

Allelic variations in phenology genes of eastern U.S. soft winter and Korean winter wheat varieties and their associations with heading date

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Abstract

Wheat heading time is genetically controlled by phenology genes including vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*) genes. Characterization of the existing genetic variation in the phenology genes of wheat would provide breeding programs with valuable information necessary for the development of wheat varieties well-adapted to the local environment and early-maturing traits suitable for double-cropping system. One hundred forty-nine eastern U.S. soft winter (ESW) and 32 Korean winter (KW) wheat varieties were characterized using molecular markers for *Vrn*, *Ppd*, *Eps* and reduced-height (*Rht*) genes, and phenotyped for heading date (HD) in eastern U.S. region. The predominant alleles in ESW and KW varieties were the *Ppd-A1b*, *Ppd-B1b* and *Ppd-D1a* alleles at the *Ppd-1* loci and the *vrn-B1-Neuse* and *vrn-D3b* alleles at the *Vrn-B1* and *Vrn-D3* loci. A single copy of the *vrn-A1* allele was observed in 81.3% of KW varieties, but only in one ESW variety. The genetic variations for HD of ESW varieties were largely contributed by the *Ppd-B1*, *Ppd-D1* and *Vrn-D3* loci. The *Rht-D1* locus largely contributed to the genetic variation for HD of KW varieties. A phenology gene profile at four major loci (*Ppd-B1b/Ppd-D1a/vrn-A1*(three or more copies)/*vrn-D3b*) was predominantly observed in 52.8% of ESW varieties, while 76.7% of KW varieties carried one of these two phenology gene profiles: *Ppd-B1b/Ppd-D1a/vrn-A1*(one copy)/*vrn-D3a* or *Ppd-B1b/Ppd-D1a/vrn-A1*(one copy)/*vrn-D3b*. KW varieties headed on average 14 days earlier than ESW varieties in each crop year, largely due to the presence of a single copy of the *vrn-A1* allele in the former.

Introduction

Double cropping increases total crop production without the expansion of cultivation acreage. Growing soybean immediately after the harvest of winter wheat is the most common double-cropping system practiced in the United States. However, the double-crop soybean yields tend to be lower by 10–40% compared to their full-season counterparts (Kyei-Boahen and Zhang 2006). Late planting is the major reason for the yield reduction of double-crop soybean and each day of delay in planting after mid-June reduces soybean yield by 0.65% and 1.7% in North Carolina and Maryland, respectively (Parvej et al. 2020), and by 34 kg ha⁻¹, 36 kg ha⁻¹ and 67 kg ha⁻¹ in Virginia, Georgia and Ohio, respectively (Holshouser 2014; Boerma and Ashley 1982; Beuerlein, 2004). The development of early-maturing wheat varieties with high yield potential is, therefore, crucial for avoiding delayed soybean planting and yield loss and for improving the profitability of the double-cropping system. The identification and acquisition of the genetic resources for the early-maturing trait, and selection of the effective molecular markers for marker-assisted selection, are prerequisite for the development of early-maturing wheat varieties. Early maturity has been a major trait considered by Korean wheat breeding programs for the development of winter wheat varieties suitable for implementing the wheat-rice double-cropping system (Cho et al. 2015). Cho et al. (2015) observed that all Korean winter (KW) wheat varieties released after 1991 carried the photoperiod insensitive allele at the photoperiod (*Ppd*)-*D1* locus, which was associated with early maturity (Guo et al. 2010), and they headed on average 4 days earlier than those released before 1991. However, only 12.5–50.0% of U.S. winter wheat varieties that carried the photoperiod insensitive allele at

the *Ppd-D1* locus were observed in several worldwide and regional collections of wheat (Guo et al. 2010; Grogan et al. 2016; Tessmann et al. 2019). KW wheat varieties would provide bountiful genetic resources needed for the development of early-maturing U.S. winter wheat varieties.

Wheat heading time is genetically controlled by the phenology genes including vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*) genes (Grogan et al. 2016). Vernalization requirements of wheat are controlled by three major loci including *Vrn-1*, *Vrn-2*, and *Vrn-3*. The *Vrn-1* and *Vrn-3* genes are dominant in spring wheat, whereas the *Vrn-2* genes are dominant in winter wheat (Yan et al. 2004a). In addition, genotypes with an increased copy number of the winter allele *vrn-A1* at the *Vrn-A1* locus require a longer vernalization, thereby delaying wheat heading (Díaz et al. 2012). Wheat photoperiod response is mainly controlled by the *Ppd-A1*, *Ppd-B1* and *Ppd-D1* loci (Díaz et al. 2012; Grogan et al. 2016). In general, genotypes with the photoperiod insensitive allele tend to head earlier than those with the photoperiod sensitive allele (Beales et al. 2007; Díaz et al. 2012; Nishida et al. 2013). *Eps* genes influence wheat heading and flowering time when both vernalization and photoperiod requirements are satisfied and act in fine tuning the heading and flowering time (Zikhali et al. 2016). However, the effects of *Eps* genes on wheat heading are usually inconsistent across environments (Griffiths et al. 2009). Wheat heading time could be affected also by the reduced-height (*Rht*) loci through the gibberellin pathway (Wilhelm et al. 2013). Compared with the wild type, the semi-dwarf alleles *Rht-B1b* and *Rht-D1b* each have a single nucleotide substitution that causes a premature stop codon and reduces the plant's ability to respond to gibberellic acid. Gibberellin insensitivity has been found to be associated with early heading in wheat (Wilhelm et al. 2013).

The diversity of phenology genes has been observed in several worldwide and regional collections of wheat (Guo et al. 2010; Wilhelm et al. 2013; Grogan et al. 2016). Substantial variation in the presence of phenology genes and their geographic distribution have been observed among continents and countries (Guo et al. 2010; Wilhelm et al. 2013; Grogan et al. 2016). However, information is lacking on the diversity of phenology genes of eastern U.S. soft winter (ESW) wheat and their effects on wheat heading date under eastern U.S. environments. A better understanding of the allelic variation in the phenology genes of ESW wheat and their effects on heading dates would provide a valuable tool for wheat breeders in the development of early-maturing ESW wheat varieties suitable for the double-cropping system. The objectives of this study were to characterize the allelic variation in the phenology genes of ESW and KW wheat varieties and to assess their effects on heading date under eastern U.S. environments.

Materials And Methods

Materials

Two sets of varieties, including 149 eastern U.S. soft winter (ESW) wheat and 32 Korean winter (KW) wheat varieties, were used in this study. ESW wheat varieties, including both currently grown cultivars and recently developed varieties from over 20 ESW wheat breeding programs, were grown in four consecutive crop years (2015, 2016, 2017 and 2018) in Wooster, Ohio (40.8051° N, 81.9351° W). The heading dates

for a total of 121 ESW wheat varieties were determined over four crop years; the remaining 28 ESW wheat varieties over 2–3 crop years. The seeds of KW wheat varieties were obtained from the CIMMYT and were grown in two consecutive crop years (2017 and 2018) in Wooster, Ohio. A complete list of ESW and KW wheat varieties can be found in Supplemental Table S1. Wheat varieties were planted in a randomized complete block design with two replications. Heading date (HD) was recorded as the Julian date when the 50% of the spikes in a plot had fully emerged from the flag leaf sheath.

