Gene regulatory network and abundant genetic variation play critical roles in heading stage of polyploidy wheat

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Abstract

Background: The extensive adaptability of polyploidy wheat is attributed to its complex genome, and accurately controlling heading stage is a prime target in wheat breeding process. Wheat heading stage is an essential growth and development processes since it starts at a crucial point in the transition from vegetative phase to reproductive phase.

Main body: Heading stage is mainly decided by vernalization, photoperiod, hormone (like gibberellic acid, GA), and earliness per se (Eps). As a polyploidy species, common wheat possesses the abundant genetic variation, such as allelic variation, copy number variation etc., which have a strong effect on regulation of wheat growth and development. Therefore, understanding genetic manipulation of heading stage is pivotal for controlling the heading stage in wheat. In this review, we summarized the recent advances in the genetic regulatory mechanisms and abundant variation in genetic diversity controlling heading stage in wheat, as well as the interaction mechanism of different signals and the contribution of different genetic variation. We first summarized the genes involved in vernalization, photoperiod and other signals cross-talk with each other to control wheat heading stage, then the abundant genetic variation related to signal components associated with wheat heading stage was also elaborated in detail.

Conclusion: Our knowledge of the regulatory network of wheat heading can be used to adjust the duration of the growth phase for the purpose of acclimatizing to different geographical environments.

Keywords: Polyploidy wheat, Heading stage, Vernalization, Photoperiod, Gene regulatory network, Genetic variation

Background

Wheat (Triticum aestivum L.) is widely cultivated across the globe, where it has adapted to different environments as a result of its natural diversity and complex genome. Bread wheat is a polyploid species with an AABBDD genome, and genome A, B, and D respectively originated from T. urartu, Aegilops speltoides, and Ae. Tauschii [1–3]. Various cultivars of wheat possess different growth characteristics to endure external stress and adapt to different climatic conditions and geographical environments through regulating their heading stage [4].

Heading occurs in the transition of wheat from the vegetative stage to the reproductive stage. As one of the most important agronomic traits of wheat, the heading days of each cultivar were calculated from the sowing date to the date of more than half of the plants has been heading [5]. The duration of the heading stage determines the flowering time of wheat, which subsequently impacts wheat yield. Vernalization, photoperiod, and exogenous hormones constitute exogenous factors that influence heading stage, while endogenous hormone and narrow-sense earliness per se (Eps) as an endogenous factor influences the duration of the wheat heading stage [6–12].

Main text

Part 1. Different signals cross-talk to control wheat heading stage

Interaction among the genes related to vernalization, photoperiod, hormone, and Eps to construct a regulatory network determine wheat growth and development as
The wheat vernalization regulatory network is mainly controlled by three pivotal genetic loci: Vernalization1 (Vrn-A1, Vrn-B1, Vrn-D1), Vernalization2 (Vrn-A2), and Vernalization3 (Vrn-B3) [16–18]. These genes have been cloned and validated to directly or indirectly take part in the vernalization pathway. Vrn1 was cloned by chromosome walking in diploid wheat and encodes a MADS-box transcription factor, which is homologous to the Arabidopsis APETALA1 with a protein-binding site (CArG-box) in the promoter region [16]. Vrn1 can directly activate Vrn-B3 expression under LD conditions [19, 20]. It could be significantly up-regulated by prolonged exposure to a cold environment and directly accelerates the transition to reproductive development at the shoot apex, but Vrn1 is not essential gene of wheat flowering since Vrn1 mutants (with premature stop codon of Vrn-A1, Vrn-B1 and Vrn-D1) can flower and produce seeds under both vernalized and unvernalized conditions, suggesting the existence of other wheat flowering association genes that make wheat cultivars preferable adapted to vagaries of climate [21]. When a winter wheat carries the 3 recessive vrn-1 (Vrn-A1, Vrn-B1 and Vrn-D1) alleles, transition is significantly accelerated by vernalization treatment [22, 23]. Voss-Fels et al. (2018) showed that six SNP markers of a highly significant QTL for nodal root angle index on chromosome 5B was identified to have strong linkage disequilibrium to Vrn-B1, and thus speculate that Vrn1 was possibly involved in the regulation of plant morphology in wheat and barley [24]. In addition, Wheat vegetative to reproductive transition-1 (TaVRT-1) and Wheat APETALA1 (WAP1) were identified to be highly similar to Vrn1, and these three genes maybe the same locus. They were identified on chromosome 5A, 5B and 5D, and encode an APETALA1 (API)-like MADS box gene. They are all not only induced by vernalization and photoperiod but also are involved in the phase transition and are maintained during the reproductive phase [16, 22, 23].

Histone modification is an important post-translational process in epigenetic regulation, and it can influence various phenotype by changing gene expression [25, 26]. Methylation modification of histone lysine mainly occurs...
in the 4, 9, 27, and 36 lysine residues of histone H3 in eu-
karyotes. H3K9me and H3K27me have been confirmed to be related to the inhibition of gene expression, but H3K4me and H3K36me were related to the activation of gene expression [27]. Vernalization activated the Vrn1 transcript by changing the ratio of H3K4me3 and H3K27me3. Vernalization enhanced the H3K4me3 expression level of Vrn1 and Vrn3 with no concurrent change in H3K27me3 in winter wheat. Vrn2 repression is associated with methylation and a lower H3K27me3 methylation level, but the enrichment of H3K27me3 may have a role in repressing Vrn2 [28]. In barley, however, Vrn1 transcripts were up-regulated during vernalization regardless of whether under long or short days, whereas the expression levels of Vrn3 and Vrn2 had no obvious changes under short days. It is thus speculated that Vrn1 is a direct target gene of vernalization. Researchers found that vernalization started the epigenetic regulation of the Vrn1 chromatin state, but no similar phenomenon was observed in Vrn2 or Vrn3, further verifying that Vrn1 is a direct target gene of vernalization. In Arabidopsis, vernalization-induced flowering is mediated by the epi-
genetic regulation of the floral repressor FLC, but in barley or other cereals, is mediated by the epigenetic regulation of the floral activator Vrn1. Vernalization alters the methylation level of Vrn1 and increases the levels of the active histone 3 lysine 4 trimethylation (H3K4me3) and suppresses the levels of histone 3 lysine 27 trimethylation (H3K27me3) [29].

