QTL Analysis of Wheat Grain Yield Components and Agronomic Traits Using Advanced Genotyping Platforms

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Authorization to Submit Thesis

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Abstract

The genetic manipulation of major yield components and agronomic traits is an important approach to increase wheat grain yield. Phenotyping of these traits is cost-effective but is time-consuming and the output is also confounded by environmental conditions. In the present study, we aimed to identify quantitative trait loci (QTL) and tightly linked, friendly used molecular markers to select for productive tiller number (PTN), fertile spikelet number per spike (fSNS), thousand kernel weight (TKW), grain yield (GY), height (HT), and heading date (HD). These traits were assessed in eight field trials over three years in a double haploid (DH) population that were derived from two adapted high yielding spring wheat cultivars 'UI Platinum' and 'LCS Star'. The DH population of 181 lines was genotyped using the 90K iSelect SNP platform and markers for known genes (*Ppd*, *Vrn*, *Rht,* and *FT*) that affect plant adaptation. The genotypic data was used in linkage analysis and QTL analysis for yield components and agronomic data using JMP Genomics Software (V9.0). To consider spatial variation, the best linear unbiased prediction (BLUP) was calculated for each trait across all trials. QTL analyses were conducted separately for each trait in individual environments and in trait BLUP across all environments. A total of 48 linkage groups were constructed with a total length of 3892.81 cM and a marker density of 0.33 marker/cM. A total of nineteen QTL were detected, including five for fSNS on chromosomes 5D, 6A, 7B (two QTL), and 7D; two for PTN on chromosomes 4A and 6A; three QTL for TKW on chromosomes 4A, 6A, and 7D; one QTL for GY on chromosome 7D; four QTL for HD on chromosomes 4B, 6A, 7B, and 7D; and four QTL for HT on chromosomes 4A (two QTL), 5D, and 7D. The two parents have complementary and additive QTL effects in all traits evaluated, providing opportunities to improve each trait through pyramiding. However, four QTL, *QPTN.uia2-6A*, *QfSNS.uia2-6A*, *QTKW.uia2-6A,* and

QHD.uia2-6A were clustered on chromosome 6A; five other QTL, *QTKW.uia2-7D*, *QfSNS.uia2-7D, QHT.uia2-7D, QGY.uia2-7D, and QHD.uia2-7D* were clustered in a small region on chromosome 7DS. The two QTL clusters each control traits that were negatively correlated, suggesting that the trade-off effects pose a challenge and further dissecting of the two clusters is necessary in order to use them in yield improvement. Using the exosome capture data, linkage maps of interest were saturated with additional KASP markers, which helps to dissect the identified QTL clusters. A few of QTL in the two cluster regions were further validated in an elite spring wheat panel, confirming the realty and effectiveness of the identified QTL. KASP markers developed in the present study may useful to pyramid multiple yield components to enhance yield improvement in wheat.

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Dedication

First and foremost, I would like to dedicate this thesis to my Lord and Savior Jesus Christ, because without Him none of this would have been possible. I would also like to dedicate this thesis to my Grandpa Isham for his countless hours of hard work on establishing Isham farms and creating a scholarship for his grandchildren to use. Without his faith and dedication to the farm, none of this would have been possible. I would like to thank my two uncles and dad that continued the countless hours of hard work and sleepless nights on Isham farms to support the scholarship fund that I was able to use to get this degree. I would also like to thank my parents, for their love and support they showed me along the way and finally, I would like to thank all my family and friends that have helped me while attending the University of Idaho

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Introduction

Wheat production

Common wheat *(Triticum aestivum*) is an important food crop that provides about 20% of the calories consumed by the world's population, and it is only behind rice as the primary source of calories of those in developing countries (Breiman and Graur, 1995). Wheat has a wide range of adaptability and is grown in many different environments, making it an important crop for global food security (Reynolds et al., 2012). It is grown on more land acreage than any other commercial crop including rice, maize, and potatoes (Curtis et al., 2002). Wheat occupies more than 240 million hectares (Curtis et al., 2002) and in 2018, total wheat production was approximately 700 million tons (FAO, 2017). Nevertheless, global demand of wheat is expected to double by 2050 due to the increasing population worldwide (Tilman et al., 2011). However, the supply of wheat acreage is being negatively affected by the reduction of arable land due to climate change and the rising population (Peña-Bautista et al., 2017).

The world's population is expected to grow, specifically in developing countries in Asia and Africa, and by 2050, the population could reach 9.3-10 billion people (Peña-Bautista et al., 2017). To meet this projected population, wheat production will need to increase by at least 1.1% per year which would result in a 60% increase in yield by 2050 (Peña-Bautista et al., 2017). Yield and agronomic improvements are crucial to meet this demand and to ensure the stability of the future population.

Wheat evolution

The major wheat species that is grown throughout the world is *Triticum aestivum,* commonly known as bread wheat (or common wheat). *T. aestivum* is an allohexaploid with three genomes (AA, BB, and DD) and 42 chromosomes (2n=6X=42) (Peng et al., 2011) making it very difficult to dissect on a genetic basis. This difficult understanding is due to the large genome size compared to other crops. The haploid DNA content of *T. aestivum* is approximately 16,000 Mb, which is approximately 100 times larger than the *Arabidopsis thaliana* genome, which contains 135 Mb, and is approximately 40 times larger than the rice (*Oryza sativa*) genome, which contains 430 Mb (Eckardt et al., 2012*).*

Previous studies have found that the hybridization of wheat took place from two different major events. The first hybridization event created the tetraploid species *T. turgidum* ssp. *diccocoides* (2n = 28, genome AA BB) which is commonly known as durum wheat (or wild emmer). Durum wheat was a cross product of two diploid wild grasses, *T. uratu* (2n = 14, genome AA) and an unknown species that is closely related to *Aegilops speltoids* (2n =14, genome BB). This hybridization took place in the wild and was driven by natural selection, long before domestication occurred (Hancock, 2004). The next hybridization event resulted in the hexaploid species, *T. aestivum* (2n =42, genome AA, BB, DD). This species was created from a cross between the *T. diccocum* (2n = 28, genome AA BB) species and the diploid species *Aegilops tauschii* (2n = 14, genome DD) (Charmet, 2011). With this hexaploidy genome, bread wheat has outperformed the earlier progenitors with its superior properties, stable yields and quality under certain challenges such as disease and drought. The hexaploid wheat (*T. aestivum*) also has a wide range of adaptability,

allowing it to grow under different environment conditions such as low rainfall areas and higher elevations (Li et al., 2018).

Quantitative trait locus mapping

A quantitative trait locus (QTL) is a genetic region that controls a quantitative trait. The most important agricultural traits such as yield, quality and some forms of disease resistance are examples of a quantitative trait (Collard et al., 2005). Generally, quantitative traits are multifactorial and are greatly influenced by multiple minor effect genes and environmental conditions (Tian et al., 2015a). QTL mapping can help us to understand quantitative traits and assist the trait selection in cultivar development (Collard et al., 2005; Gao et al., 2015; Tian et al., 2015b). To identify a QTL, three major criteria should be met: appropriate mapping materials, abundant molecular markers that can be genotyped in the mapping materials, and good statistical software that can help in QTL analysis (Tian et al., 2015a).

Constructing linkage maps is a primary step in QTL mapping. QTL mapping relies on detecting correlations between the genetic markers and the phenotypic traits; therefore, the mapping materials should have good segregation for the trait of interest. The mapping materials can be derived from two parents, such as recombinant inbred lines, doubled haploid lines population, and backcross-derived progeny lines from multiple parents, such as diverse germplasm panel and multiparent advanced generation intercross (MAGIC) population. Using both bi-parental and diverse germplasm has become a powerful approach in the QTL mapping of many important quantitative traits such as yield and productive tiller number.

Molecular marker platform is another important factor in QTL detection. Several types of genetic marker platforms were used in the past century, each with advancements making

genotyping faster, more accurate, and cheaper (Doveri et al., 2008). These marker platforms were commonly categorized as 'First Generation Markers', 'Second Generation Markers', and 'New Generation Markers'. These three categories of markers are also classified into four different groups based on their method of detection (Tian et al., 2015a): 1) hybridization-based markers such as restriction fragment length polymorphism (RFLP), diversity arrays technology (DArT) and variable number of tandem repeats (VNTRs) (Gupta et al., 1999); 2) restriction enzyme-based markers such as amplified fragment length polymorphism (AFLP) and cleaved amplified polymorphism sequences (CAPS) (Tian et al., 2015a); 3) polymerase chain reaction (PCR) based, such as sequence-tagged sites (STS), sequence characterized amplified region (SCAR), and simple sequence repeats (SSR) (Pardo et al., 2014); 4) sequenced based DNA markers, such as single nucleotide polymorphisms (SNPs) (Tian et al., 2015a; Edwards and Pushpendra 2017).

The SNP marker system is the most common variant in the genomes of all species and thus, is more valuable than other markers in building high-density genetic maps, fine mapping of targeted genes, and gene cloning (Tian et al., 2005a). SNPs are one of the new-generation genetic markers and its polymorphism is derived from the change of a single nucleotide in the DNA sequence, which can include a single nucleotide transition, deletion, transversion, and insertion. SNPs are very efficient in genomic studies which include characterization of genetic resources, genome-wide association studies and genome selection (Rimbert et al., 2018), and are becoming the marker of choice in most plant breeding programs. They are the marker of choice because they are locus-specific and they have low error rates, high call frequency, co-dominant inheritance, high efficiency rate and high-

throughput and low-cost processing compared to previous markers (Gupta et al., 1999; Schlotterer, 2004; Thomson, 2014; Gao et al., 2015; Rimbert et al., 2018).

QTL analysis is based on the association between the phenotype and genotype (or markers) (Boopathi, 2013). The markers are used to distribute the mapping population into different groups and to determine if there are any differences between the groups and the trait of interest (Boopathi, 2013). To do this analysis, there are many methods used including single marker analysis, interval mapping, and composite interval mapping. Single marker analysis is the simplest method and can be conducted using a t-test, ANOVA, and linear regression (Tian et al., 2005a; Francis et al., 2011: Boopathi, 2013). The results from this analysis will give the linkage group containing the markers, chromosome, and the phenotypic variation value (Boopathi 2013). The advantages of single marker analysis are its simplicity, a genetic map is not required, and it can be extended to multiple loci (Boopathi, 2013); however this method cannot detect a QTL that is far from a marker, which can cause a multitude of effects of the QTL to be misunderstood (Boopathi, 2013) and it cannot estimate the QTL positions which brought forth the single-interval mapping method (Tian et al., 2005a). Single-interval mapping was developed by Lander and Botstein (1989) and is the most popular approach for detecting a QTL (Boopathi, 2013). This method uses linkage maps and relates to a pair of adjacent markers along a chromosome instead of looking at a single marker (Boopathi, 2013). This method allows for a more precise position of a QTL by statistically testing a single QTL at each increment (2 cM) across linked markers (Boopathi, 2013). This mapping method measures the logarithmic of odds (LOD) score, which is used to identify the most likely position of a QTL, gives a percentage of phenotypic variance, provides the source of desirable alleles, and it provides the estimation of additive and

dominance effects (Jansen, 1993; Boopathi, 2013). This method does have drawbacks such as, the positions of the QTL are sometimes ambiguous, and it doesn't allow for interactions between multiple QTL (Tian et al., 2005; Boopathi, 2013), and the most important drawback is the 'selection bias'. Selection bias occurs when the estimated effect of the QTL is different than the true effect (Boopathi, 2013). To overcome some of the drawbacks of single interval mapping, Zeng (1994) proposed the idea of composite interval mapping (Tian et al., 2005a; Boopathi, 2013). Composite interval mapping combines a multiple regression analysis with interval mapping to detect QTL in multiple intervals by using multiple molecular marker information (Tian et al., 2005a). This method gives the most accurate position of a QTL; it estimates the effect of the QTL (Tian et al., 2005a; Li et al., 2015) by identifying the marker with the highest statistical significance and then by adding another marker that also shows statistical significance and so on (Boopathi, 2013). Markers will continue to be added to the model until a marker is no longer statistically important (Boopathi, 2013). Some of the drawbacks of this method are that it cannot distinguish between QTL-environment interactions as well as epistasis (Tian et al., 2005a).

Once a QTL is identified, molecular markers tightly linked to the traits can be used to assist in the selection of the target traits and the candidate genes associated with the QTL can be cloned through a map-based cloning method (Kumar et al., 2009; Tian et al., 2005a). Molecular markers are useful tools that have helped the advancement of many breeding programs. They increase the rate at which crop varieties can be released (Kumar et al., 2009), aid in discovering more information about the function of a desirable gene (Gupta et al., 1999), and assist in the selection of early generation plants with desirable traits (Gupta et al., 1999; Tian et al., 2005a; Kumar et al., 2009). QTL mapping and map-based cloning in wheat

are now applied in broader traits more rapidly since the Chinese Spring Wheat sequence is published.

QTL mapping for heading date and plant height

Heading date

Heading date is an important trait that determines the regional and seasonal adaptation of wheat varieties (Tian et al., 2015b). Heading days are calculated from the sowing date, or from January 1 (Julian calendar) to the date when 50% of plants have spikes protruding from flag leaves in an assessed area. The amount of heading days generally determines the flowering time of wheat, which subsequently impacts wheat maturity and grain yield in adapted environments. Heading date is mainly influenced by two genetic pathways, vernalization and photoperiod (Zanke, 2014; Guedira et al., 2016). Vernalization occurs when plants are exposed to low temperatures for a period of time in order to accelerate flowering and seed production (Guedira et al., 2016; Yan et al., 2003). The growth habits and vernalization requirements of wheat and barley are primarily determined by three genetic loci: *Vernalization1* **(***Vrn-A1, Vrn-B1, Vrn-D1***)**, *Vernalization2* **(***Vrn-A2***)**, and *Vernalization3* **(***Vrn-B3*) (Yan et al., 2003). The *Vrn-A1, Vrn-B1*, and *Vrn-D1* are located on the long arms of chromosomes 5A, 5B, and 5D in hexaploid wheat, respectively, which directly influence flowering and maturity dates and are upregulated by vernalization treatment (Trevaskis et al., 2003; Yan et al., 2003, 2004). The *Vrn-A2* gene is located on chromosome 5A and acts as a dominant repressor of flowering. Deletions or mutations involving *Vrn-2* result in the elimination of the vernalization requirement in wheat (Dubcovsky et al., 1998; Yan et al., 2004; Distelfeld et al., 2009). The *Vrn-3* gene is a homolog of the Arabidopsis flowering time

gene and has been mapped to the short arm of chromosome 7; it is upregulated by vernalization treatment and indirectly accelerates heading and flowering by promoting the expression of the *Vrn-1* gene (Yan et al., 2006; Faure et al., 2007).

