheat can be a useful strategy to improve the narrow genetic base of wheat and accelerate productivity (Zhou et al., 2021).

2.3. Challenges and opportunities in wheat yield improvement

Demand for wheat yield and production improvement is clearly going to continue to feed the growing population. So, wheat production faces several routine challenges, such as high demand, common biotic and abiotic stresses, scarcity of natural resources, yield plateau, etc. There is a consensus that harvest index (HI) which is already close to 60%, cannot be improved much further in modern elite varieties (Curtis and Halford, 2014). In addition, climate change is threatening wheat production by increasing both biotic (aggressive diseases and insect pests) and abiotic stresses (drought, heat, salinity, cold, and waterlogging).

Grain yield is the outcome of lots of physiological and biochemical processes, directly or indirectly regulated by numerous genes and their interplay with the environment. Many agronomic and physiological traits are involved in wheat grain yield response, for example, plant height, number of productive tillers, spike length, spikelet per spike, number of kernels per spike, thousand kernel weight, canopy temperature, chlorophyll content, photosynthetic rate, etc. The cumulative knowledge from wheat research suggests that both source' and 'sink' tissues need to be improved for yield improvement

and several strategies can be followed such as strategic crossing to complement “source” with “sink” traits (Reynolds et al., 2017), reductionist approach to understand gene networks regulating individual yield component (Brinton and Uauy, 2019), strategies to increase photosynthesis (Parry et al., 2011), etc. Therefore, a comprehensive and multidisciplinary approach including both basic and applied filed of research would be necessary to further improve wheat in farmer's fields (Reynolds et al., 2009) (Figure 2.2).

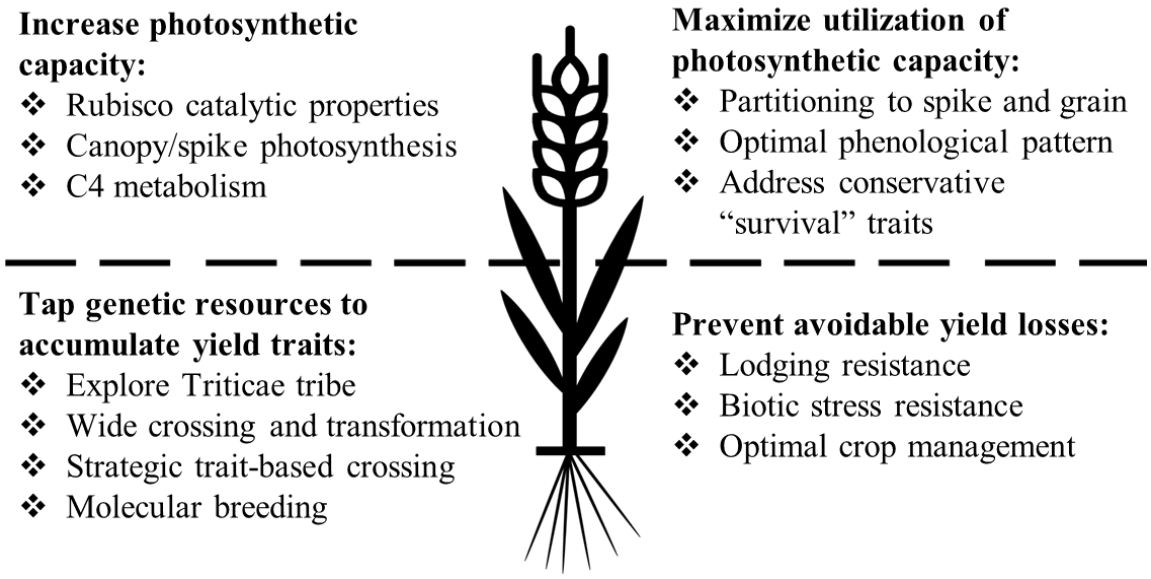

Figure 2.2. Complementary strategies including both basic and applied research areas to increase wheat yield potential (Reynolds et al., 2009).

2.4. Wheat diseases; constant threat to global food security

Wheat production faces various challenges globally from biotic and abiotic stresses and wheat breeders are creating solutions to those problems with new varieties and advanced research. The relative impact of disease and pathogen varies with the presence of resistant

cultivar, climatic conditions, and crop management. It is a continuous quest for a plant breeder and pathologist to find resistant varieties against the prevailing pests. To keep the crop safe from diseases and make a satisfactory harvest, growers use pesticides and follow cultivation practices such as crop rotation, tillage, planting density, disease-free seeds, and cleaning of equipment, but plant varieties with inherent (genetic) disease resistance are generally preferred. Most of the globally important diseases of crops are caused by either necrotrophic or biotrophic fungi (Singh et al., 2016).

2.4.1. General idea of host-pathogen interaction

Various pathogens attack plants to assimilate nutrients from them and plants, in turn, have developed a sophisticated defense mechanism against the attack as a co-evolutionary battle between plants and pathogens continues. Pathogens usually get access to the plant interior either by penetrating the leaf and root surfaces directly or by entering through wounds and natural openings such as leaf stomata. During the invasion process, pathogens degrade the plant cell wall by cell wall-degrading enzymes, then deliver pathogen effectors inside the plant cell, and eventually start interfering with the normal activities of the host (Pajerowska-Mukhtar and Dong, 2009; Tilsner and Oparka, 2010). On the other hand, plants have evolved complex defense mechanisms to combat pathogen invasion by blocking pathogen entrance and activating a range of defense responses. Plants also have preformed physical and chemical barriers as well as sophisticated two-tiered immune systems. Understanding heritability and genetics allowed researchers to identify sources of heritable resistance, called resistance genes (R genes) (Rhoades, 1935; Bushnell, 1984). R genes were further described by Harold Henry Flor’s with his

groundbreaking gene-for-gene model (Flor, 1942), where he concluded that for every resistance gene in the host there is a corresponding virulence gene in the pathogen. Recent advancement in molecular biology and genetic research of host and pathogen interaction has revealed that plant resistance relies on a complex regulatory system that is greatly building upon Flor's gene-for-gene model (Andersen et al., 2018).

2.4.2. Tan spot

2.4.2.1. Importance

Tan spot of wheat, also known as yellow leaf spot, is caused by the fungus *Pyrenophora tritici-repentis* (asexual stage: Drechslera *tritici-repentis*) is an economically important disease. This disease has been reported worldwide wherever wheat and other susceptible host crops are grown. The disease develops on wheat in the spring and summer on both the upper and lower surfaces of leaves. This fungus can be a major concern for the no-till farming practice because the inoculum overwinters in stubble residues from the previous crop (Faris et al., 2013). The disease develops on both upper and lower surfaces of susceptible host leaves, appears as tan-colored oval-shaped necrotic and/or chlorotic spots with a black pinhead spot in the center. Symptoms become severe in highly susceptible genotypes, where the lesions tend to coalesce and may cover the entire leaf surface and eventually kill the leaves (Faris et al., 2013). Severe disease development decreases the capacity for photosynthesis (Singh et al., 2011), which leads to plant stress and ultimately yields loss (Faris et al., 2013). Yield reduction may reach up to 49% in susceptible cultivars in case of favorable disease conditions (Rees et al., 1982; Dinglasan et al., 2016). Infected kernels are characterized by a pink color (pink smudge) on the seed

coat (Schilder and Bergstrom, 1994). Losses due to tan spot are attributed to lower number of kernels/spikes, low thousand kernel weight (Shabeer and Bockus, 1988), reductions in the number of tillers, dry matter accumulation, leaf area index (Rees and Platz, 1983).

2.4.2.2. Host range

Both hexaploid bread wheat (*T. aestivum*) and tetraploid durum wheat (*T. turgidum*) are the main host of *P. tritici-repentis* (Faris et al., 2013). Other than wheat, this fugus has a wide range of hosts such as *Avena sativa* (oat), *Hordeum vulgare* (barley), and *Secale cereale* (rye), and many other grass species such as *Elymus repens* (Couch grass) (Lamari and Bernier, 1989; Ali and Francl, 2002; Kastelein et al., 2002).

2.4.2.3. Disease cycle

The tan spot pathogen (*P. tritici-repentis*) overwinters on last season's wheat residue as black pinhead-sized sexual fruiting bodies (pseudothecia). During favorable weather conditions in spring and early summer, pseudothecia release sexual spores called ascospores, which is the primary source of inoculum. Asexual spores (conidia) are produced on previous crop residue and within existing leaf spots. Both types of spores can be dispersed by wind or rain and germinate and infect the wheat plant in a wide range of temperatures. During favorable weather conditions for disease, many conidia can be produced in the diseased plants, and then they can be blown to nearby plants and thereby initiate new infections (Mcmullen and Adhikari, 2009).

2.4.2.4. Race classification and Host selective toxins (HSTs)

Ptr displays a complex race structure and so far, eight different races (1 to 8) of *P. tritici-repentis* (PTR) have been identified based on the symptoms (necrosis and chlorosis) produced by host-selective toxins (HST) (Lamari et al., 2003). Out of these eight races, three races (Races 2, 3, and 5) can be designated as the basic races, while the rest of the races (Races 1, 6, 7, and 8) except race 4 (avirulent) are the combinations of the three basic races (Lamari et al., 2003). Three host-specific toxins (*Ptr ToxA*, *Ptr ToxB*, and *Ptr ToxC*) of *P. tritici-repentis* have been identified and well-characterized so far (Dinglasan et al., 2016; Kokhmetova et al., 2021). Eight *Ptr* races either induce single or multiple toxins such as Race 2 (Ptr ToxA), Race 3 (Ptr ToxC), and Race 5 (Ptr ToxB) produce single HST, while Race 1 (Ptr ToxA and Ptr ToxC), Race 6 (Ptr ToxB and Ptr ToxC), Race 7 (Ptr ToxA and Ptr ToxB), and Race 8 (Ptr ToxA, Ptr ToxB, and Ptr ToxC) produce multiple HTTs (Lamari et al., 2003; Kokhmetova et al., 2021). The races which produce Ptr ToxA are associated with necrotic symptoms in Ptr ToxA-sensitive cultivars, whereas Ptr Tox-B-sensitive cultivars induce chlorosis (Kokhmetova et al., 2021). Both Ptr ToxA and ToxB are proteins in nature, while Ptr ToxC is not a protein, rather a low-molecular-weight, nonpolar secondary metabolite (Effertz et al., 2002).

2.4.2.5. Resistance sources/genes

The disease system of *Ptr*–wheat does not follow the ‘classical’ gene-for-gene interaction, rather follow an inverse gene-for-gene manner where necrotrophic effectors (NEs) of *Ptr* interact with the corresponding host sensitivity (S) genes to induce disease

(Lamari et al., 2003; Liu et al., 2017b). Qualitative genes responsible for tan spot resistance have been given the designation "*Tsr*", while genes associated with reaction to HST are designated as "*Tsn*" or "*Tsc*" depending on the necrosis or chlorosis symptom. Eight major *Tsr* genes (*Tsrl, Tsr2, Tsr3, Tsr4, Tsr5, Tsr6, TsrHar,* and *TsrAri*) located on chromosomes 2BS, 3AS, 3BL, 3DS, and 5BL have been identified and cataloged so far (Mcintosh et al., 2013). Resistant gene *Tsr2* is from *T. turgidum*, confers resistance to race 3 isolates (Singh et al., 2006), *Tsr3* was reported in synthetic hexaploid wheat confers resistance to race 1 (Tadesse et al., 2006b), *Tsr4* comes from resistant cultivar salamouni also confers resistance to race 1 (Tadesse et al., 2006b), *Tsr5* is reported to resist race 5 isolates also coming from tetraploid wheat (Singh et al., 2008). Host plant sensitivity to each HST is conferred by a single dominant host sensitivity (S) gene, namely *Tsn1*, *Tsc1*, and *Tsc2* for Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively (Liu et al., 2017b). Sensitivity gene *Tsn1* was mapped to chromosome arm 5BL in wheat and was subsequently cloned using a map-based strategy (Faris et al., 1996, 2010), while two other *Tsc* genes (*Tsc1* and *Tsc2*), have been mapped on chromosomes 1AS (Effertz et al., 2002) and 2BS (Friesen and Faris, 2004; Abeysekara et al., 2010) that conferring sensitivity to HST Ptr ToxC and Ptr ToxB, respectively. Even though most of the resistance against tan spot coming from the tetraploid and hexaploid wheat, with few from D genome donor species *A. tauchii* (Cox et al., 1992; Siedler et al., 1994), there is still lots of scope to find the resistance sources in the different gene pool of wheat.

2.4.2.6. Disease management

A combination of various management techniques can be followed to effectively manage tan spot in the fields. Cultural practices such as removal or destruction of infested residue from previous years, crop rotation with non-hosts (other than wheatgrass, bromegrass, or rye) can be very effective. Fungicides (strobilurin and triazole classes) are labeled for the management of tan spot that provide very good to excellent control. However, host resistance against tan spot can be considered as the most cost-effective and eco-friendly way to fight against this disease (Chu et al., 2008c).

2.4.3. Stagonospora nodorum blotch (SNB)

2.4.3.1. Importance

Stagonospora nodorum blotch (SNB) is a globally important wheat disease caused by *Parastagonospora nodorum* fugus, which can infect both glumes and leaves, reducing grain yield and quality (Czembor et al., 2003), and the pathogen can affect wheat at both seedling and adult stages. *P. nodorum* is a necrotrophic fungus, which actively kills host cells during infection and subsequently lives in the dead tissue of the host plant (Laluk and Mengiste, 2010). Leaf blotches reduce the plant leaf surface area and hamper the photosynthesis process which is actively related to the plant food production mechanism and that’s how this disease reduce the overall crop growth and yield, while glume blotch is directly related to the grain quality reduction (Downie et al., 2020). Yield losses due to the SNB can reach up to 50% or more in case of severe epidemic (Shaner and Buechley, 1995; Bhathal et al., 2003). This fugus occurs in wheat-growing areas worldwide but is

more prevalent in wheat growing areas with moderate to high rainfall such as regions in the southeastern United States, Canada, South America, Australia, Scandinavia, Central and Eastern Europe (Downie et al., 2020). The infection initially shows symptom of small chlorotic water-soaked lesions on the lower leaves of the infected plants, which turn yellow and finally red brown (Mcmullen and Adhikari, 2009). Infection area on the leaf surface produce lens-shaped structure without the distinct yellow border, which is a typical symptom of tan spot lesions (Mcmullen and Adhikari, 2009).

2.4.3.2. Host range

This necrotrophic fungus has a narrow host range, is mostly known as a wheat pathogen. Except bread wheat, other hosts include *T. durum*, Triticale, barley, and wild grasses have been reported to harbour *P. nodorum* (Cunfer, 2000; Solomon et al., 2006; Downie et al., 2020).

2.4.3.3. Disease cycle

The disease cycle of *P. nodorum* fugus is very similar to that of tan spot disease. The reproductive structure (pseudothecia) or asexual structures (pycnidia) are very similar in appearance to tan spot fungus but smaller (Mcmullen and Adhikari, 2009). Ascospores forms from the reproductive structure (pseudothecia) as a part of sexual reproduction, generally cause the first infections, whereas as part of the asexual cycle, pycnidia form in lesions on the leaf that promote spore production for local infection, which can be dispersed through water-splash (Downie et al., 2020). The fungus overwinters on the wheat straw, debris, infested seed, or other overwintering crops. It was found that

sufficient moisture for a period of 12 to 18 hours is needed for infection and a temperature range between 68- and 81-degrees F is conducive for rapid disease development (Mcmullen and Adhikari, 2009).

2.4.3.4. Necrotrophic effectors (NEs) and genes

P. nodorum which is a necrotrophic fungus induces host cell death by secreting necrotrophic effectors (NEs) (typically proteins, phytotoxic metabolites), also known as host selective toxins. Once recognized by the host plant, its response to the effectors activates the programmed cell death (Friesen and Faris, 2010; Winterberg et al., 2014). However, this fungus can survive against the defense response and continue feeding on the dead tissue which makes the host susceptible to the pathogen and this phenomenon is termed as "inverse gene-for-gene" interaction, as the recognition of NEs by dominant host sensitivity genes leads necrotrophic effector-triggered susceptibly (NETS) (Friesen and Faris, 2010; Oliver et al., 2012). Instead of stopping the infection, this necrotic response by a sensitive host is believed to be helping the pathogen to colonize and continue feeding on it (Oliver and Solomon, 2010).

Research on wheat- *P. nodorum* pathosystem identified a total of nine host sensitivity gene-NE interactions that include, *Tsn1*-SnToxA (Liu et al., 2006; Zhang et al., 2009; Faris et al., 2010), *Snn1*-SnTox1(Liu et al., 2004b; Reddy et al., 2008), *Snn2*- SnTox2 (Friesen et al., 2007; Zhang et al., 2009), *Snn3*-B1-SnTox3 (Friesen et al., 2008; Liu et al., 2009b), Snn3-D1-SnTox3 (Zhang et al., 2011), *Snn4*-SnTox4 (Abeysekara et al., 2009), *Snn5*-SnTox5 (Friesen et al., 2012), *Snn6*- SnTox6 (Gao et al., 2015), and *Snn7*-

SnTox7 (Shi et al., 2015). However, only three interactions such as *Tsn1*-SnToxA, *Snn1*-SnTox1, and *Snn3*-B1-SnTox3 were studied more intensively due to the cloning of host sensitivity gene and/or pathogen NE (Haugrud et al., 2019).

2.4.3.5. Disease management

Some common practices such as crop rotation which reduces the inoculum and using fungicides can be effective to fight against disease outbreaks. However, extensive use of fungicides would increase the production cost and may not be an environment-friendly practice. Therefore, wheat cultivars with high levels of genetic resistance are more desirable, which not only decreases the cost of production but also environment friendly. However, despite decades of breeding effort, there is limited progress in improving SNB resistance as a significant level of susceptibility still retained in the modern wheat cultivars (Aguilar et al., 2005; Francki, 2013). Reduced/no tillage agricultural farming practice becoming popular throughout the globe, which was found significantly correlated with the SNB infection severity as the infected residue remains in the field (Mehra et al., 2015). So, residue management can be an effective strategy to lower the inoculum intensity in the field and lower the infection rate (Solomon et al., 2006).

2.4.4. Fusarium head blight (FHB)

2.4.4.1. Importance

FHB is mostly caused by the pathogen *Fusarium graminearum* is a widespread and powerful enemy of wheat growers throughout the globe. This disease, also known as

Scab, inflicts yield and quality losses on farms in at least 18 states of USA and other wheat growing regions of the world. For the last few decades, frequent epidemics have attracted the attention of farmers and researchers from all over the world. This pathogen not only reduces the wheat yield, but also contains mycotoxin popularly known as deoxynivalenol (DON) that is toxic to the human and animal health. Prolonged humid and wet conditions is conducive for extensive infection and due to lower test weight, yield losses can reach up to 80% (Bai and Shaner, 1994). This disease is a global concern and the combined direct and secondary economic losses due to FHB from 1993 to 2001 was estimated at $7.67 billion in the US alone (Nganje et al., 2004). Food industries throughout the U.S. incur losses from the cost of dealing with the toxin-contaminated grain that often accompanies scab infection.

2.4.4.2. Host range

Fusarium graminearum generally causes head blight in wheat and barley but can also cause disease in rice, oats and gibberella stalk and ear rot disease on maize (Goswami and Kistler, 2004). The fungus may also infect other plant species without causing disease symptoms.

2.4.4.3. Disease cycle

Fusarium graminearum overwinters as saprophytic mycelia on infested crop residues such as corn stalks, wheat straw, and other host plants (Goswami and Kistler, 2004). Fungus produces asexual spores known as macroconidia on the infested residues and can be dispersed to other host plants and debris through wind or rain. During favorable

weather (warm, humid, and wet) conditions in spring fungus produce sexual spores called ascospores, which can be windblown to infect the wheat head at the time of anthesis (Parry et al., 1995). If the environment is warm and moist, light pink/salmon-colored spores' aggregation can be visible on the rachis and glumes of individual heads of wheat.

2.4.4.4. Resistant types

Wheat resistance against head scab is a complex process, the host plant can exhibit one or multiple types of resistance against the infection. There are five different types of FHB resistance mechanisms have been described that includes Type I (resistance to initial infection), Type II (resistance to disease spread within infected heads), Type III (resistance to DON production and accumulation), Type IV (resistance to seed colonization and damage), and Type V (tolerance to yield loss) (Mesterházy et al., 1999; Yi et al., 2018). Out of these resistance mechanisms, Type I and II are more extensively studied because the host shows resistance at an early stage of infection.

2.4.4.5. Resistance sources/genes

During the past decade, numerous studies have been published on molecular mapping of FHB resistance in wheat. Fortunately, large genetic variation for FHB resistance is available in the wheat gene pool, but often the best regionally adapted, and highly productive cultivars are susceptible to FHB. Host resistance to FHB is a complex trait conditioned by oligogenic to polygenic in nature, and quantitative trait loci (QTL) have been identified in almost in every wheat chromosome. Some QTL were found in several independent mapping studies indicating that such QTL are stable and therefore useful in

breeding programs. Even though many strains or races of *Fusarium graminearum* have been identified but no specific host-strain system has been recognized, that means virulence in this pathogen is not host-specific and resistance in cultivars is not race specific, makes it a horizontal, quantitative, and non-specific in nature (Mesterházy et al., 1999). More than 200 QTL for FHB resistance have been reported for various types of resistance on all the wheat chromosomes (Buerstmayr et al., 2009), and a meta-QTL analysis clustered 43 QTL with unique chromosome locations (Liu et al., 2009a), but most of the QTLs have small to moderate effect to the FHB resistance.

A major FHB resistance QTL (*Fhb1*) from Sumai 3 has been mapped and widely used in breeding programs as this gene can significantly improve the resistance in diverse genetic backgrounds (Buerstmayr et al., 2009). However, it only provides 20-40% reduction in FHB severity in different genetic backgrounds. Several other lines from China such as Ning 7840, and Ning 8331 developed by Chinese wheat breeders, have also been the basis of the earlier projects to determine the genetic basis of Fusarium resistance. In addition, another Chinese landrace called Wangshuibai also showed high and stable resistance, made this line an alternative source for improving FHB resistance (Buerstmayr et al., 2009). Most repeatable QTL are those on chromosomes 3B (*Fhb1*), 5A (*Qfhs.ifa-5A*) (Buerstmayr et al., 2002) and 6B (*Fhb2*) (Cuthbert et al., 2007). Several other new sources of FHB resistance have recently been reported and transferred into wheat including *Fhb3* (Qi et al., 2008) from *Leymus racemosus*, *Fhb6* from *Elymus tsukushiensis* (Cainong et al., 2015) and *Fhb7* (Guo et al., 2015) from *Thinopyrum*

ponticum. Most of these genes are effective and should be utilized in the breeding programs.

2.4.4.6. Disease management

Severe FHB epidemics occur when a susceptible host genotype encounters abundant pathogen inocula during wheat flowering in presence of warm and humid weather (Osborne and Stein, 2007). There are several management practices that can be helpful in reducing losses caused by FHB (McMULLEN et al., 2012). The best way to control fhb is to practice multiple controlling strategies such as using resistant cultivars, cultural practices, and chemical controls, instead of single management practice. Plant breeders throughout the globe have been conducting research to develop fhb resistant germplasm, and using resistant germplasm is considered as the most economic and environment friendly. Timely application of available fungicides (triazole; group 3) can effectively suppress the FHB in wheat and barley (McMULLEN et al., 2012).

2.5. Characterization of wheat germplasm

2.5.1. Single nucleotide polymorphism (SNP); a powerful molecular marker

SNP is a type of polymorphism which brings variation in a single base pair in the DNA sequence, become extremely popular in genetics and breeding research because of their genome-wide abundance and abilities to capture variations quickly (Korte and Ashley, 2013). Rapid advances in next-generation sequencing technologies have significantly improved the discovery of SNPs (Allen et al., 2011; Berkman et al., 2012; Poland et al., 2012). The shift from simple-sequence repeat (SSR) marker to SNP has made an

excellent progress in the crop research (Thomson, 2014). SNP data is being widely used to detect in quantitative trait locus (QTL) for key traits in linkage mapping studies, finding marker-trait associations in genome-wide association studies (GWAS), marker assisted selection, genomic selection, fine mapping, gene clone etc. (Cook et al., 2012; Chen et al., 2016a; Halder et al., 2019; Kuzay et al., 2019; Sidhu et al., 2020; Yang et al., 2020; AlTameemi et al., 2021; Gill et al., 2021)

2.5.2. Genome-wide association study (GWAS)

Genome-wide association study (GWAS) or association mapping (AM) also known as "linkage disequilibrium mapping," is a method to uncover the association between phenotypes and genotypes and that relies on linkage disequilibrium (LD), which is the non-random co-segregation of alleles at two or more loci (Gupta et al., 2005; Breseghello and Sorrells, 2006). Compared to conventional QTL mapping or linkage analysis which is based on physical concept of distance and relies on the recombination (Xu et al., 2017), GWAS is more like a statistical concept; two loci are associated if the alleles at one locus are not independent of the alleles at another locus (allelic association). Association study, as a complement to linkage mapping, offer higher mapping effects, takes advantage of historic recombination events in broad-based diversity panels, thus providing higher resolution and a greater number of loci (Zhu et al., 2008). Because of the dramatic reduction in costs of sequence technologies, GWAS has been performed in nearly all economically important crops, including Arabidopsis, wheat, corn, rice, barley, soybean, potato, tomato, etc. (Wang et al., 2014a; Xu et al., 2017). AM has both advantages and disadvantages when compared to linkage mapping (Korte and Farlow, 2013). Its main

advantage of AM is that it exploits a mapping population with the diverse origin and utilize all the historical recombination events that occurred in its evolutionary history (Myles et al., 2009). Another advantage of GWAS is that it reduces the costs and time of creating mapping populations such as recombinant inbreed lines (RIL), and the same genotypic data of the mapping panel can be used for various studies. One common problem with AM study is that, false-positive associations can be detected between marker and traits due to the kinship that may exist among germplasm and population structure (Neumann et al., 2011; Korte and Farlow, 2013).

There are different single or multi-locus models that have been developed to increase the power of marker-trait association (MTA) detection while controlling the false positive. Single locus analysis such as General Linear Model (GLM) (Price et al., 2006) and Mixed Linear Model (MLM) (Yu et al., 2006) are the two most widely used models. Compared to GLM, which identifies a greater number of MTAs with a high risk of false-positive detection, MLM takes account of population structure and kinship in association analysis to reduce type I error, thus detecting more accurate associations. Multi-locus analysis such as Multiple Loci Mixed Linear Model (MLMM) (Segura et al., 2012) and Fixed and random model Circulating Probability Unification (FarmCPU) (Liu et al., 2016) perform better in terms of controlling the false-discovery rate and the power of QTL detection. To better apply the GWAS output in crop improvement, the output of the study should be further utilized by follow-up studies and additional experiments to investigate the genetic basis of various economically important traits.

2.5.3. Genomic selection

In conventional plant breeding, breeders identify parents with desirable characteristics, hybridized them to create favorable combinations followed by repeated phenotypic selection over several years to select lines/plants with superior performance. However, this approach has several limitations such as the variety development process may requires long period (5–12 years), mostly rely on phenotypic selection, genotype-environment interaction (G x E), complex and low heritable traits etc. To solve or minimize the shortcomings of conventional breeding, marker-assisted selection (MAS) technique was introduced which takes advantage of molecular markers to identify the lines with desirable traits (indirect selection) with less phenotypic information (Collard and Mackill, 2008). MAS technique is very effective when the marker is linked to the trait of interest and the trait of interest is governed by major effect genes/QTLs (Wang et al., 2018). However, agronomically important traits are often controlled by many small-effect genes/QTLs and are not very suitable for MAS. Therefore, to capture the effect of minor QTLs/genes, the concept of genomic selection (GS) was proposed, which is a modified form of MAS (Meuwissen et al., 2001)

The GS process starts with a set of training populations (TP), that have been genotyped with genome-wide markers and phenotyped for trait of interest. Genotypic and phenotypic data from TP is employed to develop the prediction model, which is then used to calculate the genomic estimated breeding values (GEBVs) for a set of population (validation population) (Wang et al., 2018; Tessema et al., 2020). Now a breeder can use the calculated GEBVs to select the desired lines from the validation population without

phenotypic information (Meuwissen et al., 2001). All the marker effect is taken into consideration in GS, thus capturing more genetic variation for the trait of interest, making GS more effective compared to traditional MAS (Newell and Jannink, 2014). The prediction accuracies in GS are largely depend on the relationship between training and validation population, that is, the highest prediction accuracies can be achieved when training and test data are well related (Isidro et al., 2015).

2.5.4. Fine mapping and map-based cloning

Positional cloning is a step-by-step approach to narrow down the QTL region to the shortest possible genetic interval using recombinant lines for a specific trait of interest. This cloning process includes the identification of QTLs for a trait of interest in a target environment, fine map the QTL region with markers, identifying the causal gene, characterizing the gene, and finally deploying the gene for the improvement of breeding lines (Figure 2.3). First, the original population used to identify the QTL need to exploit for recombinant inbred lines (RILs) segregating for the trait of interest. With the help of these segregating population a high-resolution map for the QTL region can be constructed. The tentative position of the gene can be located by systematically evaluating the recombination events (recombinants within the candidate interval) phenotypically. Within the shortest possible candidate region, there could be several genes potential to be the causal gene for the trait of interest. To identify the real causal gene, it is necessary to develop plants with the loss of function mutations and then reintroduce the functional version in the candidate gene and if the mutants show that the

loss/gain of the functionality of the gene significantly altering the trait of interest that indicates the candidate is the causal gene (Kuzay et al., 2019).

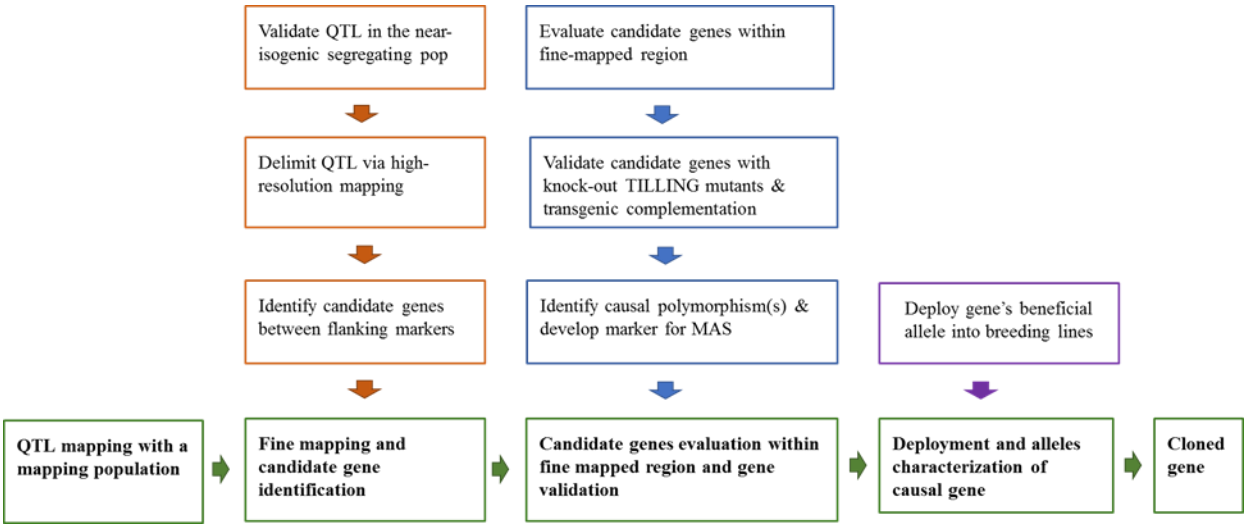

Figure 2.3. Basic steps of positional gene cloning of wheat.

Positional cloning has been successful in wheat especially for major genes controlling traits of interest such as phenology genes, quality traits, disease resistance, etc. (Yan et al., 2003; Uauy et al., 2006; Fu et al., 2009). However, using positional cloning, identification of wheat genes controlling important agronomic traits has been a challenging and time-consuming strategy because of the huge genome size, low marker density, suppressed recombination, etc. (Hatta et al., 2019). Recent development in molecular techniques and bioinformatics facilities accelerating the rapid gene cloning process (Hatta et al., 2019).