Vrn2, initially mapped on chromosome 5A, as a dom-
inant repressor of flowering time that is downregulated by vernalization, is composed of two tandem duplicated CCT domain (CONSTANS, CO-like, and TOC1) genes ZCCT1 and ZCCT2 with a putative zinc finger in the N-terminus and a CCT domain in the C-terminus, and reduction of the expression of ZCCT1 is closely associ-
ated with the acceleration of flowering time. They have no orthologs of Vrn2 in rice or Arabidopsis, so Vrn2 may be a special regulatory element during the evolution of wheat [17]. Vrn3 encodes a RAF kinase inhibitor-like protein that positively regulates wheat flowering and shows high similarity to FLOWERING LOCUS T (FT) in Arabidopsis. Vrn3 is induced by long days and acts as a bridge linked to the vernalization and photoperiod pathway, which then further accelerates reproductive apex development [18]. Therefore, an active Vrn1 or Vrn3 can accelerate flowering, but the activation of Vrn2 re-
quires vernalization in plants to flower. Vrn-D4, derived from the insertion of part of the long arm of chromo-
some 5A (including Vrn-A1) into the short arm of chromosome 5D, is a homologous gene of Vrn1. As an-
other vernalization-related gene, Vrn-D4 encodes a pro-
tein with high similarity to the Arabidopsis meristem identity protein APETALA1 (API). This gene was first identified in an Australian wheat cultivar Gabo that made an important contribution to the development of the spring growth habit in ancient wheat from South Asia [30, 31]. Detailed information on the participation of these genes in vernalization pathways was shown in Table 1.

Before vernalization, the expression level of Vrn1 and Vrn3 are low; a phenomenon that has a negative effect on wheat flowering. However, prolonged cold exposure increases Vrn1 transcripts to promote rapid flowering both under short and longer days, and the higher expression of Vrn1 at the shoot apex can facilitate the development and differentiation of wheat inflorescence meristems, whereas the expression of Vrn1 in the leaves participates in the long-day flowering response pathway. However, the expression level of Vrn2 decreases after vernalization. At the low Vrn2 expression level, long days induce the expression of Vrn3/TaFT1. Vrn1, Vrn2, Vrn3, and some other association genes are involved in a positive regulatory feedback loop that aims to balance the expression levels of positive and negative regulatory elements, finally regulating the transition of the apex from the vegetative stage to the reproductive stage [32–36]. Vrn1 can bind the promoter of Vrn3 in vernalized plants, and also can target Vrn2 that were repressors of flowering that are down-regulated in vernalized plants [33, 37]. As a duplicated copy of Vrn-D1, Vrn-D4 is pivotal for ancient wheat from South Asia to develop a spring growth habit, and expresses in the leaves and ac-
cumulates after prolonged exposure to low temperature. Furthermore, Vrn-D4 is expressed earlier than other VRN1 genes in the absence of vernalization, and induced mutations in this gene resulted in delayed flowering [31]. NUCLEAR FACTOR-Y (NF-Y) belongs to the HEME ACTIVATOR PROTEIN (HAP) transcription factor family. Previous studies showed that there were dif-
ferent NF-Y proteins involved in the integration of wheat vernalization and the photoperiod pathway [39–41]. Prior to vernalization treatment, the products of Vrn2 competed with other CCT-domain proteins (such as the photoperiod gene CO, which is the functional homolog of CO in wheat) to interact with NF-Y transcrip-
tion factors to inhibit the transcription of Vrn3. Further influence of this interaction is to facilitate or postpone flowering depending on the subunits in the NF-Y complex and the new formation of the complex includ-
ing the type of CCT domain proteins. In rice and Arabidopsis, the FT protein acts as a long-distance flow-
ering signal (florigen) that moves from the leaves to the shoot apical meristem via the phloem and promotes flowering in a wide range of plant species [42, 43]. TaFDL, a homologous gene of FD/OsFD1, exists in the stem apical meristem of bread wheat, and the VRN3 protein can form a functional protein complex with
| Gene | Chromosome location | Protein product | Description | Functions | Mutant phenotype | Gene number in IWGSC1.0 | Position in IWGSC1.0 | References |
|------|---------------------|-----------------|-------------|-----------|-----------------|-------------------------|-----------------------|------------|
| Vrn1 | 5A, 5B, 5D          | Encodes a MADS-box transcription factor, also named as WAP1 or TaVRF1 | Induced by prolonged cold, long photoperiod | Not only promotes the apex transition to generative development, but also activation of long day response in leaves | Recessive alleles at all Vrn-1 homoeoloci confer a winter growth habit (vernalization sensitive), whereas one or more dominant alleles at Vrn-1 homoeoloci result in a spring growth habit (vernalization insensitive). | TraesCS5A01G391700, TraesCS5B01G396600, TraesCS5D01G401500. | 5A:587423056–587,423,240, 5B:573815719–573,815,903, 5D:467184094–467,184,278. | [16, 22, 23] |
| Vrn2 | 5A                  | Encodes a protein containing a putative zinc finger and a CCT protein-protein interaction domain | Down-regulated when plants are vernalized | A dominant repressor of flowering | Functional mutations in the ZCCT genes result in a spring growth habit and early flowering | No | No | [17] |
| Vrn3 | 7B                  | Encodes a mobile protein, homologous to the Arabidopsis FLOWERING LOCUS T (FT) | Induced by vernalization and long days | Accelerates reproductive apex development | Transgenic wheat plants overexpressing Vrn-1B have an extra-early flowering phenotype without the need of vernalization | TraesCS7B01G013100 | 7B:9700818–9,704,363 | [18] |
| Vrn4 | 5D                  | Encodes a MADS-box transcription factor highly similar to VRN1 | VRN-D4 locus originated by the insertion of a large segment from chromosome arm 5AL into chromosome arm 5DS | Vrn-D4 likely operates upstream of the positive regulatory feedback loop connecting Vrn1, Vrn2 and Vrn3. | The mutation flowered later than plants carrying the wild type allele | No | No | [30, 31] |
| VER2 | 2D                  | Encodes a nucleocytoplasmic carbohydrate-binding protein, a jacalin-like lectin, with high affinity for glucan and galactose | After vernalization, VER2 accumulates predominantly in the nucleus in shoot tips and young leaves | Nuclear-localized VER2 interacts with O-glucan-modified TaGRP2 to relieve the repression on tavrn1 transcript accumulation and to promote flowering in hexaploid wheat | Vrn2 downregulated in winter wheat results in flowered later | No | No | [47, 50] |
| TaGRP2 | No.              | Glycine-rich RNA binding protein | TaGRP2 is dynamically O-glucanylated during vernalization | TaGRP2 binds to the pre-mRNA of Vrn1 and inhibits Vrn1 expression | TaGRP2-Ri plants accelerated flowering compared with wild type | No | No | [50, 51] |
| TaVRT2 | 7A, 7B, 7D      | Encodes a predicted protein of 226 amino acids, belongs to the hMADS 11 | Accumulate in winter wheat during the vegetative phase and decline towards the transition to the reproductive phase. | The presence of Vrn2 and TaVRT-2 transcripts early during long time exposure could reduce or delay the expression of Vrn1 | Mutation of TaVRT-2 results in the advance of flowering | No | No | [48] |
| TaGI  | 3A, 3B, 3D        | Encodes a nucleoplasmically localized protein which contains 1174 amino acid residues | The patterns of TaGI rhythmic expression in leaves are regulated by circadian clocks, but can be disturbed by light/dark | Functions in mediating photoperiodic flowering, controlling circadian rhythms and phytochrome signaling | Mutations in the TaGI gene cause delayed flowering only in long day photoperiod | TraesCS3A01G116300, TraesCS3B01G135400, TraesCS3D01G18200. | 3A:84189859–84,191,364, 3B:117978502–117,930,007, 3D:71969619–719,696,287 | [63, 64] |
| Gene | Chromosome location | Protein product | Description | Functions | Mutant phenotype | Gene number in IWGSC1.0 | Position in IWGSC1.0 | References |
|------|---------------------|-----------------|-------------|-----------|-----------------|------------------------|---------------------|------------|
| WSOC1 | 4DL | Is a member of the monocot SOC1-like gene family | WSOC1 expression is affected neither by vernalization nor photoperiod, whereas it is induced by gibberellin at the seedling stage | WSOC1 functions as a flowering activator like SOC1 in Arabidopsis | Downregulated of WSOC1 results in delayed flowering | TraesCS4D01G341700 | 4D:498394464-498398154 | [71] |
| WPCL1 | No. | Encodes a MYB transcription factor belonging to the GARP family | Maybe regulated by circadian clock | Controlling the early flowering phenotype in the einkorn wheat mutant | Deletion of WPCL1 leads to flowering even under short-day conditions | No | No | [60, 61] |
| TaHD1 | 6A, 6B, 6D | Encodes a transcription factor with zinc finger motif and nuclear localization signals, also called CO2 | Can regulated by long-day condition and circadian clock | Directly regulate vernalization gene under long-day condition | The co-mutant show a delayed flowering response under long-day environment | TraesCS6A01G289400; TraesCS6B01G319500; TraesCS6D01G269500. | 6A:586595153-586,599,481; 6D:466221190-466,223,373; 6B:573216947-573,219,055. | [44, 62] |
| TaPHYC | 5A, 5B, 5D | No | Long-day induced wheat PHYC forms signaling active homodimers and translocate into the nucleus | Promotes wheat flowering under inductive photoperiods | The loss of function of wheat PHYC results in altered expression of circadian clock and photoperiod genes and a dramatic delay in flowering under long days | TraesCS5A01G391300; TraesCS5D01G391300; TraesCS5B01G396200. | 5A:586659513-586,659,981; 5D:466221190-466,223,373; 5B:573216947-573,219,055. | [60, 61] |
| Ppd1 | 2A, 2B, 2D | Encodes a pseudo-response regulator (PRR) protein with a CCT domain, also named taprr37 | Induced by long-day | Can regulated vernalization genes and participate in circadian clock function | Knockdown of Ppd1 made the wheat delayed flowering | TraesCS2A01G081900; TraesCS2D01G079600. | 2A:36936362-36,938,400; 2D:33952488-33,955,629. | [8, 57] |
TaFDL to bind the CArG box domain which is located in the promoter region of Vrn1 in vitro, leading to transcriptional activation of Vrn1 [44].