Genetic evaluation

Genomic DNA was extracted from 10-15-day-old hypocotyls of germinating seeds using DNAzol ES (Extra Strength) reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's protocol. Molecular marker assays for phenology genes were conducted at the USDA-ARS Eastern Regional Small Grain Genotyping Laboratory, Raleigh, NC, USA. Kompetitive Allele Specific PCR (KASP) makers developed from published sequences were used to distinguish alleles at the *Vrn-A1*, *Vrn-B1*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Eps-B1*, *Eps-D1*, *Rht-B1*, and *Rht-D1* loci (Table 1). The recessive allele *vrn-A1* was distinguished from the dominant alleles *Vrn-A1a* and *Vrn-A1b* using molecular markers wMAS000033 and wMAS000035 (Yan et al. 2004b), respectively. Two molecular markers (*vrn-A1*exon4_C/T and *vrn-A1*exon7_G/A) were used to determine copy number variation (CNV) of winter allele *vrn-A1* (Díaz et al. 2012). Three markers (TaVrn-B1_D-I, wMAS000037, and *Vrn-B1_C*) were used to distinguish the dominant alleles *Vrn-B1a*, *Vrn-B1b* (Santra et al. 2009), and *Vrn-B1c* (Milec et al. 2012) at the *Vrn-B1* locus. Molecular marker (TaVrn-B1_1752) was used to detect an A/G polymorphism in intron 1 of the recessive allele *vrn-B1* (Guedira et al. 2014) and to distinguish alleles (*vrn-B1-Neuse* and *vrn-B1-AGS2000*). Genotypes were considered to carry a null allele at the *Vrn-B1* locus if no PCR amplification was detected using the molecular marker TaVrn-B1_1752. The primer pair VRN-D3-F6/VRN-D3-R8 was used to distinguish alleles at the *Vrn-D3* locus according to the method described by Chen et al. (2010).

Table 1

Alleles of vernalization (*Vrn*), photoperiod (*Ppd*), earliness per se (*Eps*) and reduced-height (*Rht*) loci governing the heading date of wheat

Locus	Allele	Marker ID	Allele effect	Reference
<i>Vrn-A1</i>	<i>Vrn-A1a</i>	wMAS000033	spring growth habit	Yan et al. 2004b
	<i>Vrn-A1b</i>	wMAS000035	spring growth habit	Yan et al. 2004b
	<i>vrn-A1</i> , CNV ^a >2	vrn-A1exon4	winter growth habit, late heading	Díaz et al. 2012
	<i>vrn-A1</i> , CNV = 2	vrn-A1exon7	winter growth habit, late heading	Díaz et al. 2012
	<i>vrn-A1</i> , CNV = 1	vrn-A1exon7	winter growth habit, early heading	Díaz et al. 2012
<i>Vrn-B1</i>	<i>Vrn-B1a</i>	Vrn-B1_I_D	spring growth habit	Santra et al. 2009
	<i>Vrn-B1b</i>	wMAS000037	spring growth habit	Santra et al. 2009
	<i>Vrn-B1c</i>	Vrn-B1_C	spring growth habit	Milec et al. 2012
	<i>vrn-B1-Neuse</i>	TaVrn-B1_1752	winter growth habit, late heading, Neuse-type	Guedira et al. 2014
	<i>vrn-B1-AGS2000</i>	TaVrn-B1_1752	winter growth habit, early heading, AGS2000-type	Guedira et al. 2014
<i>Vrn-D3</i>	<i>vrn-D3a</i>	VRN-D3-F6/VRN-D3-R8	winter growth habit with early heading	Chen et al. 2010
	<i>vrn-D3b</i>	VRN-D3-F6/VRN-D3-R8	winter growth habit with late heading	Chen et al. 2010
<i>Ppd-A1</i>	<i>Ppd-A1a.1</i>	Ppd-A1prodel	photoperiod insensitive, early heading	Nishida et al. 2013
	<i>Ppd-A1b</i>	Ppd-A1prodel	photoperiod sensitive, late heading	Nishida et al. 2013
<i>Ppd-B1</i>	<i>Ppd-B1a-Chinese Spring</i>	wMAS000027	photoperiod insensitive, early heading	Díaz et al. 2012
	<i>Ppd-B1a-Sonora 64</i>	TaPpdBJ003	photoperiod insensitive, early heading	Díaz et al. 2012
	<i>Ppd-B1b</i>	wMAS000027	photoperiod sensitive, late heading	Díaz et al. 2012

^aCNV, copy number variation.

Locus	Allele	Marker ID	Allele effect	Reference
<i>Ppd-D1</i>	<i>Ppd-D1a</i>	wMAS000024	photoperiod insensitive, early heading	Beales et al. 2007
	<i>Ppd-D1b</i>	wMAS000024	photoperiod sensitive, late heading	Beales et al. 2007
	<i>Ppd-D1b-Norstar</i>	TaPpdDD002	photoperiod sensitive, late heading	Shaw et al. 2013
<i>Eps-B1</i>	<i>TaELF3-B1a</i>	TaELF3-B1 Kasp	late heading and flowering	Zikhali et al. 2016
	<i>TaELF3-B1b</i>	TaELF3-B1 Kasp	early heading and flowering	Zikhali et al. 2016
<i>Eps-D1</i>	<i>TaELF3-D1a</i>	TaELF3-D1 Kasp2	late heading and flowering	Zikhali et al. 2016
	<i>TaELF3-D1b</i>	TaELF3-D1 Kasp2	early heading and flowering	Zikhali et al. 2016
<i>Rht-B1</i>	<i>Rht-B1a</i>	wMAS000001	tall	Ellis et al. 2002
	<i>Rht-B1b</i>	wMAS000001	semi-dwarf	Ellis et al. 2002
<i>Rht-D1</i>	<i>Rht-D1a</i>	wMAS000002	tall	Ellis et al. 2002
	<i>Rht-D1b</i>	wMAS000002	semi-dwarf	Ellis et al. 2002
^a CNV, copy number variation.				

Photoperiod insensitive allele *Ppd-A1a.1* was distinguished with the marker TaPpd-A1prodel (Nishida et al. 2013), which detects a deletion characteristic of the insensitive allele. Alleles at the *Ppd-B1* locus 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 with an inter copy junction (Díaz et al. 2012). Wheat varieties were considered to carry the null allele at the *Ppd-B1* locus if no amplification occurred with the markers. Photoperiod sensitive and insensitive alleles at the *Ppd-D1* loci were distinguished using two markers: wMAS000024, which detects a deletion upstream of the coding region responsible for the photoperiod insensitive phenotype (Beales et al. 2007), and TaPpdDD002, which identifies the ‘Norstar’-type sensitive allele (Shaw et al. 2013).