Photoperiod is another vital pathway that influences heading and flowering dates, which rely on plant responses to the length of daylight, as well as the perception of optical signals from light receptors. Photoperiod response genes in common wheat are primarily controlled by the *Ppd-1* locus on the short arm of chromosome 2 (Welsh et al., 1973), which includes the *Ppd-A1, Ppd-B1*, and *Ppd -D1* genes located on chromosomes 2AS, 2BS, and 2DS, respectively. The alleles *Ppd-A1a, Ppd -B1a*, and *Ppd-D1a* confer photoperiod insensitivity, whereas alleles *Ppd -A1b, Ppd-B1b*, and *Ppd-D1b* are responsible for photoperiod sensitivity (Pugsley, 1966; Dyck et al., 2004). *Ppd-D1a* is a deletion mutation allele that causes mis-expression of the 2D *PRR gene* and permits early flowering in both short- and long-day conditions in photoperiod-insensitive cultivars. Photoperiod insensitivity is invariably beneficial to yield in Southern Europe and Asia. Five polymorphisms in the *Ppd-D1* locus were identified by the sequencing of 2D *PRR gene* homologs in several wheat cultivars (Beales et al., 2007). Furthermore, six haplotypes were revealed, owing to these sequence polymorphisms in the wheat *Ppd -D1* gene (Guo et al., 2010), and four haplotypes were discovered in Chinese winter wheat (Chen et al., 2013a; Zhang et al., 2015a). Additionally, sequence polymorphisms of the *Ppd-A1a* gene were identified in tetraploid wheat (Wilhelm et al., 2009); while the copy number variation (CNV) of *Ppd-B1a* could influence flowering date in common wheat (Diaz et al., 2012). Analysis of gene expression and interaction among photoperiod pathways is described in detail by Beales et al., (2007) and Guo et al., (2010).

QTL mapping revealed additional genetic loci controlling heading date and flowering dates. Klahr et al., (2006) identified QTL associated with heading date on chromosomes 3B, 5A, 5B, and 7A; Zhang et al., (2008) identified QTL associated with heading date on chromosomes 1B, 2B, 5D, 6D, 7A, and 7D; Maccaferri et al., (2008) identified three QTL for heading days on chromosomes 2A, 2B, and 7B. Most recently, Zhang et al., (2018) conducted a genome-wide association mapping study and found thirteen novel genetic loci on chromosomes 2BL and 2DS, and a novel QTL within the *Ppd-D1* locus, that accounted for 20% to 34% of phenotypic variation of either heading or flowering days, was also discovered. Zhang et al., (2018) suggested that *Ppd-D1, Vrn-B1*, and *Vrn-D1*, and the novel genetic loci identified in their study should be further investigated with the desired outcome of improving heading and flowering dates in Chinese wheat.

Plant height

Plant height is a very important trait that is associated with plant adaptation (Tian et al., 2017) and positively correlates to grain yield in wheat under water limited environments (Maccaferri et al., 2008). Plant height has a direct impact on yield traits including spike length, spikelet number per spike, and main spike grain yield (Jamali and Ali, 2008).

There has been great yield improvement by genetically reducing plant height (Zhen et al., 2011), but further improvement is needed. Many *Reduced Height (Rht)* genes controlling plant height have been mapped in the wheat genome (*Rht1- Rht 22*) (Peng et al., 2011; Mcintosh et al., 2012), but only five *(Rht*) genes have been successfully used in plant breeding programs (*Rht8c, Rht9, Rht-B1b, Rht-B1d*, and *Rht-D1b*) (Li et al., 2015).

These five (*Rht*) genes are commonly grouped into two categories, the insensitive and sensitive to gibberellic acid (GA) (Chapman et al., 2007; Xiao et al., 2012). The GA-insensitive *Rht-B1* and *Rht-D1* alleles increased fertility, kernel number per spike, and yield (Chapman et al., 2007), but these genes can also reduce seedling vigor (Rebetzke et al., 2007). The GA-insensitive genes also tend to reduce overall yield under conditions of drought and high-temperature (Chapman et al., 2007). The GA-sensitive genes such as *Rht-8, Rht9, Rht12*, and *Rht-13* can greatly reduce overall plant height without negatively affecting the seedling vigor or growth (Rebetzke et al., 2012).

Two of the major dwarfing genes, *Rht-B* and *Rht-D*, which are located on chromosomes 4B and 4D, respectively, have been widely distributed worldwide through the International Maize and Wheat Improvement Center (CIMMYT) and have been used in many plant breeding programs (Li et al., 2015; Assanga et al., 2017). The height in wheat is a very important agronomic trait and due to this high importance, many QTL studies have been done to assess how plant height can be manipulated and applied to plant breeding programs. QTL associated with height have been reported on chromosomes 1B, 2B, 3A, 3B, and 7A (Maccaferri et al., 2008). Maccaferri et al., (2008) used 800 simple sequence repeat (SSR) markers on a recombinant inbred line (RIL) population consisting of 249 lines planted in sixteen environments and found that the QTL on chromosomes 2BL and 3BS positively associated with grain yield. Li et al., (2015) identified QTL associated with height on chromosomes 4D, 6A, and 6B using SSR markers on an RIL population of 207 $F_{2:4}$ lines. These three QTL explained 67% to 82% of the phenotypic variation while the QTL on 4D and 6A showed significant interactions with the environment. Assanga et al., (2017) detected QTL on chromosomes 2B, 6A, and 6B using single nucleotide polymorphism (SNP),

genotyping by sequencing (GBS), and SSR markers in an RIL population of 217 lines in eight different environments. The QTL on chromosome 6A was found to harbor the *Rht24* gene for reduced plant height. In previous studies, this gene was found to be sensitive to gibberellic acid (Tian et al., 2017; Wurschum et al., 2017). Liu et al., (2018) identified QTL associated with height on chromosome 1A, 1B, 2B, 2D, 5B, 5D, 6A, 7A, and 7B. The QTL found on chromosomes 5B, 6A, and 7A were consistently detected in two different environments and explained 9.9% to 18.3% of the phenotypic variation in their study.

QTL mapping of grain yield and yield components

Grain yield

Grain yield has consistently been a major target trait for wheat breeding programs globally; however, genetic improvement of grain yield has been slow and has decreased due, perhaps in part, to the lack of knowledge about the complex nature of grain yield and the different mechanisms that occur in specific environments (Reynolds et al., 2009; Reynolds et al., 2012). With the rapid advancements in biotechnology (molecular markers), it becomes possible to dissect QTL (or genes) controlling overall grain yield and yield components (Reynolds et al., 2012).

Grain yield is a complex trait with low heritability and is highly influenced by the environment (Bennett et al., 2012a). To improve grain yield, much effort has been made to manipulate yield components that have higher heritability and are less subject to environmental effects (Zhang et al., 2010). These yield components include increasing the fertile spikelet number per spike, the productive tiller number per unit area, and thousand kernel weight (Naruoka et al., 2011; Gao et al., 2015). Thousand kernel weight and fertile

number per spike have shown to have consistent high heritability and are less likely to be affected by the environment, which allows for a better comprehension of these traits and their location in the genome (Goel et al., 2018; Liu et al., 2018). The productive tiller number has shown to be affected greatly by the environment making it harder to understand this trait on a genetic basis (Wang et al., 2018; Goel et al., 2018; Liu et al., 2018). Understanding the genetic foundation of grain yield and yield components is critical for the advancement of the wheat industry and to improve the stability of the food supply of the future population.

QTL associated with grain yield has been extensively studied and reported on all 21 wheat chromosomes (Bennett et al., 2012a). Kato et al., (2000) detected a QTL on chromosome 5A from 118 single-chromosome RILs derived from the F_1 between Chinese Spring (*Cappelle-Desprez 5A*) and Chinese Spring (*Triticum spelta 5A*). This QTL for grain yield on chromosome 5A was also associated with grain weight, tiller number, and spikelet number per spike; Groos et al., (2003) used an RIL population of 194 F_7 lines and detected QTL on chromosomes 2B, 3B, 4A, 4B, 5A, 5B, and 7D by using RFLP and AFLP markers. The QTL on chromosomes 2B and 7D were associated with the thousand kernel weight. QTL were also detected using SSR markers on 2B and 3B using 249 RILs evaluated in ten rainfed and six irrigated environments (Maccaferri et al. 2008). Recently, Li et al., (2015) assessed a RIL population of 207 $F_{2:4}$ lines under limited irrigation and detected QTL on chromosomes 1A, 2A, 3A, 3B, 4A, 4D, 5B, and 7A. This study identified QTL clusters for grain yield, kernel number per spike, thousand kernel weight, and productive tiller number on chromosomes 1A, 2A, 3B, 4D, and 5B. Li et al., (2015) suggested that these QTL may be useful for improving grain yield under limited irrigation. Assanga et al., (2017) assessed a population of 217 RILs in eight different environments and detected QTL on chromosome

2B, 5A, 5B, 6A, and 7A using the 90K SNP iSelect platform. The QTL detected on chromosome 2B and 5B showed the most stability across the different environments, while the QTL on chromosome 2B was pleiotropic with kernel weight and productive spikes. El-Feki et al., (2018) detected QTL on 2D, 5A, 5B, and 7B using a DH population of 185 lines. The QTL on chromosome 2D was linked to kernel weight, while the most significant QTL detected, was on chromosome 5A and was in the vicinity of the vernalization gene *Vrn-A1* on 5AL (El-Feki et al., 2018). Chromosome 5A was repeatedly noted, in previous studies (Kato et al., 2000; Groos et al., 2003; Assanga et al., 2017; El-Feki et al., 2018), to contain the grain yield trait. This could solidify that the *Vrn-A1* gene could play a major role in the total grain yield that can be produced (El-Feki et al., 2018).

Productive tiller number

Productive tiller number (PTN), defined as the number of tillers that produce spikes with seed set, is a very important component of grain yield (Li et al., 2011). McMaster et al., (1994) reported the main stem, primary tiller, and secondary tiller contribute 83% to 93% of grain yield. The PTN, measured after grain filling, is a result of early tillers. Many environmental factors can affect early tillers, such as soil moisture, soil fertility, soil and air temperature, seeding date, and seeding rate (Wiersma et al., 2005). The tillers grow from the main stem and coleoptile at the base of the axil leaves. A wheat plant that has adequate nutrition and water will develop more early tillers. When a wheat plant begins its reproductive stage, those early tillers with three or fewer leaves will be aborted (Rickman and E L Klepper 1991). Loss and Siddique (1994) discovered that the older Mediterranean wheat varieties produced a larger number of tillers, but many of those tillers were incapable

of producing fertile spikes, whereas the newer varieties of wheat produce fewer tillers, but more tillers are capable of producing fertile spikes. Some earlier studies reported that lower tillering lines produces higher yields than higher tillering lines in drought situations (Richards, 1988; Donald, 1979).

There have been a few genes reported that have been known to control the tiller capacity in wheat. Spielmeyer and Richards (2004) reported a tillering inhibition gene (*tin1*) on the short arm of the 1A chromosome. Peng, (1998) reported a tillering capacity gene (*tin2*) on chromosome 2A. Kuraparthy et al., (2007) reported a tillering inhibition gene (*tin3*) on chromosome 3A, and Kato et al., (2000) found a gene on chromosome 5A (*VrnA*) that controlled tillering capacity.

When an early tiller transits to a productive tiller, there are additional genes involving the spike formation and seed set. Therefore, PTN is a very complex yield component trait that has low heritability, and it is very hard to get consistent phenotypic data across multiple environments (Li et al., 2002; Dreccer et al., 2012; Hu et al., 2017) and in different materials (Li et al., 2002).

QTL associated with PTN has been mapped on chromosomes 6A and 1D on an RIL population consisting of 111 lines from a cross between Opata 85 and a synthetic hexaploid wheat W-7984 (Li et al., 2002). The QTL mapped on chromosome 6A was the most influential of all of the locations and was mapped near the gliadin locus *Gli-2*. The QTL mapped on chromosome 1D near the *Ppd-D1* gene, which is associated with heading date, reduced the overall productive tillers. Li et al., (2002) suggested that the *Ppd-D1* gene may have influenced this lower PTN; Naruoka et al., (2011) detected QTL associated with PTN on chromosomes 1A, 2B, 3B, 3D, 4B, 4D, 5D, 6A, 6B, 6D, and 7B using 232 SSR markers

and 190 DArT markers in three different RIL populations in rain fed and irrigated environments. The QTL on chromosome 6B positively affected the overall grain yield but had a negative impact on the kernel number per spike and seed weight. Hu et al., (2017) detected QTL on 4D using SSR markers in an RIL population of 371 ($F_{11;12}$) lines. The QTL for PTN on chromosome 4D was associated with the spike formation rate and tiller number during pre-winter per unit area (Hu et al., 2017); Wang et al., (2018) detected QTL on chromosomes 4A and 6A using the 90K iSelect platform and 300 selected SSR markers in a DH population consisting of 110 lines. The QTL on chromosome 6A, in this study, was also associated with the fertile number of spikelet per spike.

Many recent studies (Li et al., 2002; Naruoka et al., 2011; Wang et al., 2018) have found a QTL for PTN on chromosome 6A which suggests this chromosome contains a major gene that influences the overall productive tiller number. This QTL on chromosome 6A could be the major key in unlocking the potential to increase productive tiller number.

Fertile spikelet number per spike

The fertile spikelet number per spike (fSNS) in wheat is defined as the number of spikelet that have produced seed. The fSNS has a higher heritability and is more stable across multiple locations (Cui et al., 2012; Zhai et al., 2016; Wang et al., 2018). Three known loci affect spike morphology, including *S, C,* and *Q* (Sourdille et al., 2000; Paillard et al., 2003; Johnson et al., 2008). If a spike has round seeds and glumes the *S* locus regulates it, while the *C* locus affects the size, number and shape of the grain (Salina et al., 2000; Johnson et al., 2008). The *Q* locus affects many traits, including rachis fragility, and spike length which is important for the number of spikelet per spike (Simons et al., 2006).

With the spikelet formation being driven by genetics, there have been several studies done for the number of fertile spikelet per spike. QTL associated with fSNS have been detected on chromosomes 1A, 2D, 3B, 6A, 7A, and 7D using SSR markers on an immortalized F_2 and RIL population consisting of 136 lines developed from a cross between Nanda2419 and Wangshuibai (Ma et al., 2007). The QTL on 2D and 7D were mapped in the same region as the total spikelet number per spike, had the largest effect, and explained 13% to 24% of the phenotypic variation (Ma et al., 2007). Li et al., (2007) detected two QTL on chromosomes 2A and 7D on an RIL population of 131 lines planted in four different environments using SSR markers; Cui et al., (2012) detected four QTL on chromosomes 2A, 4D, 5A, and 7B using two $F_{8:9}$ RIL populations. The QTL on chromosome 5A was the most stable across five different environments and explained 9% to 15% of the phenotypic variation. The QTL on chromosomes 2A, 4D, and 7B were environmental specific and explained 17% of the phenotypic variation. There is a major gene that affects the gross spike morphology located on chromosome 5A (*Q gene*) (Sourdille et al., 2000; Paillard et al., 2003; Johnson et al., 2008), but there was no evidence that showed the *Q gene* caused an increase in the fertile spikelet number (Cui et al., 2012). Zhai et al., (2016) identified QTL for fSNS on 1A, 1B, 3A, and 7A using SNP and SSR markers on an RIL population of 191 lines that were advanced to the F_9 generation. Wang et al., (2018) detected four QTL for fSNS on chromosomes 4A, 5A, 6A, and 7A in a DH population of 110 lines using the 90K iSelect SNP platform. The QTL on chromosomes 4A and 6A was also clustered with productive tiller number per spike while the QTL on chromosomes 5A and 7A was clustered with the total spikelet number per spike and sterile spikelet number per spike. The QTL on

chromosome 4A had the largest effect and explained 20% to 39% of the phenotypic variation.

