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REVIEW

Genome-wide association study of heading and flowering dates and construction of its prediction equation in Chinese common wheat

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Abstract

Heading date is one of the most important traits in wheat breeding as it affects adaptation and yield potential. A genome-wide association study (GWAS) using the 90 K iSelect SNP genotyping assay indicated that a total of 306 loci were significantly associated with heading and flowering dates in 13 environments in Chinese common wheat from the Yellow and Huai wheat region. Of these, 105 loci were significantly correlated with both heading and flowering dates and were found in clusters on chromosomes 2, 5, 6, and 7. Based on differences in distribution of the vernalization and photoperiod genes among chromosomes, arms, or block regions, 13 novel, environmentally stable genetic loci were associated with heading and flowering dates, including RAC875_c41145_189 on 1DS, RAC875_c50422_299 on 2BL, and RAC875_c48703_148 on 2DS, that accounted for more than 20% phenotypic variance explained (PVE) of the heading/flowering date in at least four environments. GWAS and *t* test of a combination of SNPs and vernalization and photoperiod alleles indicated that the *Vrn-B1*, *Vrn-D1*, and *Ppd-D1* genes significantly affect heading and flowering dates in Chinese common wheat. Based on the association of heading and flowering dates with the vernalization and photoperiod alleles at seven loci and three significant SNPs, optimal linear regression equations were established, which show that of the seven loci, the *Ppd-D1* gene plays the most important role in modulating heading and flowering dates in Chinese wheat, followed by *Vrn-B1* and *Vrn-D1*. Additionally, three novel genetic loci (RAC875_c41145_189, Excalibur_c60164_137, and RAC875_c50422_299) also show important effect on heading and flowering dates. Therefore, *Ppd-D1*, *Vrn-B1*, *Vrn-D1*, and the novel genetic loci should be further investigated in terms of improving heading and flowering dates in Chinese wheat. Further quantitative analysis of an F_{10} recombinant inbred lines population identified a major QTL that controls heading and flowering dates within the *Ppd-D1* locus with PVEs of 28.4% and 34.0%, respectively; this QTL was also significantly associated with spike length, peduncle length, fertile spikelets number, cold resistance, and tiller number.

Introduction

Common wheat (*Triticum aestivum* L.) is a staple food in various countries and regions all over the world. Heading date profoundly influences the growth and development of wheat and thus is one of the most critical traits that is evaluated to determine the adaptability of cultivars to diverse climatic environments in various regions and cropping seasons (Law and Worland 1997). Four major pathways control heading and flowering dates in plants, i.e., the vernalization, photoperiod, phytohormone gibberellic acid (GA), and autonomous pathways, with the vernalization and photoperiod pathways considered to be the two most important pathways that influence heading date in wheat (Yasuda 1984; Kato and Yamagata 1988).

Vernalization is the exposure to low temperature that is associated with seasonal variations to optimize flowering

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date and seed production, thereby preventing damage to the cold-sensitive flowering meristem during winter (Yan et al. 2003; Trevaskis et al. 2006). The growth habits and vernalization requirements of cereal plants are mainly determined by four genes; namely, *Vrn-1*, *Vrn-2*, *Vrn-3*, and *VRN-D4*. The *Vrn-1* gene encodes a MADS-box transcription factor, the homolog of the Arabidopsis meristem identity gene *APETALA1*, and is located on the long arms of chromosomes 5A, 5B, and 5D in polyploid wheat; it directly influences flowering and maturity dates and is upregulated by vernalization treatment (Trevaskis et al. 2003; Yan et al. 2003, 2004). The *Vrn-2* gene is located on chromosome 5A and consists of two completely linked zinc finger CCT domain genes (*ZCCT-1* and *ZCCT-2*) that act as dominant repressors of flowering, and deletions or mutations involving *Vrn-2* result in the elimination of the vernalization requirement in wheat (Dubcovsky et al. 1998; Yan et al. 2004; Distelfeld et al. 2009). The *Vrn-3* gene is a homolog of the Arabidopsis FT gene and has been mapped to the short arm of chromosome 7; it is upregulated by vernalization treatment and indirectly accelerates heading and flowering by promoting the expression of the *Vrn-1* gene (Yan et al. 2006; Faure et al. 2007). The *Vrn-D4* on 5DS is another important gene affecting expression of the *Vrn1* gene to modulate the heading and flowering dates in wheat (Kippes et al. 2015). The detailed pathway of the vernalization genes involved in controlling wheat flowering was summarized by Chen and Dubcovsky (2012).

Photoperiod is another vital pathway that influences heading and flowering dates, which rely on plant responses to the length of daylight, as well as the perception of optical signals from light receptors. Homology cloning has shown that the *Ppd-D1* gene is the ortholog of the *Ppd-H1* gene of barley (*Hordeum vulgare*), which is a member of the pseudo-response regulator (PRR) gene family (Beales et al. 2007); additionally, *Ppd-1* and *CO* are members of the CCT gene family (Turner et al. 2005). Photoperiod response genes in common wheat are mainly controlled by the *Ppd-1* locus on the short arm of chromosome 2 (Welsh et al. 1973), which includes the *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* genes located on 2AS, 2BS, and 2DS, respectively. The alleles *Ppd-A1a*, *Ppd-B1a*, and *Ppd-D1a* confer photoperiod insensitivity, whereas alleles *Ppd-A1b*, *Ppd-B1b*, and *Ppd-D1b* are responsible for photoperiod sensitivity (Pugsley 1966; Dyck et al. 2004). *Ppd-D1a* is a deletion mutation allele that causes mis-expression of the 2D *PRR* gene and permits early flowering in both short- and long-day conditions in photoperiod-insensitive cultivars. Photoperiod insensitivity is always beneficial to yield in Southern Europe and Asia. Five polymorphisms in the *Ppd-D1* locus were identified by sequencing of 2D *PRR* gene homologs in a number of wheat cultivars (Beales et al. 2007). Furthermore, six haplotypes were revealed, owing to these sequence polymorphisms in wheat *Ppd-D1* gene (Guo et al. 2010), and four haplotypes were discovered in Chinese winter wheat (Chen et al. 2013a; Zhang et al. 2015a). Additionally, sequence polymorphisms of the *Ppd-A1a* gene were identified in tetraploid wheat (Wilhelm et al. 2009), and copy number variation (CNV) of *Ppd-B1a* could influence flowering date in common wheat (Diaz et al. 2012). Analysis of gene expression and interaction among photoperiod pathways is described in detail by Beales et al. (2007) and Guo et al. (2010).

The autonomous pathway responds to endogenous signals from specific developmental states, and GA pathway plays a major role in flowering under short-day condition in Arabidopsis (Liu et al. 2008). Therefore, various factors (e.g., temperature, light, endogenous signals, and hormones) regulate the transition from the vegetative to the reproductive growth stages of wheat.

Heading date is a complex polygenic trait, and previous researches have identified several key regulatory genes in specific bi-parental populations using traditional positional cloning. Genome-wide association studies (GWAS) provide the opportunity to methodically analyze the genetic architecture of complex traits in plants and benefit from the high diversity and rapid linkage disequilibrium (LD) decay in species, e.g., wheat (Sela et al. 2011) and rice (Huang et al. 2010). It has been widely used for studying complex traits, including heading date in various plant species, such as *Arabidopsis* (Atwell et al. 2010; Brachi et al. 2010), rice (Huang et al. 2010; Yano et al. 2016), maize (Li et al. 2013; Yang et al. 2014), barley (Alqudah et al. 2014), and soybean (Zhang et al. 2015b).

GWAS has become an increasingly popular and efficient way of identifying genes that are responsible for quantitative variations in complex traits, which in turn facilitates the development of valuable genetic markers for molecular breeding, particularly in hexaploid wheat, which has a large genome size (≈17.0 Gb) (Borrill et al. 2015; Uauy 2017). A number of markers associated with photoperiod and vernalization genes have been identified using the 90 K assay, and one of them was homologous to the rice photoperiod gene *Hd6* that played a vital role in heading date of rice (Zanke et al. 2014).

Ain et al. (2015) identified 14 trait-associated SNPs that were linked to genes that are related to plant development through gene annotation using a 90 K SNP assay and showed the frequency of favorable alleles for some traits in modern wheat cultivars. Guo et al. (2016) quantified 54 traits in 210 European winter wheat accessions and monitored several potential target genes for selection in combination with shared QTLs by GWAS. Sun et al. (2017) showed that there were some pleiotropic SNPs that were linked to thousand kernel weight (TKW) and polygenic loci-mediated traits, such as plant height and kernel length of wheat, through GWAS using the wheat 90 K genotyping assay.