Mutations at the *Eps-B1* and *Eps-D1* loci were detected using markers TaELF3-B1 Kasp and TaELF3-D1 Kasp2, respectively (Zikhali et al. 2016). Mutations at the *Rht-B1* and *Rht-D1* loci were genotyped using wMAS000001 and wMAS000002 (Ellis et al. 2002), respectively.

Statistical analysis

Statistical analyses were performed using Statistical Analysis System software (SAS Institute, Cary, NC, USA). A general linear model (GLM) was used to determine the effects of crop years, and the vernalization, photoperiod, earliness *per se* and reduced-height loci, and their interactions, on the HD of ESW and KW wheat varieties in a combined analysis over four crop years for the former and two crop years for the latter, or in each crop year for both. The sum of squares was used to estimate the proportion of total variance contributed by crop year and each locus, and the proportion of genetic variance contributed by each locus (Grogan et al. 2016). A least significant difference (LSD) test was used to determine the differences in the effects of crop year, individual allele at each locus, and phenology gene profiles on the HD of ESW and KW wheat varieties with homozygous alleles at the phenology-related loci. Pearson's linear correlation analysis was conducted to determine the relationships between HDs of ESW wheat varieties grown in different crop years. A two-way analysis of variance (ANOVA) test was conducted to evaluate the effects of crop years and variety and their interaction effects on HD using 121 ESW wheat varieties. Broad sense heritability (H^2) was calculated over four crop years in terms of mean squares (MS): $H^2 = (MS_{\text{Variety}} - MS_{\text{Variety*Year}}) / MS_{\text{Variety}}$ (Brown et al. 2018).

Results And Discussions

Variation in heading date among ESW and KW wheat varieties

Figure 1 shows the mean and range of HDs of 121 ESW wheat varieties in 2015–2018, and 32 KW wheat varieties in 2017 and 2018, grown in Wooster, Ohio. The HDs of each variety in each of four crop years are provided in Supplemental Table S1. Significant differences were observed in the HDs of ESW and KW wheat varieties among the tested crop years. The HDs of ESW wheat varieties ranged from 138 to 148, 138–143, 127–140 and 139–149 in 2015, 2016, 2017 and 2018, respectively. The average HD of ESW wheat varieties was shortest in 2017 followed by 2016, 2015 and 2018. Early spring temperatures in Wooster, Ohio, were warmer in 2017 than in the other years when the growing degree days after January 1 accumulated more slowly (Supplemental Figure S1). This result agrees with Hu et al. (2005), who found that the HD of winter wheat is governed primarily by temperature. HDs of KW wheat varieties ranged from 113 to 119 and 130–136 in 2017 and 2018, respectively. KW wheat varieties headed on average 14 days earlier than did ESW wheat varieties in each crop year, indicating that the genetic background, in addition to crop year, significantly influences winter wheat HD.

The two-way ANOVA showed that variety, crop year and the interaction of the variety and crop year all significantly contributed to the variation in HD ($p < 0.0001$). Crop year exhibited the biggest influence on HD, followed by variety and the interaction between variety and crop year. This result agrees with the report of Würschum et al. (2018), which showed that wheat HD was mainly controlled by both genotype and environment, and much less by the interaction between genotype and environment, from the examination of 1110 winter wheat cultivars of worldwide origin. The HD was proved to be highly heritable as indicated by the significant correlations (r ranged from 0.52 to 0.73, $p < 0.001$) between HDs of ESW

wheat varieties grown in different crop years, and its high estimated heritability of 0.86. Wheat cultivars exhibited similar responses to the different environments with regards to HD.

Allelic frequencies at vernalization loci

Considerable genetic variation in the phenology gene profile was observed among ESW wheat varieties. The phenology genes of each variety are summarized in Supplemental Table S1. Among the 149 ESW wheat varieties examined, 127 varieties possessed homogeneous alleles at all the tested loci, while 22 varieties carried heterogeneous alleles at one of the tested loci. Among the 32 KW wheat varieties examined, 30 varieties carried homogeneous alleles at all the phenology-related loci evaluated. The occurrence frequencies of the alleles at the phenology-related loci in the 149 ESW and 32 KW wheat varieties are summarized in Table 2.

Table 2

Frequency of different alleles at the vernalization (*Vrn*), photoperiod (*Ppd*), earliness per se (*Eps*) and reduced-height (*Rht*) loci in 149 eastern U.S. soft winter wheat and 32 Korean winter wheat varieties

Locus	Allele	Frequency (%)	
		U.S. soft winter wheat	Korean winter wheat
<i>Vrn-A1</i>	<i>vrn-A1</i> , CNV ^a >2	94.0	12.5
	<i>vrn-A1</i> , CNV = 2	1.3	0.0
	<i>vrn-A1</i> , CNV = 1	0.7	81.3
	heterogeneous	4.0	6.3
<i>Vrn-B1</i>	<i>vrn-B1-Neuse</i>	96.0	100.0
	<i>vrn-B1-AGS2000</i>	0.7	0.0
	null	1.3	0.0
	heterogeneous	2.0	0.0
<i>Vrn-D3</i>	<i>vrn-D3a</i>	10.1	46.9
	<i>vrn-D3b</i>	89.9	53.1
<i>Ppd-A1</i>	<i>Ppd-A1a.1</i>	32.9	0.0
	<i>Ppd-A1b</i>	56.4	100.0
	heterogeneous	10.7	0.0
<i>Ppd-B1</i>	<i>Ppd-B1a-Chinese Spring</i>	12.8	6.3
	<i>Ppd-B1a-Sonora 64</i>	6.7	3.1
	<i>Ppd-B1b</i>	73.8	87.5
	null	5.4	3.1
	heterogeneous	1.3	0.0
<i>Ppd-D1</i>	<i>Ppd-D1a</i>	59.7	96.9
	<i>Ppd-D1b</i>	16.1	0.0
	<i>Ppd-D1b-Norstar</i>	12.8	0.0
	heterogeneous	11.4	3.1
<i>Eps-B1</i>	<i>TaELF3-B1a</i>	92.6	100.0

^aCNV, copy number variation.

Locus	Allele	Frequency (%)	
		U.S. soft winter wheat	Korean winter wheat
	<i>TaELF3-B1b</i>	3.4	0.0
	heterogeneous	4.0	0.0
<i>Eps-D1</i>	<i>TaELF3-D1a</i>	73.2	100.0
	<i>TaELF3-D1b</i>	19.5	0.0
	heterogeneous	7.4	0.0
<i>Rht-B1</i>	<i>Rht-B1a</i>	63.8	65.6
	<i>Rht-B1b</i>	33.6	31.3
	heterogeneous	2.7	3.1
<i>Rht-D1</i>	<i>Rht-D1a</i>	38.9	53.1
	<i>Rht-D1b</i>	56.4	46.9
	heterogeneous	4.7	0.0
^a CNV, copy number variation.			

