DISSERTATION

DISSECTING DROUGHT TOLERANCE IN WINTER WHEAT USING PHENOTYPIC AND GENETIC ANALYSES OF AGRONOMIC AND SPECTRAL TRAITS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2015

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ABSTRACT

DISSECTING DROUGHT TOLERANCE IN WINTER WHEAT USING PHENOTYPIC AND GENETIC ANALYSES OF AGRONOMIC AND SPECTRAL TRAITS

Worldwide, wheat (*Triticum aestivum* L.) is cultivated on more land than any other food crop. In 2013 wheat was grown on more than 220 million hectares worldwide, which is a larger area than the entirety of Mexico. Part of the global success of wheat can be attributed to its adaptability to diverse environmental conditions, including regions with limited water availability.

The United States is the largest exporter of wheat, and in recent years has exported 20–30% or more of its total production. Much of the wheat grown in the United States is cultivated under rainfed conditions, including regions across the Great Plains that are primarily planted to hard winter wheat. However, grain yield can be severely affected by water stress, and future climate projections predict drought will become more frequent and more severe. Therefore, it is important to characterize drought response and better understand genetic variation and genetic mechanisms of drought tolerance in winter wheat present in the U.S. Great Plains hard winter wheat.

This study used a collection of 299 hard winter wheat entries, designated the Triticeae Coordinated Agricultural Project Hard Winter Wheat Association Mapping Panel (HWWAMP), representative historic lines, recent cultivars, and experimental breeding lines present across the U.S. Great Plains. The entries were evaluated at a total of 11 Great Plains environments during 2011–2012 and 2012–2013. These environments include four

Colorado environments (paired water-stressed and non-stressed treatments in Greeley in 2011–2012 and Fort Collins in 2012–2013) with detailed phenotypic data for many agronomic traits, and seven environments in other states with data limited to heading date and grain yield.

The objectives of this study were to 1) determine allelic variation present in major developmental genes known to affect the timing of the developmental sequence and therefore adaptability, and estimate the effects of variants on heading date; 2) estimate the extent of variation and phenotypic plasticity of heading date in a range of environments representative of the U.S. Great Plains; 3) evaluate effects of water stress on grain yield and other agronomic traits, and identify underlying genomic regions affecting these drought responses, using side-by-side water-stressed and well-watered environments grown in two years; and 4) evaluate the effectiveness of water-based spectral indices calculated from hyper-spectral canopy reflectance measurements to characterize drought stress in the field.

In wheat and other small grain cereals, heading refers to the developmental stage where the spike has fully emerged from the flag leaf sheath. Heading date reflects genotypic 'earliness' and is important for regional adaptability of wheat. At heading, the developing spikelets and their sensitive reproductive structures become more exposed to changing environmental conditions, such as periods of cold, heat, or drought stress. Stress at heading and anthesis (which follows several days later) can have severe effects on grain yield.

The developmental sequence, including heading date, is affected by the vernalization and photoperiod pathways. Semi-dwarf alleles at the reduced-height genes also have an effect on the timing of plant development. We genotyped candidate genes at vernalization (*Vrn-A1, Vrn-B1,* and *Vrn-D1*), photoperiod (*Ppd-B1* and *Ppd-D1*), and

reduced-height (*Rht-B1* and *Rht-D1*) loci using polymerase chain reaction (PCR) and Kompetitive Allele Specific PCR (KASP) assays. The main effects and two-way interactions of alleles at these loci explained an average of 44% of variation in heading date across nine environments. Most of the variation was explained by *Ppd-B1*, *Ppd-D1*, and their interaction. The photoperiod sensitive and insensitive alleles were present in our germplasm in large proportions for both *Ppd-B1* and *Ppd-D1*, however, the sensitive alleles have been decreasing over time and are more common in germplasm from the northern than central or southern regions of the U.S. Great Plains.

There was significant ($P < 0.001$) genotype-by-environment interaction for heading date and growing degree-days to heading among all 11 environments. Phenotypic plasticity describes the range of possible phenotypes observed for one genotype, given different environmental conditions. We estimated phenotypic plasticity of growing degree-days to heading (GDDP) and yield for each entry, and found there was variation in our germplasm for both. We found GDDP to be negatively associated with yield (r=-0.58, P<0.001), and thus detrimental in the germplasm and environments evaluated. Greater yield plasticity was associated with increased maximum (r=0.80, P <0.001) and minimum (r=0.33, P<0.001) grain yield across environments, indicating it was a favorable trait. Over time GDDP has decreased and yield plasticity has increased, which suggests these are possible traits that could be targeted for selection.

In the Colorado environments, grain yield was reduced by similar amounts under water stress in 2011–2012 (48%) and 2012–2013 (46%), even though water stress occurred during different periods of the two growing seasons. In 2011–2012 stress occurred before anthesis and primarily reduced grain yield by limiting biomass and

tillering, and producing fewer total spikelets, fewer fertile spikelets, and fewer kernels per spike. In 2012–2013 stress occurred during grain filling and affected yield primarily by reducing kernel size. We conducted genome-wide association studies on agronomic traits in individual environments, and combined across different combinations of environments, and detected nearly 250 significant marker–trait associations for 15 agronomic traits. Most significant marker–trait associations were only detected for a single trait in one environment, had modest allelic effects, and explained a small proportion of total phenotypic variation. However, associations for kernel number explained up to 29% of variation in one environment, and associations for the proportion of fertile spikelets were stable across multiple environments.

We measured canopy spectral reflectance using a hyper-spectral radiometer in 2012–2013 and calculated spectral indices previously shown to be associated with plant water status. There was substantial spatial–temporal variation across each sampling date, which contributed to a lack of significant differences in index values among genotypes. However, values of normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4) varied gradually among developmental stages. Changes in index values were especially pronounced under water stress, when the most extreme values coincided with the period of most water stress.

In summary, there is substantial variation for agronomic and phenological traits that affect drought tolerance or susceptibility. Variation in the timing of developmental stages, such as heading date, can confer regional adaptability. Introducing additional allelic diversity at photoperiod loci could enable finer adaptation under current or future climate scenarios. Alternatively, selecting for reduced GDDP and/or increased grain yield plasticity

could result in greater yields under varying environmental conditions. We did not find evidence supporting use of canopy spectral reflectance as a selection tool, but spectral traits might be useful to monitor changes in plant water status, especially if sources of spatial and temporal variation are reduced, such as by using a sensor with an active light source or taking simultaneous estimates from an aerial vehicle.

ACKNOWLEDGEMENTS

Many different people and events shaped my doctoral research experience; it would be impossible to name them all. Thank you to my graduate advisor Dr. Patrick Byrne, for his willingness to take me on as a doctoral student with limited prior experiences in agricultural research. He was not only dedicated to my success as a student, but also to my growth as a professional. I further extend my appreciation to the other members of my graduate committee: Dr. Scott Haley, Dr. Gregory McMaster, and Dr. Patricia Bedinger. I feel privileged to have had a committee that not only worked well together, but also where every member provided me with unique guidance and mentorship. I'm grateful for the culture of support surrounding the Department of Soil and Crop Sciences. I appreciate the support from the Byrne Lab and Wheat Breeding Team that have made my field trials possible. I am especially appreciative of my graduate student peers who have always shared each other's successes and frustrations over a pitcher of beer. I am grateful to have made so many lifelong friendships over our shared experiences.

Financial support of this research was provided by the USDA National Institute of Food and Agriculture AFRI grant to the Triticeae Coordinated Agricultural Project (TCAP, grant 2011-68002-30029), the Colorado Wheat Administrative Committee, and the Colorado Wheat Research Foundation. I would like to acknowledge the support and tremendous opportunities provided to me as part of the TCAP. This includes support from and collaboration with partners at other institutions; opportunities to participate in special classes, workshops, and conferences; and many other experiences that extend beyond what I would have captured from my research at Colorado State University alone.

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CHAPTER 1:

LITERATURE REVIEW

Drought tolerance in winter wheat

The focus of most wheat breeding programs is direct selection for yield, although several other traits related to adaptability, such as plant height or flowering time, may also be selected for directly (Richards, 2006). Recent advances in next-generation sequencing, namely rapid genotyping of numerous markers at a lower cost, offer the potential to integrate genotyping into breeding decisions, such as through genomic-selection (Heffner et al., 2009). Innovations in high-throughput field-based phenotyping may also play an important role in more precise characterization of and selection for complex, quantitative traits (Cabrera-Bosquet et al., 2012; Araus and Cairns, 2014) like yield or drought tolerance.

Selection for many drought-related traits may be more effective under optimal conditions than water-stressed environments (Richards et al., 2010). In the U.S. much of the total production of winter wheat occurs from dryland wheat grown in the Great Plains region (Graybosch and Peterson, 2010), and there is substantial genotype-by-environment interaction for grain yield under water-stress (Reynolds and Tuberosa, 2008; Blum, 2011). Physiological traits associated with grain yield under water-limited conditions may have more genetic variation, respond faster to selection, or be less expensive to evaluate than yield *per se* (Richards, 2006). Incorporating selection of physiological traits into breeding decisions could increase yield potential under water stress (Blum et al., 2005). Examples of physiological traits that could be targeted include stomatal traits affecting canopy

temperature depression and carbon isotope discrimination, photosynthetic capacity, or assimilation and translocation of carbohydrates (Araus, 2002).

There are unique challenges in targeted improvements of traits associated with drought tolerance. Water-limited environments have many sources of variation and agronomic traits are not affected equally under all types or timing of water stress (Blum, 2011). The same level of stress can have different effects on grain yield depending on developmental stage at time of stress (Blum, 2011). The most severe effects of water stress on grain yield occur during reproductive development (Saini and Westgate, 2000). The severity and duration of water stress, presence of additional confounding stresses, environmental characteristics such as soil type and water-holding capacity, and agronomic practices can all influence the effects of water stress on grain yield (Passioura and Angus, 2010).

Production of grain yield under water stress can be dissected into several components, including water use, water-use efficiency, and harvest index (Passioura, 1996). Traits affecting one or more of these three elements of grain yield can be identified as breeding targets depending on the type of water-stress and preferred drought resistance or tolerance mechanism. It should be noted that Blum (2009) recommends against selecting for most traits that improve water-use efficiency, as many are inversely associated with biomass production and therefore limit grain production.

Plants exhibit three mechanisms for coping with water-limited environments: dehydration avoidance, dehydration tolerance, and drought escape. Dehydration avoidance describes mechanisms by which plants avoid water stress by maintaining high plant water status, such as though osmotic adjustment or development of extensive root systems (Blum

et al., 2005). A second mechanism of drought resistance is dehydration tolerance, which Blum et al. (2005) define as the ability of a plant to sustain function while dehydrated. Alternatively, drought escape involves timing plant development around periods of water availability. Passioura (2012) describes flowering time as "the most important physiological trait [for grain crops] in water-limited environments". In particular, winter wheat grown in the U.S. Great Plains needs to delay flowering until the risks of cold and frost damage are low, but flower before hot summer temperatures damage the developing spike. Much of the focus of this dissertation will relate back to the timing of plant development, and characterizing drought tolerance through the drought escape mechanism.

Developmental sequence of winter wheat

Growth and development of winter wheat

The developmental sequence of winter wheat consists of physiological and morphological changes to the plant. These changes begin with the initiation of organ primordia, continuing through appearance and growth of organs, and concluding with the abortion, senescence, or maturation of organs (McMaster, 2005). Development and growth of organs occur sequentially with some overlap, beginning with leaves, then tillers, spikelets, florets, and finally kernels.

The wheat canopy develops with the addition of phytomer units, each of which consists of a leaf, axillary bud, node, and internode (Wilhelm and McMaster, 1995). Phytomer units are repeated along the culm, and vegetative growth involves the addition of

new phytomer units. The axillary buds produce new shoots, and the internodes extend during stem elongation. The concept of phytomer development can be extended to reproductive (Moragues and McMaster, 2012) and below-ground (Forster et al., 2007) growth.

In the U.S. Great Plains winter wheat is typically planted in the early fall. Seeds germinate and the developing seedlings begin vegetative growth before going dormant for most of the winter. Vegetative growth continues after the vernalization requirements have been fulfilled, followed by a shift to reproductive growth in the early spring. The shift from vegetative to reproductive development ensues between single ridge and double ridge, which occurs before jointing (McMaster, 1997). At double ridge the spikelet primordia form an upper ridge above the leaf primordia at the shoot apex, and subsequent leaf primordia do not develop into leaves. The last leaf to appear is the flag leaf. Full expansion of the flag leaf occurs at booting, which can also be identified by the presence of the flag leaf collar.

Initiation of spikelet primordia begins at double ridge, and after the terminal spikelet is initiated the internodes begin to elongate (McMaster, 1997). The peduncle is the last internode to elongate, which occurs around anthesis and into grain filling. Following initiation of spikelet primordia, floret primordia begin to initiate and differentiate. However, many floret primordia are ultimately aborted. The final number of spikelets and florets are determined by anthesis, which establishes yield potential. Kernel growth occurs during grain filling, and is characterized first by cell division and then cell expansion. Kernels reach their maximum dry weight at physiological maturity.

The rate of phytomer development can be monitored by the phyllochron, or rate of leaf appearance on a culm. Phyllochrons are one method of predicting the timing of the developmental sequence, including phenological stages such as jointing, booting, heading, anthesis, and physiological maturity (McMaster and Wilhelm, 1995; Jamieson et al., 2007). Another common method to predict developmental timing is through accumulation of growing degree-days (Moragues and McMaster, 2011).

The timing of the developmental sequence varies among genotypes, environments, and management practices, and can be influenced by genotype-by-environment or genotype-by-management interactions (Longnecker et al., 1993; McMaster et al., 2005). Genotypic variation in developmental timing can affect traits like leaf number, leaf size, or growth rate. Altered developmental timing can affect the number of primordia initiated or that develop, which can affect yield potential. Environmental variables, such as water, temperature, or nitrogen availability, affect the timing and duration of developmental events, but not sequence. Water stress affects genotypes and species differently, and also varies with the pattern of stress (McMaster et al., 2005). In general, water stress accelerates the developmental timing of wheat, and continued stress can intensify the response.

Optimizing the timing of development to specific environments

The global success of wheat is in part attributed to its adaptability to a wide range of environmental conditions and management practices (Worland and Snape, 2001). Optimizing the timing of the developmental sequence to a specific target population of

environments can reduce the likelihood that sensitive reproductive organs are exposed to cold, heat, or water stress.

In wheat, heading is a developmental stage characterized by the emergence of the inflorescence from the flag leaf sheath. Heading can be scored quickly and easily in the field. Genetic variation in heading date can indicate genotypic 'earliness' and is critical for adaptation to specific environments, including to a particular type, timing, duration, or severity of stress (Kamran et al., 2014a). Reproductive development begins long before heading, with the initiation of spikelet and floret primordia at double ridge. However, heading reflects the change from vegetative growth to reproductive development, such as redirecting assimilates to the developing spike.

The flowering pathway integrates two major genetic systems: vernalization and photoperiod. Winter wheat genotypes require a vernalization period of continuous cold exposure, and photoperiod sensitive spring or winter genotypes require a critical day length, before transitioning from vegetative to reproductive growth. There is quantitative variation among winter wheat genotypes for the amount of cold temperature required to fulfill the vernalization requirement, and in the absence of cold temperatures most genotypes eventually flower.

Vernalization loci determine winter, spring, or facultative growth habit in wheat. Ancestral wheats had a winter growth habit. There is substantial co-linearity among the A, B, and D genomes of hexaploid wheat. Many genes associated with heading date have functional homoeologues on each of the three genomes. Spring growth habit is determined by one or more dominant alleles at several vernalization (*Vrn*) loci: *Vrn-1* (*Vrn-A1, Vrn-B1, Vrn-D1*) or *Vrn-3 (Vrn-A3, Vrn-B3, Vrn-D3,* Kamran et al., 2014b). Mutations at one or more

Vrn-1 gene are the predominant cause of spring growth habit (Chen and Dubcovsky, 2012). Winter growth habit requires recessive alleles at all *Vrn-1* and *Vrn-3* loci, and a dominant allele at *Vrn-2*.

The vernalization genes are closely related to the meristem identity genes in *Arabidopsis: Vrn-1* is similar to *Apetela1* (*AP1*, a MADS-box transcription factor), *Vrn-2* shares similarities with *Flowering locus C* (*FLC*), and *Vrn-3* is similar to *Flowering locus T* (*FT*) (Trevaskis et al., 2007). Flowering is accelerated by vernalization in winter wheat, but is not temperature-dependent in spring wheat.

There are many good reviews about the control of flowering time in cereals including wheat (e.g., Trevaskis et al., 2007; Distelfeld et al., 2009). Briefly, the flowering time and photoperiod pathways control expression of *Vrn*-1, the transition from vegetative to reproductive development occurs after a sufficient amount of the protein VRN1 has accumulated. After a threshold level of VRN1 has accumulated the spikelet primordia begin to form at the shoot apex. This developmental stage is know as double ridge and characterizes the beginning of reproductive development.

Most winter wheat is planted during the fall. Following seedling emergence, *Vrn-2* represses *Vrn-3* to inhibit early floral development, such as (for fall-planted winter wheat) reproductive growth and development in the fall and early winter. Expression of *Vrn-1* is low during the fall, and *Vrn-1* is also repressed by *Vrn-2.* As the vernalization requirement is fulfilled, *Vrn-1* is up-regulated, which results in accumulation of VRN1 directly, but also represses transcription of *Vrn-2*, which releases *Vrn-3* to further up-regulate *Vrn-1*. *Vrn-3* integrates both temperature and photoperiod cues, and is also up-regulated in the spring by perceiving the long days.

Genetic variation affecting developmental timing

Genetic variation in heading date can be caused both by variation in major effect genes, such as those involved in the vernalization and photoperiod pathways, and also due to small-effect *earliness per se* genes. *Earliness per se* loci are numerous, but each locus contributes a small amount of phenotypic variation, and the effects of many loci are not stable across populations or environments (Kamran et al., 2014a).

Variation in major effect genes is primarily due to single nucleotide polymorphisms (SNPs), insertion–deletions (INDELs), and copy number variation (CNV). Epigenetic modifications, such as DNA methylation, may also have an effect on phenotypic variation of important agronomic traits (King et al., 2010). Genetic variation such as SNPs and INDELs can result in many alleles at a single locus. Guo et al. (2010) identified seven haplotypes at *Ppd-D1* in a worldwide collection of mostly hexaploid wheat accessions.

Copy number variants are changes in the genome that result in the gain or loss of large (>1 kb) DNA segments (Zmienko et al., 2014). Most variation occurs within intergenic regions, but when variation occurs in the protein-coding region or regulatory element sequences of protein-coding regions it can affect the phenotype. Copy number variation (CNV) can contribute either to elevated or reduced levels of transcripts. For instance, a gene will be up-regulated if the duplication occurs in tandem but reduced or eliminated in cases of whole- or partial-gene deletion, or if an insertion interrupts gene function.

Some studies suggest CNV in plants is more common among certain types or families of genes, such as those with leucine-rich repeats (LRR) or nucleotide binding (NB) domains (Zmienko et al., 2014). Consequently, CNV is over-represented in genes associated with disease resistance and biotic stress tolerance (McHale et al., 2012). However, variation

has also been shown to significantly affect important agronomic traits (Díaz et al., 2012). Changes in copy number can be an effective method of adjusting to new or changing environmental conditions or pressures. New variants are common, but fixation requires both the resulting phenotype to be advantageous, and strong selection pressures for the new phenotype to exist for many generations (Zmienko et al., 2014). This can result in accumulation of many copies of a single gene under particular environmental conditions, such as multiple copies of *Ppd-B1* in regions where reduced photoperiod sensitivity, or a day-neutral phenotype and earlier flowering, contributes to greater yield (Díaz et al., 2012). Copy number variation associated with variation in freezing tolerance has also been described for *Vrn-A1* (Zhu et al., 2014).

Phenotypic plasticity of heading date

Phenotypic plasticity is described as the ability of a single genotype to produce variable phenotypes under different environmental conditions (Nicotra and Davidson, 2010). The extent of plasticity, as well as whether it is beneficial or detrimental, varies among traits and when evaluated on different germplasm or environments (Bradshaw, 1965). The extent of plasticity is specific to the trait evaluated, and the environments and populations in which it is evaluated. In many cases, heritability and plasticity are inversely related. Plasticity can be caused by genetic (Schlichting, 2008) and epigenetic variation (Bloomfield et al., 2014).

Many plant breeders select cultivars based on favorable mean trait values (i.e., high average grain yield) and trait stability (Eskridge, 1990). This minimizes genotype-by-environment interaction (Gauch and Zobel, 1997) and contributes to a favorable phenotype

in most years or environments. However, an alternate approach is to select genotypes with a dynamic (plastic) response under varying environmental conditions (Des Marais et al., 2013), such as moderate grain yield under water-stressed environments and high yield under optimal conditions (Juenger, 2013). There is evidence that the developmental sequence of winter wheat has underlying plasticity traits (Sadras et al., 2009). A more complete understanding of phenotypic plasticity of heading date could allow deeper understanding of crop adaptation.

Canopy spectral reflectance

Field-based platforms for high-throughput phenotyping

In field-based agriculture, high-throughput phenotyping (HTP) refers to evaluating one or more traits on multiple field plots within a small amount of time. Many traits are evaluated indirectly and non-invasively (Fiorani and Schurr, 2013). The shift towards platforms to rapidly phenotype germplasm is largely in response to recent breakthroughs in genetics and genomics, such as next-generation genotyping platforms (Mardis, 2011). Improvements in phenotyping—coupled with genetic data—are expected to increase genetic gain by reducing cycle time (Furbank and Tester, 2011). For instance, phenotypic and genotypic data can be combined for breeding of complex traits using genomic selection (Cabrera-Bosquet et al., 2012).

Advances in HTP over the past several years are in part due to improvements in technological platforms, including hardware, software, statistical methods, and community resources for instrument configuration or post-processing pipelines (Fahlgren et al., 2015).

Ground- or air-based platforms offer proximal sensing of plots, often with a sampling resolution of 1 m or finer (White et al., 2012). High-throughput phenotyping differs from remote sensing, which operates across a larger area but on a lower resolution. There are many applications for HTP. It can have many applications: researchers who wish to compare agronomic traits on different genotypes; growers who are interested in precision agriculture or understanding spatial variability; or private industries with unique interests, such as image-based mechanical weeding or health assessment for crop insurance or other purposes.

Ground-based HTP platforms, such as carts or modified farm equipment, are especially useful in a research context because they can support a heavy payload (White et al., 2012; Araus and Cairns, 2014). Most ground-based platforms are equipped with global positioning system (GPS) sensors to geo-reference measurements so they can later be assigned to individual field plots. Common sensors include sonar to estimate plant height; RGB, multispectral, hyperspectral, or thermal imaging cameras to estimate plant growth parameters or vegetation health; or an infrared thermometer to estimate canopy temperature depression (White et al., 2012), which is an indicator of stomatal conductance and water-use efficiency. Some ground-based systems extend sampling across several adjacent rows to increase the area that can be sampled in a day or reduce temporal variation (Andrade-Sanchez et al., 2014).

Air-based high-throughput phenotyping platforms, such as planes, blimps, or unmanned aerial vehicles (UAVs) further reduce, or eliminate, temporal variation across a field (White et al., 2012). A typical UAV used for remote sensing in agriculture has a low payload (about 10 kg or less), short flight time (less than one hour), travels at a low

elevation (below 300 m) and low speed (about 50 km h^{-1} or less), and is equipped with one or more cameras (Huang et al., 2013). Recently, UAVs have become more widely-used, in part due to policy and regulation changes, including exemptions for some agricultural uses (https://www.faa.gov/news/updates/?newsId=81164).

Spectral signatures of plants

Sunlight comprises a portion of the electromagnetic spectrum that includes infrared, visible, and ultraviolet light. Most of the radiation emitted by the sun is between about 200-4000 nm. However, the majority of radiation from the sun that reaches the Earth's surface is visible (400-700 nm) or near infrared (NIR, 700-1000 nm) light (Ollinger, 2011). Light reaching a plant canopy can be absorbed, transmitted, or reflected. Scattering of light, either through transmittance or reflectance, occurs any time light crosses a boundary with two different refractive indices, for instance, between a cell wall and an interior airspace (Gates et al., 1965). This results in altered patterns of light scattering between tissues with different structural properties, such as leaf angle, plant density, presence of biochemical compounds, and tissue turgidity (Ollinger, 2011; Barton, 2011).

Light absorbed by plant tissues mainly involves absorption of visible light by pigments, and absorption of wavelengths in the NIR by water (Peñuelas and Filella, 1998). Some common features in the reflectance spectra of plants include low levels of reflectance in the visible light range due to absorption by pigments such as chlorophyll, high reflectance in the NIR, and dips in the reflectance at the water absorption bands (970 nm, 1100 nm, 1400 nm, 1950 nm, Ollinger, 2011). The region between absorption in the red portion of the spectra (about 670 nm) by chlorophyll *a* and *b* and high reflectance in the

NIR is known as the "red edge" (Horler et al., 1983). A shift in the inflection point of the red edge can signify changes in nitrogen and chlorophyll content and indicate stress (Peñuelas et al., 1994).

Water-based spectral indices

Spectral indices are related to different plant characteristics, such as chlorophyll content, photosynthetic activity, pigment concentration, or canopy water content based on the amount of light reflected at two or more wavelengths (Ollinger, 2011). Indices relate the amount of light reflected at different wavelengths. Most indices are simple operations, such as ratios or differences between the amount of light reflected at two different wavelengths. Published water-based spectral indices relate the amount of light reflected at the minor water absorption band at 970 nm to regions of high reflectance in the NIR (Table 1.1).

The water index (WI) is the ratio of light reflected at 970 nm and 900 nm, and has been shown to be associated with relative water content, leaf water potential, and stomatal conductance (Peñuelas et al., 1993). The authors demonstrated WI was able to detect water stress earlier than canopy temperature depression. Normalized water-based spectral indices have also been developed (Babar et al., 2006b; Prasad et al., 2007) and shown to be associated with wheat grain yield in some environments, especially when indices were averaged across multiple sampling dates (Babar et al., 2006a) or during reproductive developmental stages (Prasad et al., 2009).

Genome-wide association analyses for agronomic traits

Marker–trait associations using genome-wide association mapping

Both genome-wide association studies (GWAS—also known as genome-wide association mapping or association analysis—and linkage analysis from bi-parental mapping populations detect marker-trait associations and identify quantitative trait loci (QTL). Identifying QTL can facilitate understanding the genetic architecture of complex, quantitative traits like grain yield or drought tolerance, that are controlled by hundreds or thousands of genes, many of which are population- or environment-specific, and only contribute a small amount of total phenotypic variation. Introgression of QTL into elite germplasm is possible using recurrent backcrossing and marker-assisted selection. However, introgression of QTL identified through QTL mapping or GWAS into breeding material is uncommon, in part due to small effect sizes, instability of QTL across environments, and large populations required to select multiple QTL (Bernardo, 2008).

Genome-wide association mapping offers several advantages over linkage analysis. Bi-parental mapping populations require a significant time investment to develop, while association mapping panels are comprised of diverse germplasm, which can include advanced breeding material that would facilitate incorporation of QTL by breeders (Würschum, 2012). Since GWAS uses a more diverse germplasm base the QTL are more likely to reflect common genotypic variants. Additionally, GWAS can be used to identify significant marker-trait associations for any trait with phenotypic variation within the population, unlike a bi-parental mapping population, which typically contrasts for only one or several traits. Association mapping capitalizes on ancestral recombination events among the germplasm, resulting in greater mapping resolution, more polymorphic alleles, and

greater power to detect numerous small-effect QTL. However, current and widely-used GWAS methods have limited ability to detect multiple allelic variants (Zhang et al., 2012) or diverse marker types, such as those associated with copy number variation, epigenetic variation, or transposons (Lipka et al., 2015). Current approaches are also not effective at detecting rare alleles, for instance, in most GWAS alleles present at levels below a chosen minor allele frequency (for instance, 5%) are not evaluated (Myles et al., 2009; Zuk et al., 2014). Genome-wide association mapping can be applied either as a hypothesis-driven candidate-gene approach, or as an exploratory whole-genome scan to identify QTL that contribute to the genomic architecture of the trait (Zhu et al., 2008). Some limitations of GWAS include statistical challenges associated with large numbers of markers, missing data, and corrections for multiple testing; instability of QTL across populations or environments; and that powerful analyses typically require very large populations and intensive phenotyping across multiple environments (Würschum, 2012).

Several technologies are responsible for the success of genetic analysis tools and techniques. The development of polymorphic marker platforms, like the Illumina 90K iSelect SNP chip and genotype-by-sequencing (GBS), expansion of tools to quickly and inexpensively detect polymorphic markers, and advances in statistical and computational methods to process very large genetic and genomic datasets have all contributed to the greater use and utility of GWAS. In particular, the increased availability and reduced cost of molecular markers, coupled with an ordered draft sequence of the wheat genome (Mayer et al., 2014) and recently published consensus maps (Cavanagh et al., 2013; Wang et al., 2014), make GWAS a viable option for wheat despite its very large and complex genome.

Tens of thousands or more molecular markers may be used in GWAS. Statistical associations occur between any markers in linkage disequilibrium (LD) with the underlying locus. Linkage disequilibrium is the nonrandom association between alleles at two loci, and is affected by genetic forces such as recombination, mutation, selection, or drift (Flint-Garcia et al., 2003). When the extent of LD is high, many markers may be linked with the locus, resulting in low mapping resolution (Myles et al., 2009; Würschum, 2012). Genome size and rate of LD decay, along with the magnitude of QTL effect, are three of the largest factors limiting our ability to detect a QTL (Lipka et al., 2015). The appropriate marker density depends on the rate of LD decay, which varies among species, populations, and even genomes. Linkage disequilibrium is broken down by many generations of recombination, however, the extent of LD is greater in self-pollinated species including wheat because there is less opportunity for recombination during meiosis (Nordborg, 2000). A greater marker density is required for the same mapping resolution when decay of LD is low. In wheat the D-genome has much higher LD than the A- or B-genomes because of its more limited genetic diversity (Chao et al., 2010; Marcussen et al., 2014; Naruoka et al., 2015).

Statistical models for genome-wide association mapping

Association mapping panels are not comprised of random individuals. Individuals are related by population structure and kinship, and the panel should be corrected for both of these elements. Population structure can be estimated either using principal components (P) or population substructure (Q) from marker data (Pritchard et al., 2000; Price et al., 2006). Population substructure is calculated first by estimating the number of

population subgroups present in the population, and then calculating the coefficient of subgroup membership for each individual (Evanno et al., 2005). Failing to account for population structure contributes to spurious associations between markers and phenotype that are based on historic relatedness (Zhu et al., 2008). Kinship (K) describes the pairwise relatedness among individuals in the population, which can also contribute to false marker associations. Kinship is estimated from the probability that two individuals share a random allele due to identity by descent (Lange et al., 2002).

Many statistical models have been proposed for GWAS. General linear models are statistical models that only account for kinship. Multiple linear models concurrently account for population structure (either using P or Q matrix) and kinship. Lipka et al. (2015) recommend that since correcting for population structure using either covariates from population substructure or principal components (PCs) are common in plant studies, PCs should be used because the methods are less computationally intensive. The authors also recommend the optimal number of covariates be fit separately for each trait and environment using model selection, such as options available in the R package Genome Association and Prediction Integrated Tool (GAPIT) using the BIC fit statistic (Lipka et al., 2012).

Multiple linear models are extended to a compressed multiple linear model that reduces processing time by clustering individuals into groups and estimating kinship separately for each group (Zhang et al., 2010). An enriched compressed multiple linear model introduces an additional parameter to choose the best-fit combination of algorithms for clustering individuals and estimating kinship (Li et al., 2014). Most research suggests incorporating kinship improves model fit compared with models that only account for

population structure (Yu et al., 2006). Other methods of applying a mixed linear model for GWAS is through efficient mixed-model association (EMMA, Kang et al., 2008), expedited EMMA (EMMAX, Kang et al., 2010), genome-wide EMMA (GEMMA, Zhou and Stephens, 2012), or population parameters previously determined (P3D, Zhang et al., 2010). These methods rely on different algorithms to speed up genetic analyses of large populations. Marker effects are estimated more accurately for GEMMA than EMMA, EMMAX, or P3D, because the model re-fits the mixed model at every marker, but is more computationally intensive (Zhou and Stephens, 2012).

Applications of genome-wide association mapping in wheat

There are many recent reports of successful use of GWAS in wheat. Genetic analyses have been used to dissect grain yield, yield components, and other agronomic traits (Yao et al., 2009; Bentley et al., 2014; Sukumaran et al., 2014; Zanke et al., 2014), including under multiple moisture regimes (Maccaferri et al., 2011; Dodig et al., 2012; Edae et al., 2014; Lopes et al., 2014; Li et al., 2015; Mora et al., 2015). Other studies have evaluated the genetic basis of quality traits (Breseghello and Sorrells, 2006; Reif et al., 2011), pre-harvest sprouting (Mohan et al., 2009; Kulwal et al., 2012), and nitrogen-use efficiency (Cormier et al., 2014). Other applications of GWAS in wheat include stripe rust resistance (Maccaferri et al., 2015; Naruoka et al., 2015), Fusarium head blight resistance (Kollers et al., 2013; Jiang et al., 2014), and insect pest resistance (Peng et al., 2009; Joukhadar et al., 2013).

The development of high-density genome-wide single nucleotide polymorphism (SNP) markers in wheat, such as the Illumina iSelect 9K (Cavanagh et al., 2013) or 90K (Wang et al., 2014) marker arrays have facilitated fine mapping resolution, and enhanced

detection of significant marker-trait associations. Prior to widespread availability of high-density markers, GWAS was conducted using other marker platforms such as simple sequence repeat (SSR) or diversity array technology (DArT) markers. SSR markers are amplified with PCT then separated with gel electrophoresis or other methods. They are simple and reliable, but time consuming (Collard et al., 2005). DArT markers are based on microarray hybridizations that detect presence–absence variation at many hundreds of loci in parallel (Jaccoud et al., 2001), and can generate a medium-density genetic map for complex species (Wenzl et al., 2004). However, there are also challenges associated with high-density marker platforms. Array-based platforms, such as SNP chips, contribute substantial ascertainment bias, especially in diverse panels or those with a limited sample size (Albrechtsen et al., 2010). Next-generation sequencing approaches, such as GBS better reflect the genetic diversity in a panel of germplasm so have less ascertainment bias, but multiplexing GBS samples can result in large amounts of missing data (Poland et al., 2012).

In one impressive study, Maccaferri et al. (2011) characterized seven agronomic and yield traits under different moisture conditions in a collection of 189 elite durum wheat accessions grown across a total of 15 Mediterranean Basin environments over two years. The environments had variable amounts of stored soil moisture, precipitation, and irrigation, ranging from 146 to 711 mm available soil moisture during the growing season. Interestingly, the panel was grown in parallel with a recombinant inbred line population, and GWAS was conducted using 179 SSR markers and three developmental genes to compare QTL detection efficiency between association mapping and bi-parental QTL mapping.

Association mapping complemented QTL detected from the bi-parental mapping population by confirming previously reported QTL and identifying new QTL. Maccaferri et al. (2011) observed that among GWAS results from different environments, the number of significant marker-trait associations was relatively stable for heading date, but variable for grain yield and other agronomic traits, which highlights the complex, quantitative genetic architecture of grain yield and significant effects of genotype-by-environment interactions for yield and yield components. For instance, an average of 10.3 significant QTL were detected in each high-yielding environment, while only 7.0 were detected in low-yielding environments. The authors concluded that increased levels of moisture stress reduce the power to detect significant QTL for grain yield, because different genotypes use a variety of adaptive strategies and gene networks in an effort to maintain grain yield. Because it can be difficult to detect QTL for grain yield, detecting QTL for other agronomic traits associated with grain yield might be a more useful approach to identifying high-yielding varieties than detecting QTL for yield *per se.*

Similar results were observed among a worldwide core collection of 96 winter wheat accessions grown under multiple moisture regimes and environments in southeastern Siberia (Dodig et al., 2012). Three moisture environments were evaluated each year for three years. The moisture treatments included full irrigation, rainfed, and drought (using rainout shelters), and in-season water availability (precipitation and irrigation) ranged from 54 to 418 mm. The germplasm was genotyped with 36 SSR markers, spaced 1-3 markers per chromosome. The markers characterized allelic diversity at 46 loci which had an average of 8 alleles per locus. Dodig et al. (2012) identified several QTL with pleiotropic effects among multiple agronomic traits and environments, and

concluded that yield components were stable and had greater heritability than grain yield. Consistent with conclusions from Maccaferri et al. (2011) that QTL for grain yield are more readily detected in the absence of moisture stress, QTL for grain yield were only detected under well-watered conditions.

The advent of DArT markers allowed for greater marker density and coverage across the genome (Wenzl et al., 2004), and have been used in several recent GWAS studies in wheat (Joukhadar et al., 2013; Edae et al., 2014). In one study, the wheat association mapping initiative (WAMI) population of 287 diverse spring wheat accessions was genotyped with 1863 DArT markers and used to identify QTL associated with grain yield, yield components, agronomic traits, and physiological traits under multiple moisture regimes. The WAMI was evaluated at five environments in the U.S. and Ethiopia, and more than 500 significant marker-trait associations were detected. These associations included five significant QTL for grain yield, including one stable QTL that was detected in three of the five environments and in the combined analysis. In a separate study, the WAMI was evaluated for grain yield and yield-components under four irrigated environments in Mexico, and genotyped with a high-density SNP array (Sukumaran et al., 2014). The authors identified 31 significant marker-trait associations for 14 traits, including four QTL for grain yield.

In summary, genome-wide association studies have provided insight on the genetic architecture of many traits of interest for plant breeders and scientists. QTL detection methods have improved due to advances in marker technologies, phenotyping platforms, and computational processes. However, many QTL are unstable across environments and have a small effect size, making incorporating novel QTL into breeding material challenging

(van Eeuwijk et al., 2010). One approach to increasing the power of GWAS is to combine population-mapping approaches with family mapping techniques, such as through developing nested association mapping panels (Zhu et al., 2008) or multi-parent advanced generation inter-cross (MAGIC) populations (Cavanagh, 2008) where individuals have structured levels of relatedness. Nested association mapping panels have been developed for maize (Yu et al., 2008), sorghum (Jordan et al., 2011), and barley (Maurer et al., 2015) and are being developed for wheat.

Tables

Table 1.1. The formula and reference for published water-based spectral indices. These indices have been associated with canopy water content and plant water status.

Spectral Index	Formula†	Reference
Water Index (WI)	R_{900}/R_{970}	Peñuelas et al., 1997
Normalized water index 1 (NWI-1)	$(R_{970}- R_{900})/(R_{970}+ R_{900})$	Babar et al., 2006
Normalized water index 2 (NWI-2)	$(R_{970}- R_{850})/(R_{970}+ R_{850})$	Babar et al., 2006
Normalized water index 3 (NWI-3)	$(R_{970}- R_{920})/(R_{970}+ R_{920}$	Prasad et al., 2007
Normalized water index 4 (NWI-4)	$(R_{970}- R_{880})/(R_{970}+ R_{880})$	Prasad et al., 2007

† R and the subscript indicate the reflectance of light at that specific wavelength (in nm)

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CHAPTER 2:

ALLELIC VARIATION IN DEVELOPMENTAL GENES AND EFFECTS ON WINTER WHEAT HEADING DATE IN THE U.S. GREAT PLAINS[1]

Summary

Heading date in wheat (*Triticum aestivum* L.) and other small grain cereals is affected by the vernalization and photoperiod pathways. The reduced-height loci also have an effect on growth and development. Heading date was evaluated in a population of 299 hard winter wheat entries representative of the U.S. Great Plains region, grown in nine environments during 2011–12 and 2012–13. The germplasm was evaluated for candidate genes at vernalization (*Vrn-A1, Vrn-B1,* and *Vrn-D1*), photoperiod (*Ppd-A1, Ppd-B1* and *Ppd-D1*), and reduced-height (*Rht-B1* and *Rht-D1*) loci using polymerase chain reaction (PCR) and Kompetitive Allele Specific PCR (KASP) assays. Our objectives were to determine allelic variants known to affect flowering time, assess the effect of allelic variants on heading date, and investigate changes in the geographic and temporal distribution of alleles and haplotypes. Our analyses enhanced understanding of the roles developmental genes have on the timing of heading date in wheat under varying environmental conditions, which could be used by breeding programs to improve breeding strategies under current and

[1] A modified version of this chapter has been submitted as follows:
Grogan, S. M., G. Brown-Guedira, S. D. Haley, G. S. McMaster, S. D. Reid, J. Smith, P. F. Byrne. 2015. Allelic variation in developmental genes and effects on winter wheat heading date in the U.S. Great Plains. PLOS One.

future climate scenarios. The significant main effects and two-way interactions between the candidate genes explained an average of 44% of variability in heading date at each environment. Among the loci we evaluated, most of the variation in heading date was explained by *Ppd-D1*, *Ppd-B1*, and their interaction. The prevalence of the photoperiod sensitive alleles *Ppd-A1b*, *Ppd-B1b*, and *Ppd-D1b* has gradually decreased in U.S. Great Plains germplasm over the past century. There is also geographic variation for photoperiod sensitive and reduced-height alleles, with germplasm from breeding programs in the northern Great Plains having greater incidences of the photoperiod sensitive alleles and lower incidence of the semi-dwarf alleles than germplasm from breeding programs in the central or southern plains.

Introduction

Hexaploid wheat is a widely cultivated, productive, and nutritionally important crop grown across most major agricultural regions of the world. In 2013 wheat was planted on more than 290 million hectares worldwide, which is more land than any other crop (http://faostat3.fao.org). Average global yield was estimated at 3.27 t ha^{-1}. In the U.S. average yield was 3.17 t ha^{-1} for all classes of wheat and 2.46 t ha^{-1} for hard red winter wheat (http://www.ers.usda.gov), the predominant market class in the Great Plains region. The global success of wheat can be attributed to its adaptability to diverse management practices and environmental conditions (Worland and Snape, 2001).

Variation in flowering time tailors wheat to a particular target environment. Floral organs are susceptible to environmental stresses, and freezing temperatures, drought stress, or heat stress can damage delicate floral structures and reduce yield. Local

adaptation can arise from direct or indirect selection of developmental timing best suited for local temperature and precipitation patterns, management practices, soil properties, or other environmental characteristics (Kamran et al., 2014a). Fine-tuning plant development may increase yield and yield stability by timing growth and development around the type, onset, duration, and severity of stresses characteristic of the region. Wheat breeders typically select (directly or indirectly) for later flowering in northern regions to protect the developing spike from cold temperatures and earlier development at southern latitudes where it is more important to flower before the onset of heat stress.

Three major genetic systems affect flowering time in wheat: vernalization, photoperiod, and earliness *per se* (EPS). Vernalization requirements control winter, spring, or facultative growth habit and are governed by at least three groups of loci (*Vrn1*, *Vrn2*, and *Vrn3*). Ancestral wheats were sensitive to vernalization and required a period of continuous cold temperature to transition from vegetative to reproductive growth. The most common source of spring growth habit is a dominant mutation at one or more *Vrn1* loci (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*), located on the long arm of the group 5 chromosomes (Yan et al., 2004a; Trevaskis, 2010). Dominant mutations at *Vrn3* (Yan et al., 2006) or recessive mutations at *Vrn2* (Yan et al., 2004b; Zhu et al., 2011) also reduce the vernalization requirement and contribute to quantitative variation in flowering time.

The photoperiod sensitivity system also provides broad adaptability to specific environmental conditions. Three homoeologous photoperiod loci (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*) are located on the group 2 chromosomes. Ancestral wheats were photoperiod sensitive and required long days to flower. Mutations at the photoperiod loci cause the photoperiod insensitive “day neutral” phenotype with earlier flowering. Variation in photoperiod

sensitivity is primarily controlled by allelic variants at *Ppd-D1* (Beales et al., 2007) and copy number variants at *Ppd-B1* (Díaz et al., 2012). A smaller amount of variation is controlled by *Ppd-A1* (Worland et al., 1998). The photoperiod and vernalization pathways are integrated and epistatic interactions between photoperiod loci and *Vrn1* are well characterized (Eagles et al., 2010).

Additional variation in flowering time after vernalization and photoperiod requirements have been fulfilled is due to EPS. These small effect loci are numerous and heritable (Kamran et al., 2014a), but generally unstable across environments (Griffiths et al., 2009). Less is known about EPS than the other genetic systems affecting flowering time. Further optimization of developmental timing is contributed by epistatic interactions among genes or QTL (Griffiths et al., 2009) and may be affected by gibberellin insensitivity through the reduced height loci (Wilhelm et al., 2013a).

The reduced-height loci are known to affect growth and development in winter wheat (Bush and Evans, 1988). Reduced-height genes encode DELLA proteins, which are growth repressors that are degraded by a process that involves gibberellin (Wilhelm et al., 2013c). *Rht-B1b* and *Rht-D1b* each have a single SNP that causes a premature stop codon, resulting in reduced sensitivity to gibberellic acid and semi-dwarf stature. Gibberellin insensitivity has been associated with earlier heading date in wheat, and the reduced height alleles *Rht-B1b* and *Rht-D1b* are the predominant semi-dwarf alleles in wheat worldwide (Wilhelm et al., 2013a). *Rht-B1b* has been reported as the leading cause of semi-dwarf stature among winter wheat in the U.S. Great Plains region (Guedira et al., 2010). Guedira et al. (2010) found the reduced height alleles *Rht-B1b* and *Rht-D1b* to be

widespread in U.S. germplasm, and *Rht8c* to be less common but linked with the photoperiod insensitive allele *Ppd-D1a.*

The allelic diversity of candidate loci known to affect heading date has been characterized in several worldwide (Wilhelm et al., 2013a; Kiss et al., 2014) and regional (Cane et al., 2013; Kamran et al., 2014b; Shcherban et al., 2014) collections of spring and winter wheat. Analyses of core collections of germplasm have indicated substantial variation in the geographic distribution of vernalization and photoperiod alleles. Variation in the presence and distribution of alleles and haplotypes has been found to vary among continents e.g.,(Kiss et al., 2014) and countries (Shcherban et al., 2014), with some haplotypes under-represented or absent from particular geographic regions. A more complete understanding of the genetic controls—including the allelic variants and effects of single genes, and distribution of favorable multi-locus genotypes—is important for plant breeders to prepare for future climate scenarios including those that are more variable or extreme than today's conditions (Beniston et al., 2007; Lobell et al., 2011).

We examined allelic variation, distribution, and effects of vernalization, photoperiod, and reduced height loci in a collection of hard winter wheat germplasm representative of the U.S. Great Plains. This is the first article to determine allelic diversity at the photoperiod loci in winter wheat germplasm from the U.S. Great Plains. The objectives of the experiment were

i) To characterize allelic variants at loci known to affect flowering time, including vernalization, photoperiod, and reduced-height genes;
ii) To assess the effect of allelic variants on heading dates in nine U.S. Great Plains environments; and

iii) To investigate temporal and geographic distribution of alleles and haplotypes among germplasm developed during different periods or from different regions of the U.S. where hard winter wheat is grown.