Another vernalization-induced gene vernalization-related 2 (VER2) was identified to encode a nucleocytoplasmic carbohydrate-binding protein. VER2 constitutes a jacalin-like lectin and has a close relationship with GlcNAc and galactose. This gene not only impacts wheat flowering, but also is involved in the regulation of spikelet development. Protracted exposure to low temperatures enhances the integral O-GlcNAcylation levels. However, if plants are subjected to high temperatures following cold treatment, the integral O-GlcNAcylation levels in plants will decrease. VER2 accumulated in the nucleus in the shoot tips and young leaves when wheat long-term exposure to low temperature [45–47], and VER2 can specifically recognize O-GlcNAc-modified proteins in vernalized wheat plants. Wheat vegetative to reproductive transition-2 (TaVRT-2) is a member of the StMADS-11 clade of flowering repressors and encodes a predicted protein of 226 amino acids that is also involved in the flowering pathway in wheat. In addition to the existing conserved domains in the TaVRT-2 protein is an MIKC structure (M, MADS domain; I, intervening region; K, K box; C, C-terminal domain), as well as another conserved bipartite nuclear targeting sequence in the MADS domain, and several putative phosphorylation sites [48].

T. aestivum glycine-rich RNA binding protein 2 (TaGRP2) encodes an RNA-binding protein and AtGRP7 is an orthologous gene in Arabidopsis involved in the direct binding to transcripts of genes that participate in flowering regulation and resistance to stress conditions [49]. In the first intron of the Vrn1 pre-mRNA, there is a critical regulatory region that is regarded as the RNA binding site following transcription. Before vernalization, TaGRP2 can directly bind to this binding site of Vrn1 to prevent transcript accumulation. After vernalization, the role of the O-GlcNAc signaling-mediated Vrn1 transcripts in the flowering transition stage in winter wheat can be activated. Phosphorylated VER2 (VER2-P) transfers into the nucleus and then gathers in the shoot tips and young leaves, physically interacting with the RNA-binding protein TaGRP2 that is O-GlcNAc modified, and the nuclear-localized VER2 interacts with the O-GlcNAc-modified TaGRP2 to decrease the inhibitory action on Vrn1 expression [50, 51]. Furthermore, SNP at the binding site of the TaGRP2 protein in the Vrn-A1 first intron was significantly associated with heading date after vernalization, and plants with 3 SNPs at this binding site headed significantly earlier than those with 1 SNP [52].