All ESW and KW wheat varieties carried the winter allele at the evaluated *Vrn* loci, which was expected since only winter wheat varieties were included in this study. Little genetic variation was observed in the evaluated *Vrn* loci among ESW wheat (Table 2). The low variation at the *Vrn* loci was also observed by Tessmann et al. (2019) in 256 ESW wheat varieties and Cho et al. (2015) in 410 Korean wheat varieties. The copy number variation (CNV) of the *vrn-A1* winter allele was significantly different between ESW and KW wheat varieties. Most ESW wheat varieties (94.0%) carried three or more copies of the *vrn-A1* winter allele, while most KW wheat varieties (81.3%) carried a single copy of the *vrn-A1* winter allele. Only three ESW wheat varieties (Jamestown, Ernie and VA08MAS-369) possessed two copies or a single copy of the *vrn-A1* allele. Increased copy number at the *Vrn-A1* locus has been reported to be associated with greater vernalization requirements, resulting in late heading (Díaz et al. 2012). The high frequency of a single copy of the *vrn-A1* allele in KW wheat varieties may be related to the early heading of KW wheat varieties.

Little variation was also observed at the *Vrn-B1* locus in ESW and KW wheats. Two winter alleles (*vrn-B1-AGS2000* and *vrn-B1-Neuse*) were identified at the *Vrn-B1* locus in ESW wheat (Table 2). The *vrn-B1-AGS2000* allele was associated with lower vernalization requirements and earlier heading than the *vrn-B1-Neuse* allele (Guedira et al. 2014). Most of the ESW wheat varieties (96%) and all the KW wheat varieties (100%) carried the *vrn-B1-Neuse* allele, indicating that the *Vrn-B1* locus may not contribute to the difference in HD between ESW and KW wheat varieties. Only one ESW wheat variety 'AGS 2060' carried the *vrn-B1-AGS2000* allele. In addition, two ESW wheat varieties were found to have a null allele at the *vrn-B1* locus. A similar result was reported in ESW wheat varieties by Tessmann et al. (2019).

Two winter alleles were identified at the *Vrn-D3* locus in both ESW and KW wheat varieties (Table 2). The *vrn-D3a* allele, known to be associated with early heading (Chen et al. 2010), was carried by 10.1% of ESW wheat varieties. Tessmann et al. (2019) observed that around 30.0% of 256 ESW wheat varieties carried the *vrn-D3a* allele; however, the frequency of the *vrn-D3a* allele varied from 3.0–59.5% in ESW wheat varieties from different states calculated from Supplemental Table S5 reported by Tessmann et al. (2019). The frequency of the *vrn-D3a* allele in ESW wheat varieties in this study was lower than that found by Tessmann et al. (2019), probably due to the differences in ESW wheat varieties used in each study. These results indicate that the *vrn-D3a* allele is common in ESW wheat varieties and its frequency varies across states in the eastern region of the U.S.

The *vrn-D3a* allele was observed in 46.9% of KW wheat varieties. In addition, most ESW wheat varieties with the *vrn-D3a* allele possessed the photoperiod sensitive allele at the *Ppd-D1* locus, while KW wheat varieties with the *vrn-D3a* allele had the *Ppd-D1a* photoperiod insensitive allele, indicating that different allelic combinations exist in ESW and KW wheat varieties.

Allelic frequencies at photoperiod loci

Allelic variations at the *Ppd-A1*, *Ppd-B1* and *Ppd-D1* loci in ESW wheat varieties were greater than those in KW wheat varieties. Most ESW wheat (56.4%) and all KW wheat (100%) carried the *Ppd-A1b* photoperiod sensitive allele. Guedira et al. (2014) found that the photoperiod insensitive allele *Ppd-A1a.1* was common in ESW wheat varieties. Tessmann et al. (2019) also observed that 57.4% of ESW wheat varieties carried the *Ppd-A1a.1* allele at the *Ppd-A1* locus. The photoperiod insensitive allele *Ppd-A1a.1* was, however, observed in 32.9% of 149 ESW wheat varieties. The *Ppd-B1b* photoperiod sensitive allele was carried by 73.8% of ESW wheat varieties and 87.5% of KW wheat varieties, and was most frequent in both ESW and KW wheat varieties. In addition, eight ESW wheat varieties and one KW wheat variety possessed a null allele at the *Ppd-B1* locus. Tessmann et al. (2019) also reported that 7.4% of ESW wheat varieties carried the null allele at the *Ppd-B1* locus. The *Ppd-D1a* photoperiod insensitive allele was the predominant allele at the *Ppd-D1* locus with a frequency of 59.7% among the 149 ESW wheat varieties, followed by the *Ppd-D1b* photoperiod sensitive allele with a frequency of 16.1% (Table 2). The *Ppd-D1b-Norstar* photoperiod sensitive allele was observed in 12.8% of the 149 ESW wheat varieties. For KW wheat varieties, 96.9% carried the *Ppd-D1a* photoperiod insensitive allele; one KW wheat variety carried heterogeneous alleles at the *Ppd-D1* locus. This result agrees with the report of Guo et al. (2010) who found that haplotype I (*Ppd-D1a* photoperiod insensitive allele) was most common in Asian wheat varieties. Cho et al. (2015) also observed that all KW wheat varieties developed since 1991 carried the *Ppd-D1a* allele.

Grogan et al. (2016) found a higher frequency of the *Ppd-A1b* photoperiod sensitive allele (98.0%) and lower frequencies of the *Ppd-B1b* photoperiod sensitive allele (57%) and the *Ppd-D1a* photoperiod insensitive allele (29.0%) in 299 U.S. great plains hard winter wheat varieties than in ESW wheat varieties. Guo et al. (2010) observed that the predominant allele at the *Ppd-D1* locus was the photoperiod sensitive allele (*Ppd-D1b*, haplotype IV) among wheat varieties from the U.S. and Canada. On the contrary, in this

study, the photoperiod insensitive allele *Ppd-D1a* was predominantly observed in ESW wheat varieties. Tessmann et al. (2019) also reported that 50.0% of ESW wheat varieties carried the *Ppd-D1a* allele. These results indicate that the occurrence of the photoperiod insensitive allele *Ppd-D1a* varies among different wheat classes in the United States.

Allelic frequencies at the earliness *per se* loci

The predominant alleles at the *Eps-B1* and *Eps-D1* loci were the late heading-related alleles *TaELF3-B1a* and *TaELF3-D1a* with frequencies of 92.6% and 73.2%, respectively, among the 149 ESW wheat varieties. All KW wheat varieties carried the late heading-related alleles *TaELF3-B1a* and *TaELF3-D1a* at both the *Eps-B1* and *Eps-D1* loci, indicating that the *Eps-B1* and *Eps-D1* loci may not be relevant to the early heading of KW wheat varieties.

Allelic frequencies at the reduced height loci

Most ESW (63.8%) and KW (65.6%) wheat varieties carried the wild type (tall) *Rht-B1a* allele at the *Rht-B1* locus. The semi-dwarfing *Rht-D1b* allele was the predominant allele at the *Rht-D1* locus in ESW wheat varieties with a frequency of 56.4%, while most KW wheat varieties (53.1%) carried the *Rht-D1a* allele at the *Rht-D1* locus. Eight ESW wheat varieties (5.4%) and seven KW wheat varieties (21.9%) carried the tall alleles *Rht-B1a* and *Rht-D1a* at the *Rht-B1* and *Rht-D1* loci (Supplemental Table S1). The semi-dwarf alleles *Rht-B1b* and *Rht-D1b* were present at similar levels to those detected by Guedira et al. (2010) in 247 ESW wheat varieties released before 2008. Wilhelm et al. (2013) and Grogan et al. (2016) reported that the *Rht-B1b* and *Rht-D1b* alleles could promote wheat heading probably through their significant associations with the reduction in gibberellin sensitivity. The similar frequencies of the *Rht-B1b* and *Rht-D1b* alleles in ESW and KW wheat varieties suggest that the *Rht-B1* and *Rht-D1* loci may not contribute to the difference in HD between ESW and KW wheat varieties.