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Chapter 3. Mining and genomic characterization of resistance to Tan spot, Stagonospora nodorum blotch (SNB), and Fusarium head blight in Watkins core collection of wheat landraces

Jyotirmoy Halder[1], Jinfeng Zhang[1], Shaukat Ali[1], Jagdeep S. Sidhu[1], Harsimardeep S. Gill[1], Shyamal K. Talukdar[2], Jonathan Kleinjan[1], Brent Turnipseed[1] and Sunish K. Sehgal[1]*

[1]Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD 57007

[2]California Cooperative Rice Research Foundation, Inc., Rice Experiment Station, Biggs, CA 95917

* Corresponding author:

Sunish K. Sehgal

sunish.sehgal@sdstate.edu

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3.1. Abstract

Background

In the late 1920s, A. E. Watkins collected about 7,000 landrace cultivars (LCs) of bread wheat (*Triticum aestivum* L.) from 32 different countries around the world. Among which 826 LCs remain viable and could be a valuable source of superior/favorable alleles to enhance disease resistance in wheat. In the present study, a core set of 121 LCs, which captures the majority of the genetic diversity of Watkins collection, was evaluated for identifying novel sources of resistance against tan spot, Stagonospora nodorum blotch (SNB), and Fusarium Head Blight (FHB).

Results

A diverse response was observed in 121 LCs for all three diseases. The majority of LCs were moderately susceptible to susceptible to tan spot Ptr race 1 (84%) and FHB (96%) whereas a large number of LCs were resistant or moderately resistant against tan spot Ptr race 5 (95%) and SNB (54%). Thirteen LCs were identified in this study could be a valuable source for multiple resistance to tan spot Ptr races 1 and 5, and SNB, and another five LCs could be a potential source for FHB resistance. GWAS analysis was carried out using disease phenotyping score and 8,807 SNPs data of 118 LCs, which identified 30 significant marker-trait associations (MTAs) with -log10 (p-value) >3.0. Ten, five, and five genomic regions were found to be associated with resistance to tan spot Ptr race 1, race 5, and SNB, respectively in this study. In addition to *Tsn1*, several novel genomic regions *Q.Ts1.sdsu-4BS* and *Q.Ts1.sdsu-5BS* (tan spot Ptr race 1) and *Q.Ts5.sdsu-1BL*, *Q.Ts5.sdsu-2DL*, *Q.Ts5.sdsu-3AL*, and *Q.Ts5.sdsu-6BL* (tan spot Ptr

race 5) were also identified. Our results indicate that these putative genomic regions contain several genes that play an important role in plant defense mechanisms.

Conclusion

Our results suggest the existence of valuable resistant alleles against leaf spot diseases in Watkins LCs. The single-nucleotide polymorphism (SNP) markers linked to the quantitative trait loci (QTLs) for tan spot and SNB resistance along with LCs harboring multiple disease resistance could be useful for future wheat breeding.

Keywords: Watkins Landrace Cultivars, Tan spot, Fusarium head blight, Stagonospora nodorum blotch, disease resistance, Genome-wide association study, QTL, biotic stress.

3.2. Background

Wheat is a staple food crop for more than 35% of the world's population (Li et al., 2015). Biotic and environmental stresses pose a serious threat to global wheat production (Tolmay, 2004; Limbalkar et al., 2018). Fungal diseases of wheat like rusts, tan spot, Stagonospora nodorum blotch (SNB), powdery mildew and Fusarium head blight (FHB) can cause up to 50% yield losses along with a significant reduction in end-use quality (Bai and Shaner, 2004; Gurung et al., 2009). Further, the FHB pathogen (*Fusarium graminearum* Schwabe) produces mycotoxins such as deoxynivalenol (DON) that accumulate in the infected grains and constitute a serious threat to food safety (Pestka, 2010). Fungicides can be used to control these diseases to some extent, but fungicide application adds additional cost to wheat growers with inadequate control over disease like FHB (McMULLEN et al., 2012). Moreover, indiscriminate use of fungicides can cause environmental contamination or may lead to the development of fungal resistance.

Growing resistant cultivars is considered as an effective and eco-friendly approach to combat foliar and spike diseases in wheat. However, resistance to FHB, tan spot, and SNB is largely quantitatively inherited and limited by additive genetic effect and genotype × environment interaction (Wolf et al., 1998; Xu et al., 2004; Gurung et al., 2009). Presently, only a couple of effective sources of resistance to FHB (*Fhb1*, *Fhb5A*) are available in cultivated bread wheat. Most of the FHB resistances have been transferred into wheat from alien species i.e. *Leymus racemosus* (*Fhb3*), *Elymus tsukushiensis* (*Fhb6*), and *Thinopyrum ponticum* (*Fhb7*) (Qi et al., 2008; Cainong et al.,

2015; Guo et al., 2015). Currently, eight different Ptr races have been identified for tan spot (Lamari and Bernier, 1989; Ali and Francl, 2003; Lamari et al., 2003; Ali et al., 2010), however, Ptr race 1 is found to be the most prevalent one (Benslimane et al., 2011; Aboukhaddour et al., 2013; Abdullah et al., 2017). Though several sources of tan spot resistance have been identified in various spring and winter wheat germplasm (Xu et al., 2004; Singh et al., 2006; Mergoum et al., 2007; Ali et al., 2008; Liu et al., 2015), a greater portion of tested germplasm, including commercial cultivars, is reported to be susceptible to Ptr race 1 (Xu et al., 2004; Ali et al., 2008; Chu et al., 2008b, 2008c; Liu et al., 2015). Similarly, SNB resistant sources also remain limited (Francki, 2013) and only a few commercial cultivars are known to be resistant to SNB (Adhikari et al., 2011). Finally, while resistance may be derived from alien species, this type of resistance is often associated with linkage drag and may hinder progress in breeding programs. Therefore, a continuous effort in identification and introgression of resistance from under-utilized landraces can offer other alternatives to help enhance the level of resistance in modern wheat.

The success of semi-dwarf wheat varieties has resulted in large areas of wheat planted to a limited number of cultivars. While the advantages of semi-dwarf wheat are well documented, their popularity has led to limited genetic diversity and increased vulnerability to pests and diseases under the threat of changing climate (Keneni et al., 2012; Fu, 2015). Previous studies showed that introgression of novel genes/alleles present in the landraces can help avert the narrowing down the genetic base of bread wheat germplasm (Smale et al., 2002; Reif et al., 2005). In general, the genetic diversity

present in various landrace collections is much higher than in modern cultivars (Wingen et al., 2017). Therefore, mining the genetically diverse bread wheat germplasm with broad resistance to multiple diseases has the potential to improve wheat resistance to diseases and pests (Polák and Bartoš, 2002).

A. E. Watkins, a scholar from Cambridge, England, initially collected over 7,000 accessions of landrace cultivars (LCs) mainly from 32 countries of Asia, Europe, Africa, and Australia in the 1930s. During the second world war, most accessions were lost, and the remaining 826 viable accessions are called Watkins collection (Wingen et al., 2014). A core set of 121 LCs was developed based on genotypic and some phenotypic evaluation that captures the majority of the genetic diversity of A.E. Watkins collection (Wingen et al., 2014). Recently, 804 accessions of Watkins collection were genotyped using 35K Wheat Breeders' Array showing that a considerable amount of novel genetic diversity is present in the Watkins collection which is yet to be fully explored (Winfield et al., 2018). Several researchers evaluated the Watkins collection and found it as a potential source for identifying new genes or alleles for leaf rust, stripe rust, eyespot, and root-lesion nematode resistance (Dyck, 1994; Bansal et al., 2011; Thompson and Seymour, 2011; Burt et al., 2014). However, these LC's are yet to be evaluated for resistance to tan spot, SNB, and FHB.

Molecular markers linked to genes or quantitative trait loci (QTLs) can facilitate simultaneous marker-assisted breeding and pyramiding for several traits avoiding laborious and time-consuming phenotyping. Previously, QTL mapping has been used to

identify marker-trait associations for *Tsr1/tsn1* (Faris et al., 1996), *Tsr2/tsn2* (Singh et al., 2006), *Tsr3/tsn3* (Tadesse et al., 2006a), *Tsr4/tsn4* (Tadesse et al., 2006b), *Tsr5/tsn5* (Singh et al., 2008) and *Tsr6/tsc2* (Friesen and Faris, 2004) and three toxin sensitivity or insensitivity loci related to SNB, *Snn1* (Liu et al., 2004a), *Snn2* (Friesen et al., 2008), and *Tsn1* (Liu et al., 2006). However, QTL studies have lower power in identifying QTLs with small effect and typically demarcate QTLs to large genomic regions (Korte and Ashley, 2013), whereas the availability of high-density SNP arrays (Wang et al., 2014b; Allen et al., 2017) and next-generation sequencing technologies (Poland et al., 2012) makes genome-wide association (GWAS) a powerful tool for dissecting the genetic architecture of complex traits. Further, GWAS can effectively identify many natural allelic variations in a large set of unrelated individuals as compared to the traditional QTL mapping (Huang and Han, 2014). The effectiveness of GWAS has already been established in several crops by identifying the genomic regions controlling a variety of traits like grain shape and flowering time in rice (Zhao et al., 2011; Feng et al., 2016), husk traits (Cui et al., 2016) and stalk lodging resistance-related traits in corn (Zhang et al., 2018b), drought stress in barley (Pham et al., 2019), and tan spot resistance in cultivated rye (Sidhu et al., 2019). In wheat, GWAS has been employed to capture genetic factors affecting complex traits like agronomic (Sukumaran et al., 2015; Sun et al., 2017a), end-use qualities (Chen et al., 2019), and disease resistance including tan spot (Gurung et al., 2011, 2014; Patel et al., 2013; Kollers et al., 2014), Stagonospora nodorum blotch (Adhikari et al., 2011; Gurung et al., 2014), Fusarium head blight (Arruda et al., 2016), spot blotch (Ayana et al., 2018), and stem and leaf rust (Edae et al.,

2018; Juliana et al., 2018). Thus, evaluating the Watkins LCs for resistance to leaf spot and head diseases and identifying linked molecular markers through GWAS is noteworthy.

The objectives of this study were to evaluate the core set of Watkins LCs for resistance to tan spot (*P. tritici-repentis* race 1 and race 5), SNB, and FHB and identify resistant LCs that can be exploited in improving resistance to tan spot, SNB, and FHB in wheat. In addition, GWAS was performed to characterize genomic regions conferring resistance to tan spot (Ptr race 1 and race 5) and SNB in Watkins core set.

3.3. Results

3.3.1. Phenotypic/resistance evaluation

The Watkins core set of 121 LCs evaluated against Ptr race 1 and race 5 and corresponding toxins Ptr ToxA and Ptr ToxB respectively, showed a diverse response (Supplementary Table S1). Genotypic variation for both the tan spot races (Ptr race 1 and 5) was significant ($p < 2e^{-16}$) among genotypes (Supplementary Table S2). The mean disease score for tan spot Ptr race 1 and Ptr race 5 among LCs was 3.6 and 1.9, respectively (Table 3.1). Of the 121 LCs, 2 (1.6%), 17 (14.0%), 54 (44.6%), and 48 (39.7%) were resistant, moderately resistant, moderately susceptible, and susceptible against Ptr race 1 respectively (Figure 3.1). On the other hand, the majority of the LCs were found to be resistant (29.7%) or moderately resistant (65.2%) against Ptr race 5 (Figure 3.1). The Pearson correlation coefficient (r) values between three repeated experiments (exp.) was 0.74 (exp. 1 and 2), 0.68 (exp. 2 and 3), and 0.75 (exp. 1 and 3)

for Ptr race 1 and 0.80 (exp. 1 and 2), 0.64 (exp. 2 and 3), and 0.67 (exp. 1 and 3) for Ptr race 5.

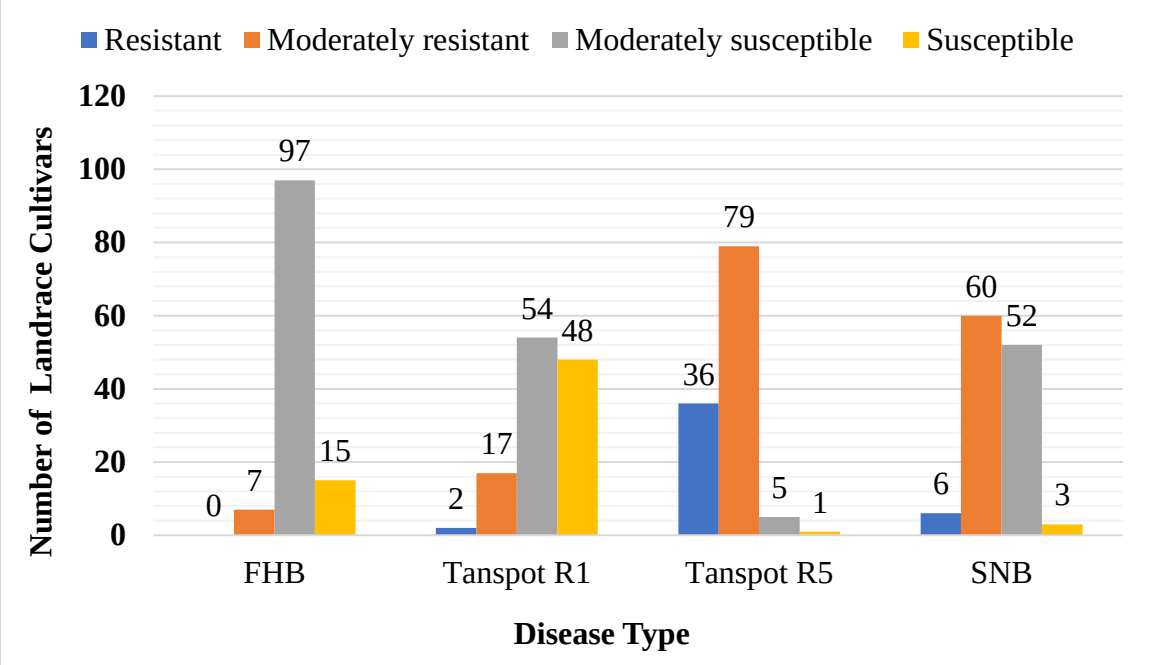

Figure 3.1. Bar graph showing the response of Watkins landrace cultivars (LCs) against Fusarium head blight (FHB), Tan spot *Pyrenophora tritici-repentis* (Ptr) race 1 (R1) and race 5 (R5), and Stagonospora nodorum blotch (SNB) evaluation. The X-axis representing the type of diseases and the Y-axis showing the number of LCs found resistant, moderately resistant, moderately susceptible, and susceptible in the evaluation. Values on the bar represents number of LCs.

A diverse response to SNB was observed among the genotypes ($p < 2e^{-16}$) (Supplementary Table S2). The mean disease score for 121 LCs was 2.8 with a range of 1.3 to 4.0 (Table 3.1). About 5% (n=6), 49% (n=60), 43% (n=52), and 2.5% (n=3) of LCs were found to be resistant, moderately resistant, moderately susceptible, and susceptible respectively against *P. nodorum* (Figure 3.1). The Pearson correlation coefficient values

between experiments were 0.76 (exp. 1 and 2), 0.69 (exp. 2 and 3), and 0.76 (exp. 1 and 3) for SNB. A variable response ($p < 2e^{-10}$) to FHB was also observed among the 119 LCs in the mist-irrigated, inoculated FHB nursery (Supplementary Table S2). The moderately resistant check Lyman showed a disease index of 15.2 and susceptible check Overley showed a disease index of 50 (Table 3.1). Out of 119 LCs, only seven (6%) demonstrated a moderately resistant response (DI: 13.4-25.3) while all other LCs (94%) showed moderately susceptible to susceptible (DI: 26.1-56.7) response to FHB in the field nursery (Figure 3.1, Supplementary Table S1). In addition to FHB response, there was also a significant variation ($p < 2e^{-16}$) between the two replications, indicating the presence of field and inoculation variation between the replications (Supplementary Table S2). The mean FHB disease severity, incidence, and index in the core set were 34.4, 98.9, and 34.1 respectively (Table 3.1). The seven moderately resistant LCs were further analyzed in the greenhouse using the point inoculation method and five of these LCs displayed percent spikelet severity (PSS) ranging from 8.6-10.2% (moderately resistant), while two LCs showed moderate susceptibility (Table 3.1).

Table 3.1: Watkins LCs found resistant/moderately resistant to leaf spot diseases and FHB.

Accession No.	Country of origin	Tan spot				Stagonospora nodorum blotch (SNB)	Fusarium head blight (FHB)			
		Ptr race 1		Ptr race 5						
		Reaction type[a] (Lesion type)	Ptr ToxA reaction	Reaction type[a] (Lesion type)	Ptr ToxB	Reaction type[a] (Lesion type)	Accession No.	Country of origin	Reaction type[b] (Disease Index)	Percent spikelet severity (PSS)
1190007	Australia	MR (2.28)	Insensitive	MR (1.56)	Insensitive	R (1.5)	1190032	India	MR (22.8)	10.2
1190042	France	MR (1.56)	Sensitive	R (1.22)	Insensitive	MR (1.67)	1190308	Iran	MR (23.0)	8.6
1190103	Italy	R (1.44)	Sensitive	R (1.0)	Insensitive	MR (2.61)	1190551	Spain	MR (23.75)	9.6
1190126	India	MR (2.28)	Insensitive	R (1.0)	Insensitive	MR (2.5)	1190662	Romania	MR (25.18)	9.6
1190160	Spain	MR (1.78)	Sensitive	R (1.44)	Insensitive	MR (1.56)	1190788	Turkestan	MR (25.35)	9.2
1190273	Spain	MR (2.0)	Insensitive	R (1.0)	Insensitive	MR (2.44)	-	-	-	-
1190292	Cyprus	MR (1.89)	Sensitive	R (1.11)	Insensitive	MR (1.72)	-	-	-	-
1190397	Portugal	MR (1.56)	Insensitive	R (1.17)	Insensitive	MR (2.56)	-	-	-	-
1190398	Palestine	MR (1.72)	Insensitive	R (1.22)	Insensitive	MR (2.94)	-	-	-	-
1190662	Romania	MR (2.56)	Insensitive	MR (2.0)	Insensitive	MR (2.0)	-	-	-	-
1190698	China	MR (1.83)	Insensitive	MR (1.61)	Insensitive	MR (2.17)	-	-	-	-
1190740	USSR	MR (1.67)	Insensitive	R (1.44)	Insensitive	MR (2.22)	-	-	-	-
1190912	Hungary	R (1.39)	Sensitive	R (1.33)	Insensitive	R (1.17)	-	-	-	-
Salamouni		1		1		1	Lyman		15.25	-
6B662		-		4.3		-	Overley		50	34.6
Glenlea		4.56		-		4	Emerson		-	9.1
Mean		3.6		1.9		2.8			34.1	
CV (%)[c]		12.7		19.4		14.3			14.7	
LSD[d]		0.7		0.6		0.6			9.9	
Range		1.3-4.4		1.1-4.0		1.3-4.0			17.4-56.7%	

[a] Tan spot Race 1 and Race 5 and stagonospora nodorum blotch (SNB) disease reaction scoring from 1 to 5.
[b] Fusarium head blight (FHB) reaction type based on disease index in field experiments. [c] CV= Coefficient of variation, d LSD= least significant difference

3.3.2. Reaction of LCs to PtrToxA and PtrToxB

All 121 Watkins LCs were also screened against Ptr ToxA and Ptr ToxB. Just over 50% of the LCs (n=61) showed sensitivity to Ptr ToxA (produced by Ptr race 1 causing tan spot) with necrotic lesions in the toxin infiltrated leaf area, while the other 49.6% LCs (n=60) were rated as toxin insensitive because they did not show any visible necrosis (Figure 3.2). Among nineteen of the resistant or moderately resistant LCs, 26% (n=5) were sensitive and 74% (n=14) were insensitive to Ptr ToxA. Out of 102 LCs that exhibited a susceptible response to Ptr race 1, 56 (55%) LCs were sensitive and 46 (45%) LCs were insensitive to Ptr ToxA (Figure 3.2).

	Ptr ToxA reaction			Ptr ToxB reaction		
		In (60)	Sen (61)		In (111)	Sen (10)
Ptr race 1 reaction	R (19)	14 (74%)	5 (26%)	Ptr race 5 reaction	R (115)	109 (95%)	6 (5%)
	S (102)	46 (45%)	56 (55%)		S (6)	2 (23%)	4 (67%)

Figure 3.2. Reaction of Watkins core set of landrace cultivars (LCs) to tan spot (Ptr race 1), Ptr ToxA and Ptr race 5, and Ptr ToxB respectively. R; resistant; S; susceptible; In; insensitive to Ptr ToxA or ToxB; Sen; sensitive to Ptr ToxA or ToxB.

In case of Ptr ToxB (produced by tan spot Ptr race 5), 111 LCs (92%) displayed as insensitive with no visible chlorosis, while the only remaining 10 LCs (8%) exhibited

sensitivity by producing chlorosis in the infiltrated area of the leaves. Of the 115 LCs showing resistance to Ptr race 5, 95% (n=109) were insensitive to the Ptr ToxB and 5% (n=6) were sensitive (Figure 3.2 and Figure 3.3). Among the six LCs susceptible to Ptr race 5, 67% (n=4) and 23% (n=2) manifested sensitive and insensitive response to Ptr ToxB respectively (Figure 3.2 and Figure 3.3). We found a significant correlation between LCs response to Ptr ToxA and Ptr race 1 (p-value=0.04) and Ptr ToxB and Ptr race 5 (p-value=$4.903e^{-06}$) (Figure 3.2).

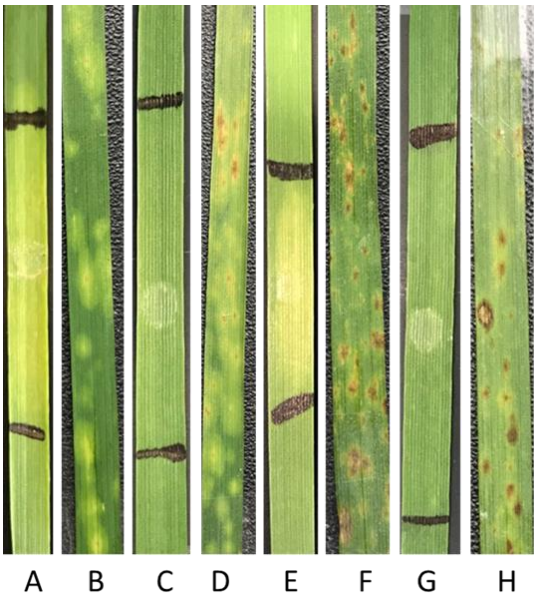

Figure 3.3. Response reaction of Watkins landrace cultivars (LCs) against *Pyrenophora tritici-repentis* (Ptr) and corresponding toxin (Ptr ToxB) at seeding stage in greenhouse. A) Ptr ToxB reaction in 6B662 (susceptible check); B) Ptr race 5 reaction in 6B662 (susceptible check); C) Insensitive reaction of Acc.1190305 to Ptr ToxB; D) Acc.1190305 showing susceptibility to race 5; E) Acc.1190352 representing sensitivity to Ptr ToxB; F) Acc.1190352 representing resistance to race 5; G) Ptr ToxB reaction in Salamouni (resistant check), and H) Ptr race 5 reaction in Salamouni (resistant check).

3.3.3. Geographical distribution of the resistant and susceptible LCs

In this study, germplasm identified as resistant to the three diseases were collected from different parts of the world. The LCs that conferred resistance to Ptr race 1 were mainly collected from different European countries (Supplementary Figure S1A). On the other hand, most of the LCs resistant to Ptr race 5 were distributed around the Mediterranean Sea and southwest Asia (Supplementary Figure S1B). Like tan spot, the resistant or moderately resistant LCs to SNB also came from two broad geographical regions in Asia and Europe (Supplementary Figure S1C). Out of the five LCs moderately resistant to FHB, three were collected from Asian counties (India, Iran, and Turkestan) and two from Europe (Spain and Romania) (Supplementary Figure S1D).

3.3.4. Genotyping and Population structure in Watkins core set

The 35,143 SNP genotype data for 118 LCs was obtained from Winfield et al. (Winfield et al., 2018). The data was filtered using a minor allele frequency (MAF) < 0.05 and missing value of >10% to obtain 10,828 high-quality SNPs. Model-based Bayesian clustering of 118 LCs using 10,828 SNPs in STRUCTURE program we determined that Watkins core set was comprised of largely two main subpopulations. However, our principal component analysis (PCA) showed that 23.4 % of the variation was explained by the first component (PC1), while 8.8% and 6.3% variations were explained by the second and third principal components, respectively (Supplementary Figure S2). Overall, a total of 38.5% of the variation was explained by the first three components. Another 2,021 SNPs with no available position (cM) on the genetic map (Allen et al., 2017) were

further removed to obtain 8,807 SNPs that were used for GWAS analysis. Out of 8,807 SNPs, 41.3% (n=3,639) were from A genome, 49.5% (n=4,356) from B genome, and 9.2% (n=812) from D (Supplementary Table S3).

3.3.5. Marker-trait associations (MTA)

Marker-trait associations revealed 20 putative genomic regions conferring resistance to tan spot (Ptr race 1 and 5) and SNB in the Watkins LCs of wheat (Figure 3.4 and Table 3.2). Quantile-quantile (Q-Q) plots of p-values for different diseases showed that the MLM model accounting for population structure and kinship fits our data (Figure 3.4).

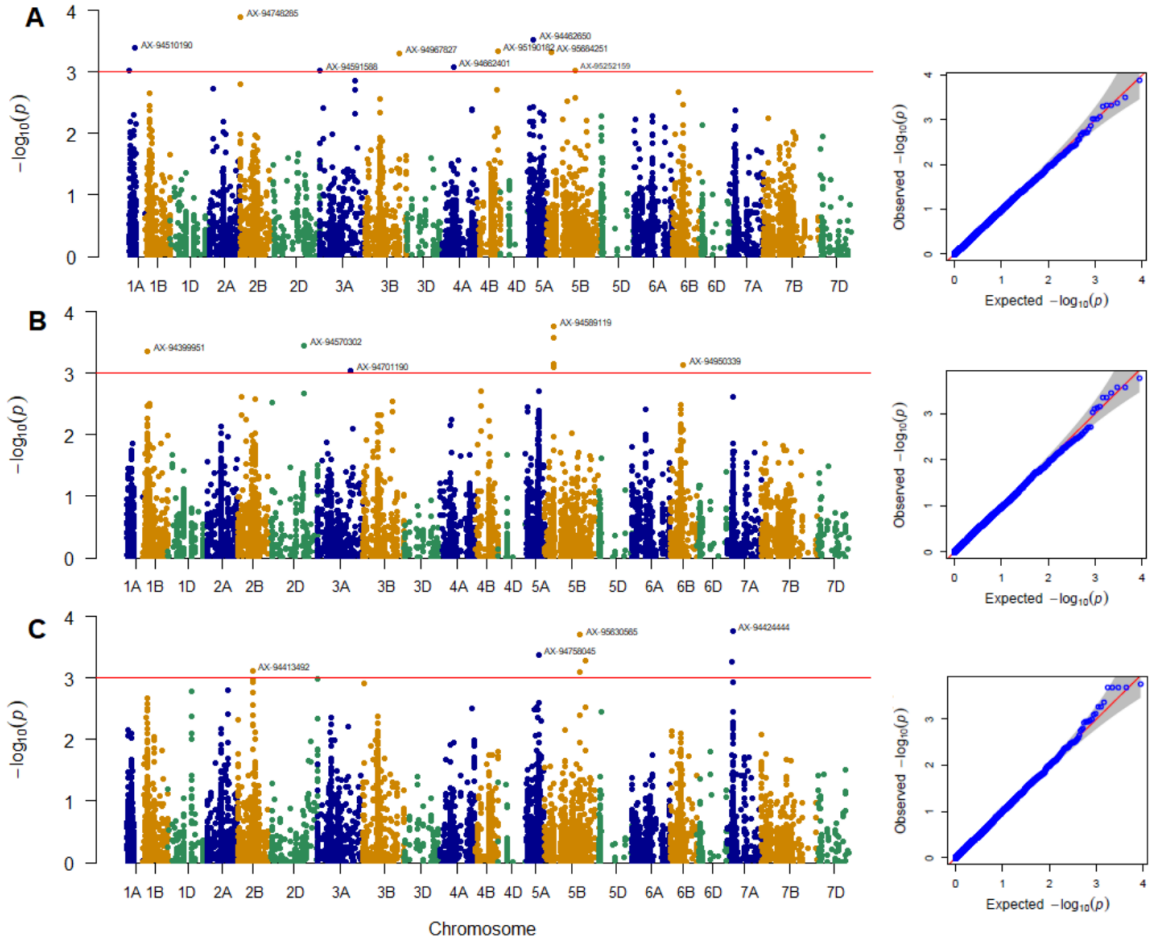

Figure 3.4. Genome-wide association scan. Mixed linear model (MLM) based Manhattan plots represent–log10 (p-value) for SNPs distributed across all 21 chromosomes of wheat. A) *Pyrenophora tritici-repentis* race 1 (Ptr race 1); B) *Pyrenophora tritici-repentis* race 5 (Ptr race 5); C) Stagonospora nodorum blotch (SNB). Y-axis:–log10 (p-value) and x-axis: wheat chromosomes. The horizontal lines stands as a threshold for significant markers with–log10 (p-value) of > 3 which correspond to a p-value <1 × 10−3. On the right side of each model, Quantile-Quantile (QQ) plots represent expected null distribution of p-values vs observed p-values.

In total, thirty significant markers with -$\log_{10}$ (*P*-value) >3.0 were identified to be associated with the traits studied. Significant markers identified ten genomic regions associated with response to Ptr race 1 that were distributed on eight chromosomes including 1A (182.2cM and 267.2cM), 2B (3.1cM), 3A (1.9cM), 3B (202.7cM), 4A (107.3cM), 4B (4.99Mbp), 5A (373.0cM), and 5B (15.7cM and 166.7cM). The significant markers explained phenotypic variation ranged from 14 to 17%. Five genomic regions associated with resistance to Ptr race 5 were identified on chromosomes 1B (50.4cM), 2D (216.1cM), 3A (198.2cM), 5B (55.3cM), and 6B (165.2cM) (Table 3.2, Figure 3.4). A QTL, *Q.Ts5.sdsu-5BS* explained the maximum variation of 20% for response to Ptr race 1. In total, six new QTLs (*Q.Ts1.sdsu-4BS*, *Q.Ts1.sdsu-5BS*, *Q.Ts5.sdsu-1BL*, *Q.Ts5.sdsu-2DL*, *Q.Ts5.sdsu-3AL*, and *Q.Ts5.sdsu-6BL*) were identified for tan spot. Association analysis for a response to SNB revealed five genomic regions on four chromosomes 2B (89.1cM), 5A (116.6cM), 5B (210.8cM and 243.0cM), and 7A (29.9cM) (Table 3.2). One SNP (AX-94394626) on chromosome 5BL (*Q.Snb.sdsu-5BL*), significantly associated with SNB resistance at the seedling stage, and explained 22% of the phenotypic variation.

Table 3.2: Significant associations between single nucleotide polymorphism (SNP) markers and Watkins LCs response to two major leaf spot diseases (tan spot Ptr race 1, race 5, and SNB).