Materials and methods

Germplasm

The germplasm was a collection of 299 winter wheat genotypes comprising the Triticeae Coordinated Agricultural Project (http://www.triticeaecap.org) hard winter wheat association mapping panel (Table S2.1). The entries included modern and historic cultivars and experimental breeding lines. Public breeding programs contributed 270 entries, private breeding programs contributed 27, and two were historic cultivars developed before 1900. The publically developed germplasm came from Nebraska (55 entries), Oklahoma (54 entries), Texas (51 entries), Colorado (34 entries), Kansas (30 entries), Montana (23 entries), South Dakota (21 entries), Michigan (1 entry), and North Dakota (1 entry). The entries were derived between 1874 and 2010, but most represent modern improvements and were derived after the Green Revolution. Only 19 were derived before 1960.

Environments and experimental design

The entries were evaluated in nine field trials conducted across the U.S. Great Plains region during 2011–2012 and 2012–2013, as described previously (Grogan et al., 2015)

and in Table 2.1**.** Each environment was a combination of location, year, and moisture treatment, with three possible moisture regimes: full irrigation, partial irrigation, and rainfed (no supplemental irrigation). Environments grown in 2011–2012 include rainfed in Bushland, TX (Bu12R), full irrigation in Greeley, CO (Gr12F), partial irrigation in Greeley, CO (Gr12P), rainfed in Ithaca, NE (It12R), and Manhattan, KS (Ma12). The 2012–2013 environments include rainfed in Ardmore, OK (Ar13R), Fort Collins, CO (Fo13), rainfed in Hays, KS (Ha13R), and rainfed in Ithaca, NE (It13R). Different environments at the same location during the same year were side-by-side treatments with a moisture differential. There were separate rainfed and irrigated treatments at Fo13 and Ma12, but irrigation was not applied until after heading date so there were no significant treatment effects on heading date and the moisture treatments were treated as replications.

Four environments (Bu12R, Gr12P, Gr12F, Ha13R) were unreplicated and arranged in an augmented row–column design with two check varieties. The experimental entries were unreplicated except for 'Wichita' (CI 11952), which was included in the panel twice. The two check varieties were replicated 15 times each and systematically placed throughout the field. Fo13 had a similar experimental design but included two replications. The check varieties at Bu12R, Fo13, Gr12P, Gr12F, and Ha13R were 'Hatcher' (Haley et al., 2005) and 'Settler CL' (Baenziger et al., 2011), and these varieties were also included as experimental entries in the trial. Irrigation was applied at Gr12P and Gr12F using drip irrigation. Gr12P was irrigated less frequently and at a reduced volume than Gr12F. Irrigation totaled 101.6 mm at Gr12P and 335.3 mm at Gr12F. It12R and It13R used a similar experimental design but plots were arranged as 15 incomplete blocks, with one plot within each block planted to each of the check varieties Settler CL and 'Jagger' (Sears et al.,

1997). Both It12R and It13R included four replications, and these trials are described in detail by Guttieri et al. (2015). Ma12 had four replications of a modified augmented design arranged as incomplete blocks using a single, locally adapted check variety, 'Everest' (http://kswheatalliance.org/varieties/everest/). The Ar13R trial was arranged as a randomized complete block design with two replications. Harvested plot area ranged from 2.2 to 4.6 m^2.

Phenotypic evaluation

Crop development was determined for each field plot using Zadoks' scale (Zadoks et al., 1974). Heading date (stage 59) was recorded when the spike had fully emerged from the flag leaf sheath in approximately 50% of tillers. Days to heading were recorded as the number of days from January 1 to heading date. Growing degree-days from 1 January to heading were determined previously, and found to be strongly correlated with days from January 1 to heading (Grogan et al., 2015). However, number of days to heading was used in these analyses because estimates of allelic effects in days are more widely understood than estimates in °C days.

Genetic evaluation

DNA preparation for genetic analyses

Genomic DNA was extracted at Colorado State University from leaf tissue from single seedlings using the phenol–chloroform method modified slightly from Riede and Anderson (Riede and Anderson, 1996) .

KASP marker analysis

Polymorphisms were identified using LGC Genomics (http://www.lgcgroup.com) KASP system fluorescent assays at the USDA-ARS Regional Small Grains Genotyping Laboratory in Raleigh, NC. Polymerase chain reaction (PCR) was run according to manufacturer's instructions, using a reaction volume of 4.0 µl, which consisted of 2 µl 2x KASPar reaction mix, 0.05 µl 72x assay mix, and 2 µl of template DNA (10 ng μl^{-1}). Endpoint genotyping was conducted from fluorescence using the software KlusterCaller (LGC Genomics, Hoddeson, UK).

Kompetitive Allele Specific PCR (KASP) assays developed from published sequences of causal genes were run to distinguish alleles at *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1, Rht-B1*, and *Rht-D1* (Table S2.2). The exception was the KASP assay wMAS000033 used for detection of the *Vrn-A1a* spring allele developed from the contextual sequences of iSelect SNP marker IWA0001 (Cavanagh et al., 2013) determined to be associated with *Vrn-A1a*.

The spring allele *Vrn-A1b* was distinguished from *Vrn-A1a* and the *vrn-A1* winter allele using codominant marker wMAS000034 (Yan et al., 2004a). Two additional markers were evaluated to determine copy number variation (CNV) of the winter allele *vrn-A1*: Vrn-A1_Exon4_C/T and Vrn-A1_Exon7_G/A (Díaz et al., 2012). The C allele at *Vrn-A1_exon4* is associated with two or fewer copies, and the G allele at *Vrn-A1_exon7* is associated with a single copy, so entries were classified as having three or more copies, two copies, or one copy of *vrn-A1*. A PCR assay for *Vrn-A1* (Chen et al., 2009) was also run that detected the same SNP polymorphism and distinguished two winter alleles—renamed *vrn-A1w* and *vrn-A1v* by Eagles et al. (2011)—associated with winter dormancy release and freezing

tolerance. Thus, the winter alleles detected *at Vrn-A1* using PCR correspond with CNV detected using KASP.

Three KASP markers used to distinguish spring alleles at *Vrn-B1*. TaVrn-B1_D-I, wMAS000037, and Vrn-B1_C are codominant markers that detect the *Vrn-B1a*, *Vrn-B1b* (Santra et al., 2009), and *Vrn-B1c* (Milec et al., 2012) spring alleles, respectively. An additional codominant marker, TaVrn-B1_1752, detected an A/G polymorphism in intron 1 of the *vrn-B1* gene associated with differences in vernalization requirement duration (Guedira et al., 2014). The spring and winter alleles at *Vrn-D1* were distinguished using a single dominant marker, wMAS000039 (Fu et al., 2005).

Photoperiod insensitive allele *Ppd-A1a.1* was assayed with the marker TaPpd-A1prodel, which detects a deletion characteristic of the insensitive allele (Nishida et al., 2013). Alleles at *Ppd-B1* were distinguished using two markers: wMAS000027, which detects the 'Chinese Spring'-type insensitive allele with a truncated copy and TaPpdBJ003, which identifies the 'Sonora 64'-type insensitive allele based on the presence of an intercopy junction (Díaz et al., 2012). Photoperiod sensitive and insensitive alleles at *Ppd-D1* were distinguished using a single codominant marker, wMAS000024 that detects a deletion upstream of the coding region responsible for the photoperiod insensitive phenotype (Beales et al., 2007).

Single markers were also used to detect point mutations at the reduced-height loci *Rht-B1* and *Rht-D1* (Ellis et al., 2002). Mutants at *Rht-B1* were genotyped using wMAS000001 that detected the causative SNP for semi-dwarf stature. Likewise, a single marker, wMAS000002 was used to detect the polymorphism at *Rht-D1* that is associated with semi-dwarf stature. Additional information about KASP assays having wMAS

designations is available at

www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php?URL=.

Diagnostic markers for candidate genes

PCR assays for *Ppd-D1, Rht-B1, Rht-D1,* and *Vrn-A1* were performed at Colorado State University and complemented results from the KASP assays by confirming allele calls, filling in missing data, and detecting alternate alleles. PCR assays for *Ppd-D1* were conducted as described previously (Beales et al., 2007) to differentiate the photoperiod insensitive *Ppd-D1a* and two sensitive alleles based on band size of the PCR product. The major photoperiod sensitive allele is *Ppd-D1b*. Detection of the reduced height alleles *Rht-B1b* and *Rht-D1b* followed the methods of Ellis et al. (2002).

Statistical analyses

Best linear unbiased predictors were calculated separately for each environment based on field design and spatial trends using SAS 9.3 (SAS Institute, Inc., Cary, NC). Six different spatial correlation models (row-column, spherical, exponential, power, anisotropic power, and Matérn, (Littell et al., 2006) were tested for environments with a modified augmented design (Bu12R, Fo13, Gr12P, Gr12F, Ha13). The best model was selected based on the AIC fit statistic. Replications were treated as a random effect for other environments with multiple replications (Ar13R, Fo13, It12R, It13R, Ma12).

Further statistical analyses were performed using the software R (version 3.1.3, R Development Core Team). Combined analyses of the effects of alleles at single genes were

evaluated on all entries with homozygous allele calls using the 'car' package (Fox and Weisberg, 2011). The ANOVA model terms consisted of the environment, gene, and when significant, interaction between the gene and environment. All terms were fit as fixed effects. Entries with heterozygous calls were treated as missing. When a gene had more than two alleles, pairwise comparisons were run using the 'lsmeans' package (Lenth, 2015) to test differences between each pair of alleles. Individual environments were analyzed using the linear model (lm) function in the 'stats' package (R Development Core Team, 2015) to evaluate the proportion of variability in heading date explained by one or more gene, and to test for interactions between genes.

Models that tested effects of multiple genes included all entries with complete genotypic data at those loci. For model comparison a subset of 280 entries with complete genotypic data and all homozygous calls at the photoperiod and reduced-height loci were analyzed (Table 2.2). First, a full model that included the main effects of all five genes and all ten two-way interactions were fit to each data set. In the combined analysis, the main effect of the environment, two-way interactions between the environment and each gene, and ten three-way interactions between the environment and pairs of genes were also included in the model. Then, through an iterative backwards stepwise process, the least significant model term was removed one-at-a-time until the best-fit model was identified based on the lowest AIC value. In some cases the best-fit model included non-significant terms, but removing these terms detracted from model fit.

Results

Allelic diversity of candidate genes

The 299 winter wheat entries were genotyped at 11 candidate genes, but sample size varied among the loci due to different amounts of missing data at each gene. Genotypic data were missing from up to 12 entries per locus. The genotypes at each candidate gene for every entry are provided in Table S2.1.

Winter growth habit is determined by recessive alleles at all three *Vrn1* genes. Spring alleles at *Vrn-A1*, *Vrn-B1*, or *Vrn-D1* were not detected for any entry, validating our assumption that this germplasm consists exclusively of winter wheat entries. There was variation among winter alleles at *vrn-A1* and *vrn-B1* The SNP in *vrn-A1* associated with copy number (Díaz et al., 2012) varied, with 265 (89%) of entries predicted to have three or more copies, four entries (1%) having two copies, and 29 entries (10%) having a single copy. Copy number variation correspond with the winter alleles at *Vrn-A1* described by Eagles et al. (2011), such that the strong winter Wichita allele (*Vrn-A1w*) is associated with three or more copies of the gene and the 'Veery' (Merker, 1982) allele (*Vrn-A1v*) with two or fewer copies. Increased copy number at *vrn-A1* has been associated with greater vernalization requirements, resulting in later flowering when the vernalization requirement is only partially fulfilled (Díaz et al., 2012).

Two winter alleles at *Vrn-B1* were previously described to affect heading date following a short vernalization duration, but with no effect when the vernalization requirement is fully satisfied (Guedira et al., 2014). The winter allele characteristic of 'AGS2000' (Johnson et al., 2002) has an A at position 1752 and was associated with lower vernalization requirements and earlier heading than the 'NC-Neuse' (Murphy et al., 2004)

allele (C at position 1752). Low variation at this locus was observed in this germplasm. Most of the germplasm (292 entries, 99%) carried *vrn-B1-Neuse*; only 'TAM 401' (Rudd et al., 2012) and TX05A001822 had *vrn-B1-AGS2000*.

Alleles at the photoperiod genes *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* were polymorphic among the wheat entries evaluated. A total of 292 entries were genotyped successfully at *Ppd-A1*, including 286 entries (98%) with the sensitive allele *Ppd-A1b* and six entries (2%) with the insensitive allele *Ppd-A1a*. The six entries all originated from breeding programs in the southern U.S. and included 'TAM 302' (Marshall et al., 1999), OK05303, OK05134, TX04M10211, TX05V7269, and TX06A001132. For *Ppd-B1*, 286 entries were genotyped, including 163 entries (56%) with the sensitive allele *Ppd-B1b* and 123 entries (43%) with the insensitive allele *Ppd-B1a*. Genotypes were obtained at *Ppd-D1* for 294 entries. Most entries (208 entries, 71%) carried the photoperiod sensitive allele *Ppd-D1b,* while 86 entries (29%) carried *Ppd-D1a*.

The KASP assay identified the photoperiod insensitive allele at *Ppd-D1*. The PCR assay for *Ppd-D1* further characterized allelic variation. Photoperiod insensitivity at *Ppd-D1* is caused by a 2089 bp deletion upstream of the coding region (Beales et al., 2007). In presence of the deletion, a 288 bp band is produced that corresponds with *Ppd-D1a*. The presence of the photoperiod sensitive *Ppd-D1b* allele is detected by amplifying a 414 bp fragment within the deletion region with primer pairs Ppd-D1_F and Ppd-D1_R1. *Ppd-D1a* was identified in 88 entries (29%), and the sensitive allele *Ppd-D1b* was found in 204 entries (68%). Guo et al. (2010) described two small (24 and 15 bp) insertions within the intact 2089 bp region characteristic of an alternate photoperiod sensitive allele that results

in a PCR fragment size of 453 bp. The alternate photoperiod insensitive allele was detected in seven entries (2%).

Both *Rht-B1* and *Rht-D1* were polymorphic among the germplasm evaluated. All 299 entries were genotyped for *Rht-D1*, while 289 entries were successfully genotyped for *Rht-B1*. For *Rht-D1,* 283 entries (95%) carried homozygous copies of the tall wild type allele *Rht-D1a*, 14 entries (5%) carried homozygous copies of the semi-dwarf allele *Rht-D1b*, and two entries (<1%) were heterozygous. For *Rht-B1*, 202 entries (70%) carried homozygous copies of the semi-dwarf allele *Rht-B1b*, 83 entries (29%) carried homozygous copies of *Rht-B1a*, and four entries (1%) were heterozygous.

Diversity of multi-locus genotypes

There were 286 entries (280 entries with all homozygous allele calls and six entries with heterozygous allele calls at one or more loci) with complete genotypic data at all five photoperiod and reduced height loci. These 286 entries were used to investigate diversity of multi-locus genotypes. Fourteen of the 32 possible combinations of five-locus genotypes with all homozygous calls were present in the germplasm (Table 2.2). Five additional genotypes had heterozygous allele calls at *Rht-B1* or *Rht-D1*. The most common genotypes were *Ppd-A1b/Ppd-B1a/Ppd-D1b/Rht-B1b/Rht-D1a* (74 entries, 26%), *Ppd-A1b/Ppd-B1b/Ppd-D1b/Rht-B1b/Rht-D1a* (54 entries, 19%), *Ppd-A1b/Ppd-B1b/Ppd-D1b/Rht-B1a/Rht-D1a* (45 entries, 16%), and *Ppd-A1b/Ppd-B1b/Ppd-D1a/Rht-B1b/Rht-D1a* (43 entries, 16%). All other five-locus genotypes had six or fewer entries each.

The most common three-locus genotype at the photoperiod loci was *Ppd-A1b/Ppd-B1b/Ppd-D1b* (104 entries, 36%, Table 2.2). There were 91 entries (32%) with *Ppd-*

A1b/Ppd-B1a/Ppd-D1b and 57 entries (20%) with *Ppd-A1b/Ppd-B1b/Ppd-D1a*. There were 28 entries (10%) with *Ppd-A1b/Ppd-B1a/Ppd-D1a*. Only six entries (2.0%) carried *Ppd-A1a* (with or without insensitive alleles at any other photoperiod gene). No entries had insensitive alleles at all three photoperiod genes.

Among the 286 entries with complete allelic data at all five photoperiod and reduced-height loci, the most common allelic combination at the two reduced-height genes was *Rht-B1b/Rht-D1a* (198 entries, 69%), followed by *Rht-B1a/Rht-D1a* (69 entries, 24%), and *Rht-B1a/Rht-D1b* (14 entries, 5%, Table 2.2). The remaining entries were heterozygous at one or both reduced-height genes. No entries had mutations at both *Rht-B1* and *Rht-D1*. These results are in agreement with those presented by Guedira et al. (2010) in a similar set of germplasm.

Changes in diversity of photoperiod alleles over time

The prevalence of the photoperiod sensitive alleles *Ppd-A1b*, *Ppd-B1b*, and *Ppd-D1b* has decreased gradually among U.S. Great Plains hard winter wheat germplasm over the past century (Table 2.3). The first appearance of *Ppd-A1a* in this germplasm collection occurred with the derivation of 'TAM 301' (Marshall et al., 1998) in 1991 (Table S2.1). *Ppd-A1a* is rare in this germplasm but the prevalence of this allele has increased over time. Among entries derived after 2000, 4% were found to carry *Ppd-A1a* (Table 2.3). The photoperiod sensitive allele *Ppd-B1b* is found in 90% of entries derived prior to 1960, 67% of entries derived between 1960 and 1979, and 58% of those derived between 1980 and 1999. Among germplasm derived after 2000, *Ppd-B1a* is the more common allele and is found in 52% of entries. The prevalence of *Ppd-D1b* remains at a constant 85% for entries

derived before 1960 and between 1960 and 1979. However, the percentage of entries carrying *Ppd-D1b* drops to 71% among entries derived between 1980 and 1999, and is further reduced to 65% among entries derived after 2000.

Effect of photoperiod alleles on heading date

Heading date had a grand mean of 130.8 days among all 299 entries and nine environments. In the combined analysis *Ppd-A1* had a significant effect ($P < 0.01$) on heading date across environments. Entries with the photoperiod sensitive allele *Ppd-A1b* reached heading an average of 2.0 days later than those with *Ppd-A1a* (Table S2.3). The effect of *Ppd-A1* was not significantly different among environments, indicating a lack of significant genotype-by-environment (G×E) interaction at *Ppd-A1*. However, in analyses of individual environments the effect was significant ($P <0.05$) at only two environments: Ar13R (where the effect size was 5.0 days) and Fo13 (where the effect size was 0.4 days). Only six entries had *Ppd-A1a* and the lack of significant effects in most environments is likely influenced by low diversity at this locus.

The effect of *Ppd-B1* on heading date was significant in the combined analysis ($P < 0.001$). *Ppd-B1b* was associated with 3.0 days later heading across environments, but there was significant G×E. *Ppd-B1* had a significant effect ($P <0.05$, Table S2.3) in all individual environments except Ma12 and It12R. The effect of *Ppd-B1b* ranged from a minimum of 0.8 days later heading date in Fo13 (latitude of 40.65 °N, Table 2.1) to a maximum of 5.2 days later in Ar13R (latitude of 34.18 °N).

There was a significant effect ($P < 0.001$) of *Ppd-D1* on heading date in the combined analysis. The average effect of *Ppd-D1b* was 3.2 days later heading, which was larger than

the effects of *Ppd-B1b* or *Ppd-A1b*. However, the effect of *Ppd-D1* varied among environments, and the effect size of *Ppd-D1b* ranged from a minimum of 1.0 day in Fo13 to a maximum of 5.2 days in Ar13R (Table S2.3). *Ppd-D1* did not have a significant effect in Bu12R, Ma12, or It12R, which were in the southern, central, and northern regions of the U.S. Great Plains.

Models including *Ppd-D1*, *Ppd-B1*, and the interaction between *Ppd-D1* and *Ppd-B1* explained up to 65% of the variability in heading date (Table S2.4). The interaction between *Ppd-B1* and *Ppd-D1* was significant in most environments (excluding Bu12R and It12R), but when the interaction was included the main effects of the loci became non-significant (Table S2.4). The interaction was such that entries with both photoperiod sensitive alleles reached heading much later than those with a single photoperiod sensitive allele or both photoperiod insensitive alleles. The largest significant effects were seen at Ar13R (8.6 days) and Ma12 (6.4 days) The effect of the significant interaction between *Ppd-B1* and *Ppd-D1* is illustrated for three Colorado environments (Gr12P, Gr12F, and Fo13) in Fig 2.1A-C. The effect of interaction between *Ppd-D1b* and *Ppd-B1b* in the Colorado environments ranged from 1.5 to 4.1 days (Table S2.4).

Effect of semi-dwarf alleles on heading date

In the combined analysis, the semi-dwarf allele *Rht-B1b* had a significant ($P < 0.001$) effect on heading date and significant ($P < 0.001$) G×E. *Rht-B1b* was associated with an average effect of 2.6 days earlier heading, but the effect ranged from 1.1 days in Ha13R to 4.4 days in Ar13R (Table S2.3). There was a low level of genetic diversity at *Rht-D1* and the

locus did not have a significant effect on heading date when it was the only genetic term in the model.

There were 285 entries with homozygous allele calls at *Rht-B1* and *Rht-D1*. When both loci were included in the model, the main effects of both *Rht-B1* and *Rht-D1* were significant ($P < 0.001$, Table 2.4). There was significant G×E for *Rht-B1* but not *Rht-D1*. The effect of *Rht-D1b* was 2.6 days later heading, and the effect of *Rht-B1b* ranged from a minimum of 1.1 days later heading in Gr12F to a maximum of 4.9 days later in Ar13R (Table 2.5). Interaction between *Rht-B1* and *Rht-D1* could not be tested because not all four allelic combinations were present in this germplasm. In most environments *Rht-B1* and *Rht-D1* had similar effects on heading (Table 2.5). *Rht-B1* had a larger effect than *Rht-D1* in six environments (Ar13R, Bu12R, Gr12P, Ha13R, Ma12, and It12R), while *Rht-D1* had the larger effect in Fo13, Gr12F, and It13R.

Effect of vernalization alleles on heading date

No entry had alleles for spring growth habit detected at *Vrn-A1, Vrn-B1*, or *Vrn-D1*. The effect of different winter alleles at *vrn-B1* was significant ($P <0.01$) in the combined analysis, with the 'Neuse' allele heading 2.2 days later (Table S2.3). However, only two entries carried the 'AGS2000' allele at *vrn*-B1. The G×E term was not significant for *vrn-B1*.

Variation observed for the winter allele *vrn-A1* had a significant effect ($P < 0.001$) on heading date that varied among environments (Table S2.3). The differential effect of three or more *vrn-A1* copies relative to a single copy ranged from a minimum of 0.5 days later heading in Gr12P to a maximum of 4.7 days later heading in Ar13R. The effect of three or more copies of *vrn-A1* relative to two copies also had a significant ($P < 0.01$) effect in the

combined analysis, such that entries with two copies reached heading 1.9 days earlier. This effect had significant G×E, and in individual environments the effects of three or more copies relative to two copies were only significant in Ar13R, where the effect size was 5.5 days (Table S2.3).

Copy number variation at *vrn-A1* has been previously shown to have a positive association with vernalization requirements and survival under freezing conditions (Eagles et al., 2011). The strong effect of *vrn-A1* in Ar13R suggests winter temperatures at this environment may not have fully satisfied the vernalization requirements of all entries. Ar13R experienced a mild winter with daily highs always above 0°C. The effect of allelic variation at *vrn-A1* did not have a significant effect at It12R, which experienced a cold winter with 11 days that had highs below 0°C and 65 days with lows below 0°C.

Multivariate analyses of alleles on heading date

Combined analyses across environments

The significance of *Ppd-A1, Ppd-B1, Ppd-D1, Rht-B1, Rht-D1*, and their interactions were tested in a multiple-gene model using a subset of 280 entries with complete genotypic data and homozygous calls at all loci (Table 2.2). For the combined analysis the full model included main effects of the environment, main effects of all five genes, all 10 two-way interactions between genes, and the interaction of each genetic term with the environment. A backwards-stepwise approach was used to identify the best-fit model based on lowest AIC value.

The best-fit model to explain heading date across environments included effects of the environment; *Ppd-D1, Ppd-B1, Rht-B1*; the interaction between *Ppd-D1* and *Ppd-B1*; the interaction between *Ppd-B1* and *Rht-B1*; two-way interactions between the environment and *Ppd-D1, Ppd-B1,* and *Rht-B1*; and a three-way interaction between the environment, *Ppd-D1*, and *Ppd-B1* (Table 2.6). This model explained 96% of the phenotypic variation in heading date, with most of the variation (77%) explained by the environment. *Ppd-D1* explained 9.0% of the variation in heading date, *Ppd-B1* explained 8.3% of the variation, and their interaction explained another 3.9% of the variation. *Rht-B1* had a small effect on heading date, explaining 0.7% of the variation. The interaction between *Rht-B1* and *Ppd-B1* had a minor effect on heading date, and explained 0.3% of the variation.

Individual analyses for each environment

Multi-gene models were fit to each environment separately using the same backwards-stepwise model selection method as for the combined analysis. The full model included the main effects and all two-way interactions between genes. The best-fit models varied among environments and are summarized in Table 2.7. When an interaction was included the main effects were always retained. In some cases the best-fit model included non-significant terms. The effects of *Ppd-D1*, *Ppd-B1*, and the interaction between *Ppd-D1* and *Ppd-B1* were included in the best-fit model for each environment. The interaction between *Ppd-D1* and *Ppd-B1* had a maximum effect size of 8.2 days in It12R. *Rht-B1* was also included for every environment except Gr12F. None of the best-fit models included the main effect of, or interactions with, *Ppd-A1* or *Rht-D1*. Some best-fit models also included interactions between *Ppd-B1* and *Rht-B1* or *Ppd-D1* and *Rht-B1.* The combined allelic effects

explained an average of 44% of the variability in heading date, but ranged from a minimum of 14.2% in Fo13 to a maximum of 69.3% in Ma12 (Table 2.7).

Geographic distribution of alleles

We investigated the geographic distribution of alleles at the vernalization, photoperiod, and reduced-height loci among 264 entries derived from public breeding programs in Colorado, Kansas, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas. Average heading date of these 264 entries, across all nine environments, was 131.0 ± 13.6 days after Jan.1, or about May 11. The distribution of alleles was expected to vary among breeding programs in different states, because the regions for those programs have variable environmental conditions known to affect heading date. For instance, Texas has much milder winter temperatures than northern locations, and Montana and North Dakota have greater changes in day length throughout the year than the central and southern plains, due to their northern latitudes.

In a combined analysis across environments there were significant differences ($P < 0.001$) in heading date among entries from different states of origin. However, not all pairs of states had significant different heading dates. The entries were divided into three broad regions within the U.S. Great Plains: northern plains (Montana, North Dakota, and South Dakota; 39 entries), southern plains (Texas and Oklahoma; 105 entries), and central plains (Colorado, Kansas, and Nebraska; 120 entries, Fig 2.2A). There were significant differences ($P < 0.01$) in heading dates among entries originating from each pair of regions. Average heading dates were earliest among entries derived in the southern plains (128.8 ± 14.3 days after Jan.1). Heading occurred about 2.8 days later in the central (131.6 ± 13.1 days)

than southern plains. Latest heading dates were observed for germplasm from the northern plains (134.9 ± 12.2 days).

All three photoperiod sensitive alleles (*Ppd-A1a, Ppd-B1a, Ppd-D1a*) were present at higher levels in germplasm from the southern plains than those from the central or northern plains (Fig 2.2B). The photoperiod insensitive allele *Ppd-A1a* was uncommon in this germplasm. *Ppd-A1a* was only present in six entries (2%), all of which originated in the southern plains. *Ppd-B1a* was much more evenly distributed—present in 42% of entries overall—but varied gradually among the three regions. *Ppd-B1a* was found at highest proportions (58%) in germplasm from the southern plains, and at much lower levels in the central (36%) or northern plains (14%). Similar patterns were observed for *Ppd-D1a*, which was found in 44% of entries from the southern plains, 19% of entries from the central plains, and only 10% of entries from the northern plains.

The semi-dwarf alleles at the reduced-height loci were more common in germplasm derived in the southern plains than those originating from the northern or central plains. *Rht-B1b* was found in 70% of entries overall, including 88% of entries from the southern plains, 61% of entries from the central plains, and only 53% of entries from the northern plains (Fig 2.2B). The semi-dwarf allele *Rht-D1b* was rare in the panel, and present in only 11 (4%) entries. However, the distribution of this allele was much higher in the southern plains (7 entries, 9%) than the northern plains (1 entry, 3%) or central plains (3 entries, 3%).

Discussion

Our first objective was to characterize allelic variants at loci known to affect developmental timing of wheat through the vernalization, photoperiod sensitivity, and reduced-height pathways. Marker analysis revealed the incidence of semi-dwarf alleles *Rht-B1b* and *Rht-D1b* in this germplasm was comparable to that reported by Guedira et al. (2010) in a similar set of germplasm. The photoperiod insensitive alleles *Ppd-B1a* and *Ppd-D1a* were present at similar levels as detected by Kiss et al. (2014) in a worldwide collection of winter wheat. We found *Ppd-A1a* to be rare in this germplasm, and present at slightly lower levels than reported among European accessions (Shcherban et al., 2014). Further allelic variation could exist in our panel as additional allelic variants (Guo et al., 2010; Nishida et al., 2013) or copy number variants (Cane et al., 2013; Zhu et al., 2014) not detected by our assays.

Guo et al. (2010) described four modern and two ancient *Ppd-D1* haplotypes present in a worldwide collection of wheat, and detected all four modern haplotypes in accessions from the U.S. and Canada. Our analyses could only differentiate two modern and one ancient haplotype, which suggests additional haplotypes could be present. Seven of our entries had an alternate photoperiod sensitivity allele at *Ppd-D1b*, described by Guo et al. (2010) as haplotype VI. The alternate allele did not have a significant effect on heading date across environments, probably due to the small sample size. The entries with haplotype VI are 'OK Rising' (PI 656382), 'Thunder CL' (Haley et al., 2009), TAM 303, KS00F5-20-3, 'Overley' (PI 634974), 'Chisholm' (Smith et al., 1985), and 'Custer' (OK88767-11). Haplotype VI is thought to be an ancient deletion and is associated with *Aegilops tauschii* accessions and synthetic wheats (Guo et al., 2010). All seven entries we characterized as

haplotype VI have pedigree contributions from *Aegilops tauschii* or Eastern European germplasm. The Eastern European parentage might also include *Aegilops tauschii*, as exotic germplasm and interspecific crosses were introduced to this region during the 1970s and 1980s (Roussel et al., 2005) .

Additional alleles have also been described at *Ppd-A1* (Nishida et al., 2013). The photoperiod insensitive allele *Ppd-A1* was expected to be associated with earlier heading, but did not have a significant effect on heading date on this germplasm. We only characterized six entries as carrying *Ppd-A1a*, all of which originated from the southern plains. This region has less variation in day length than the other U.S. Great Plains regions, so photoperiod sensitivity is expected to have less of an effect, and photoperiod insensitivity to be more common, than at more northern latitudes. Guedira et al. (2014) found the photoperiod insensitive allele *Ppd-A1a.1*—first identified in Japanese germplasm—to be common among winter wheat varieties originating in the eastern U.S. (Nishida et al., 2013). It is likely the effect of *Ppd-A1a* on heading date would be significant among germplasm with greater diversity at this locus, such as a panel that included more entries from southern or eastern regions of the United States.

Our second objective was to estimate the allelic effects of known developmental genes on heading date. We found the timing of heading date in winter wheat from the U.S. Great Plains to be more strongly affected by photoperiod loci than the vernalization or reduced height loci. Allelic effects of three developmental genes (*Ppd-B1, Ppd-D1*, and *Rht-B1*) explained 23% of the variation in heading date (Table 2.6). However, terms included in the best-fit model varied slightly among environments (Table 2.7). Differential effects among environments suggest the model might be improved if the environmental effects

were deconstructed into multiple environmental variables (such as effects of temperature, moisture, day length, etc.). Furthermore, additional variation in number of days after 1 January to heading might be improved through genotyping and inclusion of other loci known to affect developmental timing, such as *Vrn-B3* (Yan et al., 2006; Griffiths et al., 2009).

In our study, *Rht-B1* was found to have a significant effect on heading date in most environments (Table 2.7). While it is unclear how reduced sensitivity to GA affects heading date, *Rht-B1* has been previously shown to have a small effect on heading date (Wilhelm et al., 2013a). Wilhelm et al. (2013b) suggest one possibility is the role of other genes that are tightly linked with *Rht-B1*, such as *Teosinte branched 1* (*TaTb1*), which is associated with tillering and fertility. Most of the genetic effects (21.2% of total phenotypic variation) were due to the main effects and interaction of *Ppd-B1* and *Ppd-D1.* While the genetic effects on heading date are small relative to the environmental effects (Table 2.6), we observed the presence of photoperiod sensitive alleles at both *Ppd-B1* and *Ppd-D1* delayed heading date by an average of 4.7 days across all environments (Table 2.7). Even minor variation in the timing of heading date—variation of several days—may allow for more precise targeting of wheat varieties to different regions, especially if climatic patterns shift within established growing regions (Lobell et al., 2011), or wheat cultivation expands to new regions (Schmitz et al., 2014).

The allelic effects of *Ppd-B1* and *Ppd-D1* varied among environments, suggesting differential expression under varying environmental conditions such as temperature, or moisture. The greatest significant effects—more than five days at each locus—were observed at Ar13R (Table S2.3). The effect of the interaction between these loci was 8.6

days at Ar13R (Table S2.4). Variation in the size and magnitude of allelic effect size does not follow a discernable trend among environments, however, the G×E term is likely affected by different sources of environmental variability, such as climatic conditions, management practices including planting and harvest dates, or biotic and abiotic stresses. For instance, heavy rainfall during May and June, and warm maximum daily temperatures throughout the winter, spring, and summer could influence the large allelic effects at Ar13R. Interaction between *Ppd-D1* and *Ppd-B1* has been previously characterized (Tanio and Kato, 2007), is known to vary with CNV at *Ppd-B1* (Eagles et al., 2011), and is known to have much larger effects in spring than winter wheat (Kiss et al., 2014).

Allelic variation associated with CNV at *Vrn-A1* had a significant effect on heading date in this germplasm (Table S2.3). Low copy number at *Vrn-A1* is associated with earlier flowering following a short vernalization period (Díaz et al., 2012). Copy number variation at *Vrn-A1* was inferred by SNPs on exons four and seven. The SNP on exon four is associated with having two or fewer copies of the gene, and was used by Eagles et al. (2011) to distinguish *Vrn-A1v* associated with earlier heading from *Vrn-A1w* associated with greater freezing tolerance. We found the effect of CNV at *Vrn-A1* to vary among environments with contrasting winter temperatures. The effect of CNV at *vrn-A1* was largest (4.7 days) at Ar13R (Table S2.3), and that environment experienced a mild winter that had daily highs above 0°C and only three days with lows below 0°C. The second largest significant effect was seen at Bu12R, which is also located in a mild, southern environment. By contrast, variation at *Vrn-A1* did not have a significant effect at It12R, which experienced a cold winter with 65 days—including 29 continuous days—that had lows below 0°C.

Our third objective was to investigate temporal and geographic patterns of allelic distribution in the U.S. Great Plains. Changes in allele frequencies at the photoperiod genes indicate selection for photoperiod insensitivity during the last century of winter wheat breeding (Table 2.3). This could be the direct result of selecting for improved adaptability based on flowering time (Slafer, 2012). Most strikingly, the proportion of entries carrying the *Ppd-B1b* sensitive allele has gradually declined over time, from 90% of entries derived before 1960 to only 48% of germplasm derived after 2000. An increase in varieties carrying the photoperiod insensitive allele suggests a contribution to greater adaptation to specific environments.

Selection against photoperiod sensitivity is also apparent in the distribution of alleles from breeding programs in different sub-regions of the U.S. Great Plains. We saw the incidence of photoperiod sensitive alleles decrease from northern to southern latitudes (Table 2.7). Various environmental variables, such as temperature and day length vary with latitude. Extreme temperature events such as freezing temperatures and heat stress can have very pronounced and detrimental effects on yield, especially during vulnerable developmental stages. Therefore, targeting heading date could reduce the chance that extreme temperatures or stress damage the developing reproductive structures.

Conclusions

In this study we used a panel of 299 hard winter wheat entries representative of modern and historic U.S. Great Plains germplasm to evaluate allelic diversity and effects of vernalization, photoperiod, and reduced-height loci on the timing of heading date. We found most of the genetic effects of heading date to be explained by *Ppd-B1, Ppd-D1*, and

the interaction between these loci. A smaller amount of variation was explained by *Rht-B1*. Across nine environments, the interaction between *Rht-B1* and *Ppd-B1* also had a small but significant effect on heading date. Both photoperiod sensitive and insensitive alleles were common for *Ppd-B1* and *Ppd-D1*, and an alternate photoperiod sensitivity allele associated with ancestral wheats was detected at *Ppd-D1*. There was limited allelic diversity at *Ppd-A1* and *Rht-D1*, and these loci did not have a significant effect on heading date. The presence of photoperiod sensitive alleles *Ppd-A1b, Ppd-B1b,* and *Ppd-D1b* has been decreasing over the past century of wheat breeding, and these alleles are less common in the southern sub-region of the U.S. Great Plains than either the central or northern plains. Our analyses enhance the understanding of roles developmental genes have on winter wheat under varying environmental conditions, which can potentially be used to improve breeding strategies for current and future climate scenarios.

Figures.

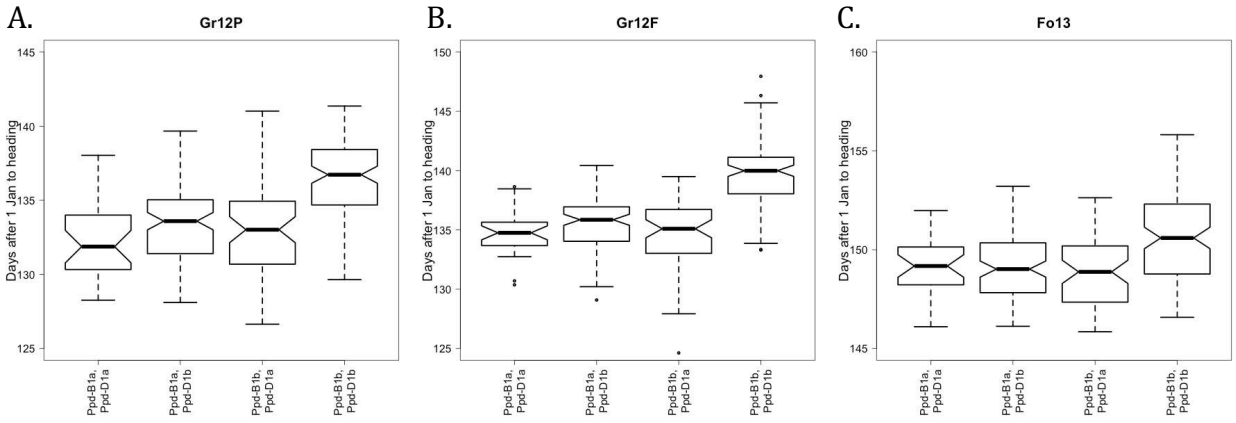

Figure 2.1. Box plot of number of days from 1 January to heading of 299 hard winter wheat entries varying for photoperiod sensitive (*Ppd-B1a, Ppd-D1a*) and insensitive alleles (*Ppd-B1b, Ppd-D1b*), evaluated in four Colorado environments. The box describes the minimum, lower quartile (25th percentile), median (50th percentile), upper quartile (75th percentile) values. The notch displays the 95% confidence interval around the median value, and if the notches don't overlap between two boxes on the same plot, there is strong evidence their medians differ. The interquartile range is described as the upper quartile minus the lower quartile. The whiskers extend to the most extreme data point that is up to 1.5 times the interquartile range from the median value. Outlying points that fall outside of this range are represented as dots. The environments are (A) partial irrigation at Greeley, CO in 2012 (Gr12P), (B) full irrigation at Greeley, CO in 2012 (Gr12F), and (C) Fort Collins, CO in 2013 (Fo13).

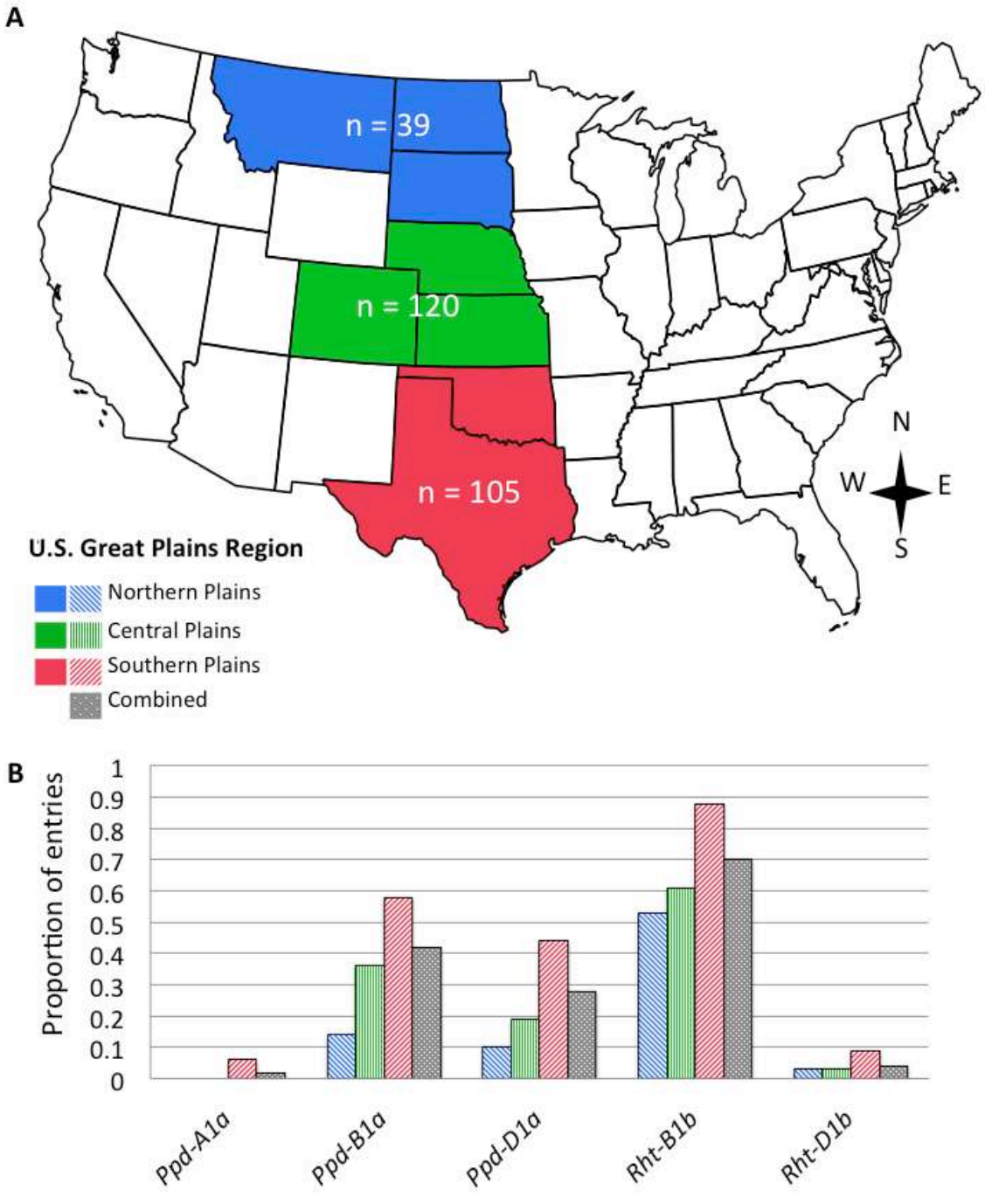

Figure 2.2. Frequency of photoperiod and reduced-height alleles in wheat entries from three regions of the U.S. Great Plains. (A) Geographic distribution of 264 winter wheat entries that originated from the northern (Montana, North Dakota, South Dakota, n = 39 entries), central (Nebraska, Colorado, Kansas, n = 120), or southern (Oklahoma, Texas, n = 105) U.S. Great Plains. (B) Proportion of wheat entries from each U.S. Great Plains sub-region, or combined across all three sub-regions, with the photoperiod insensitive alleles *Ppd-A1a*, *Ppd-B1a*, and *Ppd-D1a,* and semi-dwarf alleles *Rht-B1b*, *Rht-D1b.*

Tables.

Table 2.1. Description of environments. Environment abbreviation, location, moisture treatment, latitude and longitude, and planting and harvest dates.

Environment	Location	Moisture Treatment	Lat (°N)	Long (°W)	Planting Date	Harvest Date
Ar13R	Ardmore, OK	Rainfed	34.18	-97.09	12 Oct 2012	25 June 2013
Bu12R	Bushland, TX	Rainfed	35.18	-102.10	3 Nov 2011	10 June 2012
Fo13	Fort Collins, CO	Averaged across treatments†	40.65	-105.00	2 Oct 2012	18-22 July 2013‡
Gr12P	Greeley, CO	Partial irrigation	40.42	-104.71	19 Oct 2011	3 July 2012
Gr12F	Greeley, CO	Full irrigation	40.42	-104.71	19 Oct 2011	13 July 2012
Ha13R	Hays, KS	Rainfed	38.88	-99.33	10 Oct 2012	3 July 2013
It12R	Ithaca, NE	Rainfed	41.16	-96.43	4 Oct 2011	28 June 2012
It13R	Ithaca, NE	Rainfed	41.28	-96.41	25 Sept 2012	17 July 2013
Ma12	Manhattan, KS	Averaged across treatments	39.14	-96.64	18 Nov 2011	3 July 2012

† Averaged across treatments indicates that there were separate side-by-side rainfed and full-irrigation treatments that did not differ significantly for average heading date, so the treatments were treated as two replications.
‡ Rainfed treatment was harvested on 18 July 2013 and fully irrigated treatment was harvested on 22 July 2013.

Table 2.2. Description of multi-locus genotypes at photoperiod and reduced-height loci. The photoperiod (*Ppd*) alleles are '*a*' insensitive and '*b*' sensitive. The reduced-height (*Rht*) alleles are '*a*' tall wild type and '*b*' semi-dwarf. '*Het*' is heterozygous at the locus. Of 299 total entries, 286 entries have complete genotypic data at all five loci, and 280 have complete data with all homozygous allele calls.

n	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*
45	*b*	*b*	*b*	*a*	*a*
4	*b*	*b*	*b*	*a*	*b*
54	*b*	*b*	*b*	*b*	*a*
1	*b*	*b*	*b*	*het*	*a*
6	*b*	*b*	*a*	*a*	*a*
6	*b*	*b*	*a*	*a*	*b*
43	*b*	*b*	*a*	*b*	*a*
1	*b*	*b*	*a*	*het*	*a*
1	*b*	*b*	*a*	*het*	*het*
16	*b*	*a*	*b*	*a*	*a*
1	*b*	*a*	*b*	*a*	*b*
74	*b*	*a*	*b*	*b*	*a*
2	*b*	*a*	*a*	*a*	*a*
2	*b*	*a*	*a*	*a*	*b*
24	*b*	*a*	*a*	*b*	*a*
1	*a*	*b*	*b*	*a*	*het*
1	*a*	*b*	*a*	*a*	*b*
3	*a*	*a*	*b*	*b*	*a*
1	*a*	*a*	*b*	*het*	*a*

Table 2.3. Number of entries (n) derived during four different time periods, and the proportion of entries in each group with the photoperiod sensitive allele at *Ppd-A1, Ppd-B1*, or *Ppd-D1*.