Combined analyses of alleles on heading date of ESW and KW wheat varieties over four crop years

The significance of crop year, and the *Vrn*, *Ppd*, *Eps* and *Rht* loci, and their interactions, on the HD of ESW wheat varieties was tested using 86 ESW wheat varieties (Table 3) with homozygous alleles at each locus. The GLM with crop year and the phenology-related loci as the independent variables and HD as a dependent variable explained 92.7% of the variation in the HD of ESW wheat, with most of the variation (87.0%) explained by crop year, and a smaller amount (5.7%) explained by genetic variation (Table 3). A similarly larger influence of crop year than genetic variation on HD was also observed by Grogan et al. (2016) in hard winter wheat varieties adapted to the great plains region of the U.S. Of the genetic variation, 27.8% was contributed by the *Ppd-D1* locus, 8.9% by the *Ppd-B1* locus, 7.4% by the *Vrn-D3* locus, and 11.6% by the two-way interaction between the *Ppd-B1* locus and the *Ppd-D1* locus. A smaller influence of genetic variation on HD was explained by the *Ppd-A1* locus (2.8%) and the *Eps-D1* locus (3.7%) and by the two-way interactions between the *Ppd-B1* (5.9%) locus or *Ppd-D1* (10.4%) locus and the *Ppd-A1* locus. These results agree with the report of Huang et al. (2018) which found that the *Ppd-A1*, *Ppd-B1*, *Ppd-D1* and *Vrn-D3* loci significantly affected the HD of ESW wheat under eastern U.S.

environments. The *Vrn-A1*, *Vrn-B1* and *Eps-B1* loci exhibited insignificant effects on the HD of ESW wheat varieties, as there is limited allelic diversity at these loci.

Table 3

The influence of crop year, and the vernalization (*Vrn*), photoperiod (*Ppd*), earliness per se (*Eps*) and reduced-height (*Rht*) loci and their interactions, on the heading date of eastern U.S. soft winter wheat varieties in four crop years

Source	Degrees of freedom	Mean square	Proportion of total variance (%)	Proportion of genetic variance (%)
Crop year	3	2119.6***	87.0	
<i>Vrn-A1</i>	2	2.5	0.1	1.2
<i>Vrn-B1</i>	2	0.4	0.0	0.2
<i>Vrn-D3</i>	1	30.3***	0.4	7.4
<i>Ppd-A1</i>	1	11.7*	0.2	2.8
<i>Ppd-B1</i>	3	12.2***	0.5	8.9
<i>Ppd-D1</i>	2	57.3***	1.6	27.8
<i>Eps-B1</i>	1	5.8	0.1	1.4
<i>Eps-D1</i>	1	15.3**	0.2	3.7
<i>Rht-B1</i>	1	3.5	0.0	0.8
<i>Rht-D1</i>	1	2.7	0.0	0.6
<i>Ppd-A1*Ppd-B1</i>	3	8.1**	0.3	5.9
<i>Ppd-A1*Ppd-D1</i>	1	42.9***	0.6	10.4
<i>Ppd-B1*Ppd-D1</i>	4	11.9***	0.7	11.6
<i>Ppd-A1*Ppd-B1*Ppd-D1</i>	1	0.1	0.0	0.0
Error	262	2.0	7.3	
The sum of squares was used to calculate the proportion of total variance contributed by crop years and each locus, and the proportion of genetic variance by each locus. Eighty-six eastern U.S. soft winter wheat varieties with homozygous alleles at each locus were evaluated for heading date in four crop years and included in the analyses of variation.				
*** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$.				

The influences of crop year and the phenology-related loci on the HD variation in KW wheat were determined using 30 KW wheat varieties (Supplemental Table S2) with homozygous alleles at each locus. Most of the HD variation of KW wheat varieties (94.7%) was explained by crop year, and a smaller amount (1.7%) was explained by the phenology-related loci. Of the genetic variation in the HD of KW

wheat varieties, 63.4% was accounted for by the *Rht-D1* locus (Supplemental Table S2). Grogan et al. (2016) found that the *Rht-B1* locus significantly affected the HD of hard winter wheat varieties grown in the U.S. great plains, but not the *Rht-D1* locus. The *Rht-B1* and *Rht-D1* loci had an insignificant influence on the HD of ESW wheat varieties under the tested environments. It appears that the effects of the *Rht-1* loci on wheat HD depend on both the allelic combinations at the phenology-related loci and the environment. The *Vrn*, *Ppd* and *Eps* loci did not significantly affect KW wheat HD; this mainly resulted from the lack of genetic diversity at these loci (Table 2).

Individual analysis of alleles on heading date of ESW and KW wheat varieties for each crop year

The GLMs with the phenology-related loci as the independent variables and HD as a dependent variable were tested for each crop year separately for ESW wheat varieties (Supplemental Table S3) and KW wheat varieties (Supplemental Table S4). The best-fit GLMs varied among crop years and not all phenology-related loci were significant. The model explained on average 42.0% of the variation in the HD of ESW wheat varieties, which ranged from a minimum of 38.0% in 2018 to a maximum of 47.1% in 2016 (Supplemental Table S3). The *Ppd-D1* locus significantly affected the HD of ESW wheat in all four crop years and accounted for on average 13.3% of the variation in HD, which ranged from 9.2% in 2015 to 23.5% in 2017 (Supplemental Table S3). The largest effect of the *Ppd-D1* locus on HD was observed in 2017 (Supplemental Table S3), probably due to the warm spring weather that year. The *Vrn-D3* locus exhibited a significant influence on the HD of ESW wheat only in 2015 and 2016. The interaction between the *Ppd-D1* locus and the *Ppd-B1* locus, and between the *Ppd-D1* locus and the *Vrn-D3* locus, also contributed to small variations in the HD of ESW wheat varieties in 2018 and 2016, respectively. No significant effects of the *Vrn-A1*, *Vrn-B1*, *Ppd-A1*, *Eps-B1*, *Eps-D1*, *Rht-B1* or *Rht-D1* loci were observed on the HD of ESW wheats in any individual crop year.

Among the tested loci, only the *Rht-D1* locus showed a significant influence on the HD of KW wheat varieties in both crop years, and accounted for 21.0% of the variation in the HD in 2017 and 18.2% in 2018 (Supplemental Table S4). The other loci showed no significant influence on the HD of KW wheats in a crop year.