The present study has revealed important genetic loci for heading and flowering dates using the 90 K Illumina iSelect SNP array in Chinese winter wheat as surveyed using a combination of GWAS, linkage analysis, and polymorphism identification. We have identified genetic loci that control heading and flowering dates, with the *Ppd-D1* gene playing the most important role in regulating heading and flowering dates in wheat cultivars from the Yellow and Huai wheat region, followed by *Vrn-B1* and *Vrn-D1*. The findings of this study may facilitate the elucidation of the genetic mechanisms underlying the establishment of heading and flowering dates and marker-assisted selection during wheat breeding.

Materials and methods

Plant materials and growth conditions

A total of 375 Chinese wheat germplasm (CWG) composed of current wheat cultivars and historical cultivars were planted during the cropping seasons of years 2011–2017, and 254 Chinese landraces (CL) were planted during the cropping seasons of 2013–2014 at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University (N34.9; E113.6) following local management practices. All surveyed cultivars were vernalized through winter with an average temperature of ≈1.3 °C (December, January, and February) in 2012–2017. The wheat germplasms that were surveyed included very important landraces, historical, and current cultivars in China; these germplasms were mainly used as backbone parents and had played vital roles in wheat breeding programs in the country. The field experiment was conducted using a completely randomized design. Each plot contained 12 rows with 150 cm long and 23 cm wide and 10 cm between neighboring plants. All surveyed cultivars underwent robust growth with a supporting net and without lodging. The heading and flowering dates of each cultivar were recorded in April or May of 2012–2017, and their heading and flowering days were calculated from the sowing date to the heading and flowering dates.

Furthermore, 163 of the 375 wheat germplasms were genotyped using the wheat 90 k iSelect SNP array (Wang et al. 2014) and used in GWAS analysis as association mapping population (AMP) based on their pedigree, released regions, agronomic performance, importance (backbone parents or not), and cultivated area. The AMP was planted in the 2011–2012, 2012–2013, and 2013–2014 cropping seasons with one replicate for each year, in the 2014–2015 cropping seasons with three replicates, and in 2015–2016 and 2016–2017 cropping seasons with two replicates for each year at the Zhengzhou Scientific Research Education Center of Henan Agricultural University, as well as in 2015–2016 with one replicate and 2016–2017 with two replicates at the Zhumadian Academy of Agricultural Science (E114.1; N33.0). The field experiments were designed, and phenotypes were investigated as previously described (data in Supplemental Table 1).

A F_{10} RIL population (derived from a cross involving Proteo × Chaja) encompassing 97 lines was planted in the 2014–2015 and 2015–2016 cropping seasons at Zhengzhou Scientific Research Education Center of Henan Agricultural University. All plants grew well in the field. Seven agronomic traits, including heading date (HD), flowering date (FD), spike length (SL), peduncle length (PL), fertile spikelets number (FSN), cold resistance, and tiller number (TN), were investigated at suitable stages, respectively. Cold resistance is investigated on the first of March of each year according to the method of Zhang et al. (2017). Spike length, peduncle length, and fertile spikelet number of ten spikes and tiller number (TN) of ten different single plants for each line were investigated before harvest in middle of May.

GWAS

The AMP accessions were genotyped by Beijing Compass Technology and investment company using the 90 k Infinium Wheat Chip, and all SNP markers were filtered with missing values ≤ 10% and minor allele frequency (MAF) ≥ 5% according to the method of Purcell et al. (2007; http://pngu.mgh.harvard.edu/purcell/plink/). Finally, there are 20,890 SNPs for GWAS analysis.

The population structure of the collected 163 cultivars was assessed with unlinked markers ($r^2 = 0$) using STRUCTURE ver. 2.3.4 (Pritchard et al. 2000), based on the highest delta K value representing genetic clusters. Principle component analysis (PCA) was also conducted with the R software to assess population structure, and the results were compared to those generated by STRUCTURE.

This paper assessed population stratification in a quantile–quantile (Q–Q) plot. The overall deviation above the diagonal line at the initial stage may show the existence of population stratification. The Q–Q plot was drawn using qqman packages within the R statistical environment.

GWAS in different years or environments were performed using a mixed linear model (MLM) with PCA and kinship as covariates to estimate the association between phenotypes and genotypes (Yu et al. 2006; Zhang et al. 2010). In this study, GWAS were implemented by GAPIT packages (Lipka et al. 2012) in the R statistical environment, and variance–covariance kinship matrix (K), which reflected relationships among individuals, was automatically calculated using the VanRaden method (VanRaden 2008). To integrate the association results in different environments, we set a uniform genome-wide significance threshold (P value = $1/n$ = 1.0e−3, n = total unlinked markers).

Polymerase chain reaction (PCR) parameters

Genomic DNA was individually extracted from three pulverized kernels of all germplasms, following the protocol of Chen et al. (2011). The PCR and programs were performed according to Chen et al. (2013b). Allelic variations in vernalization and photoperiod response genes were identified in all cultivars surveyed in this study according to the method of Zhang et al. (2015a), and two new markers for identification of *Ppd-A1* and *Ppd-D1* alleles were developed based on their genomic sequences using software Primer 5.0. The PCR products were separated on 1.5–2.5% agarose gel that was stained with ethidium bromide and visualized with UV light.

QTL mapping

The genetic linkage map of the F_{10} RIL population Proteo × Chaja was composed of 2810 SNP polymorphic markers that were mapped to 767 unique loci using the 9 K iSelect Beadchip Assay (Detailed maps are given in Cavanagh et al. 2013). Genetic linkage groups were constructed with the statistical software QTL IciMapping V4.1 (http://www.isbreeding.net/). A logarithm of odds (LOD) score of 2.5 was set in the establishment of linkage groups. LOD scores for declaring significant QTLs were calculated from 1000 permutations at the $P \leq 0.05$ significance level. The inclusive composite interval mapping addition (ICIM-ADD) method was selected for QTL mapping (Li et al. 2007), and other mapping and binning parameters were to default. Finally, QTL effects estimated by the phenotypic variance explained (PVE).

Statistical analysis

Phenotype analysis and *t* test for identifying significant differences ($P < 0.05$) among heading and flowering dates of cultivars with various alleles and establishment of linear regression equation for prediction of heading and flowering dates were performed using SPSS 19.0 and Excel software 2010.

Results

Phenotypic variations in heading and flowering dates

The histogram of averaged heading and flowering dates of the association mapping population (AMP) in 13 environments showed nearly symmetrical distribution, spanning 14 and 12 days, respectively (Supplemental Figure S1A and 1B). Additionally, the correlation coefficients of the heading date among the 13 environments ranged from 0.536 to 0.897, and the correlation coefficients of the flowering date ranged from 0.533 to 0.945. The correlation coefficients between heading and flowering dates ranged from 0.882 to 0.999.

Significant and repetitive loci associated with heading and flowering dates

Principle component analysis (PCA) showed that the AMP (association mapping population) cultivars could be divided into two subgroups based on whole-genome genotyping data (Fig. 1). The number of subpopulation (*k*) was plotted against the delta k calculated from the STRUCTURE software with 2635 independent markers, and the peak of the broken line graph was observed at $k = 2$, indicating the natural population can be divided into two subpopulations (Supplemental Figure S2).

Manhattan and quantile–quantile (*Q*–*Q*) plots for heading and flowering dates are shown in Fig. 2 and Supplemental Fig. S3A, B. GWAS analysis indicated that a total of 306 significant SNPs were associated with heading and flowering dates that were distributed on all of the chromosomes (Fig. 3a, b; detailed chromosome location of each SNP in Supplemental Tables 2A–D). Of all significant SNPs, 180 and 241 were significantly associated with heading and flowering dates, respectively (Supplemental Tables 2B, C), and 115 were significantly associated with both heading and flowering dates (Supplemental Table 2D). The PVE of these SNPs in the association panels ranged from 14.1 to 31.8% for heading date and 10.9 to 29.7% for flowering date.