Thousand kernel weight

Thousand Kernel weight (TKW) is phenotypically the most stable yield component (Sun et al., 2009) and has a consistently higher heritability compared to kernels per spike and/or productive tiller numbers (Sun et al., 2009). According to Acreche and Slafer (2006), TKW contributes more to grain yield than the number of kernels per spike and/or productive tiller numbers, which is why it is a very important trait to study when seeking to improve grain yield. The QTL effects of TKW are usually additive which would make this trait beneficial for early generation selection (Wang et al., 2012).

TKW is closely related to the kernel width and the kernel length, but these two traits are controlled independently, which makes improvement difficult (Breseghello and Sorrels 2007). The determination of kernel width and length begins shortly before anthesis and continues through the grain filling process (Sinclair and Jamieson 2006). During the grain filling process, the kernel weight can be negatively affected by many environmental conditions such as increased temperatures and drought (Farooq et al., 2011). Therefore, a balance is needed between a flowering period that is long enough to increase grain number but not too long that grain filling occurs under a period of high temperature (Arjona et al., 2018). How the number of days to heading affects TKW needs to be dissected in order to understand how to manipulate TKW to increase grain yield. Lopes et al., (2013) stated that early maturity would favor the post anthesis grain growth filling periods under stress environments, which would result in increased grain size. Identifying QTL and associated

molecular markers would be more helpful than the phenotypic selection of this trait and indirectly improving grain yield.

QTL mapping associated with TKW has been detected on chromosomes 5B, 6A, 6D, and 7D (Lopes et al., 2013). All of these QTL were linked or pleotropic with heading dates and days to maturity in drought and irrigated environments. Gao et al., (2015) identified 13 QTL associated with TKW on chromosomes 1A, 2D (2), 3D, 4A, 4B, 5A (3), 5B, 6A, 7A, and 7B on an F_8 RIL population of 246 lines from a cross of Zhoa 8425B/ Chinese Spring. The QTL identified on chromosome 6A was tightly linked to the SNP marker Ku_c32392_967 at a genetic distance of 4.1 cM based on the 90K_consensus_map (Wang et al., 2014). Su et al., (2018) identified twenty-one QTL for TKW on chromosomes 1A (2), 1B, 2A (2), 2B, 2D (3), 3A (3), 3B, 4B, 5A, 5B (3), 6A, 6D, and 7A using the 90K SNP array and 225 SSR markers. These QTL explained 3.95%-15.34% of the phenotypic variation while *qTKW-5A* and *qTKW-5B.2*, which were detected in four environments, were the most significant, and the *qTKW-5B.2* accounts for kernel length. Sarma et al., (2000) previously reported two loci affecting flowering time on chromosome 5B. The two loci included the *Vrn-B1* and *Eps* and were linked to the *Xgwm604* and *Xwmc73* SSR markers. Li et al., (2007) identified five QTL associated with TKW on chromosomes 3B, 7D, 1D, 5D, and 6A. McCartney et al., (2005) identified two major QTL for TKW on the short arms of chromosomes 4B and 4D which is near the *Rht-B1b* and *Rht-D1b* genes that control plant height. The QTL on 4DS explained 31.8% of the phenotypic variations, and for both regions, the reduced thousand kernel weight directly correlated with the reduced plant height. Ramya et al., (2010) used 600 SSR primer pairs on a RIL population consisting of 185 lines and identified QTL for TKW on chromosomes 1A, 1D, 2B, 2D, 4B, 5B, and 6B. The QTL on

chromosome 4B was detected in many of the previously stated studies (McCartney et al., 2005; Ramya et al., 2010; Gao et al., 2015; Su et al., 2018), and Kumar et al., (2016) proposed that the genomic region of the detected QTL on 4B could be the ortholog of the rice gene *GS3* (Huang et al., 2015). This gene has shown to have a positive effect on the kernel weight and size. With further investigation of these QTL regions, thousand kernel weight could begin to be better understood, resulting in improvements towards increasing yield.

QTL associated with multiple yield components

It is commonly observed and accepted that the three yield components of productive tiller number, fertile spikelet number per spike, and thousand kernel weight, work independently towards grain yield. In general, the three yield components are negatively correlated; therefore, selecting higher PTN tends to have a smaller number of fSNS and TKW, and selecting higher TKW tends to have smaller fSNS and PTN. From the previous studies listed above, a few chromosomes have been mentioned to contain more than one trait of interest. These regions are known as QTL clusters and are of high importance when trying to manipulate yield and yield components.

Chromosome 6A contains a QTL cluster for grain yield, productive tiller number, fertile number per spike and thousand kernel weight **(**Li et al., 2002; Ma et al., 2007; Naruoka et al., 2011; Lopes et al., 2013; Wang et al., 2018). Li et al., (2002) and Gao et al., (2015) found that the QTL cluster for these yield traits was also associated with heading date and the height of the plant. Chromosome 5A contained a QTL cluster for grain yield, spikelet number per spike, thousand kernel weight, and the heading date (Kato et al., 2000; Zhang et al., 2008; Cui et al., 2012; El-Feki et al., 2018; Wang et al., 2018); Chromosome 4D contained QTL

clusters for grain yield, kernel number per spike, thousand kernel weight, and productive tiller number (McCartney et al., 2005; Naruoka et al., 2011; Cui et al., 2012; Li et al., 2015). This cluster on 4D could be associated with the *Rht-2* gene. These QTL clusters containing negatively correlating yield components may explain why manipulating a single yield component QTL has not always improved grain yield. Dissecting these QTL clusters could enable us to understand their relations to plant adaptation, and therefore, improve grain yield.

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Chapter 2

QTL Analysis of Wheat Grain Yield Components and Agronomic Traits Using Advanced Genotyping Platforms

Introduction

Increasing grain yield (GY) is the most important objective in cultivar development in wheat. One important approach is to manipulate major yield components (Naruoka et al., 2011; Gao et al., 2015; Wang et al., 2018), such as such as fertile spikelet number per spike (fSNS), productive tiller number (PTN), and thousand kernel weight (TKW). It is commonly accepted that the three yield component traits are quantitively inherited, controlled by multiple genes and affected by environmental conditions (Kato et al., 2000; Deng et al., 2010; Mcintyre et al., 2010 Bennett et al., 2012a). In recent years, molecular genetics, such as QTL mapping offers alternatives to the traditional phenotypic selection for yield components in response to increasing GY.

Many studies on the yield related QTL mapping have been conducted, but each only included one or two yield components in their studies and used un-adapted materials as mapping parents (Kato et al., 2000; Groos et al., 2003; Wang et al., 2011; Li et al., 2015). The plant adaptation genes, such as photoperiod genes (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*), vernalization genes (*Vrn-1* to *Vrn-3*), flowering time genes (*FT* genes), and height genes (*Rht-B1* and *Rht-D1*), are known to have effects on GY and/or yield components (McCartney et al., 2005; Assanga et al., 2017; El-Feki et al., 2018; Wang et al., 2018), but few studies included this information in their QTL mapping studies. The present study used a doubled haploid (DH) population derived from two high yielding cultivars ‘UI Platinum’ and ‘LCS

Star' that have complementary traits in fSNS, PTN, and TKW. The present study also used advanced genotyping platform, such as 90K SNP chips and exosome capture technology in the QTL analyses for fSNS, PTN, and TKW in relation to QTL analyses for GY, heading date (HD), and plant height (PH).

Materials and Methods

Plant materials used in the present study

Two sets of spring wheat lines were used in the present study. One set was used for QTL detection consisting of 181 F_1-derived doubled haploid (DH) lines from a cross between two high yielding spring wheat cultivars, 'UI Platinum' and 'LCS Star'. UI Platinum was developed by the University of Idaho Agricultural Experiment Station and was released in 2014 (Chen et al., 2016). LCS Star was developed and released by Limagrain Cereal Seeds (LCS). The DH lines were created from F_1 plants using the wheat by maize hybridization system offered by the Heartland Plant Innovation in Kansas (Laurie and Bennett, 1986). The other set was developed by the wheat breeding programs in the Pacific Northwest (PNW) and the International Maize and Wheat improvement Center (CIMMYT), which consisted of 170 spring wheat cultivars or elite lines and has been used for genome-wide association studies of disease resistance (Wang et al., 2017; Dong et al., 2018).

Phenotypic evaluation and data analysis

The mapping population was planted and assessed in eight field trials, four irrigated at Aberdeen, Idaho (42.96° N 112.83° W, elevation 1342m) in 2017, 2018, and 2019 (17-AB, 18-AB, and 19-AB) and Ashton, Idaho (44.0716° N, 111.4483° W, elevation 1603m) in 2018

(18-ASH); two high rainfall trials at Moscow, Idaho (46.7324° N, 117.0002° W, elevation 786m), in 2018 (18-MSC), and Walla Walla, Washington (46.0646° N, 118.3430° W. elevation 287m), in 2018 (18-WW); two dryland trials at Soda Springs, Idaho (42.6544° N, 111.6047° W, elevation 1760m) in 2018 and 2019 (18-SS and 19-SS).

The DH and parental lines were arranged in a randomized complete block design (RCBD) with two replications in the trials of 18-AB,19-AB, 18-ASH, 18-SS, 19-SS and with one replication in the trials of 17-AB, 18-MSC, and 18-WW. The diverse spring wheat panel were planted with one replication and assessed in three field trials at Aberdeen, ID in 2017 and 2018, and at Soda Springs, ID and Walla Walla, WA in 2018. All field trials consisted seven rows plots, 3.0 m in length, 1.5 m in width, and 0.25 m between rows. Fertilization and weeding were applied when necessary to achieve the optimal growing conditions.

Considering the availability of resources, the number of traits measured were different among the eight trials. Grain yield in bushels per acre (Bu/A) was assessed in six trials (17-AB, 18-AB, 18-ASH, 18-SS, 18-WW, and 19-SS using the following equation:

$$\text{Yield ASIS} = \text{lbs/plot} \times [43560/(5 \times 10 \times \text{TWT})])$$

$$\text{Adjusted grain yield} = \text{YieldASIS} \times (1-0.01 \times \text{moisture})/0.88)$$

The fSNS was recorded in seven trials (17-AB, 18-AB, 19-AB, 18-ASH, 18-WW, 18-SS, and 19-SS) and was measured from ten randomly selected spikes that were fully developed before harvest. PTN was recorded in four trials (18-AB, 19-AB, 18-ASH, and 18-WW) and was assessed before harvest as the number of productive tillers per 45 cm in the middle row of each plot. TKW in grams (g) was recorded in seven trials (17-AB, 18-AB, 19-AB, 18-ASH, 18-MSC, 18-SS, and 19-SS) and was assessed by weighing one hundred randomly selected seeds and multiplying it by a factor of ten to achieve the estimate of

thousand kernel weight. HD was recorded in four trials (18-AB, 19-AB, 18-WW and 19-SS) and was calculated from January 1 (Julian Calendar) to the date when 50% of plants have spikes coming out of flag leaves in an assessed area. Plant height in inches was recorded in five trials (18-AB, 19-AB, 18-WW, 18-SS, and 19-SS) and was measured from the soil surface to the tip of the spike (awns excluded) at the last stage of maturity before harvest. For the diverse spring wheat panel, the GY was collected in field trials at Aberdeen, ID in 2017 and 2018, and at Soda Springs, ID and Walla Walla, WA in 2018. The HT and HD were collected in field trials at Aberdeen, ID in 2017 and 2018 and at Soda Springs, ID and Walla Walla, WA in 2018. The fSNS was collected in the two field trials at Aberdeen, ID in 2017 and 2018. The TKW was collected at field trials at Aberdeen ID in 2017, while the PTN was collected in the field trials at Aberdeen, ID in 2017 and at Walla Walla, WA in 2018 using the same method used in the mapping population.

Phenotype data analysis, including BLUP (Best Linear Unbiased Estimates), histograms, correlations, QTL x QTL interactions, and broad-sense heritability were all conducted using JMP Genomics 9.0 (SAS Institute Inc., Cary, NC, 1989-2019). The BLUPs across different trials for each trait were calculated and the genotypes, trials, and replication were all considered as random effects in the model. The broad-sense heritability (H^2) was estimated based on the equation $H^2= \sigma^2_g/(\sigma^2_g+ \sigma^2_{gy}/y+ \sigma^2_{gl}/l+\sigma^2_{gyl}/yl+ \sigma^2_e/ylr)$ where σ^2_g is the variance of genotypes, σ^2_{gy} is the variance of genotype-year, σ^2_{gl} is the variance of genotype-location, σ^2_{gyl} is the variance of genotype-location-year, σ^2_e is the residual variance, e is environment number, and r is the number of replications in each trial. Histograms were fitted with a normal curve and tested with the Shapiro-Wilks method to check for normality.

Genotyping and linkage analysis

The DH and parental lines were genotyped at the USDA-ARS Small Grains Genotyping Laboratory, using the 90K SNP iSelect platform at Fargo, ND (Wang et al., 2014). Genotype calling and SNP clustering were conducted using the GenomeStudio V2.0 (Illumina, San Diego, CA). The number of linkage groups (LGs) was identified using the automated hierarchical and K-means clustering to reduce the number of markers in the recombination. The markers on the LGs were ordered using the cM Kosambi mapping function and the accelerated map order optimization algorithm in the function of "linkage map order" integrated in JMP Genomics. LGs were separated when the genetic distance between bordering markers was greater than 50 cM.

QTL analysis

QTL analysis was conducted using individual and the BLUP data sets for GY, HD, HT fSNS, TKW, and PTN by the composite interval mapping (CIM) method in JMP Genomics 9.0. Significant QTL was called using the expectation maximization (EM) algorithm at a threshold of 2.5 (LOD>2.5). The software output provided a proportion of phenotypic variance (R^2) and the additive effects of the parents. The source of allelic effects of the parents UI Platinum (UIP) and LCS Star (LCS) was indicated by negative and positive estimates of the additive effects, respectively.