Additionally, prior to vernalization in winter wheat, TaVRT2 (VEGETATIVE TO REPRODUCTIVE TRANSITION 2) encodes a MADS box gene with sequence similarity to the Arabidopsis SHORT VEGETATIVE PHASE (SVP) [48], which is regulated independently by photoperiod and vernalization and accumulates during the vegetative phase. It can also directly bind the CArG box of the Vrn1 promoter in vivo to inhibit its activity, and this inhibition is enhanced by VRN2. Once vernalized, the expression of TaVRT2 and Vrn2 are repressed, thus resulting in the gradual accumulation of Vrn1. In the shoot apical meristem (SAM), the activation of Vrn1 induces the transition of the stem apical meristem from the vegetative to the reproductive phase [53].

Photoperiod and other factors cross talk with vernalization signaling pathways

Based on the ability of reproductive growth to be initiated by photoperiod, crops can be classified into long day (LD) or short day (SD) species. LD plants can promote flowering by an over-threshold day length, but a sub-threshold day length can make SD plants flower. Plants growing under long day conditions can flower faster by boosting the transition to reproductive growth. As a long-day crop, wheat can adapt to a broad range of climatic and environmental conditions due to its insensitivity to photoperiod (day length). Wheat can punctually flower when growing under SD conditions (illumination time less than 10 h) under vernalization, and the plants foretell external environmental changes and adjust the flowering time appropriately through photoperiod information [54]. Plants have its own photoreceptors to perceive light signaling, and they can also accommodate the rhythm of light and dark to readjust their circadian clock. Research on flowering time controlling in Arabidopsis also not only concerned day length, but also photoreceptors and circadian clock [55, 56]. Many major components have been identified in Arabidopsis, but only a handful of homologous genes have been cloned in wheat, and the molecular mechanism of the photoperiodic network in wheat is not clear.

In wheat, the photoperiodic regulation of flowering is determined by the dominant genes Photoperoid-A1 (Ppd-A1), Photoperoid-B1 (Ppd-B1), and Photoperoid-D1 (Ppd-D1) located on chromosomes 2A, 2B, and 2D, respectively, and they can reduce the retardation of heading stage under SD conditions by controlling wheat sensitivity to photoperiod [54]. The barley Ppd-H1 gene has been cloned by positional cloning and codes for a pseudo-response regulator (PRR) protein with a CCT domain as a kind of genes were involved in circadian clock. The recessive Ppd-H1 barley mutation exhibits a later flowering time under long-day, but with no short-day phenotypic effect [8, 57]. Ppd-D1a is a homologous gene of the Ppd-H1 in hexaploid wheat with early flowering, other than barley, the semi-dominant Ppd-D1a wheat mutation has a phenotype that flowers rapidly in short-day or long-day environments [57]. In addition, Ppd-1 has a close relationship to the
development of wheat spikelet via promoting the expression level of Vrn3 that is a hub between these two pathways, and the Ppd-D1 is the main active site to regulate wheat heading and flowering dates in the Yellow and Huai winter wheat region, followed by Vrn-B1 and Vrn-D1 [5, 58]. Early studies discovered that the factors that influence photoperiodic flowering include day length and different photoreceptors. When a red/far-red-light photoreceptor has an optical signal that contains red light, this facilitates the accumulation of physiologically active Pfr (PHYB–PHYC heterodimers and PHYC:PHYC homodimers). Under far-red light, Pfr is transformed into the deactivated Pr form. PHYTOCHROME C (PHYC) can independently activate the transcription of PPD1 and the circadian clock output genes CO2/TaDH1 under irradiation [59, 60]. GZ3260 is detected in einkorn wheat and encodes a member of the MYB transcription factor family and is a homolog of PCL1 and LUX in rice and Arabidopsis, respectively. It also named WHEAT LUX ARRHYTHMO (LUX)/PHYTOCLOCK 1 (WPCL1) and might suppress Ppd-1 transcription by combining in the promoter region of Ppd-1. As observed in Arabidopsis, the WPCL1 gene expresses in the daytime and the transcriptional level of the WPCL1 gene achieve a high level at night [60, 61]. The recessive pdp-H1 allele in barley can enhance the circadian rhythm gene CO homologs and is associated with a decrease in FT expression, which is consistent with the late flowering phenotype. The T. aestivum Heading date 1 (TaHD1) gene, also called CONSTANS 2 (CO2), encodes a transcription factor with a zinc finger motif, a CCT domain, and nuclear localization signals, and is the homolog of CO in wheat, and its expression pattern follows a diurnal rhythm under long days. TaHD1 can compete with VRN2 to integrate the NF-Y transcription factors to form a complex, and plays an opposing role to VRN2 to equilibrate the decreased transcripts of Vrn3 that are inhibited by VRN2 [44, 62]. The T. aestivum GIGANTEA (TaGI) gene works upstream of CO and encodes a nucleoplasmically-localized protein that contains 1174 amino acid residues, which is initiated by photoperiod and then expressed in the leaves at the seedling stage. It is controlled by the circadian clock under a light/dark cycle and produces a bulky protein complex that binds to the critical region in the CO gene promoter [63, 64]. ODDSOC2 is another MADS box transcription factor, that can indirectly restrain flowering, but when plants express Vrn1 or long time to prolong cold, the expression level of this gene will be down-regulated [38, 65, 66].