Trait	QTLs (SNP markers)	Allele	Chr	Genetic position (cM[a])	Physical position (Mbp)	P-value	R^2
PTR1	*Q.Ts1.sdsu-1AL* (AX-94510190)	C/T	1AL	182.2	536.43	0.0004	0.16
	Q.Ts1.sdsu-1AL (AX-94932688)	C/T	1AL	267.2	589.02	0.0009	0.14
	Q.Ts1.sdsu-2BS (AX-94748285)	A/T	2BS	3.1	6.31	0.0001	0.18
	Q.Ts1.sdsu-3AS (AX-94591588)	C/T	3AS	1.9	20.00	0.0010	0.14
	Q.Ts1.sdsu-3BL (AX-94967827)	G/T	3BL	202.7	798.55	0.0005	0.16
	Q.Ts1.sdsu-4AL (AX-94662401)	C/T	4AL	107.3	543.74	0.0008	0.15
	Q.Ts1.sdsu-4BS (AX-95190182)	C/G	4BS	-*	4.99	0.0005	0.16
	Q.Ts1.sdsu-5AL (AX-94462650)	A/G	5AL	373.0	671.39	0.0003	0.16
	Q.Ts1.sdsu-5BS (AX-95684251)	A/C	5BS	15.7	13.43	0.0005	0.16
	Q.Ts1.sdsu-5BL (AX-95252159)	C/T	5BL	166.7	568.82	0.0010	0.14
PTR5	*Q.Ts5.sdsu-1BL* (AX-94399951)	C/T	1BL	50.4	352.39	0.0004	0.19
	Q.Ts5.sdsu-2DL (AX-94570302)	G/T	2DL	216.1	413.78	0.0004	0.19
	Q.Ts5.sdsu-3AL (AX-94701190)	A/G	3AL	198.2	719.76	0.0009	0.17
	Q.Ts5.sdsu-5BL (AX-94589119)	G/T	5BL	55.3	314.30	0.0002	0.20
	Q.Ts5.sdsu-6BL (AX-94950339)	C/G	6BL	165.2	678.74	0.0007	0.18
SNB	*Q.Snb.sdsu-2BS* (AX-94413492)	A/G	2BS	89.1	238.50	0.0008	0.20
	Q.Snb.sdsu-5AL (AX-94758045)	C/T	5AL	116.6	472.34	0.0004	0.21
	Q.Snb.sdsu-5BL (AX-94394626)	G/T	5BL	210.8	638.83	0.0002	0.22
	Q.Snb.sdsu-5BL (AX-94878132)	C/T	5BL	243.0	679.13	0.0005	0.21
	Q.Snb.sdsu-7AS (AX-94424444)	C/T	7AS	29.9	53.26	0.0002	0.23

[a] The cM position is based on individual genetic maps (Allen et al. 2017), * No genetic position is available

3.3.6. In silico gene annotation of the QTL regions

For response to tan spot Ptr race 1, a total of 500 genes in the 10 QTL regions with known functions in CS RefSeq v1.1 (Appels et al., 2018) were identified and 106 of those genes are predicted to have defense-related functions including major families like LRR (Leucine-rich repeat), NB-ARC (NB-ARC domain), cytochrome P450, and Pkinase (Protein kinase) (Supplementary Table S4). In addition, other proteins such as cysteine-rich secretory protein family (Pathogenesis-related protein 1), sugar transporter protein, peroxidase, ABC transporter, mitochondrial carrier protein, Barwin family (Pathogenesis-related protein PR-4), and acidic chitinase were found. In five candidate regions conferring resistance to tan spot Ptr race 5, a total of 207 genes identified of which only 26 known genes had a role in plant defense responses (Supplementary Table S4). Most of the genes belong to the protein kinase domain family. However, NBS-LRR type, NB-ARC type, and ABC transporter genes were also identified. In candidate regions conferring SNB resistance, 291 genes were identified from five QTL regions. Among them, only 36 genes were found to be associated with plant defense mechanisms. The identified proteins were mainly protein kinase domain, cytochrome P450 family, leucine-rich repeat receptor-like protein kinase family, NBS-LRR, and NB-ARC domain. (Supplementary Table S4).

3.4. Discussion

Continuous improvement in wheat varieties is needed to meet the consumer demand and ensure global food security, especially with unpredictable climatic conditions causing

new biotic and abiotic stresses. Mining novel resistant germplasm sources for wheat improvement could be a key breeding strategy to address these challenges. Evaluating the core set of Watkins LCs provided some useful insight about the distribution of resistant and susceptible germplasm to various diseases and identified potential LCs which could be a valuable source of resistant genes or alleles against tan spot, SNB, and FHB (Table 3.1).

3.4.1. Geographical distribution and characterization of resistant source

A large percentage of Watkins LCs were both susceptible to Ptr race 1 and showed a resistant response to Ptr race 5. Finding resistance against Ptr race 1 is more challenging as compared to race 5 because race 1 is the most prevalent race in Africa, Asia, Europe, North and South America (Postnikova and Khasanov Glavpochtamt, (Uzbekistan)), 1998; Ali and Francl, 2003; Lamari et al., 2003; Benslimane et al., 2011; Aboukhaddour et al., 2013). Other than its widespread presence, Ptr race 1 was also reported to contain the virulence of both race 2 and 3 (Lamari et al., 2003), making it more aggressive than other races. In this study, most of the LCs (84%) were found to be susceptible or moderately susceptible to Ptr race 1 originated from the region around the Mediterranean Sea and all over Asia (Supplementary Figure S1A). This result could be partly explained by the environmental factor such as favorable weather conditions during wheat growth in the Mediterranean Sea and Asia for disease development or lower of selection pressure. Our results are in agreement with the earlier reports where a large portion of tested wheat

germplasm was found susceptible to Ptr race 1 (Xu et al., 2004; Ali et al., 2008; Chu et al., 2008c, 2008a; Liu et al., 2015).

Two host-selective toxins (HST: Ptr ToxA and Ptr ToxB) produced by the various races and considered to be associated with the two symptoms necrosis and chlorosis respectively (Orolaza, 1995; Strelkov and Lamari, 2003), were used to evaluate the 121 LCs. All four combinations of toxin-disease reactions were observed among these LCs; tan spot Ptr race1 resistance-Ptr ToxA insensitive (74%), tan spot Ptr race 1 resistant-Ptr ToxA sensitive (26%), tan spot Ptr race 1 susceptible- Ptr ToxA sensitive (45%), tan spot Ptr race 1 susceptible-Ptr ToxA insensitive (55%) (Figure 3.2). Data from this study support the statement that the host reaction to HST does not determine the resistance or susceptibility of the host to Ptr races. These observations were consistent with previous studies (Noriel et al., 2011; Abdullah et al., 2017) and suggest that though Ptr ToxA plays a role in aggressiveness and can be used as a predictor of resistance/susceptibility, however, it is not the sole cause of pathogenicity and insensitivity to Ptr ToxA does not necessarily imply resistance to Ptr race 1 (Friesen et al., 2003). Results also suggest that other pathogenicity factors in addition to Ptr ToxA might be involved in host disease response (Noriel et al., 2011; Abdullah S, 2017).

Landrace collections response to Ptr race 5 showed a majority of LCs (95%) were resistant or moderately resistant to Ptr race 5, indicating very low virulence present in this race and those lines were mainly distributed around the region of Mediterranean Sea and in southwest Asia (Supplementary Figure S1B). Ali et al. (Ali et al., 2008) previously

reported the similar type of resistance reaction, where they found around 98% wheat genotypes resistant to Ptr race 5, however, Tadesse et al (2006b) found 84% of the tested cultivars susceptible against Ptr race 5. These differences could be attributed to the different genetic backgrounds of the germplasm evaluated.

Similar to the tan spot Ptr race 1-Ptr ToxA interaction system, all four combinations of toxin-disease reactions were observed; Ptr race 5 resistance-ToxB insensitive (95%), Ptr race 5 resistant-ToxB sensitive (5%), Ptr race 5 susceptible-ToxB sensitive (23%), and Ptr race 5 susceptible-ToxB insensitive (67%) (Figure 3.2). For example, accession 1190305 was insensitive to Ptr ToxB and susceptible to Ptr race 5, while accession 1190352 was sensitive to Ptr ToxB but resistant to Ptr race 5 (Figures 3.2 and 3.3). These four combinations of toxin-disease reaction system are fully established in Ptr race 1-ToxA interaction but the parallel relationship showing Ptr ToxB insensitivity, and Ptr race 5 susceptibility observed in this study seems to be not reported so far. Therefore, results from this study suggest that germplasm which is insensitive to Ptr ToxB is not necessarily resistant to Ptr race 5 and this could be results of multiple effector-host susceptibility interactions.

Nearly half of LCs evaluated for response to SNB in this study demonstrated resistant or moderately resistant reactions, majorly dispersed in European and Asian countries, indicating that tested LCs could be a good source of resistant genes/alleles for SNB resistant wheat breeding programs (Supplementary Figure S1C). Several other previous studies also found around 50% of tested material was resistant or moderately resistant to

SNB using both elite wheat genotypes and wheat-alien species derivatives (Mergoum et al., 2007; Oliver et al., 2008).

This study did not find any FHB resistant LCs within the core set of Watkins collection. However, five moderately resistant LCs that came from various parts of the world were identified. Three out of five moderately FHB resistant LCs identified in the field and greenhouse were originally collected from Asian countries (India, Iran, and Turkestan), indicating Asia a potential source of resistance (Supplementary Figure S1D). Previous studies have shown that a high level of resistance to FHB was mainly found in Asian sources like Chinese and Japanese cultivars (Bai and Shaner, 2004; Yu et al., 2008). Most (94%) of the tested LCs were susceptible or moderately susceptible to FHB, which implied that the resistant resources for FHB were rare in the Watkins collection. The five moderate resistance LCs could be further characterized and used in FHB resistance breeding.

3.4.2. Marker-trait association

Ten genomic regions were identified on eight chromosomes that were significantly associated with Ptr race 1 resistance. Previous studies (Faris et al., 1996; Tadesse et al., 2006; Juliana et al., 2018) have reported QTLs on eight (1AL, 2BS, 3AS, 3BL, 4AL, 5AL, and 5BL) of the 10 genomic regions, and our study supports those QTLs and identifies tightly linked SNP markers. We identified SNP AX-95252159 (*Q.Ts1.sdsu-5BL*) located on chromosome 5BL (166.7cM), which corresponds to previous known tan spot host-selective toxin (HST) insensitivity gene *tsn1* (Faris et al., 1996; Anderson et al.,

1999). A Genome-wide association study (GWAS) was also performed on the response to toxin infiltration with a purified toxin (Ptr ToxA) that produce necrosis in leaves. Infiltration study revealed three additional SNP (AX-94912015, AX-94941069, and AX-95659861) around 150 cM on chromosome 5BL co-segregating with a genomic region very close to *Tsn1* locus (Faris et al., 1996; Anderson et al., 1999). In addition to the known QTLs, two novel QTLs (*Q.Ts1.sdsu-4BS* and *Q.Ts1.sdsu-5BS*) on chromosome 4BS and 5BS were identified (Table 3.2).

Five genomic regions conferring resistance to Ptr race 5 were identified (*Q.Ts5.sdsu-1BL*, *Q.Ts5.sdsu-2DL*, *Q.Ts5.sdsu-3AL*, *Q.Ts5.sdsu-5BL*, *Q.Ts5.sdsu-6BL*) on chromosomes 1BL, 2DL, 3AL, 5BL, and 6BL (Table 2, Figure 4). Ptr race 5 produces a toxin (Ptr ToxB) and the sensitivity to this toxin is regulated by the *Tsc2* gene which was previously mapped on the short arm of chromosomes 2B (Friesen and Faris, 2004). However, no significant marker-trait association on 2BS was found where the *Tsc2* gene is located. It is also likely that due to the limited statistical power, we could not detect *Tsc2* in the Watkins core set. Furthermore, previous studies related to Ptr race 5 and tan spot non-race specific studies revealed genomic regions conferring resistance on chromosomes 2AS, 4AL, and 2BL (Friesen and Faris, 2004), 2AS and 5BL (Chu et al., 2008c), 1BS and 3BL (Faris and Friesen, 2005), 2D, 6A and 7D (Gurung et al., 2011), 3B, 5D, 6B, and 7B (Liu et al., 2015). It is clear from these independent studies that only a few common chromosomal locations have been identified related to Ptr race 5 resistance. The likely reason for rare overlap among studies could be the result of the frequency of the causal alleles in populations and small sample size. Another explanation

is that the wheat-Ptr pathosystem is complex and there may be other virulence factors in addition to toxin Ptr ToxB involved in tan spot resistance (Chu et al., 2008c).

Marker-trait associations for a response to SNB were identified in five genomic locations on chromosomes 2BS, 5AL, 5BL, and 7AS (Table 3.2, Figure 3.4). Three major genes for toxin sensitivity or insensitivity, *Snn1*, *Snn2*, and *Tsn1* were previously mapped on chromosome 1BS, 2DS and 5BL, respectively (Liu et al., 2004b, 2006; Friesen et al., 2008). In this study, no marker was found related to *Snn1* and *Snn2* genes. However, several markers were found co-segregating with a genomic region on chromosome 5BL where the major gene *Tsn1* is located (Francki, 2013). Further, we identified a SNP significantly associated with SNB resistance on chromosome 2BS, where a resistance QTL was previously identified by Czembor et al (Czembor et al., 2003).

3.4.3. In Silico functional annotation of the QTL regions

Host-pathogen interaction induces a plant defense mechanism that can be divided into two major categories, (i) constitutive defense that is triggered by pathogen-associated molecular patterns (PAMPs) and (ii) a temporarily induced more localized mechanism in which plants try to defend a specific attacked area (Howe and Jander, 2008). In plants, resistance (R) proteins are usually involved in pathogen recognition that triggers innate constitutive immune responses (Gouveia et al., 2017). There are many R genes that have been cloned so far and most resistance proteins contain a central nucleotide-binding (NB) domain fused with a C-terminal leucine-rich repeat (LRR) domain. This study found NB-ARC and NBS-LRR type genes in many of the annotated QTL regions (Supplementary

Table S4). The NB-ARC domain is a functional ATPase domain and its nucleotide-binding state is found to regulate the activity of R-proteins (Van Ooijen et al., 2008). The NBS-LRR are the most common R-genes, which detect pathogen-associated proteins, typically effector molecules of pathogens that are responsible for virulence (DeYoung and Innes, 2006). One major susceptibility gene for tan spot and SNB is *Tsn1* which encodes a protein with a leucine-rich repeat domain that is similar to the one found in NLR proteins (Keller et al., 2018). Another large family of proteins identified in this study was Receptor-like kinases (RLKs) which is involved in various functions like plant growth, development, hormone perception and response to pathogens. Most defense-related RLKs are the LRR subclass (Ramonell and Goff, 2007). The cloning of *Snn1* providing resistance against SNB identified Wall Associated kinases (WAKs), a unique class of receptor-like kinase (RLKs) which are known to drive pathways for biotrophic pathogen resistance. *Snn1* recognizes SnTox1, leading to activation of programmed cell death, thus allowing the necrotroph to gain nutrients and sporulate (Shi et al., 2016). Further, we also identified peroxidase superfamily protein which is an important component of pathogen-associated molecular pattern-triggered immunity (PTI) and plays a significant role in the production of reactive oxygen species (ROS) in response to pathogen attack (Daudi et al., 2012; Mammarella et al., 2015). Several other genes identified in this study are known to be related to plant defense-related responses including plant chitinase proteins that take part in pathogenesis-related activities (Punja and Zhang, 1993), glutathione S-transferase T3 (Wisser et al., 2005), serine/threonine-protein kinase (Zhou et al., 1995), ABC transporter (Krattinger et al., 2009),

pathogenesis-related protein 1(PR 1) (Develey-Rivière and Galiana, 2007), and disease resistance protein RPM1 (Tornero et al., 2002).

3.5. Conclusions

The mining of superior alleles is essential for continuous improvement in wheat germplasm. Recent diversity studies (Wingen et al., 2014; Winfield et al., 2018) have shown that global collections of landraces have excellent potential. Since Watkins LCs are hexaploid wheat, like modern varieties, molecular characterization and gene introgression of useful traits could be more effective due to less linkage drag as compared to introgressions from other wild relatives. In this study, after a thorough screening of the core set of LCs against tan spot (Ptr race1 and race 5), SNB, and FHB, many potential genetic resources (Table 3.1) for wheat improvement were identified. This study strengthens the fact that Watkins collection is a useful genetic resource, which may confer broad resistant gene sources against various diseases (DYCK and JEDEL, 1989; Hiebert et al., 2005; Bansal et al., 2011; Burt et al., 2014) and improving useful agronomic traits. As a recommendation, accession 1190662 (Romania) could be a valuable breeding resource because it confers resistance or moderate resistance to all the diseases evaluated (tan spot Ptr race1 and race 5, SNB, and FHB) in this study. Similarly, thirteen other LCs (acc.1190007, acc.1190042, acc.1190103, acc.1190126, acc.1190160, acc.1190273, acc.1190292, acc.1190397, acc.1190398, acc.1190662, acc.1190698, acc.1190740, and acc.1190912) showed resistance to tan spot (Ptr race1 and race 5) and SNB (Table 3.1). All these LCs could be excellent sources for current or future multi-

disease resistant germplasm improvement programs. In addition, identified resistant landraces with the diverse country of origin could be a valuable source for improving the genetic diversity in wheat. Furthermore, new QTLs and tightly linked SNPs (Table 3.2) identified in this study may be used to develop Kompetitive allele-specific PCR (KASP) assays (Supplementary Table S5) for marker-assisted breeding for tan spot and SNB.

3.6. Methods

3.6.1. Plant and fungal material

A core set of 121 Watkins land race (LC) cultivars were obtained from John Innes Centre (JIC), UK (Wingen et al., 2014). The LCs used in this study were collected from more than thirty different countries in Europe, Asia, Africa, Australia, and the USSR (Union of Soviet Socialist Republics). Most of the land races were found related to two broad geographical regions. Among which 45% of the landraces come from Asian countries and 37% from Europe (Supplementary Table S1).

All 121 LCs were evaluated for response to tan spot caused by *P. tritici-repentis* (Ptr) race 1 (isolate Pti2) and race 5 (isolate DW7) and Stagonospora nodorum blotch (SNB) caused by *Parastagonospora nodorum* (isolate Sn2K) under greenhouse conditions at the seedling stage. A set of differential lines/cultivars Salamouni (resistant to tan spot Ptr race1, race 5, and SNB), Glenlea (susceptible to tan spot Ptr race 1 and SNB), and 6B662 (susceptible to tan spot Ptr race 5) were included as checks for tan spot and Stagonospora nodorum blotch (SNB). An aggressive *Fusarium graminarum* strain (Fg1) was used to evaluate LCs for FHB in the mist-irrigated field nursery and selected moderately resistant

LCs were validated in the greenhouse. Moderately resistant cultivars Overland, Lyman, and Emerson and susceptible cultivars Flourish and Overley were used as checks for FHB.

3.6.2. Evaluation of Watkin LCs for their reaction to tan spot using Ptr race 1 and race 5 and Ptr ToxA and ToxB

3.6.2.1. Reaction to Ptr race 1 and race 5

The core set of 121 Watkin LCs was planted in a single root trainer container (Ray Leach "Cone-trainer"^{TM} Single Cell System) filled with Sunshine R 360 potting soil (Sun Gro Horticulture, Agawam, MA, USA). The cones were arranged in trays (Stuewe & Sons, Tangent, OR, USA) following a randomized complete block design with three replications, and the entire experiment was repeated three times. The inoculum was prepared by plating dry plugs of the isolate stored at -20°C in the center of petri plates containing V8PDA media (150 mL of V8 juice, 10 g of Difco PDA, 10 g of Difco agar, 3 g of calcium carbonate, and 850 mL of distilled water) (Lamari and Bernier, 1989). V8PDA plates were wrapped with aluminum foil paper and incubated for 5–6 days at room temperature. When the culture had grown about 3 cm from the center, mycelial growth was flattened with the help of a flamed sterile test tube bottom in the presence of distilled sterilized water. Excess water was removed and the plates were incubated under continuous light for 24 h at 21 °C followed by 24 h in the dark at 16 °C to induce conidiophores and conidia, respectively. Finally, 25 mL sterile distilled water was added to each plate and the conidia were dislodged with a sterile loop wired needle. Inoculum

concentration was adjusted to 3×10^3 conidia mL^{-1} using a hemacytometer. Two-week-old seedlings were spray inoculated with Ptr race 1 and 5 as described by Lamari and Bernier (1989). Following inoculation, seedlings were moved into a mist chamber to provide 100% humidity for 24 h to initiate infection. After 24 h, seedlings were transferred to a greenhouse bench at South Dakota State University, Brookings, SD. Disease response was scored 7 days after inoculation using a 1 to 5 scale lesion rating system, where scores 1–2 indicates resistant to moderately resistant, and 3–5 indicates moderately susceptible to susceptible (Lamari and Bernier, 1989).

3.6.2.2. Reaction to toxin Ptr ToxA and ToxB

Three fully expanded leaves of each accession were infiltrated with Ptr ToxA or Ptr ToxB culture filtrates using a needle-less syringe as described by Faris et al (Faris et al., 1996). Dr. Timothy Friesen, USDA-AS, Fargo, ND, kindly provided the culture filtrates. Leaves of differential genotypes such as Salamouni (insensitive to Ptr ToxA and Ptr ToxB), Glenlea (sensitive to Ptr ToxA), and 6B662 (sensitive to Ptr ToxB) were infiltrated with the equal volume (20-25 ul) of full-strength filtrate. All the infiltrated plants including differential genotypes were rated after 72 hours of toxin infiltration for necrosis (Ptr ToxA) or chlorosis (Ptr ToxB) symptoms and the leaves were rated as sensitive (+) or insensitive (–) reactions to each of the toxins (Ptr ToxA and Ptr ToxB).

3.6.3. Evaluation of Watkin LCs for their reaction to SNB

Seedlings were inoculated at the two-leaf stage in a greenhouse using the method described for tan spot. The experiment was conducted following a randomized complete block design with three replications and repeated thrice. A pure culture of Sn2k was revived on V8PDA medium by placing two dried mycelial plugs in the center of the plate. The plates were incubated at 21°C under light for 7d. The pycnidiospores were collected by adding 30 mL sterile distilled water into each plate and by scraping the plate surface using a sterile glass slide. Inoculum concentration was estimated with a hemacytometer and adjusted to 1×10^{6} mL^{-1} before inoculation. After inoculation, seedlings were moved to a humidity chamber to provide 100% humidity for 24h and then moved back to the greenhouse bench. Disease reactions were scored 8d after inoculation using a numerical scale of 0 to 5 based on the lesion type as described in Liu et al (Liu et al., 2007), where scores 0-2 were considered resistant and score 3 and above were considered susceptible.

3.6.4. Evaluation of Watkin LCs for their reaction to FHB in field and greenhouse

3.6.4.1. Field Evaluation

Watkins LCs along with checks were evaluated in mist-irrigated, inoculated FHB nurseries located in Brookings, SD. Each accession was planted in the field using a head-row planter in a 3-feet long row maintaining about 40 plants per row. The experiment was conducted following a randomized complete block design with two replications. Fusarium-infected corn kernels (scabby corn inoculum) were spread in the field at three,

two, and one-week intervals prior to heading (beginning at boot stage). In addition, direct spray inoculation was conducted at 50% anthesis for each line using a conidial suspension containing 100,000 spores/ml and a misted irrigation was applied to maintain the humidity. Twenty-one days after inoculation, disease severity was scored for 20 spikes per LC using a visual scale described by Stack and McMullen (Stack and Mcmullen, 2011). In this scale, the percentage of the infected spikelets on each of the sampled heads were visually estimated based on 10 categories of infection (0, 7%, 14%, 21%, 33%, 50%, 66%, 79%, 90%, and 100%) and disease severity was calculated by averaging all 20 heads. Disease incidence was calculated based on the number of spikes per 20 heads showing any level of disease symptoms. Disease incidence was multiplied with disease severity to calculate the FHB disease index (DI).

3.6.4.2. Greenhouse Evaluation

The Watkins LCs demonstrating moderately resistant responses were further evaluated in the greenhouse for Type II resistance using the point inoculation method described by Stack et al (Stack et al., 2002). Spore suspension was prepared from *Fusarium graminearum* (isolate Fg1) grown in ½ PDA media. The central spikelets of at least 20 spikes from each accession were inoculated at the flowering stage with l0µl of 50,000 conidia/ml. Just after inoculation, heads were lightly misted and covered with Ziploc plastic bags to maintain the relative humidity above 90% and the greenhouse temperature was kept at 20 to 26 ºC. Three days after inoculation, Ziploc plastic bags were removed. Infected spikelets of each spike were counted after twenty-one days. The total number of

spikelets in each of the inoculated spikes were used to calculate the percent spikelet severity (PSS).

3.6.5. Genotyping and SNP discovery

The Watkins collection was recently genotyped with the Axiom® Wheat Genotyping Breeders' Array platform (Winfield et al., 2018), which contains 35K SNPs (Allen et al., 2017). The genotyping data of 118 LCs were obtained from the online database CerealsDB (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/indexNEW.php). The genotype data of 118 LCs was then filtered by removing SNPs with minor allele frequency (MAF) < 0.05 and a missing value of >10%. The genetic positions of selected SNPs were obtained from the wheat 35K SNP map (Allen et al., 2017). The SNP flanking sequences were mapped using BLASTN to wheat RefSeq v1.1 assembly to identify the physical locations of the genetically mapped SNPs.

3.6.6. Statistical analyses

Descriptive statistical parameters including mean, standard deviation, and coefficient of variation of disease scores (reactions) for tan spot, SNB, and FHB were calculated using R version 3.5.3 (R Core Team, 2014). The R program was also used to perform an analysis of variance (ANOVA) to test the significance of response among LCs to different diseases. We have performed Pearson's chi-squared test to see if the toxin sensitivity/insensitivity and disease severity are correlated.

3.6.6.1. Structure analysis

Population structure within the Watkins core set of LCs (n = 118) was determined by the Principal component analysis (PCA) and STRUCTURE analysis (Pritchard et al., 2000b). Principal component analysis (PCA) among and between the LCs was performed using the R-package 'prcomp'. Structure analysis was done using STRUCTURE software version 2.3.4 (Pritchard et al., 2000b) with burn-in period and a number of Markov Chain Monte Carlo (MCMC) iterations set as 10000 and 20000, respectively. The best-fit number of clusters (DeltaK) was determined by STRUCTURE HARVESTER (Duncan et al., 2017) following Evanno et al (Evanno et al., 2005).

3.6.6.2. Marker-trait associations (MTA)

GWAS was performed to find marker-trait association using 8,807 SNP markers and the disease score data for tan spot (Ptr race 1 and race 5), and Stagonospora nodorum blotch (isolate Sn2K) with 'GAPIT' package (Tang et al., 2016) in the R program. Based on available genotypic information, a total of 118 LCs from the Watkins core set were used for GWAS analysis. Two linear models, the GLM (generalized linear model), which is based on the least square fixed effects and the MLM (mixed linear model), with both fixed and random effects, were evaluated. Marker effect and population structure (Q) were modeled as fixed effects, whereas the relatedness among the individuals (kinship) was modeled as random effect. A kinship matrix was calculated using GAPIT's default VanRaden algorithm (VanRaden et al., 2011) and population structure (Q) was obtained using PCA (Zhao et al., 2007). The MLM method was selected for analysis because of its statistical power and ability to control type I error. Significant association of markers and

traits was determined by the p-value < 1.0×10^{-3} or -log10 (p-value) > 3. The MLM for GWAS can be mathematically represented as:

$$y = X\beta + Zu + e$$

Where, y represents the vector of the phenotypic values, β represents fixed effects due to the marker and population structure, u represents the vector of the random effects, e represents the vector of residuals, and X and Z are the incidence matrices for β and u respectively.

3.6.6.3. Candidate gene annotation in QTL regions

The physical positions of all significant SNPs on Chinese spring (CS) RefSeq v1.1 were obtained from IWGSC (Appels et al., 2018). To find candidate genes associated with resistance to tan spot and SNB, the candidate regions flanking the significant SNP marker were demarcated. A 5 megabase pair (Mb) region (2.5Mb up and downstream each) from the significant SNP was selected. The CS high confidence (HC) gene annotation version 1.1 (Appels et al., 2018) was used to identify genes involved in plant defense mechanisms.

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3.8. Appendix

List of Supplementary Tables:

Supplementary Table S1. Watkins core set of wheat landrace cultivars, their country of origin and mean disease score with standard error for tan spot, SNB and FHB.

Entry number	Accession number	Country of origin	Mean Disease Score			
			Tan spot Ptr race 1	Tan spot Ptr race 5	SNB	FHB Disease Index
1	1190004	Iraq	3.7±0.31	1.1±0.11	1.5±0.29	34±1.3
2	1190007	Australia	2.3±0.15	1.6±0.22	1.5±0.29	34±6.5
3	1190023	Australia	3.8±0.22	1.8±0.29	3±0	31±5.3
4	1190032	India	3.7±0.33	1.3±0.19	3.4±0.06	23±0
5	1190034	India	3.3±0.33	2.3±0.19	2.7±0.19	35±2.8
6	1190040	France	3.7±0.33	1.9±0.11	1.3±0.33	39±8.8
7	1190042	France	1.6±0.29	1.2±0.22	1.7±0.33	38±3.6
8	1190044	Morocco	4.3±0.33	2.8±0.11	3±0	31±0.6
9	1190045	Syria	4.3±0.33	2.7±0	3.2±0.22	33±1.3
10	1190079	India	3.3±0.33	1.2±0.11	2.6±0.22	41±4.5
11	1190081	India	4.2±0.4	2.4±0.22	3.2±0.22	32±0
12	1190092	India	4±0	1±0	3.9±0.24	34±0.9
13	1190103	Italy	1.4±0.29	1±0	2.6±0.06	54±0
14	1190110	France	4.7±0.15	1.8±0.22	2.8±0.11	29±3.5
15	1190126	India	2.3±0.15	1±0	2.5±0	30±10.3
16	1190127	India	3.9±0.11	1±0	2.2±0.22	35±1.8
17	1190139	France	3.9±0.48	1.7±0.33	3.4±0.29	34±3.8
18	1190141	China	4.1±0.29	1.7±0.33	2.6±0.2	30±0
19	1190145	Spain	4±0	2.4±0.29	2.9±0.29	30±6.8
20	1190149	United Kingdom	4±0	1.6±0.4	3.3±0.15	32±0
21	1190160	Spain	1.8±0.62	1.4±0.11	1.6±0.4	36±4.5
22	1190166	India	3.8±0.17	2.7±0	3.2±0.22	36±0.5
23	1190181	Poland	3.3±0.33	2.3±0.33	3±0	41±0.8
24	1190199	India	3.9±0.11	2±0	3.7±0.19	43±0
25	1190209	Egypt	3.6±0.29	1.8±0.25	3.7±0.15	38±0
26	1190216	Morocco	2.1±0.36	1.4±0.22	3.2±0.17	43±9.3
27	1190218	Tunisia	3.6±0.22	2±0	2.8±0.11	NA

28	1190219	Spain	4±0.19	2.8±0.17	3.2±0.17	39±2.3
29	1190223	Burma	4±0.19	1.7±0.33	2.9±0.11	29±1.2
30	1190224	China	2.1±0.59	1.4±0.29	3.8±0.29	31±4.3
31	1190231	Hungary	4.2±0.4	1.2±0.22	3±0	40±0.3
32	1190238	Iran	3.8±0.17	2±0	3.3±0.19	54±2.3
33	1190239	Spain	4.5±0.29	1.7±0.33	2.2±0.17	53±0
34	1190246	India	4.3±0.38	1.4±0.11	1.3±0.33	37±8
35	1190254	Morocco	4±0.38	2.7±0.33	3.3±0.17	38±0
36	1190264	Canary Islands	4.2±0.4	1.5±0.29	3.9±0.11	48±12.3
37	1190273	Spain	2±0	1±0	2.4±0.29	33±1.6
38	1190281	Greece	3.8±0.17	1.4±0.29	2.6±0.22	34±0
39	1190291	Cyprus	4.3±0.17	2.4±0.22	2.1±0.11	33±6.5
40	1190292	Cyprus	1.9±0.22	1.1±0.11	1.7±0.15	34±0
41	1190299	Turkey	3.9±0.36	2.4±0.29	3.3±0.19	33±1.5
42	1190300	Turkey	4.3±0.19	2.1±0.34	3.4±0.06	32±0.3
43	1190305	Egypt	4±0.19	3.8±0.11	2.8±0.36	24±0
44	1190308	Iran	3.9±0.11	1.1±0.11	2.9±0.11	23±0
45	1190313	Burma	3.9±0.11	2.2±0.11	2.8±0.35	30±1.5
46	1190324	China	3.4±0.4	1±0	1.3±0.33	29±3.9
47	1190325	United Kingdom	3.6±0.29	1.9±0.4	2±0	29±2.7
48	1190349	Bulgaria	4.1±0.11	2.2±0.17	4±0	30±2.8
49	1190352	Yugoslavia	4.2±0.22	2.2±0.11	3.9±0.24	33±0.3
50	1190355	Yugoslavia	4.3±0.19	1.3±0.19	3.7±0.15	31±0.3
51	1190360	Yugoslavia	3.4±0.11	2.7±0.33	2.8±0.17	32±3.5
52	1190387	Spain	4.2±0.11	2±0.19	3.4±0.2	36±2.3
53	1190396	Portugal	3.9±0.11	2±0	3.3±0.15	32±0.3
54	1190397	Portugal	1.6±0.11	1.2±0.17	2.6±0.29	31±0.5
55	1190398	Palestine	1.7±0.15	1.2±0.22	2.9±0.24	34±1.3
56	1190406	India	3.9±0.29	1.7±0.19	3.1±0.11	34±11.5
57	1190420	India	3.5±0.29	2.1±0.11	2.8±0.35	40±0
58	1190433	India	4.1±0.11	2.2±0.22	3.6±0.2	29±4.4
59	1190440	China	4.1±0.22	2.3±0.19	3.9±0.11	31±2.8
60	1190444	China	3.8±0.17	4±0.19	2.8±0.35	37±10.9
61	1190451	Romania	3.2±0.48	2.1±0.11	1.6±0.45	30±10.5
62	1190460	Afghanistan	3.9±0.11	2.3±0.33	4±0	35±4.5
63	1190468	Afghanistan	4.1±0.44	3±0	3.7±0.33	27±1.9
64	1190471	Afghanistan	4.1±0.11	2.9±0.24	3.3±0.15	30±2.5
65	1190474	Afghanistan	4.3±0.15	2.3±0.33	2.9±0.24	35±0.1