Bioinformatic analysis of QTL regions

A BLAST search

(https://urgi.versailles.inra.fr/blast_iwgsc/?dbgroup=wheat_iwgsc_refseq_v1_chromosomes&program=blastn). was preformed to

align the QTL-associated peak and flanking SNP marker sequences with the Chinese Spring sequence (Reference Sequence v2.0, the International Wheat Genome Consortium (IWGSC). This was used to find physical positions and candidate genes for identified QTL regions. Gene lists and their annotations (IWGSC RefSeq v1.0) in the candidate regions were downloaded from the website (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/).

For the genes with multiple transcripts, only the first transcript was kept unless different protein domains exist in different transcripts.

Fine mapping

To fine map the major QTL, additional SNPs between the two parents in the target QTL regions were identified based on the gene and putative promoter capture data (Gardiner et al. 2019) that conducted in Kansas State University, which was downloaded from The Triticeae Toolbox (T3) (https://triticeaetoolbox.org/wheat/). The primers for KASP markers were designed based on each identified SNP using PolyMarker (Ramirez-Gonzalez et al. 2015). KASP primers were tested using the parents and then used to screen the whole DH population. The KASP assays were performed in a CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The reaction system and PCR conditions were based on the protocol from LGC Genomics. The plate was read at 25 ºC at the last step and the data were visualized and analyzed using allelic discrimination function in CFX Maestro software (Bio-Rad, Hercules, CA).

QTL validation

The QTL associated SNPs in the peak and flanking regions for selected major QTL were converted to KASP markers, which were then genotyped for whole validation panel. The significance between the genotypes and phenotypes was determined by T-Test in JMP Genomics 9.0.

Results

Phenotypic analysis of GY, HD, HT, fSNS, TKW, and PTN

The broad sense heritability (H^2) and the phenotypic performance of the two parents and the DH population are summarized in Table 2.1. Based on the BLUP data, the two parents had significant differences in all traits assessed except for HT. LCS had larger trait values in GY, HD, fSNS, and PTN, while greater trait values in TKW came from UIP.

The HD, HT, fSNS, and TKW showed higher broad-sense heritability at 0.79, 0.82, 0.84, and 0.68, respectively (Fig. 2.1 and Table 2.1), suggesting high levels of genetic factors are contributing to the stable effect of these traits in the population. The GY and PTN showed moderate broad sense heritability at 0.38 and 0.44, respectively (Fig. 2.1 and Table 2.1) which suggest the environmental condition have a huge effect on this trait.

GY showed low to high correlation among the irrigated locations (17-AB, 18-AB, and 18-ASH) as well as the high rainfall location (18-WW) (r^2 ranged from 0.17 to 0.74) (Table 2.2) while the dryland environments (18-SS and 19-SS) had the lowest correlation with other locations (r^2 ranged from 0.06 to 0.39) (Table 2.2) suggesting that the environment had a strong effect on grain yield. Heading date showed high correlation in 18-AB, 18-ASH, 19-AB, and 19-SS and the BLUP (r^2 ranged from 0.82 to 0.87) (Table 2.2). Height was highly

correlated in the 18-AB, 18-WW, 19-AB environments (r^2 ranged from 0.60 to0.88) while 18-SS and 19-SS had moderate to high correlation (r^2 ranged from 0.48 to 0.74) (Table 2.2). The correlation of fSNS was moderate to high among all the trials (17-AB, 18-AB, 18-SS, 18-WW, 18-ASH, 19-AB, and, 19-SS) (r^2 ranged from 0.48 to 0.89) (Table 2.2). The correlation of PTN in 18-AB, 18-ASH, 18-WW, 19-AB, and 19-SS locations were moderately correlated (r^2 ranged from 0.25 to 0.43), whereas the BLUP values were moderate to highly correlated with 18-AB, 18-ASH, 18-WW, and 19-AB (r^2 ranged from 0.50 to 0.70) (Table 2.2). Similar to the PTN, the values of TKW at 17-AB, 18-AB, 18-ASH, 18-SS, 18-MSC, 19-AB, and 19-SS were moderately correlated (r^2 ranged from 0.19 to 0.54) whereas the BLUP value was highly correlated with 17-AB, 18-AB, 18-ASH, 18-SS, 18-MSC, 19-AB, and 19-SS (r^2 ranged from 0.60 to 0.75) (Table 2.2). Correlation analysis using the BLUP data of each trait showed that GY had a moderate correlation with fSNS (0.37) which suggests that the increase in GY partially came from the fSNS. The PTN and TKW also had a moderate negative correlation with fSNS (r^2 ranged from -0.22 to -0.33) which suggest that fSNS is impacted negatively by the PTN and TKW (Table 2.2). The HD and the HT had a moderate correlation with GY (r^2 ranged from 0.26 to 0.30) and the HD also had a high correlation with fSNS (0.74) (Table 2.2), which suggests that the HD directly impacts the number of fertile spikelet per spike.

Linkage group construction and marker analysis

Of the 81,587 SNPs on the 90K iSelect SNP array, 14,236 SNPs were polymorphic between LCS Star and UIP. After excluding the markers that co-segregated at the same position and the markers that were missing in more than 10% of the lines, a total of 1,276

SNP were used to construct the linkage map. A total of 48 linkage groups (LGs) were constructed that corresponded to all 21 hexaploid wheat chromosomes. Chromosomes 6A, 2B, and 1D were represented by one LG each; chromosomes 1A, 2A, 4A, 3B, 4B, 5B, 6B, 2D, 4D, and 7D were represented by two LGs each; chromosomes 3A, 5A, 1B, 7B, 3D, 5D, and 6D were represented by three LGs each; and chromosomes 7A was represented by 4 LGs (Table 2.3). The map of the A genome included 489 markers (38%) with a total length of 1,322.46 cM and an average marker density of 0.37 marker per cM; the map of the B genome included 583 markers (46%) with a total length of 1,546.01 cM and an average marker density of 0.38 marker per cM; the map of the D genome included 204 markers (16%) with a total length of 1,024.34 cM and an average marker density of 0.2 marker per cM (Table 2.3). The D genome had the lowest marker coverage which suggests that more of the markers were polymorphic in the A and B genome. The overall length of the linkage map was 3,892.81 cM, with a marker density of 0.33 marker per cM. The highest marker density occurred on chromosome 3A with a marker density of 0.59 marker per cM. The lowest marker density occurred on chromosome 5D with a density of 0.15 marker per cM

QTL detection

QTL for GY

A QTL *QGY.uia2-7DS* on chromosome 7DS was detected in five of seven data sets (17-AB, 18-ASH, 18-WW, 19-SS, and BLUP), explained 7% to 17% of the phenotypic variation (Table 2.4). The positive allele was from LCS Star, the peak marker of this QTL is very close to the flowering time gene *Ft-D1* IWGSC RefSeq v1.0 (Fig. 2.3).

QTL for HD

A total of four QTL were detected for HD on chromosomes 4B, 6A, 7B, and 7D respectively (Table 2.4). *QHD.uia2-4B* was detected in all five data sets (18-AB, 18-WW, 19-SS, 19-AB, and BLUP), explaining 13% to 25% of the phenotypic variation. *QHD.uia2-6A* was detected in all five data sets (18-AB, 18-WW, 19-SS, 19-AB, and BLUP), explaining 6% to 13% of the phenotypic variation. *QHD.uia2-7B* and was detected in all the five data sets (18-AB, 18-WW, 19-AB, 19-SS, and BLUP) explaining 11% to 23% of the phenotypic variation. *QHD.uia2-7D* was detected in all the five data sets (18-AB, 18-WW, 19-AB, 19-SS, and BLUP), explaining 34% to 65% of the phenotypic variation. The positive allele of *QHD.uia2-7B* and *QHD.uia2-7D* came from LCS star while the positive allele of *QHD.uia2-4B* and *QHD.uia2-6A* came from UIP. The peak marker of *QHD.uia2-7D* is very close to the flowering time gene *Ft-D1* based on IWGSC RefSeq v1.0 (Fig. 2.3).

QTL for HT

A total of five QTL were detected for HT on chromosomes 4A (2), 5A, 5D, and 7D (Table 2.4). *QHT.uia2-4A-1* was detected in all six data sets (18-WW, 18-SS, 18-AB, 19-AB, 19-SS, and BLUP), explaining 7% to 20% of the phenotypic variation. *QHT.uia2-4A-2* was detected in all six data sets (18-AB, 18-WW, 18-SS, 19-AB, and BLUP), explaining 6% to 24% of the phenotypic variation. *QHT.uia2-5A* was detected in four of six data sets (18-AB, 18-SS, 18-WW, and BLUP), explaining 7% to 8% of the phenotypic variation. *QHT.uia2-5D* was detected in three of six data sets (18-WW, 18-AB, and BLUP), explaining 7% to 8% of the phenotypic variation. *QHT.uia2-7D* was detected in four of six data sets (18-AB, 18-WW, 19-AB, and BLUP), explaining 13% to 30% of the phenotypic variation. Among these

QTL, *QHT.uia2-4A-2, QHT.uia2-5D,* and *QHT.uia2-7D* had a positive allelic effect that was attributed from the parent LCS Star while the positive alleles for *QHT.uia2-4A-1* and *QHT.uia2-5A* were from the parent UI Platinum. The peak marker of *QHT.uia2-7D* is very close to the flowering time gene *Ft-D1* based on IWGSC RefSeq v1.0 (Fig. 2.3).

QTL for fSNS

A total of five QTL were detected for fSNS on chromosomes 5D, 6A, 7B (2) and 7D (Table 2.4). *QfSNS.uia2-5D* was detected in five of eight data sets (17-AB, 18-AB, 18-SS, 19-SS, and BLUP), explained 9% to 18% of the phenotypic variation. *QfSNS.uia2-6A* was detected in all eight data sets (17-AB, 18-AB, 19-AB, 18-ASH, 18-WW, 18-SS, 19-SS, and BLUP), explained 8% to 20% of the phenotypic variation. *QfSNS.uia2-7B-1* was detected in five of eight data sets (18-WW, 18-AB, 19-AB, 18-SS, and BLUP), explained 8% to 24% of the phenotypic variation. *QfSNS.uia2-7B-2* was detected in four of eight data sets (18-WW, 18-ASH, 19-SS, and BLUP), explained 13% to 24% of the phenotypic variation. *QfSNS.uia2-7D* was detected in all eight data sets, explained 15% to 48% of the phenotypic variation. Except for *QfSNS.uia2-6A,* positive alleles of all other QTL were from LCS Star. The peak marker of *QfSNS.uia2-7D* is very close to the flowering time gene *Ft-D1* based on IWGSC RefSeq v1.0 (Fig. 2.3).

QTL for TKW

A total of three QTL were detected for TKW on chromosome 4A, 6A, and 7D (Table 2.4). *QTKW.uia2-4A* was detected in five of eight data sets (17-AB, 18-AB, 18-MSC, 18-SS, and BLUP), explaining 8% to 13% of the phenotypic variation. *QTKW.uia2-6A* was detected

in three of eight data sets (18-SS, 17-AB, and BLUP), explaining 11% to 14% of the phenotypic variation. *QTKW.uia2-7D* was detected in three of eight data sets (18-AB, 19-AB, and BLUP), explaining 17% to 27% of the phenotypic variation. The positive alleles for *QTKW.uia2-4A* was contributed by LCS Star, while the positive alleles for *QTKW.uia2-6A* and *QTKW.uia2-7D* were contributed by UI Platinum. The peak marker of *QTKW.uia2-7D* is very close to the flowering time gene *Ft-D1* based on IWGSC RefSeq v1.0 (Fig. 2.3).

QTL for PTN

A total of two QTL were detected for PTN on chromosome 4A and 6A (Table 2.4). *QPTN.uia2-4A* was detected in three of five data sets (18-AB, 18-ASH, and BLUP), explaining 7% to 9% of the phenotypic variation. *QPTN.uia2-6A* was detected in all the five data sets (18-AB, 18-ASH, 18-WWA, 19-AB, and BLUP), explaining 7% to 20% of the phenotypic variation. The QTL *QPTN.uia2-6A* was mapped in the flanking region of the *QTKW.uia2-6A*.

Fine mapping of the 7D

Using genotypic data from 90K SNP, five QTL *QfSNS.uia2-7D*, *QTKW.uia2-7D, QGY.uia2-7D, QHT.uia2-7D* and *QHD.uia2-7D* were mapped in the same region on chromosome 7DS close to flowering gene *Ft-D1*, in which seven SNPs span a total length of 24.09 cM. The QTL interval for GY, HD, HT, fSNS and TKW QTL was 24.09 cM, respectively; and the peak SNP marker for these traits was located at 68417416 Mbp (Fig. 2.3). To saturate this region, ten KASP markers were designed using exosome capture data, seven of them were mapped in this QTL region. The new linkage group has a total length of

103.46 cM and the QTL interval of all of the above traits became smaller (Fig. 2.3). For example, the QTL interval of *QGY.uia2-7D* was refined from 24.09 to 7.57 cM and the QTL interval of *QfSNS.uia2-7D* was refined from 24.09 to 20.93 cM. The peak marker of *QfSNS.uia2-7D* was changed from *IWB4045* to *KASP71343*. Overall, the KASP markers that were developed for this region on chromosome 7D decreased the QTL interval for all the traits. The physical location of *QTKW.uia2-7D* and *QfSNS.uia2-7D* is at 62215000-71343000 Mbp (Fig. 2.3), and the physical position of *QGY.uia2-7D* is at 66074000 – 71343000 Mbp. The *Ft-D1* gene might have effects on the three-yield component QTL since the physical positions of this gene is at 68415945-68414871 bp.

Trade-off effect of QTL clusters on three yield components

For QTL cluster on chromosome 4A, the high number allele of *QPTN.uia2-4A* were contributed by UIP, whereas the high number allele of *QTKW.uia2-4A* was contributed by LCS, leading to a trade-off effect between PTN and TKW for this QTL cluster. For QTL cluster on chromosome 6A, the high number allele of *QPTN.uia2-6A* were contributed by LCS, whereas the high number allele of *QfSNS.uia2-6A*, *QTKW.uia2-6A*, and *QHD.uia2-6A* were contributed by UIP, leading to a trade-off effect between PTN and fSNS, HD, as well as TKW for this QTL cluster. Similarly, for QTL cluster on chromosome 7D, the high number allele of *QfSNS.uia2-7D, QGY.uia2-7D, QHT.uia2-7D,* and *QHD.uia2-7D* were contributed by LCS, whereas the high number allele of *QTKW.uia2-7D* were contributed by UIP, leading to a trade-off effect between TKW and fSNS, GY, HD, as well as HT for this QTL cluster (Table 2.4).