Furthermore, twelve stable SNPs were significantly associated with heading date in more than four environments,

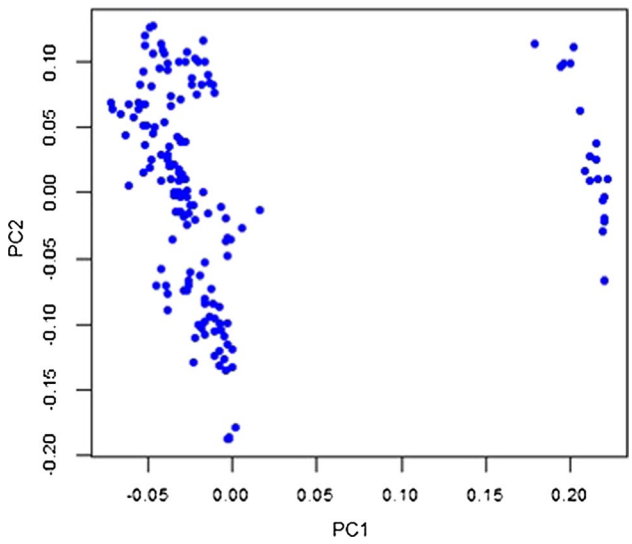

Fig. 1 Principle component analysis (PCA) for Chinese wheat cultivars based on whole-genome sequence data

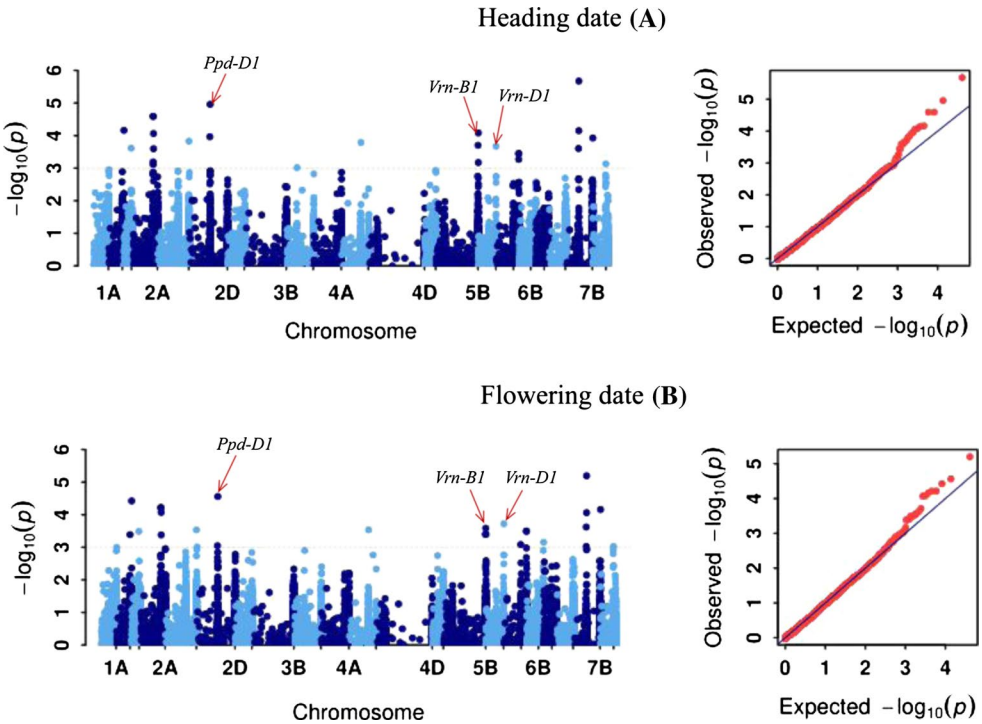

Fig. 2 Manhattan and quantile–quantile (*Q*–*Q*) plots for heading date (**a**), flowering date (**b**) in 2016_ZMD

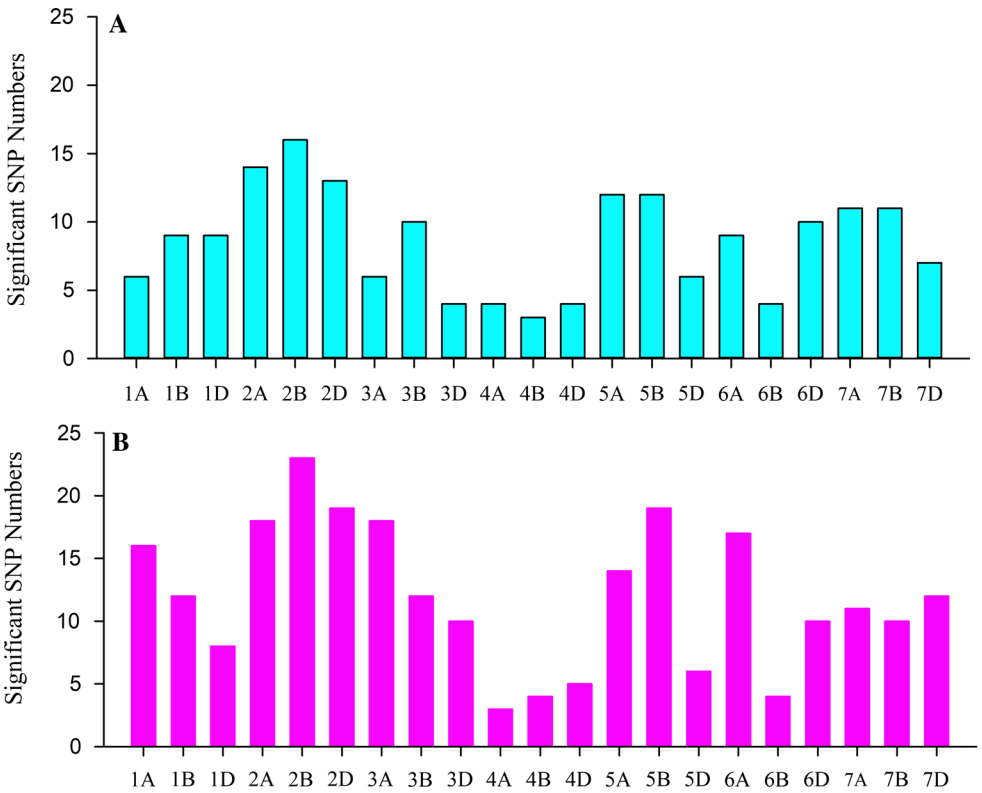

Fig. 3 Distribution of significant SNPs associated with heading date (**a**) and flowering date (**b**) in Chinese winter wheat

i.e., RAC875_c41145_189 (with 21.5% PVE in four environments) and wsnp_Ex_c17884_26647833 (with 17.2% PVE in four environments) on 1DS; RAC875_c50422_299 (with 24.0% PVE in six environments) on 2BL; and Excalibur_c60164_137 (with 21.8% PVE in seven environments), RAC875_c829_611 (with 21.4% PVE in five environments), Kukri_c5282_622 (with PVE of 21.4% in five environments), and tplb0049o19_694 (with 22.2% PVE in four environments) on 2AS; RAC875_c48703_148 (with 24.6% PVE in five environments) on 2DS; Tdurum_contig29563_183

(with 25.6% PVE in four environments) on 4BS; Excalibur_rep_c107908_308 (with 22.1% PVE in four environments) on 5BL; Excalibur_c16573_197 (with 18.8% PVE in four environments) on 5DL; and IAAV6834 (with 21.8% PVE in five environments) on 6DS. Six stable SNPs were significantly associated with flowering date in more than four environments, i.e., RAC875_c829_611 (with 20.1% PVE in five environments) on 2AS; Kukri_c5282_622 (with 20.1% PVE in five environments), tplb0049o19_694 (with 18.1% PVE in five environments), and RAC875_c48703_148 (with 18.9% PVE in six environments) on 2DS; RAC875_c50422_299 (with 19.1% PVE in five environments) on 2BL; and RAC875_c14659_1066 (with 18.7% PVE in five environments) on 6AL.

Comparison of chromosome locations, chromosome arms, or block regions with known vernalization and photoperiod genes identified a total of 13 environmentally stable SNPs (RAC875_c41145_189, wsnp_Ex_c17884_26647833, RAC875_c50422_299, Excalibur_c60164_137, RAC875_c829_611, Kukri_c5282_622, tplb0049o19_694, RAC875_c48703_148, Tdurum_contig29563_183, Excalibur_rep_c107908_308, Excalibur_c16573_197, IAAV6834, and RAC875_c14659_1066), which are novel loci that control heading and flowering dates.

Polymorphisms within vernalization and photoperiod genes in Chinese wheat germplasms

To illustrate the influence of vernalization and photoperiod genes on heading and flowering dates in Chinese wheat, we also screened for polymorphisms in the vernalization and photoperiod genes of 375 Chinese wheat germplasms (CWG) and 254 Chinese landraces (CL). To more readily distinguish *Ppd-D1a* and *Ppd-D1b* alleles, we developed a novel functional marker Ppd-P11 based on *Ppd-D1* and *Ppd-A1* sequences reported by Beales et al. (2007). The samples with double fragments of 203 bp and 185 bp belonged to *Ppd-D1b* allele and samples with a 203-bp fragment belonged to *Ppd-D1a* allele when amplified with the marker Ppd-P11 (Fig. 4a). Based on the *Ppd-A1* sequences reported by Beales et al. (2007), we also developed another novel functional marker Ppd-P12 that generates a 534-bp fragment with the Chinese Spring allele and a 231-bp fragment with the Cappelle-Desprez (null) allele (Fig. 4b). Using a series of previously developed molecular markers as well as the two novel markers (Supplemental Table 3), allelic variations in the vernalization and photoperiod genes at seven loci (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Vrn-B3*, *Ppd-D1*, *Ppd-B1*, and *Ppd-A1*) were identified 375 CWG (including AMP), and 254 CL (Supplemental Table 4).