66	1190475	Afghanistan	3.8±0.44	2.2±0.17	3.4±0.2	34±2.8
67	1190481	Poland	3.9±0.34	1.8±0.17	2.9±0.34	28±3.5
68	1190483	Poland	3.9±0.34	3±0.19	2.5±0	41±5.6
69	1190496	Morocco	4.1±0.11	2.3±0	3.4±0.22	27±0
70	1190507	Australia	3±0.51	1.6±0.29	3.2±0.22	44±6.8
71	1190546	Spain	2.7±0.19	2.2±0.11	3.6±0.22	37±13.3
72	1190551	Spain	4.4±0.2	2.1±0.11	2.4±0.22	24±0
73	1190560	Greece	3.8±0.17	1.4±0.11	3.7±0.33	57±0
74	1190562	Greece	4±0	1.7±0.19	3.2±0.29	34±7.7
75	1190566	Greece	4.2±0.17	2.1±0.11	4.2±0.29	32±9.8
76	1190568	China	4.3±0.15	2.6±0.22	3.3±0.15	38±4
77	1190579	Iran	3.9±0.11	1.7±0.15	2.4±0.29	32±0
78	1190580	Iran	2.3±0	3.4±0.22	2.6±0.22	38±0
79	1190591	Portugal	3.8±0.11	1±0	2.6±0.2	36±2
80	1190605	Greece	4±0	1.9±0.22	1.9±0.34	32±0.8
81	1190624	Bulgaria	3.9±0.24	2±0	3.1±0.24	32±7.5
82	1190627	Iran	4.3±0.15	1.8±0.62	2±0	26±0
83	1190629	Iran	3.9±0.24	2.2±0.22	2.4±0.2	34±3.5
84	1190637	Turkey	3.9±0.11	1.2±0.22	1.6±0.4	28±2.3
85	1190639	Crete	4.1±0.11	1.7±0.33	1.8±0.4	35±0
86	1190651	China	3.7±0.38	1.8±0.17	2.3±0.33	33±7.8
87	1190652	China	3.5±0.1	1.2±0.11	1.7±0.43	35±11.1
88	1190662	Romania	2.6±0.11	2±0	2±0	25±3.3
89	1190670	Poland	3.2±0.29	2.1±0.11	2.3±0.15	29±0.3
90	1190671	USSR	4±0	1.3±0.33	2.3±0.33	34±1
91	1190680	Italy	4.2±0.22	2.3±0	2±0	35±1
92	1190683	Spain	4.1±0.11	2.1±0.11	2.7±0.15	35±1
93	1190685	Spain	4.2±0.22	1.7±0.15	3.3±0.19	27±0
94	1190690	Greece	3.9±0.11	1.7±0.33	2.1±0.11	43±0
95	1190694	India	3.9±0.29	1.9±0.11	2.9±0.28	36±0.1
96	1190698	China	1.8±0.44	1.6±0.06	2.2±0.17	28±1
97	1190700	China	3.4±0.29	1.4±0.11	2.4±0.29	17±3.8
98	1190704	Iran	3.4±0.29	1.2±0.17	3.3±0.33	32±3.5
99	1190705	Iran	3.9±0.48	2.1±0.24	3.2±0.17	38±4.3
100	1190707	India	4±0	2.7±0	2.8±0.4	34±3.3
101	1190722	China	3.8±0.11	2.2±0.17	3.9±0.29	34±6.2
102	1190729	Iran	3.9±0.11	2.4±0.22	3.9±0.29	31±4
103	1190731	India	4.1±0.11	2±0.29	3.2±0.22	39±3.3
104	1190732	India	4.1±0.11	1.9±0.31	3.2±0.11	32±1.3

105	1190740	USSR	1.7±0.19	1.4±0.22	2.2±0.22	28±0.2
106	1190742	Algeria	3.8±0.11	2.8±0.11	3.9±0.29	42±3.5
107	1190746	USSR	4.1±0.11	1.9±0.31	3.5±0.1	47±8
108	1190747	Ethiopia	4±0	1±0	3.7±0.33	34±0
109	1190749	USSR	3.3±0.69	2.5±0.29	2.2±0.22	34±5.3
110	1190750	USSR	4±0	1.4±0.29	2.2±0.17	36±2.5
111	1190753	USSR	3.2±0.48	1.3±0.19	2.6±0.29	34±4
112	1190771	USSR	1.9±0.29	1.2±0.17	3.3±0.33	NA
113	1190777	Finland	2.1±0.11	1.3±0.33	3.3±0.33	49±15
114	1190784	Italy	3.9±0.11	2.5±0.29	2.4±0.29	28±0.8
115	1190788	USSR	3.7±0.15	2.6±0.22	2.8±0.33	25±0.2
116	1190789	USSR	4±0	3.3±0.15	3±0	37±13.8
117	1190811	Tunisia	3.6±0.39	1.8±0.11	2.3±0.17	27±0.5
118	1190814	Tunisia	3.3±0.44	1.7±0.15	1.7±0.31	33±2
119	1190816	Italy	4.1±0.11	2.3±0.33	2±0	37±3.5
120	1190827	China	4±0	2.8±0.11	3.9±0.11	31±0
121	1190912	Hungary	1.4±0.2	1.3±0.19	1.2±0.17	31±2.8

Supplementary Table S2. Analysis of variance (ANOVA) response to tan spot Ptr race 1, race 5, Stagonospora nodorum blotch (SNB), and Fusarium head blight (FHB).

Disease	Variable	DF	SS	MS	F value	P
Tan spot Ptr race 1	Genotype	120	634.6	5.288	16.99	<2e-16 ***
	Experiment	2	2.6	1.278	4.106	0.0168 *
	Block	2	0.9	0.47	1.51	0.2216
	Residuals	882	274.5	0.311		
Tan spot Ptr race 5	Genotype	120	390.6	3.255	14.802	< 2e-16 ***
	Experiment	2	0.4	0.222	1.008	0.36534
	Block	2	2.4	1.176	5.345	0.00492 **
	Residuals	910	200.1	0.22		
SNB	Genotype	120	516.7	4.306	15.933	< 2e-16 ***
	Experiment	2	3.7	1.873	6.933	0.00103 **
	Block	2	1	0.525	1.942	0.14399
	Residuals	855	231	0.27		
FHB	Genotype	118	9678	82	3.256	2.39e-10 ***
	Block	1	2329	2328.7	92.448	< 2e-16 ***
	Residuals	118	2972	25.2		

***, **, * Significant at p<0.001, p<0.01, p<0.05 respectively

Supplementary Table S3. SNP distribution across the three wheat genomes used for GWAS in 121 Watkins landrace cultivars (LCs).

Genome	Chromosome	Number of SNPs	% SNPs
A	1	556	
	2	565	
	3	490	
	4	360	
	5	650	
	6	461	
	7	557	
A genome	**1-7**	**3,639**	**41.3**
B	1	709	
	2	696	
	3	705	
	4	338	
	5	796	
	6	671	
	7	441	
B genome	**1-7**	**4,356**	**49.5**
D	1	204	
	2	207	
	3	99	
	4	37	
	5	123	
	6	66	
	7	76	
D genome	**1-7**	**812**	**9.2**
Total SNPs (A, B, and D)		**8,807**	**100**

Supplementary Table S4. List of genes in the candidate regions spanning the tan spot race 1, 5, and SNB resistance QTLs and their functional annotations.

S. no.	Trait	QTL	Chromosome	Most significant SNP	Candidate gene ID*	Protein
1	**Tan spot Ptr race 1**	*Q.Ts1.sdsu-1AL*	1AL	AX-94510190	TraesCS1A01G350800.1	Leucine-rich repeat receptor-like protein kinase family protein
2					TraesCS1A01G351600.1	Cytochrome P450 family protein, expressed
3					TraesCS1A01G353400.1	Acidic chitinase
4					TraesCS1A01G353900.1	NBS-LRR-like resistance protein
5					TraesCS1A01G354000.1	NBS-LRR-like resistance protein
6					TraesCS1A01G354100.1	NBS-LRR-like resistance protein
7					TraesCS1A01G354200.1	NBS-LRR-like resistance protein
8					TraesCS1A01G355300.1	Pathogenesis-related protein PR-4
9					TraesCS1A01G356100.1	Peroxidase
10					TraesCS1A01G356200.1	Mitochondrial carrier protein, expressed
11		*Q.Ts1.sdsu-1AL*	1AL	AX-94932688	TraesCS1A01G439200.1	F-box family protein
12					TraesCS1A01G439900.1	zinc finger MYM-type-like protein
13					TraesCS1A01G440300.1	Leucine-rich repeat receptor-like protein kinase family protein
14					TraesCS1A01G440400.1	Leucine-rich repeat receptor-like protein kinase family protein

15					TraesCS1A01G440600.1	Leucine-rich repeat receptor-like protein kinase family protein
16					TraesCS1A01G440700.1	Leucine-rich repeat receptor-like protein kinase family protein
17					TraesCS1A01G441000.1	Leucine-rich repeat receptor-like protein kinase family protein
18					TraesCS1A01G441500.1	LRR and NB-ARC domains-containing disease resistance protein
19					TraesCS1A01G442100.1	Cytochrome P450 family protein, expressed
20					TraesCS1A01G442800.1	Protein kinase family protein
21					TraesCS1A01G443100.1	Protein kinase
22					TraesCS1A01G443800.1	Pathogenesis-related protein 1
23					TraesCS1A01G444000.1	Pathogenesis-related protein 1
24		*Q.Ts1.sdsu-2BS*	2BS	AX-94748285	TraesCS2B01G006200.1	Cytochrome P450
25					TraesCS2B01G006600.1	Cytochrome P450
26					TraesCS2B01G006900.1	Cytochrome P450
27					TraesCS2B01G007400.3	Receptor protein kinase-related protein-like
28					TraesCS2B01G007500.1	NBS-LRR disease resistance protein, putative
29					TraesCS2B01G007600.1	NBS-LRR disease resistance protein, putative, expressed
30					TraesCS2B01G008300.1	Serine/threonine-protein kinase

31					TraesCS2B01G008400.1	Protein kinase
32					TraesCS2B01G008600.1	cytochrome P450, family 705, subfamily A, polypeptide 21
33					TraesCS2B01G009100.1	Cytochrome P450
34					TraesCS2B01G010600.1	Cytochrome P450
35					TraesCS2B01G010700.1	Leucine-rich repeat receptor-like kinase
36					TraesCS2B01G011200.1	Leucine-rich repeat receptor-like kinase
37					TraesCS2B01G011300.1	Leucine-rich repeat receptor-like kinase
38					TraesCS2B01G011600.1	NBS-LRR disease resistance protein
39					TraesCS2B01G012100.3	ABC transporter B family-like protein
40					TraesCS2B01G012200.1	ABC transporter B family-like protein
41					TraesCS2B01G012300.1	ABC transporter B family-like protein
42					TraesCS2B01G012600.1	StAR-related lipid transfer protein
43					TraesCS2B01G013700.1	Cytochrome P450
44					TraesCS2B01G013800.1	Cytochrome P450 family protein, expressed
45					TraesCS2B01G014000.1	Cytochrome P450 family protein, expressed

46					TraesCS2B01G014200.1	Cytochrome P450 family protein, expressed
47					TraesCS2B01G014300.1	Cytochrome P450
48					TraesCS2B01G014400.1	Cytochrome P450
49					TraesCS2B01G014500.1	Cytochrome P450 family protein, expressed
50					TraesCS2B01G014600.1	Cytochrome P450
51					TraesCS2B01G014700.1	Cytochrome P450, putative
52					TraesCS2B01G014800.1	Cytochrome P450
53					TraesCS2B01G014900.1	Cytochrome P450, putative
54					TraesCS2B01G015000.1	Cytochrome P450
55					TraesCS2B01G015100.1	Cytochrome P450 family protein
56					TraesCS2B01G015400.1	Cytochrome P450
57					TraesCS2B01G015700.1	Cytochrome P450
58					TraesCS2B01G015900.1	Cytochrome P450
59					TraesCS2B01G016500.1	Cytochrome P450 family protein
60					TraesCS2B01G016800.1	Cytochrome P450
61					TraesCS2B01G017000.1	Cytochrome P450 family protein, expressed
62					TraesCS2B01G017200.1	Cytochrome P450
63					TraesCS2B01G017500.1	Cytochrome P450
64					TraesCS2B01G017700.1	Cytochrome P450
65					TraesCS2B01G018200.1	NBS-LRR disease resistance protein-like protein

66					TraesCS2B01G018400.1	Disease resistance protein (NBS-LRR class) family
67					TraesCS2B01G018500.1	NBS-LRR disease resistance protein-like protein
68					TraesCS2B01G018600.1	NBS-LRR disease resistance protein-like protein
69					TraesCS2B01G018700.1	NBS-LRR disease resistance protein-like protein
70					TraesCS2B01G018800.1	NBS-LRR-like resistance protein
71					TraesCS2B01G018900.1	Cytochrome P450
72		*Q.Ts1.sdsu-4AL*	4AL	AX-94662401	TraesCS4A01G232200.1	Receptor-kinase, putative
73					TraesCS4A01G235600.2	Kinase family protein
74					TraesCS4A01G235800.1	Peroxidase
75		*Q.Ts1.sdsu-4BS*	4AS	AX-95190182	TraesCS4B01G004600.1	NBS-LRR-like resistance protein
76					TraesCS4B01G004800.1	NBS-LRR-like resistance protein
77					TraesCS4B01G004900.1	NBS-LRR disease resistance protein
78					TraesCS4B01G005000.1	NBS-LRR-like resistance protein
79					TraesCS4B01G005100.1	NBS-LRR-like resistance protein
80					TraesCS4B01G005200.1	Receptor-like protein kinase
81					TraesCS4B01G005900.1	Cytochrome P450 family protein, expressed
82					TraesCS4B01G006000.1	Cytochrome P450 family protein, expressed

83					TraesCS4B01G006100.1	NBS-LRR disease resistance protein-like protein
84					TraesCS4B01G006300.1	NBS-LRR disease resistance protein-like protein
85					TraesCS4B01G006400.1	NBS-LRR disease resistance protein-like protein
86					TraesCS4B01G007000.1	Leucine-rich repeat receptor-like protein kinase family protein
87					TraesCS4B01G007500.1	Disease resistance protein
88					TraesCS4B01G007600.1	Leucine-rich repeat receptor-like protein kinase family protein
89					TraesCS4B01G007700.1	Leucine-rich repeat receptor-like protein kinase family protein
90					TraesCS4B01G007800.1	Leucine-rich repeat receptor-like protein kinase family protein
91					TraesCS4B01G007900.1	Leucine-rich repeat receptor-like protein kinase family protein
92					TraesCS4B01G008000.1	Receptor protein kinase, putative
93					TraesCS4B01G008100.1	Receptor-like protein kinase
94					TraesCS4B01G009500.1	Receptor protein kinase, putative
95					TraesCS4B01G009700.1	Cytochrome P450 family protein, expressed
96		*Q.Ts1.sdsu-5AL*	5AL	AX-94462650	TraesCS5A01G505200.1	Pathogenic type III effector avirulence factor Avr AvrRpt-cleavage: cleavage site protein

97					TraesCS5A01G506600.1	Tetratricopeptide repeat protein 7A
98					TraesCS5A01G508100.1	kinase-like protein
99					TraesCS5A01G509000.1	cytochrome p450 78a9
100					TraesCS5A01G509400.1	Sugar transporter protein
101					TraesCS5A01G509600.1	Sugar transporter protein
102		*Q.Ts1.sdsu-5BS*	5BS	AX-95684251	TraesCS5B01G011200.1	Pathogenesis-related protein 1
103					TraesCS5B01G011300.1	Receptor protein kinase, putative
104					TraesCS5B01G012000.1	Receptor protein kinase, putative
105					TraesCS5B01G014000.1	Disease resistance protein RPM1
106					TraesCS5B01G014600.1	Serine/threonine-protein kinase
107	**Tan spot Ptr race 5**	*Q.Ts5.sdsu-1BL*	1BL	AX-94399951	TraesCS1B01G196700.3	Kinase family protein
108		*Q.Ts5.sdsu-2DL*	2DL	AX-94570302	TraesCS2D01G319400.1	Glutathione S-transferase T3
109					TraesCS2D01G320600.1	Receptor-like kinase
110					TraesCS2D01G321400.1	Receptor kinase-like protein
111					TraesCS2D01G322600.1	ABC transporter ATP-binding protein
112		*Q.Ts5.sdsu-3AL*	3AL	AX-94701190	TraesCS3A01G490000.1	F-box protein-like
113					TraesCS3A01G490100.1	Receptor-like protein kinase
114					TraesCS3A01G490200.1	protein kinase family protein
115					TraesCS3A01G491400.1	receptor kinase 1
116					TraesCS3A01G491800.1	ATPase subunit 4
117					TraesCS3A01G493100.1	Protein kinase
118					TraesCS3A01G493500.1	Protein kinase

119					TraesCS3A01G493600.1	Protein kinase
120					TraesCS3A01G493700.3	Protein kinase
121					TraesCS3A01G493800.1	Protein kinase
122					TraesCS3A01G493900.1	Protein kinase
123					TraesCS3A01G494000.1	Protein kinase
124					TraesCS3A01G494100.1	Receptor-like protein kinase
125					TraesCS3A01G495100.1	Disease resistance protein (NBS-LRR class) family
126					TraesCS3A01G495200.1	mitochondrial lipoamide dehydrogenase 1
127					TraesCS3A01G495500.1	Disease resistance protein (TIR-NBS-LRR class) family
128					TraesCS3A01G495900.1	F-box protein
129		*Q.Ts5.sdsu-5BL*	5BL	AX-94589119	TraesCS5B01G168400.1	F-box family protein
130					TraesCS5B01G170700.1	Protein kinase-like
131					TraesCS5B01G171000.1	Mitochondrial import inner membrane translocase subunit Tim22
132		*Q.Ts5.sdsu-6BL*	6BL	AX-94950339	TraesCS6B01G399900.1	Serine/threonine-protein kinase
133	**SNB**	*Q.SNB.sdsu-2BS*	2BS	AX-94413492	TraesCS2B01G236100.2	Serine/threonine-protein kinase
134					TraesCS2B01G236800.8	Serine/threonine-protein kinase
135					TraesCS2B01G236900.1	Serine/threonine-protein kinase
136					TraesCS2B01G237000.1	Serine/threonine-protein kinase
137		*Q.SNB.sdsu-5AL*	5AL	AX-94758045	TraesCS5A01G254500.1	F-box family protein
138					TraesCS5A01G254600.1	Receptor protein kinase, putative

139					TraesCS5A01G255800.1	Kinase-like
140					TraesCS5A01G257900.1	Disease resistance protein
141					TraesCS5A01G259500.1	defense protein-like protein
142					TraesCS5A01G259800.1	defense protein-like protein
143					TraesCS5A01G260100.1	defense protein-like protein
144					TraesCS5A01G261200.1	Protein kinase family protein
145					TraesCS5A01G261600.1	Disease resistance protein (NBS-LRR class) family
146		*Q.SNB.sdsu-5BL*	5BL	AX-94394626	TraesCS5B01G465400.1	Glucan endo-1,3-beta-glucosidase 1
147					TraesCS5B01G465800.1	RAC-alpha serine/threonine-protein kinase
148					TraesCS5B01G468100.1	Protein kinase family protein
149		*Q.SNB.sdsu-5BL*	5BL	AX-94878132	TraesCS5B01G511800.1	Protein kinase
150					TraesCS5B01G512500.1	Cytochrome P450
151					TraesCS5B01G512600.1	nodulin MtN21 /EamA-like transporter family protein
152					TraesCS5B01G514200.1	Cytochrome P450
153					TraesCS5B01G516300.1	Cytochrome P450 family protein
154					TraesCS5B01G517600.1	Leucine-rich repeat receptor-like protein kinase
155					TraesCS5B01G517700.1	ABC transporter ATP-binding protein YtrE
156		*Q.SNB.sdsu-7AS*	7AS	AX-94424444	TraesCS7A01G089300.1	Receptor protein kinase, putative
157					TraesCS7A01G089700.1	Peroxidase

158					TraesCS7A01G089900.1	protein kinase family protein
159					TraesCS7A01G090800.1	F-box family protein
160					TraesCS7A01G091000.1	Kinase family protein
161					TraesCS7A01G091100.1	Protein kinase
162					TraesCS7A01G091200.2	Protein kinase
163					TraesCS7A01G091300.1	Protein kinase
164					TraesCS7A01G091400.1	Protein kinase
165					TraesCS7A01G091500.1	Protein kinase
166					TraesCS7A01G091600.1	Leucine-rich repeat receptor-like protein kinase family protein
167					TraesCS7A01G091700.1	Protein kinase
168					TraesCS7A01G092400.1	Disease resistance protein (NBS-LRR class) family

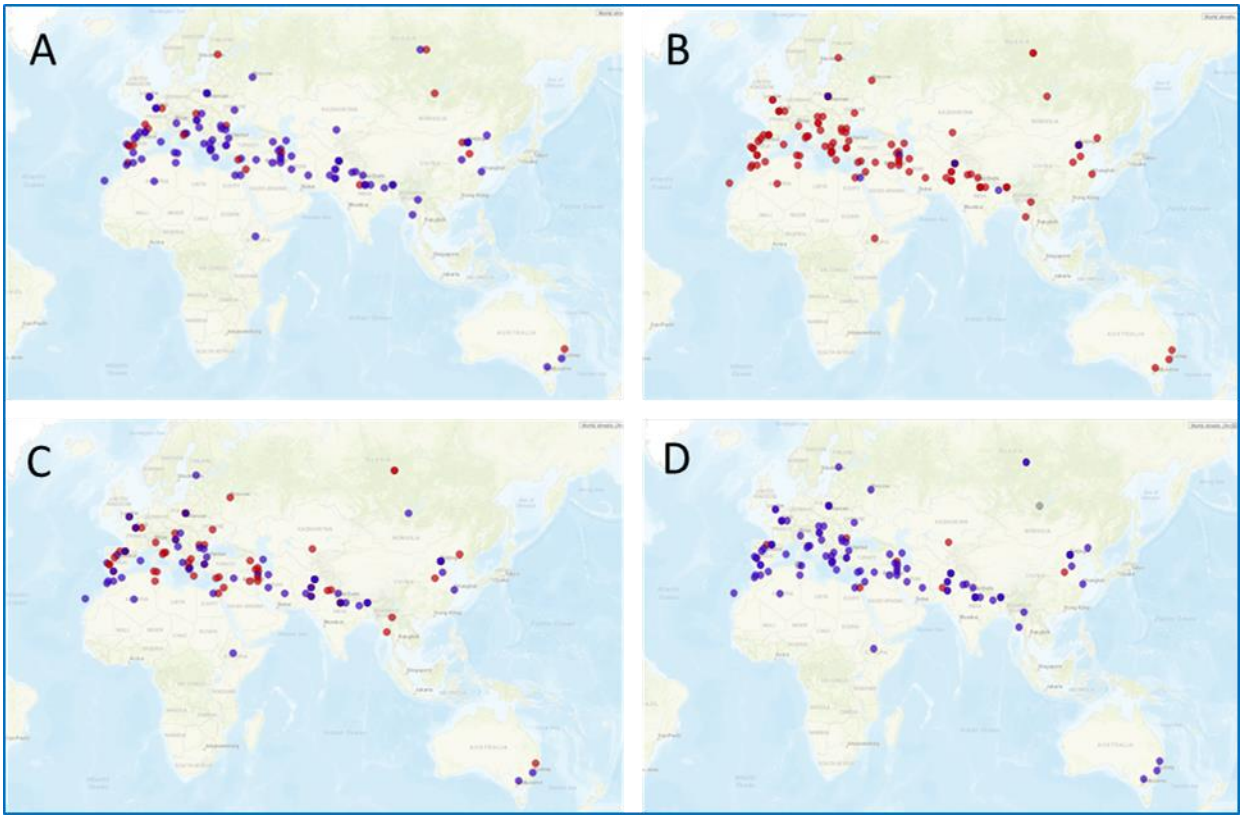
Supplementary Table S5. Flanking Sequence of the most significant SNP markers associated with two major leaf spot diseases (tan spot Ptr race 1, race 5, and SNB).

S. no.	Trait	QTL	Chr.	Most significant SNPs	Flanking sequences
1	Tan spot Ptr race 1	*Q.Ts1.sdsu-1AL*	1AL	AX-94510190	CCTGCGCGAGCACAGGAAGAACAGAGCTCCTAAAT[C/T] GTCTCATGCTCGCAATGATGATGTTGATAACTTTG
2		*Q.Ts1.sdsu-1AL*	1AL	AX-94932688	AAAAATGTGACAGATCCATGTTGTGAAGACATTGC[C/T] AGTACTATTGACAATGGAGCAGATGATAATAATCC
3		*Q.Ts1.sdsu-2BS*	2BS	AX-94748285	TCTGGCAGCCCGAAACTTGAATGAATGAAGAAAAA[A/T] TGTCTTGTATCGTCTCACCATTTTGCTACGGCCAT
4		*Q.Ts1.sdsu-3AS*	3AS	AX-94591588	GCTCAGTTGCTTCATCATCAGCAGGGAAGTTACAT[C/T] ACCTAAATTACCCTTGCACAAGAATTCTCAGCTGT
5		*Q.Ts1.sdsu-3BL*	3BL	AX-94967827	AGGGAGCGTGGTGGGAGAAAGAAATGAGCTTTTCT[G/T] ATCTCAGTTTACTCACAGGACAATGCTTACAACCA
6		*Q.Ts1.sdsu-4AL*	4AL	AX-94662401	TGTTGCAACATTTCAGCCAAGTGGAAATCCGAATG[C/T] TCCTGCCTTTCCTCCTCAAAACATGGAGGTAGCTC
7		*Q.Ts1.sdsu-4BS*	4BS	AX-95190182	ACTTCTGAAGTTTTACAACACTGTTCGGCAAATAC[C/G] TCAGATTAGCGTTGGTAGCATCCAAGACTTCCAAG
8		*Q.Ts1.sdsu-5AL*	5AL	AX-94462650	GCTGCTAATCAAGCTAAATTGAAGCCTACGGAGAT[A/G] ACATGTTGTTAACTAAGAGGTTACAGTGAGGTTGG
9		*Q.Ts1.sdsu-5BS*	5BS	AX-95684251	TACTCACTTTCAGGCCAAGACAAGGCGGACTAGAC[A/C] GGTGATGGGTCCATGTCGTCGGATATCTATGAATG

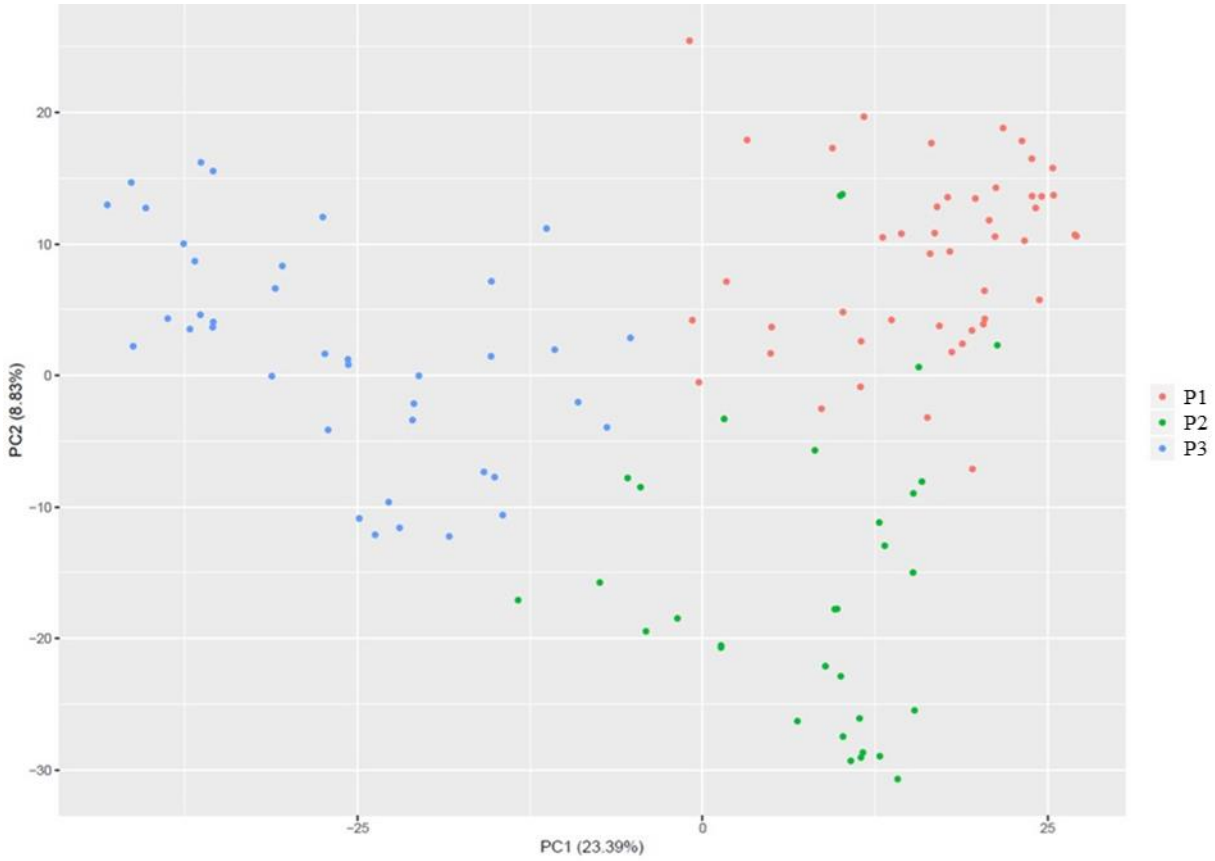
10		*Q.Ts1.sdsu-5BL*	5BL	AX-95252159	TCGCATCTGCGGGCGATGATAAGCTTGTTAAGATC[C/T]GGAAGACTGACTCGTGGCGCTGCATCCAGACTATA
11	Tan spot Ptr race 5	*Q.Ts5.sdsu-1BL*	1BL	AX-94399951	TCTAGTGCGTGCTTGACTAATCTGTATCGTCATAA[C/T]ATGGTCTCACAGAAGTAAATAAACGGTGCATATCC
12		*Q.Ts5.sdsu-2DL*	2DL	AX-94570302	GCCGGTACGTGATCTACGTCAAGGCTGGGGTCTAC[G/T]AAGAGATGGTCATGGTCCCCAAGGACAAGGTGAAC
13		*Q.Ts5.sdsu-3AL*	3AL	AX-94701190	GGAGGTGTACACGAAGCACCACAAGGCGGGGAGTG[A/G]CGAGGTGAAGCGGGAGGAGTTCGCGAAGATGAGCG
14		*Q.Ts5.sdsu-5BL*	5BL	AX-94589119	GTCCGTGTGCTGGACATCAAGTACTTACTGGTATA[G/T]TAGCAACATAATTTGTGTGGGATATGGCAATACGC
15		*Q.Ts5.sdsu-6BL*	6BL	AX-94950339	GCAAAGGAGCACAGAAGGTCCATTCCGAAGCTCGG[C/G]GAAACACCTGGGCTCACAAGGCAGGAATACCACCT
16	SNB	*Q.SNB.sdsu-2BS*	2BS	AX-94413492	GCTCTGTTTGTGAAATACCATTCTTGTGGTATACG[A/G]AGTATAAAGAGGGCCTGACCTTGGTAAATTTCTTT
17		*Q.SNB.sdsu-5AL*	5AL	AX-94758045	GCAGGCCGGAGCGAGTCGAGCTTCACTTTTTTGTT[C/T]CGTTCAGTTAGTGCTGCTGTGCATCGCAGTGTGGA
18		*Q.SNB.sdsu-5BL*	5BL	AX-94394626	CATGCTCGTTACGTTAATTGGCATTGGTGAGTTAT[G/T]TCAAACAAAAATCTCCACACAACCTTCTCTTACAG
19		*Q.SNB.sdsu-5BL*	5BL	AX-94878132	CTCAGGGGGGAAACCAAGTTCAGGAACGCCAAGAA[C/T]AATGCCACTACTGGAGGTCTGTCCATGACTTTAGC
20		*Q.SNB.sdsu-7AS*	7AS	AX-94424444	AAAATCTCCATCACTCGAGTTTGAAGGCAGATCAT[C/T]TTCCAATTGGTAATTTAATCAGATAAAGATGTTAT

Chr. Chromosome

List of Supplementary Figures:



Supplementary Figure S1. Geographical distribution of Watkins landrace cultivars (LCs) and their response to A) tan spot Ptr race 1; B) tan spot Ptr race 5; C) SNB; and D) FHB. Red and blue spots represent resistant and susceptible LCs respectively.