Influence of alleles at the *Vrn-D3*, *Ppd-B1* and *Ppd-D1* loci on heading date of ESW and KW wheat varieties

Five loci, including the *Vrn-D3*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1* and *Eps-D1* loci, were identified to significantly influence on the HDs of ESW wheat varieties in the combined analyses over four crop years (Table 3). The influences of the alleles at these five significant loci on the HD of ESW wheat were further evaluated using ESW wheat varieties with homozygous alleles at these loci. No significant differences were observed in HD between ESW wheat varieties carrying different alleles at the *Ppd-A1* and *Eps-D1* loci in any individual crop year. No significant differences in HD (except for 2018) were observed between ESW wheat varieties carrying the *vrn-D3a* allele or the *vrn-D3b* allele at the *Vrn-D3* locus (Supplemental Figure S2), likely due to the presence of the *Ppd-D1b* photoperiod sensitive allele in those ESW varieties (Supplemental Table S1). The effect of the *Ppd-D1b* allele on HD might mask the effect of the *vrn-D3a*

allele. In 2018, ESW wheat varieties carrying the *vrn-D3a* allele headed on average 1 day earlier than those carrying the *vrn-D3b* allele.

The effects of alleles at the *Ppd-B1* and *Ppd-D1* loci were also inconsistent in four crop years (Fig. 2). No significant differences in HD were observed between the ESW wheat varieties carrying different alleles at the *Ppd-B1* locus in 2016 and 2018 (Fig. 2). ESW wheat varieties possessing the 'Sonora 64'-type insensitive allele at the *Ppd-B1* locus headed on average 1 or 2 days earlier than those with other alleles at the *Ppd-B1* locus in 2015 and 2017. No significant differences in HD were also observed between ESW wheat varieties carrying different alleles at the *Ppd-D1* locus in 2015 and 2016. ESW wheat carrying the *Ppd-D1a* insensitive allele headed on average 2 days earlier than those carrying the *Ppd-D1b* or *Ppd-D1b-Norstar* sensitive allele in 2017 (Fig. 2). A significant difference was observed only between ESW wheat carrying the *Ppd-D1a* allele and the *Ppd-D1b-Norstar* allele in 2018. The allelic variation at the *Ppd-D1* locus showed variable influences on HD among crop years, suggesting differential expression under varying climatic conditions, especially temperature. The early heading of ESW wheat varieties with the photoperiod-insensitive allele *Ppd-D1a* is in agreement with results from the previous studies by Grogan et al. (2016) and Whittal et al. (2018).

For KW wheat varieties, only the *Rht-D1* locus showed a significant influence on HD in each crop year (Supplemental Table S4). KW wheat varieties carrying the *Rht-D1a* allele headed on average 2 days earlier than those carrying the *Rht-D1b* allele in each crop year (Supplemental Figure S3). Tessmann et al. (2019) also observed a slight decrease (0.6%) in the HD of ESW wheat varieties with the *Rht-D1a* allele compared to those with the *Rht-D1b* allele under eastern U.S. environments. On the contrary, several other studies reported that the *Rht-B1b* and *Rht-D1b* alleles could promote wheat heading through their significant associations with the reduction in gibberellin sensitivity (Wilhelm et al. 2013; Grogan et al. 2016). It is not evident why the *Rht-D1a* allele shows an association with the early heading of winter wheat. The significant association may be due to the association of *Rht* loci with *Ppd* loci in determining wheat heading (Wilhelm et al. 2013).

Influence of allelic combinations on the heading date of ESW and KW wheat varieties

Considering the significant influence of allelic variation at the *Vrn-D3*, *Ppd-B1* and *Ppd-D1* loci on the HD of ESW wheats, as well as significant differences in the allelic frequencies at the *Vrn-A1* locus between ESW and KW wheat varieties, the influences of allelic combinations at the *Ppd-B1*, *Ppd-D1*, *Vrn-A1* and *Vrn-D3* loci on the HD of winter wheat were determined using 127 ESW wheat varieties and 30 KW wheat varieties with homozygous alleles at these four loci. A total of 19 phenology gene profiles were observed in 127 ESW wheat varieties (Table 4). More than half (52.8%) of 127 ESW wheat varieties, however, possessed the same profile of *Ppd-B1b/Ppd-D1a/vrn-A1(CNV > 2)/vrn-D3b*. Around 26.0% of ESW wheat varieties possessed one of these four profiles: *Ppd-B1b/Ppd-D1b/vrn-A1(CNV > 2)/vrn-D3b* (10.2%); *Ppd-B1b/Ppd-D1b-Norstart/vrn-A1(CNV > 2)/vrn-D3b* (6.3%); *null/Ppd-D1a/vrn-A1(CNV > 2)/vrn-D3b* (4.7%); and *Ppd-B1a-Sonora 64/Ppd-D1a/vrn-A1(CNV > 2)/vrn-D3b* (4.7%). Fourteen other phenology gene profiles were observed in five or fewer varieties. The 30 KW wheat varieties carried six phenology gene

profiles at the *Ppd-B1*, *Ppd-D1*, *Vrn-A1* and *Vrn-D3* loci (Table 4). Most KW wheat varieties possessed one of these two profiles: *Ppd-B1b/Ppd-D1a/vrn-A1*(CNV = 1)/*vrn-D3a* (40.0%) and *Ppd-B1b/Ppd-D1a/vrn-A1*(CNV = 1)/*vrn-D3b* (36.7%). Four other phenology gene profiles were observed in three or fewer varieties.

Table 4

Phenology gene profiles of 127 eastern U.S. soft winter wheat varieties and 30 Korean winter wheat varieties carrying homozygous alleles at the *Ppd-B1*, *Ppd-D1*, *Vrn-A1* and *Vrn-D3* loci sorted by frequency and their heading dates

Loci				Frequency	Heading date			
<i>Ppd-B1</i>	<i>Ppd-D1</i>	<i>vrn-A1</i>	<i>vrn-D3</i>	(%)	2015	2016	2017	2018
CNV ^a								
U.S. soft winter wheat								
b	a	>2	b	52.8	143	140	133	145
b	b	>2	b	10.2	143	141	135	146
b	b-Nor ^d	>2	b	6.3	144	140	135	147
null	a	>2	b	4.7	143	140	134	145
a-S64 ^e	a	>2	b	4.7	140	139	131	143
a-CS ^f	b-Nor	>2	b	3.9	142	140	132	144
b	b	>2	a	3.1	141	139	133	143
a-CS	a	>2	b	3.1	143	140	133	145
b	b-Nor	>2	a	2.4	143	141	134	144
a-CS	b	>2	a	1.6	141	139	130	142
a-CS	b	>2	b	0.8	144	141	136	146
null	b	>2	b	0.8	142	140	133	142
null	b-Nor	>2	b	0.8	144	141	135	146
a-S64	b	>2	a	0.8	144	142	136	146
a-S64	b	>2	b	0.8	143	140	134	146

^aCNV, copy number variation.

^bnd, not determined.

^cLSD, least significant difference (at the 0.05 probability level).

^db-nor, 'Norstar'-type sensitive allele.

^ea-S64, 'Sonora 64'-type insensitive allele.