Screening of the AMP identified the following variations and the respective number of cultivars: three *Vrn-A1* alleles

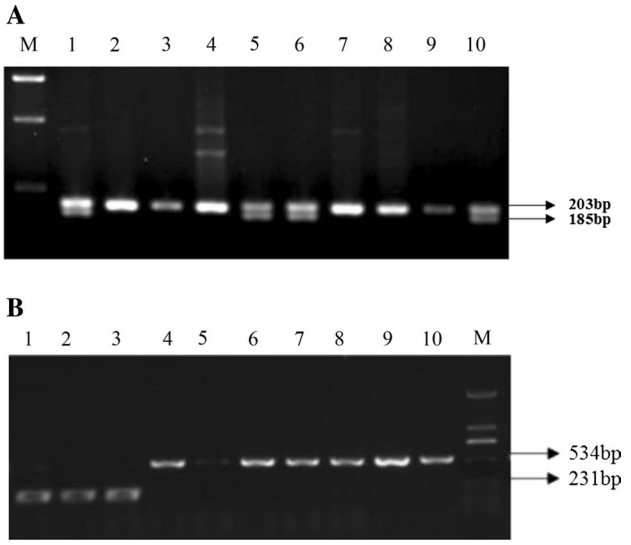

Fig. 4 Functional molecular marker developed for the identification of *Ppd-D1* alleles (**a** single fragment with the *Ppd-D1a* allele and double fragments with *Ppd-D1b* allele) and *Ppd-A1* alleles (**b** 580 bp for Chinese Spring allele and 274 bp for Cappelle-Desprez allele). A. M: 2000 Marker, 1: Chinese Spring, 2: Xichang 76-4, 3: Jinmai 50, 4: Yanzhan 4110, 5: Huabei 187, 6: Changle 5, 7: Aikang 58, 8: Yunong 035, 9: Shanhe 6, 10: Jimai 32. B. M: 2000 Marker, 1: Gaoyou 503, 2: Yuanfeng 898, 3: Yumai 47, 4: Chinese Spring, 5: Shi 84-7111, 6: Xinmai 9987, 7: Xinmai 19, 8: Gan 4589, 9: Neixiang 182, 10: Ji 923235

(*vrn-A1*, *Vrn-A1a*, and *Vrn-A1b* with 158, 3, and 2 cultivars), three *Vrn-B1* alleles (*vrn-B1*, *Vrn-B1a*, and *Vrn-B1b* with 155, 6, and 2 cultivars), four *Vrn-D1* alleles (*vrn-D1*, *Vrn-D1a*, *Vrn-D1b*, and *Vrn-D1c* with 107, 31, 24, and 1 cultivars), and two *Vrn-B3* alleles (*vrn-B3* and *Vrn-B3a* with 161 and 2 cultivars) at four *Vrn* loci; two *Ppd-D1* alleles (*Ppd-D1a* and *Ppd-D1b* with 156 and 7 cultivars), three *Ppd-D1* polymorphisms (TE insertion, 5-bp deletion, and 16-bp insertion with 3, 162, and 0 cultivars), three *Ppd-B1* polymorphisms (truncated Chinese Spring allele, intact Chinese Spring allele, and intact Sonora64/Timstein allele with 86, 49, and 48 cultivars), and two *Ppd-A1* alleles [Chinese Spring allele and Cappelle-Desprez (Null) allele with 145 and 18 cultivars]. To further verify the effect of the vernalization and photoperiod genes on heading and flowering dates, these allelic variations were subjected to a quality-controlled 90 K assay for GWAS analysis. The results showed a significant association between the *Ppd-D1* gene and heading and flowering dates in five and six environments, respectively; the *Vrn-D1* gene was significantly associated with heading and flowering dates in four and six environments, respectively; the *Vrn-B1* gene was significantly associated with heading and flowering dates in two and one environment, respectively (Table 1 and Fig. 2).

t test was further performed for the thirteen stable significant SNPs and seven vernalization and photoperiod

Table 1 *P* values of GWAS for significant SNPs, vernalization, and photoperiod genes in heading and flowering dates

Traits	FSWC contig	Allele	SNP/genes	2011–2012 ZZ	2012–2013 ZZ	2013–2014 ZZ	2014–2015 ZZ_r1	2014–2015 ZZ_r2	2014–2015 ZZ_r3	2015–2016 ZZ	2016–2017 ZZ_r1	2016–2017 ZZ_r2	2016–2017 ZZ_r3	2015–2016 ZMD	2016–2017 ZMD_r1	2016–2017 ZM_r2
	1DS_1914681	[A/G]	RAC875_ c41145_189	NS	7.28E−04	4.03E−04	NS	2.52E−04	NS	NS	NS	NS	NS	NS	NS	6.58E−04
	1DS_1885854	[T/C]	wsnp_Ex_ c17884_26647833	NS	NS	9.70E−04	NS	NS	NS	NS	6.64E−04	2.15E−04	7.71E−04	NS	NS	NS
	2BL_7991797	[A/C]	RAC875_ c50422_299	1.60E−05	2.82E−04	NS	NS	2.93E−04	1.33E−04	NS	4.04E−04	3.36E−04	NS	NS	NS	NS
	2BL_8017662	[T/C]	Excalibur_ c60164_137	NS	9.02E−04	5.16E−04	9.63E−04	7.70E−04	9.22E−04	NS	NS	NS	NS	NS	7.33E−04	3.48E−04
	2AS_5234765	[A/G]	RAC875_c829_611	NS	7.08E−05	NS	NS	NS	NS	4.33E−06	NS	7.61E−04	5.22E−04	6.05E−05	NS	NS
	2AS_5204476	[T/G]	Kukri_c5282_622	NS	7.08E−05	NS	NS	NS	NS	4.33E−06	NS	7.61E−04	5.22E−04	6.05E−05	NS	NS
	2AS_5309365	[A/G]	tplb0049o19_694	NS	4.38E−04	NS	NS	NS	NS	3.30E−04	NS	NS	8.83E−04	8.43E−05	NS	NS
	2DS_5358861	[T/C]	RAC875_ c48703_148	NS	4.75E−06	NS	NS	2.76E−05	7.70E−04	4.21E−06	NS	NS	NS	NS	NS	7.91E−04
	4BS_4947165	[T/C]	Tdurum_con-tig29563_183	NS	2.86E−05	NS	NS	NS	NS	5.94E−06	NS	NS	NS	2.94E−04	NS	NS
Heading	5BL_10867241	[A/G]	Excalibur_rep_ c107908_308	NS	7.70E−04	2.89E−04	NS	NS	NS	9.25E−04	NS	NS	NS	2.59E−04	NS	NS
	5DS_4604746	[T/G]	Excalibur_ c16573_197	NS	NS	NS	NS	NS	NS	NS	2.24E−04	2.91E−04	1.54E−04	1.92E−04	NS	NS
	6DS_2114340	[G/C]	IAAV6834	NS	7.56E−04	4.48E−05	NS	NS	NS	NS	4.93E−05	8.26E−04	NS	NS	NS	7.18E−04
	5BL	*vrn-B1/ Vrn-B1a*	*Vrn-B1*	NS	NS	NS	NS	7.98E−04	NS	NS	8.08E−03	NS	NS	NS	NS	NS
	5DL	*vrn-D1/ Vrn-D1a*	*Vrn-D1*	NS	NS	5.84E−05	2.63E−03	5.96E−04	NS	NS	NS	NS	NS	NS	NS	7.37E−05
	2DS	*Ppd-D1a/Ppd-D1 Ppd-D1b*		NS	NS	NS	NS	NS	NS	4.82E−06	NS	NS	2.80E−03	7.47E−04	NS	8.34E−03
	2AS_5234765	[A/G]	RAC875_c829_611	NS	NS	NS	NS	NS	NS	2.93E−06	5.87E−04	4.66E−05	7.91E−05	2.57E−05	NS	NS
	2AS_5204476	[T/G]	Kukri_c5282_622	NS	NS	NS	NS	NS	NS	2.93E−06	5.87E−04	4.66E−05	7.91E−05	2.57E−05	NS	NS
	2AS_5309365	[A/G]	tplb0049o19_694	NS	4.38E−04	NS	NS	NS	NS	3.30E−04	NS	NS	8.83E−04	8.43E−05	NS	NS
	2DS_5358861	[T/C]	RAC875_ c48703_148	NS	NS	NS	NS	2.32E−04	NS	1.97E−05	1.78E−04	5.53E−04	NS	1.09E−05	NS	7.91E−04
Flowering	2BL_7991797	[A/C]	RAC875_ c50422_299	5.71E−05	6.60E−04	1.48E−04	NS	NS	NS	NS	NS	NS	NS	NS	4.75E−04	3.36E−04
	6AL_5833089	[A/G]	RAC875_ c14659_1066	NS	3.46E−04	NS	NS	NS	NS	NS	5.34E−04	1.04E−04	2.66E−04	NS	NS	NS
	5BL	*vrn-B1/ Vrn-B1a*	*Vrn-B1*	NS	NS	NS	NS	NS	NS	NS	8.95E−03	NS	NS	NS	NS	NS