Supplementary Figure S2. Principal Components Analysis (PCA) of 118 Watkins LCs of wheat using 10,828, SNPs. In the PCA plot, the small colored dots representing the LCs and they were colored according to three different populations (P1: Population 1, P2: Population 2, and P3: Population 3) identified by (Winfield et al 2018) using all 804 Watkins LCs and 35K SNPs.

Chapter 4. Genome-wide association and genomic prediction for spike and kernel traits in hard winter wheat

4.1. Abstract

Wheat grain yield is the most important economic trait, and its continuous improvement is of prime importance for all the breeding programs. A better understanding of the genetic control of yield and other yield contributing traits can help in increasing the genetic gain. The present study was designed to identify the marker-trait associations (MTAs) for various spike and kernel-related traits of wheat through genome-wide association studies (GWAS). An association mapping panel comprising 297 hard winter wheat accessions was evaluated for eight spike and kernel-related traits at the three different locations in South Dakota. A wide range of phenotypic variability was found among the accessions for all the studied traits. The coefficient of variation (CV) for these traits ranged from 0.77 (kernel length) to 6.62% (kernels per spike) and high heritability estimates were obtained for each trait, ranging from 0.72 (thousand kernel weight) to 0.93 (kernel length). GWAS was performed using 15,590 SNPs distributed across all the 21 wheat chromosomes. A total of 53 significant SNPs ($P < 0.001$) for seven traits were identified, however, no MTA was detected for kernel length. The highest number of MTAs were found to be located on chromosomes 2B and 4A (6 MTAs each), followed by 1A, 2A, and 3B (5 MTAs each), while the rest of the MTAs were spread on various other chromosomes. We identified 16 MTAs for spike length, followed by spikelet per spike (15), spike density (11), and kernel per spike (5). Only six MTAs were identified for three kernel-related traits (kernel weight, kernel area, and thousand kernel weight).

Out of 53 significant MTAs, 14 were identified in two or more individual environments and were considered as stable QTLs. Five genomic regions were identified to control multiple spike/kernel traits, and these could play an important role in wheat yield enhancement. Further, we compared the predictive ability of spike and kernels traits in HWWAMP using univariate genomic selection (GS) models like GBLUP and multi-trait multi-environment models like BMTME. The multi-trait model (BMTME) outperformed the single-trait model (GBLUP) for all the traits studied in all the environments showing a prediction improvement of up to 147% for SL over the GBLUP model. The results of this study provide useful insights into the complex genetic nature of wheat yield and yield contributing traits in hard winter wheat.

Keywords: Yield, genome-wide association studies (GWAS), Marker-trait associations (MTAs), genomic selection (GS)

4.2. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important and widely grown food crops, which supplies about 20% of the daily protein and caloric requirements for billions of people (Gahlaut et al., 2019). Global food production is required to double by 2050 to feed the growing population, however, the yearly yield increase of wheat is the lowest (0.9%) among the four major food crops (maize, rice, wheat, and soybean), which is far less than the required rate (2.4%) to meet the demand (Ray et al., 2013). Therefore, to feed the ever-increasing world population with a gradual decrease in farmland, yield improvement remains the primary focus for the wheat breeding programs globally.

Wheat yield is a complex phenomenon influenced by various factors such as morphological characteristics, physiological indices, grain-related traits, and different environmental conditions, making this trait challenging to manipulate and improve (Nadolska-Orczyk et al., 2017; Liu et al., 2018c). However, grain yield is a collective output of various individual traits such as spikelet number per spike (SNS), spike length (SL), spike number, kernels per spike (KPS), kernel size (KS), and thousand kernel weight (TKW), which are less sensitive to the environment and have higher heritability than that of grain yield itself (Kato et al., 2000; Hai et al., 2008). Three major components that collectively determine the final yield of wheat are the number of spikes/unit area, kernels per spike (KPS), and thousand kernel weight (TKW) (Liu et al., 2018c). In addition, other spike-related traits like spike number per plant, spike length (SL), and spikelet number per spike (SPS) also play a significant role in wheat yield improvement (Guo et al., 2017). Therefore, identification and further deployment of

important quantitative trait loci (QTLs) that regulate different yield-related traits are essential to dissect the genetic basis of yield and overall improvement of wheat.

Grain yield and related traits are generally controlled by many small-effect QTLs. Two main approaches, traditional QTL mapping and genome-wide association studies (GWAS) have been intensively used to dissect the genetic basis of these complex traits (Liu et al., 2018a). Nevertheless, GWAS offers higher resolution due to a greater number of ancestral gene recombination present in the association mapping panel than in linkage mapping that exploits a few meiotic recombinations (Ward et al., 2019a). GWAS has shown a significant improvement in determining the genetic architecture of major food crops such as wheat (Pang et al., 2020), maize (Yu and Buckler, 2006), and rice (Huang et al., 2010). The effectiveness of GWAS has already been established to capture genetic factors affecting complex traits in wheat such as agronomic (Sukumaran et al., 2015; Sun et al., 2017b), disease resistance (Arruda et al., 2016; Juliana et al., 2018; Halder et al., 2019), and end-use qualities (Chen et al., 2019). GWAS takes advantage of high-marker density across the genome through the availability of single nucleotide polymorphism (SNP) arrays such as 90K (Wang et al., 2014b), 660K (Cui et al., 2017), and 820K making it a more robust and reliable technique (Li et al., 2019a; Tsai et al., 2020).

GWAS and linkage mapping studies have been carried out to identify significant QTLs for several yield-related traits in wheat. For example, QTLs associated with spike-related traits (Huang et al., 2006; Naruoka et al., 2011; Cui et al., 2014; Gao et al., 2015; Guo et al., 2017; Liu et al., 2017; Ward et al., 2019a; Pang et al., 2020), and kernel-related traits (Zhang et al., 2012; Jaiswal et al., 2015; Chen et al., 2016a; Liu et al., 2018c) have been

identified on all the wheat chromosomes. Several GWA studies also reported major effect genes or stable QTLs such as *TaGS5, TaSus1, TaSus2,* and *TaGW2* significantly associated with kernel size, kernel weight, spike and peduncle length, and grain weight (Bednarek et al., 2012; Hou et al., 2014; Wang et al., 2015; Ma et al., 2016). Two major genes responsible for modern wheat spike morphology (*Q* and *C*) were mapped on chromosomes 5A and 2D were also found associated with grain size, shape, grain number, grain yield, and thousand-grain weight. (Johnson et al., 2008; Xie et al., 2018). Spikelet or grain number per spike was associated with the gene *TaMOC1-7A* and *TaTEF-7A* in several studies (Zheng et al., 2014; Zhang et al., 2015). Nevertheless, a considerable number of studies dissected the genetics of yield-related traits in wheat; relatively few used winter wheat germplasm (Ward et al., 2019a). Therefore, it is likely that many new genes/QTLs affecting the yield and contributing traits have yet to be identified in hard winter wheat germplasm.

Apart from GWAS, Genomic selection (GS) is another approach that utilizes genome-wide marker data for early selection of superior individuals and has the potential to increase genetic gain per unit of time (Meuwissen et al., 2001). GS is particularly important to select for complex traits under polygenic traits. Unlike marker-assisted selection (MAS), GS does not require identifying QTLs linked to the important traits; however, it uses genome-wide markers to predict different traits of interest (Bassi et al., 2016). Furthermore, the polygenic nature of most of the agronomic traits, including grain yield, drastically limits the use of MAS in wheat breeding (Wang et al., 2019; Tsai et al., 2020). Thus, GS has been evaluated for many complex traits in wheat, such as grain yield and yield-related traits (Ward et al., 2019b; Guo et al., 2020a; Juliana et al., 2020),

disease resistance (Rutkoski et al., 2014; Arruda et al., 2015), and quality traits (Battenfield et al., 2016).

Despite several studies reporting the evaluation of GS to predict a variety of agronomic traits, the low prediction ability (PA) of GS models remains a challenge in the implementation of this approach. Recent research on GS largely focuses on the appropriate model selection and cross-validation schemes to increase the predictive ability (PA) of various traits (Belamkar et al., 2018; Guo et al., 2020b). Though single trait models, such as ridge-regression best linear unbiased prediction (rrBLUP) and genomic best linear unbiased prediction (GBLUP), are the standard models used for GS (VanRaden, 2008); several multivariate GS models have been proposed that results in an increase prediction ability for different traits (Jia and Jannink, 2012a; Jiang et al., 2015; Schulthess et al., 2016a). Montesinos-López et al. (2016) proposed a Bayesian multi-trait and multi-environment (BMTME) model that extends the conventional models to consider the correlation between multiple traits evaluated over multiple environments. Thus, the BMTME model accounts for T x G x E interaction in a unified approach and yields better predictions over single-trait models (Montesinos-López et al., 2016, 2019b). Recently, few studies reported an increase in the prediction accuracy of agronomic and end-use quality traits in wheat using the BMTME approach (Guo et al., 2020b; Ibba et al., 2020).

The present study sought to perform GWAS in a panel of hard red winter wheat from the US Great Plains region to identify QTL for various spike and kernel related traits and explore the candidate genes presumably responsible for trait of interest using the wheat

reference genome. Further, we employed genomic selection with a cross-validation scheme to evaluate if we can successfully predict various kernel- and spike-related traits. We used data for 8 traits recorded over three environments to estimate the efficacy of recent multi-trait multi-environment models.

4.3. Materials and methods

4.3.1. Plant Materials and field trials

In the current study, we used 297 accessions of the hard winter wheat association mapping panel (HWWAMP) developed under the USDA-TCAP project (Guttieri et al., 2015). This HWWAMP comprises advanced breeding lines and released varieties since the 1940s from the Great Plains region of the US, including North Dakota, Montana, Michigan, South Dakota, Nebraska, Kansas, Oklahoma, Colorado, and Texas.

The association mapping panel was evaluated at three locations E1(Aurora farm), E2 (PlantPath farm, and E3 (Felt farm)) in South Dakota, USA, during the 2019-2020 cropping season using a randomized complete block design (RCBD) with two replications. Each accession was planted in a 1.0 m long two-row plot with an inter-row spacing of 20 cm. The field was managed using recommended agronomic practices for proper growth and development.

4.3.2. Phenotypic trait evaluation and statistical analysis

Eight morphological traits were evaluated, including spikelet number per spike (SPS), spike length (SL), spike density (SD), kernels per spike (KPS), thousand kernel weight (TKW), kernel length (KL), kernel width (KW), and kernel area (KA). Ten random

spikes from each accession per replication were manually harvested at physiological maturity. SL was measured from the base of the rachis to the topmost spikelet, excluding the awns. SPS were counted from the basal sterile spikelet to the top fertile spikelet. SL and SPS were the means of measurements from 10 selected spikes in each replication. SD was calculated as the ratio of SPS to SL. TKW was measured by weighing 500 kernels from each accession with two replications. Three kernel-related traits (KL, KW, and KA) were recorded with an automatic grain analyzer Vibe QM3 (Vibe Imaging Analytics, CA 95010, USA).

META-R (Multi Environment Trail Analysis with R) version 6.04 (Alvarado et al., 2020) was used for estimating the best linear unbiased estimates (BLUE) for all the traits. The BLUEs of two replicates for individual locations were estimated using the following model:

$$y_{ij} = \mu + R_i + G_j + e_{ij}$$

where y_{ij} is the trait of interest, μ is the overall mean, R_i is the effect of the i^{th} replicate, G_j is the effect of the j^{th} genotype/accession, and e_{ij} is the residual error effect associated with the i^{th} replication and j^{th} genotype. For multi-location analysis and BLUE over three environments, we used the following statistical model:

$$y_{ijk} = \mu + E_i + R_{j(i)} + G_k + GE_{ik} + e_{ijk}$$

where y_{ijk} is the trait of interest, μ is the overall mean, E_i is the effect of the i^{th} environment, $R_{j(i)}$ is the effect of the j^{th} replicate nested within the ith environment, G_k is

the effect of the j^{th} genotype, GE_{ik} is the effect of the genotype x environment (G x E) interaction, and e_{ijk} is the residual error associated with the i^{th} replication and j^{th} genotype. The estimates from the above analyses were used to assess the broad-sense heritability (H^2) across environments as follow:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gE}^2/nLoc + \sigma_e^2/(nLoc \times nRep)}$$

where σ_g^2 genotype variance component, and σ_{gE}^2 is G × E interaction variance component, and σ_e^2 is the .error variance components. The *nLoc* term represents the number of environments in the analysis.

4.3.3. Genotyping and SNP discovery

The HWWAMP was genotyped using the wheat Infinium 90K iSelect array (Illumina Inc. San Diego, CA) under the USDA-TCAP (Cavanagh et al., 2013). We obtained the genotypic data from the T3 Toolbox (https://triticeaetoolbox.org/wheat/genotyping/display_genotype.php?trial_code=TCAP90K_HWWAMP). After removing the SNPs with more than 10% missing genotypes and minor allele frequency (MAF) of less than 0.05, 15,590 high-quality SNPs were used for further analysis. The genetic positions of the wheat Infinium 90K iSelect SNP markers were obtained from the consensus genetic map of 46,977 SNPs (Wang et al., 2014). The physical positions of the SNPs associated with various spike and kernel-related traits were obtained by blasting the flanking sequences of respective SNPs to wheat Chinese Spring RefSeq v1.1 (IWGSC et al., 2018).

4.3.4. Population Structure and Linkage Disequilibrium

Population structure among the 297 winter wheat accessions was assessed using a Bayesian model-based clustering program, STRUCTURE v2.3.4 assuming an Admixture model (Pritchard et al., 2000). The most likely number of sub-groups was inferred based on an ad-hoc statistic (Delta*K*) based on the rate of change in the log probability between runs using successive *K*-values (Evanno et al., 2005) using STRUCTURE HARVESTER (Earl and vonHoldt, 2012). We used ten subgroups (*K* =1-10) with five independent runs for each subgroup using a burn-in period of 10,000 iterations followed by 10,000 Monte-Carlo iterations. Linkage disequilibrium (LD) decay distances were calculated using TASSEL v5.0 (Bradbury et al., 2007) in our previous study (Ayana et al., 2018). The LD decay distances for individual and whole genomes were estimated by plotting the estimated r^2 values against the genetic distance (cM) between the markers.

4.3.5. Marker-trait associations (MTA)

Genome-wide associations were analyzed using two different algorithms, namely the mixed linear model (MLM) (Yu et al., 2006) and FarmCPU (fixed and random model circulating probability unification) (Liu et al., 2016). Both the models were implemented in Genomic Association and Prediction Integrated Tool (GAPIT) (Lipka et al., 2012). In brief, MLM incorporates kinship and population structure as covariates to minimize the confounding effects and control false positives. However, it leads to false negatives due to the confounding between testing markers and cofactors simultaneously. FarmCPU is an improved multiple-locus model that controls false positives by fitting the associated markers detected from the iterations as cofactors to perform marker tests within a fixed-

effect model. The quantile-quantile (QQ) plots revealed that the FarmCPU performed better than MLM for most traits. Therefore, we employed FarmCPU to report the MTAs for all the spike- and kernel-related traits. Though we found several associations to be significant based on the Bonferroni correction for multiple testing, most of these associations were limited to one or two environments. Thus, an arbitrary threshold to determine significant MTAs was set at $-\log_{10} P \geq 3.0$ (Wang et al., 2017a) and only those MTAs were reported as significant, which surpassed this threshold in the combined analysis (BLUEs over environments) and at least two of the three environments.

Allele stacking analysis was performed to study the accumulative effect of favorable alleles on the trait's phenotype. The accessions from the mapping panel were grouped based on the alleles of significant SNPs for all the spike-related traits. These groups were compared by pairwise comparison of means based on LSD with FDR corrected *P*-value at 5% level of significance to verify the additive effect of the favorable alleles on the phenotype of the traits.

4.3.6. Identification of candidate genes

For candidate gene analysis, only stable MTAs for three spike traits were selected. The candidate regions were demarcated within +/- 1Mb of the most significant SNP for each QTL to identify the candidate genes. The high confidence genes in the selected region were retrieved from wheat genome assembly IWGSC RefSeq v1.0 and gene ontology (GO) annotation information of these genes was extracted from IWGSC Functional Annotation v1.0 (IWGSC et al., 2018). Gene expression browser (http://www.wheat-

expression.com/) was used to exclude unlikely candidates and shorten the candidate list to fewer genes for each selected MTA.

4.3.7. Genomic Prediction Models and Cross-validation

We evaluated one univariate and one multivariate genomic prediction model for predicting eight spike- and kernel-related traits. The univariate genomic best linear unbiased prediction (GBLUP) model to predict the genomic estimated breeding values (GEBVs) of individuals is given below:

$$y = Xb+Zu+e$$

where y is a vector of observed phenotypes, X and Z are design matrices, b is a vector of fixed effect, u is a vector of additive genetic effects (u ~ N(0,Gσ2g), where G is a G-matrix and σ2g is additive genetic variance), and e is a vector of random residual effects (e ~ N(0,Iσe2)). The model was implemented using the 'BWGS' R package for one trait at a time.

The Bayesian multi-trait multi-environment (BMTME) model for genomic predictions can be briefly described as:

$$y = X\beta + Z_1 b_1 + Z_2 b_2 + \varepsilon$$

where y is the response matrix of order $j \times t$ (where t is the number of traits and $j = n \times I$, where n denotes the number of genotypes and I denotes the number of environments); X is of the order $j \times I$, whereas β is of the order $I \times t$. The matrices Z_1 ($j \times n$) and b_1 ($n \times t$) represent the genotype × trait interaction, while the matrices Z_2 ($j \times In$) and b_2 ($In \times t$)

represent the genotype × trait × environment interactions. The matrix ε ($j \times t$) is used to represent the BMTME model residuals. Model simulations were carried out using the R package 'BMTME' (Montesinos-López et al., 2016, 2019a) with 10,000 burn-in and 25,000 iterations.

Predictive ability was assessed as Pearson's correlation coefficient between GEBVs and observed phenotypes for the testing set. A cross-validation scheme was used to estimate the predictive ability for the GBLUP model, where the whole population was divided into five random sets of equal size. Four of the five sets (80%) were used as a training set (phenotyped and genotyped) to train the model, and the remaining set (20%) was used for prediction (genotyped only) for prediction. The cross-validation process was repeated 1,000 times, where each iteration included different lines in the training and testing sets. A similar scheme was used for the BMTME model by randomly splitting the lines into 80% training set and 20% testing set. However, the BMTME model employs a Gibbs sampler with multiple iterations and is computationally expensive; we repeated the cross-validation scheme 25 times.

4.4. Results:

4.4.1. Distribution of SNPs, population structure and LD analysis

A total of 15,590 high-quality SNPs were distributed across all 21 wheat chromosomes. Out of the 15,590 SNPs, almost 50% (7,630) markers were from the B sub-genome, while the A and D sub-genomes had 6,211 (39.84%) and 1,749 (11.22%) markers, respectively (Supplementary Table S2). Chromosomes 5B, 1B, and 6B had the highest number of markers (1,554, 1,254, and 1,237 respectively), while chromosomes 4D (52),

7D (133), and 6D (146) had the lowest number of markers (Supplementary Table S2). To identify whether the HWWAMP is structured based on the breeding programs/origin, we performed the Structure analysis in our previous study (Sidhu et al., 2020). Four sub-populations (P1, P2, P3, and P4) were identified in the HWWAMP, where P1, P2, P3, and P4 consist of 120, 34, 33, and 110 genotypes. Nevertheless, we did not observe any grouping in the HWWAMP based on the origin of germplasm. The HWWAMP was analyzed for LD decay pattern in one of our previous studies (Ayana et al., 2018) using a set of 1,842 SNPs. LD decay was estimated as the distance where LD value (r^2) falls below 0.1 or half strength of D' (D' = 0.5). For the whole genome, the LD dropped to 0.5 at 4.5 cM. LD decay was found similar for both A (3.4 cM) and B (3.6 cM) sub-genomes; however, it was much higher for the D sub-genome (14.2 cM).

4.4.2. Phenotypic variation and correlations

A wide variation was observed in the HWWAMP for all the spike and kernel-related traits (Table 4.1 and Supplementary Table S1). The linear mixed model analysis revealed low variance due to the replication effect, indicating a lower random error of field trials. The broad-sense heritability estimates for eight traits ranged from 0.72 (TKW) to 0.93 (KL). Overall, phenotypic variabilities of traits appeared to be normally distributed with noticeable variation among three environments (Figure 4.1).

Table 4.1. The combined phenotypic performance of all traits and heritability across environments.

Trait[a]	Mean	Min	Max	LSD	CV (%)	Heritability
SPS	15.68	12.73	19.87	1.17	3.60	0.88
SL	8.04	6.49	9.57	0.59	3.35	0.83
SD	1.96	1.60	2.53	0.13	5.08	0.91
KPS	39.18	26.45	54.33	5.67	6.62	0.77
KW	2.83	2.61	3.11	0.10	0.97	0.76
KL	6.11	5.46	6.80	0.17	0.77	0.93
KA	13.25	11.32	15.72	0.76	1.55	0.84
TKW	30.74	23.11	39.69	3.40	3.87	0.72

[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KL, kernel length; KA, kernel area; TKW, thousand kernel weight, CV, coefficient of variation; LSD, least significant difference; Min, minimum; Max, maximum

Pearson's correlation between eight traits was estimated using across-environment BLUE values (Figure 4.1). A moderate to high positive correlation was observed between SPS and other spike-related traits such as SL (r = 0.42), SD (r = 0.64), and KPS (r = 0.32). However, SPS was negatively correlated with all the four kernel-related traits (KW, KL, KA, and TKW). On the other hand, SL showed significant positive correlations with all the studied traits except SD (r = -0.43) (Figure 4.1). As expected, we observed a strong positive correlation within four kernel-related traits (KW, KL, KA, and TKW), and kernel-related traits showed a negative correlation with spike-related traits except for SL (Figure 4.1).

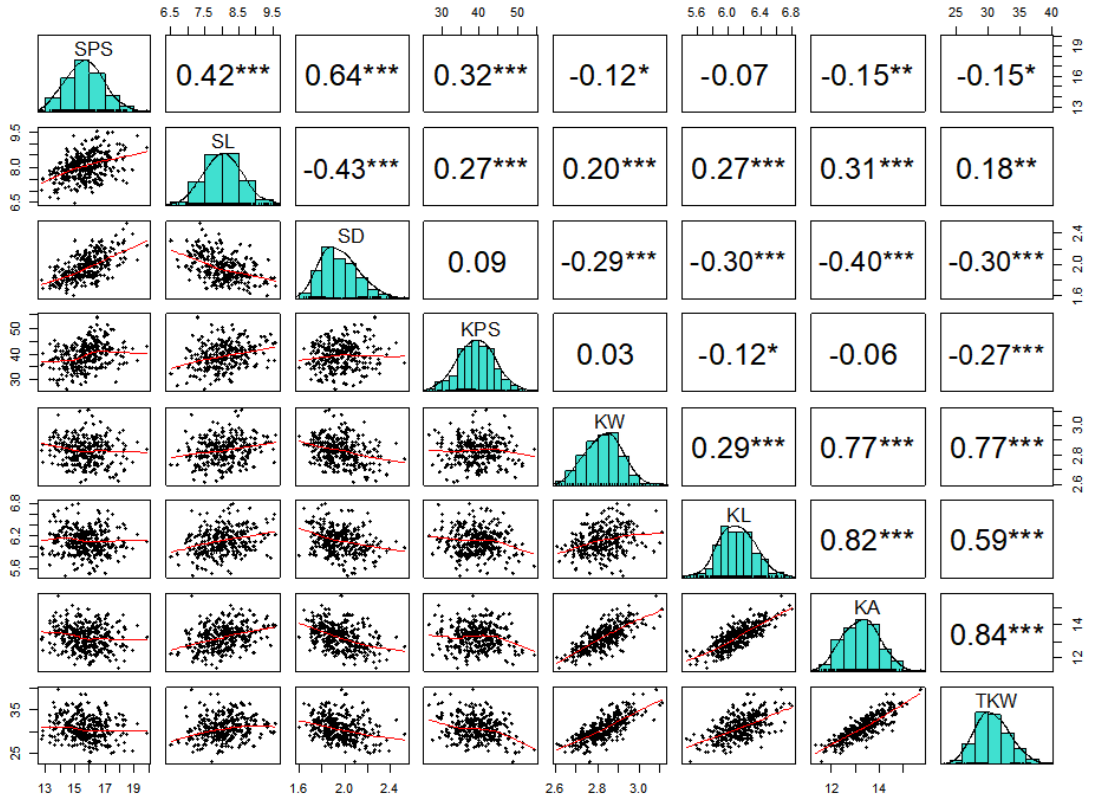


Figure 4.1. Pearson's linear correlation matrix among spike and kernel related traits based on their best linear unbiased estimates (BLUE). Values inside the rectangle represent the correlation coefficient and three symbols *, **, and ***, represent correlation coefficient significance levels at $P < 0.05$, 0.01, and 0.001, respectively. The diagonally arranged plots show the phenotypic distribution of traits based on BLUE values. Bivariate scatter plots with fitted lines are at the left side of the diagonally arranged phenotypic distribution plots. SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KL, kernel length; KA, kernel area; TKW, thousand kernel weight.

4.4.3. GWAS on spike and kernel-related traits

GWAS was performed on spike and kernel-related traits using BLUEs from across-environment analysis (combined analysis) and individual environments. Nevertheless, only those MTAs were reported that were significant in the combined analysis and at least in two of the three environments. GWAS identified a total of 53 significant MTAs ($P < 0.001$) for seven traits out of eight studied traits as no MTA was observed for KL. The MTAs were distributed across all the chromosomes except chromosomes 2D and 5D (Figure 4.3).

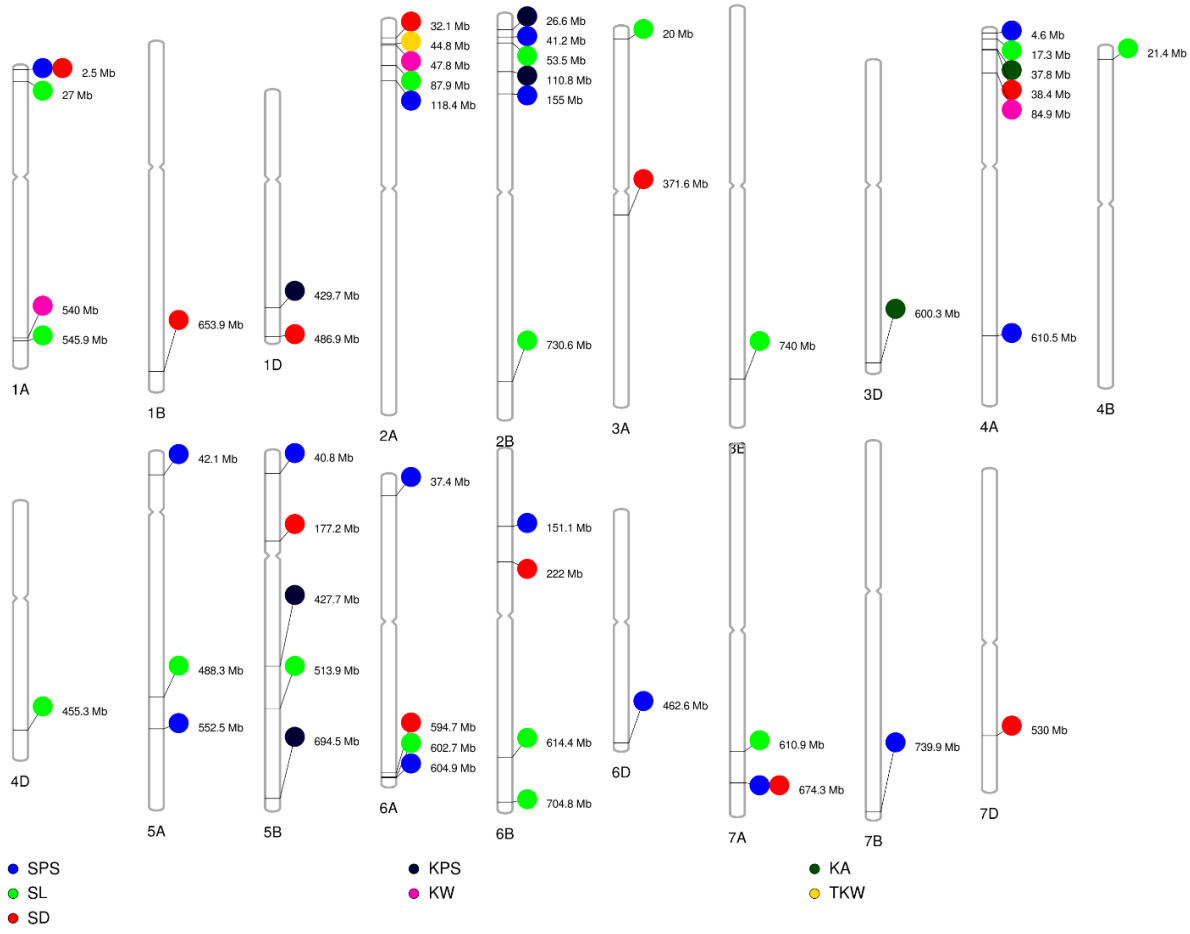


Figure 4.3. Physical positions (Mb) of the MTAs associated with 7 spike and kernel related traits identified in this study based on Chinese Spring RefSeq 1.1 (IWGSC, 2018) across chromosomes. SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KA, kernel area; TKW, thousand kernel weight.

Most of the MTAs were identified on the A sub-genome (28), followed by the B (19) and D (6) sub-genomes. The highest number of MTAs were located on chromosomes 2B and 4A (6 MTAs each), followed by 1A, 2A, and 3B (5 MTAs each) (Figure 4.3 and Supplementary Table S3). Of the 53 significant MTAs, 14 were detected in combined

analysis and in two or more individual environments (Table 4.2). Thus, these MTAs were considered stable and reported significant for further use in candidate gene analysis and employment in wheat breeding programs.

Table 4.2. Significant genomic region identified in combined and multiple locations for various kernel and spike traits.

Trait[a]	SNP[$]	Chr	Alleles*	Pos	cM	-Log10*P*	Environments*	Effect
SPS	Excalibur_c97022_396	6A	C/T	37415157	58.04	4.0	E1, E3, C	-0.266
	RFL_Contig3175_1217	6A	T/C	604877158	136.70	3.5	E2, E3, C	-0.168
	IWA4455	6D	A/G	462631946	155.56	3.8	E1, E3, C	0.061
	IWA5913	7A	A/G	674276906	152.78	14.0	E1, E2, E3, C	-0.500
SL	Kukri_c10860_1283	2A	G/A	87857405	105.89	4.7	E1, E2, C	0.163
	Tdurum_contig82393_484	2B	C/A	730562664	118.43	8.4	E1, E3, C	0.147
	IWA3639	7A	G/A	610934198	131.11	7.6	E1, E3, C	-0.138
SD	IWA2519	3A	C/T	371628644	86.16	3.3	E1, E2, C	-0.026
	IWA5913	7A	A/G	674276906	152.78	9.4	E1, E2, E3, C	-0.055
	IWA1902	7D	A/G	530035575	149.59	4.1	E1, E2, E3, C	-0.041
KPS	BS00021959_51	2B	C/T	110818850	90.97	3.2	E1, E3, C	-1.862
	Excalibur_c1921_1191	5B	G/A	427650909	51.16	4.0	E1, E2, C	1.390
KW	BS00044274_51	2A	T/G	47826702	81.90	3.3	E2, E3, C	0.024
KA	Kukri_c74409_199	4A	G/A	37773890	40.27	3.4	E1, E3, C	0.250

Chr, chromosome; Pos, physical position in base pair (based on IWGSC RefSeq); cM, genetic position in centiMorgans (based on 90K_cons2014); *favorable allele (underlined), [a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KA, kernel area
[$]SNPs/genomic region were significant in BLUE (combined) and multiple individual locations

4.4.3.1. Spike related traits

A total of 15 significant MTAs were found for SPS distributed on the chromosomes 1A, 2A, 2B, 4A, 5A, 5B, 6A, 6B, 6D, 7A, and 7B. Of the 15 MTAs, four SNPs namely *Excalibur_c97022_396* (37.4 Mb), *RFL_Contig3175_1217* (604.9 Mb), *IWA4455* (462.6 Mb), and *IWA5913* (674.3 Mb) on chromosomes 6A, 6A, 6D, and 7A, respectively (Supplementary Table S3 and Figure 4.2). For SL, a total 16 MTAs were identified on chromosomes 1A, 2A, 2B, 3A, 3B, 4A, 4B, 4D, 5A, 5B, 6A, 6B, and 7A. However, only three SNPs, namely *Kukri_c10860_1283* (87.9 Mb), *Tdurum_contig82393_484* (730.6 Mb), and *IWA3639* (610.9 Mb) on chromosomes 2A, 2B, and 7A, respectively were significant in multiple environments (Supplementary Table S3 and Figure 4.2). Eleven significant MTAs were detected for SD on chromosomes 1A, 1B, 1D, 2A, 3A, 4A, 5B, 6A, 6B, 7A, and 7D. Out of the 11 MTAs, three MTAs including *IWA2519* (371.6 Mb), *IWA5913* (674.3 Mb), and *IWA1902* (530 Mb) on chromosomes 3A, 7A, and 7D were significant in multiple environments. Similarly, we identified five MTAs for KPS on chromosomes 1D, 2B, and 5B, of which, *BS00021959_51* (110.8 Mb) and *Excalibur_c1921_1191* (427.7 Mb) on chromosomes 2B and 5B were significant in multiple environments. (Supplementary Table S3 and Figure 4.2).