Derivation Period	n	*Ppd-A1b*	*Ppd-B1b*	*Ppd-D1b*
Before 1960	19	1.00	0.90	0.85
1960-1979	34	1.00	0.67	0.85
1980-1999	114	0.99	0.58	0.71
2000 or later	132	0.96	0.48	0.65

Table 2.4. ANOVA table for two-gene model estimating the effects of *Rht-B1* and *Rht-D1* on heading date in a combined analysis across nine environments. A total of 285 entries with homozygous calls at both loci were included in the model.

Source of variation	df	Mean Square
Environment	8	55097***
Rht-B1	1	4321***
Rht-D1	1	702***
Environment**Rht-B1*	8	86.4***
Error	2546	13.6

*** indicates significance at the 0.001 probability level.

Table 2.5. Allelic effects (number of days) of *Rht-B1* in a model that estimated days after Jan.1 to heading in 285 winter wheat entries grown in nine environments. The environments are described in Table 2.1. The model terms were fit separately for each environment. The model effects included environment, *Rht-B1*, *Rht-D1*, and *Rht-B1*-by-environment interaction. The intercept (Int) describes the number of days from 1 January to heading in each environment before the allelic effects are applied. The allelic effect (number of days) at each locus is added to the Int value. The allelic effect of *Rht-D1b* was -2.59 days and did not have significant interaction with environment.

Env	**Int**	***Rht-B1b***
Ar13R	112.57	-4.89***
Bu12R	119.77	-4.32 ns†
Fo13	150.93	-2.02***
Gr12F	138.40	1.14***
Gr12P	136.47	3.83**
Ha13R	142.08	3.30***
Ma12	124.62	2.66**
It12R	125.49	3.90 ns
It13R	147.74	2.42**

, * indicates significance at the 0.01 or 0.001 probability level.
† ns indicates non-significance at the 0.05 probability level.

Table 2.6. ANOVA table for the best-fit model, considering effects of *Ppd-A1, Ppd-B1, Ppd-D1, Rht-B1, Rht-D1*, and their interactions on winter wheat heading date in a combined analysis across nine environments. A total of 280 entries with homozygous calls at each allele were included in the model.

Source of variation	**df**	**Mean Squares**
Environment	8	54154***
Ppd-D1	1	6333***
Ppd-B1	1	5847***
Rht-B1	1	519***
*Ppd-D1*Ppd-B1*	1	2754***
*Ppd-B1*Rht-B1*	1	220***
Environment**Ppd-D1*	8	218***
Environment**Ppd-B1*	8	208.1***
Environment**Rht-B1*	8	16*
Environment**Ppd-D1*Ppd-B1*	8	99.5***
Error	2474	7.1

*, *** indicates significance at the 0.05 or 0.001 probability level.

Table 2.7. Allelic effects (number of days) of gene-based terms included in the best-fit model for winter wheat heading date in each of nine environments, and the proportion of variability (R^2) in heading date explained by all terms in each model. The environments are described in Table 2.1. The model terms were fit separately for each environment. The intercept (Int) describes the number of days from 1 January to heading in each environment before the allelic effects are applied. The allelic effect (number of days) at each locus is added to the Int value.

Env	Int	*Ppd-D1b*	*Ppd-B1b*	*Rht-B1b*	*Ppd-D1b* Ppd-B1b*	*Ppd-D1b* Rht-B1b*	*Ppd-B1b* Rht-B1b*	R^2
Ar13R	105.46***	2.55*	-0.23 ns†	-0.10 ns	8.07***	-2.28 ns	--‡	0.58
Bu12R	115.78***	1.23 ns	-1.94 ns	-3.03***	6.69***	--	2.04*	0.59
Fo13	149.77***	-0.13 ns	-0.24 ns	-0.81**	1.33**	--	--	0.14
Gr12F	134.68***	0.80 ns	-0.07 ns	--	4.18***	--	--	0.41
Gr12P	133.56***	0.88 ns	0.44 ns	-1.38***	2.35**	--	--	0.31
Ha13R	140.22***	0.42 ns	0.18 ns	-0.49*	1.57***	--	--	0.32
It12R	121.71***	0.80 ns	-2.93**	-3.42***	8.21***	--	3.14***	0.64
It13R	145.77***	-0.21 ns	-0.36 ns	-1.35***	3.27***	--	--	0.31
Ma12	120.78***	0.89 ns	-1.83*	-1.87***	6.81***	--	2.24**	0.69

*, **, *** indicated significance at the 0.05, 0.01, and 0.001 probability levels, respectively.

† ns indicates non-significance at the 0.05 probability level.

‡ -- indicates term was not included in best-fit model.

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CHAPTER 3:

PHENOTYPIC PLASTICITY OF WINTER WHEAT HEADING DATE AND GRAIN YIELD ACROSS THE U.S. GREAT PLAINS[2]

Summary

Phenotypic plasticity describes the range of phenotypes produced by a single genotype under varying environmental conditions. We evaluated the extent of phenotypic variation and plasticity in thermal time to heading and grain yield in 299 hard winter wheat (*Triticum aestivum* L.) genotypes representative of the U.S. Great Plains, including both recent cultivars and breeding lines and older germplasm. The genotypes were evaluated in 11 environments in 2011/2012 and 2012/2013. The average number of days from 1 Jan.to heading across environments ranged from 109 to 150, and the cumulative growing degree-days (GDD) from 1 Jan. to heading from 730 to 1,112 °C·d. Environmental mean grain yield ranged from 1.3 to 5.3 Mg ha^{-1}. There was a strong positive correlation between plasticity of GDD (GDDP) and GDD (r=0.81, P<0.001), especially maximum GDD (r=0.90, P<0.001) across environments, indicating genotypes with a greater degree of plasticity developed later, especially within the earliest environments. Plasticity of GDD was negatively associated with yield (r=-0.58, P<0.001), and therefore detrimental in the

[2] A modified version of this chapter has been submitted as follows:
Grogan, S. M., J. Anderson, P. S. Baenziger, K. Frels, M. J. Guttieri, S. D. Haley, K.-S. Kim, S. Liu, G. S. McMaster, M. Newell, P. V. V. Prasad, S. D. Reid, K. J. Shroyer, G. Zhang, E. Akhunov, and P. F. Byrne. 2015. Phenotypic plasticity of winter wheat heading date and grain yield across the U.S. Great Plains. Crop Sci.

germplasm and environments evaluated. Yield plasticity was positively correlated with both maximum (r=0.80, P <0.001) and minimum (r=0.33, P<0.001) grain yield across environments, indicating greater plasticity was favorable under optimal conditions, without a penalty under low-yielding conditions. More than a century of wheat breeding in this region suggests GDDP has declined and yield plasticity has increased at similar rates. This is encouraging because it indicates the favorable plasticity traits (high yield plasticity, low GDDP) have been selected for indirectly, and would respond to further selection.

Introduction

Breeding crops for variable environmental conditions is inherently complex. Comstock and Moll (1963) described genotype-by-environment interaction (G×E) as occurring when "the phenotypic response to a change in environment is not the same for all genotypes." G×E results in a variable phenotype, which thereby decreases the correlation between genotype and phenotype and reduces the effectiveness of empirical selection (Comstock and Moll, 1963). G×E also makes it difficult to recommend cultivars that will be best-adapted or highest-yielding in an untested environment (Eberhart and Russell, 1966; Romagosa and Fox, 1993). Variation in genotype performance can be caused by heterogeneous environmental variances, or by a lack of correlation among environments, contributing to genotype rank changes (Lefkovitch, 1985), also known as crossover interaction (Baker, 1988).

The extent of G×E can be estimated using many different stability parameters (Eberhart and Russell, 1966; Lin et al., 1986; Cooper and DeLacy, 1994), and the choice of method can greatly affect the ranking of genotypes (Eskridge, 1990). These methods

include partitioning genotype-by-environment interaction using combined analyses of variance (Muir et al., 1992); indirect selection using genetic correlations (Itoh and Yamada, 1990), for instance, indirect selection for production in low-performing environments using production in high-performing environments (Atlin and Frey, 1990); and pattern analysis including classification and ordination methods to group similar environments (Crossa et al., 1990; DeLacy and Cooper, 1990). A comparison of many different statistical approaches to analyzing G×E has been described by Flores et al. (1998).

Plant breeders carefully characterize their target population of environments (TPE) and choose distinct breeding goals with the biotic and abiotic pressures of their region in mind (Chenu et al., 2011). Multi-environment trials within the TPE typically experience a range of environmental conditions that vary among locations and years, which contributes to G×E and can make genotypic selections difficult (Kang and Magari, 1996).

According to Becker and Leon (1988), T. Roemer in 1917 was the first to use the term 'stability' in describing a genotype with a constant yield under any environmental conditions. There are two types of stability that relate to minimizing G×E (Becker and Leon, 1988). The traditional biological concept of static stability refers to a genotype that has the same level of phenotypic expression under all environmental conditions. The agronomic concept of stability, also known as ecovalence, is more relevant to a breeding context, and refers to a dynamic stability where the phenotypic responses vary in a predictable way among environments but all genotypes are affected equally (Becker, 1981). While G×E is often viewed as detrimental to breeding progress, and an element that should be minimized or avoided, an alternative approach is to harness the variability hidden within these interactions by understanding the range of possible phenotypic responses.

Phenotypic plasticity describes the range of phenotypes produced by a single genotype under varying environmental conditions (Bradshaw, 1965). Likewise, the concept of plasticity also encompasses different genotypes that assume the same phenotype, which allows a population to maintain genetic diversity while exhibiting a similar level of phenotypic expression. Plasticity and stability are inversely related, such that a high level of plasticity indicates low stability, and vice versa. High levels of phenotypic plasticity and low levels of stability both indicate a strong effect of G×E.

The extent of plasticity could be driven by genetic factors, such as single nucleotide polymorphisms within genic regions, or variation caused by duplication and polyploidy (Schlichting, 2008). Genome duplication resulting in gene redundancy and copy number variation could impact the level of transcripts produced and facilitate more continuous phenotypic responses under variable conditions (Osborn et al., 2003; Zmienko et al., 2014). Epigenetic variation, including DNA methylation and histone modification that affect gene expression may also have an effect on phenotypic plasticity (Kalisz and Purugganan, 2004; King et al., 2010; Bloomfield et al., 2014).

The extent of plasticity varies among genotypes, environments, and traits (Bradshaw, 1965); and can have adaptive, maladaptive, or neutral effects on fitness (Des Marais et al., 2013). In a recent meta-analysis of transplant studies, Palacio-López et al. (2015) identified plastic phenotypic responses in fewer than half of the traits evaluated, which suggests phenotypic plasticity may be less common than previously thought. Traits and their plasticities are interrelated and adjusting the plasticity of one trait can influence others. Plasticities of different traits can be ranked into a hierarchy describing the tradeoffs that maximize genetic fitness (Bradshaw, 1965). Hierarchies can provide a useful

framework for understanding conditional relationships among traits (Sadras and Slafer, 2012; Alvarez Prado et al., 2014), such as the well-characterized relationship between variability of grain number and stability of grain size in grain crops (Sadras, 2007). Relationships among trait plasticities can provide insight into environmental responses of related traits. For instance, Sadras and Rebetzke (2013) identified a significant positive relationship between the plasticities of wheat grain yield and spike number in free- and reduced-tillering sister lines, even though the grain yield and spike number *per se* were not associated. This suggested yield stability was partially driven by the ability of tiller and spike production to respond to environmental stimuli, and indicated that the genetic basis of trait plasticity can be independent of the trait itself.

The relative advantage or disadvantage of phenotypic stability or plasticity varies among traits, environments, and germplasm evaluated. To assess the benefit or cost of plasticity, the relationship between trait plasticity and grain yield *per se*, as well as other traits associated with grain yield, should be considered. Plasticity of crop developmental traits, such as heading date in small grain cereals, is of special interest when breeding for changing and increasingly unpredictable environmental conditions. Heading signifies the development of delicate reproductive structures, which can be damaged by harsh environmental conditions. Developing too early may increase risk from frost or cold damage, but developing late can expose the plant to heat or drought stress during flowering or grain filling that may limit reproductive viability and yield potential (Cockram et al., 2007; Kamran et al., 2014).

A more complete analysis of phenotypic plasticity will allow deeper understanding of crop adaptation, including trade-offs between traits. Therefore, our specific research objectives were to

i) Assess variation in thermal time to heading and grain yield within a collection of 299 hard winter wheat genotypes representative of the U.S. Great Plains grown in 11 environments;

ii) Estimate plasticity of time to heading and grain yield and determine whether a high level of phenotypic plasticity is favorable or detrimental based on relationships to grain yield;

iii) Determine whether greater plasticity is most strongly associated with high, low, or mean values of accumulated thermal time at heading, or grain yield at harvest.

Materials and methods

Germplasm

Two hundred and ninety-nine winter wheat genotypes comprising the Triticeae Coordinated Agricultural Project (http://www.triticeaecap.org) hard winter wheat association mapping panel were used for this study (Supplemental Table S3.1). The genotype 'Wichita' (CI 11952) was included in the panel twice for a total of 300 experimental entries. The germplasm included 280 mostly recent cultivars and experimental breeding lines representative of the U.S. Great Plains and older cultivars derived in 1960 or earlier. The genotypes were developed by breeders in 10 public and four private breeding programs across the Great Plains. Composition of the panel reflects prevalent germplasm of the region, and genotypes with a prevailing presence in the

pedigrees of modern cultivars. Most of the genotypes have semi-dwarf stature, red grains, and awned spikes.

Environments

The germplasm was grown in a total of 11 environments across the U.S. Great Plains: six environments in 2011/2012 (hereafter referred to as 2012) and five in 2012/2013 (hereafter 2013) (Table 3.1). Only Ithaca, NE was repeated in both years. Each environment was a unique combination of location, year, and moisture treatment. The three moisture regimes were full irrigation, partial irrigation, and rainfed (no supplemental irrigation). Partially irrigated environments were always paired with a fully irrigated counterpart grown at the same location. Partial irrigation was applied to prevent total crop loss, and was less frequent and at lower levels than full irrigation (Supplemental Table S3.3). The environments were as follows: rainfed at Ardmore, OK in 2013 (Ar13R), rainfed at Bushland, TX in 2012 (Bu12R), rainfed (Fo13R) and fully-irrigated (Fo13F) using linear overhead sprinklers at Fort Collins, CO in 2013, partially (Gr12P) and fully irrigated (Gr12F) using drip irrigation at Greeley, CO in 2012, rainfed at Hays, KS in 2013 (Ha13R), rainfed at Manhattan, KS in 2012 (Ma12R), fully-irrigated using linear sprinkler irrigation at Manhattan, KS in 2012 (Ma12F), and rainfed at Ithaca, NE in 2012 (It12R) and 2013 (It13R). The trials at Ithaca, NE are described in detail by Guttieri et al. (2015).

Eight environments (Bu12R, Fo13R, Fo13F, Gr12P, Gr12F, Ha13R, It12R, and It13R) were arranged in a row–column design with two check varieties. The experimental genotypes were unreplicated at six environments (Bu12R, Fo13R, Fo13F, Gr12P, Gr12F, Ha13R), while It12R and It13R had four replications. Ten percent of the field plots were

allotted to systematically placed common and local checks. The common check for these eight environments listed above was 'Settler CL' (Baenziger et al., 2011). For It12R and It13R the local check was 'Jagger' (Sears et al., 1997). Bu12R, Fo13R, Fo13F, Gr12F, Gr12P, and Ha13R all used 'Hatcher' (Haley et al., 2005) as the local check. The Manhattan, KS environments (Ma12R and Ma12F) had two replications arranged as incomplete blocks with a single check variety: locally adapted 'Everest' (http://kswheatalliance.org/varieties/everest/). Ar13R was arranged as a randomized complete block design with two replications.

Phenotypic evaluation

Heading date was determined for each plot using the Zadoks scale (Zadoks et al., 1974) at stage 59, when the spike had fully emerged from the flag leaf sheath in at least 50% of all shoots in the plot. In It12R and It13R, anthesis was recorded instead of heading, so heading date was imputed for each genotype from the average differences between heading and anthesis dates. Heading and anthesis dates were both recorded only at six locations (Gr12P, Gr12F, Fo13R, Fo13F, Ma12R, and Ma12F). In three environments (Ar13R, Bu12R, Ha13R) only heading dates were recorded. Anthesis dates at It12R were most similar (as evaluated by principal components analysis (PCA) and Pearson's correlation coefficients, data not shown) to the anthesis dates averaged across Gr12P, Gr12F, Ma12R and Ma12F, so these environments were used to estimate heading dates at It12R. Heading and anthesis dates are very strongly related, and the phenotypic correlations between these traits were $r = 0.87$ at Gr12P, $r = 0.78$ at Gr12F, $r = 0.78$ at Ma12R, and $r = 0.78$ at Ma12F (all $P < 0.001$).

For each genotype, the average difference between anthesis and heading dates was subtracted from the anthesis date at It12R to estimate heading date. Anthesis dates at It13R were most similar to average anthesis dates at Gr12P and Gr12F. Consequently, heading dates at It13R were estimated for each genotype from the average difference between anthesis and heading dates at Gr12P and Gr12F. Imputing heading dates at It12R and It13R using these methods introduces a small amount of bias to the analyses, however, the difference between heading date and anthesis is minor, and including these environments enhances our understanding of G×E. Days to heading were calculated as the number of days between 1 Jan. and the heading date.

Thermal time to heading was calculated for each genotype in each environment using climatic data from nearby or on-site weather stations. The cumulative growing degree-days to heading (GDD) were calculated from daily minimum and maximum daily temperature ($Tmin_i$ and $Tmax_i$, °C) of n days between 1 Jan. and the heading date for each genotype, using a base temperature ($Tbase$) of 0 °C for winter wheat (McMaster and Wilhelm, 1997), as follows in Eq. 3.1:

$$\text{GDD}\ (^\circ\text{C} \cdot \text{d}) = \sum_{i=1}^{n} \frac{\text{Tmax}_i + \text{Tmin}_i}{2} - \text{Tbase}, \qquad \text{GDD} \geq 0 \qquad \text{(Eq. 3.1)}$$

Yield (kg ha^{-1}) was calculated from four-row field plots at It12R and It13R and six-row plots for all other environments, harvested by combine after plants had fully matured. The harvested plot area varied among environments: 2.2 m^2 for Bu12R, 2.6 m^2 for Ma12R and Ma12F, 3.6 m^2 for Colorado environments (Fo13R, Fo13F, Gr12P, Gr12F), 4.5 m^2 for It12R and It13R, and 4.6 m^2 for Ha12 and Ar13R.

Data analysis

Best linear unbiased predictions (BLUPs) were calculated separately for each trait and environment based on spatial patterns and field design. Six different spatial correlation models (row–column, spherical, exponential, power, anisotropic power, and Matérn) with different covariance structures were considered for environments with unreplicated row-column designs (Bu12R, Fo13R, Fo13F, Gr12P, Gr12F, Ha13R) using the PROC MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). The models are described in Supplemental Text S3.1 (Littell et al., 2006; Table 58.14, SAS/STAT 9.3 User's Guide, http://support.sas.com/documentation/cdl/en/statug/63962/HTML/default/viewer.htm #statug_mixed_sect020.htm, accessed 14 Aug. 2015). In all models, the genotypes, rows, and columns were treated as random effects. The best model was chosen based on the Akaike information criterion (AIC) fit statistic. For environments with multiple replications (Ar13R, Ma12R, Ma12F, It12R, It13R), replications were treated as a random effect. In the combined analyses location and year were treated as random effects.

To estimate the repeatability of trait values (heading date and grain yield) we evaluated the Pearson's correlation coefficient for environments with multiple replications (Ar13R, Ma12F, Ma12R, It12R, and It13R). Correlations were estimated between raw plot-level measurements of each genotype measured in different replications within the same environment. The average correlation across replicated environments was greater for heading date (r = 0.76) than for grain yield (r = 0.41, Supplemental Table S3.2), reflecting the stronger genetic control of heading date, and sensitivity of yield to small-scale spatial variation. Therefore, we expect a high level of repeatability among estimates of heading

date, and moderate repeatability among estimates of grain yield used in estimates of phenotypic plasticity.

Phenotypic plasticity was evaluated as described previously (Lacaze et al., 2009; Sadras et al., 2009). Briefly, the Finlay and Wilkinson (1963) stability parameter was estimated for each genotype as the regression coefficient of the linear regression of the genotype mean in each environment on the grand mean of each environment, using all 11 environments. The first use of this parameter to estimate adaptability or plasticity separate from stability was by Kraakman et al. (2004). Our analyses were conducted on BLUPs in SAS using the PROC REG procedure. A regression coefficient of 1.0 indicates average stability, following the dynamic stability concept (Becker, 1981), such that the trait value might differ among environments, but remains the same relative to the environmental mean. In this paper we will refer to the Finlay and Wilkinson (1963) stability parameter as a 'plasticity coefficient,' which follows terminology of Sadras et al. (2009).

Plasticity coefficients greater than 1.0 reflect above average phenotypic plasticity, whereas coefficients less than 1.0 reflect below average plasticity. The original work of Finlay and Wilkinson (1963) can be expanded to generalize that a genotype with average yield plasticity will be well-adapted to average-performing environments, while above-average plasticity can provide a dynamic response that is especially favorable in high-yielding environments, and below-average plasticity might be advantageous in low-yielding environments.

The plasticity coefficients were regressed on trait values (GDD or yield) in individual environments, average trait values across environments, and the minimum or maximum trait value for each genotype across all environments. The minimum and

maximum trait values were the earliest and latest heading dates, and lowest and highest yields, observed for each genotype across all environments. To assess whether linear models were adequate for describing a genotype's response to environment, the significance of quadratic terms was also tested using SAS PROC REG.

All other univariate and multivariate statistical analyses were conducted on BLUPs using the software JMP (version 11, SAS Institute Inc., Cary, NC). These include analyses of variance, Student's t-test, phenotypic correlations, linear regression, and PCA. Figures were generated in JMP and R (version 3.1.3, R Development Core Team). Data were analyzed both for individual environments and combined across environments.

Results and discussion

Overview of heading date data

For most unreplicated environments the best spatial adjustment models for heading date were the power model (Fo13F, Fo13R, and Gr12P) or the anisotropic power model (Gr12F and Ha13R). The row–column model was best for Bu12R. Heading date varied significantly among environments ($P < 0.001$) and ranged from a minimum of 109 d after 1 Jan. in Ar13R to a maximum of 150 d in Fo13F (Fig. 3.1, Table 3.2). The interaction between genotype and environment also had a significant ($P < 0.001$) effect on heading date. The grand mean across all environments was 132 d, or about May 12. Germplasm developed significantly earlier in 2012 than 2013 ($P < 0.001$), with average heading dates of May 5, 2012 and May 19, 2013. The hot temperatures in 2012 during the spring likely influenced earlier heading. Some unusual trends were observed for Ar13R, which developed more

quickly than other 2013 environments and was the earliest environment overall. Ar13R was much warmer than the other 2013 environments and had temperatures comparable to those of 2012 (Supplemental Figure S3.1). After excluding Ar13R the average difference between years was 21 d. Despite the earliness, heading dates at Ar13R were similar to and highly correlated (r = 0.88, P < 0.001) with Bu12R, the geographically closest site.

Growing degree-days from 1 Jan. to heading ranged from an average of 730 GDD in It13R to 1,112 GDD in Ma12R (Table 3.2). The grand mean of GDD across all germplasm and environments was 920 GDD. The 299 genotypes had means across environments that ranged from 813 to 1,067 GDD. Rapid accumulation of GDD occurs when the daily maximum and minimum temperatures are elevated, and hot temperatures are known to accelerate the developmental sequence in wheat (McMaster and Wilhelm, 2003). The 2012 season had an early spring and many hot days, so these environments accumulated GDD more rapidly than those in 2013. The effect of year on GDD was significant (P < 0.001), with an average effect of 161 °C·d more accumulated by heading in 2012 environments compared with 2013 environments. However, earlier heading dates in 2012 could also be influenced by low water availability, caused both by low precipitation (Table 3.2) and high evaporative demands from hot temperatures (Supplemental Figure S3.1). Water limitation is also known to affect the developmental sequence in wheat by altering the timing and interval between developmental stages, such that stages can occur sooner and their duration can be shorter (Frank et al., 1987; Nielsen et al., 2003; McMaster and Wilhelm, 2003; McMaster et al., 2005, 2008).

There were strong positive correlations between days to heading and GDD at each of the 11 environments (r = 0.99–1.00, P < 0.001), and pooled across all environments (r =

1.00, $P < 0.001$). The near-perfect correlation occurred because GDD were summarized at each environment on a daily basis from minimum and maximum temperatures. Because of the strong correlations between days to heading and GDD, most subsequent analyses were conducted only using GDD data, which were very similar to, but less variable across environments, than the number of days. The strong relationship indicates genotypes or environments that develop more slowly have additional days to accumulate GDD and rapid accumulation of thermal time triggers earlier heading. This result is consistent with our understanding of phenological development as a function of accumulated thermal time (Kirby, 1995; McMaster, 1997), such as through fulfillment of vernalization requirements (Yan et al., 2003).

Correlations of GDD across environments

There was widespread variation in GDD among environments, but GDD were strongly correlated between pairs of environments (average $r = 0.67$, $P < 0.001$, Supplemental Table S3.4), especially for environments grown in 2012 (average $r = 0.78$). This is likely due to the strong genetic effects influencing development and flowering time, such as major genes involved in the vernalization and photoperiod response pathways (Distelfeld et al., 2009). The strongest correlations for GDD were observed between It12R and Ma12R ($r = 0.90$, $P < 0.001$) and between It12R and Bu12R ($r = 0.90$, $P < 0.001$, Supplemental Table S3.4).

The strong correlations of GDD between all pairs of environments make PCA a useful tool for investigating relationships among environments. Most of the variation (71%) in the PCA of GDD (Fig. 3.2) was explained by a single principal component. This

may reflect large differences in temperature patterns or other environmental variables among environments. The PCA score plot (Fig. 3.2a) displays genotypes as a tight cloud of points, which demonstrates similar patterns among all germplasm. Points that are close to each other represent genotypes that performed most similarly. Points that fell near y = 0 represent stable genotypes with similar GDD across environments, because the environments primarily vary along the y-axis. Variation in genotypes along the x-axis reflects a more variable phenotypic response.

The loading plot (Fig. 3.2b) represents environments as vectors. All vectors fall in quadrants I and IV, illustrating that GDD is positively correlated between all pairs of environments. The minimal spread of vectors shows gradual variation among environments, with slight distinctions between 2012 and 2013 environments. The 2012 environments are more tightly grouped than the 2013 environments, likely because there was a wider range of environmental conditions in 2013. Between April and June 2012 the Palmer Z Index classified much of the U.S. Great Plains as experiencing moderate-to-extreme drought (http://www.ncdc.noaa.gov/sotc/drought/). During spring and early-summer (April to June) of 2013 the Palmer Z Index classified the same regions with a broader range of conditions, from very moist to extreme drought conditions. The greatest distinctions overall were between Fort Collins, CO (Fo13F and Fo13R) and Manhattan, KS (Ma12R and Ma12F), which also share low correlations (Supplemental Table S3.4). The Manhattan environments were consistently hotter and wetter than Fort Collins environments. The short vector representing Ha13R falls between Fo13R and Gr12F. This suggests that although GDD at Ha13R are similar to and strongly correlated with some environments, it has lower average correlations with environments overall. This indicates a

stronger G×E associated with Ha13R. The variation among environments, especially the large spread between locations and years, highlights challenges in breeding for diverse environment types.

Plasticity analyses of GDD

The Finlay-Wilkinson stability parameter for GDD was obtained for each genotype from the linear regression of the genotype's phenotypic BLUP on the environmental mean for each environment, and used as an indicator of phenotypic plasticity. Eight of the 299 genotypes deviated from a purely linear model. For these genotypes, the quadratic term of the environmental mean GDD was also significant ($P < 0.05$). This low proportion (< 3%) of genotypes deviating from linearity is similar to the rates observed by Sadras et al. (2009) for other phenological traits in wheat. Because deviations from linearity were rare in our germplasm, and the quadratic term was not significant across genotypes, the linear method of estimating phenotypic plasticity was considered appropriate.

The average plasticity coefficient (slope estimate) of all genotypes, by definition, is 1.00. The plasticity coefficients in our study ranged from 0.68 to 1.41 for GDD (Supplemental Table S3.1), indicating a broad range of responsiveness to environmental conditions. Growing degree-day plasticity (GDDP) was affected by the year each genotype was derived. Overall, older genotypes had higher levels of GDDP than more modern varieties. When all 299 genotypes are included in the analysis, the plasticity coefficient changed by a rate equal to a reduction of 0.028 every 10 years l (Fig. 3.3a). Two historic cultivars ('Kharkof' and 'Turkey Nebsel') appear as possible outliers but only have a minor

effect on the regression line. Excluding these two genotypes, the slope of the line increases slightly, to a rate of change equal to a reduction of 0.029 every 10 years.

The 19 genotypes derived in 1960 or earlier had an average GDDP coefficient (± standard error) of 1.11 ± 0.04, compared with mean values of 0.99 ± 0.01 for genotypes derived after 1960. Many of the more recently developed genotypes with low GDDP were developed in Oklahoma. This could be because a substantial amount of winter wheat in Oklahoma is grown for forage or for dual forage and grain use, and these genotypes have higher levels of heterogeneity than those developed strictly for grain. This could result in a wider range of heading dates, which is characterized by greater stability of GDD (and low GDDP) across environments.

Additionally, since heading was measured in each plot based on when 50% of the spikes had fully emerged from the flag leaf sheath, GDDP could be affected by genotypic differences in tillering, which was not evaluated. Environments that facilitate greater tillering might have a later heading date because spikes on secondary and tertiary tillers usually develop later than those on the main stem (McMaster, 2005).

Growing degree-day plasticity had strong positive correlations with average GDD in 2012 ($r = 0.86$, $P < 0.001$), 2013 ($r = 0.63$, $P < 0.001$), and combined years ($r = 0.81$, $P < 0.001$, Fig. 3.4). There were large differences in the magnitude of correlations between GDDP and maximum ($r = 0.90$, $P < 0.001$, Supplemental Figure S3.2a) and minimum ($r = 0.40$, $P < 0.001$, Supplemental Figure S3.2b) GDD across environments. This suggests that the environment with maximum GDD had the largest impact on GDDP of each genotype. The environment with maximum GDD varied among genotypes, but was a combination of several environments: Ma12R (n = 181 genotypes), Ar13R (n = 61), Ma12F (n = 48) and

Bu12R (n = 9). These four environments also had the earliest average heading dates (Table 3.2). Because of the robust association between GDD and days to heading, the strong positive correlation between GDDP and maximum GDD also translates to a strong positive correlation between GDDP and earliest heading date (r = 0.81, P < 0.001). Growing degree-day plasticity explained 66% of variability in average GDD (Fig. 3.4).

Overview of yield data

The best spatial adjustment models for grain yield in the unreplicated environments were the anisotropic power model (Bu12R, Fo13R, Fo13R, and Gr12F) or the power model (Gr12P and Ha13R). Yield varied significantly among environments (P < 0.001) and between years (P < 0.001). The interaction between genotype and environment was also significant (P < 0.001). Mean yield ranged from 1,332 kg ha^{-1} in Bu12R to 5,239 kg ha^{-1} in It13R (Table 3.2). There were also high average yields at Fo13F (4,958 kg ha^{-1}) and Gr12F (4,782 kg ha^{-1}). Gr12F also experienced the greatest range of yields across genotypes. Interestingly, the strongest correlation for yield among any pair of environments was between Gr12F and Bu12R (r = 0.67, P < 0.001, Supplemental Table S3.4), despite the low average yield at Bu12R. The strong correlation reflects similar ranking of genotypes at Gr12F and Bu12R, and indicates underlying similarities between the environments. The precise control of irrigation at Gr12F using drip irrigation drove a diverse yield response among genotypes that was at least partially paralleled in Bu12R.

The average correlation of yield among pairs of environments was low (r = 0.00–0.67, average 0.27), but slightly greater among 2012 environments (r = 0.08–0.67, average 0.35) than 2013 environments (r = 0.06–0.44, average 0.22, Supplemental Table S3.4). The

lower correlations among sites grown in 2013 reflect a broader range of environmental conditions, including greater diversity of the timing, severity, duration, and type of stresses. Despite the wide range of grain yields observed across environments, variation among environments is gradual and there are trends among similar environments (Supplemental Figure S3.3). The score plot (Supplemental Figure S3.3a) shows that genotypes are spread widely across the first two principal components, indicating variation in yield and yield stability. Compared with GDD, much less variation in yield (37%) is explained by the first principal component, and the vectors representing environments are shorter (Supplemental Figure S3.3b), indicating greater GxE.

Interestingly, similarities among environments (identified by high phenotypic correlations) are not explained by high- or low-yielding environments (Table 3), or by year (Supplemental Table S3.4). The strongest correlation was observed between Gr12F and Bu12R, which had strikingly different average yields: Gr12F had an average of 4,782 kg ha^{-1}, while Bu12R had an average of 1,332 kg ha^{-1}. Greater variability of yield across environments is consistent with the quantitative nature of—and lack of known stable major-effect genes for—grain yield. Although correlations for grain yield between most pairs of environments were positive, some correlations with grain yield at Ar13R or Fo13F were not significant. Lack of correlation between grain yield at Ar13R or Fo13F suggests these environments were much different from the others, possibly due to greater available soil moisture. Ar13R received the most precipitation overall and Fo13F was heavily supplemented with irrigation (Supplemental Table S3.3)

Plasticity analyses of yield

Yield plasticity, as estimated by the Finlay and Wilkinson (1963) stability parameter, had a strong positive correlation with maximum (r = 0.80, P < 0.001; Supplemental Figure S3.4a) and average (r = 0.69; P < 0.001; Supplemental Figure S3.4b) grain yield, and a weak positive correlation with minimum grain yield (r = 0.33, P < 0.001; Supplemental Figure S3.4c). This suggests a high level of yield plasticity is primarily affected by maximum yield across environments, and that genotypes with high yield plasticity have high yield potential under favorable conditions. This is consistent with original observations by Finlay and Wilkinson (1963). The relatively low, but still positive, correlation with minimum grain yield indicates that plasticity is not associated with a yield penalty under sub-optimal conditions. This is an important consideration since much of the winter wheat produced in the U.S. Great Plains is grown under rainfed conditions. Ar13R is unique in being the only environment with a negative association between yield plasticity and grain yield (Table 3.3). This is likely due to lack of similarities in yield at this environment and others (Supplemental Table S3.4, Supplemental Figure S3.3), possibly influenced by the heavy rainfall in Ardmore, OK during May 2013 (Supplemental Table S3.3).

Our results contrast with findings by Sadras et al. (2009), who identified high yield plasticity in wheat as a negative trait because it was associated with low grain yield in low-yielding conditions. However, there are substantial differences in the two studies. Our environments include more locations over a broader geographic area and the 299 wheat genotypes we evaluated are more genetically diverse than the recombinant inbred lines described in the previous study. Sadras et al. (2009) reported extreme variation in grain yield (0.6–7.8 t ha^{-1}) among the six environments and 169 genotypes evaluated, indicating

some extremely low-yielding trials that likely had a large impact on yield plasticity. The positive relationship we identified between yield plasticity and grain yield is likely influenced by continuous range of yields across environments, broader range of yield responses and yield plasticities among germplasm (our yield plasticities ranged from 0.64 to 1.37, compared with 0.74 to 1.27 by Sadras et al.), and greater genetic diversity. Our genotypes were adapted to the U.S. Great Plains, for which our environments were representative, and the genetic diversity may have included multiple sources for plasticity.

There was a trend between yield plasticity and the year each genotype was derived. Yield plasticity increased at a rate equivalent to an increase of 0.030 every 10 years (Fig. 3.3b). This was similar to, but in the opposite direction as, the rate of change observed for GDDP, which was equivalent to a decrease of 0.028 every 10 years. Excluding Kharkof and Turkey Nebsel from the analysis of yield plasticity altered the rate of change to the equivalent of an increase of 0.034 every 10 years (Fig. 3.3b). The 19 genotypes derived before 1961 had average yield plasticities (± standard error) of 0.83 ± 0.03, compared with 1.01 ± 0.01 for those derived in 1961 or later. Excluding all 19 genotypes did not have an effect on the rate of change for yield plasticity, which maintained an increase equivalent to 0.030 every 10 years. However, excluding the genotypes derived before 1961 increased the rate of change of GDDP, which was equivalent to a decrease of 0.040 every 10 years. Since high yield plasticity is associated with greater maximum yield, this suggests plant breeders have been selecting germplasm with increasingly greater yield responses under favorable conditions.

Relationships between GDD and yield

Growing degree-days to heading and grain yield were strongly and negatively correlated in most environments (Table 3.4). Growing degree-days increase linearly with temperature, so more GDD are accumulated each day under hot than cool conditions. Since GDD accumulate rapidly under hot conditions, fewer GDD at heading suggest milder temperatures during vegetative growth stages, which is characteristic of more favorable and higher-yielding environments. Low temperatures result in a longer grain filling duration than high temperatures, and although high temperatures increase grain filling rates, higher yields are usually achieved under lower temperatures (McMaster, 1997). This is one way in which high temperatures are damaging to wheat yield, and explains the strong negative correlation between GDD and yield.

Lower GDD was associated with higher grain yield in eight environments, lower yield at one environment (Ar13R), and did not have significant correlations with grain yield at Fo13F or It12R (Table 3.4). The lack of correlation at Fo13F might indicate that alleles contributing a fitness advantage in many environments did not have an effect under favorable, fully irrigated, conditions. While it is unclear why greater GDD was associated with higher grain yield at Ar13R, unusual trends are also observed in the PCA of GDD (Fig. 3.2) and yield (Supplemental Figure S3.3b). The loading plot for PCA of GDD (Fig. 3.2b) displays Ar13R as a short vector, indicating different patterns of G×E at this environment, while the loading plot for PCA of yield (Supplemental Figure S3.3b) projects Ar13R near Fo13F—indicating similarities among yield between these environments—but far from vectors representing other environments. This could be partially influenced by the substantial precipitation Ar13R received during early grain filling, totaling more than 200

mm in May (Supplemental Table S3.3). Ar13R received an additional 235 mm precipitation in June and July. All of the irrigation received at Fo13F, which totaled 220 mm, also occurred during grain filling.

Growing degree-day plasticity was most strongly associated with minimum yield across environments (r = -0.58, P < 0.001; Fig. 3.5a), and also had significant negative correlations with average (r = -0.48, P < 0.001; Fig. 3.5b) and maximum (r = -0.44, P < 0.001; Fig. 3.5c) grain yield. This indicates high GDDP is detrimental among the germplasm and environments evaluated. The negative relationship between GDDP and yield is probably related to the escape mechanism of drought tolerance (Blum, 2011), by which early flowering plants escape severe stress during their reproductive phase. We observed two competing mechanisms: plants headed around the same calendar day in all environments (high GDDP), or after a similar amount of thermal energy (low GDDP). High GDDP genotypes showed lower grain yield because these genotypes were unable to respond to environmental cues and carry out their reproductive processes before the onset of severe stresses. The negative effects were especially severe in the lowest-yielding environment, likely because stresses were more extreme, leading to reduced reproductive viability or grain filling duration. Spikelet fertility was reduced, grain filling duration was diminished, and heading, anthesis, and physiological maturity dates were earlier in Gr12P than Gr12F, and in Fo13R than Fo13F (data not shown).

Plasticity of GDD explained 34% of the variation in minimum grain yield, 23% of average grain yield, and 19% of maximum grain yield across environments (Fig. 3.5). In particular, the correlations between GDDP and grain yield were strongly negative at Bu12R (r = -0.61, P < 0.001) and Gr12F (r = -0.53, P < 0.001). The strong correlation between

GDDP and yield at Bu12R highlights the influence of the lowest-yielding environment—93% of the genotypes had their lowest yields at Bu12R, while the others had lowest yields at Ar13R. The Bu12R, Gr12F, and It13R environments were more similar for grain yield as shown by both pairwise correlations (Supplemental Table S3.4) and PCA (Supplemental Figure S3.2). Similarities among Bu12R, Gr12F, and It13R suggest shared environmental conditions, and that some environmentally-responsive genes affecting yield could have similar effects in all three environments, even if there is significant GxE.

Since there are significant relationships between GDDP and grain yield, it is important to consider association with yield plasticity. There is a weak negative correlation between GDDP and yield plasticity (r = -0.37, P < 0.001; Fig. 3.6). The negative association is encouraging because it suggests the favorable plasticity traits (high yield plasticity, low growing degree-day plasticity) could be targeted simultaneously, or one plasticity trait could be targeted for improvement without hindering the other.

It should be noted that the estimates of phenotypic stability used to draw insight on phenotypic plasticity do not comprise all possible sources of plasticity. While plasticity necessarily refers to the genotype evaluated, stability can be affected by the composition of the population, such as the degree of heterogeneity and heterozygosity, that can buffer fluctuations in environmental effects (Allard and Bradshaw, 1964).

Conclusions

We observed substantial variation for both thermal time from 1 Jan. to heading and grain yield in a population of 299 winter wheat genotypes, and across the 11 environments in which they were grown. Additionally, there were significant effects of G×E affecting the

ranking of genotypes for these traits.

Phenotypic plasticity was estimated for each genotype using the Finlay and Wilkinson (1963) stability parameter, as implemented by Sadras et al. (2009). The regression coefficients ranged from 0.68 to 1.41 for GDD and from 0.64 to 1.37 for yield (Supplemental Table S3.1).

Heading date and GDD of genotypes had varying responses under different environmental conditions. Genotypes that were most responsive to environmental conditions were able to reach heading across a wider range of calendar days. These genotypes are characterized as having high heading date plasticity and low GDDP, because the calendar day on which heading occurred was flexible, but the level of thermal time at heading was approximately constant. Plasticity of GDD was more strongly induced by maximum GDD across environments than minimum (Supplemental Figure S3.2) or mean GDD (Fig. 3.4). There was a strong negative correlation between GDDP and grain yield, indicating GDDP was detrimental among the germplasm and environments evaluated (Fig. 3.5). On the other hand, yield plasticity had a strong positive correlation with yield—especially maximum yield across environments—indicating it contributed to elevated grain yield under optimal conditions and is a favorable trait in these environments and germplasm (Supplemental Figure S3.4). Equally important, high yield plasticity was not associated with low yields in the low-yielding environments.

Variation among environments contributes to differences in the ranking of germplasm, and can make it difficult for plant breeders to make selections. However, an important goal of plant breeding should be to minimize unfavorable G×E and allow dynamic trait responses when they are advantageous. We have presented evidence that the

extent of plasticity has changed over time for two traits (GDD and grain yield, Fig. 3.3), which suggests the extent of phenotypic plasticity will respond to direct and indirect selection. Phenotypic plasticity of crops is a promising area of research to better understand effects of G×E because breeders could select for or against phenotypic plasticity, thereby tailoring the response to environmental conditions to enhance crop resilience for severe or unpredictable climate scenarios.

Figures

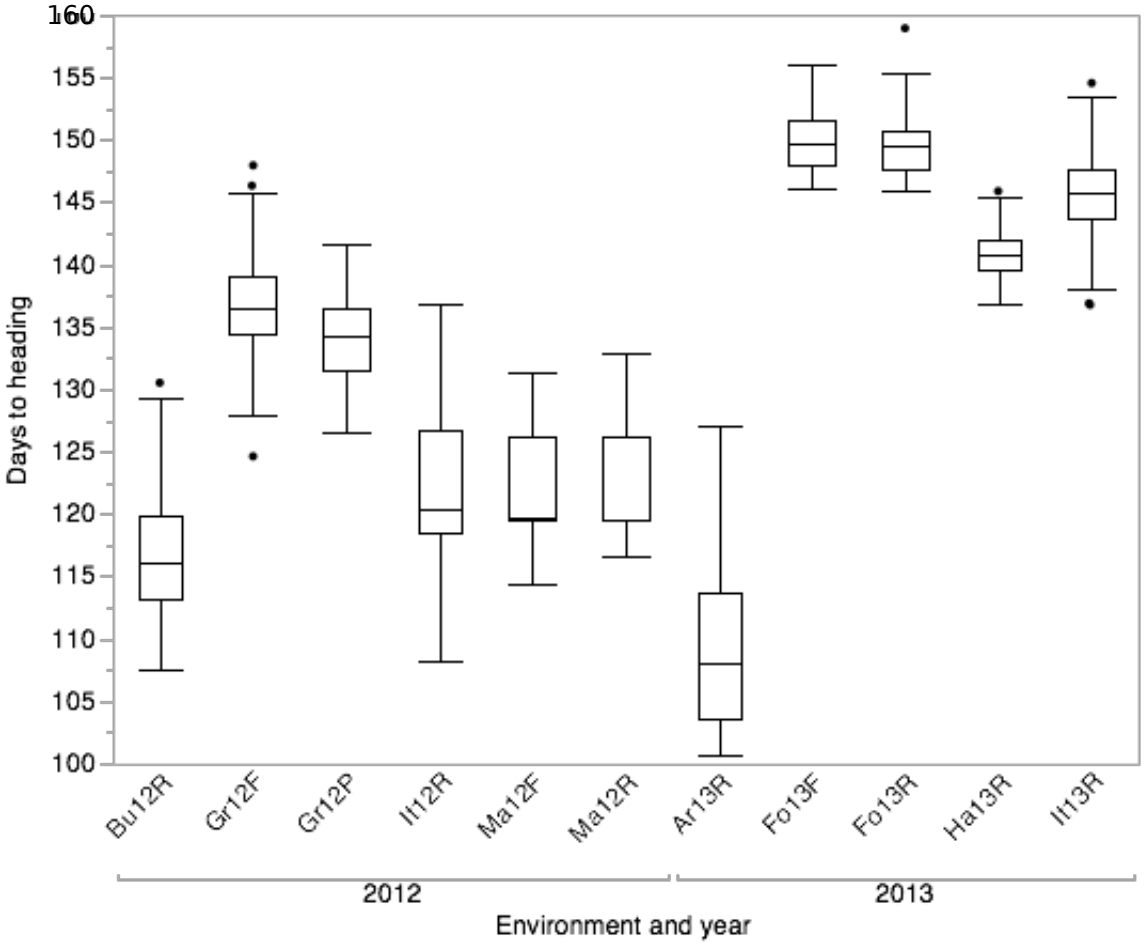

Figure 3.1. Range of days from 1 Jan.to heading among 299 hard winter wheat genotypes grown in 11 environments (defined in Table 3.1). The horizontal lines are median values for each environment. The box shows the inter-quartile range (IQR), where 50% of the genotypes fall. The horizontal lines in the boxes are median values for each environment. The whiskers extend 1.5 times the IQR in each direction. Outlying genotypes are represented as disconnected points. For Ma12R and Ma12F the median values were the same as the first quartile, which was 120 days for Ma12R and 119 days for Ma12F.