Table 1 (continued)

Traits	FSWC contig	Allele	SNP/genes	2011–2012 ZZ	2012–2013 ZZ	2013–2014 ZZ	2014–2015 ZZ_r1	2014–2015 ZZ_r2	2014–2015 ZZ_r3	2015–2016 ZZ	2016–2017 ZZ_r1	2016–2017 ZZ_r2	2016–2017 ZZ_r3	2015–2016 ZMD	2016–2017 ZMD_r1	2016–2017 ZM_r2
	5DL	*vrn-D1*/ *Vrn-D1a*	*Vrn-D1*	2.73E−03	1.29E−04	1.40E−05	2.10E−04	NS	NS	NS	NS	NS	NS	NS	9.59E−03	7.37E−05
	2DS	*Ppd-D1a*/*Ppd-D1* *Ppd-D1b*	*Ppd-D1*	NS	NS	NS	NS	9.60E−03	NS	1.25E−03	NS	3.82E−03	1.50E−04	1.49E−03	NS	8.34E−03

ZZ Zhengzhou region, *ZMD* Zhumadian region, *r1* repeat 1, *r2* repeat 2, *r3* repeat 3, *NS* indicate not significant, *FSWC contig* chromosome and contig of SNP

loci grouped by polymorphism, and the result showed that seven SNPs and three genes had significant difference of phenotype for heading and flowering dates in the AMP (Table 2). The results suggested that cultivars with the AA, CC, CC, GG, GG, TT, and CC alleles had the earlier heading and flowering dates by 2, 1, 2, 4, 4, 4, and 3 days than cultivars with the GG, CT, AC, AG, TG, TC, and TC alleles at the loci of RAC875_c41145_189, Excalibur_c60164_137, RAC875_c50422_299, RAC875_c829_611, Kukri_c5282_622, RAC875_c48703_148, and Tdurum_contig29563_183, respectively. In addition, compared to cultivars with the *Ppd-D1b*, recessive *vrn-B1*, and recessive *vrn-D1* alleles, cultivars with the *Ppd-D1a*, *Vrn-B1a*, and *Vrn-D1a* alleles had significantly early heading dates by 4 days, 2 days, and 2 days and early flowering dates by 4 days, 3 days, and 2 days, respectively.

To determine the potential utility of the *Ppd-D1*, *Vrn-B1*, and *Vrn-D1* alleles to accelerate heading and flowering in wheat breeding, we evaluated their effect on phenotype in the AMP, CWG, and CL populations. The average heading date of cultivars with the *Ppd-D1a* and *Ppd-D1b* alleles were 187.7 days and 190.8 days ($P < 0.05$) in the AMP, 188.7 days and 195.3 days ($P < 0.05$) in the CWG, and 196.6 days and 200.7 days ($P < 0.05$) in the CL, respectively. The average flowering date of cultivars with the *Ppd-D1a* and *Ppd-D1b* alleles was 193.3 days and 196.5 days ($P < 0.05$) in the AMP, 195 days and 200.9 days ($P < 0.05$) in the CWG, and 203.6 days and 206.8 days ($P < 0.05$) in the CL, respectively (Fig. 5a). The cultivars with the recessive allele *vrn-D1* showed significantly later heading and flowering dates than those with the *Vrn-D1a* allele in the AMP, CWG, and CL populations and cultivars with the *Vrn-D1b* alleles in the AMP and CWG populations (Fig. 5c). The cultivars with the recessive allele *vrn-B1* showed significantly later heading and flowering dates than those with the *Vrn-B1a* alleles in the AMP and CWG populations and cultivars with the *Vrn-B1b* alleles in the CWG population (Fig. 5b).

To better illustrate the relationship of the vernalization and photoperiod alleles and stable SNPs with heading and flowering dates, multiple linear regression equations were eventually established with eleven vernalization and photoperiod alleles and average heading and flowering dates of multiple environments (13 in the AMP, 6 in CWG, and 2 in CL), additionally, three SNPs remained to distinguish from vernalization and vernalization genes by comparing their chromosome locations in the equation below (details are presented in Supplemental Table 5). To establish a multiple linear regression equation, all recessive alleles were designated as 0, and dominant alleles were represented by values ranging from 1 to 3 (e.g., at the *Vrn-D1* locus, 0 for recessive allele *vrn-D1*, 1 for *Vrn-D1a*, 2 for *Vrn-D1b*, and 3 for *Vrn-D1c*; at the RAC875_c41145_189, 0 for GG allele, 1 for AG allele, 2 for AA allele, and 3 for NN)

Table 2 *P* values of *t* test for significant SNPs, vernalization, and photoperiod genes in heading and flowering dates

SNP/gene name	Chr	Allele	Number	HD/FD		*P* value of *t* test			
						2011–2012 ZZ	2012–2013 ZZ	2013–2014 ZZ	2014–2015 ZZ_r1
RAC875_c41145_189	1DS	AA/GG	22/99	186/192	188/194	0.001/0.000	0.000/0.000	0.000/0.000	0.001/0.000
Excalibur_c60164_137	2BL	CC/CT	26/96	186/192	187/193	0.012/0.008	0.001/0.044	0.042/0.010	0.002/0.001
RAC875_c50422_299	2BL	CC/AC	82/71	186/193	188/195	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000
RAC875_c829_611	2AS	AG/GG	10/151	191/197	187/193	0.008/0.002	0.000/0.000	0.002/0.002	0.000/0.001
Kukri_c5282_622	2AS	TG/GG	10/151	191/197	187/193	0.008/0.002	0.000/0.000	0.002/0.002	0.000/0.001
RAC875_c48703_148	2DS	TT/TC	150/9	187/193	191/197	0.011/0.001	0.001/0.000	0.029/0.000	0.001/0.000
Tdurum_contig29563_183	4BS	CC/TC	151/11	187/193	190/196	0.029/0.003	0.005/0.001	0.119/0.003	0.001/0.002
Ppd-D1	2DS	*Ppd-D1a/Ppd-D1b*	156/7	187/193	191/197	0.067/0.010	0.000/0.002	0.001/0.002	0.006/0.010
Vrn-B1	5BL	*vrn-B1/Vrn-B1a*	155/6	187/194	185/191	0.033/0.034	0.007/0.002	0.554/0.224	0.002/0.002
Vrn-D1	5DL	*vrn-D1/Vrn-D1a*	107/31	188/194	186/192	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000

SNP/gene name	*P* value of *t* test								
	2014–2015 ZZ_r2	2014–2015 ZZ_r3	2015–2016 ZZ	2015–2016 ZMD	2016–2017 ZZ_r1	2016–2017 ZZ_r2	2016–2017 ZZ_r3	2016–2017 ZMD_r1	2016–2017 ZMD_r2
RAC875_c41145_189	0.001/0.012	0.001/0.000	0.185/0.050	0.008/0.000	0.000/0.000	0.001/0.020	0.115/0.157	0.011/0.138	0.006/0.015
Excalibur_c60164_137	0.000/0.001	0.001/0.007	0.028/0.047	0.006/0.008	0.003/0.001	0.000/0.002	0.267/0.232	0.008/0.019	0.001/0.007
RAC875_c50422_299	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.001	0.000/0.001	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000
RAC875_c829_611	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000
Kukri_c5282_622	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000
RAC875_c48703_148	0.003/0.000	0.000/0.000	0.001/0.000	0.013/0.000	0.007/0.000	0.011/0.000	0.003/0.000	0.001/0.000	0.000/0.000
Tdurum_contig 29563_183	0.005/0.000	0.002/0.000	0.003/0.000	0.018/0.000	0.013/0.000	0.018/0.000	0.018/0.000	0.012/0.003	0.007/0.000
Ppd-D1	0.001/0.000	0.002/0.002	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.005/0.003	0.000/0.000
Vrn-B1	0.000/0.001	0.000/0.005	0.061/0.017	0.001/0.003	0.004/0.004	0.003/0.007	0.173/0.104	0.096/0.099	0.073/0.073
Vrn-D1	0.000/0.000	0.000/0.000	0.049/0.001	0.001/0.000	0.001/0.000	0.001/0.000	0.089/0.026	0.000/0.000	0.000/0.000