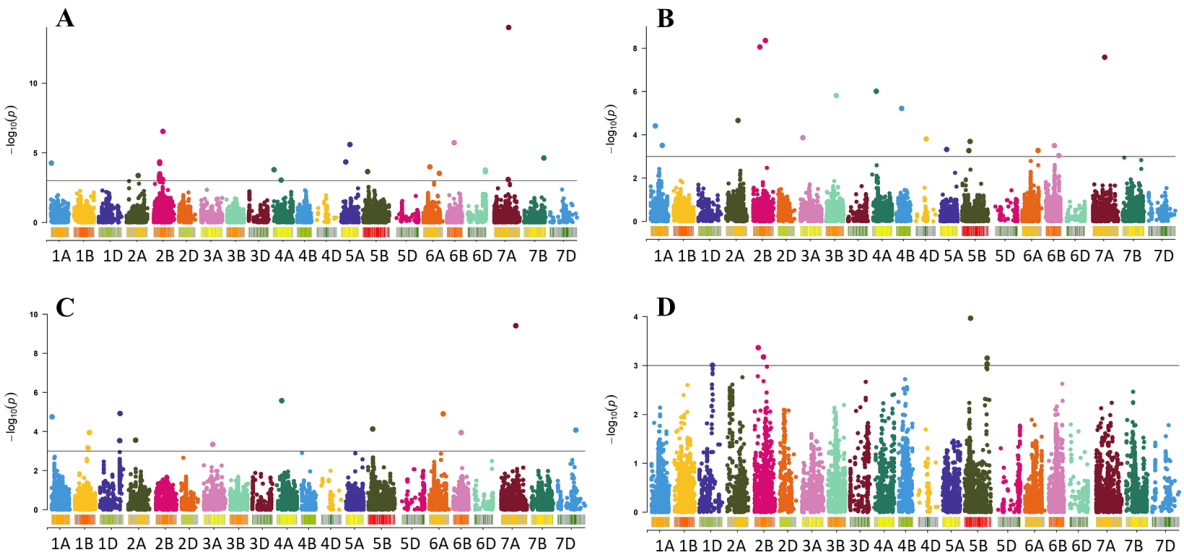


Figure 4.2. Genome-wide association scan. Fixed and random model Circulating Probability Unification (FarmCPU) based Manhattan plots represent -log10*P* for SNPs distributed across all 21 chromosomes of wheat. A) Spikelet number per spike (SPS); B) Spike length (SL); C) Spike density (SD); D). Kernel number per spike (KPS). Y-axis: -log10*P* and X-axis: wheat chromosomes. The horizontal lines stand as a threshold for significant markers with -log10*P* ≥ 3.

4.4.3.2. Kernel related traits

GWAS identified only six MTAs for three (KW, KA, and TKW) kernel-related traits (Supplementary Table S3 and Supplementary Figure S1). In total, three MTAs were detected for KW on chromosomes 1A, 2A, and 4A. Out of the three associations, only *BS00044274_51* (47.8 Mb) on chromosome 2A was significant in two environments (Table 4.2). Further, two MTAs were identified for KA on the chromosomes 3D and 4A; however, only *Kukri_c74409_199* (37.8 Mb) on chromosome 4A was found significant in multiple environments. We identified only one MTA for TKW located on chromosome 2A (Supplementary Table S3).

4.4.4. Genomic regions or SNPs affecting multiple traits

Five significant MTAs were found to be associated with more than one trait, exhibiting the pleiotropic effect of a single gene or a group of tightly linked genes (Supplementary Table S3). Two MTAs, namely *Excalibur_c35316_154* (2.5 Mb) and *IWA5913* (674.2 Mb) located on the chromosomes 1A and 7A were associated with both SPS and SD. Similarly, *BS00044274_51* (47.8 Mb) on chromosome 2A was associated with KA and KW, while *IWA6659* (84.9 Mb) on 4A was associated with several spike and kernel-related traits, including KW, KA, TKW, and SL. Another MTA *IWA6485* (600.2 Mb) on chromosome 3D was significantly associated with KA and SL (Supplementary Table S3).

4.4.5. Phenotypic effects of favorable QTL alleles

To identify the accumulative effect of favorable alleles for various traits, we grouped the HWWAMP based on the number of favorable alleles carried by the accessions for four spike-related traits (Figure 4.4).

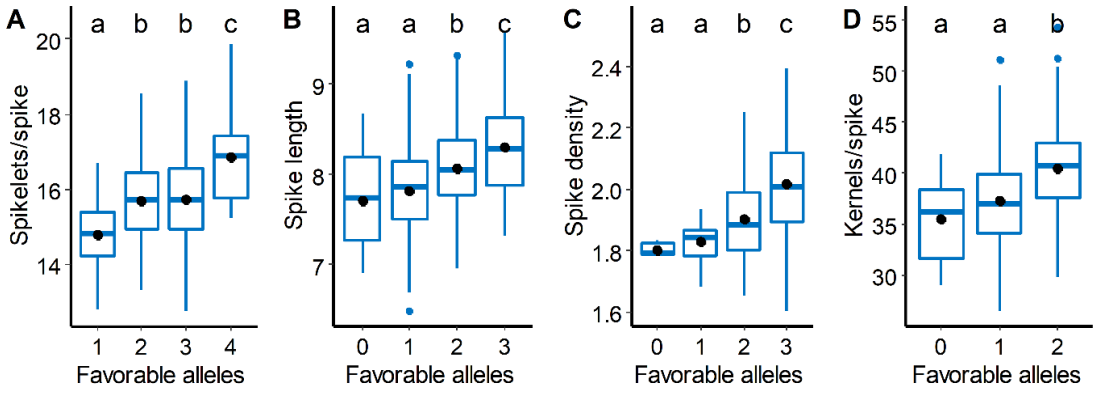


Figure 4.4. Accumulative effect of favorable alleles of the identified stable associations (MTAs identified in multiple environments) for four spikes related traits: A) spikelet/spike (count); B) spike length (cm); C) spikelet density; D) kernel/spike (count). X-axis representing the favorable allele count and Y-axis representing the respective phenotypic value. Mean was compared based on LSD (least significant difference) with FDR (false discovery rate) corrected *P*-value at 0.05 level of significance. For each trait, box plots with the same letter indicates no significant difference in mean, while different letters indicate significant difference ($P < 0.05$).

Rather than using all identified MTAs, we used only stable MTAs (Table 4.2) for this analysis. For SPS, accessions with one, two, three, and four favorable alleles had a mean value of 14.78, 15.70, 15.71, and 16.85, respectively. The accessions with three or more favorable alleles had significantly higher SPS than lines with only one favorable allele (Figure 4.4 and Supplementary Table S4). We also found a significant difference in SL with the increment of favorable alleles. HWWAMP lines that carried none of identified favorable alleles for SL had a mean of 7.7 cm, while lines with one, two, and three favorable alleles had a mean SL of 7.82, 8.06, and 8.30 cm, respectively. A similar additive effect was observed for SD, as two or more favorable alleles significantly

increased the compactness of the spike (SD) compared to lines with zero or only one favorable allele. The mean KPS was 35.49, 37.27, and 40.53 when the accessions had zero, one, and two favorable alleles identified in our study, respectively (Figure 4.4 and Supplementary Table S4).

4.4.6. Candidate gene analysis for significant MTAs

Candidate gene analysis was performed for five stable MTAs having the -log10$P \geq 4.0$. For each MTA, a window of 2 Mb was used to identify the putative candidates. A total of 120 high confidence genes were retrieved by using IWGSC RefSeq v1.0 annotation. However, we excluded the unlikely candidates using publicly available RNA-Seq expression data from Wheat Expression Browser and a thorough review of related literature. Finally, 14 putative candidate genes were selected for three different traits based on their relatively high RNA expression in the shoot, spike, and grain development at seeding, vegetative and reproductive stages of wheat (Table 4.3). The selected genes encode for various proteins, including Peptidyl-prolyl cis-trans isomerase, Cold shock protein, NADPH-cytochrome P450 reductase, Glycoprotein membrane GPI-anchored, Ubiquitin-like protein, BZIP transcription factor, and ATP-dependent RNA helicase (Table 4.3).

4.4.7. Genomic prediction on HWWAMP for various spike and kernel traits

In this study, the predictive abilities of two different genomic prediction (GP) models, namely GBLUP and BMTME, were compared using a cross-validation scheme. The prediction performance of the models for eight spike and kernel-related traits was estimated in terms of average Pearson's correlation (Figure 4.5).

Table 4.3. Putative candidate genes within the identified regions controlling wheat spike related traits.

Trait[a]	Significant SNP	Chr	Gene ID	Protein
SPS	Excalibur_c97022_396	6A	TraesCS6A01G068300	Gamma-glutamylcyclotransferase
			TraesCS6A01G068900	Peptidyl-prolyl cis-trans isomerase
			TraesCS6A01G069500	Cold shock protein
SL	Tdurum_contig82393_484	2B	TraesCS2B01G533500.1	Sulfhydryl oxidase 1
			TraesCS2B01G534200.1	NADPH--cytochrome P450 reductase
			TraesCS2B01G534700.1	UPF0136 membrane protein
	Kukri_c10860_1283	2A	TraesCS2A01G141800.1	Transcription-associated protein 1
			TraesCS2A01G141900.1	Glycoprotein membrane GPI-anchored
			TraesCS2A01G142700.1	Ubiquitin-like protein
			TraesCS2A01G142800.1	BZIP transcription factor
	IWA3639	7A	TraesCS7A01G419100.1	60S acidic ribosomal protein P3
			TraesCS7A01G419400.1	ATP-dependent RNA helicase
SD	IWA1902	7D	TraesCS7D01G411600.1	60S acidic ribosomal protein P3
			TraesCS7D01G412200.1	ATP-dependent RNA helicase

[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density, Chr, chromosome

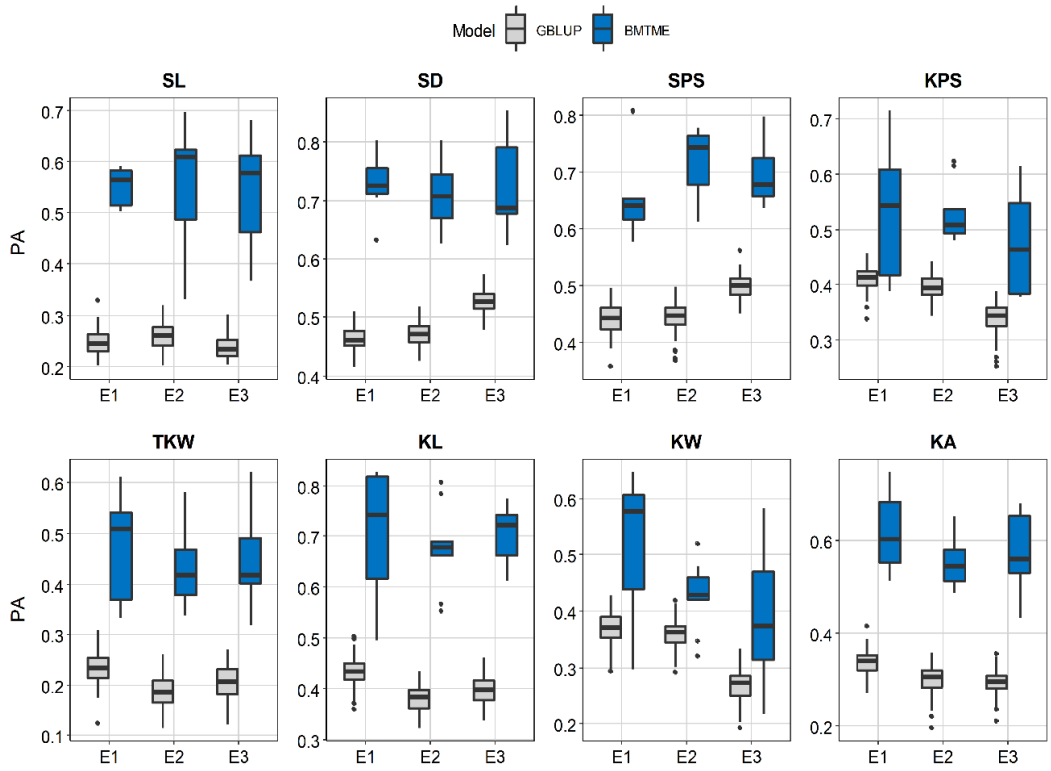


Figure 4.5. The predictive ability (PA) for spike (SL, spike length; SD, spikelet density; SPS, spikelet number per spike; KPS, kernel number per spike) and kernel (TKW, thousand kernel weight; KL, kernel length; KW, kernel width; KA, kernel area) related traits evaluated at three environments (E1, E2, and E3). Boxplots compare the PA using a single-trait prediction model (GBLUP) and a Bayesian multi-trait multi-environment prediction model (BMTME). X-axis showing the environments and Y-axis representing the % PA value.

Moderate mean predictive ability (PA) was observed for various traits using single-trait model (GBLUP). The highest PA (0.52) was for spike density (SD) at E3, while the lowest mean PA was 0.22 (E3) for SL (Figure 4.5 and Supplementary Table S6).

On the other hand, multi-trait model (BMTME) showed the highest mean PA 0.73 at E1 and E3for spike density (SD), whereas the lowest mean PA was found for KPS (0.53) at

E1 and E2. Our result showed that the multivariate model (BMTME) outperformed the single-trait model (GBLUP) for all the traits studied in all the environments (Figure 4.5 and Supplementary Table S6). In terms of a percent increase in PA, BMTME increased the mean prediction accuracy ranging from 30.5% (E1) for KPS to 147.3% (E3) for SL (Supplementary Table S6). In the case of kernel-related traits, the BMTME model outstripped the GBLUP model for all the trait-environment combinations (Figure 4.5 and Supplementary Table S6). Like spike-related traits, gain in the mean prediction accuracy using BMTME over GBLUP ranged from 22.3 to 141.1% for KW and TKW, respectively (Supplementary Table S6).

4.5. Discussion

Wheat yield is the most important and complex target trait for the wheat breeding program. Grain yield mainly depends on the accumulative effect of the different yield contributing traits, including spike- and kernel-related traits. Breeders have relied upon the identification and deployment of novel genes/QTLs governing these crucial traits. Nevertheless, a relatively lesser number of studies identified such genomic regions in winter wheat. In this study, a diverse population coming from various winter wheat breeding programs of the Great Plains region of the USA was used to dissect the genetics of yield-related traits. A wide range of phenotypic variabilities was found among the germplasm for all the traits studied, making this panel suitable for genome-wide association analysis (Supplementary Table S1).

4.5.1. Trait's heritability and correlation

High broad-sense heritability was observed for all the traits, ranging from 0.72 for TKW to 0.93 for KL (Table 4.1). The high heritability estimates were in line with several previous studies (Wang et al., 2017b; Garcia et al., 2019; Alqudah et al., 2020; Muhammad et al., 2020a). We observed a significant positive correlation between SPS and other spike-related traits (SL, SD, and KPS) (Figure 4.1). On the other hand, all the kernel-related traits were positively correlated among themselves and with SL, showing that increased SL positively affects kernel traits (Figure 4.1). Several previous studies also reported positive associations among TKW, KL, and KW (Breseghello and Sorrells, 2007; Ramya et al., 2010; Rasheed et al., 2014; Chen et al., 2016b; Muhammad et al., 2020a). We also observed that an increase in SPS, KPS, and SD causes a decrease in kernel-related traits (KW, KL, KA, and TKW), as they were negatively correlated. Muhammad et al. (Muhammad et al., 2020b) also reported a strong negative correlation for KL and KW with KPS. Further, two yield component traits, grain number (GN) and grain weight (GW) or size, are usually negatively correlated (Sadras, 2007; Bustos et al., 2013; García et al., 2013), and this might be a consequence of trade-offs between these traits. The competition for assimilates between spikelets leads to an unbalanced distribution of GN along the spike and may restrict the grain yield (Guo et al., 2017; Molero et al., 2019).

4.5.2. Marker-trait associations and comparison with previous studies

We used FarmCPU algorithm to identify the MTAs that use both fixed and random effect models iteratively to control the false discovery. In this study, a total of 53 MTAs were identified for spike- and kernel-related traits using combined BLUEs across three environments (Supplementary Table S3). However, we focused on the MTAs identified in multiple environments and MTAs associated with multiple traits (Table 4.2). We identified 47 MTAs for spike-related traits (Supplementary Table S3), while only a few associations for kernel-related traits. Some of the MTAs we identified were likely to be novel whereas many of the QTLs identified in the present study are in similar locations as reported in previous studies. Spike-related traits are generally complex in nature and most of the wheat chromosomes harbor genetic factors affecting these traits (Liu et al., 2018c). It is important to mention here that the optimization of multiple spike characteristics can effectively enhance the integrated sink capacity and ultimate yield potential of wheat (Fan et al., 2019).

The MTAs for SPS were distributed on the chromosomes 1A, 2A, 2B, 4A, 5A, 5B, 6A, 6B, 6D, 7A, and 7B (Supplementary Table S3). We compared the MTAs identified in this study with previous studies based on physical and genetic positions; though use of different types of markers and genetic maps across studies makes it difficult to precisely compare the QTLs (Ward et al., 2019a). Four stable SNPs namely *Excalibur_c97022_396* (37.4 Mb), *RFL_Contig3175_1217* (604.9 Mb), *IWA4455* (462.6 Mb), and *IWA5913* (674.3 Mb) on chromosomes 6AS, 6AL, 6DL, and 7AL (Table 4.2) were significantly associated with SPS. Of the four stable QTLs one MTA (*IWA5913*) identified for SPS on chromosome 7A was reported in earlier studies, and a promising

gene regulating SPS designated as WHEAT ORTHOLOG OF APO1 (*WAPO1*) was reported on wheat chromosome arm 7AL (~674 Mb) in a recent study (Kuzay et al., 2019). Further, Sun et al. (Sun et al., 2017) identified a SNP (Kukri_c264_539) for SPS on 6A (29.5 Mb) in four environments, that co-localized with a stable marker Excalibur_c97022_396 (37.4 Mb) detected in this study. In addition, using combined environment, two significant associations were identified for SPS (*BS00011235_51* (552 Mb) and *GENE-4848_95* (740 Mb) on chromosomes 5A and 7B, respectively. Liu et al., (2018b) have also reported QTLs for SPS in the similar region. It is important to mention that the marker *BS00011235_51* is ~35 Mb away from the vernalization gene (*Vrn-A1*) (Yan et al., 2003), located on chromosome 5A at ~587 Mb. Previous studies have reported that *Vrn-A1* is also involved in increasing the SPS (Whitechurch and Snape, 2003).

For SL, 16 MTAs were identified on chromosomes 1A, 2A, 2B, 3A, 3B, 4A, 4B, 4D, 5A, 5B, 6A, 6B, and 7A. Out of the 16 MTAs, three SNPs, namely *Kukri_c10860_1283* (87.9 Mb), *Tdurum_contig82393_484* (730.6 Mb), and *IWA3639* (610.9 Mb) on chromosomes 2A, 2B, and 7A were found to be stable over multiple environments (Table 4.2). MTAs (*Kukri_c10860_1283* and *Tdurum_contig82393_484*) for SL have been previously reported in various studies (Liu et al., 2017, 2018b; Mwadzingeni et al., 2017). Another significant marker (*Excalibur_rep_c68588_1196*) identified on chromosome 4BS (21 Mb) for SL, corresponds to the physical location of semi-dwarfing gene *Rht-B1*(~30 Mb). Other than reducing plant height, *Rht-B1* was reported to exhibit pleiotropic effects on grain yield and yield components, including SL (Okada et al., 2019; Guan et al., 2020). Several MTAs identified on chromosomes 5A, 6A, and 6B for SL were found to be co-

localized with MTAs reported by previous studies focusing on wheat yield component traits (Yu et al., 2014; Mwadzingeni et al., 2017; Liu et al., 2018b) (Supplementary Table S3).

We identified five MTAs for KPS distributed on chromosomes 1D, 2B, and 5B (Supplementary Table S3). One stable association on chromosome 2B (*BS00021959_51*) was detected at 110.8 Mb (90.9 cM) for KPS (Table 2). Shi et al., (2017) also reported an association for KPS at 122.3Mb and suggested that this marker may be linked to the photoperiod insensitive gene *Ppd-B1* in common wheat. A similar association was detected by Gao et al. (2015) at 92 cM for the same trait on chromosome 2B in a bi-parental mapping population in two environments. Further, we identified a stable MTA (*Excalibur_c1921_1191*) for KPS on chromosome 5B at 427.6 Mb (Table 4.2). Tang et. al. (2011) mapped three QTLs for KPS spanned by markers *Xgwm499* and *Xgwm213* (418.8-477.5 Mb), overlapping the physical position identified in our study. Two other MTAs were identified using combined environment analysis for KPS on chromosomes 2B (CAP8_c5108_139) and 5B (IWA4329), which co-localized with the previously reported MTAs in several studies (Neumann et al., 2011; Guo et al., 2017; Liu et al., 2018b) (Supplementary Table S3).

Eleven significant MTAs were detected for SD on chromosomes 1A, 1B, 1D, 2A, 3A, 4A, 5B, 6A, 6B, 7A, and 7D, however, MTAs on 3A, 7A, and 7D were significant in multiple environments (Table 4.2 and Supplementary Table S3). Similar to our finding, several previous studies reported MTAs for SD on chromosomes 1A, 1B, 3A, 4A, 6A, and 6B (Jantasuriyarat et al., 2004; Liu et al., 2018c). One stable SNP (*IWA5913*) on 7A

at 674 Mb for SD was also identified for SPS and could be a pleiotropic locus (Table 4.2).

A total of three MTAs were identified for KW on chromosomes 1A, 2A, and 4A. Several studies have reported associations in similar physical locations on chromosomes 1A and 2A for KW (Wu et al., 2015; Li et al., 2019b). Our study identified MTAs for KA on chromosomes 3D and 4A and TWK on 2A, however, previous studies also identified significant association for KA and TKW on chromosomes 4A and 2A, respectively (Liu et al., 2018b; Su et al., 2018) (Table 4.2 and Supplementary Table S3).

4.5.3. Multi-trait QTL regions

Our result revealed the pleiotropic nature of the QTLs/MTAs for the spike and kernel-related traits and assumed this phenomenon might be due to the complex relationships among these traits. Spike is a complex and multi-component trait, and its overall expression is comprehensively determined by a series of correlated traits, such as SPS, SL, SD, etc. and major genes which control these components generally show pleiotropic effects or linkage at the QTL level (Fan et al., 2019). Our study identified two MTAs (Excalibur_c35316_154 and IWA5913) responsible for SPS, also found to be associated with the trait SD (Supplementary Table S3). In many previous studies also reported numerous pleiotropic QTL clusters simultaneously affecting various spike-related traits and involving major genes (Heidari et al., 2011; Cui et al., 2012; Zhai et al., 2016; Fan et al., 2019). In case of kernel-related traits, MTAs for KW was found having a pleiotropic relationship with other kernel and spike traits (KA, TKW, and SL) (Supplementary Table S3) and multi-trait QTLs have also been previously reported for grain yield, TKW, KW,

SPS, SL and KPS in wheat (Prashant et al., 2012; Patil et al., 2013; Wu et al., 2015; Chen et al., 2016b). Pleiotropic effects may also partially be explained by correlations between agronomic traits (Chen et al., 2016b; Kumar et al., 2016; Liu et al., 2017), as shown in our study, a strong correlation within kernel-related traits and with SL (Figure 4.1).

4.5.4. Staking favorable alleles for wheat yield enhancement

In our study favorable alleles showed significant additive effects on the phenotype of four spike-related traits (Figure 4.4 and Supplementary Table S4), indicating the importance of favorable alleles staking to improve the performance of yield contributing traits of wheat (Sun et al., 2017; Li et al., 2019b). It was found that the germplasm with 3 or more favorable alleles showed significant improvement in SPS count compared to germplasm with only one favorable allele (Figure 4.4 and Supplementary Table S4). Several best and worse lines based on the highest and lowest phenotypic value for spike-related traits were compared to examine the cumulative allele effect on the individual germplasm phenotypes (Supplementary Table S5). Line with the highest number of SPS in our mapping population was MT9982 (19.87), followed by OK05108 (19.23), had all four favorable alleles, while lines with only 1 favorable allele such as TAM400 (12.80), TAM109 (13.27), and HV906-865 (13.27) were among the worse (Supplementary Table S5). This phenomenon of better performance with a higher number of favorable alleles was true for all other traits (SL, SD, and KPS) as well, such as a line with the highest mean SL in all the environments was OK1067274 (9.57) that had all the 3 favorable alleles, whereas line MT85200 (6.49) which showed the lowest SL, had only one favorable allele. It was also observed that some lines in HWWAMP had higher number

of favorable alleles for multiple traits such as line OK05108 was among the best for SPS and SL with all the 7 favorable alleles studied for these two traits (Supplementary Table S5). Therefore, breeders could use such materials with higher number of favorable alleles for multiple traits as parents to develop new high-yielding varieties.

4.5.5. Putative candidate genes for several important MTAs

Fourteen putative candidate genes were identified for 5 chosen MTAs (-log10$P \geq 4.0$) for 3 different spike related traits (SPS, SL, and SD) based on their potential involvement and higher expression in shoot, spike, and kernel development at vegetative and reproductive stages of wheat (Table 4.3). Gene (*TraesCS6A01G068900*) on chromosome 6A was identified as a putative candidate for SPS, that code for protein Peptidyl-prolyl cis-trans isomerase (PPIase), which was found to have a significant role in the deposition of storage proteins in wheat (Dutta et al., 2011). It was also found that PPIase activity in the wheat was regulated by the developmental stages and was also cultivar-dependent (Dutta et al., 2011). Another gene *TraesCS6A01G069500,* was also identified as a putative candidate for SPS that code for cold shock proteins (CSPs). CSP family have their known roles in cold acclimation, gene expression regulation, developmental processes such as flower and seed development, etc. (Behl et al., 2020). In a field experiment conducted under drought stress conditions by Yu et al., (2017), found that the cold shock protein *SeCspA* transgenic wheat lines showed significant improvement in the 1000-grain weight and grain yield compared to the control genotype. Several putative candidate genes were identified for SL such as *TraesCS2B01G534200*, *TraesCS2A01G141900*, *TraesCS2A01G142700,* and *TraesCS2A01G142800* code for

proteins NADPH-cytochrome P450 reductase, Glycoprotein membrane glycosylphosphatidylinositol-anchored, Ubiquitin-like protein, and BZIP transcription factor, respectively (Table 4.3). Cytochrome P450s (CYPs) are the largest and versatile enzymes family involved in NADPH- and/or O2-dependent hydroxylation reactions and play a significant role in multiple processes of plant growth and development (Pandian et al., 2020). *TaMs1*(glycosylphosphatidylinositol-anchored lipid transfer protein) is a wheat fertility gene, was found to express in pollen development and encodes a GPI-LTP targeted to the plasma membrane (Kouidri et al., 2018). Protein ubiquitination is a major post-translational modification that occurs in eukaryotes and regulates diverse biological processes, such as lysine ubiquitination in common wheat regulating proteasome composition ribosome assembly/translation, carbohydrate metabolism, signal transduction, and photosynthesis (Zhang et al., 2017). Another important gene *TraesCS7D01G412200* code for protein ATP-dependent RNA helicase was identified for SD (Table 4.3) which was one of the top candidates with a very high level of gene expression. Most of the members of the DEAD-box enzymes family have putative ATP-dependent RNA helicase activity, play important roles in all types of processes in RNA metabolism (Zhang et al., 2014). Recent studies showed that DEAD-box RNA helicases have various roles in growth, development, and stress responses in various crops such as wheat, rice, maize, tomato, etc. (Nawaz and Kang, 2019).

4.5.6. Genomic prediction on HWWAMP

Our GWAS results show that yield contributing traits are highly quantitative therefore genomic selection for multiple yield contributing traits could be another promising strategy to improve wheat yield. We compared the predictive abilities of both single- and

multi-trait and multi-environment models (GBLUP and BMTME) to predict spike and kernel-related traits of wheat (Figure 4.5). Our result demonstrated that, compared to common single-trait model (GBLUP), multi-trait model (BMTME) showed much higher mean prediction abilities for all the studied traits and all three environments (Figure 4.5). The mean prediction abilities spike traits increased in range of 30.5% (E1) for KPS up to 147.3% for SL in E3 BMTME model (Supplementary Table S5). Similarly, improved mean prediction abilities were found for kernel-related traits as well, ranging from 22.3% (E2) to 141.13% (E2) for KW and TKW, respectively. Several previous studies reported an increase in the prediction accuracies for various agronomic and end-use quality traits by using Bayesian-based model for multi-trait and multi-environment analysis (Jia and Jannink, 2012b; Schulthess et al., 2016b; Guo et al., 2020; Ibba et al., 2020). BMTME model is found effective for both high and low heritability traits and can be used in plant breeding programs to predict economically important traits when they are inter-correlated (Guo et al., 2020; Ibba et al., 2020).

In conclusion, our study showed that the diversity panel we used is a valuable source for exploiting genetic variation for various yield contributing traits. The negative relationships between spike and kernel-related traits indicate the moderate physiological trade-off between primary grain yield components. GWAS effectively identified both stable and environment-specific QTLs for various spike and kernel-related traits. The QTLs identified or validated in the current study can be tracked in the hard winter wheat breeding programs using linked SNP markers and could also be incorporated into multivariate GS models for enhancement of yield-related traits in wheat.

4.6. References

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4.7. Appendix

List of Supplementary Tables:

Supplementary Table S1. Mean (BLUE) phenotypic value of HWWAMP for 8 different spike and kernel related traits.