When given wheat enough vernalization and photoperiod, differences exist among wheat varieties that are regulated by Eps genes. Narrow-sense earliness per se (Eps) of wheat is the third effector that influences wheat heading stage equivalently to the autonomous flowering pathway in Arabidopsis. Wheat always displays Eps when the seeds are subjected to full vernalization and are grown under long days [12, 67]. No major genes have been cloned for this character, and the molecular mechanisms and wheat cultivars of different alleles/allelic combinations have scarcely been reported. Eps genes are supposed to be involved in various periods of wheat heading stage and work independently. The Eps-Am1 gene was identified as the wheat ortholog of circadian clock regulator EARLY FLOWERING 3 (ELF3) in T. monococcum [12]. Meanwhile, an Eps quantitative trait locus (QTL) mapped on the 1DL based on different types of molecular markers also included TaELF3, which had functional similarity to the T. monococcum Eps-Am1 locus. Premature stop codons or deletion of the TaELF3 gene results in its failure to function and an early flowering phenotype, and thus may be a repressor of flowering [67–70]. The molecular mechanism by which Eps regulates heading stage is unknown, and the location of these genes suggests that they are possibly cross-talking with vernalization genes and photoperiod genes, which may overspread their influence. Another important factor influencing wheat heading stage is plant hormones. In Arabidopsis, GAs, as a class of hormones, can up-regulate the transcription of SOC1 (SUPPRESSOR OF OVEREXPRESSION OF CO1), which encodes a MADS-box gene to accelerate flowering [70]. Wheat SOC1 (WSOC1) in polyploid wheat is homologous with SOC1 in Arabidopsis thaliana, can partially restore the Arabidopsis SOC1 mutation. Its expression is induced by GA at the seedling stage, but cannot be regulated by vernalization and photoperiod. This gene was expressed in young spikes initiated before the reproductive transition, but was preferentially expressed in leaves and was associated with the expression of WAP1/Vrn1 in the flowering pathway [71]. The existence of both GA and Vrn1 precipitate the up-regulation of WSOC1 and WFL (Wheat FLO/LFY) to accelerate process of wheat spike development [72]; WFL is a gene identified in the floral meristem associated with young spike development that is widely conserved in both monocots and dicots. In conclusion, both Vrn1 and GA are important to wheat shoot apical meristem and spike development under short days by accelerating the expression of SOC1–1 and WFL [73, 74]. The detailed information and function of genes related to wheat heading stage are described in Table 1.

Part 2. Abundant genetic variation of signal components associated with wheat heading stage
As a heterogeneous hexaploid crop, wheat genome possesses the abundant genetic variation, such as insertions, deletions, point mutations, copy number variation and haplotype diversity etc. Many studies
indicated that genetic variants can alter vernalization requirement and photoperiod response to regulate heading stage and flowering times in wheat [75, 76].

**Genetic variation of vernalization-related genes influence wheat heading stage**

As a pivotal regulatory element, the allelic diversity of genes regulating the wheat growth life cycle is extensively adapted towards dealing with constant changes and pressure. The variation in Vrn1 is the most abundant. The existence of one dominant allele of at least one Vrn1 gene homolog (Vrn-A1, Vrn-B1, Vrn-G1, or Vrn-D1) determines the spring growth habit in wheat. Several important domains exist in the promoter region of the Vrn1 gene, for example, CArG-box is a common binding site for MADS-box proteins located 180 bp upstream of the transcription initiation site, and Vrn-box is a sequence (TTAA AAACCCCTCCCC) of 16 bp that determines whether the wheat requires vernalization to promote flowering. Between the CArG box and Vrn-box is a G-box (CACGTG), which is a binding site for bZIP transcription factors [16].

In the pre-mRNA of Vrn1, a crucial region contributes to the interaction between TaGRP2 and VRN1. Therefore, the allelic variation of Vrn1 relies on mutations in the promoter region or the intron1 of the A, B, and D genome. An analysis of the mutations in the Vrn-A1, Vrn-B1, and Vrn-D1 genes in accessions with a spring growth habit are shown in Table 2. The number of distinct mutations that were reported in the promoter region or intron1 of Vrn1 can be described as follows. The Vrn-A1a gene has three alleles termed Vrn-A1a.1, Vrn-A1a.2, and Vrn-A1a.3. Vrn-A1a.1 and Vrn-A1a.3, also known as Vrn-A1a, which were identified in hexaploid and tetraploid wheat separately. They are associated with a foldback repetitive element insertion and a duplicated region in the promoter, resulting in an intense impact on the vernalization response. Vrn-A1a.2 displays a 16-bp deletion and four single nucleotide deletions within the MITE insertion compared to Vrn-A1a.1 [76, 77]. Six types of Vrn-A1b allele variants exist that have 20-bp deletions at 157 bp, and Vrn-A1b.1-Vrn-A1b.5 differ from each other only on account of a polymorphism of the A-tract within the Vrn-box, and are indicated in turn as AAAAA, AAACC, AAACC, AAAC, and AAACA. Nevertheless, Vrn-A1b.6 takes a “C > G” transversion in the C-rich fragment than vrn-A1b.4 and Vrn-A1b.1. Then Vrn-A1b.2, Vrn-A1b.5, Vrn-A1b.6 detected for “spring” but vrn-A1b.3, vrn-A1b.4 detected for “winter” variants [76, 77]. Vrn-A1c, Vrn-A1d, Vrn-A1e, Vrn-A1f, and a series of allele variants have been characterized in polyploid wheat. Compared with the recessive vrn-A1 allele, Vrn-A1c, Vrn-A1l, and Vrn-A1h have a deletion in intron1, respectively. A variant with a 7.2-kb deletion in intron1 was identified in the tetraploid cultivar ‘Langdon,’ and this allele was named Vrn-A1l. Vrn-A1u is the variant of the CArG-box is a common binding site for MADS-box proteins located 180 bp upstream of the transcription initiation site, and Vrn-box is a sequence (TTAA AAACCCCTCCCC) of 16 bp that determines whether the wheat requires vernalization to promote flowering. Between the CArG box and Vrn-box is a G-box (CACGTG), which is a binding site for bZIP transcription factors [16].

In the Vrn-B1 locus, one distinct deletion of 6850 bp occurs in intron1 in the Vrn-B1a mutant, position is counting start with intron1 in TDB (Triple Dirk B, vrn-A1/Vrn-B1/vrn-D1). TDB is a spring wheat that carries a dominant Vrn-B1 allele, and TDC (Triple Dirk C, vrn-A1/vrn-B1/vrn-D1) carries recessive alleles at the three Vrn-1 loci with a winter growth habit. In addition to this large deletion, two single nucleotide polymorphisms (SNPs) in intron1 and intron2 were detected in TDB and TDC (Fu et al., 2005). Vrn-B1b is a mutation with a 6850-bp deletion at + 386 bp and a 37-bp deletion at + 7992 bp in TDC [80]. An 817-bp deletion from 798 to 1614 bp, and a 432-bp duplication upstream of this region was found in the T. aestivum cultivar Saratovskaya29, suggesting that distinct alterations within the Vrn-B1c allele may influence the flanking sequence of intron1 by breaking a putative binding site [81, 82].