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + b_{11}X_{11} + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14},$$

where Y is heading/flowering date; X_1 is *Vrn-A1*; X_2 is *Vrn-B1*; X_3 is *Vrn-D1*; X_4 is *Vrn-B3*; X_5 is *Ppd-D1a/Ppd-D1b*; X_6 is a TE insertion at the *Ppd-D1* locus; X_7 is a 5-bp insertion/deletion at the *Ppd-D1* locus; X_8 a 425-bp insertion/deletion at the *Ppd-B1* locus; X_9 is 994-bp insertion/deletion at the *Ppd-B1* locus; X_{10} is a 223-bp insertion/deletion at the *Ppd-B1* locus; X_{11} is *Ppd-A1*; X_{12} is RAC875_c41145_189; X_{13} is Excalibur_c60164_137; X_{14} is RAC0875_c50422_299; b_0 is the regression intercept; and b_1–b_{14} are the multiple linear regressive coefficients.

By eliminating insignificant loci using a stepwise regression method, the optimal multiple linear regression equations were established (Table 3), and path coefficients (p_i)

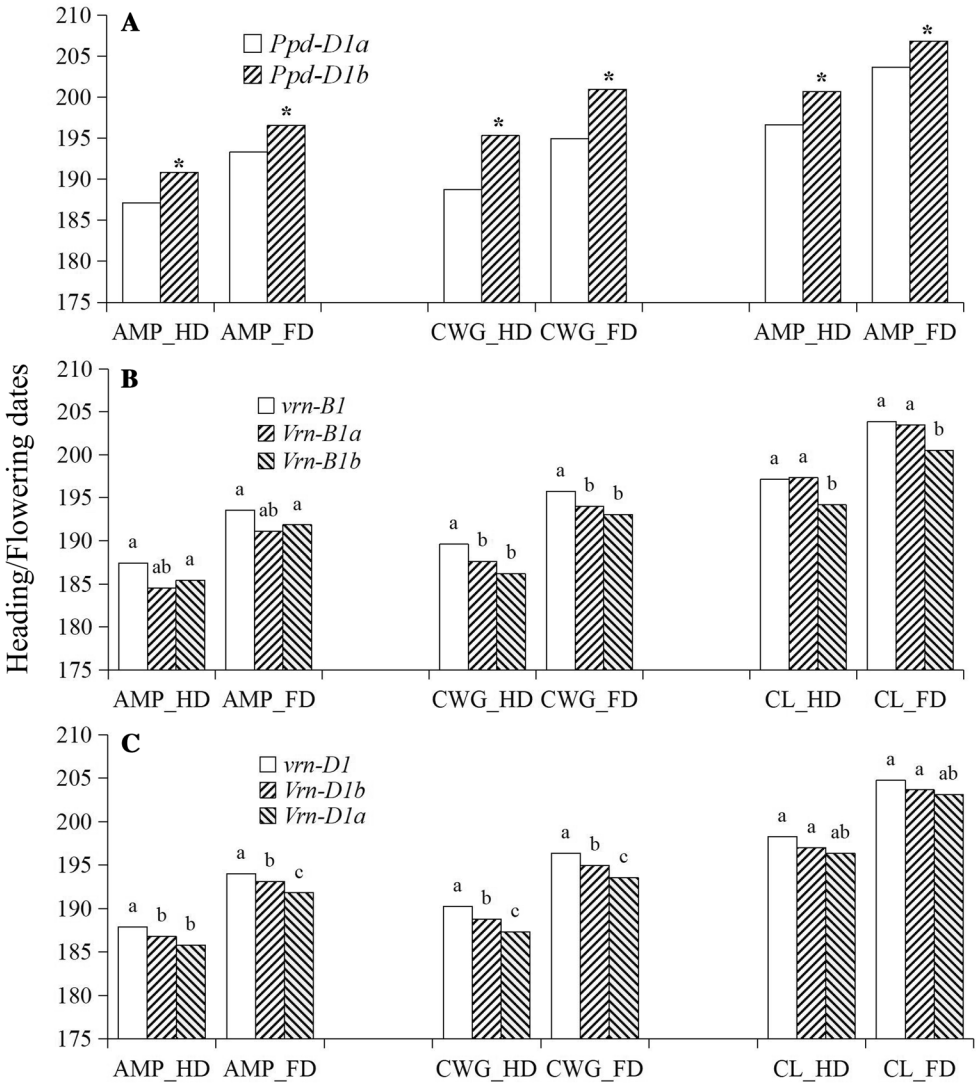

Fig. 5 The heading and flowering dates of different *Ppd-D1* (**a**), *Vrn-B1* (**b**), and *Vrn-D1* (**c**) alleles in three wheat populations (AMP, CWG, and CL). **a**, **b** Significant level. *AMP* association mapping population, *CWG* Chinese wheat germplasm, *CL* Chinese landrace, *HD* heading date, *FD* flowering date

were calculated to evaluate the effects of significant loci, and partial regression and path coefficients of the *Ppd-D1* gene were the largest in AMP and CWG populations. Taken together, results suggested that the *Ppd-D1* gene plays the most important role in modulating heading and flowering dates in modern Chinese winter wheat.

QTL mapping indicates that the *Ppd-D1* gene plays a key role in the RIL population

To further determine the influence of the *Ppd-D1* alleles on heading and flowering dates in common wheat, the F_{10} RIL population Proteo × Chaja that is composed of 97 lines was further used to map QTLs that control heading and flowering dates (Supplemental Table 6). Linkage analysis mapped a major QTL for heading and flowering dates to chromosome 2DS, between the markers Ppd-D1 and WSNP_CAP11_c3842_1829821 that were derived from a 9 K chip, which explained 28.8% of the heading date and 34.0% of the flowering date, with a logarithm of odds (LOD) score of 8.6 and 10.3 in this F_{10} population. Additionally, this QTL was also significantly associated with spike length, peduncle length, fertile spikelets number, cold resistance, and tiller number (Fig. 6 and Table 4). Identification of *Ppd-D1* alleles using the Ppd-P11 marker showed that 53 and 44 out of 97 lines belonged to the *Ppd-D1a* and *Ppd-D1b* alleles, respectively. Analysis of association of the *Ppd-D1* alleles with heading and flowering dates indicated that the average heading date (189.9 days) and flowering date (197.9 days) of the lines with the *Ppd-D1a* allele were significantly earlier than those of lines with the *Ppd-D1b* allele (193.2 days and 200.1 days, respectively; $P < 0.05$).

Table 3 The partial regression intercept and coefficient and path coefficient in the optimal linear regression equation of AMP, CWG, and CL populations

b_i/p_i	HD/FD	Intercept	*Vrn-A1*		*Vrn-B1*		*Vrn-D1*		*Vrn-B3*		*Ppd-D1a/b*		*Ppd-D1*			*Ppd-B1*				*Ppd-A1*		RAC875_c41145_189		Excalibur_c60164_137		RAC875_c50422_299	
													TE insertion		5 bp deletion	425 bp		994 bp	223 bp							
		($b0$)	b_1	p_1	b_2	p_2	b_3	p_3	b_4	p_4	b_5	p_5	b_6	p_6	b_7	p_7	b_8	p_8	b_9	p_9	b_{10}	p_{10}	b_{11}	p_{11}	b_{12}	p_{12}	b_{13}	p_{13}	b_{14}	p_{14}
AMP	HD (Y_1)	187.5	–	–	−1.78	−0.22	−0.70	−0.23	–	–	3.42	0.30	–	–	–	–	–	–	–	–	–	–	1.20	0.16	−0.53	−0.19	−0.56	−0.17	−0.67	−0.17
	FD (Y_2)	193.6	–	–	−1.51	−0.22	−0.63	−0.24	–	–	2.99	0.31	–	–	–	–	–	–	–	–	–	–	0.93	0.15	−0.54	−0.23	−0.52	−0.19	−0.46	−0.14
CWG	HD (Y_3)	191.1	–	–	−1.85	−0.2	−0.76	−0.16	–	–	3.48	0.26	−3.46	−0.23	2.36	0.13	0.92	0.1	–	–	1.22	0.14	1.5	0.13	–	–	–	–	–	–
	FD (Y_4)	196.9	–	–	−1.55	−0.18	−0.68	−0.15	–	–	3.14	0.25	−3.06	−0.22	2.28	0.14	0.98	0.12	–	–	117	0.15	1.35	0.13	–	–	–	–	–	–
CL	HD (Y_5)	191.2	–	–	−2.23	−0.13	–	–	–	–	3.59	0.25	–	–	–	–	3.73	0.27	–	–	3.79	0.24	–	–	–	–	–	–	–	–
	FD (Y_6)	199.7	–	–	−2.51	−0.18	–	–	–	–	2.84	0.24	–	–	–	–	2.95	0.25	–	–	2.65	0.20	–	–	–	–	–	–	–	–