Sl. no.	Entry name	SPS	SL	SD	KPS	KW	KL	KA	TKW
1	2145	15.33	7.73	1.99	34.65	2.74	5.96	12.49	28.53
2	2180	15.97	8.62	1.85	39.92	2.72	6.23	12.75	27.64
3	2174-05	16.83	7.75	2.17	34.77	2.86	5.74	12.55	30.63
4	ABOVE	15.53	7.95	1.96	43.45	2.83	5.95	13.06	28.57
5	AGATE	14.83	8.25	1.80	28.92	2.95	6.77	15.16	36.40
6	AKRON	16.23	8.71	1.86	36.90	2.86	5.92	13.12	32.14
7	ALICE	16.07	8.62	1.86	42.48	2.81	6.00	13.12	29.16
8	ALLIANCE	16.33	8.15	2.01	41.12	2.83	6.26	13.56	30.56
9	ANTELOPE	15.87	8.34	1.90	38.37	2.75	5.85	12.31	29.04
10	ANTON	16.57	8.05	2.06	37.95	2.86	6.06	13.54	32.28
11	ARAPAHOE	15.17	8.50	1.78	34.65	2.73	6.42	13.30	30.29
12	ARLIN	14.80	8.36	1.77	37.08	2.90	6.07	13.51	32.78
13	AVALANCHE	15.37	8.20	1.87	41.70	2.86	6.19	13.68	30.06
14	BAKERS_WHITE	16.07	8.06	1.99	40.58	2.81	6.33	13.53	31.19
15	BENNETT	14.53	8.00	1.82	34.03	2.96	6.47	14.64	34.74
16	BIG_SKY	16.60	8.15	2.04	44.93	2.78	5.86	12.43	27.75
17	BILL_BROWN	15.40	8.29	1.87	41.12	2.82	6.18	13.47	29.23
18	BILLINGS	14.47	8.80	1.64	42.03	2.87	6.70	14.87	36.31
19	BISON	15.20	8.03	1.89	32.57	2.83	6.43	13.89	34.21
20	BOND_CL	14.97	8.77	1.71	44.47	2.82	5.97	13.05	28.15
21	BRONZE	15.70	8.25	1.90	29.28	2.68	6.09	12.50	28.73
22	BUCKSKIN	17.03	7.68	2.22	36.60	2.89	6.02	13.40	32.12

23	BURCHETT	16.30	8.08	2.02	38.03	2.73	6.00	12.43	29.23
24	BYRD	15.47	8.96	1.73	42.98	2.88	6.09	13.54	32.85
25	CAMELOT	16.70	8.61	1.94	40.37	2.91	6.39	13.98	34.28
26	CAPROCK	12.73	7.04	1.81	35.50	2.83	5.96	13.09	28.26
27	CARSON	16.93	7.71	2.20	42.77	2.81	5.92	12.55	28.85
28	CENTERFIELD	15.23	7.70	1.97	38.43	2.84	6.07	13.13	29.89
29	CENTURA	16.63	7.23	2.30	38.03	2.79	6.03	12.93	29.08
30	CENTURK78	15.70	7.16	2.19	31.37	2.63	5.94	11.94	26.66
31	CENTURY	15.67	8.22	1.91	41.40	2.70	6.03	12.51	28.36
32	CHENEY	15.40	8.26	1.87	32.47	2.71	6.11	12.67	28.36
33	CHEYENNE	16.40	7.49	2.19	31.27	2.73	6.08	12.67	29.96
34	CHISHOLM	14.30	7.91	1.81	34.32	2.89	6.35	13.88	34.73
35	CO03064	16.93	9.13	1.86	46.60	2.82	6.09	13.21	29.58
36	CO03W043	14.67	8.15	1.80	38.60	2.86	6.33	13.92	32.45
37	CO03W054	16.57	9.28	1.79	44.77	2.89	6.51	14.38	31.75
38	CO04025	15.15	8.50	1.78	43.55	2.91	6.00	13.55	30.23
39	CO04393	16.10	9.21	1.75	40.22	2.93	6.21	13.93	32.66
40	CO04499	13.83	7.75	1.78	42.83	2.94	6.18	13.91	31.96
41	CO04W320	16.47	9.22	1.79	46.57	2.88	6.14	13.77	31.70
42	CO050337-2	16.07	7.85	2.05	43.83	2.85	6.24	13.58	31.60
43	CO07W245	16.20	8.22	1.97	42.85	3.01	6.07	14.05	33.85
44	CO940610	15.10	8.43	1.79	38.28	2.95	6.39	14.47	35.56
45	COLT	15.30	7.19	2.13	33.60	2.73	6.18	12.59	30.99
46	COMANCHE	15.33	7.77	1.97	35.30	2.80	6.20	13.26	30.83
47	COSSACK	18.27	7.43	2.46	44.63	2.88	6.08	13.27	32.66
48	COUGAR	15.43	8.43	1.83	36.62	2.88	6.19	13.52	33.89
49	CREST	18.30	7.93	2.31	36.48	2.69	5.81	11.78	25.57

50	CRIMSON	17.90	7.83	2.28	34.12	2.62	5.72	11.32	26.96
51	CULVER	15.03	7.56	1.99	33.45	2.78	6.39	13.41	29.87
52	CUSTER	15.63	8.79	1.78	38.18	2.81	6.16	13.34	31.64
53	CUTTER	13.33	7.50	1.77	43.82	2.80	6.24	13.47	29.14
54	DANBY	17.17	8.50	2.02	44.28	2.84	6.12	13.39	31.33
55	DARRELL	14.93	8.23	1.82	35.85	2.79	6.41	13.37	30.31
56	DAWN	14.93	7.92	1.88	33.38	2.76	5.98	12.68	29.67
57	DECADE	15.10	8.13	1.86	44.10	2.76	6.09	12.83	28.80
58	DELIVER	17.83	8.96	1.99	40.37	2.91	6.36	14.11	31.64
59	DENALI	17.03	8.55	1.99	47.13	2.84	6.26	13.71	31.22
60	DODGE	14.80	8.03	1.84	31.67	2.73	6.04	12.49	28.88
61	DUKE	16.57	7.60	2.18	45.17	2.72	5.91	12.30	29.05
62	DUMAS	17.03	8.27	2.06	42.20	2.87	5.50	12.08	28.14
63	DUSTER	16.80	8.38	2.01	40.75	2.90	6.22	13.79	31.75
64	E2041	15.90	8.42	1.89	41.80	2.91	6.21	13.77	30.30
65	EAGLE	14.20	7.92	1.79	29.87	2.76	6.34	13.32	30.11
66	ENDURANCE	16.38	8.70	1.89	34.57	2.91	6.41	14.13	32.17
67	ENHANCER	15.53	8.02	1.94	44.78	2.68	6.23	12.87	27.37
68	EXPEDITION	13.93	7.33	1.91	34.05	2.82	6.28	13.45	32.37
69	FULLER	14.63	7.86	1.86	37.75	2.83	6.04	12.83	30.65
70	G1878	15.43	7.65	2.01	28.08	2.97	6.31	14.59	38.58
71	GAGE	15.87	8.28	1.92	32.52	2.81	6.13	13.02	31.04
72	GALLAGHER	18.33	8.44	2.17	44.68	2.84	6.39	13.83	31.72
73	GARRISON	14.17	7.54	1.88	41.12	2.86	5.87	13.07	29.40
74	GENOU	18.30	8.71	2.10	40.62	2.70	6.07	12.46	27.67
75	GENT	14.37	7.51	1.92	33.92	2.83	6.22	13.64	31.73
76	GOODSTREAK	14.13	7.56	1.87	38.82	2.85	6.25	13.65	33.76

77	GUYMON	17.23	9.49	1.82	43.87	2.84	6.51	13.97	29.73
78	HAIL	17.50	9.51	1.84	37.42	2.75	6.59	13.83	30.10
79	HALLAM	14.80	7.62	1.94	34.47	2.90	6.13	13.64	31.26
80	HALT	13.30	7.68	1.73	37.30	2.87	5.86	12.92	31.22
81	HARDING	16.73	8.06	2.08	38.43	2.78	6.31	13.33	31.72
82	HARRY	17.07	8.04	2.12	48.43	2.93	6.30	14.10	32.02
83	HATCHER	14.70	8.13	1.80	37.92	2.84	6.21	13.79	32.84
84	HEYNE	15.90	7.87	2.03	38.92	2.81	6.03	13.01	30.55
85	HG-9	18.03	8.98	2.01	35.85	2.95	6.35	14.24	34.20
86	HOMESTEAD	13.83	6.69	2.07	27.55	2.73	5.87	12.15	28.01
87	HONDO	15.23	7.57	2.01	34.20	2.94	6.30	14.14	35.02
88	HUME	17.33	7.96	2.18	36.73	2.65	5.84	11.77	26.59
89	HV906-865	13.27	6.72	1.98	34.70	2.83	5.65	12.34	27.62
90	HV9W03-1379R	16.07	7.14	2.25	47.20	2.68	5.70	11.72	25.41
91	HV9W03-1551WP	14.40	8.09	1.78	36.13	2.78	6.41	13.72	28.90
92	HV9W03-1596R	16.57	8.69	1.91	41.73	2.89	5.98	13.19	31.51
93	HV9W05-1280R	15.43	8.58	1.80	38.08	2.97	6.21	14.15	36.45
94	HV9W06-504	16.47	7.27	2.26	54.33	2.84	5.63	12.17	25.89
95	INFINITY_CL	15.77	7.40	2.13	40.43	2.82	5.85	12.69	29.42
96	INTRADA	14.73	7.79	1.89	31.87	2.77	6.10	12.97	31.44
97	JAGALENE	16.27	8.12	2.01	45.00	2.78	5.94	12.58	27.25
98	JAGGER	14.80	8.10	1.83	41.67	2.68	6.00	12.19	26.70
99	JERRY	16.60	7.54	2.20	34.57	2.82	6.61	14.38	35.02
100	JUDEE	17.17	8.54	2.01	36.23	2.69	5.87	12.03	27.07
101	JUDITH	17.17	8.47	2.03	41.65	2.83	6.66	14.56	30.18
102	JULES	16.37	8.22	1.99	43.38	2.79	6.14	13.14	27.96
103	KARL_92	15.20	8.14	1.87	34.35	2.78	6.34	13.45	32.00

104	KAW61	17.77	8.30	2.14	28.97	2.80	5.97	12.80	33.30
105	KEOTA	15.83	8.57	1.85	41.57	2.94	6.26	14.15	33.36
106	KHARKOF	16.67	8.94	1.86	35.32	2.84	5.92	12.98	32.40
107	KIOWA	14.87	7.81	1.90	29.68	2.85	6.31	13.69	33.00
108	KIRWIN	15.57	7.92	1.97	32.53	2.88	6.29	13.81	33.85
109	KS00F5-20-3	14.43	8.05	1.80	42.90	2.96	6.25	14.17	34.39
110	LAKIN	13.87	8.00	1.74	37.83	2.83	6.21	13.46	29.52
111	LAMAR	16.63	7.50	2.22	38.53	2.82	6.47	13.95	33.08
112	LANCER	15.73	7.24	2.17	30.90	2.71	5.90	12.12	29.24
113	LARNED	14.53	8.12	1.79	32.90	2.82	6.35	13.84	32.23
114	LINDON	15.30	8.17	1.87	41.13	2.74	5.90	12.50	29.40
115	LONGHORN	14.73	8.41	1.75	35.52	2.89	5.84	13.01	30.75
116	MACE	17.07	8.17	2.09	39.80	2.70	6.13	12.67	26.74
117	MCGILL	14.47	8.29	1.74	42.67	2.78	6.07	12.81	28.98
118	MILLENNIUM	15.20	6.98	2.18	34.57	2.85	5.85	12.60	30.08
119	MIT	13.47	7.75	1.74	37.77	2.74	5.91	12.60	28.97
120	MT0495	17.30	8.93	1.94	41.77	2.71	5.99	12.31	26.61
121	MT06103	17.10	8.06	2.12	40.42	2.87	6.15	13.30	32.84
122	MT85200	15.00	6.49	2.31	32.58	2.73	5.81	12.27	26.90
123	MT9513	18.90	7.90	2.39	42.28	2.75	5.94	12.45	25.71
124	MT9904	18.53	7.33	2.53	36.62	2.77	5.98	12.71	29.51
125	MT9982	19.87	8.83	2.25	43.08	2.84	6.09	13.23	29.81
126	MTS0531	16.00	8.49	1.88	38.72	2.75	6.41	13.46	26.39
127	NE02558	16.83	8.60	1.96	40.37	2.78	6.27	13.33	29.17
128	NE04490	15.77	7.83	2.01	42.88	2.78	6.14	13.20	29.90
129	NE05430	14.10	7.28	1.94	39.77	2.73	5.79	12.26	26.97
130	NE05496	17.33	8.40	2.06	41.68	2.84	5.97	13.02	29.57

131	NE05548	16.57	8.04	2.06	42.98	2.89	6.66	14.61	35.42
132	NE06545	15.07	8.22	1.83	35.95	2.89	6.03	13.45	30.97
133	NE06607	13.60	7.94	1.72	38.57	2.85	6.55	14.27	31.91
134	NE99495	16.40	8.76	1.87	38.80	2.80	6.44	13.64	29.97
135	NEKOTA	13.80	7.79	1.77	29.68	2.88	6.13	13.52	33.22
136	NELL	15.13	7.05	2.15	31.32	2.80	5.92	12.71	30.61
137	NEOSHO	17.20	9.42	1.83	47.68	2.89	5.80	12.77	30.01
138	NEWTON	16.07	8.71	1.85	39.88	2.79	6.06	13.00	29.05
139	NI06736	14.87	8.20	1.81	34.05	2.86	6.30	13.75	33.55
140	NI06737	14.83	7.83	1.89	43.32	2.78	5.96	12.61	28.03
141	NI07703	16.30	7.34	2.22	45.65	2.70	6.22	12.81	29.59
142	NI08707	15.27	8.23	1.86	46.17	2.91	6.62	14.66	34.32
143	NI08708	15.00	8.08	1.86	42.57	2.80	6.20	13.17	28.28
144	NIOBRARA	15.80	7.55	2.09	37.27	2.79	6.29	13.27	27.99
145	NORKAN	14.40	7.87	1.83	31.57	2.73	6.15	12.71	29.31
146	NORRIS	15.77	7.85	2.01	35.02	2.89	5.91	13.07	28.19
147	NUFRONTIER	17.60	8.01	2.20	49.33	2.65	6.02	12.17	25.26
148	NUHORIZON	15.97	8.05	1.98	38.92	2.76	5.84	12.35	28.76
149	NUPLAINS	15.77	7.92	2.00	37.12	2.69	5.92	12.22	28.05
150	NUSKY	16.77	7.45	2.25	40.90	2.74	5.90	12.29	26.96
151	NW03666	15.97	8.47	1.88	41.02	2.84	6.27	13.65	31.74
152	OGALLALA	14.97	7.51	1.99	36.62	2.70	5.58	11.59	26.96
153	OK_BULLET	18.25	8.93	2.04	42.80	2.97	6.42	14.55	33.77
154	OK_RISING	15.67	8.32	1.88	39.18	2.92	5.91	13.39	32.74
155	OK02405	17.00	8.13	2.10	43.30	2.89	5.83	12.91	31.14
156	OK04111	16.33	8.67	1.89	37.68	2.90	6.00	13.25	33.18
157	OK04415	16.83	7.30	2.31	35.90	2.89	5.96	13.30	31.40

158	OK04505	15.40	7.90	1.95	45.67	2.88	6.24	13.74	31.90
159	OK04507	17.10	8.27	2.07	40.00	2.86	6.13	13.45	32.79
160	OK04525	15.17	7.28	2.08	33.68	2.82	5.59	11.92	26.97
161	OK05108	19.23	9.34	2.06	37.33	2.89	6.21	13.89	31.96
162	OK05122	14.60	7.43	1.97	41.13	2.91	5.98	13.40	32.00
163	OK05134	15.93	8.56	1.86	41.90	3.11	6.27	14.97	36.77
164	OK05204	15.33	8.41	1.83	38.85	3.10	6.05	14.47	36.02
165	OK05303	15.50	8.28	1.87	34.54	2.98	6.11	14.08	34.94
166	OK05312	15.60	8.01	1.95	40.92	2.99	6.02	13.88	34.28
167	OK05511	17.37	8.38	2.07	42.78	2.97	5.79	13.24	32.75
168	OK05526	13.90	7.09	1.97	38.28	3.08	6.67	15.72	39.69
169	OK05711W	15.50	7.96	1.95	39.65	2.83	5.66	12.38	29.19
170	OK05723W	17.17	8.49	2.02	47.82	2.99	6.38	14.63	34.19
171	OK05830	16.33	8.19	1.99	40.13	2.91	5.55	12.43	29.37
172	OK06114	16.30	8.29	1.97	44.73	2.89	5.97	13.25	30.70
173	OK06210	14.97	8.27	1.81	38.40	2.97	6.18	14.07	34.28
174	OK06318	15.47	7.96	1.94	36.73	2.82	5.77	12.49	28.96
175	OK06319	15.67	8.42	1.86	38.92	2.94	5.90	13.36	33.10
176	OK06336	14.03	8.19	1.72	41.13	2.90	6.47	14.50	34.91
177	OK07231	15.60	7.83	2.00	42.27	2.80	6.15	13.15	30.99
178	OK07S117	16.30	8.57	1.90	39.25	2.86	6.29	13.76	29.94
179	OK08328	17.50	7.63	2.30	42.08	2.86	6.03	13.18	29.91
180	OK09634	13.63	7.05	1.93	40.98	2.79	6.11	12.81	28.82
181	OK101	15.20	8.67	1.76	36.45	2.76	6.24	12.94	29.39
182	OK10119	16.27	8.68	1.87	39.62	2.74	6.01	12.67	29.16
183	OK102	15.47	7.37	2.10	33.75	2.80	5.89	12.64	29.82
184	OK1067071	16.87	8.33	2.03	42.07	2.77	6.00	12.82	30.19

185	OK1067274	16.43	9.57	1.72	44.55	2.94	6.22	13.95	31.93
186	OK1068002	15.63	8.09	1.93	44.65	2.86	6.09	13.38	30.57
187	OK1068009	16.73	7.73	2.17	38.17	3.03	5.99	13.97	34.79
188	OK1068026	16.17	8.48	1.91	46.43	3.07	5.91	13.74	32.66
189	OK1068112	17.10	8.60	1.99	40.28	2.95	6.41	14.59	35.98
190	OK1070267	17.07	8.63	1.98	47.70	2.89	6.09	13.36	28.92
191	OK1070275	16.23	8.24	1.97	43.17	2.87	6.03	13.07	28.02
192	ONAGA	14.37	7.13	2.02	34.68	2.92	5.46	12.35	28.29
193	OVERLAND	14.77	7.03	2.11	36.30	2.76	6.21	13.20	30.03
194	OVERLEY	13.63	6.91	1.97	38.32	2.96	6.06	13.65	35.59
195	PARKER	16.47	7.35	2.24	34.03	2.79	6.02	12.66	30.63
196	PARKER76	15.67	7.60	2.06	33.97	2.79	6.11	12.75	30.41
197	PETE	14.93	9.32	1.60	34.58	2.93	5.84	13.28	31.05
198	PLATTE	16.33	8.68	1.88	48.98	2.83	5.84	12.64	27.27
199	POSTROCK	14.93	7.88	1.89	41.42	2.81	5.67	12.28	28.65
200	PRAIRIE_RED	13.70	7.48	1.83	38.73	2.89	6.19	13.81	31.21
201	PRONGHORN	15.97	7.38	2.16	38.65	2.88	6.38	14.12	33.95
202	PROWERS	17.03	7.89	2.16	34.85	2.94	6.45	14.37	36.68
203	RAWHIDE	16.10	6.95	2.32	42.28	2.79	6.08	12.76	27.18
204	REDLAND	16.00	7.58	2.11	34.50	2.75	6.02	12.66	29.42
205	RIPPER	14.47	8.76	1.66	36.12	2.87	6.46	14.29	31.63
206	RITA	16.40	8.00	2.05	39.10	2.67	5.92	12.09	26.68
207	ROBIDOUX	16.50	8.84	1.87	40.98	2.92	5.92	13.45	33.24
208	RONL	16.73	8.33	2.01	49.65	2.86	5.87	12.99	29.02
209	ROSE	17.80	7.51	2.37	39.93	2.66	5.91	11.88	28.02
210	ROSEBUD	18.23	7.96	2.29	35.87	2.82	6.01	12.83	31.49
211	SAGE	15.10	8.75	1.73	35.28	2.78	6.36	13.60	30.98

212	SANDY	15.90	7.76	2.05	37.33	2.73	5.93	12.41	28.27
213	SANTA_FE	13.70	7.73	1.77	41.65	2.88	6.06	13.33	30.91
214	SCOUT66	14.53	8.65	1.68	36.43	2.86	6.44	14.20	35.96
215	SD00111-9	15.23	8.32	1.83	37.25	2.89	6.80	14.96	35.87
216	SD01058	18.07	8.71	2.08	39.27	2.74	6.00	12.70	29.32
217	SD01237	14.13	7.85	1.80	37.50	2.88	6.31	14.10	34.09
218	SD05118	16.77	8.25	2.03	44.05	2.78	6.29	13.31	30.44
219	SD05210	16.80	8.37	2.01	35.03	2.79	6.10	12.96	30.84
220	SD05W018	15.57	7.40	2.11	43.25	2.78	6.17	12.99	28.67
221	SETTLER_CL	15.53	8.48	1.83	38.93	2.84	6.18	13.56	31.82
222	SHAWNEE	17.90	8.55	2.27	41.65	2.90	6.16	13.54	31.67
223	SHOCKER	14.23	7.51	1.90	43.40	2.92	5.89	13.12	30.75
224	SIOUXLAND	15.97	8.34	1.92	37.37	2.85	6.25	13.50	32.82
225	SMOKYHILL	16.50	7.68	2.15	51.17	2.74	5.87	12.34	27.54
226	SPARTAN	14.47	7.67	1.89	39.95	2.81	6.37	13.79	31.94
227	STANTON	15.00	7.62	1.97	38.35	2.86	6.21	13.51	30.85
228	STURDY	12.80	7.51	1.70	40.07	2.87	5.94	13.24	30.35
229	STURDY_2K	14.27	7.60	1.88	36.32	2.87	6.20	13.58	32.81
230	TAM105	14.07	8.03	1.75	31.77	2.84	5.99	13.18	29.76
231	TAM107	13.67	8.00	1.71	43.20	2.95	6.46	14.69	34.07
232	TAM107-R7	13.33	7.50	1.78	34.55	2.92	6.21	14.03	32.51
233	TAM109	13.27	7.83	1.70	30.02	2.90	6.46	14.18	33.83
234	TAM110	13.50	7.85	1.72	37.08	2.93	6.21	14.16	30.67
235	TAM111	15.77	7.69	2.05	43.90	2.83	5.94	12.72	29.87
236	TAM112	14.57	7.59	1.92	37.77	2.86	6.01	13.28	30.89
237	TAM200	14.17	7.32	1.94	37.80	2.72	5.87	12.41	29.61
238	TAM202	14.97	8.12	1.84	39.32	2.78	6.08	13.17	29.74

239	TAM203	13.70	7.04	1.95	41.33	2.84	6.18	13.47	28.15
240	TAM302	15.40	8.32	1.85	39.37	2.80	6.28	13.55	28.64
241	TAM303	13.73	7.63	1.83	39.07	2.97	6.14	14.23	32.75
242	TAM304	17.47	7.86	2.22	49.42	2.74	5.97	12.36	25.06
243	TAM400	12.80	7.57	1.69	36.18	2.75	5.86	12.49	29.11
244	TAM401	15.87	8.58	1.85	47.52	2.77	6.24	13.24	28.27
245	TAMW-101	13.53	7.35	1.84	30.13	2.92	6.59	14.51	36.59
246	TANDEM	14.40	7.48	1.92	26.45	2.94	6.19	14.02	36.14
247	TARKIO	15.03	7.09	2.12	39.23	2.91	6.22	13.72	30.35
248	TASCOSA	14.43	7.77	1.86	35.02	2.84	6.16	13.59	31.26
249	THUNDER_CL	15.77	9.11	1.73	43.55	2.85	6.24	13.64	28.60
250	THUNDERBOLT	14.60	7.56	1.93	37.67	2.85	5.83	12.80	31.48
251	TREGO	15.40	8.28	1.85	37.67	2.89	6.11	13.78	32.47
252	TRISON	15.70	7.87	2.00	32.23	3.02	6.24	14.30	38.50
253	TRIUMPH64	13.80	6.95	1.99	28.60	2.88	6.12	13.47	33.58
254	TURKEY_NEBSEL	17.30	8.65	2.01	36.60	2.85	6.28	13.55	33.23
255	TX00V1131	14.43	7.95	1.81	40.40	2.68	6.02	12.38	29.06
256	TX01A5936	16.13	8.74	1.85	46.92	2.86	6.29	13.85	32.95
257	TX01M5009-28	16.00	7.71	2.08	48.72	2.76	5.50	11.60	23.11
258	TX01V5134RC-3	13.90	7.96	1.75	44.05	2.92	6.08	13.60	32.19
259	TX02A0252	14.27	7.76	1.84	39.95	2.73	6.14	12.98	29.87
260	TX03A0148	16.40	9.30	1.76	45.27	2.92	6.45	14.49	32.16
261	TX03A0563	14.60	8.01	1.82	39.67	2.78	6.11	12.98	30.19
262	TX04A001246	15.13	8.56	1.77	41.20	2.90	6.05	13.67	34.31
263	TX04M410211	15.12	7.83	1.93	39.58	2.86	6.20	13.56	33.03
264	TX04V075080	14.47	8.19	1.77	40.63	2.85	6.71	14.61	32.35
265	TX05A001188	14.63	7.19	2.04	39.68	2.69	5.87	12.05	27.02

266	TX05A001822	15.67	8.19	1.92	45.25	2.89	5.91	13.33	32.10
267	TX05V7259	16.27	7.98	2.04	39.82	2.78	6.07	12.86	31.41
268	TX05V7269	17.23	8.30	2.08	51.23	2.72	6.03	12.46	26.38
269	TX06A001132	15.57	7.34	2.12	41.90	2.94	6.35	14.05	33.71
270	TX06A001263	16.33	7.77	2.10	43.03	2.79	5.92	12.85	28.92
271	TX06A001281	15.73	7.98	1.98	45.28	2.79	6.18	12.99	29.72
272	TX06A001386	16.20	8.83	1.84	41.47	2.82	5.93	12.76	30.66
273	TX06V7266	16.03	8.02	2.00	50.45	2.76	5.73	12.15	25.32
274	TX07A001279	16.20	7.43	2.18	46.73	2.70	5.60	11.76	26.21
275	TX07A001318	14.73	8.08	1.82	42.43	2.82	6.33	13.80	32.60
276	TX07A001420	15.87	8.57	1.85	42.52	2.85	5.77	12.68	30.34
277	TX86A5606	16.73	8.30	2.02	34.98	2.93	6.37	14.20	34.77
278	TX86A6880	18.43	8.84	2.09	42.38	2.86	6.32	13.71	32.70
279	TX86A8072	13.57	7.79	1.74	34.30	2.95	6.06	13.84	32.26
280	TX96D1073	14.17	7.63	1.86	35.05	2.88	5.81	12.89	30.43
281	TX99A0153-1	14.43	8.51	1.70	34.37	2.74	6.41	13.48	31.57
282	TX99U8618	15.33	7.99	1.92	36.97	2.72	5.84	12.11	29.14
283	VENANGO	16.43	8.32	1.98	42.20	2.79	6.13	13.22	31.96
284	VISTA	15.43	7.48	2.07	33.15	2.61	6.00	12.00	27.15
285	VONA	15.57	7.50	2.08	43.87	2.79	5.78	12.40	27.98
286	W04-417	13.60	7.32	1.86	45.17	2.79	5.84	12.44	26.88
287	WAHOO	16.27	7.74	2.11	36.93	2.75	5.80	12.17	27.69
288	WARRIOR	16.17	7.95	2.04	34.85	2.80	5.91	12.65	29.19
289	WB411W	15.93	7.96	2.00	42.88	2.85	6.26	13.49	31.14
290	WENDY	15.23	8.16	1.87	35.37	2.85	5.86	12.93	29.33
291	WESLEY	15.73	7.29	2.16	36.67	2.73	6.20	12.90	28.27
292	WICHITA	13.97	8.22	1.70	30.00	2.88	6.17	13.71	34.55

293	WINDSTAR	16.03	7.82	2.05	40.65	2.79	6.15	13.02	29.30
294	WINOKA	15.62	7.34	2.13	37.07	2.71	5.95	12.38	26.01
295	YELLOWSTONE	18.23	8.37	2.18	38.80	2.79	5.99	12.75	29.70
296	YUMA	15.30	7.88	1.94	37.90	2.84	6.16	13.40	31.63
297	YUMAR	15.73	8.00	1.97	45.20	2.82	5.98	13.06	27.96

SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KA, kernel area; TKW, thousand kernel weight

Supplementary Table S2. SNP distribution across the three wheat sub-genomes used for GWAS in HWWAMP.

Sub-genome	Chromosome	Number of SNPs	% SNPs
A	1	1036	
	2	956	
	3	773	
	4	744	
	5	790	
	6	958	
	7	954	
Subtotal A	1-7	6211	39.8396
B	1	1254	
	2	1162	
	3	1117	
	4	451	
	5	1554	
	6	1237	
	7	855	
Subtotal B	1-7	7630	48.9416
D	1	438	
	2	581	
	3	240	
	4	52	
	5	159	
	6	146	
	7	133	
Subtotal D	1-7	1749	11.2187
Total SNPs (A, B, and D)			100

Supplementary Table S3. Significant SNPs/MTAs identified with BLUE (combined) value for various traits.

Trait[a]	SNP[$]	Chr	Allele	Pos	cM	-Log10*P*	effect	Environment*	Pleiotropic effect
SPS	Excalibur_c35316_154	1A	T/C	2540768	16.672	4.3	0.20455	E2, C	SD
	Excalibur_c11398_913	2A	C/T	118446604	102.437	3.4	-0.2223	C	
	Kukri_c36783_91	2B	A/C	154985272	93.282	6.5	0.21808	E3, C	
	Tdurum_contig42153_1190	2B	T/C	41198679	66.196	4.3	-0.2434	C	
	BS00069271_51	4A	A/G	4620085	8.607	3.8	-0.2732	C	
	BS00037357_51	4A	T/C	610493930	67.921	3.0	-0.2019	C	
	BS00011235_51	5A	C/A	552515237	76.809	5.6	-0.3999	C	
	IAAV4072	5A	C/A	42106388	42.476	4.3	-0.2106	E3, C	
	Ex_c23426_1546	5B	A/G	40786594	39.4	3.6	0.27005	E2, C	
	Excalibur_c97022_396	6A	C/T	37415157	58.038	4.0	-0.2661	E1, E3, C	
	RFL_Contig3175_1217	6A	T/C	604877158	136.701	3.5	-0.1681	E2, E3, C	
	IWA7896	6B	C/T	151130562	59.159	5.7	-0.3324	E3, C	
	IWA4455	6D	A/G	462631946	155.557	3.8	0.06089	E1, E3, C	
	IWA5913	7A	A/G	674276906	152.783	14.0	-0.5	E1, E2, E3, C	SD
	GENE-4848_95	7B	G/T	739931213	165.047	4.6	0.20834	C	
SL	TA006139-0953	1A	T/C	26959396	51.943	4.4	-0.107	E1, C	
	RAC875_c60162_206	1A	T/C	545891404	110.678	3.5	-0.0836	C	
	Kukri_c10860_1283	2A	G/A	87857405	105.892	4.7	0.16259	E1, E2, C	
	Tdurum_contig82393_484	2B	C/A	730562664	118.432	8.4	0.14718	E1, E3, C	
	IWA7916	2B	T/C	53464964	71.999	8.1	0.1535	E3, C	
	IWA8127	3A	A/C	20004385	33.664	3.9	-0.0872	C	

	Ra_c35_3184	3B	C/T	739984089	88.313	5.8	0.12681	E1, C	
	Ex_c883_2618	4A	A/G	17259209	33.77	6.0	0.12594	E3, C	
	Excalibur_rep_c68588_1196	4B	G/A	21377973	45.719	5.2	0.10603	C	
	Kukri_c4210_480	4D	T/C	455252652	94.22	3.8	-0.1023	C	
	CAP8_c1066_309	5A	A/G	488262170	57.929	3.3	0.15612	C	
	JD_c63005_896	5B	T/C	513873396	58.443	3.3	-0.0947	E1, C	
	IWA6116	6A	A/G	602708877	135.858	3.3	-0.1316	E2, C	
	GENE-4204_311	6B	T/C	614437173	71.972	3.5	0.09673	C	
	Tdurum_contig569_263	6B	T/C	704793345	108.86	3.0	0.08076	C	
	IWA3639	7A	G/A	610934198	131.114	7.6	-0.1382	E1, E3, C	
SD	Excalibur_c35316_154	1A	T/C	2540768	16.672	4.8	0.02645	C	SPS
	BobWhite_c14362_86	1B	T/C	653888017	125.263	3.9	0.05179	E3, C	
	IAAV618	1D	T/C	486879128	167.108	4.9	-0.0346	C	
	Kukri_rep_c104307_905	2A	A/G	32144831	65.649	3.6	-0.0244	C	
	IWA2519	3A	C/T	371628644	86.158	3.3	-0.0258	E1, E2, C	
	BobWhite_rep_c66057_98	4A	G/T	38369643	40.27	5.6	0.0307	C	
	Kukri_c19760_2091	5B	A/G	177188132	38.495	4.1	-0.0194	E1, C	
	RFL_Contig5037_560	6A	G/A	594748679	117.771	4.9	-0.0323	C	
	TA015451-0472	6B	A/G	222005447	64.57	3.9	0.02838	E1, C	
	IWA5913	7A	A/G	674276906	152.783	9.4	-0.0548	E1, E2, E3, C	SPS
	IWA1902	7D	A/G	530035575	149.588	4.1	-0.0411	E1, E2, E3, C	
KPS	IWA6805	1D	A/G	429698652	111.969	3.0	1.22743	C	
	CAP8_c5108_139	2B	G/A	26567970	46.763	3.4	-2.3829	C	
	BS00021959_51	2B	C/T	110818850	90.971	3.2	-1.8616	E1, E3, C	
	Excalibur_c1921_1191	5B	G/A	427650909	51.159	4.0	1.38977	E1, E2, C	

	IWA4329	5B	C/T	694520891	188.578	3.2	-1.3803	E2, C	
KW	RAC875_c21411_162	1A	A/G	539964977	105.742	3.2	0.03634	C	
	BS00044274_51	2A	T/G	47826702	81.895	3.3	0.02359	E2, E3, C	KA
	IWA6659	4A	C/T	84934131	47.532	3.2	-0.0304	E1, C	KA, TKW, SL
KA	IWA6485	3D	A/G	600261870	149.826	3.3	0.20109	C	SL
	Kukri_c74409_199	4A	G/A	37773890	40.27	3.4	0.25033	E1, E3, C	
TKW	Tdurum_contig46797_585	2A	T/C	44836524	81.489	3.3	0.725	E3, C	

Chr, chromosome; Pos, physical position in base pair (based on IWGSC RefSeq); cM, genetic position in centiMorgans (based on 90K_cons2014); *Environment 1(E1), Environment 2 (E2), Environment 3 (E3), Combined locations (C)

[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; KW, kernel width; KA, kernel area; TKW, thousand kernel weight

Supplementary Table S4. Phenotypic value with the no of favorable alleles.