Additive effect of different QTL on three yield components

The PTN QTL on chromosome 4A and 6A; the fSNS QTL on chromosome 5D, 6A, and 7D; and the TKW QTL on chromosome 4A, 6A, and 7D are additive towards increasing PTN, fSNS, and TKW (Fig. 2.4 and Table 2.5). The lines with all positive alleles showed 5.31 more PTN per 45-cm row compared to the lines that didn't contain these QTL (Fig. 2.4 and Table 2.5). The lines with positive alleles showed 2.15 more fSNS than those lines without these QTL (Fig. 2.4 and Table 2.5). The lines with all the QTL for TKW showed 4.92g more than those without these QTL (Fig. 2.4 and Table 2.5).

Validation of the QTL effects in the diverse spring wheat panel

The developed KASP markers with the highest LOD score on the chromosomes 6A and 7DS were successfully genotyped in the diverse spring wheat panel. The allelic analyses were conducted based on the BLUP data for each trait (Table 2.6). For the 7DS QTL cluster, allelic effects of *QHD.uia2-7D* and *QYLD.uia2-7D* were significant in this diverse panel with *p* values less than 0.001, while *QTKW.uia2-7D* and *QfSNS.uia2-7D* was not significant with *p* values at 0.91 and 0.73, respectively. For the 6A QTL cluster, the positive allele of *QPTN.uia2-6A* showed 13.21 more PTN than the negative allele, which was significant at $P < 0.001$ (Table 2.6), while *QfSNS.uia2-6A* show a 0.3 more fertile spikelet per spike compared to the negative allele with P value at 0.16.

Discussion

Phenotyping analysis

The present study used genome-wide linkage mapping to identify QTL associated with GY, HD, HT, fSNS, PTN, and TKW in a DH population derived from a cross between 'UI Platinum' and 'LCS Star'. The DH population used in this study was phenotyped for GY, HD, HT, fSNS, PTN, and TKW under irrigated, high-rainfall, and dryland conditions in two years. The traits HT, HD, fSNS, and TKW showed high heritability which is consistent with previous studies (Goel et al., 2018; Liu et al., 2018; Wang et al., 2018). On the other hand, PTN and GY had a moderate heritability but is also consistence with previous studies (Hu et al., 2017 and Wang et al., 2018). Grain yield had a moderate correlation with fSNS (r^2 at 0.37) which suggests that the increase in grain yield partially came from the fSNS. The PTN and TKW had a moderate negative correlation with fSNS (r^2 ranged from -0.22 to -0.38) which suggests that fSNS is negatively impacted by the PTN and TKW (Table 2.2). The heading date had a very high correlation with fSNS (0.74) (Table 2.2), which suggests that the heading date has a huge impact on the number of fertile spikelets a spike may contain.

QTL analysis

There have been many studies done on QTL mapping of major traits in wheat such as GY, HD, HT, fSNS, TKW, and PTN (Li et al., 2002; Kumar et al., 2007; Maccaferri et al., 2008; Wang et al., 2011; Gao et al., 2015; Sukumaran et al., 2015; Liu et al., 2018; Wang et al., 2018). In the present study, we detected a QTL for grain yield on chromosome 7D that explains 7% to 17% of the phenotypic variation (Fig. 2.2). This location on chromosome 7D for grain yield is in the same region as the flowering time gene *Ft-D1* (68417416 bp), as well

as HD, HT, fSNS, and TKW which could suggest that flowering time has an effect on the individual traits that impact grain yield (Table 2.4). Maccaferri et al., (2008) also reported a QTL on 7D for grain yield using RFLP and AFLP markers, but its position was different from ours, therefore, the *QGY.uia2-7D* is a novel QTL.

There were four QTL identified for the heading date trait on chromosomes 4B, 6A, 7B, and 7D (Table 2.4 and Fig. 2.2). *QHD.uia2-4B* was mapped on the long arm on chromosome 4B and can explain 13% to 25% of the phenotypic variation. *QHD.uia2-6A* was mapped on the long arm on chromosome 6A and explains 6% to 13% of the phenotypic variation and was mapped in the same region harboring QTL for PTN, TKW, and fSNS, suggesting that HD has some sort of interaction with individual yield components. *QHD.uia2-4B* and *QHD.uia2-6A* have not been previously reported which suggests these two QTL could be novel QTL. *QHD.uia2-7B* and *QHD.uia2-7D* were both mapped on the short arms of the chromosomes 7B and 7D, respectively, and explained 11% to 65% of the phenotypic variation. Both QTL have been previously reported by recent studies (Maccaferri et al., 2008 and Zhang et al., 2018). *QHD.uia2-7D* had the largest LOD and the largest allelic effect for HD (Table 2.4), making this QTL an important region to look at.

There were five QTL identified for height on chromosome 4A (2), 5A, 5D, and 7D (Table 2.4 and Fig. 2.2). *QHT.uia2-4A-1* spans both the short arm and long arm on chromosome 4A and explains 7% to 20% of the phenotypic variation. *QHT.uia2-4A-2* was mapped on the long arm of chromosome 4A and explains 6% to 24% of the phenotypic variation. *QHT.uia2-5A* was mapped on the long arm on chromosome 5A and explains 7% to 8% of the phenotypic variation. *QHT.uia2-5D* was mapped on the short arm on chromosome 5D and explains 7% to 8% of the phenotypic variation. Lastly, *QHT.uia2-7D* was mapped on

the short arm on chromosome 7D and explains 13% to 30% of the phenotypic variation (Table 2.4) and this QTL had the largest LOD. This location is the same region that harbors QTL for GY, HD, fSNS, and TKW, which is close to the flowering time gene *Ft-D1*. Out of the five QTL detected for HT, three have not been previously reported (*QHT.uia2-4A-1, QHT.uia2-4A-2,* and *QHT.uia2*-7D) which suggests these are novel QTL.

There were five QTL for fSNS identified on chromosomes 5D, 6A, 7B (2), and 7D, explaining 8% to 48% of the phenotypic variation (Table 2.4 and Fig. 2.2). *QfSNS.uia2-5D* was mapped on the long arm of chromosome 5D while Li et al., (2007) also reported a QTL for fSNS on the long arm of chromosome 5D. *QfSNS.uia2-6A* was located in a very large region on the short arm as well as the long arm of chromosome 6A (Fig. 2.2) and Kumar et al., (2007) and Wang et al., (2011) also detected a QTL associated with fSNS on the short arm of chromosome 6A. *QfSNS.uia2-7B* and *QfSNS.uia2-7D* were mapped on the short arm of the chromosomes 7B and 7D, respectively, close to the region of the *Ft-B1* and *Ft-D1* genes (Bonnin et al., 2008) (Fig. 2.3). Wang et al., (2011) reported QTL associated with fSNS on the long arms of both chromosomes 7B and 7D which suggests that the two QTL identified in this study could be novel QTL. The QTL for chromosome 7D had the highest phenotypic variation with a range of 15% to 44% and was directly associated with the *Ft-D1* gene (Yan et al., 2006; Bonnin et al., 2008) (Fig. 2.3), which suggest, that the flowering time has a direct impact on the number of florets that will form.

The QTL for TKW were detected on chromosomes 4A, 6A, and 7D, explaining 8% to 27% of the phenotypic variation (Table 2.4 and Fig. 2.2). *QTKW.uia2-4A* was mapped in a large region of the short arm and part of the long arm (16.04-43.34 cM) of chromosome 4A (Fig. 2.2), whereas Gao et al., (2015) detected a QTL for TKW on the long arm of

chromosome 4A. *QTKW.uia2-6A* was mapped on the short arm and long arm (17.13-27.42 cM) of chromosome 6A (Fig. 2.2). Li et al., (2007) and Gao et al., (2015) also detected a QTL on chromosome 6A in the same region. *QTKW.uia2-7D* was mapped on the short arm (6.66 cM) of chromosome 7D and Li et al., (2007 and Lopes et al., (2013) also mapped a QTL for TKW on chromosome 7D. The QTL on chromosome 7D had the highest phenotypic variation with a range of 17% to 27% and was associated with the *Ft-D1* gene polymorphism (Fig. 2.3). Bonnin et al., (2008) suggests that the time the plant flowers determines the overall size of the kernel.

The QTL that was detected for PTN were found on chromosomes 4A and 6A, explaining 7% to 20% of the phenotypic variation. *QPTN.uia2-4A* was mapped in a very large region on the short arm and long arm of chromosome 4A (Fig. 2.2) and Wang et al. (2018) also reported QTL associated with PTN on chromosome 4A. *QPTN.uia2-6A* was mapped in a large region of the short and long arm of chromosome 6A (Fig. 2.2). Sukumaran et al. (2015) reported a linkage block in this region between 77–81 cM, which encompasses 63% of the entire 6A chromosome (100-500 Mbp). Our results based on the DH population showed the 6A QTL located at 90-530 Mbp and there are no obvious peaks. Wang et al., (2018) used a DH population with a common parent (UI Platinum) and found a QTL for PTN in the same region 90-530 Mbp and was contributed by the SY Capstone parent which suggest that LCS star and SY Capstone could contain the same allele for PTN on chromosome 6A.

In summary, a total of 19 QTL were detected for two agronomic traits and four yield-related traits on 48 linkage groups that represented all 21 wheat chromosomes. Four major QTL (*QTKW.uia2-6A, QfSNS.uia2-6A, QfSNS.uia2-7B, and QPTN.uia2-6A*) may be common with the previously published (Li et al., 2007; Wang et al., 2011; Lopes et al., 2013;

and Gao et al., 2015; Wang et al., 2018), while other four QTL (*QGY.uia2-7D, QHT.uia2-7D, QfSNS.uia2-7D, QTKW.uia2-7D*) are novel ones identified in the present study. Pyramiding the known and novel QTL may be an important approach to increase wheat grain yield in cultivar improvement using molecular marker assisted selection.

QTL clusters and challenges for marker-assisted selection

The present study used a DH population derived from two adapted high-yielding hard white spring wheat cultivars that have three complementary yield component traits, fSNS, PTN, and TKW. This population allowed us to detect QTL for the three traits simultaneously and to explore their relationship in response to grain yield. Out of the 19 QTL identified, positive alleles of the eleven QTL were from LCS Star and eight were from UI Platinum. Additive effects of these QTL suggest that pyramiding of positive alleles from the both parents may increase the values of each yield components and grain yield. However, the genetic architecture and regulating network for spike-related traits is very complicated. In the present study, we found a trade-off relationship between fSNS, TKW, and PTN based on the phenotypic correlation and QTL identification. Three QTL clusters were identified on chromosomes 4A, 6A and 7D, and each contains negatively correlated traits. This observation explains the complexity of selection for yield and yield components that breeders have encountered for a long time. This may be a challenge for a wheat breeder to use molecular markers in yield improvement. Based on the results derived from the present study, we propose the following selection strategies to manipulate and improve yield components in response to increasing yield.

QTL cluster on chromosome 7D

The QTL for yield, two yield components (TKW and fSNS), and two agronomic traits (HD and HT) were all detected on the chromosome 7D in the flanking region of *Ft-D1*. Based on the positions of these QTL and the correlations among GY, fSNS, HD, and TKW, the effect of these QTL may be the results from different closely linked genes or from the pleiotropic effects of *Ft-D1,* later heading and flowering favor more fSNS development towards increasing GY, as reported in Finnegan et al., (2018) for *Ft-D1* gene. Dissecting of the 7DS QTL cluster will help us to understand whether *Ft-D1* has pleiotropic effects on fSNS and TKW. Allelic analysis indicated that the LCS Star allele of this QTL cluster increased HD, fSNS, and GY, but decreased TKW (Table 2.4). Furthermore, *QfSNS.uia2-7D* was detected in all environment (Table 2.4), with the average additive effect of 1.16 spikelets more than *QfSNS.uia2-7B, QfSNS.uia2-6A,* and *QfSNS.uia2-5D***.** The correlation value between GY and fSNS was 0.37, which was highest comparing to the values with other traits (Table 2.2). Correlation value between TKW and GY was not significant, but with fSNS was negatively correlated. These results suggest that selecting LCS allele at *QfSNS.uia2-7D* locus may increase GY.

QTL cluster on chromosomes 4A and 6A

Two QTL for TKW and PTN (*QTKW.uia2-4A* and *QPTN.uia2-4A*) were identified in the same QTL region on chromosome 4A, which suggests that this region include more than one tightly linked genes, or a single gene with pleotropic effects (Fig. 2.2). Further allelic analysis indicated that the LCS Star allele of this QTL cluster decreased PTN but increased TKW by 1.07 g (Table 2.4). Three QTL for the three yield components (*QfSNS.uia2-6A,*

QPTN.uia2-6A, and *QTKW.uia2-6A*) were mapped on chromosome in the same flanking region that spans a very large region over the short and long arms (Fig. 2.2). Further allelic analysis indicated that the LCS Star allele of this QTL cluster increased PTN but decreased TKW and fSNS (Table 2.4). These results are consistent with the negative correlations between PTN and TKW as well as fSNS, while UIP allele increased TKW and fSNS. Because PTN has much smaller heritability than TKW and fSNS, selecting UIP allele at this QTL may be a good approach towards increasing grain yield..

Precision mapping

For a long time, the low coverage and low density of the molecular markers in wheat D genome prevent the effective QTL identification in this genome (Chen et al., 2016; Wang et al., 2017; Liu et al. 2018). Recently, the utilization of capture technology on wheat regulatory and exosome sequences (Gardiner et al. 2019), combined with KASP technology, provides a possibility to develop more markers on D genome to be used in the whole mapping population. In this study, by developing more KASP markers converted from capture SNPs in the QTL-7D region, the candidate region for the QTL were refined to less than 10 Mbp, compared with the original 60 Mbp using 90K SNP only. Moreover, the peak marker for *QfSNS.uia2-7D* was away from *Ft-D1* gene after more KASP markers added to the QTL region. This demonstrated the advantages of using the released wheat reference sequence and the new developed wheat genotyping methods, such as exosome capture and KASP. As far as the authors know, this study is the first one to use the combination of capture and KASP platforms in a QTL mapping analysis.

QTL effects in the diverse spring wheat panel

Three QTL (*QPTN.uia2-6A*, *QHD.uia2-7D* and *QYLD.uia2-7D)* identified in the bi-parental population were validated, while *QTKW.uia2-7D* and *QfSNS.uia2-7D* were not validated in the diverse panel. This result suggests that the LCS alleles at loci of *QTKW.uia2-7D* and *QfSNS.uia2-7D* are not related to the LCS allele at locus of *QYLD.uia2-7D*. To confirm this, it is necessary to conduct additional validation studies or increase size of the validation panel or evaluate this panel in more diverse environments in the future. This result also suggests that it is possible to dissect those QTL that were clustered on the 7DS in the present study. Although *QfSNS.uia2-6A* was not significant, p value was 0.16, the position and higher value allele of this QTL was different from *QPTN.uia2-6A*. It is possible that we can pyramid UIP allele of fSNS and LCS allele of PTN on 6A.