AMP association mapping population, *CWG* Chinese wheat germplasm, *CL* Chinese landrace, *HD* heading date, *FD* flowering date, b_0 regression intercept, b_i partial regression coefficient, p_i path coefficient, X_1 *Vrn-A1*, X_2 *Vrn-B1*, X_3 *Vrn-D1*, X_4 *Vrn-B3*, X_5 *Ppd-D1*, X_6 TE insertion, X_7 5 bp deletion, X_8 425 bp, X_9 994 bp, X_{10} 223 bp, X_{11} *Ppd-A1*, X_{12} is RAC875_c41145_189, X_{13} is Excalibur_c60164_137, X_{14} is RAC875_c50422_299, Y_1 AMP_HD, Y_2 AMP_FD, Y_3 CWG_HD, Y_4 CWG_FD, Y_5 CL_HD, Y_6 CL_FD

'–' indicated that data were missing

Discussion

In China, wheat is mainly planted in 10 agro-ecological zones that are further divided into 26 sub-zones, with winter, facultative, and spring wheat sown in autumn or spring (Zhuang 2003), and winter wheat occupying more than 85% of the total area and production of Chinese wheat. Of all agro-ecological zones, the Yellow and Huai wheat production region is the most important and largest wheat production zone, contributing 60–70% of both total harvested area and total wheat production (Chen et al. 2013a, b). In the Yellow and Huai wheat production region, the grain-filling stage of winter wheat cultivars usually lasts approximate 45 days from late April to early June; this may fluctuate according to the environmental characteristics among different regions or provinces. Therefore, farmers would generally plant early-maturing wheat cultivars in their fields to avoid the frequent dry-hot winds in late May or early June and to meet the next crop in June, particularly in Henan, which is the most important wheat production province in China.

Heading and flowering dates influence wheat adaptation and yield, and they are complex quantitative traits that mainly affected by photoperiod response genes, vernalization response genes, and earliness per se genes (*Eps*). Previous findings showed that genes associated with heading and flowering are mainly distributed on chromosomes 5A (Yan et al. 2003, 2004), 5B and 5D (Yan et al. 2004), 7B (Yan et al. 2006), 2A, 2B and 2D (Welsh et al. 1973; Law and Worland 1997; Beatles et al. 2007), 1A, and 3A (Lewis et al. 2008; Gawroński et al. 2014). Recently, GWAS has become an efficient way for identifying multiple genes that are responsible for heading and flowering in common wheat, which in turn facilitates in the development of valuable genetic markers for molecular breeding. Previously, some SNPs on chromosomes 1B, 3D, and 7D have been identified to be significantly associated with heading date except for photoperiod and vernalization loci (Zanke et al. 2014; Ain et al. 2015). In this study, SNPs significantly associated with heading date were identified on almost all of the chromosomes and were mainly distributed on chromosomes 2A, 2B, 2D, 5A, 5B, 6A, 6D, 7A, and 7B, and those identified on 2AS, 2BL, 2DS, 5AL and 6DS had significant effects on flowering date.

Of all the significant SNPs identified in this study, there are 13 environmentally stable genetic loci (*P* values indicating statistical significance in at least four environments) that may require further investigation. Furthermore, we compared the heading and flowering dates of cultivars with different alleles of the above-mentioned novel, stable SNPs. At the RAC875_c41145_189 locus on 1DS, cultivars with the AA allele both headed and flowered

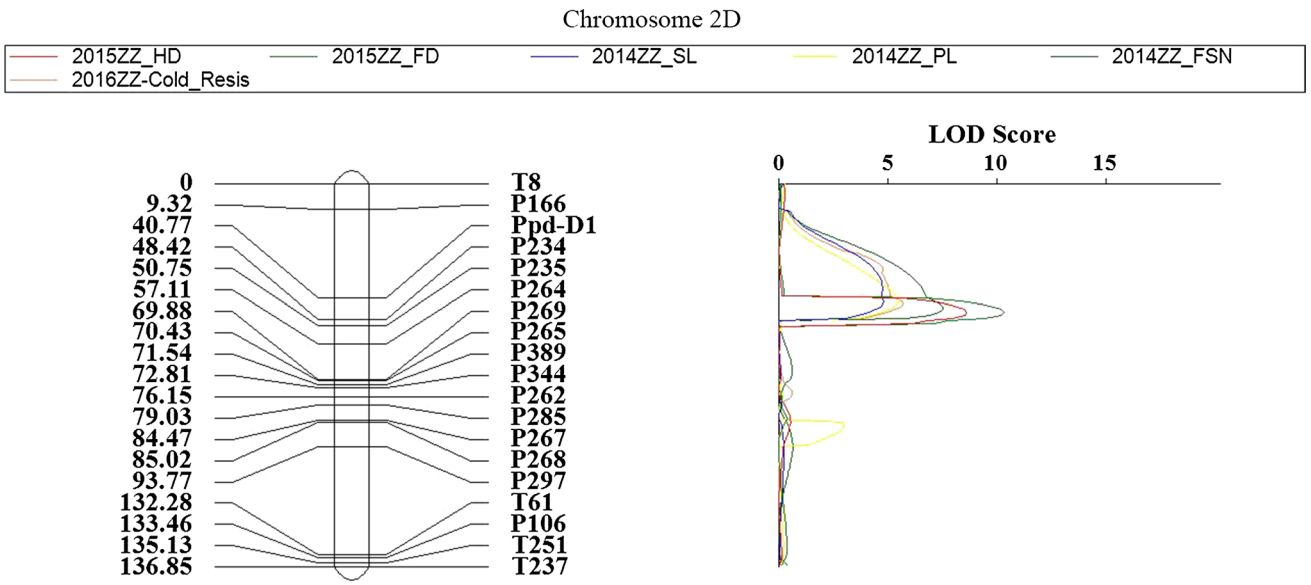

Fig. 6 QTL mapping of the *Ppd-D1* gene on chromosome 2D in the F_{10} RIL population of Proteo × Chaja. *HD* heading date, *FD* flowering date, *SL* spike length, *PL* peduncle length, *FSN* fertile spikelets number, *TN* tiller number. *T8* wsnp_Ex_rep_c70458_69393028, *P166* wsnp_JD_c69_109951, *P235* WSNP_CAP12_c812_428290, *P234* WSNP_CAP11_c3842_1829821, *P264* WSNP_CAP12_c1503_764765, *P269* wsnp_Ex_c1944_3664205, *P265* wsnp_Ku_c12022_19520410, *P389* WSNP_BM140538D_Ta_2_1, *P344* wsnp_BE488779D_Ta_1_2, *P262* wsnp_Ra_c67199_65253620, *P285* wmc18, *P268* WSNP_RFL_Contig3960_4401914, *P267* WSNP_RFL_Contig4134_4692458, *P297* gwm539, *T61* wsnp_Ex_c10411_17037327, *P106* wsnp_Ex_c53729_56868062, *T251* wsnp_Ex_c979_1874338, *T237* wsnp_RFL_Contig2324_1803878

Table 4 QTL mapping on the Proteo × Chaja (PC) population

Chromosome	Marker	Trait	Years	LOD	PVE (%)	Add
2D	Ppd-D1-WSNP_CAP11_c3842_1829821	HD	2015	8.6	28.8	2.1
	Ppd-D1-WSNP_CAP11_c3842_1829821	FD	2015	10.3	34.0	1.5
	Ppd-D1-WSNP_CAP11_c3842_1829821	SL	2014	4.8	14.1	0.5
	Ppd-D1-WSNP_CAP11_c3842_1829821	PL	2014	5.5	4.1	1.5
	Ppd-D1-WSNP_CAP11_c3842_1829821	FSN	2014	7.5	24.9	0.8
	Ppd-D1-WSNP_CAP11_c3842_1829821	Cold_Resistance	2016	5.7	24.3	−0.5
	Ppd-D1-WSNP_CAP11_c3842_1829821	TN	2016	2.7	11.3	2.4

HD heading date, *FD* flowering date, *SL* spike length, *PL* peduncle length, *FSN* fertile spikelets number, *TN* tiller number

earlier by 2 days than cultivars with the GG allele; at the Excalibur_c60164_137 locus on 2BL, cultivars with the CC allele both headed and flowered earlier by 1 day than cultivars with the CT allele; at the RAC875_c50422_299 locus on 2BL, cultivars with the CC allele both headed and flowered earlier by 2 days than cultivars with the AC allele; at the RAC875_c829_611 locus on 2AS, cultivars with the AG allele both headed and flowered earlier by 4 days than cultivars with the GG allele; at the Kukri_c5282_622 locus on 2AS, cultivars with the TG allele both headed and flowered earlier by 4 days than cultivars with the GG allele; at the RAC875_c48703_148 locus on chromosome 2DS, cultivars with the TT allele both headed and flowered earlier by 4 days than the TC allele; and at the Tdurum_contig29563_183 locus on chromosome 4BS, cultivars with the CC allele both headed and flowered earlier by 3 days than the TC allele. These environmentally stable genetic loci could be utilized in marker-assisted selection in adaption and high-yield breeding programs in Yellow and Huai wheat.