Based on the Vrn-D1 genome, four alleles have been identified and three of them occur in intron1, while only one allele was found in the promoter region. Vrn-D1a was first identified in TDE (vrm-A1/vrn-B1/vrn-D1), which is a spring wheat with the dominant Vrn-D1 allele. It has a 4235-bp deletion (625–4859 bp) in intron1 compared to TDC [79]. Vrn-D1b has the same deletion in intron1 in comparison with the Vrn-D1a allele, and in addition, a single nucleotide C to A mutation at 161 bp in the CArG box results in 32-day-later heading [83]. Vrn-D1s has an 844-bp insertion in intron1 of T. spelta strain PI 348700 and this fragment is a novel transposable DNA element (named DTA_Chimera_KF800714) [84]. The fourth allele constitutes the only one insertion mutant in the promoter region of Vrn-D1c. Cultivars with Vrn-D1c exhibit earlier heading and flowering than others with the recessive allele vrn-D1 without vernalization, but display extension heading when
| Gene     | Accession number of NCBI | Mutation region       | Mutation pattern                                                | Phenotype    | Ployploid wheat                                      | Reference |
|----------|--------------------------|-----------------------|----------------------------------------------------------------|--------------|-----------------------------------------------------|-----------|
| vrn-A1   | AY747600                 | Promoter              | A 222-bp foldback element insertion in the promoter region     | Later heading| T. aestivum cultivar Triple Dirk C                    | [79]      |
| Vrn-A1a  | AY616458                 | Promoter              | A 222-bp foldback element insertion in the promoter region     | Early heading| T. aestivum cultivar Triple Dirk C                    | [17]      |
| Vrn-A1a.1| AY616458                 | Promoter              | A 222-bp foldback element insertion in the promoter region     | Early heading| T. aestivum cultivar Triple Dirk C                    |           |
| Vrn-A1a.2| KR782255                 |                       | A 16-bp deletion and 4 single nucleotide deletions within the   | Early heading| Triticum compactum cultivar Tiroler Frihe Binkel     | [76]      |
| Vrn-A1b  | AY616461                 | A-tract within the VRN-box: AAAAT | A 20-bp deletion at−157 bp                                      | Early heading| T. aestivum cultivar Spring Marquis                  |           |
| Vrn-A1b.1| KM047646                 | A-tract within the VRN-box: AACCC | A 20-bp deletion at−157 bp                                      | Early heading| Triticum dicocoides strain PI 264954                | [76]      |
| Vrn-A1b.2| KM047641                 | A-tract within the VRN-box: AAACC | A 20-bp deletion at−157 bp                                      | Early heading| Triticum dicocoides strain PI 233288                | [76]      |
| Vrn-A1b.3| KM047647                 | A-tract within the VRN-box: AAACC | A 20-bp deletion at−157 bp                                      | Early heading| Triticum dicocoides strain PI 466941                | [76]      |
| Vrn-A1b.4| KM047651                 | A-tract within the VRN-box: AAAAC | A 20-bp deletion at−157 bp                                      | Early heading| Triticum dicocoides strain PI 1000212               |           |
| Vrn-A1b.5| KM047652                 | A-tract within the VRN-box: AAACA | A 20-bp deletion at−157 bp                                      | Early heading| Triticum dicocoides strain PI 1000212               |           |
| Vrn-A1b.6| KT692944                 |                       | Takes along a "C- > G" transversion in the C-rich fragment than Vrn-A1b | Early heading| Triticum dicocoides strain PI 1000212               |           |
| Vrn-A1c  | AY747599                 | Intron1               | Intron 1 deletion in the A genome copy                          | Early heading| T. aestivum cultivar IL369                           | [79]      |
| Vrn-A1d  | AY616462                 | Promoter              | A 32-bp deletion, which included the complete HDD region and   | Early heading| Triticum turgidum subsp. dicocoides                 |           |
| Vrn-A1e  | AY616463                 | Promoter              | A 54-bp deletion, which included the CARG box and HDD regions  | Early heading| Triticum turgidum subsp. dicocoides                 |           |
| Vrn-A1f  | DQ146421                 | Promoter              | An 8-bp deletion in the region between −128 and −120, and a   | Early heading| Triticum monococcum subsp. aegilopoides             |           |
| Vrn-A1h  | GQ451745                 | Promoter              | A 20-bp deletion near the CARG box                              | Early heading| Triticum monococcum subsp. aegilopoides             |           |
| Vrn-A1i  | KM016790                 | Promoter              | Nucleotide substitution in promoter region                      | Weak effect on the vernalization response               | Triticum turgidum strain Ps53874                  |           |
| VRN-A1u  | GQ451737                 | Intron1               | A 1.4-kb deletion in intron1                                    | Later heading| Triticum urartu isolate Tu54                        | [78]      |
| vm-B1    | AY747604                 | Promoter              | A 20-bp deletion near the CARG box                              | Early heading| T. aestivum cultivar Triple Dirk C                   | [79]      |
| vm-B1a   | AY747603                 | Intron1               | A 20-bp deletion near the CARG box                              | Early heading| T. aestivum cultivar Triple Dirk B                   | [79]      |
| vm-B1b   | FJ66015                  | Intron1               | A 20-bp deletion near the CARG box                              | Early heading| T. aestivum                                          | [80]      |
| vm-B1c   | HQ593668                 | Intron1               | A 20-bp deletion near the CARG box                              | Early heading| T. aestivum                                          | [81]      |
treated with prolonged low temperature. The Vrn-D1c genotype is important to bread wheat cultivars from the Yellow and Huai Valley areas, and was discovered in Yunong 876 with a 174-bp fragment insertion in the 5′-UTR at 601 bp of the Vrn-D1 gene [85].

Copy number variation (CNV), as a type of gene mutation, play the vital role in the regulation of extensive adaptability. The CNV of Vrn-A1 alleles investigated also influenced gene expression and showed a slight influence on wheat heading stage and flowering time. One, two, and three Vrn-A1 copies dictate the time under longer period of exposure to cold conditions that a plant requires to accelerate flowering, and plants with an increased CNV require longer cold treatment to reinforce flowering [75]. A SNP in exon4 of vrn-A1 affected the QTL of winter wheat development genes, and the alleles vrn-A1a and vrn-A1b resulted in early flowering in the wheat cultivars Jagger and 2174, respectively [86]. The diverse variation in VRN1 can be seen in Table 2.