Conclusion

The present study used a unique DH population that was derived from two high yielding spring wheat cultivars and advanced genotyping platform in QTL analysis for three major yield components in relation to grain yield in the background of segregation of *Ft-D1*gene. Three QTL clusters, controlling major QTL effects, associated SNP-derived KASP markers, and DHLs have a great potential to be used in yield improvement and cultivar development. Our results suggest that selecting of yield component architecture may achieve the best pyramiding effect towards increasing grain yield per se.

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Table 2.1 Phenotypic summary of DH lines and the two parents and the broad-sense heritability (H^2) estimated in eight trials

Traits	Parents[a]			DHLs[b]				H^2
	UIP	LCS	Diff	Mean	Std. Dev.	Min.	Max.	
GY	83.03	89.01	-5.98	85.52	2.31	78.01	91.28	0.38
HD	171.2	173.54	-2.34	173.92	1.12	171.28	176.45	0.79
HT	27.41	27.63	NS	27.59	1.15	24.98	30.59	0.82
fSNS	16.74	18.18	-1.44	17.57	0.92	14.5	20.19	0.84
PTN	47.7	57.7	-10	55.86	3.49	47.8	65.8	0.44
TKW	38.12	30.86	7.26	33.6	2.39	29	39.57	0.68

[a] UIP: UI Platinum; LCS: LCS Star; Diff: difference between UIP and LCS (UIP–LCS): NS means not significant at $\alpha = 0.05$, numbers mean significant at $\alpha = 0.05$; [b] Std. Dev.: standard deviation; Min.: minimum value in the population; Max.: maximum value in the population.

Table 2.2 Correlations among traits of interest among tested environments

Grain Yield

	18-AB	18-ASH	18-SS	18-WW	17-AB	19-SS
18-ASH	0.17*					
18-SS	0.06	0.12				
18-WW	0.34***	0.47***	0.38***			
17-AB	0.30***	0.37***	0.26**	0.49***		
19-SS	0.19*	0.16*	0.24**	0.39***	0.27***	
BLUP	0.43***	0.57***	0.26**	0.54***	0.74***	0.37***

Heading Date

	18-AB	18-WW	19-AB	19-SS
18-WW	0.83***			
19-AB	0.87***	0.82***		
19-SS	0.85***	0.83***	0.93***	
BLUP	0.85***	0.83***	0.86***	0.85***

Height

	18-AB	18-SS	18-WW	19-AB	19-SS
18-SS	0.60***				
18-WW	0.81***	0.63***			
19-AB	0.82***	0.59***	0.77***		
19-SS	0.48***	0.66***	0.74***	0.68***	
BLUP	0.88***	0.75***	0.85***	0.82***	0.80***

fSNS

	17-AB	18-AB	18-SS	18-WW	18-ASH	19-AB	19-SS
18-AB	0.59***						
18-SS	0.49***	0.69***					
18-WW	0.48***	0.69***	0.53***				
18-ASH	0.53***	0.69***	0.60***	0.59***			
19-AB	0.57***	0.83***	0.66***	0.63***	0.66***		
19-SS	0.55***	0.81***	0.69***	0.63***	0.68***	0.77***	
BLUP	0.59***	0.89***	0.85***	0.79***	0.86***	0.81***	0.89***

TKW							
	18-ASH	18-AB	18-SS	18-MSC	17-AB	19-AB	19-SS
18-AB	0.33***						
18-SS	0.28**	0.31***					
18-MSC	0.37***	0.45***	0.24**				
17-AB	0.41***	0.43***	0.34***	0.29**			
19-AB	0.33***	0.54***	0.19**	0.44***	0.40***		
19-SS	0.45***	0.34***	0.53***	0.30***	0.45***	0.21**	
BLUP	0.68***	0.75***	0.60***	0.65***	0.70***	0.72***	0.63***

PTN				
	18-AB	18-ASH	18-WA	19-AB
18-ASH	0.39***			
18-WW	0.40***	0.41***		
19-AB	0.43***	0.25**	0.34***	
BLUP	0.70***	0.64***	0.60***	0.50***

Trait BLUPs					
	TKW	PTN	fSNS	GY	HD
PTN	-0.37***				
fSNS	-0.33***	-0.22**			
GY	-0.04	0.002	0.37***		
HD	-0.38***	-0.15*	0.74***	0.30***	
HT	0.21**	-0.21**	0.20**	0.26***	0.25***

Significance level: ***, **, and * indicate $P < 0.0001$, 0.01, and 0.05, respectively.

Table 2.3 Genetic linkage groups of the DH population from UI Platinum and LCS Star

Chromosome	No of groups	No. of SNP	Length (cM)	Density (marker/cM)
1A	2	55	159.9	0.34
2A	2	105	283.51	0.37
3A	3	66	111.68	0.59
4A	2	50	107.02	0.47
5A	3	69	162.68	0.42
6A	1	58	101.57	0.57
7A	4	86	396.1	0.22
1B	3	61	118.23	0.52
2B	1	113	229.24	0.49
3B	2	81	220.88	0.37
4B	2	62	179.99	0.34
5B	2	111	248.3	0.45
6B	2	62	289.14	0.21
7B	3	93	260.23	0.36
1D	1	35	111.36	0.31
2D	2	45	260.29	0.17
3D	3	32	155.55	0.21
4D	2	14	94.24	0.15
5D	3	31	213.37	0.15
6D	3	22	48.47	0.45
7D	2	25	141.06	0.18
A genome	17	489	1322.46	0.37
B genome	15	583	1546.01	0.38
D genome	16	204	1024.34	0.20
Total	48	1276	3892.81	0.33

Table 2.4 QTL detected for grain yield (GY), heading date (HD), height (HT), fertile spikelet number per spike (fSNS), thousand kernel weight (TKW), and productive tiller number (PTN) in the DH population.

Trait	QTL	Env.	Interval	Positions	Peak marker	Peak position (cM)	Physical position (bp)	LOD	Effect[a]	R^2 (%)
GY	***QGY.uia2-7D***	18-ASH	*IWB2380-IWB40232*	2.55-25.59	*IWB4045*	6.66	68417416	4.52	7.82	11
		18-WW	*IWB2380-IWB40120*	2.55-24.09	*IWB4045*	6.66	68417416	7.22	6.33	17
		17-AB	*IWB18914-IWB42766*	2.55-24.09	*IWB4045*	6.66	68417416	6.15	18.08	14
		BLUP	*IWB2380-IWB40120*	0.55-24.09	*IWB4045*	8.66	68417416	7.13	1.89	17
	KASP Markers: QGY.uia2-7D	19-SS	*Kasp66074-Kasp66074*	48.2-48.2	*Kasp66074*	48.2	66074000	4.61	9.28	11
		18-ASH	*Kasp62215-Kasp71343*	42.52-53.88	*Kasp71343*	49.88	71343000	6.31	9.8	15
		18-WW	*Kasp66074-IWB4045*	48.2-55.45	*IWB4045*	57.45	68417416	5.29	9.75	13
		17-AB	*IWB4045-IWB4045*	55.45-55.45	*IWB4045*	57.45	68417416	6.88	19.59	16
		BLUP	*Kasp71343-IWB4045*	49.88-55.45	*IWB4045*	57.45	68417416	2.74	2.24	7
HD	***QHD.uia2-4B***	18-AB	*IWB73001-IWB13349*	45.7-69.19	*IWB10847*	68.63	609515871	7.1	-1.66	17
		18-WW	*IWB73001-IWB23337*	45.7-74.29	*IWB23968*	64.21	518682966	6.92	-1.4	16
		19-SS	*IWB73001-IWB23337*	45.7-69.19	*IWB10847*	68.63	609515871	6.63	-1.39	16
		19-AB	*IWB73001-IWB23337*	45.7-74.29	*IWB11884*	65.32	548120919	11.24	-1.93	25
		BLUP	*IWB73001-IWB23337*	45.7-69.19	*IWB23968*	64.21	518682966	5.67	-0.54	13
	QHD.uia2-6A	18-AB	*IWB39323-IWB10738*	8.63-36.83	*IWB10644*	12.16	57728545	3.82	-1.12	9
		18-WW	*IWB3945-IWB34744*	6.08-71.35	*IWB11102*	14.92	61024039	4.89	-1.18	12
		19-SS	*IWB39323-IWB34744*	6.63-69.35	*IWB11102*	14.37	61024039	5.24	-1.16	13
		19-AB	*IWB11102-IWB10738*	14.37-34.83	*IWB76736*	23.76	NA	2.68	-0.9	6
		BLUP	*IWB3945-IWB2006*	6.08-49.8	*IWB11102*	34.83	61024039	2.5	-0.35	6

	QHD.uia2-7B	18-AB	*IWB76332-IWB76332*	18.06-22.06	*IWB76332*	22.06	5922944	4.61	1.29	11
		18-WW	*IWB10879-IWB76332*	11.08-22.06	*IWB76332*	22.06	5922944	8.13	1.55	19
		19-AB	*IWB53325-IWB76332*	13.84-22.06	*IWB76332*	22.06	5922944	7.49	1.56	17
		19-SS	*IWB76332-IWB76332*	18.06-22.06	*IWB76332*	22.06	5922944	5.19	1.25	12
		BLUP	*IWB10879-IWB76084*	11.08-22.06	*IWB76332*	22.06	5922944	10.16	0.76	23
	QHD.uia2-7D	18-AB	*IWB18914-IWB40120*	0-24.09	*IWB4045*	6.66	68417416	27.19	3.71	50
		18-WW	*IWB18914-IWB40120*	0-24.09	*IWB4045*	6.66	68417416	16.12	2.33	34
		19-AB	*IWB18914-IWB40120*	0-24.09	*IWB4045*	6.66	68417416	41.44	4.8	65
		19-SS	*IWB18914-IWB40120*	0-24.09	*IWB4045*	6.66	68417416	34.87	4.04	59
		BLUP	*IWB18914-IWB40120*	0-24.09	*IWB4045*	6.66	68417416	27.81	1.45	50
	KASP Markers: QHD.uia2-7D	18-AB	*Kasp62215-IWB4045*	42.52-63.45	*Kasp71343*	51.88	71343000	19.74	4.61	39
		18-WW	*Kasp62215-IWB4045*	42.52-63.45	*Kasp71343*	53.88	71343000	7.06	2.65	16
		19-AB	*Kasp62215-IWB4045*	42.52-63.45	*Kasp71343*	51.88	71343000	14.47	2.54	31
		19-SS	*Kasp62215-IWB4045*	42.52-63.45	*Kasp66074*	48.2	66074000	12.71	2.69	28
		BLUP	*Kasp62215-IWB4045*	42.52-63.45	*Kasp66074*	48.2	66074000	21.4	1.69	42
HT	***QHT.uia2-4A-1***	18-AB	*IWB1375-IWB56811*	30.63-50.68	*IWB12737*	37.86	629475880	8.63	-1.61	20
		18-WW	*IWB37469-IWB1375*	30.08-36.63	*IWB1375*	34.63	689853869	2.74	-1.04	7
		19-AB	*IWB47072-IWB10595*	19.52-40.07	*IWB37469*	30.08	622236655	8.49	-1.77	19
		18-SS	*IWB20951-IWB1375*	24.28-36.63	*IWB20951*	26.28	618040255	3.33	-0.92	8
		19-SS	*IWB20951-IWB19112*	26.28-47.09	*IWB37106*	38.41	629476117	5.23	-1.31	12
		BLUP	*IWB5019-IWB12351*	49.01-60.09	*IWB11801*	59.1	713120773	6.78	-0.75	16

QHT.uia2.4A-2	18-AB	*IWB11778-IWB18325*	5.53-32.33	*IWB33052*	20.46	102614749	9.92	1.76	22
	18-WW	*IWB-11778-IWB18325*	5.53-34.33	*IWB20649*	22.67	102614749	6.76	1.75	16
	19-AB	*IWB11778-IWB18325*	5.53-34.33	*IWB33052*	20.46	102614749	10.96	2.04	24
	19-SS	*IWB18250-IWB18250*	14.17-14.17	*IWB18250*	14.17	11917064	2.55	0.94	6
	18-SS	*IWB11778-IWB18325*	5.53-28.33	*IWB31143*	16.04	38447212	5.47	1.18	13
	BLUP	*IWB19937-IWB21625*	2.21-22.12	*IWB31143*	16.04	38447212	5.71	0.68	14
QHT.uia2.5A	19-AB	*IWB40074-IWB3232*	37.78-40.11	*IWB10909*	38.45	664273116	3.32	-1.01	8
	18-SS	*IWB64718-IWB11245*	29.57-41.76	*IWB3232*	40.11	665471359	2.69	-1.08	7
	18-WW	*IWB70049-IWB70049*	19.26-19.26	*IWB70049*	19.26	626607513	2.56	-1.08	6
	BLUP	*IWB15328-IW40074*	14.75-31.38	*IWB35391*	25.47	645412111	2.86	-0.47	7
QHT.uia2-5D	18-AB	*IWB6557-IWB6557*	82.37-106.37	*IWB6557*	84.37	107583992	3.37	2.31	8
	18-WW	*IWB6557-IWB6557*	82.37-106.37	*IWB6557*	84.37	107583992	2.64	6.9	7
	BLUP	*IWB6557-IWB6557*	82.37-100.37	*IWB6557*	82.37	107583992	2.6	1.19	7
QHT.uia2-7D	18-AB	*IWB23802-IWB40120*	0.55-22.09	*IWB4045*	6.66	68417416	14.23	2.28	30
	18-WW	*IWB23802-IWB40120*	0.55-22.09	*IWB4045*	8.66	68417416	5.4	1.57	13
	19-AB	*IWB23802-IWB40120*	0.55-22.09	*IWB4045*	8.66	68417416	13.43	2.39	28
	BLUP	*IWB23802-IWB40120*	0.55-24.09	*IWB4045*	10.66	68417416	8.8	0.89	20
KASP Markers: QHT.uia2-7D	18-AB	*Kasp51953-IWB4045*	36.9-63.45	*Kasp66074*	48.2	66074000	4.99	1.95	12
	18-WW	*Kasp71343-IWB4045*	49.88-63.45	*IWB4045*	57.45	68417416	5.56	1.81	13
	19-AB	*Kasp62215-IWB4045*	42.52-63.45	*IWB4045*	57.45	68417416	8.14	2.43	19
	BLUP	*Kasp62215-Kasp62215*	45.52-46.52	*Kasp62215*	46.52	62215000	4.09	0.94	10