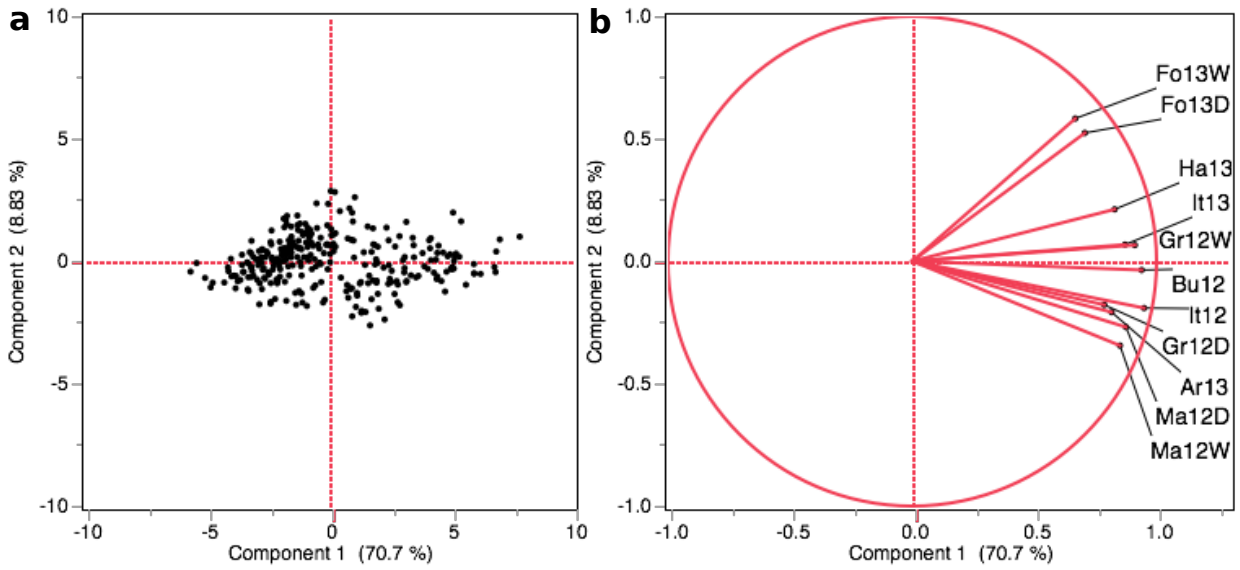

Figure 3.2. Principal components analysis of growing degree-days from 1 Jan. to heading among 299 hard winter wheat genotypes grown in 11 environments, showing the (a) score plot and (b) loadings plot. Environments are described in Table 3.1.

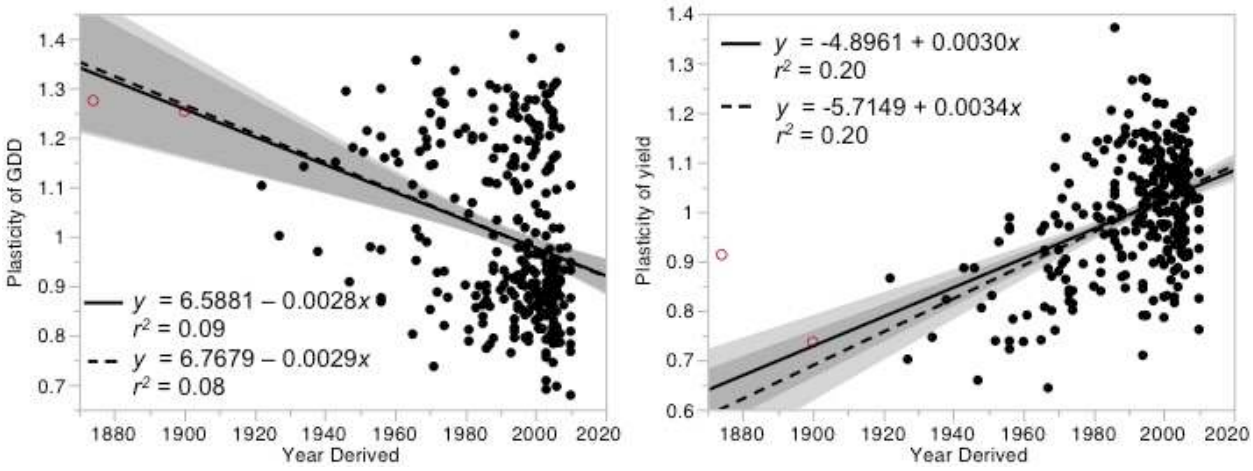

Figure 3.3. Linear regression and 95% confidence interval of plasticity coefficients by year of derivation for each of 299 hard winter wheat genotypes (solid line) or 297 hard winter wheat genotypes (dashed lines) that exclude Turkey Nebsel and Kharkof (open circles). (a) Plasticity of growing degree-days (GDD) from 1 Jan.to heading, where the narrower, lower confidence interval corresponds with the solid line. (b) Plasticity of grain yield, where the upper confidence interval corresponds with the solid line.

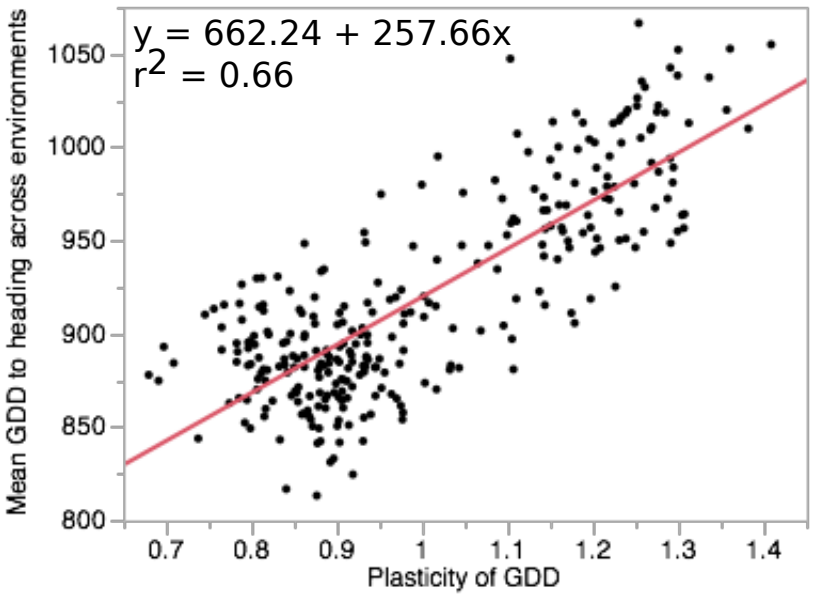

Figure 3.4. Linear regression of average growing degree-days (GDD) from 1 Jan.to heading of 299 hard winter wheat varieties grown in 11 environments on plasticity of GDD to heading.

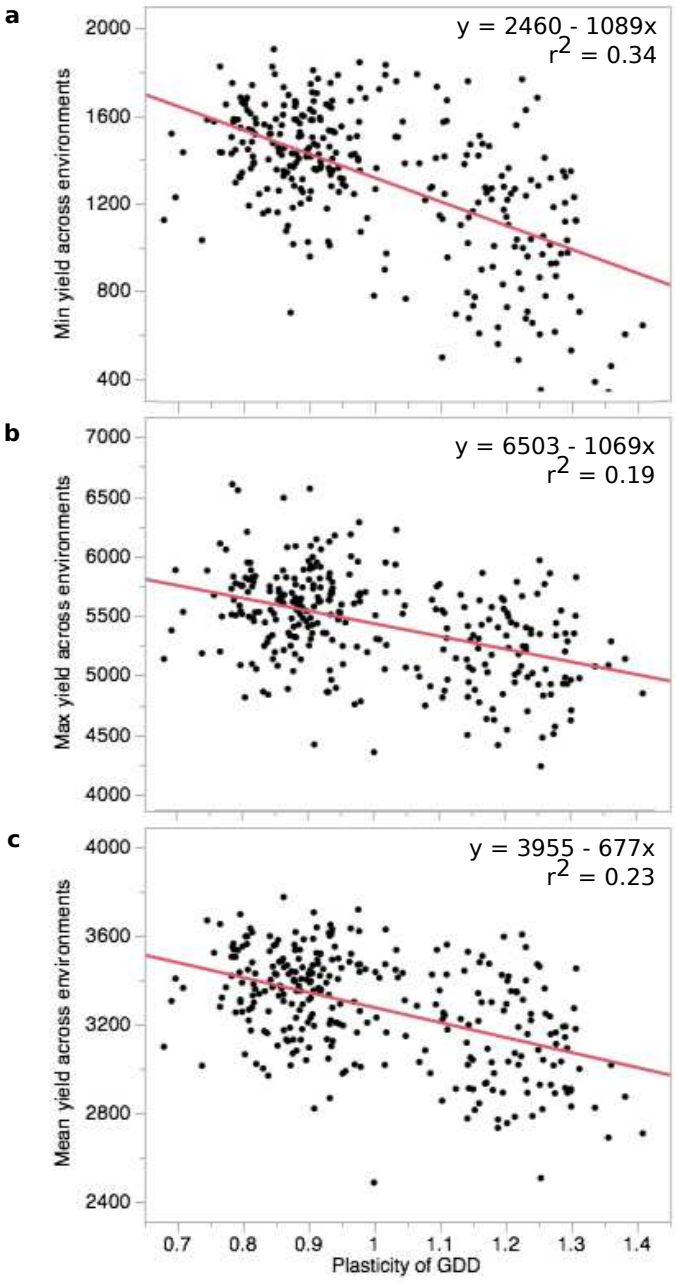

Figure 3.5. Linear regression of (a) minimum, (b) maximum, and (c) mean yield (kg ha^{-1}) of 299 hard winter wheat varieties grown in 11 environments on plasticity of growing degree-days (GDD) from 1 Jan. to heading.

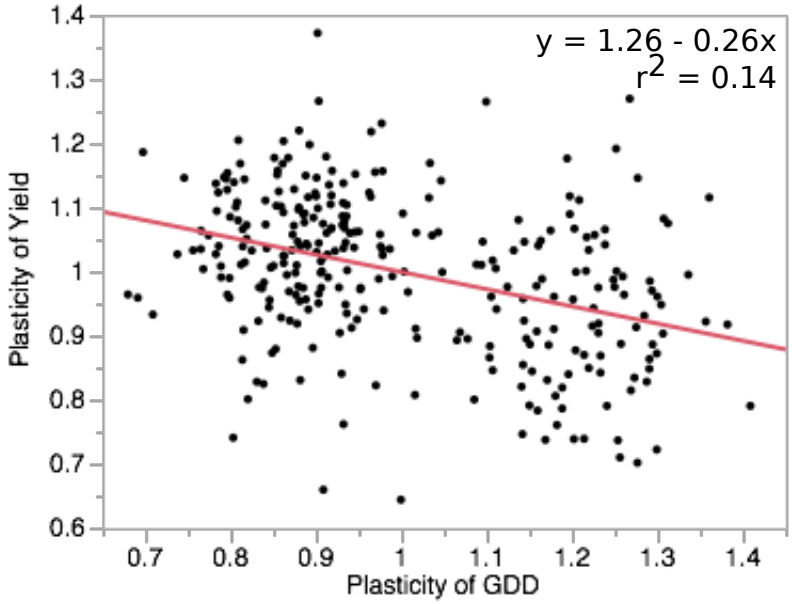

Figure 3.6. Linear regression of plasticity of growing degree-days (GDD) from 1 Jan. to heading of 299 hard winter wheat varieties grown in 11 environments on plasticity of grain yield.

Tables

Table 3.1. Environment abbreviations, location, moisture treatment, latitude, longitude, and planting and harvest dates of environments used in the study. Each environment is a unique combination of location, year, and moisture treatment. The moisture treatment is denoted at the end of the environment name (R = rainfed, F = full irrigation, and P = partial irrigation).

Environment	Location	Moisture Treatment	Lat (°N)	Long (°W)	Planting Date	Harvest Date
Ar13R	Ardmore, OK	Rainfed	34.18	-97.09	12 Oct 2012	25 June 2013
Bu12R	Bushland, TX	Rainfed	35.18	-102.10	3 Nov 2011	10 June 2012
Fo13R	Fort Collins, CO	Rainfed	40.65	-105.00	2 Oct 2012	18 July 2013
Fo13F	Fort Collins, CO	Full irrigation	40.65	-105.00	2 Oct 2012	22 July 2013
Gr12P	Greeley, CO	Partial irrigation	40.42	-104.71	19 Oct 2011	3 July 2012
Gr12F	Greeley, CO	Full irrigation	40.42	-104.71	19 Oct 2011	13 July 2012
Ha13R	Hays, KS	Rainfed	38.88	-99.33	10 Oct 2012	3 July 2013
It12R	Ithaca, NE	Rainfed	41.16	-96.43	4 Oct 2011	28 June 2012
It13R	Ithaca, NE	Rainfed	41.28	-96.41	25 Sept 2012	17 July 2013
Ma12R	Manhattan, KS	Rainfed	39.14	-96.64	18 Nov 2011	3 July 2012
Ma12F	Manhattan, KS	Full irrigation	39.14	-96.64	18 Nov 2011	3 July 2012

Table 3.2. Mean, standard deviation (s.d.), and range of growing degree-days (GDD) between from 1 Jan. to heading, days from 1 Jan. to heading, and yield (kg ha^{-1}) in each of 11 environments where a panel of 299 hard winter wheat genotypes were grown in 2011/2012 and 2012/2013. The environments are described in Table 3.1. Genotype min and max are the minimum and maximum values of each genotype across all environments.

Environment	GDD†		Days to heading†		Grain yield	
	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.	Range
	---------- °C·d ----------		---------- days ----------		---------- kg ha^{-1}----------	
It13R	730 ± 59	571–903	145.7 ± 3.0	137–155	5,239 ± 443	4,050–6,204
Fo13R	739 ± 31	684–890	149.4 ± 2.0	146–159	2,503 ± 132	2,140–2,989
Fo13F	744 ± 32	684–837	149.9 ± 2.2	146–156	4,958 ± 398	3,980–5,999
Gr12P	870 ± 44	783–986	134.4 ± 3.2	127–142	2,532 ± 217	1,961–3,093
Ha13R	892 ± 32	816–999	140.9 ± 1.8	137–146	3,444 ± 339	1,592–4,474
Gr12F	903 ± 52	753–1,089	136.8 ± 3.4	125–148	4,782 ± 969	1,711–6,604
It12R	925 ± 96	706–1,192	122.3 ± 5.2	108–137	3,597 ± 374	2,378–4,522
Bu12R	1,042 ± 96	873–1,306	116.6 ± 4.8	107–131	1,332 ± 321	338–1,904
Ar13R	1,057 ± 94	922–1,339	109.0 ± 5.8	101–127	2,119 ± 419	701–3,024
Ma12F	1,105 ± 80	949–1,276	122.2 ± 3.9	114–131	2,872 ± 337	2,113–3,861
Ma12R	1,112 ± 79	1,010–1,309	122.5 ± 4.0	117–133	2,663 ± 333	1,846–3,728
Genotype Mean	920 ± 55	813–1,067	131.8 ± 3.0	119–140	3,276 ± 242	2,489–3,777
Genotype Min	712 ± 42	571–824	109.0 ± 5.7	101–125	1,318 ± 321	338–1,904
Genotype Max	1,129 ± 81	1,010–1,339	150.3 ± 2.1	146–159	5,431 ± 415	4,238–6,604

† Beginning from 1 Jan.

Table 3.3. Pearson's correlation coefficient (*r*) between grain yield and yield plasticity among 299 hard winter wheat genotypes grown in 11 environments during 2011/2012 and 2012/2013. The environments are described in Table 3.1.

Environment	***r***
Ar13R	-0.16**
Bu12R	0.36***
Fo13R	0.25***
Fo13F	0.47***
Gr12P	0.20**
Gr12F	0.78***
Ha13R	0.43***
It12R	0.40***
It13R	0.76***
Ma12R	0.16**
Ma12F	0.27***
Minimum yield	0.33***
Maximum yield	0.80***
Mean yield	0.69***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3.4. Pearson's correlation coefficient (*r*) between growing degree-days (GDD) from 1 Jan. to heading date and grain yield among 299 hard winter wheat genotypes grown in 11 environments during 2011/2012 and 2012/2013.

Environment	*r*
Ar13R	0.13*
Bu12R	-0.71***
Fo13R	-0.31***
Fo13F	0.03 ns†
Gr12P‡	-0.39***
Gr12F	-0.56***
Ha13R	-0.42***
It12R	0.11 ns
It13R	-0.43***
Ma12R	-0.40***
Ma12F	-0.39***
Pooled¶	-0.55***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† ns, nonsignificant at the 0.05 probability level.
‡ Gr12P only had 298 entries with both GDD and grain yield data.
¶ The sample size is 3,288 when entries from all environments are pooled.

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CHAPTER 4:

PHENOTYPIC ANALYSES AND GENOME-WIDE ASSOCIATION MAPPING FOR AGRONOMIC TRAITS IN WINTER WHEAT GROWN UNDER CONTRASTING MOISTURE TREATMENTS

Summary

Genome-wide association mapping is an effective method of dissecting the genetics of complex, quantitative traits like grain yield and drought tolerance. The objectives of this study were to conduct genome-wide association studies (GWAS) on 15 traits, including yield, yield components, and agronomic and phenological traits, in 299 winter wheat entries representative of breeding material across the U.S. Great Plains region. The study was conducted under well-watered and water-stressed environments in Greeley, CO in 2011–2012 and in Fort Collins, CO in 2012–2013. Grain yield was reduced by 48% under water stress in 2012, and 46% under water stress in 2013. During 2012 the timing of water stress occurred before anthesis and reduced grain yield by limiting biomass production including tillering, producing fewer spikelets and kernels per spike, and reducing the proportion of fertile spikelets. In 2013 stress occurred post-anthesis and primarily impacted grain fill duration and kernel size.

Genotypic data included 16,052 single nucleotide polymorphism (SNP) markers genotyped with the Illumina 90K iSelect chip. Markers for photoperiod sensitivity and reduced-height genes, including *Ppd-D1*, *Ppd-B1*, and *Rht-B1* were used as covariates in the GWAS. We identified 173 significant marker–trait associations in individual environments, , 35 significant marker–trait associations were detected in a combined analysis of year, and

33 were detected in a combined analysis of moisture treatment. Genome-wide association mapping is a powerful tool to detect genomic regions affecting a trait, but we found relatively few QTL with large effects or that were stable across environments, years, or moisture treatments. The number of significant marker–trait associations detected depends on the statistical criteria used. We used the Benjamini–Hochberg procedure with a false discovery rate of 50% to identify significant associations, but a different approach or significance threshold would yield different results. A best method for establishing significant marker–trait associations is yet to be established for agricultural research. Using a larger population, incorporating denser marker data, or improving the genetic map could improve GWAS results.

Abbreviations**:** ANOVA, analysis of variance; BH, Benjamini–Hochberg procedure for multiple comparisons; BLUPs, best linear unbiased predictors; BM, above-ground biomass; DTB, days to booting; DTF, days to flowering; DTH, days to heading; DTJ, days to jointing; DTM, days to maturity; GBM, grain biomass; GF, grain fill duration; G×E, genotype-by-environment interaction; GWAS, genome-wide association study; GY, grain yield; HI, harvest index; HWWAMP, Hard Winter Wheat Association Mapping Panel; KPS, kernels per spike; KN, kernel number; LD, linkage disequilibrium; MAF, minor allele frequency; PFS, proportion fertile spikelets; PH, plant height; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; SPS, spikelets per spike; KW, kernel weight; SN, Spike number.

Introduction

Bread wheat (*Triticum aestivum* L.) is an important component of human diets worldwide, including in the United States. However, acreage planted to wheat in the United States has declined by about 30% since the early 1980's, in part due to increased demand for ethanol and corresponding increases in the acreage planted to maize, and the United States Department of Agriculture projects it will continue to decline over the next decade or more (USDA, 2015). At the same time, wheat consumption in the U.S. is expected to increase at the same rate as population growth, and demands on wheat production for export are expected to rise (Westcott and Hansen, 2015). Grain yield can be increased either using improved crop management techniques or increases in genetic gain (Tester and Langridge, 2010). However, the rates of improvements in yield potential may not be high enough, especially among water-limited environments, to meet the projected demands of wheat and other cereals in 2050 (Hall and Richards, 2013). Some research suggests the rate of genetic gain may be slowing in wheat (Graybosch and Peterson, 2010), at least for some types of wheat in particular regions of the U.S. (Graybosch et al., 2014). Therefore, new methods of yield improvement need to be addressed in order to meet demands for wheat production.

Yield is a complex trait controlled by many genes, most of which contribute only a small amount of phenotypic variation and are specific to a population or environment (van Eeuwijk et al., 2010). Association analysis, such as genome-wide association studies (GWAS), is one approach to identify the genetic architecture of complex traits (Zhu et al., 2008). Genome-wide association mapping can identify quantitative trait loci (QTL) through marker–trait associations (Zhu et al., 2008). Quantitative trait loci can be introduced to

elite germplasm through recurrent backcrossing using marker-assisted selection, but to date, introgression of QTL into breeding materials using these methods is rare, in part due to instability of QTL across environments (Bernardo, 2008).

The development of polymorphic marker platforms (Shendure and Ji, 2008; Metzker, 2010), expansion of tools to rapidly and inexpensively detect polymorphic markers (Varshney and Dubey, 2009; Poland and Rife, 2012), and advances in statistical and computational methods (Lipka et al., 2015) to process very large genetic and genomic datasets have all contributed to recent increases in the use and utility of GWAS. In particular, the increased availability and reduced cost of molecular markers, coupled with an ordered draft sequence of the wheat genome (Mayer et al., 2014) and recently published consensus maps (Cavanagh et al., 2013; Wang et al., 2014) make GWAS a viable option to identify sources of genetic variation for grain yield or agronomic traits in wheat.

There are many recent reports of successful use of GWAS in wheat. Genetic analyses have been used to dissect grain yield, yield components, and other agronomic traits (Yao et al., 2009; Bentley et al., 2014; Sukumaran et al., 2014; Zanke et al., 2014), including under multiple moisture regimes (Maccaferri et al., 2011; Dodig et al., 2012; Edae et al., 2014; Lopes et al., 2014; Li et al., 2015; Mora et al., 2015). Other studies have evaluated the genetic basis of quality traits (Breseghello and Sorrells, 2006; Reif et al., 2011), pre-harvest sprouting (Mohan et al., 2009; Kulwal et al., 2012), and nitrogen-use efficiency (Cormier et al., 2014). Other applications of GWAS in wheat include stripe rust resistance (Maccaferri et al., 2015; Naruoka et al., 2015), Fusarium head blight resistance (Kollers et al., 2013; Jiang et al., 2014), and insect or virus resistance (Peng et al., 2009; Joukhadar et al., 2013). Some benefits of additional GWAS in wheat include evaluation of novel traits, utilization of

different or more dense genetic markers to detect QTL, identification of new QTL or validation of previously identified QTL in a different set of germplasm, and identification of beneficial alleles in locally-adapted breeding lines.

The primary objective of this study was to identify QTL using marker–trait associations between markers from the Illumina 90K iSelect assay platform and phenotypic traits in winter wheat accessions evaluated under contrasting moisture regimes. Secondary objectives were to estimate the extent of linkage disequilibrium across the A-, B-, and D-genomes; evaluate phenotypic variation among accessions and environments; estimate phenotypic and genetic correlations between traits; and estimate broad-sense heritability of traits.

Materials and methods

Germplasm and experimental design

The germplasm used in this study was a collection of 299 winter wheat accessions representative of the U.S. Great Plains region, and includes recent cultivars, experimental breeding lines, and historic varieties. The germplasm has been designated the Triticeae Coordinated Agricultural Project (TCAP, http://www.triticeaecap.org) Hard Winter Wheat Association Mapping Panel (HWWAMP), which has been described previously (Grogan et al., 2015a; Guttieri et al., 2015b).

The HWWAMP was evaluated in four field trials in Colorado during 2011–2012 (referred to as the 2012 season) and 2012–2013 (2013 season). In 2011–2012 the panel was grown at the USDA-Agricultural Research Service Limited Irrigation Research Farm in

Greeley, CO (40° 27'N, -104° 38'W; elevation 1,427 m) under full (Gr12F) and partial (Gr12P) irrigation. During the 2012 season, trials were planted on 19 Oct 2011 and harvested on 3 July 2012 (Gr12P) and 13 July 2012 (Gr12F). Supplemental moisture was applied using drip irrigation. Irrigation was applied 10 times between spring green-up and physiological maturity at Gr12F, and five times at Gr12P. The moisture differential was applied beginning during vegetative growth such as tillering or stem expansion, in early April 2012 (Table 4.1). The moisture treatment had a significant effect on phenological development by booting, which was the first developmental stage evaluated. Supplemental irrigation totaled 101.6 mm at Gr12P and 335.3 mm at Gr12F. Both Gr12P and Gr12F received 82.1 mm precipitation between 1 Jan and harvest.

During 2012–2013 the panel was grown at the Colorado State University Agricultural Research Development and Education Center in Fort Collins, CO (40° 39'N, -105° 00'W; elevation 1,534 m). The trials included full irrigation (Fo13F) and rainfed (Fo13R) treatments that were planted on 2 Oct 2012 and harvested on 18 July 2013 (Fo13R) and 22 July 2013 (Fo13F). Irrigation at Fo13F began at anthesis, and was applied seven times, totaling 222.3 mm using overhead linear sprinklers. Thus, there was no treatment effect between Fo13F and Fo13R until anthesis. Both Fo13F and Fo13R received 124.4 mm precipitation between 1 Jan and harvest.

All four trials had an experimental design similar to a type II modified augmented design (Lin and Poushinsky, 1985). The experimental entries were unreplicated, except for 'Wichita' (CI 11952), which was included in the panel twice. Two check varieties, 'Hatcher', (Haley et al., 2005) and 'Settler CL' (Baenziger et al., 2011) were replicated 15 times each per trial, and were also included as experimental entries.

Phenotypic trait evaluation

Fifteen phenotypic traits (Table 4.2) were evaluated in this study and were estimated as follows. Mature plant height (PH) was measured in cm from the soil surface to the tip of the spike excluding awns. Above-ground biomass (BM) was obtained by cutting a 1 m strip of all plants at the soil surface after physiological maturity, and recording the dry weight of the samples after drying for at least 72 hours in a 40°C oven. Each BM sample was threshed and the grain was weighed to obtain grain biomass weight (GBM). Harvest index (HI) was calculated as the ratio of dry grain weight to dry biomass weight.

Spikelets per spike (SPS) were determined from the average of 10 spikes collected at physiological maturity. The proportion of fertile spikelets (PFS) was estimated by first counting the total number of spikelets on each spike, then counting the number of sterile spikelets (those not filled with grain) on the top and base of each spike, and finally taking the ratio of spikelets filled with grain to total number of spikelets. Kernel number per spike (KPS) was determined by threshing each group of 10 spikes, counting the number of resulting kernels, and dividing the number of kernels by the number of spikes. Each group of kernels was weighed and divided by the kernel number and then multiplied by 1000 to estimate kernel weight (KW). Grain yield (GY) was the total weight of all grain from each plot, divided by the plot area (adjusted for length and large gaps), and was expressed as kg ha^{-1}. Spike number (SN) was estimated by dividing GY by kernel weight per spike (estimated from KPS and KW) and is reported as spikes m^{-2}. Kernel number (KN) was estimated as the product of SN and KPS and is reported as kernels $m^{-2.}$

Crop developmental stages were determined for each plot using the Zadoks scale (Zadoks et al., 1974). Calendar days to each developmental stage was calculated as the

number of days after 1 Jan, and included days to jointing (DTJ, stage 31), booting (DTB, stage 45), heading (DTH, stage 59), flowering (DTF, stage 65), and physiological maturity (DTM, stage 90). Grain filling duration (GF) was calculated as the difference between DTM and DTF. Developmental stages were defined when approximately 50% of tillers in interior rows of the plot exhibited characteristic traits. Jointing date was only evaluated at Fo13F. There was no treatment effect observed at 2013 environments until anthesis.

Phenotypic analyses

Best linear unbiased predictions (BLUPs) were calculated separately for each trait and environment using SAS 9.3 (SAS Institute, Inc., Cary, NC). Genotypes were treated as random. Six models: row-column and five different spatial models (row-column, spherical, exponential, power, anisotropic power, and Matérn) were tested, and the best model was selected based on the Akaike information criterion test statistic. Combined analyses were conducted on BLUPs using mixed models where genotypes were treated as random, and environment or year was treated as a fixed effect. Combined analyses include estimates across all four environments, estimates across years, and estimates across well-watered or water-stressed treatments. Further analyses were performed using the software R (version 3.1.3, R Development Core Team), as specified below.

Variance components, genetic correlations, and heritability

Phenotypic correlations (r) were estimated from BLUPs using the Pearson product-moment correlation coefficient, estimated using the 'stats' package (R Development Core

Team, 2015). Estimates of *r* measure the magnitude and direction of the linear association between two traits.

Variance components were estimated from BLUPs for each trait based on mean squares from an analysis of variance (ANOVA) table. Analyses of variance were conducted in the 'car' package (Fox and Weisberg, 2011). For each agronomic trait the main effects of genotype and environment, and the interaction between genotype and environment (G×E), were included as fixed effects. Genotype-by-environment interaction was only retained in the model when the effect was significant at a probability level of 0.05. Broad-sense heritability (H^2), the proportion of total phenotypic variance due to genetic effects, was estimated as follows in Eq. 4.1:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r \cdot e} + \frac{\sigma_{ge}^2}{e}} \qquad \text{(Eq. 4.1, Hallauer et al., 1988)}$$

Where σ_g^2, σ_{ge}^2, and σ_e^2, are the genetic, G×E, and error variance components, *e* is the total number of environments, and *r* is the number of replications at each environment.

The genetic correlation estimates the degree to which two traits are affected by the same genetic factors, such as pleiotropic genes or linkage disequilibrium (LD) and was estimated between each pair of agronomic traits, as described in Williams et al. (2002). Estimating the genetic correlation required creating a new combined variable for each pair of traits. For two traits: *x* and *y*, a third combined variable, *z*, was calculated as *x* + *y*. Genetic variance of *z* was calculated from mean squares as described above.

The genetic variances of traits are related and can be computed using Eq. 4.2 and Eq. 4.3, where σ_x^2, σ_y^2, and σ_z^2 are the genetic variance components for traits *x*, *y*, and *z*:

$$\sigma_z^2 = \left(\sigma_x + \sigma_y\right)^2 = \sigma_x^2 + \sigma_y^2 + 2\sigma_{xy} \qquad \text{(Eq. 4.2)}$$

Rearranging Eq. 4.2 enabled computing the genetic variance of *xy* directly:

$$\sigma^2_{xy} = \frac{\sigma^2_z - \sigma^2_x - \sigma^2_y}{2} \quad \text{(Eq. 4.3)}$$

The genetic variances of traits *x, y,* and *xy* were used to estimate the genetic correlation (r_G) between *x* and *y*, as follows in Eq. 4.4:

$$r_{G(xy)} = \frac{\sigma^2_{xy}}{\sqrt{\sigma^2_x \cdot \sigma^2_y}} \quad \text{(Eq. 4.4)}$$

Genotype data

Single nucleotide polymorphism markers for all 299 accessions were assayed using a 90K Illumina iSelect genotyping platform (Wang et al., 2014) at the USDA-ARS Small Grains Genotyping Laboratory in Fargo, ND. Genotyping was repeated for 12 accessions ('Akron', 'Camelot', CO04W320, 'Lindon', 'Millenium', 'NuFrontier', 'OK Rising', OK10119, OK1068002, 'Overland', 'Pete', and 'PostRock') due to concerns with initial genotyping based on comparing kinship estimates of known pedigree-based relatedness. TAM 105 was genotyped twice and only one genotype for TAM 105 was retained, which was chosen based on kinship with TAM 107, a TAM 105 backcross. TAM 105 was expected to share about 75% of allele calls with TAM 107. The retained genotype of TAM 105 was 77% identical to TAM 107, and the eliminated genotype had greater levels of missing data and was only 62% identical to TAM 107

Allele calls were made using the Illumina GenomeStudio Polyploid Clustering Module v1.0 software (www.illumina.com). The DBSCAN algorithm was used to identify genotype clusters assuming cluster distance 0.07, five data points per cluster, and confidence score limit of 0.8. Cluster separation was filtered to have no less than two

standard deviations of Theta value of each cluster. Semi-automatically curating the markers eliminated monomorphic SNPs, multi-allelic SNPs, SNPs with low call rate, SNPs that produced diffuse clusters, and SNPs with smaller cluster distances to produce a set of 26,553 SNPs. Manually curating the markers resulted in 21,555 SNP calls on 299 genotypes, of which 21,546 SNPs were missing < 10% of calls. A total of 5,497 SNPs had a minor allele frequency (MAF) < 5%. Markers with MAF <5% or more than 10% missing calls were removed to produce a set of 16,052 filtered SNPs. Map positions were obtained from http://wheatgenomics.plantpath.ksu.edu/ (download date: 22 May 2015). Of the 16,052 filtered SNPs, 14,829 mapped to a unique chromosome position and 1,223 were unanchored. Distribution of the markers varied among genomes and chromosomes, and included 5,971 on the A-genome, 7,244 on the B-genome, and 1,614 on the D-genome.

Linkage disequilibrium

The extent of LD among markers was generated using the software TASSEL (Trait Analysis by aSSociation, Evolution, and Linkage) v. 5 (Bradbury et al., 2007) with a sliding window size of 200 cM. The extent of LD was estimated on a set of 10,778 markers that included 4,114 markers on the A-genome, 5,291 markers on the B-genome, and 1,373 markers on the D-genome. Decay of r^2 was estimated using the Hill and Weir method (Hill and Weir, 1988), as implemented by Marroni et al. (2011).

Association analyses

Association analyses were conducted in the GAPIT (Genomic Association and Prediction Integrated Tool) package (Lipka et al., 2012) for R using 16,052 filtered SNPs. The analyses were conducted separately for each environment and combined across environments using a multiple linear model with principal components (Price et al., 2006) and kinship (P+K). The model selection option was used to account for population structure, and allowed between zero and four principal components for each trait and environment. Three functional genes associated with plant development through the photoperiod and gibberellin sensitivity pathways (*Ppd-D1*, *Ppd-B1*, and *Rht-B1*) were also included as covariates using model selection to reduce confounding. Genotyping at these loci has been described previously (Grogan et al., 2015b). Covariate files require complete genotypic data; so missing data at *Ppd-D1*, *Ppd-B1*, and *Rht-B1* were imputed as the major allele. The major alleles were photoperiod sensitive alleles *Ppd-D1b* and *Ppd-B1b*, and the semi-dwarf allele *Rht-B1b*. In preliminary analyses missing data were imputed both as the major allele and as heterozygotes, and there were not significant differences in model fit between the two imputation methods for any of the agronomic traits evaluated.

Best model fit was assessed based on the largest Bayesian information criterion value (Schwarz, 1978). Kinship was estimated using the realized additive relationship matrix (Endelman and Jannink, 2012) from the R package 'rrBLUP' (Endelman, 2011) with missing data imputed using the EM algorithm with tolerance = 0.2, as described previously (Guttieri et al., 2015a).

Significant marker–trait associations were identified using the Benjamini–Hochberg (BH) procedure to correct for multiple comparisons (Benjamini and Hochberg, 1995). The

false-discovery rate threshold was set to 50%. A high false-discovery rate was used because the BH procedure is known to be conservative when correcting for large numbers of multiple comparisons (Bradbury et al., 2011). The BH threshold critical *P*-value is determined from the total number of markers (m), rank of markers (i) from lowest to highest un-adjusted *P*-value, and false-discovery rate (Q), and can be described as follows in Eq. 4.5:

$$\mathrm{P_{crit}} < \left(\frac{\mathrm{i}}{\mathrm{m}}\right) \cdot \mathrm{Q} \qquad \text{(Eq. 4.5)}$$

Thus, the critical value for the BH procedure is not a static number, but varies for each marker based on the rank of the un-adjusted *P*-value for each marker relative to all markers. The highest significant *P*-value that meets the BH criteria is identified as the largest unadjusted *P*-value that is less than P_{crit}. Markers with an unadjusted *P*-value less-than or equal-to the highest significant *P*-value are considered to have significant marker–trait associations.

Results

Phenotypic analyses of agronomic traits

Variability of agronomic traits across environments

A summary of phenotypic trait values is provided for Gr12F and Gr12P in Table 4.3, and for Fo13F and Fo13R in Table 4.4. Similar reductions in grain yield were achieved under water stress in 2012 and 2013. Grain yield was reduced by 48% under water stress in 2012, from 4802 ± 979 kg ha^{-1} at Gr12F to 2510 ± 251 kg ha^{-1} at Gr12P. In 2013, grain

yield was reduced by 46%, from 5008 ± 402 kg ha^{-1} at Fo13F to 2684 ± 133 kg ha^{-1} at Fo13R.

In 2012 the reduction of grain yield under water stress was primarily caused by a large reduction in plant size, including reduced BM (52% reduction), SN (30% reduction), and PH (36% reduction). Other traits not related to plant size were also reduced under water stress, such as KN (36% reduction). Smaller plants had fewer assimilates available to partition to the developing grain. Reduced BM resulted in a greater HI under water stress (0.38 ± 0.04 in Gr12P and 0.34 ± 0.05 in Gr12F), but lower GBM overall (45% reduction under water stress). Additionally, spikes produced many fewer spikelets (9% reduction) and kernels (15% reduction) under water-stressed than non-stressed conditions. At Gr12F high GBM was associated with increased KW (r = 0.49, P < 0.001) and SN (r = 0.43, P <0.001), while at Gr12P high GBM was more strongly associated with KN (r = 0.26, P < 0.001) and KPS (r= 0.25, P <0.001, Table 4.5). This suggests that high yield at Gr12F was due to more spikes and larger kernel size, while high yield at Gr12P was more strongly impacted by kernel number. Many other traits also had strong positive genetic correlations (Table 4.5).

Different effects on grain yield and yield components due to water stress were observed in 2013: grain yield decreased due to reductions in kernel size (KW was reduced by 27%) and KPS (reduced by 21%). Biomass was reduced by 25% under water stress in 2013, which was less than the 52% reduction observed in 2012. However, this difference is primarily due to greater BM production under full irrigation in 2012 (477.9 ± 36.7 g at Gr12F) than in 2013 (305.0 ± 10.6 g at Fo13F), rather than a large difference in biomass at stressed environments (231.2 ± 19.6 g at Gr12P and 228.2 ± 16.6 g at Fo13R). Number of

spikes was also greater at Gr12F (454.7 ± 17.7 spikes m^{-2}) than Fo13F (373.8 ± 32.9 spikes m^{-2}). At Fo13F high GBM was associated with more KPS (r = 0.26, P <0.001) and greater KW (r = 0.22, P <0.001, Table 4.5), but at Fo13R GBM was most strongly associated with SN (r = 0.24, P <0.001, Table 4.5). This suggests that overall, high grain yield at Fo13F relative to Fo13R was mostly influenced by kernel size. Entries with the highest grain yield at Fo13F produced relatively more kernels and had larger kernels than other entries grown in that environment, while entries with the highest grain yield at Fo13R produced relatively more total spikes than other entries grown in that environment.

Combined analyses across environments

Results from the combined analyses of variance across environments are summarized in Table 4.6. The main effect of environment was significant for all traits. The main effect of genotypes was significant for all traits except BM. Genotype-by-environment interaction was significant for GY, GBM, HI, DTB, and DTF.

Estimates of broad-sense heritability were lower for GY (H^2 = 0.43) than other yield components, including KN (H^2 = 0.56), SN (H^2 = 0.69), KPS (H^2 = 0.78), SPS (H^2 = 0.84), and PFS (H^2 = 0.65, Table 4.6). Total BM had the lowest heritability (H^2 = 0.08) and GBM also had low heritability (H^2 = 0.20). Among the developmental traits, DTH was more heritable (H^2 = 0.89) than DTB (H^2 = 0.87), DTM (H^2 = 0.82), or DTF (H^2 = 0.69).

Strong positive genetic correlations with GY were observed for GBM (r_G > 1), HI (r_G > 1), and KPS (r_G = 0.69, Table 4.7). Strong negative genetic correlations were observed between GY and PH (r_G = -0.77), and between GY and phenological stages (r_G = -0.76 to -

0.79). Plant height had positive genetic correlations with DTB (r_G = 0.62), DTH (r_G = 0.58), DTF (r_G = 0.55), and DTM (r_G = 0.39).

There were negative genetic correlations between SN and some yield components (r_G = -0.14 to < -1.0 for HI, KPS, KW, SPS, GBM, PFS), and positive correlations between SN and vegetative growth traits BM (r_G = 0.36) and PH (r_G = 0.16, Table 4.7). This highlights the genetic control affecting partitioning of assimilates to either vegetative structures or the developing spike, such that the same genes either directed assimilates towards tiller and biomass production, or towards yield components in the spike, but that these processes were in competition with each other.

Additional sources of environmental variance

When the environment was deconstructed into two environmental variables: year (2012 or 2013) and moisture treatment (water-stressed or full irrigation), the effect of treatment was larger than year for most traits (Table 4.8). This is not surprising because the moisture treatments each year were intended to be very different, whereas the locations were in the same geographic region and expected to experience a similar set of environmental conditions both years. Despite differences in the timing of water stress, yield reduction under stress was similar both years (Table 4.3 and Table 4.4). Year had a larger effect than treatment for phenological traits (DTB, DTH, DTF, DTM) and PFS. Treatment had a significant effect on all traits and year had a significant effect on every trait except KPS. In individual environments, genotype had a significant effect on every trait except for BM. Two-way interactions between either genotype and treatment or genotype and year were significant for most traits, but not for SN, KW, BM, or GBM. The interaction

between treatment and year was significant for every trait except for GY. The three-way interaction between genotype, treatment, and year was a significant source of variation for GBM, HI, TW, DTB, and DTF. These results indicate there was substantial phenotypic variation in the HWWAMP for most agronomic and phenological traits and genotype-by-environment interaction affected the traits in complex ways.

Genetic analyses of agronomic traits

Linkage disequilibrium

The extent of LD varied among genomic regions, chromosomes, and whole genomes (Flint-Garcia et al., 2003). We found the extent of LD to vary substantially on the A-, B-, and D-genomes. Genome-level LD decayed below r^2 = 0.20 at 6 cM in the A-genome, 4 cM in the B-genome, and 28 cM in the D-genome. Across all three genomes, LD decayed below r^2 = 0.20 at 9 cM. These results differ from those reported by Edae et al. (2014) in a spring wheat panel from CIMMYT ("Centro Internacional de Mejoramiento de Maíz y Trigo"), where LD was found to decay below r^2 = 0.20 at 1.7 cM for the A-genome, 2 cM for the B-genome, and 6.8 cM for the D-genome. However, Chao et al. (2010) reported that among worldwide spring and winter wheats, CIMMYT spring wheat lines had the lowest extent of LD. In a separate spring wheat panel, Mora et al. (2015) found LD decayed to below 50% initial values at 22 cM for the D-genome, and 2 cM for the A- and B-genomes. Results by Chao et al. (2010) differed from our study, Edae et al. (2014), and Mora et al. (2015) in that they found extent of LD in winter wheat to be greater for the B-genome (7 cM) than the D- (6 cM) or A-genome (5 cM).

Marker–trait associations in individual environments

A total of 173 significant marker–trait associations were detected for agronomic traits in individual environments (Table 4.9), including associations with 124 mapped and 49 unmapped markers. Associations were detected on every chromosome. The marker–trait associations involved 108 unique, mapped markers. Most markers (96 markers) were only detected for a single trait and environment, while 8 markers were significant in two associations, and four markers were significant in three associations. The 108 unique mapped markers mapped to 64 unique QTL, defined as unique genomic regions separated by at least 10 cM. Most of the QTL (51 of 64 total QTL) were separated by at least 20 cM.

There were more QTL detected in 2012 than 2013, and this is influenced in part by the large number of QTL (20 significant marker–trait associations) detected for SPS in Gr12P, and for DTH (17 significant marker–trait associations) in Gr12F (Table 4.9). The most QTL (57 significant marker–trait associations) were detected at Gr12P, while the fewest (32 significant marker–trait associations) were detected at Fo13F. Up to 28 QTL were detected per trait.

Most markers with significant effects were only associated with a single trait in one environment. The GWAS results are summarized separately for mapped markers at Fo13F (Table 4.10), Fo13R (Table 4.11), Gr12F (Table 4.12), and Gr12P (Table 4.13). In both years, more significant marker–trait associations were detected in the water stressed than non-stressed treatment (Table 4.9). In Fo13R and Gr12P, the greatest number of marker–trait associations were detected for traits most affected by water stress, including 15 associations each for DTF and GF in Fo13R, and 20 associations for SPS in Gr12P (Table 4.9). The GWAS results for unmapped markers are summarized in Table 4.14. Some

markers were associated with multiple traits in one or more environment, or the same trait across several environments. Furthermore, in some cases different markers at the same chromosomal position, or within the same genomic region, had significant marker–trait associations across environments.

Several markers were associated with a phenological trait and vegetative growth traits like HI or PH. *Tdurum_contig42153_1190* (chromosome 2B) was associated with PFS and HI at Gr12P and with PFS at Fo13F. A second marker located at the same position, *Tdurum_contig42153_891*, was associated with PFS at Fo13R and DTB at Gr12F. Three unmapped markers: *BS00012081_51*, *RAC875_c15844_348*, and *RAC875_c31358_214v* were associated with DTH and PH at Gr12F, PFS at Fo13R and/or Fo13F. The allelic effects of these markers were similar on all traits. In addition, *BS00012081_51* was associated with SPS at Gr12P, and both *RAC875_c15844_348* and *RAC875_c31358_214v* were associated with HI at Gr12P.

Five significant marker–trait associations were detected between either DTB or DTH and HI. *Ex_c9615_1202* (chromosome 7A) and *GENE.4008_418* (unmapped) were associated with DTB at Fo13F and HI at Gr12P. *BobWhite_rep_c63363_160*, *Kukri_c25245_998*, and *Kukri_c29807_713* (all unmapped) were associated with DTH at Gr12F and HI at Gr12P. A sixth marker, *Excalibur_c35316_154* (chromosome 1A) was associated with DTB, SPS, and KN at Gr12F.

Many markers were associated with one or more phenological stage, in one or more environment. *GENE.1125_32* (unmapped) had an effect of 1.0 d on DTF at Fo13R, and an effect of 1.7 d on DTB at Gr12F. Other markers with large effects on DTB include *BobWhite_c19554_544* (chromosome 2B, with an effect of 1.7 d at Gr12F), *BS00067096_51*

(chromosome 5A, effect of 1.5 d at Gr12P), *CAP12_c590_307* (chromosome 1B, effect of 1.4 d at Gr12P), and *BobWhite_c37935_124* (chromosome 1A, effect of 1.4 d at Gr12F). *BobWhite_c37935_124* also had an effect of 0.6 d on DTF at Fo13R. No QTL had an allelic effect greater than 0.8 d on DTF. *Tdurum_contig15734_221* (chromosome 7B) had an effect of 1.0 d on DTB at Gr12F, and an effect of 0.9 d on DTH. *Wsnp_Ex_c9779_16145653* (chromosome 6A) had an effect of 1.1 d on DTB at Gr12P and an effect of 0.5 d on DTF at Fo13R. *BS00034554_51* (chromosome 6B) also affected the timing of vegetative growth, with similar effect sizes of 0.7 d for both DTB at Gr12P and DTH at Gr12F. Other markers with large effects on DTH include: *wsnp_Ku_c5693_10079278* (chromosome 7A, effect of 1.3 d at Gr12F), *wsnp_Ku_c3956_7237707* (chromosome 4A, effect of 1.0 d at Gr12F), and *wsnp_Ex_rep_c70756_69644826* (chromosome 9B, effect of 1.0 d at Gr12F).