^fa-CS, 'Chinese Spring'-type insensitive allele.

Loci				Frequency	Heading date			
<i>Ppd-B1</i>	<i>Ppd-D1</i>	<i>vm-A1</i>	<i>vm-D3</i>	(%)	2015	2016	2017	2018
CNV ^a								
a-S64	b-Nor	>2	b	0.8	nd ^a	141	136	146
b	a	2	b	0.8	139	139	130	144
b	b-Nor	2	b	0.8	nd	139	134	nd
b	b	1	a	0.8	143	139	135	146
				LSD (0.05) ^c	4	2	4	4
Korean winter wheat								
b	a	1	a	40.0	nd	nd	116	133
b	a	1	b	36.7	nd	nd	117	133
b	a	>2	b	10.0	nd	nd	118	134
a-CS	a	1	b	6.7	nd	nd	119	135
b	a	>2	a	3.3	nd	nd	117	132
null	a	1	a	3.3	nd	nd	119	135
				LSD (0.05)			4	4
^a CNV, copy number variation.								
^b nd, not determined.								
^c LSD, least significant difference (at the 0.05 probability level).								
^d b-nor, 'Norstar'-type sensitive allele.								
^e a-S64, 'Sonora 64'-type insensitive allele.								
^f a-CS, 'Chinese Spring'-type insensitive allele.								

Within ESW wheat varieties, the average HD of 19 phenology gene profiles ranged from 139 to 144 in 2015, 139–142 in 2016, 130–136 in 2017 and 142–147 in 2018 (Table 4). Within KW wheat varieties, the average HD of six phenology gene profiles ranged from 116 to 119 in 2017 and 132–135 in 2018 (Table 4). Within ESW wheat varieties or KW wheat varieties, relatively small variations in HD were observed among the 19 or six phenology gene profiles in each crop year, respectively, whereas each phenology gene profile exhibited considerable variation in HD among the crop years (Table 4). It appears

that wheat varieties adapted to a specific location have HDs appropriate to the local climatic conditions to ensure the maximum yield potential.

Five predominant profiles of ESW wheat varieties had the same winter alleles at the *Vrn-A1* and *Vrn-D3* loci. Among these five predominant profiles, ESW wheat varieties carrying the profile of *Ppd-B1a-Sonora 64/Ppd-D1a/vrn-A1(CNV > 2)/vrn-D3b* headed 1–4 days earlier than those carrying one of the remaining four profiles. Two predominant profiles of KW wheat varieties had the same alleles at the *Ppd-B1*, *Ppd-D1* and *Vrn-A1* loci. No significant differences were observed in the average HD of KW wheat varieties between different phenology gene profiles.

The copy number variation at the *Vrn-A1* locus was the major difference in the predominant phenology gene profiles between ESW and KW wheat varieties and might be responsible for the different HDs between ESW and KW wheat varieties under eastern U.S. environments. Additional variation in the HD of ESW wheat varieties could be achieved through the inclusion of other loci known to affect HD, such as the *Vrn-A1* locus.

Conclusions

In this study, we used 149 ESW and 32 KW wheat varieties to evaluate the allelic diversity and influences of *Vrn*, *Ppd*, and *Rht* on HD. We found that the genetic effects on the HD of ESW wheat varieties were largely explained by the *Ppd-B1*, *Ppd-D1* and *Vrn-D3* loci. The low levels of genetic diversity at the *Vrn-A1*, *Vrn-B1*, *Eps-B1* and *Eps-D1* loci contributed to their insignificant influences on ESW wheat HD. The genetic variation for the HD of KW wheat varieties was mostly explained by the *Rht-D1* locus. The *Vrn*, *Ppd* and *Eps* loci didn't significantly affect KW wheat HD, mainly due to the lack of genetic diversity at these loci. A phenology gene profile (*Ppd-B1b/Ppd-D1a/vrn-A1(CNV > 2)/vrn-D3b*) was predominantly observed in ESW wheat varieties with a frequency of 52.8%. Two phenology gene profiles (*Ppd-B1b/Ppd-D1a/vrn-A1(CNV = 1)/vrn-D3a* and *Ppd-B1b/Ppd-D1a/vrn-A1(CNV = 1)/vrn-D3b*) were predominantly observed in KW wheat varieties, making up 76.7% of the total observed profiles. ESW wheat varieties exhibited on average a HD 14 days later than that of KW wheat varieties in each crop year, largely due to the absence of one copy of the *vrn-A1* allele in the former. The development of early-maturing ESW wheat varieties could be achieved by selecting for the one-copy *vrn-A1* and *vrn-D3a* winter alleles in combination with photoperiod insensitive alleles at the *Ppd-B1* and *Ppd-D1* loci. These results enhance our understanding of the effects of phenology genes on the HD of winter wheat under eastern U.S. environments and could be useful for the development of early-maturing wheat varieties suitable for wheat-soybean double-cropping systems and wheat varieties well-adapted to the current and future climate conditions.

Declarations

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Declaration of competing interest

The authors declare no conflict of interest.

Data availability The data that support the findings of this study are available from the corresponding author on reasonable request.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fengyun Ma, Moonseok Kang and Gina Brown-Guedira. The first draft of the manuscript was written by Fengyun Ma. Byung-Kee Baik supervised the study and revised the manuscript. All authors approved the final manuscript.

Supplemental material

Supplemental tables and figures are available online. Tables present the phenology genes and heading dates of all winter wheat varieties evaluated (Supplemental Table S1), along with the influence of crop year and the phenology-related loci on heading date of ESW and KW wheat varieties over two crop years or in each crop year (Supplemental Tables S2, S3 and S4). Figures depict cumulative growing degree days in Wooster, Ohio, during 2015-2018 (Supplemental Figure S1), the heading dates of ESW wheat varieties with different alleles at the *Vrn-D3* locus (Supplemental Figure S2), and the heading dates of KW wheat varieties with different alleles at the *Rht-D1* locus (Supplemental Figure S3).