fSNS		17-AB	*IWB7620-IWB3446*	3.88-24.01	*IWB17912*	18.43	464633623	3.51	0.8	9
		18-AB	*IWB7620-IWB49479*	3.88-38.12	*IWB17912*	22.43	464633623	4.81	0.63	12
	QfSNS.uia2-5D	18-SS	*IWB7620-IWB34466*	3.88-28.01	*IWB17912*	20.43	464633623	4.65	0.91	11
		19-SS	*IWB7620-IWB49479*	13.88-38.12	*IWB34466*	24.01	586352242	5.56	0.68	13
		BLUP	*IWB7620-IWB34466*	7.88-28.01	*IWB17912*	22.43	464633623	7.56	0.54	18
		17-AB	*IWB10644-IWB45465*	13.82-44.81	*IWB10738*	34.83	535894664	3.68	-0.855	9
		18-AB	*IWB39323-IWB2006*	6.63-49.8	*IWB63176*	16.92	63562964	4.89	-0.66	12
		18-WW	*IWB-63176-IWB10738*	16.92-36.83	*IWB10321*	32.62	288411565	3.17	-0.5	8
	QfSNS.uia2-6A	18-ASH	*IWB10738-IWB45465*	34.83-46.81	*IWB45465*[b]	44.81	646630596	3.97	-0.97	10
		19-AB	*IWB10644-IWB34744*	13.82-55.35	*IWB76733*	33.17	454649338	5.13	-0.75	12
		18-SS	*IWB35333-IWB2006*	33.17-49.8	*IWB45465*[b]	44.81	646630596	4.21	-0.85	10
		19-SS	*IWB35333-IWB45465*	33.17-46.81	*IWB35333*	23.76	NA	4.75	-0.66	11
		BLUP	*IWB35333-IWB2006*	33.17-53.8	*IWB45465*[b]	44.81	646630596	8.55	-0.56	20
		18-AB	*IWB26212-IWB35038*	193.17-204.78	*IWB35038*	204.78	15304629	10.7	1.01	24
		18-WW	*IWB51594-IWB20673*	169.37-184.31	*IWB32502*	174.9	104301334	4.06	0.59	10
	QfSNS.uia2-7B	19-AB	*IWB26212-IWB35038*	193.17-204.78	*IWB35038*	204.78	15304629	11.22	0.93	25
		18-SS	*IWB51594-IWB35038*	168.81-204.78	*IWB32502*	176.9	104301334	3.44	0.78	8
		BLUP	*IWB1480-IWB25504*	172.69-205.89	*IWB35038*	204.78	15304629	4.26	0.52	10
		18-WW	*IWB10879-IWB76332*	11.08-22.06	*IWB76332*	16.06	5922944	9.33	0.94	21
		18-ASH	*IWB10879-IWB76332*	11.08-22.06	*IWB76332*	16.06	5922944	5.81	1.2	14
	QfSNS.uia2-7B-2	19-SS	*IWB10879-IWB76332*	11.08-22.06	*IWB76332*	16.06	5922944	5.27	0.69	13
		BLUP	*IWB10879-IWB76332*	11.08-22.06	*IWB76332*	16.06	5922944	10.72	0.66	24

	QfSNS.uia2-7D	17-AB	*IWB18914-IWB40120*	0.55-24.09	*IWB4045*	6.66	68417514	6.58	1.17	15
		18-AB	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	21.74	1.59	42
		18-WW	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	7.19	0.83	17
		18-ASH	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	7.66	1.31	17
		19-AB	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	25.78	1.57	48
		18-SS	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	12.55	1.59	27
		19-SS	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	14.5	1.25	31
		BLUP	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	22.3	1.04	43
	KASP Markers: QfSNS.uia2-7D	17-AB	*Kasp62215-Kasp71343*	42.52-53.88	*Kasp62115*	46.52	62115000	6.03	1.5	15
		18-AB	*Kasp62215-IWB4045*	42.52-59.45	*Kasp71343*	53.88	71343000	9.63	1.72	22
		18-ASH	*Kasp62215-Kasp66074*	42.52-48.2	*Kasp66074*	48.2	66074000	5.01	1.47	12
		19-AB	*Kasp51953-IWB40120*	36.9-63.45	*Kasp66074*	48.2	66074000	19.4	2.05	39
		18-SS	*Kasp62215-Kasp66074*	42.52-48.2	*Kasp62115*	46.52	66074000	8	1.51	18
		19-SS	*Kasp62215-IWB4045*	42.52-63.45	*Kasp71343*	51.88	71343000	10.91	1.37	24
		BLUP	*Kasp62215-IWB4045*	42.52-63.45	*Kasp71343*	53.88	71343000	15.81	1.16	33
TKW	***QTKW.uia2-4A***	17-AB	*IWB23168-IWB18325*	18.8-28.33	*IWB18325*[c]	24.33	394968199	3.12	1.72	8
		18-AB	*IWB31143-IWB18325*	16.04-34.33	*IWB18325*[c]	24.33	394968199	5.3	2.52	13
		18-MSC	*IWB18250-IWB20649*	14.17-22.67	*IWB31143*	16.04	38447212	3.36	1.75	8
		18-SS	*IWB18325-IWB35445*	24.33-59.34	*IWB35445*	43.34	21063715	4.02	1.77	10
		BLUP	*IWB65970-IWB18325*	11.19-32.33	*IWB46934*	19.91	488252088	5.31	1.69	13
	QTKW.uia2-6A	18-SS	*IWB12868-IWB1754*	17.13-39.6	*IWB38557*	27.42	201348573	6.14	-2.12	14
		17-AB	*IWB10644-IWB38557*	13.82-27.42	*IWB12868*	17.13	73723540	4.61	-2.2	11
		BLUP	*IWB34488-IWB1754*	19.9-39.6	*IWB38557*	25.42	201348573	4.49	-1.50	11

	QTKW.uia2-7D	18-AB	*IWB18914-IWB40120*	0.00-24.09	*IWB4045*	6.66	68417514	12.12	-4.28	27
		19-AB	*IWB18914-IWB40120*	0.00-22.09	*IWB4045*	6.66	68417514	8.09	-3.32	19
		BLUP	*IWB18914-IWB40120*	0.00-22.09	*IWB4045*	6.66	68417514	7.3	-1.8	17
	KASP Markers: QTKW.uia2-7D	18-AB	*Kasp51953-IWB4045*	36.9-63.45	*Kasp62215*	46.52	62215000	12.45	-5.33	27
		18-SS	*IWB40120-IWB40232*	68.96-74.46	*IWB40120*	72.96	92570641	2.68	-2.18	7
		19-AB	*Kasp62215-IWB4045*	42.52-59.45	*Kasp62215*	46.52	62215000	5.14	-4.11	12
		BLUP	*Kasp62215-IWB40120*	42.52-72.96	*IWB4045*	55.45	68417514	5.49	-2.12	13
PTN	***QPTN.uia2-4A***	18-AB	*IWB11778-IWB-18325*	5.53-24.33	*IWB31143*	16.04	38447212	3.67	-4.42	9
		18-ASH	*IWB54290-IWB35445*	40.99-47.34	*IWB77282*	42.78	24755594	2.65	-2.99	7
		BLUP	*IWB23168-IWB18325*	18.8-26.33	*IWB20649*	22.67	56501948	3.5	-1.58	9
	QPTN.uia2-6A	18-AB	*IWB34957-IWB10321*	10.5-32.62	*IWB38557*	25.42	201348573	8.96	6.93	20
		18-ASH	*IWB9036-IWB10321*	12.16-32.62	*IWB68246*	22.11	410915261	4.43	3.91	11
		18-WWA	*IWB10321-IWB-11269*	32.62-41.47	*IWB35333*	33.17	454649338	3.02	3.57	7
		19-AB	*IWB34957-IWB10321*	10.5-29.30	*IWB68246*	22.11	410915261	7.6	4.81	18
		BLUP	*IWB34957-IWB10321*	10.5-32.62	*IWB38557*	25.42	201348573	8.7	3.04	20

Env.: environment; LOD: logarithm of the odds ratio; effect: additive effect; R2: the phenotypic variation explained by a QTL; [a] The effect contribution from LCS or UIP was indicated by positive or negative number, respectively; * Doesn't meet the requirements set for being a major QTL (LOD > 2.5); [b] Peak marker was referenced to the 6B chromosome based on the Chinese Spring sequence (Reference Sequence v1.0, the International Wheat Genome Consortium (IWGSC) [c] Peak marker was referenced to the 4D chromosome based on the Chinese Spring sequence (Reference Sequence v1.0)

Table 2.5 Additive QTL effects for fSNS, TKW, and PTN

Trait	QTL[a]	BLUP	No. of lines
fSNS	7DS+6A+5D	18.46A	35
	7DS+6A	18.06AB	20
	7DS+5D	17.89B	31
	6A+5D	17.28C	29
	7DS	17.33C	29
	6A	16.97CD	9
	5D	16.60D	13
	None	16.31D	15
TKW	7DS+6A+4A	36.40A	12
	7DS+6A	34.75AB	27
	7DS+4A	35.39AB	10
	6A+4A	34.38ABC	23
	7DS	33.29BCD	17
	6A	33.89CD	32
	4A	33.20BC	31
	None	31.48D	29
PTN	6A+4A	58.74A	42
	6A	56.34B	45
	4A	54.87BC	59
	None	53.43C	35

[a] Peak marker with additive effects for different QTL associated with fSNS, TKW, and PTN.

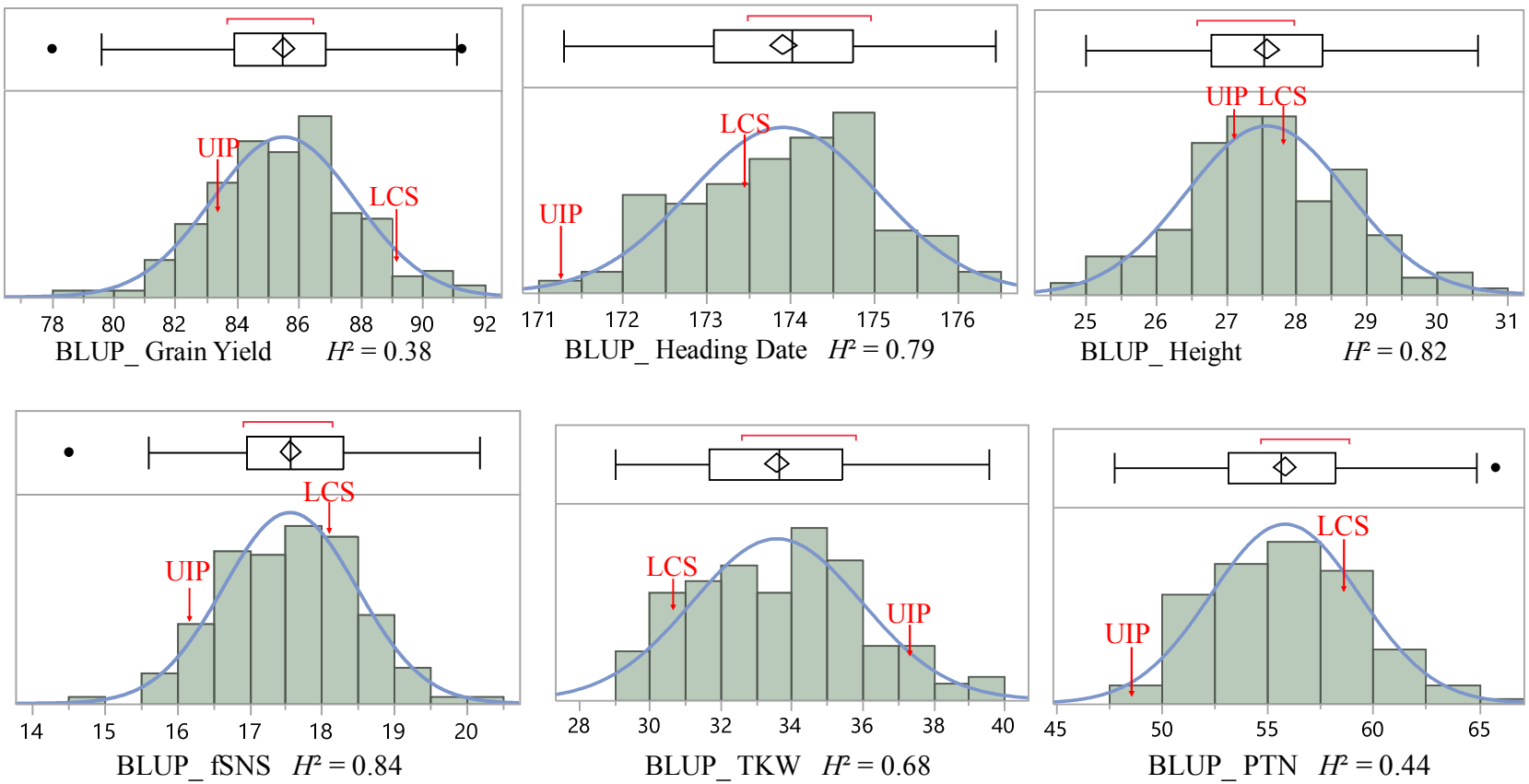
Table 2.6 Effects of *QPTN.uia2-6A*, *QfSNS.uia2-6A* and *Q.uia2-7D* cluster in a diverse spring wheat panel using KASP markers.

QTL	Trait	Mean	*P* value	Sample size
QPTN.uia2-6A				
UIP allele[a]	PTN	445.4	< 0.001[b]	86
LCS allele		458.6		83
QfSNS.uia2-6A				
UIP allele[a]	fSNS	17.2	0.16	106
LCS allele		16.9		62
Q.uia2-7D cluster				
UIP allele	fSNS	17	0.73	60
LCS allele		17.12		108
UIP allele	HD	133.8	< 0.001	60
LCS allele		135		108
UIP allele	YLD	64.86	< 0.001	60
LCS allele		69.49		108
UIP allele	TKW	36.09	0.91	60
LCS allele		36.14		108

[a] UIP or LCS allele group stands for the lines with the allele of designed KASP markers for different QTL come from UIP or SYC.