Many QTL were detected for spike traits, especially SPS (28 associations) and PFS (18 associations, Table 4.9). *Wsnp_Ku_rep_c104159_90704469* (chromosome 7A) was associated with SPS at Fo13F, Fo13R, and Gr12P and affected the number of spikelets by 0.3–0.4. *Wsnp_JD_c20555_18262317* is positioned near *wsnp_Ku_rep_c104159_90704469*, and was associated with KN at Gr12F, with an effect size of 213 kernels m^{-2}. *Wsnp_Ex_c53843_56941644* (unmapped) was associated with SPS in Gr12P and Fo13R, and also had an effect size of 0.3–0.4 spikelets. It also had an effect on KN at Gr12F. *Excalibur_c29255_404* (chromosome 5A) was associated with PFS at Fo13F, Fo13R, and Gr12P and changed fertility by 1.3–2.2%. *Wsnp_JD_c2128_2930150* (chromosome 5A) had an effect on PFS at both Fo13F and Fo13R, and the effect size was similar (0.7–0.8%) in both environments.

Marker–trait associations across environments

Many traits did not vary significantly among genotypes in the combined analysis across all four environments. Only four traits: HI, KPS, PFS, and SPS had significant variability and could be evaluated in a GWAS. Among these traits, QTL were detected for PFS and SPS (Table 4.16).

Three QTL were detected for PFS, including two on chromosome 5A (at 15.9 and 139.8 cM), and one on chromosome 2D. The largest allelic effect, a 1.3% change in spike fertility, was observed for *Excalibur_c29255_404* (chromosome 5A), and significant associations at this position were also detected for PFS at Fo13F, Fo13R, and Gr12P. Two significant marker–trait associations were detected for SPS, including with *wsnp_Ku_rep_c104159_90704469* (chromosome 7A). QTL at this position were also detected for SPS at Fo13F, Fo13R, and Gr12P.

Marker-trait associations across treatments and years

Seven traits had significant variation in combined analyses of water-stressed or non-stressed treatments: GY, HI, KPS, PFS, PH, SPS, and KW. Genome-wide association studies detected 33 QTL, including 11 for PFS, 10 for HI, seven for KW, three for PH, and two for SPS (Table 4.17). Four markers had significant associations with multiple traits: *BS00012081_51*, *RAC875_c15844_348*, and *RAC875_c31358_214* (all unmapped) were associated with PFS and PH, while *Tdurum_contig29087_757* (chromosome 1B) was associated with HI and KW.

Eight traits varied significantly in combined analyses of 2011 or 2012 environments: DTB, DTF, DTH, HI, KPS, PFS, and SPS. A total of 35 QTL were detected,

including 11 for DTF and HI, 10 for PFS, two for SPS, and one for KPS (Table 4.18). Three markers had significant associations with multiple traits: *Kukri_c22513_1780* (chromosome 2B) and *GENE.1273_395* (unmapped) associated with HI and DTF, and *Tdurum_contig42153_1190* (chromosome 7B) was associated with HI and PFS.

Discussion

Phenotypic analyses of agronomic traits

Variability of agronomic traits across environments

Grain yield was reduced by a similar amount under water stress in 2011-12 and 2012-13 even though the timing of water stress varied (Table 4.1). Stress occurred pre-anthesis in the summer of 2012, and post-anthesis in 2013. The timing of water stress resulted in grain yield being reduced by different yield components in each year. In 2012, grain yield was reduced mostly due to lower SN (30% reduction) and fewer SPS (9% reduction, Table 4.3) because the developmental processes occurring before anthesis include initiation of vegetative and reproductive structures including tillers and spikelets. In 2013, grain yield was reduced at Fo13R relative to Fo13F primarily due to smaller seed size (27% reduction in KW, Table 4.4), which is consistent with our understanding that final kernel number is determined at anthesis, and further improvements of grain yield are due to increases in kernel size. Additionally, environmental conditions between the two years contributed to an average of 15 days earlier heading in the water stressed treatment, and 12 days earlier heading in the non-stressed treatment, in 2012 than 2013 (Tables 4.2,

4.3). Earlier heading in 2012 was likely facilitated by hot and dry conditions beginning in the early spring and extending through the summer (Grogan et al., 2015b).

Combined analyses across environments

In the combined analysis across all four environments, G×E only had a significant effect on a few traits (GY, GBM, HI, TW, DTB, and DTF; Table 4.6), which suggests most traits had similar rankings of germplasm across environments. However, when environment was partitioned into effects of moisture treatment and year, the interaction between genotype and treatment and/or year was significant for most traits (Table 4.8). This illustrates the complexity of G×E and that variation among years or moisture treatments affected the ranking of germplasm. The interaction between treatment and year was also significant for most traits, which is partially explained by variation in the timing of water stress. In 2013 the moisture differential was not applied until May (Table 4.1) and stress occurred post-anthesis, so yield components such as SN, KN, KPS, and SPS are not expected to differ significantly between Fo13F and Fo13R, although since stress occurred pre-anthesis in 2012, these traits are expected to vary significantly for Gr12F and Gr12P. This type of variation among years contributes to significant treatment-by-year interaction.

Environmental effects due to year were greater than those due to treatment for most of the phenological traits (DTB, DTH, DTF, DTM, Table 4.8). This is likely because plant development is largely dependent on temperature accumulation (Moragues and McMaster, 2011), and there were many very hot days in 2012, including many in the early spring. Similarly, PFS had a larger effect due to year than treatment, likely because high temperatures in 2012 elevated the rate of floret abortion, reducing spike fertility (Cossani

and Reynolds, 2012). The number of spikelets per spike and other spike measurements were the most highly heritable traits (H^2 = 0.85 for SPS, H^2 = 0.84 for PFS, and H^2 = 0.78 for KPS, Table 4.6). Each of these traits was measured from a collection of 10 spikes per plot. High heritability for these traits is likely due to strong genetic control and a high degree of replication.

The main causes underlying a genetic correlation between two or more traits are linkage and pleiotropy. Like all correlations, genetic correlations fall within a theoretical limit of 1 and -1. However, some of our genetic correlations exceeded the theoretical limits (Table 4.8). The genetic correlations were > 1 or < -1 when the genetic variance of one trait was much smaller than the other. A small genetic variance sometimes resulted in r_G > |1| because taking the product of both genetic variances, and then the square root of that value, results in a very small value in the denominator of Eq. 4.4. In some cases the denominator was smaller than the numerator.

There were positive genetic correlations between PH and the phenological traits (DTB, DTH, DTF, and DTM). The correlation was strongest at booting (r_G = 0.62) and decreased steadily at each successive developmental stage (Table 4.7). This highlights the possible role of the *Reduced-height* (*Rht*) loci on wheat development, which has been identified previously (Berry et al., 2014) but not fully explained.

Genetic analyses of agronomic traits

Associations between traits can be confounding and result in false associations or distort the magnitude of an association (Thomas, 2010). The effects of phenology are known to confound some agronomic traits, especially under drought stress (Reynolds et al.,

2009). We attempted to minimize confounding by correcting for population structure and kinship, and including three major developmental genes: *Ppd-B1* (chromosome 2B), *Ppd-D1* (chromosome 2D), and *Rht-B1* (chromosome 4B) as covariates in the analysis. Previously, we found these loci to have significant effects on heading date in the HWWAMP (Grogan et al., 2015b). We detected the largest number of significant marker–trait associations on chromosome 2B (Table 4.15, some of which could be caused by *Ppd-B1*, also located on chromosome 2B, such as through genetic linkage. Our genotyping methods only detected allelic variants caused by a SNP. However, a single SNP variant could be associated with multiple alleles. Spurious associations could result from the presence of allelic variants (Cane et al., 2013), copy number variants (Díaz et al., 2012), epigenetic variation (King et al., 2010), or marker imputation (He et al., 2015). Copy number variants and epigenetic variation are not detected in SNP markers. Other developmental genes that could have confounding effects include *Rht-D1* (chromosome 4D), and *Ppd-A1* (chromosome 2A), and *Vrn-B3* (chromosome 7B). The vernalization loci *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* located on the group 5 chromosomes are unlikely to have a large effect within the HWWAMP, since the accessions are believed to be exclusively winter wheats and thus do not vary at these loci (Grogan et al., 2015b).

The power to detect associations depends on the marker density, extent of LD, sample size, and QTL effect size (Long and Langley, 1999; Zhu et al., 2008). Linkage disequilibrium is the non-random association between alleles at different loci (Flint-Garcia et al., 2003). The extent of LD is greater in self-pollinated crops like wheat than in cross-pollinating species, because there is less opportunity for recombination during meiosis. High levels of selfing, coupled with a large genome size, means wheat requires a very large

number of molecular markers for high mapping resolution. The extent of LD has been shown to extend much further on the D- than A- or B-genomes (Chao et al., 2010; Edae et al., 2014; Lopes et al., 2014). The extent of LD and level of genetic diversity in a population can be affected by a number of different processes, such as inbreeding, natural selection, domestication, outcrossing with wild relatives, and admixture (Chao et al., 2010). In hexaploid wheat, a higher extent of LD on the D-genome can be explained by polyploid speciation resulting in a genetic bottleneck, and reduced diversity on the D-genome (Dubcovsky and Dvorak, 2007). We found LD to extend more than four times further on the D- than A- or B-genome. Therefore, it is not surprising that many fewer significant marker–trait associations were detected on the D-genome (15 associations) than were detected across the A-genome (63 associations) and B-genome (61 associations, Table 4.15).

Comparable numbers of QTL were detected in 2012 compared to 2013 (Table 4.9). In the water-stressed treatments (Gr12P and Fo13R), the traits with the greatest numbers of QTL reflect the traits most affected by drought stress each year. At Gr12P reductions in yield were due to limitations imposed by small biomass production and a reduction in number of spikelets and kernels produced per spike. A large number of QTL were detected for SPS, HI, and BM at Gr12P. At Fo13R yield was reduced due to smaller kernel size, which was affected by the timing of phenological development, including grain fill duration. At Fo13R many QTL were detected for DTF and GF.

As expected, the traits for which significant marker–trait associations co-localized tended to have strong genetic correlations. For instance, several markers were shared between HI and DTH (r_G = < -1.0), HI and PFS (r_G = 0.51), and PFS and DTH (r_G = 0.58) or DTB (r_G = 0.62).

Many significant QTL were detected for DTB, DTH, and DTF in one or more environment (Table 4.9), and QTL were detected on 18 different chromosomes (Table 4.15). The greatest number of phenological QTL was detected on chromosomes 2B (8 QTL), 3A (5 QTL), and 7B (4 QTL, Table 4.15). The effect of these QTL could not be evaluated in a combined analysis across environments, because DTB, DTH, and DTF did not have significant variation in the combined analysis. However, these traits were evaluated in a GWAS estimating the effects on year. Interestingly, QTL were detected for DTF but not DTB or DTH. In the combined analysis of year, QTL for DTF were detected on chromosomes 1A, 3A, 7A, 2B, 4B, 7B, and 1D (Table 4.18). Some of these genomic regions could indicate genetic variation at earliness *per se* loci, which are known to have inconsistent effects across environments (Laurie et al., 2004). Griffiths et al. (2009) have reported a number of QTL associated with wheat development and heading date, including QTL that may be consistent with the QTL we detected on chromosomes 3A, 7A, 4B, 7B, and 1D. A QTL with a large effect on thermal time to heading was also detected on chromosome 4B by Langer et al. (2014).

Many traits did not vary significantly in combined analyses across environments, moisture treatments, or years and GWAS could only be performed on traits with significant variation. Five QTL were detected in the combined analysis across all four environments (Table 4.16).

No significant marker–trait associations were detected for GY, GBM, KW, or DTM. This could be due to the complex and quantitative nature of these traits, low trait heritability, or lack of detection power due to sample size or marker density. Additionally, there may be more sources of error intrinsic in measuring these traits than some others.

Some of these sources of error include variation in plot size or missed harvestable plot area in estimating GY; error in KN caused by either incomplete separation of grain from spikes—which could be caused by thresher instrument settings, spike size, or spike dryness—or error in counting number of seeds due to variation in seed cleanness or seed counter speed and size detection settings; or error estimating GBM due to variation in biomass cutting and losses during threshing or seed cleaning. By contrast, many of the most highly-significant associations were detected for traits related to spike fertility, including SPS, DTB, DTF, and PFS (Table 4.9). Numerous QTL were also detected for DTB and DTF. The high number of QTL detected for these traits could be due to both the presence of large-effect QTL and rigorous phenotyping with fewer opportunities to introduce error.

Conclusions

Our results indicate substantial phenotypic and genetic variation of agronomic traits among winter wheat germplasm from the U.S. Great Plains when evaluated under contrasting moisture regimes. There were considerably different patterns of water stress in the two years the germplasm was evaluated. Water-stress occurred pre-anthesis in 2012 and post-anthesis in 2013. Despite this, the percent reduction in grain yields under stress, and total number of QTL detected for all traits in both moisture treatments, was similar in 2012 and 2013. We found that the extent of LD varied substantially among genomes, with a much greater extent along the D- than the A- or B- genomes. The higher extent of LD along the D-genome can be attributed to relatively low diversity along this genome, which

explains why there are fewer SNP markers and why a lower number of significant marker–trait associations were detected.

Genome-wide association mapping is a powerful tool to detect genomic regions affecting a trait, and we detected nearly 250 significant marker–trait associations in individual environments and combined across moisture treatments, years, or environments. However, most QTL were only detected for a single trait in one environment, and only explained a small proportion of total phenotypic variability. The number of significant marker–trait associations detected depends on the statistical criteria used. We used the Benjamini–Hochberg procedure with a false discovery rate of 50% to identify significant associations, but a different approach or significance threshold would yield different results. A best method for establishing significant marker–trait associations is yet to be established. Considerable challenges still exist in conducting and analyzing a GWAS, and results could be improved by using a larger population, incorporating denser marker data with greater coverage across the D-genome and across all chromosome regions. Applications of GWAS for wheat will be enhanced as the genetic and physical maps are improved and better annotated. Further validation of these QTL, such as understanding the underlying polymorphism and verifying the effects in an independent population is needed before incorporating these QTL into breeding material using marker-assisted selection.

Tables.

Table 4.1. Total monthly precipitation (P, mm) and irrigation (I, mm) each environment received. The environments are Greeley, CO full irrigation (Gr12F) and partial irrigation (Gr12P) in 2012, and Fort Collins, CO full irrigation (Fo13F) and rainfed (Fo13R) in 2013. No irrigation was applied to any environment in January or February. The last month only includes precipitation that occurred before harvest, and all 2012 environments were harvested in June.

	Jan	Feb	Mar		Apr		May		June		July		Total		
Env	P	P	P	I	P	I	P	I	P	I	P	I	P	I	P+I
Gr12F	0.3	10.7	0.5	25.4	23.1	50.8	28.2	144.8	19.3	114.3	.	.	82.1	335.3	417.4
Gr12P	0.3	10.7	0.5	25.4	23.1	12.7	28.2	50.8	19.3	12.7	.	.	82.1	101.6	183.7
Fo13F	0.3	2	5.3	--	30.2	--	35.8	63.5	11.9	146.1	38.9	12.7	124.4	222.3	346.7
Fo13R	0.3	2.0	5.3	--	30.2	--	35.8	--	11.9	--	38.9	--	124.4	--	124.4

Table 4.2. List of agronomic traits evaluated, abbreviations for traits, and units of measurement. All agronomic traits except for developmental traits were measured at physiological maturity.

Trait	Abbreviation	Unit
Grain yield	GY	kg ha^{-1}
Spike number	SN	n m^{-2}
Kernel number	KN	n m^{-2}
Kernels per spike	KPS	n $spike^{-1}$
Spikelets per spike	SPS	n $spike^{-1}$
Proportion fertile spikelets	PFS	ratio as a decimal
Kernel weight	KW	mg $kernel^{-1}$
Above ground biomass†	BM	g
Grain biomass†	GBM	g
Harvest index†	HI	ratio as a decimal
Plant height	PH	cm
Days to booting after 1 Jan	DTB	d
Days to heading after 1 Jan	DTH	d
Days to flowering after 1 Jan	DTF	d
Days to maturity after 1 Jan	DTM	d
Grain fill duration	GF	d

†From a 1 m sample cut from of one row of each field plot.

Table 4.3. Summary of phenotypic trait means, standard deviations (s.d.), and ranges for 299 hard winter wheat accessions grown at two environments grown in 2011–2012. The two environments are Greeley, CO full irrigation (Gr12F) and partial irrigation (Gr12P). Traits and units are described in Table 4.2. The percent reduction is the amount the average trait value was reduced by at Gr12P relative to Gr12F.

	Gr12F		Gr12P		
Trait	**Mean ± s.d.**	**Range**	**Mean ± s.d.**	**Range**	**% Reduction**
GY	4801.89 ± 978.54	1631.06–6704.98	2510.21 ± 251.38	1787.45–3112.53	47.72
SN	454.74 ± 17.65	396.69–513.44	318.05 ± 37.55	237.47–606.74	30.06
KN	7286.30 ± 522.71	6000–8638	4651.48 ± 530.20	3128–9022	36.17
KPS	37.17 ± 3.87	26.11–48.3	31.72 ± 4.76	17.05–49.58	14.66
SPS	16.04 ± 1.25	13.02–20.49	14.67 ± 1.09	11.72–18.32	8.54
PFS	0.89 ± 0.03	0.79–0.97	0.90 ± 0.04	0.74–0.99	-1.12
KW	33.07 ± 0.03	32.99–33.13	29.57 ± 2.28	20.42–36.4	10.58
BM	477.92 ± 36.66	277.34–588.53	231.22 ± 19.56	174.15–300.34	51.62
GBM	161.87 ± 30.05	65.85–260.78	88.32 ± 9.11	63.14–128.09	45.44
HI	0.34 ± 0.05	0.15–0.43	0.38 ± 0.04	0.26–0.52	-11.76
PH	89.91 ± 9.30	67.77–117.74	57.97 ± 3.89	47.36–72.69	35.52
DTB	128.49 ± 3.59	122.07–139.78	124.89 ± 2.95	117.55–136.20	2.80
DTH	136.69 ± 3.38	124.7–148.05	134.34 ± 3.21	126.87–141.47	1.72
DTF	143.42 ± 2.53	136.83–152.27	137.71 ± 2.05	133.94–143.17	3.98
DTM	174.15 ± 2.28	168.66–180.50	166.44 ± 1.89	161.44–171.84	4.43
GF	32.79 ± 2.28	25.67–38.75	28.75 ± 0.63	26.87–30.46	12.32

Table 4.4. Summary of phenotypic trait means, standard deviations (s.d.), and ranges for 299 hard winter wheat accessions grown at two environments grown in 2012–2013. The two environments are Fort Collins, CO full irrigation (Fo13F) and rainfed (Fo13R). Traits and units are described in Table 4.2. The percent reduction is the amount the average trait value was reduced by at Fo13R relative to Fo13F.

	Fo13F		Fo13R		
Trait	**Mean ± s.d.**	**Range**	**Mean ± s.d.**	**Range**	**% Reduction**
GY	5008.33 ± 401.80	3984.50–6042.30	2684.49 ± 132.85	2318.60–3171.40	46.40
SN	373.82 ± 32.88	276.35–459.94	355.05 ± 17.53	320.69–449.27	5.02
KN	5966.17 ± 665.79	4061–8133	5569.00 ± 395.18	4548–6976	0.07
KPS	38.21 ± 2.46	32.49–46.42	30.28 ± 3.61	17.84–40.00	20.75
SPS	15.97 ± 1.27	11.96–19.28	15.69 ± 0.88	13.31–18.70	1.75
PFS	0.92 ± 0.03	0.84–0.98	0.91 ± 0.03	0.77–0.98	1.09
KW	35.62 ± 2.87	27.58–45.45	25.85 ± 1.86	19.84–31.17	27.43
BM	304.96 ± 10.56	279.33–338.15	228.21 ± 16.55	190.28–270.99	25.17
GBM	122.24 ± 8.38	101.70–147.52	74.88 ± 5.40	62.91–90.38	38.74
HI	0.40 ± 0.02	0.30–0.49	0.33 ± 0.03	0.24–0.40	17.50
PH	79.2 ± 6.83	65.56–99.82	61.86 ± 4.42	48.43–74.11	21.89
DTJ	NA	NA	131.85 ± 0.12	131.38–132.29	NA
DTB	145.56 ± 1.69	140.92–148.88	145.63 ± 1.81	142.55–151.43	-0.05
DTH	149.84 ± 2.16	146.19–156.08	149.45 ± 2.13	145.56–159.51	0.26
DTF	155.35 ± 1.60	152.19–159.05	154.36 ± 1.63	151.94–159.37	0.64
DTM	185.91 ± 1.68	180.19–189.96	178.95 ± 1.90	172.76–183.41	3.74
GF	30.57 ± 1.87	25.07–36.72	24.60 ± 1.85	19.58–29.93	19.53

Table 4.5. Pearson's correlation coefficient (*r)* between agronomic traits. Traits and units are described in Table 4.2. Shaded rows represent water-stressed environments (Fo13R or Gr12P), while non-shaded rows represent fully-irrigated environments (Fo13F or Gr12F). The environments grown in 2013 are in the upper diagonal (Fo13R, upper shaded; Fo13F, upper non-shaded) and environments grown in 2012 are in the lower diagonal (Gr12P, lower shaded; Gr12F, lower non-shaded).

	GY	SN	KN	KPS	SPS	PFS	KW	BM	GBM	HI	PH	DTH	GF
GY		0.44***	0.40***	0.18**	0.09 ns	0.08 ns	0.23***	0.37***	0.49***	0.30***	0.09 ns	0.04 ns	0.15**
		0.17**	0.14*	0.37***	0.03 ns	0.12*	0.35***	0.42***	0.58***	0.51***	0.32***	-0.32***	0.00 ns
SN	0.18**		0.70***	-0.59***	-0.12***	-0.35***	-0.25***	0.31***	0.24***	-0.01 ns	0.25***	0.07 ns	-0.08 ns
	0.50***		0.62***	-0.56***	-0.09 ns	-0.2***	-0.34***	0.17**	0.11 ns	-0.11 ns	0.17**	0.01 ns	-0.06 ns
KN	0.37***	0.79***		-0.15**	0.62***	-0.49***	-0.35***	0.28***	0.19***	-0.06 ns	0.39***	0.36***	-0.09 ns
	0.00 ns	0.13*		-0.09 ns	0.72***	-0.31***	-0.44***	0.20***	0.12*	-0.16**	0.26***	0.24***	0.09 ns
KPS	0.50***	-0.52***	-0.11 ns		0.45***	0.37***	-0.09 ns	-0.03 ns	0.09 ns	0.17**	-0.21***	0.10 ns	0.15*
	0.32***	-0.37***	0.29***		0.38***	0.31***	0.03 ns	0.13*	0.26***	0.35***	-0.01 ns	-0.08 ns	0.06 ns
SPS	0.27***	-0.37***	0.27***	0.65***		-0.30***	-0.21***	0.07 ns	0.01 ns	-0.07 ns	0.26***	0.42***	-0.02 ns
	-0.26***	-0.39***	0.86***	0.45***		-0.21***	-0.26***	0.11 ns	0.06 ns	-0.11 ns	0.19**	0.29***	0.17**
PFS	0.37***	-0.29***	-0.01 ns	0.57***	0.45***		0.06 ns	-0.13*	0.01 ns	0.16**	-0.44***	-0.18**	0.12*
	0.21***	-0.09 ns	0.25***	0.44***	0.27***		0.07 ns	-0.10 ns	-0.01 ns	0.16**	-0.18**	-0.17**	0.03 ns
KW	0.13*	-0.41***	-0.45***	0.02 ns	-0.03 ns	0.07 ns		0.05 ns	0.12*	0.16**	0.10 ns	-0.20***	0.14*
	0.57***	0.10 ns	-0.38***	0.03 ns	-0.41***	0.07 ns		0.08 ns	0.22***	0.33***	0.25***	-0.28***	0.06 ns
BM	0.11 ns	0.22***	0.22***	-0.03 ns	-0.01 ns	-0.12*	-0.1 ns		0.87***	-0.02 ns	0.35***	0.07 ns	0.07 ns
	0.13*	0.09***	0.09 ns	0.12*	0.04 ns	0.02 ns	-0.02 ns		0.86***	0.13*	0.25***	-0.19**	-0.12*
GBM	0.52***	0.15**	0.26***	0.25***	0.14*	0.13*	0.08 ns	0.71***		0.43***	0.17**	0.03 ns	0.11 ns
	0.75***	0.43***	0.01 ns	0.27***	-0.21***	0.14*	0.49***	0.53***		0.54***	0.27***	-0.25***	-0.13*
HI	0.61***	-0.04 ns	0.11 ns	0.46***	0.22***	0.40***	0.24***	-0.08 ns	0.56***		-0.19***	-0.05 ns	0.08 ns
	0.81***	0.43***	-0.06 ns	0.24***	-0.27***	0.17***	0.59***	-0.02 ns	0.80***		0.08 ns	-0.27***	-0.05 ns

Table 4.5. Continued.

	GY	SN	KN	KPS	SPS	PFS	KW	BM	GBM	HI	PH	DTH	GF
PH	-0.27***	0.03 ns	0.01 ns	-0.13*	-0.03 ns	-0.18**	-0.08 ns	0.26***	-0.03 ns	-0.34***		0.28***	-0.09 ns
	-0.71***	-0.39***	0.10 ns	-0.18***	0.29***	-0.23***	-0.47***	0.08 ns	-0.50***	-0.67***		0.00 ns	-0.01 ns
DTH	-0.54***	-0.29***	0.31***	0.02 ns	0.44***	-0.10 ns	-0.60***	0.02 ns	-0.45***	-0.55***	0.67***		0.05 ns
	-0.39***	0.16**	0.06 ns	-0.34***	-0.15**	-0.34***	-0.30***	0.33***	-0.16**	-0.60***	0.35***		0.35***
GF	0.26***	-0.11 ns	-0.06 ns	0.17**	0.07 ns	0.15**	0.25***	-0.13*	0.11 ns	0.29***	-0.13*	-0.44***	
	0.56***	0.21***	-0.01 ns	0.28***	-0.13*	0.17**	0.32***	0.03 ns	0.43***	0.52**	-0.55**	-0.43***	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels.

† ns, nonsignificant at the 0.05 probability level.

Table 4.6. Summary of ANOVA results testing the effects of genotype, environment, and genotype-by-environment interaction (G×E) in agronomic traits evaluated on 299 hard winter wheat accessions in four environments. The table includes mean square values and significance level of each term. Broad-sense heritability (*H2*) is the proportion of phenotypic variance that is due to genetic effects, and is estimated using actual mean squares. The mean square values for G×E are only presented for traits where the interaction term was significant. Traits are described in Table 4.2.

Trait	G	E	G×E	H^2
GY	440026.8*	534505169.7***	256200.7*	0.43
SN	1346.1***	998046.0***	-- ‡	0.69
KN	43315.4***	26739237.0***	--	0.56
KPS	34.081***	4633.0***	--	0.78
SPS	3.474***	120.7***	--	0.84
PFS	0.003***	0.05***	--	0.85
KW	9.11***	5414.5***	--	0.71
BM	564.6 ns†	4095665.7***	--	0.08
GBM	319.9**	450585.3***	256.5*	0.20
HI	0.002**	0.314***	0.001*	0.63
PH	100.3***	67138.3***	--	0.77
DTB	20.134***	36365.3***	2.583**	0.87
DTH	23.232***	20231.0***	--	0.89
DTF	9.735***	22055.7***	2.059**	0.79
DTM	9.983***	20131.3***	--	0.82
GF	4.685***	3611.2***	--	0.44

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels.
† ns, nonsignificant at the 0.05 probability level.
‡ Double-dash (--) indicates G×E did not have a significant effect so only main effects were included in the model.

Table 4.7. Genetic correlations (r_G) between traits, estimated from genetic variance components of each trait. The genetic variance components are estimated using all four environments. Traits and units are described in Table 4.2.

	GY	SN	KN	KPS	SPS	PFS	KW	BM	GBM	HI	PH	DTB	DTH	DTF	DTM	GF
GY		-0.27	<-1.0†	0.69	-0.14	0.69	0.45	0.14	>1.0†	>1.0	-0.77	-0.79	-0.77	-0.76	-0.56	0.36
SN			<-1.0	-0.69	-0.35	-0.14	-0.42	0.36	-0.34	<-1.0	0.16	0.15	0.20	0.13	-0.07	-0.40
KN				-0.91	<-1.0	-0.93	<-1.0	-0.93	<-1.0	-0.89	-0.92	<-1.0	-0.91	<-1.0	-0.92	<-1.0
KPS					0.50	0.47	-0.11	0.05	0.71	0.08	-0.44	-0.21	-0.21	-0.13	0.07	0.41
SPS						-0.04	-0.35	0.27	-0.22	-0.42	0.17	0.41	0.40	0.50	0.50	0.02
PFS							0.01	-0.11	0.74	0.51	-0.48	-0.38	-0.34	-0.33	-0.14	0.37
KW								0.15	0.71	0.45	-0.05	-0.43	-0.45	-0.46	-0.38	0.17
BM									-0.49	<-1.0	0.81	0.47	0.53	0.49	0.50	0.00
GBM										>1.0	-0.76	<-1.0	<-1.0	<-1.0	-0.87	0.22
HI											<-1.0	<-1.0	<-1.0	<-1.0	-0.72	0.27
PH												0.62	0.58	0.55	0.39	-0.30
DTB													0.97	0.94	0.83	-0.22
DTH														>1.0	0.01	>1.0
DTF															-0.55	-0.32
DTM																0.28

† r_G exceeded the theoretical range [1, -1] for some pairs of traits. This occurred when the genetic variance component for one trait was very small.

Table 4.8. Summary of ANOVA results testing the main effects of genotype (G), moisture treatment (T, full or partial irrigation), year (Y, 2012 or 2013); two-way interactions between genotype and treatment (G×T), genotype and year (G×Y), and treatment and year (T×Y); and the three-way interaction between genotype, treatment, and year (G×T×Y). ANOVA models were run separately for each trait, and only include terms that had a significant effect: with the exceptions that if an interaction term had a significant effect the main effects of those terms were always included. The table includes mean square values and the significance level of each term. The traits are described in Table 4.2.

Trait	G	T	Y	G×T	G×Y	T×Y	G×T×Y
GY	440003.6***	1590701682.0***	10770542.0***	--‡	303959.3**	--	--
SN	1346.1***	1804320.0***	145449.0***	--	--	1042078.0***	--
KN	43315.4***	53065419.0***	106672.0**	--	17436.3***	27045619.0***	--
KPS	12.4 ns†	13423.0***	34.1***	--	9.789***	463.3***	--
SPS	3.474***	205.8***	67.72***	0.584***	0.717***	88.6***	--
PFS	0.003***	0.008***	0.122***	--	0.001**	0.021***	--
KW	9.1***	13195.4***	100.8***	--	--	2947.3***	--
BM	--	7820057.0***	2318572.0***	--	--	2162446.0***	--
GBM	279.3*	3927.8***	210656.0***	236.8*	283.5*	51138.0***	247.3*
HI	0.002**	0.001*	0.006***	0.001*	0.001**	0.89***	0.001*
PH	100.3***	181808.0***	3527.0***	32.111***	21.332***	15945.0***	--
DTB	10.815***	4.629***	107148.0***	1.497**	4.705***	1012.0***	1.550**
DTH	23.232***	569.0***	59847.0***	--	4.668***	289.0***	--
DTF	5.662***	12.862***	61108.0***	1.567*	3.034**	1675.0***	1.569*
DTM	9.983***	16138.0***	44214.0***	1.896*	1.896*	43.0***	--
GF	4.685***	7484.9***	3037.1***	2.760*	2.908**	282.2***	--

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels.
† ns, nonsignificant at the 0.05 probability level.
‡ Double-dash (--) indicates term did not have a significant effect so was not included in model.

Table 4.9. Summary of significant marker–trait associations for agronomic traits evaluated in four individual environments: full irrigation in Fort Collins, CO during 2013 (Fo13F), rainfed in Fort Collins, CO during 2013 (Fo13R), full irrigation in Greeley, CO during 2012 (Gr12F), and partial irrigation in Greeley, CO during 2012 (Gr12P). No significant marker–trait associations were detected for GY, KW, GBM, or DTM. Traits are described in Table 4.2. Significant marker–trait associations include quantitative trait loci (QTL) with mapped markers, and also significant associations with unmapped markers. Significant QTL are separated from each other by a minimum of 10 cM, and corrected for multiple comparisons using the Benjamini–Hochberg procedure at a false discovery rate of 50%.

Trait	Fo13F	Fo13R	Gr12F	Gr12P	Total
GY	0	0	0	0	0
SN	1	0	0	2	3
KN	5	0	6	0	11
KPS	1	0	0	1	2
SPS	1	5	2	20	28
PFS	3	10	0	5	18
KW	0	0	0	0	0
BM	1	0	0	10	11
GBM	0	0	0	0	0
HI	3	1	2	14	20
PH	0	0	4	0	4
DTB	11	0	7	5	23
DTH	0	0	17	0	17
DTF	6	15	0	0	21
DTM	0	0	0	0	0
GF	0	15	0	0	15
Total	**32**	**46**	**38**	**57**	**173**

Table 4.10. Summary of GWAS results involving mapped markers at Fo13F. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
BM	Tdurum_contig66604_927	5B	183.0	4.41E-05	0.47	0.06	2.648
DTB	wsnp_Ex_rep_c102067_87314043	1A	107.7	1.29E-03	0.38	0.03	0.371
	wsnp_Ex_c15269_23491104	3A	89.2	5.61E-04	0.26	0.04	0.522
	tplb0049a09_1302	5A	139.8	2.22E-04	0.07	0.04	0.706
	Ex_c9615_1202	7A	82.7	1.13E-04	0.32	0.05	0.460
	BS00066271_51	1B	21.0	1.20E-03	0.50	0.03	0.337
	BS00057153_51	4B	75.6	2.46E-04	0.11	0.04	0.674
	Tdurum_contig81113_395	4B	112.2	1.07E-04	0.28	0.05	0.458
	IAAV8378	5B	76.9	1.90E-04	0.06	0.04	0.751
	BobWhite_c11495_120	5B	112.4	6.58E-05	0.09	0.05	0.684
	Excalibur_c6871_217	7B	98.6	2.57E-04	0.21	0.04	0.451
	Ex_c8238_637	7D	137.6	4.73E-04	0.11	0.04	0.569
DTF	Excalibur_rep_c101767_219	3A	76.4	5.02E-04	0.25	0.04	0.441
	BobWhite_c2988_2161	2B	56.3	5.73E-04	0.16	0.04	0.481
	BS00023023_51	7B	120.8	2.54E-04	0.26	0.04	0.412
	Ra_c11906_1618	1D	107.1	2.73E-04	0.35	0.04	0.412
	Excalibur_c17039_436	2D	36.5	1.03E-04	0.28	0.05	0.437
	RAC875_c51595_177	3D	142.3	2.36E-04	0.46	0.04	0.401
HI	IAAV880	2A	149.6	2.18E-04	0.17	0.05	0.006
	BobWhite_c22728_78	2B	109.2	3.50E-04	0.18	0.04	0.006
	BS00095515_51	3B	106.7	3.38E-04	0.34	0.04	0.005

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.10. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
KN	Excalibur_c57840_227	6B	100.1	2.27E-05	0.06	0.06	349.6
	BS00093252_51	3A	181.5	1.44E-04	0.08	0.05	278.4
	wsnp_JD_c43684_30430706	5D	69.1	1.95E-04	0.29	0.04	177.4
	CAP11_c3666_426	7A	211.7	4.36E-04	0.05	0.04	294.6
	Kukri_c40035_258	2A	99.3	4.38E-04	0.18	0.04	202.8
KPS	IACX6337	2A	113.3	7.59E-05	0.25	0.04	0.781
PFS	wsnp_JD_c2128_2930150	5A	15.9	1.79E-04	0.29	0.04	0.007
	Excalibur_c29255_404	5A	139.8	1.10E-04	0.06	0.04	0.013
	Tdurum_contig42153_1190	2B	66.2	1.22E-04	0.43	0.04	0.008
SPS	wsnp_Ku_rep_c104159_90704469	7A	152.8	3.99E-05	0.26	0.05	0.405
SN	wsnp_Ex_c28942_38018762	2A	113.3	5.55E-05	0.27	0.05	10.192

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.11. Summary of GWAS results involving mapped markers at Fo13R. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
DTF	BobWhite_c37935_124	1A	74.8	6.52E-04	0.09	0.04	0.618
	BobWhite_c9249_564	3A	42.6	2.56E-04	0.48	0.04	0.371
	wsnp_Ku_c5378_9559013	3A	90.6	2.52E-04	0.47	0.05	0.405
	wsnp_Ex_c9779_16145653	6A	21.1	7.26E-04	0.12	0.04	0.519
	Kukri_c65887_282	6A	85.1	5.49E-04	0.05	0.04	0.778
	BS00088489_51	2B	108.0	1.87E-05	0.05	0.06	0.959
	BS00062304_51	4B	110.8	2.36E-04	0.17	0.05	0.496
GF	wsnp_Ex_c2181_4089788	1A	68.8	1.39E-03	0.19	0.03	0.513
	Ex_c101416_378	4A	70.6	1.41E-03	0.07	0.03	0.705
	BobWhite_c534_837	6A	58.5	2.74E-04	0.15	0.04	0.634
	CAP8_c702_377	7A	124.3	5.70E-04	0.09	0.04	0.639
	IAAV5863	1B	114.6	1.30E-03	0.44	0.03	0.398
	RAC875_c52774_135	4B	75.6	9.24E-04	0.10	0.04	0.691
	IACX7649	5B	79.6	8.62E-04	0.09	0.04	0.676
	Kukri_c12901_706	7B	98.7	3.08E-04	0.22	0.04	0.492
	Kukri_c8913_655	3D	119.4	7.77E-04	0.13	0.04	0.573
	Kukri_c64744_698	4D	17.7	1.52E-03	0.25	0.03	0.420
	RAC875_c98242_422	6D	22.9	9.96E-04	0.22	0.04	0.451
HI	wsnp_Ex_c3963_7179957	1A	70.1	3.71E-06	0.21	0.07	0.012
PFS	wsnp_BE442666A_Ta_2_1	4A	51.7	1.08E-04	0.08	0.04	0.012
	wsnp_JD_c2128_2930150	5A	15.9	4.41E-05	0.29	0.05	0.008
	Excalibur_c29255_404	5A	139.8	3.73E-05	0.06	0.05	0.014
	BS00040600_51	7A	122.9	7.02E-04	0.13	0.03	0.010
	Tdurum_contig42153_891	2B	66.2	3.44E-04	0.43	0.04	0.008
	wsnp_Ex_rep_c67471_66073729	5B	39.4	6.01E-04	0.06	0.03	0.012
	Jagger_c3235_381	6B	88.7	2.67E-05	0.05	0.05	0.017

Table 4.11. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
SPS	tplb0032i02_1435	2A	25.0	5.55E-05	0.47	0.05	0.233
	wsnp_Ku_rep_c104159_90704469	7A	152.8	8.19E-05	0.26	0.05	0.269
	JD_c5200_835	7B	72.7	1.81E-04	0.06	0.04	0.416

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.12. Summary of GWAS results involving mapped markers at Gr12F. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
DTB	Excalibur_c35316_154	1A	16.7	2.08E-05	0.40	0.05	0.97
	BobWhite_c37935_124	1A	74.8	3.34E-04	0.09	0.03	1.39
	BobWhite_c25163_178	4A	153.0	3.18E-04	0.26	0.03	0.87
	Tdurum_contig42153_891	2B	66.2	3.95E-04	0.43	0.03	0.92
	BobWhite_c19554_544	2B	107.5	3.17E-04	0.05	0.03	1.70
	Tdurum_contig15734_221	7B	120.9	5.22E-05	0.24	0.04	1.01
DTH	wsnp_Ex_c905_1748920	2A	151.3	8.33E-04	0.26	0.03	0.76
	wsnp_Ku_c3956_7237707	3A	86.7	2.76E-04	0.25	0.04	1.00
	wsnp_Ku_c5693_10079278	7A	208.7	8.14E-04	0.06	0.03	1.29
	Kukri_c22513_1780	2B	26.5	6.13E-04	0.19	0.03	0.84
	wsnp_Ex_rep_c70756_69644826	2B	65.0	1.36E-04	0.40	0.04	0.95
	BobWhite_c29596_649	2B	115.0	9.83E-04	0.31	0.03	0.78
	BS00034554_51	6B	93.5	8.26E-04	0.35	0.03	0.71
	Tdurum_contig15734_221	7B	120.9	1.44E-04	0.24	0.04	0.88
	Kukri_rep_c73094_348	5D	72.3	5.98E-04	0.35	0.03	0.74
	Excalibur_c55782_55	7D	127.7	8.27E-04	0.49	0.03	0.63
HI	Tdurum_contig29087_757	1B	136.0	1.41E-05	0.06	0.05	0.03
	wsnp_Ra_c4321_7860456	2B	41.4	1.02E-04	0.27	0.04	0.01
KN	Excalibur_c35316_154	1A	16.7	2.62E-04	0.40	0.04	130.5
	BobWhite_c26374_339	2A	73.9	2.16E-04	0.29	0.04	137.7
	BS00046261_51	6A	133.7	2.40E-04	0.05	0.04	263.8
	wsnp_JD_c20555_18262317	7A	152.8	2.75E-07	0.26	0.26	213.5
	IACX938	4B	76.2	1.74E-04	0.06	0.06	263.0

Table 4.12. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
PH	wsnp_Ra_c407_862316	2B	72.0	1.22E-04	0.49	0.03	2.37
SPS	Excalibur_c35316_154	1A	16.7	2.07E-04	0.40	0.04	0.31
	BS00021657_51	7A	152.2	7.56E-06	0.23	0.06	0.45

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.13. Summary of GWAS results involving mapped markers at Gr12P. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
BM	Ra_c42858_91	3A	109.9	6.53E-04	0.10	0.04	7.494
	wsnp_Ex_c18941_27840714	5A	74.8	9.44E-04	0.10	0.04	6.573
	BobWhite_c534_837	6A	58.5	7.57E-04	0.15	0.04	6.372
	Kukri_c15151_249	7A	175.7	1.08E-03	0.47	0.04	4.233
	Kukri_c21270_1870	4B	59.9	2.06E-04	0.14	0.05	7.015
	tplb0026o15_1634	4B	74.3	4.95E-04	0.27	0.04	5.379
	BobWhite_c26082_239	5B	170.5	7.40E-04	0.28	0.04	4.824
	BS00101364_51	7B	120.1	4.64E-04	0.46	0.04	4.484
DTB	BS00067096_51	5A	39.0	9.18E-05	0.05	0.04	1.461
	wsnp_Ex_c9779_16145653	6A	21.1	2.91E-05	0.12	0.05	1.107
	CAP12_c590_307	1B	136.0	3.17E-04	0.06	0.03	1.387
	Kukri_c16479_765	2B	76.7	1.52E-05	0.22	0.05	0.935
	BS00034554_51	6B	93.5	1.38E-04	0.35	0.04	0.726
HI	Excalibur_c24511_1196	4A	74.5	8.62E-04	0.21	0.03	0.009
	Ex_c9615_1202	7A	82.7	8.08E-04	0.31	0.03	0.008
	Excalibur_c20062_195	7A	121.4	5.61E-04	0.06	0.03	0.015
	Tdurum_contig42153_1190	2B	66.2	3.03E-05	0.43	0.05	0.011
	D_GBUVHFX02JKG4A_54	2D	22.5	2.44E-04	0.35	0.04	0.009
KPS	wsnp_Ex_c23383_32628864	6D	82.1	2.57E-05	0.23	0.05	1.382
PFS	Excalibur_c29255_404	5A	139.8	3.72E-05	0.06	0.05	0.022
	BS00103846_51	7A	139.9	1.19E-04	0.32	0.04	0.012
	RAC875_c5243_206	1B	43.9	1.49E-04	0.05	0.04	0.024
	Tdurum_contig42153_1190	2B	66.2	2.40E-04	0.43	0.04	0.012
	RAC875_rep_c114200_428	5B	116.8	2.48E-05	0.10	0.05	0.019

Table 4.13. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
SPS	wsnp_Ex_c5412_9564046	2A	78.0	7.89E-04	0.15	0.03	0.325
	BobWhite_c32226_104	2A	102.0	4.87E-04	0.08	0.04	0.490
	BS00065734_51	3A	148.0	2.01E-04	0.49	0.04	0.251
	RAC875_c42756_168	4A	49.0	7.92E-04	0.24	0.03	0.298
	Excalibur_c43822_370	4A	164.1	1.49E-03	0.40	0.03	0.234
	Kukri_c108256_381	5A	106.1	1.51E-03	0.09	0.03	0.377
	wsnp_Ku_rep_c110993_94857161	7A	133.5	5.39E-04	0.18	0.04	0.347
	wsnp_Ku_rep_c104159_90704469	7A	152.8	1.67E-07	0.26	0.08	0.444
	BS00012071_51	2B	106.6	1.16E-03	0.22	0.03	0.286
	Excalibur_c62990_114	5B	206.1	1.10E-03	0.16	0.03	0.307
	RAC875_c17182_600	7B	3.3	1.08E-03	0.41	0.03	0.222
	wsnp_Ex_c13629_21411429	3D	146.5	1.02E-03	0.38	0.03	0.265
SN	wsnp_Ex_c6664_11531767	7D	137.3	3.17E-05	0.08	0.05	17.087

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.14. Summary of significant marker–trait associations detected in individual environments with unmapped markers. Traits and units are described in Table 4.2.

Trait	Env.	Marker	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
BM	Gr12P	GENE.2847_1060	1.71E-04	0.13	0.05	7.311
		TA004394.0527	3.34E-04	0.12	0.04	7.257
DTB	Fo13F	GENE.4008_418	7.68E-04	0.11	0.03	0.563
		GENE.4045_141	2.05E-04	0.33	0.04	0.463
		wsnp_Ex_c18915_27811736	5.89E-04	0.43	0.04	0.374
	Gr12F	GENE.1125_32	3.93E-04	0.05	0.03	1.679
DTF	Fo13R	BS00041922_51	9.79E-05	0.08	0.05	0.718
		GENE.0609_166	5.79E-04	0.07	0.04	0.665
		GENE.0675_104	3.33E-04	0.12	0.04	0.563
		GENE.0675_161	8.37E-04	0.12	0.04	0.522
		GENE.1125_32	1.87E-05	0.05	0.06	0.959
		Kukri_c20939_226	3.58E-04	0.05	0.04	0.801
		RAC875_c817_2282	5.10E-04	0.34	0.04	0.377
		TA002269.1202	8.24E-04	0.12	0.04	0.523
DTH	Gr12F	BobWhite_rep_c63363_160	2.06E-04	0.40	0.04	0.912
		BS00012081_51	2.51E-04	0.34	0.04	0.859
		GENE.1273_395	7.40E-04	0.19	0.03	0.820
		Kukri_c25245_998	3.84E-04	0.41	0.03	0.880
		Kukri_c29807_713	2.06E-04	0.40	0.04	0.912
		RAC875_c15844_348	7.13E-04	0.35	0.03	0.780
		RAC875_c31358_214	3.05E-04	0.35	0.03	0.839
GF	Fo13R	GENE.2538_158	1.04E-03	0.36	0.04	0.410
		GENE.4796_109	1.11E-03	0.16	0.04	0.497
		GENE.4796_65	1.36E-03	0.17	0.03	0.483
		GENE.4996_592	1.55E-03	0.12	0.03	0.541
HI	Fo13F	BS00065253_51	3.52E-04	0.09	0.04	0.008
		Tdurum_contig55699_246	3.50E-04	0.18	0.04	0.006
KN	Gr12F	wsnp_Ex_c53843_56941644	4.18E-06	0.25	0.25	193.3

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.