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Figures

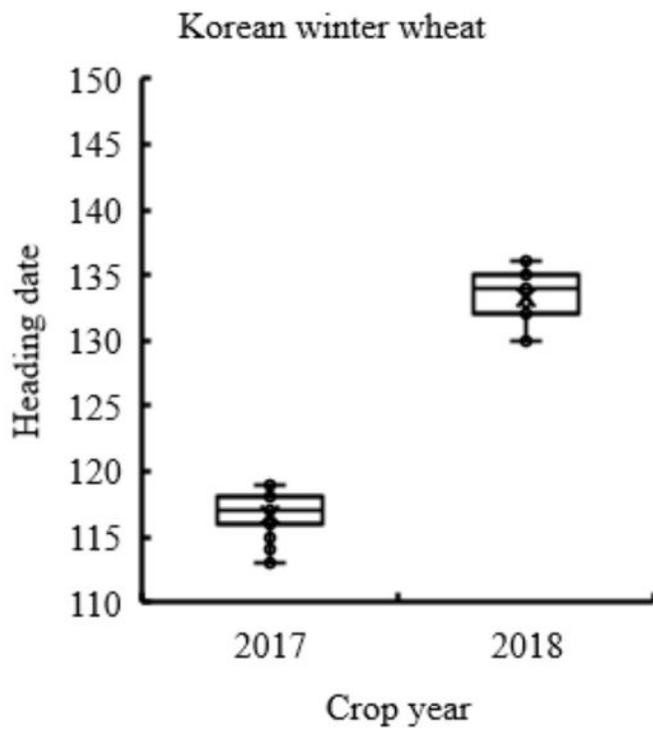
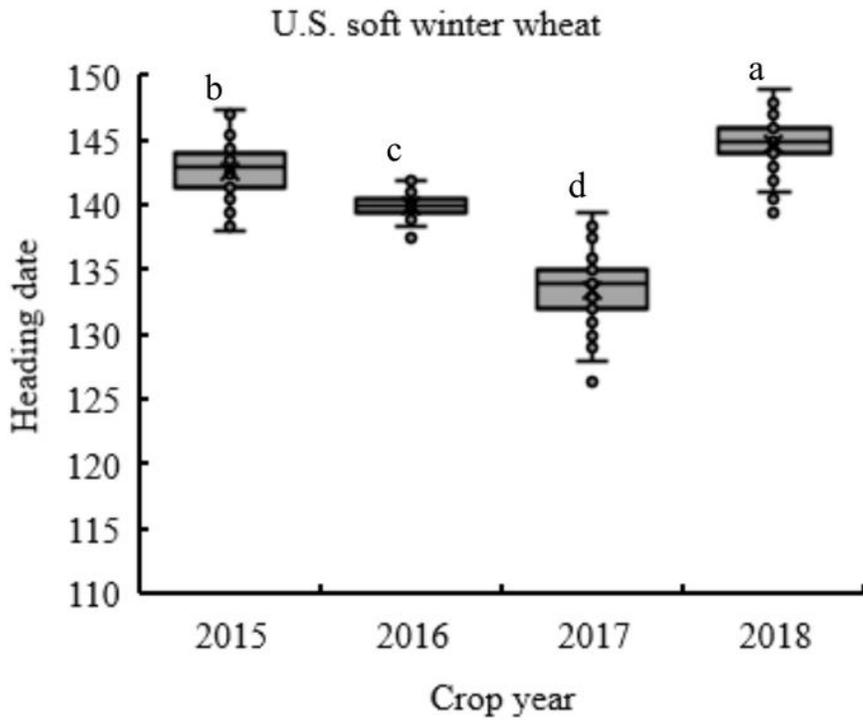


Figure 1

Mean heading dates of 121 eastern U.S. soft winter wheat varieties grown in 2015-2018 and 32 Korean winter wheat varieties grown in 2017 and 2018 in Wooster, Ohio. x indicates the mean. Means with different letters are significantly different at the 0.05 probability level.

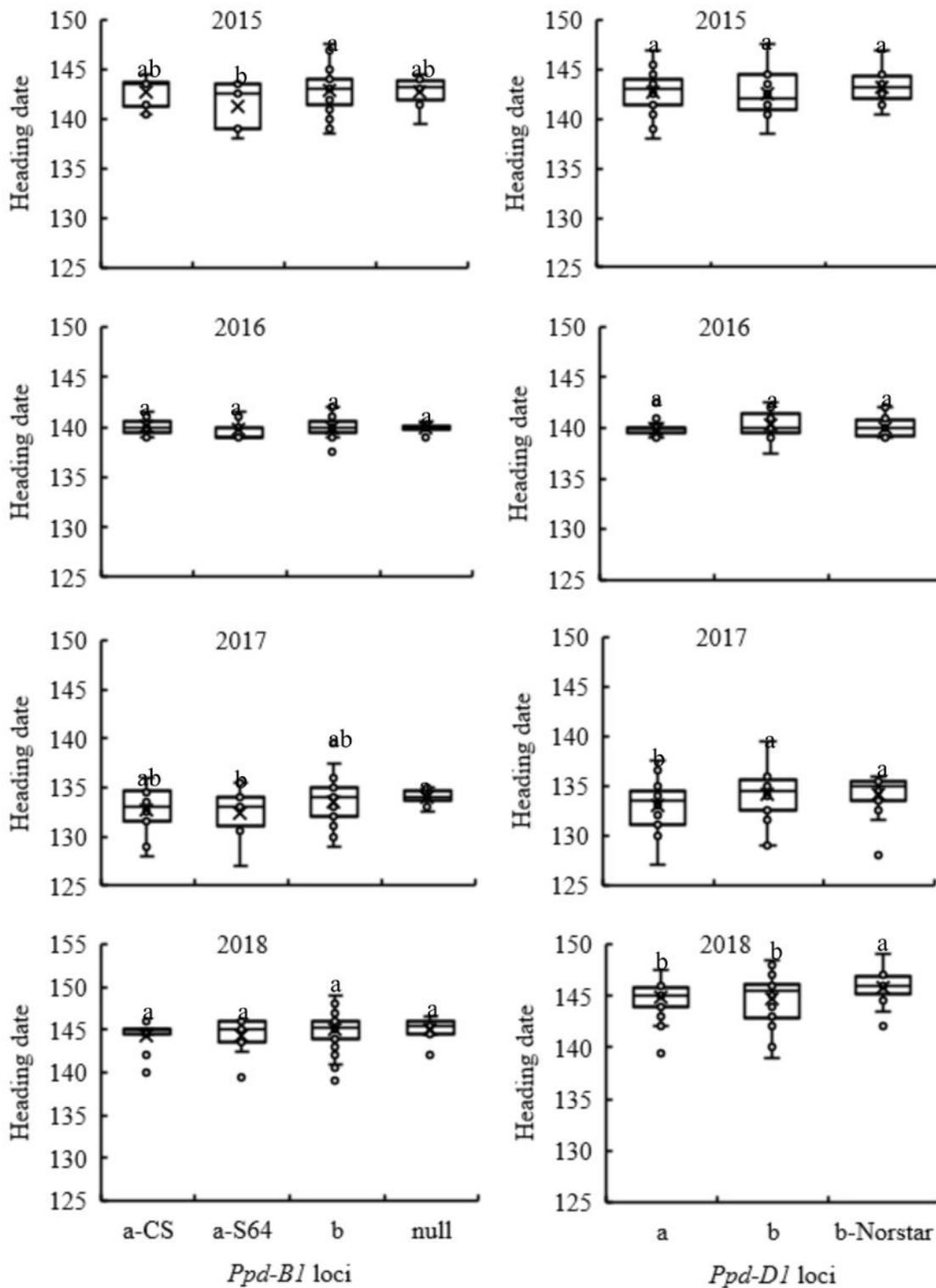


Figure 2

Heading dates of 127 eastern U.S. soft winter wheat varieties with different alleles at the *Ppd-B1* and *Ppd-D1* loci in four crop years. a-S64, 'Sonora 64'-type insensitive allele. a-CS, 'Chinese Spring'-type insensitive allele. b-Norstar, 'Norstar'-type sensitive allele. x indicates the mean. Means with different letters are significantly different at the 0.05 probability level.

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