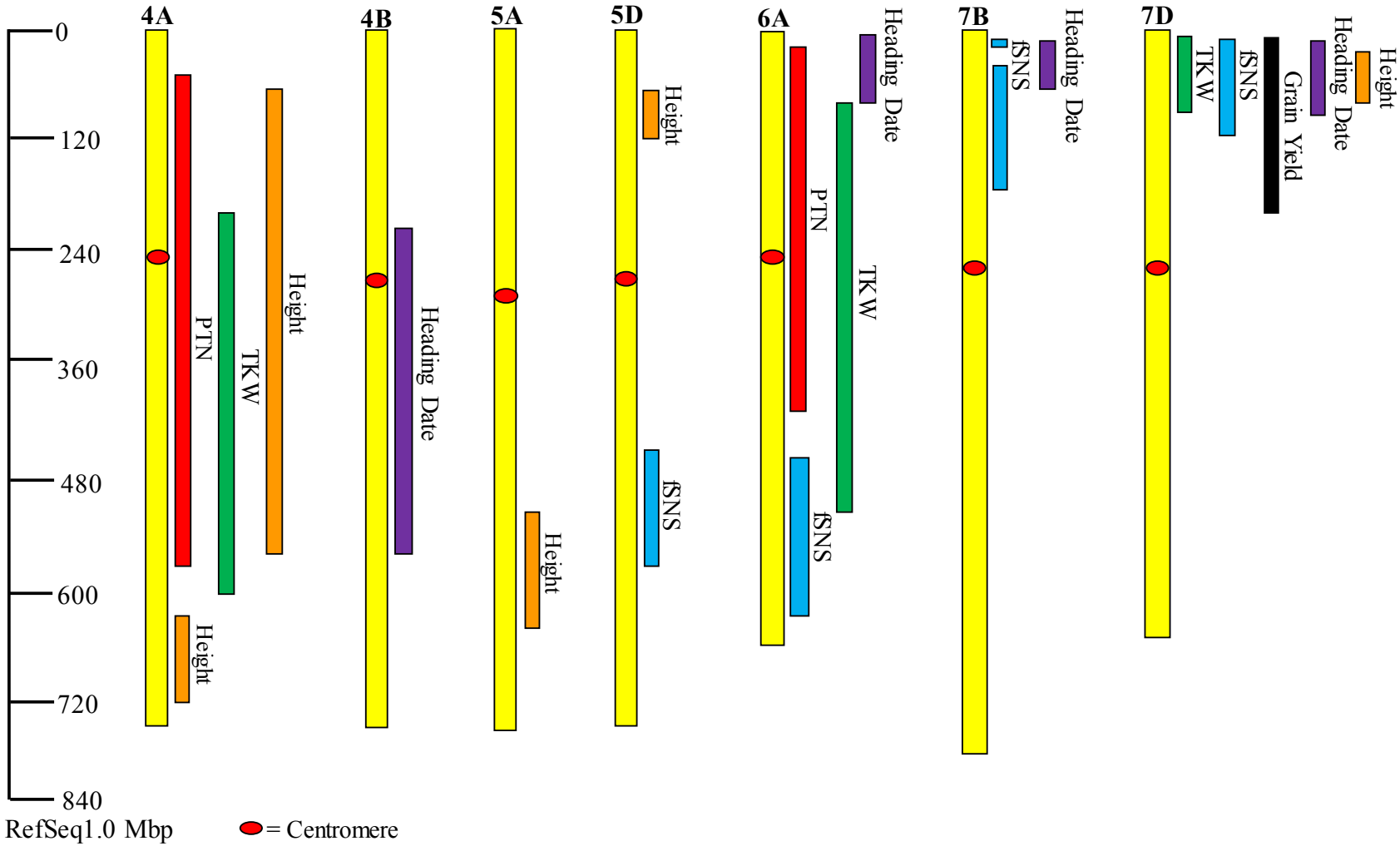
[b] T-test analyses were used to compare the two different allele groups.

Fig. 2.1 Histogram and H^2 of GY, HD, HT, fSNS, TKW, and PTN



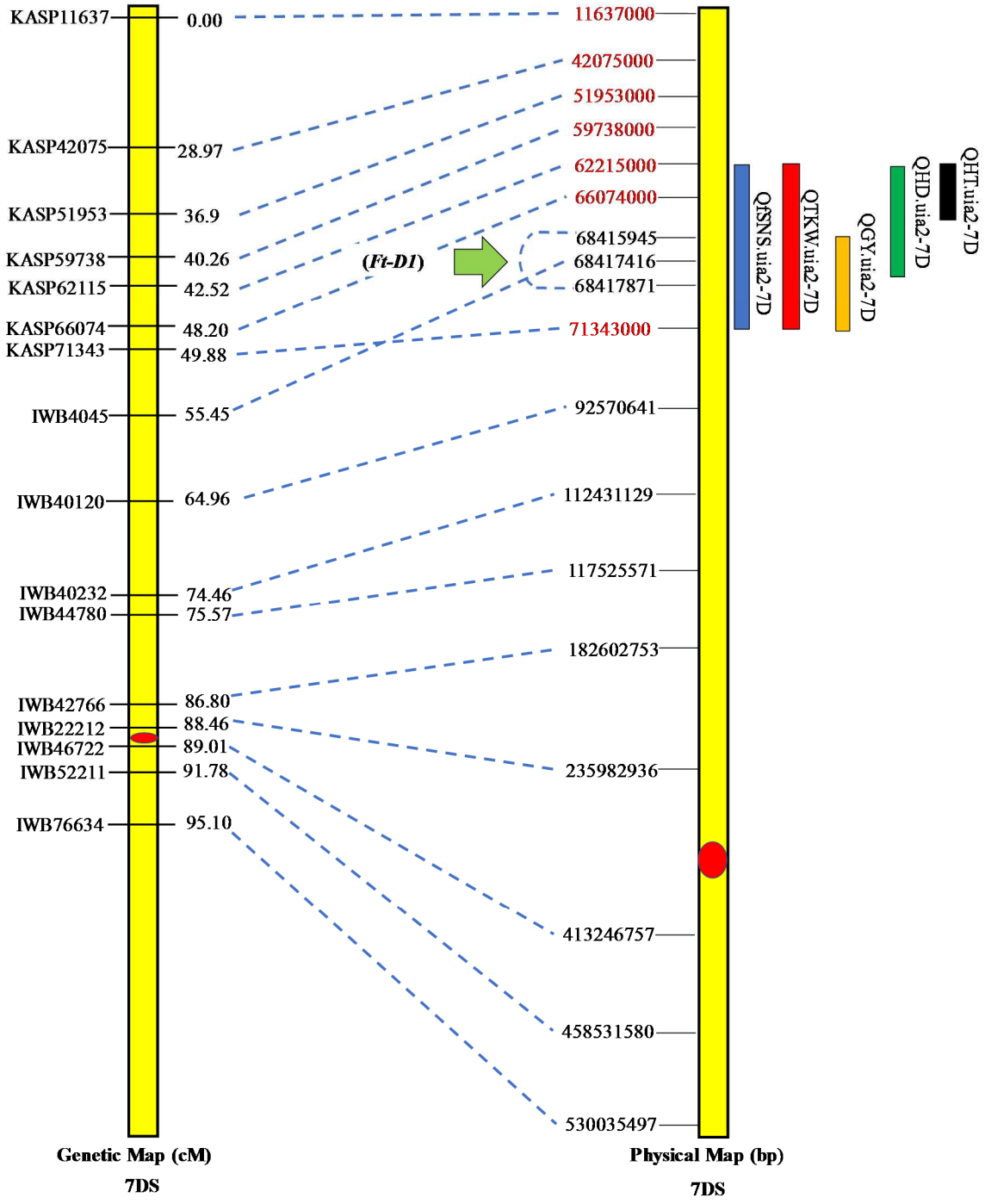
The BLUP values of the parents are indicated on the histogram plots in red. The broad sense heritability (H^2) for each trait is shown below each histogram.

Fig. 2.2 Significant QTL for GY, HD, HT, fSNS, TKW, and PTN in the DH population



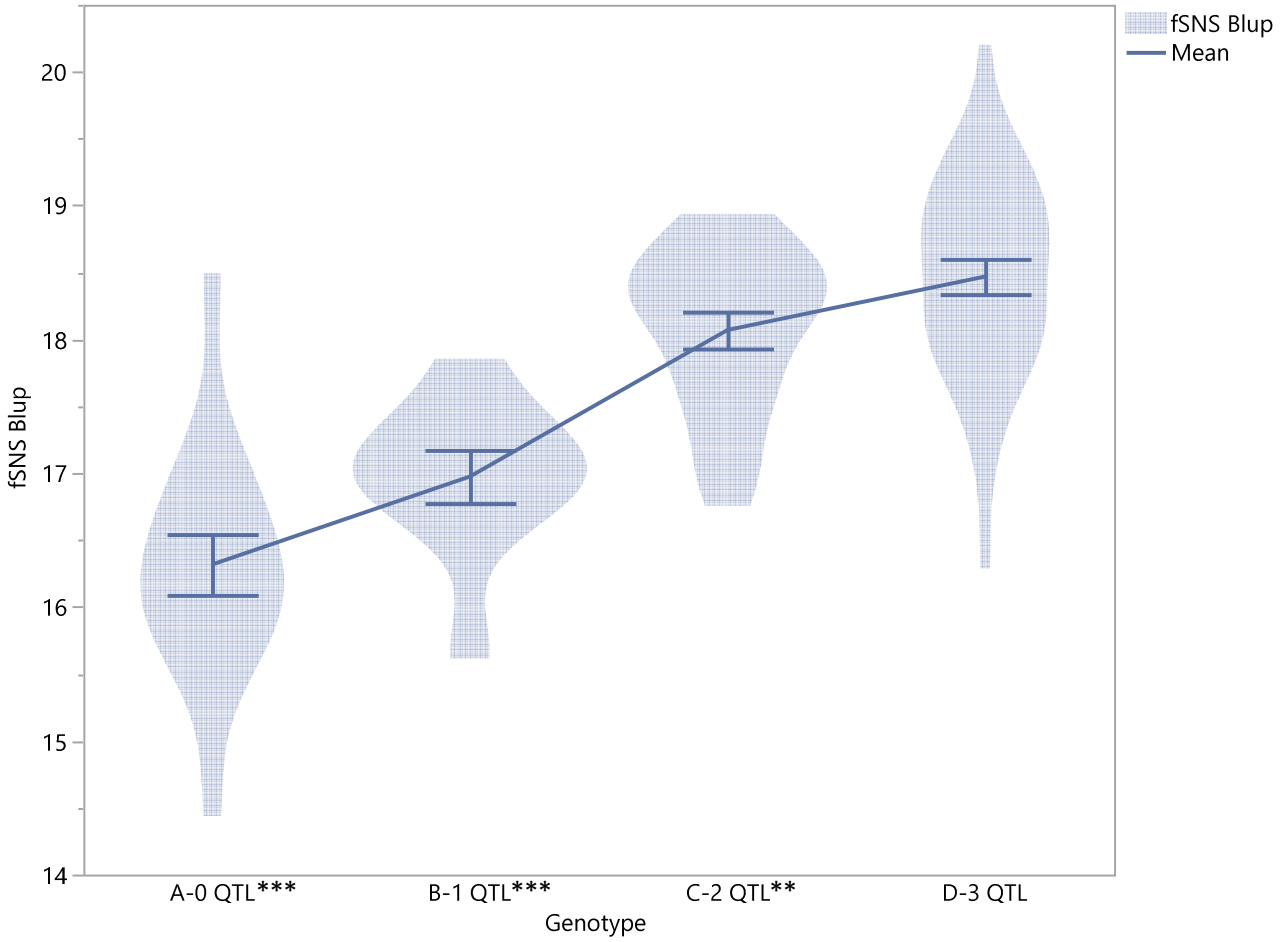
Physical positions of the QTL identified for GY, HD, HT, fSNS, PTN and TKW based on the Chinese Spring sequence (Reference Sequence v1.0, the International Wheat Genome Consortium (IWGSC).

Fig. 2.3 Genetic and Physical positions of the 7DS QTL and the position of the *Ft-D1* gene on chromosome 7D



Collinearity relationships among the genetic map from the present study and the physical map for the identified QTL/QTL pairs were indicated by dash lines on the corresponding chromosomes. The fSNS is indicated by blue bars; TKW is indicated by red bars; grain yield indicated by orange bars; heading date indicated by green bars; height indicated by black bars. The positions are based on the genetic positions detected in the BLUP dataset

Fig. 2.4 Boxplots showing additive effect of the identified QTL for fSNS, TKW and PTN based on the BLUP data for each trait



Analysis of Variance (ANOVA) test and Tukey-Kramer HSD method for multiple comparison analysis were used for the comparisons among different allele groups. Significance level less than 0.001 is indicated by *** and ** indicate significance level at 0.05. The number in the X-axis indicated the number of major QTL for that yield component trait

Fig. 2.4 continued

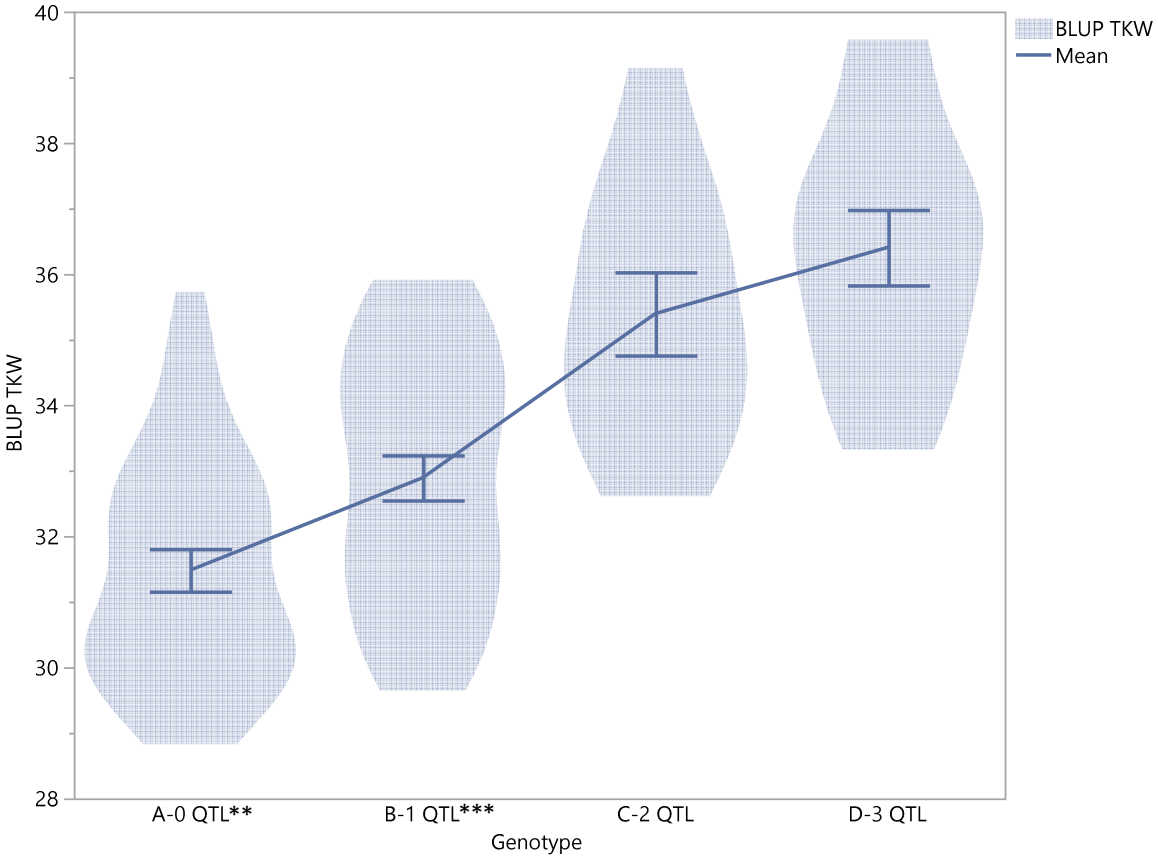


Fig. 2.4 continued