Vrn2 encodes a zinc finger transcription factor and includes two tandem-duplicated CCT domains, ZCCT1 and ZCCT2, with 76% analogy to a putative zinc finger, CONSTANS (CO), CONSTANS-LIKE (CO-like), or TIMING OF CAB EXPRESSION1 (TOC1) [17, 29]. In Arabidopsis, the CCT domain and yeast HEME ACTIVATOR PROTEIN2 (HAP2) act similarly as key elements for the HAP2/HAP3/HAP5 complex to bind to CCAAT boxes in the promoter region of their target genes to regulate their expression. Variation in the CCT domain impacting on the functions of proteins CO, TOC1, Vrn2, and PPD-H1 has been identified several in plants, but rare alleles in the CCT domain have been detected in bread wheat [87]. Plants with homozygous recessive vrn-2 alleles have a spring growth habit, but RNA interference of ZCCT1 in the hexaploid winter wheat cultivar Jagger

### Table 2 The detailed information of genes association with the wheat heading-flowering regulatory network (Continued)

| Gene      | Accession number of NCBI | Mutation region | Mutation pattern | Phenotype | Ployploid wheat | Reference |
|-----------|--------------------------|-----------------|------------------|-----------|-----------------|-----------|
| Vrn-B1ins | HQ130482                 | Intron1         | 817 bp deletion from 798 to 1614 bp, and 432 bp (from 798 to 1614 bp upstream) duplicated | Early heading | T. aestivum cultivar Saratovskaya29 | [82] |
| vrn-D1    | KR782252                 | Promoter        | A retrotransposon insertion in the promoter | Early heading | Triticum turgidum cultivar Zerdakia | [76] |
| Vrn-D1a   | AY747606 No.             | No.             | No.              | Later heading | T. aestivum cultivar Triple Dirk C | [79] |
| Vrn-D1b   | AY747597                 | Intron1         | A 4235-bp deletion in intron1 (625–4859 bp) | Later heading | T. aestivum cultivar Triple Dirk E | [79] |
| Vrn-D1c   | JQ406528                 | Intron1         | Deletion in intron 1 identical to Vrn-D1a allele and a single nucleotide mutation C to A at −161 bp in CArG box in promoter region | Later heading | T. aestivum | [83] |
| Vrn-D1s   | KP721800 Promoter        | An 174-bp fragment was inserted into the 5′-UTR at −601 bp (relative to ATG) of the Vrn-D1 gene | Early maturity | T. aestivum | [85] |
| Vrn-D1s   | KF800714                 | Intron1         | An 844-bp insertion which is a novel transposable DNA element in intron1 | Early heading | Triticum spelta strain PI 348700 | [84] |
| vrn-B3    | DQ890162                 | Promoter        | A 5295-bp repetitive element insertion that is absent in the CS allele associated with late flowering | Later heading | T. aestivum cultivar Chinese Spring | [18] |
| Vrn-B3a   | DQ890165                 | Intron1         | An exact 5295-bp fragment inserted 591 bp upstream from the start codon | Later heading | T. aestivum cultivar CS(Hope7B) | [18] |
| Vrn-B3b   | JN627519                 | Promoter        | An exact 890-bp fragment was inserted into the 5′ UTR (untranslated region) at −429 bp | Later heading | T. aestivum | [93] |
| Vrn-B3c   | JQ082311                 | Promoter        | A 20-bp deletions at −3543 bp and a 4-bp deletion at −3591 bp compared with Vrn-B3a | Later heading | T. aestivum cultivar J874–109 | [93] |

Note, No. Means there are no more information refer to Shi et al. BMC Plant Biology (2019) 19:6
can also accelerate flowering [88, 89]. If alleles for winter growth habit are present at all VRN1 loci, then allelic variation for VRN2 would be detected. The ZCCT1 and ZCCT2 proteins from nonfunctional vrn-2 alleles have mutations at positions 16, 35, or 39 of the CCT domain that are conserved between the CCT and HEME ACTIVATOR PROTEIN2 (HAP2) proteins. Therefore, both ZCCT-B2 gene mutations are sufficient to make tetraploid wheat exhibit a spring growth habit [89].

Non-vernalized synthetic vrn2-null plants flowered 118 days earlier than the winter control, and showed a limited vernalization response. In the mutant, Vrn-B2 expressed higher than the Vrn-D2 and displayed an inhibiting action under partial vernalization, but without vernalization, it only carried Vrn-B2 or Vrn-D2 in the heterozygous condition. This suggested that different combinations of Vrn-B2 and Vrn-D2 may participate in regulating the vernalization network in mild winter regions [89].

The T. aestivum cultivar Chinese Spring (Hope7B) allele Vrn-B3a has a 5295-bp repetitive element inserted 591 bp upstream in the promoter region and resulted in early flowering, while the vrn-B3 allele in Chinese Spring makes wheat flower later. Vrn-B3b is an exact 890-bp fragment inserted into the 5’ untranslated region (UTR) at 429 bp identified in one Chinese landrace cultivar Chadianhong. Then, a 20-bp deletion at 3543 bp and a 4-bp deletion at 3591 bp when compared with Vrn-B3a in Ji874-109 were discovered and designated as the Vrn-B3c allele [18]. The condition of the Vrn3 alleles is illustrated in Table 2.

Recent studies relating to the characterization of the Vrn-D4 locus, which arose via the duplication of Vrn-A1, discovered the existence of a SNP in the conserved TaGRP2 RIP-3 region that influences the binding ability of TaGRP2 with Vrn1 [30].

**Diverse variants of photoperiod (Ppd-1) genes play different roles in wheat heading stage regulation**

Large deletions, insertions, SNP, and copy number variations in Ppd-D1a, Ppd-D1b, and Ppd-D1c have been reported to influence wheat sensitivity to photoperiod [57, 90, 91].

A 2089-bp deletion in the promoter region of **Ppd-D1** in the photoperiod-insensitive wheat cultivar ‘Ciano 67’, regarded as the **Ppd-D1a** allele, was detected and co-segregated with wheat early flowering and has four varieties-Ppd-D1a.1, Ppd-D1a.2, Ppd-D1a.3 [8, 57, 91]. These four alleles with different length deletions in the promoter region appear in Winter-Abukumawase, “GS-100”, “GS-105”, and Kanak, respectively. Other than barley, the semi-dominant **Ppd-D1a** mutation in wheat permits plants to flower earlier under both SD and LD conditions, while in barley there is no phenotypic effect under SD conditions [8, 57]. Three SNPs were detected in the promoter of wheat cultivars of Winter-Abukumawase (Ppd-A1b.2) and Chinese Spring (Ppd-A1b.1), with two In/Dels of 1-bp more in Ppd-A1b.2. In the 5’ UTR, a 308-bp insertion was identified in Winter-Abukumawase, which results in an insensitive phenotype regarded as **Ppd-B1a.1, Ppd-B1b.1, and Ppd-B1a.2** separately represent a SNP mutation in the promoter region of Chihokukomugi (Ppd-B1b.1) and Chinese Spring (Ppd-B1a.2) [57, 91] (see Additional file 1). Moreover, some cultivars possessed the photoperiod insensitive **Ppd-D1a** allele and other cultivars had the photoperiod sensitive **Ppd-D1b** allele had been also identified in Huanghui wheat region [92].