Vernalization and photoperiod response genes influence wheat flowering and maturity. Cultivars with the *Vrn-A1a* allele flowered earlier than cultivars with the *Vrn-B1* or *Vrn-D1* alleles in non-vernalizing conditions in Pakistani wheat (Iqbal et al. 2012), wherein *Vrn-A1a* is the predominant allele in CIMMYT wheat (Yan et al. 2004) and *vrn-A1* is the predominant allele in Chinese winter wheat (Chen et al. 2013a). Cultivars with the *Vrn-D1a* allele

headed and flowered earlier than the cultivars with other *Vrn-D1* alleles in Australian wheat (Eagles et al. 2010; Cane et al. 2013) and Chinese wheat (Zhang et al. 2015a). Cultivars with the recessive *vrn-B3* headed and flowered later than cultivars with the dominant *Vrn-B3* in Chinese winter wheat (Chen et al. 2013a). The combination of *vrn-A1/vrn-B1b/vrn-D1a/vrn-B3* is predominant in the Yellow and Huai winter wheat, and the cultivars with this combination show relatively later heading and flowering dates than those with other combinations (Zhang et al. 2015a). The photoperiod-insensitive *Ppd-D1a* allele is predominant in CIMMYT and Chinese wheat, and cultivars with the *Ppd-D1a* allele headed and flowered early under both long days and short days. Among the five *Ppd-D1* haplotypes (*Ppd-HapI-V*) reported by Guo et al. (2010), *Ppd-Hap-III* is expressed at a very low level and showed later heading, whereas *Ppd-Hap-I* is highly expressed and showed earlier heading. Further studies showed that *Ppd-Hap-I* is predominantly presented and headed earlier than other haplotypes in the Yellow and Huai wheat region, and cultivars with *Ppd-B1_Hapl-VI* had the earliest heading and flowering dates among all *Ppd-B1* haplotypes (Chen et al. 2013a; Zhang et al. 2015a). In this study, we identified seven loci on vernalization and photoperiod alleles associated with heading and flowering dates, and our results show that the *Ppd-D1* gene had the most important effect on heading and flowering, followed by the *Vrn-B1* and *Vrn-D1* genes. At the *Ppd-D1* locus, cultivars with the *Ppd-D1a* allele both headed and flowered earlier by 4 days than cultivars with the *Ppd-D1b* allele. At the *Vrn-B1* locus, cultivars with the *Vrn-B1a* allele headed and flowered earlier by 2 and 3 days than those with the *vrn-B1* allele. At the *Vrn-D1* locus, cultivars with the *Vrn-D1a* allele both headed and flowered earlier by 2 days than cultivars with the recessive allele *vrn-D1*. Therefore, further screening of the relatively superior genotypes in view of vernalization and photoperiod response genes would be beneficial in improving the adaptability of bread wheat.

The present study conducted linkage analysis, which indicated that the major LOD at the *Ppd-D1* locus controlling heading and flowering was also significantly associated with peduncle length, spike length, fertile spikelets, cold resistance, and tiller number in the F_{10} RIL population (Proteo × Chaja). Previous studies (Strampelli 1932; Worland et al. 2001; Ellis et al. 2007; Wurschum et al. 2017) showed that the *Ppd-D1* gene was intimately linked to other agronomically important genes on 2DS, such as the *Rht8* gene, a gibberellic acid-responsive dwarfing gene. Therefore, the QTL containing *Ppd-D1* and *Rth8* genes not only affects heading and flowering but also reduces plant height and tiller number, and which is also associated with other adaptation-related phenotypes (Borner et al. 1993; Worland et al. 1998a, b; Yang et al. 2009). However, independent influence of *Ppd-D1* and *Rht8* on differently agronomic traits still needs to be further researched.

Based on the association of vernalization and photoperiod alleles and three novel significant SNPs with heading and flowering dates in wheat cultivars, we propose an optimal linear regression equation that could predict heading and flowering dates by vernalization and photoperiod alleles and significant SNPs in Chinese wheat cultivars. We further evaluated the effects of the seven loci and seven SNPs by path coefficients (p_i), and found that *Ppd-D1*, *Vrn-B1*, *Vrn-D1* genes, and three stable SNPs (RAC875_c41145_189, Excalibur_c60164_137, and RAC0875_c50422_299) are significant and vital factors, suggesting that these three vernalization and photoperiod loci and three novel SNPs should be given priority in efforts to improve heading and flowering dates of wheat cultivars in the Yellow and Huai wheat region. Among these above six loci, the path coefficient of the *Ppd-D1* gene was the highest in the AMP populations, indicating that the *Ppd-D1* gene plays the most important role in heading and flowering dates in Chinese wheat.

Genetic studies have shown that the most effective photoperiod response gene is the *Ppd-D1* gene, followed by *Ppd-B1* and *Ppd-A1* (Worland et al. 1998a), although the theory that *Ppd-B1a* could be as strong as *Ppd-D1* remains controversial (Tanio and Kato 2007). In this study, we compared the effect of *Ppd-B1a* with that of the *Ppd-D1* genes in Chinese wheat based on the following two aspects. (1) Cultivars with the *Ppd-D1a* allele exhibit significantly earlier heading and flowering dates by 4 days than the *Ppd-D1b* allele, and this difference is statistically significant based on the *t* test involving the three populations (AMP, CWG, and CL), whereas the heading or flowering dates of cultivars with three *Ppd-B1a* polymorphisms (Truncated Chinese Spring allele, Intact Chinese Spring allele, and Intact Sonora64/Timstein allele) had only 0.3, 0.5, and 0.3 days difference between the presence and absence at the three *Ppd-B1a* loci, respectively. (2) We established the multiple linear regression equations and optimal multiple linear regression equations based on the vernalization and photoperiod alleles and calculated the average heading and flowering dates in multiple environments, which showed that the path coefficient of *Ppd-D1* is higher than the three *Ppd-B1a* loci in three populations surveyed, suggesting that *Ppd-D1* plays the most important role in heading and flowering dates in Chinese wheat. In addition, GWAS and regression analysis indicate that the role of *Ppd-A1* is as important as that of *Ppd-B1* in CWG. Masako et al. (2011) indicated that *Ppd-B1a* has a significant effect in the genetic background with *Ppd-D1a* for the *Ppd-B1a/Ppd-D1a* genotype heading 6.7 days earlier than the *Ppd-B1b/Ppd-D1a* genotype on Japanese wheat. The *Ppd-D1a* allele occupies 66.0% in all cultivars, and the frequency of improved cultivars is as high as 90.6% in Chinese wheat (Yang et al. 2009), suggesting that the *Ppd-D1* gene plays crucial roles in the adaptation of common wheat in China.

The present study identified a large number of genetic loci that were related to heading and flowering dates in wheat with the 90 K iSelect SNP genotyping assay using GWAS and found 13 possibly novel environmentally stable loci different from vernalization and photoperiod genes. Among the seven common vernalization and photoperiod loci, the *Ppd-D1*, *Vrn-B1*, and *Vrn-D1* genes showed greater influence on heading and flowering dates; among these novel loci, RAC875_c41145_189, Excalibur_c60164_137, and RAC0875_c50422_299 showed important effect on heading and flowering dates; thus, they should be given greater consideration in the selection of heading and flowering dates of wheat cultivars in the Yellow and Huai wheat region.

Author contribution statement FC designed the research. XZ, JC, YY, and CS performed genotyping and phenotyping. XZ and LZ performed data analysis. XZ and FC wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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