Table 4.15. Number of significant marker–trait associations detected between anchored markers and agronomic traits in individual environments. No significant marker–trait associations were detected for GY, KW, GBM, or DTM. There are 124 significant marker–trait associations total. Traits are defined in Table 4.2.

Trait	A-Genome							B-Genome							D-Genome						
	1A	**2A**	**3A**	**4A**	**5A**	**6A**	**7A**	**1B**	**2B**	**3B**	**4B**	**5B**	**6B**	**7B**	**1D**	**2D**	**3D**	**4D**	**5D**	**6D**	**7D**
GY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SN	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KN	1	2	1	-	-	1	2	-	-	-	1	-	1	-	-	-	-	-	1	-	-
KPS	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
SPS	1	3	1	2	1	-	5	-	1	-	-	1	-	2	-	-	1	-	-	-	-
PFS	-	-	-	1	5	-	2	1	3	-	-	2	1	-	-	-	-	-	-	-	-
KW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BM	-	-	1	-	1	1	1	-	-	-	2	2	-	1	-	-	-	-	-	-	-
GBM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HI	1	1	-	1	-	-	2	1	3	1	-	-	-	-	-	1	-	-	-	-	-
PH	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
DTB	3	-	1	1	2	1	1	2	3	-	2	2	1	2	-	-	-	-	-	-	1
DTH	-	1	1	-	-	-	1	-	3	-	-	-	1	1	-	-	-	-	1	-	1
DTF	1	-	3	-	-	2	-	-	2	-	1	-	-	1	1	1	1	-	-	-	-
DTM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GF	1	-	-	1	-	1	1	1	-	-	1	1	-	1	-	-	1	1	-	1	-
Total	**8**	**9**	**8**	**6**	**9**	**6**	**15**	**5**	**16**	**1**	**7**	**8**	**4**	**8**	**1**	**2**	**3**	**1**	**2**	**2**	**3**

Table 4.16. Summary of GWAS results with mapped and unmapped markers in a combined analysis across four environments. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
PFS	wsnp_Ex_c3620_6612231	5A	15.9	6.43E-05	0.30	0.04	0.006
	Excalibur_c29255_404	5A	139.8	1.19E-06	0.06	0.06	0.013
	wsnp_Ku_c14251_22503965	2D	47.1	5.93E-05	0.09	0.04	0.009
SPS	wsnp_Ku_rep_c104159_90704469	7A	152.8	5.88E-08	0.26	0.09	0.281
	wsnp_Ex_c53843_56941644	NA§	NA§	1.08E-06	0.25	0.07	0.257

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.
§ NA indicates unmapped chromosome.

Table 4.17. Summary of GWAS results with mapped and unmapped markers in a combined analysis of moisture treatment across two years. Markers had a significant effect that varied between water-stressed or non-stressed environments. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value[†]	MAF	Marker R^2	Allelic Effect[‡]
HI	RAC875_c19718_551	4A	64.1	1.04E-03	0.13	0.03	0.001
	Ex_c9615_1202	7A	82.7	1.32E-04	0.32	0.04	0.001
	Tdurum_contig29087_757	1B	136.0	6.29E-05	0.06	0.04	0.003
	Kukri_c22513_1780	2B	26.5	6.23E-04	0.19	0.03	0.001
	Ex_c12051_875	2B	69.8	3.91E-04	0.49	0.03	0.001
	Excalibur_c18966_804	2B	130.3	1.17E-03	0.33	0.03	0.001
	Tdurum_contig50954_1264	3B	51.1	1.07E-03	0.40	0.03	0.001
	Tdurum_contig50954_1095	3B	134.8	7.47E-04	0.40	0.03	0.001
	D_GBUVHFX02JKG4A_54	2D	22.5	6.27E-04	0.35	0.03	0.001
	GENE.1273_395	NA[§]	NA[§]	2.16E-04	0.19	0.04	0.001
PFS	wsnp_Ex_c3620_6612231	5A	15.9	8.68E-05	0.30	0.04	0.006
	RAC875_rep_c106118_339	5A	39.0	5.23E-04	0.13	0.03	0.007
	Excalibur_c29255_404	5A	139.8	1.26E-05	0.06	0.05	0.012
	wsnp_Ex_c965_1845447	6A	99.4	5.05E-04	0.11	0.03	0.007
	Tdurum_contig42153_1190	2B	66.2	3.42E-05	0.44	0.05	0.007
	Tdurum_contig18858_324	2B	109.5	4.18E-04	0.27	0.03	0.006
	Tdurum_contig17697_771	7B	131.1	4.45E-04	0.22	0.03	0.006
	wsnp_Ku_c14251_22503965	2D	47.1	9.33E-05	0.09	0.04	0.009
	BS00012081_51	NA	NA	1.32E-04	0.35	0.04	0.006
	RAC875_c15844_348	NA	NA	2.44E-04	0.36	0.04	0.006
	RAC875_c31358_214	NA	NA	1.13E-04	0.36	0.04	0.006

Table 4.17. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
PH	BS00012081_51	NA	NA	8.86E-05	0.35	0.04	0.766
	RAC875_c15844_348	NA	NA	4.83E-05	0.36	0.04	0.781
	RAC875_c31358_214	NA	NA	3.01E-05	0.36	0.04	0.810
SPS	wsnp_Ku_rep_c104159_90704469	7A	152.8	1.60E-07	0.25	0.09	0.305
	wsnp_Ex_c53843_56941644	NA	1193.0	1.30E-06	0.24	0.07	0.285
KW	RAC875_c21411_162	1A	105.7	1.86E-04	0.09	0.05	0.205
	Tdurum_contig46797_585	2A	81.5	2.37E-04	0.42	0.05	0.124
	Tdurum_contig29087_757	1B	136.0	1.29E-04	0.06	0.05	0.262
	wsnp_Ex_rep_c67926_66647362	6B	68.4	2.72E-04	0.09	0.04	0.215
	Ra_c2557_2531	6B	104.6	3.57E-04	0.13	0.04	0.164
	wsnp_Ex_rep_c67164_65655648	5D	67.5	2.25E-05	0.21	0.06	0.176
	TA002565.0478	NA	NA	2.25E-05	0.21	0.06	0.176

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.
§ NA indicates unmapped chromosome.

Table 4.18. Summary of GWAS results with mapped and unmapped markers in a combined analysis of year (2012 or 2013) across two moisture treatments. Markers had a significant effect that varied between years. Traits and units are described in Table 4.2.

Trait	Marker	Chr.	Pos. (cM)	Raw *P*-value†	MAF	Marker R^2	Allelic Effect‡
DTF	BobWhite_c37935_124	1A	74.8	1.09E-03	0.09	0.03	0.123
	wsnp_Ku_c8334_14181247	3A	87.4	1.42E-03	0.20	0.03	0.099
	CAP11_c3666_426	7A	211.7	9.73E-05	0.05	0.04	0.159
	Kukri_c22513_1780	2B	26.5	4.95E-04	0.19	0.03	0.088
	BobWhite_c2988_2161	2B	56.3	2.71E-04	0.15	0.04	0.104
	Tdurum_contig54925_202	2B	107.4	2.07E-04	0.06	0.04	0.168
	BS00048794_51	4B	74.6	6.44E-04	0.21	0.03	0.092
	wsnp_Ku_rep_c103690_90365429	7B	120.8	6.48E-04	0.27	0.03	0.076
	Ra_c11906_1441	1D	107.1	1.16E-03	0.36	0.03	0.078
	GENE.1125_32	NA§	NA§	2.08E-04	0.06	0.04	0.168
	GENE.1273_395	NA	NA	4.35E-04	0.19	0.04	0.089
HI	Excalibur_c24511_1196	4A	74.5	2.30E-04	0.22	0.04	0.001
	Ex_c9615_1202	7A	82.7	1.30E-04	0.32	0.04	0.001
	wsnp_Ex_c6354_11053460	7A	178.4	5.64E-04	0.32	0.03	0.001
	Tdurum_contig29087_757	1B	136.0	6.30E-05	0.06	0.04	0.002
	Kukri_c22513_1780	2B	26.5	6.31E-04	0.19	0.03	0.001
	Tdurum_contig42153_1190	2B	66.2	5.77E-04	0.44	0.03	0.001
	Excalibur_c18966_804	2B	130.3	1.18E-03	0.33	0.03	0.001
	Tdurum_contig50954_1264	3B	51.1	1.05E-03	0.40	0.03	0.001
	Tdurum_contig50954_1095	3B	134.8	7.34E-04	0.40	0.03	0.001

Table 4.18. Continued.

Trait	Marker	Chr.	Pos. (cM)	Raw P-value†	MAF	Marker R2	Allelic Effect‡
HI	D_GBUVHFX02JKG4A_54	2D	22.5	6.24E-04	0.35	0.03	0.001
	GENE.1273_395	NA	NA	2.20E-04	0.19	0.04	0.001
KPS	BS00079440_51	2D	43.7	3.14E-05	0.19	0.05	0.308
PFS	wsnp_Ex_c3620_6612231	5A	15.9	8.66E-05	0.30	0.04	0.006
	Excalibur_c29255_404	5A	139.8	1.25E-05	0.06	0.05	0.012
	wsnp_Ex_c965_1845447	6A	99.4	5.03E-04	0.11	0.03	0.008
	Tdurum_contig42153_1190	2B	66.2	3.43E-05	0.44	0.05	0.007
	Tdurum_contig18858_324	2B	109.5	4.18E-04	0.27	0.03	0.006
	Tdurum_contig17697_771	7B	131.1	4.46E-04	0.22	0.03	0.007
	wsnp_Ku_c14251_22503965	2D	47.1	9.33E-05	0.09	0.04	0.009
	BS00012081_51	NA	NA	1.32E-04	0.35	0.04	0.007
	RAC875_c15844_348	NA	NA	2.45E-04	0.36	0.04	0.006
	RAC875_c31358_214	NA	NA	1.13E-04	0.36	0.04	0.007
SPS	wsnp_Ku_rep_c104159_90704469	7A	152.8	1.61E-07	0.25	0.09	0.287
	wsnp_Ex_c53843_56941644	NA	NA	1.30E-06	0.24	0.07	0.269

† Unadjusted *P*-values are provided for reference but significance of marker–trait associations was determined using the Benjamini–Hochberg procedure with a false discovery rate of 50%.
‡ Allelic effects are magnitude of effect size only, and don't signify effect of major or minor allele.
§ NA indicates unmapped chromosome.

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CHAPTER 5:

APPLICATIONS OF CANOPY SPECTRAL REFLECTANCE TO EVALUATE DROUGHT TOLERANCE

Summary

Improvements in phenotyping are needed to match advances in genotyping to conduct powerful analyses that may help researchers or plant breeders increase grain yield or the rate of genetic gain under drought stress. Recent advances in genotyping include tools and technologies such as high-throughput marker platforms and genomic selection. Spectral tools, such as canopy spectral reflectance, could be used to facilitate high-throughput phenotyping, especially for complex traits like grain yield or drought tolerance that are difficult to accurately evaluate in the field. Spectral tools could have a role evaluating drought tolerance by indirectly estimating water status or other physiological traits. Published water-based spectral indices estimate plant water status based on the relative amount of light reflected at the minor water absorption band at 970 nm and other regions in the near infrared between 850 and 920 nm. Reflectance at 970 nm is a useful indicator of plant water status because radiation at this wavelength is absorbed by water in the plant canopy. We evaluated five published water-based spectral indices (WI, NWI-1, NWI-2, NWI-3, and NWI-4) on multiple dates and during different developmental stages of both rainfed and irrigated environments in Fort Collins, CO during 2013. Under water stress estimates of NWI-1, NWI-3, and NWI-4 followed a distinctive growth curve with their most extreme values coinciding with the period of highest water stress in the field. Thus,

these tools and indices could be useful on a regional or farm level to make management decisions about resource allocation for dryland crops. NWI-3 detected more differences among developmental stages than the other water-based indices evaluated, and is recommended over the other water-based spectral indices for winter wheat. However, the spectral tools were not able to detect significant differences among entries, so the current technology showed little promise in our study as a selection tool.

Introduction

Wheat breeders have benefited from recent advances in marker technologies (Elshire et al., 2011; Poland and Rife, 2012) and genomics (Cavanagh et al., 2013; Wang et al., 2014) to select for beneficial traits (Liu et al., 2014), estimate breeding value (Heffner et al., 2009; Poland et al., 2012), and speed up the selection cycle (Heffner et al., 2010). However, implementing technologies like genomic selection and genome-wide association mapping requires phenotypic data. Obtaining accurate, inexpensive, and high-throughput phenotypic data in the field has remained a major constraint to plant breeders (Araus and Cairns, 2014). Drought tolerance is a complex trait that is especially difficult and time-consuming to accurately evaluate in the field (Richards et al., 2010). Estimating agronomic traits such as yield or drought tolerance indirectly, using spectral traits measured proximally with hand-held, aerial, or vehicle-mounted sensors, offers a possible solution to relieve the phenotyping bottleneck. Measuring spectral traits can speed up the selection cycle by reducing time spent evaluating germplasm, selecting genotypes earlier in the season, or evaluating more genotypes during each round of selection (Andrade-Sanchez et al., 2014).

Spectral indices are constructed based on known spectral properties of plant tissues and canopy structure (Ollinger, 2011). One such relationship is lower levels of reflectance at the minor water absorption band located at 970 nm relative to nearby regions in the near infrared. Less light is reflected at 970 nm because some of that energy is absorbed by water within the plant tissues. Several water-based spectral indices have been developed to estimate plant water status in agricultural fields during the growing season using the 970 nm band. These indices include the water index (WI, Penuelas et al., 1997), normalized water index 1 (NWI-1, Babar et al., 2006b), normalized water index 2 (NWI-2, Babar et al., 2006b)), normalized water index 3 (NWI-3, Prasad et al., 2007b), and normalized water index 4 (NWI-4, Prasad et al., 2007b).

The water index and normalized water indices relate the amount of light reflected at the minor water absorption band at 970 nm to regions in the NIR that have higher reflectance. The water index is the ratio of light reflected at 970 nm and 900 nm, and has been shown to be associated with relative water content, leaf water potential, and stomatal conductance (Penuelas et al., 1993, 1997). Normalized water-based spectral indices have been developed using the amount of light reflected at the 970 nm minor water absorption band, and different reflectance bands in the NIR (between 880–920 nm), and have previously been used to explain up to 88% of variation in wheat grain yield when evaluated during the milk stage of grain filling (Bandyopadhyay et al., 2014). The water-based spectral indices have also been previously used to select up to 83% of the 25% highest-yielding winter wheat genotypes across two years and populations, which was a greater selection efficiency than widely-used vegetative indices such as the red- or green normalized difference vegetation indices (Prasad et al., 2007b).

A better understanding of the relationships between five water-based spectral indices and agronomic traits is needed to evaluate the use of canopy spectral reflectance to characterize drought response in wheat. Therefore, our specific objectives were to evaluate how water-based spectral indices vary among genotypes and to evaluate changes in indices when measured on different sampling dates or during different developmental stages.

Materials and methods

Germplasm and experimental design

The germplasm includes 299 winter wheat entries belonging to the Triticeae Coordinated Agricultural Project (TCAP) hard winter wheat association mapping panel. The entries were a diverse collection of cultivars, experimental lines, and historic varieties identified by wheat breeders from across the U.S. Great Plains as having made a significant contribution to the current germplasm pools of that region. The entries were contributed by both public and private breeding programs, and reflect a long history of wheat breeding.

The panel was evaluated at the Colorado State University Agricultural Research Development and Education Center in Fort Collins, CO (40° 39'N, 105° 00'W; elevation 1,534 m) during 2012–2013. The trials were unreplicated and arranged in an augmented row–column design with two check varieties. Each field plot was a 3.1 m-long, six-row plot. The experimental entries were unreplicated, except for 'Wichita' (CI 11952), which was included in the panel twice. Two check varieties were replicated fifteen times each and systematically placed throughout the field. The check varieties were 'Hatcher' (Haley et al., 2005) and 'Settler CL' (Baenziger et al., 2011), and these varieties were also included as

experimental entries in the trial. The trials were planted on 2 Oct 2012 and harvested on July 18 (Fo13R) and 22 (Fo13F) 2013. The germplasm and experiment have been described in detail previously (Grogan et al., 2015). There were separate rainfed (Fo13R) and full irrigation (Fo13F) environments. These environments are discussed in detail in Chapter 4 of this dissertation and monthly precipitation and irrigation are detailed in Table 4.1. Irrigation at Fo13F began around heading; thus, there was no treatment effect between Fo13F and Fo13R until anthesis. Irrigation at Fo13F was applied seven times using overhead linear sprinklers, totaling 222.3 mm. The first irrigation event was on DOY 140. Fo13R did not receive any supplemental irrigation. Both Fo13F and Fo13R received 124.4 mm precipitation between 1 Jan and harvest.

Spectral measurements

Canopy spectral reflectance was measured with a Jaz (Ocean Optics, Dunedin, FL) hyper-spectral radiometer. The radiometer was equipped with a passive sensor optimized to measure reflectance in the near infrared (NIR) from 600 to 1050 nm. The sampling interval ranges from 0.25 – 0.36 nm, with an average interval of 0.32 nm, thus each measurement recorded reflectance at 2047 different wavebands. Prior to sampling, the sensors were normalized against a $BaSO_4$–coated white board. The radiometer was mounted to a pole that held the fiber optic sensor (field of view = 25.4°) perpendicular over the plot. The sensor was centered 20 cm over an interior row for a sampling spot size 9 cm in diameter. Measurements were collected at walking speed and averaged about 80 scans per plot. Sampling time took about 30 seconds per plot. We collected spectral

measurements at Fo13F on six dates (day of year, DOY 151, 160, 162, 170, 178, and 182), and at Fo13R on five dates (DOY 151, 157, 160, 170, and 177).

Substantial effort was taken to minimize environmental variation among sampling dates. For example, sampling was restricted to within about two hours of solar noon to reduce temporal variation associated with changes in zenith angle. Spectral measurements were limited to relatively clear, still days to minimize the effects of clouds affecting light scattering and wind affecting leaf angle and canopy architecture. Despite our efforts to minimize variation within the field, spatial-temporal trends were present in the data. Spectral patterns in reflectance values did not necessarily result in spatial patterns of spectral index values, since changes in irradiance can affect reflectance at all wavebands. Data were excluded from analysis when outlying estimates were extreme and persisted for multiple indices. Typically excluded measurements included several sequentially measured plots.

Five water-based spectral indices were calculated using raw plot-level data and a 1.0 nm window width. These indices are WI, NWI-1, NWI-2, NWI-3, and NWI-4, and are described in Table 5.1. Additional window widths ranging from 0.5 to 3.0 nm were evaluated, but for most indices the strongest correlations with agronomic traits were obtained using a 1.0 nm window (data not shown).

Phenological development

Crop development was determined for each plot using the Zadoks scale (Zadoks et al., 1974). Developmental stages were defined when approximately 50% of tillers in interior rows of the plot exhibited characteristic traits. Each plot was evaluated at least

three times a week. Booting date was determined at stage 45, when the flag leaf collar was visible and the boot was very swollen. In some instances the flag leaf sheath was beginning to split or awns were just barely visible. This variability in booting date adds to some error to whether measurements were accurately classified as booting versus early vegetative growth (prior to booting). Heading date was evaluated at stage 59, when the spike had fully emerged from the flag leaf sheath. Irrigation at Fo13F began on DOY 140, which was during heading at Fo13F and Fo13R. There was no significant differences in heading dates at Fo13F and Fo13R. Anthesis date was characterized at stage 65 when florets in the middle of the spike were receptive, indicated by a "fluffy" stigma, or when anthers were extruded and shedding a moderate amount of pollen. In some instances anthesis was back-estimated after desiccated anthers were visible. Average anthesis dates were DOY 155 at Fo13R and DOY 156 at Fo13F. Physiological maturity (stage 90) was determined when the peduncle lost all green coloration and the spikes retained little or no greenness. Calendar days to each developmental stage was calculated as the number of days after January 1, and included days to booting, heading, flowering, and physiological maturity. Grain filling duration was calculated as the number of days between anthesis and physiological maturity.

Phenological dates were also used to group measurements from each plot and sampling date into one of seven developmental categories to analyze spectral indices by developmental stage. On most sampling dates multiple developmental stages were present across the field, reflecting early- and late-maturing entries. Because of the genotypic diversity within the panel, not all entries were measured at each developmental stage, and some entries have multiple measurements during the same developmental stage. The

categories included: early vegetative growth including tillering and stem expansion (before booting), booting, heading, anthesis (including anthesis date and two days prior), grain filling (three-or more days after anthesis date until one week before physiological maturity), late grain filling (measurements after anthesis date that were within one week of physiological maturity), and physiological maturity. Grain filling and late grain filling were distinguished to sub-divide the large number of measurements between anthesis and physiological maturity (and provide more-equal number of measurements for each developmental stage bin).

Statistical analysis

Statistical analyses were conducted in the software R (version 3.1.3, R Core Team, 2015) on raw plot-level data using the 'stats' (R Core Team, 2015) and 'car' (Fox and Weisberg, 2011) packages. Tests for pairwise significant differences were conducted using Tukey HSD in JMP (version 11.0, SAS Institute Inc., Cary, NC). Heat maps were created in JMP and all other figures were generated in R.

Results and discussion

Effects of environment and genotypes

There were significant differences between spectral estimates at Fo13F and Fo13R for NWI-1, NWI-3, and NWI-4 ($P < 0.001$ each), but not for WI or NWI-2. No significant differences among entries were observed for any of the indices evaluated. Because genotypic variance was not significant, broad-sense heritability, phenotypic and genetic correlations with agronomic traits, and selection efficiency were not evaluated.

The lack of significant genotypic variation was influenced by substantial spatial–temporal variation (Figure 5.1a-c). Localized spatial trends observed in nearby plots could be due to underlying spatial variation due to environmental conditions like soil type, water availability, and presence of weeds, insect, mite, or rodent pests, or disease. Variation among sequentially-measured plots was most likely due to changes in solar conditions or deteriorating calibration efficiency (Figure 5.1c). These spatial patterns were not corrected for because making spatial adjustments eliminated variation among entries, such that all measurements of each index were estimated to be the same for some indices and dates. This is problematic because it does not allow for summary statistics, such as standard deviation or variance, for measurements collected on the same day.

The amount of light reflected at every waveband should range between 0-100%, and as such, estimates of the normalized water indices should range from -1 to 1. Because some light is absorbed by water at 970 nm, reflectance is expected to be greater at other estimates (850 nm, 880 nm, 900 nm, 920 nm) in the near infrared than at 970 nm (Table 5.1). Therefore, most estimates of the normalized water indices are expected to fall from 0 to 1, with estimates of 0 indicating the same amount of light reflected at each waveband, and negative estimates indicating more light was reflected at 970 nm than the second waveband. However, not all spectral index values fell between -1 and 1. This indicates that reflectance at one or more waveband was greater than 100% or less than 0%, which could be caused by changes in incoming irradiance (relative to levels used for calibration) or by instrumentation error.

Minor fluctuations in solar conditions, such as changes in total incoming irradiance or cloud coverage, had minor or negligible effects on spectral indices. This can be explained

by changes in irradiance increasing or decreasing the amount of light reflected by equal or near-equal amounts across the entire spectra. This would result in a very minor change to indices because the relationship between wavebands would not be substantially changed.

There was also major spatial–temporal variation resulting in gradual changes of index values between the first and last plots measured (or between first plot and re-calibration). This variation is likely due to changes in zenith angle, or the sensor calibration efficiency deteriorating over time, or both (Figure 5.1c). The effects of calibration efficiency and changes in irradiance could be reduced or eliminated by using an active sensor that emits its own light source.

Estimates of canopy spectral reflectance were very sensitive to instrumentation error. Instrumentation error was identified as spatial trends among sequentially sampled plots and varied from underlying effects of spatial variation by the very extreme and outlying measurements. Instrumentation error ranged from several plots to an entire pass, and was most likely caused by deviating the angle at which the sensor was held.

Effects of sampling date

The amount of light reflected across the entire spectra varied among sampling dates (Figure 5.2). Variation in the total amount of irradiance and reflectance can be most easily observed by comparing the NIR from approximately 800–900 nm in Figure 5.2. Particular features within the spectra also varied among dates. For instance, the amount of light reflected from the visible light portion of the spectrum (<700 nm) is diminished as the plant begins to senesce. This is especially visible for Fo13R on DOY 170 and 177 (Figure 5.2a). The change to the red edge (700–750 nm) is less pronounced at Fo13F because all

measurements were taken before physiological maturity (Figure 5.2b). Reflectance at the 970 nm minor water absorption band also varies among sampling dates. Lower reflectance at 970 nm is due to absorption of light by water in the tissue, so higher levels of reflectance suggest less water in the plant tissue. Reflectance at 970 nm is lower for Fo13F than Fo13R, due to higher plant water status in the irrigated environment.

Both WI and NWI-2 had significant differences among sampling dates in the pooled data across environments, although Tukey's honest significance test did not detect pairwise differences for WI (Table 5.2). Much of the variation in NWI-2 was influenced by the large spread of measurements at DOY 151 relative to other sampling dates (Figure 5.3a,b). Many estimates of NWI-2 were greater than |1| on DOY 151, and several estimates were greater than |1| on DOY 182. Plots with aberrant estimates of NWI-2 were distributed across the field, did not follow spatial patterns, and did not have outlying values for other water-based indices. This indicates reflectance at 850 nm used to estimate NWI-2 was very different from reflectance at the wavebands used to estimate NWI-1, NWI-3, and NWI-4, and suggests NWI-2 may not be an appropriate index for early- or late-season measurements on winter wheat.

There were significant differences among sampling dates for NWI-1, NWI-3, and NWI-4 at both Fo13F (Table 5.3) and Fo13R (Table 5.4). Variation among sampling dates is likely due to differences in environmental conditions (total irradiance, zenith angle, wind speed, etc.) and physiological characteristics (plant water status, canopy architecture, senescence, etc.). The sampling dates with the most similar average values of spectral indices are not necessarily the dates that are closest to each other, which suggests changing environmental conditions introduce large sources of variation. Average spectral index

values from a single date were significantly different from measurements on most other dates, even when the dates were only separated by several days. This is concerning because it suggests single measurements may not be reliable indicators of spectral index values.

Effects of developmental stage

There were significant differences in estimates of NWI-2 ($P < 0.001$, Table 5.5) among seven developmental stages when measurements were pooled across Fo13F and Fo13R (Figure 5.3c, 5.3d). There were no significant differences among developmental stages for WI in the pooled data. There were significant differences in estimates of NWI-2 when evaluated at booting and heading, and all estimates evaluated before anthesis were significantly different from those measured during grain filling (Table 5.5). The highly variable estimates of NWI-2 before anthesis (such as on DOY 151) could indicate reflectance at 850 nm is more strongly influenced by canopy structure than wavebands used by the other normalized water indices. For instance, NWI-2 could be influenced by light refracted from the soil before the canopy closes, or NWI-2 could be affected by changes in spike architecture.

There were also significant differences among developmental stages for NWI-1, NWI-3, and NWI-4 when analyzed separately for Fo13F (Table 5.6) and Fo13R (Table 5.7). Most changes occurred gradually among sequential developmental stages (Figure 5.4a-f). The interaction between sampling date and developmental stage was significant ($P < 0.001$) for NWI-1, NWI-3, and NWI-4. For most indices, the effect from sampling date was greater than that from developmental stage (Table 5.8). Since indices changed gradually

across sequential developmental stages, but not sequential sampling dates, this suggests it is more important to group measurements by common sampling date and highlights the importance of controlling for phenology in spectral estimates. It also suggests the most appropriate way to collect meaningful spectral data might be to focus on one or few developmental stages, and average multiple estimates collected on different days but within the same developmental stage.

The three-way interaction between environment, sampling date, and developmental stage was tested for NWI-1, NWI-3, and NWI-4. The three-way interaction was not evaluated for NWI-2 or WI because the main effect of environment was not significant for these indices. The effect of the three-way interaction and two-way interaction between environment and development stage were significant for each of NWI-1, NWI-3, and NWI-4, (Table 5.9). The two- and three-way interactions are significant because variation among developmental stages is much more pronounced at Fo13R than Fo13F (Figure 5.4), which was expected because plant water status should vary among moisture treatments.

Differences among water-based spectral indices

The water index did not vary significantly among entries, environments, sampling dates, or developmental stages and is therefore not a useful indicator of plant water status or drought stress. Normalized water index 2 had many noisy estimates during early- and late-season growth that suggest this index is unreliable for winter wheat (Figure 5.3). However, NWI-1, NWI-3, and NWI-4 had significant and gradual differences among developmental stages, and followed similar growth curves with developmental stage at Fo13R (Figure 5.4). The indices were expected to provide similar results, because index

values were derived using similar formulas and wavebands. Among the normalized water-based indices, NWI-3 detected slightly more differences among developmental stages than the other indices, which suggests NWI-3 is most sensitive to detecting changes in winter wheat. For this reason, NWI-3 is recommended over the other water-based spectral indices for winter wheat.

Many more differences were detected for Fo13R than Fo13F. Some of the gradual changes observed among developmental stages at Fo13R were mimicked to a lesser degree at Fo13F (Table 5.6). Variation in responses at Fo13R and Fo13F was expected because we evaluated indices that are associated with plant water status, in trials that vary for water availability. A treatment effect was not imposed on Fo13R until anthesis, and consequently there are greater changes in NWI-1, NWI-3, and NWI-4 after this point than in the early season (Figure 5.4). Less of a response over developmental stages was seen at Fo13F because the plants were receiving regular irrigation.

While it is insightful to evaluate and compare water-based spectral indices using particular wavebands, this is not the only approach for analyzing hyperspectral data. An alternative approach to using spectral indices is to characterize drought through partial-least squares regression (Weber et al., 2012; Dreccer et al., 2014). Partial-least squares regression uses reflectance data from all available wavebands and might be a more effective method of capturing genotypic variation.

Conclusions

Values of water-based spectral indices changed gradually with plant development, following a distinctive growth curve, when evaluated under water stress at Fo13R. The

lowest values of NWI-1, NWI-3, and NWI-4 were observed during grain filling, which coincided with the period of most severe water stress. Therefore, it is likely these water-based spectral indices are detecting changes in plant water status and this type of technology could be useful on a regional- or farm-level to improve management decisions about resource allocation, especially for dryland crops or in drought years. However, we were unable to detect significant differences in index values among wheat entries. This indicates our instrument is not appropriate as a selection tool. The lack of significant differences among entries was likely influenced by substantial spatial–temporal variation, and this could be addressed by sampling using an aerial or vehicle-mounted sensor to reduce or eliminate temporal variation among measurements.

Figures

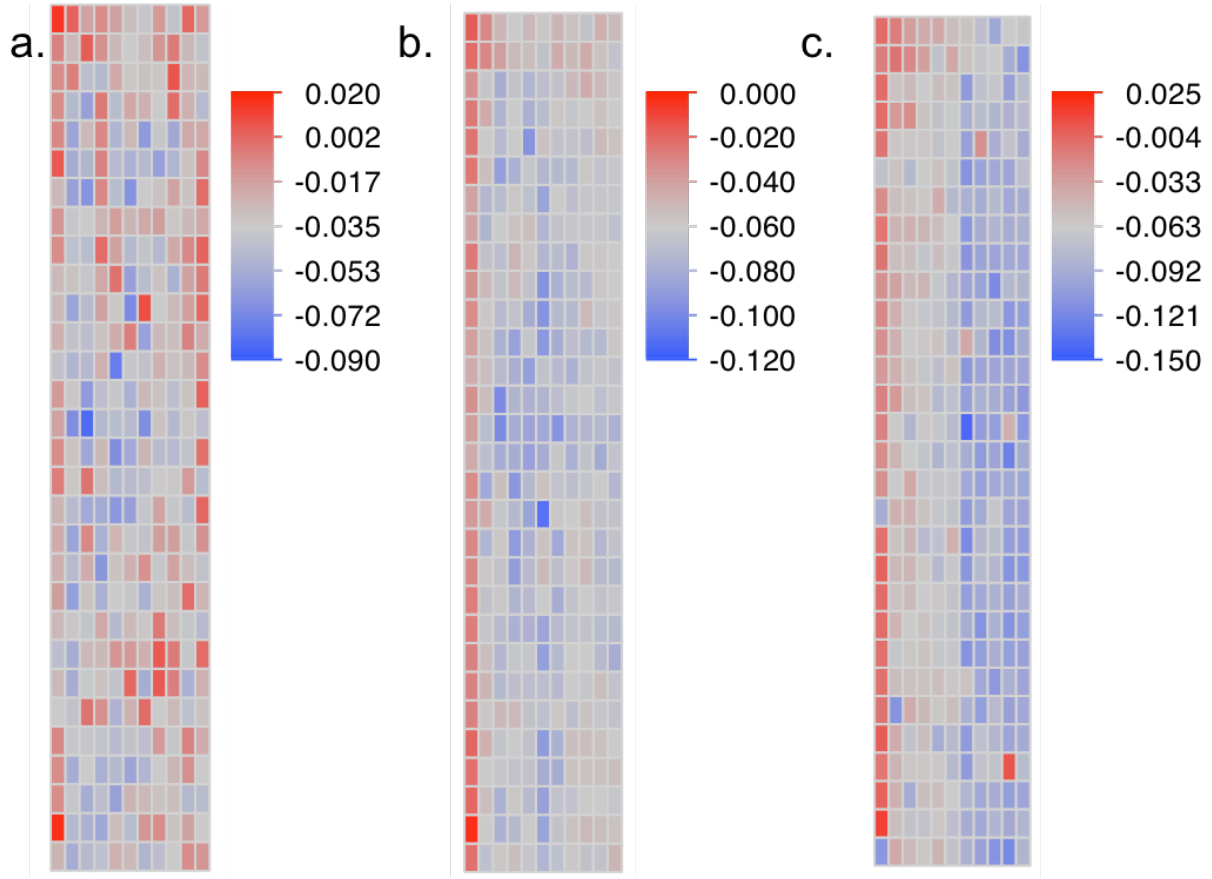

Figure 5.1. Visualizing spatial trends among winter wheat entries grown in Fort Collins, CO under rainfed conditions during 2013 (Fo13R) using heat maps of spectral index values. There are 330 3.1 m long, six-row field plots at Fo13R. **a.** Heat map showing the mean value of normalized water index 1 (NWI-1) for each field plot, evaluated at Fo13R on day of year (DOY) 151. There is little or no temporal variation. Small spatial patterns can indicate underlying environmental variation, changes in solar conditions, or instrumentation error. Variation in the heat map reflects differences in plots during early season growth. **b**. Heat map showing the average value of NWI-1 across five sampling dates (DOY 151, 157, 160, 170, 177) at Fo13R provides a good estimate of underlying field variation in Fo13R. The west (left) most pass and north (top) end of the field had low biomass and total grain yield at physiological maturity. **c.** Heat map plotting all estimates of NWI-1 in Fo13R on DOY 157. Sampling was north–south (up–down), and the sensor was re-calibrated halfway through the field.

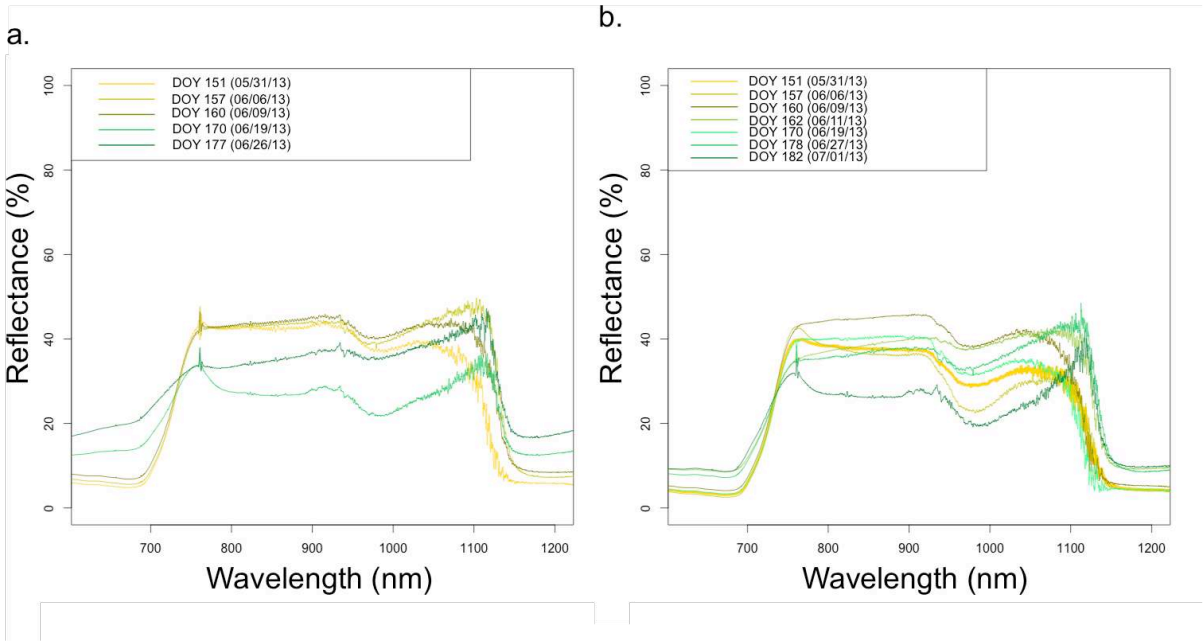

Figure 5.2. Variation in mean reflectance spectra for the check variety 'Hatcher' across sampling dates when evaluated in Fort Collins, CO under rainfed (Fo13F; Fig. A) or full irrigation (Fo13R; Fig. B) conditions in 2013. The first irrigation event occurred on DOY 140. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Each spectrum is the average of measurements from 15 six-row, 3.1 m long field plots. **a.** Average reflectance across five sampling dates (days of year, DOY) at Fo13R. **b.** Average reflectance across eight sampling dates at Fo13F.

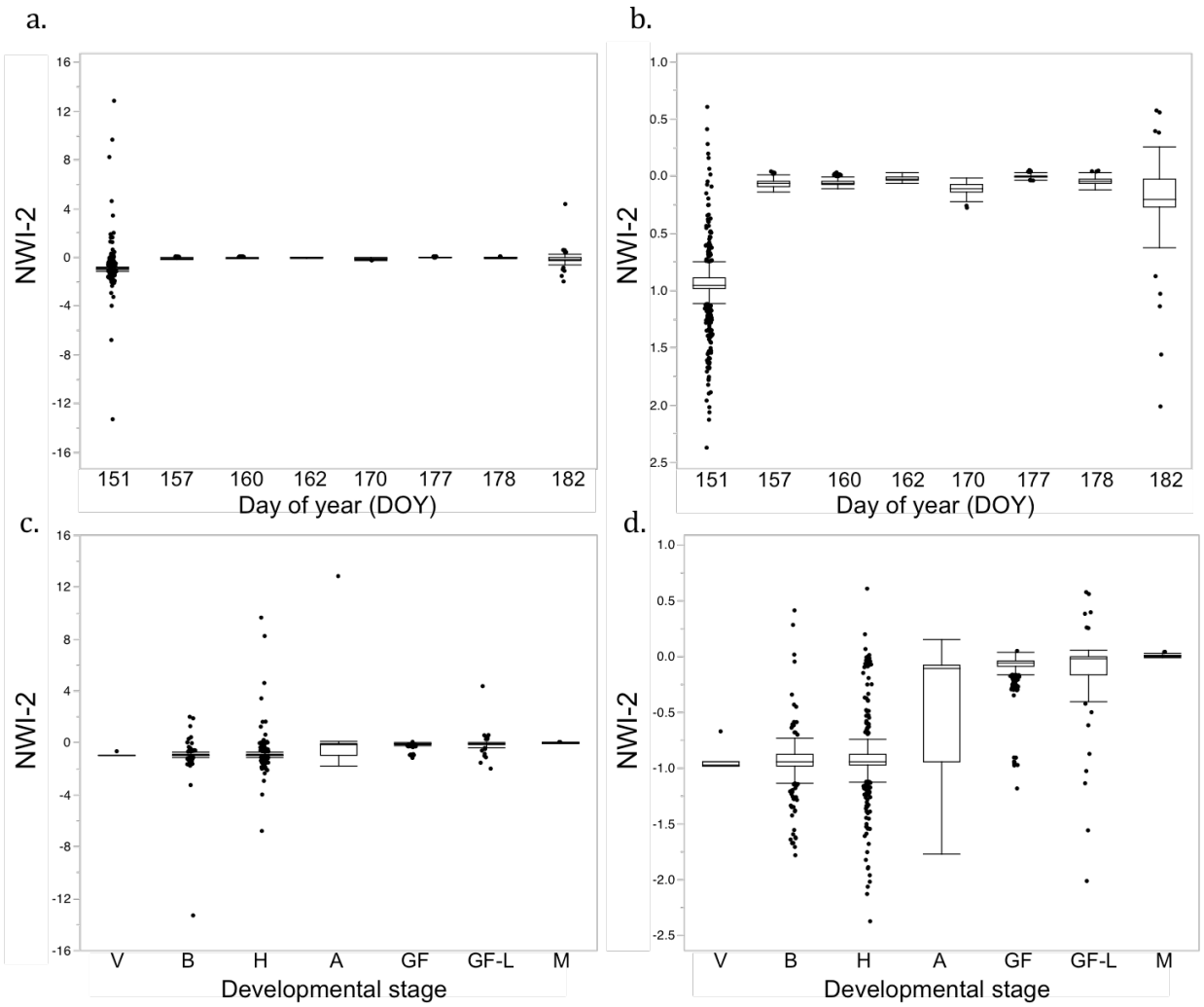

Figure 5.3. Variation of normalized water index 2 (NWI-2) values for winter wheat entries pooled across two environments. The horizontal lines are median values for each environment. The box shows the inter-quartile range (IQR), where 50% of the genotypes fall. The horizontal lines in the boxes are median values for each environment. The whiskers extend 1.5 times the IQR in each direction. The environments are full irrigation in Fort Collins, CO during 2013 (Fo13F) and rainfed in Fort Collins, CO during 2013 (Fo13R). The first irrigation event occurred on DOY 140. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R.NWI-2 is defined in Table 5.1. **a.** Variation in NWI-2 across sampling dates, showing all measurements. **b.** Finer resolution of variation in NWI-2 across sampling dates, trimming some measurements on DOY 151 and DOY 182. **c.** Variation in NWI-2 across developmental stages, showing all measurements. The developmental stages are early vegetative growth (V), booting (B), heading (H), anthesis (A), grain filling (GF), late grain filling (GF-L, within one week of physiological maturity), and physiological maturity (M). **d.** Finer resolution of variation in NWI-2 across developmental stages, trimming some measurements at heading and late grain filling.

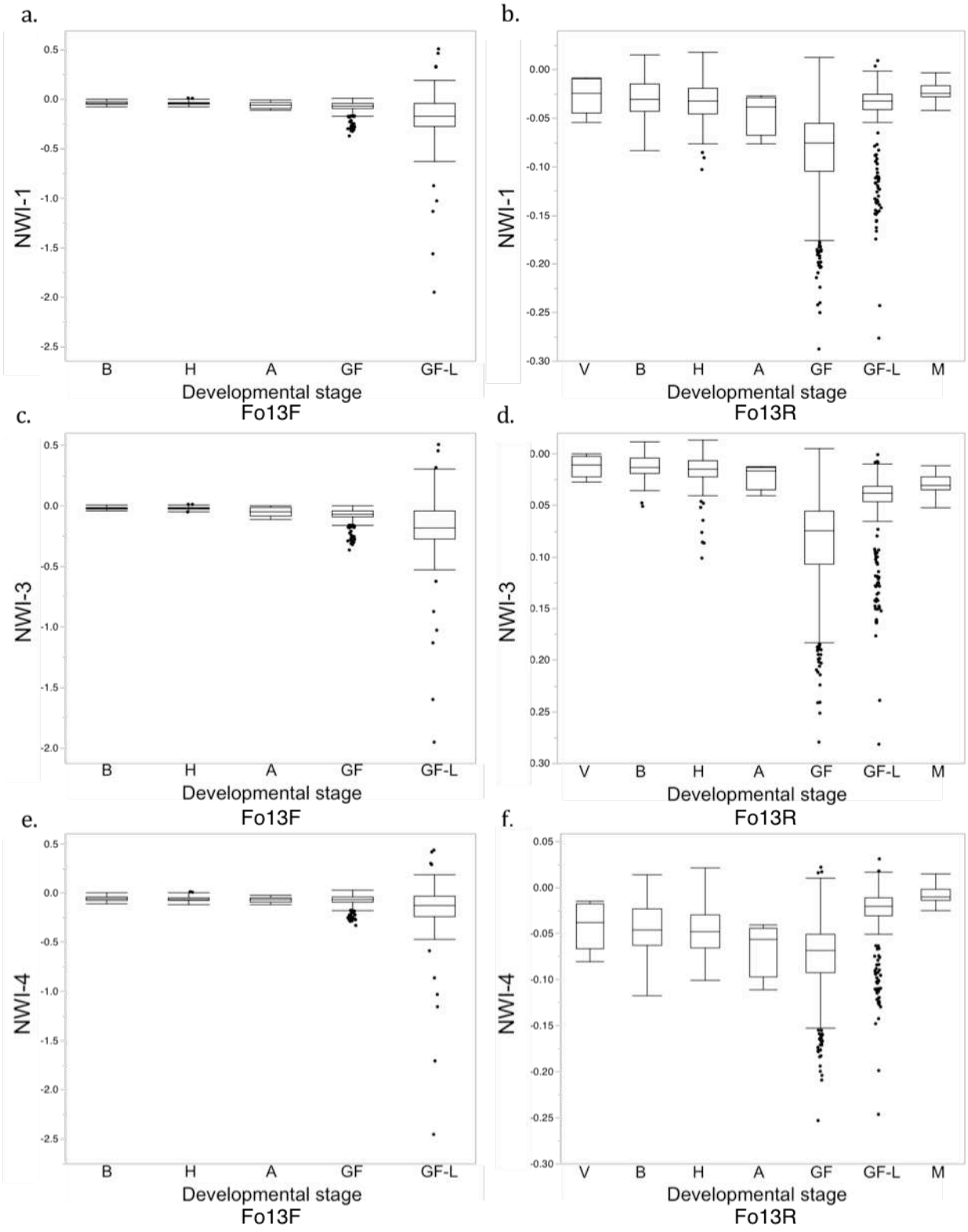
a.
NWI-1
B
H
A
GF
GF-L
Developmental stage
Fo13F
b.
NWI-1
V
B
H
A
GF
GF-L
M
Developmental stage
Fo13R
c.
NWI-3
B
H
A
GF
GF-L
Developmental stage
Fo13F
d.
NWI-3
V
B
H
A
GF
GF-L
M
Developmental stage
Fo13R
e.
NWI-4
B
H
A
GF
GF-L
Developmental stage
Fo13F
f.
NWI-4
V
B
H
A
GF
GF-L
M
Developmental stage
Fo13R

Figure 5.4. Variation of water-based spectral index values for wheat entries grown in Fort Collins, CO under full irrigation (Fo13F) or rainfed (Fo13R) conditions in 2013. The horizontal lines are median values for each environment. The box shows the inter-quartile range (IQR), where 50% of the genotypes fall. The horizontal lines in the boxes are median values for each environment. The whiskers extend 1.5 times the IQR in each direction. Measurements were taken at different developmental stages, including early vegetative growth (V), booting (B), heading (H), anthesis (A), grain filling (GF), late grain filling (GF-L, within one week of physiological maturity), and physiological maturity (M). The first irrigation event occurred on DOY 140. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R.The water-based spectral indices are described in Table 1. **a.** Estimates of normalized water index 1 (NWI-1) at Fo13F. **b.** Estimates of NWI-1 at Fo13R. **c.** Estimates of normalized water index 3 (NWI-3) at Fo13F. **d.** Estimates of NWI-3 at Fo13R. **e.** Estimates of normalized water index 4 (NWI-4) at Fo13F. **f.** Estimates of NWI-4 at Fo13R.