Trait[a]	0	1	2	3	4
SPS	NA	14.78	15.7	15.71	16.85
SL	7.7	7.82	8.06	8.3	NA
SD	1.8	1.83	1.9	2.02	NA
KPS	35.49	37.27	40.53	NA	NA

[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike

Supplementary Table S5. Five best and worse lines in terms of phenotypic performance for spike related traits.

Trait[a]	Entry name	No of fav. alleles	Pheno. value	Trait	Entry name	No of fav. alleles	Pheno. value
SPS	CAPROCK	3	12.73	SD	SAGE	0	1.73
	TAM400	1	12.80		CO04025	0	1.78
	STURDY	3	12.80		LARNED	0	1.79
	TAM109	1	13.27		EAGLE	0	1.79
	HV906-865	1	13.27		NORKAN	0	1.83
	TX86A6880	4	18.43		RAWHIDE	3	2.32
	MT9904	2	18.53		ROSE	3	2.37
	MT9513	3	18.90		MT9513	3	2.39
	OK05108	4	19.23		COSSACK	3	2.46
	MT9982	4	19.87		MT9904	3	2.53
SL	MT85200	1	6.49	KPS	TANDEM	1	26.45
	HOMESTEAD	1	6.69		HOMESTEAD	1	27.55
	HV906-865	1	6.72		G1878	1	28.08
	OVERLEY	0	6.91		TRIUMPH64	1	28.60
	TRIUMPH64	2	6.95		AGATE	1	28.92
	OK05108	3	9.34		RONL	2	49.65
	NEOSHO	3	9.42		TX06V7266	2	50.45
	GUYMON	3	9.49		SMOKYHILL	1	51.17
	HAIL	3	9.51		TX05V7269	2	51.23
	OK1067274	3	9.57		HV9W06-504	2	54.33

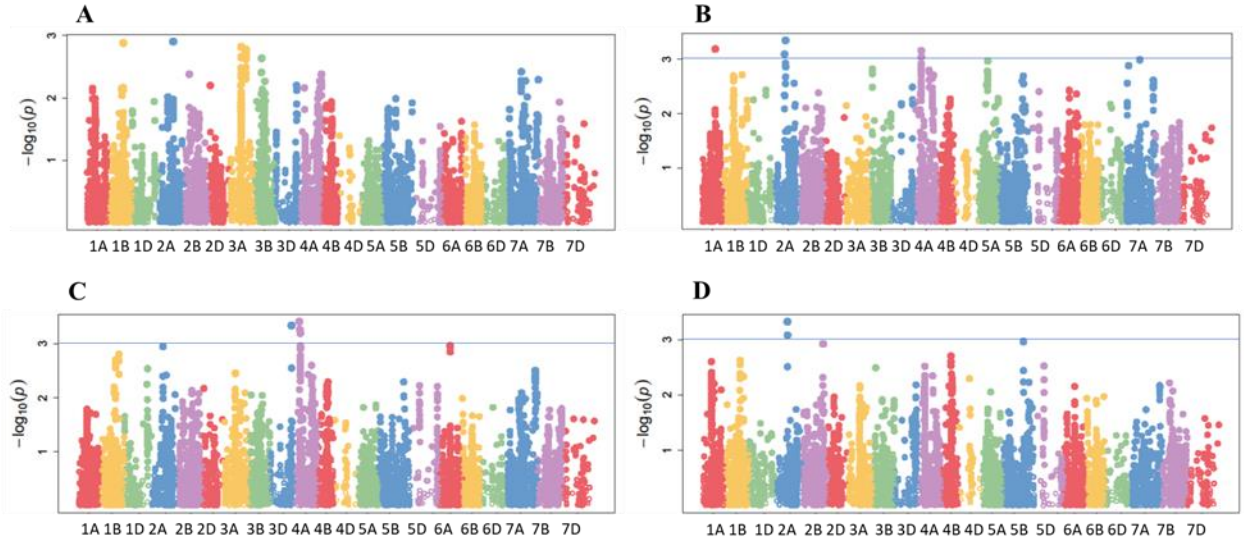
[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike; fav., favorable; Pheno., Phenotypic

Supplementary Table S6. Performance of the two models for 8 different spike and kernel related traits.

Trait[a]	Env	GBLUP		BMTME		Improvement (%)
		Mean PA	SE	Mean PA	SE	
SPS	E1	0.44	0.002	0.66	0.026	50.00
	E2	0.44	0.002	0.71	0.020	62.25
	E3	0.49	0.002	0.70	0.018	42.53
SL	E1	0.23	0.002	0.55	0.011	139.76
	E2	0.25	0.003	0.56	0.041	122.34
	E3	0.22	0.003	0.54	0.035	147.3
SD	E1	0.46	0.002	0.73	0.014	58.41
	E2	0.47	0.002	0.71	0.018	50.87
	E3	0.52	0.002	0.73	0.026	40.22
KPS	E1	0.41	0.002	0.53	0.040	30.48
	E2	0.39	0.002	0.53	0.016	35.19
	E3	0.33	0.002	0.48	0.030	44.18
TKW	E1	0.23	0.003	0.47	0.033	105.44
	E2	0.18	0.003	0.43	0.026	141.13
	E3	0.20	0.003	0.45	0.034	124.94
KL	E1	0.43	0.002	0.70	0.040	63.55
	E2	0.38	0.002	0.68	0.025	78.11
	E3	0.39	0.002	0.70	0.017	80.60
KW	E1	0.36	0.002	0.52	0.042	43.30
	E2	0.35	0.002	0.43	0.019	22.29
	E3	0.27	0.002	0.39	0.040	45.71
KA	E1	0.33	0.002	0.62	0.027	87.38
	E2	0.29	0.002	0.56	0.018	91.63
	E3	0.29	0.002	0.57	0.028	97.19

[a]SPS, spikelet number per spike; SL, spike length; SD, spikelet density; KPS, kernel number per spike, KW, kernel width; KA, kernel area; TKW, thousand kernel weight

List of Supplementary Figures:



Supplementary Figure S1. Genome-wide association scan. Fixed and random model Circulating Probability Unification (FarmCPU) based Manhattan plots represent -log10P for SNPs distributed across all 21 chromosomes of wheat. A) Kernel length (KL); B) Kernel width (KW); C) Kernel area (KA); and D). Thousand kernel weights (TKW). Y-axis: -log10*P* and X-axis: wheat chromosomes. The horizontal lines stand as a threshold for significant markers with -log10$P \geq 3$.

Chapter 5: Fine mapping a grain yield QTL introgressed into bread wheat from D-genome donor *Aegilops tauschii*

5.1. Abstract

Wheat (*Triticum aestivum* L.) is one of the most important food crops supplying one-fifth of all calories consumed worldwide. A steady wheat yield improvement is essential to feed the continually rising human population. Modern wheat can take advantage of genetic variation for agronomically important traits present in *Aegilops tauschii*, the D-genome donor of modern wheat. A D-genome Nested Association Mapping (DNAM) population in a hexaploid hard white winter wheat (HWWW) breeding line KS05HW14-3 was evaluated to identify grain yield QTLs. We identified a yield QTL from *Ae. tauschii* (ac. TA1615) transferred to wheat (KS05HW14-3) that was located on the distal 11 Mb region of chromosome 7DS. This 7D QTL explained ~4% of the phenotypic variation in grain yield. We developed and mapped 11 high-quality co-dominant SNP markers to 7DS QTL region by screening 29 homozygous recombinants (7 haplotype groups) lines. The homozygous recombinants were evaluated in the greenhouse and field for yield and yield contributing traits in 2020-21. No consistent variation in yield was found between the recombinant lines at Brookings, likely due to some winter kill and a limited number of replications. However, variation for TKW between parental lines was intriguing and could be further explored along with other yield contributing traits.

Keywords: Wheat, *Aegilops tauschii*, D genome, grain yield, QTL, recombinants

5.2. Introduction

Wheat including both bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* subsp. *durum* is one of the most important food crops that provide about 20% of the calories and protein for human consumption (Shiferaw et al., 2013). Wheat yield improvement is the prime interest of wheat breeding which is very crucial to meet the demands of the growing human population. Yield is the final output of a complex process that is directly and multilaterally determined by various yield-component traits, such as tiller number, spikelet per spike, grains per spike, thousand kernel weight, etc., and also largely influenced by the environmental factors (Wu et al., 2012). Even though yield is a low heritable trait, several yield-associated traits are less sensitive to environmental conditions i.e., higher heritability compared to grain yield (Cuthbert et al., 2008). Therefore, it is useful to study yield-associated traits for a better understanding of the genetic control and relationship between yield and related traits.

Wheat gene pool is a huge reservoir of diverse genes/alleles however, accessing this resource from secondary, or tertiary gene pool can be challenging due to the lack of viability of the hybrids and limited or no recombination. Therefore, genetic resources from various gene pools remain under-utilized, however, when effectively transferred to elite breeding materials, have resulted in improvement in economically important traits such as grain yield, biotic and abiotic stress resistance, improved nutritional and processing qualities, etc. (Trethowan and Mujeeb-Kazi, 2008).

The D genome of modern wheat has narrow genetic diversity as compared to its diploid progenitor (*Ae. tauschii* Coss.) because of the genetic bottleneck from the hybridization between tetraploid *Triticum turgidum* L. (2n = 4x = 28, AABB) and diploid *Ae. tauschii* Coss. (2n = 2x = 14, DD) (Dvorak et al., 1998; Wang et al., 2013, Strauss et al., 2021). *Ae. tauschii* is a valuable genetic resource for bread wheat improvement and has been most widely used in wheat breeding (Rakszegi et al., 2020). Useful genes or alleles of *Ae. tauschii* can be transferred to the bread wheat through direct hybridization (Sehgal SK., 2006; Sehgal et al. 2011; Olson et al., 2013) and this additional genetic diversity in the D genome could bring higher grain yield, better end-use quality, and improved stress tolerance (Cox et al., 1995; Yang et al., 2009; Lopes and Reynolds, 2010; Jia et al., 2013). Numerous useful genes for stress-related traits have been identified and introgressed from *Ae. tauschii* to modern wheat including leaf rust (Cox et al., 1994; Raupp et al., 2001), stem rust (Assefa and Fehrmann, 2004; Olson et al., 2013), powdery mildew (Miranda et al., 2006; Wiersma et al., 2017), root lesion nematode (Thompson and Haak, 1997), tan spot (Tadesse et al., 2006), Hessian fly (Cox and Hatchett, 1994) etc. Therefore, it is important to exploit the D genome donor (*Ae. tauschii*) of wheat for other economically important traits including yield.

Mapping the quantitative trait loci (QTL) and identification linked molecular markers can facilitate rapid transfer and pyramiding of several genes and QTLs for various agronomic traits including yield. Subsequence cloning of the respective genes can help in better understanding the regulatory mechanisms of genes controlling wheat yield and this information could be used to wheat improvement.

Olson et al., (2013) develop a wheat D-genome Nested Association Mapping (DNAM) population by directly hybridizing eight *Ae. tauschii* accessions (TA1615, TA1617, TA1642, TA1662, TA1693, TA1718, TA10171, and TA10187) to a hexaploid hard white winter wheat (HWWW) breeding line KS05HW14-3 (Supplementary figure S1). A subset of 420 $BC_2F_{4:6}$ lines (DNAM Core) from 1,200 BC_2F_4 RILs were evaluated for 3 years in 6 different winter wheat growing regions across the United States to identify several grain yields contributing QTLs, including an important QTL in chromosome 7DS. The most significant marker linked to the 7DS QTL is at 11,665,611 bp and explained ~4% of the phenotypic variation in grain yield (Supplementary figure 2). In the current study, we further mapped the grain yield QTL on the short arm of chromosome 7D and identified new molecular markers spanning the region.

5.3. Material and methods

5.3.1. Development of heterogeneous inbred families (HIFs)

Three BC_1F_5 lines from the cross KS05HW14-3/TA1615 that were heterozygous across the 7DS QTL region were identified and self-pollinated to develop two heterogeneous inbred families (HIFs).

5.3.2. DNA extraction and quantification

DNA was extracted manually from leaf samples of 7-10 days old seedlings using MagMAX™ Plant DNA Isolation Kit based on MagMAX magnetic bead technology that eliminates the need for phenol/chloroform extraction or alcohol precipitation (MagMAX™ Plant DNA Isolation Kit). The complete DNA extraction protocol can be

found in the following website: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0015954_MagMAX_Plant_DNA_Kit_UG.pdf. The concentration of the extracted DNA was measured with the Synergy™ H1 multi-mode microplate reader (BioTek Instruments, Winooski, VT 05404, United States).

5.3.3. Identification of SNPs and development of KASP™ assays

To saturate the target regions with markers, the parents of the mapping population KS05HW14 (HWWW) and TA1615 (*Ae. tauschii*) were genotyped using wheat exome capture to identify many polymorphisms in the selected HIFs. SNPs located in the high confidence genes were selected using JBrowse (https://wheat-urgi.versailles.inra.fr/Tools/JBrowse) for primer designing (KASP assays). Initially, the markers polymorphic among parents were identified for further genotyping of the HIF populations (Supplementary figure 3). KASP™ primers were designed using Kraken™ software from LGC genomics (https://www.biosearchtech.com/) and from exome capture data of the parents using the Triticeae Toolbox (T3) (https://shiny.triticeaetoolbox.org/primer_filter/).

The total reaction volume for preparing KASP genotyping mix was 10 µL (per well), where 5 µL of genomic DNA (~50 ng), 5 µL of 2X KASP-TF master mix and 0.14 µL of KASP assay mix was used. The details KASP genotyping thermal cycle protocol is provided in tabular format (Table 5.1) and a qPCR machine (CFX96 Touch Real-Time PCR Detection System) was used for running PCR reaction and read the fluorescence signal generated after cycle completion. More details about KASP thermal cycle protocol

can be found in KASP genotyping manual (https://biosearch-cdn.azureedge.net/assetsv6/KASP-genotyping-chemistry-User-guide.pdf).

Table 5.1. KASP thermal cycling protocol used in this study.

Protocol stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Hot-start Taq activation	94 °C	15 minutes	× 1 cycle
Stage 2 Touchdown	94 °C	20 seconds	× 10 cycles
	61 °C (61 °C decreasing 0.6 °C per cycle to achieve a final annealing/extension temperature of 55 °C).	60 seconds	
Stage 3 Amplification	94 °C	20 seconds	× 26 cycles
	55 °C	60 seconds	
Stage 4 (read the plate)	35 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	× 1 cycle

5.3.4. Genotyping and Phenotyping

Initial seed production from HIFs was carried out in the greenhouse. All individuals in HIF were screened with two SNP markers (at 3Mb and 17 Mb) to identify recombinants. Candidate recombinants were screened with 11 KASP markers. As some recombinant lines were heterozygous in the target region, the recombinants were further grown in the greenhouse to identify potential homozygous recombinants in the advanced generations, and later seed increased for phenotyping. Homozygous recombinants were evaluated in replicated yield trials at one location (Brooking, SD) 2020-21 field season due to a limited quantity of seed. Data was recorded for spikelet number per spike (SNS), spike length (SL), spike number, row yield, and thousand kernel weights (TKW) in the

greenhouse, while only plot yield and thousand kernel weights (TKW) were recoreded in the field.

5.4. Results

5.4.1. Identifying recombinants

Parents of the mapping population KS05HW14 (HWWW) and TA1615 (*Ae. tauschii*) were genotyped using exome capture and a total of 1,150 SNPs were identified in the yield QTL region (3-17 Mb) on chromosome 7DS. Selected SNPs were used to develop KASP assays to screen the recombinant lines and their progeny. More than 80 KASP markers spanning the 7DS QTL region were evaluated and 11 high-quality co-dominant markers were identified. More than 2,500 progenies F_6, F_7, and F_8 progenies were screened with KASP markers to identify lines carrying recombination events in the target region (Table 5.2). We identified 70 lines with 15 potential recombination events (recombinants) between marker 7D_3793951 and 7D_17345795 including both homozygous and heterozygous recombinants. Only a few recombinants were homozygous in the target region in F_6 lines and heterozygosity in the recombinant lines was a common problem and had to be selfed for two more generations to identify homozygous recombinants. A total of 29 homozygous recombinants lines were finally identified and grown in the greenhouse for seed increase.

Table 5.2. Selected markers frequently used for the genotyping of heterogeneous inbred families (HIFs) to identify heterozygous and homozygous recombinants.

Sl. No.	Marker_name	Allele	Sequence	A1	A2	Chr.	Position
	NCB_7D1710406_A1	A1	GAAGGTGACCAAGTTCATGCTGgttcattacagcatgaggttcaGTatttA	A	G	7D	1710406
	NCB_7D1710406_A2	A2	GAAGGTCGGAGTCAACGGATTGgttcattacagcatgaggttcaGTatttG	A	G	7D	1710406
1	NCB_7D1710406_C1	C1	gctagCatttccttcattgctgcaaG	A	G	7D	1710406
	NCB_7D3785762_A1	A1	GAAGGTGACCAAGTTCATGCTcttcctcctcacacaaatctgctG	C	A	7D	3785762
	NCB_7D3785762_A2	A2	GAAGGTCGGAGTCAACGGATTgtcttcctcctcacacaaatctgctT	C	A	7D	3785762
2	NCB_7D3785762_C1	C1	cggcttgcaaaggcttagatcTtcaT	C	A	7D	3785762
	NCB_7D4289467_A1	A1	GAAGGTGACCAAGTTCATGCTactttggcgtcatcactatccacaA	T	G	7D	4289467
	NCB_7D4289467_A2	A2	GAAGGTCGGAGTCAACGGATTactttggcgtcatcactatccacaC	T	G	7D	4289467
3	NCB_7D4289467_C1	C1	gtcagcctcgttgatgatggcaC	T	G	7D	4289467
	NCB_7D5304440_A1	A1	GAAGGTGACCAAGTTCATGCTgacgaagaaccaaatgataggaggatTG	C	T	7D	5304440
	NCB_7D5304440_A2	A2	GAAGGTCGGAGTCAACGGATTggacgaagaaccaaatgataggaggatTA	C	T	7D	5304440
4	NCB_7D5304440_C1	C1	gggggaaccttgggcactcG	C	T	7D	5304440
	NCB_7D6696159_A1	A1	GAAGGTGACCAAGTTCATGCTgtctcctaacaagggaagaaactcaagT	T	A	7D	6696159
	NCB_7D6696159_A2	A2	GAAGGTCGGAGTCAACGGATTgtctcctaacaagggaagaaactcaagA	T	A	7D	6696159
5	NCB_7D6696159_C1	C1	gcagcgactgatgagtgtggattAC	T	A	7D	6696159
	NCB_7D7073644_A1	A1	GAAGGTGACCAAGTTCATGCTcatgttcaggttgggtcaatcgcT	A	C	7D	7073644
	NCB_7D7073644_A2	A2	GAAGGTCGGAGTCAACGGATTgttcaggttgggtcaatcgcG	A	C	7D	7073644
6	NCB_7D7073644_C1	C1	agggcaaacatgacaaattctagtgataC	A	C	7D	7073644
	NCB_7D10021983_A1	A1	GAAGGTGACCAAGTTCATGCTgggattccagagaggccggtT	A	C	7D	10021983
7	NCB_7D10021983_A2	A2	GAAGGTCGGAGTCAACGGATTAgggattccagagaggccggtG	A	C	7D	10021983

	NCB_7D10021983_C1	C1	caaacaccactgatgcagctccC	A	C	7D	10021983
	NCB_7D12546901_A1	A1	GAAGGTGACCAAGTTCATGCTcgagcctcttgattcttccccG	G	A	7D	12546901
	NCB_7D12546901_A2	A2	GAAGGTCGGAGTCAACGGATTccgagcctcttgattcttccccA	G	A	7D	12546901
8	NCB_7D12546901_C1	C1	atcggctatcaatcagcaggaggaG	G	A	7D	12546901
	NCB_7D14783159_A1	A1	GAAGGTGACCAAGTTCATGCTgccatCGtccCtTctgaccgT	T	C	7D	14783159
	NCB_7D14783159_A2	A2	GAAGGTCGGAGTCAACGGATTccatCGtccCtTctgaccgC	T	C	7D	14783159
9	NCB_7D14783159_C1	C1	gtggcgAttcgacatggaggcT	T	C	7D	14783159
	NCB_7D17276946_A1	A1	GAAGGTGACCAAGTTCATGCTgtaatcaagcatctccccGgagtatT	A	C	7D	17276946
	NCB_7D17276946_A2	A2	GAAGGTCGGAGTCAACGGATTgtaatcaagcatctccccGgagtatG	A	C	7D	17276946
10	NCB_7D17276946_C1	C1	agtgttgggtacgaaaccttccttcT	A	C	7D	17276946
	NCB_7D17860294_A1	A1	GAAGGTGACCAAGTTCATGCTcaggccaggtatacgttgttatgcA	A	G	7D	17860294
	NCB_7D17860294_A2	A2	GAAGGTCGGAGTCAACGGATTaggccaggtatacgttgttatgcG	A	G	7D	17860294
11	NCB_7D17860294_C1	C1	tccagcagcataggcacccatC	A	G	7D	17860294

Chr. chromosome

5.4.2. Haplotype map of the QTL region

The yield QTL used in this study was mapped in the distal 11 Mb on the short arm of chromosome 7D and the yield contributing allele came from the TA1615 (*Ae. tauschii*). To delimit the QTL region, we developed KASP makers between 3-17 Mb on chromosome 7DS. We identified 29 homozygous recombinant lines representing 7 haplotypes (3-17 Mb) (Figure 5.1). On average, a marker was identified every 1.6 Mb.

Line names/ID	7D_3793951	7D_4016285	7D_6491812	7D_8250872	7D_11428557	7D_12546901	7D_14783159	7D_17345795	Haplotypes
Wheat (KS05HW14)	W	W	W	W	W	W	W	W	Wheat
WCAP-R-06	W	W	T	T	T	T	T	T	HAP1
WCAP-R-14	T	T	W	W	W	W	W	W	HAP2
WCAP-R-36	T	T	T	W	W	W	W	W	HAP3
WCAP-R-24	T	T	T	T	T	T	W	W	HAP4
WCAP-R-32	T	T	T	T	T	T	T	W	HAP5
WCAP-R-64	T	T	W	W	W	T	T	W	HAP6
WCAP-R-63	T	W	W	W	W	W	H	H	HAP7
W-T introgression line	T	T	T	T	T	T	T	T	*Tauschii*

W = Wheat allele, T = *Tauschii* allele, W-T = Wheat-*Tauschii*, H = Heterozygotes, Mb = Mega base

Figure 5.1. The haplotype map of the recombinant lines in the 7DS QTL region (3-17 Mb)

5.4.3. Field and greenhouse evaluation in 2020-21 field season

Data was recorded for yield and yield contributing traits from both greenhouse and field in 2020-21 season. In the greenhouse the wheat (KS06HW14) had a grain yield of 52 g and the wheat-*tauschii* introgression line had a grain yield of 47 g. The seven haplotypes demonstrated a grain yield ranging from 22 g to 67g. The number of spikelets/spikes (SPS) ranged 18.2- 22.0 and spike length (SL) ranged 8.6- 10.5 among the seven haplotypes. The thousand kernel weight (TKW) ranged 32.0 g to 37.9 g among the haplotypes whereas, KS06HW14 and wheat-*tauschii* introgression line had TKW of 31 and 36.8g, respectively (Table 5.3). We did not find any significant difference among

different haplotypes based in the greenhouse for grain yield, SL, and SPK. In the field Wheat -*tauschii* introgression line that carries the QTL region produced a higher grain yield (533.7g) as compared to the recipient wheat line KS06HW14 (503.0 g) (Table 5.3), however, the yield variation was not statistically significant. The mean yield (4 replications) in seven haplotypes ranged from 322 g to 504 g, however, these were not statistically significant due to variability among replicates.

Table 5.3. Phenotypic performance of parents and recombinant haplotypes for various yield contributing traits at greenhouse and field.

Parents & Haplotypes	Mean (greenhouse)					Mean (field)	
	SPS	SL	Tillers / Plant	Grain Yield (g)	TKW (g)	Grain Yield (g)	TKW (g)
Wheat (KS05HW14)	20.4	9.3	4.4	52.1	31.0	503.0	29.0
Wheat-*Tauschii* introgression lines	21.3	9.9	3.8	47.1	36.8	533.7	31.7
Haplotype 1	20.8	9.2	4.2	44.1	33.0	398.0	30.0
Haplotype 2	20.8	10.2	3.8	44.0	37.9	492.7	33.0
Haplotype 3	22.0	10.5	5.1	67.4	35	485.0	32.0
Haplotype 4	19.6	9.7	3.9	46.5	36.5	504.3	32.0
Haplotype 5	20.7	9.6	4.3	47.5	33.4	500.0	30.3
Haplotype 6	18.2	8.6	2.6	22.2	32.0	322.7	30.7
Haplotype 7	21.5	10.0	3.8	35.0	33	409.0	31.0

SPS; spikelet number per spike, SL; spike length, TKW; thousand kernel weights

5.5. Discussion

A total of 29 homozygous recombinants from 7 haplotype groups were identified and evaluated in the greenhouse and field. Heterozygosity and reduced recombination rate were evident in the QTL region. Finding more recombination events will be necessary to delimit the QTL region as suggested previously the biggest challenge for the use of

Aegilops is low recombination rates between *Aegilops* and wheat chromosomes in certain parts of the genome (Kishii, 2019). Field data from the 2020-21 season did not show any significant variation in yield which could be attributed to limited replications required proper statistical analysis. Further, unexpected variation among the biological replicates for various traits due to winter kill and drier conditions in the 2020-21 season, limited the statistical analysis. Grain yield perse has low heritability trait because it is highly regulated by environmental factors and interactions, this tends to hamper the progress in understanding the yield-related genes and gene network (Kuzay et al., 2019). Partitioning the gross yield into various yield contributing traits such as SPS, seeds per spike, TKW, etc. that have higher heritability can be an effective strategy to deal with this problem (Zhang et al., 2018). Significant variation for TKW between parental lines would be interesting to explore further along with other high heritable traits. Data from future (2021-22) replicated trials should help in demarcating the location grain yield QTL.

5.6. References

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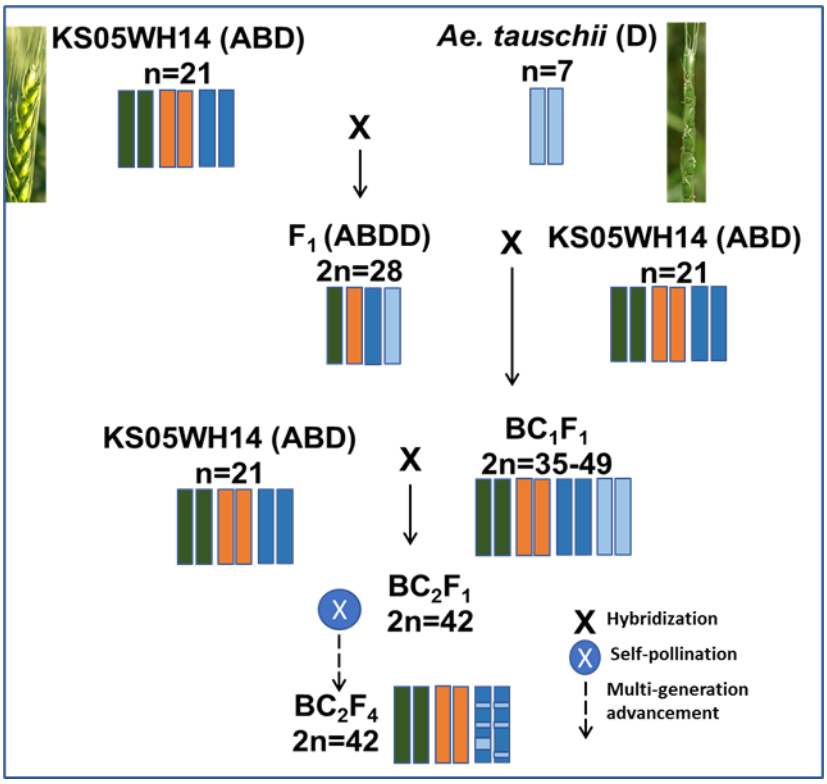
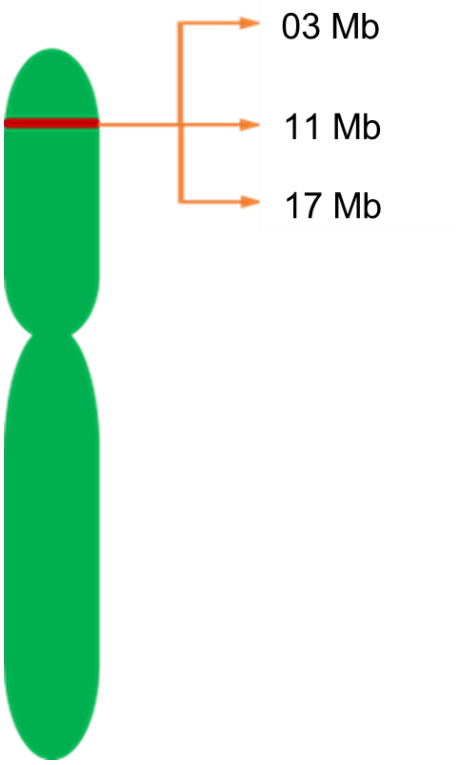
5.7. Appendix

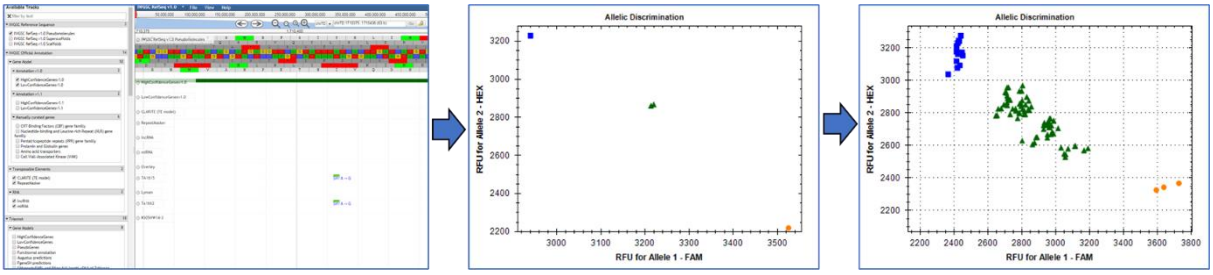
List of Supplementary Figures:



Supplementary Figure S1. Development of the DNAM population by direct hybridization of wheat and *tauschii*



Supplementary Figure S2. The physical location of 7D QTL and the most significant marker linked to the QTL is at 11,665,611 bp.



SNPs located in the high confidence genes were selected using Jbrowse for primer designing

Markers polymorphic among parents were identified

The polymorphic markers were used to genotype the F_6 and F_7 progenies

Supplementary Figure S3. Primer development and KASP genotyping of HIFs.