Other modalities of mutations have subsequently been reported. ‘Mercia’ flowers earlier under SD conditions than some photoperiod-sensitive varieties due to the existence of a mariner-like transposable element (MLTE) in intron1, which differs distinctly from the 2089-bp deletion in ‘Ciano 67’ [57].

A recent study indicated that some connection exists between the distribution frequency of **Ppd-D1** haplotypes and the geographical environment of the wheat cultivation regions. A total of eight **Ppd-D1** haplotypes were detected. **Haplotype (Hapl-I)** was characterized by a 5-bp deletion in exon 7, and the addition of a 2-kb upstream deletion distinguished **Hapl-II** from **Hapl-I**. **Hapl-III** has an extra TE insertion in intron1 compared with **Hapl-II**, and **Hapl-IV** only possessed the 5-bp deletion in exon7. **Hap-V** carries a 2-kb deletion in the promoter region, a 5-bp deletion in exon7, and a 16-bp insertion in exon8. **Hap-VI** has a 24-bp plus a 15-bp insertion in the 2-kb upstream region [93]. Two new polymorphism combinations in **Ppd-D1** designated as **Hapl-VII** and **Hapl-VIII** were identified in cultivars that originate from the Yellow and Huai Valley of China. **Hapl-VII** is characterized by the absence of a 2089-bp in exon8 and the presence of both TE in intron1 and 5-bp deletion in exon7, while these are absent in **Hapl-VIII** [92]. An additional file shows the haplotypes of **Ppd-1** in wheat in more detail (see Additional file 2).

Some genes also influence flowering time regulation when they exhibit CNV, but no sequence changes. CNV is closely associated with gene expression and there is less chance of CNV occurring in independent alleles of **Ppd1**. However, copy number variations in the **Ppd-B1** locus have been detected with increased gene numbers that results in earlier flowering [75].

It is clear that diverse mutations of these genes associated with wheat heading stage and flowering influence their expressions, resulting in earlier or later flowering under certain circumstances.

**Conclusions**

Based on the current research in wheat, four major pathways exist that regulate wheat heading-flowering...
Fig. 2 (See legend on next page.)
time. Vernalization and photoperiod pathways integrate environmental signals to determine the transition from the vegetative phase to the reproductive phase, while \( \text{Eps} \) and GA pathways act as internal stimuli independently of the environmental signals. GA and \( \text{Eps} \) perform throughout wheat growth and development, while vernalization occurs during the transition from the vegetative phase to the reproductive phase during winter, and directly influences spikelet initiation. In addition to vernalization, photoperiod plays a leading role to control flowering begins from spikelet initiation and determines floret initiation and spikelet development, as indicated in Fig. 1.

A better understanding of the genetic regulatory mechanism of wheat heading stage assisted breeding. In the case of vernalization, \( \text{Vrn1}, \text{Vrn2}, \) and \( \text{Vrn3} \) generate a positive feedback loop and were assisted by \( \text{TaGRP2}, \text{VER2}, \text{TaVRT2}, \) and \( \text{TaFDL2}, \) constituting a vernalization regulatory network initiating wheat heading stage and flowering. Then, the photoperiodic pathway cross-talks with the vernalization pathway via \( \text{Ppd1}, \text{TaPHYC}, \text{TaHD1}, \text{WPCLI}, \) and \( \text{TaGI}. \) \( \text{WSOC} \) and \( \text{LHY} \) also regulate wheat heading stage and flowering time independently via the GA pathway. A schematic summary of the wheat heading stage regulatory network is depicted in Fig. 2. These genes encode members of multitudinous transcription factors, for instance, MADS-box families, some MYB transcription factors, types of Myc and zinc finger families, RNA-binding proteins, and other protein families, and can directly or indirectly interact with each other, thereby supporting the entire wheat heading stage and flowering regulatory network. In spite of lots of researches about heading-flowering based on \textit{Arabidopsis} constructing a relatively prefect network, \textit{Arabidopsis} cannot fully reflect wheat and its relative. Some other components may also exist that participate in the pathway, and the identified genes in the wheat heading-flowering time regulation network are still unclear. Therefore, there are lots of tasks on unknown gene cloning by rapidly growing modern biotechnology, such as high-throughput sequencing, multi-omics profiling, mutant screening and association mapping to offer new exciting insights into future researches. For instance, the \( \text{Vrn2} \) protein interacts with \( \text{CO2} \) (\text{CONSTANTS2}) in vitro and can competitively bind with members of the \text{NUCLEAR FACTOR-Y} (\text{NF-Y}) transcription factor family, but the detailed integration and competitive process of these interactions remains unclear. And if any other hormones participate in the wheat heading stage regulation pathway? The genetic regulation mechanism of hormone and \( \text{Eps} \) is not clear in wheat maybe because of the masking by strong influence of vernalization and photoperoid, \( \text{Eps} \) will be important for fine-tuning some of these traits and understanding their basis is the next major step.

Our knowledge of the regulatory network of wheat heading-flowering can be applied to targeted breeding or other means, including adjusting the duration of the growth phase for the purpose of acclimatizing to different geographical environments.

### Additional files

**Additional file 1:** Table S1
Diversity variations of \( \text{Ppd-D1} \) gene in wheat. (DOC 37 kb)

**Additional file 2:** Table S2
Haplotype information of \( \text{Ppd-D1} \) gene in wheat. (DOC 26 kb)

### Abbreviations

- \( \text{AP1}: \text{APETALA1} \)
- \( \text{CNV}: \text{Copy number variation} \)
- \( \text{CO2}: \text{CONSTANS2} \)
- \( \text{EFL3}: \text{Early Flowering 3} \)
- \( \text{Eps}: \text{Earliness per se} \)
- \( \text{FT}: \text{Flowering time} \)
- \( \text{GA}: \text{Gibberellic acid} \)
- \( \text{HAP}: \text{HEME ACTIVATOR PROTEIN} \)
- \( \text{HRP}: \text{Hemeactivator Protein} \)
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