Tables

Table 5.1. The formula and reference for published water-based spectral indices associated with canopy water content and plant water status.

Spectral Index	Formula†	Reference
Water Index (WI)	R_{900}/R_{970}	Peñuelas et al., 1997
Normalized water index 1 (NWI-1)	$(R_{970}- R_{900})/(R_{970}+ R_{900})$	Babar et al., 2006
Normalized water index 2 (NWI-2)	$(R_{970}- R_{850})/(R_{970}+ R_{850})$	Babar et al., 2006
Normalized water index 3 (NWI-3)	$(R_{970}- R_{920})/(R_{970}+ R_{920})$	Prasad et al., 2007
Normalized water index 4 (NWI-4)	$(R_{970}- R_{880})/(R_{970}+ R_{880})$	Prasad et al., 2007

† R and the subscript indicate the reflectance of light at that specific wavelength (in nm)

Table 5.2. Mean and standard deviation of the water index (WI) and normalized water index 2 (NWI-2) values evaluated on winter wheat entries on eight different days (day of year, DOY) in 2013. The water-based spectral indices are described in Table 5.1. Data are pooled across two environments: full irrigation (Fo13F) and rainfed (Fo13R) conditions in Fort Collins, CO. The first irrigation event occurred on DOY 140. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R. Means not connected by the same letter are significantly different at $P < 0.05$.

DOY	N	WI		NWI-2	
151	659	1.072 ± 0.047	a	-0.908 ± 0.018	d
157	330	1.152 ± 0.067	a	-0.062 ± 0.025	abc
160	659	1.145 ± 0.047	a	-0.059 ± 0.018	ab
162	330	1.068 ± 0.067	a	-0.018 ± 0.025	ab
170	653	1.271 ± 0.048	a	-0.107 ± 0.018	bc
177	328	1.058 ± 0.067	a	-0.002 ± 0.025	a
178	330	1.121 ± 0.067	a	-0.043 ± 0.025	ab
182	330	1.287 ± 0.067	a	-0.164 ± 0.025	c

Table 5.3. Mean and standard deviation of normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4) values evaluated on winter wheat entries on six different days (day of year, DOY) in Fort Collins, CO under full irrigation (Fo13F). The water-based spectral indices are described in Table 5.1. Means not connected by the same letter are significantly different at $P < 0.05$.

DOY	N	NWI-1		NWI-3		NWI-4	
151	329	-0.039 ± 0.008	a	-0.020 ± 0.009	a	-0.058 ± 0.006	b
160	329	-0.077 ± 0.008	c	-0.077 ± 0.009	bc	-0.075 ± 0.006	b
162	330	-0.033 ± 0.008	a	-0.035 ± 0.009	a	-0.026 ± 0.006	a
170	330	-0.109 ± 0.008	c	-0.113 ± 0.009	c	-0.114 ± 0.006	c
178	330	-0.056 ± 0.008	ab	-0.057 ± 0.009	ab	-0.054 ± 0.006	b
182	330	-0.192 ± 0.008	d	-0.187 ± 0.009	d	-0.170 ± 0.006	d

Table 5.4. Mean and standard deviation of normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4) values evaluated on winter wheat entries on five different days (day of year, DOY) in Fort Collins, CO under rainfed (Fo13R) conditions. The water-based spectral indices are described in Table 5.1. Means not connected by the same letter are significantly different at $P < 0.05$.

DOY	N	NWI-1	NWI-3	NWI-4
151	330	-0.030 ± 0.001 a	-0.013 ± 0.001 a	-0.046 ± 0.001 b
157	330	-0.070 ± 0.001 c	-0.067 ± 0.001 d	-0.066 ± 0.001 c
160	330	-0.058 ± 0.001 b	-0.061 ± 0.001 c	-0.050 ± 0.001 b
170	323	-0.128 ± 0.001 d	-0.132 ± 0.001 e	-0.107 ± 0.001 d
177	328	-0.028 ± 0.001 a	-0.035 ± 0.001 b	-0.015 ± 0.001 a

Table 5.5. Mean and standard deviation of normalized water index 2 (NWI-2) values evaluated on winter wheat entries at seven developmental stages. NWI-2 is described in Table 5.1. Measurements were pooled across two environments: full irrigation (Fo13F) and rainfed (Fo13R) conditions in Fort Collins, CO in 2013. The first irrigation event occurred on DOY 140. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R. The developmental stages are early vegetative growth (V), booting (B), heading (H), anthesis (A), grain filling (GF), late grain filling (GF-L, within one week of physiological maturity), and physiological maturity (M). Means not connected by the same letter are significantly different at $P < 0.05$.

Level	N	NWI-2	
V	8	-0.931 ± 0.165	bc
B	218	-0.987 ± 0.032	c
H	412	-0.860 ± 0.023	b
A	50	-0.261 ± 0.066	a
GF	2185	-0.072 ± 0.010	a
GF-L	699	-0.079 ± 0.018	a
M	47	0.006 ± 0.068	a

Table 5.6. Mean and standard deviation of normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4) evaluated on winter wheat entries during five developmental stages in Fort Collins, CO under full irrigation (Fo13F). The water-based spectral indices are described in Table 5.1. The developmental stages are booting (B), heading (H), anthesis (A), grain filling (GF), and late grain filling (GF-L, within one week of physiological maturity). Means not connected by the same letter are significantly different at $P < 0.05$.

Level	N	NWI-1		NWI-3		NWI-4	
B	114	-0.038±0.014	ab	-0.019±0.017	a	-0.057±0.011	a
H	193	-0.040±0.010	a	-0.021±0.013	a	-0.059±0.008	a
A	46	-0.060±0.021	ab	-0.051±0.026	ab	-0.068±0.017	a
GF	1249	-0.074±0.004	b	-0.076±0.005	c	-0.072±0.003	a
GF-L	376	-0.158±0.008	c	-0.154±0.009	b	-0.140±0.006	b

Table 5.7. Mean and standard deviation of normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4) evaluated on winter wheat entries during seven developmental stages in Fort Collins, CO under rainfed (Fo13R) conditions. The water-based spectral indices are described in Table 5.1. The developmental stages are early vegetative growth (V), booting (B), heading (H), anthesis (A), grain filling (GF), late grain filling (GF-L, within one week of physiological maturity), and physiological maturity (M). Means not connected by the same letter are significantly different at $P < 0.05$.

Level	N	NWI-1		NWI-3		NWI-4	
V	8	-0.026±0.013	ab	-0.012±0.013	ab	-0.041±0.012	abcd
B	104	-0.029±0.004	a	-0.013±0.004	a	-0.044±0.003	c
H	219	-0.032±0.002	a	-0.016±0.002	a	-0.047±0.002	c
A	4	-0.045±0.018	abc	-0.022±0.018	ab	-0.066±0.017	bcd
GF	936	-0.082±0.001	c	-0.084±0.001	c	-0.073±0.001	d
GF-L	323	-0.043±0.002	b	-0.049±0.002	b	-0.028±0.002	b
M	47	-0.023±0.005	a	-0.029±0.005	a	-0.009±0.005	a

Table 5.8. Mean squares and significance level of the main effects and interaction of sampling date (day of year, DOY) and developmental stage (DEV) of water-based spectral indices. Measurements were pooled across two environments: full irrigation (Fo13F) and rainfed (Fo13R) conditions in Fort Collins, CO in 2013. The first irrigation event occurred on DOY 140. There was no significant difference in phenological development of Fo13F and Fo13R until anthesis. Average anthesis dates were DOY 156 in Fo13F and DOY 155 in Fo13R. The indices include the water index (WI), and normalized water indices 1 (NWI-1), 2 (NWI-2), 3 (NWI-3), and 4 (NWI-4), which are described in Table 5.1.

Model term	WI	NWI-1	NWI-2	NWI-3	NWI-4
DOY	5.00 ns	1.15***	0.04 ns	1.21***	0.98***
DEV	0.82 ns	0.07***	36.51***	0.09***	0.09***
DOY*DEV	0.05 ns	0.29***	3.60***	0.23***	0.27***

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. ns indicates not significant at the 0.05 probability level

Table 5.9. Mean squares and significance level of main effects and interactions of environment (ENV,), sampling date (day of year, DOY) and developmental stage (DEV) on water-based spectral indices. The two environments are full irrigation (Fo13F) or rainfed (Fo13R) in Fort Collins, CO during 2013. Measurements were collected on eight sampling dates representing seven developmental stages. The spectral indices include normalized water indices 1 (NWI-1), 3 (NWI-3), and 4 (NWI-4), which are described in Table 5.1.

Model term	NWI-1	NWI-3	NWI-4
ENV	0.02 ns	0.02 ns	0.01 ns
DOY	0.88***	0.97***	0.62***
DEV	0.03*	0.07***	0.02**
ENV *DOY	0.02 ns	0.05 ns	0.00 ns
ENV * DEV	0.21***	0.20***	0.16***
DOY * DEV	0.00 ns	0.01 ns	0.00 ns
ENV *DOY* DEV	1.88***	1.78***	1.40***

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. ns indicates not significant at the 0.05 probability level

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APPENDICES

Supplemental Text.

Supplemental Text S3.1

The six spatial correlation models were analyzed with the PROC MIXED procedure of SAS (version 9.3, SAS Institute, Inc., Cary, NC) and are described by Littell et al. (2006) and in the SAS/STAT 9.3 User's Guide, Table 58.14 (http://support.sas.com/documentation/cdl/en/statug/63962/HTML/default/viewer.htm#statug_mixed_sect020.htm). In all spatial correlation models the correlation between measurements decreases as a function of increasing distance (Hu and Spilke, 2009).

The row–column model accounts for positive spatial variation in two perpendicular directions, as in Eq. S3.1, where Y_{ij} is the ij^{th} observation, μ is the mean, x_i explains north–south variation, y_j explains east–west variation, and e_{ij} describes the corresponding error:

$$Y_{ij} = \mu + x_i + y_j + e_{ij} \quad \text{(Eq. S3.1)}$$

The power, spherical, exponential, and Matérn models are isotropic, thus the variation properties are the same along the *x*- and *y*-axes (Hu and Spilke, 2009). For isotropic models, the distance between locations s_i *and* s_i is described as d_{ij} and the parameter ρ is related to the range of the process, where the strength of the correlation between measurements decreases with distance, such that at some distance measurements are no longer correlated.

In the power model the correlation between measurements decreases with distance. The covariance structure of the power model is described in Eq. S3.2:

$$f(d_{ij}) = \sigma^2 \rho^{d_{ij}} \quad \text{(Eq. S3.2)}$$

The exponential model is an extension of the power model where the correlation decreases exponentially. The covariance structure of the exponential model is described in Eq. S3.3:

$$f(d_{ij}) = \sigma^2 \exp\left(\frac{-d_{ij}}{\rho}\right) \quad \text{(Eq. S3.3)}$$

In the spherical model the correlation between measurements is symmetrical in all directions, such that variation extends in the shape of a sphere from the point where the measurement was collected. The covariance structure of the spherical model is described in Eq. S3.4:

$$f(d_{ij}) = \sigma^2 \left[1 - \left(\frac{3d_{ij}}{2\rho}\right) + \left(\frac{{d_{ij}}^3}{2\rho^3}\right)\right] I(d_{ij} \leq \rho) \quad \text{(Eq. S3.4)}$$

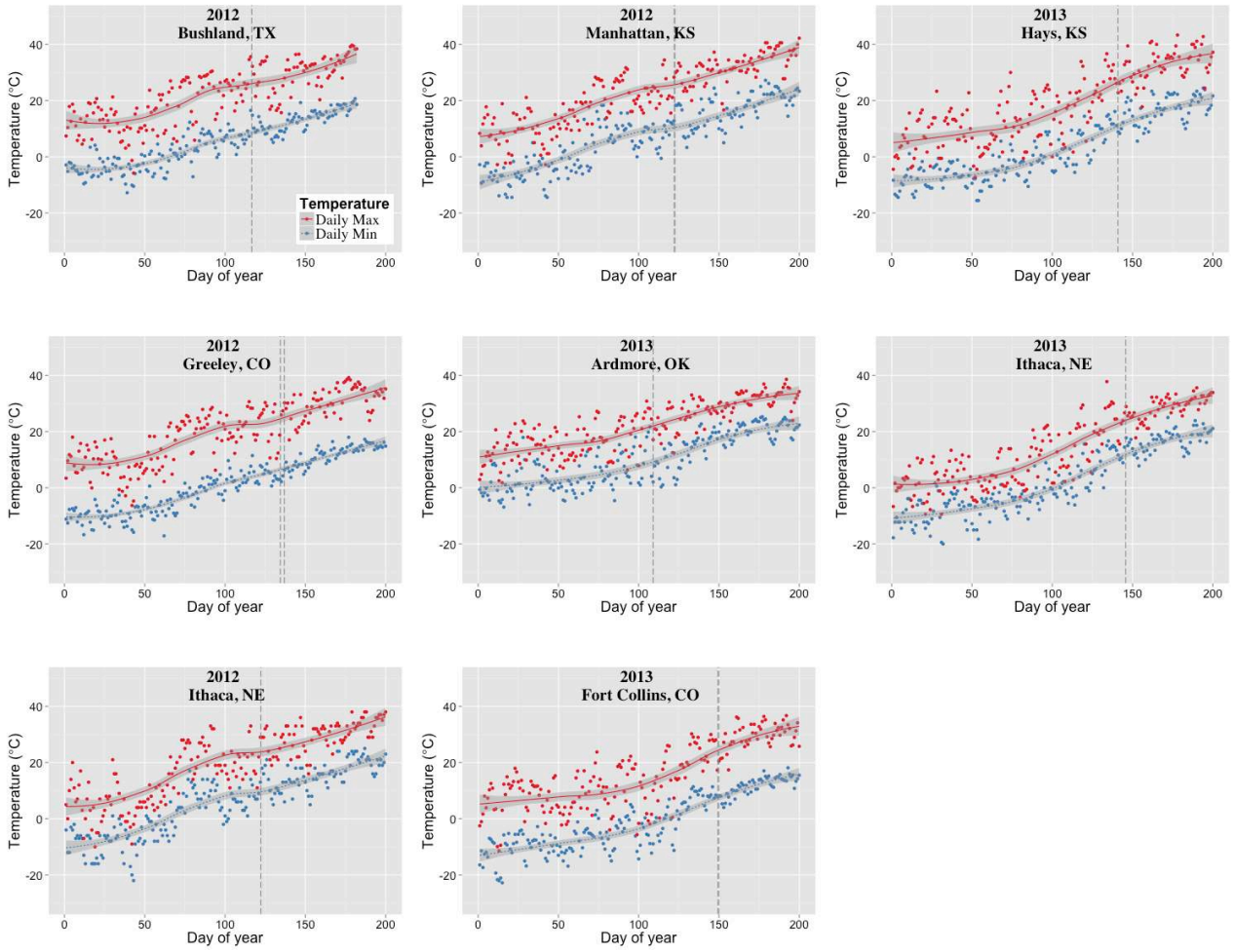
Covariance structure of the Matérn model is described in Eq. S3.5, where the parameter v describes smoothness and K_v is a modified Bessell function frequently associated with cylindrical or spherical coordinates, and $\Gamma_{(v)}$ is the gamma function. When $v = 0.5$, the Matérn model is equal to the exponential model.

$$f(d_{ij}) = \frac{1}{\Gamma(v)}\left(\frac{d_{ij}}{2\rho}\right)^{v} 2K_v\left(\frac{d_{ij}}{\rho}\right) \quad \text{(Eq. S3.5)}$$

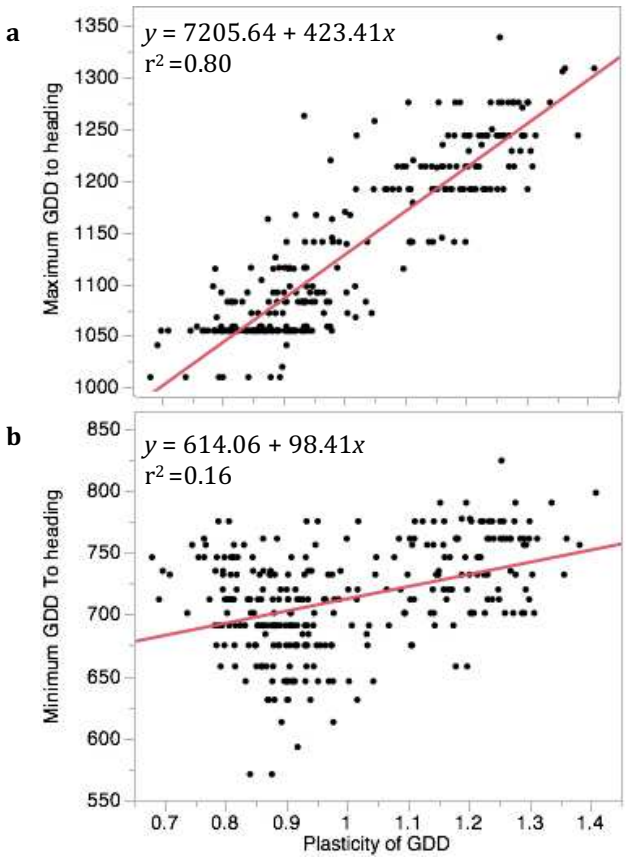
Covariance structure of the anisotropic power model (using covariance structure type=sp(powa)) is described in Eq. S3.6, where there are c coordinates and $d(i, j, k)$ describes the absolute distance between the k^{th} coordinate, $k = 1, \ldots, c$, of the i^{th} and j^{th} observations in the data set. Anisotropic models allow the variation properties to be different along the x- and y-axes.

$$f(d_{ij}) = \sigma^2 \rho_1^{d(i,j,1)} \rho_2^{d(i,j,2)} \ldots \rho_c^{d(i,j,c)} \quad \text{(Eq. S3.6)}$$

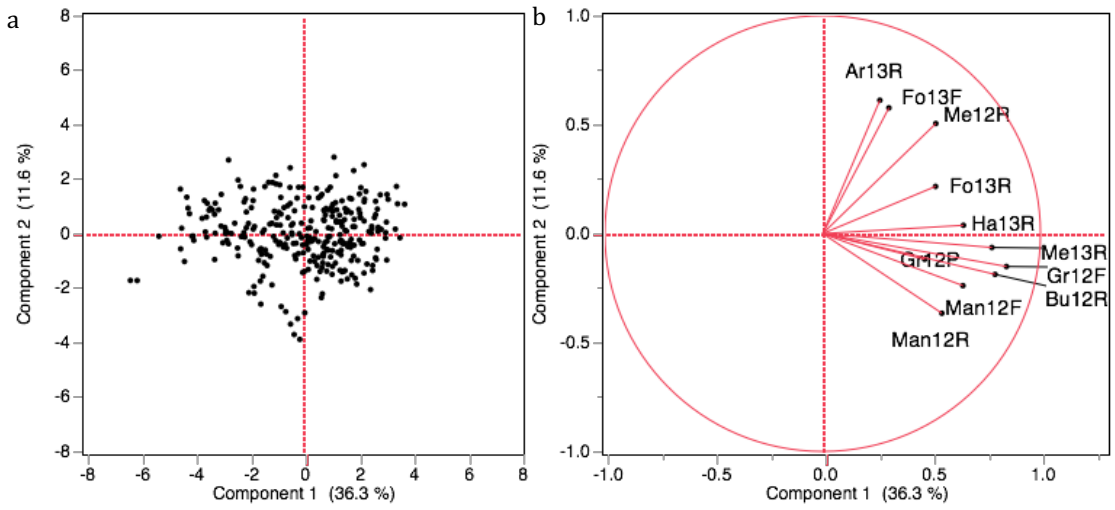
Supplemental Figures.



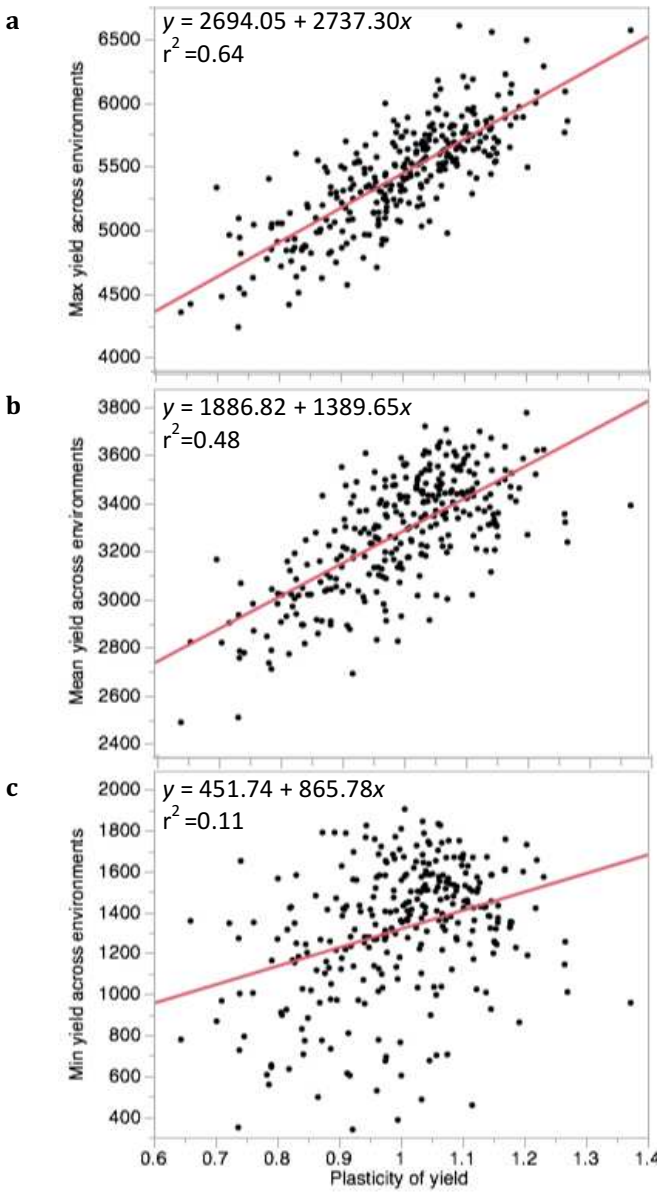
Supplemental Figure S3.1. Daily maximum and minimum temperatures (°C), and fitted loess curves for eight environments where the hard winter wheat panel was grown during 2011–12 or 2012–13. Greeley, CO, Manhattan, KS, and Fort Collins, CO each include two environments, which reflect different moisture treatments. The 11 total environments are described in Table 3.1. Average heading dates are identified by a vertical line, and are described in Table 3.3.



Supplemental Figure S3.2. Linear regression of (a) maximum, and (b) minimum growing degree-days (GDD) from 1 Jan. to heading across environments, for each of 299 hard winter wheat varieties grown at 11 environments, on plasticity of GDD.



Supplemental Figure S3.3. Principal components analysis of grain yield among 299 hard winter wheat genotypes grown in 11 environments, showing the (a) score plot and (b) loadings plot. Environments are defined in Table 3.1.



Supplemental Figure S3.4. Linear regressions of (a) maximum, (b) mean, and (c) minimum grain yield (kg ha^{-1}) across 11 environments, for each of 299 hard winter wheat varieties, on plasticity of yield..

Supplemental Tables.

Supplemental Table S2.1. Description of U.S. winter wheat entries evaluated. Description of winter wheat entries evaluated, including entry name, year derived, state breeding program derived in, region of the U.S. Great Plains derived in, and allele calls. The genotyped loci include photoperiod (*Ppd*) genes where alleles are '*a*' insensitive and '*b*' sensitive, reduced-height (*Rht*) genes where alleles are '*a*' tall and '*b*' semi-dwarf, and vernalization (*Vrn*) genes. *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* allele calls are '*W*' for winter growth habit. Variation in winter alleles at vernalization loci include copy number variation (CNV) at *vrn-A1*, Wichita-type ('*w*') or Veery-type ('v') alleles at *vrn-A1*, and Neuse-type ('*N*') or AGS2000-type ('*A*') alleles at *vrn-B1*. Missing genotypic data are indicated with a dash (-).

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
AGATE	1969	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
AKRON	1988	CO	Central	b	b	b	b	a	W	W	W	> 2	w	N
ALLIANCE	1988	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
ANTON	1998	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
ARAPAHOE	1982	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
BENNETT	1973	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
BISON	1946	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
BUCKSKIN	1968	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
CAMELOT	2001	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
CARSON	1981	CO	Central	b	b	b	a	a	W	W	W	> 2	w	N
CHENEY	1973	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
CHEYENNE	1922	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
CO03W054	2003	CO	Central	b	b	b	b	a	W	W	W	1	v	N
CO04025	2004	CO	Central	b	b	b	b	a	W	W	W	> 2	w	N
CO04393	2004	CO	Central	b	b	b	b	a	W	W	W	> 2	w	N
CO04W320	2004	CO	Central	b	b	b	b	a	W	W	W	1	v	N
CO07W245	2007	CO	Central	b	b	b	a	a	W	W	W	> 2	w	N
CO940610	1994	CO	Central	b	b	b	b	a	W	W	W	> 2	w	N
COLT	1978	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
COMANCHE	1934	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
COUGAR	1993	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
DODGE	1982	KS	Central	b	b	b	b	a	W	W	W	1	v	N
EAGLE	1967	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
GAGE	1952	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
GOODSTREAK	1997	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
HAIL	1977	CO	Central	b	b	b	a	b	W	W	W	> 2	w	N
HALLAM	1998	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
HOMESTEAD	1968	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
INFINITY_CL	2001	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
JULES	1986	CO	Central	b	b	b	a	b	W	W	W	> 2	w	N
KAW61	1947	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
KIOWA	1943	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
LANCER	1957	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
LARNED	1970	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
MCGILL	2001	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
MILLENNIUM	1994	NE	Central	b	b	b	a	a	W	W	W	1	v	N
NE02558	2002	NE	Central	b	b	b	b	a	W	W	W	1	v	N
NE04490	2004	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
NE05496	2005	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
NE05548	2005	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
NE06545	2006	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
NE06607	2006	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
NE99495	1999	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
NEKOTA	1988	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
NEWTON	1973	KS	Central	b	b	b	b	a	W	W	W	1	v	N
NIOBRARA	1989	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
NUPLAINS	1994	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
NW03666	2003	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
OVERLAND	2001	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
REDLAND	1985	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
RIPPER	2000	CO	Central	b	b	b	b	a	W	W	W	> 2	w	N
SAGE	1970	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
SCOUT66	1956	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
VISTA	1987	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
WAHOO	1994	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
WARRIOR	1948	NE	Central	b	b	b	a	a	W	W	W	> 2	w	N
WESLEY	1995	NE	Central	b	b	b	b	a	W	W	W	> 2	w	N
WICHITA	1927	KS	Central	b	b	b	a	a	W	W	W	> 2	w	N
2145	1997	KS	Central	b	b	a	b	a	W	W	W	> 2	w	N
ABOVE	1998	CO	Central	b	b	a	b	a	W	W	W	2	v	N
ARLIN	1992	KS	Central	b	b	a	b	a	W	W	W	> 2	w	N
CULVER	1993	NE	Central	b	b	a	a	a	W	W	W	> 2	w	N
DENALI	2005	CO	Central	b	b	a	a	b	W	W	W	> 2	w	N
KARL_92	1983	KS	Central	b	b	a	b	a	W	W	W	> 2	w	N
LAKIN	1996	KS	Central	b	b	a	b	a	W	W	W	> 2	w	N
NI06736	2006	NE	Central	b	b	a	b	a	W	W	W	> 2	w	N
NI06737	2006	NE	Central	b	b	a	b	a	W	W	W	> 2	w	N
ROBIDOUX	2004	NE	Central	b	b	a	b	a	W	W	W	> 2	w	N
SIOUXLAND	1986	NE	Central	b	b	a	a	a	W	W	W	> 2	w	N
YUMAR	1994	CO	Central	b	b	a	b	a	W	W	W	2	v	N
BOND_CL	2000	CO	Central	b	a	b	b	a	W	W	W	> 2	w	N
BYRD	2006	CO	Central	b	a	b	a	a	W	W	W	> 2	w	N
CENTURA	1977	NE	Central	b	a	b	a	a	W	W	W	> 2	w	N
CENTURK78	1969	NE	Central	b	a	b	a	a	W	W	W	> 2	w	N
CO03064	2003	CO	Central	b	a	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
CO050337-2	2005	CO	Central	b	a	b	b	a	W	W	W	> 2	w	N
DANBY	2002	KS	Central	b	a	b	b	a	W	W	W	> 2	w	N
DUKE	1974	CO	Central	b	a	b	a	a	W	W	W	> 2	w	N
FULLER	2000	KS	Central	b	a	b	b	a	W	W	W	> 2	w	N
HEYNE	1985	KS	Central	b	a	b	b	a	W	W	W	1	v	N
JAGGER	1984	KS	Central	b	a	b	b	a	W	W	W	1	v	N
KIRWIN	1966	KS	Central	b	a	b	a	a	W	W	W	> 2	w	N
KS00F5-20-3	2000	KS	Central	b	a	b	b	a	W	W	W	1	v	N
LAMAR	1982	CO	Central	b	a	b	a	a	W	W	W	> 2	w	N
MACE	2002	NE	Central	b	a	b	b	a	W	W	W	> 2	w	N
NI07703	2007	NE	Central	b	a	b	b	a	W	W	W	> 2	w	N
OVERLEY	1994	KS	Central	b	a	b	b	a	W	W	W	1	v	N
PARKER	1953	KS	Central	b	a	b	b	a	W	W	W	1	v	N
PARKER76	1974	KS	Central	b	a	b	a	a	W	W	W	> 2	w	N
PLATTE	1989	CO	Central	b	a	b	b	a	W	W	W	> 2	w	N
PRAIRIE_RED	1994	CO	Central	b	a	b	b	a	W	W	W	> 2	w	N
PRONGHORN	1988	NE	Central	b	a	b	a	a	W	W	W	> 2	w	N
PROWERS	1994	CO	Central	b	a	b	a	a	W	W	W	> 2	w	N
RONL	2003	KS	Central	b	a	b	b	a	W	W	W	> 2	w	N
SETTLER_CL	2003	NE	Central	b	a	b	b	a	W	W	W	> 2	w	N
SHAWNEE	1960	KS	Central	b	a	b	a	a	W	W	W	> 2	w	N
STANTON	1995	KS	Central	b	a	b	b	a	W	W	W	> 2	w	N
TAM107-R7	1994	NE	Central	b	a	b	b	a	W	W	W	> 2	w	N
TRISON	1965	KS	Central	b	a	b	a	a	W	W	W	> 2	w	N
WINDSTAR	1990	NE	Central	b	a	b	b	a	W	W	W	1	v	N
BILL_BROWN	2001	CO	Central	b	a	a	b	a	W	W	W	2	v	N
HALT	1991	CO	Central	b	a	a	b	a	W	W	W	> 2	w	N
LINDON	1972	CO	Central	b	a	a	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
NE05430	2005	NE	Central	b	a	a	b	a	W	W	W	> 2	w	N
NI08707	2008	NE	Central	b	a	a	b	a	W	W	W	> 2	w	N
NI08708	2008	NE	Central	b	a	a	b	a	W	W	W	> 2	w	N
RAWHIDE	1983	NE	Central	b	a	a	b	a	W	W	W	> 2	w	N
TREGO	1995	KS	Central	b	a	a	b	a	W	W	W	> 2	w	N
VONA	1972	CO	Central	b	a	a	b	a	W	W	W	> 2	w	N
YUMA	1985	CO	Central	b	a	a	b	a	W	W	W	2	v	N
ANTELOPE	1997	NE	Central	b	-	b	b	a	W	W	W	> 2	w	N
SANDY	1961	CO	Central	b	-	b	-	a	W	W	W	> 2	w	N
THUNDER_CL	2003	CO	Central	b	-	b	b	a	W	W	W	> 2	w	N
HARRY	1997	NE	Central	-	-	b	-	a	W	W	W	> 2	w	N
AVALANCHE	1994	CO	Central	-	-	-	-	-	W	W	W	> 2	w	-
CO03W043	2003	CO	Central	-	-	-	-	-	W	W	W	-	-	-
CO04499	2004	CO	Central	-	-	-	-	-	W	W	W	> 2	w	-
HATCHER	1998	CO	Central	-	-	-	-	-	W	W	W	-	-	-
NORKAN	1982	KS	Central	-	-	-	-	-	W	W	W	> 2	w	-
BIG_SKY	1994	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
BRONZE	1967	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
CREST	1966	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
DARRELL	1998	SD	North	b	b	b	b	a	W	W	W	> 2	w	N
DECADE	2005	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
GENOU	2000	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
GENT	1971	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
HARDING	1992	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
HUME	1956	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
JERRY	1992	ND	North	b	b	b	b	a	W	W	W	> 2	w	N
JUDEE	2007	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
JUDITH	1980	MT	North	b	b	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
MT0495	2004	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
MT06103	2006	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
MT85200	1985	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
MT9513	1995	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
MT9904	1999	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
MT9982	1999	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
MTS0531	2005	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
NELL	1973	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
NUSKY	1994	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
RITA	1973	SD	North	b	b	b	b	a	W	W	W	> 2	w	N
ROSE	1972	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
ROSEBUD	1974	MT	North	b	b	b	a	a	W	W	W	> 2	w	N
SD00111-9	2000	SD	North	b	b	b	b	a	W	W	W	> 2	w	N
SD05118	2005	SD	North	b	b	b	b	a	W	W	W	> 2	w	N
SD05210	2005	SD	North	b	b	b	b	a	W	W	W	> 2	w	N
TANDEM	1989	SD	North	b	b	b	a	a	W	W	W	> 2	w	N
YELLOWSTONE	2000	MT	North	b	b	b	b	a	W	W	W	> 2	w	N
ALICE	1997	SD	North	b	b	a	b	a	W	W	W	> 2	w	N
SD01058	2001	SD	North	b	b	a	b	a	W	W	W	> 2	w	N
WENDY	1997	SD	North	b	b	a	b	a	W	W	W	> 2	w	N
EXPEDITION	1997	SD	North	b	a	b	b	a	W	W	W	> 2	w	N
NORRIS	2003	MT	North	b	a	b	a	a	W	W	W	> 2	w	N
SD01237	2001	SD	North	b	a	b	b	a	W	W	W	> 2	w	N
WINOKA	1966	SD	North	b	a	b	a	b	W	W	W	> 2	w	N
DAWN	1970	SD	North	b	a	a	a	a	W	W	W	> 2	w	N
CRIMSON	1989	SD	North	b	-	b	a	a	W	W	W	> 2	w	N
SD05W018	2005	SD	North	-	-	b	-	a	W	W	W	> 2	w	N
BAKERS_WHITE	1998	-	Other	b	b	b	b	a	W	W	W	1	v	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
HONDO	1995	-	Other	b	b	b	b	a	W	W	W	> 2	w	N
KHARKOF	1900	-	Other	b	b	b	a	a	W	W	W	> 2	w	N
NUHORIZON	1995	-	Other	b	b	b	b	a	W	W	W	> 2	w	N
SMOKYHILL	1999	-	Other	b	b	b	b	a	W	W	W	> 2	w	N
TURKEY_NEBSEL	1874	-	Other	b	b	b	a	a	W	W	W	> 2	w	N
BURCHETT	1996	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
COSSACK	1987	-	Other	b	b	a	a	a	W	W	W	> 2	w	N
E2041	2000	-	Other	b	b	a	a	b	W	W	W	> 2	w	N
ENHANCER	1998	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
HV9W03-1551WP	2003	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
HV9W03-1596R	2003	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
HV9W05-1280R	2005	-	Other	b	b	a	a	a	W	W	W	> 2	w	N
KEOTA	1998	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
ONAGA	1991	-	Other	b	b	a	b	a	W	W	W	> 2	w	N
W04-417	2004	-	Other	b	b	a	a	b	W	W	W	> 2	w	N
WB411W	1998	-	Other	b	b	a	b	a	W	W	W	1	v	N
CUTTER	1997	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
G1878	1996	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
HV9W06-504	2006	-	Other	b	a	b	b	a	W	W	W	1	v	N
JAGALENE	1998	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
LONGHORN	1988	-	Other	b	a	b	a	a	W	W	W	> 2	w	N
NEOSHO	1996	-	Other	b	a	b	a	a	W	W	W	> 2	w	N
NUFRONTIER	1994	-	Other	b	a	b	a	a	W	W	W	> 2	w	N
OGALLALA	1989	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
POSTROCK	1995	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
SANTA_FE	2003	-	Other	b	a	b	b	a	W	W	W	1	v	N
SHOCKER	1999	-	Other	b	a	b	b	a	W	W	W	1	v	N
SPARTAN	1994	-	Other	b	a	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
TARKIO	1999	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
THUNDERBOLT	1995	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
VENANGO	2000	-	Other	b	a	b	b	a	W	W	W	> 2	w	N
DUMAS	1995	-	Other	b	a	a	a	b	W	W	W	> 2	w	N
HV906-865	2006	-	Other	b	a	a	b	a	W	W	W	> 2	w	N
HV9W03-1379R	2003	-	Other	b	a	a	b	a	W	W	W	1	v	N
HG-9	<2000	-	Other	-	-	-	-	-	W	W	W	> 2	w	-
LOCKETT	1991	TX	South	b	b	b	b	a	W	W	W	> 2	w	N
OK07S117	2007	OK	South	b	b	b	b	a	W	W	W	> 2	w	N
OK10119	2010	OK	South	b	b	b	b	a	W	W	W	> 2	w	N
OK1067071	2010	OK	South	b	b	b	het	a	W	W	W	> 2	w	N
TAM105	1969	TX	South	b	b	b	b	a	W	W	W	> 2	w	N
TAM109	1987	TX	South	b	b	b	a	b	W	W	W	> 2	w	N
TAMW-101	1965	TX	South	b	b	b	a	b	W	W	W	> 2	w	N
TASCOSA	1951	TX	South	b	b	b	a	a	W	W	W	> 2	w	N
TX04V075080	2004	TX	South	b	b	b	b	a	W	W	W	> 2	w	N
TX06V7266	2006	TX	South	b	b	b	b	a	W	W	W	> 2	w	N
TAM302	1991	TX	South	a	b	b	a	het	W	W	W	> 2	w	N
2180	1987	TX	South	b	b	a	a	b	W	W	W	> 2	w	N
BILLINGS	2003	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
CAPROCK	1956	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
CHISHOLM	1975	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
CUSTER	1988	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
DELIVER	1998	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
DUSTER	1993	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
ENDURANCE	1994	OK	South	b	b	a	a	b	W	W	W	> 2	w	N
GALLAGHER	2007	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
INTRADA	1998	OK	South	b	b	a	het	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
MIT	1971	TX	South	b	b	a	b	a	W	W	W	1	v	N
OK02405	2002	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK05312	2005	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK05526	2005	OK	South	b	b	a	het	het	W	W	W	> 2	w	N
OK06114	2006	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK06336	2006	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK07231	2007	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK08328	2008	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK101	1995	OK	South	b	b	a	a	b	W	W	W	> 2	w	N
OK1067274	2010	OK	South	b	b	a	a	a	W	W	W	> 2	w	N
OK1068026	2010	OK	South	b	b	a	b	a	W	W	W	> 2	w	N
OK1070267	2010	OK	South	b	b	a	a	a	W	W	W	1	v	N
STURDY	1956	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
STURDY_2K	1956	TX	South	b	b	a	b	a	W	W	W	1	v	N
TAM304	2001	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX00V1131	2000	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX03A0148	2003	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX04A001246	2004	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX06A001281	2006	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX06A001386	2006	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
TX96D1073	1996	TX	South	b	b	a	b	a	W	W	W	> 2	w	N
OK05303	2005	OK	South	a	b	a	a	b	W	W	W	> 2	w	N
CENTURY	1981	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
GUYMON	2000	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK_BULLET	2000	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK_RISING	2006	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK04505	2004	OK	South	b	a	b	b	a	W	W	W	1	v	N
OK04507	2004	OK	South	b	a	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
OK05108	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05122	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05204	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05711W	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05723W	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05830	2005	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK06210	2006	OK	South	b	a	b	b	a	W	W	W	1	v	N
OK09634	2009	OK	South	b	a	b	b	a	W	W	W	1	v	N
OK1068002	2010	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
OK1070275	2010	OK	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM110	1988	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM111	1995	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM112	1998	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM200	1981	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM202	1986	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM203	2001	TX	South	b	a	b	b	a	W	W	W	1	v	N
TAM303	1998	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM400	1993	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TAM401	2003	TX	South	b	a	b	b	a	W	W	W	> 2	w	A
TRIUMPH64	1938	OK	South	b	a	b	a	a	W	W	W	> 2	w	N
TX01A5936	2001	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX01V5134RC-3	2001	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX02A0252	2002	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX03A0563	2003	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX04M410164	2004	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX05A001188	2005	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX05A001822	2005	TX	South	b	a	b	b	a	W	W	W	> 2	v	A
TX05V7259	2005	TX	South	b	a	b	b	a	W	W	W	> 2	w	N

Entry Name	Year Derived	State†	Region‡	*Ppd-A1*¶	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	CNV *vrn-A1*	*vrn-A1*	*vrn-B1*
TX06A001263	2006	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX07A001279	2007	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX07A001318	2007	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX07A001420	2007	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX86A5606	1986	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX86A6880	1986	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX86A8072	1986	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
TX99A0153-1	1999	TX	South	b	a	b	b	a	W	W	W	> 2	w	N
OK05134	2005	OK	South	a	a	b	het	a	W	W	W	> 2	w	N
TX04M410211	2004	TX	South	a	a	b	b	a	W	W	W	1	v	N
TX05V7269	2005	TX	South	a	a	b	b	a	W	W	W	> 2	w	N
TX06A001132	2006	TX	South	a	a	b	b	a	W	W	W	> 2	w	N
2174-05	1997	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
CENTERFIELD	2003	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
GARRISON	2005	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK04111	2004	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK04415	2004	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK04525	2004	OK	South	b	a	a	a	b	W	W	W	> 2	w	N
OK05511	2005	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK06318	2006	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK06319	2006	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK102	1997	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
OK1068009	2010	OK	South	b	a	a	a	a	W	W	W	> 2	w	N
PETE	2003	OK	South	b	a	a	b	a	W	W	W	> 2	w	N
TX01M5009-28	2001	TX	South	b	a	a	b	a	W	W	W	1	v	N
TX99U8618	1999	TX	South	b	a	a	b	a	W	W	W	1	v	N
OK1068112	2010	OK	South	b	-	b	b	a	W	W	W	1	v	N
TAM107	1980	TX	South	b	-	b	b	a	W	W	W	> 2	w	N

Supplemental Table S2.2. Description of KASP markers used to genotype 299 U.S. Great Plains hard winter wheat entries. KASP detected allelic variants at *Vrn-A1, Vrn-B1, Vrn-D1, Ppd-A1, Ppd-B1, Ppd-D1, Rht-B1,* and *Rht-D1.*

Locus	Allele(s) assayed	Marker ID	Primer name	Primer Sequence
Vrn-A1	Vrn-A1a	wMAS000033	Vrn-A1_9K0001_AL2	GAGTTTTCCAAAAAGATAGATCAATGTAAAC
			Vrn-A1_9K0001_AL1	AGAGTTTTCCAAAAAGATAGATCAATGTAAAT
			Vrn-A1_9K0001_C1	GTTAGTAGTGATGGTCCAATAATGCCAAA
	Vrn-A1b	wMAS000035	Vrn-A1b-Marq_AL2	GTTTTGGCCTGGCCATCCTCA
			Vrn-A1b-Marq_AL1	GTTTTGGCCTGGCCATCCTCC
			Vrn-A1b-Marq_C1	TATCAGGTGGTTGGGTGAGGACGT
	vrn-A1 exon 4_C/T	vrn-A1exon4	Vrn-A1_Exon4_F1	AGGCATCTCATGGGAGAGGATC
			Vrn-A1_Exon4_F2	CAGGCATCTCATGGGAGAGGATT
			Vrn-A1_Exon4_R	CCAGTTGCTGCAACTCCTTGAGATT
	vrn-A1 exon 7_G/A	vrn-A1exon7	Vrn-A1_Exon7_F1	TGAGTTTGATCTTGCTGCGCCG
			Vrn-A1_Exon7_F2	CTGAGTTTGATCTTGCTGCGCCA
			Vrn-A1_Exon4_R	CTTCCCCACAGCTCGTGGAGAA
Vrn-B1	Vrn-B1a	Vrn-B1_I_D	Vrn-B1_D_A2	GGCAGCTAATGTGGGGTAGTCT
			Vrn-B1_D_C1s	ATTCGTATTGCTAGCTCCGGCCAT
			Vrn-B1_I_ALG	CAACCTCCACGGTTTCAAAAAGTAG
			Vrn-B1_I_C1	ATATTTACTAAGCAGCGGTCATTCCGAT
	Vrn-B1b	wMAS000037	Vrn-B1_B_ALC	GCGCAAGCGGGAGCTACATC
			Vrn-B1_B_ALG	TGCGCAAGCGGGAGCTACATG
			Vrn-B1_B_C1	GCCATGAACAACAAAGGGGGTGGT
	Vrn-B1c	Vrn-B1_C	Vrn-B1_C _ALT	CCTAAACAGGGGCAGAACACTA
			Vrn-B1_C _ALG	CCTAAACAGGGGCAGAACACTG
			Vrn-B1_C _C	GACCCCAGGGCCTATGAATGTAATT
	vrn-B1_intron1_A/C	TaVrn-B1_1752	TaVrnB1_1752_AF2	GGAATGACCGCTGCTTAGTAAATATA
			TaVrnB1_1752_CF1	GGAATGACCGCTGCTTAGTAAATATC
			TaVrnB1_1752_R	GATTTAGCACCTCAACATACAGGTCT

Vrn-D1	Vrn-D1a	wMAS000039	Vrn-D1-D1a_A_ALC	ATCATTCGAATTGCTAGCTCCGC
			Vrn-D1-D1a_A_ALG	ATCATTCGAATTGCTAGCTCCGG
			Vrn-D1-D1a_A_C	GCCTGAACGCCTAGCCTGTGTA
Ppd-A1	Ppd-A1a.1	Ppd-A1prodel	Ppd-A1prodel_AL2	GCGGCGAGCCGGTTAATCG
			Ppd-A1prodel_AL1	TTTCGGTGTTTGACTTCAGGCG
			Ppd-A1prodel_C1	GTGGCGTACTCCCTCCGTTTCTT
Ppd-B1	Ppd-B1a Chinese Spring truncated copy	wMAS000027	TaPpdBJ001tR	GACGTTATGAACGCTTGGCA
			TaPpdBJ001iR	CCGTTTTCGCGGCCTT
			TaPpdBJ001tF	GGGTTCGTCGGGAGCTGT
	Ppd-B1a Sonora64 type intercopy	TaPpdBJ003	TaPpdBJ003F	CGTGAAGAGCTAGCGATGAACA
			TaPpdBJ003R	TGGGCACGTTAACACACCTTT
Ppd-D1	Ppd-D1a Ciano67 promoter deletion	wMAS000024	TaPpdDD001RI	CAAGGAAGTATGAGCAGCGGTT
			TaPpdDD001RD	AAGAGGAAACATGTTGGGGTCC
			TaPpdDD001FL	GCCTCCCACTACACTGGGC
Rht-B1	Rht-B1b	wMAS000001	RhtB1_SNP-AL1	CCCATGGCCATCTCSAGCTG
			RhtB1_SNP-AL2	CCCATGGCCATCTCSAGCTA
			RhtB1_SNP-C	TCGGGTACAAGGTGCGGGCG
Rht-D1	Rht-D1b	wMAS000002	RhtD1_AL1	CATGGCCATCTCGAGCTRCTC
			RhtD1_AL2	CATGGCCATCTCGAGCTRCTA
			RhtD1_C1	CGGGTACAAGGTGCGCGCC

Supplemental Table S2.3. Allelic effects (number of days) of photoperiod, reduced-height, and vernalization loci on heading date in each of nine environments. The environments are described in Table 2.1. The model terms were fit separately for each locus. The intercept (Int) describes the number of days from1 January to heading in each environment before the allelic effect is applied. The allelic effect at each locus is added to the Int value. Allelic effects were fit separately for each environment when there was significant genotype-by-environment interaction (G×E) at that locus in the combined analysis across all environments. Allelic effects from the combined analyses are reported for *Ppd-A1* and *vrn-B1* because significant G×E was not observed at these loci. The grand mean heading date across all germplasm and environments was 131.8 ± 0.3 days.

	CNV *vrn-A1*			***vrn-B1***	
Environment	**Int**	**CNV=1**	**CNV=2**	**Int**	**'*Neuss*' allele**
Ar13R	109.51	-4.67***	-5.53**	106.81	2.22**
Bu12R	116.86	-2.30*	-1.48 ns†	114.43	2.22**
Fo13	149.76	-1.13***	-0.45 ns	147.43	2.22**
Gr12F	136.93	-1.65**	-1.29 ns	134.56	2.22**
Gr12P	134.41	-0.46***	-0.68 ns	132.17	2.22**
Ha13R	140.97	-1.10***	-0.74 ns	138.66	2.22**
Ma12	122.53	-1.47**	-3.54 ns	120.14	2.22**
It12R	122.66	-2.91 ns	-3.19 ns	120.14	2.22**
It13R	145.80	-1.27**	-0.27 ns	143.48	2.22**

Supplemental Table S2.3. Continued.

	Ppd-A1		***Ppd-B1***		***Ppd-D1***	
Environment	**Int**	***'b'* allele**	**Int**	***'b'* allele**	**Int**	***'b'* allele**
Ar13R	107.02	1.97***	105.95	5.23***	105.31	5.23***
Bu12R	114.66	1.97***	114.23	4.04*	113.15	4.93 ns[1]
Fo13	147.70	1.97***	149.17	0.77***	148.94	0.98***
Gr12F	134.81	1.97***	135.29	2.50***	134.65	2.99***
Gr12P	132.41	1.97***	133.11	2.07***	132.77	2.27***
Ha13R	138.93	1.97***	140.14	1.23***	139.96	1.27***
Ma12	120.37	1.97***	119.93	4.10 ns	119.24	4.39 ns
It12R	120.36	1.97***	119.50	4.81 ns	118.64	5.24 ns
It13R	143.73	1.97***	144.47	2.04***	144.40	1.82***

*, **, *** indicates significance at the 0.05, 0.01, and 0.001 probability levels, respectively.
† ns indicates non-significance at the 0.05 probability level.

Supplemental Table S2.4. Allelic effects (number of days) of photoperiod sensitive alleles *Ppd-D1b* and *Ppd-B1b.* Allelic effects and interaction of *Ppd-D1b* and *Ppd-B1b* on winter wheat heading date in each of nine environments, and proportion of variability (R^2) in heading date explained by all terms in each model. The environments are described in Table 2.1. The model terms were fit separately for each environment. The intercept (Int) describes the number of days from 1 January to heading in each environment before the allelic effects are applied. The allelic effect (number of days) at each locus is added to the Int value.

Env	Int	*Ppd-D1b*	*Ppd-B1b*	*PpdD1b*PpdB1b*	R^2
Ar13R	105.45	-0.20 ns†	0.65 ns	8.59***	0.54
Bu12R	113.18	-0.05 ns	1.35 ns	6.65 ns	0.55
Fo13	149.07	-0.19 ns	0.13 ns	1.53***	0.12
Gr12F	134.68	-0.05 ns	0.79 ns	4.13***	0.40
Gr12P	132.38	0.57 ns	0.94 ns	2.52***	0.26
Ha13R	139.79	0.25 ns	0.45 ns	1.61***	0.30
Ma12	119.18	0.09 ns	0.97 ns	6.43*	0.61
It12R	118.77	-0.20 ns	0.94 ns	7.99 ns	0.27
It13R	144.60	-0.31 ns	-0.18 ns	3.62***	0.65

*, **, *** indicates significance at the 0.05, 0.01, and 0.001 probability levels, respectively.
† ns indicates non-significance at the 0.05 probability level.

Supplemental Table S3.1. Description of 299 winter wheat genotypes evaluated across environments, including genotype name, Germplasm Resources Information Network (GRIN) identifier, year of derivation, year of release, breeding program that contributed the genotype, pedigree, and plasticity coefficients for yield and growing degree-days (GDD) from 1 Jan. to heading. Genotypes are sorted by year of derivation, which describes when the genotype was first selected from a cross. Not all genotypes have been released as cultivars. Breeding programs contributing germplasm include: Agripro Biosciences (AB), Colorado State University (CSU), Goertzen Seed Research (GSR), Hardeman Grain & Seed (HGS), Kansas State University (KSU), Michigan State University (MSU), Montana State University (MTSU), North Dakota State University (NDSU), Oklahoma State University (OSU), South Dakota State University (SDSU), Texas A&M University (TAM), University of Nebraska-Lincoln (UNL), WestBred (WB), and unknown or historic variety (UNK).

Genotype Name	GRIN Identifier	Year Derived	Year Released	Breeding Program	Pedigree	Plasticity of Yield	Plasticity of GDD
Turkey Nebsel	.	1874	1874	UNK	Turkey	0.913	1.275
Kharkof	PI 5641	1900	1900	UNK	Kharkof	0.737	1.253
Cheyenne	CItr 8885	1922	1933	UNL	Selection from Crimean	0.866	1.103
Wichita	CItr 11952	1927	1944	KSU	Early Blackhull/Tenmarq	0.702	1.002
Comanche	CItr 11673	1934	1942	KSU	Oro/Tenmarq	0.746	1.142
Triumph 64	CItr 13679	1938	1964	OSU	Danne-Beardless-Blackhull/3/Kanred/Blackhull//Florence/4/Kanred/Blackhull//Triumph	0.822	0.970
Kiowa	CItr 12133	1943	1950	KSU	Chiefkan//Oro/Tenmarq	0.887	1.150
Bison	CItr 12518	1946	1956	KSU	Chiefkan//Oro/Tenmarq	0.886	1.294
Kaw 61	CItr 12871	1947	1960	KSU	purification and re-release of Kaw Oro//Mediterranean/Hope/3/Early-Blackhull/Tenmarq	0.660	0.908
Warrior	CItr 13190	1948	1960	UNL	Pawnee/Cheyenne	0.806	1.180
Tascosa	CItr 13023	1951	1959	TAM	Kanred/HardFederation//Tenmarq/3/Mediterranean/Hope/4/Cimarron	0.831	1.171
Gage	CItr 13532	1952	1963	UNL	Ponca/3/Mediterranean/Hope//Pawnee	0.739	1.214

Parker	CItr 13285	1953	1966	KSU	Quivira/3/Kanred/HardFederation//Prelude/Kanred/4/Kawvale/Marquillo//Kawvale/Tenmarq	0.939	0.979
Hume	CItr 13526	1956	1965	SDSU	Crosses involving: Minter, Kharkof, Wichita, Nebred, Cheyenne, and others	0.739	1.202
Caprock	CItr 14516	1956	1969	TAM	Sinvalocho/Wichita//Hope/Cheyenne/3/Wichita/4/Seu Seun27	0.989	0.973
Sturdy	CItr 13684	1956	1966	TAM	Sinvalocho/Wichita//Hope/Cheyenne/3/2*Wichita/4/Seu Seun27	0.969	0.868
Sturdy 2K	PI 636307	1956	2005	TAM	Selection from Sturdy	0.964	0.876
Scout 66	CItr 13996	1956	1967	UNL	Composite of 85 selections from Scout	0.722	1.299
Lancer	CItr 13547	1957	1963	UNL	Turkey Red/Cheyenne//Hope/2*Cheyenne	0.783	1.159
Shawnee	CItr 14157	1960	1967	KSU	Mediterranean/Hope//Pawnee/3/Oro/IllinoisNo.1//Comanche	0.737	1.168
Sandy	CItr 17857	1961	1981	CSU	Sonora64A/TezanosPintosPrecoz/Yaqui54//(Frontana/Kenya58/Newthatch)/Norin10/Brevor/Gabo55B/Trapper//Centurk	0.791	1.150
Trison	CItr 17278	1965	1973	KSU	Triumph/Bison	0.741	0.803
TAMW-101	CItr 15324	1965	1971	TAM	Norin10/3/Nebraska60//Mediterranean/Hope/4/Bison	0.961	1.105
Kirwin	CItr 17275	1966	1973	KSU	Parker*3/Bison	0.808	1.016
Crest	CItr 13880	1966	1967	MTSU	Westmont*2/PI178383	0.922	1.356
Winoka	CItr 14000	1966	1969	SDSU	Selection from Winalta	0.972	0.952
Eagle	CItr 15068	1967	1970	KSU	Selection from Scout	0.885	1.172
Bronze	CItr 14013	1967	1974	SDSU	Hume/Gage/4/Hume/3/Mida/Kenya117A//2*Hope/2*Turkey Red	0.644	0.999

Buckskin	CItr 17263	1968	1973	UNL	Scout/3/Quivera/Tenmarq//Marquillo/Oro	0.800	1.085
Homestead	CItr 17264	1968	1973	UNL	Scout/4/Kenya/Newthatch//Cheyenne/Tenmarq/Mediterranean/Hope/3/Pawnee/Cheyenne	0.870	1.214
TAM 105	CItr 17826	1969	1979	TAM	Sturdy composite bulk selection	1.090	1.197
Agate	CItr 17463	1969	1979	UNL	Ponca/3*Cheyenne//Kenya58/Newthatch//2*(Cheyenne/Tenmarq/Mediterranean/Hope)/3/Scout	0.761	1.182
Centurk 78	CItr 17724	1969	1978	UNL	Selection from Centurk	0.993	0.989
Dawn	CItr 17801	1970	1982	CSU	II21031/Trapper/4/Warrior//Kenya58/Newthatch/2*(Cheyenne/Tenmarq/Mediterranean)/Hope/3/Parker	0.879	0.852
Larned	CItr 17650	1970	1976	KSU	Ottawa/5*Scout	0.923	1.143
Sage	CItr 17277	1970	1973	KSU	Agent/4*Scout	0.976	1.250
Gent	CItr 17293	1971	1974	SDSU	Agent/4*Scout	0.895	1.146
Mit	CItr 17896	1971	1980	TAM	Sinvalocho/Wichita//Hope/Cheyenne/3/Wichita/4/Seu Seun27/6/T.dicoccoides/Aeg. speltoides, amphiploid//2*Austin/3/Supremo/4/Bison/5/Caddo/7/Frontana/Westar	1.027	0.737
Lindon	CItr 17440	1972	1975	CSU	Andes64A/Sonora64//Tacuari/4/Warrior2/Kenya58/Newthatch//Cheyenne/Tenmark/Mediterranean/Hope/3/Parker/5/Lancer/3/Norin16/CI12500//Kaw	0.904	0.928

Vona	CItr 17441	1972	1976	CSU	II21183/CO652363//Lancer/KS 62136; II21183=Andes 64A/Sonora 64//Tacuari; CO652363=Warrior//Kenya58/ Newthatch/2*(Cheyenne/Tenma rq/Mediterranean)/Hope/3/Par ker	1.150	0.888
Rose	CItr 17795	1972	1979	SDSU	Seu Seun/Denton8//Westmont/4/Hu me/3/NE63265	0.864	1.290
Cheney	CItr 17765	1973	1978	KSU	Scout/Tascosa	0.819	1.188
Newton	CItr 17715	1973	1978	KSU	Pitic62/Chris sib//2*Sonora64/3/KleinRendid or/4/Scout	1.057	1.226
Nell	CItr 17803	1973	1981	SDSU	Scout selection/Capitan	0.834	1.273
Rita	CItr 17799	1973	1980	SDSU	SeuSeun/Denton8//Westmont/3 /Ponca//3*Cheyenne/Kenya58/ Newthatch//2*(Cheyenne/Tenm arq//Mediterranean/Hope)	0.842	1.233
Bennett	CItr 17723	1973	1978	UNL	Scout/3/Quivira/Tenmarq//Mar quillo/Oro/4/Homestead	0.970	1.293
Duke	CItr 17856	1974	1981	CSU	Sonora 64*3/Warrior//Selkirk/2*Cheye nne/5/Scout/4/Quivera/3/Tenm ark//Marquis1/Oro	0.801	0.820
Parker 76	CItr 17685	1974	1976	KSU	Parker*5/Agent	0.841	0.930
Rosebud	PI 473570	1974	1981	MTSU	Rego/Cheyenne, Sel. 39-18- 7)//Winalta	0.815	1.269
Chisholm	PI 486219	1975	1983	OSU	Sturdy sib/Nicoma	1.010	0.877
Hail	PI 470927	1977	1982	CSU	Mexican/USA//Scout/3/Mara/4/ Scout/5/Ciano/6/Trapper/7/Par ker	0.995	1.336

Centura	PI 476974	1977	1983	UNL	Warrior*5/Agent/NE68457/3/Centurk78	0.895	1.077
Colt	PI 476975	1978	1983	UNL	Agate sib (NE69441)//391-56-D8/Kaw	1.112	1.208
Judith	PI 584526	1980	1989	MTSU	Lancota/Froid//NE69559/Winoka	0.850	1.219
TAM 107	PI 495594	1980	1984	TAM	TAM105*4/Amigo	1.099	0.902
Carson	PI 501534	1981	1986	CSU	Anza/Scout//Centurk	1.142	1.046
Century	PI 502912	1981	1986	OSU	Payne//TAMW-101/Amigo	1.011	0.812
TAM 200	PI 578255	1981	1986	TAM	Sturdy-sib/Tascosa//Centurk*3/3/Amigo	0.991	0.789
Lamar	PI 559719	1982	1988	CSU	74F878 (Mexican dwarf)/Wings//Vona	0.877	1.204
Dodge	PI 506344	1982	1986	KSU	KS73H530 (Newton sib)/KS76HN1978-1 (Arkan sib)	0.905	1.068
Norkan	PI 506345	1982	1986	KSU	Plainsman V/3/2*(KS76H3705)Larned/Eagle//Sage	0.985	1.291
Arapahoe	PI 518591	1982	1988	UNL	Brule/3/Parker*4/Agent//Belocerkovskaja 198/Lancer	0.840	1.196
Karl 92	PI 564245	1983	1992	KSU	Atlass50//Park85/Agent	0.954	0.881
Rawhide	PI 543893	1983	1990	UNL	Warrior*5/Agent//Kavkaz/4/Parker*4/Agent//Belocerkovskaja198/Lancer/3/Vona	1.125	0.856
Jagger	PI 593688	1984	1994	KSU	KS82W418/Stephens	1.146	0.900
Yuma	PI 559720	1985	1992	CSU	NS14/NS25//2*Vona	1.205	0.824
Heyne	PI 612577	1985	2001	KSU	KS82W422/SWM754308/KS831182/KS82W422	0.983	0.839
MT85200	.	1985	.	MTSU	Froid/Winoka/3/TX55-391-56-D8/Westmont//Trader	1.013	0.862
Redland	PI 502907	1985	1986	UNL	Selection from Brule	0.957	1.201
Jules	PI 564851	1986	1993	CSU	Warrior*5/Agent//Agate sib(NE76667)/3/Hawk	0.941	1.111

TAM 202	PI 561933	1986	1992	TAM	Siouxland outcross	1.372	0.902
TX86A5606	PI 587028	1986	.	TAM	TAM105*4/Amigo*4//Largo	1.057	0.774
TX86A6880	.	1986	.	TAM	TAM105*4/Amigo*4//Largo	1.034	0.871
TX86A8072	PI 587029	1986	.	TAM	TAM105*4/Amigo*4//Largo	1.044	0.888
Siouxland	PI 483469	1986	1984	UNL	Warrior*5/Agent//Kavkaz/3/Warrior*5/Agent	0.957	0.888
Cossack	PI 606780	1987	1998	GSR	BCD1828/83	0.928	0.859
2180	PI 532912	1987	1989	OSU	TAMW-101/Pioneer W603//Pioneer W558	1.138	0.782
TAM 109	PI 554606	1987	1991	TAM	TAMW-101*5/CI9321	1.006	1.110
Vista	PI 562653	1987	1992	UNL	Warrior//Atlas66/Comanche/3/Comanche/Ottawa/5/Ponca/2*Cheyenne/3/Illinois No. 1//2*Chinese Spring/T. timopheevii/4/Cheyenne/Tenmarq//Mediterranean/Hope/3/Sando60/6/Centurk/Brule	1.083	1.307
Longhorn	PI 552813	1988	1991	AB	NS2630-1/Thunderbird	1.000	1.003
Akron	PI 584504	1988	1994	CSU	TAM107/Hail	0.869	1.233
Custer	.	1988	1994	OSU	F-29-76/TAM105//Chisholm	1.107	0.931
TAM 110	PI 595757	1988	1996	TAM	TAM107*5/Largo	1.145	0.794
Alliance	PI 573096	1988	1993	UNL	Arkan/Colt//Chisholm sib.	0.993	1.259
Nekota	PI 584997	1988	1994	UNL	Bennett/TAM107	0.907	1.158
Pronghorn	PI 593047	1988	1996	UNL	Centura/Dawn//Colt sib	0.912	0.941
Ogallala	PI 573037	1989	1993	AB	TX81V6187/Abilene	1.169	1.033
Platte	PI 596297	1989	1997	AB	Tesia79/Chat'S'//Abilene	1.155	0.969
Crimson	PI 601818	1989	1997	SDSU	TAM105/Winoka	0.872	1.299
Tandem	PI 601817	1989	1997	SDSU	Brule/Agate	0.846	1.107
Niobrara	PI 584996	1989	1994	UNL	TAM105*5/Amigo/Brule	0.961	1.179
Windstar	PI 597379	1990	1996	UNL	TAM103/Newton-sib (TX79A2729)//Caldwell/Brule field sel 6/3/Siouxland	1.198	0.892

Halt	PI 584505	1991	1994	CSU	Sumner/CO820026//PI372129/3/TAM107	1.266	0.903
Lockett	PI 604245	1991	2001	TAM	TX86V1540/TX78V2430-4	0.910	1.178
TAM 302	PI 605910	1991	1998	TAM	Probrand812/Caldwell//TX86D1310 (TAM300 sib)	1.072	0.874
Onaga	.	1991	1998	WB	HT43-231-19 (Pioneer bulk)	1.138	0.932
Arlin	PI 564246	1992	1992	KSU	Selection from population of intercrossed hard red winter wheat and hard red spring wheat genotypes	1.157	0.919
Jerry	PI 632433	1992	2001	NDSU	Roughrider//Winoka/NB66425/3/Arapahoe	0.961	1.300
Harding	PI 608049	1992	1999	SDSU	Brule//Bennett/Chisholm/3/Arapahoe	0.976	1.124
Duster	PI 644016	1993	2006	OSU	W0405D/NE78488//W7469C/TX81V6187	1.064	0.765
TAM 400	PI 614876	1993	2001	TAM	TAM200//(TX82D5668) Era/TAMW-101	1.041	0.890
Cougar	PI 613098	1993	2000	UNL	Warrior*5/Agent//Kavkaz/4/NE63218/Kenya58/3/Newthatch/2*(Cheyenne/Tenmarq/Mediterranean/Hope)//Ponca/2*Cheyenne/5/Thunderbird	1.046	1.144
Culver	PI 606726	1993	1999	UNL	NE82419/Arapahoe	0.987	1.248
Nufrontier	PI 619089	1994	2002	AB	2180/HBZ356A//Mesa	1.005	1.110
Avalanche	PI 620766	1994	2001	CSU	RL6005/RL6008//Larned/3/Cheney/Larned/4/Bennett sib/5/TAM107/6/Rio Blanco	1.040	0.881
CO940610	GSTR 10702	1994	.	CSU	KS87H22/MWO9	1.041	1.160
Prairie Red	PI 605390	1994	2000	CSU	CO850034/PI372129//5*TAM107	0.991	0.914
Prowers	PI 605389	1994	1997	CSU	CO850060/PI372129//5*Lamar	0.855	1.142

TAM107-R7	GSTR11601	1994	.	CSU	CO850034/PI372129//5*TAM107	0.975	0.833
Yumar	PI 605388	1994	2000	CSU	Yuma/PI372129,F1//CO850034/3/4*Yuma	1.161	0.809
Overley	PI 634974	1994	2004	KSU	TAM107*3/TA2460//Heyne 'S'/3/Jagger	1.015	0.816
Big Sky	PI 619166	1994	2001	MTSU	NuWest/Tiber	0.844	1.153
Nusky	PI 619167	1994	2001	MTSU	NuWest/Tiber	0.790	1.409
Endurance	PI 639233	1994	2004	OSU	HBY756A/Siouxland//2180	1.128	0.796
Millennium	PI 613099	1994	2000	UNL	Arapahoe/Abilene//NE86488	0.931	1.284
Nuplains	PI 605741	1994	1998	UNL	Abilene/KS831872 = Abilene/3/Plainsman V//Newton/Arthur 71	1.270	1.267
Wahoo	PI 619098	1994	2000	UNL	Arapahoe*2/Abilene	0.710	1.256
Spartan	.	1994	2007	WB	RL8400193/PL2180	1.105	0.917
Dumas	PI 619199	1995	2001	AB	WI90-425/WI89-483	1.157	0.978
Hondo	PI 603958	1995	1999	AB	WI88-028/WI89-339	1.118	1.197
Nuhorizon	PI 619198	1995	2001	AB	WI89-282/Arlin	1.265	1.099
Postrock	PI 643093	1995	2006	AB	Ogallala/KSU94U261//Jagger	1.115	1.032
Thunderbolt	PI 608000	1995	2000	AB	Abilene/KS90WGRC10	1.057	1.036
Stanton	PI 617033	1995	2002	KSU	PI220350/KS87H57//TAM200/KS87H66/3/KS87H325	1.032	0.922
Trego	PI 612576	1995	1999	KSU	RL6005/RL6008//Larned/3/Cheney/Larned/4/Bennett-sib/5/TAM107(KS87H325)/6/RioBlanco	1.151	0.854
MT9513	.	1995	.	MTSU	NuWest/MT8030	0.790	1.241
OK101	PI 631493	1995	2001	OSU	OK87W663/Mesa//2180	1.231	0.977
TAM111	PI 631352	1995	#N/A	TAM	TAM107/4/Sturdy sib/Kaw//Centurk/3/Centurk78/5/Sturdy sib/Kaw//Centurk/3/Jupetaco/Bluejay	1.025	0.862

Wesley	PI 605742	1995	1998	UNL	KS831936-3//Colt/Cody	1.011	1.094
Burchett	PI 633863	1996	2004	AB	W91-126/WI88-052-05	0.975	0.906
G1878	PI 591622	1996	1995	GSR	Hawk//Sturdy/Plainsman V	0.951	0.903
Lakin	PI 617032	1996	2002	KSU	KS89H130/Arlin	1.103	0.853
Neosho	PI 639739	1996	2006	MTSU	W91-376-20/W95-084	1.119	0.918
TX96D1073	.	1996	.	TAM	TX86D1310/Kavkaz//TX86D1308	0.975	0.952
Cutter	PI 631389	1997	2002	AB	Jagger/WI89-189-14	1.035	0.977
2145	PI 631087	1997	2002	KSU	HBA142A/HBZ621A//Abilene	1.154	0.796
2174-05	PI 602595	1997	1998	OSU	IL71-5662/PL145 (Newton sib)//2165	1.073	0.931
OK102	PI 632635	1997	2002	OSU	2174/Cimarron	1.038	0.944
Alice	PI 644223	1997	2006	SDSU	Abilene/Karl	1.036	0.842
Expedition	PI 629060	1997	2002	SDSU	Tomahawk/Bennett	1.062	0.945
Wendy	PI 638521	1997	2004	SDSU	SD89333/Abilene	1.118	0.900
Antelope	PI 633910	1997	2005	UNL	Pronghorn/Arlin	0.820	1.141
Goodstreak	PI 632434	1997	2002	UNL	Len//Butte/ND526/6/Agent/3/ND441//Waldron/Bluebird/4/Butte/5/Len/7/KS88H164/8/NE89646	0.897	1.018
Harry	PI 632435	1997	2002	UNL	Brule/4/Parker*4/Agent//Beloterkovskaia198/Lancer/3/Newton/Brule/5/Newton//Warrior*5/Agent/3/Agate sib	0.964	1.261
Jagalene	PI 631376	1998	2002	AB	Jagger/Abilene	1.086	0.935
Above	PI 631449	1998	2001	CSU	TAM110*4/FS2	1.129	0.874
Hatcher	PI 638512	1998	2004	CSU	Yuma/PI372129//TAM200/3/4*Yuma/4/KS91H184/Vista	1.085	0.799
Enhancer	PI 606779	1998	1998	GSR	HT43H-331-9 (Nebraska winterhardy selection)	1.220	0.880
Bakers White	PI 633865	1998	2004	MTSU	Ponderosa/Jagger	1.017	1.107
Deliver	PI 639232	1998	2004	OSU	Yantar/Chisholm*2//Karl	1.008	0.788

Intrada	PI 631402	1998	2000	OSU	RioBlanco/TAM200	1.035	0.986
Darrell	PI 644224	1998	2006	SDSU	2076-W12-11/Karl92	1.146	1.277
TAM 112	PI 643143	1998	2007	TAM	TAM200/TA2460// TAM107*5/Largo	1.117	0.964
TAM 303	.	1998	2006	TAM	TX89D1253*2/TTCC404 (=WX93D208-9-1-2)	1.009	0.850
Anton	PI 651044	1998	2007	UNL	Brevor/CI15923//Nugaines/4/P I 559717/3/Platte	1.177	1.194
Hallam	PI 638790	1998	2006	UNL	Brule/Bennett//Niobrara	1.042	1.238
Keota	PI 648007	1998	2007	WB	Custer/Jagger	1.006	0.847
WB411W	.	1998	.	WB	G3006/Arlin	0.949	0.934
MT9904	.	1999	.	MTSU	MT85200/Tiber	0.975	1.230
MT9982	.	1999	.	MTSU	Promontory/Judith	1.116	1.360
TX99A0153-1	.	1999	.	TAM	Ogallala/TAM202	1.078	0.933
TX99U8618	.	1999	.	TAM	TX84V1237/TX71C8130R	1.102	0.859
NE99495	.	1999	.	UNL	Alliance/Karl 92	1.048	1.163
Shocker	PI 646185	1999	2006	WB	Freedom/Tomahawk/Jagger	1.123	0.899
Smoky Hill	PI 646184	1999	2006	WB	97 8/64 MASA (Population developed by combining several crosses with a common female "G2500")	0.904	1.231
Tarkio	.	1999	2006	WB	OK90604/KS6397//Snowwhite	0.998	0.905
HG-9	PI 614118	<2000	2000	HGS	TAM200 outcross selection	0.828	0.830
Bond CL	PI 639924	2000	2004	CSU	Yumar//TXGH12588-120*4/FS2	1.218	0.964
Ripper	PI 644222	2000	2006	CSU	PI220127/P5//TAM200/CO9406 06/3/TAM107R-2/PI372129//5*TAM107	1.064	1.175
Fuller	PI 653521	2000	2007	KSU	Selected from a population with an unknown pedigree.	1.030	0.900
KS00F5-20-3	.	2000	.	KSU	Unknown	1.021	0.907

E2041	.	2000	.	MSU	Pioneer Brand 2552/Pioneer Brand 2737W; sib line to Red Ruby	1.090	0.885
Genou	PI 640424	2000	2004	MTSU	Lew/Tiber//Redwin/3/Vanguard/Norstar	0.786	1.188
Yellowstone	PI 643428	2000	2005	MTSU	Selected from a composite of F2 seed from two closely related populations: Promontory/Judith and Judith-phenotypic dwarf selection/Promontory	1.033	1.276
Guymon	PI 643133	2000	2005	OSU	Intrada/Platte	0.893	1.065
OK Bullet	PI 642415	2000	2005	OSU	KS96WGRC39/Jagger	0.975	0.927
Lyman	PI 658067	2000	2009	SDSU	KS93U134/Arapahoe	1.054	1.217
TX00V1131	.	2000	.	TAM	TX87V1613/KS91WGRC11	1.093	0.861
Venango	.	2000	2000	WB	HBE1066-105/HBF0551-131	0.968	1.007
Bill Brown	PI 653260	2001	2007	CSU	Yumar/Arlin	1.158	0.855
SD01058	.	2001	.	SDSU	XH1877/NE967430	0.831	0.881
SD01237	.	2001	.	SDSU	Unknown	1.066	0.814
TAM 203	PI 655960	2001	2009	TAM	TX89V4132/704 L I-2221	0.919	0.878
TAM 304	PI 655234	2001	2009	TAM	TX92U3060/TX91D6564	1.091	0.886
TX01A5936	.	2001	.	TAM	Jagger/3/PSN 'S'/Bow 'S'//TAM200	1.144	0.816
TX01M5009-28	.	2001	.	TAM	Mason/Jagger//Pecos	0.974	0.837
TX01V5134RC-3	.	2001	.	TAM	TAM200/Jagger	0.924	0.869
Camelot	PI 653832	2001	2008	UNL	KS91H184/Arlin sib//KS91HW29/3/NE91631/4/VBF0168	1.067	1.202
Infinity CL	PI 639922	2001	2006	UNL	Windstar//Millennium sib/Above sib	1.001	1.216

McGill	PI 659689	2001	2010	UNL	Vona//Chisholm/PlainsmanV (OK83201)/3/Redland (NE92458)/4/Ike	0.999	1.048
Overland	PI 647959	2001	2007	UNL	Millennium sib//Seward/Archer	0.943	1.225
Danby	PI 648010	2002	2007	KSU	Trego/KS84063-9-39-3-8W	1.074	0.841
OK02405	.	2002	.	OSU	Tonkawa/GK50	0.862	0.813
TAM 113	PI 666125	2002	2013	TAM	Unknown	1.123	0.962
Mace	PI 651043	2002	2007	UNL	Yuma//T-57/3/CO850034/4/4*Yuma/5/(KS91H184/Arlin S/KS91HW29//NE89526)	1.077	0.886
NE02558	.	2002	.	UNL	Jagger/Alliance	0.903	1.306
CO03064	.	2003	.	CSU	CO970547/Prowers99	1.091	1.001
CO03W043	.	2003	.	CSU	KS96HW94/CO980352	0.816	1.268
CO03W054	PI 658597	2003	.	CSU	KS96HW94//Trego/CO960293	0.828	1.287
Thunder CL	PI 655528	2003	2008	CSU	KS01-5539/CO99W165	1.033	1.131
RonL	PI 648020	2003	2007	KSU	Trego/CO960293	0.956	0.845
Norris	PI 643430	2003	2005	MTSU	BigSky//TXGH12588-26*4/FS2	1.033	0.756
Billings	PI 656843	2003	2009	OSU	N566/OK94P597	1.147	0.792
Centerfield	PI 644017	2003	2006	OSU	TXGH12588-105*4/FS4//2*2174	1.087	0.931
Pete	PI 656844	2003	2009	OSU	N40/OK94P455	0.881	0.896
TAM 401	PI 658500	2003	2010	TAM	Mason/Jagger	0.933	0.709
TX03A0148	.	2003	.	TAM	TX89A7137/Tipacna	0.959	0.691
TX03A0563	.	2003	.	TAM	X96V107/Ogallala	1.062	1.043
NW03666	.	2003	.	UNL	N94S097KS/NE93459	1.011	1.088
Settler CL	PI 653833	2003	2009	UNL	Wesley sib//Millennium sib/Above sib	1.001	0.907
HV9W03-1379R	.	2003	.	WB	B1127/3/B1551W//Rowdy/RWA 671 MONT	1.035	0.765
HV9W03-1551WP	.	2003	.	WB	B1043/PL2180	1.111	0.840

HV9W03-1596R	.	2003	.	WB	B1397-1/WGRC33	1.178	0.867
Santa Fe	PI 641772	2003	2006	WB	Hawk//Sturdy/Plainsman V/3/Jagger	1.152	0.946
W04-417	.	2004	.	AB	Bulk population	1.135	0.915
CO04025	.	2004	.	CSU	CO940610/CO960293//CO99W189	1.000	1.204
CO04393	.	2004	.	CSU	Stanton/CO950043	1.081	1.137
CO04499	.	2004	.	CSU	Above/Stanton	1.178	0.851
CO04W320	.	2004	.	CSU	CO950635/CO99W1126	0.848	1.290
MT0495	.	2004	.	MTSU	MT9640/NB1133	0.887	1.257
OK04111	.	2004	.	OSU	2174*2/Jagger	1.067	0.918
OK04415	.	2004	.	OSU	N563/OK98G508W	1.058	0.975
OK04505	.	2004	.	OSU	OK91724/2*Jagger	0.975	0.885
OK04507	.	2004	.	OSU	OK95593/Jagger//2174	1.097	0.879
OK04525	.	2004	.	OSU	FFR525W/Hickok//Coronado	0.990	0.798
TX04A001246	.	2004	.	TAM	TX95V4339/TX94VT938-6	1.047	0.878
TX04M410164	.	2004	.	TAM	Mit/TX93V5722//W95-301	0.995	0.866
TX04M410211	.	2004	.	TAM	Mason/Jagger//Ogallala	0.944	0.845
TX04V075080	.	2004	.	TAM	Jagger/TX93V5722//TX95D8905	0.958	1.142
NE04490	.	2004	.	UNL	NE95589/NE94632(=Abilene/Norkan//Rawhide)//NE95510(=Abilene/Arapahoe)	1.002	0.936
Robidoux	PI 659690	2004	2010	UNL	OdesskayaP/Cody//Pavon76/3*Scout66/3/Wahoo-sib	0.966	0.903
CO050337-2	.	2005	.	CSU	CO980829/TAM111	1.146	0.745
Denali	PI 664256	2006	2011	CSU	CO980829/TAM111	1.061	1.017

Decade	PI 660291	2005	2010	MTSU	Composite of three single crosses made in 1996: N95L159/CDC Clair, N95L159/MT9602 (NuWest/Tiber), and N95L159/MT9609 (Froid/SD1287//Redwin/3/Nu West). N95L159 is a sib of Wesley.	1.001	1.252
MTS0531	.	2005	.	MTSU	L'Govskaya167/Rampart//MT9409	0.915	1.223
Garrison	PI 661992	2005	2011	OSU	OK95616-1/Hickok//Betty	1.045	0.934
OK05108	.	2005	.	OSU	Lut13686/2174//Jagger	0.935	0.936
OK05122	.	2005	.	OSU	KS94U337/NE93427	1.075	0.908
OK05134	.	2005	.	OSU	OK97411/TX91D6825	0.942	0.956
OK05204	.	2005	.	OSU	SWM866442/OK95548	1.039	0.814
OK05303	.	2005	.	OSU	OK95548/TXHBG0358	1.168	0.861
OK05312	.	2005	.	OSU	TX93V5919/WGRC40//OK94P549/WGRC34	1.044	0.903
OK05511	.	2005	.	OSU	TAM110/2174	1.109	0.806
Ruby Lee	PI 661991	2005	2011	OSU	KS94U275/OK94P549	1.060	0.940
OK05711W	PI 661991	2005	.	OSU	G1878/OK98G508W	1.068	0.933
OK05723W	.	2005	.	OSU	SWM866442/Betty	1.101	0.806
OK05830	.	2005	.	OSU	OK93617/Jagger	0.923	0.832
Ideal	.	2005	.	SDSU	Wesley/NE93613	1.192	1.251
SD05210	.	2005	.	SDSU	SD98444/SD97060	1.066	1.238
SD05W018	.	2005	.	SDSU	SD98W302/SD98W175	0.948	1.304
TX05A001188	.	2005	.	TAM	TAM107//TX98V3620/CENTURK78/3/TX87V1233/4/N87V106//TX86V1540/TAM200	1.073	0.818
TX05A001822	.	2005	.	TAM	2145/X940786-6-7	1.180	0.912

TX05V7259	.	2005	.	TAM	TAM107//TX78V3620/CENTURK78/3/TX87V1233/4/ARAPAHOE//TX86V1540/TAM200	0.977	0.879
TX05V7269	.	2005	.	TAM	HBG0358/4/TAM107//TX78V3620/Centurk78/3/TX87V1233	1.108	0.807
NE05430	.	2005	.	UNL	IN92823A1-1-4-5/NE92458	1.058	0.872
NE05496	.	2005	.	UNL	Trego/Hallam	0.978	1.158
Panhandle	.	2005	2014	UNL	NE97426	0.919	1.231
HV9W05-1280R	.	2005	.	WB	Spartanka/G980761	1.095	0.784
Byrd	PI 664257	2006	2011	CSU	TAM112/CO970547-7	1.104	0.936
MT06103	.	2006	.	MTSU	MT9409/W94-137	1.075	1.312
OK Rising	PI 656382	2006	2009	OSU	KS96WGRC39/Jagger	1.016	0.905
OK06114	.	2006	.	OSU	KS97P0630-4-5/CM95560//X920879-C15-1/3/X84WO63-9-18/U1324-25-1-4	1.169	0.811
OK06210	.	2006	.	OSU	KS90175-1-2/CMSW89Y271//Karl92/3/ABI86*3414/X86035*-BB-34//HBC302E	0.964	0.796
OK06318	.	2006	.	OSU	HBG0358/2174//2145	0.909	0.815
OK06319	.	2006	.	OSU	Enhancer/2174	0.941	0.891
OK06336	.	2006	.	OSU	Magvars/2174//Enhancer	1.051	0.818
TAM 204	.	2006	2014	TAM	TX99U8617/TX97U2001	0.873	0.848
TAM 305	.	2006	2014	TAM	Unknown	1.060	0.912
TX06A001132	.	2006	.	TAM	HBG0358/4/TAM107//TX78V3620/Centurk78/3/TX87V1233	1.124	0.785
TX06A001281	.	2006	.	TAM	TX98VR8422/U3704A-7-7	0.959	0.798
TX06A001386	.	2006	.	TAM	TX99A6030/Custer	1.068	0.918
Freeman	PI 667038	2006	2013	UNL	BI86*3414/Jagger/Karl92//Alliance	1.047	1.095

NE06607	.	2006	.	UNL	NE98466=(KS89H50-4/NE90518(=BRL//SXL/BENN))/WESLEY	0.989	1.165
NI06736	.	2006	.	UNL	NW97S312=(KM602-90/NE89657//Arlin) X KS96HW10-3=(KS91HW29// Rio Blanco/KS91H184)	1.084	0.897
NI06737	.	2006	.	UNL	NW97S312=(KM602-90/NE89657//Arlin) X KS96HW10-3=(KS91HW29// Rio Blanco/KS91H184)	1.063	0.950
HV906-865	.	2006	.	WB	G980039/Onaga	1.116	0.890
HV9W06-504	.	2006	.	WB	G982231/G982159//KS920709W	1.186	0.697
Antero	PI 667743	2007	2013	CSU	KS01HW152-1/TAM111	1.039	0.975
Judee	PI 665227	2007	2011	MTSU	Vanguard/Norstar//Judith/3/Nu Horizon	0.918	1.382
Gallagher	.	2007	2013	OSU	OK-99711/Duster	1.074	0.908
OK07231	.	2007	.	OSU	OK92P577-(RMH 3099)/OK93P656-(RMH 3299)	1.040	0.786
OK07S117	.	2007	.	OSU	Altar84/AE.SQ//Opata/3/OK98G508W	1.034	1.219
TX07A001279	.	2007	.	TAM	X930332-4-1/TX97V2838	1.139	0.804
TX07A001318	.	2007	.	TAM	TX98VR8431/TX95A3091	0.911	1.017
TX07A001420	.	2007	.	TAM	U1254-1-5-2-1/TX81V6582//Desconocido	1.027	0.913
NI07703	.	2007	.	UNL	R-148 (G97343) =(919021/B725//K92)/NI00436 =(WI89-273-13/NE93427 (=Bez 1/CTK78//Arthur/CTK78/3/Bennet/4/Norkan)	1.100	0.880
OK08328	.	2008	.	OSU	GKKeve/Ok101//OK93P656-RMH3299	1.037	0.932

NI08707	.	2008	.	UNL	CO980829=(Yuma/T-57//CO850034/3/4*Yuma/4/NEWS12)/Wesley	1.025	0.862
NI08708	.	2008	.	UNL	CO980829=(Yuma/T-57//CO850034/3/4*Yuma/4/NEWS12)/Wesley	1.204	0.862
OK09634	.	2009	.	OSU	OK95616-98-6756/Overley	1.026	0.978
OK10119	.	2010	.	OSU	JEI110/Overley	0.884	1.104
OK1067071	.	2010	.	OSU	TX98V9437/OK00316//Farmec	0.964	0.679
OK1067274	.	2010	.	OSU	GA961912-8-4-5/OK02129//Kristi-K.K	0.998	0.876
OK1068002	.	2010	.	OSU	Efect/Jagalene//Deliver	0.925	0.948
OK1068009	.	2010	.	OSU	Lada/Jagalene//G980122	1.004	0.768
OK1068026	.	2010	.	OSU	ERYTHROSPERMUM270/TAM111//OK99212	1.027	0.782
OK1068112	.	2010	.	OSU	Farmec/Jagalene	1.080	0.808
OK1070267	.	2010	.	OSU	VI.9/Guymon//G980411W	0.825	0.838
OK1070275	.	2010	.	OSU	KNJAZHNA/KS00HW175-4//OK00611W	0.762	0.932

Supplemental Table S3.2. Pearson's correlation coefficients of days to heading (DTH) or grain yield (GY) between replications within the same environment. Three environments had two replications each and thus a single pairwise correlation. Two environments had four replications each, and the correlation is the average of all six pairwise comparisons. The overall mean reflects the average correlation across all environments when equally weighted. Environmental abbreviations are provided in Table 1.

	n	n pairwise	Average *r*	
Env	**reps**	**comparisons**	**DTH**	**GY**
Ar13R	2	1	0.96	0.70
Ma12F	2	1	0.72	0.35
Ma12R	2	1	0.74	0.20
Me12R	4	6	0.88	0.44
Me13R	4	6	0.76	0.36
Env. Average			0.82	0.41

Supplemental Table S3.3. Total monthly precipitation (P, mm) and irrigation (I, mm) received by each environment. Total precipitation and irrigation is summarized separately for 1 Jan. to average heading date (H), and total accumulation between 1 Jan and harvest. Environments are described in Table 1. No irrigation was applied to any environment in January or February. The last month only includes precipitation that occurred before harvest, and all 2012 environments were harvested in June.

Env	Jan	Feb	Mar		Apr		May		June		July		To H	Total		
	P	P	P	I	P	I	P	I	P	I	P	I	P+I	P	I	P+I
	mm															
Bu12R	2.3	2.6	34	--	16.2	--	29	--	33.1	--	.	.	54.1	117.2	--	117.2
Gr12P	0.3	10.7	0.5	25.4	23.1	12.7	28.2	50.8	19.3	12.7	.	.	106.7	82.1	101.6	183.7
Gr12F	0.3	10.7	0.5	25.4	23.1	50.8	28.2	144.8	19.3	114.3	.	.	199.4	82.1	335.3	417.4
It12R	0	26.9	19.3	--	71.1	--	87.4	--	100.8	--	.	.	118.4	305.5	--	305.5
Ma12R	0.8	47.5	55.1	--	63.8	--	34	25.4	97.8	--	.	.	167.1	299.0	25.4	324.4
Ma12F	0.8	47.5	55.1	--	63.8	--	34	114.3	97.8	--	.	.	167.1	299.0	114.3	413.3
Ar13R	41.1	62.2	36.6	--	49.8	--	212.6	--	141	--	93.7	--	188.2	637.0	--	637.0
Fo13R	0.3	2.0	5.3	--	30.2	--	35.8	--	11.9	--	38.9	--	73.7	124.4	--	124.4
Fo13F	0.3	2	5.3	--	30.2	--	35.8	63.5	11.9	146.1	38.9	12.7	137.2	124.4	222.3	346.7
Ha13R	19.3	30.2	19.8	--	26.9	--	54.9	--	69.3	--	179.8	--	133.1	400.2	--	400.2
It13R	9.4	1.8	23.4	--	87.6	--	152.7	--	116.3	--	20.3	--	194.3	411.5	--	411.5

Supplemental Table S3.4. Pearson's correlation coefficient (*r*) between pairs of environments of growing degree-days from 1 Jan. to heading (above the diagonal) and grain yield (below the diagonal) of 299 hard winter wheat genotypes grown at 11 environments. Environments are described in Table 1.

	Ar13R	Bu12R	Fo13R	Fo13F	Gr12P	Gr12F	Ha13R	Ma12R	Ma12F	It12R	It13R
Ar13R		0.75***	0.48***	0.44***	0.70***	0.68***	0.59***	0.70***	0.66***	0.77***	0.72***
Bu12R	0.14*		0.63***	0.60***	0.69***	0.86***	0.78***	0.83***	0.81***	0.90***	0.75***
Fo13R	0.16**	0.36***		0.63***	0.50***	0.62***	0.64***	0.47***	0.44***	0.58***	0.64***
Fo13F	0.11 ns†	0.16**	0.27***		0.41***	0.63***	0.60***	0.48***	0.40***	0.53***	0.57***
Gr12P	0.09 ns	0.41***	0.17**	0.11 ns		0.62***	0.52***	0.63***	0.64***	0.73***	0.79***
Gr12F	0.10 ns	0.67***	0.34***	0.16**	0.48***		0.78***	0.78***	0.75***	0.84***	0.79***
Ha13R	0.20***	0.40***	0.31***	0.09 ns	0.39***	0.55***		0.67***	0.64***	0.75***	0.67***
Ma12R	0.09 ns	0.47***	0.25***	0.11 ns	0.26***	0.46***	0.27***		0.86***	0.90***	0.64***
Ma12F	0.06 ns	0.40***	0.17**	0.00 ns	0.18**	0.39***	0.25***	0.48***		0.89***	0.64***
It12R	0.33***	0.24***	0.15**	0.28***	0.12*	0.35***	0.28***	0.26***	0.19***		0.79***
It13R	0.06 ns	0.56***	0.31***	0.20 ***	0.08 ns	0.67***	0.44***	0.43***	0.33***	0.41**	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† ns, nonsignificant at the 0.05 probability level.